Pergamon

Pharmacology Biochemistry and Behavior, Vol. 52, No. 1, pp. 1–6, 1995

0091-3057/95 $9.50 + .00

0091-3057(94)00402-1

Role of 5-HT Receptor Subtypes in the Modulation of Dorsal Periaqueductal Gray Generated Aversion

R. L. NOGUEIRA AND F. G. GRAEFF[1]

Laboratório de Neuropsicofarmacologia, FFCLRP e Núcleo de Neurociências e Comportamento da Universidade de São Paulo, 14040-901 Ribeirão Preto, SP, Brazil

Received 11 July 1994; Revised 18 October 1994; Accepted 18 October 1994

NOGUEIRA, R. L. AND F. G. GRAEFF. *Role of 5-HT receptor subtypes in the modulation of dorsal periaqueductal gray generated aversion.* PHARMACOL BIOCHEM BEHAV **52**(1) 1–6, 1995.—To explore the role of 5-HT receptor subtypes in controlling aversion, we measured the effect of 5-HT_{1A} and 5-$HT_{2A/2C}$ receptor agonists microinjected into the dorsal periaqueductal gray (DPAG) of rats on aversive behavior induced by electrical stimulation of the same brain area. The 5-HT_{1A} agonists 8-OH-DPAT (4–16 nmol) and BAY-R-1531 (4–16 nmol) raised the threshold of aversive electrical stimulation in a dose-dependent way. Similarly, microinjection of the 5-$HT_{2A/2C}$ agonist DOI (4–16 nmol) increased the aversive threshold in a dose-dependent way. In contrast, the 5-HT_{2C} agonist mCPP (16 and 32 nmol) was ineffective. Previous intra-DPAG administration of the 5-HT_{1A} receptor blocker NAN-190 (40 nmol) antagonized the antiaversive effect of 8-OH-DPAT (8 nmol), whereas pretreatment with the 5-HT_{2A} receptor blocker spiperone (10 nmol) antagonized the effect of DOI (16 nmol). Spiperone also counteracted the effect of 8-OH-DPAT and NAN-190 counteracted the effect of DOI. These results indicate that activation of 5-HT_{1A} and 5-HT_{2A} receptors inhibits aversion in the DPAG and that both receptors have to be functional for the expression of each one's activation to occur.

Aversive brain stimulation Rat Dorsal periaqueductal gray 5-HT_{1A} receptor 5-HT_{2A} receptor

THE PERIAQUEDUCTAL gray matter of the midbrain integrates behavioral and neurovegetative manifestations of the defense reaction, and electrical stimulation of its dorsal division has strong aversive effects. Several neurotransmitters have been implicated in the modulation of aversion generated in the dorsal periaqueductal gray (DPAG), among them GABA, excitatory amino acids, serotonin (5-HT), and opioid peptides [for a review, see (14)].

In regard to 5-HT, there is abundant experimental evidence indicating that this amine inhibits aversion in the DPAG. Early results obtained by Kiser and coworkers (26,27) have shown that intraperitonial injection of the 5-HT precursor 5-hydroxitryptophan (5-HTP), as well as of the 5-HT uptake inhibitor chlorimipramine, decreased rat lever-pressing behavior that reduced, step by step, the intensity of aversive electrical current applied to the DPAG. Conversely, the 5-HT synthesis inhibitor PCPA facilitated lever-pressing escape from DPAG electrical stimulation. The latter effect was also caused by nonselective 5-HT receptor antagonists, such as methysergide and cyproheptadine (11,33). More recently, Jenck and coworkers (23–25) reported that the relatively selective 5-HT_{2A} receptor antagonists ketanserin, trazodone, and spiperone or the 5-HT_{2C} receptor agonists mCPP and DOI (also a 5-HT_{2A} agonist) had antiaversive effects in rats trained to jump from one side of a shuttle-box to the other to switch off DPAG electrical stimulation. Conversely, the 5-HT_{1A} receptor agonist 8-OH-DPAT or the mixed 5-$HT_{2A/2C}$ receptor antagonists ritanserin, cyproheptadine, and mianserin had proaversive effects. With systemic administration, however, it is not possible to ascertain whether these drugs were acting in the DPAG or elsewhere in the brain.

