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Alteration of the Neurochemical Effects of Fenfluramine by Previous Treatment With *d*-Amphetamine

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HUNSINGER, R N AND M C WILSON *Alteration of the neurochemical effects of fenfluramine by previous treatment with d-amphetamine* PHARMACOL BIOCHEM BEHAV 22(1) 127–134, 1985 —It was found in an earlier study that a 15-day IP subacute *d*-amphetamine treatment rendered an apparent "tolerance" to the food intake suppressant effects of fenfluramine The purpose of the present study was to determine if the neuronal substrate on which fenfluramine supposedly acts to produce a decrement in food consumption was altered by the previous treatment with *d*-amphetamine. The time course effects (0–90 min) of a single IP injection of 10 mg/kg fenfluramine on brain monoamines in saline-treated rats included a significant lowering of serotonin in all brain regions examined Although the serotonin depleting actions of fenfluramine in the *d*-amphetamine-treated rats generally paralleled those seen in the saline group (i e , as in the hypothalamus), no such effects were noted in regions where baseline values (see below) were already lowered by *d*-amphetamine treatment (i e , pons-medulla, thalamus, and substantia nigra) Norepinephrine and dopamine depletions were observed in the pons-medulla and hypothalamic areas after fenfluramine administration in the saline-treated rats, but fenfluramine caused no decreases in hypothalamic dopamine or in pons-medulla and hypothalamic norepinephrine content in rats previously treated with *d*-amphetamine Analysis of the baseline amine levels (i e , values in the *d*-amphetamine and saline-treated rats before fenfluramine was given) indicated that the repeated *d*-amphetamine treatment generally lowered norepinephrine in most brain regions, serotonin in the pons-medulla, substantia nigra and thalamus, and dopamine in the striatum The overall results of this study suggest that the anorectic tolerance conferred toward fenfluramine by previous subacute treatment with *d*-amphetamine does not result from a *d*-amphetamine related change in hypothalamic biogenic amines

d-Amphetamine Biogenic amines Brain Fenfluramine Rats Serotonin Dopamine Norepinephrine

THERE are reports suggesting that fenfluramine and *d*-amphetamine elicit their anorectic effects by different mechanisms, fenfluramine being primarily serotonergic and *d*-amphetamine acting mainly through noradrenergic systems [3, 4, 12, 17, 31] Garattini [13] in a review of anorexic agents succinctly presents the evidence for a distinct mode of action for each of the two drugs

Because of the differences in neuronal substrates involved in the anorectic action of these agents, theoretically pharmacodynamic cross-tolerance between the two drugs should not exist However, very little information is available at the present time in regard to the possibility of such cross-tolerance Kandel *et al* [19] and Lewander [21] have reported that no anorectic cross-tolerance existed between *d*-amphetamine and fenfluramine, but in both of these studies the test criterion was based upon the fact that fenfluramine's effect on food intake after subacute *d*-amphetamine dosing was no different than after subacute saline administration A closer examination of the Kandel and Lewander data suggests that the effects of fenfluramine following subacute *d*-amphetamine were indeed less than the food intake suppression seen on the initial day of fenfluramine treatment in the same group A previous report from this laboratory [16] demonstrated that 15-day treatment with *d*-amphetamine rendered an apparent anorectic tolerance toward fenfluramine In that study, *d*-amphetamine was given after the feeding period in order to minimize the potential contribution of behavioral tolerance [5,30]. Therefore, it is likely that the tolerance observed was pharmacokinetic and/or pharmacodynamic in nature. The purpose of this study was to determine if the neuronal substrate on which fenfluramine supposedly acts to produce a decrement in food consumption was altered by previous treatment with *d*-amphetamine Particularly relevant to this intent was the 5-HT content of the hypothalamus, an area believed to play a critical role in the serotonergic modulation of feeding [15,23]

METHOD

Animal Care and Housing

Male Wistar rats weighing 175–200 g, purchased from Harlan Industries (Cumberland, IN), were used in this study Upon arrival, the rats were group-housed (5–6 per cage) for 3

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TABLE 1

THE TIME COURSE EFFECT OF AN IP DOSE OF FENFLURAMINE (10 MG/KG) ON SEROTONIN CONTENT (NG/G) IN VARIOUS BRAIN REGIONS OF RATS PREVIOUSLY EXPOSED TO REPEATED INJECTIONS OF SALINE (S) OR *D*-AMPHETAMINE (AM)

