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Comparison of the Effects of Repeated Oral Versus Subcutaneous Fenfluramine Administration on Rat Brain Monoamine Neurons: Pharmacokinetic and Dose-Response Data

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DE SOUZA, E. B., R. ZACZEK, S. CULP, N. M. APPEL AND J. F. CONTRERA. *Comparison of the effects of repeated oral versus subcutaneous fenfluramine administration on rat brain monoamine neurons: Pharmacokinetic and dose-response data.* PHARMACOL BIOCHEM BEHAV **39**(4) 963–969, 1991.—The importance of the route of drug administration (oral vs. subcutaneous) on the neurochemical effects and pharmacokinetics of repeated d,l-fenfluramine administration in rats (1–24 mg/kg b.i.d., i.e., 2–48 mg/kg/day for 4 days) was examined. Overall, comparable dose-dependent alterations in brain monoamine markers were observed following repeated oral (PO) and subcutaneous (SC) administration of fenfluramine. Doses of 1 and 2 mg/kg fenfluramine were without significant effects on the density of ^{3}H-paroxetine-labeled serotonin (5-HT) uptake sites. Higher doses of fenfluramine (4, 12 and 24 mg/kg) produced dose-dependent decreases in 5-HT, 5-hydroxyindoleacetic acid and 5-HT uptake sites with maximal decreases (80–90%) occurring at the 12 mg/kg dose. Fenfluramine administration produced dose-dependent and biphasic effects on brain dopamine markers with increases in homovanillic acid (HVA) observed at 2 hours, whereas decreases in the levels of dopamine, HVA and dihydroxyphenylacetic acid were evident at 18 hours posttreatment. Norepinephrine levels were only decreased at the highest dose of fenfluramine. Significantly higher levels of brain fenfluramine were observed following SC than following PO administration of the drug. On the other hand, comparable levels of its active metabolite norfenfluramine were present in the brain following the two routes of fenfluramine administration. These data suggest the importance of norfenfluramine levels in the brain in determining the high-dose neurotoxic effects of fenfluramine on brain 5-HT neurons in rats.

Amphetamine Anorexia Dopamine Catecholamines Neurotoxicity Norepinephrine Obesity Serotonin

FENFLURAMINE, a substituted amphetamine derivative which lacks the psychostimulant effect of amphetamine, is used clinically in the treatment of obesity (7, 22, 26). The anorectic effects of fenfluramine are believed to be mediated through the effects of fenfluramine or its active N-dealkylated metabolite norfenfluramine in the brain to cause both release and block reuptake of serotonin (5-HT) from nerve terminals (9,10).

Neurochemical data in rats demonstrating long-lasting reductions in brain serotonergic markers after acute (6, 13, 15, 27, 29) or short-term (16, 28, 32) administration of the drug in some studies, but not others (8), have led to a controversy over whether or not fenfluramine can cause damage to 5-HT neurons. More recent neuroanatomical studies demonstrating selective decreases in the density of 5-HT-like-immunolabeled nerve terminals and morphology characteristic of degenerating neurons (1,20) and decreases in the autoradiographic distribution of ^{3}H-paroxetine-labeled 5-HT uptake sites (2) following a high-dose fenfluramine treatment regimen in rats have added further to concerns of a "neurotoxic" potential of fenfluramine on brain 5-HT terminals.

Very recent studies suggest that the high-dose brain 5-HT-depleting effects of fenfluramine are probably a consequence of the nonlinear kinetics of the drug and the enormous capacity of the rat to concentrate both fenfluramine and its active metabolite

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norfenfluramine in the brain, resulting in an inordinate brain exposure to these compounds (32). Furthermore, the hypothesis has been put forth that the long-term serotonergic deficits observed following fenfluramine administration may result primarily from its metabolite norfenfluramine (15). The relationship of the high-dose toxic effects of systemically administered fenfluramine in rats to the therapeutic use of the drug is unclear, in view of the differences in the prescribed dose, route of administration, kinetics and metabolism of fenfluramine in humans versus rats. The present study was designed to assess the importance of the route of drug administration (oral vs. subcutaneous) on the effects of repeated d,l-fenfluramine administration in rats on brain monoamine markers, and to relate the neurochemical effects to the pharmacokinetics of the drug and its metabolite.

METHOD

Studies in this report were carried out in accordance with the Declaration of Helsinki and with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institute of Health.

