UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

FACULTAD DE MEDICINA

SEIZURES INDUCED BY PENICILLLIN MICROINJECTIONS IN THE MESENCEPHALIC TEGMENTUM

TESIS

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PRESENTA:

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Received 30 March 1999; received in revised form 14 June 1999; accepted 18 June 1999

Abstract

The location and extension of a convulsive area in the brain stem in cats was determined through penicillin microinjections (0.5–1.0 μl) of a concentrated sodium penicillin solution (500 IU/μl), stereotactically oriented to multiple structures, in fully awake animals, partially restrained through a rod fixation system that avoided pain, allowed the observation of clinical seizures and simultaneous recording of EEG, EMG and multiple unit activity (MUA) from the injected site and the motor cortex (Cx). Clinical and EEG seizure patterns in relation to the injected sites and penicillin doses were studied in another group of animals using doses from 12.5 IU/0.1μl to 125 IU/1.0 μl. The time relationship between muscular clonus, EEG spikes and MUA at the injected site and Cx were analyzed. The only area in which penicillin induced seizures was the mesencephalic tegmentum (MT). The amount of penicillin but not the setereotactic coordinates determined the seizure type. MT EEG and MUA paroxysms anticipated clinical seizure and Cx EEG spikes. When Cx EEG appeared, they were accompanied by an increase in MUA beginning in the Cx and EMG, followed by significant increase in MT MUA. The sequence of events suggest that MT seizure activity propagates via alternative pathways not involving direct reticulospinal or pyramidal tract pathways.

Keywords: Brain stem; EEG; Multiple unit recording; Penicillin; Reticular formation; Seizure pattern

1. Introduction

Experimental data has provided evidence of the participation of the brain stem in the genesis and propagation of convulsive activity (for review, see Fromm et al., 1987; Gale and Browning, 1988; Velasco and Velasco, 1990).

From those observations a number of controversies have been raised in regard to: (1) the anatomical structures involved in the genesis of seizures; (2) the clinical type of seizures induced in the brain stem; and (3) the possible mechanisms by which epileptic activity 'originated' in the brain stem propagates to the forebrain, spinal cord and muscles.

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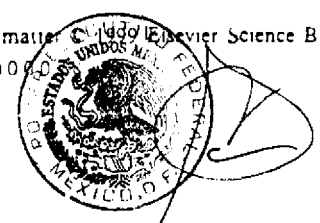

Discrepancies are probably related to the methods used to induce seizure, as many observations were made using systemic convulsants (PTZ, penicillin, bicuculline, maximal electroshock seizure or MES, etc.) or by topical applications designed so that the convulsant may reach several structures simultaneously (pledgets, push pull perfusion, powder, large volume of solution, etc.), rendering the correlation of the anatomical site with the effect obtained practically impossible. On the other hand, many observations have been made in anesthetized or paralized preparation allowing EEG and unitary or multiunit activity (M UA) recordings but certainly interfering in the correlation of such recordings with clinical events. Finally, many of the convulsant methods have a poorly defined action mechanism (electrical stimulation, MES, cobalt, aluminum hydroxide, etc.) or have been used in amounts and concentrations that cast doubts on their possible diffusion and the participation of other physiological variables (pH, osmolarity, electrolytic content, etc.) in the epileptic event studied.

Sodium penicillin (NaPC) is a convulsant with well defined intracellular (Ayala and Vasconsetto, 1970; Wong and Prince, 1979), topical (Pockberger et al., 1984; Chatt and Ebersole, 1988) and propagated (Crowell, 1970; Gloor et al., 1977; Redecker et al., 1984; Horn and Gehring, 1996) mechanism, and maybe prepared in stable, buffered solutions approaching interstitial fluid constants.

The present study describes the effects of NaPC injected in small volumes of a solution resembling the interstitial fluid in pH, osmolarity and sodium content within the brain stem of awake cats, partially restrained to allow EEG and MUA recordings during seizures and at the same time the observation of the clinical events elicited. Special attention was paid to the identification of the injected sites.

2. Material and methods

Twenty-five adult cats weighting 2.8 to 3.6 kg were used for the experiments. All had an initial surgery under general anesthesia with intraperitoneal sodium penthotal (20 mg/kg) for implanting a stereotactically oriented 16-gauge cannula guide, 0.5 cm short of the intended target to inject penicillin solution into the brain stem, as well as a 45° angled cannula convergent to the previous target to allow the passage of a microelectrode for recording EEG and multiple unit activity (MUA) of the injected area. Burr holes were performed to allow placement of silver balls for EEG recordings from right and left suprasylvian cortices (RSS and LSS) and left anterior sigmoid gyrus (LAS) and a craniotomy window over the right anterior sigmoid (RAS) gyrus to allow passage of a microelectrode for MUA and EEG recordings of the subjacent cortex (Cx) was also performed. Stainless steel wires isolated but 3 mm from their tips were inserted in the posterior neck muscles on both sides to record EMG. A screw drilled on the frontal bone served as reference lead to all recordings, and all recording electrodes were wired to a connector (Amphenol). The connector, cannulas and a pair of rods placed anteriorly and posteriorly on the skull to replace the head in the stereotactic frame for future experiments without inducing pain in an awake animal (Rod fixation system by Kopf Inst, Tujuma, CA) were cemented to a plastic skull cap.

