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Effects of the Putative Anxiolytic SM-3997 on Central Monoaminergic Systems

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TATSUNO, T., H. SHIMIZU, A. HIROSE, H. TANAKA, Y. KUMASAKA AND M. NAKAMURA. *Effects of the putative anxiolytic SM-3997 on central monoaminergic systems*. PHARMACOL BIOCHEM BEHAV **32**(4) 1049–1055, 1989.—The effects of SM-3997 on central monoaminergic systems were evaluated by ex vivo measurement of monoamines and their metabolite levels in rat brain after intraperitoneal treatment of drugs and by in vitro measurement of monoamine uptake into rat brain slices. The effects of SM-3997 were also compared with those of other new nonbenzodiazepine anxiolytic compounds. SM-3997, buspirone, gepirone and ipsapirone showed no effects on serotonin uptake and dopamine uptake, and a weak inhibition of norepinephrine uptake at the concentration of 100 μM. SM-3997 decreased the serotonin metabolite (5-hydroxyindole-3-acetic acid) level without changing the serotonin level in hippocampus and increased dopamine metabolite (3,4-dihydroxyphenylacetic acid, homovanillic acid) level with no effect on the dopamine level in striatum. SM-3997 also produced an increase in the norepinephrine metabolite (3-methoxy-4-hydroxyphenylglycol) level with a decrease in the norepinephrine levels in hippocampus. Similar effects on serotonin metabolites and norepinephrine metabolites were observed in several other regions. Although the serotonergic effect of SM-3997 was similar to that of buspirone, gepirone and ipsapirone, the dopaminergic effect of SM-3997 was much weaker than that of buspirone.

SM-3997 Nonbenzodiazepine anxiolytics Monoamine Serotonin Dopamine Norepinephrine Monoamine metabolite Uptake Buspirone Gepirone Ipsapirone 5-HT_{1A} receptor

SM-3997 (3aα, 4β, 7β, 7aα-hexahydro-2-(4-(4-(2-pyrimidinyl)1-piperazinyl-butyl)-4,7-methano-1H-isoindole-1,3-(2H)-dionedihydrogen citrate) is a novel putative anxiolytic compound which has a chemical structure and pharmacological profile different from that of benzodiazepine (35). It shows potent anticonflict activity in animal models, but lacks muscle-relaxant, sedative-hypnotic and anticonvulsant effects (35). Although the mechanism of action of SM-3997 is as yet unclear, it is considered to be different from that of benzodiazepine because SM-3997 binds to neither benzodiazepine receptors nor GABA receptors (17, 36, 37) and the anticonflict action of SM-3997 is not inhibited by benzodiazepine antagonists (17). Moreover, we have shown that SM-3997 preferentially binds to a serotonin $(5\text{-HT})_{1A}$ receptor subtype with high affinity in spite of low affinity for the dopamine $(DA)_2$ receptor and 5-HT_2 receptor (36,37).

Recently, the 5-HT_{1A} receptor-related mechanism has been implicated in anxiolytic actions of buspirone and several new putative anxiolytics (40). However, buspirone has been reported to influence serotonergic, dopaminergic and noradrenergic systems in complex manners (30,39). On the other hand, these monoaminergic systems have also been suggested to be involved in the anticonflict activity and other actions of benzodiazepines (10, 13, 14). Therefore, to clarify the effects of SM-3997 on monoaminergic neurotransmission, we examined the effects of SM-3997 on monoamine uptake in vitro and measured the monoamines and their metabolite levels in rat brain after intraperitoneal injection of SM-3997. We also compare the serotonergic and dopaminergic effects of SM-3997 with those of several new putative anxiolytics.

METHOD

Male Sprague-Dawley rats (body weight, 230–300 g) were used for all experiments.

