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ESTUDIOS SOBRE REGULACION ALFA ADRENERGICA

T E S I S

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ESTUDIOS SOBRE REGULACION ALFA ADRENERGICA

El sistema neuroendocrino participa en la coordinación de los procesos del metabolismo mediante la liberación de moléculas llamadas hormonas y/o neurotransmisores (primeros mensajeros) las cuales provocan un conjunto de respuestas en las "células blanco". Se ha observado que éstas moléculas (a excepción de las hormonas esteroideas y tiroideas) no requieren penetrar a la célula ya que su efecto lo ejercen "a distancia", por interacción con moléculas presentes en la membrana plasmática de las células blanco, que han recibido el nombre de receptores. Estos receptores transducen la señal hormonal, generándose una molécula (segundo mensajero) encargada de dar la señal intracelular que va a alterar el metabolismo a corto plazo (glucogenólisis, contracción, lipólisis, etc.)[1].

Los mecanismos de transducción involucrados en la respuesta a muchas hormonas permanecen indefinidos hasta el momento, sin embargo, se han desarrollado modelos que explican los efectos mediados por estas hormonas en el metabolismo celular[2,3]. En este caso se encuentran las aminas adrenérgicas.

La distinción de dos patrones de respuesta (potencia de agonistas) para las aminas adrenérgicas en función de la respuesta fisiológica observada, condujo a Ahlquist[4] a proponer una nomenclatura que define a dos tipos de receptores; los dividió en receptores alfa (α) y beta (β) adrenérgicos. A partir de ese momento, se ha acumulado una gran cantidad de evidencia experimental que apoya su existencia[1]. Posteriormente, Lands[5] propuso la subdivisión de los receptores β en β_1 y β_2 en función de la potencia relativa de una serie de aminas simpatomiméticas en los siguientes parámetros: movilización de ácidos grasos libres del tejido adiposo, estimulación cardíaca, broncodilatación y vasodepresión. Esta

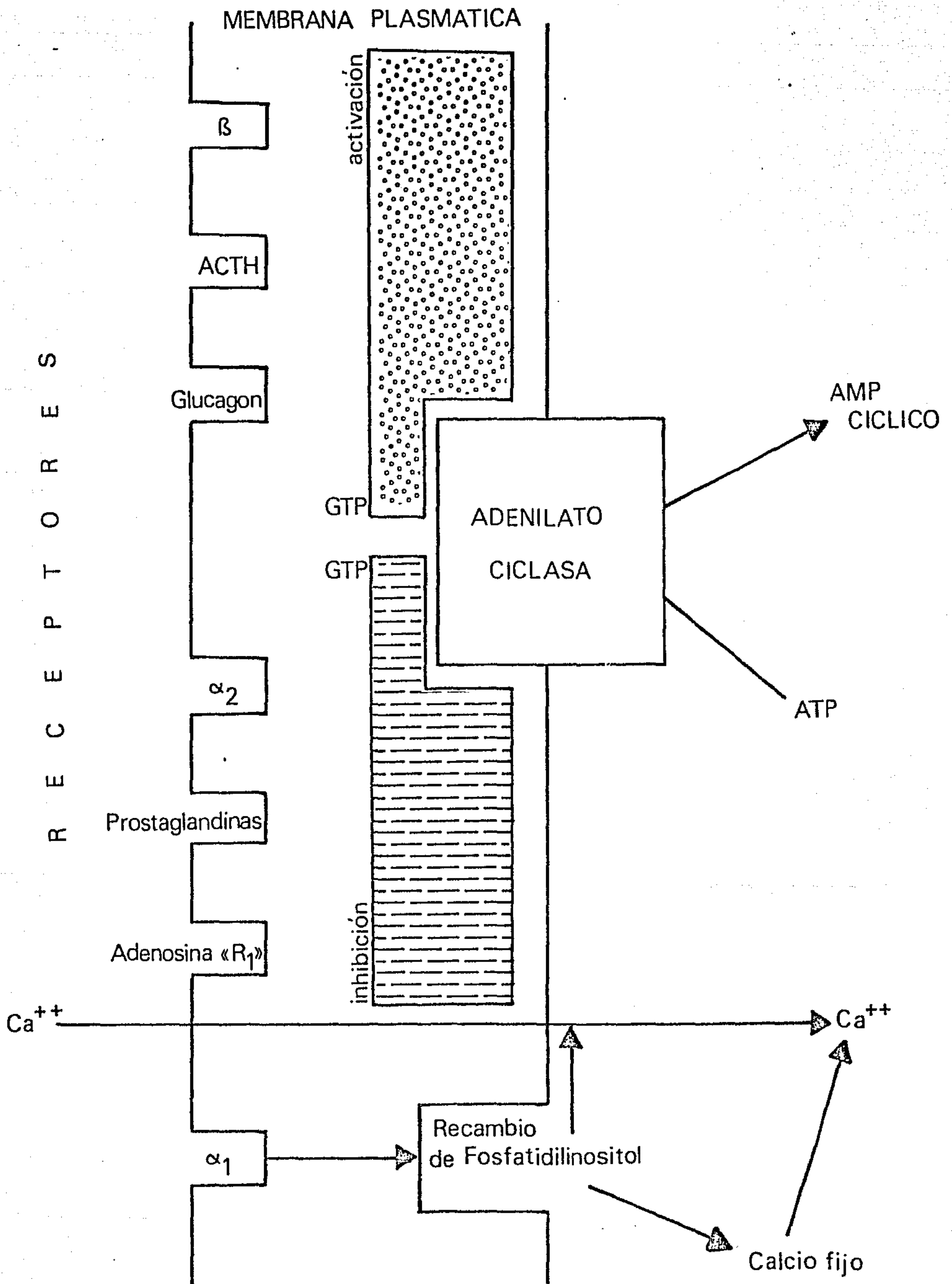
diferenciación empírica coincide con la propuesta por Ariens et al[6] donde los receptores β_1(noradrenérgicos) se encuentran en las terminales sinápticas o en tejidos bajo inervación directa (tejido adiposo), mientras que los receptores β_2 se encuentran en tejidos bajo la acción de la epinefrina circulante. En otras palabras, los receptores β_1 responden a moléculas tipo el neurotransmisor norepinefrina; mientras que los receptores β_2 responden a moléculas tipo la hormona epinefrina. Sin embargo, este punto de vista es aún controvertido.

El efecto producido por la unión de una hormona a un receptor β adrenérgico está asociado a un aumento de la actividad enzimática de la adenilato ciclasa y por consiguiente a elevación de los niveles intracelulares de AMP cíclico (AMPc), molécula llamada comunmente segundo mensajero, que se ha involucrado en una gran cantidad de efectos metabólicos[1,7].

Se considera que la generación de AMPc como respuesta a la interacción de diferentes ligandos (glucagon, ACTH, epinefrina, dopamina, histamina) con los receptores membranales es producto del mismo mecanismo de transducción en la bicapa lipídica que involucra un sistema formado esquematicamente de la siguiente manera: el receptor que mira hacia la parte externa de la célula acoplado a una proteína que une GTP y que mira hacia el citoplasma (para tener acceso al GTP) y que actúa de una manera activadora sobre la adenilato ciclasa, la cual sintetiza AMPc a partir de Mg-ATP (fig. 1), además, también se ha involucrado la metilación de los fosfolípidos de la membrana (lo cual le daría una diferente fluidez) como un evento asociado a la activación β adrenérgica[2]. Se ha considerado, sin embargo, que los 2 subtipos de receptores (β_1 y β_2) sean verdaderos isorreceptores puesto que se tiene una sola señal intracelular (aumento de AMPc)

Fig. 1.

Modelo que representa los tipos de interacción entre los diferentes ligandos y los receptores membranales, así como el efecto que hay sobre la adenilato ciclasa a excepción del receptor α_1 cuya acción es a través de la movilización de calcio.


MEMBRANA PLASMATICA
RECEPTORES
ß
ACTH
Glucagon
α2
Prostaglandinas
Adenosina «R1»
α1
activación
inhibición
GTP
GTP
ADENILATO CICLASA
AMP CICLICO
ATP
Ca++
Ca++
Recambio de Fosfatidilinositol
Calcio fijo

que parece provenir del mismo mecanismo de transducción.

