CONTRIBUCION DEL NUCLEO ARCUATO HIPOTALAMICO PARA LA FUNCION DEL ORGANO SUBFORNICAL EN LA REGULACION DEL BALANCE DE LOS LIQUIDOS CORPORALES Y EL SISTEMA CARDIOVASCULAR

Tesis que para obtener el grado de
Doctor en Ciencias Biomédicas: Fisiología

p r e s e n t a

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Tesis apoyada por D.G.A.P.A., U.N.A.M. y I.C.C.S. de Canadá

1994

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RESUMEN

El núcleo arcuato del hipotálamo (Arc) es conocido tradicionalmente por el importante papel que desempeña en la función neuroendócrina. Sin embargo, recientemente se ha sugerido que está involucrado en el control de la presión arterial y en el balance de los líquidos corporales. En el presente trabajo se realizaron cinco estudios para determinar la función del Arc en dichos mecanismos homeostáticos mediante la determinación de la contribución de este núcleo sobre la función del órgano subfornical (SFO). En el primer estudio se determinó el efecto de infusiones intracerebroventriculares de soluciones hipertónicas de NaCl sobre la actividad de las neuronas del Arc mediante la detección inmunohistoquímica de proteinas tipo Fos en la rata conciente. Se observó marcado de tipo Fos en el Arc después del estímulo hipertónico pero no después de un estímulo hiperosmótico al fluido cerebroespinal. Lo anterior indica que el Arc está involucrado en la homeostasis de los líquidos corporales. En el segundo estudio se determinaron las proyecciones eferentes de los tres subnúcleos que componen al Arc mediante la detección inmunohistoquímica del trazador anterogrado leucoaglutinina Phaseolus vulgaris (PHA-L) en la rata. Fibras y terminales conteniendo PHA-L se observaron en el SFO y en diversas estructuras del encéfalo bien conocidas por participar en el control cardiovascular y en el balance de los líquidos corporales. Las fibras conteniendo PHA-L en el SFO también mostraron neurotensina como se muestra en el tercer estudio. En el cuarto estudio se investigó el efecto de la estimulación del Arc sobre la frecuencia de descarga de las neuronas del SFO en la rata anestesiada con uretano. La estimulación de la región medial del Arc inhibió la frecuencia de descarga de neuronas del SFO localizadas preferentemente en la región dorsal del SFO. Por otro lado, la estimulación de la región dorsal del Arc excitó las neuronas de la región ventral del SFO. En el estudio final, se exploró en la rata anestesiada con uretano el efecto de la estimulación del Arc sobre las respuestas de las neuronas del SFO ante la infusión intrarterial de Angiotensina II o de solución salina hipertónica. La estimulación del Arc resultó en una inhibición o en una potenciación de los efectos de dichas substancias sobre las neuronas del SFO. Estos efectos dependieron de la localización rostrocaudal del sitio de estimulación en el Arc. Todos estos resultados sugieren que el Arc está involucrado en la modulación de los eventos humorales y neurales relacionados al control cardiovascular y del balance de los líquidos corporales.

ABSTRACT

The arcuate nucleus (Arc) has traditionally been known to play an important role in neuroendocrine function. However, recently it has been suggested to be involved in the control of the arterial pressure and body fluid balance. In this research thesis, five studies were done to detemine the role of Arc in these homeostatic mechanisms by investigating the contribution of this nucleus to the function of subfornical organ (SFO). In the first study, the effect of intracerebroventricular infusions of hypertonic NaCl solutions on the activity of Arc neurons was investigated using the immunohistochemical detection of Fos-like proteins in the conscious rat. Fos-labelling in Arc was observed after a hypertonic NaCl stimulus, but not after a hyperosmotic challenge to the cerebrospinal fluids suggesting a role of the Arc in the body fluid homeostasis. In the second study, the efferent projections of the three subnuclei of Arc were determined by means of the immunohistochemical detection of the anterogradely transported tracer Phaseolus vulgaris leucoagglutinin (PHA-L) in the rat. PHA-L labelled fibers and presumptive terminals were observed in several structures previously implicated in cardiovascular control and body fluid balance, including SFO. Additionaly, the PHA-L fibers in SFO were also labelled for the neurotensin peptide, as shown in the third study. In the fourth study, the effect of stimulating Arc on the spontaneous frequency of SFO neurons was investigated in the urethane anesthetized rat. Stimulation of sites in the medial aspect of Arc inhibited the firing frequency of neurons found primarily in the dorsal aspect of SFO. On the other hand, stimulation of sites in the dorsal aspect of Arc excited neurons in the ventral aspect of SFO. In the final study, the effect of Arc stimulation on the response of SFO neurons to intraarterial injections of Angiotensin II or hypertonic saline was investigated in the urethane anesthetized rat. Stimulation of Arc either inhibited or potentiated the effects of these blood borne substances on SFO neurons. These effects on SFO units was dependent on the rostrocaudal location of the site of stimulation in Arc. Taken together, these data suggest that Arc is involved in the modulation of humoral and neuronal events related to body fluid balance and cardiovascular regulation.

INTRODUCCION

Las células de toda la materia viva están constituidas de soluciones de diferentes substancias en agua. La integridad de las funciones de las células depende de la constancia de la composición de las soluciones dentro y alrededor de ellas. Hasta ahora, todos los organismos han desarrollado estructuras sofisticadas así como sistemas y mecanismos para asegurar que la composición de estas soluciones se mantengan dentro de límites estrictos. Cuando se perturba esta composición más allá de sus límites normales, el organismo exhibe una disfunción y puede morir. Esto fue establecido claramente en el siglo XIX por Claude Bernard quien señaló que "... existen muchos mecanismos que cambian y restauran la cantidad de agua y que ponen en marcha un gran número de sistemas para la secreción, exhalación, ingestión y circulación del líquido ingerido y absorbido. A pesar de su diversidad, estos mecanismos convergen para lograr un resultado común: la presencia de una proporción de agua casi constante en el medio interno, condición necesaria para la vida libre..." [Bernard, 1878]. Al respecto, dos mecanismos complejos se implican en el mantenimiento de la constancia del medio interno: uno relacionado al sistema cardiovascular y otro que corrige las perturbaciones en la tonicidad de los líquidos corporales [Brody y Johnson, 1980; Buggy et al., 1984; Mangiapane, 1987; Johnson et al., 1992]. Estos mecanismos son activados cuando estructuras especializadas que detectan cambios en diferentes parámetros fisiológicos proporcionan señales al cerebro que a su vez las integra junto con una variedad de entradas aferentes y entonces inicia la mobilización de acciones efectoras apropiadas para resolver o minimizar los efectos de cualquier perturbación.

Las perturbaciones del medio interno ocurren constantemente, de un momento a otro. Algunas perturbaciones suceden diariamente y algunas otras ocurren quizás solo una

vez a lo largo de la vida de un organismo. Además, estas perturbaciones pueden ser tan triviales que pasan inadvertidas, o tan criticas como para poner en peligro el buen funcionamiento de la vida cotidiana. Por ejemplo, la ingestión de una comida muy jugosa representa una ingestión innecesaria de agua que será excretada. Un segundo ejemplo, la sudoración abundante después de un largo caminar en un dia asoleado representa una pérdida inesperada de agua por lo que disminuye el volumen de los liquidos corporales y por consequencia se tiene una reducción de presión en el sistema de baja presión de la circulación, esta pérdida de agua puede ser remplazada después de satisfacer el apetito por un vaso de limonada fresca. Un último ejemplo, la pérdida de sangre en un accidente disminuye el contenido de los liquidos corporales, baja la presión arterial y desencadena una necesidad intensa de beber agua [Russell et al., 1975]. En estas tres situaciones anteriores, parámetros vitales como la cantidad de agua en el organismo, la concentración de solutos del liquido que baña a las células y del mismo fluido celular así como la presión de la sangre perfundiendo el tejido, son alterados en menor o mayor grado y serán restaurados a sus valores normales por la activación de los mecanismos de regulación de los liquidos corporales y cardiovascular.

Como se indicó anteriormente con estos ejemplos simples, es claro que el balance de los liquidos corporales es un aspecto vitalmente importante de la regulación del sistema cardiovascular. Complementariamente, la regulación de la función cardiovascular es un componente determinante para el mantenimiento del balance de los liquidos corporales. En otras palabras, estos dos mecanismos están acoplados armonicamente y en forma simultanea [Morris, 1982].

Cada vez más, la investigación en este campo fundamenta la existencia de un exquisito acoplamiento entre los mecanismos de regulación de la actividad cardiovascular y los mecanismos de regulación del balance de los liquidos

corporales que se puede encontrar desde los primeros momentos de detección de señales así como durante la integración de la información, hasta el momento mismo de la emisión de las acciones efectoras. Un ejemplo claro de ésto está dado en la función de la zona cerebral conocida como lámina terminalis, la cual puede ser activada por dos tipos de señales, aquellas relacionadas al balance de los líquidos corporales (i.e. cambios en la osmolaridad del plasma) así como por señales relacionadas a la función cardiovascular (i.e. hipovolemia). Adicionalmente, ante perturbaciones del tipo de las descritas arriba, la integridad anatómica de la lámina terminalis es esencial para la expresión de respuestas compensadoras como el beber, el apetito de sal, la liberación de vasopresina (VP) y aldosterona, y la excreción renal de agua y electrólitos [Johnson et al., 1992]. De las estructuras localizadas en la lámina terminalis, se ha demostrado que el órgano subfornical (SFO) es la estructura sensitiva central determinante para el enlace entre la actividad cardiovascular y la actividad de regulación del balance de los líquidos corporales debido a que del SFO surge la intensa conducta de bebida, cambios presores e incremento en la secreción de VP [Mangiapane y Simpson, 1980; Mangiapane et al., 1984] en respuesta al octapéptido exógeno angiotensina II (ANG II) [Ishibashi et al., 1985; Nicolaides et al., 1983; Simpson y Routtenberg, 1973], hormona que es liberada en la circulación por perturbaciones como hemorragia [Russell et al., 1975] o deshidratación [Abdelaal et al., 1976]. Adicionalmente, se ha mostrado que en el SFO al parecer existen natriosensores [Gutman et al., 1988] u osmosensores [Giovannelli y Bloom, 1992; Sibbald et al., 1984] cuya activación por hipernatremia desencadena también las respuestas anteriores [Mangiapene et. al., 1984; McKinley et al., 1986].

Por otro lado, en los últimos años, han aparecido algunas evidencias experimentales que sugieren que el núcleo arcuato del hipotálamo (Arc) puede ser otro ejemplo de una estructura

cerebral enlazada al balance de los líquidos corporales y de la regulación de la presión arterial. Sin embargo, trabajos enfocados al estudio de la participación de esta estructura en dichas funciones no se han realizado. Por lo tanto, el propósito del presente trabajo fué el explorar la participación del Arc en el control de la homeostasis de los líquidos corporales y su contribución como estructura moduladora en la actividad del SFO para la regulación del sistema cardiovascular y del balance de los líquidos corporales.

A. OSMORECEPCION CENTRAL.

Los mamíferos frente a perturbaciones de su medio interno producidas por ejemplo por hipovolemia [Russell et al., 1975], hiperosmolaridad [McKinley et al., 1987; 1988] y/o deshidratación [McKinley et al., 1983; 1988] producen respuestas compensadoras coordinadas de naturaleza cardiovascular [Phillips, 1988; Kawano et al., 1991b], neuroendocrina [Morris, 1982], renal [Andersson et al., 1984; Kawano et al., 1991b] y conductual [Miselis et al., 1987]. Estas respuestas compensadoras que consisten en cambios de la presión arterial, de la secreción de VP y/o aldosterona, de la excreción renal de agua y/o sodio (Na^+), conducta de bebida, apetito de sal, son organizadas y reguladas por el sistema nervioso central [Robertson, 1983]. Considerables evidencias han indicado que en el sistema nervioso central existen osmosensores y/o natriosensores los cuales detectan los cambios en la tonicidad del plasma y desencadenan la activación de los mecanismos que mantienen la estabilidad cardiovascular y de los líquidos corporales. Sin embargo, la localización de estos sensores no ha sido determinada [Andersson, 1953; Jewell y Verney, 1957; Andersson et al., 1967; Mouw y Vander, 1970; Erikson, 1974; McKinley et al., 1974, 1978, 1980; Leksell et al., 1981; Pierce y Mouw, 1984].

Los experimentos clásicos de Verney [1947] sugirieron la

existencia de osmosensores en el cerebro, cuya función es detectar el aumento en la presión osmótica efectiva del plasma y producir la liberación de la hormona antidiurética o VP. En un trabajo subsecuente, mediante una ligadura selectiva de los vasos sanguíneos intracerebrales, Jewell y Verney [1957] demostraron en perros que la región sensitiva para la osmoregulación era el hipotálamo anterior y/o la región preóptica. Esta idea fué también seguida por Andersson [1952; 1953], quien mostró en cabras la existencia de un "área de bebida" a través de infusiones selectivas de soluciones hipertónicas de NaCl en diferentes regiones del hipotálamo y posteriormente proporcionó evidencias que indicaban la existencia de natriosensores. Con esos datos surgió un debate acerca de si dichos sensores centrales responden a la osmolaridad o especificamente responden a los cambios de la concentración extracelular de Na^+ [Fitzsimons, 1989]. Durante cerca de dos décadas, Andersson y sus colegas proporcionaron abundantes evidencias que indicaron que: 1) las respuestas homeostáticas producidas por activación de los natriosensores son más drásticas que las producidas por activación de los osmosensores [Andersson, 1971; 1977; 1978; Andersson et al., 1967; 1969a]; 2) los natriosensores pueden estar localizados a lo largo del tejido de la pared anterior del tercer ventrículo cerebral (AV3V) asi como en regiones más posteriores hipotalámicas [Andersson, 1977; 1978; Andersson et al., 1975a]; 3) los natriosensores también pueden estar involucrados en la regulación de la presión sanguínea, de la excreción renal de Na^+, y del apetito de sal [Andersson et al., 1969b; 1972; 1984; Andersson y Olsson, 1973]; 4) la disminución en la concentración de Na^+ del líquido cerebroespinal puede inhibir la liberación de VP [Andersson, 1978]; y finalmente, 5) la infusión intracerebroventricular (ICV) de inhibidores del transporte de Na^+ puede reducir tanto la sed como la antidiuresis producidas por infusión ICV de solución hipertónica de NaCl y/o ANG II [Andersson et al.,

1975b]. Experimentos semejantes han sido reproducidos en diferentes especies, incluyendo humanos [Wolf, 1950], ratas [Dorn y Porter, 1970; Bealer, 1983a; 1983b], perros [Dorn et al., 1969; Mouw y Vander; 1970; Pierce y Mouw, 1984; Thrasher et al., 1980a; 1980b], ovejas [Mckinley et al., 1974; 1978; 1980], y monos [Swaminathan, 1980]. Sin embargo, la precisa localización de los natriosensores y/o osmosensores, así como los circuitos neurales a los cuales están asociados estos sensores permanecen desconocidos.

Durante las décadas pasadas, tres abordajes experimentales han sido usados para localizar estas áreas osmosensitivas y/o natriosensitivas. Primero, la región anterior ventral del tercer ventriculo (3V) o AV3V la cual incluye a la lámina terminalis pueden ser una de estas áreas [Bealer et al., 1983a; 1983b; Brody y Johnson, 1980; Lichardus et al., 1987; McKinley et al., 1988; 1989] puesto que la lesión de esta región atenúa el incremento de la excreción de Na^+ producido por la inyección ICV de NaCl hipertónico, y afecta severamente la ingesta de agua inducida por cambios osmóticos. Segundo, la perfusión ICV de soluciones hipertónicas de NaCl limitadas a la parte dorsal, a la parte ventral o a la posterior del 3V, mediante el bloqueo selectivo de las otras partes con un tapón de vaselina, mostraron que los natriosensores involucrados en la respuesta de natriuresis inducida por dicho estímulo ICV, podían no estar localizados en la región AV3V solamente, sino también a lo largo de la región ventral posterior del hipotálamo [Pierce y Mouw, 1984; Kawano et al., 1991a]. Y tercero, experimentos de perfusión de soluciones hipertónicas de NaCl con la técnica empuje-desagüe (push-pull) realizadas en cuatro sitios diferentes del 3V indicaron que los natriosensores están localizados cerca de la superficie ventricular en la parte dorsal anterior del 3V [Cox et al., 1987]. De lo anterior, quedó planteada la posibilidad de que existan varias regiones en las paredes ventriculares y/o zonas periventriculares que contengan

elementos sensitivos a los cambios de la concentración de Na^+.

Por otro lado, está bién establecido que los órganos circunventriculares (CVOs) del tejido de la lámina terminalis contenidos en la AV3V son elementos sensitivos para la detección de señales humorales transmitidas tanto por la circulación como por el líquido cerebroespinal y relacionadas con los circuitos neurohumorales implicadas en la homeostasis cardiovascular y de los líquidos corporales [Jonhson et al., 1992; McKinley et al., 1992]. Así, hay evidencias que indican que las neuronas de los CVOs aumentan su actividad metabólica [Gross et al., 1985] así como su síntesis proteica [Lepetit et al., 1988; 1992a] ante una deshidratación crónica. Adicionalmente, se ha mostrado que en las neuronas de la lámina terminalis se induce expresión de Fos por infusión intravenosa de soluciones de NaCl hipertónico [Oldfield et al., 1991] o de ANG II (McKinley et al., 1992) así como después de una hemorragia en ratas concientes (Badoer et al., 1992). Más aún, se ha mostrado que los CVOs, los cuales carecen de barrera hematoencefálica, contienen sitios ligando para la ANG II circulante la que normalmente no entra del plasma al cerebro [Lind et al., 1985].

En suma, la integridad anatómica de los CVOs del tejido de la lámina terminalis es esencial para la expresión de respuestas compensadoras tales como la conducta de bebida, apetito de sodio, liberación de VP y aldosterona, y la excreción renal de agua y Na^+ para mantener el balance de los líquidos corporales y la regulación cardiovascular [Johnson et al., 1992].

Uno de los CVOs del tejido de la lámina terminalis, es el SFO que es la estructura determinante que contiene elementos y vias neurales involucrados en la detección, la integración y el procesamiento de la información humoral que se asocia con la homeostasis cardiovascular y de los líquidos corporales [Ciriello y Gutman, 1991; Ferguson, 1992; Ferguson et al., 1992; Gross, 1985; Renaud et al., 1985; Summy-Long,

1987].

B. EL ORGANO SUBFORNICAL.

B.1. LOCALIZACION Y MORFOLOGIA.

El SFO, reconocido como uno de los CVOs de la lámina terminalis [Hofer, 1958], es una protusión pequeña semiesférica dentro del cuadrante dorsorostral del 3V, a nivel del foramen interventri-cular, entre las ramificaciones de los plexos coroideos del 3V. El SFO se deriva embriologicamente del epéndima telencefálico en el punto donde surge la tela coroidea y la lámina terminalis [Akert, et. al., 1961]. En estudios citológicos en la rata, el SFO ha sido dividido rostrocaudalmente en cuatro subregiones: 1) rostral, 2) transicional, 3) central y 4) caudal (donde el SFO es cubierto por los plexos coroideos). Todas estas subregiones, excepto para la región rostral, han sido subdivididas en tres zonas en el plano sagital: zona dorsal, zona ventromedial y zona lateral [Gross, 1992; Sposito y Gross, 1987].

Su red capilar y endotelio capilar tienen seis distintas caracteristicas no comunes en la mayoría de las regiones cerebrales, pero tipicas de los otros CVOs y glándulas endócrinas périfericas: 1) alta densidad de capilares; 2) células endoteliales tortuosas de forma irregular, las cuales ofrecen una extensa superficie de contacto con los elementos circulantes de la sangre sistémica; 3) fenestraciones; 4) uniones intercelulares que pueden ser canales patentes; 5) numerosas vesículas citoplásmicas; y, 6) anchos espacios pericapilares invadidos por numerosas dendritas y procesos axonales [Bouchaud, 1974; Dellman, 1985; 1987; Gross, 1992]. Todos estos factores probablemente contribuyen a la alta permeabilidad endotelial a los solutos del plasma. Además, la combinación de estas caracteristicas morfológicas en los cuerpos capilares proporciona el sustrato microanatómico para la quimiorecepción humoral por el SFO. Así, el SFO esta idealmente conformado para detectar señales humorales

transportadas en la circulación destinadas para ser transducidas a mensajes neurales. Otras características morfológicas interesantes del SFO, son la variedad de células ependimarias que contiene así como las neuronas supraependimarias y células gliales sobre la superficie ventricular que permitem un contacto con el líquido cerebroespinal [Dellman, 1985; 1987; Leonhardt y Lindemann, 1973; Gross, 1992]. En la rata, las células ependimarias son numerosas en las subregiones rostral y caudal, y escasas o ausentes en la región central [Dellman, 1987]. Las células ependimarias tienen una superficie fina de microvellosidades y procesos ciliares que sobresalen hacia el espacio ventricular y que pueden optimizar el intercambio de substancias entre el fluido y el tejido. Tanicitos y patentes hendiduras interependimarias, que pueden ser conductos especializados para transferir solutos entre el líquido cerebroespinal y la sangre capilar también son comunes en el SFO [Broadwell et al., 1983; Dellman y Simpson, 1979; Gross, 1992].

Cuerpos neurales están presentes en todas partes del SFO. En la rata, estos son particularmente numerosos en la región central, que es un área que también tiene alta densidad capilar [Dellman, 1985]. Un detallado exámen del SFO de la rata y del gato revela dos tipos diferentes de neuronas: las neuronas no vacuoladas y las vacuoladas [Dellman, 1987; Dellman y Simpson, 1979]. Las neuronas no vacuoladas estan caracterizadas por su tamaño relativamente grande, un núcleo esferico u ovoide a menudo identado por una o más invaginaciones, muchos o pocos poliribosomas y cisternas de reticulo endóplasmico rugoso (rER), y un prominente Aparato de Golgi multilocular con vesiculas granuladas y electrotranslúcidas en su vecindad. Los cuerpos neurales vacuolados han sido caracterizados por sus cisternas rER y el Aparato de Golgi que pueden aparecer vacios o contener material granular fino electrodenso. Los cuerpos neurales no

vacuolados y vacuolados así como sus procesos son comunmente, pero no consistentemente, observados en localizaciones supraependimarias. Por otro lado, procesos neurales de calibre y configuraciones variados están presentes consistentemente y forman diversas redes complejas con sinapsis intraventriculares axodendríticas y axoependimarias especialmente en las regiones rostrales del SFO de la rata [Dellman, 1987; Dellmann y Linner, 1977; Phillips et al., 1974]. Además de los procesos neurales de origen extraSFO, dendritas y axones emergen entre las células ependimarias y proyectan dentro del lumen ventricular, y aparentemente terminan, después de un curso intraventricular corto, como bulbos terminales. Es posible que estas terminales intraventriculares en la rata ayuden a la función quimioreceptora como se ha sugerido para las proyecciones bulboso dendríticas ventriculares de las neuronas que establecen contacto con el líquido cerebroespinal en el SFO de la rana [Dellman, 1987].

Cambios estructurales finos en las neuronas del SFO han sido reportados en varias condiciones experimentales, tales como la deshidratación a través de privación de agua, administración oral de solución salina hipertónica, ligadura de la vena cava caudal, y adrenalectomía [Dellman, 1987; Dellman y Simpson, 1979]. Únicamente cuerpos neurales no vacuolados reaccionan a estas intervenciones con un aumento en la extención del rER, dilatación eventual de su cisterna, y aparición de material granular fino electrodenso, además de Aparatos de Golgi expandidos y un aumento numeroso de vesículas asociadas al Golgi. Si se administra colchicina intraventricularmente a animales deshidratados por deprivación de agua, hay un aumento marcado en el número de vesículas granuladas en muchos cuerpos neurales. Cambios finos estructurales son particularmente obvios en el centro del cuerpo neural evidenciando así una activación de la síntesis de proteína [Dellman, 1985; 1987].

Las sinapsis halladas en el SFO son principalmente axodendríticas y el resto son axosomáticas [Akert et al., 1967; Dellman et al., 1985]. Los contactos sinápticos de los axones terminales y de las dendritas con la lámina basal perivascular, con el tejido conectivo de los espacios pericapilares, y con las células ependimarias proyectando dentro del líquido cerebroespinal [Dellman et al., 1985; Dellman y Simpson, 1979; Leonhardt y Lindemann, 1973] han sido identificados como serotoninérgicos [Bouchaud, 1975]. Esta organización morfológica sugiere que las salidas autónomicas y neurosecretoras del sistema nervioso central pueden ser moduladas por la actividad de células del SFO que están bajo la influencia de péptidos, aminas o iones que ganan acceso al interstisio del SFO a través de la sangre o del líquido ventricular [Gross et al., 1990].

B.2. CONECCIONES NEURALES.

B.2.1. Proyecciones eferentes y neurotransmisores.

Estudios neuroanatómicos en el cerebro de la rata han mostrado que el SFO envia proyecciones eferentes a varias estructuras del cerebro tales como: el núcleo paraventricular del hipotálamo (PVH), el núcleo supraóptico (SON), el área lateral hipotálamica, el órgano vasculoso de la lámina terminalis (OVLT), el núcleo del lecho de la estría medularis (BST), el núcleo paraventricular del tálamo (PV), el núcleo mediano (NM) y el tejido periventricular adyacente [Lind, 1987; Lind et al., 1982; Miselis, 1981; Miselis et al., 1979]. Recientemente, Swanson y Lind (1986) utilizando el método de rastreo anterógrado de la leucoaglutinina del Phaseolus vulgaris (PHA-L) mostraron que el SFO de la rata proyecta a la corteza infralimbica, a las partes rostrales y ventrales del SFO, a la substancia inonimata, al núcleo periventricular anteroventral, al Arc [Gruber et al., 1987], al núcleo dorsomedial del hipotálamo (DMH), a la zona incerta (ZI), a los núcleos tálamicos de la línea media, y a los núcleos del

rafé dorsal y mediano.

A través de técnicas combinadas de transporte retrógrado con tinción imunohistoquímica se ha obsevado que las proyecciones del SFO al SON [Jhamandas et al., 1989], NM y PVH son imunoreactivas a la ANG II [Lind et al., 1984b; 1985]. Procesos axonales que presentan imunoreactividad a la ANG II originados en el SFO [Lind et al., 1984a], así como alta densidad de receptores a la ANG II [Gehlert et al., 1986] han sido también localizados dentro del PVH en la rata. Este hallazgo ha sido confirmado utilizando técnicas electrofisiológicas [Ferguson et al., 1984; Tanaka et al., 1986; 1987].

Subsecuentemente, varios estudios en la rata han mostrado que la conección del SFO al PVH está involucrada en la acción presora central de la ANG II circulante, involucrando probablemente tanto salida humoral como neural del PVH [Gutman et al., 1988a; Tanaka et al., 1985], posiblemente a través de vías monosinápticas [Fergurson et al., 1984; Lind et al. 1982; 1984a; Miselis, 1981; Tanaka et al 1985] bidireccionales [Gutman et al., 1986; 1988b]. Evidencias recientes muestran que la proyección eferente del SFO al PVH utiliza ANG II como neurotransmisor sugiriéndose que a través de esta vía el SFO podría tener el potencial para influir significativamente en el control central de las funciones neuroendócrinas y autónomicas [Li y Ferguson, 1993]. Más aún, también se cree que las proyecciones del SFO al NM usan ANG II como neurotransmisor para la regulación de la sed [Lind y Johnson, 1982; Nelson y Johnson, 1985]. Por otro lado, se ha sugerido que estas diferentes vias participan en los componentes motor, afectivo, neuroendócrino y cognositivo para la conducta de bebida [Lind, 1987].

B.2.2. Proyecciones aferentes y neurotransmisores.

Las fibras aferentes que recibe el SFO han sido determinadas usando técnicas de rastréo retrógrado de la

peroxidasa del rábano (HRP), por técnicas de rastréo anterógrado usando PHA-L así como por métodos de visualización de fibras degeneradas. Las estructuras que proyectan al SFO son el séptum medial, la parte rostral ventral de la estría terminalis, el núcleo periventricular anteroventral, el OVLT, el DMH, el NM, la zona periventricular preóptica del hipotálamo anterior [Hernesniemi et al., 1972], el PVH [Larsen, 1991], el LHA, la ZI, el núcleo reuniens del tálamo, los núcleos del rafé dorsal y medial, el núcleo tegmental laterodorsal y el núcleo parabraquial lateral [Lind, 1987; Lind et al., 1982; 1984b; 1985].