Using microinjection of drugs directly into the DPAG, Schütz et al. (34) have shown that 5-HT administration raised in a dose-dependent way the threshold of electrical current inducing flight when applied to the same brain area. The nonselective 5-HT receptor agonist 5-MeODMT was more potent

[1] Requests for reprints should be addressed to F. G. Graeff, Laboratório de Neuropsicofarmacologia, FFCLRP, Campus USP, Ribeirão Preto, SP, Brazil, 14040-901.

and more effective than 5-HT. Both the nonselective 5-HT antagonist methergoline and the 5-$HT_{2A/2C}$ antagonist ketanserin prevented the antiaversive effect of 5-HT at a dose level that did not affect the aversive threshold when the antagonists were given alone. Similarly, increasing the activity of 5-HT from nerve fibers innervating the DPAG (8) by either inhibiting 5-HT reuptake with zimelidine or promoting 5-HT release with the presynaptic receptor [5-HT_{1B} (13)] blockers propranolol and isamoltane had antiaversive effects that were blocked by ketanserin or ritanserin (3,30). Furthermore, in conformity with the results obtained using aversive brain stimulation, microinjection of either 5-MeODMT or propranolol into the DPAG of the rat had an anxiolytic effect in the elevated plus-maze (19), antagonized by local pretreatment with ritanserin (4,17). These results indicate that activation of postsynaptic 5-$HT_{2A/2C}$ receptors in the DPAG inhibits aversion.

Although the evidence described to date strongly implicates 5-$HT_{2A/2C}$ receptors, reported behavioral as well as electrophysiological results suggest that 5-HT_{1A} receptors also participate in the regulation of aversion generated in the DPAG (7,10,28). To further analyze the role of 5-HT receptor subtypes in aversion, we measured the effect of selective agonists of 5-HT_{1A} (8-OH-DPAT, BAY-R-1531) and of 5-$HT_{2A/2C}$ (DOI) receptors microinjected into the DPAG of rats on aversive threshold of electrical stimulation applied to the same brain region. The 5-HT_{2C} agonist mCPP was also studied. In addition, we investigated the combination of 8-OH-DPAT with the selective 5-HT_{1A} antagonist NAN-190, and that of DOI with spiperone, a 5-HT antagonist that has nearly 1000 times more affinity for 5-HT_{2A} than for 5-HT_{2C} receptors (32). Finally, the interaction between 5-HT_{1A} and 5-HT_{2A} receptors was analyzed by combining 8-OH-DPAT with spiperone and DOI with NAN-190.

METHOD

Animals and Housing

Male albino Wistar rats, weighing 200–250 g, were housed in pairs, with free access to food and water, under a 12L : 12D cycle (lights on at 0600 h). The temperature in the animal room was maintained at 23 ± 1°C.

Surgery

Rats anesthetized with sodium pentobarbital (40 mg/kg, IP) were operated in a stereotaxic instrument (David Koft, USA) and a chemitrode was implanted in the DPAG. The chemitrode was made of stainless steel guide cannula (outside diameter 0.6 mm, length 12.5 mm) glued to a brain electrode made of stainless steel wire (diameter 250 μm), enamel insulated except at the cross-section of the tip, reaching 1 mm below the lower end of the cannula. The electrode wire was connected to a male pin, parallel to the outer end of the cannula, that could be plugged into an amphenol socket at the end of a flexible electrical cable and used for brain stimulation. Holding the incisor bar 2.4 mm below the interaural line, the chemitrode was introduced 1.9 mm lateral to lambda, at an angle of 22° with the sagittal plane, until the electrode tip was 5.2 mm below the surface of the skull. The chemitrode was attached to the bone with stainless steel screws and methylmethacrylate polymer cement. A stylet with the same length of the guide cannula was introduced inside it to prevent obstruction.

Apparatus

A shuttle-box consisting of two compartments of 25 × 20 × 22 cm, with no barrier between them, was placed inside an insulating chest provided with fan and a wide-angle lens allowing one-way vision. During the experiments, the compartments were indirectly illuminated by a 5-W red lamp. The grid floor of the shuttle-box tilted around the midline axis whenever a rat passed from one compartment to the other. This movement closed a microswitch placed under the floor, which was connected to electromechanical programming and recording equipment (Grason-Stadler, USA).

Brain stimuli were generated by a sine-wave stimulator (29). The stimulation current (peak to peak) was monitored on the screen of an oscilloscope (Beckman Industrial, Korea). The brain electrode was connected to the stimulator by means of a mercury swivel and a flexible cable, allowing ample movement of the animal inside the box.

