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BRIEF COMMUNICATION

Behavioral Effects of Intrahippocampal Injections of Clonidine, Yohimbine and Salbutamol in the Rat

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VERLEYE, M. AND F. BERNET. *Behavioral effects of intrahippocampal injections of clonidine, yohimbine and salbutamol in the rat.* PHARMACOL BIOCHEM BEHAV **26**(2) 421–424, 1987.—Intrahippocampal injections of adrenergic drugs, clonidine (an α_2-agonist), yohimbine (an α_2-antagonist), and salbutamol (a β_2-agonist) were performed in the awake rat. The injection of a high dose of clonidine caused a depression in locomotion in the open-field. Yohimbine partially antagonized the clonidine-induced hypomotility. The intrahippocampal injection of salbutamol had no effect on ambulatory behavior of the rat. These results suggest that the role played by the anterodorsal hippocampus in modifying behavior in novel situations is dependent on the specific sub-population of adrenoceptors that is stimulated.

Hippocampus α_2 and β_2 adrenoceptors Open-field Ambulation Clonidine Yohimbine Salbutamol Rat

THE hippocampal formation in the rat receives an important contingent of noradrenergic fibers which stem principally from a nucleus of the brainstem, the locus coeruleus. Vinogradova [17] has shown in this animal the presence of hippocampal neuronal systems for which unit activity is evoked by a novel stimulus. Therefore, the hippocampal formation appears to be implicated in the integration, control and expression of neurovegetative and somatomotor responses to novel anxiogenic situations. Van der Wolf *et al.* [15] have shown that the increase in exploratory activity of rats correlates with the occurrence of the theta rhythm; these behavioral changes might reflect an increase in activity of the hippocampal neurons. The exploratory and ambulatory activities in novel situations increase in the rat as a result of hippocampal lesions [6] or noradrenaline intrahippocampal injections [3]. Furthermore, U'Prichard *et al.* [14] have demonstrated the coexistence of α- and β-adrenoceptors on the soma of hippocampal pyramidal neurons.

The purpose of the present investigation is to study the effects of stimulation of hippocampal α_2- and β_2-adrenoceptors sub-populations on ambulatory and exploratory behavior of the rat in the open-field. We intrahippocampally injected adrenergic drugs; the α_2-agonist, clonidine, the α_2-antagonist, yohimbine, and the β_2-agonist, salbutamol. While the behavioral effects of these drugs, injected intraperitoneally and intracerebrally, are known, this study makes it possible to precisely determine the role of the hippocampal formation in the rat's locomotor behavior.

METHOD

Fifty male Sprague Dawley rats, weighing 300–350 g, were used. The animals were housed in propylene cages (43×43×20 cm), five per cage, on a 12:12 light-dark cycle with food and water available ad lib. Animals were anesthetized with equithesine (0.4 ml/100 g IP) and bilaterally implanted with cannulae in the anterodorsal hippocampus. Cannulae implants were double barreled and the set was composed of an outer cannulae guide (26 gauge) and a removal mandril to avoid occlusion. The implantations were carried out using the following coordinates: +3.2 mm anteroposterior, 2 mm mediolateral and 3 mm dorsoventral according to the data of the König and Klippel atlas [7] and preliminary histological controls. The skull was flat between bregma and lambda which was the reference point. The dorsoventral coordinates were measured from the top of the level skull.

After the operation, the rats were caged individually. A week later, the mandril was extracted from the implanted cannulae guide and the rats were bilaterally injected in the hippocampus for three minutes through a cannula (32 gauge) with drugs dissolved in 2 μl of saline solution. The doses were chosen based on published dose-response relations of these α- and β-adrenoceptors on the rat's exploratory and ambulatory activities [9,11]. The animals were divided into five groups of ten rats. The injected substances and the doses used were: group clo, 80 nmoles of clonidine; group yo, 90

