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Monoamines and Self-Stimulation of the Medial Prefrontal Cortex in the Rat

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The participation of noradrenaline (NE) and serotonine (5-HT) in self-stimulation (SS) of the medial prefrontal cortex (MPC) in the rat has been studied. Three groups of rats with bilateral electrodes implanted into the MPC were used in these experiments. In one of the groups, electrodes were also implanted into the locus coeruleus. In the first group, the rats received systemic injections of the following drugs: clonidine (α-agonist), phenoxybenzamine (α-antagonist), isoproterenol (β-agonist) and propranolol (β-antagonist). In the second group, p-chlorophenylalanine (a 5-HT synthesis inhibitor) was administered intragastrically and SS measured during the following 16 days. In these two groups of rats and previous to every SS session, spontaneous motor activity (SM) was measured as control for non specific effects of the drugs. In a third group of rats, lesions of the locus coeruleus were performed unilaterally and SS measured in both prefrontal cortex during the following 16 days post-lesion. SS contralateral to the lesioned side served as control for non-specific effects of the lesions. After all these treatments, SS of the MPC was not specifically affected. Our results suggest the non participation of NE and 5-HT terminals in the neural substrates underlying SS of the MPC.

Although dopamine (DA) has been suggested to participate in self-stimulation (SS) of the medial prefrontal cortex (MPC) in the rat (11), this catecholamine does not seem to be the exclusive neurochemical substrate of SS in this area of the brain (10). Since DA terminals coexist with noradrenaline (NE) and serotonine (5-HT) terminals in the MPC (5, 8, 9, 14, 20) and both of these monoamines seem to be involved in SS of other areas of the brain (3, 21, 22) we have investigated whether or not 5-HT and NE participate in the mediation of SS of the MPC together with DA.

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Materials and Methods

Twenty eight male Wistar rats, weighing 250-300 g at the time of the operation, were used. The rats were divided into

three groups and, throughout all the experiments, they were housed individually in a room with controlled light and temperature and with food and water *ad libitum*.

Under the effects of Equithesin anesthesia (2 mg/kg) bilateral monopolar electrodes were implanted into the MPC of all rats. The electrodes were made of stainless steel insulated except for 0.5 mm at the tip. The stereotaxic coordinates derived from the atlas of KÖNIG and KLIPPEL (7) were as follows: 2.5 mm anterior to bregma; 0.8 mm lateral to the midline and 4 mm beneath the dura. In one group of rats, moreover, they were also implanted with electrodes aimed at the locus coeruleus (LC) following the stereotaxic coordinates derived from the atlas of PELLEGRINO and CUSHMAN (15): 10.5 mm posterior to bregma; 1.1 mm lateral to the midline and 6.5 mm beneath the dura. At the end of the experiments, the electrode location was verified histologically with the aid of frozen 40 μm cresyl violet stained sections.

The experimental protocol consisted of measuring spontaneous motor activity (SM) during a period of 10 min. This was followed by another 10 min period in which SS was measured. The animals were trained for SS by lever pressing. Each lever press yielded a simple 0.3 s train of 100 Hz square waves of 0.5 ms pulse duration. The current intensity was selected individually after a rate-intensity curve was performed in each animal (12). SM was measured via contacts lining the floor of a 26 × 29 × 36 cm motility chamber. SM was used as control for non-specific effects of the drugs such as sedation or motor impairment of the animals.

Drugs. The following drugs were administered to the first group: Clonidine (α-agonist, 0.008, 0.016, 0.032, 0.075, 0.15 and 0.3 mg/kg). Phenoxybenzamine (α-antagonist, 5, 10, 15 and 20 mg/kg). Isoproterenol (β-agonist, 0.03, 0.62, 0.125, 0.25, 0.5, 1 and 2 mg/kg). Propranolol (β-antagonist, 0.125, 0.25, 0.5 and 1 mg/kg). After each dose, the rats were given 48 h to recover.

A single dose of 400 mg of P-chlorophenylalanine (PCPA) (an inhibitor of 5-HT synthesis) was administered intragastrically to the rats of the second group.