Por otra parte, Langer[8] revisa el concepto de que las terminales nerviosas simpáticas poseen α-adrenoceptores y los subdivide en receptores α adrenérgicos presinápticos y postsinápticos de acuerdo a localización y función en la terminal sináptica. En época mas reciente, Berthelson y Pettinger[9] presentan evidencia experimental que sugiere la subdivisión de los receptores α adrenérgicos en α_1 y α_2 de acuerdo a bases funcionales mas que morfológicas; a partir de esta clasificación se han realizado estudios que conducen a sostener la existencia de 2 subtipos de α-adrenoceptores tanto in vivo[10], en tejidos aislados[11,12], en células aisladas[3] y en membranas aisladas[13].

Sin embargo, estos α receptores no parecen ser isorreceptores puesto que involucran diferentes mecanismos de transducción[3]. Los receptores α_1 están involucrados con la movilización de calcio y como evento primario a este efecto, se ha observado que hay un recambio de fosfatidilinositol (PI). Mientras que los receptores α_2 están asociados a la adenilato ciclasa de una manera inhibidora, participando en este proceso una proteína que une GTP y que actúa como modulador negativo de la ciclasa haciendo que los niveles intracelulares de AMPc disminuyan[3] (fig. 1).

El trabajo desarrollado aquí reporta dos investigaciones; una que estudia la repercusión que sobre el metabolismo lipídico ejerce el desacoplar los receptores α_2 adrenérgicos del adipocito en el animal íntegro y otra que caracteriza los receptores α_1 adrenérgi-

cos en músculo liso. Estos trabajos representan dos enfoques diferentes tendientes a conocer el "fenómeno adrenérgico".

La metodología y los reactivos empleados en este trabajo se encuentran descritos en cada una de las partes experimentales.

RESUMEN DE RESULTADOS REFERENCIA 14.

Se tiene evidencia de que las células adiposas de hamster tienen receptores α_2 adrenérgicos, los cuales están ligados a la adenilato ciclasa de una manera inhibidora[3,15]; por lo tanto, cuando se activan estos receptores, se observa una disminución en los niveles de AMPc y de la lipólisis. Al inocular a hamsters con vacuna pertussis se pierde la respuesta a la estimulación α_2 medible por una elevada lipólisis junto con ausencia en la disminución de los niveles de AMPc intracelulares[16].

La administración intraperitoneal de una sola dosis de la vacuna pertussis a hamsters produce un severo hígado graso que se desarrolla a partir del segundo día de la inyección, observándose también, una marcada alteración en el metabolismo y movilización de lípidos (hiperlipemia y cetosis). Se encontró un incremento en la concentración de triacilgliceroles (TG) hepáticos y de ácidos grasos libres séricos (AGL) 2 días después de administrada la vacuna (figs. 2 y 3[14]) que se mantiene proporcional con respecto al tiempo hasta el cuarto día experimental. Estos resultados indican una mayor movilización de lípidos en tejido adiposo (lipólisis) y el consecuente aumento en la esterificación de los AGL por el hígado (fig. 2[14]), así como su oxidación, medida como niveles circulan-

tes de cuerpos cetónicos (CC que comprenden el acetoacetato,AA y el β-hidroxibutirato, βOHB). Estos metabolitos elevan su concentración en sangre aproximadamente 1 orden de magnitud con respecto al control un día después de la inoculación, y durante el transcurso del tratamiento alcanzan valores entre 30 y 50 vrces el basal (fig. 5[14]).

La esterificación de AGL por el hígado, es seguida por la liberación de los TG sintetizados que viajan hacia los depósitos para su almacenamiento. Al cuantificar los valores de TG séricos en los animales inoculados con vacuna pertussis, se encontró un aumento significativo desde el tercer día del tratamiento y continuo hasta el final del experimento (fig. 4[14].).

El curso temporal del efecto producido por la presencia de *B. pertussis* se monitoreó hasta el quinto día después de administrar la vacuna, sin embargo, la mortalidad de los animales en este tiempo sobrepasó el 75%, por lo que los resultados presentados consideran el efecto encontrado hasta el cuarto día de tratamiento.

Para comprobar que el fenómeno observado se debe a una molécula producida por la vacuna de *B. pertussis*, se comparó la potencia del efecto probando diferentes lotes de la vacuna (Tabla 1). Como se observa, se mantiene la alteración de los metabolitos lipídicos en los animales tratados aunque hay diferencias en la magnitud del efecto.

DISCUSION GENERAL DE LA REFERENCIA 14.

Recientemente se ha aislado una proteína (PM=77,000) proveniente del medio de cultivo de *Bordetella pertussis* que produce una esti-

TABLA 1. Comparación del efecto producido por diferentes lotes de la vacuna pertussis sobre el metabolismo lipídico de hamsters.

	TG hepáticos (mg/gr)	TG séricos (mg/dl)	CC (nmol/ml)		AGL séricos (µmol/dl)
			AA	βOHB	
Control	3.3±0.58	94.2±14.2	10.8 ±0.87	6.1 ±1.3	116±11
Lote 29-090	53±15	997±280	298 ±79	389 ±103	421±2
Lote 27-212	63±10	237±118	193 ±61	294 ±106	141±36
Lote 29-127	50.5±13	120±40	152 ±38	245 ±65	147±19

Los datos representan el promedio ± S.E. de por lo menos 4 determinaciones.

mulación de los islotes de Langerhans para liberar insulina. Esta respuesta se ve potenciada cuando se ponen diferentes secretagogos para insulina, manteniéndose el efecto por largos períodos[17]. Este fenómeno se presenta al usar Proteína Activadora de Insulina (IAP) purificada o vacuna de B. pertussis (ambas administradas en una sola dosis) in vivo, o IAP en células aisladas[18,19].

En estudios muy recientes, García-Sáinz[16] demostró que la administración de la vacuna pertussis a hamsters produce una disminución notable en la sensibilidad de los adipocitos a agentes α_2 adrenérgicos, adenosina y prostaglandinas, cuya acción esta mediada vía inhibición de la adenilato ciclasa[3]. En forma independiente y simultánea, Ui[19] administró IAP sobre miocitos cardiácos aislados y midió su efecto sobre la regulación en la acumulación de AMPc estimulada por diferentes agentes (epinefrina, glucagon, prostaglandinas y adenosina). Ambos autores concluyen que la IAP puede bloquear o modificar el mecanismo de acoplamiento en la transferencia de la modulación negativa del receptor α_2 hacia la adenilato ciclasa.

El empleo de células aisladas para conocer la regulación metabólica es muy útil ya que los resultados obtenidos con estos modelos probablemente se pueden extrapolar al organismo íntegro. Esta situación se presenta en este reporte puesto que se sabe que la lipólisis in vivo está regulada vía la activación del proceso a través de diferentes hormonas (efecto de catecolaminas, glucagon, ACTH, etc.), mientras que su inhibición está mediada por efecto $\alpha_2$adrenérgico, insulina, prostaglandinas, adenosina, etc..

Al existir un bloqueo o desacoplamiento entre la ocupación del receptor α_2 y la señal que éste envía hacia la ciclasa, se tiene que la enzima no responde a la inhibición por los diferentes agentes y por lo tanto puede seguir sintetizando AMPc que es el encargado de activar la lipólisis[1]. Por otra parte, el aumento de AGL (fig.3[14]) y glicerol (fig.3[16]) provenientes de la lipólisis son reutilizados por el hígado para reesterificarlos y formar TG (fig.2[14]) que a su vez los libera a la circulación (fig.4[14]) para que se almacenen o para oxidarlos dando como producto cuerpos cetónicos que también libera (fig.5[14]) para que se puedan utilizar por otros tejidos. Estos 2 procesos se encontraron muy aumentados (aunque desfasados en tiempo) lo que indica que el tejido adiposo ha perdido el mecanismo de regulación de sus procesos metabólicos y constantemente está liberando AGL y glicerol que el hígado debe eliminar de la circulación.

RESUMEN DE RESULTADOS REFERENCIA 20.

El empleo de preparaciones *in vitro* ha sido ampliamente usado para tratar de discernir el efecto metabólico y el mecanismo de acción de los diferentes moduladores de la homeostasis corporal (neurotransmisores y hormonas). Tal es el caso de las preparaciones vasculares con las cuales se desarrolló el segundo enfoque de éste trabajo.

La determinación de fosfato inorgánico (Pi) en los fosfolípidos de la membrana, en condiciones basales, muestra la proporción que de estas moléculas existe en el músculo liso (Tabla 1[20]). La mayor

incorporación de $[^{32}P]$Pi encontrada en los fosfolípidos, siguió el orden Fosfatidilcolina (PC)>Acido Fosfatídico (PA)>Fosfatidilinositol (PI)>Fosfatidiletanolamina (PE), estos resultados semejan los descritos para adipocitos por García-Sáinz & Fain[21] .