The animals were left to recover, and one week later their heads were replaced in the stereotactic frame, using the rod system and maintaining the body inside a transparent plastic cage that allowed the follow up of the animal's behavior, with an exit for the neck to restrict motion and avoid excessive traction on the rod fixation system.

2.1. Injections of supramaximal doses of penicillin

Fourteen animals were used to determine the location and extension of the convulsive areas in the brain stem. In those animals, 32 target points along the brain stem were injected on the right side with a solution containing 500 UI Na penicillin/μl (850 mM, pH 6.8 to 7.2, 484 mEq Na/L) at a rate of 0.1 μl/5s while EEG from RAS, LAS, RSS and LSS as well as EMG-MUA from right and left posterior neck muscles were recorded. A careful time lock chart of the animal's behavior was kept throughout the experiment. The solution

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is injected by means of a Hamilton syringe amilton Co, Reno, NV) beginning with 0.5 μl 50 IU NaPC) and increasing 0.5 μl every 30 min til a convulsive effect was observed or a maxium of 2.0μl (1000 NaPC) were injected in each rget point. At the end of the experimental sesn, the site of injection was marked with 10% ver nitrate, injecting a volume similar of that of penicillin solution used during the experiment, determine the precise area covered by the injecn. Animals used for more than one injection d an interval of 4 to 8 days between injections ring which EEG and clinical observations did t show residual effects.

2. Dose–response curves

Once the convulsive area for penicillin injection the brain stem was determined (see results), 11 imals were used to determine the convulsive reshold for penicillin, the pharmacological dose sponse curves and the relationship between the fferent type of convulsive effects induced with amount of penicillin injected and the precise reotactic coordinates of the injections within convulsive area.

Animals were prepared as described above, ding recordings of EEG and MUA from the ected place and ipsilateral anterior sigmoid rus. MUA recordings were performed through ipolar concentric stainless steel electrode, made m a 25 gauge cannula, isolated but at a distal g of 2 mm and a inside wire isolated but at the that was electrolytically sharpened to a size of -80 μ (40 Kilohms of impedance). Recordings re amplified and filtered though a 3000 Hz ndpass filter coupled with a stair cage generator d audiomonitor. Through a Smith trigger UA over 100% background noise were selected d counted by means of the stairs cage generat. Each reset represented the count of 25 of the cted units (Velasco et al., 1975). Signal from EMG recording was analyzed in the same nners as MUA.

In these animals, penicillin solution injected was usted to approach physiological parameters: 5 mM, 272 mOsm.l, pH 6.8 to 7.2 and Na ntent of 150 mEq l. The solution contained 125 IU of penicillin/μl and was injected in volumes of 0.1 μl (12.5 IU) at the same rate of 0.1 μl/5s repeated every 10-min until a convulsive effect was obtained or a maximum of 1.0 μl (125 IU) was injected. The place of injection was marked with silver nitrate as previously described.

The relationship between the injected site determined by their stereotactic coordinates (AP, lateral and height) and the seizure type obtained (focal motor, myoclonic tonic and tonic clonic) was statistically evaluated obtaining a mean value for each stereotactic coordinate in mm for each one of the seizure patterns and using Friedman test (F-test) to determine differences in mean values between different seizure patterns. Student t-test served to determine the significance of differences in the position of injection in the three coordinates that induced a given seizure pattern in regard to the coordinates of injections that gave other seizure patterns. Relationships of NaPC dose (in IU) with different clinical and EEG seizures were evaluated through Student t-test.

Determination of ED 50 for generalized tonic-clonic seizure (GTC) and dose response curve were performed with observations made using the different concentration of sodium penicillin in all 25 animals.

2.3. EEG, EMG and MUA relationships

When EEG, MUA and EMG paroxysmal discharges were not evidently time locked they will be only described in results. When they appeared time locked temporal relationship between the cortical EEG spike with the increments and decrements of MUA at the cerebral cortex (Cx MUA), brain stem convulsive area (MT-MUA) and EMG (EMG-MUA) were analyzed.

The increments and decrements of MUA were determined in relation to the apex of the EEG spike while the polygraphic recording was speeded at 50 mm/s. Increments or decrements in MUA occurring every 100 ms were quantified along 1 s, starting 500 ms before the apex of the EEG spike. Significance of changes was determined through a Student t-test.

At the end of the experiments animal were sacrificed by a lethal dose of penthobarbital and

their brains perfussed through intracarotid injection of 10% buffered formaldehide. The injected sites were examined in 10 μ thick section stained with Luxol-fast blue.

3. Results

3.1. Location and extension of the convulsive area

A total of 36 injections using penicillin solution 500 UI/μl were performed in 12 different targets along the brain stem of 14 cats. All injected sites were identified in histological sections. At the mesencephalon they included: Mesencephalic tegmentum (MT) (from $A = 1.0$ to 3.5, $L = 1.5$ to 3.5 and $H = +2.0$ to -4.0) covering mostly the area of the mesencephalic reticular formation (MRF) and surrounding tegmentum dorsal and lateral to the red nucleus ($n = 10$); the pes peduncule including substantia nigra (SN) and cerebral peduncule ($n = 3$) and tectal area in the medial geniculate body, superior and inferior colliculi and periaqueductal gray ($n = 4$). In the pons and medulla they included the pontine reticular formation (magnocellular nucleus) ($n = 7$), raphe nucleus ($n = 5$), superior cerebellar peduncule ($n = 2$), facial nucleus ($n = 1$), vestibular nucleus ($n = 1$) and bulbar reticular formation (BRF) ($n = 3$).

