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Opposed Regulation by Dorsal Raphe Nucleus 5-HT Pathways of Two Types of Fear in the Elevated T-maze

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GRAEFF, F. G., M. B. VIANA AND P. O. MORA. *Opposed regulation by dorsal raphe nucleus 5-HT pathways of two types of fear in the elevated T-maze.* PHARMACOL BIOCHEM BEHAV **53**(1) 171-177, 1996.—To investigate the influence of dorsal raphe nucleus (DRN) 5-HT pathways on different types of fear, we microinjected into the rat DRN the benzodiazepine inverse agonist FG 7142 and the excitatory amino acid kainic acid. In addition, we systemically administered the 5-HT releasing drug D-fenfluramine. The behavioral effects of these drugs were measured in an elevated T-maze, consisting of three arms of equal dimensions (50 × 10 cm), elevated 50 cm from the floor. One arm is enclosed by walls (40 cm) and stands perpendicular to the two open arms. Inhibitory (passive) avoidance—representing learned fear—was measured by placing a rat at the end of the enclosed arm and recording the time to withdraw from this arm with the four paws during three consecutive trials. Soon afterwards, the same animal was placed at the end of one of the open arms and the time to withdraw from this arm with the four paws was recorded. This one-way escape response represents unconditioned fear. Intra-DRN FG 7142 (40 pmol) facilitated inhibitory avoidance (anxiogenic effect), but did not affect one-way escape. Kainic acid (60 pmol) also facilitated inhibitory avoidance and, in addition, impaired one-way escape (anxiolytic effect). These effects are unlikely to be due to motor deficit, because intra-DRN kainate did not change locomotor activity and rearing behavior of rats placed inside a circular arena for 10 min. Finally, D-fenfluramine (0.03, 0.1, and 0.3 mg/kg, IP) tended to enhance inhibitory avoidance while depressing one-way escape in a dose-dependent way. Because the three drug treatments are believed to increase 5-HT release from DRN nerve terminals, these results support the hypothesis that ascending DRN 5-HT pathways facilitate learned fear while inhibiting unconditioned fear. The former may be related to generalized anxiety and the latter to panic disorder.

5-HT Dorsal raphe nucleus Types of fear/anxiety Elevated T-maze FG 7142 Kainic acid D-Fenfluramine

ALTHOUGH the participation of 5-HT in anxiety is generally acknowledged, there is no agreement of whether 5-HT facilitates or, conversely, decreases anxiety. In animal models that produce response inhibition, such as conflict tests, drugs or brain lesions that reduce 5-HT output have an anxiolytic effect, like the benzodiazepines [for reviews, see (9,11,17,19, 25)]. Also, microinjection of 5-HT receptor antagonists into the amygdala has been shown to release punished behavior, whereas 5-HT receptor stimulation increases response suppression (23,34,36). These results support the classical view that 5-HT is anxiogenic (44).

In contrast, animal models in which rats actively escape or avoid aversive brain stimulation point to an anxiolytic role for 5-HT (16). Thus, systemic administration of PCPA or 5-HT antagonists facilitates learned escape from electrical stimulation of the dorsal periaqueductal gray (DPAG) of the rat, while 5-HT reuptake blockers, 5-HTP and some 5-HT receptor agonists impair the same behavior. Even more clearly, intra-DPAG administration of 5-HT, postsynaptic 5-HT receptor agonists, 5-HT uptake blockers, and nerve ending autoreceptor blockers all raise the threshold of aversive electrical stimulation of the DPAG following their microinjection into the same brain structure. Therefore, 5-HT seems to inhibit aversion generated in the DPAG, a brain structure related to anxiety [for reviews and specific references see (11,13)].

Reported clinical evidence is also contradictory. Although

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the anxiolytic effect of benzodiazepine (BZD) agonists has been attributed to a decrease of 5-HT in pathways mediating punishment (44), antidepressant drugs that are likely to increase the efficacy of 5-HT neurotransmission (3) are clinically used to treat anxiety disorders (25,31). In double-blind, placebo-controlled studies, the 5-HT_2 receptor antagonist ritanserin has been shown to significantly improve generalized anxiety disorder (5), whereas panic disorder was either not affected (8) or significantly worsened (7) by the drug. Also, in human experimental anxiety, ritanserin markedly accelerated the extinction of skin conductance responses to an auditory conditioned aversive stimulus (20), whereas the rise in anxiety caused by speaking in front of a videocamera was prolonged (7).

