Pharmacology Biochemistry & Behavior, Vol 20, pp 859–862, 1984 © Ankho International Inc Printed in the U S A 0091-3057/84 $3 00 + 00

Serotonergic Mediation of Habenular Self-Stimulation in the Rat[1]

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Received 22 August 1983

NAKAJIMA, S *Serotonergic mediation of habenular self-stimulation in the rat* PHARMACOL BIOCHEM BEHAV 20(6) 859–862, 1984 —The possible involvement of serotonergic neurons in self-stimulation of the habenular complex was examined in 16 rats Animals were implanted with bipolar electrodes into the habenula (Hb), lateral hypothalamus (LH), or median raphe (MR), and trained to touch a dry spout to receive electrical stimulation of the brain Metergoline (5 mg/kg, IP), a serotonergic receptor blocking agent, produced a complete suppression of self-stimulation with Hb and MR electrodes, but significantly less suppression with LH electrodes, suggesting that the rewarding effect of habenular stimulation is mediated by serotonergic neurons In contrast to the differential effects of metergoline, chlorpromazine (2 mg/kg, IP), a catecholamine receptor blocking agent, suppressed both Hb and LH self-stimulation in a similar manner

Chlorpromazine Habenula Lateral hypothalamus Median raphe Metergoline Self-stimulation Serotonin 5-Hydroxytryptamine

SUTHERLAND and Nakajima [13] found that stimulation of the habenular complex (the medial and lateral habenular nuclei and the fasciculus retroflexus) produces a rewarding effect. The effect could be mediated by orthodromic excitation of an efferent pathway or antidromic excitation of an afferent pathway which makes a collateral connection elsewhere. One of the structures that maintain heavy connections with the habenular complex is the median raphe nucleus. A large proportion of ascending and descending fibers in the fasciculus retroflexus passes through the interpenduncular nucleus and connects the habenula with the raphe [12]. Sutherland and Nakajima [13] placed electrolytic lesions centered around the median raphe and observed a suppression of habenular self-stimulation lasting more than four weeks. These data suggest that the rewarding effect of habenular stimulation may be mediated by neurons in the median raphe. It should be noted that the raphe nuclei are the major source of serotonergic neurons projecting to various structures in the forebrain. The present experiment was conducted to examine whether the rewarding effect of habenular stimulation is mediated by serotonergic neurons.

A widely used technique for testing serotonergic involvement in the brain is to inject p-chlorophenylalanine (PCPA), which inhibits tryptophan hydroxylase thereby suppressing biosynthesis of 5-hydroxytryptamine (5HT). There are, however, some problems associated with the interpretation of results obtained with PCPA. First, there is often a discrepancy in the time course between behavioral and biochemical effects. For example, Stark and associates [11] found that the rate of hypothalamic self-stimulation was most suppressed 6 hours after PCPA injection, and that the animals resumed responding 24 hours after injection when the concentration of 5HT in the brain had reached the lowest level Therefore, they proposed that the behavioral effect was produced, not by the depletion of transmitter substance, but by the presence of PCPA metabolites such as 3-chlorotyrosine and 3-chlorotyramine [10].

With median raphe self-stimulation, however, Miliaressis and co-workers [8] found a maximal suppression of bar-pressing 24–48 hours after intraperitoneal injection of PCPA. Van der Kooy and associates [15] found that the bar-pressing rate changes in parallel with the time course of the 5HT-level change in the brain, with maximal effects observed 4 days after intragastric administration of PCPA. These results seem to indicate that the rewarding effect of median raphe stimulation is suppressed by cerebral 5HT depletion.

One reservation has to be made about this interpretation: rats do not demonstrate a complete suppression of responding even under a maximal depletion of 5HT. Animals show normal or above normal rates of responding at the beginning of a 2-hour session, and then gradually reduce their response rate as the test session continues [8,15]. A similar slow decline of responding under PCPA has been reported with self-stimulation of the hippocampus [14]. In contrast, animals responding for stimulation of the caudate nucleus [9] or the dorsal raphe nucleus [15] show a suppression under PCPA from the beginning of a test session. There has been no explanation offered to account for these phenomena.

