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Differential Pharmacological Reactivity of Aversion Induced by Stimulation of Periaqueductal Gray or Mesencephalic Locomotor Region

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DEPOORTERE, R., G. DI SCALA, M. J. ANGST AND G. SANDNER. *Differential pharmacological reactivity of aversion induced by stimulation of periaqueductal gray or mesencephalic locomotor region.* PHARMACOL BIOCHEM BEHAV **37**(2) 311–316, 1990.—Rats were trained to switch-off aversive electrical brain stimulations applied to the periaqueductal gray (PAG) or mesencephalic locomotor region (MLR) by pressing a bar (switch-off behavior). We investigated the effects of IP injections of the benzodiazepine (BZ) receptor inverse agonist FG 7142 (2.5, 5, 10 mg/kg) or BZ receptor agonist chlordiazepoxide (CDP: 5 mg/kg) on the switch-off latency, i.e., the time elapsed between the onset of the stimulation and its offset by a press of the bar. It was found that FG 7142 decreased, whereas CDP increased the mean switch-off latency for electrical stimulation of the PAG, which is interpreted as a potentiating effect of FG 7142 and a reducing effect of CDP on the electrically induced aversive state. By contrast, neither FG 7142 nor CDP were found to affect the mean switch-off latency for MLR stimulations. These results suggest a difference in the pharmacological sensitivity to BZ receptor ligands between aversive states elicited by electrical stimulation of the PAG or MLR.

Aversion Switch-off test Mesencephalic locomotor region Periaqueductal gray Electrical brain stimulation
FG 7142 Chlordiazepoxide Rat

IN the rat, electrical stimulation of the dorsal part of the periaqueductal gray (PAG) is known to elicit escape reactions, mainly characterized by violent running and jumps out of the experimental environment (1,3). These escape reactions are thought to represent the overt expression of an aversive state generated by the electrical stimulation, since such stimulations support the learning of an operant task, like pressing a bar (switch-off behavior), in order to interrupt the stimulation (9, 28, 31). The latency to switch-off the stimulation, which is inversely related to the strength of the electrical stimulation, is considered as a reliable measure of the aversiveness of the stimulation (28,33). Several studies have shown that the aversive state induced by electrical stimulations of the PAG could be decreased by antiaversive/anxiolytic drugs acting on the GABA/benzodiazepine (GABA/BZ) receptor complex, also referred to as $GABA_A$ receptor. For example, IP injections of chlordiazepoxide, a benzodiazepine receptor agonist, were observed to increase the switch-off latency of electrical stimulations applied to the PAG (32). This antiaversive effect is thought to be mediated through an enhancement of the GABAergic inhibition at the level of the PAG. This is supported by the observations that microinjections into the PAG of BZ receptor agonists or $GABA_A$ agonists were shown to raise the threshold of electrical current inducing escape reactions (1–3).

We have recently shown that in intact and freely moving rats electrical stimulation of the mesencephalic locomotor region (MLR), a brain area classically considered as involved in the control of locomotion (35) and comprising the cuneiform nucleus and pedunculopontine tegmental nucleus (6,36), generates aversive effects since it elicits escape reactions and switch-off behavior (10). However, the aversive state produced by electrical stimulation of the MLR has not yet been compared on a pharmacological basis, in particular with respect to BZ receptor ligands, to the aversive state induced by PAG stimulation.

In the present study, we compared the effects of IP injections of a BZ receptor inverse agonist, FG 7142, and a BZ receptor agonist, chlordiazepoxide (CDP), on the switch-off behavior induced by electrical stimulation of the PAG or of the MLR. So far, the effects of BZ receptor inverse agonists on switch-off behaviors have never been characterized. In particular, it is unknown if in that experimental situation, they produce effects opposite (i.e., decrease switch-off latencies) to the effects of BZ receptor agonists, as it is the case for other experimental situations (7, 21, 22, 26). To that end, we tested the effects of nonconvulsive (24,26) doses of FG 7142 (2.5, 5, 10 mg/kg) on switch-off latencies of rats stimulated in the PAG or in the MLR. For CDP, we chose a single dose of 5 mg/kg. This dose was chosen because

it has been shown to be within the range of doses effective in decreasing aversion induced by electrical stimulation of the PAG, as well as to cause very little sedation/ataxia (32).