A solution for these contradictions may lie in the heterogeneity of both 5-HT functions and anxiety conditions. In this respect, it was suggested (6,7,10) that the ascending 5-HT pathway originating in the dorsal raphe nucleus (DRN) and innervating the amygdala and frontal cortex facilitates active escape and avoidance behaviors that occur in response to potential or distal threat. Because these behavior strategies largely rely on learning, they may be related to conditioned or anticipatory anxiety and, perhaps, to generalized anxiety disorder. In contrast, the DRN–periventricular pathway that innervates the periventricular and periaqueductal gray matter would inhibit inborn flight or fight reactions arising in response to proximal danger, presumably related to panic disorder.

The experimental testing of this hypothesis in laboratory animals requires conditions in which different types of fear are generated. Because current animal models of anxiety do not clearly address to this question [for a critical review, see (16)], a new model has been recently developed (12,41). The apparatus, named the elevated T-maze, is derived from the elevated X- or plus-maze (18,33) and consists of three arms of equal dimensions elevated 50 cm from the floor. One of the arms is enclosed by walls and stands perpendicular to the two open, opposed arms. The procedure allows measurement, in the same animal, of both inhibitory (passive) avoidance of the open arms—thought to represent learned fear—and one-way escape from one of the open arms, a behavior that is seemingly motivated by the species-typical fear of openness and/or elevation (30). In validating studies (12,41), appropriate doses of the BZD anxiolytic diazepam and of the 5-HT_{1A} ligand ipsapirone markedly impaired inhibitory avoidance, but did not significantly change one-way escape behavior. These results indicate that different types of fear are indeed at play in the elevated T-maze.

The dual 5-HT fear hypothesis (6,7,10) outlined above predicts that simultaneous activation of the 5-HT pathways arising from the DRN will both increase learned anxiety and decrease innate fear. Thus, in the elevated T-maze inhibitory avoidance is expected to be enhanced, whereas one-way escape will be impaired. Three ways of promoting DRN 5-HT activity were used in this study: a) microinjection of the BZD inverse agonist FG 7142 into the DRN, to counteract tonic GABAergic inhibition of 5-HT neurons (24,40); b) microinjection into the DRN of a subtoxic dose of kainic acid, a glutamate analog that stimulates local neuron cells, but not fibers of passage (42); c) systemic administration of D-fenfluramine, a drug that seems to release 5-HT selectively from terminals of the DRN as compared to nerve fibers from the median raphe nucleus (MRN) (35). As a control for nonspecific motor impairment, we measured the effect of intra-DRN kainate on locomotor activity in a circular arena.

METHOD

Animals and Housing

Male Wistar rats, 220–270 g in weight, were housed in groups of five with food and water freely available. After brain cannula implantation, rats were housed in pairs. Lights were on from 0600 to 1800 h, and temperature was maintained at 23 ± 1°C.

Apparatus

The elevated T-maze was made of wood and had three arms of equal dimensions (50 × 10 cm). One arm, enclosed by walls 40 cm high, was perpendicular to two opposed open arms. To avoid falls, the open arms were surrounded by a Plexiglas rim 1 cm high. The whole apparatus was elevated 50 cm above the floor. The experiments were performed with an observer inside the room.

The arena consisted of a white circular floor, 72 cm in diameter, divided by black lines into 12 equal areas, and was surrounded by a 60-cm high wall made of transparent plastic. A videocamera placed above the center of the arena allowed videotape recording of the experimental session.

In both cases, illumination was provided by a fluorescent lamp of 40 W in the ceiling of the room, above the center of the apparatus. Environmental temperature was kept at 23 ± 1°C with an air conditioner that also produced background noise.

Surgery

Rats to be used in Experiments 1, 2, and 3 were anesthetized with sodium pentobarbital (50 mg/kg, IP) and received atropine (1 mg/kg) to block airway secretion. Their skull was fixed to a stereotaxic frame (David Kopf, Tujunga, CA) with the incisor bar held 2.5 mm below the interaural line. A cannula (outside diameter 0.6 mm, length 13.0 mm) was introduced at an angle of 26°C with the saggital plane, 3.2 mm lateral to lambda, until the tip was 5.5 mm below the surface of the skull. The cannula was attached to the bone with stainless steel screws and methylmethacrylate polymer cement. A stylet was introduced into the cannula to prevent obstruction.