Nine out of ten injections in MT induced clinical and EEG seizures with amounts between 250–500 IU (mean 428 IU). Seizure started 7.3/2.6 min after injection as a focal contralateral myoclonus, evolving to generalized myoclonus in all, associated to generalized spikes or polyspikes EEG complexes and seven cases ended with repeated generalized tonic clonic (GTC) clinical and EEG seizures. None of the injections outside MT induced seizures even when the total dose injected reached 1000 IU of penicillin. Eight out of ten injections in PRF and BRF induced EEG synchronization with persistent spindle bursts associated with sleep in animals otherwise reactive and 3/5 injections in the raphe nucleus induced sleep, in one case associated to the whole clinical, EEG and EMG picture of REM sleep. Injections in the vestibular nuclei induced tonic contraction in extension of the four extremities and in the facial nerve tonic facial ipsilateral contraction and salivation, neither one associated to any EEG paroxysmal discharges. The rest of the injections did not elicited detectable effects (Fig. 1)

3.2. Clinical and EEG seizure patterns in relation to penicillin dose and injected site.

Injection of sodium penicillin solution 82.5 mM (272 mOsm, 125 IU μl) was performed in 12 targets within the previously defined convulsive area. Injections started at 12.5 IU (0.1 μl) and were progressively increased 0.1 μl every 10 min until GTC were obtained or a maximun of 1.0 μl (125 IU) were injected. All injections induced a contralateral facial myoclonus and EEG spikes more prominent in the ipsilateral Cx. As injections proceeded 10/12 times facial myoclonus extended to contralateral limbs, more prominent in the anterior limb and generalized myoclonus accompanied by generalized spikes or polyspikes; in 7/12 a generalized tonic clinical and EEG seizure developed; finally, in 6/12 injections a GTC clinical and EEG convulsion developed. The amount of penicillin necessary to induce the different clinical seizure patterns was significantly larger for contralateral myoclonus ($P > 0.02$), generalized myoclonus, tonic and GTC ($P < 0.001$) than for facial myoclonus. There were also significant differences between generalized myoclonus and tonic seizure ($P < 0.05$) and GTC ($P < 0.002$). DE 50 for GTC seizures was 126 IU. The amount of penicillin necessary to induce EEG isolated spikes was significantly lower than for tonic ($P < 0.01$) or GTC ($P < 0.001$) EEG paroxysms. (Table 1 and Figs. 2 and 3). None of the seterotactic coordinates (AP, lateral and height) correlated with any particular type of clinical or EEG pattern.

It was remarkable that clinical facial myoclonus preceded by seconds or minutes the onset of Cx EEG spikes.

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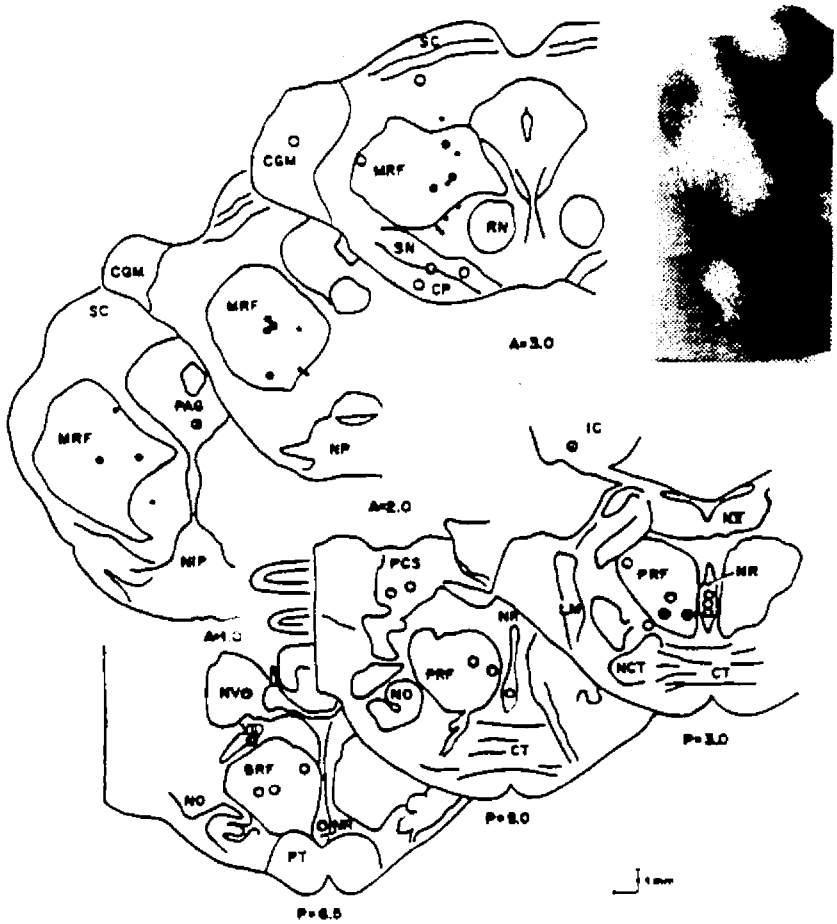

