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Role of the Central Serotonergic System in the Anticonflict Effect of *d*-AP159

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TAKAO, K., T. NAGATANI, K.-I. KASAHARA AND S. HASHIMOTO. *Role of the central serotonergic system in the anticonflict effect of* d-*AP159*. PHARMACOL BIOCHEM BEHAV 43(2) 503–508, 1992.—*d*-AP159 is a *d*-optical isomer, and in rats it has a high affinity for 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) receptors and potent anticonflict activity, equal to that of buspirone. The anticonflict effects of *d*-AP159 and buspirone were investigated in animals in which lesions of the serotonergic neurons were caused by intradorsal raphe (d-RA) injection of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT). The anticonflict effect of buspirone, but not that of *d*-AP159, was attenuated in 5-HT neuron-lesioned rats. The anticonflict effect of *d*-AP159 injected into various brain sites was also studied. *d*-AP159 and buspirone microinjected into the d-RA caused significant anticonflict activity in rats. There was a significant anticonflict effect of *d*-AP159 injected into the amygdala centralis (ACE), but not the dorsal hippocampus (d-HC). The anticonflict effect of *d*-AP159 injected into the d-RA was antagonized by systemic administration of (−)propranolol but not Ro 15-1788. This effect of *d*-AP159 injected into the ACE was antagonized by systematic administration of Ro 15-1788 but not (−)propranolol. These results suggest that the d-RA and the ACE play important roles in the anticonflict effects of *d*-AP159 but that the mechanisms by which this drug acts at these sites are different.

d-AP159 5-HT$_{1A}$ 5,7-DHT lesion Microinjection Dorsal raphe Amygdala Conflict Rat

RACEMIC AP159 [(*N*-cyclohexyl-1,2,3,4-tetrahydrobenzo-(*b*)thieno-(2,3*c*)pyridine)-3-carboamide, HCl] is a novel putative anxiolytic with a pharmacological profile different from that of benzodiazepines (BDZs) (13). We previously reported that racemic AP159 binds to 5-hydroxytryptamine$_{1a}$ (5-HT$_{1A}$) receptors with high affinity despite the low affinity with which it binds to other neurotransmitter receptors, including BDZ receptors. Racemic AP159 can increase 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) contents in some regions of the brain (13).

The BDZs are widely used to treat anxiety, but because they have undesirable side effects, including drowsiness, ataxia, and memory impairment, their clinical utility is limited (11,14). Recently, 5-HT$_{1A}$ agonists, including buspirone, ipsapirone, and tandospirone, have been developed as anxiolytics because they are expected to have less severe side effects than BDZs and to be anxioselective (21,24,26). The central serotonergic system has been reported to play an important role in the control of anxiety (8,25). In particular, the 5-HT$_{1A}$ receptor has been said to be closely related to anxiety (9,24). Radioligand binding studies indicate that the 5-HT$_{1A}$ receptor sites are located on presynaptic as well as postsynaptic structures (16).

In the present study, we tried to clarify the role of the central serotonergic system in the anticonflict effect of AP159. First, the anticonflict effect of AP159 was studied in animals in which presynaptic serotonergic neurons have been destroyed by intradorsal raphe nucleus (d-RA) injection of 5,7-dihydroxytryptamine (5,7-DHT). Second, AP159 was microinjected directly into various brain areas to determine the site of its anticonflict effect.

Because AP159 has an asymmetrical carbon in its chemical structure, it has optical isomers. *d*-AP159 has a higher affinity for 5-HT$_{1A}$ receptors than dose racemic AP159 (Ki; *d*-AP159 $= 2.8 \times 10^{-8}$ M, racemic AP159 $= 2.8 \times 10^{-7}$ M), and the two have similar pharmacological profiles, including anticonflict activity. Because the purpose of this study is to clarify the role of serotonergic mechanisms in the anticonflict effect of AP159, we used *d*-AP159 because it may affect serotonergic transmission more strongly than the racemic form.