Monoamine Uptake

The 0.3 mm thick slices of hippocampus for [^{3}H]-5-HT uptake, cerebral cortex for [^{3}H]-norepinephrine (NE uptake and striatum for [^{3}H]-DA uptake were prepared with a McIlwain-type tissue chopper. These slices were incubated for 30 min at 30°C in 95% O_2–5% CO_2 saturated Krebs-Ringer bicarbonate buffer (KR) composed of 118 mM NaCl, 1.85 mM KCl, 1.15 mM $MgSO_4$, 1.15 mM KH_2PO_4, 1.2 mM $CaCl_2$, 25 mM $NaHCO_3$, 11.1 mM

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TABLE 1

EFFECTS OF VARIOUS DRUGS ON [^{3}H]-5-HT, [^{3}H]-DA AND [^{3}H]-NE UPTAKE IN BRAIN SLICES

Drugs (100 μM)	Uptake (% of control) [^{3}H]-5-HT (n=4)	[^{3}H]-DA (n=4)	[^{3}H]-NE (n=3)	[^{3}H]-NE[1] (n=4)
Control	100.0 ± 5.9[a]	100.0 ± 6.7[b]	100.0 ± 1.5[c]	100.0 ± 8.7[d]
Imipramine	33.9 ± 6.6†	55.1 ± 7.1†	ND	30.7 ± 5.2†
SM-3997	96.4 ± 10.2	96.3 ± 12.8	76.7 ± 4.8*	103.1 ± 14.3
Buspirone	87.5 ± 6.4	95.4 ± 14.7	57.7 ± 4.4†	75.1 ± 11.1
Gepirone	87.9 ± 9.7	105.2 ± 12.1	82.0 ± 5.9*	ND
Ipsapirone	87.9 ± 9.1	85.1 ± 6.6	59.0 ± 10.3†	ND

Uptake assays were performed as described in the Method section. Values are means ± SE expressed as % of control. Each control value was: [a]1.40 pmol/mg protein/5 min; [b]3.12 pmol/mg protein/2.5 min; [c]2.62 pmol/mg protein/15 min; and [d]2.94 pmol/mg protein/15 min. [1]The data in this column represent the values at the drug concentrations of 10 μM. *$p<0.05$; †$p<0.01$ vs. control (Duncan's test). ND: not determined.

glucose, 0.03 mM ethylenediaminetetraacetic acid (EDTA) and 0.06 mM ascorbic acid. The slices were then transferred to small test tubes (1.0–2.0 mg protein/tube) containing 100 μM pargyline and test drugs. After the additional incubation for 5 min at 37°C the uptake of monoamine was determined under the following conditions. [^{3}H]-5-HT uptake: Hippocampal slices were incubated with 0.2 μCi (100 nM) of [^{3}H]-5-HT for 5 min at 37°C, [^{3}H]-NE uptake: Cerebral cortical slices were incubated with 0.2 μCi (100 nM) of [^{3}H]-NE for 15min at 37°C, [^{3}H]-DA uptake: Striatal slices were incubated with 0.1 μCi (100 nM) of [^{3}H]-DA for 2.5 min at 37°C. The reactions were terminated by the addition of 5 ml of ice-cold KR and a subsequent centrifugation (1000 × g for 1 min). The medium was decanted, and the slices were washed twice with fresh ice-cold KR. After the slices were solubilized in 1 N NaOH, the radioactivity incorporated into the slices was counted by a liquid scintillation counter and the protein content of slices was determined by the method of Lowry *et al.* (23). Parallel incubations with radiolabeled amines at 0°C were performed in each experiment, and the activity of uptake was estimated by subtracting the value obtained at 0°C from that obtained at 37°C.

Monoamines and Their Metabolites Levels

Drugs were dissolved in saline and administered intraperitoneally. Control rats received equivalent volumes of saline. After the drug treatment, the rats were killed by decapitation and the brain was quickly removed, rinsed in ice-cold saline, and dissected into eight regions: cerebral cortex, hippocampus, striatum, thalamus, hypothalamus, mesencephalon, cerebellum, pons + medulla oblongata. Immediately the tissues were weighed, frozen in ethanol-dry ice, and stored at −24°C until assay (within a week). Monoamines and their metabolites were analyzed by high performance liquid chromatography with electrochemical detection (HPLC-ECD). Frozen tissues were homogenized in 4–9 volumes of cold 0.1 M $HClO_4$ containing 5 mM EDTA and isoproterenol as an internal standard. After the homogenates were centrifuged at 10000 × g for 20 min at 4°C, the clear supernatant was filtered through a 0.22 μm pore, and then applied to the HPLC-ECD system.