Procedure

Five days after surgery, the rats were handled for 10 min and placed inside the shuttle-box for 20 min. The experiment was performed on the next day. For the determination of the aversive threshold, a method modified from Audi and Graeff (2) was used. Five minutes after the rat was placed inside the shuttle-box a sham intracerebral injection was made (see below). Ten minutes after the injection, a series of 10 electrical stimuli (AC, 60 Hz) was presented through the implanted electrode. The interstimulus interval was 10 s whereas the interval between successive series was 1 min. Whenever the rat crossed the midline of the shuttle-box, ongoing brain stimulation was automatically switched off. If no switch-off response occurred, the stimulus lasted 10 s. Switch-off responses were recorded by a digital counter. The current intensify started at the subthreshold level of 20 μA, peak to peak, equal to 7.1 μA root mean square (RMS). The current intensity was increased by steps of 2.8 μA RMS until the rat made a switch-off response. Thereafter, the current intensity was increased if the rat failed to switch off twice within a series of 10 stimuli. Otherwise, the current intensity was kept constant along the series of 10 stimuli. The aversive threshold was the lowest current intensity causing 9 or 10 switch-off responses in a series of 10 stimuli. Animals with a basal threshold above 42.4 μA RMS were discarded. With this method, determination of the aversive threshold took nearly 15 min. Following the threshold determination, rats stayed inside the shuttle-box and received one or two intracerebral injections. The aversive threshold was redetermined either 10 or 20 (DOI) min after the last (or the only) injection.

Intracerebral Injection

For drug injection into the DPAG, a needle (outside diameter 0.3 mm) was introduced through the guide cannula until its tip was 1 mm below the cannula end. A volume of 0.5 μl was injected in 30 s (for 16 nmol 8-OH-DPAT, 1 μl in 60 s), using a 10-μl microsyringe (Hamilton, USA), its embolus being pushed by a micrometer. The same procedure was used for sham injections, except that the tip needle did not penetrate into nervous tissue and no volume was injected. 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 60 s after the injection was finished. When a second injection was given, a 10-min interval was allowed between the injections.

Histology

After the experiment the rat was sacrificed under deep anesthesia with ether. The brain was perfused through the heart

with saline solution (0.9% NaCl), followed by 10% formalin solution, before being removed and fixed in 10% formalin. Frozen sections of 55 μm were placed on a glass slide and examined with a microscope under low magnification. Electrode placements were localized in diagrams of the Paxinos and Watson (31) rat brain atlas. Only the data from rats having electrode tips inside or near the border of the DPAG were used for calculations.

Drugs

The following drugs were used: (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT, RBI, USA), 6-methoxy-4-(di-*n*-propylamino)-1,3,4,5-tetrahydrobenz(*c,d*) indole hydrochloride (BAY-R-1531, Bayer, Germany), (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI, RBI), 1-(*m*-chlorophenyl)piperazine dihydrochloride (mCPP, RBI), 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine hydrobromide (NAN-190, RBI), and spiperone hydrochloride (RBI).

A microsuspension of NAN-190 and spiperone in saline containing 2% Tween 80 was used for intracerebral injection. The remaining drugs were dissolved in sterile saline.

Statistical Analysis

Single factor analyses of variance (ANOVA) were performed. For post hoc comparisons the multiple range test of Newman-Keuls was used. A value of $p \leq 0.05$ was required for significance.

RESULTS

Behavioral Effects of DPAG Electrical Stimulation

Electrical stimulation of the DPAG caused the following sequence of behavioral changes as the intensity of electrical current increased: first alert, then freezing, and finally running, which stopped as soon as the brain stimulation was switched off. The basal aversive threshold, measured after the sham injection, was 20.82 ± 0.54 μA (RMS) for all 287 rats used.