Estudios inmunohistoquímicos y bioquímicos han mostrado que tanto en las neuronas como en las terminales neurales del SFO existen diversos tipos de neurotransmisores y neuropéptidos: acetilcolina [Achaval y Schneider, 1984; Buranarugsa y Hubbard, 1979a; Felix, 1976; Lewis y Shute, 1967; Lind, 1987; Mangiapane y Simpson, 1983; Saavedra et al., 1976; Simpson y Routhenberg, 1974; Summy-Long, 1987], norepinefrina, dopamina [Saavedra et al., 1976; Lind, 1987], serotonina [Lichtensteiger, 1967; Summy-Long, 1987; Takeuchi y Sano, 1983], adrenalina [Nakajima et al., 1968], LHRH (factor liberador de la hormona luteinizante) [Bennett-Clarke y Joseph, 1982; Pelletier et al., 1976], TRH (factor liberador de tirotropina) [Kizer et al., 1976; Lechan et al., 1983; Palkovits et al., 1976; Pelletier et al., 1976] y somatostatina (factor inhibidor de la liberación de la hormona del crecimiento) [Bennett-Clarke et al., 1980; Krisch y Leonhardt, 1980; Pelletier et al., 1976, Palkovitz et al., 1976]. Adicionalmente, estudios inmunohistoquímicos recientes han descrito proyecciones aferentes hacia el SFO de naturaleza angiotensinérgica originadas en neuronas del NM, el LHA, la ZI y del núcleo reuniens del tálamo [Lind, 1987]. La importancia funcional de estas entradas aferentes aún se desconoce, sin embargo, es interesante puntualizar la existencia de una convergencia en el SFO de dos rutas de

diferente naturaleza, neural y humoral, que hacen uso del mismo mensajero neuroquímico, la angiotensina.

B.3. PARTICIPACION DEL SFO EN LA REGULACION CARDIOVASCULAR Y DE LOS LIQUIDOS CORPORALES.

Tradicionalmente se tomaba al SFO como un CVO que funciona en la regulación del sistema cardiovascular y en el balance de los líquidos corporales [Johnson et al., 1992]. En la actualidad, se reconoce con certeza que el SFO influye al sistema cardiovascular a través del mantenimiento del balance de los líquidos corporales. Esta pequeña estructura parece ser esencial para la organización neural de respuestas conductuales y fisiológicas ante una variedad de perturbaciones del medio interno. El SFO esta involucrado en la detección tanto de la ANG II circulante como de la osmolaridad plasmática [Gutman et al., 1988a], señales ambas que se producen por perturbaciones como hipovolemia (i.e. hemorragia) o deshidratación (i.e. deprivación de agua, ingestión crónica de Na^+) [Abdelaal et al., 1976; Fitzimons, 1972; Simpson y Routtenberg, 1975; Russell et al., 1975; Weisinger et al., 1990]. En respuesta a estas perturbaciones, en el SFO se inicia la activación de un complejo circuito neural que produce aumento en la ingestión de agua [Lind et al., 1984c], liberación de VP, y vasoconstricción vascular [Mangiapani y Simpson, 1980; Mangiapane et al., 1984], acciones mismas que compensan los cambios en el volumen sanguineo, en la presión arterial y en la osmolaridad plasmática [Ciriello y Gutman, 1991; Ferguson, 1992; Gross, 1985; Johnson et al., 1992]. Estas respuestas también han sido obtenidas por estimulación eléctrica y química del SFO [Ferguson et al., 1984; Gutman et al., 1985; Ishibashi y Nicolaidis, 1981; Robertson et al, 1983]. Adicionalmente, se ha sugerido la existencia de elementos sensores al Na^+ [Gutman et al., 1988a] o aún osmosensores en el SFO [Sibbald et al., 1984; 1988] que pudieran estar involucrados en las respuestas

presoras y de ingesta de agua así como en la liberación de VP ante la hipernatremia [Knepel et al., 1982; Mangiapane et al., 1984].

El octapeptido ANG II participa en el control de la presión arterial, y en la homeostasis de los fluidos y electrolitos a través de sus efectos sobre el SFO y sobre otras estructuras participantes en los mecanismos centrales de control de los líquidos corporales [Felix et al., 1988; Lind, 1988; Phillips, 1988]. El efecto más importante de la ANG II consiste en acciones asociadas a la regulación del volumen de los líquidos corporales en respuesta a la hipovolemia [Abdelaal et al., 1976; Phillips, 1988]. Esto es debido a la capacidad que tiene la ANG II de estimular la sensación de sed, incrementar la presión arterial, aumentar la liberación de hormonas como la adrenocorticotropica (ACTH), la VP y la aldosterona así como de potenciar el apetito por Na^+ [Bickerton y Buckley, 1961; Fitzimons, 1980; Joy y Lowe, 1970; Lind, 1988; Ramsay et al., 1978]. Otra propiedad de la ANG II es su capacidad de interacción con diferentes neurotransmisores a nivel sináptico como catecolaminas, serotonina, prostaglandinas y otros péptidos. La importancia de estas interaciones podría radicar en la posible participación en funciones superiores que auxilien en el control de la homeostasis como serian las de tipo motivacional (sed, dolor), de memoria (y posiblemente aprendizaje) y de control motor asociado por ejemplo, a la búsqueda y a la ingesta de agua. Finalmente, la ANG II parece paticipar en la función reproductiva debido a que se ha implicado tanto en la modulación de la actividad de factores reguladores y hormonas de la pituitaria durante el ciclo reproductivo, en alteraciones de la sensación de sed durante el ciclo estral como en la retención de agua durante la preñez [Phillips, 1988].

Respecto a los efecto de la ANG II circulante en el sistema nervioso central, existen evidencias que sugieren que

la ANG II actúa en receptores en el SFO resultando ésto en la ingesta de agua en la rata [Lind, 1988; Lind et al., 1984c; Simpson et al., 1978]. Asi, se ha visto que tanto la destrucción del SFO como la inyección de antagonistas de la ANG II en el SFO producen una reducción del agua ingerida en respuesta a la ANG II circulante o a un estímulo hipovolémico [Simpson et al., 1978]. Aún más, tanto células como fibras inmunoreactivas a la ANG II se han localizado en el SFO de muchas especies de mamíferos [Allen et al., 1988], incluyendose la rata [Lind et al., 1985]. Por otro lado, datos electrofisiológicos han mostrado que la ANG II excita directamente a las neuronas del SFO [Felix y Schlegel, 1978; Gutman et al., 1988a; Ishibashi, et al., 1985].

La ANG II sistémica, cuyos niveles se incrementan en respuesta a la hipovolemia, produce una elevación de presión sanguinea y un incremento de secreción de aldosterona [Phillips, 1988]. Adicionalmente, se piensa que la ANG II sistémica actuando en el cerebro induce sed y liberación de VP [Mangiapane, 1987]. Ya que los niveles fisiológicos de ANG II circulantes necesarios para estos efectos centrales son altos, es muy posible que en condiciones normales la ANG II sistémica no funcione como vector regulador excepto en emergencias patofisiológicas como la hipovolemia que produce sed no osmótica [Abdelaal et al., 1976; Phillips, 1988]. La respuesta de ingesta de agua ante la ANG II inyectada ICV es una de los efectos biológicos más sorprendentes producidos por un péptido [Epstein, 1978; Lind, 1988]. La cuestión de que tan verdaderamente fisiológica es la acción dipsogénica de la ANG II no ha sido totalmente esclarecida, sin embargo, se ha mostrado que dosis muy bajas inyectadas en el 3V producen ingesta de agua [Mangiapane, 1982; Phillips, 1978]. La hipovolemia pudiera afectar la sensación de sed, bién por el aumento de los niveles de renina y angiotensina en el plasma o bién por la activación de aferencias vagales originadas en voloreceptores. Una teoria propone que la renina plasmática

actua directamente en los CVOs [Epstein, 1976; Eriksson y Fyhrguist, 1976]. Las acciones de la ANG II incluyen: efectos directos en receptores a la ANG II a través de estructuras que carecen de barrera hematoencefálica, efectos en la contracción de la musculatura lisa vascular, la estimulación de la biosíntesis de aldosterona en la corteza adrenal, aumento en la liberación de VP, e interacciones con el sistema nervioso periférico para potenciar la vasoconstricción neurogénica y la liberación de catecolaminas de la médula adrenal [Mendelsohn, 1985].

En conclusión, esta información indica que el aumento de los niveles de ANG II y la hipernatremia producidos por perturbaciones del medio interno resultan en una modificación de la actividad neuronal del SFO y que esta estructura tiene el potencial de influir importantemente en el control central de funciones neuroendócrinas y autonómicas para la regulación cardiovascular y el balance de los líquidos corporales.

C. EL NUCLEO ARCUATO.

C.1. LOCALIZACION Y MORFOLOGIA.

El núcleo arcuato del hipotálamo surge de la proliferación lateral del núcleo periventricular. El Arc es una estructura par que rodea la parte ventral del 3V, con una extensión rostrocaudal que inicia en la rata en el área retroquiasmática y concluye en el receso inframamilar del 3V [Krieg, 1932; Palkovits, 1975; Paxinos y Watson, 1986].

El Arc sobresale como una zona de notable densidad celular, bordeada por una zona de escasas células que lo separan del núcleo ventromedial del hipotálamo (VMH). Las arborizaciones dendríticas de las neuronas del Arc son diferenciadas del VMH por su forma y tamaño pequeño. Las neuronas del Arc generalmente tienen dos o tres dendritas primarias cubiertas con espinas. Algunas neuronas con cuatro dendritas primarias pueden ser observadas también en el Arc, sin embargo, éstas frecuentemente están localizadas más

lateralmente tanto en la parte rostral como en la caudal del núcleo. Las neuronas del Arc presentan un núcleo oval y en el citoplasma abunda substancia Nissl. Axones originados en neuronas del Arc terminan en dos áreas principales: como colaterales locales dentro del mismo Arc y en la eminencia media [Chronwall, 1985]. Sin embargo, otros axons originados en neuronas del Arc se han observado que salen del núcleo. Algunos axons cruzan al lado contralateral por debajo del 3V y se dirijen hacia la parte anterior del Arc. Algunos de estos axons tiene ramas que aparecen no más lejos de 50 μm del cuerpo celular con una parte del axón en el Arc y la otra ramificación proyectando fuera del núcleo. La orientación del árbol dendritico como se observa en secciones horizontales es generalmente rostrocaudal. Sin embargo, esta organización dendritica es menos pronunciada lateralmente en contraste con las áreas mediales [van den Pol y Cassidy, 1982].

Un razgo prominente de la organización del Arc es la presencia de tanicitos cuyo cuerpo celular está localizado en la pared del 3V y que proyectan sus prolongaciones a través del cuerpo del Arc hacia la superficie ventral del hipotálamo [Bleier, 1971; Rodriguez et al., 1979; van de Pol y Cassidy, 1982]. Estudios mediante la técnica de impregnación de Golgi y de microscopia electrónica muestran una red paralela densa de procesos de los tanicitos que sugiere que pueden estar involucrados en el transporte de neurohormonas del sistema sanguineo hacia el sistema ventricular o vice versa [Bruni, 1974]. Los tanicitos en el Arc pueden quizás actuar también como una barrera permeable para la difusión lateral. Las neurohormones liberadas de la gran cantidad de pequeños axones que se encuentran cerca de la base de los tanicitos pudieran tener una mayor facilidad para difundir a través del neuropilo hacia el interior del Arc y de esta forma influir su actividad neural [van den Pol y Cassidy, 1982].

Recientemente, en estudios inmunohistoquímicos en la rata, se propuso una subdividisión del Arc a nivel medial que

consiste en tres subregiones: dorsomedial, ventrolateral y ventromedial [Meister y Hökfelt, 1988; Meister et al., 1989; Hökfelt et al., 1989]. La parte ventromedial ha sido definida como el componente parvocelular mientras que las partes dorsomedial y ventrolateral se definen como el componente magnocelular [Fuxe et al., 1985; Meister y Hökfelt, 1988].

El Arc tiene un gran número de capilares de caracteristicas convencionales con diametros luminales entre 3 y 7.5 μm pero, además exhibe un tipo especial de capilares sinusoidales con diametros luminales mayores de 7.5 μm. Se encuentran espacios perivasculares en la vecindad del endotelio capilar donde pueden estar contenidos procesos neurales y terminales nerviosa que presentan baja y moderada densidad de vesículas [Shaver et al., 1992]. Este hecho es de cierta importancia ya que sugiere que la regulación de neuronas del Arc que sintetizan aminas y péptidos, como la de aquellas que sintetizan dopamina [Hökfelt y Fuxe, 1972] y proopiomelanocortina (POMC) [Kiss et al., 1985], o bién la actividad de las terminales axónicas que penetran los espacios de Virchow-Robin de los capilares de la eminencia media [Krisch et al., 1978; van den Pol y Cassidy, 1982] pudieran estar bajo cierto grado de control por subtancias originadas en la sangre sistémica. Una observación novedosa es que el Arc ventromedial y parte del ventrolaterl exhiben un producto permeabilidad x superficie de 69 μl g^{-1} mm^{-1}, que es 34 veces mayor que el presentado por zonas más distales del tuber en donde existe barrera hematoencefálica [Shaver et al., 1992].

C.2. CONECCIONES NEURALES.

C.2.1. Proyecciones eferentes y neurotransmisores.

El Arc envia proyecciones eferentes hacia: el núcleo acumbens (NA), núcleo lateral septal [Anschel et al., 1982], BST, ZI, área hipotalámica anterior (AHA), LHA, hipotálamo dorsal (DH), tálamo medial (MH), habénula dorsal (DH) [Sim y Joseph, 1991], eminencia media (ME) [Szentagothai, 1964;

Rethelyi y Halasz, 1970; Raisman, 1972; Záborszky y Palkovits, 1978; Wiegand y Price, 1980; van den Pol y Cassidy 1980; Heimer y Robards, 1981; Lechan et al., 1982], VMH [Zaborszky y Makara, 1979; Pelletier, 1980], SON [Leng et al., 1988], núcleo anterior periventricular (APN) [Zaborszky y Makara, 1979], núcleo preóptico medial [Renaud, 1977; Zaborszky y Makara, 1979; Eskay et al., 1979; Joseph, 1980], PVH [Harris y Sanghera, 1974; Ricardo y Koh, 1978; Joseph, 1980; Pelletier, 1980; Sim y Joseph, 1991], núcleo premamilar [Zaborszky y Makara, 1979; Joseph, 1980], núcleo supraquiasmático (Sch), región preóptica [Anschel et al., 1982; Makara y Hodács, 1975], núcleo periventricular del tálamo (TPV), DMH, complejo amygdalino [Anschel et al., 1982], porción medial del núcleo del tracto solitario (mNTS) [Ricardo y Koh, 1978; Sim y Joseph, 1991], substancia gris periaqueductal (PAG), núcleos del rafé dorsal (DRN), núcleos del rafé magnus (NRM), núcleos del rafé pallidus, locus coeruleus (LC), núcleo parabraquial, nucleus reticularis gigantocellularis pars alpha, y núcleo dorsal motor del nervio vago [Sim y Joseph, 1991]. Recientemente se realizó un exhaustivo trabajo de localización de péptidos y neurotransmisores en el Arc, encontrándose que la subregión dorsomedial contiene dopamina, GABA, galanina y neurotensina, que la región ventromedial contiene somatoestatina, y neuropeptido Y (NPY), que la región ventrolateral contiene dinorfina, GABA, factor de crecimiento, neurotensina, colinacetiltransferasa, tirosina hidroxilasa y derivados de la propiomelanocortina, y por último, que la zona envuelta por estas tres regiones contiene encefalina y neuropeptido K. Sin embargo, con todo y ésto, los campos especificos de proyección de estas neuronas no han sido determinados [Everitt et al., 1986; Hökfelt et al., 1989; Meister y Hökfelt, 1988; Meister et al, 1989].

En las neuronas del Arc que proyectan a la región preóptica, al Sch, a la PAG, al NTS, a los DRN y a los NRM se

han identificado inmunohistoquimicamente tanto al péptido derivado de la propiomelanocortina α-MSH como al NPY [Chronwall, 1985; Gray et al., 1984; O'Donohue et al. 1985]. Las fibras terminales de dichas neuronas establecen contactos sinápticos con neuronas serotoninérgicas de la región ventrolateral del PAG, del DRN y del NRM asi como con neuronas noradrenérgicas del LC [Strahlendorf et al., 1982].

C.2.2. Proyecciones aferentes y neurotransmisores.

Se han observado proyecciones aferentes al Arc originadas en la amigdala [Ricardo y Koh, 1978], en el BST, el LSN, el PVH, la AHA [Conrad y Pfaff, 1976a; 1976b], el LHA [Saper et al., 1979], el VMH, el área preóptica medial [Krieger et al., 1979], el Sch [Swanson y Cowan, 1975], el OVLT [Camacho y Phillips, 1981], el SFO, los nucleos del rafé mediano [Gruber et al., 1987], los DRN [Azmitia y Segal,'78; Gruber et al., 1987], el LC [Jones and Moore, 1977; Kobayashi et al., 1974], el NTS [Ricardo y Koh, 1978], y la formación reticular [O'Donohue et al., 1979; Palkovits et al., 1980a; 1980b]. El Arc recibe una proyección serotoninérgica proveniente del DRN [Azmitia y Segal, 1978; Palkovits et al., 1977], una múltiple proyección noradrenérgica originada en el LC [Jones y Moore, 1977], en el NTS [Ricardo y Koh, 1978] y en la formación reticular [Palkovits et al., 1980a, 1980b].

C.3. PARTICIPACION DEL Arc EN LA REGULACION CARDIOVASCULAR Y DE LOS LIQUIDOS CORPORALES.

Aunque tradicionalmente el Arc es conocido por su participación en la función neuroendócrina pues está directamente involucrado en la mediación de la secreción de hormonas hipofisiarias [Antoni et al., 1982; Chen, 1983; Chronwall, 1985; O'Donohue y Dorsa, 1982; Raisman, 1972; Sawyer, 1979] y tiene importantes interacciones con la regulación del ciclo estral, de la conducta sexual [Brawer, 1971; Gallo y Oland, 1976; Knuth et al., 1983] y de la

diferenciación sexual [Lamperti y Baldwin, 1983], su participación en el control cardiovascular y de los líquidos corporales ha sido escasamente explorada.

La estimulación eléctrica de las regiones rostrales del Arc producen una respuesta presora mediada por VP [Brody et al., 1986; Kunos et al., 1991; Mastrianni et al., 1989], mientras que la estimulación de las regiones caudales del Arc produce bradicardia y una respuesta depresora. Además, la estimulación de esta misma region caudal durante la activación del reflejo baroreceptor ocasiona la potenciación de la bradicardia provocada cuando unicamente se estimula el Arc [Kunos et al., 1991; Mastrianni et al., 1989].

Existen hasta ahora solo dos trabajos cuyos datos sugieren una participación del Arc en la regulación de los líquidos corporales. Así, se ha observado que la privación crónica de agua durante 3-4 días produce en el Arc un aumento de la síntesis de proteinas [Lepetit et al., 1992b] y un aumento de los niveles del RNAm-prepro-NPY [O'Shea y Gundlach, 1991]. Además, la ingestión prolongada (3 días) de solución salina hipertónica también ocasiona un aumento de la síntesis de proteinas en las neuronas del Arc [Lepetit et al., 1992b].

JUSTIFICACION DEL ESTUDIO Y OBJETIVOS.

De acuero a lo discutido anteriormente, puede considerarse al SFO como el substrato anatómico principal tanto, para la transducción, procesamiento y relevo de señales acarreadas por la circulación relacionadas a la regulación cardiovascular y de los líquidos corporales en el sistema nervioso central, como para el desencadenamiento de las correspondientes respuestas compensadoras. Por otro lado, evidencia experimental escasa y fragmentada apoya una posible participación del Arc en el control de la regulación cardiovascular y de los liquidos corporales. A pesar de que la citoarquitectura intrínseca del Arc ha sido estudiada en detalle [van den Pol y Cassidy, 1982] y una exahustiva

descripción de la localización de neurotransmisores en el Arc ha sido hecha [Everitt et al., 1986; Meister et al., 1989; Hoekfelt et al., 1989], no se han descrito aún en detalle las proyecciones aferentes y eferentes del Arc. Por consiguiente, la posible participación del Arc en la regulación cardiovascular y de los líquidos corporales queda por explorarse. Para abordar este problema diversas preguntas deben ser contestadas. Si es el caso de que el Arc está involucrado en la regulación cardiovascular y de los líquidos corporales, ¿el Arc está conectado con estructuras involucradas en esas funciones? ...como por ejemplo ¿el SFO?. ¿Son funcionales estas conecciones en cuanto a que su activación afecte la actividad de las neuronas sobre las que se proyecta?. ¿Cuales son los neurotransmisores involucrados en estas vías?. ¿Cual es el efecto de la activación de dichas vías en la actividad normal de las neuronas sobre las que se proyecta?. Y aún más, ¿cual es el efecto de la activación de dichas vías sobre la actividad de estas neuronas que reciben proyecciones cuando el organismo es perturbado o expuesto a circunstancias extremas?. ¿Cuales son los circuitos neurales participantes en estos efectos?. Y finalmente, ¿como puede hacerse manifiesto el significado funcional de la actividad del Arc en estas funciones?.

Para contestar las anteriores preguntas, se efectuó una serie de cinco estudios usando técnicas neuroanatómicas, inmunohistológicas y electrofisiológicas, para investigar la posible participación del Arc en la regulación cardiovascular y de los líquidos corporales.

En el primer estudio, tomando en consideración que el Arc es una estructura a través de la cual muy posiblemente se detecten señales humorales en el líquido cerebroespinal puesto que está localizado anatomicamente en una posición unica a lo largo de la zona ventral del tercer ventriculo y puesto que exhibe caracteristicas anatómicas similares a las observadas en los CVOs, se decidió explorar la posibilidad de que la

actividad del Arc pudieran ser afectada por cambios en la tonicidad del Na^+ del liquido cerebroespinal, efecto mediado posiblemente a través de elementos natrioresponsivos. Así pues, se aplicó la técnica inmunohistoquímica para la detección de proteinas del tipo Fos inducidas en el núcleo de neuronas después de estimulación selectiva.

En el segundo estudio, considerando que el Arc muy posiblemente mantiene conecciones neurales con estructuras involucradas en la regulación cardiovascular y de los liquidos corporales, ya que cambios metabólicos se observan en el Arc después de deshidratación y ya que la estimulación del Arc produce cambios en la presión arterial, se decidió investigar sus conecciones eferentes anatomicamente. Así pues, se aplicaron técnicas inmunohistoquimicas para la localización de la leucoaglutinia Phaseolus Vulgaris (PHA-L) transportada anterogradamente por los axones de las neuronas del Arc.

En el tercer estudio, se determinó uno de los posibles neurotransmisores putativos mediante el cual el Arc ejerce sus influencias a través de sus proyecciones eferentes hacia los CVOs demostradas en el segundo estudio. Así pues, se aplicaron técnicas inmunohistoquímicas de detección combinada de péptidos y proteinas.

En el cuarto estudio, tomando en cuenta que el Arc proyecta axones hacia el SFO como lo indicó el segundo estudio, se decidió estudiar el efecto de la activación del Arc sobre la actividad neural del SFO. Así pues, se aplicaron técnicas electrofisiológicas para el registro de actividad unitaria extracelular en el SFO ante la estimulación eléctrica del Arc.

En el quinto y último estudio, considerando que las neuronas del SFO responden y median señales circulantes en la sangre relacionadas con la regulación cardiovascular (i.e. hipernatremia plasmática y ANG II), se decidió investigar la interacción de la activación del Arc sobre la respuesta producida en neuronas del SFO ante la hipernatremia plasmática

y la ANG II sistémica usando técnicas electrofisológicas de registro de actividad unitaria extracelular en el SFO.

MANUSCRITOS

1. C-fos expression in arcuate nucleus following intra-cerebroventricular hypertonic saline injections.
2. Direct innervation of circumventricular organs by arcuate nucleus neurons.
3. Neurotensin projection to subfornical organ from arcuate nucleus.
4. Effect of arcuate nucleus activation on neuronal activity in subfornical organ.
5. Effect of arcuate nucleus on responses of subfornical organ neurons to plasma hypernatremia and angiotensin II.

Neuroscience Letters, 164 (1993) 217–220

NSL 10074

C-fos expression in arcuate nucleus following intracerebroventricular hypertonic saline injections

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(Received 26 July 1993; Revised version received 30 September 1993; Accepted 2 October 1993)

Key words: Sodium-sensitive neuron; Fluid regulation; Osmoregulation; Subfornical organ; Hypothalamus; Organum vasculosum laminae terminalis; Cardiovascular regulation

Experiments were done in conscious rats to investigate the effect of i.c.v. infusions of hypertonic NaCl solutions on the induction of the protein Fos in the arcuate nucleus (Arc). Neurons containing Fos-like immunoreactivity were observed throughout the rostrocaudal extent of Arc after i.c.v. infusions of hypertonic saline solutions (337–744 mM). However, most of the labelled neurons were confined to the middle third of the nucleus, in the region of the dorsomedial and ventromedial subnuclei. Few, if any Fos-labelled neurons were observed in Arc of animals that received i.c.v. infusions of isotonic (142 mM) or mild hypertonic (173 mM) saline solutions or a hyperosmotic (660 mOsm/kg) saline solution of mannitol. No Fos-labelled neurons were found in the subfornical organ, although a few were observed scattered throughout the organum vasculosum laminae terminalis (OVLT) in all the animals studied. The density nor the distribution pattern of Fos-labelled neurons in OVLT was altered in animals receiving i.c.v. infusions of hypertonic saline or hyperosmotic solutions. These data demonstrate that Arc neurons are activated during a hypertonic saline challenge and suggest that Arc may function as a sodium-sensitive structure that is involved in body-fluid and circulatory homeostasis.

The role of arcuate nucleus (Arc) in the control of anterior pituitary function through its connections with the median emminence is well documented [8, 21]. Additionally, on the basis of its neuronal organization, Arc has been suggested to play an important role in integrating autonomic and endocrine homeostatic mechanisms [5].

Recently, data have been obtained suggesting that Arc may play a role in body-fluid homeostasis. After water deprivation, levels of prepro-NPY mRNA have been shown to be selectively increased in Arc [24]. Water deprivation has also been shown to result in increased protein synthesis in Arc [17]. Furthermore, neurons in Arc have been demonstrated to directly innervate [26] and to alter the firing frequency [27] of the subfornical organ (SFO), a circumventricular structure known to be involved in body-fluid and cardiovascular regulation [11, 12, 15, 18].

The present study was done to investigate the effect of i.c.v. infusions of hypertonic saline into the anterior third ventricle (3V), a method widely used to study mechanisms controlling body-fluid balance [1, 2, 6, 22, 25], on the activity of Arc neurons by monitoring c-fos induction using the immunohistochemical detection of the phosphoprotein Fos. The detection of the protooncogene c-fos and Fos-related proteins in neurons have been used as indicators of neuronal activation after application of a specific stimulus [7, 28]. In particular, this method has been used to identify structures participating in the control of body-fluid regulation after osmotic stress resulting from i.p. injections of hypertonic saline [10], hemorrhage [3], i.c.v. and i.v. infusions of angiotensin II [13, 19], or hypertonic saline [14, 23].

Experiments were done in 14 male Wistar rats (300–380 g). The animals were anesthetized with chloral hydrate (400 mg/kg i.p.) and a 23-gauge guide cannula was stereotaxically implanted ~ 0.7 mm above the dorsal border of the anterior 3V and cemented to the skull [1, 13]. The animals were given postoperative care and monitored to ensure that food and water intake had returned to presurgical levels before the experiment.

3 days after surgery, infusions of either 136 ($n = 1$), 142 ($n = 3$), 173 ($n = 4$), 337 ($n = 2$) or 744 mM ($n = 1$) NaCl solutions were made into the 3V (3 μl/min over 30 min) through a 31-gauge injection cannula 1.5 mm longer than the guide cannula using a Harvard infusion pump. In two additional animals, a hyperosmotic (660 mOsm/kg) solution of mannitol in 142 mM NaCl was

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similarily infused i.c.v. The osmolality of the hyperosmotic mannitol solution and the 337 mM NaCl solution was similar. All the infusions were made at ~ 12:00. During the infusions, water bottles were removed and the animals were carefully monitored. No signs of pain or discomfort were detected. To determine background Fos activity in Arc, one animal was similarly prepared and handled but no infusion was made.

