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Involvement of Serotonin in the Central Regulation of Blood Pressure: Evidence for a Facilitating Effect on Sympathetic Nerve Activity

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ABSTRACT

The effects of serotonin (5-HT) receptor agonists and antagonists on sympathetic nervous discharge (SND) recorded from the external carotid and splanchnic nerves were studied in baroreceptor-denervated cats. Intravenous administration of the 5-HT antagonists methysergide (0.025–1.6 mg/kg), metergoline (0.01–0.32 mg/kg), cyproheptadine (0.05–1.6 mg/kg) and cinanserin (0.2–6.4 mg/kg) was associated with a prolonged dose-related inhibition of SND. Maximum reductions in SND produced by methysergide, metergoline, cyproheptadine and cinanserin were 90, 72, 71 and 50%, respectively. In contrast, a progressive increase in SND was observed in vehicle control animals. Methysergide, metergoline and cyproheptadine failed to reduce SND in cats pretreated with the 5-HT synthesis inhibitor *p*-chlorophenylalanine. Clonidine (20 μg/kg i.v.) significantly inhibited SND (−73%) in *p*-chlorophenylalanine-treated cats. The selective 5-HT agonists 5-methoxydimethyltryptamine and lisuride also reduced SND in a dose-dependent manner. The time course of the depressor effects of 5-methoxydimethyltryptamine and lisuride correlate well to their ability to inhibit 5-HT cell firing. These data indicate that 5-HT agonists which act presynaptically to inhibit 5-HT cell firing and antagonists which act postsynaptically to block the effect of synaptically released 5-HT both mediate a central reduction in SND. It is concluded that central 5-HT neurons facilitate transmission in central sympathetic pathways.

In recent years evidence has accumulated to indicate that central serotonergic neurons participate in the regulation of blood pressure. Anatomically, areas of the brain stem and spinal cord involved in vasomotor control (*i.e.*, the intermediolateral cell column, nucleus tractus solitarius and areas of the hypothalamus) are heavily innervated by 5-HT-containing neurons (Dahlstrom and Fuxe, 1965; Fuxe, 1965; Palkovits *et al.*, 1974; Bobillier *et al.*, 1976). The turnover rate and/or content of 5-HT in these areas is altered during experimental hypertension (Wing and Chalmers, 1974; Smith *et al.*, 1979; Chemerinski *et al.*, 1980). Pharmacologic evidence also suggests that central serotonergic neurons participate in cardiovascular control. However, the nature of the interaction between 5-HT pathways and central sympathetic networks is not well understood (Kuhn *et al.*, 1980a). For example, the 5-HT precursor 5-hydroxytryptophan has been shown to produce a fall in MAP in the conscious and anesthetized rat (Henning and Rubenson, 1971; Nolan, 1977), in the cat (Baum and Shropshire, 1975; Tadepalli *et al.*, 1977) and in the dog (Antonaccio and Robeon, 1973, 1975). The hypotension was accompanied by bradycardia and a decrease in splanchnic sympathetic nerve activity (Baum and Shropshire, 1975). The depressor response produced by i.v. or i.c.v. 5-hydroxytryptophan was potentiated by monoamine oxidase inhibition and blunted by decarboxylase inhibition (Antonaccio and Robeon, 1973, 1975; Tadepalli *et al.*, 1977). In agreement with these observations, i.c.v. injection of 5-HT decreases MAP, HR and SND in cats and dogs (McCubbin *et al.*, 1960; Dunkley *et al.*, 1972; Baum and Shropshire, 1975). In addition, several studies have shown that depletion of brain 5-HT with the 5-HT synthesis inhibitor PCPA either fails to alter or increases blood pressure (Ito and Schanberg, 1972; Yamori *et al.*, 1972; and Helke *et al.*, 1978). Collectively, these observations suggest that 5-HT neurons normally inhibit transmission in central sympathetic pathways.

However, an equally impressive body of evidence suggests that 5-HT facilitates central sympathetic nervous activity. First, i.c.v. injections of 5-HT in the rat typically result in a pressor response (Lambert *et al.*, 1975; Smits and Struyker-Boudier, 1976; Nahmod *et al.*, 1978). Similarly, microinjections of 5-HT into the anterior hypothalamus/preoptic area or the nucleus tractus solitarius also increase MAP (Smits and Struyker-Boudier, 1976; Wolf *et al.*, 1981b). These pressor effects were enhanced by the 5-HT uptake inhibitor fluoxetine and blocked by the 5-HT antagonist metergoline. Second, i.v. or i.c.v. administration of the 5-HT antagonist methysergide decreases blood pressure in the rat, cat and dog (Antonaccio *et al.*, 1975;

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ABBREVIATIONS: 5-HT, serotonin; MAP, mean arterial pressure; HR, heart rate; SND, sympathetic nervous discharge; PCPA, *p*-chlorophenylalanine; UML, methysergide; MET, metergoline; CIN, cinanserin; CYPRO, cyproheptadine; 5-MeODMT, 5-methoxydimethyltryptamine.

Smits and Struyker-Boudier, 1976; Antonaccio and Taylor, 1977). In the cat, the depressor response produced by UML was associated with a decrease in SND (Antonaccio and Taylor, 1977). Third, pretreatment with the 5-HT neurotoxins 5,6- or 5,7-dihydroxytryptamine typically results in a decrease in blood pressure (Myers *et al.*, 1974; Wing and Chalmers, 1974; Finch, 1975; Gothert and Klupp, 1978). Finally, electrical stimulation of 5-HT nuclei (*e.g.*, posterior portions of raphe pallidus and obscuris, anterior portion of raphe magnus and dorsal and median raphe nuclei) elicits pressor responses (Gillis *et al.*, 1976; Adair *et al.*, 1977; Smits *et al.*, 1978; Kuhn *et al.*, 1980b; Wolf *et al.*, 1981b). These data suggest that 5-HT neurons facilitate activity in central sympathetic pathways.

