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Effects of Quipazine and of Tryptamine on Self-Stimulation of Median Raphé Nucleus and of Lateral Hypothalamus in Rats

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BROADBENT, J. AND A. J. GREENSHAW. *Effects of quipazine and of tryptamine on self-stimulation of median raphé nucleus and of lateral hypothalamus in rats.* PHARMACOL BIOCHEM BEHAV 23(6) 943–947, 1985.–Separate groups of male Wistar rats were trained to lever press on a continuous reinforcement schedule under which behaviour was maintained by electrical stimulation of the median raphé nucleus (N=6) or the lateral hypothalamus (N=6). The effects of several doses of quipazine (2.5–7.1 mg/kg) and of tryptamine (10–80 mg/kg) were assessed with each group. Administration of quipazine resulted in a decrease of median raphé self-stimulation at 5.0 and 7.1 mg/kg. This compound had no statistically significant effect on lateral hypothalamic self-stimulation. Administration of tryptamine resulted in significant decreases in self-stimulation at both sites, however, whereas the effects of this drug were significant at 20, 40 and 80 mg/kg with median raphé self-stimulation, a significant decrease in lateral hypothalamic self-stimulation was only observed at 80 mg/kg. As baseline response rates differed in the two self-stimulation sites, a second group of animals with lateral hypothalamic sites (n=6) were tested with quipazine (2.5–7.1 mg/kg) at an overall baseline response rate matched to that of the median raphé group. Although a tendency to decrease self-stimulation rates was found in this group, these results were not significant. These data suggest, therefore, that median raphé self-stimulation is more sensitive than lateral hypothalamic self stimulation to disruption by the effects of quipazine and tryptamine.

Quipazine Tryptamine Self-stimulation Median raphé nucleus Lateral hypothalamus Reinforcement

OPERANT behaviour may be maintained by electrical stimulation of a variety of local brain areas in a variety of species [18]. Considerable evidence suggests that a number of neural substrates may be involved in this phenomenon [10]. Catecholamine and particularly dopamine activity in the central nervous system (CNS) may be principally involved in the reinforcing effects of brainstem stimulation [24]. Several studies, however, suggest a role for 5-hydroxytryptamine (5HT) activity in the reinforcing effects of midbrain raphé stimulation, particularly in relation to self stimulation of the median raphé nucleus [13, 15, 22].

Evidence for a dissociation of dopamine and 5HT mediation of reinforcement has been provided by studies of selective manipulations of dopamine and 5HT activity at brainstem and midbrain sites respectively. Katz and Baldrighi [13] have reported that the 5HT antagonist methysergide increased medial forebrain bundle self-stimulation but decreased self-stimulation at median raphé sites. Conversely, Miliaressis [15] has reported that depletion of catecholamines with α-methyl-*para*-tyrosine or facilitation of catecholamine activity with methamphetamine respectively decrease or facilitate self-stimulation of the ventral tegmental area without affecting median raphé self-stimulation in the same animals. In the same study the 5HT depletor *para*-chlorophenylalanine (PCPA) selectively decreased self-stimulation at median raphé sites. Although equivalent results have been observed with PCPA in some other studies, there are conflicting reports of effects of PCPA on self-stimulation [2, 16, 22]. A recent report indicates a lack of site-specificity for effects of PCPA, as a decrease in self-stimulation at both brainstem and median raphé was observed after PCPA treatment [5]. Furthermore, poor correlations between the degree of 5HT depletion and the effects of PCPA on self-stimulation have been reported (see [5]).

Compounds which may directly act as agonists at 5HT receptors have not been investigated in the present context. In view of the conflicting reports of PCPA effects in the literature we have investigated the effects of the 5HT agonist quipazine [6,7] on self-stimulation maintained at median raphé and lateral hypothalamic sites. Although some authors have suggested that quipazine may also interact with dopamine receptors [7,8], this contention has not received substantive or consistent support [4, 8, 23]. In view of the increasing interest in the possible role of tryptamine as a neurotransmitter or neuromodulator in the CNS [12], we have also assessed the effects of this compound in the pres-

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TABLE 1

BASELINE RESPONSE RATES MAINTAINED BY STIMULATION OF LATERAL HYPOTHALAMUS OR BY MEDIAN RAPHÉ STIMULATION

Rat No.	Lateral Hypothalamus	Rat No.	Median Raphé
1	84.4 ± 1.1	7	29.5 ± 1.1
2	50.5 ± 1.4	8	17.8 ± 2.2
3	51.7 ± 4.8	9	50.5 ± 2.7
4	79.9 ± 4.2	10	27.1 ± 1.4
5	93.6 ± 3.6	11	36.2 ± 3.5
6	63.8 ± 2.7	12	58.4 ± 2.9
Group Mean ± S.E.	70.7 ± 6.4		36.6 ± 5.4

Res/Min: Mean ± S.E., N=8 pre-drug control session.