The HPLC-ECD system was equipped with a graphite working electrode and Eicompak MA-ODS reverse phase column (25 × 0.46 cm) which was protected by a precolumn. The working electrode was maintained at a potential of +750 mV relative to the reference electrode. The mobile phase was made of 0.1 M citrate phosphate buffer, pH 3.7, containing 0.1 mM EDTA, 150 mg/l sodium octyl sulfate, and 15% (v/v) of methanol, filtered through 0.22 μm pores and passed through an apparatus for degassing before pumping into the HPLC system. All separations were performed with a flow rate of 1.3 ml/min at 30°C. Under this condition, good separation of monoamines and their metabolites was obtained with each typical retention time in minutes as follows: NE; 3.76, 3-methyoxy-4-hydroxyphenylglycol(MHPG); 4.71, DA; 7.39, 3,4-dihydroxyphenylacetic acid (DOPAC); 8.75, isoproterenol (an internal standard); 9.88, 5-hydroxyindole-3-acetic acid (5-HIAA); 13.83, 5-HT; 16.59 and homovanillic acid (HVA); 21.05. The amount of each compound in a sample was determined by measuring peak heights and comparing them with the known standards. In this report, a total of free and conjugated MHPG (MHPG-SO_4) was determined as MHPG level. For the hydrolysis of conjugated MHPG, the supernatant of the tissue homogenate adjusted to pH 4.5 was incubated for 2–4 hr at 37°C with sulfatase (15 units/tube). After the hydrolysis, the samples were filtered and applied to the HPLC system. The data from the tissue samples were expressed in nanograms per grams of tissue wet weight. Duncan's test was used for multiple comparison to a control.

Drug and Chemicals

SM-3997 citrate, buspirone HCl, gepirone HCl, ipsapirone and 1-pyrimidinyl piperazine (1-PP) HCl were synthesized at the Sumitomo Pharmaceutical Co., Ltd. [^{3}H]-5-HT (14.9 Ci/mmol), [^{3}H]-DA (49.7 Ci/mmol) and [^{3}H]-NE (32 Ci/mmol) were purchased from Amersham. The sources of other materials used in this work were as follows: L-NE bitartrate, DA HCl, HVA, imipramine HCl and sulfatase (type H-1) from Sigma, DL-MHPG piperazine, DOPAC, 5-HIAA and 1-octane sulfonic acid Na from Aldrich Chem. Co., DL-isoproterenol HCl from Nakarai Chem. Ltd., Kyoto, Japan, 5-HT creatinine sulfate from Merck.

RESULTS

The effects of SM-3997, buspirone, gepirone, ipsapirone and imipramine on [^{3}H]-5-HT, [^{3}H]-DA and [^{3}H]-NE uptake were examined in hippocampal, striatal and cerebral cortical slices respectively (Table 1). Imipramine, a tricyclic monoamine uptake