	Post-Fenfluramine Injection Time (Minutes)					
	0		30		90	
	Treatment					
Brain Region	S[1]	AM[1]	S	AM	S	AM
Cortex	15 05± 1 31	15 06± 3 21	10 23± 2 52	14 16± 3 33	4 14±0 57†	4 71± 1 18†
Pons-Medulla	77 38± 4 77	61 50± 6 68*	48 90± 2 68†	54 26± 3 56	46 46±1 57†	52 26± 5 10
Hypothalamus	98 03±22 28	89 24±21 09	47 50± 6 02†	48 23±11 89†	32 83±4 81†	34 92± 3 53†
Thalamus	139 84±31 91	41 54±13 99*	51 87±18 85†	40 59± 8 38	ND	ND
Substantia N	123 74±20 27	71 66±14 08*	64 30±12 31†	41 30±10 55	38 51±4 13†	34 90±12 66
Striatum	87 93±12 51	76 12±11 48	54 35± 7 44†	51 92±10 58†	35 14±3 00†	39 26± 4 54†
Amygdala	89 69±12 83	74 23±12 43	49 67± 5 16†	44 28± 8 79†	22 33±6 19†	38 74± 4 44†
Hippocampus	66 35±16 05	70 87± 9 74	27 69± 6 22†	31 69± 6 06†	ND	ND

[1]The abbreviation "S" represents animals that received saline (0 5 ml/day) for 15 days prior to fenfluramine (10 mg/kg) administration, whereas, "AM" represents animals that had similarly received *d*-amphetamine (4 0 mg/kg) prior to the fenfluramine injection Each value represents the mean of 5–8 determinations ±S E M

*Indicates a significant difference between the S and AM groups at the respective sampling time via the paired Student's *t*-test ($p \leq 0$ 05)

†Indicates a significant difference between serotonin content at a particular sampling time versus the "0" time control value for that brain area in the respective treatment group via the Duncan's Multiple Range Test ($p \leq 0$ 05)

ND indicates non-detectable amine levels

days, during which time the animal's general state of health was assessed according to the guidelines set forth by Fox [10] Animals displaying signs of disease or sickness were not used in any of the subsequent phases of the study Feeding and dosing procedures as well as housing conditions during the experiment were the same as described previously [16]

Drugs and Chemical Reagents

Dextro-amphetamine sulfate (Dexedrine®) was generously supplied by Smith, Kline, and French Laboratories (Philadelphia, PA), while fenfluramine hydrochloride was a gift from the A H Robins Company (Richmond, VA) Aldrich Chemical Company (Milwaukee, WI) supplied ninhydrin and tricine (N-[tris (hydroxymethyl)-methyl] glycine) Alumina (activity grade I) was obtained from Alupharm Chemicals (New Orleans, LA) Fisher Scientific Company (Fair Lawn, NJ) was the source of boric acid, hydrochloric acid, iodine, N-heptanol, perchloric acid, potassium carbonate, potassium hydroxide, potassium iodide, sodium chloride, sodium hydroxide, sodium phosphate dibasic, sodium phosphate monobasic, sodium metabisulfate, sodium sulfite, and sulfuric acid Dopamine hydrochloride, norepinephrine hydrochloride, and serotonin hydrochloride were purchased from Sigma Chemical Company (St Louis, MO)

Neurochemical Studies

Rats were injected IP 10 min after a daily 3 hr feeding period with either 0 5 ml saline (i e , saline regimen) or 4 0 mg/kg *d*-amphetamine (i e , *d*-amphetamine regimen) for 15 consecutive days as described previously [16] In order to reasonably distribute the workload on the days of sacrifice, subacute dosing was initiated in a "staggered" manner over an 8-day period

Twenty-one hours after the last subacute dose of *d*-amphetamine or saline, the rats were injected with fenfluramine (10 mg/kg, IP) and killed by decapitation either 30- or 90-min post-dosing (N=7–8 per time per subacute regimen) In addition, eight rats from each of the two subacute regimens received no fenfluramine, but were sacrificed immediately after an injection of 0 5 ml saline These saline injected rats provided zero baseline control data

Upon decapitation, the brains were quickly excised, rinsed with ice-cold saline, blotted dry, and weighed Following the weight determinations, the brains were dissected into 8 discrete areas (i e , cortex, striatum, hypothalamus, thalamus, substantia nigra/ventral tegmentum, amygdala, hippocampus, and pons-medulla), using the cutting-block method of Heffner *et al* [14] The dissections were performed while the temperature of the aluminimum alloy block was maintained at 4°C in an ice bath Following dissection, the samples were weighed, placed in polyethylene, screw-cap vials and frozen at −70°C until the biogenic amine analysis could be performed

Norepinephrine, dopamine, and serotonin content in each brain region was determined by the fluorometric assay of Shellenberger and Gordon [28] The alumina used to absorb the catecholamines in this study was Woelm neutral grade I and was activated to pH 3 4 by a combination of the procedures described by Shellenberger and Gordon [28] and Anton and Sayre [2] Approximately 250 g of alumina were placed in a 2000 ml graduated cylinder and were washed with flowing tap water for 12 hr The remaining alumina particles were then washed twice with 500 ml portions of 2 N HCl at 100°C for 45 min, twice with 500 ml portions of 2 N HCl at 70°C for 10 min, and once with a 500 ml portion of 2 N HCl at 50°C for 10 min Following this acid treatment, the alumina was washed 25 times with distilled water in order to adjust the pH to 3 4 The alumina was then placed in an evaporation dish and was heated at 120°C for 1 hr, followed by 200°C for 2 hr The dried alumina was stored in an incubator at 37°C until use