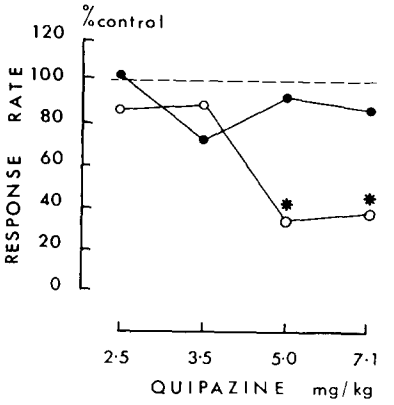

FIG. 1. The effects of quipazine on rates of lever pressing maintained by median raphé (○—○) or lateral hypothalamic (●—●) stimulation. Each data point represents the median performance of the group expressed as a percentage of baseline responding (*$p<0.05$).

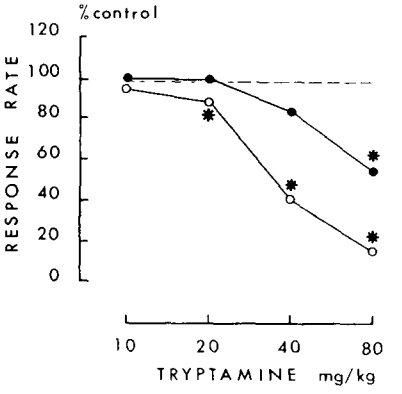

FIG. 2. The effects of tryptamine on rates of lever pressing maintained by median raphé (○—○) or lateral hypothalamic (●—●) stimulation. Details as for Fig. 1.

ent study. Tryptamine is an indoleamine which is present in mammalian tissue in very small amounts. This trace amine exhibits an extremely fast turnover rate [25] and may interact with 5HT receptors and/or postulated tryptamine receptors in the CNS [12].

A differential effect of quipazine, and possibly tryptamine, on median raphé and lateral hypothalamic self-stimulation may provide further evidence for a neuropharmacological dissociation of midbrain and brainstem reinforcement systems. As response-contingent neurotransmitter release is critical for the maintenance of reinforcement in the self-stimulation paradigm [1,20] we hypothesised that, if indoleamines (i.e., 5HT and possibly tryptamine) mediate reinforcement resulting from median raphé stimulation, then administration of indoleamine agonists should selectively decrease self-stimulation at this site. More specifically, the agonists should abolish the possible contingency between indoleamine receptor activation and electrical stimulation by interacting with these receptors independently of self-stimulation.

The effects of quipazine and of tryptamine respectively on self-stimulation behaviour have, to the best of our knowledge, not previously been reported in the literature.

EXPERIMENT 1

METHOD

Subjects

Twelve male Wistar rats weighing 170–200 g at the time of surgery were used. These animals were individually housed under a 12 hr light/dark cycle at a temperature of 20±1°C. Food and water were freely available in the home cages.

Procedure

Surgery and histology. The subjects were each implanted with a twisted bipolar electrode (Plastic Products, Roanoke, VA, U.S.A. MS303/2, 250 μ bore diameter) insulated except for a cross sectional area at the tips. The electrodes were directed to the medial forebrain bundle at the level of the lateral hypothalamus (Rats 1–6: co-ordinates in mm from bregma AP +1.50 Lat +1.50 Vent −8.50 from skull surface) or to the median raphé nucleus (Rats 7–12 co-ordinates in mm from bregma AP −8.00 Lat 0.00 VENT −8.0 from skull surface). Standard stereotaxic surgical procedures were employed; the co-ordinates were based on the Atlas of Konig and Klippel [14]: the incisor bar being positioned at 2.4 mm below interaural zero.

On completion of behavioural testing the animals were deeply anaesthetised and perfused intracardially with 0.9% saline followed by 10% formalin. The skulls with the implants intact were then placed in a solution of 10% formalin for seven days after which the brains were removed and placed in 10% formalin for a further seven days. The brains were then frozen and coronal sections were taken at 50 μ mounted and stained using the Kluver-Berrera technique [3]. Electrode sites were microscopically verified to be located in and around the medial forebrain bundle at the level of the lateral hypothalamus (rats 1–6) and directly in, or adjacent to the borders of, the median raphé nucleus (rats 7–12) (Fig. 3).

