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Behavioural and Electroencephalographic Effects of Intracerebral Microinjections of Sodium Pentobarbital *

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The effects of intracerebral microinjections of Nembutal into different brain areas, with the purpose of reproducing the behavioural and EEG sings of barbiturate narcosis, have been studied and analyzed. Cats were implanted for polygraphic study of the sleep-wakefulness cycle and bilateral brain canulae were also placed in strategic brain areas. The most striking results was the controlateral turning after unilateral injections of Nembutal in the nigra, suggesting a loss of balance among the actions of the nigro-striatal-cortical pathways on both sides. The sequence of events produced by intravestibular injections of Nembutal suggests that the drug, when injected intraperitoneally, acts also upon the vestibular system. This data stresses the importance of localized intracerebral microinjections of drugs to determine and differentiate their action mechanisms when injected in an intraperitoneal or intravenous way.

Sodium pentobarbital (Nembutal) is the commonest drug used in experimental work for inducing anesthesia. After intraperitoneal injections the stereotyped sequence of phenomena before the animal is completely anesthetized, is well known. The effects of Nembutal upon physiological parameters such as evoked potentials (5) and unit activity in different brain areas have been widely studied (13, 14). However, it has been demonstrated that the routes of administration may alter the distribution of substances in the brain (7). Moreover, in the case of Nembutal, the minimal brain levels which exert a significant behavioural action cover very small range of dose (9).

Studies have searched for more localized effects of Nembutal by cortical application

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of the drug (3) or injection in the N. Caudatus (6), and changes in feeding behaviour of several species were induced after intraventricular injections of this drug (15, 18). Therefore we thought it was interesting to study the behavioural effects of localized intracerebral microinjections of Nembutal in different brain areas. A particular aim in our study was to determine the brain areas in which we could provoke behavioural and EEG aspects of barbiturate narcosis.

Materials and Methods

Sixteen adults cats were used in this work. Under Nembutal anesthesia there were implanted bilateral cortical screw epidural electrodes and electrodes in the neck muscles. Two nichrome wires were implanted bilaterally in the rectus muscles of the eyes. Bipolar electrodes were implanted in the lateral geniculate bodies.

A electrooculogram was registered through screws inserted in the orbital bone. This preparation was performed to record polygraphic signs of sleep stages. Small canulae (internal diameter = 0.15 mm; external diameter = 0.33 mm) were stereotactically placed, according to the atlas of REINOSO-SUÁREZ (16), in the following areas: 1) Anterior Hypothalamus; 2) Posterior Hypothalamus; 3) Septum; 4) N. Caudatus; 5) Hippocampus; 6) Nucleus Ruber; 7) N. Pontis oralis; 8) N. Raphe; 9) Mesencephalum; 10) Substantia Nigra; 11) Brachium conjunctivum; 12) N. Vestibularis superior; 13) N. Deiters.

After recovering from anesthesia, control studies of sleepwakefulness stages were performed on an 8 channel Grass polygraph. Ulteriorly we injected 10 μl of a Nembutal solution in the different brain areas (50 mg/ml). The injection time was ten minutes (1 μl/min). Behavioural and EEG responses were analyzed for six hours following the injection. Each animal had four to six canulae. Therefore the effects produced in different brain areas in the same animal, could be differentiate. Control NaCl 0.9 % injections were always performed before the Nembutal injections. An injection of Nembutal in subcortical white matter served also as a control. After several injections in each canula (at four to six days intervals) and after studying the animals for a period of up to two months they were sacrified with an overdose of Nembutal. Histological control confirmed accurate placement of the canulae and electrodes.

Results

General considerations. All the animals showed normal sleepwakefulness cycles. Behaviour was also normal. The effects of intracerebral injections of NaCl 0.9 % were ineffective as were the control injections in the subcortical white matter.

The effects produced by Nembutal microinjections were transitory, disappeared after a certain time, and they were reproduced in subsequent sessions (in each animal, and for each canula). None of the injections produced the behavioural and EEG aspects of Barbiturate narcosis, nor created the normal sequence of sleep stages.

The behavioural results of intracerebral microinjections of Nembutal with the stereotactic coordinates of the histological controlled canula tips are as follows:

Anterior Hypothalamus. Bilateral injections (3 cats). (A = 15; L = 1.5; H = —2.5). (4 cats) (14; 1.5; —3). Immediate effects. Vomiting, defecation and micturition (does not clean up) and ataxia (posterior limbs particularly). No sleep. Effects last for 45 minutes.