Standard curves were generated by carrying known free-

TABLE 2

THE TIME COURSE EFFECT OF AN IP DOSE OF FENFLURAMINE (10 MG/KG) ON NOREPINEPHRINE CONTENT (NG/G) IN VARIOUS BRAIN REGIONS OF RATS PREVIOUSLY EXPOSED TO REPEATED INJECTIONS OF SALINE (S) OR *D*-AMPHETAMINE (AM)

	Post-Fenfluramine Injection Time (Minutes)					
	0		30		90	
	Treatment					
Brain Region	S[1]	AM[1]	S	AM	S	AM
Cortex	184 53±13 29	120 15± 7 52*	153 72±10 32	121 48± 7 02*	168.21±18 18	119 10±11 72*
Pons-Medulla	384 34±10 24	252 82±20 08*	315 37±22 67†	257 75±10 21*	296 32±21 12†	243.29±12.26*
Hypothalamus	1168 88±66 72	687 44±63 17*	820 32±69 18†	447 98±49 85*†	941 17±29 51†	544 48±46.25*
Thalamus	383 23±42 21	260 86±23 34*	287 44±42 04	173 13±10 36*†	304 49±40 91	213 41±29.13
Substantia N	344 18±41 13	291 52±26 53	329 05±25 85	194 50±10 87*	339 75±38 31	241 91±27 66
Striatum	177 42±16 83	117 05±13 05*	158 28±11 50	111 14±20 76*	131 59±13.91	101 66±13 55
Amygdala	340 15±37 95	206 73±18 02*	275 08±25 57	185 19±18 48*	243 00±10 45	106 40±29 02
Hippocampus	183 22±18 78	154 05±28 30	176 29±17.65	112 53±12 73	150.01±16.89	134 78±20 23

[1]The abbreviation "S" represents animals that received saline (0 5 ml/day) for 15 days prior to fenfluramine (10 mg/kg) administration, whereas, "AM" represents animals that had similarly received *d*-amphetamine (4 0 mg/kg) prior to the fenfluramine injection Each value represents the mean of 5–5 determinations ±S E M

*Indicates a significant difference between the S and AM groups at the respective sampling time via the paired Student's *t*-test ($p \leq 0$ 05)

†Indicates a significant difference between norepinephrine content at a particular sampling time versus the "0" time control value for that brain area in the respective treatment group via the Duncan's Multiple Range Test ($p \leq 0$ 05)

TABLE 3

THE TIME COURSE EFFECT OF AN IP DOSE OF FENFLURAMINE (10 MG/KG) ON DOPAMINE CONTENT (NG/G) IN VARIOUS BRAIN REGIONS OF RATS PREVIOUSLY EXPOSED TO REPEATED INJECTIONS OF SALINE (S) OR *D*-AMPHETAMINE (AM)

	Post-Fenfluramine Injection Time (Minutes)					
	0		30		90	
	Treatment					
Brain Region	S[1]	AM[1]	S	AM	S	AM
Cortex	148 00± 15 15	102 58± 11 59	97 36± 18 30	108 58± 17 92	73 67± 19 58	60.93± 9.04
Pons-Medulla	91 83± 9 74	82 49± 12 80	62 39± 9 07†	54 22± 2 77†	38 14± 2 87†	42 08± 7 01†
Hypothalamus	507 77±112 07	397 39± 95 65	378 14± 81 28	340 22± 58 18	280 28± 43.87†	343 15± 52 36
Thalamus	ND	ND	ND	ND	ND	ND
Substantia N	470 73± 72 99	492 89± 98 54	408 29± 77 58	321 84± 91 75	363 95± 54 00	304 52± 51.54
Striatum	7178 31±917 65	5281 66±554 66*	4909 67±409 35	5170 72±836 71	5655 88±612 58	4402 25±686 55
Amygdala	273 83± 38 28	292 52± 51 54	365 30± 77 21	326 93± 80 00	252 91± 67 58	287 61± 37 30
Hippocampus	ND	ND	ND	ND	ND	ND

[1]The abbreviation "S" represents animals that received saline (0 5 ml/day) for 15 days prior to fenfluramine (10 mg/kg) administration, while, "AM" represents animals that had similarly received *d*-amphetamine (4 0 mg/kg) prior to the fenfluramine injection Each value represents the mean of 5–8 determinations ±S E M

*Indicates a significant difference between the S and AM groups at the respective sampling time via the paired Student's *t*-test ($p \leq 0$ 05)

†Indicates a significant difference between dopamine content at a particular sampling time versus the "0" time control value for that brain area in the respective treatment group via the Duncan's Multiple Range Test ($p \leq 0$ 05)

ND indicates non-detectable amine levels

base concentrations of norepinephrine, dopamine, and serotonin through the entire extraction procedure

RESULTS

As can be seen in Table 1, subacute *d*-amphetamine treatment resulted in a significant lowering of serotonin in the pons-medulla, thalamus, and substantia nigra/ventral tegmental areas, as compared to the serotonin content in these same areas in saline-treated rats Likewise, the *d*-amphetamine treatment caused a decrease in norepinephrine content in the cortex, pons-medulla, hypothalamus, thalamus, striatum, and amygdala (Table 2); and in dopamine in the striatum (Table 3) Dopamine content in the thalamic and hippocampal areas in both subacute treatment groups was below the detection limit of the assay (i e , 2–3 ng per sample) and therefore, could not be determined (Table 3).