METHOD

Animals

Male Wistar rats weighing 180–200 g (Seiwa Experimental Animals Ltd., Fukuoka, Japan) were used. They were housed

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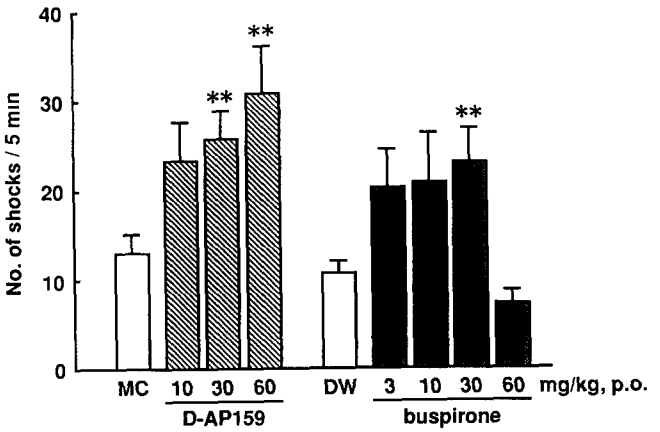

FIG. 1. Effects of *d*-AP159 and buspirone, given by systemic injection in the anticonflict drinking test. Each drug was injected 1 h prior to the test. MC, methylcellulose; DW, distilled water, $^{**}p < 0.01$, significantly different from the value in the respective vehicle group. For each dose tested, 14–16 rats were used.

in groups of five and had free access to food and water in humidity- and temperature-controlled rooms (23 ± 3°C and 60 ± 5%, respectively).

5,7-DHT Lesions

Rats were anesthetized with pentobarbital sodium (40 mg/kg, IP) and placed in a stereotaxic apparatus. Thirty minutes after injection of desmethylimipramine (25 mg/kg, IP), 5,7-DHT (50 μl of isotonic saline containing 0.1% ascorbic acid) was slowly infused into the d-RA. Stereotaxic coordinates according to Paxinos and Watson (15) were A = 0.2 mm from lambda, L = 0 mm from the midline, and 7.8 mm from the surface of the skull. The injection cannula was introduced into the brain at an angle of 20° to the sagittal plane to avoid damaging the sagittal sinus. Sham lesions were performed by the same procedure with the exception of 5,7-DHT infusion. Seven days after surgery, 5,7-DHT-treated rats were tested with the conflict test and brain monoamines were analyzed.

Conflict Test

A modified thirsty rat conflict test (10) was used in this study. Rats were deprived of water for 24 h prior to the test. Rats were placed in a test box enclosed in a soundproof cage and allowed to drink water from a nipple for 30 s after finding it. During this trial, an electric shock (1 mA, 1 s) was delivered through the nipple every time the rat drank one drop (0.03 ml) of water (0.1% NaCl solution) from it. We selected those rats that drank water 3–10 times during this trial. After 5 h of this selection, we performed the conflict test. The selected rats were removed from the chamber, drugs were administered, and rats were returned to the home cage. After drugs were given, rats were placed in the test box again. The 5-min test period started at the time when rats began to drink the water. During this period, rats received a shock (2.0 mA, 1 s) for every drop they drank.

Microinjection Studies

Under anesthesia with pentobarbital sodium (40 mg/kg, IP), guide cannulae (stainless steel, external diameter 0.7 mm) were implanted bilaterally 1 mm above the target structure. The coordinates, anterior (A) from the bregma, lateral (L) from the midline, and horizontal (H) from the surface of the skull, were selected according to the atlas of Paxinos and Watson (15). They were A, − 2.0; L, ± 4.0; H,7.5 mm for the amygdala centralis (ACE) and A, − 3.0; L, ± 2.0; H, 4.0 mm for the dorsal hippocampus (d-HC). The coordinates for the d-RA were the same as those used for the 5,7-DHT lesion. One week after cannulae were implanted, the drug experiment was performed. An injection cannula extending 1.0 mm below the tip of the guide cannula was used for microinjection of drugs. One microliter of each drug was injected into the d-RA and bilaterally injected into the ACE. Two microliters of each drug was injected bilaterally into the d-HC.