Los resultados obtenidos en la fig.1[20] demuestran que hay un efecto dependiente de la dosis de epinefrina en la incorporación de $[^{32}P]$Pi a fosfatidilinositol y a su precursor, el ácido fosfatídico, mientras que los otros fosfolípidos (PC y PE) no se ven afectados por la epinefrina. El aumento observado en el recambio de PI a la máxima concentración usada en este experimento (10^{-5}M epinefrina) corresponde a 7 veces el valor del basal, mientras que, el ácido fosfatídico se incrementa 4 veces (fig.1[20]).

Para caracterizar el tipo de receptor involucrado en la respuesta descrita, se emplearon agentes con diferente selectividad y potencia, obteniéndose que la epinefrina y la metoxamina producen la misma cinética de incorporación de fosfato radiactivo a PI, siendo agonistas totales para este efecto. La estimulación inducida por norepinefrina sigue un patrón discretamente diferente pero también se comporta como agonista total (fig.2A[20]).

Al emplear fenilefrina como agente adrenérgico, se encontró que es un agonista parcial para el efecto descrito ya que no se alcanza el máximo de incorporación a PI obtenido con los ligandos anteriores. La clonidina, siendo un agonista preferencial α_2, tiene un ligero efecto a concentraciones elevadas (10^{-4}M) mientras que el B-HT 933 (agente agonista α_2) no produce efecto a ninguna concen-

tración (fig. 2A[20]).

Posteriormente se exploró la inhibición del marcaje a PI (estimulado por epinefrina) al usar antagonistas α adrenérgicos. Como se observa en la fig. 3A[20] la prazosina (antagonista α_1 altamente selectivo) inhibió la incorporación de fosfato radiactivo a PI, causada por 10^{-5}M epinefrina, en forma dependiente de la dosis, alcanzando el valor basal a una concentración de 10^{-5}M prazosina. Cuando se usó yohimbina (antagonista α_2) la inhibición fue menos potente, encontrándose desplazada 3 órdenes de magnitud con respecto a prazosina y no se abate la estimulación adrenérgica aún a la dosis de 10^{-3}M yohimbina (fig. 3A[20]).

DISCUSION DE RESULTADOS REFERENCIA 20.

El recambio de PI estimulado por catecolaminas se ha asociado a la activación de receptores α_1[3]. Los resultados presentados en la referencia 20 indican que el aumento en el recambio de PI mediado por epinefrina es consistente con el encontrado para otros tejidos[21], y que este efecto es reproducido si se usan diferentes agonistas para los receptores α_1 (fig.1[20]); a su vez, no se observa un incremento significativo con los agonistas α_2 (clonidina y B-HT 933).

Un dato curioso es el obtenido con metoxamina, pues se sabe que este agonista es muy débil cuando se emplean como modelos los hepatocitos[22] o los adipocitos[21] lo que hace sugerir la presencia de isorreceptores α_1 en los diferentes tejidos.

Cuando se emplean antagonistas para bloquear el aumento de marcaje en PI causado por 10^{-5}M epinefrina, se observó la selectividad

y potencia del agente utilizado (fig.3[20]). La prazosina que es un agente α_1 altamente selectivo[23] inhibe el efecto producido por la epinefrina de una manera dosis-dependiente, obteniéndose la inhibición total a 10^{-5}M prazosina; mientras que al usar yohimbina (agente antagonista α_2) el desplazamiento de la curva es de 3 órdenes de magnitud, sin alcanzar el nivel basal, aún a concentración de 10^{-3}M yohimbina (fig. 3A[20]). Estos datos sugieren fuertemente que la incorporación de fosfato radiactivo a PI está mediado a través de adrenoceptores α_1 y que es un evento primario a la respuesta fisiológica final (ver correlación con contracción en el trabajo).

DISCUSION GENERAL.

El efecto biológico que resulta de la interacción de moléculas liberadas por el sistema neuroendócrino con las células blanco, está mediado por la unión de mensajeros químicos con sus sitios de acción específicos (receptores) que pueden estar presentes en la membrana celular o en el citoplasma. El complejo hormona-receptor transmite información que induce la alteración de la función celular[1]. La interacción entre el mensajero y el receptor inicia la transducción de la señal recibida del exterior y posteriormente se tiene el efecto intracelular.

La presencia de 2 tipos de receptores α adrenérgicos propuesta por Langer[8] y Berthelson & Pettinger[9] en diferentes tejidos ha conducido a tratar de esclarecer el mecanismo de acción de cada receptor. Debido a que los receptores α_1 actuan a través de un aumento en la movilización de calcio intracelular asociado a un recambio

previo de PI y los receptores α_2 lo hacen a través de la inhibición de la adenilato ciclasa[3], sugiere que el mecanismo de acoplamiento entre el complejo hormona-receptor y el efecto intracelular son diferentes.

Los datos presentados en este reporte, en el cual se utilizan 2 modelos experimentales, demuestran que ambos tipos de receptores α adrenérgicos desempeñan un papel muy importante en la regulación del metabolismo celular que puede tener repercusiones a nivel de organismo íntegro.

La presencia de receptores α_2 adrenérgicos en los adipocitos del hamster controlan la lipólisis, vía metabólica que al perder su regulación por desacoplamiento del receptor (α_2) y la adenilato ciclasa (evidenciado por la presencia de la vacuna pertussis[16]), constantemente está liberando AGL y glicerol al medio, con lo cual el metabolismo hepático se ve alterado y por lo tanto se generan situaciones anormales como el hígado graso, la cetosis y la hiperlipemia. Incluso el organismo puede morir (como se observó en 14) después de un corto periódo de tratamiento.

Por otra parte, la activación de los receptores α_1 adrenérgicos en el tejido vascular conduce al recambio de PI, movilización de calcio y debido a que este ión es un agente inotrópico, se obtiene la contracción muscular (tanto en músculo estriado[7], como en músculo liso[7,24]). Sin embargo, el esclarecimiento de la inducción contráctil por los agonistas no se ha establecido, ya que algunas drogas actuan despolarizando la membrana y permitiendo la entrada

de calcio extracelular y otras lo hacen en forma independiente del potencial de membrana y se sugiere la movilización de calcio a partir de los reservorios intracelulares (retículo endoplásmico y mitocondria) para inducir la contracción[24].

Estos hechos presentan una gran correlación entre la acción de los agentes adrenérgicos norepinefrina (neurotransmisor) y epinefrina (hormona) en la regulación de la contracción vascular[20].

Sin embargo, los participantes del mecanismo de acoplamiento entre el complejo hormona-receptor y el efecto intracelular aún no se han definido claramente por lo que su identificación así como el mecanismo por el cual interaccionan con el receptor y la señal intracelular son uno de los objetivos que se persiguen en la actualidad.

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EFFECTS OF PERTUSSIS VACCINE ON THE LIPID METABOLISM OF HAMSTERS

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Summary

Administration of pertussis vaccine to hamsters markedly affected their lipid metabolism. Four days after the administration of the vaccine a severe fatty liver was observed. Concomitantly, a rise in the serum levels of free fatty acids, triacylglycerols and ketone bodies was detected. It is suggested that an altered regulation of adipose tissue lipolysis might be at least partially responsible for the observed effects.

It has recently been shown that administration of pertussis vaccine to hamsters markedly decreases the sensitivity of their fat cells to antilipolytic agents such as $alpha_2$ adrenergic amines, adenosine and prostaglandins which act through receptors coupled to fat cell adenylate cyclase in an inhibitory fashion (1). It was suggested that vaccine administration might alter a common intermediate or coupling mechanism for the transfer of inhibitory information from receptors to adenylate cyclase (1). The present study was undertaken in an effort to determine the metabolic consequences in the whole animal of the altered regulation of adipose tissue lipolysis produced by the vaccine. The results show that pertussis vaccine administration to hamsters increases the blood level of free fatty acids, ketone bodies and triacylglycerols. Furthermore it induces a severe fatty liver.

Materials and Methods

Pertussis vaccine (strain 509, lot 29-090) was obtained from the National Institute of Hygiene (Secretaría de Salubridad y Asistencia, México). Beta-hydroxybutyrate dehydrogenase, coenzymes and 1-nitroso-2-naphtol were obtained from Sigma Chemical Co. Other chemicals used were analytical reagents of the best quality available.