These extremely variable results undoubtedly reflect the complexity of 5-HT neuronal pathways and their interaction with the central sympathetic system. In this regard, at least three types of 5-HT receptors exist in the central nervous system (Haigler and Aghajanian, 1974; DeMontigny and Aghajanian, 1977; Aghajanian and Wang, 1978; McCall and Aghajanian, 1979, 1980a). The first type of 5-HT receptor mediates an inhibition of neuronal activity in areas such as the suprachiasmatic nucleus, the ventrolateral geniculate and the cortical and basolateral nuclei of the amygdala (Aghajanian and Wang, 1978). A second postsynaptic 5-HT receptor has been shown to facilitate excitatory inputs to motor nuclei (McCall and Aghajanian, 1979; White and Neuman, 1980). Finally, a third 5-HT receptor mediates a 5-HT-induced inhibition of serotonergic neurons in the dorsal raphe nucleus (*i.e.*, a presynaptic or autoreceptor; DeMontigny and Aghajanian, 1977; Aghajanian and Wang, 1978). Classical 5-HT antagonists (*e.g.*, UML, MET, CIN and CYPRO) fail to block either the presynaptic or postsynaptic inhibitory action of 5-HT (Haigler and Aghajanian, 1974; Aghajanian and Wang, 1978). In contrast, these antagonists act selectively on 5-HT receptors to block the facilitating action of 5-HT in the central nervous system (McCall and Aghajanian, 1979, 1980a; White and Neuman, 1980).

The possibility exists that UML inhibits SND by blocking a facilitating action of 5-HT on central sympathetic pathways. However, the central sympatholytic action of methysergide has never been shown to be related to its 5-HT-blocking properties. If UML produced its depressor effect *via* an action on the 5-HT system, then this would suggest that central 5-HT normally enhances sympathetic activity. Therefore, to test this hypothesis, a number of structurally unrelated 5-HT antagonists were studied in control and PCPA-treated cats to determine their effect on central sympathetic outflow. In addition, the effects of 5-MeODMT and lisuride on SND were also studied. These two agents have been shown to be selective 5-HT autoreceptor agonists which act to inhibit serotonergic firing (DeMontigny and Aghajanian, 1977; Kehr, 1977; Rogawski and Aghajanian, 1979). The present study indicates that agents which act presynaptically to inhibit 5-HT cell firing and agents which act postsynaptically to antagonize the effect of synaptically released 5-HT both mediate a central reduction in SND. These data suggest that central 5-HT neurons normally facilitate transmission in central sympathetic pathways.

Methods

Animal preparation. Sixty-two cats weighing between 2.0 and 4.0 kg were anesthetized by an i.p. injection of a mixture of sodium diallylbarbiturate (60 mg/kg), urethane (240 mg/kg) and monoethylurea (240 mg/kg). The cats were placed in a David Kopf Instruments stereotaxic apparatus, and a glass trachea tube was inserted. The femoral artery was cannulated to measure MAP with a Statham transducer and a Grass polygraph (model 7D). A femoral vein catheter was inserted to permit i.v. drug or vehicle administration. HR was recorded continuously with a Grass 7P4 tachograph triggered by the electrocardiogram (lead I, II or III). Rectal temperature was maintained between 36 and 38°C with a heating pad and/or lamp.

The cats were baroreceptor-denervated in order to assess more accurately a possible central reduction in SND produced by 5-HT agonists and antagonists. Briefly, the carotid sinus, aortic depressor and vagus nerves were exposed from a ventral aspect after reflection of the cephalad portion of the trachea and esophagus into the mouth. The carotid sinus nerve was identified at its junction with the glossopharyngeal nerve, while the aortic depressor nerve was located at its junction with the superior laryngeal nerve. The vagus nerve was isolated in the midcervical region. Baroreceptor denervation was accomplished by bilaterally sectioning these nerves. The denervation was judged complete if SND was not inhibited during the pressor response to i.v. norepinephrine (0.1 μg/kg).

Sympathetic nerve recording. The sympathetic postganglionic external carotid nerve was isolated distal to its junction with the superior cervical ganglion, ligated and sectioned near the carotid sinus. Nerve potentials were recorded monophasically under mineral oil with a bipolar platinum electrode after capacity coupled preamplification (low and high half-amplitude responses at 1 and 500 Hz, respectively). Nerve activity was displayed on the Grass polygraph and quantitated with a Grass P710B cumulative integrator. In several experiments, SND was similarly recorded from the left sympathetic preganglionic greater splanchnic nerve following a retroperitoneal approach. These cats were artificially respired after immobilization with gallamine triethiodide (4 mg/kg i.v.). Pneumothoracotomy was performed to minimize untoward respiratory movements.

Acute drug treatments. MAP, HR and SND were allowed to equilibrate for 1 hr after baroreceptor denervation. Pretreatment values for MAP, HR and SND were averaged over the last 10 min of the equilibration interval. Twelve vehicle control cats were then treated with an i.v. injection of distilled water ($n = 9$) or 2.5% ascorbic acid ($n = 3$). MAP, HR and SND were monitored over the ensuing 2 hr with supplemental vehicle being given at 20, 40, 60, 80 and 100 min. Since no differences were noted between these two vehicles, the values obtained from these two groups of experiments were pooled for comparison with the drug-treated animals.

The 5-HT antagonists UML, MET, CYPRO, and CIN were examined under these same conditions. UML, CYPRO and CIN were dissolved in 1 mg/ml of distilled water. The MET solution (0.5 mg/ml in 2.5% ascorbic acid) was used immediately since this compound is relatively unstable. The starting doses of UML, MET, CYPRO and CIN were 0.025, 0.01, 0.05 and 0.2 mg/kg, respectively, and the cumulative dose of each antagonist was doubled at 20-min intervals. The effects of these agents on MAP, HR and SND were determined 15 to 20 min after each dose. In some instances the effects of the *alpha*-2 adrenergic receptor agonist clonidine (20 μg/kg i.v.), the *alpha*-2 adrenergic receptor antagonist piperoxane (0.5 mg/kg i.v.) and the selective 5-HT autoreceptor agonists lisuride (0.005–0.01 mg/kg i.v.) and 5-MeODMT (0.01–0.05 mg/kg i.v.) were also determined. These compounds were dissolved in 0.5 mg/ml of distilled water.

PCPA-pretreated cats. In a separate series of experiments, the effects of these same 5-HT antagonists on MAP, HR, and SND were determined in PCPA-pretreated cats. These cats received 400 mg/kg (i.p.) of PCPA methyl ester hydrochloride once daily for 2 consecutive days. The 5-HT antagonists were studied in the manner described above 2 days after the last injection of PCPA.