To avoid the problems associated with PCPA, metergoline was used in the present experiment. Metergoline is a centrally acting 5HT-receptor blocking agent [3,4]. It has a high affinity to 5HT-binding sites and LSD-binding sites in the homogenate of rat cerebral cortex [5], but it has no effect on dopaminergic or adrenergic system [4]. Metergoline has been tested in rats bar-pressing for stimulation of the dorsal and median raphe nuclei: it reduced response rate to about

[1]This research was supported by the Natural Sciences and Engineering Research Council of Canada, Grant A0233

50% of pre-injection level, and the effect was maximal in a period 15–30 min after intraperitoneal injection [2] The data are consistent with the results with PCPA [15] except that metergoline is much more fast-acting and short-lasting than PCPA.

METHOD

Male hooded rats of the Holtzman strain, procured from Charles River Canada, were implanted with one or two bipolar electrodes. The body weight at the time of surgery ranged from 292 to 555 g. The electrode (Plastic Products Co) consisted of twisted stainless-steel wire insulated except for the tip cross-sections. Under pentobarbital anesthesia (50 mg/kg, IP), electrodes were implanted into either the habenular complex (Hb, n=7), the lateral hypothalamic area (LH, n=3), both Hb and LH sites (n=3), or the median raphe nucleus (MR, n=3). The stereotaxic coordinates for the electrodes were, with the incisor bar 5.0 mm above the interaural line, 2 0 mm posterior to bregma, 0.7 mm lateral to the midsagittal suture, and 5 0 mm below the dural surface for the Hb electrodes, 0.5 mm posterior, 1 7 mm lateral, and 8.3 mm deep for the LH electrodes, and 6.1 mm posterior on the midline, and 7.2 mm deep for MR electrodes.

After a recovery period of 7 days, the animals were trained to self-stimulate by touching a dry spout according to the method described by White [16] Each rat was placed in a box 20×35 cm and 33 cm deep with a drinking spout protruding about 3 cm from one of the narrow walls 4 cm above the grid floor. The spout was stainless steel tubing about 8 mm in diameter and similar to the water spout in the animal's home cage, but it did not deliver any water Contact with the spout closed an electrical circuit through the grid floor and activated a stimulator (Grass, Model SD-9). Most animals touched the spout with the snout or a forepaw, but some of them bit it.

Stimulation was a train of 0 3 msec rectangular pulses at 100/sec. The duration of the train was 0 5 sec for LH stimulation, but it was reduced to 0 2–0.1 for Hb and MR stimulation in order to minimize motoric effects. The response rate was recorded every 10 min period, and the intensity of stimulation was individually adjusted to produce minimal fluctuation in the rate. A sample of 60-min response record was examined from time to time. If none of the rates at a certain intensity was above 140% or below 60% of the mean, the animal was trained at that intensity to continue responding for a session of 150 min Actual intensities ranged from 80 to 800 μA peak to peak for Hb electrodes, 140–600 μA for LH, and 240–800 μA for MR

Metergoline was freshly prepared by dissolving it in a 2% ascorbic acid solution and injected intraperitoneally at a dosage of 5 mg/kg after 30 min of baseline responding in the test box Chlorpromazine hydrochloride (2 mg/kg, IP) was given to animals with Hb electrodes and those with LH electrodes to block catecholamine receptors Physiological saline was used as a control. Response rate was recorded for two hours after injection There were intervals of 2 to 7 days between injections At the end of the drug tests, the animals were anesthetized with sodium pentobarbital (50 mg/kg, IP) and their brains removed for histological confirmation of the electrode sites