TABLE 2

EFFECT OF SM-3997 ON THE LEVELS OF MONOAMINES AND THEIR METABOLITES IN VARIOUS BRAIN REGIONS OF RATS

		Contents (ng/g tissue)						
		Monoamines			Metabolites			
Brain Regions		NE	DA	5-HT	MHPG	DOPAC	HVA	5-HIAA
Cerebral cortex	Control	314 ± 12	417 ± 15	350 ± 17	83 ± 5	94 ± 5	82 ± 4	193 ± 9
	SM-3997	248 ± 6†	453 ± 15	427 ± 10†	127 ± 5†	156 ± 9†	170 ± 7†	151 ± 10†
Striatum	Control	160 ± 6	7713 ± 271	521 ± 31	144 ± 29	1154 ± 44	858 ± 44	435 ± 8
	SM-3997	152 ± 9	8385 ± 464	511 ± 19	123 ± 23	2142 ± 76†	1831 ± 59†	425 ± 24
Hippocampus	Control	380 ± 19	29 ± 5	378 ± 29	77 ± 4	7 ± 1	9 ± 2	261 ± 9
	SM-3997	288 ± 18†	32 ± 7	431 ± 28	124 ± 8†	10 ± 0†	16 ± 1†	183 ± 8†
Thalamus	Control	395 ± 34	129 ± 32	469 ± 38	107 ± 4	27 ± 6	24 ± 7	405 ± 23
	SM-3997	304 ± 24	145 ± 32	544 ± 48	176 ± 10†	43 ± 7	43 ± 6	283 ± 15†
Hypothalamus	Control	1365 ± 75	274 ± 35	679 ± 53	119 ± 9	34 ± 4	27 ± 4	422 ± 10
	SM-3997	1043 ± 53†	310 ± 36	811 ± 61	203 ± 13†	80 ± 7*	44 ± 3†	296 ± 8†
Mesencephalon	Control	476 ± 19	130 ± 10	670 ± 40	119 ± 9	34 ± 4	39 ± 3	531 ± 34
	SM-3997	381 ± 22†	124 ± 19	827 ± 45*	157 ± 17	37 ± 5	41 ± 5	389 ± 16†
Cerebellum	Control	282 ± 16	17 ± 8	58 ± 8	58 ± 4	5 ± 0	14 ± 1	41 ± 2
	SM-3997	175 ± 9†	24 ± 9	58 ± 3	106 ± 4†	10 ± 1†	33 ± 2†	30 ± 2†
Pons,	Control	565 ± 19	49 ± 4	486 ± 22	117 ± 3	24 ± 2	27 ± 1	308 ± 14
Medulla oblongata	SM-3997	499 ± 17*	69 ± 7*	594 ± 27*	170 ± 7†	47 ± 2†	57 ± 2†	266 ± 20

Vehicle (control) or SM-3997 (10 mg/kg, IP) was administered 1 hr before decapitation. Values are means ± SE from 5 rats. *$p<0.05$, †$p<0.01$ vs. each control (Student's t-test).

inhibitor (6, 20, 22, 46), potently inhibited the uptake of [^{3}H]-5-HT and [^{3}H]-NE by about 70% and showed a relatively weak inhibition of [^{3}H]-DA uptake as has been known (6). SM-3997 and related compounds showed no significant effects on the [^{3}H]-5-HT and [^{3}H]-DA uptakes even at the high concentration of 100 μM, whereas these drugs inhibited [^{3}H]-NE uptake by 20–40%. At the concentration of 10 μM, however, SM-3997 and buspirone did not inhibit [^{3}H]-NE uptake (Table 1).

The effects of SM-3997 (10 mg/kg, IP) on monoamine and their metabolites levels in various brain regions were studied (Table 2). The level of 5-HIAA was reduced significantly by SM-3997 in a variety of regions including cerebral cortex, hippocampus, thalamus, hypothalamus, mesencephalon and cerebellum with elevation of 5-HT level in cerebral cortex, mesencephalon and pons + medulla oblongata. MHPG was significantly elevated in six regions except striatum and mesencephalon while DA metabolite (DOPAC, HVA) levels were increased only in striatum and cerebral cortex. A significant decrease in NE was also seen in cerebral cortex, hippocampus, hypothalamus, mesencephalon, cerebellum and pons + medulla oblongata.

SM-3997 (1–30 mg/kg, IP) produced a dose-dependent decrease in the 5-HIAA level in hippocampus with no effect on 5-HT, the effect reaching maximum at 30 to 60 min after administration and returning to control levels at 2 hr (Fig. 1). Figure 2 shows that doses over ten mg/kg of SM-3997 induced marked elevation of DA metabolites (both DOPAC and HVA) in striatum and these effects did not reach a plateau even with the 30 mg/kg dose. The time course of the increase in DOPAC and HVA was similar to that of the decrease in 5-HIAA. Furthermore, SM-3997 (3–30 mg/kg, IP) produced a dose-dependent increase in MHPG levels in hippocampus accompanied by a reduction of NE levels (Fig. 3). The duration of SM-3997- (10 mg/kg, IP) induced increase in MHPG was more than two hours after administration.