Posterior Hypothalamus. Bilateral injections (2 cats) (8; 2.5; —2.5). There were no signs of sleep or barbiturate spindles in the EEG. One cat which was normally moving before the injection showed afterwards a clear cut arrest of the motility and resumed a sitting down position.

Septum. Bilateral injections (2 cats) (16; 1; 5). They presented unsteady gait and a clear cut diminution of mobility and the animals resumed a sitting down position.

N. Caudatus. Bilateral injections (2 cats) (16; 3.8; 4.5). Slight ataxia. They presented a clear cut arrest of normal mobility and they resumed a sitting down position.

Hippocampus. Bilateral injections (2 cats) (3; 9, +3). They seemed to present hallucinations or as if they were suddenly placed in an unknown unpredicted situation to which they reacted by continuous searching movements of the head in order to assume and define it. They acted as if they felt insecure. They presented an overall «attitude of fear» which was not directly related to the presence or approach of the investigator.

Nucleus Ruber. Bilateral injections (2 cats) (3; 2; —3.5). Forward propulsive running. Strike any abstacle in their way and keep pushing against it. Ataxia.

Nucleus Pontis Oralis. Bilateral injections (2 cats) (—4.5; 2; —5.5). No particular effects. No sleep.

N. Raphe. (2 cats) (—3; 0; —5). No particular effects. No sleep.

Mesencephalic tegmentum. Bilateral injections (3 cats) (3; 1.5; —3). The effects were of marked sedation of the animal but no clear cut signs of sleep. No EEG signs of barbiturate narcosis. Presented bilateral midriasis.

Bilateral injections (4 cats) (—2; 1.5; —4.5). Ataxia of body and posterior limbs.

Substantia Nigra. Unilateral injections (5 cats) (5; 4; —4). These effects were the most reliable of all these series. Twenty unilateral injections were made and it was always observed the same effects. They started two - three minutes after the injection and consisted in a controlateral circling movement with a slow and continuous motion (fig. 1). This controlateral turning lasted for one hour. If after the start of the circling, the controlateral side was injected the movement would stop, and if one hour after injecting one side, a controlateral injection was performed the direction of the circling movement could be inverted. Bilateral injections were ineffective.

Brachium conjunctivum. Bilateral injections (2 cats) (0; 3.5; —2.5). Ataxia. Tendency to fall to either side. Unilateral injections produced ataxia of the limbs in the injected side.

N. Vestibulariis Superior. (5 cats) (—6; 3.5; —2.5). 15 injections were performed in this site in different cats in different days. Together with the substantia nigra, this point was the most effective in producing constant effects. Moreover these

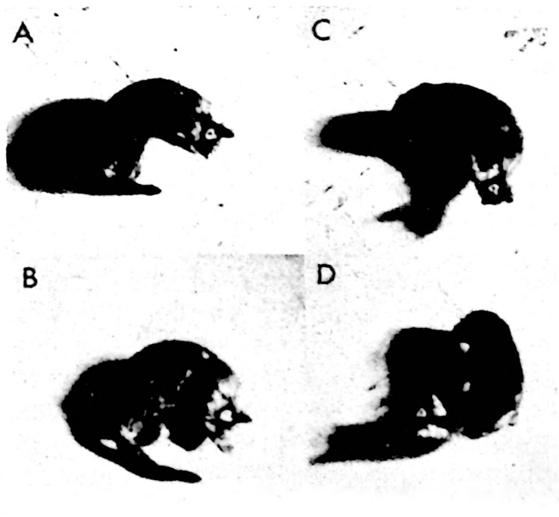

Fig. 1. *Sequential images of circling behavior to the right side elicited by Nembutal microinjection (10 μl of solution 50 mg/ml) into the left substantia nigra.*

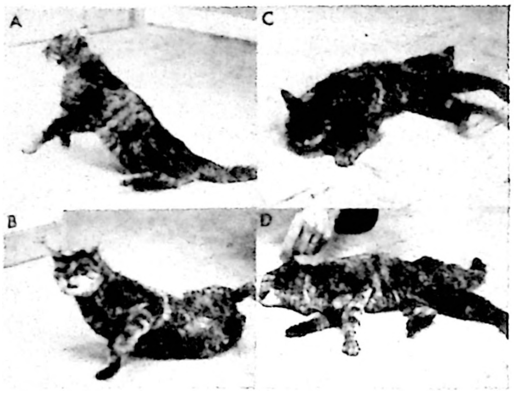

Fig. 2. *Sequential effects on behaviour following bilateral microinjections (rigth side, then left side) of Nembutal into the S.V.N.* The injection in this area mimicked closely the effect of intraperitoneal injection of Nembutal (except the total loss of consciousness). Another cat different from figure 1.