The time course effects of fenfluramine on brain

monoamines in the saline-treated rats included a significant lowering of serotonin in all brain regions examined at both the 30- and 90-min sampling times, with the exception of the cortex (Table 1) In the cortical areas, no significant differences in serotonin levels were noted, with respect to the baseline values, until 90-min post-fenfluramine treatment

In the *d*-amphetamine-treated animals, fenfluramine depleted serotonin only in the hypothalamus, striatum, amygdala, and hippocampus at the 30-min sampling time (Table 1) At 90 min post-fenfluramine treatment, serotonin was significantly lowered, as compared to the respective baseline values, in the cortex, hypothalamus, thalamus, striatum, amygdala, and hippocampus

There were no significant differences in serotonin content noted between the two regimens at either the 30- or 90-min sampling times in any brain region examined (Table 1) In addition, serotonin levels were below the limit of detection (i e , <1–2 ng per sample) in the thalamic and hippocampal regions from animals in both regimens at 90-min post-fenfluramine dosing

Table 2 indicates that fenfluramine significantly lowered norepinephrine below control values at 30 min post-treatment in the pons-medulla and hypothalamic area of the saline-treated rats This effect persisted at the 90-min sampling time

In *d*-amphetamine-treated animals, norepinephrine levels were found to be decreased 30 min after fenfluramine administration in the hypothalamic and thalamic areas However, by 90-min post-fenfluramine dosing, norepinephrine content in these regions had returned to baseline control levels

Significant differences in norepinephrine content were noted between the saline and *d*-amphetamine groups at the 30- and 90-min sampling times (Table 2) For example, at 30 minutes post-fenfluramine treatment, norepinephrine in the *d*-amphetamine group was significantly lowered, as compared to the saline group, in all brain areas except the hippocampus This pattern was seen only in the cortex, pons-medulla, and hypothalamus at the 90-min sampling time

Dopamine in the pons-medulla of both saline-treated and *d*-amphetamine-treated animals was decreased by fenfluramine, as compared to the baseline control level, at both the 30- and 90-min measurements (Table 3) In addition, fenfluramine lowered dopamine in the hypothalamic regions of saline-treated rats at the 90-min sampling period There were no significant differences in dopamine content noted between regimens in any brain region at either the 30- or 90-min sampling times Dopamine levels were non-detectable (i e , <2–3 ng per sample) at all sampling times in the thalamic and hippocampal regions in both regimens

Figures 1–4 represent a summary of the time course effects of fenfluramine on biogenic amine levels in the saline-treated animals and at the same time indicate where previous treatment with *d*-amphetamine altered such effects A complete description of the specific changes observed in each regimen has already been given in the above explanation of the data in the tables However, graphic representation of the data in these figures highlights several important comparative aspects of the treatments First of all, from Fig 1 it is evident that the only amine in the hypothalamus lowered by the subacute *d*-amphetamine treatment was norepinephrine Secondly, the serotonin depleting action of fenfluramine in the hypothalamic area of the *d*-amphetamine-treated rats paralleled those seen in the saline group Thirdly, in spite of the lower baseline levels of norepinephrine in the hypothalamic areas of the