METHOD

Animals and Surgery

All experiments were performed on male Wistar rats (350–500 g, Centre de Neurochimie animal breeding unit) kept on a 12-12 hr light/dark cycle (light on: 8 a.m.–8 p.m.). Rats were anesthetized with pentobarbital (60 mg/kg IP) and fixed in a stereotaxic frame with the bite-bar 2 mm below the earbars. They were implanted on each side with an electrode made up of 2 intertwisted 125 μm nichrome enamel insulated threads, with a 0.3 to 0.6 mm dorso-ventral intertip distance. A total of 34 rats were implanted: 15 rats were implanted in the MLR, the remaining 19 rats were implanted in the dorsal PAG. The following coordinates were used, the lambda point serving as the reference for each plane: for the MLR: 0.8 to 1 mm posterior, 2 mm lateral, 6.5 mm ventral; for the PAG: 1 mm anterior, 1.7 mm lateral, 5.8 mm ventral. For the PAG, electrodes were implanted with a 15 degree mediolateral angle. Threads were soldered onto a 5 pin female connector which was then embedded in acrylate resin anchored to the skull by means of 4 stainless steel screws. The most rostral screw was used as the common anode. A one week postoperative period was observed before starting the experiments.

Apparatus

Electrical brain stimulation. The electrical brain stimulation (EBS) consisted of a continuous train of monopolar, cathodal pulses delivered by WPI 1850A isolation units connected to a UNIXSYS PC/AT286 computer via a RTI 800 Analog Device general purpose interface card. The timer output of the card was used to set the pulse duration (0.1 msec) and to generate variable interpulse intervals (IPI's). Stimulation parameters were continuously monitored on a differential oscilloscope across a 100 kΩ resistor put in series in the stimulation circuit. Each rat was connected to the stimulation circuit by means of a 5 lead cable terminated by a 5 pin male connector.

The three switch-off cages (25 × 25 × 35 cm) were made of Plexiglas and were fitted with a bar (4 × 4 cm) on the front side, 5 cm above the floor. Cages were controlled by the same hardware and software as the ones used to pilot the WPI 1850A isolation units for generation of EBS. The program controlled the following operations independently for each cage: 1) setting of an IPI value randomly chosen within a range predetermined by the experimenter at the beginning of each session. Each IPI was set by the "randomize" function of the TURBO PASCAL programming language, and could hence take any discrete value (± 1 msec) in between or equal to the minimum and maximum IPI values of the predetermined range; 2) onset of the electrical stimulation at the IPI set by the program; 3) offset of the stimulation following a press of the bar by the rat; 4) recording of the time elapsed between the onset of the stimulation and its offset by the rat, referred to as the switch-off latency (SOL), with a precision of 20 msec; 5) resetting of an IPI value, and so on.

If the rat did not press the bar within 30 sec of stimulation onset, the stimulation was then stopped, but the switch-off latency was not recorded. A press of the bar interrupted the stimulation for 15 sec. During this period, a press of the bar had no effect.

Protocol

Selection of stimulation site. Each rat was first put into the switch-off cage and allowed to habituate for 10 min. Each of the 4 sites was then stimulated in order to assess the aversive nature of the stimulation. Sites were tested in a randomized order, with a 2 min pause in between each site testing. For that part of the experiment, the IPI of the EBS was set at 20 msec. The intensity was manually incremented by steps of 10 μA over 5 sec, with a cutoff point of 150 μA and a time limit of 15 sec, until a jump out of the cage was elicited, at which point the stimulation was immediately stopped. The site to be used for the switch-off training was randomly chosen among those sites that revealed positive for escape reaction.