The concentration of brain stem and spinal cord 5-HT and norepinephrine were determined in cats receiving PCPA ($n = 4$) or the saline vehicle ($n = 4$) for 2 consecutive days. Two days after the last injection the animals were anesthetized with Dial-urethane and mounted in a stereotaxic apparatus. After exposure, the third thoracic segment of the cord and a 4-mm slice of the medulla at the level of the obex were removed and frozen on Dry Ice. Tissue samples were weighed and homogenized in two 3-ml aliquots of cold butanol-1 in a centrifuge tube

containing 750 μl of 0.025 N HCl, 100 μl, of 0.1 N EDTA, 10 μl of an ascorbic acid solution (11 mg/ml in 0.01 N HCl) and 3 g of NaCl. The homogenizing tube was then washed with 6 ml of cold butanol-1, shaken for 30 min and centrifuged at 3000 rpm for 10 min. The butanol-1 phase was transferred to a glass-stoppered conical centrifuge tube containing 17 ml of *n*-heptane and 500 μl of 0.01 N HCl. Tubes were then vortexed and the phases were allowed to separate. The upper organic layer was aspirated and discarded. The aqueous phase was collected and an internal standard was added. Biogenic amine levels were determined using a high pressure liquid chromatographic system and an electrochemical detector (Bioanalytical Systems, West Lafayette, IN).

Statistical analysis. Differences in mean arterial pressure, heart rate and sympathetic nerve discharge between the vehicle and drug-treated groups were analyzed for statistical significance by an unpaired *t* test. Both the absolute values and the changes from pretreatment were evaluated. A probability level of $P \leq .05$ was presumed to reflect statistical significance. Values are expressed as mean ± S.E.

Results

Effects of 5-HT antagonists in baroreceptor-denervated cats. The effects of the peripheral 5-HT antagonists UML, MET, CYPRO and CIN on external carotid SND are illustrated in figures 1 and 2. UML (0.025–1.6 mg/kg, $n = 5$), MET (0.01–0.32 mg/kg, $n = 5$), CYPRO (0.05–1.6 mg/kg, $n = 5$) and CIN (0.2–6.4 mg/kg, $n = 4$) dramatically reduced SND in a dose-dependent manner (fig. 1). Maximum reductions in SND from pretreatment produced by UML, MET, CYPRO and CIN were 90, 72, 71 and 50%, respectively. Similar reductions in SND were observed in four experiments in which activity was recorded from the sympathetic preganglionic splanchnic nerve. The effects of 5-HT antagonists on SND are even more striking when compared to the progressive increase in nerve activity observed in vehicle control animals. The onset of the reduction in SND occurred within 1 min after the i.v. injection of UML, CYPRO and CIN, with maximum inhibition observed 5 to 10 min after dosing. The onset of the sympatholytic action of MET was delayed (*i.e.*, ≥10 min) and the maximum effect was not observed until 15 to 20 min after each dose. The delay in the onset of the sympatholytic action of MET correlates well with the delay of onset of its 5-HT blocking action (McCall and Aghajanian, 1980a). Figure 2 illustrates the reduction in external carotid sympathetic activity and integrated SND produced by i.v. MET (20 μg/kg). The *alpha*-2 adrenergic antagonist piperoxane (0.5 mg/kg i.v.) failed to reverse the inhibition of SND produced by MET (fig. 2C) and the other 5-HT antagonists. In contrast, numerous investigators have found that piperoxane reverses the clonidine-mediated central reduction in SND (Schmitt *et al.*, 1973; Kobinger, 1978). These results indicate that the decrease in SND produced by 5-HT antagonists is not due to any central *alpha*-2 adrenergic agonist activity.

Table 1 summarizes the effects of these 5-HT antagonists on MAP and HR. The decreases in external carotid nerve activity produced by UML and CYPRO were associated with significant, dose-dependent reductions in MAP and HR. Those doses of CIN which significantly reduced SND also produced mild

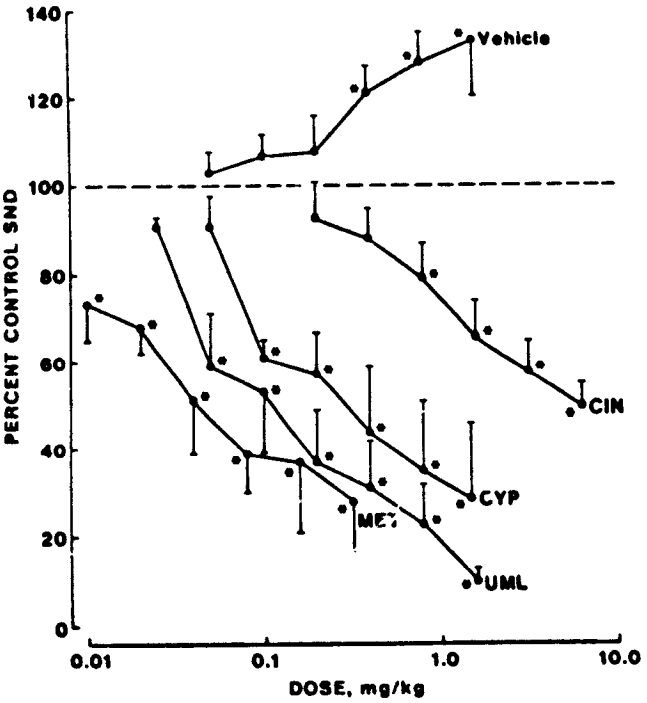

Fig. 1. Log dose-response curve of the effects of MET, UML, cyproheptadine (CYP) and CIN on external carotid SND. The 5-HT antagonists reduced SND significantly (*) while the vehicle increased SND. Data were obtained 15 to 20 min after each dose. See text for further details regarding dosing regimen.