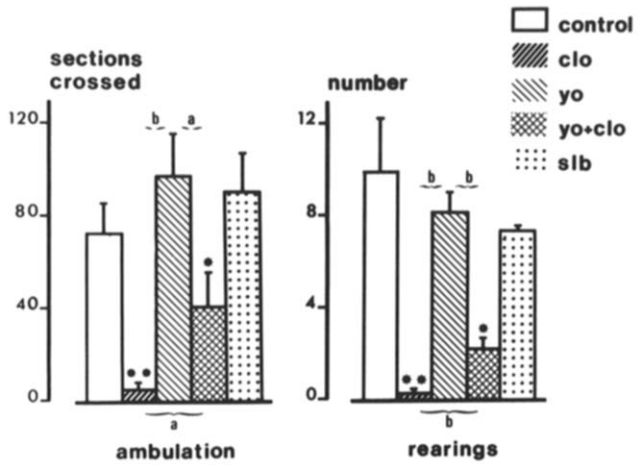

FIG. 1. Effects of microinjections of clonidine (clo), yohimbine (yo), yohimbine + clonidine (yo+clo), salbutamol (slb) in the anterodorsal region of the hippocampus on the behavior on the rat in the open-field. The mean values of the two parameters are indicated with S.E.M. in one direction. *$p<0.05$, **$p<0.001$, treated vs. control; $^{a}p<0.05$, $^{b}p<0.001$. treated vs. treated.

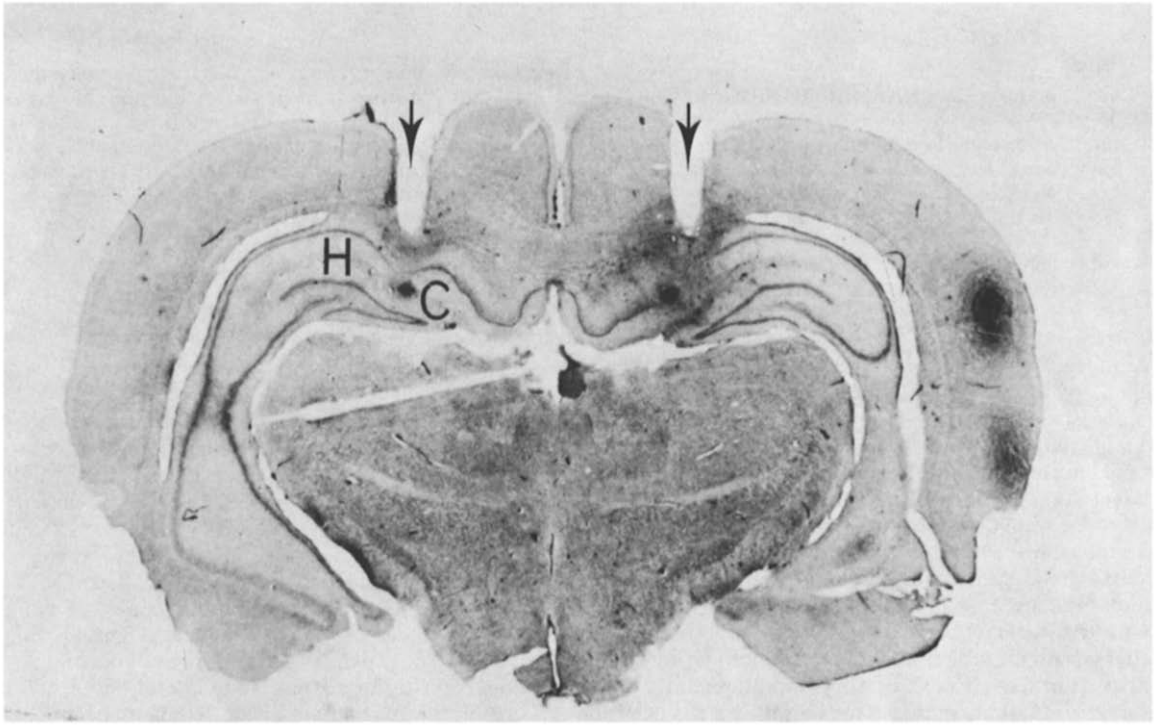

FIG. 2. Frontal section of the brain showing the localization of cannulae guide (↓) and the extremities of injection cannulae (C) in the hippocampus (H). The section shown is taken from about 3 mm in front of lambda.

nmoles of yohimbine; group yo+clo, 90 nmoles of yohimbine plus 80 nmoles of clonidine; group slb, 220 nmoles of salbutamol.

Control animals were given 2 μl saline solution (0.9% NaCl), instead of drug solutions.

Clonidine hydrochloride was donated by Boehringer-Ingelheim (Reims, France). Yohimbine hydrochloride was obtained from Sigma (St. Louis, MO) and Salbutamol sulfate from Glaxo (Paris, France).

Fifteen minutes after drug administration, the rats were examined in an arena or open-field (O.F.) as described elsewhere [1]. Each animal was placed in the center of the area and its behavioral activity was recorded continuously over a five minute period. Two parameters were measured, the locomotor activity in arbitrary units (each unit corresponds to the movement from one sector where the animal is placed to the next sector) and the number of rearings on hind feet.

