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DECREASED HYPOTHALAMIC EPINEPHRINE CONCENTRATION BY QUIPAZINE AND OTHER SEROTONIN AGONISTS IN RATS

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Abstract—Epinephrine concentrations in rat hypothalamus were decreased after the injection of quipazine, a direct-acting serotonin (5-HT) agonist. The decrease was statistically significant and dose-dependent from 0.1 to 10 mg/kg, s.c., was apparent within 1 hr, and persisted for 8 hr but not 24 hr. There was no decrease in epinephrine concentrations in rat medulla oblongata, a region containing epinephrine cell bodies. Epinephrine concentrations in rat hypothalamus were also decreased by 1-(*m*-trifluoromethylphenyl)-piperazine (TFMPP) and by 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT), other direct-acting 5-HT agonists, and by *d*-fenfluramine, a 5-HT-releasing drug. The decrease evoked by quipazine was prevented by pretreatment with metergoline, ketanserin or LY53857 (6-methyl-1-[methylethyl]-ergoline-8-carboxylic acid 2-hydroxy-1-methyl-propyl ester), centrally acting 5-HT antagonists. The lowering of rat hypothalamic epinephrine concentrations by 8-OH-DPAT was prevented by pretreatment with pindolol, a centrally acting 5-HT_{1A} receptor antagonist. These data suggest that serotonergic drugs affect epinephrine concentrations in rat hypothalamus.

Key words: rat hypothalamic epinephrine; quipazine; 8-OH-DPAT; serotonin antagonists

Epinephrine-containing neurons in the rat brain have cell bodies, mainly in the medulla oblongata, which project upward to the hypothalamus as well as downward to the spinal cord [1, 2]. These epinephrine-forming neurons have been suggested to participate in the hypothalamic control of endocrine function [3–6] and in other hypothalamic functions [1]. Relatively little is known about neurotransmitter input to epinephrine-forming neurons. We report here pharmacologic findings that serotonergic drugs affect epinephrine neurons in rat hypothalamus.

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing 130–160 g were purchased from Charles River Breeding Laboratory, Portage, MI. Quipazine maleate was purchased from Miles Laboratories, Elkhart, IN. 8-OH-DPAT† hydrobromide was obtained from Research Biochemicals, Natick, MA. (±)-Pindolol was purchased from the Sigma Chemical Co., St. Louis, MO. Metergoline was a gift from Farmitalia Carlo Erba, Milan, Italy. TFMPP was purchased from the Aldrich Chemical Co., Milwaukee, WI. LY53857 and *d*-fenfluramine were synthesized in the Lilly Research Laboratories. The doses of direct- and indirect-acting 5-HT agonists used in this study (quipazine, 8-OH-DPAT, TFMPP and *d*-fenfluramine) have been shown previously to increase rat serum corticosterone levels [7–9]. The doses of the antagonists used (metergoline, ketanserin, LY53857 and pindolol) have been shown previously to block agonist-induced increases in rat serum corticosterone concentrations [7, 9, 10].

At specified times after drug injection, rats were killed by cervical dislocation, brains were removed quickly, and hypothalamus and ventrolateral medulla oblongata were dissected, frozen on dry ice, and stored at −70° prior to analysis. After alumina adsorption, epinephrine concentrations were measured using high pressure liquid chromatography with electrochemical detection [11]. Statistical analyses were performed by analysis of variance followed by Tukey's method ($P < 0.05$) based on the mean square error.

RESULTS

Table 1 shows rat hypothalamic and ventrolateral medulla oblongata epinephrine concentrations 3 hr after injection of quipazine maleate (2.5 mg/kg, s.c.). Epinephrine concentrations in the hypothalamus were decreased significantly (26%) after quipazine injection, whereas there was no effect on concentrations of epinephrine in the medulla oblongata.

Figure 1 shows the dose-dependent lowering of rat hypothalamic epinephrine concentrations 2 hr after subcutaneous (s.c.) administration of quipazine (3 mg/kg). Quipazine from 0.1 to 10 mg/kg decreased epinephrine concentrations significantly. Figure 2 shows that epinephrine concentrations were decreased up to 8 hr but less than 24 hr after quipazine.