In a further study, serotonergic and dopaminergic actions of several anxiolytic agents were compared (Fig. 4). SM-3997, buspirone, gepirone and ipsapirone (10 mg/kg, IP) caused a similar decrease in 5-HIAA. Although these agents induced elevations of DA metabolites, SM-3997 as well as gepirone and ipsapirone induced much less effect on DA metabolites than buspirone.

DISCUSSION

The present results indicate that the anxiolytic candidate SM-3997 can affect three monoaminergic systems in a complex manner. In various brain regions, not only a decrease in 5-HIAA and NE levels but also an increase in DOPAC, HVA and MHPG levels were observed with an intraperitoneal injection of SM-3997.

It is unlikely that SM-3997 inhibits monoamine oxidase since SM-3997 generally does not increase monoamine levels and has varied effects on monoamine metabolite levels.

With regard to the effects on monoamine uptake, SM-3997, buspirone, gepirone and ipsapirone did not influence 5-HT or DA uptake, and showed a weak inhibition of NE uptake relative to imipramine. However, these agents may be virtually inactive as inhibitors of NE uptake when systemically administered, because high concentrations (100 μM) are required for the inhibition of NE uptake (Table 1).

SM-3997 produced a dose-dependent reduction of 5-HIAA levels in hippocampus. This effect seems to reflect an agonistic action of SM-3997 on 5-HT receptors. Recent receptor binding studies (36,37) have shown that SM-3997 selectively binds to 5-HT$_{1A}$ receptors with high affinity in rat brain and it binds as an agonist since the affinity of binding is decreased by GTP and a nonhydrolyzable GTP analogue. Taken together, these data suggest that SM-3997 is acting as an agonist for 5-HT$_{1A}$ receptors. Further, the observation that a 5-HT$_{1A}$ selective agonist 8-OH-DPAT (41) also reduced the 5-HIAA level (18) supports this view.

The decrease in 5-HIAA suggests the suppression of the firing

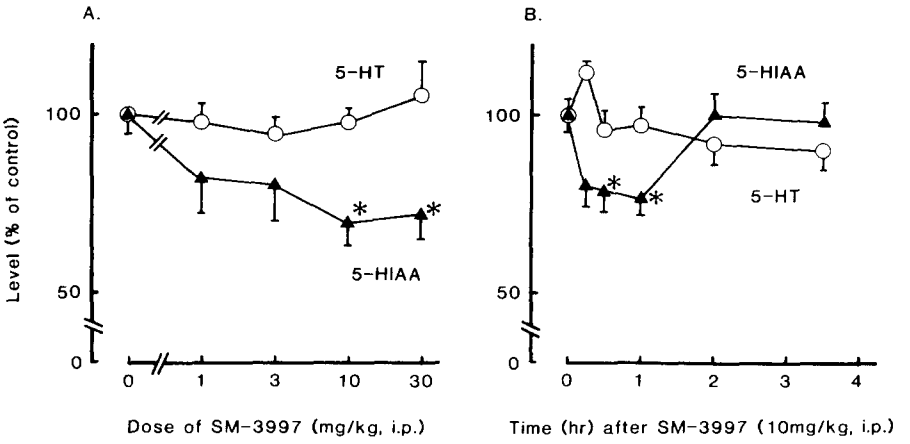

FIG. 1. Effect of SM-3997 on the levels of 5-HT and 5-HIAA in hippocampus of rat brain. (A) Dose-response. Assays were performed 1 hr after administration of SM-3997. Values are means ± SE from 5 rats expressed as % of control (vehicle). (B) Time course. Values are means ± SE from 5 rats expressed as % of control (1 hr after vehicle). *$p<0.05$ vs. control (Duncan's test).

rate of 5-HT neurons (33). Several 5-HT_{1A} agonists such as 8-OH-DPAT, buspirone, gepirone and ipsapirone have been reported to inhibit firing of 5-HT containing dorsal raphe neurons by acting on somatodendritic 5-HT autoreceptors which are probably of the 5-HT_{1A} subtype (3, 5, 19, 42, 45). Similarly, SM-3997 has also been shown to inhibit the spontaneous activity of serotonergic neurons in the dorsal raphe nucleus with an ED_{50} value of 5 μg/kg (IV) (16). Alternatively, SM-3997 has no interaction with 5-HT_{1B} receptors (36) which appear to be presynaptic terminal 5-HT autoreceptors modulating 5-HT release (11, 29). Therefore, although in this study no significant decrease in 5-HIAA as a biochemical index could be detected at doses of SM-3997 below 10 mg/kg (IP), the electrophysiological data suggest that SM-3997 suppresses release of 5-HT as a result of the inhibited firing of 5-HT neurons via somatodendritic 5-HT_{1A}-type autoreceptors. Thus, overall concentrations of 5-HIAA within a given structure may not be a precise reflection of extracellular or synaptic concentrations.