Drugs and Injections

FG 7142 (FG, Ferrosan, Denmark) was dissolved in a saline-Tween 80 2% solution (vehicle). Kainic acid (KAIN, Sigma, St. Louis, MO) and D-fenfluramine (FEN, Servier, Brazil) were dissolved in saline (0.9% NaCl) for injection (pH: KAIN = 5.5, saline = 6.0). FEN was injected, IP, in a volume of 1 ml/kg body weight. FG and KAIN were microinjected intracerebrally. For this, a needle (outside diameter 0.3 mm) was introduced into the guide cannula until its tip was 2 mm below the cannula end. A volume of 0.2 μl was injected over a period of 2 min, using a 10 μl microsyringe (Hamilton, USA) attached to a microinfusion pump (Harvard Apparatus, USA). The displacement of an air bubble inside the polyethylene catheter connecting the syringe needle to the intracerebral needle was used to monitor the microinjection. The intracerebral needle was removed 1 min after the injection was finished.

Procedures

Experiment 1. On the fifth and sixth days after the surgery, animals were gently handled for 5 min. On the seventh day, each animal was microinjected with either 40 pmol FG (n =

11) or vehicle (n = 10). Rats were tested in the elevated T-maze as previously reported (41). Ten minutes after drug injection, the rat was placed at the end of the enclosed arm of the T-maze and the time taken to withdraw from this arm with the four paws was recorded (baseline latency). The same measurement was repeated in two subsequent trials (avoidance 1 and avoidance 2) performed at 30-s intervals. Following avoidance training (30 s), the rat was placed at the distal end of the right open arm of the maze, and the time taken to withdraw from this arm with the four paws to enter the enclosed arm was recorded (escape).

Experiment 2. The same as in Experiment 1, except that the rats were injected with either 60 pmol of KAIN (n = 8) or saline (n = 10) and placed in the maze immediately after the injection.

Experiment 3. Rats were injected with either 60 pmol KAIN (n = 5) or saline (n = 5). Immediately after the injection each rat was placed in the center of the circular arena and allowed to explore the environment for 10 min. Their behavior was recorded on videotape. Later, the tape was played back and the behavior of the rat observed on a television screen. The number of crossings of the lines dividing adjacent floor areas and number of rearings were counted.

Experiment 4. On the last 2 days before the experiment, naive rats were gently handled for 5 min. Animals were randomly assigned to four treatment groups of 12–13 rats, given 0.03, 0.1, and 0.3 mg/kg FEN or saline, respectively. Twenty five minutes after the injection, each animal was tested in the elevated T-maze, as described in Experiment 1.

