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GABA-Benzodiazepine Modulation of Aversion in the Medial Hypothalamus of the Rat

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MILANI, H. AND F. G. GRAEFF. *GABA-benzodiazepine modulation of aversion in the medial hypothalamus of the rat.* PHARMACOL BIOCHEM BEHAV **28**(1) 21–27, 1987.—Earlier results indicate that the neurons of the midbrain central gray (CG) responsible for the elaboration and/or expression of aversive states are tonically inhibited by the GABA-benzodiazepine system. In the present study, chemitrodes were implanted in the medial hypothalamus (MH) of the rat, another aversive area of the brain deeply interrelated with the dorsal CG. Microinjection of the benzodiazepine receptor agonist midazolam raised the aversive threshold of electrical stimulation of the MH in a dose-dependent way, though in only about half of the animals tested. In the remaining rats, midazolam was ineffective. Similar antiaversive effects were caused by the GABA-A receptor agonist THIP. In contrast, microinjection of the GABA-A receptor blocker bicuculline induced aversive-like behavioral and autonomic changes. The effects of bicuculline were antagonized by pretreatment with either THIP or midazolam, the latter being counteracted by the competitive benzodiazepine receptor blocker Ro 15-1788. These results extend to the MH, the hypothesis of GABA-benzodiazepine modulation of neurons integrating aversive motivational states.

Aversion Medial hypothalamus Brain electrical stimulation Intracerebral injection THIP Bicuculline Midazolam GABA Benzodiazepine receptors

EXPERIMENTAL evidence reviewed elsewhere [10] indicates that the medial hypothalamus (MH), the dorsal midbrain central gray (CG) and parts of the amygdala constitute an integrated neural system responsible for the elaboration and/or expression of aversive states.

Previously reported results have shown that microinjection in the dorsal CG of two benzodiazepine anxiolytics, chlordiazepoxide and midazolam, of the sedative-hypnotic pentobarbital, as well as of the inhibitory neurotransmitter GABA and several direct GABA-A receptor agonists raises the aversive threshold of electrical stimulation of the same brain area [1, 2, 13]. The antiaversive effect of the benzodiazepine anxiolytics is likely to be mediated by benzodiazepine receptors since it was abolished by pretreatment with the competitive benzodiazepine receptor blocker Ro 15-1788 [15], also microinjected in the dorsal CG [1]. Therefore, increase of GABAergic activity in the dorsal CG attenuates the aversive effect of its electrical stimulation.

Complementary results were obtained with drugs that impair GABAergic neurotransmission. Thus, the competitive GABA-A receptor blockers, bicuculline [4,27] and SR 95103 [25], the non-competitive GABA antagonist picrotoxin, and three different inhibitors of GABA synthesis have been shown to induce either aversive-like behaviors or neurovegetative changes characteristic of the defense reaction when microinjected into the dorsal CG of the rat [2, 3, 6, 23, 25, 26]. The above results led to the suggestion that the GABA-benzodiazepine system tonically inhibits neuronal groups in the dorsal CG, commanding aversive-defensive behaviors and elaborating negative motivational states [2, 6, 12, 13, 23].

The MH is anatomically interconnected with the dorsal CG [8, 9, 21, 28] and its electrical stimulation is similarly aversive [5, 7, 18, 29, 22, 24]. In addition, flight behavior has been described following microinjection of GABA-A receptor antagonists as well as of inhibitors of GABA synthesis in the MH of the rat, indicating that GABA modulates aversion in the MH in the same way as in the CG [3, 6, 25, 26].

In the present study, the role of the GABA-benzodiazepine system in the MH was further explored. For this purpose, the effect of microinjections of the benzodiazepine receptor agonist midazolam [14] as well as of the GABA-A receptor agonist THIP [17] on the aversive threshold of electrical stimulation of the MH of the rat was measured. The behavioral and neurovegetative changes induced by intrahypothalamic injection of bicuculline were also assessed. In addition, the antagonism of bicuculline effects by

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local pretreatment with either midazolam or THIP was evidenced. Finally, reversal by Ro 15-1788 of the antibicuculline action of midazolam was demonstrated.