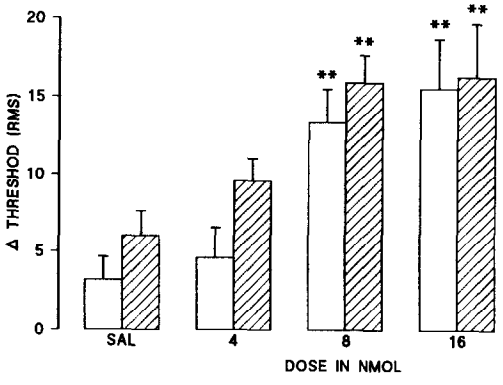

FIG. 1. Dose-dependent increases in aversive threshold produced by microinjection (0.5 μl in 30 s) into the dorsal periaqueductal gray (DPAG) of the 5-HT_{1A} receptor agonists 8-OH-DPAT (open columns) and BAY-R-1531 (striped columns). The aversive threshold was the lowest current intensity inducing at least nine midline crossings (switch-off responses) in 10 successive trials with electrical stimulation (AC, 60 Hz) applied to the DPAG of rats placed inside a shuttle-box. For each animal, the drug-induced change in threshold (in μA, RMS) was the difference between the value measured 10 min after drug administration and the basal threshold determined 10 min after a sham intracerebral injection. Columns represent the mean and vertical lines the SEM for 12 rats. SAL indicates control saline injection. **$p < 0.01$ compared to respective control by the Newman-Keuls test.

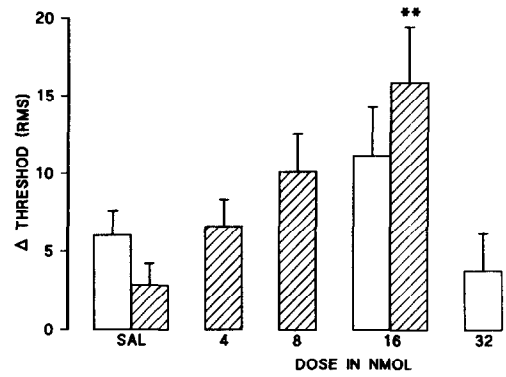

FIG. 2. Effect on aversive threshold of intra-DPAG microinjection of the 5-$HT_{2A/2C}$ receptor agonist DOI (striped columns) and of the preferential 5-HT_{2C} receptor agonist mCPP (open columns). For each animal, the drug-induced change in threshold (in μA, RMS) was the difference between the value measured 10 min (mCPP) or 20 min (DOI) after drug administration and the basal threshold determined 10 min after a sham intracerebral injection. Columns represent the mean and vertical lines the SEM for 12 rats. SAL indicates control saline injection. **$p < 0.01$ compared to respective control by the Newman-Keuls test.

Effect of 5-HT Receptor Agonists on Aversion

As shown in Fig. 1, the two 5-HT_{1A} agonists used raised the aversive threshold of DPAG electrical stimulation in a dose-dependent way. ANOVA showed a significant drug effect for BAY-R-1531, $F(3, 44) = 5.40, p = 0.003$, and 8-OH-DPAT, $F(3, 44) = 7.34, p = 0.0004$.

It can be seen in Fig. 2 that the 5-HT_2 agonist DOI also had a dose-dependent antiaversive effect following its microinjection into the DPAG. ANOVA showed a significant drug effect, $F(3, 44) = 5.03, p = 0.0044$. In contrast, the doses of 16 and 32 nmol of the 5-HT_{2C} agonist mCPP did not significantly change the aversive threshold, $F(2, 33) = 2.45, p = 0.1021$.

Antagonism of the Antiaversive Effect of 8-OH-DPAT and DOI

The upper panel of Fig. 3 shows that pretreatment with either NAN-190 or spiperone antagonized the antiaversive effect of 8-OH-DPAT. ANOVA showed a significant overall drug effect, $F(4, 55) = 6.96, p = 0.0001$. Multiple comparisons with the Newman-Keuls test showed a significant antiaversive effect of 8 nmol of 8-OH-DPAT (VEH + DPAT vs. VEH + SAL, $p < 0.01$) and a significant attenuation of this effect by 40 nmol of NAN-190 (NAN + DPAT vs. VEH + DPAT, $p < 0.01$) and by 10 nmol of spiperone (SPIPER + DPAT vs. VEH + DPAT, $p < 0.01$).