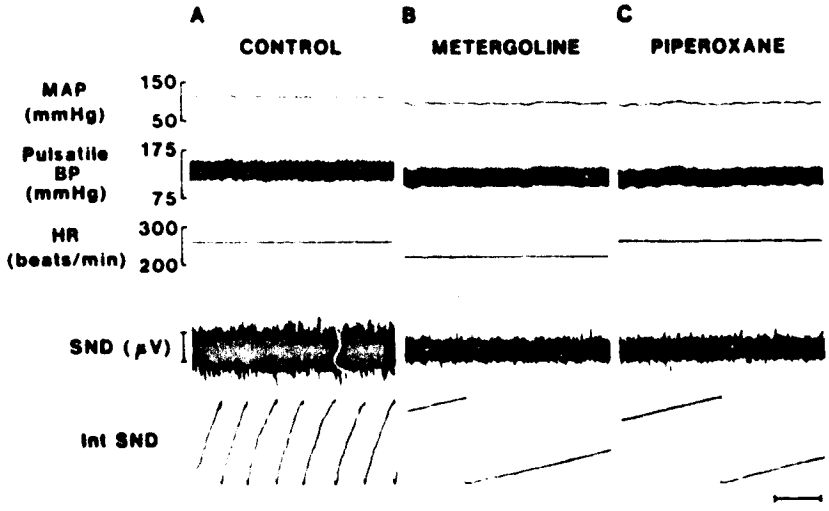

Figure 2. Effects of MET (20 μg/kg i.v.) in baroreceptor-denervated cat. Tracings from top to bottom are: mean arterial pressure, pulsatile blood pressure, heart rate, external carotid sympathetic nervous discharge and integrated SND. A, control; B, 15 min after metergoline administration; C, 15 min after piperoxane (0.5 mg/kg i.v., 30 min after MET). Piperoxane failed to reverse the reduction in SND produced by MET. Vertical calibration is 40 μV. Horizontal calibration is 1 min.

TABLE 1

Effect of serotonin antagonists on MAP and HR in baroreceptor-denervated cats

All values are expressed as mean ± S.E. PT, pretreatment

Expt Time	Vehicle Control			UML			MET			CYPRO			CIN		
	Dose	MAP	HR	Dose	MAP	HR	Dose	MAP	HR	Dose	MAP	HR	Dose	MAP	HR
min	*ml/kg*			*mg/kg*			*mg/kg*			*mg/kg*			*mg/kg*		
PT		120	248		119	251		104	255		122	260		135	243
		±6	±10		±4	±8		±7	±5		±10	±9		±6	±9
20	0.05	120	248	0.05	98***	216***	0.01	102	253	0.05	107***	253	0.2	131	238
		±7	±10		±3	±7		±6	±6		±9	±11		±5	±10
40	0.10	120	248	0.1	95***	204***	0.02	97	250	0.1	103***	250**	0.4	128	235
		±6	±10		±4	±6		±8	±8		±7	±13		±5	±9
60	0.20	118	248	0.2	91***	192***	0.04	95	248	0.2	100***	241***	0.8	124**	233
		±6	±10		±6	±7		±8	±14		±4	±11		±6	±10
80	0.40	117	249	0.4	87***	183***	0.08	95	245	0.4	98***	233***	1.6	124**	230
		±6	±10		±6	±8		±9	±14		±3	±12		±8	±9
100	0.80	114	249	0.8	77***	178***	0.16	94**	237	0.8	98**	227***	3.2	122**	226
		±7	±10		±5	±12		±9	±10		±3	±11		±7	±10
120	1.60	112*	249	1.6	75***	161***	0.32	86**	225	1.6	98**	219***	6.4	120**	219**
		±6	±10		±3	±8		±6	±12		±3	±10		±7	±9

* Indicates a significant difference ($P \leq .05$) from pretreatment values; ** indicates a significant difference ($P \leq .05$) between the magnitude of the change from PT in vehicle- and drug-treated animals.

reductions in MAP which were significantly different from the vehicle control treated cats. HR declined with all doses of CIN, but significance was limited to the highest dose (6.4 mg/kg). MET reduced MAP only at the two highest doses (0.16 and 0.32 mg/kg i.v.) and failed to significantly alter HR. MET differed from the other 5-HT antagonists in that it produced an immediate pressor response which lasted approximately 5 min after i.v. administration. If due to a direct vasoconstrictor action, this observation could help to explain the relatively mild hypotensive effect of MET despite its marked inhibition of SND.

Effects of 5-HT antagonists in baroreceptor denervated, PCPA-pretreated cats. The data presented in figures 1 and 2 indicate that UML, MET, CYPRO and CIN all reduce SND. There is no direct evidence, however, to suggest that this effect is due to their 5-HT-blocking action. Therefore, the effects of UML, MET and CYPRO on SND were tested in baroreceptor-denervated cats chronically pretreated with the 5-HT synthesis inhibitor PCPA. In agreement with other investigators (Koe and Weissman, 1966), we found that the concentration of 5-HT and 5-hydroxyindoleacetic acid in the spinal cord and medulla was significantly reduced in cats treated for 2 days with 400 mg/kg/day of PCPA i.p. (table 2). This PCPA regimen, however, did not affect NE concentrations in the medulla or the spinal cord significantly. The PCPA-pretreated cats ($n = 4$) had significantly lower starting MAP values (100 ± 7 mm Hg) than nonpretreated control cats (119 ± 3 mm Hg). In contrast, there was no significant difference in the heart rate of control (254 ± 4 beats/min) and PCPA cats (245 ± 11 beats/min).

The effects of UML, MET and CYPRO on SND in PCPA-pretreated cats are illustrated in figure 3. Intravenous UML (0.025–1.6 mg/kg, $n = 5$) and MET (0.01–0.32 mg/kg, $n = 5$) failed to reduce SND. In fact, external carotid sympathetic nerve activity actually increased significantly from pretreatment with high doses of UML (0.4–0.8 mg/kg i.v.) and low doses of MET (0.01–0.04 mg/kg i.v.). The changes in SND from pretreatment seen in the PCPA-pretreated cats were uniformly significant from the reductions in SND previously noted in nonpretreated cats with each dose of UML and MET. Low and middle doses of CYPRO (0.05–0.8 mg/kg, $n = 4$) likewise failed

TABLE 2

Concentration of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in control ($n = 4$) and PCPA-pretreated ($n = 4$) cats

Values are expressed as mean ± S.E.M.