METHOD

Animals

Male Wistar rats, weighing 230–250 g were housed in individual glass-walled cages and given free access to food and water throughout the experiment. Temperature in the animal room was maintained at 24±1°C.

Surgery

Rats were anesthetized with 40 mg/kg sodium pentobarbital, IP, and operated in a stereotaxic instrument (David Kopf Instr.). A chemitrode made of stainless steel guide cannula (o.d. 0.7 mm) glued to a brain electrode was implanted in the hypothalamus, aiming at the dorsomedial hypothalamic nucleus. The electrode was made with two twisted stainless steel wires, 160 μm in diameter, electrically insulated except for the cross section of the tips, reaching 4.5 mm below the lower end of the cannula. With the incisor teeth holder 2.5 mm below the interaural line, the chemitrode was vertically introduced 0.5 mm lateral and 4.2 mm anterior to lambda, until the tip reached 7.6 mm below the dura. The chemitrode was attached to the bone with stainless steel screws and methyl-methacrylate polymer cement. A stylette was introduced inside the guide cannula to prevent obstruction. The upper end of each electrode wire was connected to a male pin. The pins could be plugged into an Amphenol socket for electrical stimulation.

Apparatus

Brain stimuli were generated by a constant current, sine-wave stimulator [19]. The stimulation current was monitored on the screen of an oscilloscope (LABO, Brazil).

A shuttle-box consisting of two compartments of 25×20×20 cm without any barrier between them was placed inside an insulating chest provided with a fan and a wide-angle lens allowing one-way vision of both compartments. The grid-floor of the shuttle-box oscillated within a narrow angle around a midline axis whenever a rat passed from one compartment of the box to the other. The movement of the grid-floor opened a microswitch connected with standard electromechanical equipment, recording the crossing responses. During the experiment, the shuttle-box was illuminated by a 30W lamp painted red, placed at the posterior wall. The rat inside the experimental chamber had its brain electrode connected with the stimulator by means of a mercury swivel and a flexible cable, allowing ample movement inside the box. Environmental temperature was kept at 24±1°C.

Procedure

Seven to nine days after the surgery, the rat was placed inside the shuttle-box and allowed to habituate for 30 min. On the next day, the animal was replaced inside the experimental chamber and its brain was electrically stimulated (AC, 60 Hz). Ten-second periods of brain stimulation alternated with 10 sec intervals without stimulation. Only animals showing the following sequence of behaviors with increasing current intensity were used: enhanced locomotion, standing up against the walls of the shuttle-box, running around and leaping forward.

Twenty-four hours later, the selected rats returned to the experimental chamber for determination of the aversive threshold. Several series of 10 electrical stimuli were applied to the rat's brain. Inter-stimulus interval was 10 sec while the interval between successive series of stimuli was 1 min. Whenever the rat crossed the midline separating the two compartments of the shuttle-box, ongoing brain electrical stimulation was switched-off (escape). Otherwise, the brain stimulus lasted for 10 sec. Switch-off responses were automatically recorded in a digital counter. The current intensity of brain stimulation started at a low, subthreshold level and was increased by steps of 1.4 μA, expressed as the root mean square (RMS) of the peak-to-peak current intensity (4 μA). The lowest current causing 9 or 10 switch-off responses in a series of 10 brain stimuli was considered the aversive threshold. Thus, whenever the rat failed to switch-off twice in a series, brain stimulation was interrupted and the current intensity was increased for the next series. Each threshold determination was based upon a single ascending sequence of current intensities and took from 5 to 10 min. Animals with a threshold above 54 μA (RMS) were discarded.