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† Abbreviations: 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)-tetralin; 5-HT, serotonin; TFMPP, 1-(*m*-trifluoromethylphenyl)-piperazine; and LY53857, 6-methyl-1-[methylethyl]-ergoline-8-carboxylic acid 2-hydroxy-1-methyl-propyl ester.

Table 1. Decrease of epinephrine concentration in the terminal region but not in the cell body region after quipazine injection

Treatment	Epinephrine (pmol/g)	
	Hypothalamus	Medulla oblongata
Control	95.1 ± 5.7	27.7 ± 1.4
Quipazine	70.4 ± 5.6* (−26%)	27.2 ± 2.5

Quipazine was injected at 2.5 mg/kg, s.c., and rats were killed 3 hr later. Means and standard errors for 5 rats per group are shown. *$P < 0.05$ vs the control group.

Table 2 shows the lowering of rat hypothalamic epinephrine concentrations 2 hr after several direct-acting and indirect-acting serotonin agonists. Quipazine (3 mg/kg, s.c.) lowered rat hypothalamic epinephrine 38%, TFMPP (10 mg/kg, i.p.) lowered epinephrine concentrations 35%, 8-OH-DPAT (0.5 mg/kg, s.c.) lowered epinephrine concentrations 39% and *d*-fenfluramine (10 mg/kg, i.p.) lowered rat hypothalamic epinephrine concentrations 49%.

Figure 3 shows the effects of various serotonin receptor antagonists on the lowering of rat hypothalamic epinephrine concentrations 2 hr after a 3 mg/kg, s.c., dose of quipazine. One-hour pretreatments with metergoline (1 mg/kg, i.p.), ketanserin (10 mg/kg, i.p.) or LY53857 (10 mg/kg, i.p.) completely antagonized the quipazine-induced decreases in rat hypothalamic epinephrine concentrations. The selective 5-HT_{1A} receptor antagonist

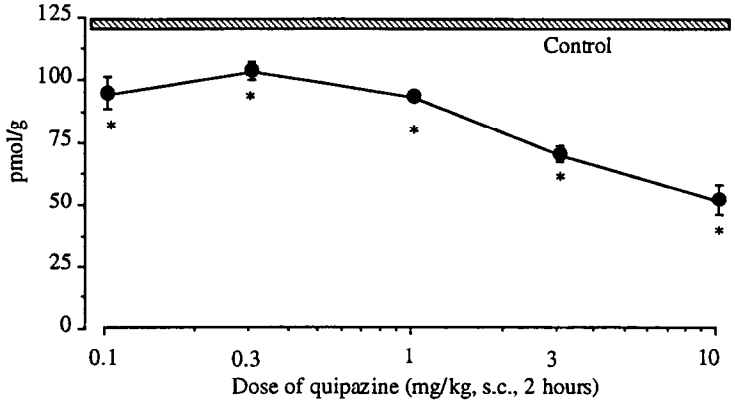

Fig. 1. Dose response for the lowering of rat hypothalamic epinephrine concentrations by quipazine. Quipazine was injected s.c. at the doses shown, and rats were killed 2 hr after injection. Means and standard errors for hypothalamic epinephrine concentrations (6 rats per group) are shown. Key: (*) $P < 0.05$ vs the control group.