Drugs

The following drugs were used in this study: *d*-AP159, Ro 15-1788, (Asahi Chemical Ind. Co., Ltd.), buspirone HCl, and (−)propranorol HCl (Sigma Chemical Co., St. Louis, MO). *d*-AP159 was suspended in 1% methylcellulose. Ro 15-1788 was suspended in 0.1% Tween-80. Buspirone and (−)propranolol were dissolved in distilled water. *d*-AP159 and buspirone were given 60 min (systemic administration) or 10 min (microinjection) before the test. Ro 15-1788 and (−)propranolol were given IP 15 and 30 min, respectively, before the drug microinjection.

Monoamine Analysis

One week after the 5,7-DHT lesions, rats were decapitated, their brains were quickly removed, and the amygdala, hippo-

TABLE 1

EFFECT OF 5,7-DHT (50 μg INTO THE d-RA 7 DAYS BEFORE KILLING ON 5-HT AND 5-HIAA CONTENTS IN VARIOUS BRAIN REGIONS OF RATS

Drug	Amygdala	Hippocampus	Hypothalamus
5-HT (ng/mg protein)			
Sham lesion	4.68 ± 0.56	2.01 ± 0.10	6.21 ± 0.50
5,7-DHT lesion	0.29 ± 0.12*	0.31 ± 0.04†	0.67 ± 0.14†
5-HIAA (ng/mg protein)			
Sham lesion	4.51 ± 0.23	2.22 ± 0.07	4.12 ± 0.32
5,7-DHT lesion	0.54 ± 0.05†	0.25 ± 0.01	0.54 ± 0.06†

*$p < 0.01$, †$p < 0.005$, significantly different from the value in the sham-lesioned group.

campus, and hypothalamus were dissected on dry ice. Each sample of tissue was placed in a 1.5-ml polypropylene tube and sonicated in 300 μl 0.1 M perchloric acid solution (PCA) containing $Na_2S_2O_5$ (1 g/l) and EDTA·2 Na (0.1 g/l), then centrifuged at 4,000 rpm for 20 min at 0°C. After centrifugation, the supernatant was removed and placed in another polypropylene tube and stored at −80°C until taken for analysis.

The concentrations of 5-HT and its metabolite, 5-HIAA, were analyzed by high-pressure liquid chromatography (HPLC) with electrochemical detection. A 20-μl aliquot of the supernatant was injected into the HPLC system. The HPLC was performed with a model EP-10 pump (Eicom, Tokyo, Japan) equipped with Eicompak MA-ODS (4.6 × 250 mm, Eicom), and protected by a precolumn (Eicom Prepak). The column was operated at 25°C and the flow rate was set at 1 ml/min. Electrochemical detection was accomplished with a model ECD-100 (Eicom), and the applied voltage was maintained at 750 mV.

The mobile phase consisted of 0.1 M/l citric acid–0.1 M/l sodium acetate (10:7, pH 3.9) containing methanol (17%), EDTA·2Na (3 mg/l), and sodium 1-octanesulfonate (320 mg/l), and was continuously degassed by a model DG-100 (Eicom).

Histology

After tests, rats were killed and their heads were perfused with saline and 10% formalin. Then, 5-μm sections of brains were prepared and stained with hematoxylin & eosin. The placements of the guide cannulae were verified histochemically. Only the data obtained from rats with correctly placed cannula tips were used.

Statistical Analysis

The data were analyzed with Student's *t*-test.

RESULTS

Systemic d-*AP159*

d-AP159 clearly caused a dose-dependent increase in the number of shocks rats tolerated. The minimum significant effective dose of *d*-AP159 was 30 mg/kg PO. Buspirone also caused a significant increase in the number of shocks at 30 mg/kg PO. (Fig. 1).