At the end of the experiment, all subjects were intrahippocampally and bilaterally injected with 2 μl of 1% Bromophenol blue to ascertain the exact location of the cannulae and the extent of injections. After these last injections, all the rats were sacrificed with an overdose of nembutal and intracardially perfused with 10% formalin. After decapitation, brains were removed, deep-frozen and cut into 50 μm frontal sections for microscopic examination.

Statistical analyses were performed by Mann-Whitney U test (two-tailed).

RESULTS

The results are shown in Fig. 1. After the intrahippocampal injection of clonidine, ambulation and rearing are significantly reduced ($p<0.001$). The yohimbine-treated rats appear to be more active than the controls because they cover a greater number of O.F. sectors but the difference is not statistically significant. Rearing scores appear identical in the two groups of rats.

The simultaneous injection of yohimbine and clonidine does not reduce ambulation or rearings to the same extent as clonidine alone. The differences between yohimbine + clonidine and clonidine treatments are significant ($p<0.01$).

Compared with controls, the yohimbine-plus-clonidine treated rats have a significantly lower score for locomotor activity ($p<0.05$) and rearings ($p<0.05$).

There is no difference between control and salbutamol treated rats, or between yohimbine and salbutamol treated rats.

Histological Control

A microscopic examination of the implant area shows that the tips of the cannulae had reached the hippocampus in all animals (Fig. 2). Injection of the dye indicates that the product spreads primarily through the Gyrus dentatus and to about 1 mm on either side of the implantation area.

DISCUSSION

Isaacson [4] reported a greater amount of ambulation activity in the open-field in rats with bilateral dorsal hippocampal lesions. Our results show that the injection of an α_2-agonist, clonidine, in the dorsal hippocampus causes a depression in ambulatory and exploratory activities in the intact rat placed in the open-field. The rats were examined in the open-field fifteen minutes after drug administration to eliminate the effects of the three minutes of manual immobilization but the clonidine treated rats showed typical sedation immediately after the injection. This sedation lasts approximately thirty minutes. We suspect that through the activation of α_2-adrenoceptors, this region of the hippocampus formations in the mediation of hypomotility in the rat when placed in a novel environment. Recently, Flicker and Geyer [3] provoked ambulatory hyperactivity in the rat in the open-field by injecting noradrenaline (NA) in the dorsal hippocampus. The NA binds like clonidine to the α-adrenoceptors. NA stimulates the α_1-adrenoceptors whereas clonidine binds with high affinity to α_2-adrenoceptors [5]. Various authors have shown that a low dose of clonidine had selective agonist activity at prejunctional α_2-adrenoceptors while a high dose of this substance stimulated the postjunctional α_2-adrenoceptors [12]. The dose of clonidine injected into each dorsal hippocampus (80 nmoles/site) is high compared with doses used in the literature [2]. Therefore, the behavioral perturbations in the animals treated with clonidine can be associated with the activation of the α_2-adrenoceptors located on the soma of the pyramidal neurons. Previous results [16] support this hypothesis. Indeed, we have observed that an intrahippocampal injection of 6-OHDA (8 μg/2 μl of solvent/site), a neurotoxic substance selectively destroys the noradrenergic fibers and hence the presynaptically located α_2-andrenoceptors, did not reduce the ambulatory hypomotility induced by the injection of a high dose of clonidine. The topical injection of yohimbine partially antagonizes the behavioral effects induced by clonidine. It is likely that this action takes place in two ways. On the one hand, yohimbine which is a compound that blocks prejunctional α_2-adrenoceptors, facilitates the synaptic release of NA by a positive feed-back mechanism [13]; on the other hand, our results lead us to suspect that the high dose of yohimbine (90 nmoles/site) can also block the postjunctional α_2-adrenoceptors located on the pyramidal neurons and directly attenuate the effects of clonidine.

The absence of behavioral effects induced by intrahippocampal injection of salbutamol, a β_2-adrenergic agonist, leads us to think that the β_2-adrenoceptor subtype is not implicated in the mediation of open-field behavior in the rat. The studies of U'Prichard *et al.* [14] using the radiolabelling of receptors with specific ligands, revealed the coexistence of α- and β-adrenoceptors in the hippocampus. However, our results agree with those of Minneman *et al.* [10], who showed an unequal distribution of the β_1- and β_2-adrenoceptors subtypes in numerous brain regions with a predominance of β_1-adrenoceptors except in the cerebellum where the density of the β_2-subtype is higher. Nevertheless, the implication of the central β-adrenoceptors in the etiology of behavioral problems and the antidepressant activity of salbutamol has been suggested recently by Lerer *et al.* [8] from clinical trials.