Electrolytic lesions. To the rats of the third group, unilateral electrolytic lesions of the LC were performed by sending an anodal current of 2 mA for 10 s through an electrode of similar characteristics as the ones described for SS. SS contralateral to the lesioned side served as control for non specific effects of the lesion.

Statistical analysis was performed using an analysis of variance (ANOVA).

Results

Figure 1 shows a schematic representation of the stereotaxic planes at which the electrode tips for SS in the MPC were located (Figure 1A) and the extent of the lesions in the LC (Figure 1B). The intraperitoneal injections of clonidine on SS and SM produced a dose-related decrease of both parameters except at the doses of 0.008 and 0.16 mg/kg on SS (Fig. 2). No statistical differences with control values were found on SS under the effects of phenoxybenzamine which produced a significant decrease on SM at the higher two doses of 15 and 20 mg/kg (Figure 3). Isoproterenol produced a significant decrease of SS and SM, except at the doses of 0.03 and 0.06 mg/kg (Fig. 4). On the other hand, propranolol and PCPA had no effect on SS and SM (Figures 5 and 6). The same can be said for the effects of unilateral lesions of LC (Figure 7). During the 16 days post-lesion, there were no differences on SS of the MPC either ipsilateral or contralateral to the lesioned side (Figure 7).

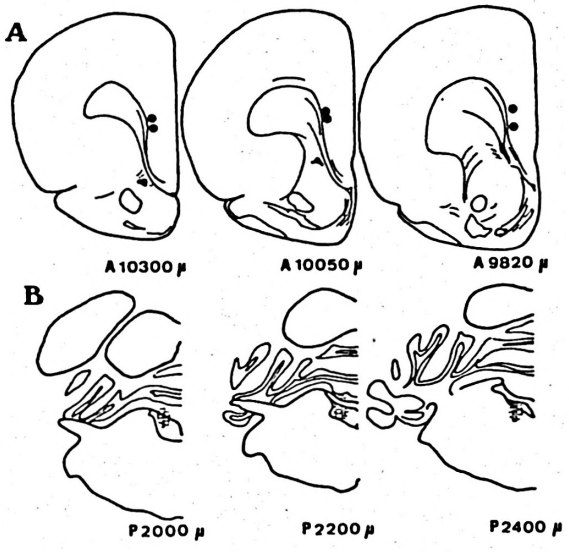

Fig. 1. *Schematic representation of the stereotaxic planes at which the electrode tips were located (dots) in the MPC (A) and the extent of lesions in the LC (B) (horizontal lines).*
The outlines of the MPC were taken from the atlas of König and Klippel (7) and those for LC from Pellegrino and Cushman (15). Numbers indicate the distance, in μm, from the interauricular line, anterior (A) or posterior (P).

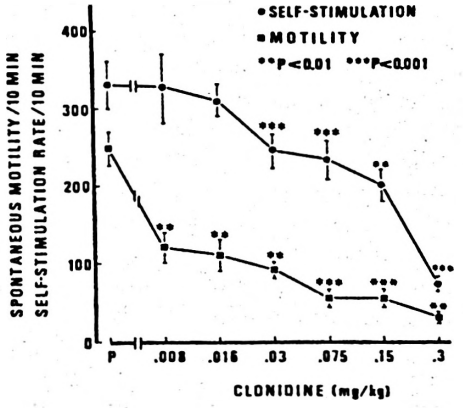

Fig. 2. *Effects of intraperitoneal injections of clonidine on SS of the MPC and SM in a group of 8 rats.*
The vertical lines indicate the standard error of the mean. P means placebo.

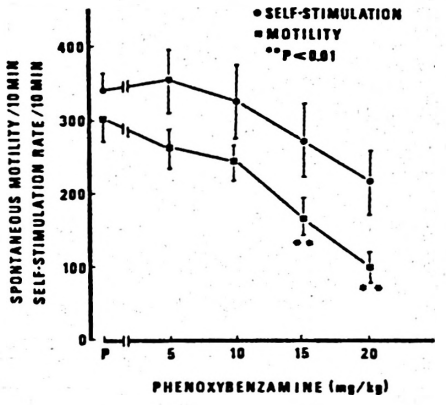

Fig. 3. *Effects of intraperitoneal injections of phenoxybenzamine on SS of the MPC and SM in a group of 8 rats.*
The vertical lines indicate the standard error of the mean. P means placebo.