Fig. 1. Diagrams of coronal sections of the cats brain stem from $A = 3.0$ to $P = 6.5$ taken from the stereotactic atlas by Snider and Niemer (1961), showing the injected sites with high concentrated penicillin solution (big filled and clear dots) and low concentrated solution (small dots). All injection but one within the mesencephalic tegmentum elicited some pattern of clinical and EEG seizure (filled dots), whereas none outside this area induced clinical and EEG seizures (clear dots). Abbreviations: BRF, bulbar reticular formation; CGM, medial geniculate body; CP, cerebral peduncle; CT, trapezoid body; IC, inferior colliculus; LM, medial lemniscus; MRF, mesencephalic reticular formation; NCT, nucleus trapezoid body; NIP, nucleus interpeduncularis; NO, nucleus olivaris; NR, raphe nucleus; NV, vestibular nucleus; NV, trigeminal nucleus; PAG, periaqueductal gray; PCS, superior cerebellar peduncule; RN, red nucleus; SC, superior colliculus; SN, substantia nigra. In the left upper corner a microphotograph of a colorant mark using 0.5 μl is presented to give an idea of the diffusion along the needle tract of the injected solution.

and were accompanied by clinical and EMG focal myoclonus, before any paroxysmal activity appeared in the motor Cx. Increments in MUA in MT and EMG did not correlated in time with MT EEG spikes (Fig. 4).

When Cx EEG spikes appeared, paroxysmal discharges in MUA at Cx, and EMG became synchronic with the EEG spikes. In order to determine the precise time relationship between these events, EEG individual spikes were analyzed in relation to MUA increments in different structures. Taking the apex of cortical EEG spike as time '0', it was observed that Cx-MUA had a significant increase 200 ms before the apex, accompanied by a progressive increase in EMG-MUA. Increments in MT-MUA occurred at time '0' and accompanied by further increase in EMG-MUA and decrease in Cx-MUA (Fig. 5).

Table 1
Summarizes the observation ($n = 12$) when injecting 82.5 mM NaPC[a]

Seizure type	Number of events	Range of penicillin dose in international units (IU)	X	SE	$P<$
Facial myoclonus	12	12.5–35.0	19.4	2	-
Contralateral myoclonus	10	25.0–50.0	38.5	7.8	0.018
Generalized myoclonus	10	25.0–100.0	54.2	9	0.001
Generalized tonic	7	35.0–125.0	94.1	18.3	0.0001
Generalized tonic-clonic	6	62.5–125.0	117.5	14.8	0.0001
Egg pattern					
Isolated spikes	9	12.5–87.5	33	8.2	-
Polyspikes	10	25.0–87.5	57	8.8	NS
Tonic	8	25.0–125.0	91.7	17.5	0.001
Tonic-clonic	7	87.5–125	108.1	15.6	0.0001

[a] In all instances, facial myoclonus and isolated EEG spikes and/or polyspikes were induced. Since the maximal volume of penicillin was 1.0 μl (125 IU) only half of the injections were followed by GTC convulsions. The mean values of the amount of penicillin injected inducing different clinical and EEG patterns are presented and one may observe that they were significantly lower for facial myoclonus and isolated spikes that for other clinical and EEG patterns.

4. Discussion

4.1. Anatomical location of the convulsive area

One limitation of the present study is the lack of control on the diffusion of NaPC away form the injected site as well as the possibility of back reflux along the needle tract. However, spreading to neighbor structures as a cause of the convulsive effect is unlikely as doses of NaPC eight times larger those required to induce GTC seizure and up to 80 times the threshold dose for facial myoclonus, failed to induce any seizure event when injected 2–3 mm away form the MT. Spreading through the blood stream to distant sites as the cause of seizures is even more remote as systemic administration of NaPC requires about 30 000 IU/kg of animals weight to induce GTC seizures (Quesney et al., 1977), i.e. over 7000 times the amount used in this study. On the other hand, the cannula guide used to orient the needle of the Hamilton syringe within brain stem was intended to prevent spreading of NaPC as the needle traversed other cortical and subcortical areas and although the colorant used (silver nitrate) and NaPC solution may have different diffusion rateshan NaPC, visual inspection of the anatomical sections gave an idea of how little colorant reflux is obtained injecting from 0.5 to 0.1 μl, that in no instance exceeded 2 mm and most likely injections of 0.1 μl cover even smaller areas (Fig. 1). Finally, studies on the diffusion within the cerebral parenchyma using (C14) labeled NaPC solution shows a very slow diffusion rate of about 1.5 mm/h (Noebels and Pedley, 1977) while epileptic events in present experiments develop within 10 min after the injection of NaPC. Using the technique of current-source-density analysis, Eiselt et al. (1998) demonstrated that NaPC applied subpially induce a focus of spikes within an area of 3 mm, beyond which electrical potential seem to be involved in inhibition rather than excitation of epileptic activity.