injections were the most impressive because they produced from the second to third minute following bilateral injection, a stereotyped sequence of events which was characteristic of the events observed in the Nembutal narcosis obtained by intraperitoneal injections. They are shown in figure 2 and consist namely of a) ataxia of posterior limbs and trunk, followed by ataxia and weakness of the forelimbs and finally inability to move the head, even with strong painful stimuli (figure 2-D). With the exception that the animal remained awake, this sequence is observed in cats in barbiturate anesthesia and with intraperitoneal injections of Nembutal it is followed by complete sedation and deep narcosis. These effects which were obtained only after biateral injections, lasted for ninety minutes. Unilateral injections produced stark ataxia with tendencies to fall towards the injected side.

Nucleus of Deiters. Bilateral injections were only performed (2 cats) (—7; 5; —4.5). It was obtained stark ataxia of posterior limbs which lasted for one hour.

Discussion

The results show that localized intracerebral microinjection of Nembutal can produce different effects when injected in different brain areas. The effects were specific as saline control injections were ineffective. The effects were related probably to inactivation of the specific injected brain areas as it has been proved that carefully controlled intracerebral microinjection produce only limited spread of the chemicals injected (2). They suggest a methodological tool for studying the intrinsic phenomena which appear in a sequential way in barbiturate narcosis. The changes in feeding behaviour observed after intraventricular Nembutal injections (15, 17) were not confirmed, nor the EEG and behavioural sedation signs after Nembutal injection in N. Caudatus (6).

Injections in the mesencephalic tegmentum were not repeated in a particular session, and therefore it was not obtained the comatose state produced by repeated injections of Nembutal (12). Autonomic effects obtained after hypothalamic injections were in agreement with previous results (11). The ataxia obtained here could be interpreted as inactivation of the medial forebrain bundle whose coordinates are in the hypothalamic areas injected.

The results obtained with injections of the substantia nigra (SN) are very spectacular. It has been shown that the SN projects mainly unilaterally to the striatum (8) and to the cortex (10). On the other hand it has been advanced that the nigro-striatal dopaminergic pathways modulate and inhibit the activity of cholinergic striatal neurons (17). Therefore inhibition of the nigro-striatal dopaminergic pathways by Nembutal injections in the nigra, create an imbalance between both sides and produce the controlateral turning. That such an imbalance exists is suggested by the fact that bilateral injections in the SN were ineffective. The ataxia and motor effects produced by injections in the Nucleus Ruber, Brachium conjunctivum, and N. Deiters, could be due to inactivation of known cerebellar projections to these areas (1, 4).

Perhaps the most interesting findings are produced with injections in the area of the superior vestibular nuclei (SVN) (1). Except for the final narcosis it was reproduced the sequence of events observed after intraperitoneal injection of Nembutal (ataxia and paralysis of posterior limbs, trunk, anterior limbs and head with final inability to react to noxious stimuli). This may suggest that the effects of intraperitoneal Nembutal injections are partially executed through the area which was injected (SVN) and stress the importance of localized brain microinjections to differentiate the site of action of the drug.

It is interesting that isolated bilateral injections did not produce the EEG and behavioural signs of sleep in any of the sites injected. This suggest that the different aspects of sleep stages need the combined action of several brain structures. This study is by no means exhaustive and can be completed with systematical analysis of the effects obtained in other brain areas.

Resumen

Se analizan los efectos de microinyecciones intracerebrales de Nembutal en áreas cerebrales diferentes, con el fin de reproducir los signos comportamentales y EEG de la narcosis barbitúrica. Los gatos fueron implantados para estudio poligráfico del ciclo vigilia-sueño y también se colocaron cánulas en áreas cerebrales particulares. Los resultados más notables consistieron en giro controlateral después de inyecciones unilaterales en la sustancia negra que sugieren una pérdida del equilibrio entre las acciones de las vías nigro-striato-corticales de ambos lados. La secuencia de acontecimientos obtenida por inyecciones intravestibulares sugiere que el Nembutal, inyectado intraperitonealmente, actúa sobre el sistema vestibular. Estos hechos subrayan la importancia de microinyecciones intracerebrales de drogas, para determinar y diferenciar sus mecanismos de acción cuando son inyectadas por vía intraperitoneal o intravenosa.

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