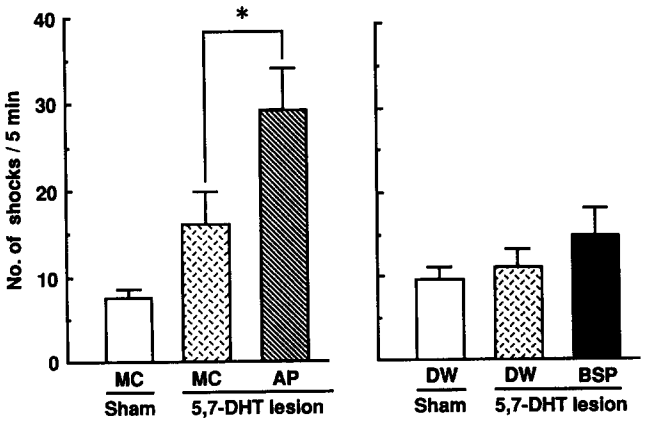

FIG. 2. Effects of *d*-AP159 and buspirone in the anticonflict drinking test of 5,7-DHT-treated rats. 5,7-DHT (50 μg/μl) was injected into the d-RA 7 days before the test. Each drug was injected 1 h prior to the test. MC, methylcellulose; AP, *d*-AP159 (60 mg/kg, PO); DW, distilled water; BSP, buspirone (30 mg/kg, PO). *$p < 0.05$, significantly different from the value in the 5,7-DHT lesioned MC group. Each column represents the mean of 12–14 rats.

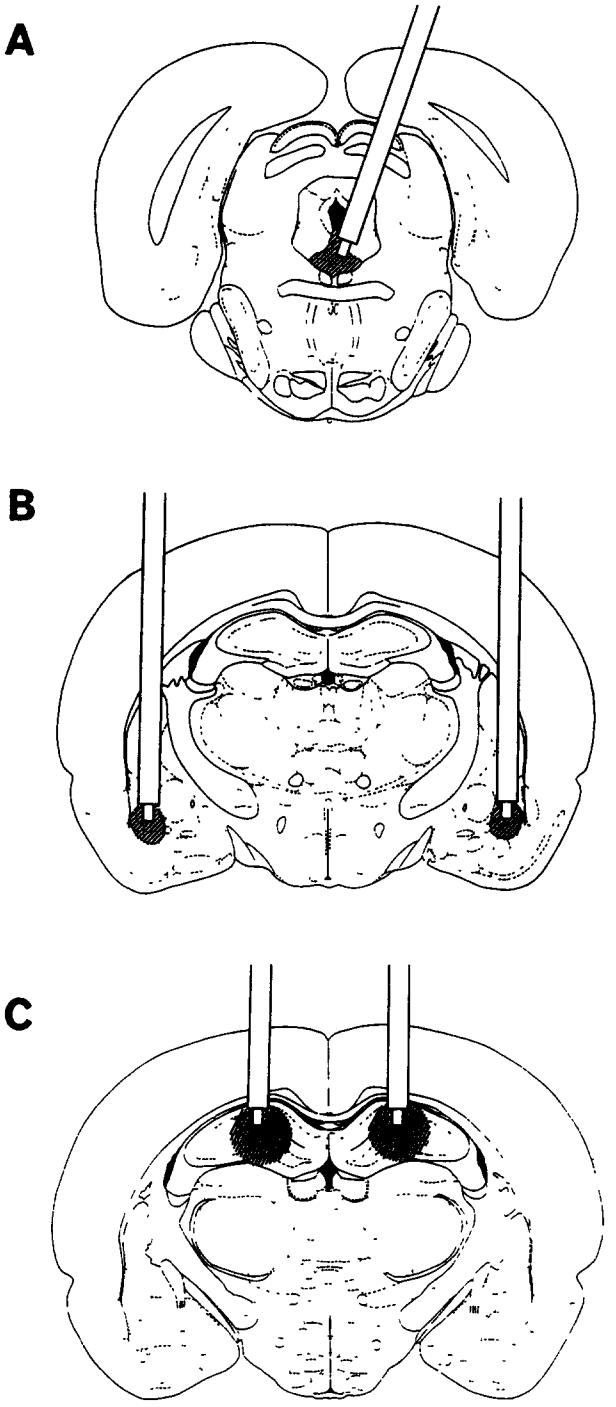

FIG. 3. Schematic representation of the injection sites: (A) dorsal raphe (d-RA); (B) amygdala centralis (ACE); (C) dorsal hippocampus (d-HC).