The convulsive area in the MT of the cat herein described closely corresponds to the convulsive area described in the rabbit mesencephalon using threshold electrical stimulation (Bergmann et al., 1963). Apparently, higher intensity current (Kreindler et al., 1958) or larger volumes and doses of penicillin (Ralston and Langer, 1968) induce seizures from other parts of the reticular formation. NaPC up to 1000 IU did not produce seizures when injected into bulbar and pontine reticular formation and instead animals developed

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Tonic	8	25.0–125.0	91.7	17.5	0.001
Tonic-clonic	7	87.5–125	108.1	15.6	0.0001

[a] In all instances, facial myoclonus and isolated EEG spikes and/or polyspikes were induced. Since the maximal volume of penicillin was 1.0 μl (125 IU) only half of the injections were followed by GTC convulsions. The mean values of the amount of penicillin injected inducing different clinical and EEG patterns are presented and one may observe that they were significantly lower for facial myoclonus and isolated spikes that for other clinical and EEG patterns.

4. Discussion

4.1. Anatomical location of the convulsive area

One limitation of the present study is the lack of control on the diffusion of NaPC away form the injected site as well as the possibility of back reflux along the needle tract. However, spreading to neighbor structures as a cause of the convulsive effect is unlikely as doses of NaPC eight times larger those required to induce GTC seizure and up to 80 times the threshold dose for facial myoclonus, failed to induce any seizure event when injected 2–3 mm away form the MT. Spreading through the blood stream to distant sites as the cause of seizures is even more remote as systemic administration of NaPC requires about 30 000 IU/kg of animals weight to induce GTC seizures (Quesney et al., 1977), i.e. over 7000 times the amount used in this study. On the other hand, the cannula guide used to orient the needle of the Hamilton syringe within brain stem was intended to prevent spreading of NaPC as the needle traversed other cortical and subcortical areas and although the colorant used (silver nitrate) and NaPC solution may have different diffusion rateshan NaPC, visual inspection of the anatomical sections gave an idea of how little colorant reflux is obtained injecting from 0.5 to 0.1 μl, that in no instance exceeded 2 mm and most likely injections of 0.1 μl cover even smaller areas (Fig. 1). Finally, studies on the diffusion within the cerebral parenchyma using (C14) labeled NaPC solution shows a very slow diffusion rate of about 1.5 mm/h (Noebels and Pedley, 1977) while epileptic events in present experiments develop within 10 min after the injection of NaPC. Using the technique of current-source-density analysis, Eiselt et al. (1998) demonstrated that NaPC applied subpially induce a focus of spikes within an area of 3 mm, beyond which electrical potential seem to be involved in inhibition rather than excitation of epileptic activity.

The convulsive area in the MT of the cat herein described closely corresponds to the convulsive area described in the rabbit mesencephalon using threshold electrical stimulation (Bergmann et al., 1963). Apparently, higher intensity current (Kreindler et al., 1958) or larger volumes and doses of penicillin (Ralston and Langer, 1968) induce seizures from other parts of the reticular formation. NaPC up to 1000 IU did not produce seizures when injected into bulbar and pontine reticular formation and instead animals developed