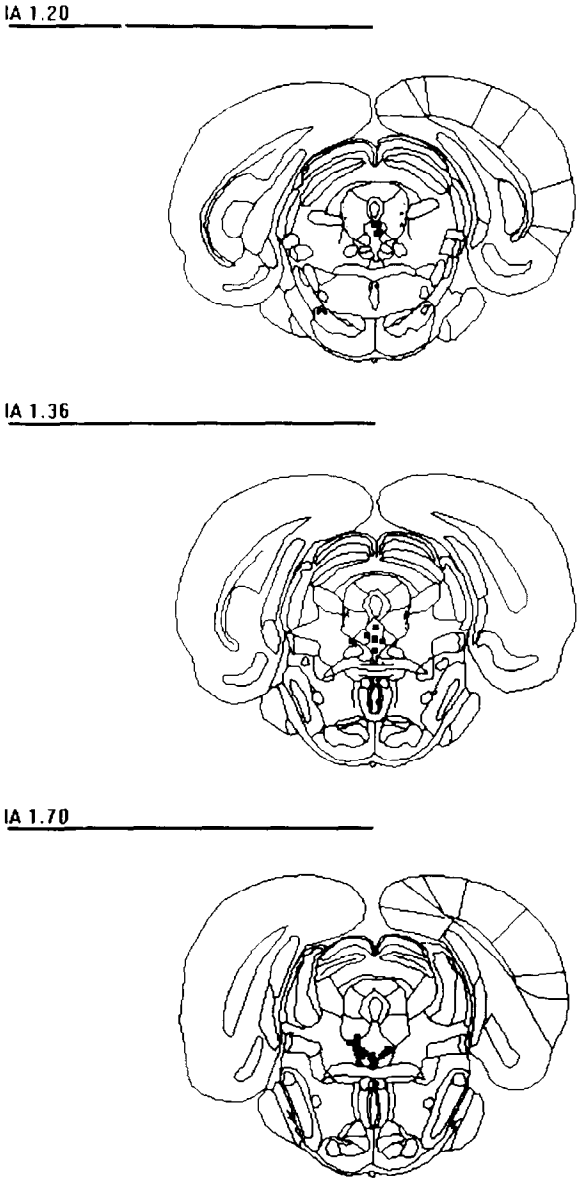

FIG. 1. Localization of injection sites inside diagrams from computer based atlas of the rat brain (4). Figures represent coordinates from Paxinos and Watson (32) rat brain atlas, anterior to the interaural line (IA).

Histology

Rats with an intracerebral cannula were sacrificed under deep anesthesia with ether. The brain was perfused through the heart with saline, followed by 10% formalin solution. The brains were removed and, after a minimum of three days, immersed in a 10% formalin solution, frozen sections of 60 μm were obtained in a cryostat (Cryocut 1800, Leica, Germany). The sections were placed on a glass slide and examined with a microscope under low magnification. Injection sites were localized in diagrams from the rat brain atlas of Paxinos and Watson (32).

Data Analysis

For avoidance latencies, a two-way between-within ANOVA was used. Escape latencies were analyzed by the unpaired Student's t-test (Experiments 1 and 2) or by one-way ANOVA followed by the Newman-Keuls test (Experiment 4). To analyze locomotor activity and rearings, unpaired Student's t-test was used (Experiment 3). A p-value of 0.05 or less was required for significance.

RESULTS

Localization of Injection Sites

The diagrams of Fig. 1 show the injection sites of rats intracerebrally injected with either KAIN, FG or respective vehicles and tested in the elevated T-maze. It may be seen that injection sites were either inside or at the border of the DRN. The sites of KAIN or saline microinjection in rats tested in the circular arena (not shown in Fig. 1) were similarly localized.

Experiment 1: Effect of Intraraphe FG 7142 in the Elevated T-Maze

As illustrated in Fig. 2, FG increased inhibitory avoidance latencies. Two-way ANOVA showed a significant effect of trials, $F(2, 38) = 12.59$, $p < 0.001$, and of drug treatment, $F(1, 19) = 6.99$, $p = 0.016$, but a nonsignificant drug × trial interaction, $F(2, 38) = 1.03$, $p = 0.366$. Thus, performance of inhibitory avoidance was facilitated, but avoidance learning was not affected by FG.

In contrast to inhibitory avoidance, one-way escape was not changed by FG. Escape latency was equal to 14.10 ± 1.36 s in the control group and 17.45 ± 2.95 s in the group treated with FG. The difference between the two means was not statistically significant, $t(19) = 1.00$, $p = 0.331$.

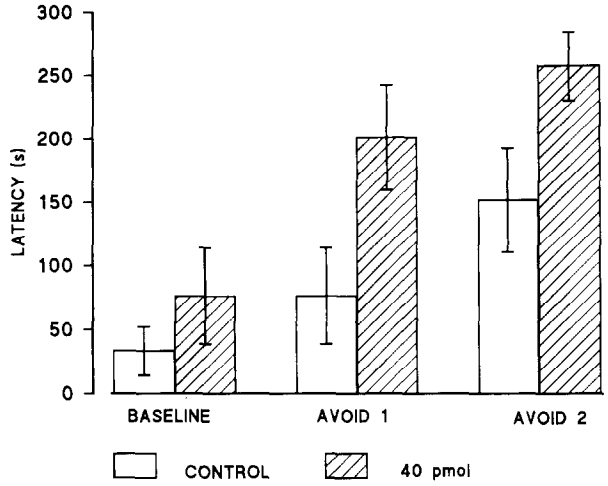

FIG. 2. Effect of FG 7142 (FG), microinjected into the dorsal raphe nucleus, on inhibitory (passive) avoidance of open arms in the elevated T-maze. Bars represent mean ± SEM of 10 rats for the control group and 11 rats for the group treated with 40 pmol FG. BASELINE, avoidance 1 (AVOID 1), and avoidance 2 (AVOID 2) latencies were measured at 30 s intervals, beginning 10 min after the intracerebral injection of either drug or vehicle.