Materials

d,l-Fenfluramine HCl was purchased from Sigma Chemical Co. (St. Louis, MO). Citalopram was obtained from Lundbeck (Copenhagen, Denmark). ^{3}H-Paroxetine (specific activity, 19 Ci/mmol) and Formula 963 liquid scintillation mixture were purchased from DuPont-New England Nuclear (Boston, MA). All other reagents were purchased from Sigma Chemical Company.

Animal Treatment Regimens and Sample Collection Procedures

Male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN), weighing 200 to 300 g, were used in all studies. Rats were housed under standard environmental conditions (12 h light/12 h dark; 23°C) and had access to food (Purina Rodent Chow #5001) and water ad libitum.

Rats were administered either fenfluramine HCl (1, 2, 4, 12 or 24 mg/kg) (i.e., 2, 4, 8, 24, or 48 mg/kg/day) or saline vehicle (1 ml/kg) subcutaneously (SC) (32) or orally (PO) dissolved in water (10 ml/kg) with water serving as control, twice daily (at approximately 12-h intervals) for 4 days. Animal weights were recorded in the morning prior to drug or vehicle administration. Rats were sacrificed by decapitation at 2 or 18 h after the final drug treatment. Trunk blood was collected in ice-chilled heparinized tubes, and plasma was separated by centrifugation. Plasma was decanted and stored at −70°C for subsequent determination of fenfluramine and norfenfluramine concentrations. Brains were removed, and frontal pole areas, containing frontal cerebral cortex and striatum, were dissected out by making a vertical cut just anterior to the optic chiasm and trimming away remaining elements of the olfactory bulb. This combined brain area enriched in dopamine (striatum), norepinephrine and 5-HT (cerebral cortex) neurons enabled us to optimally detect all three monoamines and their respective metabolites in the same tissue. Frontal pole areas were stored at −70°C until assay of monoamine markers. The remainder of the brain was stored at −70°C for subsequent fenfluramine and norfenfluramine determinations.

Measurement of Fenfluramine and Norfenfluramine

Plasma and brain concentrations of fenfluramine and its metabolite, norfenfluramine, were measured using the method of Richards et al. (25). Briefly, brain tissues (1–2 g) were homogenized in 5 to 10 volumes of 0.05 M sulfuric acid; 100 μl of the internal standard, diethylanaline (100 ng), were added to the homogenate. The sample was then made basic by the addition of 100 μl of 5 M NaOH and extracted with 200 μl of butyl acetate. After separation by centrifugation, the extract was injected directly into a capillary gas chromatograph equipped with a nitrogen/phosphorous specific detector. Plasma (1 ml), to which the internal standard, diethylanaline (65 ng), had been added, was made basic with 0.5 ml of 5 N NaOH and extracted with 8 ml of diethylether. The organic phase was then back-extracted into 0.5 ml of 0.5 M sulfuric acid. After the aqueous phase was made basic by the addition of 0.5 ml of 5 N NaOH, compounds were extracted into butyl acetate (100 μl) and injected into the gas chromatograph as described above.

HPLC Determination of Biogenic Amines

Brain regions were homogenized in 20 volumes of ice-cold 50 mM monobasic sodium phosphate using a Sonfier cell disrupter (setting 6 for 10 s). The homogenate was then separated by centrifugation at 20,000 × g for 20 min at 4°C. Ascorbate oxidase (5 units) was added to 1.0 ml of the resulting supernatant and, after a 10-min incubation at room temperature, the supernatant was assayed for biogenic amines using the HPLC-electrochemical detection method of Zaczek and Coyle (30).

Measurement of Serotonin Uptake Sites

Brain regions were homogenized in 40 volumes of ice-cold 50 mM Tris HCl, pH 7.1, containing 120 mM NaCl and 5 mM KCl using a Brinkmann Polytron (setting 6 for 20 s). The resulting homogenate was separated by centrifugation at 48,000 × g for 10 min at 4°C and washed by rehomogenization in fresh buffer and subsequent centrifugation. The resulting pellet was resuspended in buffer to a final concentration of 15 mg original wet weight/ml. Protein was measured by the method of Lowry et al. (17) using bovine serum albumin as the standard.