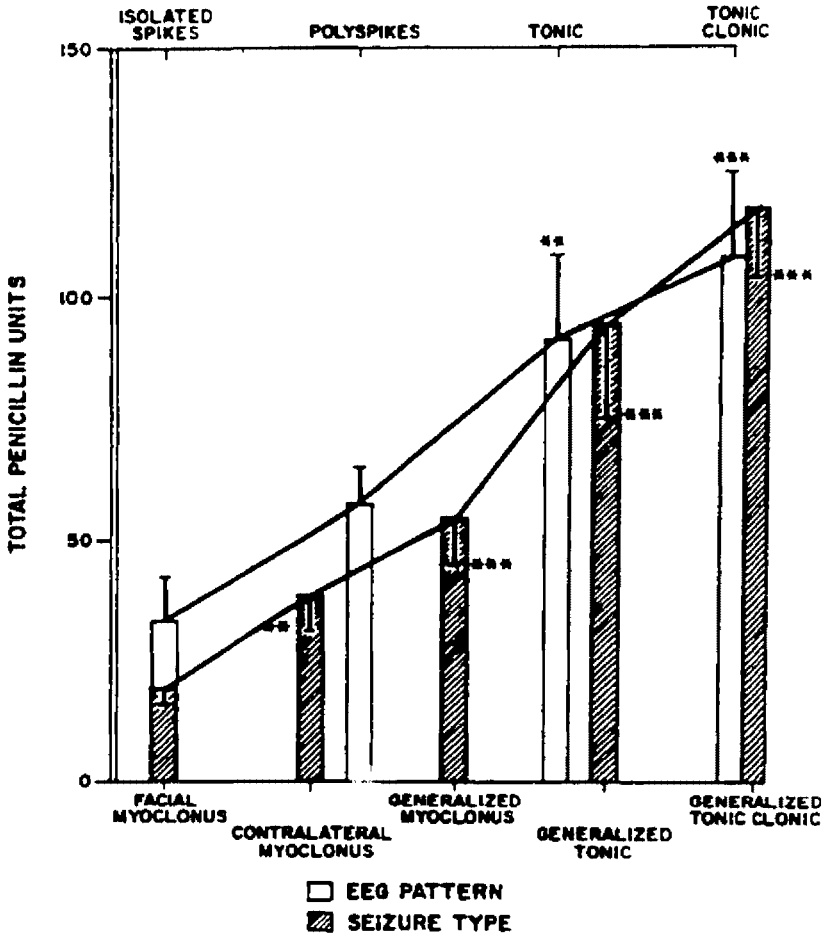

Fig. 2. Dose response curve in relation to penicillin dose. Filled bars, clinical seizure pattern; empty bars, EEG pattern, showing the mean and standard errors to elicit facial myoclonus and other clinical patterns and also to elicit spikes and tonic or GTC-EEG seizures. $^{**}P < 0.02$, $^{***}P < 0.001$

a clinical and EEG pattern of sleep, as had been previously described injecting pentylenetetrazol in the same areas (Velasco et al., 1983). When injected in the raphe nucleus NaPC induced either spindle bursts or paradoxical sleep reported by others (Martinez et al., 1997).

The convulsive area was restricted to the intermediate part of the MRF (Van der Kooy, 1987) and the tegmental area dorsal and lateral to the red nucleus. It was identified using both isoosmotic, low sodium content NaPC solution, that has been described to have specific antigabaergic effect (Wong and Prince, 1979; McCandless and Kinesmith, 1992) as well as hyperosmolar, high sodium content solution that may have other depolarizing effects (Ayala and Vasconsetto, 1970). Injections ventral to this area did not induce either clinical or EEG epileptic events, as has been described before using other antigabaergic substances in the substantia nigra (SN) (Arnt and Scheel-Kruger, 1980; Gunne et al., 1988). GABA activation in the SN seems to be related to the control of seizures initiated elsewhere or induced by systemic convulsants (Iadarola and Gale, 1982). On the contrary, Kainic acid induce strong motor seizures when injected in the SN, but not in the MT (Maggio et al., 1990) and recent experiments in our laboratory replicate most of the

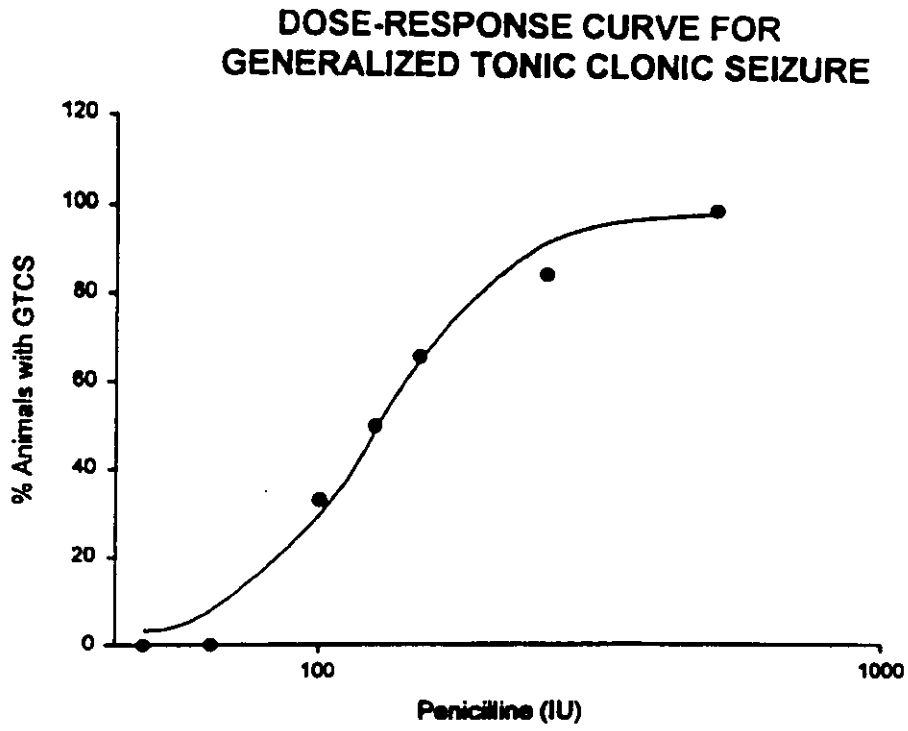

Fig. 3. Dose-response curve for GTC. This curve was obtained using the information derived from all animals that received NaPC in MT, in iso and hyperosmolar concentrations and through a non-lineal regression sigmoidal curve estimated by means of a computer program named Sigma Plot for Windows

observations made with NaPC in the MT using bicuculline, a specific GABA A receptor antagonist (Velasco et al in preparation), suggesting that within the mesencephalon there are different biochemical mechanisms to induce and control seizures. In the cerebral peduncle injection of 500 IU did not produce clinical or EEG paroxysms, which makes unlikely that the convulsive effects produced by mesencephalic NaPC are due to antidromic excitation of the motor cortex via pyramidal tract.

Injection in the colliculi did not produce detectable EEG or clinical changes, contrary to that described injecting bicuculline in rats (Tsutsui et al., 1992).