Switch-off training. Following the selection of the stimulation site, the rat was allowed a 10-min resting period. The rat was then shaped to press the bar in order to interrupt the electrical stimulation. The intensity was adjusted so as not to induce too violent a behavioral response. This initial shaping phase lasted until the rat stopped jumping out of the cage and proved able to reliably press the bar in order to stop the stimulation. The time limit was set at 1 hour for this initial shaping session. Rats that failed to be shaped during this initial session were shaped again on the next day. During following days, each rat was subjected to daily training sessions, in which it had to interrupt a series of 80 stimulations, at a fixed intensity of 150 μA. Initially, the IPI was fixed and individually adjusted for each rat, so as to produce a SOL of 6 to 8 sec for each stimulation. Once variability was no greater than 20%, the range of IPI's was progressively extended, according to individual performances, in order to obtain a final array of SOL's in the range of 1 sec (shorter IPI's) to 25 sec (longer IPI's). At the end of each of these variable IPI sessions, a plot of the SOL against the IPI was computed by the program. Intersessions stability was assessed by visual comparison of these plots. Once stability was acquired, rats were subjected to the pharmacological part of the experiment.

Pharmacological treatment. For this part of the study, data were obtained during measurement sessions, in all respects similar to the variable IPI sessions described above (80 stimulations, intensity: 150 μA, IPI range individually set for each rat so as to induce SOL's in the range of 1 to 25 sec). Two consecutive measurement sessions were separated by a minimum of 24 hr. Drugs or vehicle were injected IP (1 ml/kg body weight) 15 min before the start of the measurement session.

FG 7142 treatment was administered to 9 rats implanted in the PAG and 8 rats implanted in the MLR, at 3 doses: 2.5, 5 and 10 mg/kg body weight. FG 7142 was suspended in its vehicle (saline with addition of Tween 80, 1 drop/2 ml of saline) and thoroughly stirred. Injections (drug or vehicle) were made in a randomized order, with the only restriction that 2 drug injections were always separated by one vehicle injection.

CDP (5 mg/kg body weight) was administered to 10 rats with electrodes in the PAG and 7 rats implanted in the MLR. CDP was dissolved in its vehicle (saline). Rats implanted in the MLR were submitted to the vehicle injection first, PAG electrodes rats to the CDP injection first.

Data Analysis

For each measurement session, the program computed a logarithmic transformation of the SOL versus IPI function, yielding a linear relationship of the form: Ln (SOL) = a (IPI) + b (Ln: Napierian logarithm; a: slope; b: ordinate at the origin). Top panel of Fig. 1 gives a typical example of the SOL versus IPI relationship obtained during a measurement session, after logarithmic transformation. In addition, the program also calculated the mean SOL (mSOL) (see caption of top panel of Fig. 1 for definition and details of calculation).

For each rat, a differential switch-off latency (dSOL) was calculated for each dose of FG 7142 by subtracting the mSOL of