Serotonin uptake sites were labeled with ^{3}H-paroxetine as previously described (11). Paroxetine is the most potent and selective inhibitor of serotonin uptake in vitro (14), and ^{3}H-paroxetine has been shown to selectively label only sites associated with the serotonin transporter complex in the brain (3). Briefly, 100 μl of tissue homogenate were added to tubes containing 4.9 ml of homogenization buffer containing 0.25 nM ^{3}H-paroxetine. Nonspecific binding was measured in the presence of 2 μM citalopram. After an incubation period of 120 min at room temperature, the mixture was filtered and washed (3 × 3 ml) rapidly over Whatman GF/C filter paper (presoaked in 0.05% polyethyleneimine) using a Brandel Cell Harvester (Brandel, Gaithersberg, MD). The filters were then placed in 7.0 ml counting vials and equilibrated with 5.0 ml of Formula 963 scintillation mixture. Radioactivity was measured in a Beckman LS 3801 liquid scintillation counter.

Data Analysis

Data were analyzed for statistical significance using Student's *t*-test and one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range test or regression analysis. Significant relationships between the various parameters were determined using Pearson's correlation matrix and Bonferroni-adjusted probabilities.

RESULTS

Dose-Related Effects of Oral Versus Subcutaneous Administration on Fenfluramine and Norfenfluramine Levels in Plasma and Brain

The relationships between plasma and brain levels of fenfluramine or norfenfluramine after the 4-day PO and SC fenflu-

ramine treatment regimens are shown in Fig. 1. Significant linear relationships were found between brain and plasma levels of both fenfluramine ($r^2 = .95$, $p<0.001$) and norfenfluramine ($r^2 = .95$, $p<0.001$). Of note, the regression lines defining the relationship between plasma and brain concentrations of fenfluramine were virtually identical following PO and SC administration of the drug, suggesting that fenfluramine was concentrated in the brain to the same extent when compared to plasma following the two routes of administration (brain:plasma 37:1) (Fig. 1A). With regard to norfenfluramine (Fig. 1B), the brain to plasma ratio was higher following SC (54:1) than following PO (41:1) administration of the drug. Given the highly significant positive correlations between plasma and brain levels of fenfluramine and norfenfluramine, the remainder of the report will be limited to description of brain levels of the drug and its metabolite.

Figure 2 illustrates dose-dependent increases in brain concentrations of fenfluramine and norfenfluramine observed at 2 and 18 hours following the 4-day treatment regimen. Significantly higher levels of fenfluramine were present in the brain at 2 hours following SC than following PO administration of the drug. In contrast, at 18 hours, significantly higher levels of fenfluramine were present in the brain following PO than SC administration of the drug. These data suggest that while the peak levels of fenfluramine may be higher, the clearance rates of the drug may be increased following SC dosing when compared to those following PO administration of the drug. Comparable dose-dependent increases in brain levels of norfenfluramine were seen following PO and SC administration of the drug regardless of the time point examined (2 hours or 18 hours).

Effects of Oral Versus Subcutaneous Fenfluramine Administration on Body Weight

Overall, comparable dose-dependent decreases in body weight were observed following both routes (PO and SC) of fenfluramine administration (Fig. 3). The weight reduction was somewhat greater in rats administered the drug orally than in animals receiving the drug by the SC route of administration. No significant differences in body weight were noted among control rats and those treated with either 1 or 2 mg/kg fenfluramine at any of the time points examined. Rats treated with 4 mg/kg fenfluramine showed weight reduction during the first two days following onset of the treatment, and showed some recovery of lost body weight on days 3 and 4 of the treatment regimen; however, their weight never approached that seen in saline-treated animals. Progressively greater decreases in body weight were observed at 12 and 24 mg/kg doses following both routes of administration. The decreases in body weight were dose related and showed significant relationships with brain levels of fenfluramine and norfenfluramine. Of note, the reductions in body weight correlated somewhat better to brain levels of norfenfluramine than to brain levels of fenfluramine.

Dose-Dependent Effects of Fenfluramine Administration on Monoamine Indices in the Brain: Comparison of Oral and Subcutaneous Routes of Administration

Serotonergic markers. Dose-dependent reductions of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) (Table 1), and ^{3}H-paroxetine-labeled serotonin uptake sites (Fig. 4) in the frontal pole of the brain were observed following SC and PO fenfluramine treatment with doses of 4 mg/kg and higher regardless of the time after treatment. While maximal and comparable decreases in all three brain 5-HT markers (80–90% reductions) were evident in rats treated with 12 mg/kg and 24 mg/kg fenfluramine following both routes of administration, there were some quantitative differences observed at the 4 mg/kg dose of fenfluramine. At this dose (4 mg/kg) of fenfluramine, the reductions in 5-HIAA (Table 1) and 5-HT uptake sites (Fig. 4) were significantly greater following the PO than following the SC treatment regimen. In addition, some subtle differences were noted in the low-dose (1 mg/kg) fenfluramine treatment effects on brain 5-HT markers following PO and SC treatment. In the 1 mg/kg SC treatment group, significant increases in 5-HT and 5-HIAA were noted at 2 hours after treatment; these changes were reversed at 18 hours posttreatment. However, no significant alterations in either 5-HT or 5-HIAA were noted in the 1 mg/kg group fol-