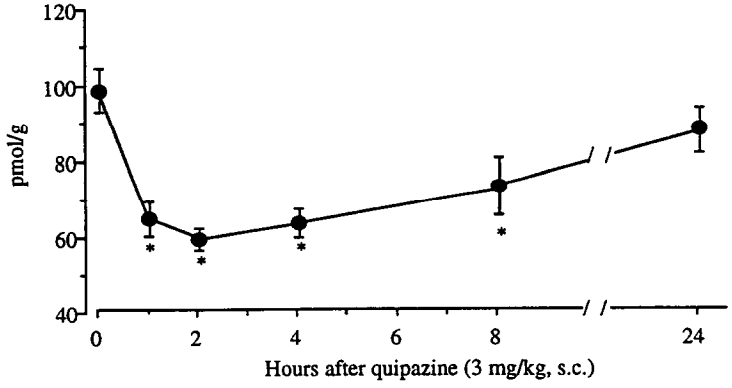

Fig. 2. Time course for the lowering of rat hypothalamic epinephrine concentrations by quipazine. Quipazine was injected at 3 mg/kg, s.c., and rats were killed at the times specified. Means and standard errors for hypothalamic epinephrine concentrations (6 rats per group) are shown. Key: (*) $P < 0.05$ vs the control group.

Table 2. Effects of direct-acting and indirect-acting serotonin receptor agonists on rat hypothalamic epinephrine concentrations

Treatment	Hypothalamic epinephrine (pmol/g)
Control	108.1 ± 4.3
Quipazine	66.8 ± 4.0* (−38%)
Control	125.7 ± 4.6
8-OH-DPAT	76.8 ± 1.8* (−39%)
Control	106.5 ± 5.2
TFMPP	69.0 ± 6.8* (−35%)
Control	89.3 ± 8.8
d-Fenfluramine	45.2 ± 6.8* (−49%)

Rats were killed 2 hr after 3 mg/kg s.c. quipazine, 10 mg/kg i.p. TFMPP, 0.5 mg/kg s.c. 8-OH-DPAT or 10 mg/kg i.p. *d*-fenfluramine. Means and standard errors for 5–6 rats per group are shown. *P < 0.05 vs the control groups.

pindolol (10 mg/kg, s.c.), however, had no effect on the quipazine-induced decrease in rat hypothalamic epinephrine concentrations.

Rat hypothalamic and ventrolateral medulla oblongata epinephrine concentrations were measured 2 hr after 8-OH-DPAT (0.5 mg/kg, s.c.). Epinephrine concentrations in hypothalamus were decreased significantly (39%) after 8-OH-DPAT injection, whereas there was no effect on concentrations of epinephrine in the medulla oblongata (data not shown).

Figure 4 shows the effect of various serotonin receptor antagonists on the lowering of rat hypothalamic epinephrine concentrations 2 hr after 0.5 mg/kg, s.c. 8-OH-DPAT. Metergoline, ketanserin and LY53857, antagonists with high affinity for the 5-HT_2 receptor, had no effect on the 8-OH-DPAT-induced decreases in rat hypothalamic epinephrine, whereas pindolol significantly antagonized the 30% decrease in epinephrine concentrations caused by 8-OH-DPAT injection.

DISCUSSION

The depletion of hypothalamic epinephrine by quipazine and other serotonergic drugs may occur because of increased release of epinephrine. It is