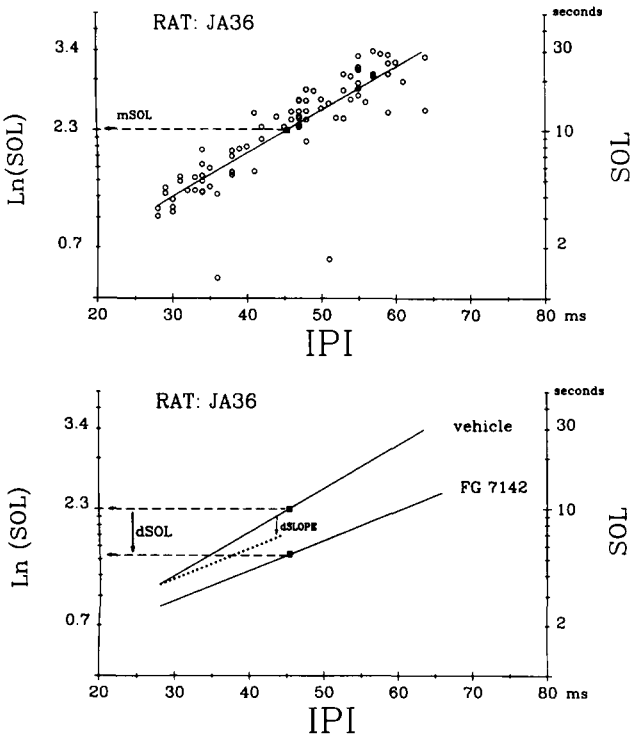

FIG. 1. Example of raw data and data treatment for measurement sessions. (Top panel) Napierian logarithm of switch-off latency [Ln (SOL)] as a function of interpulse interval (IPI), obtained during one vehicle session for a rat stimulated in the PAG. Each circle represents a SOL for a given IPI. The square in the middle represents the center of gravity of the cloud of circles, its "Y" coordinate corresponding to the mean switch-off latency (mSOL, with mSOL = 1/80 Σ Ln (SOL). A regression line (here, slope = 0.06) was fitted to the 80 individual circles. (Bottom panel) Regression lines of Ln (SOL) versus IPI functions for a vehicle session and the FG 7142 (5 mg/kg) session for the same rat. For clarity, individual circles have been omitted. The differential switch-off latency (dSOL) was calculated by subtracting the vehicle mSOL from the FG 7142 mSOL (here, dSOL = 1.68–2.31 = −0.63). The differential slope (dSLOPE) was calculated on the same principle (here, dSLOPE = 0.041 − 0.060 = −0.019). For both graphs, an additional "Y" axis in linear scale was reported on the right to give an indication of the SOL's values in seconds.

the immediately preceding or following (depending on the order of injection of each dose) vehicle session from the mSOL of the considered dose [dSOL = mSOL(dose) − mSOL(vehicle)]. dSOL for CDP was obtained by subtracting the mSOL of the vehicle session from the mSOL of the CDP session [dSOL = mSOL(CDP) − mSOL(vehicle)]. A differential slope (dSLOPE) was calculated on the same principle both for FG 7142 and CDP. An example of dSOL and dSLOPE calculations is treated in caption of bottom panel of Fig. 1.

For FG 7142, dSOL's and dSLOPE's were submitted to a one-way analysis of variance for repeated measures [P2V, BMDP Statistical Software, (14)] with one 3 levels within factor (3 doses of FG 7142). In addition, to check for stability of baseline responding, mSOL's obtained during all vehicle sessions were submitted to a similar P2V analysis. Lastly, to assess if FG 7142 differentially affects dSOL's and dSLOPE's depending on the structure stimulated (PAG or MLR), both parameters were subjected to a two-way analysis of variance for repeated measures [P2V, BMDP Statistical Software, (14)] with one 3 levels within factor (3 doses of FG 7142) and one in between factor (structure stimulated).

For CDP, dSOL's and dSLOPE's were submitted to a pairwise *t*-test [P3d, BMDP Statistical Software, (14)], as well as to a one-way analysis of variance [P2V, BMDP Statistical Software, (14)], with the structure stimulated (PAG or MLR) as the in between factor, again to assess if CDP differentially affects dSOL's and dSLOPE's according to the structure stimulated.

Histology

On completion of the experiments, rats were killed by an overdose of pentobarbital and perfused intracardially with NaCl 0.9% followed by 4% formalin. Serial 20 μm brain sections were stained with cresyl violet in order to localize the 34 stimulation sites used in the pharmacological treatment experiments. These sites were drawn on corresponding frontal planes of the Paxinos and Watson atlas (29).