The animals were anesthetized with pentobarbital sodium (60 mg/kg i.p.) 90 min after the completion of the infusion and perfused transcardially as previously described [14]. The brains were removed and stored in 10% sucrose in PBS at 4°C overnight. Serial, frozen transverse sections (50 μm) of the forebrain were cut and placed in rabbit polyclonal c-fos antisera (lot H172, Santa Cruz Biotechnology, Santa Cruz, CA; raised against the N-terminal epitope) and processed for c-fos immunoreactivity as previously described [14]. Forebrain sections were analyzed using bright-field microscopy. The total number of Fos-labelled nuclei in Arc were counted on every section (38–48) through the nucleus of each animal and an average value/section was calculated for each group (control group, 136–142 mM saline; group 2, 173 mM saline; group 3, 337–744 mM saline; and group 4, hyperosmotic mannitol-saline solution). Means ± S.E. were calculated and compared using an ANOVA followed by Bonferroni posthoc test. A P value of < 0.05 was considered statistically significant.

Controls for Fos immunoreactivity were processed as previously described [14]. In these control sections of the forebrain, no Fos-immunoreactive neurons were observed.

In all animals, the guide cannula tracts were histologically verified. In 12 animals, the tips of the infusion cannulas were located in the lumen of the 3V at the level of the rostral anterior commissure. In the animal infused with 136 mM and in the one infused with 744 mM solutions, the cannula tracts were located in the lumen of the 3V at the level of the middle third of Arc.

Fos-immunoreactive neurons (304 ± 50/section) were observed throughout the rostrocaudal extent of Arc (Fig. 1a) in all animals that received i.c.v. infusions of hypertonic saline (337–744 mM) solutions. However, it was evident that in animals that received infusions of 173 mM saline solutions, the number of Fos-labelled neurons (25 ± 8/section) was significantly smaller (Fig. 1b) compared with that from animals that received infusions of ≥ 337 mM saline solutions (Fig. 1a). Most of the Fos-labelled neurons in Arc were found within the middle third of the nucleus, in the dorsomedial and ventromedial subnuclei (Fig. 1a). The ventrolateral subnucleus of Arc had a few Fos-labelled neurons that were primarily located along its ventral aspect.

In control animals that received infusions of hypotonic (136 mM) or isotonic (142 mM) saline solutions, the number of Fos-labelled neurons (21 ± 12/section) was not statistically different from animals that received the 173 mM saline solutions (Fig. 1c). Similarly, in animals that received i.c.v. infusions of a hyperosmolar mannitol (660 mOsm/kg)-saline (142 mM) solution Arc was not distinguishable from that in animals that received infusions of isotonic saline with regards to the number of Fos-labelled neurons (13 ± 9/section) (Fig. 1d). In the one animal that did not receive an i.c.v. infusion of saline, Arc contained <1 cell/section.

A small and inconsistent number of Fos-labelled neurons was observed scattered thoughout organum vasculosum laminae terminalis (OVLT) in all animals studied. On the other hand, no Fos-labelling was observed in SFO. Infusions of hypertonic saline did not alter these patterns in OVLT or SFO (Fig. 1e,f). However, the ependymal layer covering the ventricular surface of both structures contained numerous Fos-labelled cells (Fig. 1e,f).

This study has provided immunohistochemical evidence of a forebrain structure that may participate in the pathway involved in the regulation of sodium concentration of the cerebrospinal fluid (CSF). I.c.v. infusions of hypertonic saline solutions resulted in c-fos induction in Arc neurons. This c-fos induction was likely not due to a nonspecific activation of the neurons as a result of local distortion of neuronal tissue from the small volumes infused i.c.v., as infusions of similar volumes of isotonic saline did not increased Fos-labelling in Arc. Furthermore, the possibility that a change in osmolarity was responsible for the c-fos induction is unlikely as the infusion of a hyperosmotic solution of mannitol that was as osmotically active as the 337 mM NaCl solution, did not alter c-fos activity in Arc. Finally, it is interesting to note that Fos-labelling in the two circumventricular organs classically recognized as central osmosensitive sites, OVLT and SFO [20], was not altered during the i.c.v. infusions of hypertonic saline solutions. This suggests that these two structures were likely not responsible for the activation of Arc neurons. Therefore, taken together, these data suggest that Arc neurons were activated as a result in the change in the sodium concentration of the CSF. The suggestion that the sodium concentration of the CSF may be detected by a sensor in the forebrain has previously been made [1, 2, 6, 22, 25]. It has been shown that vasopressin release, antidiuresis and drinking behaviour can be induced after i.c.v. infusions of hypertonic saline solutions, but not with equiosmolar solutions of mannitol, D-glucose or fructose [2, 9, 16]. Although the mechanism by which sodium exerts its stimulatory effect on the cells is not known, a prominent ana-

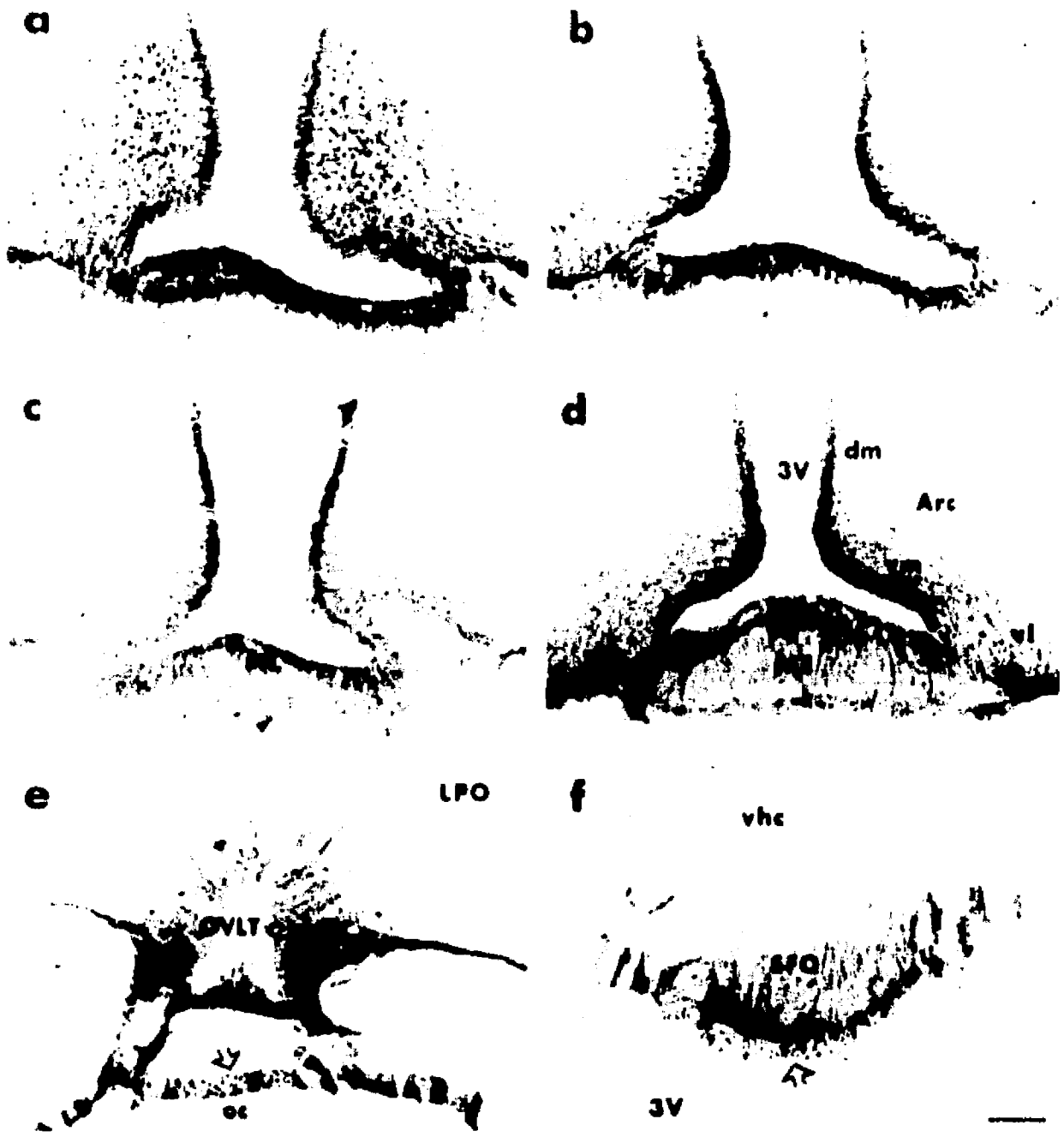

Fig. 1. Bright-field photomicrographs of transverse sections of rat forebrain taken through region of Arc (a–d), OVLT (e) and SFO (f) after i.c.v. infusions of 337 (a,e,f), 173 (b) and 142 mM (c) saline solutions and a hyperosmotic (660 mOsm/kg) solution of mannitol in 142 mM saline (d). Note dense pattern of Fos-immunoreactive neurons in dorsomedial (dm) and ventromedial (vm) subnuclei of Arc after 337 mM NaCl infusions (a). In addition, note very few Fos-labelled neurons after 173 (b) and 142 mM (c) saline infusions and in OVLT and SFO in hypertonic saline-infused animals (e,f). Arrows in (e) and (f) point to Fos-labelled cells in ependymal layer of 3V surrounding OVLT and SFO. LPO, lateral preoptic area; ME, median eminence; vhc, ventral hippocampal commissure; oc, optic chiasm; vl, ventrolateral subnucleus of Arc. Calibration mark of 100 μm in (f) applies to all photomicrographs.

tomical feature of Arc is that it is invaginated by tanycytes [29]. It has previously been suggested that the tanycyte may act as an interface that aids in the diffusion of substances from the ventricular lumen [4, 29].

In summary, it has been demonstrated that hypertonic saline solutions infused i.c.v. into the anterior 3V induces c-fos activity in Arc neurons. These data suggest that Arc may function as detector of sodium concentration in the CSF and are consistent with the idea that forebrain sodium sensors exit, in addition to osmoreceptors, both of which are involved in osmoregulation.

The technical assistance of Z.M. Zhang is gratefully acknowledged. This work was supported by the Heart and Stroke Foundation of Ontario. J. Ciriello is a Career Investigator of the Heart and Stroke Foundation of Ontario. L.P. Solano-Flores and M.P. Rosas-Arellano are visiting scientists from the Departamento de Fisiologia, Facultad de Medicina, Universidad Nacional Autónoma de México and holders of DGAPA awards.

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Direct Innervation of Circumventricular Organs by Arcuate Nucleus Neurons.

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Key words: Arcuate nucleus; circumventricular organs; cardiovascular regulation; body fluid homeostasis; PHA-L neuronal tracing; rat.

ABSTRACT

The efferent projections to the circumventricular organs (CVOs), originating in the hypothalamic arcuate nucleus (Arc), were studied in the rat by using the anterograde tracer Phaseolus vulgaris leuccoagglutinin (PHA-L). New observations with regard to the axonal projections and terminals from the Arc could be examined by the merits of the PHA-L. PHA-L was microiontophoresed into either of the three subregions of the Arc: dorsomedial (dm), ventromedial (vm) and ventrolateral (vl). After a survival period of 9-14 days, transverse sections of the forebrain and the brainsteim were processed immunohistochemistry to visualize the PHA-L labelling extension in the injection sites, and the distribution pattern of axons and presumptive terminals fields in the CVOs. Specifically, two predominant efferent projections (rostromedial and dorsocaudal) and one minor (ventral) originating within the boundary that form the three subdivisons of the Arc are projecting towards the organum vasculosum of the lamina terminalis (OVLT), the subfornical organ (SFO), the median eminence (ME), the subcomissural organ (SCO) and pineal recess (PiRe). The labelled axons of the rostromedial projection coursed across the periventricular nucleus to the preoptic area in parallel to the fornix towards the lamina terminalis and at the anterior commissure level the fibers taken two directions, a dorsal direction terminating primordially into

the (SFO), and other descending towards to the OVLT. Along the midline of the posterior hypothalamus, labelled fibers running through of the paraventricular thalamic posterior following around the wall of the third ventricle (3V) towards the subcomissural organ (SCO) and then through habenular commissure to the pineal recess (PiRe). Others fibers originating from this projection was extending caudally through the periaqueductal grey following through of the wall of 3V and 4V and terminating axons into the AP. The labelled fibers of the minor ventral projection shown a number dense in the ME. The apparent terminals fields and axons within all CVOs were usually observed in the vicinity of the pericapillaries.

These new anatomical data demonstrate that medial neurons of the Arc may be involved in modulating the function of circumventricular organs with regards to drinking behavior, sodium excretion and release of vasopressin to systemic changes in circulating levels of Angiotensin II and plasma osmolality. These findings are discussed in terms of the CVO's role within a neural network mediating the body fluids behavioral and physiologically.

Abbrevations

12M	hypoglossal motor nucleus
3V	third ventricle
4V	fourth ventricle
ac	anterior commissure
AHA	anterior hypothalamic nucleus
AP	area postrema
Arc	arcuate nucleus
bsc	brachium of the superior colliculus
CC	central canal
com	nucleus of the solitary tract, commissural part
dm	dorsomedial part of the arcuate nucleus
DMV	dorsal motor nucleus of vagus
F	fornix
Gr	gracile nucleus
hbc	habenular commissure
HDB	nucleus of the horizontal limb of the diagonal band
ME	median eminence
MPA	medial preoptic area
NTS	nucleus of the solitary tract
oc	optic chiasm
OVLT	organum vasculosum of the lamimae terminalis
pc	posterior commissure
Pe	periventricular hypothalamic nucleus
PiRe	pineal recess of third ventricle
SCO	subcommissural organ

Sg	nucleus of the solitary tract, gelatinous part
Sm	nucleus of the solitary tract, medial part
St	solitary tract
SFi	septofimbrial nucleus
SFO	subfornical organ
subAP	subpostrema area
TC	tuber cinereum area
TS	triangular septal nucleus
vhc	ventral hippocampal commissure
vl	ventolateral part of the arcuate nucleus
vm	ventromedial part of the arcuate nucleus
VMH	ventromedial hypothalamic nucleus

INTRODUCTION

The arcuate nucleus (Arc) of the hypothalamus is a paired structure localized surrounding of the ventral wall of the third ventricle (3v) with a rostrocaudal extension from the retrochiasmatic area to the mammillar recess of the 3V. Arc is thought to play an important role in integrating emotionality, vegetative, homeostatic and autonomic functions, as well as in neuroendocrine functions (Swayer, '79; Chronwall, '85). It is well known its important role in the modulation in the neuroendocrine axis through its direct connections with both the median eminence and the pituitary portal system (Bodoky and Réthely,'77; Everitt et al.,'86; Meister and Hökfelt,'88). Some of neurotransmitters and neuropeptides contained in either of the three components of the Arc: dorsomedial (dm), ventromedial (vm) and ventrolateral (vl) (Everitt et al.,'86; Hökfelt et al., '89; Meister et al.,'89; Meister and Hökfelt,'88) have been suggesting to be involved in the central regulation of blood pressure control (Kunos et al.,'91; Mastrianni et al.,'89; Mok et al.,'90). Furthermore, Arc has recently been implicated in the control of the cardiovascular system because of its effects on the arterial pressure, baroreceptor reflex and release of vasopressin (Brody et al., '86; Kunos et al., '91; Mastrianni et al., '89; Mok et al., '90; O'Neil and Brody, '84; '85). In addition, other studies about the control of body fluid balance in chronic dehydration in the rat have shown that by chronic water deprivation or hypertonic saline result an

increased protein synthesis in Arc neurons (Lepetit et al., '92), and also, by water deprivation that is increased level of prepro-NPY mRNA in the Arc (O'Shea and Gundlach, '91).

The CVOs have been considered members of a family by to have usually dense and permeable capillary networks that facilitate secretion of or tissue penetration by circulating substances (except for the subcomissural organ), impaired ependymal structures, lacked of a blood-brain-barrier and located in the median portion of the brain at strategic positions on the ventricular surface (see Gross,' 92). Recently, it has been confirmed that CVOs including the OVLT, the SFO, the ME and the area postrema are implicated in central regulation of body fluids and in cardiovascular regulation. This regulation of these central mechanisms trough CVOs is a multifactorial physiological process and then its participation is probably not essential to life but may be important for the basal state and/or fine homeostatic regulation (Thrasher,'89). Physiological studies suggest that they are involved in neurohemal communication between the central nervous system and the systemic blood circulation, because there are in its network of capillaries responsive sites to angiotensin II (ANGII) and/or to plasma tonicity (Fitzsimons,'87; Ciriello and Gutman,'91; Gutman et al.'88; Hatori and Koizumi,'90; MacKinley et al.,'87; Simpson, '81; Thrasher,'89). Although the CVOs share some ultrastructural characteristics, their functional roles are highly distinct (see Gross, '92).

Many studies by autoradiography, retrograde and anterograde

axonal transport have shown afferents to Arc arise from central structures as the SFO (Lind,'87; Gruber et al.,'87), the OVLT (Gruber et al.,'87), and the paraventricular nucleus of the hypothalamus (PVH)(Conrad and Pfaff,'76; Csiffáry,'92;). In addition, to its well known direct projection to the ME, it has been showing electrophysiologically and immunohistochemically that the Arc neurons send efferents to the supraoptic nucleus (Leng et al.,'88; Saphier and Feldman,'86), the PVH (Joseph,'80; Pelletier, '80), the VM (Pelletier,'80; Zaborszky and Makara,'79), and others brainstem regions which modulate nociception (Sim and Joseph,'91). However, at the present there are not studies focused on the existence of Arc efferents to the CVOs. Therefore, in this study was used the anterograde tracer PHA-L combined with immunohistochemestry to provide evidence for direct projections from the three different subregions of the Arc to the CVOs, and provide a mapping of the distribution pattern of these projections within the each area of the CVOs. A preliminary account of these data has been presented elsewhere (Rosas-Arellano and Ciriello,'92).

MATERIALS AND METHODS

Experiments were done in 26 wistar rats (250-420 g; Charles River Canada Inc., St Constant, Canada) under equithesin anesthesia (0.3 ml/100g, i.p.; Gandal,'69) and placed in a Kopf stereotaxic frame. Access to the either of the subregions (dm, vm and vl) of

the Arc was obtained by drilling a 2 mm hole into the parietal bone following stereotaxic coordinates from 2.7- 3.4 caudal to bregma, 0.2 - 0.5 lateral to sagittal sen and 9.5 - 10.3 below dura that were obteined from a stereotaxic atlas of the rat brain (Paxinos and Watson, '86). The iontophoretic injections into the either of the subregions of the Arc were done using a glass microppipette (internal tip diameter 30 μm) filled with 2.5% solution of anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L; Vector Laboratories, Burlingame, CA) in 10 mM potassium phosphate-buffered (PBS; pH 7.2-7.4). The current (5 μA, cathodal) was delivered through a Grass PSIU stimulus isolation unit using 7 s pulses on-of every 14 s for 30 min (Gerfen and Sawchenko, '84). Animals were given post-operative care and allowed to survive for 9-12 days. After the survival period animals were reanesthetized with pentobarbital sodium (65 mg/Kg i.p.) and perfused transcardially with 300 ml of 0.9% physiological saline followed by 300 ml of fixative at 4 °C following the procedure described by Roder and Ciriello ('93). The brains were removed and placed in the same fixative for 5 hours and after changed to a solution containing 10 % sucrose in PBS (pH 7.2-7.4) at 4°C for 20 hours. Serial transverse sections (50 μm) of the forebrain (FB) and brainstem (BS) were cut in a cryostat (-18°C) and collected in two series on PBS for PHA-L immunoreactivity. For each rat, FB and BS, one in two sections were placeted in normal rabbit serum (Vector Laboratories) diluted 1:50 in PBS containing 0.3% Triton X-100 for 30 min. The sections were then rinsed in PBS and placed

for 72 hours at 4 °C in primary antisera to PHA-L (goat polyclonal anti-PHA-E + L; Vector Laboratories) diluted 1:200 for immunofluorescence and 1:1000 for immunoperoxidase in PBS/0.3% Triton X-100. In a second set, FB and BS sections for immunofluorescence were then washed three times in PBS and placed for 30 min into rabbit biotinylated anti-goat IgG (Vectastain Kit) diluted at 1:100 in PBS/0.3% Triton X-100. After the sections were then washed in PBS and incubated in streptavidin Texas Red diluted at 1:100 in PBS for 15 min to visualize the PHA-L labelling. The sections were then rinsed and mounted onto glass slides, dried and placed into an acid alcohol solution for 10 min and later coverglassed with a glycerol/PBS solution for analysis by fluorescence microscopy.

A third set, FB and BS sections processed for immunoperoxidase were then rinsed in PBS and placed also for 30 min in rabbit biotinylated anti-goat IgG (Vectastain Kit) diluted 1:200 in PBS/0.3% Triton X-100. After a wash the sections were placed in a solution which consisted of methanol and hydrogen peroxide (29:1) for 30 min. Two washes in PBS were followed by placement of the sections for 60 min in ABC reagent (Vectastain Kit) in PBS/0.3% Triton X-100. The sections were removed from ABC reagent and returned to the rabbit biotinylated anti-goat IgG that was previously used for an additional 30 min, rinsed and then placed into ABC reagent for another 30 min. The sections were rinsed in PBS, and the horseradish peroxidase contained in the ABC reagent was visualized by placing the sections for 20-30 min in a solution

of 0.006% hydrogen peroxidase and 0.02% 3,3'-diaminobenzidine tetrahydrochloride (Sigma) in PBS. The sections were rinsed in PBS and mounted out of water distilled onto glass microscope slides, dried, counterstained with Neutral red and coverglassed for analysis. Controls for PHA-L immunoreactivity included FB and BS sections that were placed in primary antisera to antigen or sections in which the reaction of the tissue with the primary antisera was omitted. Under these conditions no PHA-L immunoreactivity was demonstrated. Transverse sections of the FB and BS for each animal were systematically analyzed using brightfield and darkfield microscopy, as well as fluorescent microscopy. The location PHA-L deposits of tracer, labelled fibers and presumptive axon terminals was mapped onto camera lucida projection drawings of the Arc and CVOs. A representative example of injection site and projections to each area of the CVOs were used for fluorescent photography.

The atlas of the rat brain and the nomenclature of Paxinos and Watson ('86) were used for the identification of brain structures.

RESULTS

Injections sites and general comments on axonal labelling

This study provides neuroanatomical findings, by iontophoretic injection of PHA-L restricted to the Arc, the direct projections to the CVOs from Arc neurons. The location and extent of the PHA-L deposits were determined by the distribution of the darkly

stained PHA-L immunoreactive cell bodies in either of the three components of the Arc. Of the 26 injections of PHA-L, 18 were located virtually limited in Arc extending from the level 2.7 - 3.4 caudal to bregma, the remaining injections sites were founded outside of the Arc. In all cases the region containing PHA-L deposits had a range from 200-450 μm as was delineated from contrerstained sections with DAB immunoreactivity (Fig. 1). Coordinates for the injection site were varied in attempt to sample at different rostromedial levels where it has been reported that Arc contain a major number of neurons (Van den Pol and Cassidy, '81; Meister and Hökfelt,'88) The results of injections out area of the Arc were used as controls because not any shown projections to either area of the CVOs and by the possibility that spread of the tracer into adjacent structures to the Arc could have accounted for the projections attributed to the subregions of the Arc. Differences in staining intensity were noted, in some cases as #4, #6, #8, #16, #18-20, #22, #24-26 where the PHA-L deposits were found within the three or two subregions (dm and vm) of the Arc (at the boundary area that form the three subdivisions of the subregions in the Arc). The majority of these cases presented a strong density of labelled axons and axon terminals in all the circumventricular organs. The PHA-L positively stained neurons observed in each case were the small and ovoid or the medium elongated size (< 30 μm). The most important an popular population were neurons of the medium size found in central and medial area in the boundary that form the three subregions of the

Arc. The small ovoid neurons were more frequently observed close to the ventricular wall and oriented parallelly to the ependymal of the 3V. Arc axons were found which branch within of the cell body with one part of the axon going out to the Arc an the other ramifying within the nucleus (Fig. 2). Some axons could to be observed crossing to the contralateral side under third ventricle most in the anterior part of the 3V (Fig. 5b and c). A representative example of an injection site within dm subregion of the Arc extending to the boundary of the three subregions of the Arc is shown in Figs. 2 and 5a. The PHA-L deposits in Arc neurons were located at the ventral level of the dm component of the Arc extending to the dorsal level of the vm and vl components of the Arc.

PHA-L immunoreactive axons from Arc neurons were observed to course in three directions: rostromedial, dorsomedial and ventromedial, terminating in each target of the circumventricular organs. The rostromdial projection coursed within the anterior periventricular nucleus to the bed nucleus of the stria terminalis following to the anterior medial preoptic area, passing both ventrally and dorsally to the body of the anterior commissure along the fornix. At this level of the anterior commissure, the rostromedial projection subdivided in several fiber projections to distint areas of the basal forebrain. One bundle of this pathway coursed in the periventricular strata in a rostral direction to the supraoptic recess and terminated in the OVLT. Another subdivision followed the medial corticohypothalamic tract (Palkovits and

Zaborsky,'79) medial to the fornix, to run in a dorsal direction in the periventricular strata of the preoptic area to the stalk of the SFO.

The dorsomedial projection coursed from Arc neurons through the mediodorsal thalamic nucleus to the periventricular zone and the diencephalon the fibers coursed by two directions, a dorsal and a dorsocaudal. The dorsal part coursed in the midline through the thalamic paraventricular nucleus into a cell poor area dorsal to this nucleus. Beneath the ventral surface of the dorsal part of the third ventricle, beaded fibers could be followed ventral and dorsocaudally to body of the posterior commissure terminating some of them in the SCO and other following the medial habenular nucleus to the PiRe. The dorsocaudal fibers descending joined the posterior part of the periventricular tract, which they followed in the dorsal longitudinal fasciculus of Schütz (Krieg,'32). The fibers passing through the thalamus coursed further caudally, and together with the fibers cursing in the periventricular strata of the 3v constituted a dense pathway entering the masecephalic periaqueductal grey. More caudally, the major descending fiber projection passed through the periaqueductal grey to the pons. At the level of the locus coeruleus, labeled fibers were observed to descend in two separate tracts of equal density. One tract coursed medially through the pontine central grey and proceeded caudally beneath the ventral surface of the fourth ventricle following more distally through central canal to the nucleus of nucleus of the solitary tract (NTS) and termining in the AP. The other tract

penetrated to the lateral parabrachial nucleus and coursed ventrally around the lateral margin of the cerebelar peduncle to other areas of the brainstem.

The less prominent ventral projection coursed ventrally in the periventricular strata of the third ventricle in a ventrocaudal directions towards the ME.

The organum vasculosum of the laminae terminalis (OVLT)

The distribution pattern from the rostral pathway in the majority of the experiments resulted in a high number of varicose axons of ipsilateral predominance that coursed through the anteroventral periventricular nucleus following a medial course along the dorsal surface of the optic chiasm and subsequently just above the ventral surface of the brain. Some of these fibers end in the OVLT (Fig. 6b and 6c), while the rest turns dorsally and arches around the nucleus horizontal limb diagonal band (Fig. 3a). Furthermore, many PHA-L immunoreactive fibers and presumtive axon terminals were always observed in and araound of the fenestrated capillaries near the ventricular surface of the 3V, and occasionally in body cells of the neuropil or the ependyma of the OVLT (Figs. 6b and 6c). The labeled fibers always exhibited a beaded appearence and were predominantly located in the midline of the organ. According to Weindl and Sofroniew ('78), the external (subpial) and the internal (subventricular) layers had the same density of immunoreactive fibers and terminal boutons.

The subfornical organ (SFO)

PHA-L immunoreactive axons running through of the medial corticohypothalamic tract cursing dorsally in direction of the periventricular strata of the 3V to the SFO. These inputs appear to enter the SFO by all possible routes. Fibers were present in both the dorsal and ventral stalk of the SFO, and others appeared to enter by piercing the overlying ventral hippocampal commissure (Fig. 3b). Interestingly, these fibers were most prominent at rostral levels of the SFO where they were scattered among randomly to central cell bodies and perivascular vassels. The distribution patterns of axons in central part of the SFO were oriented to dorsal bodies and terminlas boutons were observed in blood vassels located peripherally (Figs. 7a-d). Further caudally, the fibers were absent in the cell layer of the SFO. In the superficial part of the organ, the fibers were delicated and beaded.