Male golden hamsters (75-100 g) were maintained with Purina lab chow and water *ad libitum*. Pertussis vaccine was administered intraperitoneally in a single dose (0.1-0.2 ml). Animals were sacrificed by decapitation and exsanguinated. Hepatic and serum triacylglycerols were determined by the method of Gottfried and Rosenberg (2). Serum free fatty acids were quantified according to Novak (3). Blood ketone bodies were determined enzymatically (4,5).

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Results

It was observed that 3-4 days after the administration of pertussis vaccine to hamsters, the livers presented marked color changes similar to those produced by agents that induce fatty liver, such as ethanol, cycloheximide, ethionine or carbon tetrachloride (6-8). Consistent with this observation a dose-dependent and time-dependent accumulation of triacylglycerols in the liver was produced by the vaccine (Figs. 1 and 2). The maximal accumulation was obtained at a dose of 10^{11} cells/hamster (Fig. 1). Higher doses did not further increase the accumulation of triacylglycerols but were associated with a very high mortality. A dose of 10^{11} cells/hamster was used in all the following studies. It is noteworthy that the onset of fatty liver was very slow, taking at least two days to produce a significant accumulation of triacylglycerols in the liver (Fig. 2). Five days after treatment with the vaccine most of the hamsters died (60-75%) and the survivors usually had lower values of triacylglycerols in the liver than those observed four days after treatment (data not shown).

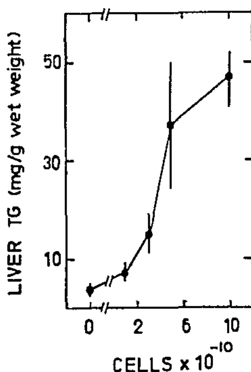

FIG.1.
Dose-response curve for the effect of pertussis vaccine on the amount of triacylclycerols (TG) in the liver. Hamsters were injected with different amounts of bacteria 3 days before sacrifice. Results are the means and vertical lines represent the S.E.M. of at least 5 hamsters.

In order to gain further information about the actions of the vaccine on lipid metabolism and to get some insight on the mechanism of induction of fatty liver, the levels of free fatty acids, triacylglycerols and ketone bodies in the blood were quantified (Figs. 3-5). The serum level of free fatty acids was significantly increased 2-4 days after treatment (2-4 fold increases) (Fig. 3). Serum triacylglycerols were also significantly increased after 3 and 4 days (approx. 10-fold increase on the 4th day) (Fig. 4). Ketone bodies also markedly increase in the blood in response to pertussis vaccine administration. One day after treatment blood ketone bodies had risen about 5-fold and a 40-fold

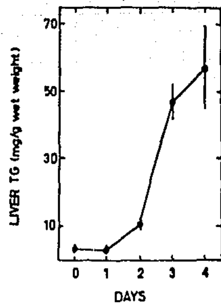

FIG.2.
Time-course of the effect of pertussis vaccine on the accumulation of triacylglycerols (TG) in the liver. The animals were injected with the vaccine at a dose of 10^{11} bacteria/hamster. Results are the means and vertical lines represent the S.E.M. of at least 4 hamsters.

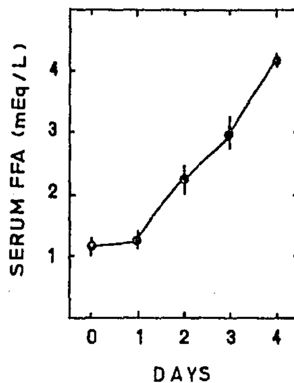

FIG.3.
Time-course of the effect of pertussis vaccine on the serum level of free fatty acids (FFA). Indications as in Fig. 2.

increase was detected 4 days after treatment (Fig. 5). The beta-hydroxybutyrate acetoacetate ratio was only slightly increased (from 0.7 to about 1) which suggests that the vaccine did not modify significantly the hepatic mitochondrial redox state (Fig. 5).

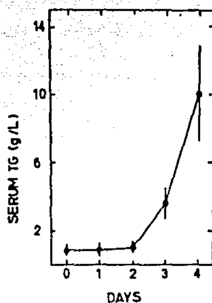

FIG.4.
Time-course of the effect of pertussis vaccine on the serum level of triacylglycerols (TG). Indications as in Fig. 2.

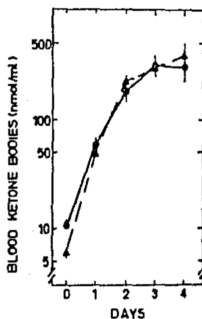

FIG.5.
Time-course of the effect of pertussis vaccine on the blood level of ketone bodies in hamsters. Beta-hydroxybutyrate (Triangles, broken line), acetoacetate (circles, solid line). Other indications as in Fig. 2.

Discussion

It was previously found that the administration of pertussis vaccine to hamsters decreases the sensitivity of their fat cells to antilipolytic agents that inhibit adenylate cyclase (1). It was also found that basal lipolysis was higher in fat cells from treated hamsters than in the controls which suggests that there is a decreased sensitivity to endogenous regulator(s) of lipolysis (1). *In vivo*, lipolysis is regulated through a balance between activators

(catecholamines (beta-adrenergic action), glucagon, ACTH, etc.) and inhibitors (catecholamines (alpha$_2$ adrenergic action), adenosine, free fatty acids, prostaglandins, ketone bodies, etc.). It is possible that the altered sensitivity to antilipolytic agents produced by the vaccine in fat cells (1) may significantly contribute in the pathogenesis of the alterations here reported.

The uptake of fatty acids by the liver is directly proportional to the concentration to which it is exposed (9). Fatty acids taken up by the liver can be esterified or oxidized. Ketone bodies reflect the oxidation of fatty acids by the liver (10). The oversupply of free fatty acids, reflected by the serum level (Fig. 3) might be at least partially responsible for the ketosis (Fig. 5; increased oxidation), hypertriacylglycerolemia and fatty liver (Figs. 4, 1 and 2 respectively; increased esterification in both cases). The high levels of triacylglycerols in the serum argue against any role of defective secretion of this type of lipid by the hepatocyte in the genesis of fatty liver by the vaccine. The level of triacylglycerols in the blood results from a balance between production, mainly by the liver, and utilization, mainly by adipose tissue and muscle. Thus, the possibility that a decrease in the utilization of triacylglycerols might contribute to the hypertriacylglycerolemia can not be ruled out.

The time-course of the effects indicate that the first parameter affected is the level of ketone bodies in blood. A significant increase was detected as soon as 24 hours after treatment. At this time the serum level of free fatty acids was not significantly modified. A 4-fold increase in the level of free fatty acids is associated with a 40-fold increase in the serum level of ketone bodies. It is possible that a small undetected increase in the serum level of free fatty acids might be responsible for the rise in ketone bodies observed the first day after treatment. However, the possibility that the vaccine could have a direct action on hepatic ketogenesis, in addition to its action on the supply of fatty acids, can not be ruled out.

A moderate fatty liver was observed 2 days after treatment without any increase in the serum level of triacylglycerols. Hypertriacylglycerolemia was only observed under conditions of severe fatty liver. This has also been observed in ethanol induced fatty liver and it has been suggested that a hepatic threshold may exist for the production of hyperlipemia by ethanol (6).

It has been observed that in mice, pertussis vaccine administration decreases the response of adipose tissue to beta adrenergic stimulation (11). Furthermore, it has been suggested that some actions of pertussis vaccine may be due to beta adrenergic blockade (11, 12). Our results are different and can not be explained by a beta adrenergic blockade. It is possible that the effects are produced by different components of the vaccine. The difference in species might also be important. We are using a higher dose of vaccine than Keller and Fishel (11). The action of epinephrine on adipose tissue lipolysis results from the balance between beta adrenergic sensitivity (activation of adenylate cyclase) and alpha$_2$ adrenergic sensitivity (inhibition of adenylate cyclase) (13). Species difference with respect to alpha$_2$ adrenergic sensitivity of adipocytes has been observed (13). Other significant differences between our results and those of Keller and Fishel (11) is that our effects on adipose tissue are of slow onset and they are not lost during the fat cell isolation procedure (compare 1 and 11).