3.2. Seizure pattern

NaPC isoosmolar solution injected in progressive doses in the MT invariably induced focal motor seizures before other seizure patterns. Although in our experiments we remained attached to the use of 82.5 mM solution (isoosmolar) to avoid the effect of osmolar or Na content variables as cause of depolarization, in 2 instances using 8.2 mM solution and 2.5 IU NaPC dose, we could induce focal EEG spikes and focal myoclonus, which is the same dose used in cortical NaPC injection to induce focal spikes (Chatt and Ebersole, 1988). Since there is not anatomical or physiological evidence of a topographical organization in the MT, one many speculate that focal myoclonus resulted from orthodromic or antidromic excitation to the motor cortex. However, as illustrated in Fig. 4, distinct MT EEG spikes and muscular myoclonus were present before EEG or MUA paroxysms appeared in the motor Cx. Myoclonus has been produced by NaPC injected in the brain stem (Ralston and Langer, 1968) and other circumstances affecting the brain stem (Hallett et al., 1979; Chung and Van Woert, 1986) and have been proposed to result from

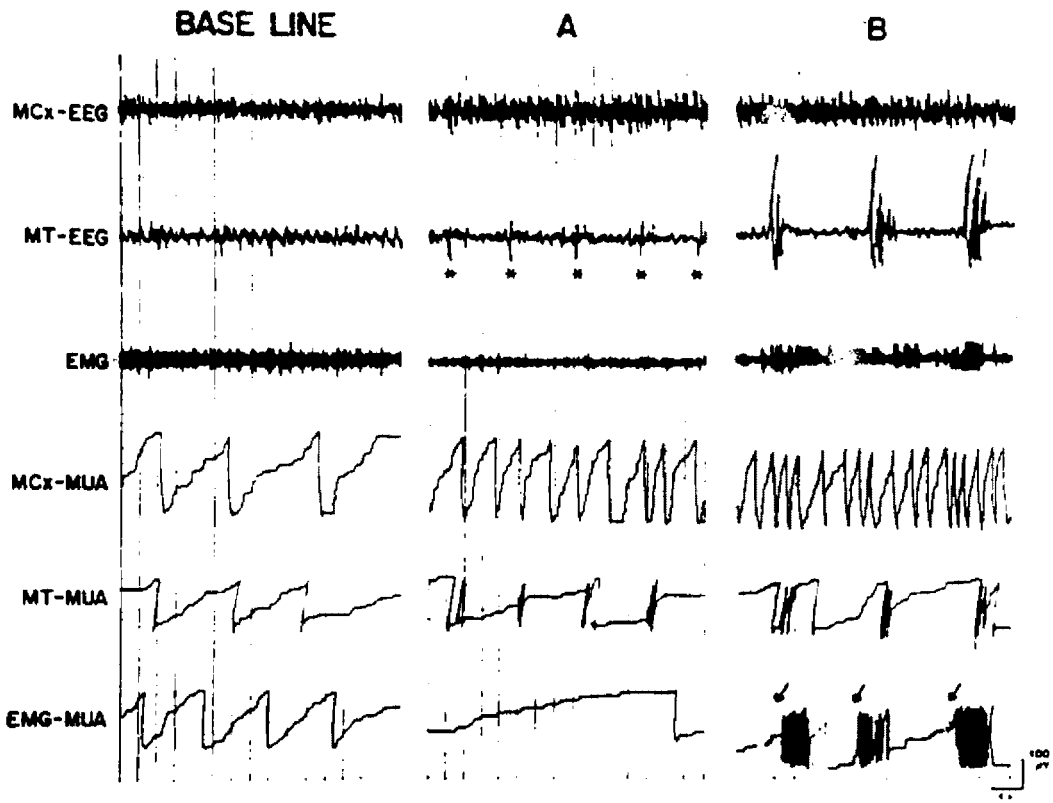

Fig. 4. Sequence of events induced by NaPC in MT. EEG Cx, motor cortex; EEG-MT, mesencephalic tegmentum; EMG, muscular recording from neck muscles; MUA, integration of multiple unit recording from the Cx, MT and EMG, each reset of the staircase represents the count of 25 of the selected units which were those with an amplitude above 100% the noise level. A: 3 min after injection of 12.5 IU penicillin EEG spikes accompanied by MUA paroxysms at the MT (asterisks) appear in the absence of Cx-EEG, and MUA and EMG-MUA changes. B: As NaPC dose increase (25 IU), MT EEG and MUA paroxysms became more prominent and EMG discharges appear (arrows); there is a steady increase in Cx-MUA but still in the absence of Cx-EEG paroxysms. Notice that MT- EEG and MUA and EMG discharges are not precisely related in time.

propagation of brain stem epileptic activity to the spinal cord, through midline descending reticulo-spinal fibers originated in the pontine and bulbar reticular formation and raphe nucleus (Newman, 1995). Since no clinical seizure were induced injecting NaPC in those nuclei, it is unlikely that MT paroxysmal activity used these pathways to propagate to the spinal cord. Besides, experimental evidence has been provided that focal myoclonus originated in the forebrain (for review see Gale and Browning, 1988) and therefore, it is possible that myoclonus result from propagation through other forebrain cortical or subcortical structures different from the motor Cx.