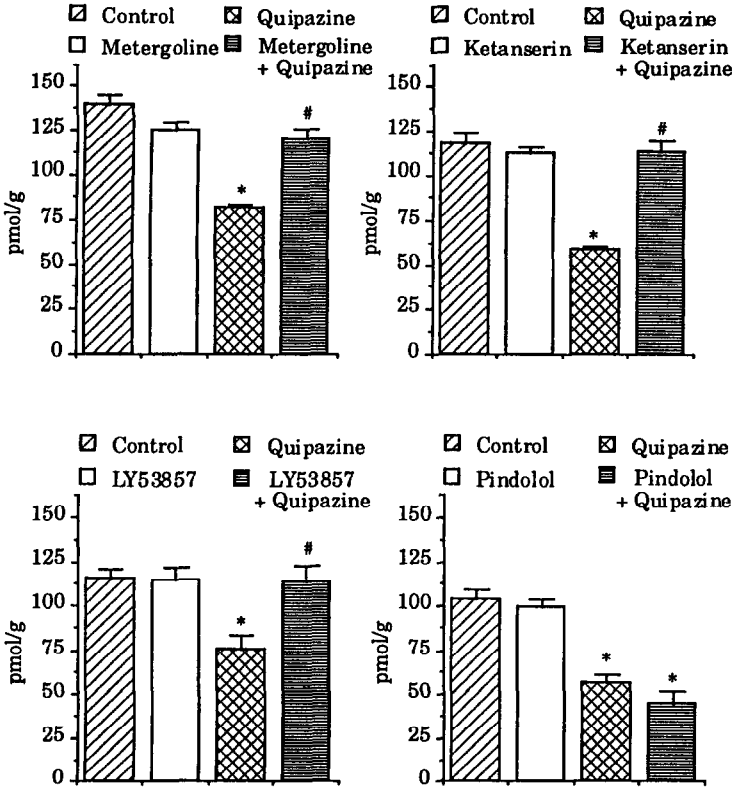

Fig. 3. Effect of 5-HT receptor antagonists on the quipazine-induced lowering of rat hypothalamic epinephrine concentrations. Metergoline (1 mg/kg, i.p.), ketanserin (10 mg/kg, i.p.) and LY53857 (10 mg/kg, i.p.) were injected as 1-hr pretreatments, and pindolol (10 mg/kg, s.c.) was injected 15 min prior to quipazine (3 mg/kg, s.c.). Rats were killed 2 hr after quipazine administration. Means and standard errors for hypothalamic epinephrine concentrations (5–6 rats per group) are shown. Key: (*) $P < 0.05$ vs the control group; and (#) $P < 0.05$ vs quipazine alone.

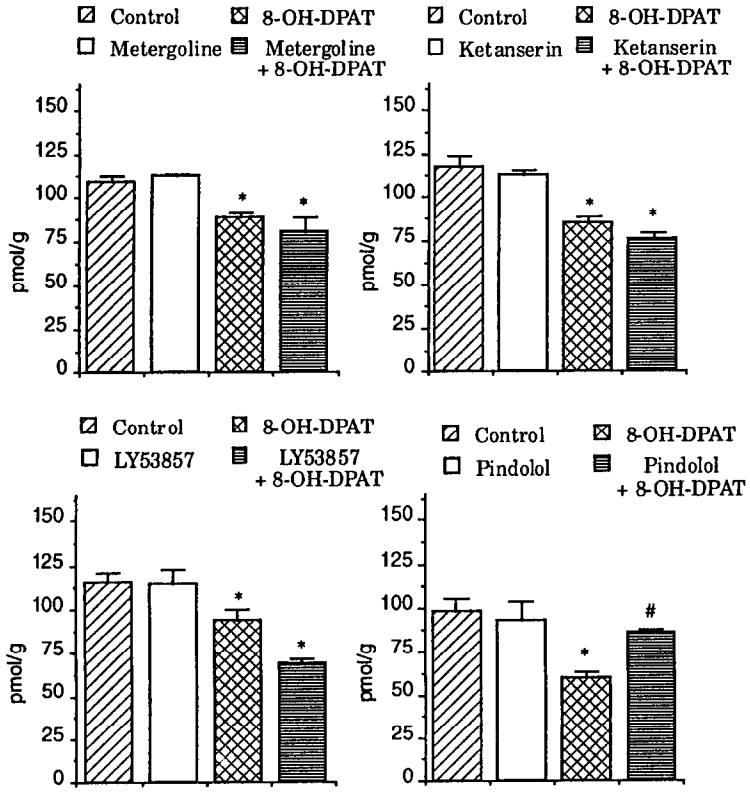

Fig. 4. Effect of 5-HT receptor antagonists on the 8-OH-DPAT-induced lowering of rat hypothalamic epinephrine concentrations. Metergoline (1 mg/kg, i.p.), ketanserin (10 mg/kg, i.p.) and LY53857 (10 mg/kg, i.p.) were given as 1-hr pretreatments and pindolol (10 mg/kg, s.c.) as a 15-min pretreatment; rats were killed 2 hr after 8-OH-DPAT (0.5 mg/kg, s.c.). Means and standard errors for hypothalamic epinephrine concentrations (5 rats per group) are shown. Key: (*) $P < 0.05$ vs the control group; and (#) $P < 0.05$ vs 8-OH-DPAT alone.

