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EFFECT OF CHRONIC D-FENFLURAMINE ADMINISTRATION ON RAT HYPOTHALAMIC SEROTONIN LEVELS AND RELEASE

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Summary

D-fenfluramine, an anorectic agent in rats and man, was administered daily at doses 1.25, 2.5, 5 or 10 mg/kg/day for 10 days, and sacrificed 6 days later. Hypothalamic serotonin (5-HT) levels were unchanged in rats receiving 1.25-5 mg/kg/day of d-fenfluramine but reduced by 22% ($p<0.01$) at the highest drug dose (10 mg/kg/day); hypothalamic 5-hydroxyindole acetic acid (5-HIAA) levels were reduced by 15% ($p<0.05$) or 28% ($p<0.01$) in rats receiving 5 or 10 mg/kg/day of the drug, respectively. Hypothalamic slices prepared from rats which were previously treated with any of the drug doses spontaneously released endogenous 5-HT at rates that did not differ from those of vehicle-treated rats. 5-HT released with electrical field-stimulation was unaffected by prior d-fenfluramine treatment at doses of 1.25-5 mg/kg/day, and was reduced by 20% ($p<0.05$) from slices prepared from rats which received 10 mg/kg/day. 5-HIAA efflux was also attenuated by the highest drug dose. These data indicate that chronic administration to rats of customary anorectic doses of d-fenfluramine (i.e. 0.06-1.25 mg/kg) fail to cause long-lasting reductions in brain 5-HT release.

Racemic dl-fenfluramine (1, 2) and, more recently, d-fenfluramine (3, 4) have been shown to be clinically effective in reducing appetite and facilitating weight loss. The anorexigenic effect of d-fenfluramine, which accounts for the action of the racemic mixture (3, 5), probably results from an enhancement in serotonin-mediated brain neurotransmission since the drug both potentiates 5-HT release from nerve terminals and inhibits its inactivation by reuptake (6).

Numerous studies have described long-lasting depletions in brain 5-HT levels when a high dose of dl-fenfluramine (15-25 mg/kg) (7-10) or d-fenfluramine (6 mg/kg/day) (11) was administered to rats. However, these doses produce elevations in plasma and brain fenfluramine concentrations (12, 13) which far exceed those needed to reduce food intake in deprived rats (ED_{50} for d-fenfluramine = 1.3 mg/kg; for l-fenfluramine = 3.0 mg/kg) (13). Lower dose(s) of d-fenfluramine administered to rats either acutely (1.25-2.5 mg/kg) (14) or chronically (3 mg/kg/day for 6 days) (11) have not been reported to deplete brain 5-HT levels. Apparently, no data are available on possible effects of chronic d-fenfluramine treatment on the ability of serotoninergic neurons to release their neurotransmitter. The study that follows has addressed this relationship using a superfused, electrically-stimulated, rat hypothalamic slice preparation.

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Tissue levels of tryptophan, 5-HT and 5-HIAA were determined using this chromatographic system, though the applied potential was set at 0.85 V and the sensitivity at 5 nA/V. Frozen tissue samples were sonicated in 0.2 N $HClO_4$, containing 0.5 mM ascorbate and internal standards (approximately 0.4 ml/mg protein), and centrifuged (17,000 rpm, 10 min). An aliquot of this supernatant was injected over the reverse-phase column. The amounts of indole measured in each sample, superfused medium and tissue supernatant, were normalized by the amount of protein in the tissue pellets, as determined by the Lowry method (16).

The amounts of 5-HT released from the slices, under basal conditions and with electrical field-stimulation, were calculated as the average rate (fmol/mg protein/min) during the four rest periods and the three periods of electrical stimulation, respectively. The rate of 5-HIAA efflux was taken as the average across the 80 minute (16 fraction) collection period. Results were evaluated by the Student's paired t-test. Values are reported here as means ± the standard error of the mean (s.e.m.).

Results

Hypothalamic contents of tryptophan, 5-HT and 5-HIAA in vehicle-treated rats were 194±9, 81±3 and 51±2 pmol/mg protein, respectively. The levels of 5-HT in the hypothalamus of rats treated with d-fenfluramine for 10 days at a dose of 1.25, 2.5 or 5 mg/kg/day were not different, 6 days after the last injection, from those of vehicle-treated rats (Table I). Only those rats which received the highest dose of d-fenfluramine, 10 mg/kg/day, showed a significant decrease in