However, 5-HT_{1A} receptors are also known to be located postsynaptically especially in the hippocampus as indicated by the persistence of 5-HT_{1A} binding sites after lesioning serotonergic terminals (15, 43, 44). SM-3997 can induce part of the 5-HT behavioral syndrome including a flat body posture (34) which has been thought to result from the stimulation of postsynaptic 5-HT_{1A} receptors (9,41). Furthermore, in rat hippocampal slices direct activation of the 5-HT_{1A} receptor decreases CA_1 population spike amplitude (8) and some 5-HT_{1A} agonists elicit a hyperpolarization of hippocampal pyramidal cells (1). Thus, a direct agonistic effect of SM-3997 on the postsynaptic 5-HT_{1A} receptors may be involved in the decrease in 5-HIAA level through a local feed-back mechanism.

Interestingly, in striatum, which also receives serotonergic neuronal projection from the dorsal raphe nucleus (38), SM-3997 (10 mg/kg, IP) did not reduce the 5-HIAA level. A similar observation has been reported with buspirone, which decreases 5-HIAA levels in hippocampus(30) but not in striatum (7). These

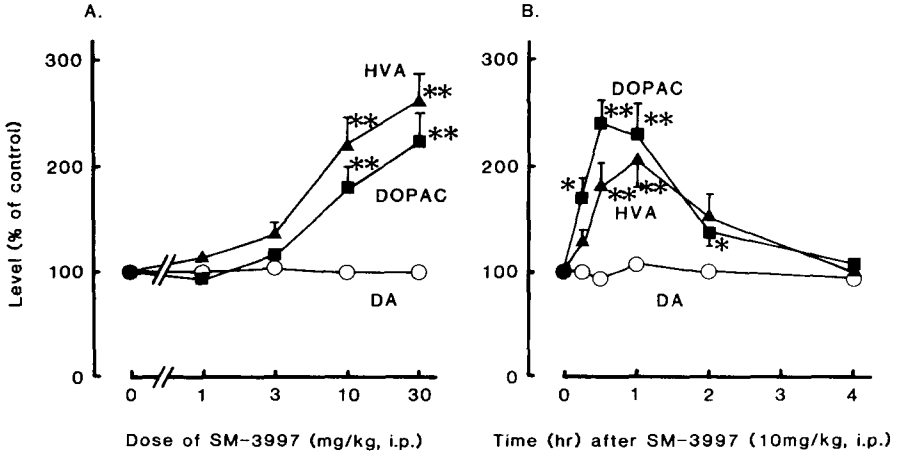

FIG. 2. Effect of SM-3997 on the levels of DA, DOPAC and HVA in striatum of rat brain. (A) Dose-response. Assays were performed 1 hr after administration of SM-3997. Values are means ± SE from 5 rats expressed as % of control (vehicle). (B) Time course. Values are means ± SE from 5 rats expressed as % of control (1 hr after vehicle). *$p<0.05$, **$p<0.01$ vs. control (Duncan's test).