5,7-DHT Lesions

Intra-*d*-RA administration of 5,7-DHT produced about 80% reduction of 5-HT and 5-HIAA contents in the amyg-

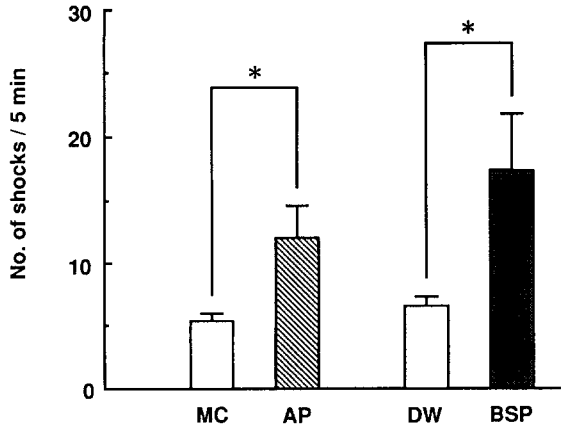

FIG. 4. Effects of *d*-AP159 and buspirone, given by intra-d-RA injection, in the anticonflict drinking test. Each drug was injected 10 min prior to the test. MC, methylcellulose; AP, *d*-AP159 (30 μg/μl); DW, distilled water; BSP, buspirone (30 μg/μl). *$p < 0.05$, significantly different from the value in the respective vehicle group. Each column represents the mean of 9–11 rats.

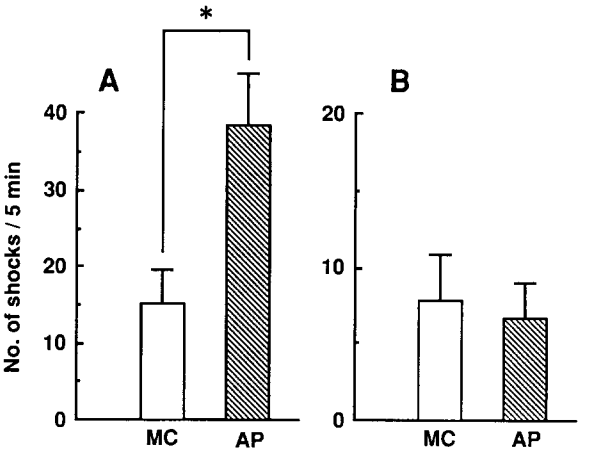

FIG. 6. Effects of *d*-AP159 in the anticonflict drinking test. (A) intra-ACE administration, (B) intra-d-HC administration. Each drug was injected 10 min prior to the test. MC, methylcellulose; AP, *d*-AP159 (30 μg/μl). *$p < 0.05$, significantly different from the MC group. Each column represents the mean of 8–13 rats.

dala, hippocampus, and hypothalamus (Table 1). The 5,7-DHT lesion had no significant effect on the conflict behavior of methylcellulose-treated rats. The anticonflict activity of *d*-AP159 was still observed in 5,7-DHT-treated rats. The anticonflict effect of buspirone was attenuated in 5,7-DHT-treated animals (Fig. 2).

Microinjection Studies

Figure 3 shows representative examples of d-RA(A), ACE(B), and d-HC(C) cannulae placements. Only data from animals with cannula tips in these regions were included in the analysis. Microinjection of *d*-AP159 and buspirone into the d-RA clearly had anticonflict effects (Fig. 4). (−)Propranolol (10 mg/kg, IP) significantly inhibited the increase in the number of shocks that occurred after *d*-AP159 (30 μg/μl) was injected into the d-RA. Ro 15-1788 (10 mg/kg, IP) did not attenuate the anticonflict effect of *d*-AP159 injected into the d-RA (Fig. 5A). Significant anticonflict activity was produced by intra-ACE injection of *d*-AP159 (Fig. 6A). The anticonflict effect of *d*-AP159 injected into the ACE was antagonized by Ro 15-1788 (10 mg/kg, IP) but not by (−)propranolol (10 mg/kg, IP) (Fig. 5B). *d*-AP159 had no significant effect after microinjection into the d-HC (Fig. 6B).