For intracerebral injections, a thin dental needle (o.d. 0.3 mm), at the end of a 20 cm long PE 10 polyethylene catheter, was introduced through the guide cannula until its tip was 4.5 mm below the cannula end. Thus, the tip of the injection needle reached the same horizontal level of the electrode tip. A polyethylene sleeve occluded the upper extremity of the guide cannula, avoiding reflux of the injected solution. After the introduction of the intracerebral needle, the animal was allowed to move freely inside a 26×17×33 cm box with transparent walls. As the exploratory activity subsided and the animal became motionless, a volume of 0.5 μl was injected over 30 sec, using a Hamilton (USA) 10 μl microsyringe. The needle was held in place for 3 min following the injection.

The aversive threshold was redetermined 10 min after the intracerebral injection. The effect of drug treatments was measured as the difference (Δ) between the last threshold and the pre-injection threshold in the same rat.

Feces and urine were collected in a tray under the grid floor. The tray was cleaned before and observed after the threshold determination in order to detect the occurrence of defecation and/or urination.

For measurement of bicuculline effects, rats were placed inside the shuttle-box and microinjected in the medial hypothalamus. The rats were systematically observed from 2 to 12 min following the drug administration. The total number of rearing and forward leaps in 10 min was recorded by the experimenter. In addition, the midline-crossing responses were automatically recorded in a digitial counter. Drug pretreatments, also microinjected into the MH, were made inside the transparent cage 10 and 20 min before the administration of bicuculline. Only animals responding to the first bicuculline injection were used in the experiments with drug combinations. When three injections were made in the same experiments, volumes of 0.25 μl, 0.5 μl and 0.25 μl were used for the 1st, 2nd and 3rd injection, respectively. Otherwise, injection volume was always 0.5 μl. A minimum interval of 48 hr elapsed between successive bicuculline injections. A maximum of 5 intracerebral injections was made in the same rat.

The 10 min interval used between intracerebral drug injection and threshold determination or subsequent drug injection was chosen on the basis of previously reported observations [1].

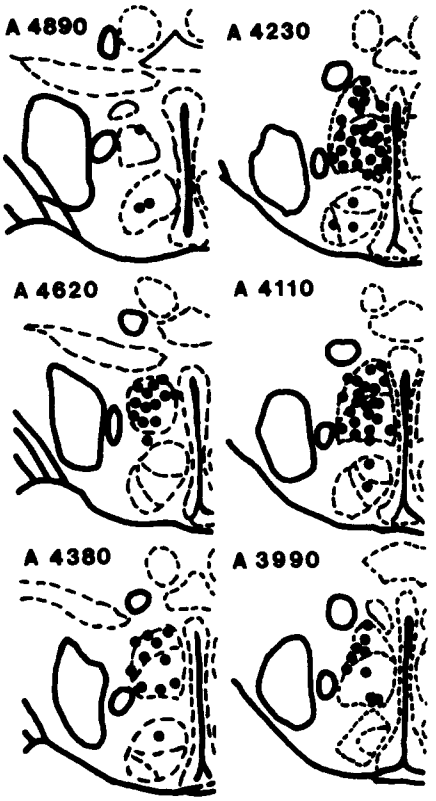

FIG. 1. Localization of electrode sites (●) inside diagrams from König and Klippel's rat brain atlas [16]. Figures represent the atlas coordinates in μm, anterior (A) to the interaural line. The number of points in the figure is less than the total number of rats used (255) because of several overlaps.