The median eminence (ME)

PHA-L labeled fibers coursed ventrally to the ME. Axons which can be followed into the ME usually enter as a single axon and give off collaterals branches within the ME; with much less frequency axons may divide into collaterals within the Arc, with both collaterals entering to ME (Fig. 5b). Axons originating from medial Arc neurons can be observed into both medial and lateral external zone of the ME (Fig. 2 and 6a). Some axons are found which enter the rostral part of the ME descend parallel to the ventral tanycytes to more external parts of the ME. Axons terminals were

observed in the full rostro-caudal extent to both the internal and external zone of the ME, and the projections were always ipsi and contralaterals (Fig. 2). In the palisade and reticular layers of the external zone of the ME, the labeled terminals were usually located in the portal vassels (Fig. 6a). Within the internal zone of the ME, the axon terminals were observed in the fiber layer.

The subcommissural organ (SCO)

A medium density of beaded PHA-L labeled fibers could be seen to penetrate directly from periventricular surface of the 3V and from ventral part of the posterior commissure into the SCO (Fig. 4a). labeled fibers were observed crossing through of the apical region to ependymal cells (Fig. 8a). A low density of axons terminals were observed in the ependymal cells and blood vassels.

The Pineal Recess (PiRe)

Within the deep of the pineal gland, identified as a small cluster of pinealocytes located between the habenular and posterior commissurres, the labeled fibers were located intraparenchymally between the pinealocytes. A high density of beaded PHA-L labeled fibers were observed penetrate directly from the dorsal part the posterior commissure crossing the habenular commissure to the PiRe (Figs. 4 and 8b). Labelled fibers were observed from the rostral extent of the deep of the pineal gland, surrounding the PiRe (Fig. 8c), to the most caudal part of the deep of the pineal gland.

The area postrema (AP)

The descending periventricular projections from Arc neurons crossed just to the obex nevel through the central canal, where were observed primarily in the ipsi and contralateral NTS complex, (Fig. 4b). Within the ipsilateral NTS complex rostral a large number of labelled fibers was observed to follow through of the gelatinous part of the NTS to the area subpostrema and terminating in the lateral and ventral zones of the AP. Additionally, a moderate number of labelled fibers and terminal boutons was observed around of the blood vassels (Figs. 4b and 9a). The location of a major density of fibers in the AP was at medial and caudal level. Fibers coursed through the medial subnucleus of NTS were mostly observed within the medial and ventral zone of the AP and termining in the vicinity of the blood vassels (Figs. 9b and c). Along the ventromedial axis of the organ the innervating fibers arborized towards the superficial parts. Interestingly, the area subpostrema was always moderatelly innervated.

DISCUSSION

The present study provides anatomical evidence for the existence of direct pathways from Arc neurons to the CVOs in the rat. These pathways originating in or around of the boundary that form the three subdivisions of the Arc and that showen a strong tendency for send axons rostrally or caudally to the CVOs.

The observation in the present results that neurons located in

the boundary zone of the Arc project out of the nucleus is consistent with an earlier report in the Golgi preparations in the rat (Van den Pol and Cassidy, '82). The present data, however, considerably extend these earlier findings by identify the anterograde axonal tracts and its distribution pattern of the terminals boutons in the CVOs.

As a neuroanatomical tool, the PHA-L has advantages over earlier anterograde tracers in its specificity for uptake by cell bodies and their dendrites, in its sensitivity, and in the morphological detail that it provides (Gerfen and Sawchenko,'84). The principal findings of this study is that the PHA-L labelling found in different zones of the Arc neurons results in a variety in the distribution pattern of the axons and its terminals boutons in the CVOs. Efferent fibers from Arc neurons took three different courses, rostral, caudal y ventral. The rostral pathway that emerged from the periventricular area and passed anteriorly toward the anterior commissure on the midline and along the anterior border of the nucleus medianus following an direction ascendent to the SFO and an direction descendent to OVLT has been described previously (Larsen et al., '91), using the same PHA-L technique as employed here, but they did not included any of the distributions patterns of the axons and terminals boutons into the CVOs that are atributed to the Arc neurons in this study. The caudal projections from Arc neurons to brinstem areas in our results are also consistent with the descending pathways to brainstem regions reported by Sim and Joseph ('91). In contrast, in the present

study, PHA-L labelled axons were found to have a heavy and bilateral innervation to the AP, when the injection site included Arc neurons located within the boudary of the three subdivisions of the Arc. Injections of PHA-L into vl subregion of the Arc resulted with axon labelled only to NTS complex. The distribution ventral of fibers to the ME and its axons terminals on portal capilaries described in the present study are consistent with earlier neuroanatomical evidences in the rat (Bodoky and Rethelyi,'77; Everitt et al.,'86; Lechan et al.,'84; Van den Pol and Cassidy,'82; Wiegand and Price,'80). The present data considerably extend findings that may be reflected the complex network which exist between the different cerebral structures implicated in the control of the cardiovascular system and body fluid balance.

The pathway from the caudal projetion of the Arc towards the SCO and the PiRe may be consistent with the innervation of the SCO by gaba-containing nerve fibers described by Gamrani et al. ('81).

The projections from the 8 cases where the PHA-L deposit was delivered outside of the subregions of the Arc did not shown fibers in the CVOs. In the ME injections resulted light axons and terminal field in the lateral area of the hypothalamus and ventromedial nucleus. Additionally, in the ventromedial injections were observed efferent projections towars others brain structures, but never shown projections to the CVOs as have reported in other studies.

Functional considerations.

The pattern of Arc efferent projections compels the notion of its involvement in a neural circuit mediating the body fluids and cardiovascular regulation in both behaviorally and physiologically. The evidence supporting this hypothesis comes from a wide variety of studies which implicate to the Arc in the cardiovascular system and body fluids homeostasis. First, the Arc has been implicated in the control of the cardiovascular system by its effects on the arterial pressure, baroreceptor reflex and release of vasopressin (Brody et al.,'86; Kunos et al.,'91; Mastrianni et al.,'89; O'Neill and Brody, '84;'85). It has also been implicated in the control of body fluid balance by a progressive increase of protein synthesis during chronic dehydration (Lepetit et al.,'92), an increase of prepro-NPY mRNA levels in water deprivation (O'Shea and Gundlach,91), and c-fos immunoreactivity after a hipertonic saline injection (Giovannelli et al.,'90; Guldenaar et al.,'92). Based on the prominent projections from Arc and the select distribution of its axon terminals in the OVLT, the SFO, the ME and the AP and we recent evidences that neurons to Arc exert an influence on the discharge rate of neurons in the SFO (Rosas-Arellano et al.,'93) obviously we suggest that these neuronal efferents may be convey information to CVOs for to maintain a basl state and/or fine homeostatic regulation. The hypothesis about of Arc may be an area of integration has been discussed by others previously, mostly in regard to its endocrine function which depends both on hormonal

and neural. The Arc and CVOs can be viewed as a family of neuroendocrine transducers for integrating neural and humoral stimuli that may arise simultaneously under certain conditions. These small structures appear to be essential for neuronal organization of behavioral and physiological responses to a variety of endocrine disturbances.

These results encourage further anatomical studies to precisely define the bidirectional neural connections of the Arc with the SFO, the OVLT and the AP as well as their neurotransmitters. This information will permit a fine and more sophisticated functional analysis about the neural network implicated in a fine homeostatic regulation.

ACKNOWLEDGMENTS

The authors thank Dr. L. Pastor Solano-Flores for comments on the manuscript. J. Nichols is acknowledged for their technical assistance. This work was supported by the Hearth and Stroke Foundation of Ontario and the Medical Research Council of Canada. M.P. Rosas-Arellano was the holder of a ICCS of Canada Studentship and a DGAPA scholarship from the Universidad Nacional Autónoma de México and J. Ciriello is a Heart and Stroke Foundation of Ontario Career Investigator.

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Figure Legends

Fig. 1. Reconstructions of PHA-L injections sites of the experiments used for further evaluation in the present study. A serie of transverse sections of the rat forebrain taken through the mid-part of the arcuate nucleus at 2.7 to 3.4 mm caudal to bregma. The injection area containing PHA-L deposits in body cells is shaded. Not all cases gave rise to labeled fibers in the circumventricular organs, but these are included as controls. Calibration mark, 1 mm.

Fig. 2. Series of camera lucida projection drawings through the region of the arcuate nucleus (Arc) of the rat, extending from 2.7 to 3.4 mm caudal to bregma show the distribution pattern of PHA-L labelled cells and immunoreactive varicosities in either of the three subregions of the Arc. Each section drawn at the different rostrocaudal levels was taken from adjacent

sections (50 μm). Note the bilateral distribution of PHA-L anterograde labelled neurons in the dm subregion of the Arc. Calibration mark, 1mm.

Fig. 3. Series of camera lucida projection drawings of transverse sections of the rat, taken through the region of the OVLT at 0.1 rostral to 0.1 mm caudal to bregma (a) and through the region of the SFO at 0.7 to 1.2 mm caudal to bregma (b). Note the distribution pattern of labelled fibers and presumptive terminals labelling resulting from a PHA-L injection in the dm Arc, show also its predominance ipsilateral. Calibration mark, 1 mm.

Fig. 4. Series of camera lucida projection drawings of transverse sections of the rat, taken through the region of the habenular commissure at 4.5 to 4.9 mm caudal to bregma (a) and through the region of the AP at 13.6 to 14.0 mm caudal to bregma. Note that the distribution pattern of labelled axons and its presumptive terminals were in both ipsilateral and contralateral direction for all regions the PiRe, the SCO and the AP, it results from PHA-L injection in the dm subregion of the Arc. Calibration mark, 1 mm.

Fig. 5. Fluorescent photomicrographs of the PHA-L labelled neurons taged with Tx Rd in the dm component of the Arc. Note the extension of the PHA-L deposits in body cells and tanycites (arrow in a) and the resulting anterograde labelling of fibers in and around of the nucleus following to the ME (b) or running to the contralateral site (c and d). Calibration bar in a= μm and b-d μm.

Fig. 6. Fluorescent photomicrographs show PHA-L labelled fibers and terminals boutons taged with Tx Rd in the ME (a) and the OVLT (b and c). Note in a: the dense innervation from Arc to the internal and external layer of the ME. In b it is shown an ellargament from c, where the labelled fibers and terminals fields are seen in and around of a capillar (asterisk). Calibration bar in a and c = μm, in b = μm.

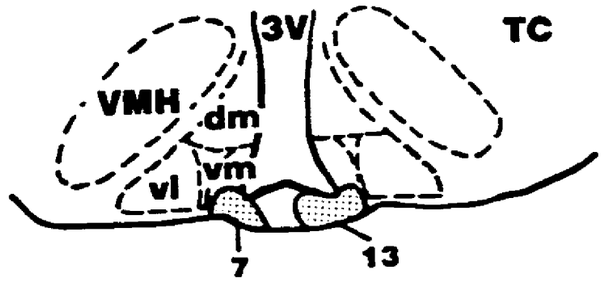
Fig. 7. Fluorescent photomicrographs of the coronal sections taken in and around the SFO after an injection in the dm component of the Arc. a: PHA-L labelled fibers taged with TxRd shown a predominant distribution ipsi and contralateral in the rostral part of the SFO. b: Fiber and terminal -like labelling entering towards the capillaries of the SFO. c: A distribution dense of fibers and terminals fields localited in the perypheria of the medial part of the SFO. d: An allargement area from (c) showing a dense network of PHA-L labelled terminals

boutons aroun of the capilar (asterisk). Calibration bar in a = μm, b and c = μm and d = μm.

Fig. 8. Fluorescent photomicrographs show PHA-L labelled fibers and presumptive terminals boutons (a) in the SCO and (b and c) in the PiRe taged with TxRd. a: Labelled fibers entering to SCO trough the strata periventricular of the 3V towards the ependymal zone. b and c: Fibers and terminals boutons running through of the hbc towards the PiRe. Calibration bar in a = μm and aplies to b and c.

Fig. 9. Fluorescent photomicrographs show PHA-L labelled fibers and terminals boutons taged with TxRd in the AP. a and b: Distribution pattern of the fibers and terminals boutons (small arrows) in and around of the blood vassels (asterisk). c: Labelled fibers across the Sg. Calibration bar in a = μm and aplies to b and c.

-2.7



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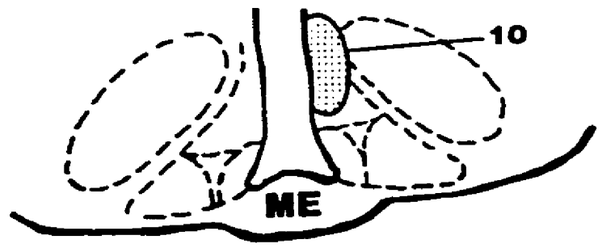

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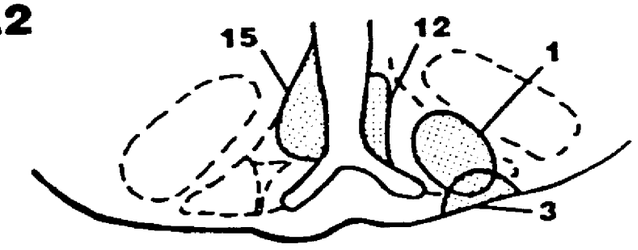

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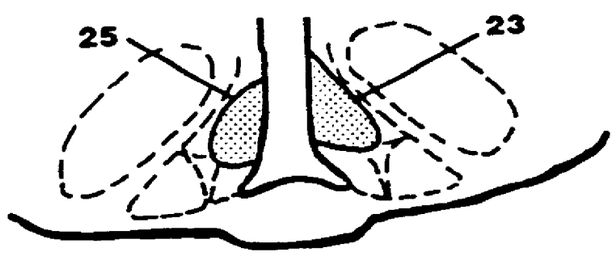

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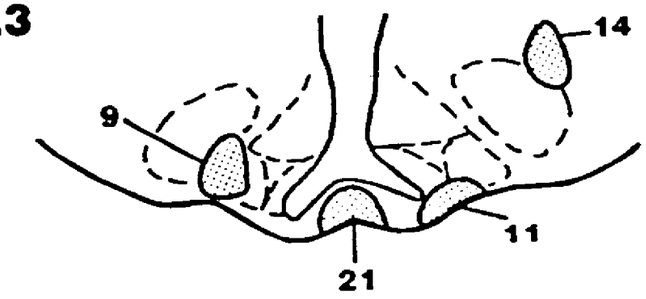

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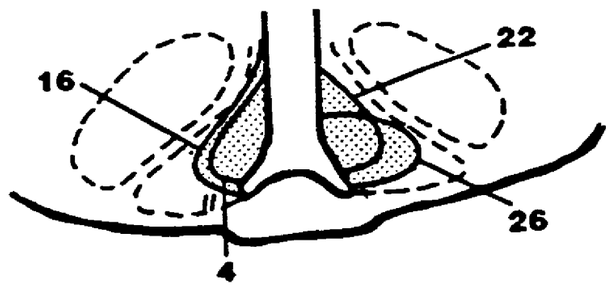

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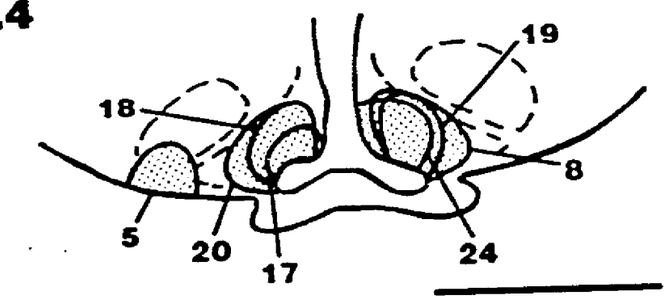

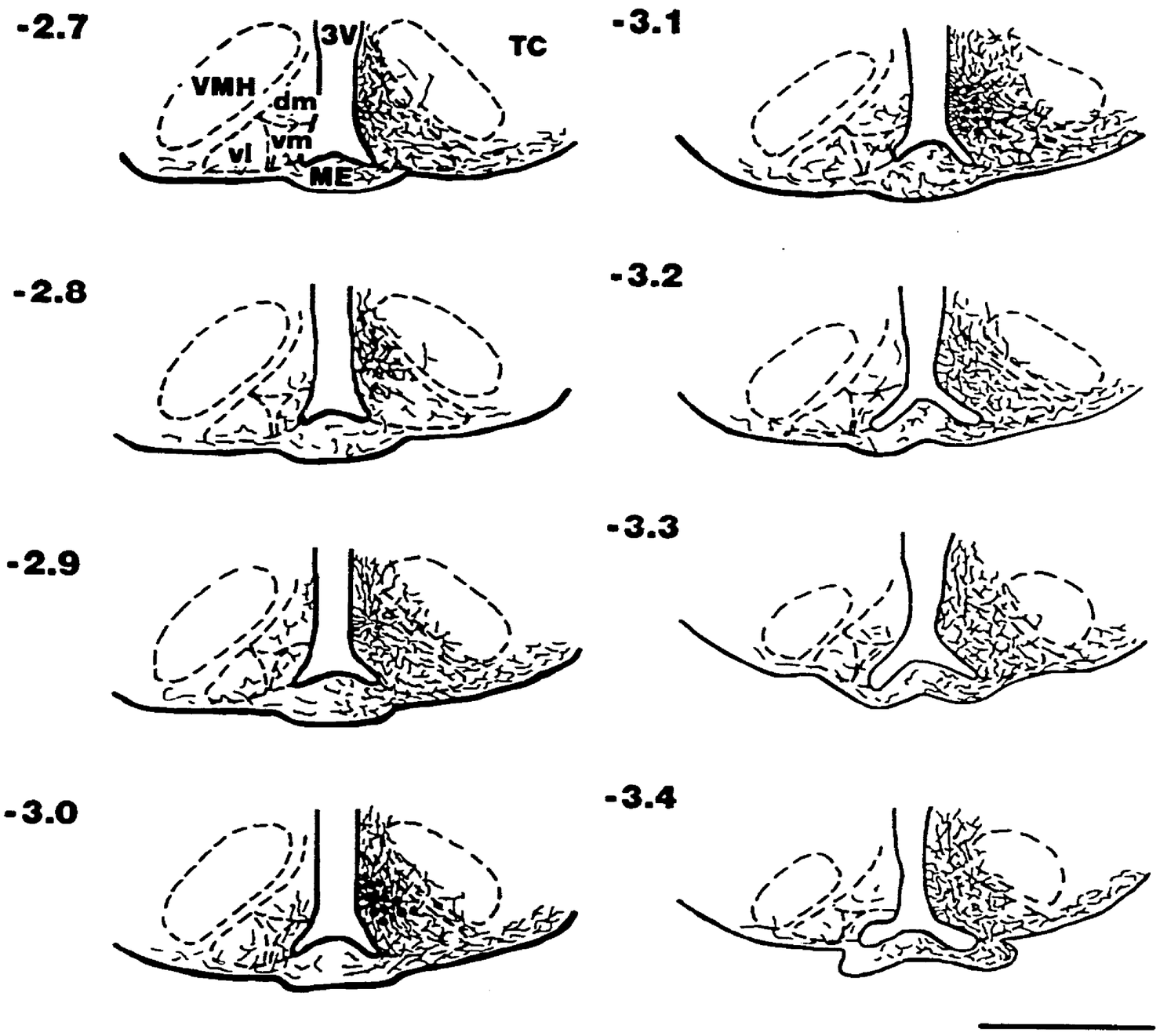
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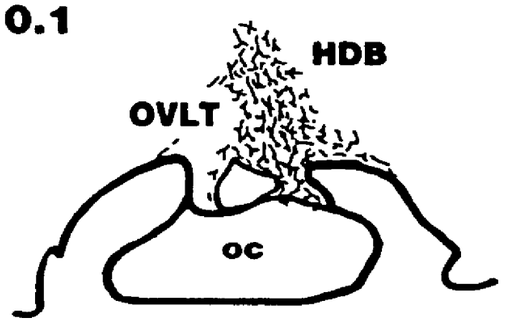

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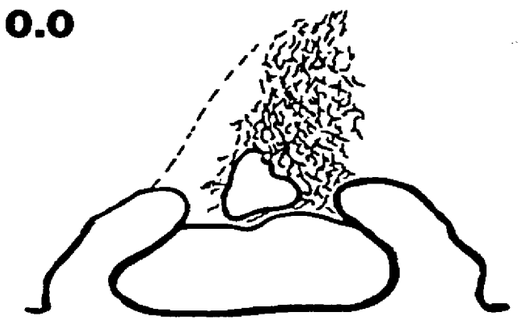

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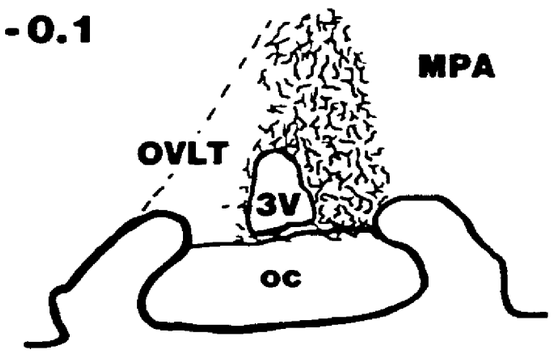

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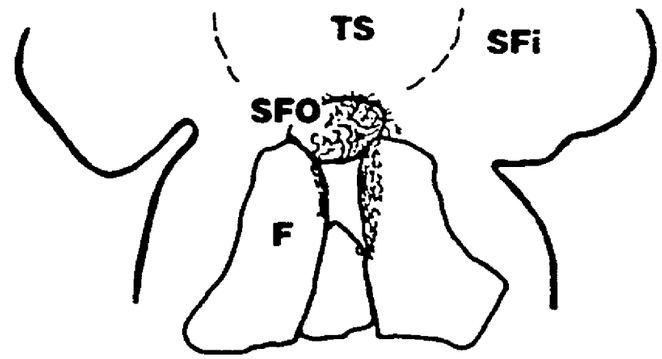

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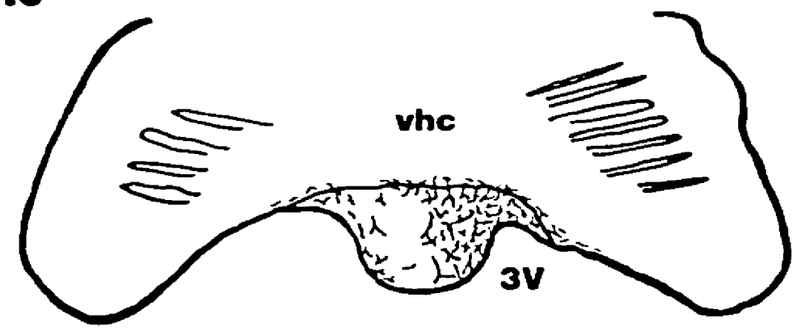

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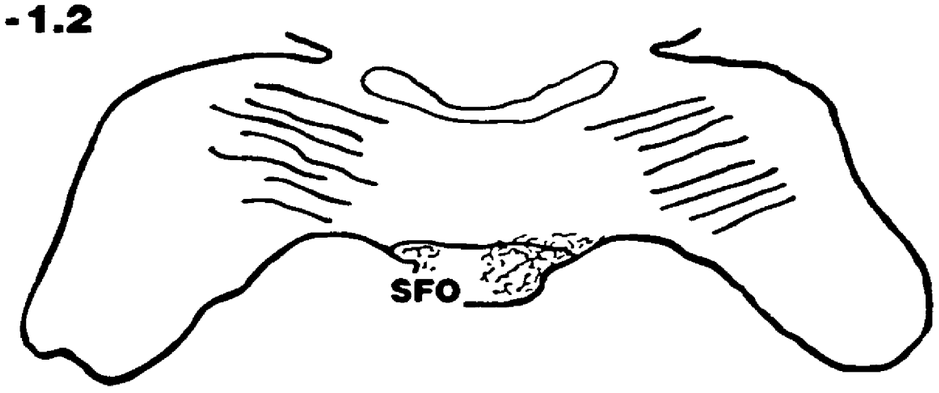