possible that serotonin receptors occur directly on epinephrine neurons to modulate epinephrine synthesis and/or release. Nicholas and Hancock [12] have shown that neural projections from raphe nuclei are contiguous with phenylethanolamine *N*-methyltransferase-immunoreactive neurons in the C1, C2 and C3 cell groups in rat brain and have identified serotonin-containing boutons in contiguity with catecholamine-containing medullary cells. An alternative possibility is that these doses of serotonergic drugs lower epinephrine concentration through nonspecific "stressful" effects, since hypothalamic epinephrine concentration has been reported to be decreased by stress [13–15].

Our data suggest that at least two different serotonin receptor subtypes may influence epinephrine neurons projecting to rat hypothalamus. The lowering of epinephrine concentration by quipazine may be mediated by 5-HT_{2A} receptors.* Metergoline, ketanserin and LY53857, antagonists with high affinity for 5-HT_{2A} receptors [16], blocked the quipazine-induced decrease in epinephrine concentration. Involvement of 5-HT_{2C} receptors cannot be excluded, however, because these antagonists also have high affinity for that receptor [16]. Indeed, the ability of TFMPP to mimic quipazine in lowering hypothalamic epinephrine concentration might implicate 5-HT_{2C} receptors, inasmuch as TFMPP may lack agonist efficacy at 5-HT_{2A} receptors but is an agonist at 5-HT_{2C} receptors [17–19].

The direct-acting agonist 8-OH-DPAT has high affinity for the 5-HT_{1A} receptor subtype [16, 20] and has been shown to increase serum corticosterone concentrations at doses reported here to decrease rat hypothalamic epinephrine [21]. Pindolol, a direct 5-HT_{1A} antagonist, has been shown to block the increase in serum corticosterone elicited by 8-OH-DPAT but not quipazine, whereas metergoline has no effect on the 8-OH-DPAT-induced increase in serum corticosterone [9, 21]. In our experiments, pindolol antagonized the decrease in rat hypothalamic epinephrine concentrations after 8-OH-DPAT but not quipazine, suggesting involvement of the 5-HT_{1A} receptor subtype in mediating this effect.

These same two receptor subtypes, 5-$HT_{2A/2C}$ and

* Current recommended nomenclature uses 5-HT_{2A} to designate the receptor previously called the 5-HT_2 receptor and 5-HT_{2C} to designate the receptor previously called the 5-HT_{1C} receptor (TIPS Receptor Nomenclature Supplement, *Trends Pharmacol Sci* pp. 1–43, 1993).

5-HT_{1A}, can mediate serotonin agonist-induced increases in serum corticosterone concentration in rats [22]. Quipazine and other direct- and indirect-acting serotonin agonists are known to increase serum corticosterone concentrations in rats at the doses shown here to decrease hypothalamic epinephrine [9, 22]. The increase in serum corticosterone concentrations in rats may be mediated by direct activation of 5-HT_{2A} receptors, since serotonin receptor antagonists with a high affinity for 5-HT_{2A} receptors block the increase in serum corticosterone elicited by quipazine [9].

There is no evidence showing whether the 5-HT agonist-induced increases in rat serum corticosterone and the decrease in hypothalamic epinephrine concentrations are related. These two effects—elevation of serum corticosterone and decrease in hypothalamic epinephrine concentrations—may be separate and unrelated effects of serotonin receptor activation. It is possible, though, that increased release of hypothalamic epinephrine may trigger the increase in serum corticosterone by activating the pituitary-adrenocortical axis. There is evidence that epinephrine neurons make synaptic connections with corticotrophin-releasing factor (CRF)-containing neurons [23] and modulate CRF release [3, 24–26]. Thus, it could be that increased release of hypothalamic epinephrine may be involved in mediating the elevation of serum corticosterone by serotonergic drugs. Further studies will be necessary to elucidate a possible interrelationship between these two actions of serotonin agonists.

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