RESULTS

Histology

The 34 stimulation sites used in this study are drawn on Fig. 2. All stimulation sites of rats bearing electrodes aimed for the PAG were found within the dorsal PAG. Stimulation sites for rats implanted in the MLR were lying in the cuneiform nucleus, pedunculopontine tegmental nucleus and immediately adjacent dorsolateral tegmentum.

Injection of Vehicle

No significant difference was detected in mSOL's between the 3 vehicle sessions.

Injection of FG 7142

FG 7142 decreased switch-off latencies for electrical stimulation of the PAG but not for electrical stimulation of the MLR. Average dSOL's for the 3 doses of FG 7142, for electrical stimulation of the PAG and the MLR, are shown in Fig. 3. For electrical stimulation of the PAG, FG 7142 significantly, $F(1,8) = 14.34$, $p<0.01$, affected the dSOL (average dSOL decrease for the 3 doses = −0.20), meaning that mSOL was significantly decreased under FG 7142. Although this decrease appears to be orderly related to the dose, the dose effect was found to be nonsignificant. By contrast, dSOL's were nonsignificantly affected by the treatment for MLR stimulation (average dSOL for the 3 doses = −0.01). FG 7142 was also found to differentially affect average dSOL's according to the structure stimulated, $F(1,15) = 8.59$, $p = 0.01$. Statistical analysis of dSLOPE's showed no significant change for the PAG (average dSLOPE for the 3 doses = −0.004), but revealed a very small (average dSLOPE for the 3 doses = −0.013) though significant decrease for the MLR, $F(1,6) = 14.68$, $p<0.01$.

Injection of Chlordiazepoxide

CDP increased switch-off latencies for electrical stimulation of the PAG but not for electrical stimulation of the MLR. Effects of 5 mg/kg of CDP on average dSOL's for electrical stimulation of the PAG and the MLR are represented in Fig. 3. CDP significantly affected dSOL's for electrical stimulation of the PAG only, $t(9) = 2.32$, $p<0.05$, which translates into a significant increase of mSOL under CDZ. dSOL's for electrical stimulation of the MLR were not significantly changed. Although the difference in effect of CDP on the dSOL produced either by PAG or MLR stimulation is of a magnitude of 12 (0.12 against 0.01), this differential effect turned out to be nonsignificant. For both structures, dSLOPE's were not significantly affected by CDZ.