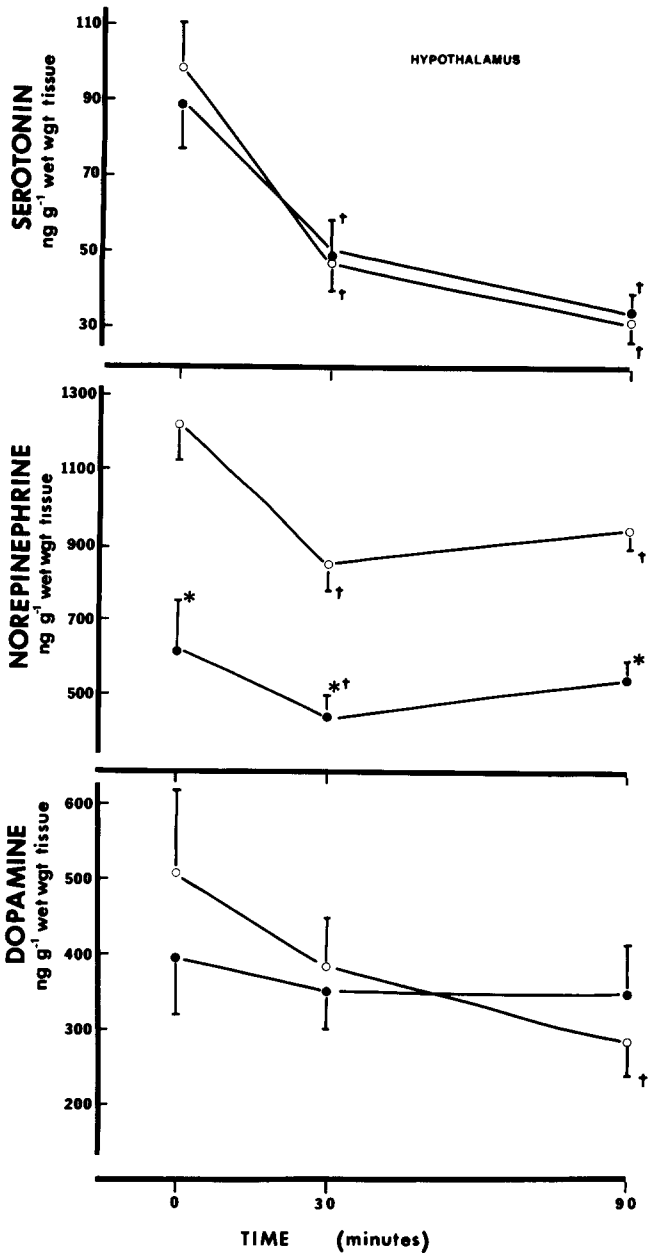

FIG 1 The time course effects of an IP dose of fenfluramine (10 mg/kg) on hypothalamic serotonin, norepinephrine, and dopamine levels in rats previously exposed to repeated IP injections of saline (○—○) or *d*-amphetamine (●—●) An (*) indicates a significant difference between monoamine content with respect to the two regimens at each sampling time via the paired Student s *t*-test ($p \leq 0.05$) A (†) indicates a significant difference between monoamine content at the respective sampling times versus the "0" time baseline control value for that treatment regimen via the Duncan's Multiple Range Test ($p \leq 0.05$) Each point represents the mean of 5–8 determinations and the vertical bars depict the S E M

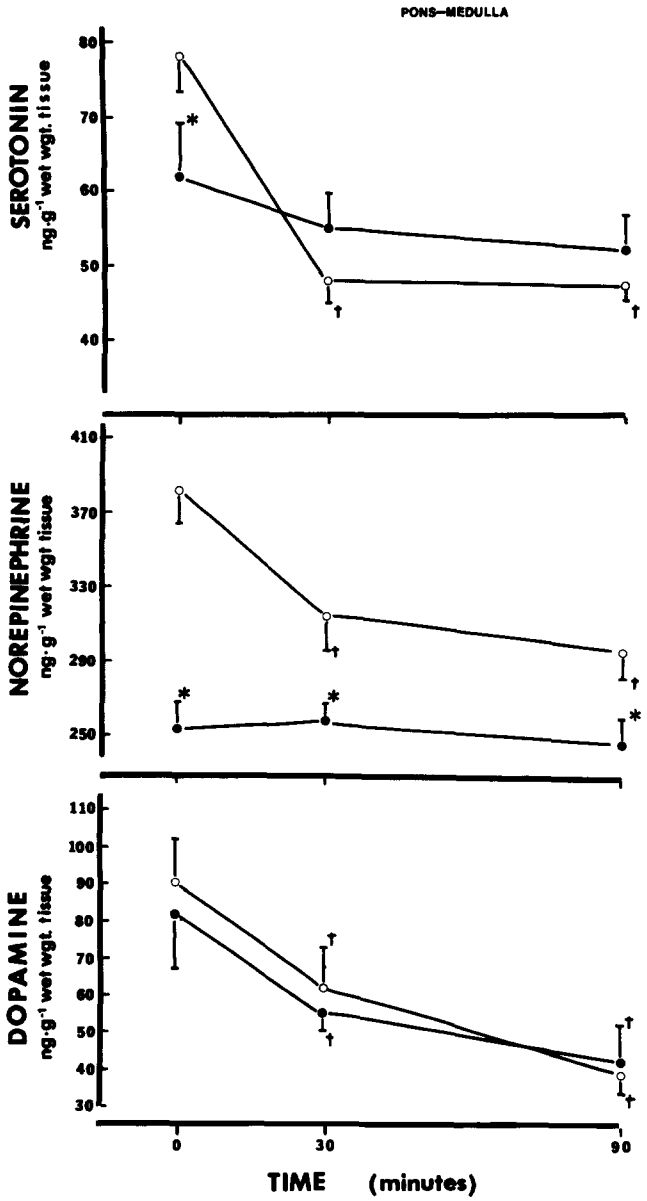

FIG 2 The time course effects of an IP dose of fenfluramine (10 mg/kg) on serotonin, norepinephrine, and dopamine levels in the pons-medulla region of rats previously exposed to repeated IP injections of saline (○—○) or *d*-amphetamine (●—●) An (*) indicates a significant difference between monoamine content with respect to the two regimens at each sampling time via the two-tailed paired Student's *t*-test ($p \leq 0$ 05) A (†) indicates a significant difference between monoamine content at the respective sampling time versus the "0" time control value for that treatment group via the Duncan's Multiple Range Test ($p \leq 0$ 05) Each point represents the mean of 5–8 determinations and the vertical bars depict the S E M

d-amphetamine-treated group, fenfluramine did elicit a slight, but significant, decrease in norepinephrine content at the 30-min sampling time, however, no fenfluramine effect was evident in this area at 90-min after treatment. Lastly, dopamine was significantly decreased in the hypothalamus below baseline levels at the 90-min measurement in the saline-treated subjects, but no such effect was seen in the *d*-amphetamine-treated rats at either the 30- or 90-min sampling times.