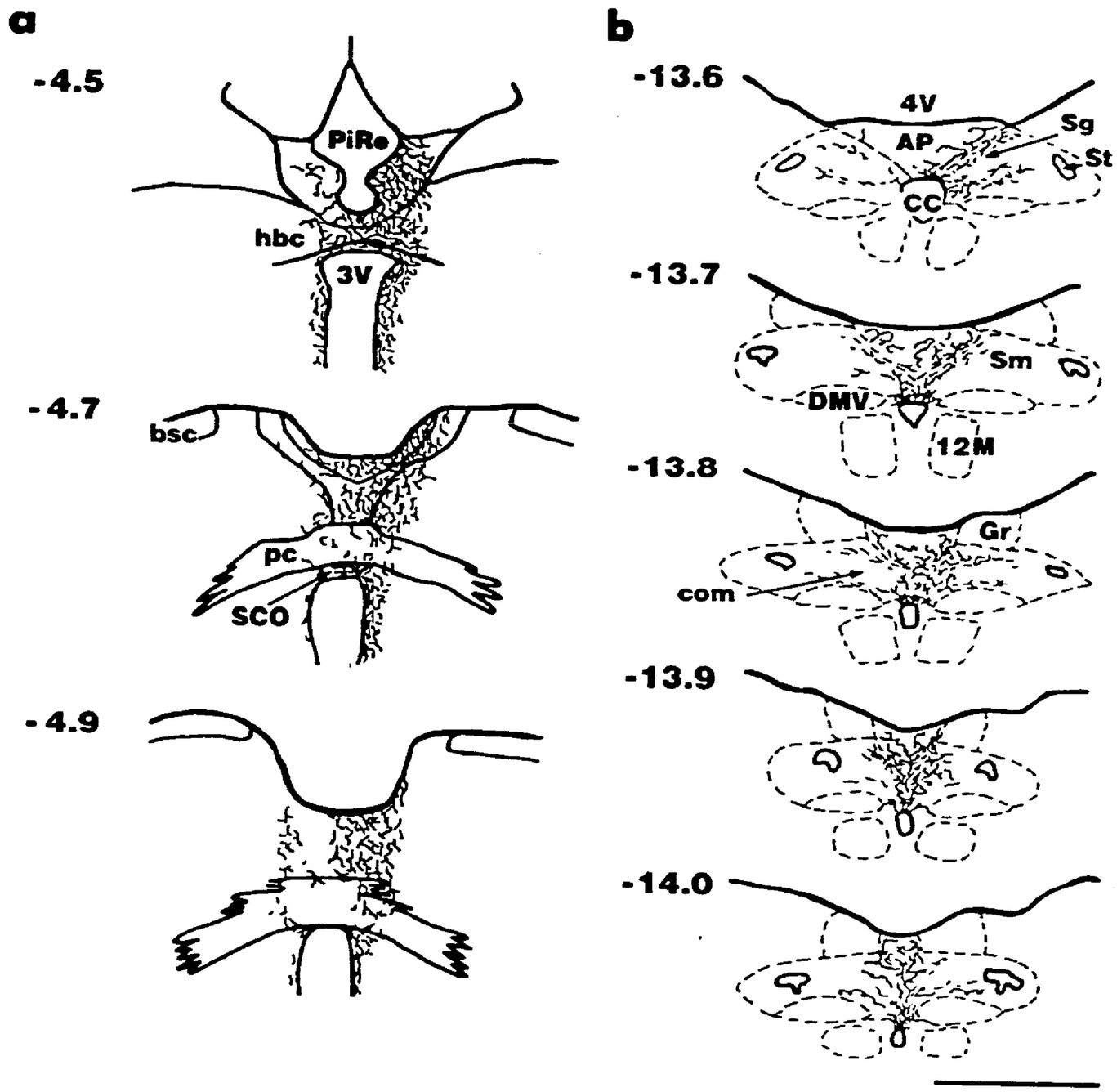
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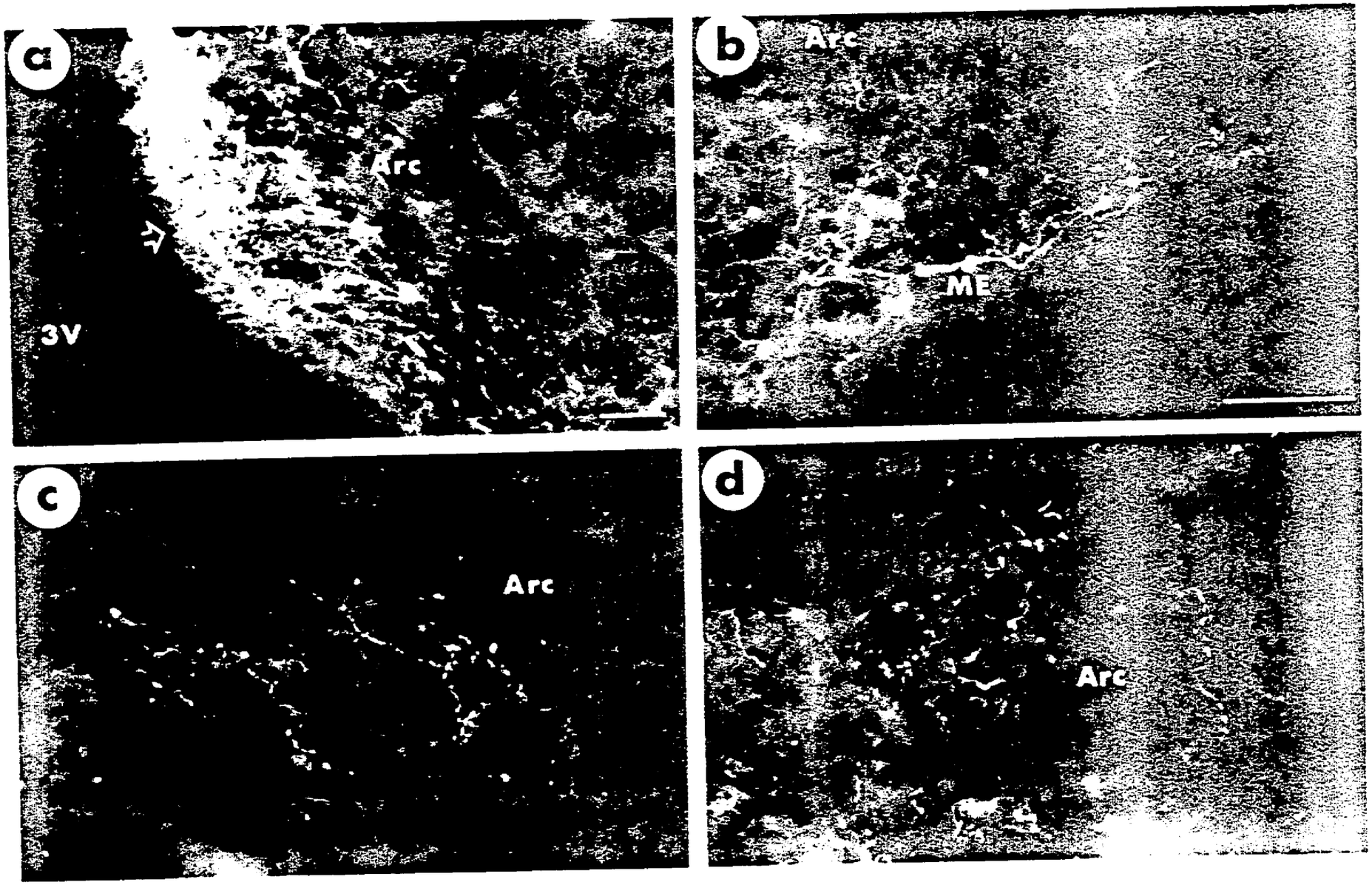
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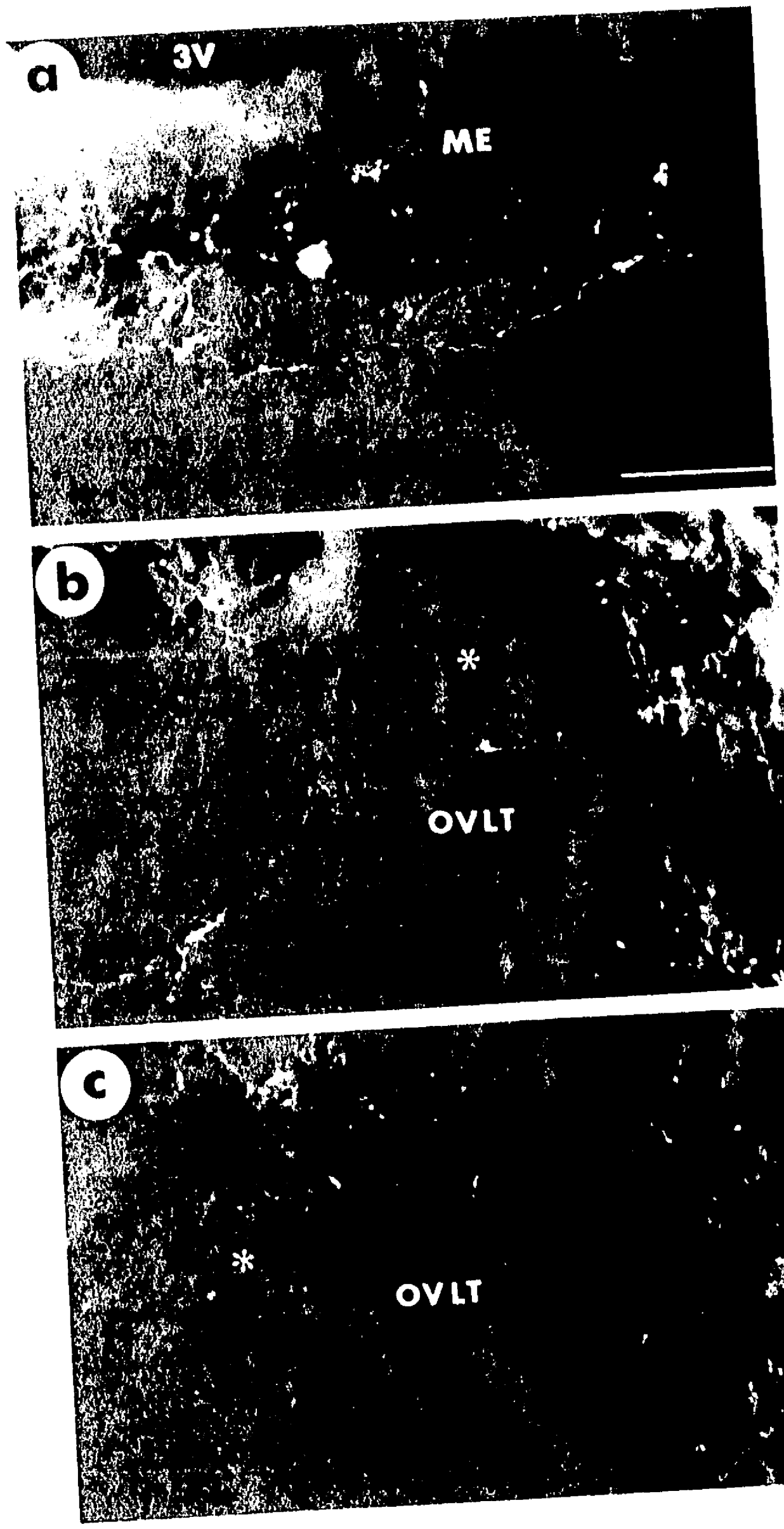
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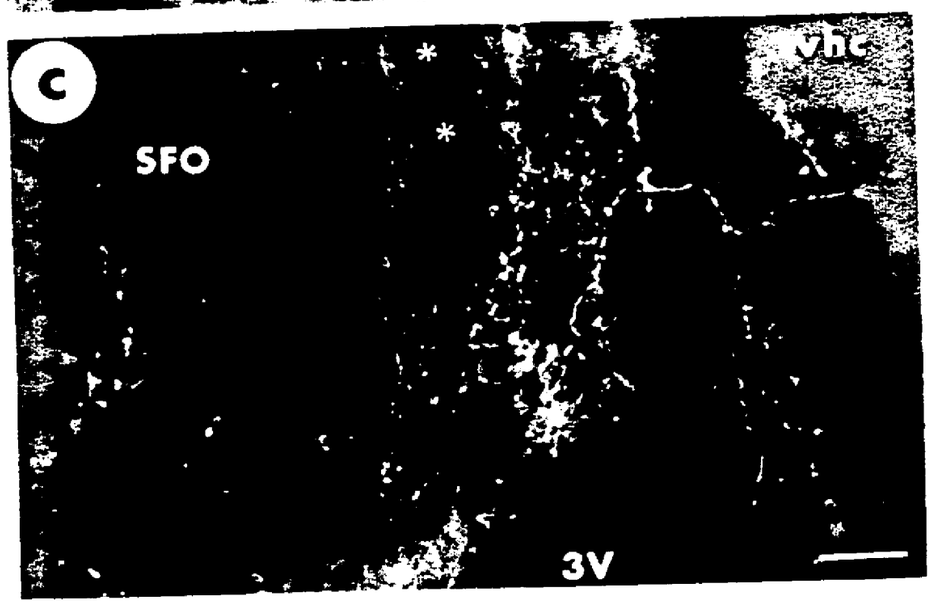
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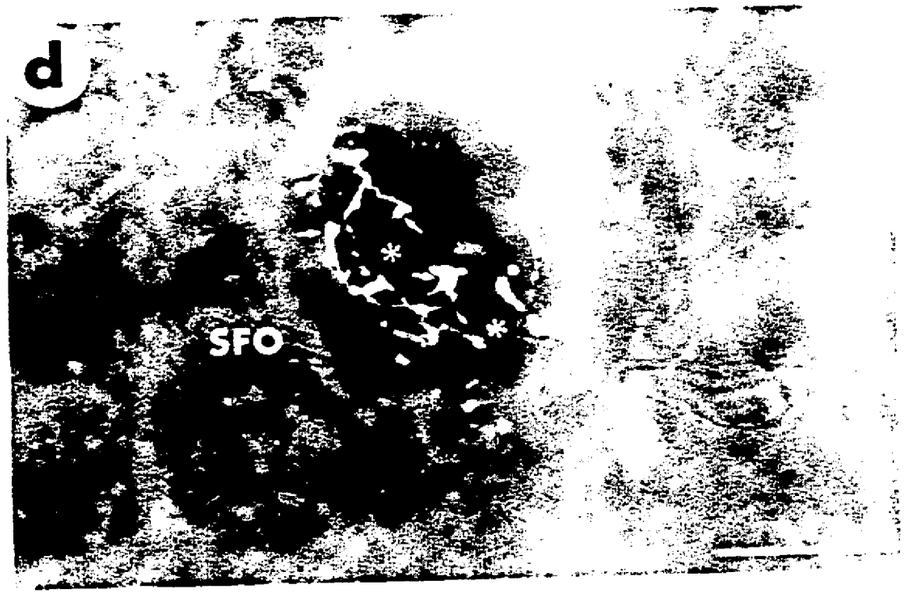
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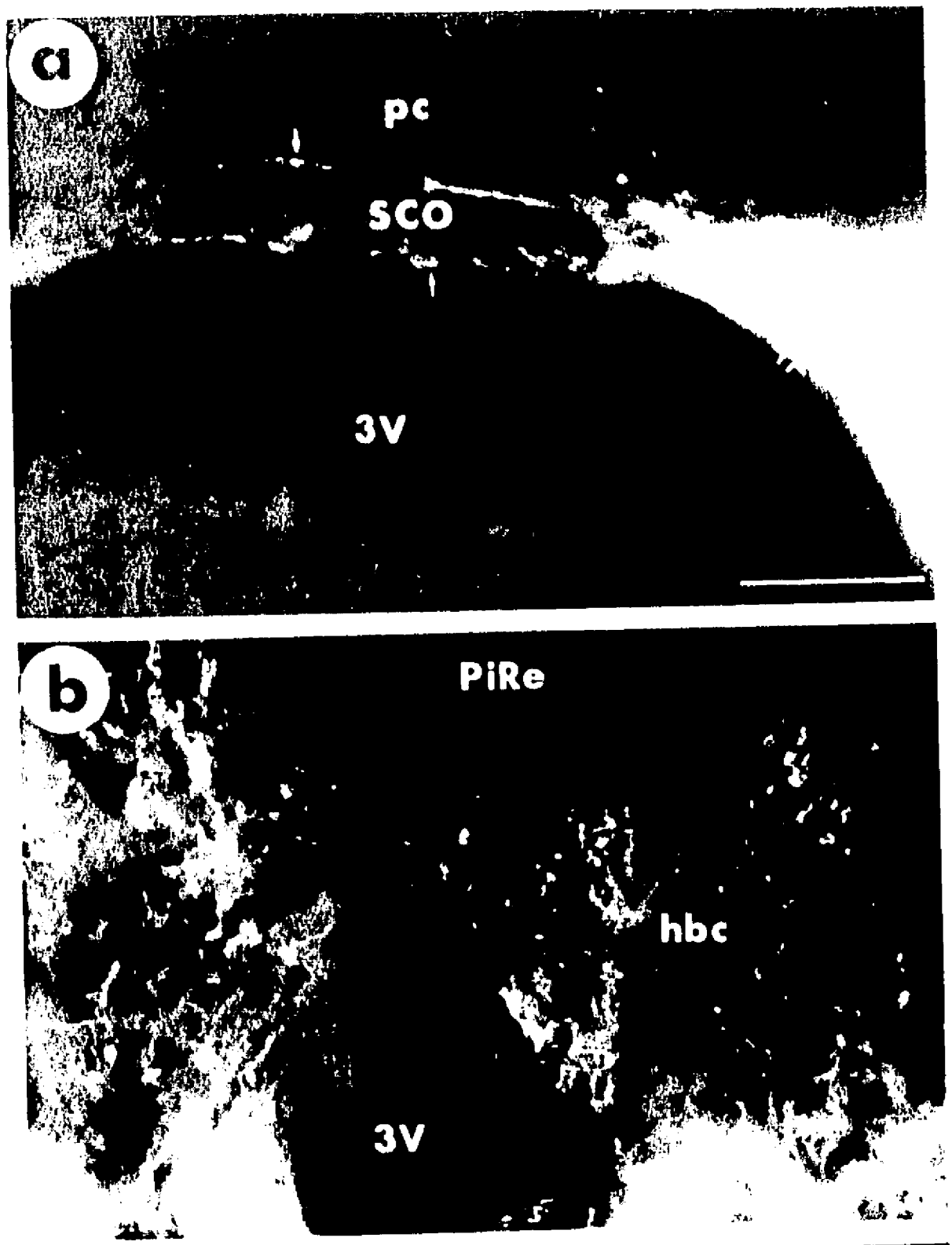
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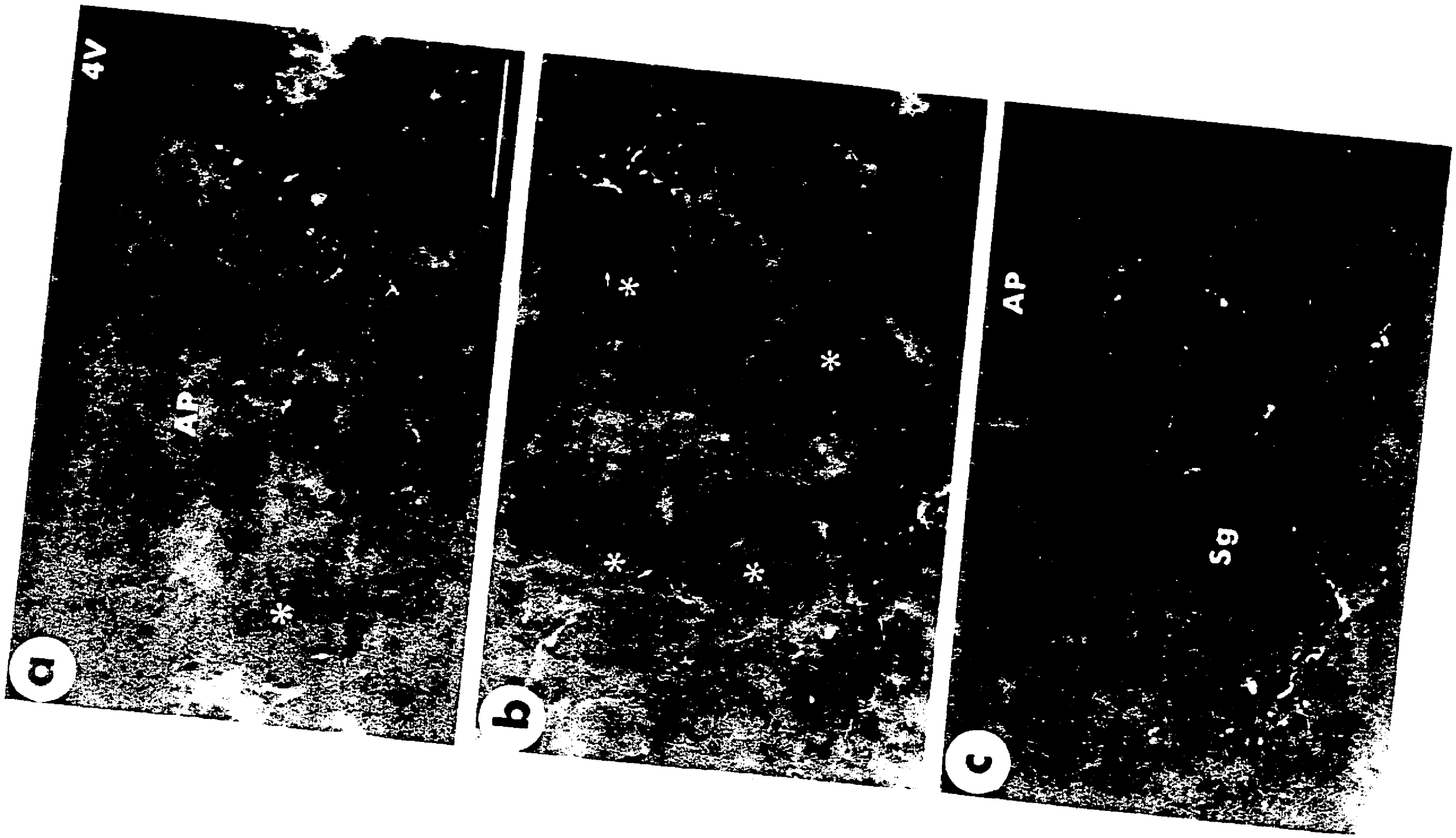
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Neurotensin Pathway From Arcuate Nucleus to Subfornical Organ

by

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Key Words: Circumventricular organ, Cardiovascular regulation, Body fluid homeostasis, Plasma osmolality.

The projections of arcuate nucleus of the hypothalamus (Arc) containing neurotensin (NT)-like immunoreactivity to subfornical organ (SFO) were studied in the rat. The anterograde tract-tracer Phaseolus vulgaris leucoagglutinin (PHA-L) was microiontophoresed into the region of Arc that contains NT neurons and after a 9-12 day survival period the animals were sacrificed and forebrain sections were processed for combined PHA-L and NT immunoreactivity. PHA-L labelled fibers and presumptive terminal boutons were found in SFO primarily on the side ipsilateral to the site of injection. A small number of the PHA-L labelled fibers in the lateral aspect of SFO was also immunoreactive to NT. These data demonstrate the existence of a direct pathway from Arc to SFO that contains the putative neurotransmitter NT and suggest that this pathway may function in the modulation of neural and/or humoral events related to cardiovascular regulation and body fluid homeostasis by influencing the activity of SFO neurons.

In addition to its well know role in neuroendocrine function [5, 31], the hypothalamic arcuate nucleus (Arc) has been suggested to play an important role in the central control of the circulation [2, 16]. Stimulation of Arc has been shown to elicit changes in arterial pressure, inhibit the baroreceptor reflex and increase the release of vasopressin [2, 16, 21, 24, 25]. Recently, Arc has also been implicated in the control of body fluid homeostasis. Chronic water deprivation and hypertonic saline ingestion has been reported to increase protein synthesis in Arc neurons [17]. In addition we have recently demonstrated that intracerebroventricular injections of hypertonic saline resulted in the expression of the phosphoprotein fos in Arc neurons [32]. Furthermore, stimulation of Arc has been shown to alter the discharge rate of subfornical organ (SFO) neurons [28], and to modulate the response of SFO neurons to changes in plasma hypernatremia and angiotensin II [29]. The SFO is thought to function as a central site for the detection of bloodborne signals of dehydration and hypovolemia [1, 13, 19, 20].

The present study was done to investigate whether Arc neurons project directly to SFO using the anterograde tract-tracer Phaseolus vulgaris leucoagglutinin (PHA-L). In addition, as neurons containing the tridecapeptide neurotensin (NT) [3, 4] have been identified throughout Arc in the rat [7, 14, 22], and NT has been implicated as a putative transmitter in central pathways that function in cardiovascular control [9], the anterograde tracing

technique was combined with immunohistochemistry for NT.

Experiments were done in 7 male Wistar rats (275-350 g) under equithesin (0.3 ml/100g, i.p.; [8]) anesthesia. The head of the rat was fixed in a Kopf stereotaxic instrument and a 2 mm hole was made in the bone overlying the region of Arc. A glass micropipette (internal tip diameter, 10-30 μm) filled with 2.5% PHA-L in 10 mM potassium phosphate buffered saline (pH 7.2-7.4) was lowered stereotaxically into the dorsal region of Arc. The PHA-L was iontophoresed as previously described [10, 11]. The current (5 μA, anodal) was delivered through a Grass PSIU stimulus isolation unit using 7 s pulses every 14 s for 20-30 min. The rats were given postoperative care and allowed to survive for a period of 9-12 days. After the survival period the rats were anesthetized with pentobarbital sodium (65 mg/Kg, i.p.) and perfused transcardially with 300 ml of physiological saline followed by 300 ml of fixative at 4 °C that consisted of 4% paraformaldehyde in 0.4 M phosphate buffer with lysine (3.3 g/L) and sodium m-periodate (0.55 g/L) at pH 7.2-7.4. Serial transverse sections of the forebrain were cut at 50 μm in a cryostat (-17 °C) and collected in phosphate buffered saline. One in every two sections was treated with a mixture of primary antisera in equal volumes of goat anti-PHA-E+L (Vector Laboratories, Burlingame, CA) and polyclonal rabbit anti-NT (Instar, Stillwater, MN) diluted at 1:200 in phopsphate buffered saline/0.3% Triton X-100. The immunohistochemical procedures described previously by Roder and Ciriello [27] for the combined demonstration of PHA-L and neuropeptide labelled fibers was used.

PHA-L labelling was visualized by placing the tissue sections in Streptavidin Texas red (Amersham, Oakville, ON, Canada), whereas NT was visualized by placing the sections in Streptavidin 7-amino-4 methyl coumarin-3-acetic acid (AMCA; Jackson Immunoresearch Laboratories, West Grove, PA). Controls for double labelling for PHA-L and NT included sections that were placed in only one of the primary antisera or in which one of the primary antisera had been preadsorbed by one of the antigens. Immunoreactivity for PHA-L could not be observed if the tissue sections were only incubated in primary antisera for NT. The converse was also true. The sections were examined and photographed by fluorescence microscopy.

The location and extent of PHA-L injections sites were determined by the distribution of PHA-L immunoreactive neuronal perikarya in Arc. All PHA-L injections were located in the middle third of the rostrocaudal extent of Arc, and overlapped primarily the dorsomedial aspect of the nucleus (Fig. 1a), the region where NT neurons have previously been located [7, 22], and where NT neurons were also observed in this study. Some of the cell bodies in Arc labelled with PHA-L were also found to be immunoreactive to NT (Fig. 1b).

PHA-L labelled fibers and presumptive terminal boutons were found throughout the rostral half of SFO. Most were observed in the lateral aspect of the nucleus on the side ipsilateral to the injection site, although an occasional PHA-L labelled fiber was found in the contralateral SFO. These fibers were scattered among neuronal cell bodies and near perivascular spaces (Fig. 1c-f). A

small number of PHA-L labelled fibers were also found to contain NT-like immunoreactivity (Fig. 1c-f). Most of these double labelled fibers were observed in close proximity to blood vessels in the superficial layers of SFO (Fig. c and f). Few, if any, were observed to extend into the deeper part of SFO.

These data provide the first anatomical evidence of a direct projection from neurons in Arc to SFO. In addition, the results have shown that some of the fibers in this tract to SFO contain the putative neurotransmitter NT. These observations are in agreement with the earlier demonstration that activation of Arc neurons alters the discharge rate of neurons in SFO [28], and provides evidence for the anatomical substrate and possible putative neurotransmitter by which Arc exerts these effects on SFO. It is interesting to note that the Arc projections were observed to those areas of SFO that have previously been shown to contain neurons that respond to plasma hypernatremia and angiotensin II [13, 29] and that project directly to hypothalamic structures that have magnocellular neurosecretory neurons [6, 23, 30]. As SFO is known to also project directly back to the Arc [12, 18, 15], these findings suggest the possibility of a feedback loop by which SFO may alter the excitability of Arc neurons to afferent neuronal inputs and Arc, in turn, may influence the sensitivity of SFO output neurons to bloodborne substances [29].

The observation of PHA-L and NT immunoreactive fibers scattered around neuronal cell bodies and in close proximity to blood vessels in the lateral aspects of the superficial layer of

SFO suggests that this neuroactive peptide may not only exert a modulatory effect on neuronal elements [33], but may also be released into the blood stream where it may exert an effect on local blood vessels. NT administered systemically has been shown to result in a decrease in arterial pressure [3, 9, 26]. Therefore, the possibility exists that NT released into SFO vessels may produce a local vasodilatation that could result in an increase in blood flow that would therefore allow a greater exposure of receptors/neurons in SFO to bloodborne substances. This also could occur if the NT released into the blood vessels changed the permeability of the vessels to bloodborne substances. This possibility is supported by the recent observation that activation of Arc potentiates the response of SFO neurons to plasma angiotensin II [29].

In summary, these data have provided evidence for a direct NT pathway from Arc to SFO. Although the function of this pathway is not known, it may be involved in influencing the activity and/or sensitivity of SFO neurons to bloodborne substances.

Acknowledgements

This work was supported by The Heart and Stroke Foundation of Ontario. J.C. is a Career Investigator of the Heart and Stroke Foundation of Ontario, and M.P.R-A. and L.P.S-F. are visiting scientists from the Departamento de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México and holders of D.G.A.P.A. awards.

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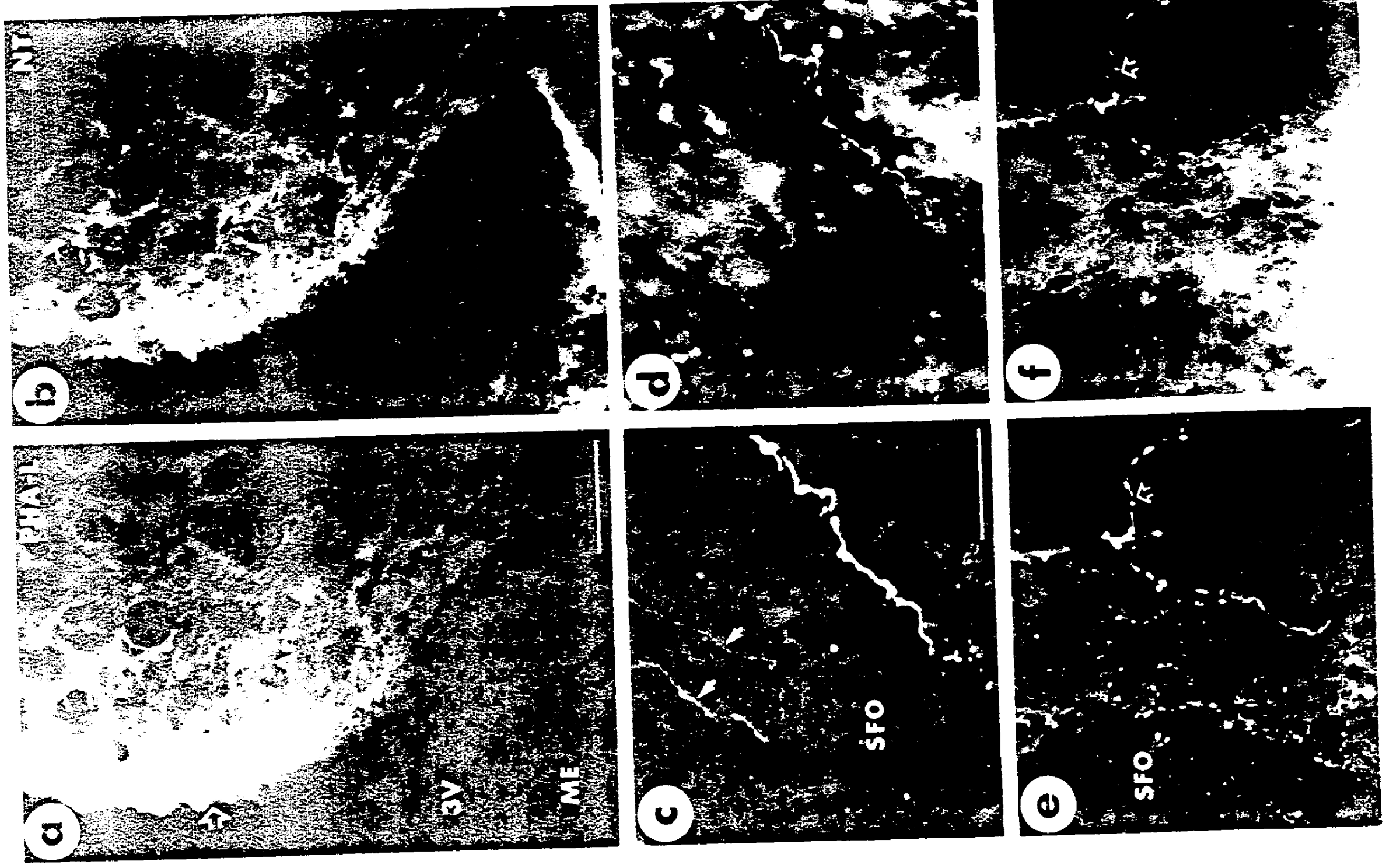
FIGURE LEGEND

Figure 1.