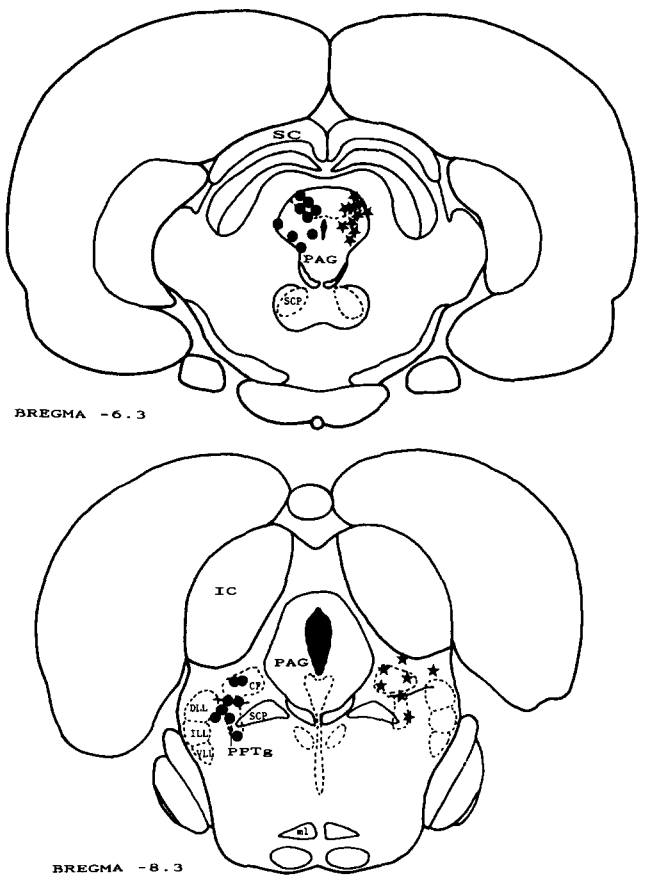

FIG. 2. Histological localization of the 34 stimulation sites. (●) Sites used in the FG 7142 treatment; (★) sites used in the CDP treatment. For clarity, all stimulation sites have been reported on the same side of the drawings, adapted from the atlas of Paxinos and Watson (29). Abbreviations: CF: cuneiform nucleus; DDL: dorsal nucleus of lateral lemniscus; IC: inferior colliculus; ILL: intermediate nucleus of lateral lemniscus; ml: medial lemniscus; PAG: periaqueductal gray; PPTg: pedunculopontine tegmental nucleus; SC: superior colliculus; SCP: superior cerebellar peduncle; VLL: ventral nucleus of lateral lemniscus.

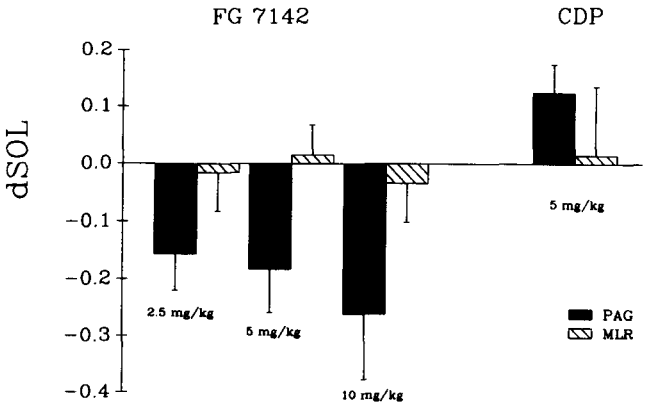

FIG. 3. Average effects of FG 7142 and chlordiazepoxide (CDP) treatments on the differential switch-off latency (dSOL). This figure shows the histogram of the average (±sem) values of dSOL's for FG 7142 (2.5, 5 and 10 mg/kg) and CDP (5 mg/kg) treatments for rats implanted in the PAG and rats implanted in the MLR.

DISCUSSION

The present results demonstrate that the mean switch-off latency (mSOL) induced by electrical stimulation of the PAG is decreased by FG 7142, a benzodiazepine (BZ) receptor inverse agonist, and increased by chlordiazepoxide, a BZ receptor agonist. By contrast, neither drug at the doses tested affected the mSOL induced by electrical stimulation of the MLR. These results suggest a difference in the pharmacological reactivity to BZ receptor ligands between the aversive states produced by electrical stimulation of the MLR or of the PAG.

Treatment with the beta carboline FG 7142, an inverse agonist of the BZ receptor, decreased the mSOL for electrical stimulation of the PAG. This decrease in mSOL can be interpreted as a potentiating effect of FG 7142 on the aversion generated by the electrical stimulation. This potentiating effect of FG 7142 is interesting from two points of view. 1) It is the first time, to our knowledge, that a BZ receptor inverse agonist is reported to potentiate the aversion induced by electrical stimulation of the PAG. 2) This potentiating effect of FG 7142 seems to be specific, and not the consequence of a drug-induced general excitatory effect or facilitatory effect on the effection of the operant response, since FG 7142 treatment did not decrease the mSOL of rats stimulated in the MLR. In contrast to FG 7142, 5 mg/kg of chlordiazepoxide (CDP), a BZ receptor agonist, increased the mSOL for electrical stimulation of the PAG. This CDP-induced increase in mSOL suggests an antiaversive effect of this drug on the electrically generated aversive state. Our results confirm those of a previous study in which low doses of CDP (3 to 10 mg/kg) were shown to be effective in increasing mean escape latencies from electrical stimulation of the PAG (32). The authors favored the hypothesis of a specific antiaversive effect of the drug, arguing that the doses they used were under the range causing major sedative or ataxic effects. Our present result is of nature to reinforce this interpretation since the same dose of CDP failed to affect the mSOL of rats stimulated in the MLR.