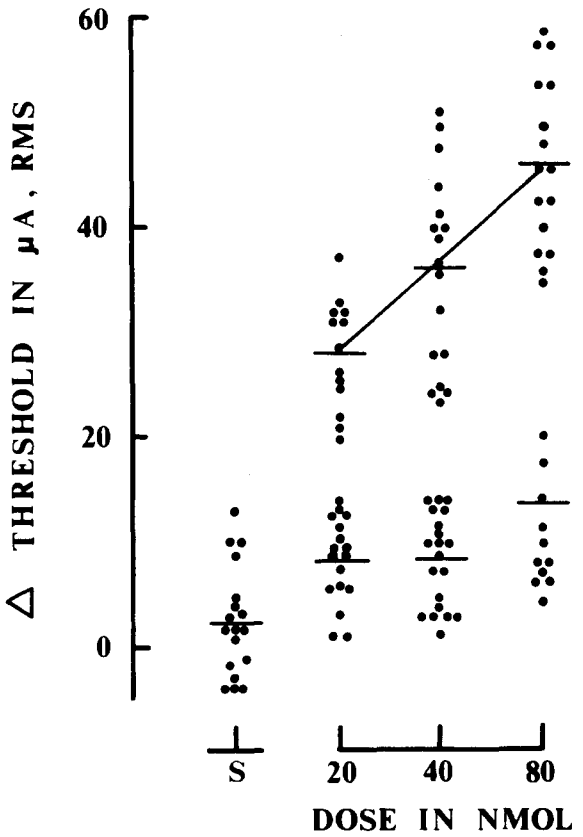

FIG. 2. Changes in aversive threshold caused by microinjections (0.5 μl in 30 sec) of midazolam in the medial hypothalamus of the rat. The aversive threshold was the lowest current intensity inducing at least 9 midline crossings (escapes) in 10 successive trials of electrical stimulation, applied to the medial hypothalamus of rats placed inside a shuttle-box. The change in threshold (Δ) was the difference between the threshold determined 10 min after the intracerebral injection and the threshold measured immediately before the injection in the same animal. Each point in the figure represents individual data and the horizontal lines, the means. Note that rats were either sensitive or insensitive to midazolam. The straight line in the upper part of the figure is the calculated linear regression and represents the dose-effect curve for the sensitive animals. S stands for saline (0.9% NaCl) injection.

Data Analysis

One-way analyses of variance were used for the experiments in which the aversive threshold was measured. The significance levels of specific comparisons were determined by means of the multiple range test of Tukey. The dose-effect relationships of midazolam and THIP on aversive threshold were submitted to linear regression analysis.

For the statistical analysis of the behavioral changes caused by bicuculline, Mann-Whitney's U-test as well as the signed rank test and the two-way, multiple-range test of Wilcoxon were used whenever appropriate. Occurrence of defecation or of urination was analyzed by means of the test of independence, using a 2×2 contingency table.

Histology

After the experiment, rats were sacrificed under deep anesthesia and their brains perfused through the heart with saline solution (0.9% NaCl), followed by 10% formalin, buffered at pH 7.3–7.5. The brains were removed and fixated in 10% formalin for at least 3 days. Frozen sections of 100 μm were placed on a glass slide, coated with a thin layer of albumin and glycerin (1:1). Four hours later, the sections were stained with cresyl violet, according to Nissl. The stained sections were examined with a stereoscopic microscope. Electrode placements were localized in diagrams of König and Klippel's [16] rat brain atlas. Only the results of animals with electrodes localized inside the medial hypothalamus, comprehending the dorsomedial nucleus, the ventromedial nucleus and the area in between these nuclei were analyzed.

Drugs

The following drugs were used: midazolam maleate (Roche), 4,5,6,7-tetrahydroisoxazol (4,5-C) piridine-3-ol (THIP, Sandoz), bicuculline methiodide (Sigma), Ro 15-1788 (Roche).

A microsuspension of Ro 15-1788 in saline containing 2% Tween 80 was used. The remaining drugs were dissolved in saline for intracerebral injection.