Fluorescent photomicrographs that show the location of a PHA-L injection site (a; open arrow) that overlapped NT immunoreactive neurons in Arc (b) and the resulting PHA-L labelled fibers and presumptive terminal boutons in SFO (c and e) that were also immunoreactive to NT (d and f). In (a), (c) and (e), PHA-L is tagged with Texas red and in (b), (d) and (f) the NT is tagged with AMCA. Arrows in (a) and (b) point to PHA-L-positive cells also immunoreactive to NT. Calibration mark of 100 μm in (a) applies to both (a) and (b). Arrows in (c) point to PHA-L labelled fibers that were not immunoreactive to NT (d). Arrow in (e) points to PHA-L labelled fiber that was also immunoreactive to NT (f). Calibration mark in (c) of 50 μm applies to (c) to (f).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Brain Research, 619 (1993) 352–356

BRES 25776

Effect of arcuate nucleus activation on neuronal activity in subfornical organ

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(Accepted 11 May 1993)

Key words: Cardiovascular regulation; Body fluid homeostasis; Plasma osmolality; Angiotensin II; Circumventricular organ

Experiments were done in urethane anesthetized rats to investigate the effect of activation of the arcuate nucleus (Arc) on the discharge rate of single units in the subfornical organ (SFO). Extracellular recordings were made from 51 spontaneously active neurons histologically verified in the SFO. Of these units, 19 (37%) were inhibited (mean latency 7.3 ± 1.1 ms) and 14 (28%) were excited (mean latency 12.8 ± 3.2 ms) during electrical stimulation of the Arc. The remaining 18 units did not alter their firing frequency during stimulation of the Arc. These data suggest that the Arc may be involved in the modulation of neural and humoral events related to body fluid homeostasis and cardiovascular regulation by altering the activity of SFO neurons.

It is well established that the hypothalamic arcuate nucleus (Arc) plays an important role in neuroendocrine function[20]. In addition, the Arc has been suggested to be involved in the control of the cardiovascular system because of its effects on the arterial pressure, baroreceptor reflex and release of vasopressin[1,9,13–15]. Furthermore, the Arc has been implicated in the control of body fluid balance as chronic water deprivation and hypertonic saline ingestion results in increased protein synthesis in Arc neurons[10].

The subfornical organ (SFO), a circumventricular structure that lacks a blood–brain barrier, is thought to be an important site for the detection of bloodborne signals of hypovolemia and dehydration[5]. The SFO in turn has been shown to activate a neuronal circuit that corrects these changes in blood volume, arterial pressure and plasma osmolality[3,7,8,12,17].

Recently, we have demonstrated using the anterograde transport of phaseolus vulgaris leucoagglutinin, that neurons in the Arc project directly to the SFO[18]. This finding suggests that the Arc may contribute to the control of arterial pressure and to body fluid balance by influencing the activity of neurons in the SFO. The present study was done to investigate electrophysiologically the effect of activation of the Arc on the discharge rate of neurons in the SFO.

Experiments were done in six adult male Wistar rats (320–350 g) under urethane anesthesia (1.5 g/kg i.p.) instrumented with a femoral artery cannula for the recording of arterial pressure. Rectal temperature was monitored and maintained at 35–37°C using a heating pad controlled by a Yellow Springs temperature controller. All animals were allowed to breathe spontaneously. The head of the rat was fixed in a stereotaxic frame and access to the Arc and the SFO was obtained by a partial craniotomy. All exposed nervous tissue was covered with warm Dow Corning 360 medical fluid to prevent drying.

Bipolar stainless steel microelectrodes (SNEX-100; David Kopf, Tujunga, CA; 0.25 mm tip diameter; 50–70 kΩ initial resistance in saline) were stereotaxically placed into the region of the left Arc. The stimulus applied was a rectangular pulse of 0.5-ms duration at a current intensity of 0.1 mA. The threshold current required to activate each unit was also determined. The stimulation pulse was delivered from a Grass S88 stimulator through a Grass PS1U6D stimulus isolation and constant current unit.

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The region of the SFO was explored for spontaneously active single units using glass microelectrodes filled with 2% pontamine sky blue in 0.5 M Na acetate (initial impedance 10–14 MΩ). The reference point for positioning the electrode was the longitudinal fissure at ~ 0.9–1.6 mm posterior to bregma according to a stereotaxic atlas[16]. Single unit activity was recorded extracellularly, amplified using an AXOPROBE-1A (Axon Instruments, Foster City, CA) differential DC preamplifier, filtered using a 2004-F Signal Conditioner (Intronix Technologies, Woodbridge, Ontario, Canada), displayed on a Tektronix 5113 oscilloscope for observation and photography and led through a window discriminator (ME04011; Frederick Haer, Brunswick, ME), to a A/D multiplexer (DAM0405; CY Electronics, London, Ontario, Canada) and an AST 286 microcomputer with an A/D conversion board from Data Translation, for the generation of peristimulus time histograms.

Most central sites of recording and all stimulation sites were marked at the end of each experiment. Recording sites were determined by interpolation between at least two Pontamine sky blue deposits (10-μA negative current for 30 min) for each recording penetration in each animal. Stimulation sites were marked by depositing iron from the electrode tip (10 μA for 20 s, electrode tip positive). The animals were perfused with 50 ml of a 0.9% saline solution followed by 50 ml of a 3% K ferrocyanide in 10% buffered formalin to reveal the marked stimulation sites by the Prussian blue reaction. The brains were further fixed in 3% K ferrocyanide in 10% buffered formalin for at least 12 h and 50-μm frozen transverse sections of the forebrain were cut and stained with Neutral red. Recording and

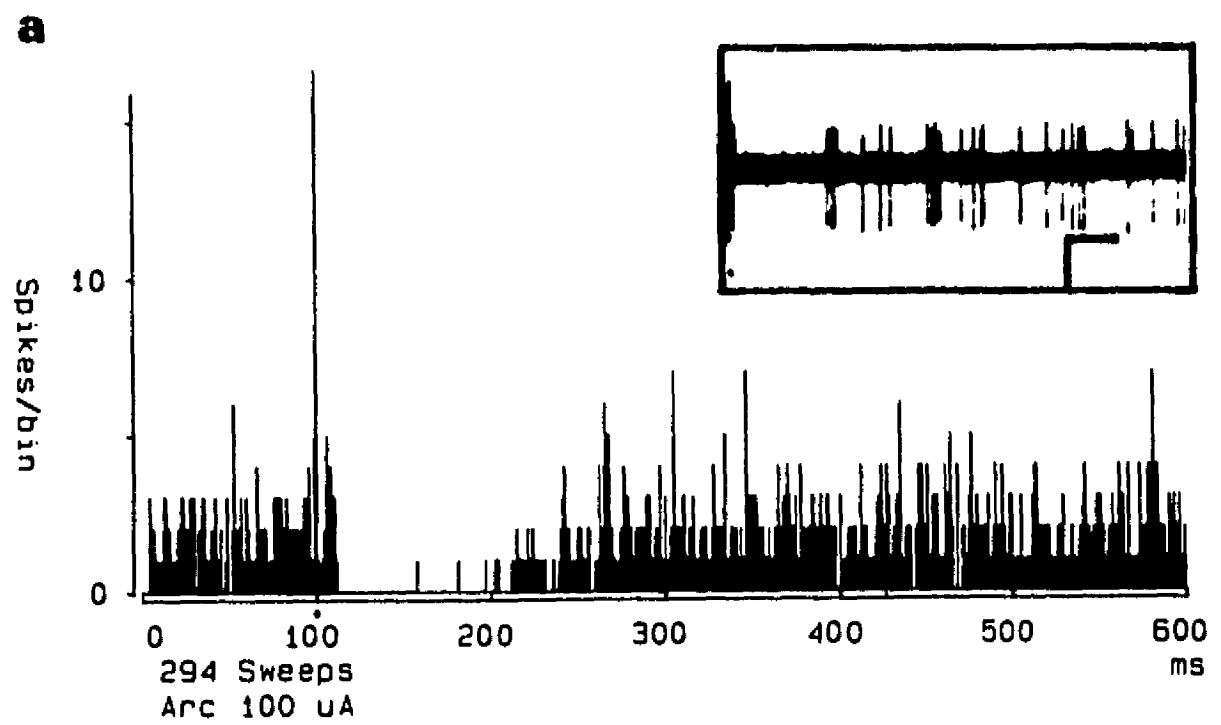

Fig. 1. Peristimulus time histograms (1 ms/bin) of units in SFO inhibited (Fig. 1a) or excited (Fig. 1b) by stimulation of Arc. Insets in Fig. 1a and 1b are 15 superimposed oscilloscope tracings of orthodromic response of units to Arc stimulation. Stimuli were delivered at dots. Calibration is 50 ms and 100 μV in Fig. 1a and 5 ms and 100 μV in Fig. 1b.

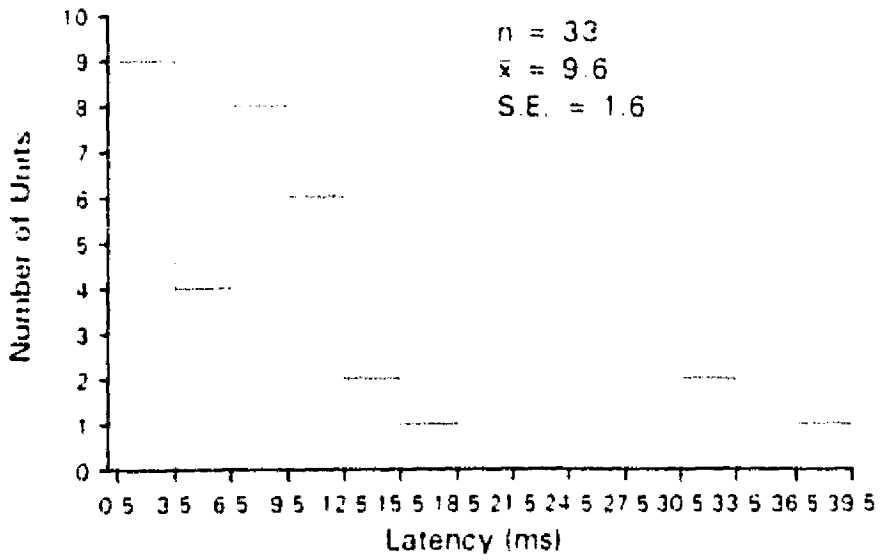

Fig. 2. Histogram showing distribution of latencies of single units in SFO responding to stimulation of Arc.

stimulation sites were mapped on projection drawings of the forebrain of the rat.

The criteria for establishing that an orthodromic response had occurred were similar to those of Yang and Mogenson[22]. Changes in the firing rate of neurons in SFO after Arc stimulation were identified and quantified by comparing the height of each poststimulus bin of the peristimulus time histogram with the average height of the bins before the stimulus. The average bin height for the period of 100 ms before the stimulus was considered as the baseline discharge rate of the SFO neuron. Responses were defined as the occurrence of a period of time after the stimulus during which the mean height of the peristimulus time histogram was 30% above or below the mean baseline discharge rate. The beginning (onset latency) and end boundaries of possible periods of significant responses (response duration) were defined by the occurrence of five consecutive bins with heights that were >2 S.D. values from the baseline mean. Latencies, duration and spontaneous discharge rates were compared statistically by an ANOVA and Student's t test. A P value of <0.05 was considered statistically significant.

The SFO was explored for spontaneously active (0.02–3.64 spikes/s) single units that altered their discharge rates to electrical stimulation of histologically verified sites in the Arc. Of 51 units recorded extracellularly from histologically verified sites in the SFO, 33 (65%) responded orthodromically to stimulation of the Arc: 19 (37%) were inhibited (Fig. 1a) with a mean latency of 7.3 ± 1.1 ms (mean duration of response 90.1 ± 13.4 ms), and 14 (28%) were excited (Fig. 1b) with a mean latency of 12.8 ± 3.2 ms (mean duration of response 19.4 ± 5.6 ms). The remaining 18 units (35%) did not alter their discharge rate during stimulation of the Arc. A histogram showing the distribution of latencies of single unit responses in SFO to Arc stimulation is shown in Fig. 2.

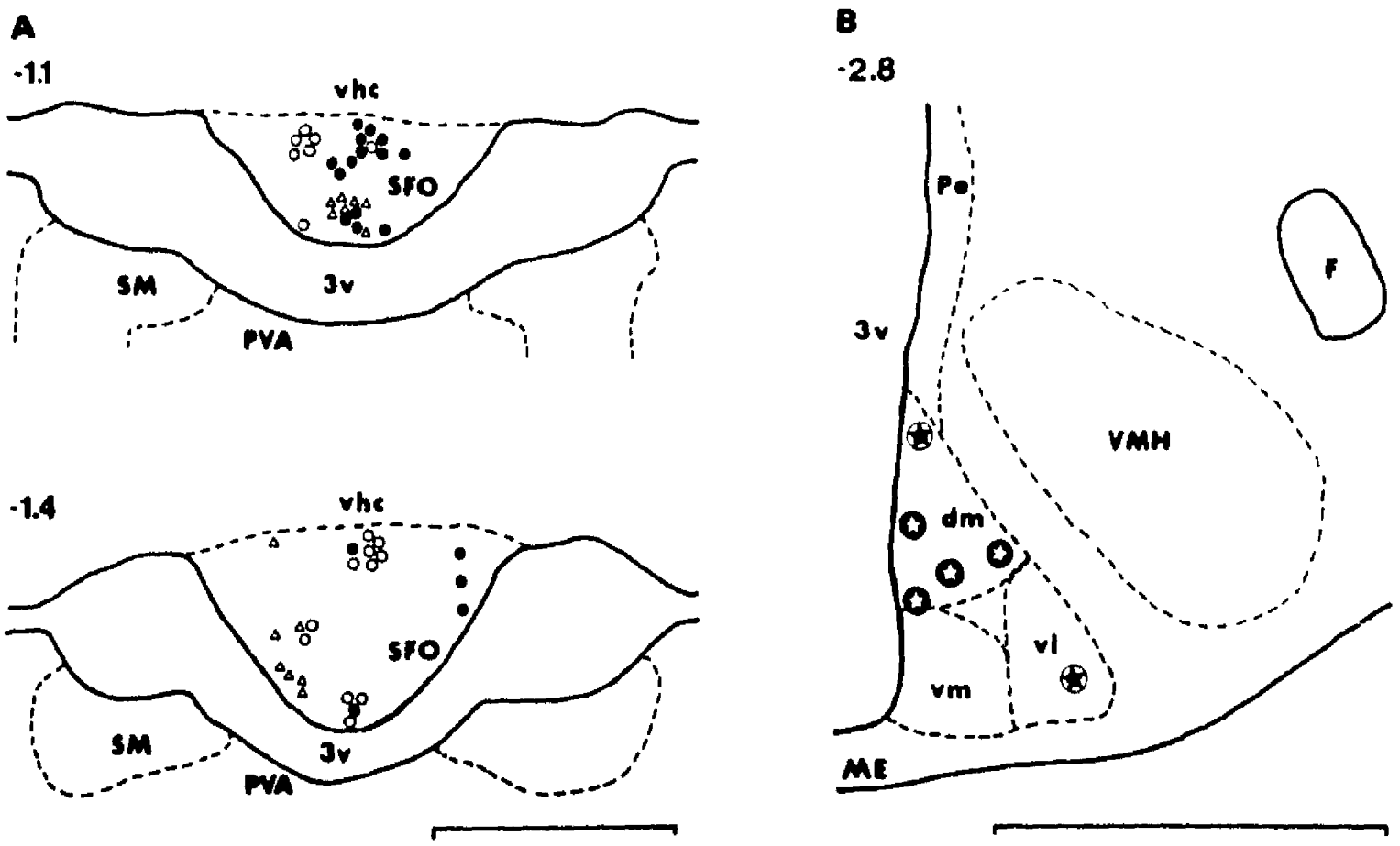

Fig. 3. Representative transverse sections of rat forebrain taken through region of SFO at 1.1 and 1.4 mm caudal to bregma (A) and through region of Arc at 2.8 mm caudal to bregma (B). A, location of histologically verified sites of single units responding to electrical stimulation of Arc: ●, units inhibited; △, units excited; ○, no response. B, location of histologically verified sites of stimulation in Arc: circle with filled star ✪, sites that elicited excitatory single unit responses in SFO only; filled circle with star ✪, sites that elicited predominantly inhibitory responses in SFO. Calibration marks, 1 mm. 3v, third ventricle; dm dorsomedial component of Arc; F, fornix; ME, median eminence; Pe, periventricular hypothalamic nucleus; PVA, paraventricular nucleus of thalamus; SM, stria medullaris thalami; vhc, ventral hippocampal commissure; vl, ventrolateral component of Arc; vm, ventromedial component of Arc; VMH, ventromedial hypothalamic nucleus.

Although the range of latencies of the SFO units that were inhibited by Arc stimulation overlapped with that of the units that were excited, the inhibited units responded with a significantly shorter latency. On the other hand, the duration of the inhibitory response was significantly longer than that of the excitatory response. Similarly, the mean spontaneous discharge rate of SFO units inhibited by Arc stimulation was significantly greater (0.67 ± 0.18 spikes/s) than that of either the excited units (0.12 ± 0.02 spikes/s) or that of those units that did not respond to Arc stimulation (0.22 ± 0.04 spikes/s).

The location of all units in the SFO tested for Arc inputs is shown in Fig. 3A and that of the stimulation sites in the Arc in Fig. 3B. Most of the units inhibited by Arc stimulation were found in the dorsal half of the SFO whereas most of those excited were found within the ventral half of the nucleus (Fig. 3A). Stimulation sites in the Arc that elicited primarily inhibitory single unit responses in the SFO were localized to the ventral aspect of the dorsomedial subnucleus of the Arc (Fig. 3B). Stimulation sites in either the dorsal aspect of the dorsomedial subnucleus of the Arc or in the lateral aspects of the ventrolateral subnucleus of the Arc elicited excitatory responses only in SFO.

These data provide the first electrophysiological evidence that neurons in the Arc exert an influence on the discharge rate of neurons in the SFO. This finding supports the earlier demonstration of a direct anatomical projection from Arc to the SFO[18].

The anatomical distribution of the single units in the SFO responding to Arc stimulation overlaps that of SFO neurons that have been shown to project to the supraoptic and paraventricular hypothalamic nuclei and to the median preoptic nucleus[6,19,21], and which responded to changes in angiotensin II in the systemic circulation or in plasma osmolality[5]. Although no attempt was made in this study to identify the output projection system of the SFO neurons responding orthodromically to stimulation of the Arc or their response to bloodborne substances, the finding of populations of neurons that were either excited or inhibited by Arc stimulation and that have different spontaneous discharge rates, may reflect different physiological roles of these neurons. Data suggesting that different functions are mediated by separate populations of neurons in the SFO have previously been presented[5,6,11].

The finding in this study that Arc stimulation alters the activity of SFO neurons, taken together with the earlier demonstration that the SFO projects directly to the Arc[4], suggests the possibility of a feedback loop by which the Arc may influence the excitability of SFO output neurons to bloodborne substances. In addition, through these bidirectional connections, the SFO may alter the sensitivity of Arc neurons to incoming afferent inputs[2,4] which than in turn may alter the sensitivity of SFO neurons. Therefore, on the basis of these data it is not unreasonable to suggest that the Arc may function as a modulatory structure in the neuronal and humoral events related to cardiovascular regulation and body fluid homeostasis.

This work was supported by the Medical Research Council of Canada and Heart and Stroke Foundation of Ontario. The authors acknowledge the technical assistance of J. Nichols and Z.M. Zhang. J. Ciriello is a Heart and Stroke Foundation of Ontario Career Investigator and M.P. Rosas-Arellano was the holder of a ICCS of Canada Studentship and a DGAPA scholarship from the Universidad Nacional Autónoma de México. L.P. Solano-Flores is a visiting scientist from the Universidad Nacional Autónoma de México.

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EFFECT OF ARCUATE NUCLEUS ON RESPONSES OF SUBFORNICAL ORGAN NEURONS TO PLASMA HYPERNATREMIA AND ANGIOTENSIN II

by

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Pages: 23
Figures: 7

Running title: Arcuate nucleus effects on SFO neurons.

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ABSTRACT

ROSAS-ARELLANO, M. PATRICIA., L. PASTOR SOLANO-FLORES, AND JOHN CIRIELLO. Effect of arcuate nucleus on responses of subfornical organ neurons to plasma hypernatremia and angiotensin II. Am. J. Physiol. (Regulatory Integrative Comp. Physiol.) : R -R

, 1994. -Experiments were done in urethane anesthetized rats to investigate the effect of arcuate nucleus (Arc) stimulation on the discharge rate of subfornical organ (SFO) neurons during changes in plasma hypernatremia and angiotensin II (ANG II). Extracellular recordings were made from 110 histologically verified single neurons in SFO that responded with either excitation (90/110; 82%) or inhibition (20/110; 18%) to Arc stimulation. Thirty (33%) of the units excited by Arc were also excited by intracarotid infusion of hypertonic (0.5 M) NaCl. Similarly, 30 (33%) units excited by Arc were also excited by intracarotid infusion of ANG II. Additionally, 5 (2%) of the units inhibited by Arc were found to be excited by ANG II. Of the 30 neurons activated by Arc and NaCl infusion, 20 were tested for their response during combined activation by Arc and NaCl. All 20 neurons showed a decreased (96%) response to the NaCl infusion after Arc stimulation. Of the 35 neurons responding to ANG II, 24 were tested for their response during combined stimulation of Arc and ANG II infusion. The response to ANG II of 10 (42%) neurons activated by Arc stimulation was abolished, whereas that of 9 (38%) neurons was potentiated (39%). In the remaining 5 neurons inhibited by Arc stimulation, the ANG II response was attenuated by approximately

66%. These data indicate that Arc alters the firing rate of SFO neurons during changes in plasma concentration of Na^+ and ANG II, and suggest that Arc may be involved in body fluid balance and circulatory regulation by modulating the activity of SFO neurons that function in the detection of blood-borne signals from the depletion of intra- and extra-cellular fluid volumes.

Key words: cardiovascular regulation; body fluid homeostasis; hypothalamus; circumventricular organs.

INTRODUCTION

TRADITIONALLY, ARCUATE NUCLEUS OF THE HYPOTHALAMUS (Arc) has been thought to be involved in neuroendocrine function (5, 31). However, experimental evidence has recently been obtained suggesting an important integrative role for Arc in the control of cardiovascular and body fluid homeostasis. Stimulation of the rostral Arc has been shown to elicit a vasopressin mediated pressor response (2, 17, 21), whereas stimulation of the caudal Arc elicits a depressor and bradycardia response and potentiates the bradycardia response during activation of the baroreceptor reflex (17, 21). With regards to body fluid balance, chronic water deprivation has been shown to result in elevated protein synthesis (18) and prepro-NPY mRNA levels (23) in Arc neurons. In addition, chronic hypertonic saline ingestion resulted in increased protein synthesis in Arc neurons (18). Finally, we have recently

demonstrated that intracerebroventricular infusions of hypertonic saline results in the induction of the phosphoprotein Fos in Arc neurons (33).

The subfornical organ (SFO), a circumventricular organ that lacks a blood-brain barrier (6, 11), is thought to be involved in detecting blood-borne signals of body fluid volume perturbations (1, 24). In turn, SFO activates neuronal circuits that restore body fluid balance and arterial pressure (AP) (7, 13, 16, 22). Activation of SFO neurons has been shown to increase drinking (20, 32), AP (12, 15, 20), and the release of vasopressin (9, 10).

Recently, we have shown that SFO receives a direct projection from Arc (26). In addition, activation of Arc alters the firing rate of SFO neurons (27). Taken together, these data suggest that Arc may be involved in altering the excitability of SFO neurons to plasma hypernatremia and angiotensin II (ANG II).

Therefore, in this study, experiments were done to investigate the effects of Arc stimulation on the response of SFO neurons to intracarotid infusion of hypertonic saline or ANG II. A preliminary account of this investigation has been presented elsewhere (28).

METHODS

Surgical procedures. Experiments were done in 30 adult male Wistar rats weighing 300-430 g under urethane anesthesia (1.5 g/kg, i.p.). The animals were instrumented with femoral arterial and venous cannulae for the recording of AP and administration of drugs, respectively. AP was recorded through a Statham transducer

(P23Db) and a 7P4FG Grass tachograph triggered by the AP pulse was used to monitor heart rate (HR). Both AP and HR were continuously recorded on a Grass model 7D polygraph. The internal carotid artery was cannulated, for infusions of solutions, by insertion proximal to the carotid bifurcation after denervation of that carotid sinus. The external carotid artery on the same side was ligated, and the cannula was sutured to the underlying muscle. The trachea was cannulated and the animals were allowed to breathe spontaneously. The head of the animal was fixed in a Kopf stereotaxic frame, and access to the forebrain was obtained by partial craniotomy. The sagittal sinus was isolated and tied off, and the dura was retracted. All exposed nervous tissue was covered with Dow Corning 360 medical fluid to prevent drying. Rectal temperature was monitored and maintained by a heating pad at 36-37 °C controlled by a Yellow Springs 73A temperature controller.

Electrical stimulation of Arc. Concentric bipolar stainless steel electrodes (SNEX-100, David Kopf, Tujunga, CA; 0.25 mm tip diameter; 0.25 mm tip to concentric ring distance; 50-70 KΩ initial DC resistance in saline) were stereotaxically placed into Arc at 2.6-3.2 caudal to bregma (25). The stimulus applied to Arc was a 20 Hz train of rectangular pulse of 0.5 ms duration at a current intensity of 500 μA for 10 s. The stimulus train was delivered from a Grass S88 stimulator through a Grass PSIU6 stimulus isolation and constant current unit.

Recording of single-unit activity in SFO. The region of SFO was explored for spontaneously active single units using glass

microelectrodes filled with 0.5 M sodium acetate containing 2% pontamine sky blue and that had an initial impedance of 4-6 MΩ. The reference point for positioning the microelectrode was the longitudinal fissure at approximately 0.9-1.4 mm posterior to bregma (25). Single unit activity was recorded extracellularly, amplified using an Axoprobe-1A (Axon Instruments; Foster City, CA) differential DC preamplifier, filtered using a 2004-F Signal Conditioner (Intronix Technologies, Woodbridge, ON, Canada), displayed on a Tektronix 5113 storage oscilloscope for observation and photography and led through a window discriminator (model S/H 74-60-1; Frederick Haer, Brunswick, ME) to an AST 286 microcomputer (A/D conversion board from Data Translation).

Spontaneously active single units encountered in SFO during an electrode penetration were tested for their response to stimulation of Arc, and intracarotid infusion of 0.1 ml (0.01 ml/s) of hypertonic NaCl solution (0.5 M) or ANG II (0.5 μg), and isotonic saline (0.9%). Infusion of isotonic saline was made to eliminate the possibility that changes in firing rate of SFO neurons were due to alterations in cerebral blood flow. In addition, as the hypertonic NaCl and ANG II infusion were observed to transiently raise systemic AP (mean AP, 10 - 48 mmHg), the effect of a pressor dose of phenylephrine (10 μg/kg in 0.1 ml) on the firing rate of the neurons was also tested to determine whether changes in neuronal firing frequency were due to AP changes rather than the direct effect of hypertonic NaCl or ANG II on the neurons.

The return of base-line firing rate and pattern was required

before a second test solution was infused. Time of each test was simultaneously marked on the polygraph AP and HR responses, and on computer-generated rate meter records of neuronal firing.

In some cases single units activated by either the hypertonic NaCl solution or ANG II were further tested after a conditioning-stimulus was applied to Arc 15 s prior to the response of the SFO neurons to the hypertonic NaCl or ANG II test-stimulus. This was done as the peak mean latency for the response of the SFO neuron to the test stimuli was 29.25 ± 2.11 s (range, 17-79 s).