Experiment 2: Effect of Intraraphe Kainic Acid in the Elevated T-Maze

Figure 3 shows the effect of KAIN on inhibitory avoidance. It may be seen that KAIN generally increased avoidance latencies. Two-way ANOVA showed a significant effect of trials, $F(2, 32) = 4.14$, $p = 0.025$, and of drug, $F(1, 16) = 10.51$, $p = 0.005$, but a nonsignificant drug × trial interaction, $F(2, 32) = 0.49$, $p = 0.618$. Therefore, in the same way as FG, KAIN enhanced inhibitory avoidance performance without affecting avoidance acquisition.

As shown in Fig. 4, one-way escape was impaired by KAIN because escape latency was significantly increased by the drug, $t(16) = 4.92$, $p = 0.041$.

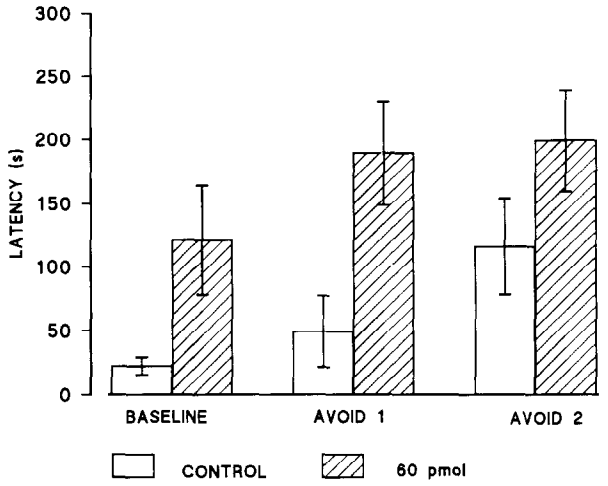

FIG. 3. Effect of kainic acid (KAIN), microinjected into the dorsal raphe nucleus, on inhibitory (passive) avoidance of open arms in the elevated T-maze. Bars represent mean ± SEM of 10 rats for the control group and 8 rats for the group treated with 60 pmol KAIN. BASELINE, avoidance 1 (AVOID 1) and avoidance 2 (AVOID 2) latencies were measured at 30 s intervals, beginning immediately after the intracerebral injection of either drug or saline.

Experiment 3: Motor Activity in the Circular Arena Following Intraraphe Kainic Acid

Table 1 shows that neither locomotor activity nor rearing behavior was significantly changed by intra-DRN injection of 60 pmol of KAIN. Statistical analysis showed nonsignificant differences between means for crossings, $t(8) = 0.53$, $p = 0.611$, as well as for rearings, $t(8) = 0.79$, $p = 0.452$. In two rats, the injection site was localized inside the MRN. In these animals, locomotion was markedly decreased: crossings equal to 11 and 2, respectively; rearings were absent in one rat and equal to 10 in the other.

Experiment 4: Effect of Systemic D-Flenfluramine in the Elevated T-Maze

The effect of FEN on inhibitory avoidance is shown in Fig. 5. Two-way ANOVA revealed a significant effect of trials, $F(2, 90) = 85.42$, $p < 0.001$, a nearly significant effect of drug, $F(3, 45) = 2.42$, $p = 0.079$, but a nonsignificant drug × trial interaction, $F(6, 90) = 1.57$, $p = 0.165$. A tendency for a dose-dependent facilitation of inhibitory avoidance may be seen in Fig. 5, except for the dose of 0.1 mg/kg.

As illustrated in Fig. 6, escape latency was increased in a dose-dependent way by FEN. One-way ANOVA showed a significant effect of treatment, $F(3, 45) = 3.39$, $p = 0.026$, and the Newman-Keuls test showed a significant difference between the control group and the group treated with 0.3 mg/kg FEN ($p < 0.05$).