RESULTS

Localization of the Brain Electrodes

As shown in Fig. 1, most of the electrode tips were lo-

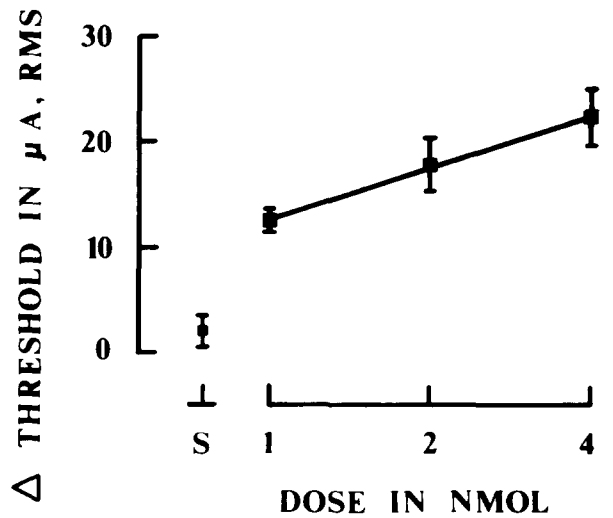

FIG. 3. Dose-effect curve of the changes in aversive threshold caused by microinjections of THIP in the medial hypothalamus of the rat. Each point in the figure represents the mean and the vertical bars the SEM of the least 11 rats. The straight line is the calculated linear regression. Other specifications in the legend of Fig. 2.

TABLE 1

EFFECT OF INTRAHYPOTHALAMIC INJECTION OF MIDAZOLAM AND THIP ON DEFECATION AND URINATION INDUCED BY ELECTRICAL STIMULATION OF THE MEDIAL HYPOTHALAMUS OF THE RAT

Drug Treatment (Dose in nmol)	Occurrence of Defecation	Urination	N
Saline	55.6	27.8	18
Midazolam			
(20)	35.7	15.4	13
(40)	17.6*	11.8	17
(80)	0.0†	0.1	16
THIP			
(1)	63.6	27.3	11
(2)	50.0	25.0	12
(4)	46.7	6.7	14

Figures represent percent of rats showing defecation or urination during the period of determination of the aversive threshold (see Fig. 2). Drugs were injected 10 min before the test. Significant differences from saline are indicated by *$p<0.05$ and †$p<0.01$.

calized inside the dorsomedial hypothalamic nucleus. A few electrode placements were in the ventromedial hypothalamic nucleus and one electrode site was in between these nuclei.

No difference between the anatomical distribution of electrode sites of rats sensitive and unsensitive to midazolam (see below) was noticed.

Behavioral Effects of Brain Electrical Stimulation

In the rats selected for this study, the following sequence of behavioral changes was observed as the intensity of brain stimulation was increased. The first reaction was a brief period of arrest, followed by locomotion and sniffing at the floor and walls of the shuttle-box. Also, standing up at the corners of the box, and nose-poking between the bars of the grid floor were often observed. The speed of locomotion increased with the current intensity, followed by leaping forward with both hind legs thrusting together. At still higher current intensities, jumping up against the ceiling of the box occurred. Whenever this happened, the brain stimulation was turned off. No freezing behavior was observed during hypothalamic stimulation, in contrast to what has been described following electrical stimulation of the dorsal CG [1,2]. Freezing nevertheless occurred during the interval between successive brain stimulations. Defecation and urination were always observed either during or in between brain stimulation periods. The pre-injection escape threshold was 24.0±3.5 μA (RMS) for the 113 animals used.

Antagonism by Midazolam and THIP of the Aversive Effects of Electrical Stimulation of the Medial Hypothalamus

As shown in Fig. 2, the intrahypothalamic injection of midazolam increased the aversive threshold in a dose-dependent way. However, this effect occurred in nearly half of the rats tested, the remaining rats being insensitive to midazolam. Thus, for statistical analysis animals were divided according to their response to midazolam, based on the scatter diagram of Fig. 2. Variance analysis indicated an overall significance of drug effects, $F(6,109)=122.9$, $p<0.001$. For the rats sensitive to midazolam, comparison with control (saline) using Tukey's multiple range test showed significant effects ($p<0.01$) for the doses of 20, 40 and 80 nmol of midazolam. A significant linear regression was obtained when log dose was plotted against drug-induced change in aversive threshold, $F(1,44)=40.4$, $p<0.001$. The calculated equation for the regression was $Y=-9.88+20.12X$. As for the animals insensitive to midazolam, no significant difference from saline control was found at any dose used (test of Tukey, $p>0.05$).