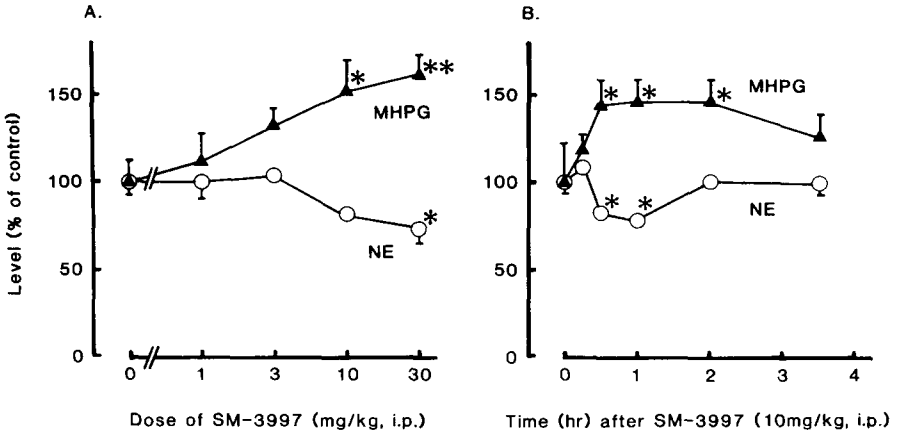

FIG. 3. Effect of SM-3997 on the levels of NE and MHPG in hippocampus of rat brain. (A) Dose-response. Assays were performed 1 hr after administration of SM-3997. Values are means ± SE from 5 rats expressed as % of control (vehicle). (B) Time course. Values are means ± SE from 5 rats expressed as % of control. *$p<0.05$, **$p<0.01$ vs. control (Duncan's test).

regional differences might be related to the characteristic regional distribution of 5-HT_{1A} receptors, which were particularly abundant in hippocampus but could not be detected in striatum (43).

In contrast with the effect on 5-HIAA, SM-3997 increased the concentrations of DA metabolites particularly in striatum, but the potency for this effect seemed to be lower than that for the serotonergic effect because the dopaminergic effect did not reach a maximum at doses up to 30 mg/kg. DA_2 receptor antagonists such as chlorpromazine, haloperidol, etc., have been reported to induce elevation of DOPAC and HVA levels potently (24, 27, 32). Our previous study (36) showed that SM-3997 has a relatively low affinity for DA_2 receptors ($K_i = 650$ nM) compared with its affinity for 5-HT_{1A} receptors ($K_i = 25$ nM) and behaviorally it exhibits DA antagonistic action only at high doses (35). Accordingly, SM-3997 may increase DA metabolite levels by blocking DA_2 receptors with low potency. Alternatively, the increase in DA metabolites could be due, at least in part, to a modification of the serotonergic system by SM-3997. Reduced serotonergic neurotransmission leads to an increase in the activity of dopaminergic transmission as shown by the prevention of haloperidol-induced catalepsy (2,21). Because SM-3997 preferentially acts on 5-HT_{1A} receptors (16,36), its inhibition of the firing rate of dorsal raphe serotonergic neurons may be largely responsible for the increase in DA metabolites rather than the direct DA blocking effect of SM-3997, whereas the latter effect may account for the much weaker effects on DA metabolites of SM-3997 than that of buspirone which can bind not only to 5-HT_{1A} receptors but also to DA_2 receptors with equal ($IC_{50} = 24$ nM) and higher ($IC_{50} = 380$