TABLE I

Effect of chronic d-fenfluramine administration on hypothalamic indole levels

	d-fenfluramine dose (mg/kg/day)			
	1.25	2.5	5.0	10.0
TRYPTOPHAN				
vehicle	182.1±19.0	196.9±29.6	185.8±17.1	207.7±12.4
drug	207.7±19.9*	205.4±24.2	184.8±14.3	226.7±19.2
%	115.4±4.4	107.6±7.2	101.7±8.0	108.8±5.3
5-HT				
vehicle	78.9±4.7	75.7±7.8	85.2±6.8	83.7±5.1
drug	78.1±6.7	72.3±5.9	72.2±5.3	65.8±5.5**
%	98.3±5.5	97.2±6.9	86.0±6.4	78.4±3.8
5-HIAA				
vehicle	45.8±3.6	46.6±4.2	51.2±5.1	57.9±3.6
drug	47.7±4.0	49.7±4.8	42.8±3.1*	41.3±3.3**
%	103.9±3.6	107.1±6.3	85.1±5.0	71.6±4.8

Rats received d-fenfluramine dissolved in 0.9% NaCl (drug) or 0.9% NaCl (vehicle) for 10 days; tissues were taken 16 days from the onset of treatment. Values are given as group means ± s.e.m. for N = 5-8 pairs; levels are in pmol/mg protein. * $p<0.05$, ** $p<0.01$ differs from vehicle-treated group by Student's paired t-test.

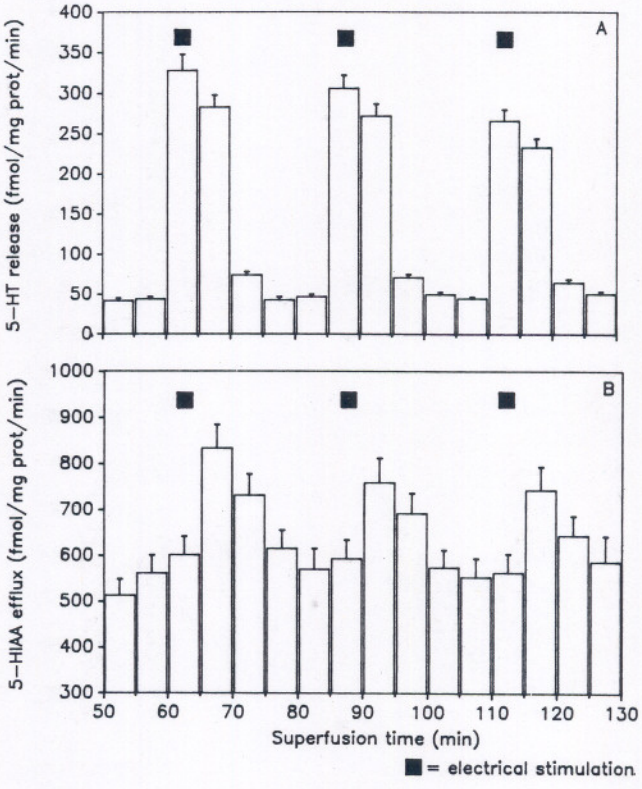

FIG. 1

Time-course of 5-HT release (A) and 5-HIAA efflux (B) from hypothalamic slices prepared from control rats. Rats were given daily intraperitoneal injections of saline for 10 days. On day 16 following the onset of treatment, slices were prepared and equilibrated in physiologic medium for 50 minutes; 5 minute fractions were collected during the subsequent 80 minutes. The slices were electrically field-stimulated for 3 periods. The amounts of 5-HT and 5-HIAA (fmol/mg protein/min) released into the medium were monitored. N = 24.

Discussion

These results indicate that chronic administration of d-fenfluramine to rats, even in doses of 5 mg/kg/day, which are considerably higher than those needed to produce anorexia (17), does not cause long-lasting decreases in hypothalamic 5-HT levels (that is an effect which persists beyond the period of drug administration for at least 6 days). This treatment also has no effect on the ability of hypothalamic nerve terminals to release endogenous 5-HT, basally or during membrane depolarization. Repeated injections of even higher doses (10 mg/kg/day) can reduce hypothalamic 5-HT content (by 21.6±3.8%), the evoked release of 5-HT (by 20±5%) and the efflux of 5-HIAA (by 37±4%). However, basal 5-HT release remains unaltered even after such doses, a finding in accord with the demonstration by Mennini that basal ^{3}H-5-HT release from cortical synaptosomes prepared from rats chronically treated (for 14 or 28 days) with d-fenfluramine was unaffected, but the release evoked by in vitro challenge with d-fenfluramine was reduced (18).

The dose of d-fenfluramine that we found to cause a long-lasting reduction in hypothalamic 5-HT levels and release, 10 mg/kg/day, is much greater than doses used to inhibit food intake in a variety of test paradigms, 0.06-1.25 mg/kg (17). Caccia et. al. has shown that the concentration of d-fenfluramine reaching the brain is nonlinearly related to dose, such that an 8-fold increase in dosage (i.e. from a potent anorectic dose of 1.25 mg/kg to an excessive dose of 10 mg/kg) causes a greater than 30-fold increase in the area under the curve relating brain d-fenfluramine levels to

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