DISCUSSION

It is well known that agents with clinical anxiolytic activity, including BDZs, are effective in the Vogel-type conflict para-

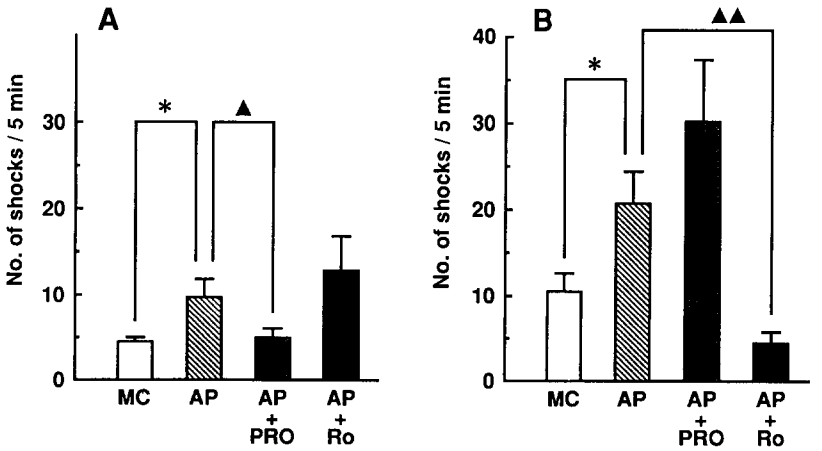

FIG. 5. Effects of (-)propranolol and Ro 15-1788 on the anticonflict action of *d*-AP159 given by (A) intra-d-RA and (B) intra-ACE injection. Each blocker was injected IP 15 and 30 min prior to microinjection of *d*-AP159 respectively. MC, methylcellulose; AP, *d*-AP159 (30 μg/μl); PRO, (-)propranolol (10 mg/kg); Ro, Ro 15-1788 (10 mg/kg). *$p < 0.05$, significantly different from the value in MC-treated animals. ▲$p < 0.05$, ▲▲$p < 0.01$, significantly different from the value in AP-treated animals.

digm (4). Buspirone, a nonbenzodiazepine 5-HT_{1A}-type anxiolytic, has also been reported to be effective in this paradigm (6,17,21). In the present study, *d*-AP159, as well as buspirone, both of which have high affinity for 5-HT_{1A} receptors, had significant anticonflict activities. The anticonflict effect of buspirone was attenuated at the dose of 60 mg/kg PO. The ED_{50} of reduction in the spontaneous activity and induction of motor incoordination in rats has been reported to be 66 and 24.5 mg/kg PO (12). The attenuation of anticonflict effect of buspirone may be caused by these nonselective effects at this dose. Thus, we tried to study the role of the central serotonergic system in the mechanism of the anticonflict effect of *d*-AP159.

Lesions of presynaptic 5-HT neurons in the d-RA caused at least 80% depletion of 5-HT and 5-HIAA contents in the amygdala, hippocampus, and hypothalamus. The d-RA is the primary source of 5-HT innervation to limbic structures including the amygdala, hippocampus, and hypothalamus (1). Intraventricularly injected 5,7-DHT has been reported to decrease 5-HT and 5-HIAA levels in some brain regions (2,5).

The anticonflict activity of *d*-AP159 was still observed in 5,7-DHT-treated rats. The anticonflict effect of buspirone was attenuated in such animals. Eison et al. (5) and Carli et al. (3) demonstrated that 5-HT neuron lesions caused by 5,7-DHT completely eliminated the anxiolytic effect of buspirone, as measured by the conflict drinking test and the two-compartment exploratory test. These results suggest that presynaptic, rather than postsynaptic, 5-HT_{1A} receptors are involved in the anxiolytic activity of buspirone. However, the lack of effect of 5-HT neuron lesions on the anticonflict action of *d*-AP159 suggests that the mechanism responsible for the effects of *d*-AP159 also includes a role for postsynaptic 5-HT_{1A} receptors. The differences in the anticonflict effects of *d*-AP159 and buspirone in 5,7-DHT-treated animals might be due to differences in the roles of pre- and postsynaptic 5-HT_{1A} receptors in the anxiolytic activities of these drugs.