Brain Region	Control		PCPA-Pretreated	
	5-HT	5-HIAA	5-HT	5-HIAA
	ng/ml protein		*ng/ml protein*	
Medulla	12.1 ± 0.54	7.47 ± 0.73	2.85 ± 0.70*	1.15 ± 0.32*
Spinal cord	16.4 ± 1.8	4.12 ± 0.23	4.34 ± 0.62*	1.48 ± 0.45*

* Indicates a significant difference ($P \leq .05$) between saline- and PCPA-pretreated animals.

to effect SND in PCPA-pretreated cats. The highest dose of CYPRO (1.6 mg/kg i.v.) did reduce SND by 35% from pretreatment, but this reduction was nevertheless significantly less than the 71% reduction seen with the same dose of CYPRO in nonpretreated cats.

In addition to severely attenuating the inhibitory action of the 5-HT antagonists on SND, PCPA pretreatment also minimized their hypotensive action. Low doses of UML (0.05–0.4 mg/kg i.v.) failed to reduce MAP and HR. A significant depressor response (−23 mm Hg) was observed with the 0.8 and 1.6 mg/kg doses of UML. This was accompanied by a significant bradycardia (−57 beats/min). MET failed to alter MAP or HR in PCPA-pretreated cats. Higher doses of CYPRO (0.2–0.8 mg/kg i.v.) produced a small but significant reduction in MAP (−15 mm Hg). The highest dose of CYPRO (1.6 mg/kg) failed to reduce MAP when compared to pretreatment values. HR was not altered by any doses of CYPRO.

To confirm that SND could indeed be inhibited in these PCPA-pretreated cats, the *alpha*-2 adrenergic receptor agonist clonidine was administered at the conclusion of each experiment. As previously reported (McCall and Gebber, 1976; Kobinger, 1978), i.v. clonidine (20 μg/kg) produced a transient pressor response which was followed by a long-lasting decrease in MAP (−18 mm Hg) and HR (−75 beats/min). As illustrated in figure 4, clonidine significantly decreased external carotid SND (−73%) in PCPA-pretreated cats. This observation supports the contention that activity recorded in PCPA-pretreated cats was of sympathetic neuronal origin.

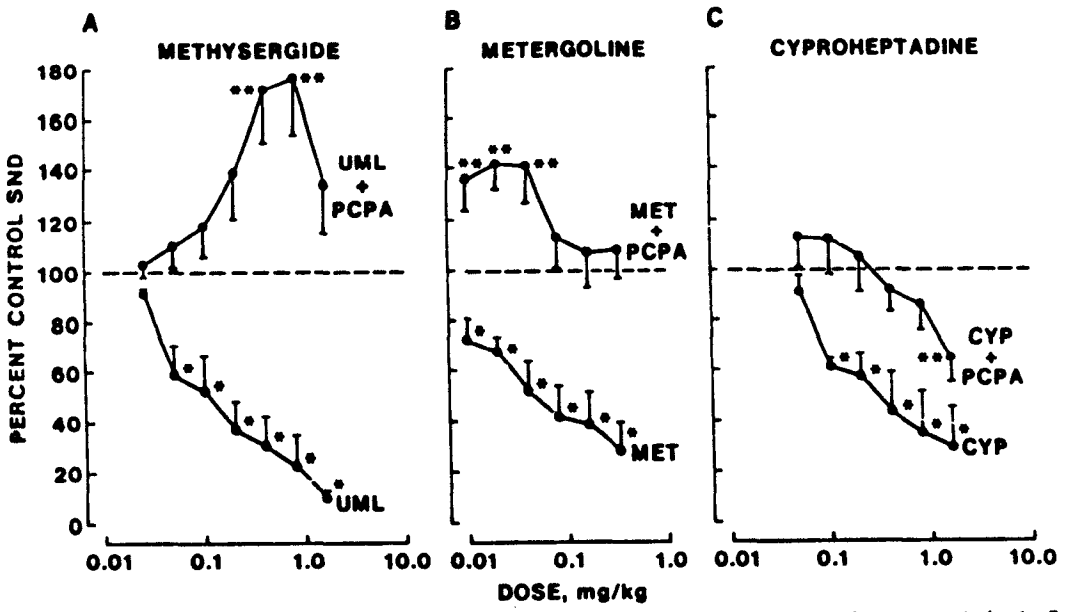

Fig. 3. Log dose-response curves of the effects of UML, MET and CYPRO on SND in PCPA-pretreated and nonpretreated cats. 5-HT antagonists failed to reduce SND in PCPA-pretreated animals. *Indicates statistical difference from pretreatment.

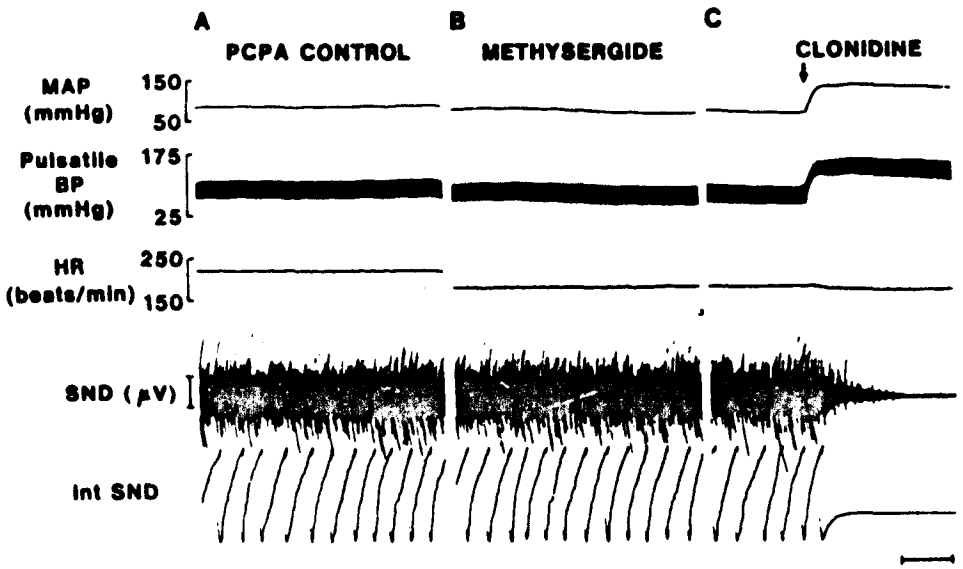

Fig. 4. Effects of UML (1.6 mg/kg i.v.) and clonidine (20 μg/kg i.v.) in PCPA-pretreated cat. Tracings from top to bottom are: mean arterial pressure, pulsatile blood pressure, heart rate, sympathetic nervous discharge and integrated SND. A, PCPA-pretreated control; B, 15 min after UML; C, clonidine, 20 min after UML. UML failed to reduce SND. Clonidine inhibited SND. Vertical calibration is 60 μV. Horizontal calibration is 1 min.