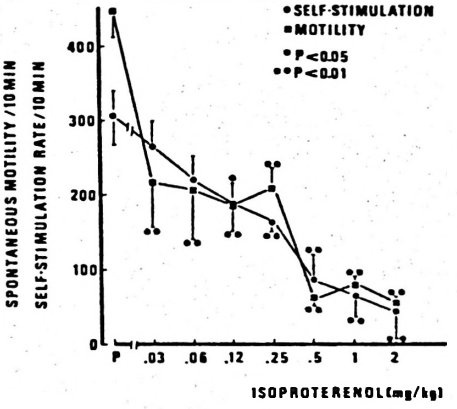

Fig. 4. *Effects of subcutaneous injections of isoproterenol on SS of the MPC and SM in a group of 6 rats.*
The vertical lines indicate the standard error of the mean. P means placebo.

Discussion

The present study was performed in order to investigate whether or not NE, 5-HT or both participate as part of the neurochemical substrate underlyng SS of the MPC in the rat. In reference to noradrenaline, two different experimental approaches were used. First, systemic in-

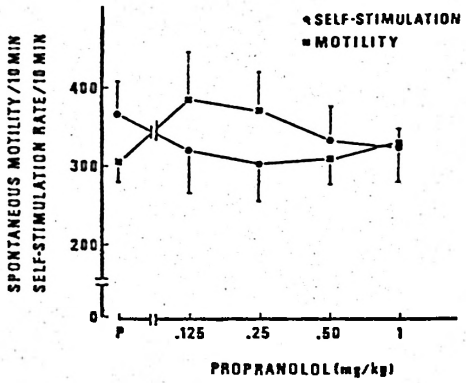

Fig. 5. *Effects of intraperitoneal injections of propranolol on SS of the MPC and SM in a group of 8 rats.*
P means placebo.

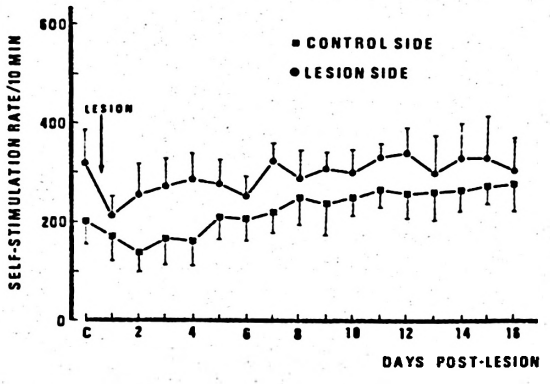

Fig. 7. *Effects of unilateral electrolytic lesions of the LC on SS of the MPC in a group of 5 rats.*
The vertical lines indicate the standard error of the mean. C means pre-lesion rate.

jections of α and β agonist and antagonist of NE receptors and second electrolytic lesions of the LC. This last experimental approach was undertaken after it had been reported that lesions of LC, produce a depletion of NE in the cortex in more than 98 % of control values (2, 20).

Since it has been reported that systemic injections of some drugs that act on noradrenergic systems have some effect on arousal (16, 17) it was important in these experiments to have a control in order to evaluate the specificity of effects on SS. We and others (13, 17) have previously shown that SM is a very sensitive measure to evaluate the motor capacity or arousal of an animal. Therefore, SM was used as an index to assess the possible non specific effects of drugs such as sedation or motor impairment.