Both *d*-AP159 and buspirone had significant anticonflict effects after microinjection into the d-RA in rats. The d-RA, where the presynaptic 5-HT_{1A} receptors are located, provides extensive 5-HT innervation to limbic structures including the amygdala and hippocampus (22). When the 5-HT_{1A} receptor-related anxiolytics buspirone and ipsapirone were microinjected into this area, they had anxiolytic effects, as measured by the social interaction test and the Vogel-type conflict test (7). In addition, the d-RA has recently been shown to be important as the site of the anxiolytic activity of BDZs (23). All these reports indicate that the d-RA is important in the neuronal system that controls anxiety. The anticonflict effect of *d*-AP159 microinjected into the d-RA was antagonized by pretreatment with the 5-HT_{1A} antagonist (−)propranolol but not by the BDZ antagonist Ro 15-1788. Accordingly, the anticonflict effect of *d*-AP159 injected into the d-RA is probably mediated via somatodendritic 5-HT_{1A} but not BDZ receptors. However, the anticonflict effect of *d*-AP159 administered systemically may be exerted mainly through the postsynaptic 5-HT_{1A} receptor because that effect of *d*-AP159 did not disappear after 5,7-DHT treatment.

Electrolytic lesions of the ACE had anticonflict effects in Vogel- and Geller-type paradigms (20,27). Significant anticonflict effects were also caused by intra-ACE injection of some BDZs (18,19). The ACE may be important in the control of conflict behavior in rats. In the present study, the anticonflict effect of *d*-AP159 was antagonized by Ro 15-1788 but not by (−)propranolol. Thus, the effect of *d*-AP159 injected into the ACE might not be involved in the 5-HT_{1A} mechanism. In fact, very low concentrations of 5-HT_{1A} receptors labeled with 8-OH-[^{3}H]DPAT can be found in the ACE (16). We have also found that racemic AP159, as well as diazepam, enhanced [^{3}H]GABA binding in vitro and in both cases the enhancement was antagonized by Ro 15-1788 in vitro (data not shown). These results suggest that the anticonflict effect of *d*-AP159 injected into the ACE may involve some action of the BDZ-GABA complex through the Ro 15-1788-sensitive site.

In summary, the present study has three main results. The first is that the d-RA and ACE are important in the mechanism of the anticonflict activity of *d*-AP159. The second is that the anticonflict effect of *d*-AP159 in the d-RA might be mediated by presynaptic 5-HT_{1A} receptors and that in the ACE it might be mediated by the BDZ-GABA complex. Finally, we found that the anticonflict effect of *d*-AP159 is different from that of buspirone, namely, the former involves the pre- and postsynaptic 5-HT_{1A} mechanisms. Further study is in progress to clarify the role of postsynaptic 5-HT_{1A} receptors in the anticonflict activity of *d*-AP159.

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REFERENCES

1. Azmitia, E. C.; Segal, M. An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. J. Comp. Neurol. 179:641–668; 1978.
2. Briely, M.; Chopin, P.; Moret, C. Effect of serotonergic lesion on "anxious" behaviour measured in the elevated plus-maze test in the rat. Psychopharmacology (Berl.) 101:187–189; 1990.
3. Carli, M.; Prontera, C.; Samanin, R. Evidence that central 5-hydroxytryptaminergic neurones are involved in the anxiolytic activity of buspirone. Br. J. Pharmacol. 96:829–836; 1989.
4. Cook, L.; Davidson, A. B. Effects of behaviorally active drugs in a conflict-punishment procedure in rats. In: Garattini, S.; Mussini, E.; Randall, L. O., eds. The benzodiazepines. New York: Raven Press; 1973:327–345.
5. Eison, A. S.; Eison, M. S.; Stanley, M.; Riblet, A. R. Sero tonergic mechanisms in the behavioral effects of buspirone and gepirone. Pharmacol. Biochem. Behav. 24:701–707; 1986.
6. Gower, A. J.; Tricklebank, M. D. α_2-Adrenoceptor antagonist activity may account for the effects of buspirone in an anticonflict test in the rat. Eur. J. Pharmacol. 155:129–137; 1988.
7. Higgins, G. A.; Bradbury, A. J.; Jones, B. J.; Oakley, N. R. Behavioural and biochemical consequences following activation of 5-HT_1-like and GABA receptors in the dorsal raphe nucleus of the rat. Neuropharmacology 27:993–1001; 1988.
8. Iversen, S. D. 5-HT and anxiety. Neuropharmacology 23:1553–1560; 1984.
9. Kahn, R. S.; van Praag, H. M.; Wetzler, S.; Asnis, G. M.; Barr, G. Serotonin and anxiety revisited. Biol. Psychiatry 23:189–208; 1988.
10. Lippa, A. S.; Coupet, J.; Greenblatt, E. N.; Klepner, C. A.; Bear, A. A synthertic non-benzodiazepine ligand for benzodiazepine receptors: A probe for investigating neuronal substrates for anxiety. Pharmacol. Biochem. Behav. 11:99–106; 1979.
11. Lister, R. G. The amnesic action of benzodiazepines in man. Neurosci. Behav. Rev. 9:87–94; 1985.