Data analysis. The criteria for establishing that a response had occurred were similar to those previously described (13, 35). Changes in the firing rate of SFO neurons following stimulation of Arc or following the intravascular injections of various solutions were identified and quantified by comparing the height of each post-stimulus bin of the rate meter histogram with the average height of the bins before the stimulus. The average bin height for the period of 50 s before the stimulus was considered as the baseline firing rate of the SFO neuron. Responses were defined as the occurrence of a period of time following the application of the stimulus during which the mean height of the rate meter histogram was 30% above or below the mean baseline firing rate. The beginning (onset latency) and end boundaries of possible periods of significant responses (duration) were defined by the occurrence of 3 consecutive bins with heights that were more than one standard deviation from the baseline mean.

To determine whether a conditioning stimulus to Arc had an

effect on the response of the SFO unit to hypertonic NaCl or ANG II infusions, the sum of all bin heights of the SFO unit response to the test-stimuli alone was compared to the sum of all bin heights of the SFO unit response to the test-stimuli when a conditioning-stimulus was applied to Arc. Changes in the magnitude of the response were statistically evaluated using an ANOVA for repeated measures followed by a post-hoc paired t-test. A p value of less than 0.05 was considered statistically significant.

Histological localization of recording and stimulation sites. Most central sites of recording were marked by Pontamine sky blue deposits (10 μA negative current for 45 min) and all stimulation sites were marked by depositing iron from the electrode tip (3 μA for 10s, electrode tip positive) at the end of each experiment. Recording sites were also determined by interpolation between marked sites in an electrode penetration in each animal. The animals were perfused with 50 ml of a 0.9% saline solution followed by 50 ml of a 3% potassium ferrocyanide in 10% buffered formalin solution to reveal the marked stimulation sites by the Prussian blue reaction. The brains were further fixed in 3% potassium ferrocyanide in 10% buffered formalin for at least 12 h. Fifty μm frozen transverse sections of the forebrain were cut and stained with Neutral red. Recording and stimulation sites were mapped onto projection drawings of the forebrain for each animal. The nomenclature from the stereotaxic atlas of Paxinos and Watson (25) was used. A representative example of a recording site in SFO and a stimulation site in Arc is shown in Figure 1.

RESULTS

The region of SFO was explored for spontaneously active (0.2-4.8 spikes/s) single units that responded to electrical stimulation of histologically verified sites in Arc (Figs. 1b and 2). A total of 110 histologically verified single units were recorded extracellularly in SFO that responded to stimulation of Arc (Figs. 1a and 3). Of these responsive units, 82% (90/110) were excited and 18% (20/110) were inhibited by Arc stimulation. In addition, 30 of these units responded to hypertonic saline infusions and 35 to intracarotid administration of ANG II. The remaining 45 SFO units did not respond to the intravascular infusions of the solutions. In general, as shown in Figure 2, sites that elicited changes in single unit activity in SFO were located in the lateral aspects of Arc, primarily in a region including the ventral aspect of the dorsomedial subdivision of Arc and the dorsal aspect of the ventrolateral subdivision of Arc. Sites that excited SFO units and potentiated the excitatory effect of ANG II on single unit activity in SFO were located primarily in the rostral Arc. On the other hand, sites that inhibited SFO single unit activity and inhibited the excitatory effect of ANG II on single unit activity in SFO were localized to the caudal Arc. Sites located in the medial aspect of Arc, in the median eminence or along the ventromedial border of the ventromedial hypothalamic nucleus were ineffective in evoking changes in single unit activity in SFO. The response of SFO units to Arc stimulation was apparent immediately after the application of the stimulus (Figs. 4 and 6) and lasted 15 - 138 s.

Thirty of the 110 (27%) SFO units responsive to Arc stimulation were also activated by intracarotid infusion of hypertonic NaCl (Figs. 4 and 5A) with a mean latency (from onset of the infusion to the peak of the response) of 30.25 ± 3.98 s (range, 17 - 79 s) and had a response duration of 42.95 ± 6.09 s (range, 15 - 106 s). Of these 30 units, 20 were also tested for their response to hypertonic saline after application of a conditioning stimulus to Arc (Fig. 4). The response of the 20 units to the hypertonic saline stimulus after Arc stimulation was not significantly different from the response of the units to the hypertonic saline stimulus alone (Fig. 5A). However, the units response to the hypertonic saline stimulus after Arc stimulation was significantly smaller than the algebraic summation of the Arc and hypertonic saline responses alone (Fig. 5A). Similarly, the units response to the hypertonic saline stimulus during the combined stimulation was found to be significantly reduced (96%) following subtraction of the units response to Arc alone from the combined response (Fig. 5A).

Thirty-five of the 110 (32%) responsive units in SFO to Arc stimulation also responded to intracarotid infusion of ANG II with a mean latency (from the onset of the infusion to the peak of the response) of 28.42 ± 2.07 s (range 17 - 56 s) and a duration of 53.7 ± 6.44 s (range, 13 - 120 s) (Figs. 5B, C and D, and 6). Twenty-four of these units were further tested for their responses to ANG II after application of a conditioning stimulus to Arc. The response of 10 units to the ANG II after Arc stimulation was

reduced compared to the response of the units to the ANG II stimulus alone (Fig. 5B). In addition, the units response to the ANG II stimulus during the combined stimulation was found to be abolished after subtraction of the units response to Arc alone from the combined response (Fig. 5B).

The response of 9 units to ANG II after Arc stimulation was potentiated compared to the units response to the ANG II stimulus alone (Fig. 5C and 6). The response of the units to ANG II after Arc stimulation was found to be significantly greater than the algebraic summation of the Arc and ANG II responses alone (Fig. 5C). Similarly, the units response to the ANG II during the combined application of the ANG II and Arc stimuli was significantly greater (39%) after subtraction of the response to Arc alone from the combined response (Fig. 5C).

Finally, 5 units were found to be inhibited by Arc stimulation and excited by ANG II (Fig. 5D). The response of the units to ANG II after Arc stimulation was abolished compared to the response of the units to ANG II alone (Fig. 5D). The response to ANG II during the combined stimulation was found to be significantly reduced (66 %) after subtraction of the units response to Arc alone from the combined response (Fig. 5D).

Forty five of the 110 (41%) units recorded in SFO that were activated by Arc stimulation did not respond to either infusion of hypertonic saline or ANG II.

It was consistently observed that systemic AP increased during the infusion of hypertonic saline or ANG II. To determine whether

the change in firing rate of SFO neurons during the infusion of the solutions were due to changes in AP, neurons were also tested for their response to an intravenous pressor dose of phenylephrine and intracarotid infusion of isotonic saline. In all cases, the firing rate of SFO neurons excited by hypertonic saline or ANG II was not altered by the rise in AP after phenylephrine injection or the isotonic saline infusions.

The location of units in SFO tested for Arc inputs is shown in Figure 3. Note that units responding to Arc stimulation and NaCl were found predominantly in the lateral aspect of SFO (Fig. 3a), whereas those that responded to both Arc stimulation and ANG II were found primarily in the medial aspects of the nucleus (Fig. 3b). Furthermore, neurons in SFO inhibited by Arc and excited by ANG II were found in the central parts of the nucleus, whereas those excited by Arc and excited by ANG II appear to surround the former group (Fig. 3b). Neurons responsive to Arc stimulation, but non-responsive to either the hypertonic saline or ANG II infusions were found predominantly along the dorsal border of SFO (Fig. 3a).

DISCUSSION

These data have provided electrophysiological evidence to suggest the existence of a pathway from Arc that may be involved in altering body fluid balance and the circulation by modulating the excitability of SFO neurons that respond to blood-borne ANG II or plasma hypernatremia. This is based on the demonstration that inputs from Arc converge on neurons that receive information about

plasma levels of ANG II or sodium and that the magnitude of the excitatory response of SFO neurons to change in plasma sodium concentrations or ANG II was altered by the application of a conditioning stimulus to Arc.

The finding that SFO neurons alter their firing rate to stimulation of Arc is consistent with our previous demonstration of a neuronal input from Arc to SFO neurons (27), and of a direct anatomical projection from Arc to SFO (26). Similarly, the finding that neurons in SFO increase their firing rate to plasma hypernatremia or ANG II is consistent with previous electrophysiological data (13).

It was observed that the excitatory response of SFO neurons to intracarotid infusion of hypertonic saline or ANG II was in most cases attenuated when a conditioning stimulus was applied to Arc. This inhibitory interaction occurred regardless if the SFO neuron was excited or inhibited during Arc stimulation. Although the present results do not directly address the mechanisms involved in these inhibitory interactions, it is likely that a postsynaptic mechanism was involved. It may be argued that in the situation where an inhibitory interaction occurs between the two inputs that independently excite the same SFO neuron, the SFO neuron may become hyperpolarized as a result of the initial excitation by the conditioning stimulus. Thus, the SFO neuron may be refractory at the time the hypertonic saline or ANG II response occurs. Intracellularly recorded action potentials from SFO neurons have shown large and prolonged after-hyperpolarizations (4). However,

as inhibitory post synaptic potentials have also been recorded in SFO neurons (4), it is also possible, as schematically shown in Figure 7, that the conditioning stimulus applied to Arc excited inhibitory interneurons that hyperpolarize the membrane of SFO cells that monitor changes in the plasma sodium concentration or ANG II. Therefore, the hypertonic saline or ANG II stimulus would not be of sufficient strength to bring the membrane potential of the SFO neuron to threshold. This latter possibility is supported by the demonstration of glutamic acid decarboxylase immunoreactivity in fiber varicosities and neurons in SFO (34). In addition, electrophysiological evidence exists suggesting the possibility of inhibitory interneurons in SFO (3).

A small number of SFO neurons was also found in this study to increase their firing rate to ANG II after a conditioning stimulus was applied to Arc. These data suggest that specific groups of neurons in Arc may be involved in the facilitation of a homeostatic response to elevated circulating levels of ANG II.

It is interesting to note that stimulation of the rostral Arc has been shown to elicit a vasopressin dependent pressor response (2, 17, 21). In addition, stimulation of SFO has been shown to result in the increased release of vasopressin (9) and in the increase of excitability of vasopressin secreting neurons in the paraventricular nucleus of the hypothalamus (PVH) (8). These data, taken together with the observation that systemic ANG II increases the activity of neurons in SFO that project to magnocellular neurosecretory neurons in the supraoptic and PVH (13), suggests

that the rostral Arc may modulate the release of vasopressin by altering the excitability of SFO neurons to systemic ANG II levels. This suggestion is further supported by the fact that stimulation sites in Arc that potentiated the excitatory effect of ANG II in single unit activity in SFO were located primarily in the rostral Arc.

On the other hand, stimulation of the caudal Arc elicits a depressor response mediated through a neural pathway to the dorsal vagal complex (17, 21). Stimulation sites in Arc that inhibited SFO single unit activity and inhibited the excitatory effect of ANG II on single units in SFO were localized to the caudal Arc. Taken together, these data suggest that the enhancing and/or dampening-actions of Arc on the excitability of SFO neurons may account for the dual nature of Arc in cardiovascular regulation (i.e. preventing a pressor effect by decreasing the excitability of SFO neurons to ANG II when a depressor effect mediated by the caudal Arc-dorsal vagal complex pathway has been evoked).

The anatomical distribution of the single units in SFO responding to Arc stimulation and to plasma hypernatremia or ANG II overlaps that area of SFO that contains neurons that project to hypothalamic regions containing magnocellular neurosecretory neurons and to the median preoptic nucleus (13, 29, 30). Although no attempt was made in this study to identify the output projections of the SFO neurons responding to Arc and blood-borne substances, the finding of populations of neurons in which their response to blood-borne substances was either attenuated or

facilitated by Arc stimulation, likely reflects the different physiological roles of these neurons (13, 14, 19).

In summary, these data suggest that Arc may be involved in body fluid and circulatory homeostatic mechanisms by modulating the activity of SFO neurons that monitor blood-borne signals from the depletion of intracellular and extracellular fluid volumes.

ACKNOWLEDGEMENTS

This work was supported by the Heart and Stroke Foundation of Ontario. J. Ciriello is a Heart and Stroke Foundation of Ontario Career Investigator. M.P. Rosas-Arellano was the holder of a ICCS of Canada Studentship. M.P.R.A and L.P. Solano-Flores are visiting scientists from the Universidad Nacional Autónoma de México and holders of DGAPA awards.

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FIGURE LEGENDS

Figure 1.
Bright-field photomicrographs of the rat forebrain stained with Neutral red showing the location of a pontamine sky blue deposit (arrow) in subfornical organ (SFO;a) corresponding to a recording site, and of an iron deposit (arrow) in arcuate nucleus (Arc;b) corresponding to a stimulation site. 3V, third ventricle; ME, median eminence; vhc, ventral hippocampal commissure; VMH ventromedial hypothalamic nucleus. Calibration mark in (b) of 500 μm applies to both (a) and (b).

Figure 2.
Transverse sections of the basomedial hypothalamus extending from 2.6 to 3.2 mm caudal to bregma showing the location of sites of stimulation in arcuate nucleus (Arc). , sites that elicited changes in single unit activity in subfornical organ (SFO). ▲, sites that excited SFO units and potentiated the excitatory effect of ANG II on units in SFO. $, sites that inhibited SFO units and inhibited the excitatory effect of ANG II on units in SFO. ○, sites that did not evoke changes in single unit activity in SFO. 3V, third ventricle; dm, dorsomedial subnucleus of Arc; vl, ventrolateral subnucleus of Arc; vm, ventromedial subnucleus of Arc; VMH, ventromedial hypothalamic nucleus. Calibration mark, 1 mm.

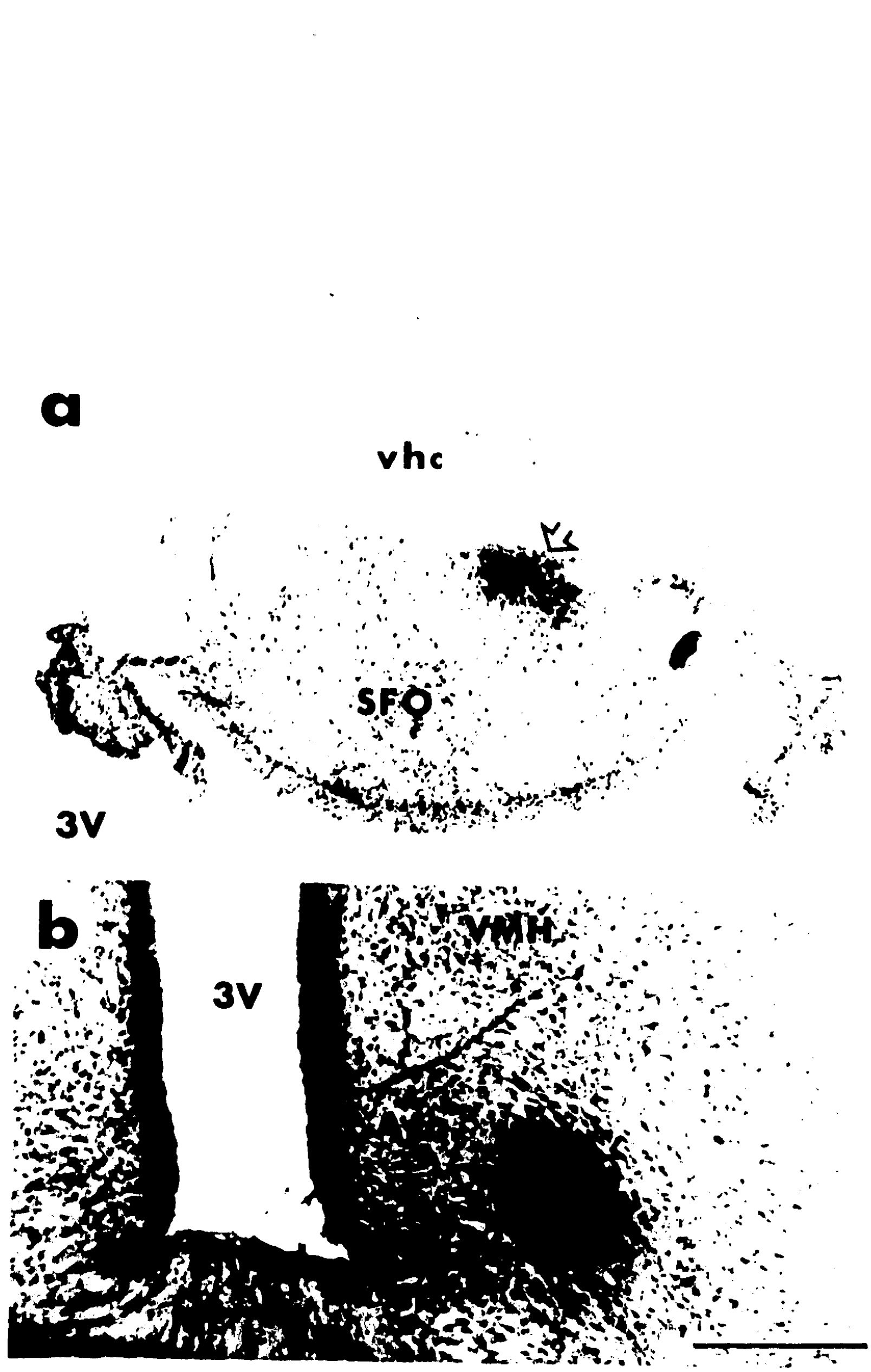
Figure 3.
Location of single units plotted on representative transverse sections of subfornical organ (SFO) extending from 1.0 to 1.3 caudal to bregma that responded to stimulation of arcuate nucleus (Arc). In (a), ▼, units excited by Arc and by hypertonic saline infusion and in which the excitatory response to hypertonic saline was inhibited after Arc stimulation; ▯, units excited by Arc and hypertonic saline infusion; ○, units responsive to Arc but not to the intravascular infusion of hypertonic saline or ANG II. In (b), $, units excited by Arc and by ANG II infusion and in which the excitatory response to ANG II was inhibited after Arc stimulation; ▲, units excited by Arc and excited by ANG II infusion and in which the excitatory response to ANG II was potentiated after Arc stimulation; , units inhibited by Arc stimulation, excited by ANG II and in which the ANG II response was inhibited by Arc stimulation; ι, units excited by Arc and ANG II infusion. 3V, third ventricle, vhc, ventral hippocampal commissure. Calibration bar, 500 μm.

Figure 4.
Rate meter record (2 s/bin) showing the effect of stimulation of arcuate nucleus (Arc), the infusion of hypertonic saline (Na^+), and the combination of the two stimuli (Arc & Na^+) on the firing rate of a subfornical organ neuron. Each horizontal bar indicates the time and duration of the stimuli (10 s). Note that there was a marked increase in the neurons firing frequency after each stimulus alone and a decrease in the neurons response to hypertonic saline after the application of both stimuli. Insets (top) show 10 superimposed oscilloscope tracings of firing rate of the unit corresponding to the period marked by the letters on the rate meter record: a, control; b, after stimulation of Arc; c, after infusion of hypertonic saline; d, after the combination of Arc stimulation and hypertonic saline infusion. Calibration mark is 50 ms and 50 μV.

Figure 5.
A: Histograms showing the changes in firing rate of single units in subfornical organ during stimulation of arcuate nucleus (Arc), infusion of hypertonic saline (Na^+), during the combination of the two stimuli (Arc & Na^+), the algebraic subtraction of Arc response from the response during the combination of the two stimuli ((Arc & Na^+)-Arc) (crosshatch), and the algebraic summation of the units responses to arcuate nucleus stimulation and hypertonic saline infusion (Arc + Na^+) (crosshatch). B, C and D: Histograms showing the firing rate of single units in subfornical organ during stimulation of arcuate nucleus (Arc), infusion of angiotensin II (ANG II), during the combination of the two stimuli (Arc & ANG II), the algebraic subtraction of Arc response from the response during the combination of the two stimuli ((Arc & ANG II)-Arc) (crosshatch), and the algebraic summation of the units responses to arcuate nucleus stimulation and angiotensin II infusion (Arc + ANG II) (crosshatch). Asterisks, show significance ($p < 0.005$) between different groups.

Figure 6.
Rate meter records (2 s/bin) showing the effect of stimulation of arcuate nucleus (Arc), the infusion of angiotensin II (ANG II), and during the combination of the two stimuli (Arc & ANG II) on the firing rate of a subfornical organ neuron. Each horizontal bar indicates the time and duration of the stimuli (10 s). Note that there was a marked increase in the cells firing rate after the combination of both stimuli. Insets (top) show 10 superimposed oscilloscope tracings of the unit activity corresponding to the period marked by the letters on the rate meter record: a, control; b, after stimulation of Arc; c, after infusion of ANG II; d, after the combination of Arc stimulation and ANG II infusion. Calibration mark is 50 ms and 50 μV.