DISCUSSION

The present results show that inhibitory avoidance and one-way escape in the elevated T-maze were differentially affected by the drug treatments used. Thus, microinjection into the region of the DRN of the BZD inverse agonist FG facilitated inhibitory avoidance or, in other words, had an anxio-

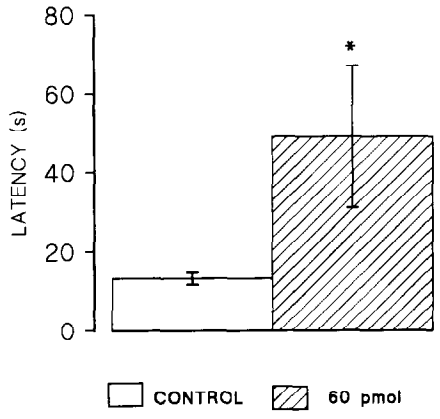

FIG. 4. Effect of kainic acid (KAIN), microinjected into the dorsal raphe nucleus, on escape from the open arm of the elevated T-maze. Escape latencies were measured 30 s after inhibitory avoidance training (see legend to Fig. 3). *$p < 0.05$ compared to control.

TABLE 1

LOCOMOTOR ACTIVITY AND REARINGS IN THE CIRCULAR ARENA DISPLAYED BY RATS MICROINJECTED WITH EITHER 60 pmol KAINIC ACID OR SALINE INTO THE DORSAL RAPHE NUCLEUS

Treatment	Crossings	Rearings	*n*
Kainic acid	94.0 ± 11.14	29.4 ± 8.11	5
Saline	85.0 ± 12.73	21.6 ± 5.52	5

genic effect. At the same time, escape behavior was not significantly changed by FG. Like the latter drug, intra-DR injection of KAIN markedly enhanced inhibitory avoidance performance. At variance with FG, however, KAIN simultaneously reduced one-way escape from the open arms, an effect that may be viewed as anxiolytic. Finally, the 5-HT releaser and uptake blocker FEN, given IP, tended to facilitate inhibitory avoidance while markedly increasing escape latencies in a dose-dependent way. In addition, previously reported results obtained under the same experimental conditions showed that the BZD agonist diazepam as well as the azapirone derivative ipsapirone impaired inhibitory avoidance, but did not change one-way escape behavior (12,41). Such pharmacological specificity of inhibitory avoidance and one-way escape behaviors, strongly suggests that two types of fear are generated by these tasks in the elevated T-maze.

The present anxiogenic effect of FG on inhibitory avoidance agrees with previously reported evidence (24) showing that another BZD inverse agonist β-CCM, similarly microinjected into the DRN, selectively reduced social interaction, an effect consistent with increased anxiety. Conversely, microinjection of BZD agonists into the same brain area has anxiolytic effects [e.g., (40)]. An anxiogenic effect on inhibitory avoidance was also determined by KAIN. Nevertheless, latencies of one-way escape were similarly prolonged by KAIN. Therefore, the drug could be nonspecifically impairing motor

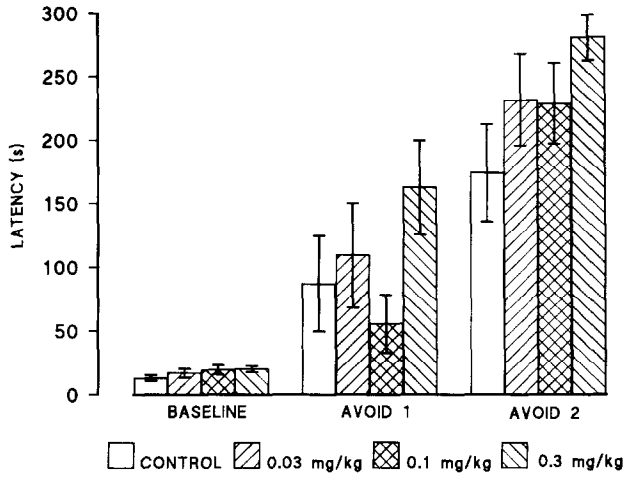

FIG. 5. Effect of D-fenfluramine (FEN), injected IP, on inhibitory avoidance of open arms in the elevated T-maze. Bars represent mean ± SEM of 13 rats for the group treated with 0.3 mg/kg FEN and of 12 rats for the remaining groups. BASELINE, avoidance 1 (AVOID 1) and avoidance 2 (AVOID 2) latencies were measured at 30 s intervals, beginning 25 min after the injection of either drug or saline.