Figure 2 indicates that subacute *d*-amphetamine treatment lowered serotonin in the pons-medulla area, and that fenfluramine administration failed to deplete serotonin below this already sub-baseline value Previous treatment with *d*-amphetamine also significantly lowered norepinephrine as compared to the saline control in the pons-medulla and apparently prevented any further decrease in this neurotransmitter by fenfluramine *d*-Amphetamine did not alter baseline dopamine levels in the pons-medulla, and the time course effects of fenfluramine, with regard to this monoamine, did not differ between the two regimens.

Figure 3 shows that *d*-amphetamine was also effective in lowering serotonin content in the thalamus and apparently prevented any further depletion by fenfluramine at the 30-min measurement Serotonin levels were decreased at the 90-min measurement below detectable limits. Fenfluramine had no effect on thalamic norepinephrine content Subacute *d*-amphetamine treatment was effective in significantly lowering baseline values of norepinephrine and apparently increased the sensitivity of this monoamine system to fenfluramine, as evidenced by a significant decrease in norepinephrine 30 min after fenfluramine dosing

d-Amphetamine also lowered serotonin content in the substantia nigra (Fig 4), however, no further decrease in this monoamine was seen after fenfluramine injection This pattern is in contrast with fenfluramine's effect on serotonin in the substantia nigra of saline-treated rats, where serotonin was significantly lowered below baseline control levels at both the 30- and 90-min measurements *d*-Amphetamine did not alter baseline levels of norepinephrine or dopamine in the substantia nigra region Fenfluramine was also without effect on norepinephrine and dopamine in the substantia nigra

DISCUSSION

The purpose of this study was to determine if the anorectic tolerance previously seen with fenfluramine after repeated post-feeding session *d*-amphetamine treatment was pharmacodynamic in nature and involved alterations in the neuronal substrate available to fenfluramine In this regard, the literature indicates that acute neurochemical effects of fenfluramine mainly involve serotonin. For example, fenfluramine has been shown to decrease serotonin levels in the telencephalon and diencephalon of rats [7]. Furthermore, this effect lasts longer than the half-life of fenfluramine would indicate This action does not extend to the serotonin stores of the brainstem, heart, and stomach unless doses in the range of 20 mg/kg are used [7] Fenfluramine may also effect other biogenic amines Reuter [24] reported that fenfluramine decreased rat brain tel- and di-encephalic serotonin content as expected, but also lowered norepinephrine and to some extent, dopamine

Despite the evidence for different neurochemical mechanisms for fenfluramine and *d*-amphetamine, there are data which suggest that the two drugs might share certain neurochemical effects For instance, the behavioral responses in