Behavioural Testing

Following a minimum recovery period of seven days after surgical implantation the rats were trained to lever press in standard operant test chambers (Coulbourn Insts. E10-10) each equipped with a single lever. Behaviour was maintained

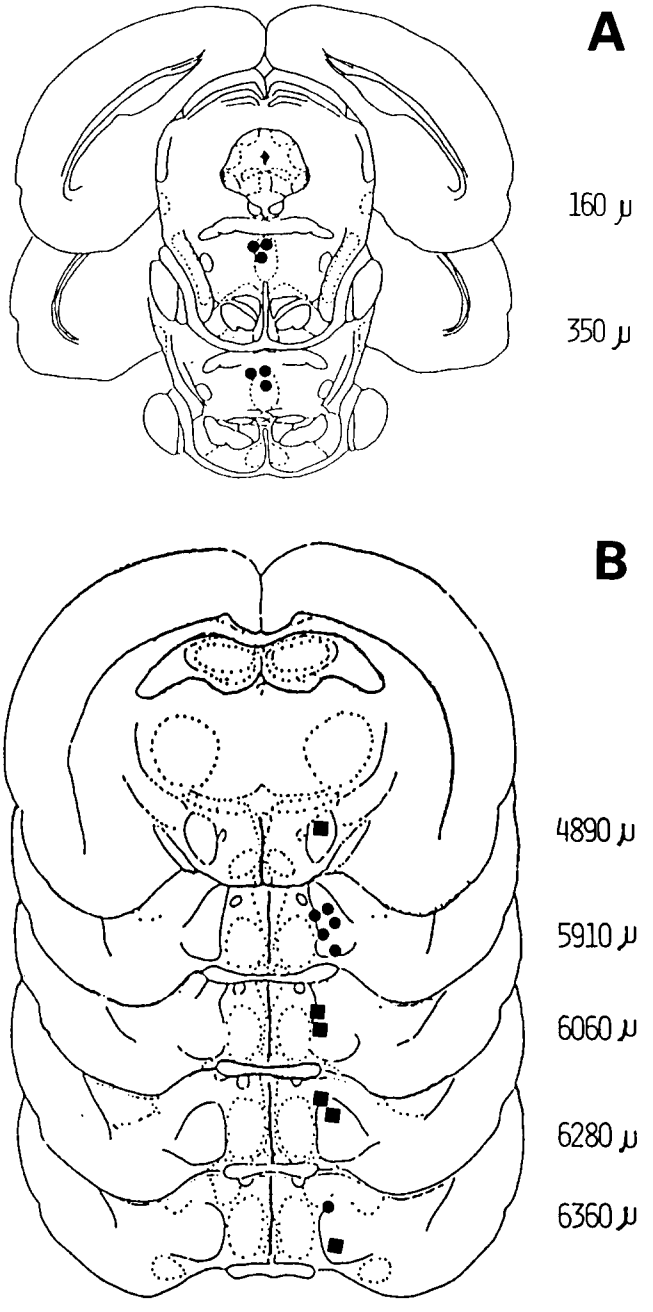

FIG. 3. Electrode sites for the six MR subjects (A), and the twelve LH animals (B). Animals from Experiment 1 are depicted by closed circles. Subjects from Experiment 2 are indicated by squares. All sites were found to be in or near the intended site.

by electrical stimulation of the lateral hypothalamus (rats 1–6) or median raphé nucleus (rats 7–12). Each lever press resulted in the delivery of a 200 msec train of sinusoidal stimulation (60 Hz) to the electrodes from a constant current source. Current intensity (36–106 μA rms) was continuously monitored on an oscilloscope connected to the rat through a 10 kΩ series resistor. Commodore 4016 microcomputers [9] served to control the delivery of electrical brain stimulation and to record responses during the session. The daily sessions were of 20 min duration. A houselight illuminated the test chambers during each session, and at the end of each session this light was extinguished. After stable lever pressing behaviour was established, each animal was tested with different stimulation intensities and a working intensity chosen which would give maximal response rates. The animals were exposed to this continuous reinforcement schedule for at least seven days until responding had stabilised, after which the effects of several doses of quipazine and of tryptamine were assessed.

Drug Administration

Quipazine maleate (Miles Research Products Div., Elkhart, IN) and tryptamine hydrochloride (Sigma Ltd., St. Louis, MO) were dissolved in 0.9% saline and injected intraperitoneally in a volume of 1 ml/kg. Quipazine was injected ten minutes prior to testing and tryptamine was injected immediately prior to testing. Each animal received each dose of quipazine (2.5, 3.5, 5.0, 7.1 mg/kg) once in a mixed order. At least three control days separated each drug day. On each control day an equivalent volume of 0.9% saline was administered according to the same protocol. Seven days after the completion of the quipazine dose response estimation the effects of several doses of tryptamine (10, 20, 40, 80 mg/kg) were investigated. Each animal received each dose of tryptamine once, the doses were administered in a mixed order. As with quipazine, each drug day was separated by at least three saline control days. Drug doses are expressed as the salts.