Pertussis vaccine is not a purified preparation. It is a suspension of died bacteria which obviously contains multiple molecular components. Sumi and Ui (14) initially reported that the administration of pertussis vaccine to rats abolishes the ability of alpha adrenergic agonists to decrease blood insulin. Ui and coworkers have been able to purify a protein (Islet-Activating-Protein) from the culture medium of *Bordetella pertussis* which is able to mimick the effects of the vaccine *in vivo* and in isolated pancreatic islets (15-17). Two characteristics are common to the effects of pertussis vaccine on lipid metabolism in the hamster and the action of the Islet Activating

Protein on rat pancreatic islets: hyposensitivity to agents that decrease cyclic AMP levels and a slow onset of action. Experiments are in progress to purify the molecule(s) responsible for these effects of the vaccine.

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The authors want to thank Dr. M. González-Pacheco for the donation of the vaccine and the authorities of the IMSS for their gift of hamsters. The authors also thank Drs. E. Piña and A. Peña for letting them use some of the equipment in their laboratories and for the generous gift of some reagents. This research was partially supported by a Grant from the Fondo de Estudios e Investigaciones "Ricardo J. Zevada".

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DECREASED SENSITIVITY TO α_2 ADRENERGIC AMINES, ADENOSINE AND PROSTAGLANDINS IN WHITE FAT CELLS FROM HAMSTERS TREATED WITH PERTUSSIS VACCINE

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1. Introduction

The release of insulin from pancreatic islets is inhibited by α-adrenergic activation and is increased by stimulation of β-adrenoceptors [1]. α-Adrenoceptors have been divided into 2 subtypes, α_1 and α_2, by their affinities for agonists and antagonists [2] and their underlying mechanism of signal transduction [3]. The α-adrenoceptor involved in the regulation of plasma insulin level is of the α_2 subtype [4]. α_2-Adrenoceptors are linked in an inhibitory fashion to adenylate cyclase [3,5,6].

The decrease in blood insulin level due to α-adrenoceptor activation is effectively blocked by pertussis vaccination in rats [7]. This effect of the vaccine seems to be due to a protein produced by *Bordetella pertussis* [8,9]. Hamster adipocytes have α_2-adrenergic receptors linked in an inhibitory fashion to adenylate cyclase [3,5]. Activation of these receptors decreases basal and stimulated cyclic AMP levels and lipolysis [3,5,10]. The possibility that administration of pertussis vaccine to hamsters may alter the sensitivity of their adipocytes to α_2-adrenergic amines was studied. The results show that administration of pertussis vaccine to hamsters markedly decreases the sensitivity of their adipocytes to α_2-catecholamines. Furthermore, it was found that such decrease in sensitivity is not exclusive to α_2-adrenergic amines but it is common to other agents such as adenosine and prostaglandins.

2. Materials and methods

Epinephrine, isoproterenol, theophylline and prostaglandin E_2 (PGE_2) were obtained from Sigma Chemical Co. N^6-(L-2-phenyl-isopropyl)-adenosine (PIA), adenosine deaminase, glycerokinase, glycerophosphate dehydrogenase and NAD^+ were obtained from Boehringer-Mannheim. Collagenase from *Clostridium histolyticum* (lot 40C190) and bovine serum albumin (fraction V) (lot U13707) were obtained from Worthington and Armour Pharmaceutical Co., respectively. Clonidine was a generous gift of Boehringer-Ingelheim. Pertussis vaccine (strain 509, lot 29-090) was obtained from the National Institute of Hygiene (Secretaría de Salubridad y Asistencia, México).

Golden hamsters were fed ad libitum. Pertussis vaccine was injected intraperitoneally ($\sim 10^{11}$ organisms). Animals were used 3–9 day after a single injection. White adipocytes were isolated and incubated as in [5,10]. Cyclic AMP accumulation and glycerol release were determined as in [5,10].

3. Results

The level of cyclic AMP in the absence of hormones was slightly higher in adipocytes from pertussis-treated hamsters than in control adipocytes (42 ± 10 compared to 29 ± 2 pmol/10^6 cells, respectively; means ± SEM of 6 determinations in duplicate in each case). Isoproterenol, a pure β-adrenergic agonist, produced a dose-dependent increase in the level of cyclic AMP (fig.1). The maximal accumulation of the cyclic nucleotide was not affected in adipocytes from pertussis-treated hamsters. However, a decreased accumulation of cyclic AMP in response to the lowest dose of isoproterenol used (10^{-8} M) was observed in fat cells from pertussis-sensitized hamsters (fig.1). Epinephrine, an α–β-adrenergic agonist, produced a

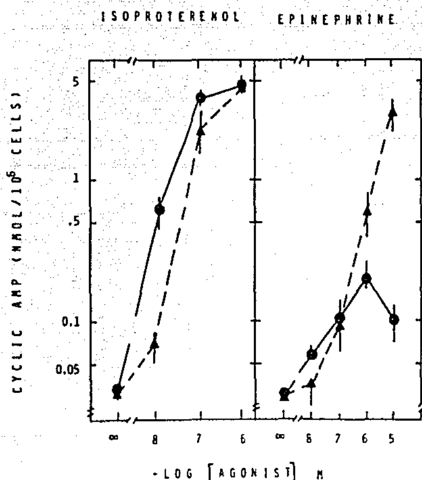

Fig.1. Dose–response curves for the effects of isoproterenol or epinephrine on the accumulation of cyclic AMP in adipocytes from hamsters. Fat cells ($1-2 \times 10^5$) were incubated for 10 min in 1 ml Krebs-Ringer phosphate buffer containing 3% albumin, 0.5 μg adenosine deaminase, 100 μM theophylline and different concentrations of either isoproterenol or epinephrine. (•) Adipocytes from control hamsters; (▲) adipocytes from hamsters treated with pertussis vaccine. Results are the means and vertical lines represent the SEM of 6 expt performed in duplicate. Results are plotted on a log scale.

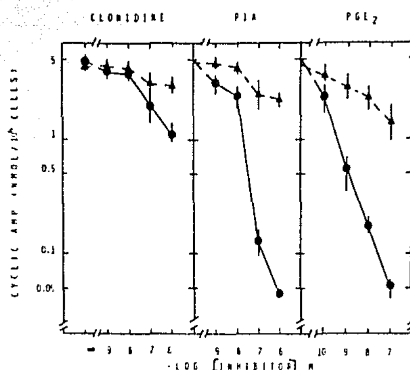

Fig.2. Dose–response curves for the effects of clonidine, N^6-(2-phenyl-isopropyl) adenosine (PIA) and prostaglandin E_2 (PGE_2) on the accumulation of cyclic AMP due to isoproterenol in adipocytes from hamsters. Fat cells ($1-2 \times 10^5$) were incubated for 10 min in 1 ml Krebs-Ringer phosphate buffer containing 3% albumin, 0.5 μg adenosine deaminase, 100 μM theophylline, 10 μM isoproterenol and different concentrations of clonidine, PIA or PGE_2. (•) Adipocytes from control hamsters; (▲) adipocytes from hamsters treated with pertussis vaccine. Results are the means and vertical lines represent the SEM of 6 expt performed in duplicate. Results are plotted on a log scale.

dose-dependent accumulation of cyclic AMP in control adipocytes (fig.1). However, the maximal accumulation was much smaller than that due to isoproterenol (~10-fold increase compared to ~150-fold increase, respectively). This is due to inhibition of adenylate cyclase by activation of α_2-adrenoceptors [3,5,6]. In adipocytes from pertussis-treated hamsters the accumulation of cyclic AMP due to epinephrine was much higher, reaching levels similar to those obtained with isoproterenol (fig.1). The data suggest that in adipocytes from hamsters treated with pertussis vaccine, there is a decreased α_2-adrenergic sensitivity.

To further test this point, the ability of the α_2-adrenergic agonist, clonidine, to decrease the level of cyclic AMP due to isoproterenol was assayed. The sensitivity to clonidine was significantly decreased in adipocytes from treated animals (fig.2). In order to determine if the action of pertussis vaccine was exclusively on α_2-adrenoceptors, the ability of other agents, which inhibit adenylate cyclase, to decrease the levels of cyclic AMP due to isoproterenol was tested. PIA and PGE_2 decreased the accumulation of cyclic AMP due to β-adrenergic stimulation to a much lesser extent in adipocytes from treated hamsters than in control adipocytes (fig.2).