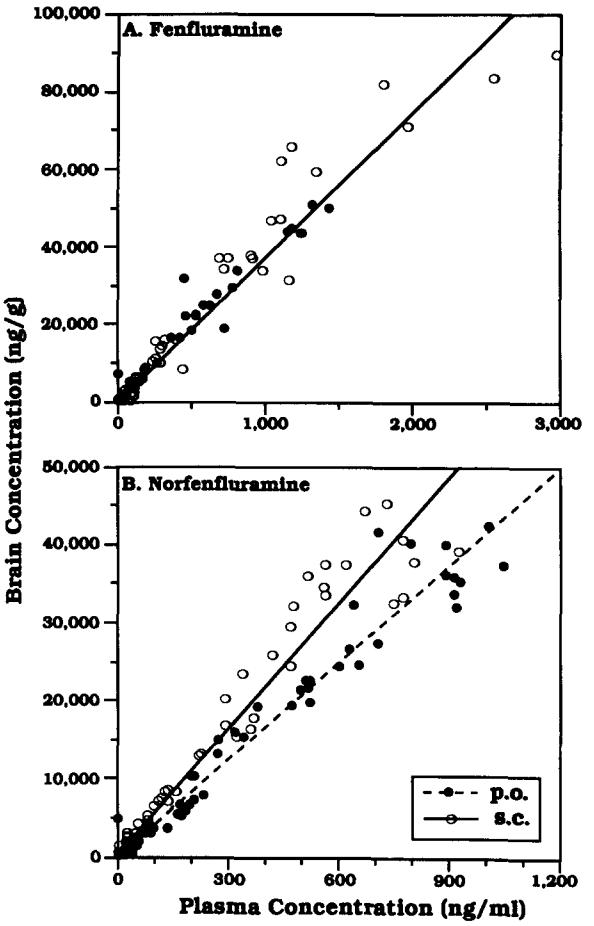

FIG. 1. Relationships between plasma and brain concentrations of fenfluramine and norfenfluramine after repeated administration of various doses of fenfluramine in rats: comparison of oral versus subcutaneous routes of administration. Rats were administered fenfluramine either orally (PO) or subcutaneously (SC) twice daily for four days with doses ranging from 1 to 24 mg/kg, and sacrificed at 2 or 18 hours after final drug treatment. Brain and plasma were processed for fenfluramine and norfenfluramine as described in the Method section. Significant linear relationships were found between plasma and brain levels of both fenfluramine ($r^2 = .97$, $p<0.001$) and norfenfluramine ($r^2 = .97$, $p<0.001$). The slopes of the lines defining the relationships between plasma and brain levels of fenfluramine were not different from each other, while the slopes of the norfenfluramine lines were significantly different ($p<0.01$) following the two routes of drug administration as determined by the Test for Parallelism and Student's *t*-test. Each point represents the average of values from 4–8 rats at each dose.