12. Michael, S. E. Behavioral effects of buspirone in laboratory animals. In: Godfrey, T.; Arlene, S. E.; Duncan, P. T., eds. Buspirone: Mechanism and clinical aspects. San Diego, CA: Academic Press; 1991:81–95.
13. Nagatani, T.; Yamamoto, T.; Takao, K.; Hashimoto, S.; Kasahara, K.; Sugihara, T.; Ueki, S. Pharmacological profile of a potential anxiolytic: AP159, a new benzothieno-pyridine derivative. Psychopharmacology (Berl.) 104:432–438; 1991.
14. Palva, E. S.; Linnolia, I.; Sarrio, I.; Mattila, M. Acute and subacute effects of diazepam on psychomotor skills: Interaction with alcohol. Acta Pharmacol. Toxicol. 45:257–264; 1979.
15. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. New York: Academic; 1986.
16. Pazos, A.; Palacios, J. M. Quantitative autoradiographic mapping of serotonin receptors in the rat brain. 1. Serotonin-1 receptors. Brain Res. 346:205–230; 1985.
17. Pich, E. M.; Samanin, M. Disinhibitory effects of buspirone and low doses of sulpiride and haloperidol in two experimental anxiety models in rats: Possible role of dopamine. Psychopharmacology (Berl.) 89:125–130; 1986.
18. Shibata, K.; Kataoka, Y.; Gomita, Y.; Ueki, S. Localization of the site of the anticonflict action of benzodiazepines in the amygdaloid nucleus of rats. Brain Res. 234:442–446; 1982.
19. Shibata, K.; Kataoka, Y.; Yamashita, K.; Ueki, S. An important role of the central amygdaloid nucleus and mammillary body in the mediation of conflict behavior in rats. Brain Res. 372:159–162; 1986.
20. Shibata, S.; Yamashita, K.; Yamamoto, E.; Ozaki, T.; Ueki, S. Effects of benzodiazepine and GABA antagonists on anticonflict effects of antianxiety drugs injected into the rat amygdala in a water-lick suppression test. Psychopharmacology (Berl.) 98:38–44; 1989.
21. Shimizu, H.; Hirose, Al; Tatsuno, T.; Nakamura, M.; Katsube, J. Pharmacological properties of SM-3997: A new anxioselective anxiolytic candidate. Jpn. J. Pharmacol. 45:493–500; 1987.
22. Soubrie, P. Reconciling the role of central serotonin neurons in human and animal behavior. Behav. Brain Sci. 9:319–364; 1986.
23. Thiebot, M. H.; Hamon, M.; Soubrie, P. Attenuation of induced anxiety in rats by chlordiazepoxide: Role of raphe dorsalis benzodiazepine binding sites and serotonergic neurons. Neuroscience 7: 2287–2294; 1982.
24. Traber, J.; Glaser, T. 5-HT_{1A} receptor-related anxiolytics. Trends Pharmacol. Sci. 8:432–437; 1987.
25. Tye, N. C.; Iversen, S. D.; Green, A. R. The effects of benzodiazepines and serotonergic manipulations on punished responding. Neuropharmacology 18:689–695; 1979.
26. Weissmann, B. A.; Barrett, J. E.; Brady, L. S.; Witkin, J. M.; Mendelson, W. B.; Paul, S. M.; Skolnick, P. Behavioral and neurochemical studies on the anticonflict actions of buspirone. Drug Dev. Res. 4:83–93; 1984.
27. Yadin, E.; Thomas, E.; Strickland, C. E.; Grishkat, H. L. Anxiolytic effects of benzodiazepines in amygdala-lesioned rats. Psychopharmacology (Berl.) 103:473–479; 1991.