Basal lipolysis (in the absence of adenosine deaminase and theophylline) was higher in adipocytes from treated hamsters than in the control adipocytes (0.80 ± 0.02 and 0.33 ± 0.01 μmol glycerol/10^6 cells in 60 min incubation, respectively; results are the means ± SEM of 4 expt in each case). However, lipolysis due to adenosine deaminase plus theophylline was only slightly higher in adipocytes from treated hamsters than in control fat cells (fig.3). This suggests that in fat cells from treated hamsters the sensitivity to endogenously released adenosine was significantly reduced. However, the possibility that the sensitivity to another endogenous regulator(s) may also be decreased can not be ruled out. The ability of clonidine, PIA and PGE_2 to inhibit lipolysis due to adenosine deaminase plus theophylline was also significantly reduced in pertussis-sensitized hamsters (fig.3).

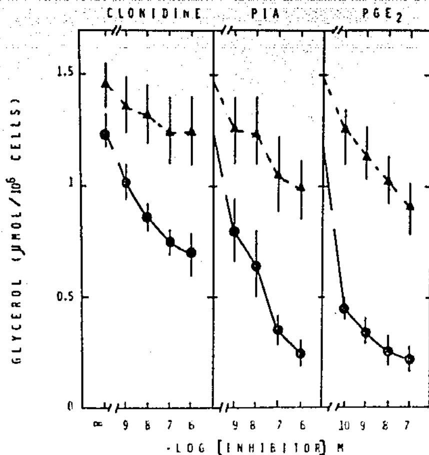

Fig.3. Dose–response curves for the effects of clonidine, N^6-(2-phenyl-isopropyl) adenosine (PIA) and prostaglandin E_2 (PGE_2) on lipolysis due to adenosine deaminase and theophylline. Fat cells ($1-2 \times 10^5$) were incubated for 60 min in 1 ml Krebs-Ringer phosphate buffer containing 3% albumin, 0.5 μg adenosine deaminase, 100 μM theophylline and different concentrations of clonidine, PIA or PGE_2. (•) Adipocytes from control hamsters; (▲) adipocytes from hamsters treated with pertussis vaccine. Results are the means and vertical lines represent the SEM of 8 expt.

4. Discussion

Administration of pertussis vaccine to hamsters produced a marked decrease in the sensitivity of their adipocytes to α_2-adrenergic amines, PIA and PGE_2 as reflected by both cyclic AMP levels and lipolysis. α_2-Adrenergic amines, PIA, and PGE_2 share the property of inhibiting adenylate cyclase through receptor-mediated processes. Inhibition of adenylate cyclase by hormones and neurotransmitters seems to involve at least 3 molecular entities: the catalytic subunit of adenylate cyclase; the receptor; and a guanine nucleotide binding protein [11,12]. Calcium does not seem to be involved in inhibition of adenylate cyclase by these agents.

It is unlikely that treatment with pertussis vaccine may decrease the level of receptors for α_2-adrenergic amines, adenosine and prostaglandins. Therefore, it is possible that the action of pertussis vaccine may occur at the level of the coupling between the receptor and the cyclase.

These data are consistent with the data in [7–9] where pertussis sensitization or administration of the purified 'islet-activating protein' abolished the ability of α-adrenergic agents and somatostatin to inhibit glucose-induced insulin release, cyclic AMP accumulation and $^{45}Ca^{2+}$ uptake by pancreatic islets from rats. It was concluded that the action of the 'islet-activating protein' is the result of sustained activation of native calcium ionophores present on the cell membrane [8]. The present findings suggest a complementary or alternative explanation:

> It is possible that the 'islet-activating protein', present in the vaccine, may block the transfer of inhibitory information from the receptor to adenylate cyclase.

Calcium and cyclic AMP are known to play important roles as a link in the stimulus–secretion coupling in the pancreatic β-cell [13]. Changes in the level of cyclic AMP may explain the altered calcium fluxes and insulin secretion due to epinephrine in islets from rats treated with the 'islet-activating protein'.

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The author wants to express his gratitude to Dr A. Peña and Dr E. Piña for the generous gift of some substances used in this research. He is also grateful to the authorities of the Medical Center of the IMSS for their generous gift of hamsters and to Dr M. González-Pacheco for the donation of the vaccine.

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December 28, 1981

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Re: Ms# 11249
"Correlation between phosphatidylinositol labeling and contraction in rabbit aorta: Effect of α_1 adrenergic activation"

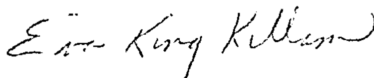
Dear Dr. Garcia-Sainz:

Your manuscript referenced above was received on December 23, 1981. It has been sent for review to Specific Field Editor:

Dr. Robert E. Stitzel
Department of Pharmacology
West Virginia University
Medical Center
Morgantown, West Virginia 26506

All correspondence regarding the manuscript should be addressed directly to Dr. Stitzel. Thank you for submitting your work to the Journal.

Sincerely,



Eva King Killam, Ph.D.

EKK:mk

December 14, 1981.

Dr. Eva King Killam,
Departament of Pharmacology,
School of Medicine,
University of California,
Davis, California 95616.

Dear Dr. Killam:

Please find enclosed the manuscript "Correlation Between Phosphatidylinositol Labeling and Contraction in Rabbit Aorta: Effect of α_1 Adrenergic Activation", to be considered for publication in The Journal of Pharmacology and Experimental Therapeutics.

Thank you very much for your attention and courtesy

Yours Sincerely

J. Adolfo García-Sáinz, MD, PhD.

CORRELATION BETWEEN PHOSPHATIDYLINOSITOL LABELING AND CONTRACTION IN RABBIT AORTA: EFFECT OF α_1 ADRENERGIC ACTIVATION.

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PI-EFFECT AND CONTRACTION

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ABSTRACT

Villalobos-Molina, Rafael, Mirna Uc, Enrique Hong and J. Adolfo García-Sáinz: Correlation Between Phosphatidylinositol Labeling and Contraction in Rabbit Aorta: Effect of α_1 Adrenergic Activation.

Activation of rabbit aortic strips with α adrenergic agonists increased the labeling(with $[^{32}P]$Pi) of phosphatidylinositol(PI) and phosphatidic acid and contracted the vascular preparations in dose-related fashions. Epinephrine, norepinephrine and methoxamine produced maximal effects, while clonidine behaved as partial agonist and B-HT 933 was almost without activity in the two experimental models used. Phenylephrine was a full agonist in contraction but failed to elicit the maximal increase in PI labeling. The EC_{50}'s to produce contraction of aortic strips were lower for all agonists than those required to increase the incorporation of radioactive phosphate into PI, but there was a good correlation between the two sets of data. The increased PI labeling and contraction of aortic strips induced by epinephrine were antagonized by prazosin and yohimbine in dose-related fashions but the first α blocker was about three orders of magnitude more potent than the second in the two effects. The present results indicate that both stimulation of PI labeling and contraction, are mediated through activation of α_1 adrenoceptors in rabbit aorta.

Activation of hormone receptors that control intracellular responses by increasing the concentration of ionized calcium in the cytosol produces the so-called phosphatidylinositol(PI)-effect (Michell and Kirk,1981). This PI response is characterized by an initial breakdown of this phospholipid followed by a compensatory increase in the rate of its synthesis(Michell and Kirk,1981). Interestingly, in most tissues the intracellular responses(such as glycogenolysis, exocytosis or contraction) are calcium dependent and mimicked by calcium ionophores but the PI-effect is not, suggesting that the turnover of PI might be involved in the calcium signalling process.

Alpha(α) adrenoceptors have been divided in α_1 and α_2 adrenergic subtypes(Berthelson and Pettinger,1977; Fain and García-Sáinz,1980). However these adrenoceptors do not seem to be isoreceptors since they involve different mechanism of signal transduction(Fain and García-Sáinz,1980). Alpha$_2$ adrenoceptors are linked in an inhibitory fashion to adenylate cyclase whereas α_1 adrenoceptors are not linked to the cyclase but involved in PI turnover and calcium mobilization. Alpha adrenergic amines contract rabbit aorta in a dose-dependent manner and stimulate PI turnover(Lapetina et al,1976). However the effect of adrenergic agonists on PI turnover, the type of α adrenoceptor involved and its correlation with contraction has received little attention.

This work presents a pharmacological characterization of the adrenergic receptor involved in PI labeling and contraction in rabbit aorta.

METHODS

Rabbits of either sex(New Zealand strain) weighing between 1.5 and 3 kg were used. The animals were sacrificed by a blow on the head and the thoracic aorta(from aortic arch to diaphragm) was excised rapidly and immediately placed in cold buffer, cleaned and freed of surrounding connective tissue.