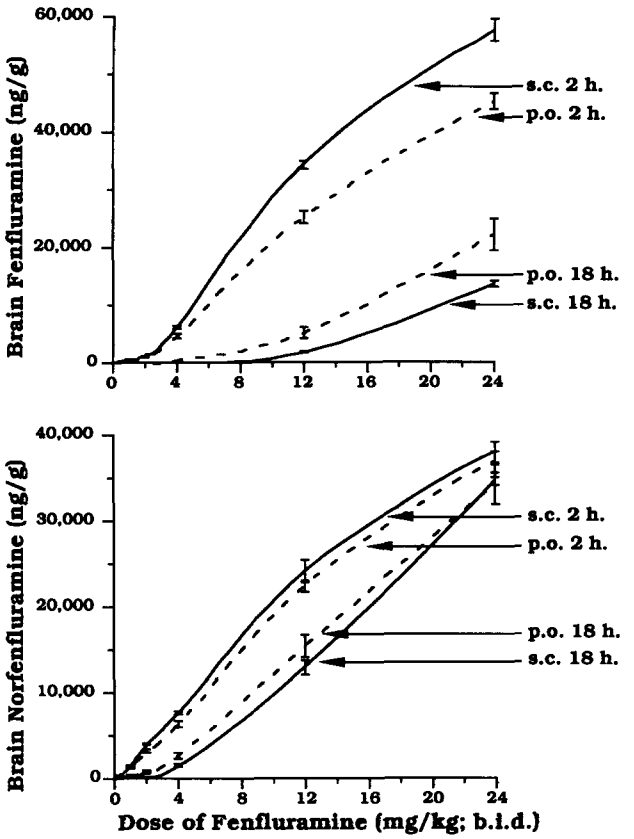

FIG. 2. Dose-related increases in fenfluramine and norfenfluramine levels in brain: comparison of oral versus subcutaneous routes of administration. Rats were administered fenfluramine either subcutaneously (SC) or orally (PO) twice daily for four days with various doses of fenfluramine (1–24 mg/kg). Animals were sacrificed at 2 or 18 hours after final drug administration. Brains were processed for fenfluramine and norfenfluramine as described in the Method section. Each value represents the mean ± SEM from 4–8 rats at each dose.

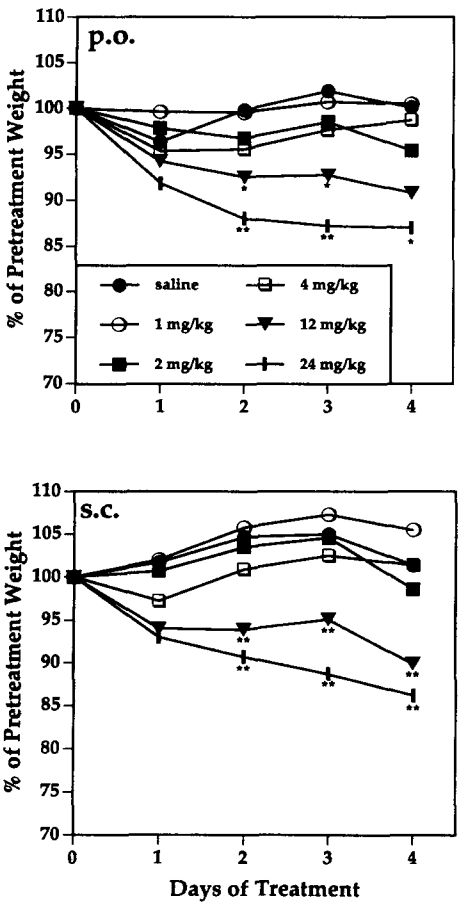

FIG. 3. Comparison of the effects of oral versus subcutaneous repeated administration of various doses of fenfluramine on body weight in rats. Rats were administered either vehicle (water or saline) or fenfluramine (1–24 mg/kg) orally (PO) or subcutaneously (SC) twice daily (at approximately 12-hour intervals) for four days. Rats were weighed each morning prior to drug administration (Days 0 to 3) or sacrifice (Day 4). Values indicate percent of Day 0 (211 ± 2 g; n = 13 for SC-treated rats and 277 ± 4 g; n = 16 for PO-treated rats). * and ** indicate significant differences at $p<0.05$ and $p<0.01$, respectively, from the vehicle control group.

lowing PO administration of the drug (Table 1). No significant alterations were observed in the density of 5-HT uptake sites in the 1 and 2 mg/kg fenfluramine treatment groups regardless of whether the drug was administered PO or SC (Fig. 4). Negative correlations were evident between alterations in all three serotonergic markers measured and the dose of fenfluramine administered, the brain concentrations of fenfluramine or its metabolite norfenfluramine.

Since both fenfluramine and norfenfluramine are relatively potent in inhibiting serotonin uptake (5,19), we examined whether the decreases in ^{3}H-paroxetine binding following the high-dose 4-day fenfluramine treatment regimen were due to residual high levels of fenfluramine and norfenfluramine present in the brain. Specifically, we measured the density of ^{3}H-paroxetine binding sites in the frontal pole in groups of rats that were sacrificed at 30 minutes following SC administration of single doses of fenfluramine varying from 1 to 24 mg/kg. Previous studies have demonstrated that peak levels of fenfluramine and norfenfluramine are present in the brain at 30 minutes following administration of the drug (32). As seen in Fig. 4, acute administration of doses of fenfluramine identical to those used in the 4-day treatment regimen did not alter the density of ^{3}H-paroxetine-labeled uptake sites in the brain.