The lower panel of Fig. 3 similarly shows that pretreatment with either spiperone or NAN-190 antagonized the antiaversive effect of DOI. ANOVA showed a significant overall drug effect, $F(4, 54) = 5.15, p = 0.0014$. Multiple comparisons with the Newman-Keuls test showed a significant antiaversive

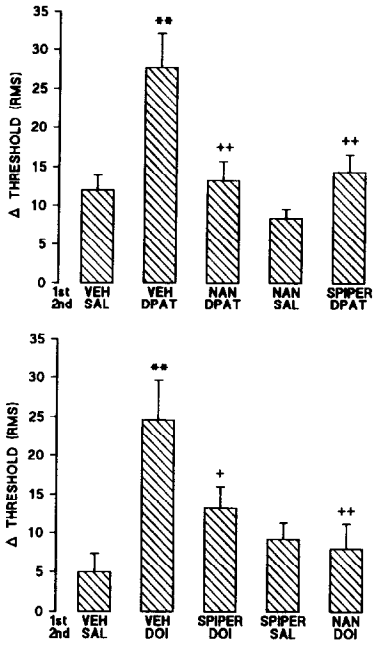

FIG. 3. Antagonism of the antiaversive effect of 8 nmol 8-OH-DPAT (upper panel) or of 16 nmol DOI (lower panel) by NAN-190 (40 nmol) and spiperone (SPIPER, 10 nmol). The drug antagonists or vehicle (VEH, 2% Tween in saline) were injected 10 min before the agonists or saline (SAL). The posttreatment threshold was determined 10 min after 8-OH-DPAT and 20 min after DOI injection. Columns represent the mean and vertical lines the SEM of 11 rats for the NAN + DOI group and 12 for the remaining groups. Other specifications are in the legend of Fig. 1. $^{**}p < 0.01$ compared to control (VEH + SAL), $+p < 0.05$, $++p < 0.01$ compared to 8-OH-DPAT (VEH + DPAT) or DOI (VEH + DOI) by the Newman-Keuls test.

effect of 16 nmol of DOI (VEH + DOI vs. VEH + SAL, $p < 0.01$) and a significant reduction of this effect by spiperone (SPIPER + DOI vs. VEH + DOI, $p < 0.05$) and by NAN-190 (NAN + DOI vs. VEH + DOI, $p < 0.01$).

Neither NAN-190 nor spiperone, alone, significantly changed the aversive threshold (NAN + SAL or SPIPER + SAL vs. VEH + SAL, $p > 0.05$).

DISCUSSION

In the present study, two 5-HT_{1A} agonists, 8-OH-DPAT and BAY-R-1531, raised the aversive threshold of DPAG electrical stimulation in a dose-dependent way following their microinjection into the same brain area. Because components of the 5-HT behavioral syndrome, such as flat body posture, did not occur following microinjection of these drugs into the DPAG, the observed drug effect does not seem to be due to locomotion impairment. Moreover, the present results agree with reported evidence showing that intra-DPAG administration of 5-HT_{1A} agonists (8-OH-DPAT and 5-CT) attenuated escape behavior induced by local microinjection of the excitatory amino acid D,L-homocisteic acid (7). Therefore, activation of 5-HT_{1A} receptors is likely to inhibit aversion generated in the DPAG. Furthermore, these receptors may be located postsynaptically, because the antiaversive effect of 8-OH-DPAT was antagonized by NAN-190, at a dose that was ineffective when given alone. Reported evidence indicates that NAN-190 acts as an antagonist on postsynaptic 5-HT_{1A} receptors, yet behaves as a partial agonist on autosomic receptors of the dorsal raphe nucleus, causing cell firing inhibition, per se (18).

Nevertheless, stimulation of 5-$HT_{2A/2C}$ receptors also seems to determine antiaversive effects, because in the present study the 5-$HT_{2A/2C}$ agonist DOI caused dose-dependent increases in aversive threshold when microinjected into the DPAG. In addition, it has been shown that the antiaversive effect of either 5-HT or drugs that increase 5-HT concentration in the synaptic cleft is antagonized by 5-$HT_{2A/2C}$ receptor blockers, such as ketanserin and/or ritanserin (3,4,30,34). In addition, the antiaversive effect of DOI was counteracted by spiperone, a drug that has nearly 1000 times more affinity for 5-HT_{2A} than for 5-HT_{2C} receptors (32). Therefore, 5-HT_{2A} receptors are likely to be mainly responsible for the inhibition of aversion in the DPAG.