On the other hand, as NaPC dose was increased, seizure progression closely resembled 'kindling' induced by temporal lobe amygdala stimulation and although electrical stimulation of MRF has been unsuccessful in eliciting kindling (Goddard et al., 1969), the possibility that MT paroxysmal activity propagates using the same forebrain structures of amygdala kindling remains. Further experiments using the epilepsy model herein described and recording EEG and MUA of different cortical and subcortical structures in relation to clinical events are necessary to elucidate this matter.

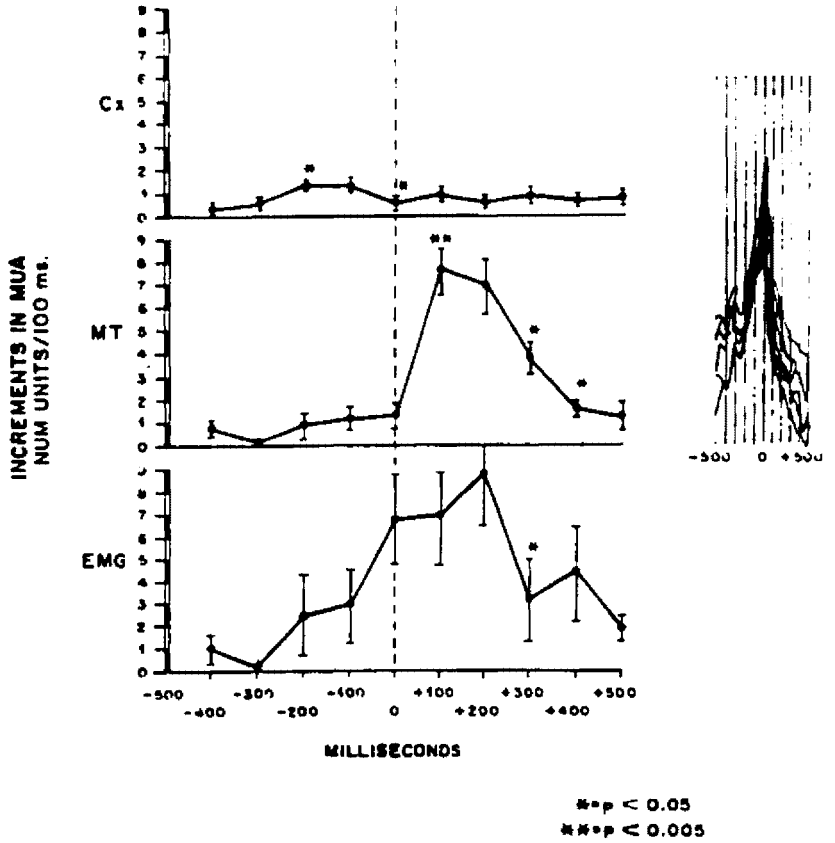

Fig. 5. Increments and decrements in cortical (Cx), mesencephalic tegmentum (MT) and EMG multiple unit activities (MUA) in relation to Cx-EEG spikes (right side). EEG spike apex was considered time '0' and changes in MUA evaluated for each structure in 100 ms epochs along 1 s, starting 500 ms before the apex. From 20 observations, the mean value of MUA for each epoch and each structure was obtained and compared with that of the immediately preceding epoch, and the significance of changes evaluated through a Student *t*-test. It can be seen that discrete but significant increase in Cx-MUA anticipated EEG spike apex, while more prominent increments in EMG-MUA and MT-MUA occurred at time '0' and 100 ms after, respectively. Increase in MT-MUA was coincident with further increase in EMG-MUA and decrease in Cx-MUA.

4.3. EEG, EMG, MUA and clinical seizures relationships

Focal EEG spikes and increased MUA resulting form local application of NaPC in the brain stem have been reported before (Ralston and Langer, 1968, Mameli et al., 1991). Since those experiments were performed in anesthetized or paralyzed animals by means of muscular relaxants or brain stem transections, temporal relationships between electrophysiological and clinical events were difficult to assess.

In our experiments particular interest was paid to these temporal relationships and it was clear that focal EEG spikes and increased MUA in the MT anticipated clinical and EMG seizure and Cx EEG spikes. As NaPC dose was increased, focal MT EEG spikes became accompanied by EMG and clinical myoclonus before Cx EEG spikes appeared. EEG spikes and increase MUA in MT increase in EMG-MUA and clinical seizures were not time locked, which again makes unlikely that seizure activity in MT propagates by direct spinoreticular fibers or through the motor Cx.

When Cx EEG spikes appeared, the clinical seizure patterns was generalized myoclonus and at that moment Cx EEG spikes and increase in MUA in Cx, MT and EMG and clinical seizure

became timelocked. Fine analysis of time correlation indicates that increase in Cx MUA anticipated increase in MUA at MT and EMG and that was a simultaneous increase in MUA at MT and EMG at the apex of EEG spike, which indicates that Cx is facilitatory of myoclonic seizure activity in the brain stem.

5. Uncited references

Chung and Von Woert, 1986; Hallet et al., 1977; Lüders et al., 1980; Ralston and Langer, 1965; Redecker et al., 1997; Velasco, 1968

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