Effects of 5-HT agonists in baroreceptor-denervated cats. 5-MeODMT and lisuride have been shown to be 5-HT agonists which possess a relatively high degree of specificity for the 5-HT autoreceptor. Thus, low doses of these agents dramatically inhibit 5-HT cell firing, but have little effect on postsynaptic inhibitory or facilitatory receptors (DeMontigny and Aghajanian, 1977; Aghajanian and Wang, 1978; McCall and Aghajanian, 1979, 1980b; Rogawski and Aghajanian, 1979). Figure 5 illustrates that 5-MeODMT (10–50 μg/kg i.v.; $n = 8$) dramatically decreased MAP, HR and external carotid SND. SND was maximally reduced within 1 min after 5-MeODMT and remained depressed for 10 to 20 min. Thus, the time course of this depressor response correlates well to the published time course of depressed 5-HT cell firing elicited by 5-MeODMT (DeMontigny and Aghajanian, 1977). Lisuride (5–10 μg/kg i.v.; $n = 4$) also rapidly depressed MAP, HR, and SND (fig. 6). Like 5-MeODMT, maximum reductions were observed within 1 to 2 min after dosing. However, MAP, HR and SND remained depressed throughout a 1-hr observation period. Previous studies have indicated that lisuride (5–10 μg/kg i.v.) produces a similar long-lasting inhibition of 5-HT cell discharges in the dorsal raphe nucleus (Rogawski and Aghajanian, 1979). Thus, the sympatholytic time course of both of these 5-HT agonists correlates well with their duration of action on central 5-HT autoreceptors. The sympatholytic actions of 5-MeODMT and lisuride could not be prevented or reversed by piperoxane. However, as illustrated in figure 7, lisuride (5–10 μg/kg i.v.) failed to reduce external carotid SND in cats pretreated with

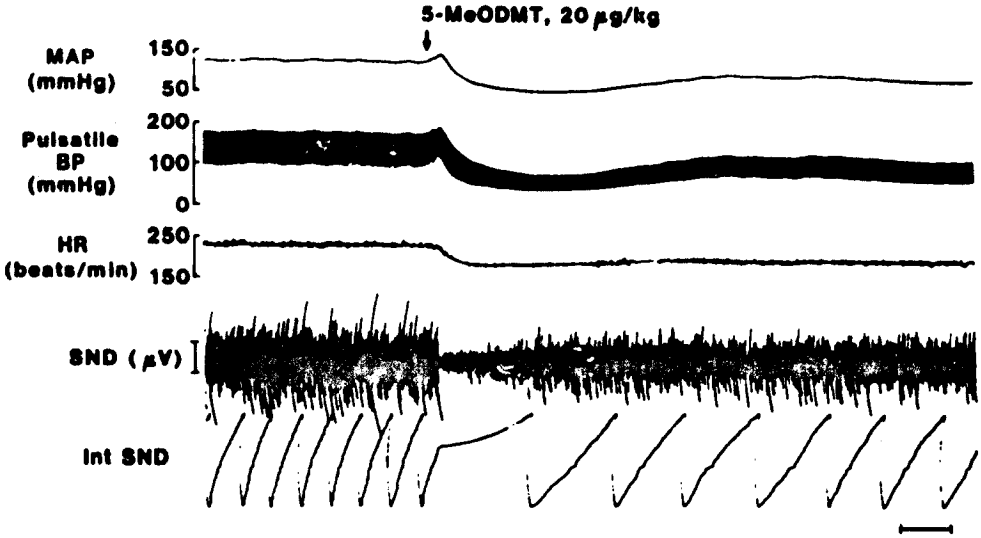

Fig. 5. Effects of 5-MeODMT in baroreceptor-denervated cat. Tracings from top to bottom are: mean arterial pressure, pulsatile blood pressure, heart rate, sympathetic nervous discharge and integrated SND. 5-MeODMT (i.v.) reduced MAP, HR and SND. Vertical calibration is 50 μV. Horizontal calibration is 1 min.

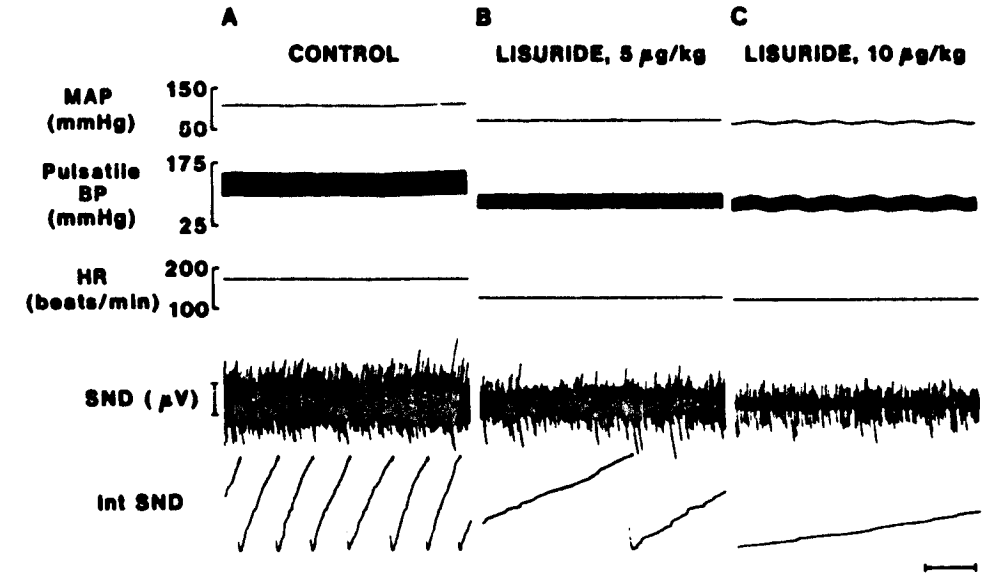

Fig. 6. Effects of lisuride (i.v.) in baroreceptor-denervated cat. Tracings from top to bottom are: mean arterial pressure, pulsatile blood pressure, heart rate, sympathetic nervous discharge and integrated SND. Lisuride reduced MAP, HR and SND in a dose-dependent manner. Vertical calibration is 40 μV. Horizontal calibration is 1 min.