Norepinephrine. No significant alterations in the brain norepinephrine concentration were observed following SC or PO administration of low doses of fenfluramine (1 and 2 mg/kg). At the 4 mg/kg dose of fenfluramine, there were nonsignificant decreases in the concentration of norepinephrine at 2 hours following both PO and SC drug administration; the levels recovered to those seen in saline-injected controls at 18 hours posttreatment. At the highest dose of 24 mg/kg fenfluramine, there was a 50% reduction in norepinephrine levels at 2 hours following both PO and SC administration which persisted for at least 18 hours (Table 1). There were significant negative correlations between norepinephrine concentrations in the brain and dose of drug administered, brain fenfluramine levels and also brain norfenfluramine levels (Table 1).

Dopaminergic markers. Overall, there was a trend for dopamine levels to be decreased with increasing doses of the drug following both routes of drug administration. The decreases were more evident following PO than following SC administration of the drug, and were greater at 2 hours than at 18 hours following final drug administration (Table 1). In contrast, there was a trend for the levels of the dopamine metabolite homovanillic acid

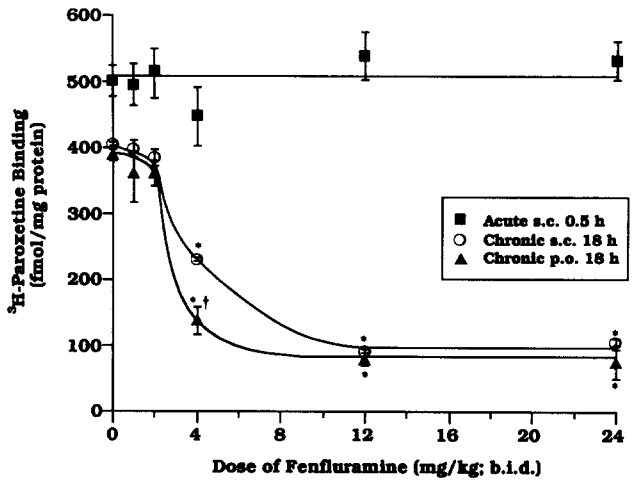

FIG. 4. Dose-dependent effects of fenfluramine administration on the density of ^{3}H-paroxetine-labeled serotonin uptake sites in frontal pole of rat brain: comparison of routes of administration and treatment regimens. Rats were administered fenfluramine either subcutaneously (SC) or orally (PO) twice daily for four days (chronic) with indicated doses of fenfluramine and sacrificed at 18 hours after final drug administration. Additional groups of rats received a single acute SC injection of indicated doses of fenfluramine and were sacrificed 0.5 hour following the injection. ^{3}H-Paroxetine-labeled 5-HT uptake sites were measured as described in the Method section. Mean values ± SEM from 4–8 rats/group. *Indicates significant differences at $p<0.0001$ from corresponding values in vehicle-treated controls are determined by one-way ANOVA and Duncan's Multiple Range test. †Indicates significant differences at $p<0.0001$ from chronic SC treatment group as assessed using Student's t-test.

(HVA) to increase at 2 hours after treatment. The increases appeared to be dose dependent following SC administration of the drug, in that they were only evident in the high-dose treatment goup (24 mg/kg) following PO administration of the drug. Levels of another dopamine metabolite, dihydroxyphenylacetic acid (DOPAC), were unchanged at 2 hours after fenfluramine treatment, but were reduced at 18 hours posttreatment. Again, the dose-dependent decreases in DOPAC levels were more evident following SC administration of the drug (Table 1). There were no significant correlations between brain fenfluramine or norfenfluramine and the levels of dopamine, HVA or DOPAC.

DISCUSSION

Fenfluramine is an anorectic agent used in the control of obesity (7, 22, 26). Suggestions of fenfluramine-induced neurotoxicity in rats have led to concern over its safety. In view of the differences in the prescribed dose, route of administration, kinetics and metabolism of fenfluramine in humans versus rats, the relevance of the high-dose toxic effects of systemically administered fenfluramine in rats to the therapeutic use of the drug is at present unclear. The present report addresses the importance of the route of drug administration (PO vs. SC) on the dose-dependent effects of repeated d,l-fenfluramine administration in rats to potential neurotoxic effects of the drug on brain monoamine neurons. In addition, the fenfluramine-induced alterations in brain monoamine markers following both routes of drug administration are related to the plasma and brain levels of the drug and its active metabolite norfenfluramine.