RESULTS

Self-stimulation with LH electrodes became stable for individual rats at various rates ranging from 29 to 77 per min There was a larger variation in response rates with Hb electrodes, ranging from 7 to 99 per min. The rates were from 19 to 66 per min with MR electrodes The results of drug tests are shown in Fig. 1. During the 150-min no-injection trial, some animals showed minor fluctuations in their response rates, but there was no consistent tendency. The number of responses for each of the three electrode sites was subjected to an analysis of variance There was no statistically significant change in response rates over 5 consecutive 30-min periods with any electrode. $F(4,36)<1\ 0$ for Hb, $F(4,20)=2.79$ for LH, and $F(4,8)=1\ 58$ for MR The effect of saline injection was negligible The animals were minimally disturbed by intraperitoneal injection and returned to spout-responding immediately

The effect of metergoline was very different depending on the electrode sites. In all Hb animals, without exception, responding was completely interrupted for a period of 10–60 min The interruption started within 10 min after injection in 5 rats and after 10–30 min in the remaining 5 rats. The animals moved away from the spout and engaged in activities unrelated to self-stimulation, such as grooming, exploration, and in some cases crouching. There was no indication that metergoline induced sleep in any of the rats: the eyes remained open and the head stayed upright. At the end of this interruption, the animals spontaneously returned to the spout, gave a burst of responses for a few minutes, and then started another period of non-responding All animals resumed steady responding in the second hour after injection though none of them recovered to the pre-injection level

Metergoline slowed down but did not completely stop LH self-stimulation. The animals looked around and groomed themselves but did not go too far from the spout, and the majority of them managed to perform at least 200–300 responses in a 10 min period at the peak of the drug effect The differential effect of metergoline was clear in those animals which had both Hb and LH electrodes: the same animals making the same response under the same drug condition showed an entirely different degree of response suppression depending on the site of stimulation. The differential effect was not related to the pre-injection rate of responding. Hb rats which had higher pre-injection rates than some of LH rats still showed complete suppression just like those which had lower pre-injection rates The effect of metergoline on MR self-stimulation was similar to the effects on Hb self-stimulation, though the process of recovery was faster

Chlorpromazine suppressed responding for both Hb and LH stimulation In the majority of cases (7 out of 10 Hb sites and 4 out of 6 LH sites), responding was suppressed below 25% by 10–30 min after injection In 2 other rats (1 Hb and 1 LH) only partial suppression was obtained, and in the remaining 3 cases (2 Hb and 1 LH) no suppression was observed.

A relative response rate for a 30 min period, starting 10 min after injection (and the corresponding period in the no-injection trial), for each electrode site was calculated as a percentage of the pre-injection rate The results of analyses of variance were as follows: $F(3,36)=30\ 65$ for Hb, $F(3,20)=7.65$ for LH, and $F(2,6)=33\ 73$ for MR, all significant at $p<0.01$ The difference between the saline and metergoline effects was significant in Hb and MR but not in LH: $t(9)=12\ 62$ for Hb and $t(2)=11\ 19$ for MR, both $p<0.01$, and $t(5)=2\ 17$ for LH The chlorpromazine effect was significant in both Hb and LH: $t(9)=5\ 40$, $p<0\ 01$ for Hb and $t(5)=2\ 79$, $p<0\ 05$ for LH. The metergoline effects in Hb and LH sites were significantly different from each other: $t(14)=5\ 32$, $p<0\ 01$

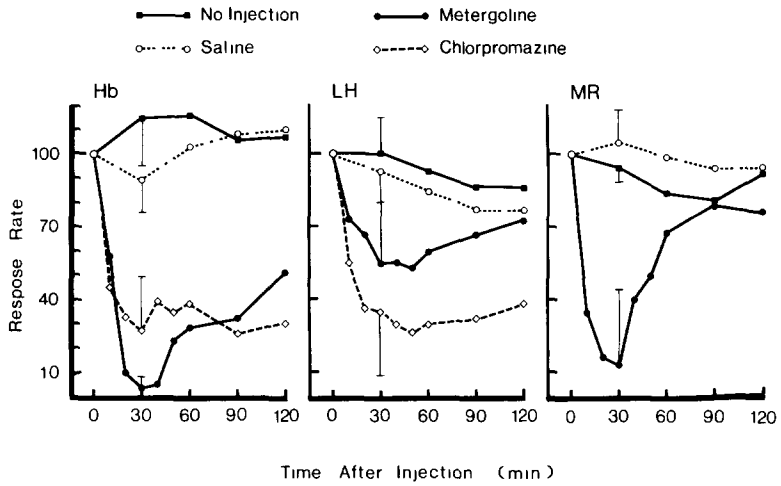

FIG 1 Effects of drug injections on responding for habenular (Hb), lateral hypothalamic (LH), or median raphe (MR) stimulation Response rate is expressed as a percentage of pre-injection rate. Vertical lines at the 30 min position indicate the confidence limits at $p=0$ 95