PCPA ($n = 3$). This observation supports the contention that the inhibition of SND produced by lisuride and 5-MeODMT was secondary to a decrease in 5-HT cell firing.

Discussion

The present investigation was designed to determine the role of central 5-HT neurons in the regulation of blood pressure by studying the action of 5-HT agonists and antagonists on sympathetic nerve activity. We found that the 5-HT antagonists UML, MET, CYPRO and CIN dramatically reduced SND recorded from the external carotid nerve of baroreceptor-denervated cats. The fact that these agents also inhibited nerve activity recorded from the preganglionic splanchnic nerve indicates that a sympatholytic effect of these 5-HT antagonists at sympathetic ganglia is negligible. In this regard, hexamethonium fails to inhibit SND recorded from the splanchnic nerve. Taken together, these observations indicate that UML, MET, CYPRO and CIN act centrally to reduce sympathetic nerve activity.

Previous studies indicate that i.c.v. administered UML produces hypotension and bradycardia (Antonaccio *et al.*, 1975). In addition, UML has previously been shown to reduce SND (Antonaccio and Taylor, 1977). However, these studies did not indicate whether UML was acting to decrease sympathetic activity *via* its 5-HT antagonist property. In the present investigation, several observations suggest that UML, MET, CYPRO and CIN reduced central sympathetic outflow *via* their 5-HT-blocking actions. First, each of these agents has been shown to antagonize both the peripheral and central actions of 5-HT (Aghajanian *et al.*, 1975; McCall and Aghajanian, 1980a). Second, the fact that UML, MET, CYPRO and CIN all reduced sympathetic nerve activity suggests that they did so by a common mechanism (*i.e.*, 5-HT receptor antagonism). In this

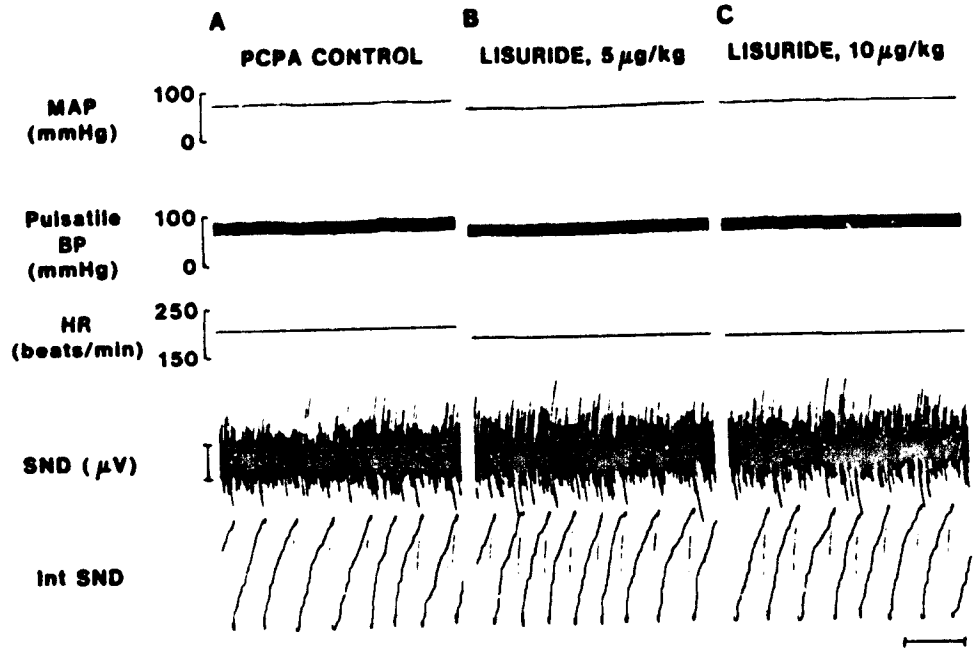

Fig. 7. Effects of lisuride (i.v.) in PCPA-pretreated, baroreceptor-denervated cat. Tracings from top to bottom are: mean arterial pressure, pulsatile blood pressure, heart rate, sympathetic nervous discharge and integrated SND. Lisuride (5–10 μg/kg) failed to reduce SND in PCPA-pretreated animals. Vertical calibration is 40 μV. Horizontal calibration is 1 min.

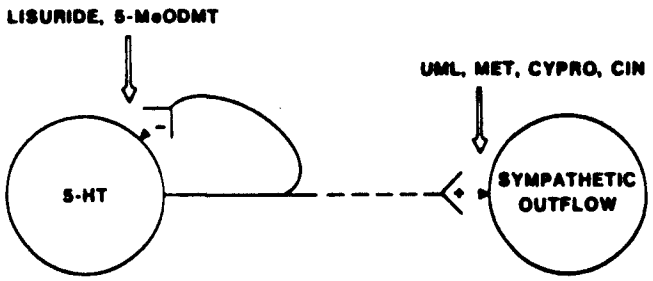

Fig. 8. Model depicting a possible interaction between serotonergic and central sympathetic neurons. Dashed line indicates a possible polysynaptic pathway. The sympathetic neuron represents a neuronal network involved in the genesis of sympathetic outflow. Lisuride and 5-MeODMT act at the 5-HT autoreceptor to inhibit serotonin firing resulting in a decrease in SND (disfacilitation). 5-HT antagonist acts postsynaptically to block the excitatory effect of synaptically released 5-HT on sympathetic pathways. Thus, 5-HT neurons facilitate SND.

regard, there are no other known pharmacologic properties common to all four of these structurally dissimilar agents. Third, we found the onset of the sympatholytic action of UML, CYPRO and CIN to be rapid, while the onset of action of MET was delayed. This agrees well with the time course of the 5-HT antagonist action of these agents in the central nervous system (McCall and Aghajanian, 1980a). Fourth, the effects of UML, MET and CYPRO were significantly attenuated or prevented in cats depleted of 5-HT by pretreatment with the 5-HT synthesis inhibitor, PCPA. In contrast, the *alpha*-2 adrenergic agonist clonidine reduced SND in PCPA-pretreated animals. Finally, the *alpha*-2 adrenergic receptor agonist piperoxane failed to reverse the central reduction in SND produced by the 5-HT antagonists. Thus, UML, MET, CYPRO and CIN did not produce their central sympatholytic effects *via* an *alpha* adrenergic mechanism. Taken together these observations strongly suggest that UML, MET, CYPRO and CIN reduced SND by antagonizing the actions of 5-HT in the central nervous system.