The most profound neurochemical effects of short-term fenfluramine administration in rats were changes in brain 5-HT markers. For the most part, comparable dose-dependent decreases in 5-HT and 5-HIAA levels and the density of ^{3}H-paroxetine-labeled 5-HT uptake sites were observed following PO and SC administration of the drug. In view of the presence of residual fenfluramine and its active metabolite norfenfluramine in the brain following the high-dose drug treatment protocol, one must consider the possibility that the observed decreases in ^{3}H-paroxetine binding are simply a result of fenfluramine/norfenfluramine competing with ^{3}H-paroxetine for the 5-HT uptake site. This is, however, unlikely since no significant decreases in ^{3}H-paroxetine binding were evident at a time (30 minutes) following acute administration of the drug when peak levels of fenfluramine and norfenfluramine are present in the brain. Thus, as evidenced by decreases in the density of 5-HT uptake sites, it appears that repeated SC or PO administration of fenfluramine at doses ≥4 mg/kg may produce neurodegeneration of serotonin neurons.

There were dose-dependent increases in the concentration of fenfluramine and its metabolite norfenfluramine in the brain following SC or PO fenfluramine administration. Doses of fenfluramine (≥4 mg/kg) that produced significant reductions in all three 5-HT markers resulted in significantly higher peak concentrations (at 2 hours) of fenfluramine and comparable concentrations of norfenfluramine in the brain following SC administration than when following PO administration of the drug. These data suggest that norfenfluramine levels in the brain may be more important than fenfluramine in mediating the high-dose neurotoxic effects of fenfluramine in rats. In support of this hypothesis, it has recently been reported that d-norfenfluramine produced significantly greater long-term serotonergic deficits than comparable doses of d-fenfluramine (15). The more potent neurotoxic effects of norfenfluramine than fenfluramine may relate to the fact that norfenfluramine is a much more potent releaser of serotonin than fenfluramine (5,19). Other amphetamine-related serotonergic neurotoxins such as p-chloroamphetamine (12,18), methamphetamine (24,31), 3,4-methylenedioxyamphetamine (MDA) (3, 21, 23), and 3,4-methylenedioxymethamphetamine (3,21) are also relatively potent releasers of serotonin. There were significantly higher concentrations of fenfluramine in the brain at 18 hours following the final drug treatment in rats given the drug orally than following SC administration of the drug. In view of the lower peak levels of fenfluramine in the brain following PO than following SC administration of the drug, these data suggest that the clearance of fenfluramine may be slower following PO than following SC administration of the drug resulting in an overall comparable brain exposure to fenfluramine (area under the curve) following the two routes of administration. The relative contribution of this mechanism to the neurotoxic effects of fenfluramine must also be considered.

The effects of fenfluramine treatment on catecholamines were, for the most part, comparable following both routes of administration. Furthermore, the effects of the drug on catecholamines were acute and were evident primarily at higher doses of the drug. High-dose fenfluramine treatment (24 mg/kg) caused acute reductions in norepinephrine content. With regard to dopamine markers, biphasic acute and subacute changes were observed following the SC fenfluramine treatment regimen. At 2 hours, HVA concentrations in the brain were increased, whereas dopamine, DOPAC and HVA levels were all decreased 18 hours after fenfluramine treatment. Following PO administration of the drug, the early increases in HVA and later decreases in DOPAC and dopamine were only evident following the high-dose (24 mg/kg) treatment regimen. In contrast to the long-term effects of high-dose fenfluramine treatment on the integrity of 5-HT neurons, catecholamine neuronal integrity appears to be unaffected, since previous studies demonstrated no long-term ef-

TABLE 1

DOSE-RELATED EFFECTS OF REPEATED FENFLURAMINE ADMINISTRATION ON LEVELS OF BIOGENIC MONOAMINES AND THEIR METABOLITES IN FRONTAL POLE OF RAT BRAIN