The tips of the Hb electrodes were distributed within the habenular complex. Six electrodes were in the lateral nucleus, one of them touching the medial nucleus, and 4 on the fasciculus retroflexus. The LH electrodes were all in the lateral hypothalamic area at the level of the ventromedial nucleus. The MR electrodes were all in the median raphe nucleus. No systematic relation was found between the degree of drug effects and minor variation in electrode locations within the target structures

DISCUSSION

Metergoline produced a complete suppression of responding in those rats working for habenular stimulation and significantly less suppression in those working for hypothalamic stimulation. The presence of a differential effect can be interpreted in either of the following two ways. First, metergoline may have made the animals totally incapable of performing the operant response while leaving the ability for grooming and exploration intact, and LH stimulation somehow prevented this incapacitation from occurring. This interpretation is theoretically possible, but it is difficult to identify exactly what causes the specific incapacitation. The other interpretation is that metergoline diminished rewarding effect of habenular stimulation. Metergoline may have produced perceptual or motoric disturbance or a change in arousal level, but any of these effects would have interfered with hypothalamic self-stimulation as much as with habenular self-stimulation. A complete suppression of habenular self-stimulation, far beyond the extent to which hypothalamic self-stimulation was interfered, indicates that metergoline has blocked a mechanism critical for the generation of rewarding effect from the habenula. Since metergoline selectively blocks 5HT receptors, one could safely conclude that the rewarding effect of habenular stimulation is mediated by serotonergic neurons

The suppression of median raphe self-stimulation by metergoline is similar in time course to the results of Deakin [2], though the degree of suppression was more complete in the present experiment The present results are consistent with findings of Miliaressis [7,8] and Van der Kooy [15] that median raphe self-stimulation is suppressed by PCPA. Combined with the previous findings by Sutherland and Nakajima [13] that habenular self-stimulation is suppressed after placing lesions in the region of the median raphe nucleus, the present results suggest that the serotonergic neurons mediating the rewarding effect of habenular stimulation may originate in the median raphe nucleus. A branch of the median raphe forebrain tract ascends the fasciculus retroflexus to reach the habenula [12], while the other branch joins the medial forebrain bundle to terminate in the lateral hypothalamus, septal area, and the hippocampus [1]. Another possibility is that habenular self-stimulation is mediated by the dorsal raphe forebrain tract, which passes through the lateral aspect of the median raphe The dorsal tract also bifurcates to join the fasciculus retroflexus and the medial forebrain bundle [1].

The effects of chlorpromazine were similar on habenular and hypothalamic self-stimulation. the drug suppressed responding. Miliaressis [8] has also reported a suppression of bar-pressing for median raphe stimulation under chlorpromazine One could assume that the absence of regional specificity implies the presence of a common underlying mechanism. It may be that habenular stimulation, by way of the median raphe region, eventually excites the lateral hypothalamic area where catecholamines play an important role in producing a rewarding effect. However, this interpretation is difficult to reconcile with the fact that lesions placed in the preoptic and lateral hypothalamic areas do not suppress but enhance habenular self-stimulation [13] Taking a different view, one could argue that the absence of regional specificity reflects an interference with performance without a reduction in rewarding effect Whether the suppression of hypothalamic self-stimulation by chlorpromazine, and other neuroleptics, is caused by a loss of rewarding effect or by an interference with motor function is still a controversial issue [6,17].

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Prof. L Valzelli and to Farmitalia Carlo Erba for a supply of metergoline, to H A Schellinck for her technical assistance, and to B Fantie for his critical comments on the manuscript

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