Measurement of[^{32}P]Pi incorporation into phospholipids.

Aortic rings of 2.5 mm wide and weighing approximately 10 mg were incubated in plastic tubes containing 1 ml of Krebs-Ringer tris buffer(pH 7.4 at 37°C) of the following composition: NaCl,120 mM; KCl,4.75 mM; $MgSO_4$,1.2 mM; $CaCl_2$, 1.2 mM; tris,5 mM containing 10 μCi/ml of[^{32}P]Pi. Rings were incubated for 1 hr and lipids were extracted with 6 ml of chlorophorm/methanol. The single phase system obtained was separated by the addition of 4 ml 100 mM-KH_2PO_4. The cholephorm layer was evaporated in a vacuum centrifuge to dryness. Phospholipids were separated by one-dimension t.l.c. on glass plates coated with silica gel H(García-Sáinz and Fain,1980). Lipids were identified by using I_2-vapour staining, the phosphorus content of phospholipids was determined by the micromodification of the procedure of Bartlett(1959) after acid hydrolysis of silica gel scrapings containing each individual phospholipid. Incorporation of radioactive phosphate into each phospholipid was measured in silica gel scrapings added to a liquid scintillation fluid (Fricke,1975) in a Beckman LSC-100 spectrometer.

The results are expressed as a percentage of control incorporation into each phospholipid. Each experiment was repeated at least three times on different days and the data are expressed as the means ± S.E.

Contraction

Helicoidal aortic strips were prepared as described by Furchgott and Bhadrakom(1953) and placed in a 25 ml tissue bath filled with Krebs-bicarbonated

solution, warmed at 37°C and bubbled with a mixture of 95% O_2 and 5% CO_2. Contraction was assessed by means of an isometric FT 03 Grass force-displacement transducer connected to a 7P Grass polygraph. A resting tension of 4 g was applied repeatedly until strips remained without relaxation under such resting tension. Then, responses to epinephrine(5.5 x 10^{-8} M) were obtained every 20 min until maximal reactivity was observed(usually 2 hours). In one type of experiments, accumulated dose-response curves were obtained with one of the α adrenergic agonists used(due to the high concentrations used, some strips did not recover full reactivity, as it had been previously described for epinephrine by Pardo et al,1967). In the other type of experiments, responses to epinephrine(10^{-7}M) were obtained every 20 min before and five minutes after contact of preparations with increasing concentrations of prazosin or yohimbine. Chambers were washed four times after obtention of each response. Each drug concentration was administered in a 0.1 ml volume. Results from dose-response curve to agonists were expressed in grams in order to show differences in intrinsic activity, while the effect of the α-blockers in terms of percent of control responses. Each experiment was replicated six times and the data are expressed as the mean ± S.E.

Chemicals

Epinephrine-HCl or bitartrate, norepinephrine-HCl, phenylephrine-HCl and yohimbine-HCl were obtained from Sigma Chemical Co.; methoxamine-HCl, clonidine-HCl, B-HT 933 and prazosin were generous gifts from Burroughs Wellcome Co., Boehringer Ingelheim and Pfizer respectively; [^{32}P] Pi as orthophosphoric acid(carrier free) was obtained from New England Nuclear.

RESULTS

Incorporation of $[^{32}P]$ Pi into aorta phospholipids

As shown in table 1, a substantial incorporation of $[^{32}P]$ Pi into major phospholipids was obtained within 1 hr of incubation. The phospholipid composition of rabbit aorta was similar to that reported by Lapetina et al (1976) for cat aorta and resembles that of other tissues(White,1973). Phosphatidylcholine and phosphatidylethanolamine contribute about 65% of the total phosphate content. Incorporation of radioactive phosphate was mainly into phosphatidylcholine followed by phosphatidic acid and phosphatidylinositol (table 1). The incorporation into phosphatidylethanolamine was very little as compared to other phospholipids. These results are similar to those reported in adipocytes(García-Sáinz and Fain,1980). No clearcut change in the amount of any phospholipid was produced by any of the agents employed over the 1 hr incubation period used in the present studies.

Effect of epinephrine on the incorporation of $[^{32}P]$ Pi into phospholipids

Epinephrine produced an increased incorporation of $[^{32}P]$ Pi into phospholipids (fig 1). The effect of the amine was exclusively on the labeling of PI and its precursor phosphatidic acid and it was dose-dependent (fig.1).

A significant(two-fold) increase in the labeling of PI was observed at a concentration as low as 3×10^{-7}M. The effect increased linearly with the dose up to 10^{-5}M(roughly 7-fold), higher concentrations did not increase further the effect(fig.1). A similar pattern was observed for the labeling of phosphatidic acid although the magnitude of the epinephrine action was smaller (2-4 fold increases, fig.1).

Effect of α adrenergic agonists on the incorporation of radioactive phosphate into PI and contraction

The effect of different α adrenergic agonists on PI labeling was tested and the data presented in figure 2. Epinephrine, methoxamine and norepinephrine

were full agonists whereas phenylephrine and clonidine were partial agonists for this effect. The selective α_2 adrenergic agonist B-HT 933 was without effect on this parameter.

Alpha adrenergic agonists contracted rabbit aorta in a dose-dependent fashion (fig.2). The maximal responses elicited by epinephrine, norepinephrine, methoxamine and phenylephrine were of large magnitude(3.9-4.3 g), indicating a complete agonist effect. On the contrary, clonidine produced a much smaller effect and B-HT 933 elicited a maximal contraction of only 1.0 g at the highest concentration tested(10^{-4}M).

A correlation between the effects of adrenergic agents on PI labeling and aorta contraction seems to exist(fig.2).

Effect of α adrenergic antagonists on epinephrine-mediated PI labeling and contraction.

The effect of epinephrine on PI labeling was antagonized by prazosin(a highly selective α_1 antagonist) or yohimbine(an α_2 antagonist). The results are presented in figure 3. Prazosin inhibits the PI response caused by 10^{-5}M epinephrine in a dose-dependent fashion, the total inhibition of the effect was observed with 10^{-5}M prazosin and the IC_{50} obtained for this drug was 1.0×10^{-7}M. Yohimbine was much less potent in antagonizing epinephrine action (fig.3). The IC_{50} for yohimbine(1.0×10^{-4}M) is 3-orders of magnitude greater than that obtained for prazosin. Under the conditions used here, no total inhibition was reached with yohimbine up to 10^{-3}M. The selective inhibition by prazosin suggests that the PI effect induced by epinephrine is mediated through activation of α_1 adrenoceptors. Prazosin(up to 10^{-5}M) and yohimbine (up to 10^{-3}M) were without effect on the basal labeling of phospholipids(data not shown).

The contraction of aorta produced by 10^{-7}M epinephrine was antagonized by both α adrenergic blockers, prazosin and yohimbine(fig.3). However, the first was much more potent than the second; thus, the IC_{50} for prazosin was 1.6 x 10^{-9}M whereas that for yohimbine was 1.8 x 10^{-6}M.

The stimulation of PI labeling produced by 10^{-4}M methoxamine or 10^{-5}M norepinephrine were completely abolished by 10^{-5}M prazosin and only partially inhibited by 10^{-4}M yohimbine(data not shown). The small effect of clonidine was inhibited by 10^{-6}M prazosin whereas yohimbine up to 10^{-5}M had no effect (data not shown).

DISCUSSION

PI turnover is stimulated by adrenergic agonists in many tissues and this effect is generally mediated through α_1 adrenoceptors(Fain and García-Sáinz, 1980). Lapetina et al(1976) have previously reported that α adrenergic stimuli selectively enhance PI labeling in isolated cat aorta. The stimulation of PI labeling observed in this study resembles that produced by other ligands whose final actions on target tissues are mediated by a rise in intracellular Ca^{++} concentration like vasopressin(V_1 receptor), angiotensin, histamine(H_1 receptor)and many other agents(Michell and Kirk,1981). The effect of catecholamines on vascular smooth muscle contraction has been established long time ago(Furchgott,1955). However, more recently an increased research on the characterization of the effectors involved in this response has been focused(Altura and Altura,1970; Sheys and Green,1972; Altura and Altura,1977; Ruffolo et al,1979; Ruffolo et al,1981). The present results indicate that both adrenergic responses(PI turnover and contraction of aorta) are mediated through α_1 adrenoceptors. Similarly, it has been observed that vasopressin stimulates PI labeling and contraction in rat aorta(Takhar and Kirk,1981; Altura and Altura,1977) through activation of receptor of the V_1 subtype. Our data and those of Takhar and Kirk(1981) suggest a role of calcium signalling in aorta contraction induced by vasopressin and epinephrine. In our experiments the dose-response curve for the PI effect appears shifted to the right in relation to the dose-response curve for the final physiological event (contraction), this has been observed in most tissues that exhibit a PI response. Such finding has been interpreted as a consequence of the presence of spare receptors(Michell and Kirk,1981).