Figure 7.
Schematic representation of the neuronal circuits between arcuate nucleus (Arc) and subfornical organ (SFO) that may be involved in the modulation of SFO activity in response to blood-borne ANG II and increases in plasma sodium (Na^+). dm, vl and vm, dorsomedial, ventrolateral and ventromedial subnuclei of Arc, respectively.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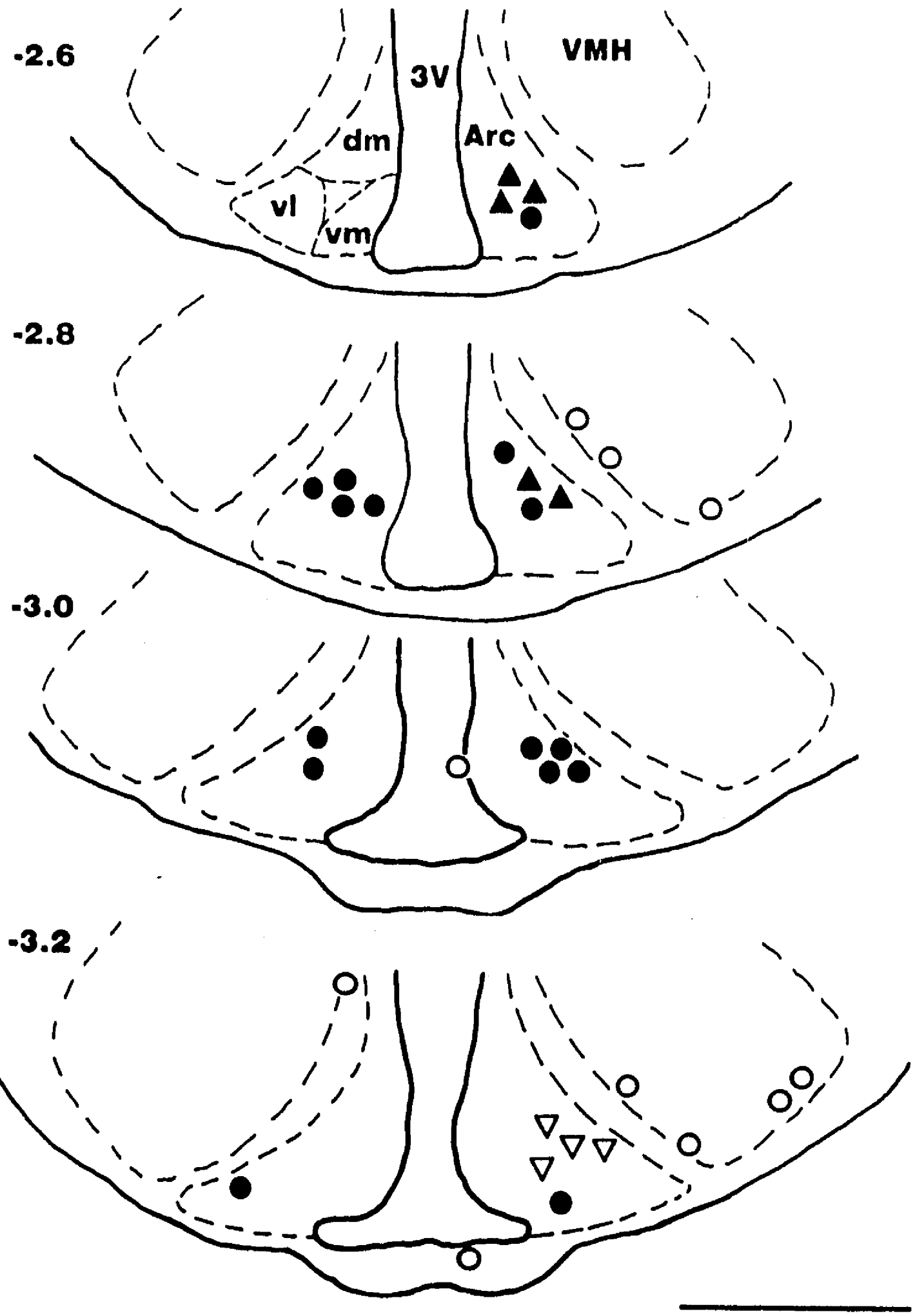
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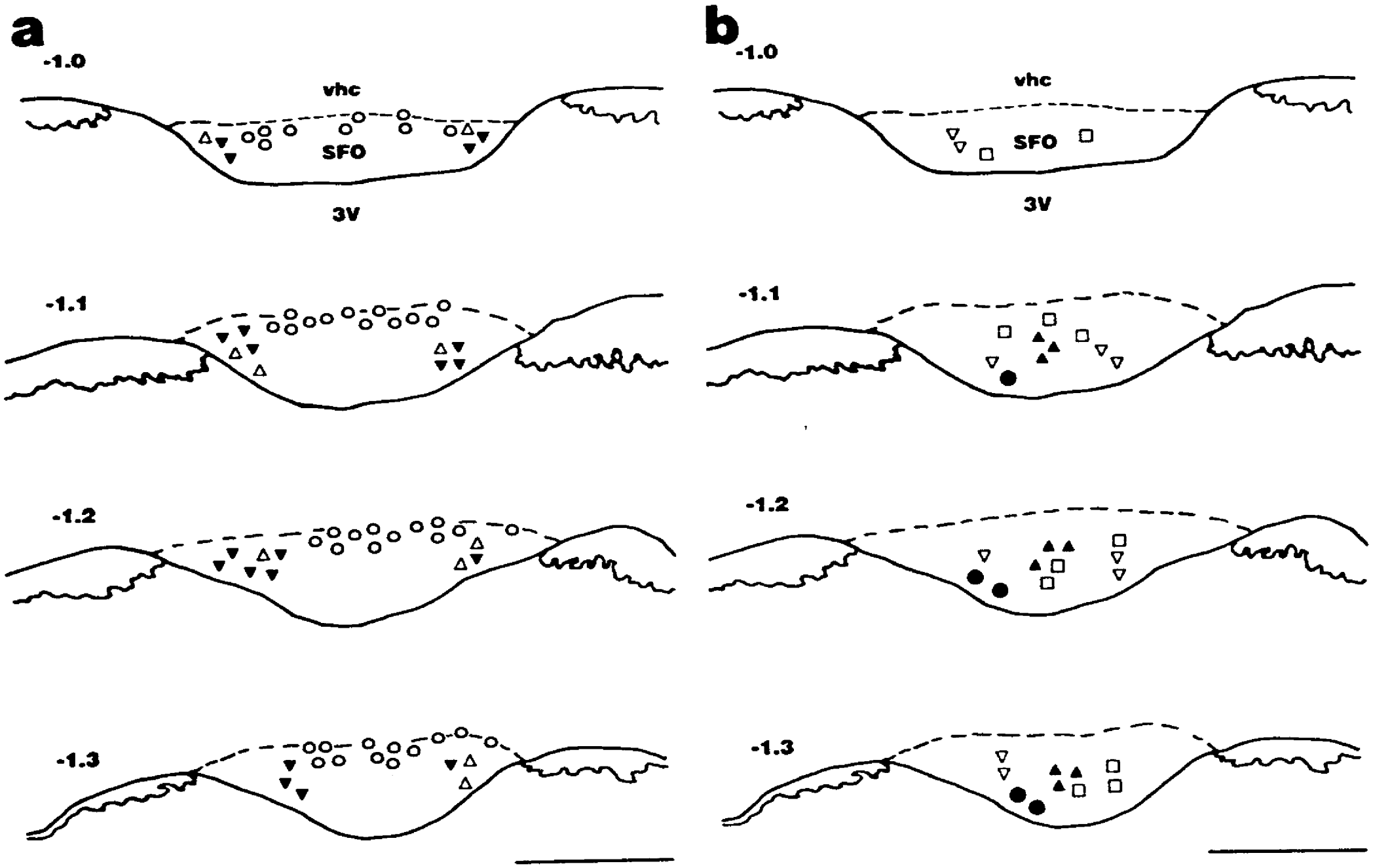
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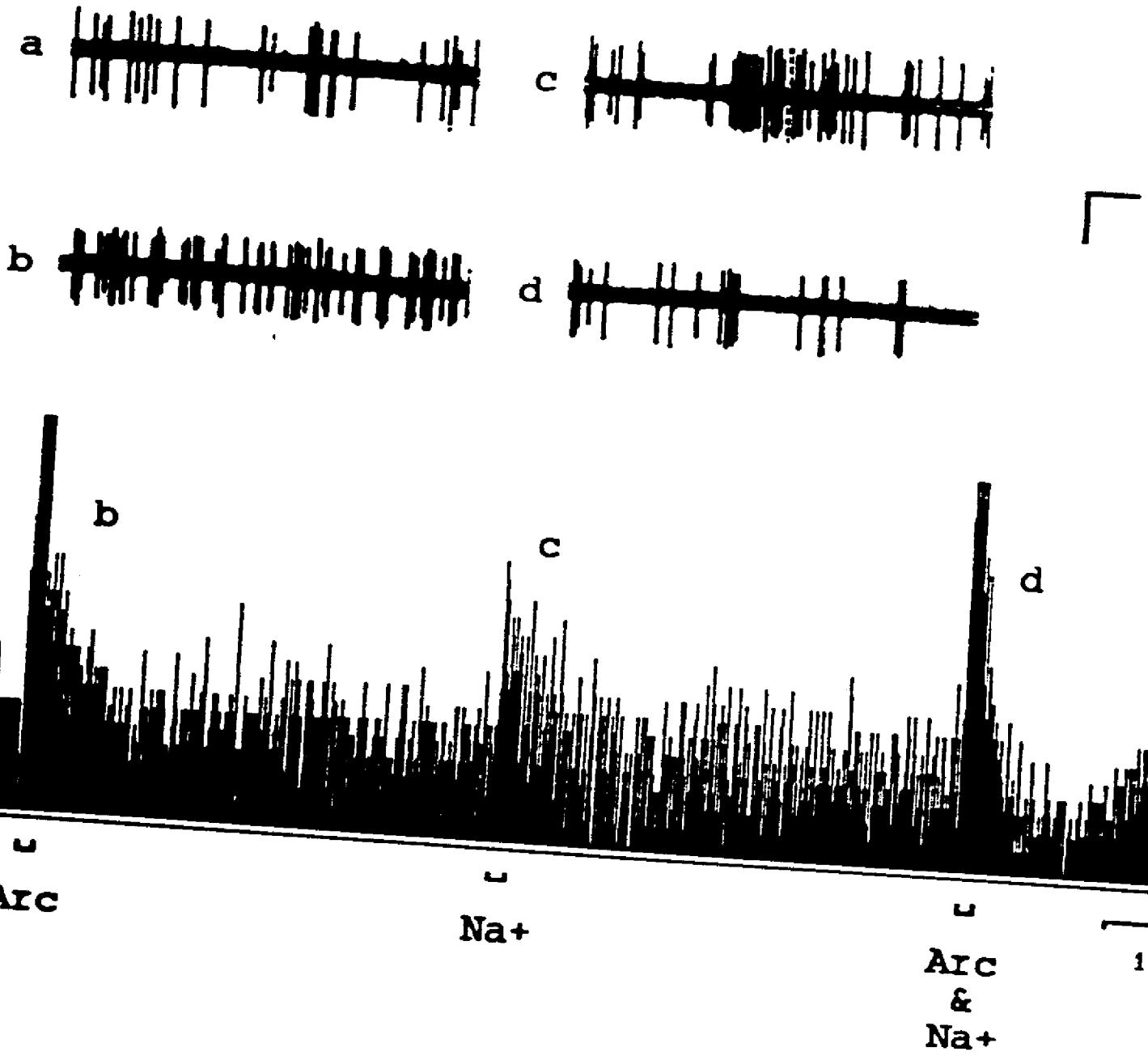
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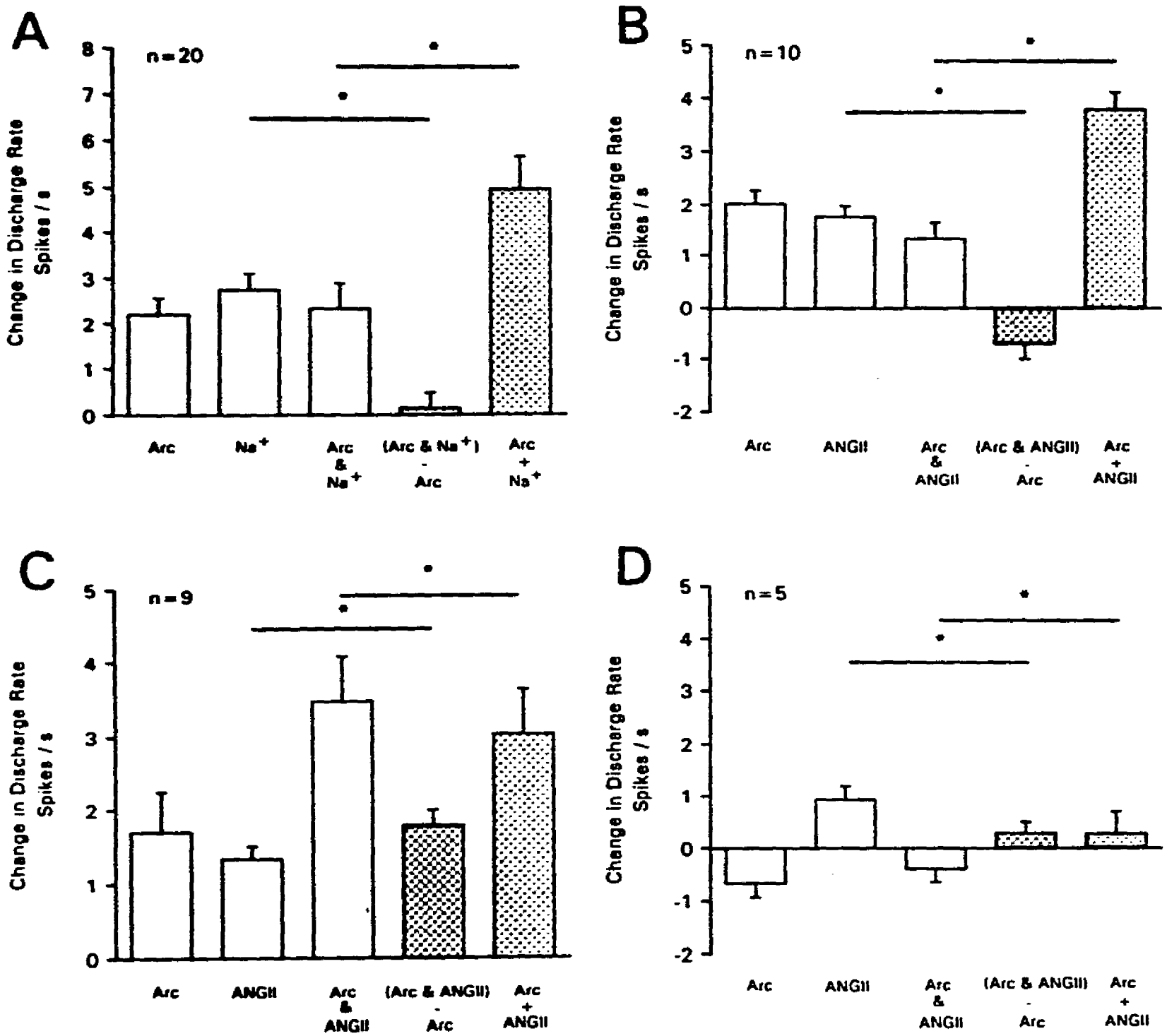
A
n=20
Change in Discharge Rate
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Na+
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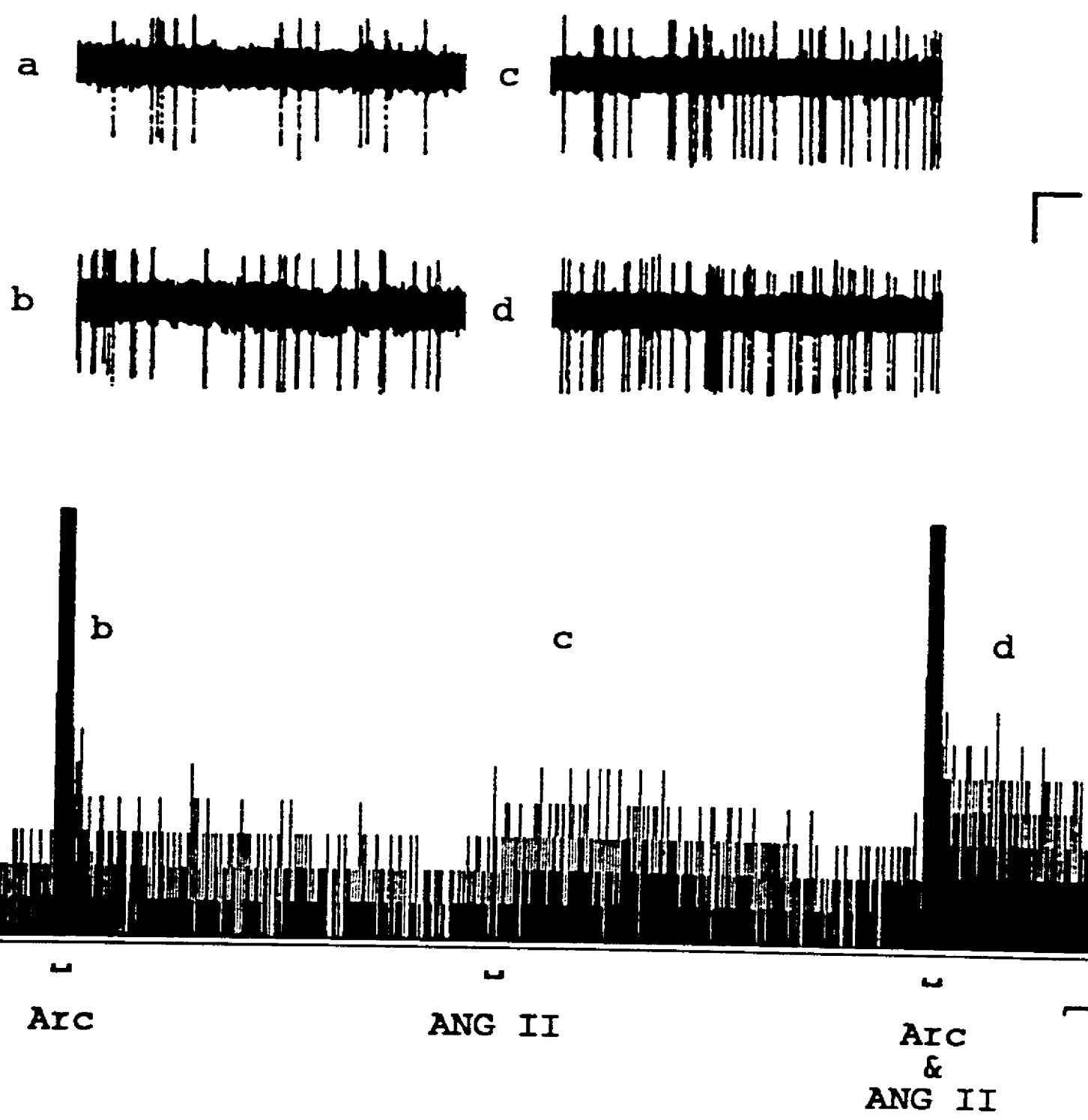
(Arc & Na+) - Arc
Arc + Na+
B
n=10
Change in Discharge Rate
Spikes / s
Arc
ANGII
Arc & ANGII
(Arc & ANGII) - Arc
Arc + ANGII
C
n=9
Change in Discharge Rate
Spikes / s
Arc
ANGII
Arc & ANGII
(Arc & ANGII) - Arc
Arc + ANGII
D
n=5
Change in Discharge Rate
Spikes / s
Arc
ANGII
Arc & ANGII
(Arc & ANGII) - Arc
Arc + ANGII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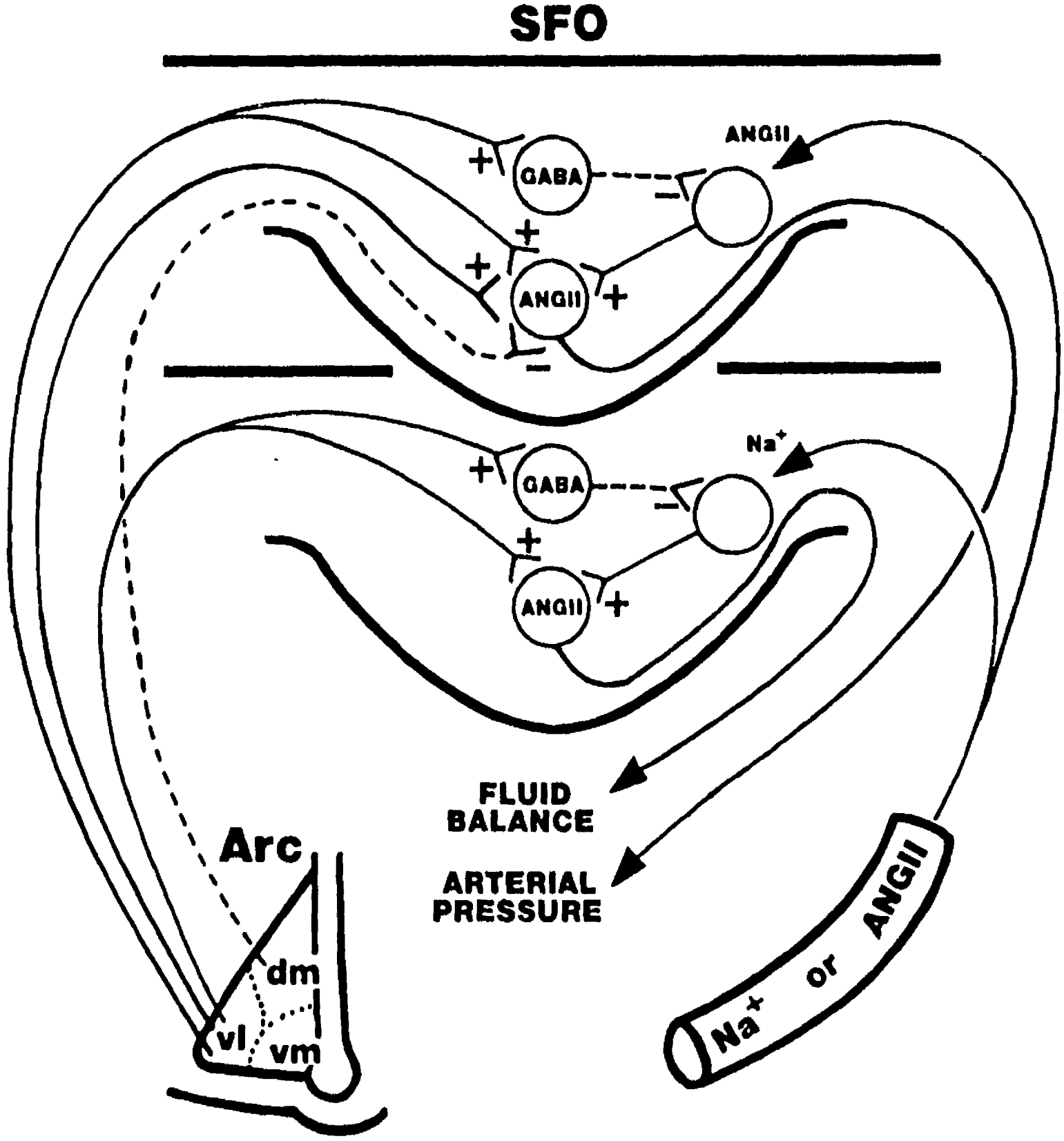
SFO
ANGII
GABA
ANGII
Na+
GABA
ANGII
FLUID
BALANCE
ARTERIAL
PRESSURE
Arc
dm
vl
vm
Na+ or ANGII

DISCUSION

CONTRIBUCIONES ORIGINALES DEL PRESENTE TRABAJO.

Los presentes datos ofrecen evidencias neuroanatómicas y electrofisiológicas que indican que el Arc participa en las funciones de acoplamiento entre los mecanismos de control del balance de los líquidos corporales y los mecanismos de control cardiovascular. Más especificamente, estos resultados indican que su función se lleva a cabo cuando menos mediante dos mecanismos: el primero consiste en responder a los cambios de concentración de Na^+ en el líquido cerebroespinal, y el segundo consiste en influir sobre la respuesta que uno de los CVOs, el SFO, presenta a las señales humorales transmitidas a través de la circulación relacionadas con la homeostasis de los líquidos corporales y cardiovascular. Estos efectos sobre el SFO parecen estar mediados por proyecciones directas organizadas topograficamente originadas en dos de los subnúcleos del Arc los cuales ejercen diferentes efectos sobre la actividad de las neuronas del SFO. Uno de los neurotransmisores putativos involucrados en esta proyección es el péptido neurotensina.

ORIGEN DE LA IDEA DE LA EXISTENCIA DE OSMOSENSORES Y NATRIOSENSORES CENTRALES.

La idea de la existencia de estructuras centrales que actuan como sensores para los mecanismos que controlan el balance de los líquidos corporales fue propuesta inicialmente por Verney (1947) quien sugirió la existencia de osmosensores hipotalámicos cuya activación resulta en la secreción de VP. Posteriormente, Anderson y colaboradoress (1953-1975) sugirieron que el control de los líquidos corporales se inicia con la detección de la concentración del Na^+ más que con la de la osmolaridad de los líquidos. Recientemente existen datos que sugieren la existencia de ambos tipos de sensores centrales, los natriosensores y los osmosensores [McKinley,

1987]. Finalmente, los datos presentados en el primer estudio que muestran expresión de proteinas tipo Fos en el Arc ante la infusión ICV de solución salina hipertónica, pero no por infusión de solución hiperosmótica, refuerzan la idea de que existe un sistema central para la detección de cambios en la concentración de Na^+ del liquido cerebroespinal.

LOCALIZACION DE LOS OSMOSENSORES CENTRALES.

El SFO, contenido en la lámina terminalis, ha sido propuesto como el substrato anatómico que contiene los elementos sensoriales para los mecanismos que controlan el balance de los líquidos corporales [Sibbald et al., 1984; 1988]. Esto está apoyado por el hecho de que la destrucción del SFO de la rata resulta: 1) en un deterioro pronounciado de la liberación de VP en respuesta a la deshidratación celular [Mangiapane et al., 1984]; 2) en un deterioro de la respuesta de ingesta de agua ante la reducción de la osmolaridad [Hosutt et al., 1981; Lind et al., 1984c]; 3) en una reducción de los niveles de VP plasmática y de la ingesta de agua en respuesta a la infusión intracarotídea de solución salina hipertónica [Knepel et al., 1982]; y, 4) en una reducción de la dramática conducta de ingesta de agua en respuesta al suministro de ANG II [Mangiapane, 1987; Simpson y Routtenberg, 1975; Simpson et al., 1978].

La idea de que las estructuras de la lámina terminalis eran las únicas que contenian a los elementos sensitivos para los mecanismos que controlan el balance de los líquidos corporales fue debilitada cuando Pierce y Mouw (1984) demostraron que respuestas compensadoras pudieron ser producidas por soluciones hipertónicas salinas inyectadas ICV aún cuando el acceso de dichas soluciones a la región anterior del tercer ventrículo fue bloqueado mediante un tapón de vaselina. Esto les llevo a concluir que el tejido hipotalámico periventricular ventral puede también llevar a cabo una importante función en la detección de disturbios de naturaleza

hipertónica en el liquido cerebroespinal. Lo anterior fue consistente con la previa propuesta de Anderson en 1978 quien planteó que las respuestas compensadoras originadas por perturbación de la tonicidad del Na^+ en el líquido cerebroespinal podrian estar bajo control hipotalámico y que el tejido periventricular que se extiende desde el cerebro anterior hasta el tallo cerebral pudiera ser parte de un sistema sensor central para el Na^+. Finalmente, los datos presentados en el primer estudio adicionalmente sugieren que el Arc es una de las estructuras que son parte de dicho sistema sensor central.

EL REQUERIMIENTO DE LA EXISTENCIA DE MULTIPLES SENSORES PARA EL MECANISMO DE CONTROL DE LOS LIQUIDOS CORPORALES.

En cualquier momento, la composición de los liquidos corporales esta determinada por la relación de dos componente: el soluto y el disolvente. Esta composición puede ser alterada al modificarse la entrada o bién la salida de uno de estos componentes o de ambos a la vez. Tomando como punto de partida el estado de composición normal de los fluidos, este puede ser transformado en un estado hiperconcentrado por la adición de soluto al sistema o por pérdidad de disolvente. De modo similar, un estado hipoconcentrado puede obtenerse mediante la pérdida de soluto o por la adición de disolvente. Así pues, los mecanismos de control de los líquidos corporales requieren contar con diferentes estrategias y diversos tipos de sensores especializados para detectar especificamente el origen de una perturbación. En forma complementaria, para el mantenimiento de la tonicidad dentro de los límites normales, los mecanismos que controlan el balance de los líquidos corporales requieren de la orquestración de diferentes sistemas efectores que compensan disturbios de diferente naturaleza. Por ejemplo, el contenido corporal de Na^+ depende del equilibrio establecido entre la ingesta de Na^+ inducida por el apetito por el Na^+, la pérdida de Na^+ por la actividad natriurética renal y la

retención de Na^+ resultado de la actividad antinatriurética renal. Complementando ésto, el contenido corporal de agua depende a su vez del equilibrio establecido entre la ingesta de agua producidad por la sensación de sed, las pérdidas de agua debidas a la actividad diurética renal y la retención de agua debida a la actividad antidiurética renal.

En conclusión, la amplia variedad de perturbaciones posibles que los fluidos corporales pueden sufrir y la múltiple variedad de respuestas compensadoras que el mecanismo de control de los líquidos corporales puede ofrecer, sugieren la existencia de diferentes substratos anatómicos sensores que funcionen en el muestreo constante del estado de los fluidos y que localizen especificamente la naturaleza de las perturbaciones. El Arc, de acuerdo a los datos del primer estudio, al parecer es una estructura en la que se lleva a cabo este tipo de función.

COORDINACION DE DOS MECANISMOS DE CONTROL.

Como ya se ha mencionado anteriormente, dos mecanismos complejos se implican en el mantenimiento de la constancia del medio interno: uno relacionado al sistema cardiovascular y otro que corrige las perturbaciones en la tonicidad de los líquidos corporales [Brody y Johnson, 1980; Buggy et al., 1984]. Por lo tanto, para que se logre dicha constancia del medio interno, debe existir una armonía entre las acciones de ambos mecanismos. Por ejemplo, la compensación de un disturbio que afecta al sistema cardiovascular muy bién pudiese llevarse a cabo alterando parámetros relacionados al balance de los liquidos corporales. Sin embargo, dicha acción pudiera amenazar al mismo tiempo la estabilidad del balance de los líquidos corporales. Más concreto aún, en una situación de hemorragia con perdida del 10 al 15 por ciento del volumen sanguineo, el volumen del fluido total corporal es disminuido isosmoticamente y la función cardiovascular es amenazada por el abatimiento de la presion arterial. En este caso la

secreción de VP es estimulada vigorosamente debido a la reducida actividad de los baroreceptores atriales y arteriales. El gran aumento de los niveles de VP causa entonces retención de agua lo que previene pérdidas de volumen. Adicionalmente, se estimula la liberación de ANG II que a través de sus acciones sobre el SFO induce ingesta de agua. Sin embargo dichas acciones resultan en un decremento de la osmolaridad del plasma. Así, en este caso, se tiene que la función osmoreguladora es abandonada en favor a la función cardiovascular.

En conclusión, el complejo proceso de establecimiento de prioridades de acción en función de las situaciones particulares en que se encuentre el organismo y que considere el origen del disturbio detectado, requiere de estructuras que realizen la exquisita coordinación de estos dos mecanismos.

EL Arc COMO UNA ESTRUCTURA INTEGRADORA DE LOS MECANISMOS DE REGULACION CARDIOVASCULAR Y DE LOS LIQUIDOS CORPORALES.

La estimulación eléctrica de la parte rostral del Arc resulta en una respuesta presora mediada por VP. En contraste, la estimulación de la parte caudal del Arc produce una respuesta depresora mediada a través de una vía neural que proyecta al complejo vagal dorsal [Brody et al., 1986; Kunos et al., 1991; Mastrianni et al., 1989]. Aunque el significado funcional de la participación del Arc en los mecanismos de control cardiovascular queda aún por explorarse, los hechos anteriores, indican que el Arc es capaz de participar en el control central cardiovascular. Por otro lado, la deprivación crónica de agua resulta en una elevada síntesis de proteinas (Lepetit et al., 1992b) y de los niveles del mRNA-prepro-NPY (O'Shea y Gundlach, 1991) en las neuronas del Arc. Adicionalmente, la ingestión crónica de solución salina hipertónica produce un incremento en la síntesis de proteinas en las neuronas del Arc (Lepetit et al., 1992b). Es importante resaltar que en estos estudios, adicionalmente a los efectos

observados en el Arc, solo los CVOs presentaron síntesis elevada de proteinas por la deshidratación crónica [Lepetit et al., 1988; 1992b]. Uniendo todo lo anterior con el hallazgo de la existencia en el Arc de elementos sensibles a los cambios de la concentración de Na^+ en el líquido cerebroespinal, como lo indican los datos obtenidos en el primer estudio, es posible plantear entonces que el Arc se encuentra en una posición singular como una estructura susceptible de producir respuestas cardiovasculares ante cambios en los niveles de Na^+ de los líquidos corporales. No solo ésto, los datos del segundo estudio que muestran que el Arc proyecta directamente a los CVOs, sugieren que el Arc es una estructura capaz de afectar la entrada de información de señales humorales relacionadas a la regulación cardiovascular y del balance de los fluidos corporales. Finalmente, uno de los neurotransmisores putativos involucrados en esta proyección es el péptido neurotensina como lo muestran los datos presentados en el tercer estudio.

MODULACION DEL Arc SOBRE LA RESPUESTA DEL SFO A SEÑALES HUMORALES.

El SFO es un CVO sobre el cual las señales humorales relacionadas a la regulación cardiovascular y del balance de los fluidos corporales son transducidas en información neural. El presente trabajo indica que el Arc es capaz de modular la actividad de las neuronas del SFO. Así, se ofrecen evidencias en el cuarto y quinto estudio que indican que: 1) las neuronas del SFO son inhibidas cuando la región medial del Arc es estimulada electricamente; 2) las neuronas del SFO son excitadas cuando la región dorsal del Arc es estimulada electricamente; 3) la estimulación eléctrica del Arc resulta en una inhibición de las neuronas del SFO que responden con excitación a infusiones hipertónicas de Na^+; 4) la estimulación eléctrica del Arc resulta en una variedad de efectos en las neuronas del SFO que responden a la ANG II

(potenciación de la respuesta de excitación, inhibición de la respuesta de excitación a la ANG II). Adicionalmente, los sitios de estimulación en el Arc que potencian el efecto excitador de la ANG II en la actividad unitaria del SFO estuvieron localizados preferentemente en el Arc rostral, mientras que los sitios de estimulación en el Arc que inhibieron la actividad unitaria en el SFO y que inhibieron o atenuaron el efecto excitador de la ANG II en la actividad unitaria del SFO estuvieron localizados en el Arc caudal.

De estas observaciones es posible sugerir que las acciones del Arc sobre la excitabilidad de las neuronas del SFO pueda representar un mecanismo para prevenir un efecto presor al abatir la excitabilidad de las neuronas del SFO a la ANG II cuando se está llevando a cabo un efecto depresor mediado por la proyección del Arc caudal hacia el complejo vagal dorsal. Por otro lado el abatimiento de la excitabilidad de las neuronas del SFO al aumento de la concentración de Na^+ pudiera representar el abandono de la osmoregulación en favor de la función cardiovascular para evitar una oposición de acciones. Así pues, el significado funcional de la proyección del Arc hacia el SFO pudiera ser el de optimizar la respuesta del circuito neural en el SFO a las señales acarreadas por la sangre durante ciertas condiciones emergentes.

En conclusión, las evidencias experimentales ofrecidas en el presente trabajo fundamentan la idea de que el Arc es una estructura central capaz de ejercer una función de encadenamiento y coordinación de las acciones de los mecanismos que regulan la función cardiovascular con las acciones de los mecanismos que controlan el balance de los liquidos corporales para lograr una respuesta compensadora óptima después de una perturbación al volumen sanguineo o de un cambio en la tonicidad de los fluidos corporales.

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