Concerning 5-HT_{2C} receptors, the present results show that neither 16 nor 32 nmol of mCPP decreased the aversive threshold of DPAG electrical stimulation. However, Marsden and coworkers reported that intra-DPAG administration of mCPP (20 nmol in 250 nl, 10 min before the test) enhanced the defense response induced by local application of the excitatory amino acid D,L-homocisteic acid (6), indicating a proaversive role of 5-HT_{2C} receptors in the DPAG. The different methods used in these studies may explain the discrepancy between the latter and the present results. So far, only nonsignificant trends towards aversive threshold decrease have been observed with the procedure used in the present study [e.g., (30)], indicating that this method may not be able to detect proaversive drug effects. Nonetheless, the interpretation of mCPP action is obscured by the low specificity of this drug as regards 5-HT receptor subtypes. In binding assays mCPP displays considerable affinity for 5-HT_{2A} and 5-HT_{1B}, in addition to 5-HT_{2C} receptors (13,21). For instance, it has been shown that presumed 5-HT_{1B} receptor blockade with propranolol or isamoltane produces antiaversive or anxiolytic effects (3,4,30). Therefore, stimulation of 5-HT_{1B} receptors localized on nerve endings may decrease 5-HT release (13), resulting in proaversive effects.

Altogether, the experimental evidence discussed to date indicates that activation of both 5-HT_{1A} and 5-HT_{2A} receptors mediates the antiaversive effect of 5-HT in the DPAG. Two electrophysiological studies (10,28) strongly support this assumption, because they revealed the existence of two types of neurons responsive to 5-HT in the DPAG and adjacent midbrain tectum. The first type displays a very low level of spontaneous activity and is excited by 5-HT. Because this excitatory effect was blocked by pretreatment with ketanserin and mimicked by α-methyl-5-HT, it is likely to be mediated by 5-$HT_{2A/2C}$ receptors. In contrast, the second type of neuron has its baseline activity—either spontaneous or evoked by iontophoretic application of D,L-homocisteic acid—inhibited by 5-HT as well as by the 5-HT_{1A} agonists, 8-OH-DPAT and gepirone. Why both an excitatory effect and an inhibitory effect at the cellular level result in attenuation of aversion is not clear, although a likely possibility is the excitation of inhibitory interneurons through 5-HT_{2A} receptors. This arrangement has already been described in the cerebral cortex (35).

Admitting that 5-HT can affect either 5-HT_{1A} or 5-HT_{2A} receptors to produce antiaversive effects in the DPAG, it is quite puzzling that in previous studies the direct or indirect effect of 5-HT was blocked by 5-$HT_{2A/2C}$ antagonists alone (3,4,30,34). In this regard, however, the present results show that the effect of 8-OH-DPAT—a 5-HT_{1A} agonist—was blocked by the 5-HT_{2A} antagonist spiperone, whereas that of

the 5-$HT_{2A/2C}$ agonist DOI was similarly affected by previous administration of the 5-HT_{1A} antagonist NAN-190. Unless receptor specificity is abolished at the high concentrations of drug achieved with intracerebral injection, such reciprocal antagonism indicates that both receptor subtypes have to be functional for the expression of each one's activation to occur. Although several examples of 5-HT_{1A}/5-HT_2 receptor interaction have been reported (5,9,36), none fits the presently evidenced pattern. Therefore, the interplay between 5-HT_{1A} and 5-HT_{2A} receptors regulating aversion in the DPAG seems to be worth further investigation.

There is an apparent contradiction between the evidence with peripherally and centrally administered drugs concerning the role of different 5-HT receptor subtypes in aversion. Although reported results obtained with systemic injection suggest that stimulation of either 5-HT_{1A} or 5-HT_{2A} receptors enhances aversion (23–25), the above discussed evidence with intra-DPAG injection indicates that the same receptors mediate antiaversive effects in the DPAG. However, systemically injected drugs may be acting outside the DPAG, in regions where 5-HT facilitates aversion (e.g., the amygdala) (12,17, 20). Also, 5-HT_{1A} agonists, when given at the periphery, can stimulate autosomic 5-HT receptors that inhibit raphe neuron firing (1).

On the basis of preclinical and clinical evidence it has been suggested that 5-HT mechanisms regulating aversion in the DPAG are involved in panic disorder and in the mode of action of antipanic drugs (12,15–17). Therefore, the present results indicating that both 5-HT_{1A} and 5-HT_{2A} receptors inhibit aversion in the DPAG may have clinical implications. For instance, the above-discussed evidence pointing to an antiaversive role of 5-HT_{1A} receptors in the DPAG indicates that the therapeutic potential of 5-HT_{1A} receptor ligands such as ipsapirone and gepirone in panic disorder is worth careful investigation (17).