In contrast to the bimodal response to midazolam, rats reacted homogeneously to intrahypothalamically injected THIP. As shown in Fig. 3, THIP raised the aversive threshold in a dose-dependent way. Variance analysis indicated an overall significance for drug effects, $F(3,54)=18.4$, $p<0.001$. Comparison with control, using Tukey's multiple range test, showed significant effects for the doses of 1 ($p<0.05$), 2 ($p<0.01$) and 4 ($p<0.01$) nmol. A significant linear regression was obtained $F(1,35)=9.2$, $p<0.01$, when log dose was plotted against drug-induced change in aversive threshold. The calculated equation for the regression was $Y=12.64+16.20X$. Observation of the animals evidenced that 4 nmol of THIP induced sedation and ataxia, no such changes being observed after midazolam.

The effects of midazolam and THIP on the occurrence of defecation and urination during the threshold determination period are shown in Table 1. In the rats showing an increase in aversive threshold following midazolam, the occurrence of stimulus-induced defecation was significantly decreased by 40 (test of independence, $\chi^2(1)=10.0$, $p<0.01$, nmol of the benzodiazepine. Only a non-significant tendency to decrease urination was caused by midazolam, although this tendency was quite strong after the highest dose of the benzodiazepine. In the rats which did not show an increase in aversive threshold following midazolam, defecation and urination were not affected by the drug (not shown in Table 1). In contrast to midazolam, THIP did not significantly change defecation induced by brain electrical stimulation. A non-

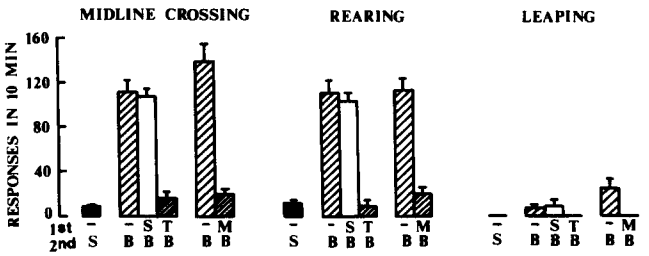

FIG. 4. Antagonism by THIP (T, 2 nmol) and midazolam (M, 80 nmol) of the increases in locomotion, rearing and leaping forward caused by bicuculline methiodide (B, 0.07 nmol). All drugs were microinjected (0.5 μl, 30 sec) into the medial hypothalamus of the rat. The 1st injection was made 10 min before the 2nd one. S stands for saline. N=10 in every group. The 10-min observation period started 2 min after the injection of bicuculline.

significant trend to decrease urination was observed following 4 nmol of THIP (Table 1).

Behavioral Changes Caused by Intrahypothalamic Injection of Bicuculline

The microinjection of 0.07 nmol of bicuculline methiodide into the medial hypothalamus induced behavioral changes similar to those caused by electrical stimulation of the same brain area. Locomotion and rearing were more or less increased. In some rats, vigorous leaps forward and, less frequently, vertical jumps were observed. Intense grooming often occurred following bicuculline administration; nevertheless, this behavioral item was not systematically recorded. While not walking or running, rats usually stayed at one corner of the shuttle-box, biting vigorously or poking their noses in between the metal bars of the grid floor. Alternatively, they stood up and intensively explored the openings made in the walls of the chamber for the house lamp and the mercury swivel, occasionally biting at their edges. Most of the rats defecated and/or urinated following bicuculline injection. The effects of bicuculline started 1 to 2 min after the drug injection, reached a maximum after 4 to 5 min and gradually subsided after 10 min. For this reason, systematic observation and recording of locomotion were made between the 2nd and the 12th min from the injection. Only rats showing at least four of the following effects were selected for further experiments with drug combinations: increased locomotion, increased rearing, leaping forward, defecation and urination.