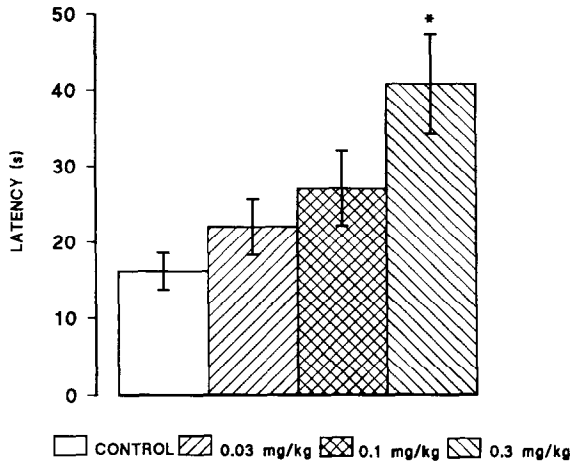

FIG. 6. Effect of D-fenfluranime, injected IP, on escape from the open arm of the elevated T-maze. Escape latencies were measured 30 s after inhibitory avoidance training (see legend to Fig. 5). *$p < 0.05$ compared to control.

function or alertness. This is unlikely, however, because the present results show that the same drug treatment did not change motor activity in rats placed inside a circular arena. This negative result contrasts with the depressant effect on locomotor and nose poking behaviors reported by Wirtshafter and McWilliams (43) following microinjection of KAIN into the MRN. Accordingly, in two rats presently injected into the MRN locomotor activity was markedly decreased. Therefore, neurons in the DRN do not seem to be involved in motor regulation. This conclusion agrees with reported results showing that intracerebral injection of the 5-HT_{1A} agonist 8-OHDPAT in the MRN, but not in the DRN caused hypermotility. Because this drug stimulates 5-HT_{1A} autosomic receptors, the last results indicate that 5-HT neurons in the MRN inhibit motor activity in a tonic way (22).

Because the MRN is anatomically close to the DRN, it may also be deduced from the above evidence that, in the present results, KAIN activated neurons within a relatively short range from the injection site. More direct evidence for this comes from results of a study with c-*fos* immunocytochemistry recently performed by Silveira et al. (37). Using the same volume and rate of injection as in the present work, these authors found that 60 pmol of KAIN caused intense labeling of cell nuclei—indicating neuronal activation—only within approximately a 0.5 mm radius from the center of the injection site. In contrast, 120 pmol KAIN caused a clear zone of about 200 μm around the injection site—indicating functional impairment—surrounded by a halo of darkened nuclei, signaling neuronal activation. Hence, the choice of 60 pmol as the dose of KAIN suitable for the present study.

In terms of 5-HT mechanisms, intraraphe FG and KAIN as well as systemically administered FEN supposedly increase 5-HT release from terminals of axons arising from neurons in the DRN. On the basis of the dual 5-HT fear hypothesis (6,7,10), summarized in the introductory paragraphs, it was expected that inhibitory avoidance—representing learned fear/anxiety—would be enhanced, whereas one-way escape—representing unconditioned fear—would be impaired by these drug treatments. The effects of KAIN and, seemingly, of the highest dose of FEN (see Figs. 5 and 6) entirely fulfill these

expectations. However, FG enhanced inhibitory avoidance, but did not affect escape behavior. This may be due to insufficient dosage, but may also result from differences in mode of action among the drugs. The excitatory amino acid KAIN acts on specific receptors stimulating all kinds of neurons (45). Due to the high density of 5-HT-containing cell bodies in the DRN, a major activation of the 5-HT systems arising from this nucleus is likely to happen. In turn, FEN is uptaken into nerve terminals where it actively releases 5-HT and, in addition, blocks 5-HT reuptake, thus prolonging the availability of 5-HT in the synaptic cleft (2). In both these instances, 5-HT is released independently of predrug firing rate of the neuron. In contrast, intra-DRN FG is believed to attenuate GABA-mediated inhibition, which exerts a tonic inhibitory action on 5-HT neurons of the DRN (24,40). As a consequence, the cell rate of firing is increased, promoting impulse-dependent 5-HT release. It is possible that rats forcefully exposed to the open arm of the T-maze have DR 5-HT cells already activated (or disinhibited). Hence, the lack of effect of FG on one-way escape behavior shown by the present results.