ACKNOWLEDGEMENTS

The present work was funded by FAPESP (94/0821-1) and CNPq (520247/94-9). R.L.N. was the recipient of a CNPq research fellowship. We are indebted to Dr. J. Traber, from Troponwerke, Germany, for the gift of BAY-R-1531.

REFERENCES

1. Aghajanian, G. K. Central 5-HT receptor subtypes: Physiological responses and signal transduction mechanisms. In: Marsden, C. A.; Heal, D. J., eds. Central serotonin receptors and psychoptropic drugs. Oxford: Blackwell Scientific Publications; 1992:39–55.
2. Audi, E. A.; Graeff, F. G. Benzodiazepine receptors in the periaqueductal grey mediate anti-aversive drug action. Eur. J. Pharmacol. 103:279–285; 1984.
3. Audi, E. A.; de Aguiar, J. C.; Graeff, F. G. Mediation by serotonin of the antiaversive effect of zimelidine and propranolol injected into the dorsal midbrain central grey. J. Psycopharmacol. 2:26–32; 1988.
4. Audi, E. A.; de Oliveira, R. M. W.; Graeff, F. G. Microinjection of propranolol into the dorsal periaqueductal gray causes an anxiolytic effect in the elevated plus-maze antagonized by ritanserin. Psychopharmacology (Berlin) 105:553–557; 1991.
5. Backus, L. I.; Sharp, T.; Grahame-Smith, D. G. Behavioural evidence for functional interactions between central 5-HT_2 and 5-HT_{1A} receptor. Br. J. Pharmacol. 100:793–799; 1990.
6. Beckett, S. R. G.; Marsden, C. A.; Marshal, P. W. Intra periaqueductal grey administration of mCPP potentiates a chemically induced defence response. Br. J. Pharmacol. 107:8P; 1992.
7. Beckett, S. R. G.; Lawrence, A. J.; Marsden, C. A.; Marshal, P. W. Attenuation of chemically induced defence response by 5-HT_1 receptor agonists administered into the periaqueductal gray, Psychopharmacology (Berlin) 108:110–114; 1992.
8. Beitz, A.; Clements, J. R.; Mullett, M.; Ecklund, L. Differential origin of brainstem serotonergic projections to the midbrain periaqueductal gray and superior colliculus of the rat. J. Comp. Neurol. 250:498–509; 1986
9. Berendsen, H. H. G.; Broekkamp, C. L. E. Behavioural evidence for functional interactions between 5-HT-receptor subtypes in rats and mice. Br. J. Pharmacol. 101:667–673; 1990.
10. Brandão, M. L.; Lopez-Garcia, J. A.; Graeff, F. G.; Roberts, M. H. T. Electrophysiological evidence for excitatory 5-HT_2 and depressant 5-HT_{1A} receptors on neurones of the rat midbrain tectum. Brain Res. 556:259–266; 1991.
11. Clarke, A.; File, S. Effects of ACTH, benzodiazepines and 5-HT antagonists on escape from periaqueductal grey stimulation in the rat. Prog. Neuropsychopharmacol. Biol. Psychiatry 6:27–35; 1982.
12. Deakin, J. W. F.; Graeff, F. G. 5-HT and mechanisms of defence. J. Psychopharmacol. 5:305–315; 1991.
13. Engel, G.; Gothert, M.; Hoyer, D.; Schlieker, E.; Hillebrand, K. Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) auto-receptors in the rat brain cortex with 5-HT 1B binding sites. Naunyn Schmiedebergs Arch. Pharmacol. 332:1–7; 1986.
14. Graeff F. G. Brain defense systems and anxiety. In: Roth, M.; Burrows, G. D.; Noyes, R., eds. Handbook of anxiety vol. 3. The neurobiology of anxiety. Amsterdam: Elsevier Science Publishers; 1990:307–354.
15. Graeff, F. G. Neurotransmitters in the dorsal periaqueductal gray and animal models of panic anxiety. In: Briley, M.; File, S. E., eds. New concepts in anxiety. London: Macmillan Press; 1991: 288–312.
16. Graeff F. G. Role of 5-HT in defensive behavior and anxiety. Rev. Neurosci. 4:181–211; 1993.
17. Graeff, F. G.; Silveira, M. C. L.; Nogueira, R. L.; Audi, E. A.; De Oliveira, R. M. W. Role of the amygdala and periaqueductal gray in anxiety and panic. Behav. Brain Res. 58:123–131; 1993.
18. Gruel, J. M.; Glaser, T. The putative 5-HT_{1A} receptor antagonists NAN-190 and BMY 7378 are partial agonists in the dorsal raphe nucleus in vitro. Eur. J. Pharmacol. 211:211–219; 1992.
19. Handley, S. L.; Mithani, S. Effects of alpha-adrenoceptor agonists in a maze-exploration model of "fear-motivated behaviour. Naunyn Schmiedebergs Arch. Pharmacol. 327:1–5; 1984.
20. Hodges, H.; Green, S.; Glenn, B. Evidence that the amygdala is involved in benzodiazepine and serotonergic effects on punished responding but not in discrimination. Psychopharmacology (Berlin) 92:491–504; 1987.
21. Hoyer, D. Functional correlates of serotonin 5-HT_1 recognition sites. J. Recep. Res. 8:59–81; 1988.
22. Humphrey P. P. A.; Hartig P.; Hoyer D. A proposed new nomenclature for 5-HT receptors. Trends Pharmacol. Sci. 14:233–236; 1993.
23. Jenck, F.; Broekkamp, C. L. E.; Van Delft, A. M. L. Effects of serotonin receptor antagonists on PAG stimulation induced aversion: Different contributions of 5-HT_1, 5-HT_2 and 5-HT_3 receptors. Psychopharmacology (Berlin) 97:489–495; 1989.
24. Jenck, F.; Broekkamp, C. L. E.; Van Delft, A. M. L. Opposite control mediated by central 5-HT_{1A} and non-5HT_{1A} (5HT_{1B} or 5-HT_{1C}) receptors on periaqueductal gray aversion. Eur. J. Pharmacol. 161:219–221; 1989.
25. Jenck, F.; Broekkamp, C. L. E.; Van Delft, A. M. L. 5-HT_{1C} receptors in the serotonergic control of periaqueductal gray induced aversion in rats. Psychopharmacology (Berlin) 100:372–376; 1990.