In conclusion, the response to novelty in the rat is expressed differently depending on whether we stimulate or block a certain type of adrenoceptors in the hippocampus. The stimulation of α_1-adrenoceptors located on the pyramidal neurons by NA [3] is associated with an increase in locomotor activity, whereas the stimulation of α_2-adrenoceptors promotes a decrease in ambulation. On the other hand, the β_2-adrenoceptors do not seem to be involved.

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REFERENCES

1. Bernet, F. and J. Denimal. Ráctivité émotionnelle et activité adrénosympathique chez le rat. *Physiol Behav* **20:** 135–141, 1978.
2. Davis, M. and D. Astrachan. Spinal modulation of acoustic startle: opposite effects of clonidine and d-amphetamine. *Psychopharmacology (Berlin)* **75:** 219–225, 1981.
3. Flicker, C. and M. A. Geyer. The hippocampus as a possible site of action of increased locomotion during intracerebral infusions of norepinephrine. *Behav Neural Biol* **34:** 421–426, 1982.
4. Isaacson, R. L. *The Limbic System.* New York: Plenum, 1974.
5. Johansson, P. α-Adrenoceptors: recent development and some comparative aspects. *Comp Biochem Physiol* **78:** 253–261, 1984.

6. Kimble, D. P. The effects of bilateral hippocampal lesions in rats. *J Comp Physiol Psychol* **56:** 273–283, 1963.
7. König, J. F. and R. A. Klippel. *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brainstem,* vol 1, Baltimore: Williams and Wilkins, 1963.
8. Lerer, B., R. P. Ebstein and R. H. Belmaker. Salbutamol treatment of depression induces subsensitivity of beta-adrenergic adenylate cyclase. Göteborg: Abstract for the 12th C.I.N.P.-Congress. *Prog Neuropsychopharmacol* (Suppl) p. 224, 1980.
9. Liebman, J. M., N. R. Hall, J. Prowse, S. Gerhardt, L. Noreika and H. W. Fenton. Comparative effects of β_2-adrenoceptor agonists on intracranial self stimulation Sidman avoidance, and motor activity in rats. *Psychopharmacology (Berlin)* **84:** 336–341, 1984.
10. Minneman, K. P., L. R. Hegstrand and P. B. Molinoff. Simultaneous determination of Beta-1- and Beta-2-adrenergic receptors in tissues containing both receptor subtypes. *Mol Pharmacol* **16:** 34–36, 1979.
11. Spyraki, C. and H. C. Fibiger. Clonidine-induced sedation in rats: Evidence for mediation by postsynaptic α_2-adrenoceptors. *J Neural Transm* **54:** 153–163, 1982.
12. Starke, K. and K. P. Altmann. Inhibition of adrenergic neurotransmission by clonidine: an action on prejunctional alpha-receptors. *Neuropharmacology* **12:** 339–347, 1973.
13. Starke, K., E. Borowski and T. Endo. Preferential blockade of presynaptic α-adrenoceptors by Yohimbine. *Eur J Pharmacol* **34:** 385–388, 1975.
14. U'Prichard, D., T. E. Reisine, S. T. Mason, H. C. Fibiger and H. Yamamura. Modulation of rat brain α- and β-adrenergic receptor population by lesion of the dorsal noradrenergic bundle. *Brain Res* **187:** 143–154, 1980.
15. Vanderwolf, C. H., R. Kramis, L. A. Gillepsie and B. H. Bland. Hippocampal rhythmic slow activity and neocortical low-voltage fast activity: Relationship to behavior. In: *The Hippocampus,* vol 2, edited by R. L. Isaacson and K. H. Primbram. New York: Plenum Press, 1975, p. 101.
16. Verleye, M. Rôle des systèmes noradrénergiques centraux dans l'expression des réactions émtionnelles chez le rat. Thèse Docteur 3e Cycle, Univ. Lille I, 1 vol., 1982.
17. Vinogradova, O. S. Investigation of habituation in single neurons of different brain structures with special reference to the hippocampus. In: *Orienting Reflex, Alertness and Attention.* Moscow: XVIII Int Congr Psychol, 1966.