Antagonism by THIP and Midazolam of the Aversive Effects of Intrahypothalamic Bicuculline

As shown in Fig. 4, bicuculline significantly increased locomotion, recorded as the number of midline crossings, as well as the frequency of rearing and leaping (Mann-Whitney's U-test, $p<0.05$). The comparisons refer to the two leftside columns in the three panels of Fig. 4, representing different groups of animals treated with saline (S) and bicuculline (B), respectively.

Figure 4 also shows that pretreatment with saline did not significantly change the effects of bicuculline on locomotion, rearing and leaping (SB × B, $p>0.05$), whereas 2 nmol of THIP blocked the increases in locomotion ($p<0.05$), rearing ($p=0.01$) and leaping ($p=0.01$) induced by the GABA antagonist (TB × B). These within group comparisons were made using Wilcoxon's two-way, multiple range test. In a different group of animals, pretreatment with 80 nmol of midazolam similarly antagonized the effect of bicuculline on locomotion, rearing and leaping (MB × B, Wilcoxon's signed rank test, $p<0.01$).

TABLE 2

INFLUENCE OF THIP AND MIDAZOLAM ON DEFECATION AND URINATION INDUCED BY INTRAHYPOTHALAMIC BICUCULLINE

Drug Treatment (Dose in nmol)	Occurrence of Defecation	Occurrence of Urination
Saline	10	10
Bicuculline (0.07)	70*	60*
Saline + Bicuculline (0.07)	60	40
THIP (2) + Bicuculline (0.07)	20	20
Midazolam (80) + Bicuculline (0.07)	30	20

Figures represent percent of rats showing defecation or urination during the 10 min observation period starting 2 min after the injection of bicuculline. Significant differences from saline are indicated by $*p<0.01$. THIP and midazolam were microinjected inside the medial hypothalamus 10 min before bicuculline. N=10 in every group.

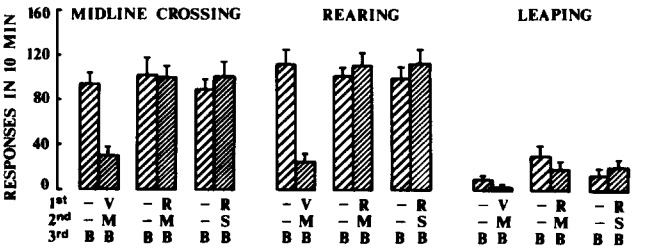

FIG. 5. Reversal by Ro 15-1788 (80 nmol) of the anti-bicuculline action of midazolam. V stands for vehicle (2% Tween 80 in saline). The interinjection interval was 10 min. The following injection volumes were used: 1st injection=0.25 μl, 2nd=0.50 μl and 3rd=0.25 μl. The injection time was always 30 sec. Other specifications in the legend of Fig. 4.

As shown in Table 2, bicuculline significantly increased defecation and urination during the 10-min observation period following its intracerebral injection as compared to saline (test of independence, $p<0.01$). Pretreatment with saline did not significantly affect the occurrence of defecation and urination caused by bicuculline. Both THIP and midazolam tended to decrease bicuculline-induced defecation and urination. However, these tendencies did not become statistically significant.

The effect of the benzodiazepine antagonist, Ro 15-1788, on midazolam-induced attenuation of the behavioral changes caused by intrahypothalamic bicuculline are illustrated in Fig. 5. This figure shows that pretreatment with Ro 15-1788 (80 nmol), intracerebrally injected 10 min before midazolam (80 nmol), reversed the antagonism of bicuculline-induced increases in locomotion and rearing caused by midazolam (RMB × VMB, Mann-Whitney's U-test, $p<0.05$). Leaping was not significantly affected. By itself, Ro 15-1788 did not interfere with the effects of bicuculline (RSB × B, Wilcox-

on's signed rank test, $p>0.05$). Also, pretreatment with the 2% Tween vehicle did not prevent midazolam from blocking the effects of bicuculline (VMB × B, signed rank test, $p<0.01$).