Experimental evidence recently reported by Maier et al. (26) further supports the notion that DR 5-HT neurons exert opposing actions on learned and nonlearned fear, respectively. Having established that rats exposed to inescapable shock developed both escape deficit and enhanced fear conditioning, these workers assumed—in conformity with the dual 5-HT fear hypothesis (6,7,10)—that this could be due to sensitization of DRN 5-HT neurons. Attempting to reverse this condition, Maier et al. (26) gave microinjections of chlordiazepoxide into the DRN and blocked both enhanced fear conditioning and escape deficit, regardless of the drug being administered before the inescapable shocks or before testing. They attribute these effects to restoration of GABA-mediated inhibition of DRN 5-HT neurons, resulting in less 5-HT being released in both the amygdala and the DPAG.

The present use of FEN as a tool for testing the dual 5-HT fear hypothesis relies on the assumption that FEN releases 5-HT selectively from DRN terminals. Mamounas et al. (27) have shown that nerve endings from the DRN and from the MRN differ not only morphologically, but also in the sensitivity to the neurotoxic effect of halogenated amphetamines, among which are para-chloramphetamine (PCA) and FEN; only DRN terminals are lesioned by these compounds. Using in vivo microdialysis, Grahame-Smith and co-workers (35) showed that FEN-induced 5-HT and 5-HIAA release in the frontal cortex of anesthetized rats was abolished after PCA treatment, indicating that only DRN 5-HT terminals were affected by FEN. In agreement, unpublished results obtained by two of us (M.B.V. and F.G.G.), in collaboration with R. Silveira, from the Instituto de Investigaciones Biologicas Clemente Estable, Montevideo, Uruguay, showed that 10 mg/kg FEN, IP—the same dose used by Series et al. (35)—markedly increased the concentration of 5-HT in the dialysate from the amygdala-pallidum region, which is chiefly innervated by the DRN (1). In contrast, the amount of these compounds in the dialysate of the dorsal hippocampus, a structure that receives 5-HT terminals mainly from the MRN (1), remained unchanged. Therefore, systemically administered FEN seems to release 5-HT preferentially from fibers of the DRN. However, the dose used in the neurochemical experiments was many times higher than those presently used. Although anesthesia and lower sensitivity of biochemical as compared to behavioral measurements may explain the discrepancy, further microdialysis studies, specially in awake animals, are needed to verify whether low doses of FEN release 5-HT selectively from DR fibers.

Because FEN is currently utilized clinically for reducing excessive food intake (29), the drug has been recently used to test the dual 5-HT fear hypothesis (6,7,10) in healthy volunteers. The subjects were exposed to two experimental situations intended to generate different types of anxiety. One was a conditioned fear test that measures changes in skin conductance in response to a tone associated once with an aversive noise (15). The other was a simulated public speaking test—speaking in front of a videocamera—which is supposed to represent unconditioned anxiety (14,28). It was found that FEN markedly attenuated the rise in anxiety produced by public speaking (21) while having a mild anxiogenic effect in the conditioned fear test (Hetem, De Souza, Guimarães, Zuardi, and Graeff, unpublished observations).

The correspondence between these human experimental results and the effects of FEN presently described in the elevated T-maze is remarkable, and may have therapeutic implications. If it is true that one-way escape in the elevated T-maze and simulated public speaking relate to panic disorder (6,7,10,13), the anxiolytic effect of FEN in both conditions points to a therapeutic potential of this drug in panic disorder. In agreement, an open study recently reported by Solyom (38) showed that chronic administration of D,L-fenfluramine improves panic disorder. In contrast, however, acute D,L-fenfluramine has been shown to induce anxiety in panic disorder patients (39). Therefore, further studies on the effect of FEN in panic disorder are warranted.

ACKNOWLEDGEMENTS

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