Low doses of lisuride and 5-MeODMT in the present study also produced a reduction in MAP, HR and SND by an action on the central nervous system. Lisuride and 5-MeODMT are highly selective 5-HT autoreceptor agonists which inhibit the discharges of 5-HT cells. Aghajanian and co-workers (DeMontigny and Aghajanian, 1977; Rogawski and Aghajanian, 1979) have demonstrated that the duration of the 5-MeODMT-induced suppression of 5-HT neuronal firing is relatively short (*i.e.*, <20 min), whereas the duration of action of lisuride is much longer (*i.e.*, >1 hr). We found that the depressor response produced by 5-MeODMT lasted 10 to 20 min, whereas the sympatholytic action of lisuride extended for at least 1 hr. Thus, the correlation between the duration of the depression of sympathetic activity and 5-HT cell firing suggests that 5-MeODMT and lisuride depressed SND *via* a direct action on serotonergic neurons. This contention is supported by the observation that depressor responses produced by 5-MeODMT and lisuride could not be prevented or reversed by piperoxane. Thus, 5-MeODMT and lisuride were not acting *via* adrenergic mechanisms. In addition, since both 5-MeODMT and lisuride reduced SND, it is likely that they did so by their common ability to inhibit 5-HT cell firing. Finally, lisuride failed to reduce SND in PCPA-pretreated cats. Combined, these observations support the view that the inhibition of SND produced by the 5-HT autoreceptor agonists 5-MeODMT and lisuride was a consequence of their inhibitory actions on 5-HT neurons.

The present study may shed light on the role of central serotonergic pathways in the regulation of blood pressure. Our data suggest that 5-HT neurons act to enhance sympathetic outflow from the brain (fig. 8). First, 5-HT antagonists inhibit SND. Since it has been found that these agents block the postsynaptic excitatory but not the inhibitory effects of 5-HT at serotonergic receptors (Haigler and Aghajanian, 1974; Aghajanian and Wang, 1978; McCall and Aghajanian, 1979, 1980a), it is likely that UML, MET, CYPRO and CIN acted postsynaptically to antagonize the excitatory effect of synaptically released 5-HT on central sympathetic neurons. Alternatively, 5-HT might inhibit an inhibitory interneuron which projects to

sympathetic neurons. This possibility seems unlikely, however, since 5-HT antagonists do not block the inhibitory actions of 5-HT (Haigler and Aghajanian, 1974). Second, the 5-HT autoreceptor agonists lisuride and 5-MeODMT also inhibited SND. These agents have been shown to mimic the inhibitory effects of 5-HT released from the terminals of serotonergic axon collaterals (fig. 8). Our data are consistent with the possibility that lisuride and 5-MeODMT stimulate the autoreceptors and thus inhibit 5-HT cell firing. In turn, SND would be reduced through a process of disfacilitation. The observation that mean arterial pressure was significantly reduced in PCPA-pretreated cats when compared to control animals also suggests that 5-HT normally enhances activity in sympathetic pathways. Thus, the data in the present investigation support the idea that central 5-HT neurons enhance sympathetic outflow in a tonic manner. Interestingly, recent work in our laboratory indicates that highly selective *alpha*-1 adrenergic receptor antagonists (*e.g.*, prazosin and WB-4101) and *alpha*-2 adrenergic agonists (*e.g.*, clonidine) also act centrally to reduce sympathetic outflow. Thus, it appears that central noradrenergic neurons, like serotonergic neurons, facilitate sympathetic nervous discharge (McCall and Humphrey, 1981).

The model presented in figure 8 may help to explain some of the extremely variable observations that have been made regarding the role of central 5-HT neurons in blood pressure regulation. For example, the effect of 5-HT and its precursors on SND would be dependent on the site of administration. If centrally administered 5-HT acted primarily on serotonergic cell bodies, the result would be an inhibition of 5-HT cell firing and an associated disfacilitation of sympathetic outflow. In contrast, if the major action of 5-HT was on postsynaptic neurons, then one might expect to see an increase in SND and MAP. Alternatively, or in addition, the variable effects of 5-HT agonists on MAP might result from a dual action of 5-HT on sympathetic outflow. It is possible that depending on the site, 5-HT may either excite or inhibit central sympathetic activity. In this regard, Davis *et al.* (1980) have shown that 5-HT in the brain stem and spinal cord facilitates acoustic startle, while 5-HT in the forebrain inhibits startle.

In the present study, we made no attempts to localize the site(s) at which serotonergic neurons impinge on central sympathetic pathways. However, since all experiments were performed in baroreceptor-denervated animals, it appears that 5-HT agonists and antagonists do not stimulate baroreceptor afferent input. Anatomically, serotonin-containing neurons in the medulla project to sympathetic preganglionic neurons in the intermediolateral cell column of the spinal cord (Dahlstrom and Fuxe, 1965). DeGroat and Ryall (1967) found that iontophoresis of 5-HT onto sympathetic preganglionic neurons excited these cells. This observation is consistent with the view that 5-HT enhances sympathetic outflow, possibly at the level of the spinal cord. More recently, it has been demonstrated that microinjections of 5-HT into the area of the nucleus tractus solitarius and the anterior hypothalamus/preoptic area produced pressor responses (Smits and Struyker-Boudier, 1976, and Wolf *et al.*, 1981a). Clearly, more experiments are required to identify the sites at which central 5-HT neurons influence sympathetic outflow. Finally, more studies are necessary to determine whether the 5-HT receptor which mediates the enhancement of central sympathetic activity acts by facilitating excitatory inputs, as in the facial motor nucleus, or by a direct excitatory action on central sympathetic pathways.

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