26. Kiser, R. S., Jr.; Lebovitz, R. M. Monoaminergic mechanisms in aversive brain stimulation. Physiol. Behav. 15:47–53; 1975.
27. Kiser, R. S., Jr.; Lebovitz, R. M.; German, D. C. Anatomic and pharmacological differences between two types of aversive midbrain stimulation. Brain Res. 155:331–342; 1978.
28. Lovick, T. A. Serotonergic influence from nucleus raphe obscurus on neurones in the periaqueductal grey matter in the rat. Brain Res. 606:92–98; 1993.
29. Marseillan, R. F. A solid state sine-wave stimulator. Physiol. Behav. 19:339–340; 1977.
30. Nogueira, R. L.; Graeff, F. G. 5-HT mediation of the antiaversive effect of isamoltane injected into the dorsal periqueductal grey. Behav. Pharmacol. 2:73–77; 1991.
31. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1986.
32. Pierce, P. A.; Kim, J. Y.; Peroutka, S. J. Molecular structural basis of ligand selectivity for 5-HT_2 versus 5-HT_{1C} receptors. Naunyn Schmiedebergs Arch. Pharmacol. 346:4–11; 1992.
33. Schenberg, L. C.; Graeff, F. G. Role of the periaqueductal gray substance in the antianxiety action of benzodiazepines. Pharmacol. Biochem. Behav. 9:287–295; 1978.
34. Schütz, M. T. B.; de Aguiar, J. C.; Graeff, F. G. Anti-aversive role of serotonin in the dorsal periaqueductal grey matter, Psychopharmacology (Berlin) 85:340–345; 1985.
35. Sheldon, P. W.; Aghajanian, G. K. Serotonin (5-HT) induces IPSPs in pyramidal layer cells of rat piriform cortex: Evidence for the involvement of a 5-HT_2-activated interneuron. Brain Res. 506:62–69; 1990.
36. Uphouse, L.; Andrade, M.; Caldarola-Pastuszka, M.; Maswood, S. Hypothalamic infusion os the 5-HT_{2IC} agonist, DOI, prevents the inhibitory actions of the 5-HT_{1A} agonist, 8-OH-DPAT, on lordosis behavior. Pharmacol. Biochem. Behav. 47:467–470; 1994.