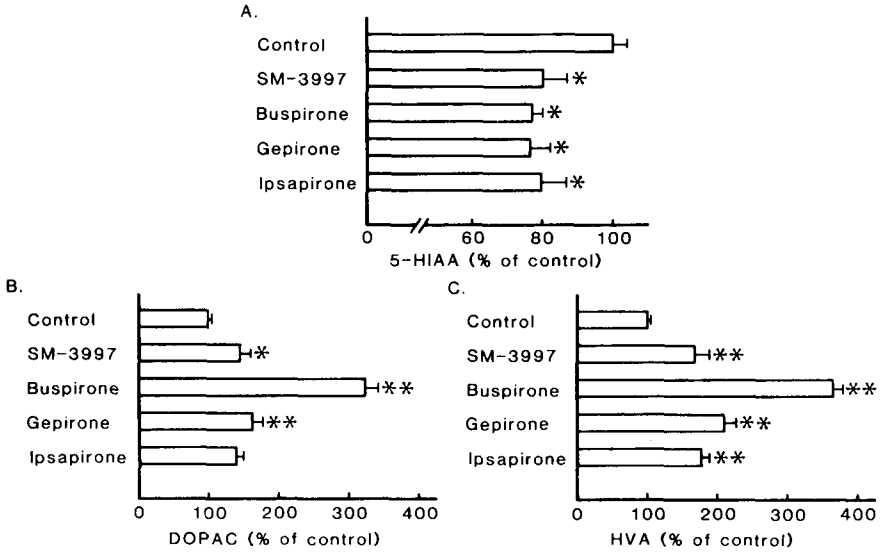

FIG. 4. Effects of SM-3997, buspirone, gepirone and ipsapirone on the levels of 5-HT and DA metabolites in hippocampus and striatum of rat brain. 5-HIAA (A) in hippocampus, DOPAC (B) and HVA (C) in striatum were determined 1 hr after administration of drugs (10 mg/kg, IP) or vehicle (control). Values are means ± SE from 8 rats expressed as % of control. *$p<0.05$, **$p<0.01$ vs. control (Duncan's test).

nM) affinity compared with SM-3997 respectively (31).

Noradrenergic effects of SM-3997 were also observed. SM-3997 increased NE metabolite (MHPG) levels and decreased NE levels dose-dependently. In a receptor binding study (36), SM-3997 did not interact with α_1, α_2 and β-adrenergic receptors. Therefore, its effects on MHPG and NE may be secondary to the actions of SM-3997 on serotonergic or dopaminergic systems. However, it should be noted that the increase in MHPG was maintained longer than the decrease in 5-HIAA and the increase in DOPAC and HVA. This suggests that metabolites of SM-3997 are important for the noradrenergic effects. As in the case of buspirone and gepirone (12), the most probable metabolite of SM-3997 is 1-pyrimidinyl piperazine (1-PP), which raises the MHPG levels in rat cerebral cortex and antagonizes the behavioral effect of clonidine, an α_2 receptor agonist (4). Moreover, the concentration of 1-PP in rat brain reached a much higher level than that of SM-3997 and remained at nearly the maximum level for six hours (Kimura *et al.*, unpublished observations). Thus, the effects of SM-3997 on MHPG and NE are possibly mediated, at least in part, by its metabolite 1-PP.

The effects of SM-3997 on the serotonergic and dopaminergic systems were common to buspirone, gepirone and ipsapirone, whereas the potencies of each drug were different (Fig. 4). Although all of the four drugs showed a similar decrease in hippocampal 5-HIAA levels, the order of potency for the dopaminergic effect was: buspirone $>>$ gepirone $\cong$ ipsapirone $\cong$ SM-3997, which nearly corresponds with the affinity of these drugs for 5-HT_{1A} receptors and DA_2 receptors (26, 31, 36). This is consistent with previous observations about the abilities of buspirone, gepirone and SM-3997 to affect monoamine metabolites (7, 16, 25, 26, 30). Furthermore, in behavioral studies, SM-3997 (35), ipsapirone (28) and gepirone (26) have been reported to have little effect on the dopaminergic system compared with buspirone, which also correspond with the present results.

In summary, our results indicate that SM-3997 shows no effects on monoamine uptake except a weak inhibition of NE uptake but produces complex effects on the brain concentrations of monoamine metabolites (5-HIAA, DOPAC, HVA and MHPG). SM-3997 probably decreases 5-HIAA levels by agonistic interaction with somatodendritic or postsynaptic 5-HT_{1A} receptors and increases DOPAC and HVA levels by an antagonistic action on DA_2 receptors, whereas the metabolite of SM-3997, 1-PP, may be involved in the increase in MHPG and in the decrease in NE produced by SM-3997. The present study also shows that SM-3997 decreases the 5-HIAA level comparable fashion to buspirone, gepirone and ipsapirone, but increases the levels of DOPAC and HVA to a much smaller extent than buspirone. Recently, the involvement of the serotonergic system in the control of anxiety has been reevaluated with the development of new anxiolytic compounds which bind to 5-HT_{1A} receptors (40). However, the exact 5-HT_{1A} receptor-related mechanism is as yet unclear and the role of noradrenergic and dopaminergic effects in the actions of these drugs remain to be elucidated. Our findings will be useful for understanding the pharmacological actions of SM-3997 and other 5-HT_{1A}receptor-related anxiolytics.

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