RESULTS

The lateral hypothalamic and median raphé electrode placements maintained stable rates of responding over the course of the experiment. This is illustrated by the mean±SE values over each of the eight pre-drug control days presented in Table 1 for each individual lateral hypothalamic and median raphé rat. A Kruskal Wallis one-way ANOVA revealed that response rates maintained by lateral hypothalamic stimulation were generally higher than those maintained by median raphé stimulation ($H=13.35$, $p<0.05$).

The effects of quipazine on rates of self-stimulation are displayed in Fig. 1. The data in this figure show that median raphé self-stimulation was markedly decreased by this drug in contrast to a slight decrease in lateral hypothalamic self-stimulation. Friedman's two-way ANOVA (subjects×dose) revealed that the effect of quipazine in the median raphé group was significant, $\chi^2_r(4)=10.78$, $p<0.05$, whereas that in the lateral hypothalamic group was not, $\chi^2_r(4)=2.48$, $p>0.05$. A randomisation test for matched pairs revealed that these effects of quipazine on median raphé self-stimulation were significant at 5.0 and 7.1 mg/kg ($p<0.05$).

The effects of tryptamine on self-stimulation at the two sites are displayed in Fig. 2. The data in this figure illustrate that administration of tryptamine resulted in a marked decrease in self-stimulation at both median raphé and lateral hypothalamic sites. These effects on median raphé self-stimulation, however, appeared to be greater than those on lateral hypothalamic self-stimulation. Friedman's two-way ANOVA (subjects×dose) revealed that the rate decreasing effects of tryptamine on self-stimulation of median raphé, $\chi^2_r(4)=18.66$, $p<0.05$, and of lateral hypothalamus, $\chi^2_r(4)=9.6$, $p<0.05$, respectively, were significant. Randomisation tests for matched pairs showed that with median raphé self-stimulation these effects were significant at 20, 40 and 80 mg/kg ($p<0.05$), whereas with lateral hypothalamic self-stimulation they were significant only at 80 mg/kg ($p<0.05$).

TABLE 2

BASELINE RESPONSE RATES AND DRUG RATES OF LATERAL HYPOTHALAMIC SUBJECTS MAINTAINED AT RESPONSE RATES MATCHED TO MEDIAN RAPHÉ RATES

Rat No.	Baseline Response Rate (Res/Min: mean ± S.E.) N=8 pre-drug control days	Quipazine (mg/kg) Dose 2.5	3.5	5.0	7.1
1	31.1 ± 5.0	24.9	31.1	10	5.3
2	37.2 ± 6.8	27.5	6	3.7	0
3	37.1 ± 2.3	44.1	34.9	16.7	3
4	42.4 ± 3.8	33.5	42.8	42.4	0.4
5	27.7 ± 3.7	28	12.5	14.7	29.6
6	37.5 ± 4.8	49.5	39	54.8	49.5
			Group Median		
Group Mean ± S.E.	35.0 ± 2.1	30.8	33	29.6	4.2

DISCUSSION

In Experiment 1 the systemic administration of quipazine resulted in a decrease in self-stimulation of median raphé sites at doses which did not significantly affect lateral hypothalamic self-stimulation. Essentially similar results were obtained after systemic administration of tryptamine. Median raphé self-stimulation was decreased by tryptamine at 20, 40 and 80 mg/kg in contrast to a significant decrease in lateral hypothalamic self-stimulation only at 80 mg/kg. The administered doses of tryptamine stand in marked contrast to the observed levels of this amine in normal rat brain [12]. Tryptamine, however, possesses an extremely high turnover rate due to its rapid catabolism by monoamine oxidase [25]. Thus, to effect behavioural changes after peripheral administration, relatively high doses of tryptamine are required.

It is evident that baseline response rates differed significantly between the lateral hypothalamic and median raphé self-stimulators. Although baseline response rate *per se* is clearly established as a determinant of the effects of certain drugs, the present selective decrease in the lower overall median raphé rates is inconsistent with the rate dependency principle [19]. Nevertheless, it is possible that the higher rates maintained by hypothalamic stimulation are less liable to motor disruption by the present drugs: although it is clear that response rate *per se* may be a poor index of the reinforcing effects of electrical stimulation of one brain site relative to another [11].