DISCUSSION

The present results, showing an antiaversive effect of the benzodiazepine receptor agonist midazolam, as well as of the GABA-A receptor agonist THIP, following their microinjection in the MH, extend similar observations made in the dorsal CG of the rat [1, 2, 13]. Also, the behavioral and autonomic changes presently induced by the intrahypothalamic injection of the GABA-A receptor blocker bicuculline, antagonized by either midazolam or THIP, confirm and extend reported results obtained in the dorsal CG as well as in the MH of the same species [2, 6, 23, 25, 26]. Furthermore, the antagonism by Ro 15-1788 of the antibicuculline action of midazolam in the MH is consistent with former observations in the CG, using electrical current as stimulating agent [1]. In the present study, however, bicuculline was used instead of electrical stimulation because with the latter, only about half the animals were sensitive to midazolam, as discussed in the following. Althogether, these results strongly suggest that in the MH as well as in the dorsal CG the GABA-benzodiazepine system exerts a tonic inhibitory influence on neurons elaborating aversive motivational states.

Despite these similarities between the MH and the dorsal CG the present results have also evidenced some differences in the neuronal substrate of aversion in the two regions. The clearest of all was the bimodal distribution of the effect of midazolam on the aversive threshold of hypothalamic electrical stimulation, contrasting with the unimodal response to the drug found with the same experimental procedure in the dorsal CG [1]. This difference between the CG and the MH is difficult to explain. Although subtle differences among sites of injection and stimulation between animals sensitive and resistant to midazolam might exist, they could not be detected with the techniques used in the present study. On the other hand, midazolam uniformly antagonized the aversive effects of bicuculline injected in the MH, indicating that the kind of stimulation is a crucial factor. However, the present results also show that the response to THIP was unimodal in animals electrically stimulated at the MH. Because, in the contrast to midazolam, sedation was observed following the intrahypothalamic injection of THIP, the latter effect could have interfered with the measurement of the aversive threshold, preventing the detection of a bimodal distribution.

In addition to the above qualitative difference in drug response between the MH and the dorsal CG, the present results have also evidenced quantitative differences between the two aversive brain areas. Considering only the group of rats which responded to midazolam in a dose-dependent way and comparing the presently obtained log dose-response function with a similar function generated when log dose of midazolam was plotted against drug-induced increase in aversive threshold of electrical stimulation applied to the dorsal CG [1], the MH was 4.7 (1.9–12.5) times more sensitive to midazolam than the CG. A similar comparison made with THIP evidenced that the MH was 2.3 (1.9–9.1) times more sensitive to this drug than the dorsal CG [13].

The neuropharmacological differences between the MH and the dorsal CG shown by the present results agree with an earlier suggestion that the neurochemical substrates of aversion in the MH and in the dorsal CG are not identical [26]. This suggestion was based on published results using morphine, GABA agonists and antagonists, pentobarbital, chlordiazepoxide, d-tubocurarine and excitatory aminoacids, injected either into the dorsal CG or in the MH. Experiments with electrical stimulation have also shown that rats can discriminate between aversive states evoked from these two periventricular sites [18].

In spite of the likely differences between the neurochemical substrates of aversion in the MH and in the dorsal CG, the present as well as previously reported evidence [1–3, 6, 23, 25] indicates that in both structures the neurons responsible for the elaboration and/or expression of aversive states are under tonic inhibitory control of the GABA-benzodiazepine system. The implications of this GABA-benzodiazepine modulation of aversive periventricular-periaqueductal regions for the pathophysiology of anxiety and for the mode of action of anxiolytics have been extensively discussed elsewhere [11,12].

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