BZ receptor ligands are known to produce their pharmacological effects through a modulation of GABAergic neurotransmission (8,25). Consequently, it is possible that FG 7142 and CDP exert their respective aversivogenic and antiaversive effects by reducing and increasing GABAergic inhibition at the level of the PAG. There is in fact a bulk of evidence concerning the implication of a tonic GABAergic control exerted at the level of the PAG on the expression of aversively motivated behaviors. For example, local blockade of GABAergic transmission, by microinjections into the PAG of $GABA_A$ receptors antagonists, is known to produce escape reactions (3, 11, 12, 34). Also, microinjection into the PAG of semicarbazide, a GABA synthesis blocker, has been shown to induce a conditioned place aversion suggestive of the existence of an aversive state (13). On the other hand, enhancement of GABAergic transmission by microinjection into the PAG of BZ or $GABA_A$ receptor agonists was shown to increase the switch-off latency (11) or raise the PAG threshold electrical current for inducing escape reactions (1–3).

Unlike for PAG electrical stimulation, FG 7142 and CDP did not affect the mSOL of rats stimulated in the MLR. A slight but nonetheless significant decrease in the slope of the regression line of the Ln(SOL) versus IPI function was observed with MLR stimulations. Although this might reflect a tendency of FG 7142 to decrease SOL's for the longer IPI values, longer IPI's also produce greater dispersion of SOL's and therefore this effect has to be considered with caution. This lack of effect suggests that BZ receptors are not involved in the control of aversion produced by

MLR stimulation. Nonetheless, BZ receptors have been implicated in the control of locomotion, since MLR microinjections of diazepam were shown to suppress locomotion in mesencephalic animals (17, 18, 30). Additionally, $GABA_A$ receptors at the level of the MLR have been implicated in the genesis of escape reactions in the rat. In effect, microinjections into the MLR of $GABA_A$ antagonists such as bicuculline at the same doses as those used for the PAG (unpublished) or of picrotoxin (4) induced escape reactions.

Taken together, our results suggest a differential pharmacological sensitivity to BZ receptor ligands between aversive states induced by MLR stimulation or PAG stimulation. In a previous study (10), we already hypothesized the existence of a lower inhibition at the level of the MLR substrate than at the level of the PAG substrate, on the basis of a comparative parametric study of the switch-off latency. These differences may reflect differential activities of GABAergic neurotransmission within these two structures, but supportive comparative neurochemical studies are needed. It is interesting to mention, though, that the density of glutamic acid decarboxylase (GAD) immunoreactive terminals was found to be higher in the PAG than in both the cuneiform nucleus and pedunculopontine tegmental nucleus [see figures p. 479 and 480, (27)].

One step further, these results suggest that the aversive states elicited by electrical stimulation of each of the two structures are different in nature. A differential pharmacological sensitivity between two brain structures whose electrical activation induces aversive states (PAG and ventral reticular formation) has previously been reported in the literature (23). In this study, it was found that the aversive nature of PAG stimulation was increased by para-chlorophenylalanine, a serotonin-depleting drug. Conversely, alpha-methyl-para-tyrosine, a catecholamine-depleting drug, was only effective in reducing aversiveness of ventral reticular formation stimulation. A wealth of experimental data have confirmed the implication of the serotoninergic and GABA/BZ systems in the control of anxiety states (5, 7, 15, 16, 19, 20, 37, 38). In the light of such information, it would be reasonable to speculate that the aversive state generated by electrical stimulation of the PAG possesses an anxiogenic dimension which might be less pronounced or absent in the case of MLR stimulation.

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