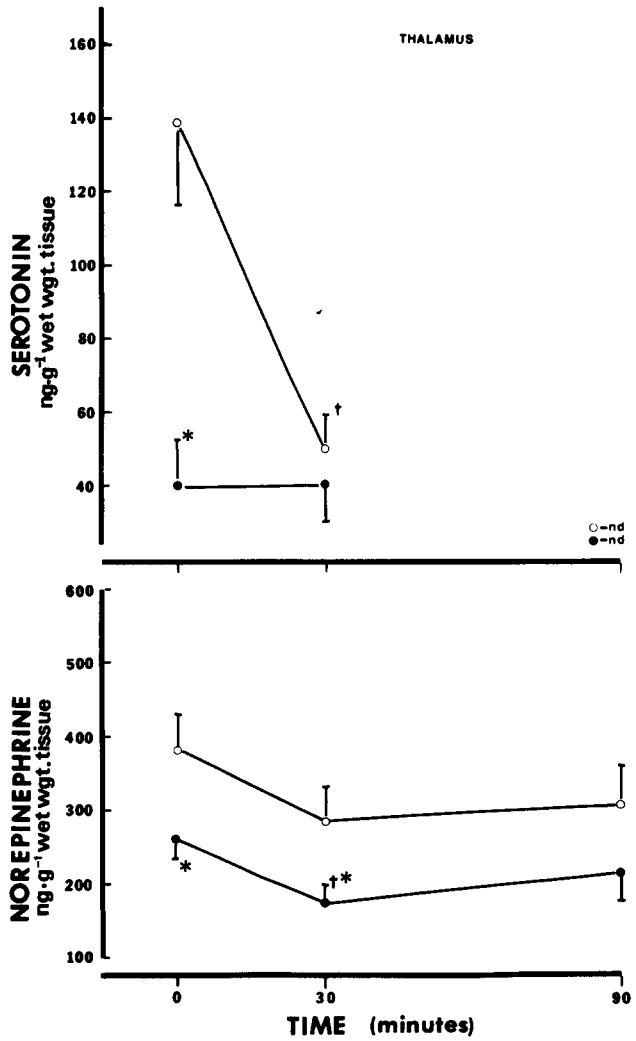

FIG 3 The time course effects of an IP dose of fenfluramine (10 mg/kg) on serotonin and norepinephrine levels in the thalamus of rats previously exposed to repeated IP injections of saline (○—○) or *d*-amphetamine (●—●) An (*) indicates a significant difference between monoamine content with respect to the two regimens at each sampling time via the paired Student's *t*-test ($p \leq 0$ 05) A (†) indicates a significant difference between monoamine content at the respective sampling times versus the "0" time baseline control value for that treatment group via the Duncan's Multiple Range Test ($p \leq 0$ 05) Each point represents the mean of 5–8 determinations and the vertical bars depict the S E M

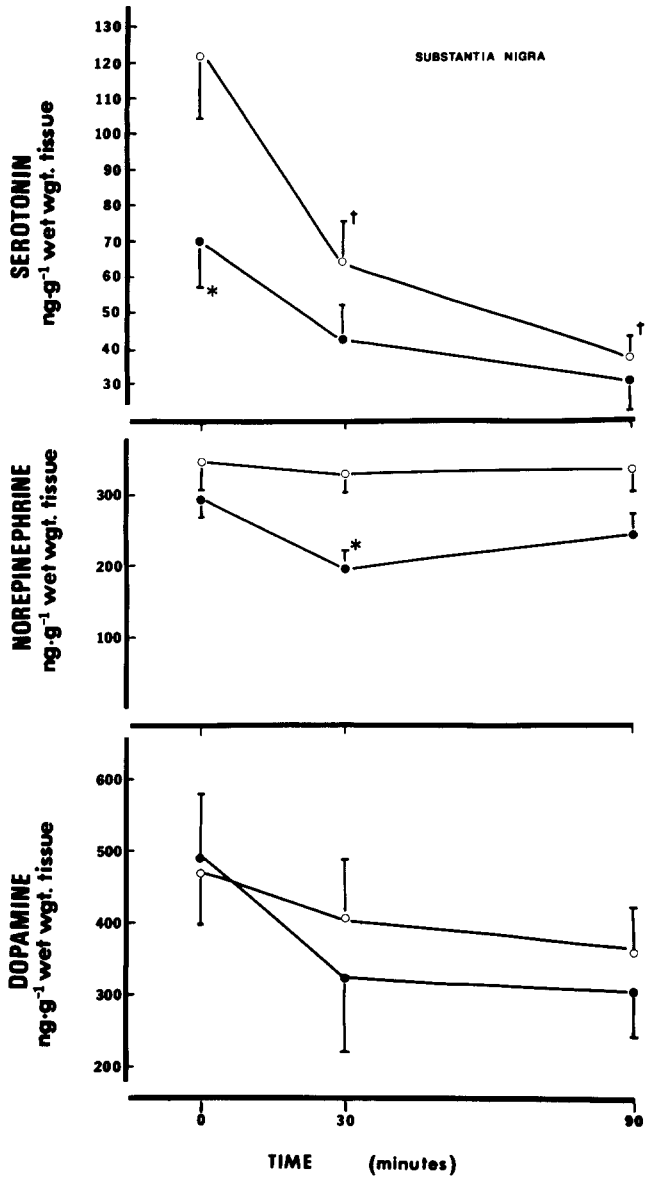

FIG 4 The time course effects of an IP dose of fenfluramine (10 mg/kg) on serotonin, norepinephrine, and dopamine levels in the substantia nigra of rats previously exposed to repeated IP injections of saline (○—○) or *d*-amphetamine (●—●) An (*) indicates a significant difference between monoamine content with respect to the two regimens at each sampling time via the paired Student's *t*-test ($p \leq 0$ 05) A (†) indicates a significant difference between monoamine content at the respective sampling times versus the "0" time baseline control value for that treatment group via the Duncan's Multiple Range Test ($p \leq 0$ 05) Each point represents the mean of 5–8 determinations and the vertical bars depict the S E M

the rat associated with moderate doses of *d*-amphetamine include increases in forward locomotion, rearing, head bobbing, and gnawing At higher doses, the above stereotypy is altered so that only head bobbing, backward walking, and circling are seen [31] All of the latter responses may be caused by concurrent release of dopamine and serotonin [1]

Possible dopamine and serotonin interactions could also explain findings of Franklin and Robertson [11], who report that *d*-amphetamine-induced locomotor activity in rats was significantly increased by the serotonergic blocker, methysergide. When given subacutely or chronically, *d*-amphetamine has been shown to significantly affect both central serotonin and dopamine stores. Trulson and Jacobs [32] reported that a 10-day treatment with *d*-amphetamine in cats resulted in decreased serotonin levels in all brain regions examined Segal *et al* [27] found that in rats treated with *d*-amphetamine every 4 hr for 5 days, norepinephrine was decreased in the hippocampus and hypothalamus, and dopamine levels were lowered in the caudate nucleus Eight days after the last injection, all central amines had returned to normal, however Ellison *et al* [8] found that several days after a *d*-amphetamine containing pellet was implanted subcutaneously in rats, the caudate dopaminergic axons were swollen and there was a significant decrease in tyrosine hydroxylase activity Both effects were confined to the caudate region Sparber and Tilson [30] investigated the releasability of central norepinephrine and serotonin by injecting *d*-amphetamine in rats before and after development of tolerance to the drug's disruptive effects on operant behavior It was found that *d*-amphetamine given acutely before tolerance developed was capable of releasing significant amounts of norepinephrine, but not serotonin, however, after tolerance had developed, the same doses of *d*-amphetamine were able to release significant amounts of both norepinephrine and serotonin.