	Dose of Fenfluramine							
	1 mg/kg		2 mg/kg		4 mg/kg		24 mg/kg	
	PO	SC	PO	SC	PO	SC	PO	SC
5-HT								
2 h	119 ± 24	160 ± 8*‡	75 ± 6	105 ± 11	48 ± 13	56 ± 5	30 ± 25*	12 ± 5†
18 h	86 ± 22	81 ± 3	81 ± 14	69 ± 7	55 ± 9	37 ± 5*	32 ± 20*	9 ± 3†
5-HIAA								
2 h	81 ± 9	198 ± 19†‡	53 ± 9†	139 ± 10*	21 ± 2†	76 ± 11	13 ± 5†	21 ± 3†
18 h	82 ± 6	94 ± 5	63 ± 5*	100 ± 7	33 ± 1†	50 ± 4†	22 ± 5†	29 ± 12†
NE								
2 h	109 ± 5	109 ± 2	94 ± 10	94 ± 8	79 ± 8	88 ± 9	44 ± 13	49 ± 2†
18 h	98 ± 7	100 ± 2	102 ± 3	101 ± 4	107 ± 12	100 ± 1	66 ± 9	58 ± 5†
DA								
2 h	93 ± 1	106 ± 3	76 ± 8	104 ± 1	77 ± 4	97 ± 4	65 ± 8	77 ± 3†
18 h	85 ± 10	98 ± 2	93 ± 4	111 ± 6	90 ± 6	86 ± 3	80 ± 8	72 ± 3†
DOPAC								
2 h	85 ± 7	107 ± 1	95 ± 10	108 ± 6	91 ± 13	103 ± 8	94 ± 21	88 ± 10
18 h	101 ± 7	80 ± 2	93 ± 7	77 ± 2*	111 ± 7	63 ± 6†	77 ± 4	56 ± 3†
HVA								
2 h	93 ± 9	140 ± 11†	80 ± 3	158 ± 8†	96 ± 7	154 ± 12†	138 ± 7†	181 ± 16†
18 h	90 ± 8	75 ± 3	97 ± 10	73 ± 8	85 ± 6	76 ± 12	97 ± 8	42 ± 10†

Rats were administered vehicle (water or saline) or fenfluramine (1–24 mg/kg) orally (PO) or subcutaneously (SC) twice daily for 4 days and sacrificed at 2 h or 18 h after final drug administration. Brain levels of serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), norepinephrine (NE), dopamine (DA), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured as described in the Method section. Values represent percent of vehicle-treated control (mean ± SEM). * and † indicate significant differences at $p<0.05$ and $p<0.01$ respectively from controls.

‡Indicate significant difference at $p<0.01$ from corresponding values in rats given a corresponding dose of fenfluramine orally. No significant differences in monoamines and their metabolites were noted between groups of rats administered saline SC or PO. The pooled control values expressed as pmol/mg tissue were 5-HT 0.99±0.11; 5-HIAA 0.92±0.08; NE 2.86±0.25; DA 13.6 ± 0.83; DOPAC 2.28±0.17; HVA 1.06±0.05.

fects of drug treatment on the density of norepinephrine and dopamine uptake sites in the brain (2,32) and on tyrosine hydroxylase-immunolabeled neurons (1). The effects of d,l-fenfluramine administration on catecholamines may primarily relate to properties of the l-isomer since l-fenfluramine has been reported to be an antidopaminergic agent which has neuroleptic-like effects on the release of striatal dopamine (4).

While comparable neurochemical and pharmacokinetic effects were observed following SC and PO administration of fenfluramine, some subtle differences were evident. Fenfluramine was concentrated in the brain (approximately 37-fold) to the same extent following PO and SC administration of the drug, while norfenfluramine was concentrated in the brain to a greater extent following SC (54-fold) than following PO (41-fold) administration of the drug. The reasons for the higher brain to plasma ratio of norfenfluramine following SC administration of the drug are at present unclear. Other subtle differences between the two routes of drug administration include significantly greater reductions in 5-HIAA and the density of 5-HT uptake sites in the 4 mg/kg-treated group following PO than following SC administration of the drug. The greater decreases in serotonergic markers following PO administration of this "intermediate" dose of fenfluramine do not appear to relate to brain concentrations of fenfluramine or its metabolite norfenfluramine, since somewhat higher concentrations of the parent drug and its metabolite were found in SC-treated than in the orally treated group.

In summary, the present study assessed the importance of the route of drug administration on the neurotoxic effects of repeated fenfluramine administration in rats on brain monoamine neurons. The data demonstrate the relevance of the active metabolite norfenfluramine to the high-dose neurotoxic effects of fenfluramine on brain serotonin neurons, and underscore the importance of relating the neurochemical effects of the drug to the pharmacokinetics of the drug and its metabolite.

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