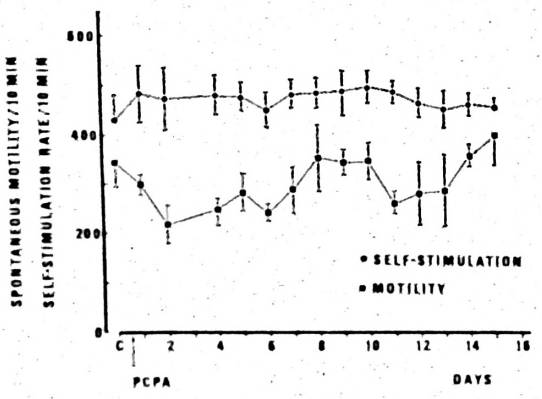

Fig. 6. *Effects of intragastrical administration of 400 mg of PCPA on SS of the MPC and SM in a group of 4 rats.*
The vertical lines indicate the standard error of the mean. C means control rate.

As it has been shown in the results, neither propranolol nor phenoxybenzamine had any significant effects on SS. These experiments were extended also using the α and β agonists clonidine and isoproterenol. Although systemic injections of these last two drugs produced a decrease on SS, such effects were probably non-specific, since a large decrease of SM relative to SS was also produced. It has been reported that clonidine produces a decrease of SS in other areas of the brain (6, 19). But in those papers, either the results appeared not to be specific on SS or the authors did not report for non-specific effects. Other drugs such as

phentolamine, an α-antagonist, have also been reported to produce a decrease on SS although it also induces sedation and therefore produces a decrease on SM (17). Our results on the effects of α and β NE-agonist and antagonists together with those suggested in the literature (6, 13, 17, 19) for other areas of the brain, suggest that NE receptors do not participate in SS of the MPC. This suggestion is also consistent with the finding of a lack of effect produced by lesions of the LC (see above), and it is in agreement with other similar reports showing a lack of effect on SS of other areas of the brain after lesions of LC (1, 2, 18, 21, 22). Therefore the present series of results, taken together, suggest that SS of the MPC is not dependent upon NE-receptors or NE terminals present in the MPC.

In regard to 5-HT, the dose of 400 mg/kg of PCPA used in these experiments, has previously been shown to deplete the whole brain of 5-HT, 2-3 days after administration. Therefore, it would be expected to find some evidence on whether 5-HT was part of the neurochemical substrate of SS in the MPC after administration of PCPA. Contrary to that expectation, during the 16 days after administration of this synthesis inhibitor of 5-HT, SS was unaffected. With a similar experimental design as the one reported here, VAN DER KOOY *et al.* (21, 22) found a differential effect of PCPA on SS of the lateral hypothalamus (LH) and hippocampus. Thus, VAN DER KOOY *et al.* (21, 22) found that PCPA produced no effect on SS of the LH but it produced a specific effect on SS of the hipocampus. It is therefore suggested that although 5-HT could participate in SS of some areas of the brain (21, 22) it does not participate in others such as LH (21) or MPC.

In conclusion, the present series of results suggest that neither NE or 5-HT seem to participate on the neurochemical substrates of SS in the medial prefrontal cortex of the rat.

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Resumen

Se estudia la posible participación de la noradrenalina y serotonina en la autoestimulación (SS) de la corteza prefrontal medial (MPC) de la rata. Se han empleado tres grupos de ratas, todas ellas con electrodos implantados en la MPC. En uno de los grupos se implantaron, además, electrodos en el locus coeruleus. A las ratas del primer grupo se les administró sistémicamente Clonidina (α-agonista), Fenoxibenzamina (α-antagonista), Isoproterenol (β-agonista) y Propranolol (β-antagonista). Al segundo grupo se le administró P-clorofenilalanina (inhibidor de la síntesis de serotonina, intragástricamente, midiéndose la SS durante los 16 días siguientes a la administración. En estos dos grupos de ratas, y antes de cada sesión de SS, se determinó también la actividad motora espontánea (SM) como control de los posibles efectos colaterales de los fármacos administrados. A las ratas del tercer grupo se les lesionó unilateralmente el núcleo locus coeruleus y la SS se midió en la MPC de ambos lados durante los 16 días postlesión. En este caso, la SS del lado contralateral no lesionado, fue utilizado como control de los efectos no específicos de la lesión. Ninguno de los tratamientos experimentales precedentes alteró específicamente la SS de la MPC. Los resultados sugieren que las terminales noradrenérgicas y serotoninérgicas no tienen un papel importante en los substratos neurales que median la SS de la MPC.

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