Several aspects of our data generally agree with the studies cited above First of all, the depleting effects of the subacute *d*-amphetamine treatment were found to be widespread in regard to brain areas affected for norepinephrine, but confined to the striatum with respect to dopamine. Secondly, the *d*-amphetamine treatment also decreased serotonin levels, but this effect was restricted to the pons-medulla, substantia nigra, and thalamic regions Thirdly, acute injections of fenfluramine caused rapid decreases in serotonin content in all brain regions examined in saline-treated rats Lastly, fenfluramine administration to saline-treated rats also resulted in a decrease in norepinephrine and dopamine in some brain regions

These data indicate that significant differences in the neuronal substrate available to fenfluramine may have existed between the two regimens For example, serotonin, the major substrate for fenfluramine, was markedly lowered to below pre-drug baseline and saline control-values by *d*-amphetamine treatment in the pons-medulla, thalamus, and substantia nigra However, the direct contribution of such serotonin decreases to the anorectic tolerance seen with fenfluramine in these animals is uncertain, since the involvement of these anatomical regions with feeding behavior is not precisely known

It should be noted that serotonin levels in all brain regions of the control animals were lower than those generally reported in the literature This is most likely a result of the 15-day limited food access paradigm used in the present study and the fact that the animals were sacrificed 21-hr after the last feeding session In this regard, it has been shown that brain serotonin content is proportional to the dietary intake of tryptophan [9,22]. The salient feature of these data is that existing serotonin levels in the region where fenfluramine might be expected to exert its main central anorectic effects (i.e , the hypothalamus) were not affected by the *d*-amphetamine treatment. Furthermore, serotonin depleting action of fenfluramine in this area was not significantly different between the two regimens. These findings tend to rule out serotonin availability in the hypothalamus as a factor in the anorectic tolerance conferred to fenfluramine by *d*-amphetamine, but they do not eliminate the possible contribution of more subtle differences in the hypothalamic serotonergic systems between the two regimens.

Sedation commonly occurs after fenfluramine administration [6,20] and is probably the result of serotonin release in the cortex [18]. Since cortical serotonin levels were not altered by the *d*-amphetamine treatment, and since fenfluramine induced depletion of serotonin was not significantly different between the two regimens, it is unlikely that different degrees of sedation, due to fenfluramine, existed in the animals Therefore, this factor would not have been expected to alter the feeding behavior of the animals in the earlier study [16]

Norepinephrine and dopamine are thought to exert primarily an inhibitory influence on feeding [15] At least two aspects of the present data seem to suggest that a regimen-related difference in these transmitters could have influenced the degree of fenfluramine-induced anorexia The first is that norepinephrine levels were markedly lowered in the hypothalamic regions of the *d*-amphetamine-treated animals versus the saline group Furthermore, the action of fenfluramine on hypothalamic norepinephrine in the *d*-amphetamine group appeared to be less pronounced than in the saline group. The second regimen-related difference in the neurochemical effects of fenfluramine was seen in the depleting action of fenfluramine on hypothalamic dopamine Although baseline hypothalamic dopamine levels were not found to be significantly different between the two regimens, fenfluramine caused a statistically significant decrease of hypothalamic dopamine in the saline-treated animals, such an effect was not observed in the *d*-amphetamine group The functional significance of these findings with norepinephrine and dopamine is questionable however, because the vast majority of literature indicates that fenfluramine still exerts powerful anorectic effects even after blockade or depletion of norepinephrine and dopamine [13]

In conclusion, it was found in a previous study that subacute *d*-amphetamine treatment resulted in a diminution in the food intake suppressant effects of fenfluramine Since the primary anorectic action of fenfluramine might be expected to involve serotonin release in the hypothalamus, a major hypothesis tested in this study was to determine if the subacute *d*-amphetamine treatment lowered the hypothalamic availability of serotonin The effects of subacute *d*-amphetamine treatment on one index of availability, i e , hypothalamic serotonin levels, suggested that this hypothesis was not valid, since no significant differences in this parameter existed between animals in the two regimens. Furthermore, the time course effects of fenfluramine on serotonin in the hypothalamic regions of animals in the two regimens did not differ Data from these neurochemical experiments also suggested that other factors such as the state of arousal of the subjects in the two regimens did not differ at the time of fenfluramine dosing and therefore, did not likely contribute to the apparent cross-tolerance

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