It is also notewhorty that phenylephrine is a partial agonist for the PI response whereas it is a full agonist for contraction(fig.2). This relationship

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has also been observed in other tissues such as the liver, where the PI response was compared with phosphorylase activation as final event (Tolbert <u>et al</u>,1980) and suggests that the PI response might be "closer" to the generation of the second messenger than the other effect. In other words, a minimal amount of "signal" might be required to produce the maximal final response. Therefore, an agonist which may activate a minimal proportion of the receptors could behave as a full agonist for the final effect. Similar relationships have been observed for agents which act through the adenylate cyclase system.

Recently, Wikberg(1979) has revised the α adrenergic effects on smooth muscle(namely vascular and intestinal) from a physiological point of view and concluded that it is possible that two factors (calcium and cyclic AMP) might be involved in the response. Wikberg suggested that α_1 adrenoceptors could act through calcium whereas α_2 adrenoceptors exert their action by decreasing cyclic AMP levels. Similarly, Fain and García-Sáinz(1980) have suggested that α_1 adrenoceptors are not linked to adenylate cyclase but mediate those effects of catecholamines which involve PI turnover and calcium signalling whereas α_2 adrenoceptors are linked to adenylate cyclase in an inhibitory fashion. This obviously suggest that α_1 and α_2 adrenoceptors are not isoreceptors since they do not have the same system of signal transduction. This has been clearly shown in two systems:adipocytes(García-Sáinz <u>et al</u>,1980;Burns <u>et al</u>,1981) and hepatocytes(Tolbert <u>et al</u>,1980;Jard <u>et al</u>,1981).

In the present studies, methoxamine was a potent and complete agonist in both effects studied(PI response and contraction). Although these findings are in complete agreement with the well known pharmacological properties of methoxamine, it should be mentioned that methoxamine

is a very weak agonist in fat cells(García-Sáinz and Fain,1980) and liver cells(Aggerbeck *et al*,1980). The reason for this significant difference is at present unknown, but suggests the possibility that true subtypes of α_1 adrenergic receptors might exist in different tissues.

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TABLE 1. Incorporation of $[^{32}P]$ Pi into phospholipids of rabbit aorta.

	Pi content (% of total)	$[^{32}P]$Pi incorporation (% of total)
Phosphatidylethanolamine	26.4 ± 2.0	5.6 ± 0.6
Phosphatidylcholine	39.5 ± 2.2	40.1 ± 1.4
Phosphatidylinositol	14.7 ± 1.5	26.8 ± 1.0
Phosphatidic Acid	20.5 ± 2.0	27.9 ± 1.3

Total phospholipid phosphorus was 1.20 ± 0.08 μg; total incorporation was 3539 ± 270 cpm/10 mg aorta wet weight. The results are expressed as means ± S.E. for thirty different determinations.

Figure 1. Effect of epinephrine on the labeling of phospholipids. Plotted are the means and vertical lines represent the S.E.

PE= Phosphatidylethanolamine

PC= Phosphatidylcholine

PI= Phosphatidylinositol

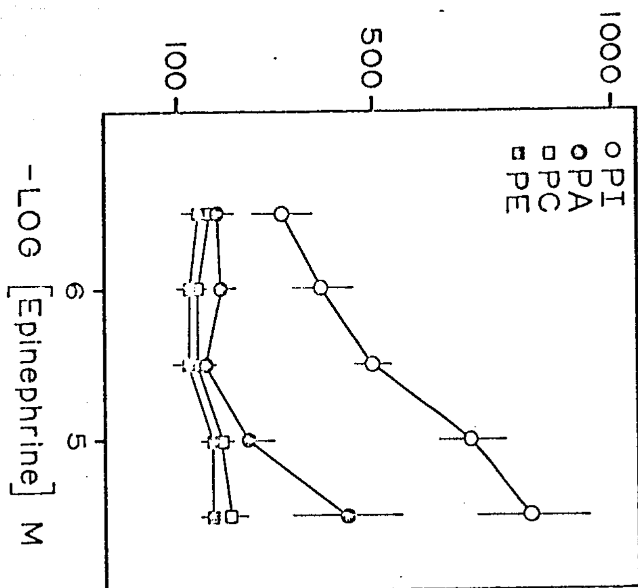
PA= Phosphatidic Acid

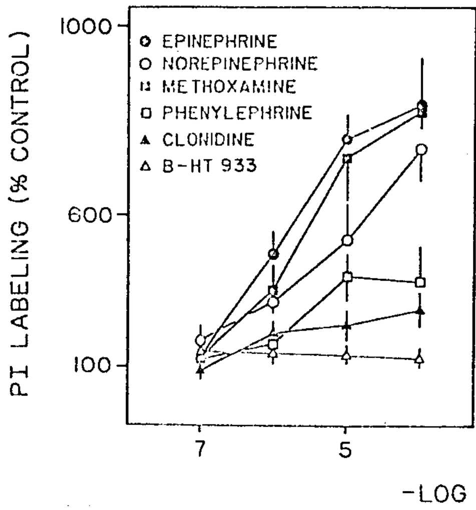
Basal values for incorporation are shown in Table 1.

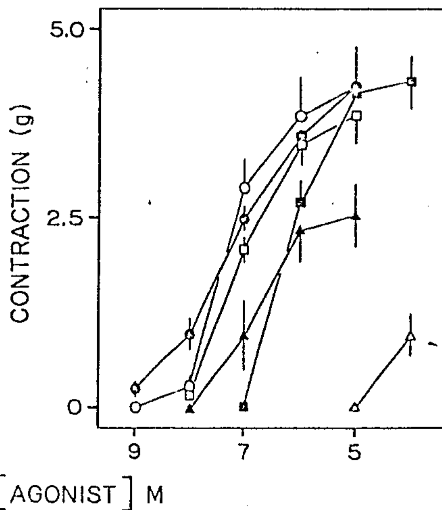
Figure 2. Effect of α adrenergic agonists on the labeling of phosphatidylinositol and on contraction. Basal incorporation of label into PI was 930 cpm/10 mg aorta wet weight. Plotted are the means and vertical lines represent the S.E.

Figure 3. Effect of prazosin and yohimbine on epinephrine-induced phospatidylinositol labeling and contraction. Epinephrine was present at a concentration of 10^{-5}M in the labeling experiments and 10^{-7}M when contraction was studied. Control labeling refers to the amount of label incorporated into PI in the absence of drugs and

was 930 cpm/10 mg aorta wet weight. Control contraction refers to the tension produced by 10^{-7}M epinephrine in the absence of adrenergic antagonists and was 2.3 g. Plotted are the means and vertical lines represent S.E.







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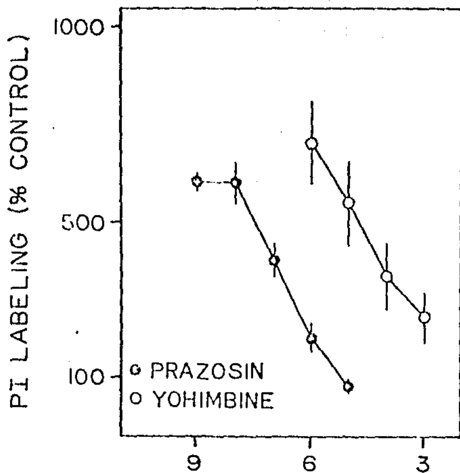

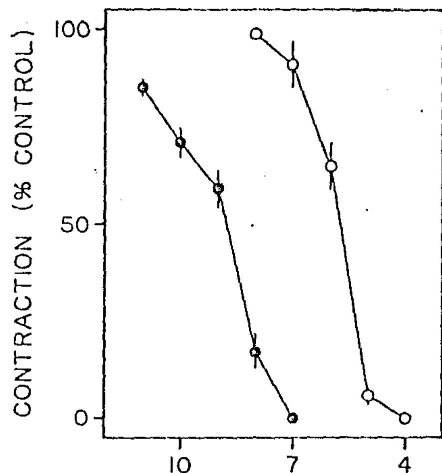

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