EXPERIMENT 2

In order to assess the possible contribution of differential baseline rates to the effect of quipazine in Experiment 1 further analysis was conducted. In this experiment the effect of several doses of quipazine were assessed in animals self-stimulating at lateral hypothalamic sites at response rates equivalent to those of the median raphé animals in the previous experiment.

Subjects

Six male Wistar rats weighing 170–200 g were used; housing conditions were identical to those in Experiment 1.

Procedure

Subjects were implanted with bipolar stimulating electrodes aimed at the medial forebrain bundle at the level of the lateral hypothalamus using the technique described in Experiment 1. As described earlier electrode sites were verified after completion of drug testing. These sites were located in or close to the medial forebrain bundle and are shown in Fig. 3.

Behavioural Testing

Each subject was trained to lever press on a continuous reinforcement schedule for sinusoidal stimulation of 200 msec duration as described in Experiment 1. Response rates of each animal at different stimulation intensities were measured and a working intensity was chosen to maintain a baseline response rate close to that of the median raphé group.

Drug Administration

Four doses of quipazine maleate (2.5–7.1 mg/kg) were tested in animals with stable response rates. The drug was administered as described in Experiment 1.

RESULTS

It may be seen from the data displayed in Table 2 that the mean response rate, based on eight pre-drug control days, for the group closely corresponds to that of the median raphé group in Experiment 1. The effects of quipazine on the overall response rate of each animal are also displayed in Table 2. It may be seen from these data that, although the group median scores are decreased at the two highest doses of quipazine, considerable individual variation was observed in response to the drug. Friedman's two-way ANOVA (subjects×dose) revealed no significant effects of quipazine on response rate, $\chi^2_r(4)=7.47$, $p>0.05$. The lack of statistical significance is due to the large degree of variation associated with the animals' responses to the drug.

DISCUSSION

In this experiment animals self-stimulating at lateral hypothalamic sites at rates matched to those of the median raphé animals of Experiment 1 did not exhibit a consistent response to quipazine. Although it is clear that the responding of individual animals was markedly disrupted, the overall effect of quipazine was not significant. These data, in comparison to those of Experiment 1, indicate a greater sensitivity to quipazine with median raphé self-stimulation even when compared to lateral hypothalamic stimulation at equivalent response rates.

GENERAL DISCUSSION

The data from Experiments 1 and 2 suggest that median raphé self-stimulation is consistently disrupted by quipazine. Lateral hypothalamic responding, however, is not consistently altered. The failure of quipazine to attenuate lateral hypothalamic self-stimulation is, however, not necessarily inconsistent with an inhibitory role of 5-HT [1,18]. The present lack of effect may be related to the choice of quipazine doses.

Tryptamine induced a reduction in response rates with both groups of animals in Experiment 1. The effects of this compound were, therefore, not assessed in Experiment 2.

Significant effects of tryptamine on lateral hypothalamic self-stimulation were only observed at the highest dose. The significance of this tryptamine-induced decrease in lateral hypothalamic self-stimulation is questionable as the effect may simply be secondary to a disruption of response performance. Further work with tryptamine is necessary to address this possibility.

The quipazine induced decrease in response rate with the median raphé group observed in Experiment 1 may not simply be explained in terms of a drug-induced disruption of response performance, as the response requirement was identical for self-stimulation at both sites. It is possible, however, that the unconditioned responses to stimulation at the two sites may differ and, therefore, interact differently with drug administration. A recent report by Tasman and Simon has shown that amphetamine-induced stereotyped behaviour may be affected by midbrain stimulation [21], indicating that unconditioned responses to electrical stimulation of CNS sites may interact with the behavioural effects of drugs. This represents a largely unexplored possibility for an alternative interpretation of the interaction of drugs with self-stimulation at particular sites, representing a viable alternative to the 'reinforcement' interpretation of site-dependent drug effects. This is clearly an avenue of research which must be explored further in the analysis of effects of drugs on self-stimulation.

Nevertheless, it is apparent from the results of the present experiments that there is a greater consistency in the response to quipazine and to tryptamine of median raphé self-stimulation. These findings, therefore, support the hypothesis that non-contingent stimulation of indoleamine receptors will selectively attenuate median raphé self-stimulation. Nakajima has recently reported differential effects of a 5-HT antagonist metergoline on lateral hypothalamic self-stimulation and behavior maintained by habenular and median raphé stimulation [17]. These data are consistent with the results of the present study and with previous evidence for a neuropharmacological dissociation of self-stimulation at respective midbrain and brain stem sites [13, 15, 22].

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