Molecular Neurobiology

ISSN0893-7648/95/11(1-3): 165-175/$6.20

The Nature of *d,l*-Fenfluramine-Induced 5-HT Reuptake Transporter Loss in Rats

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Abstract

The administration of the anorexigenic drug *d,l*-fenfluramine (Ponderax®) to laboratory animals results in a dose-dependent reduction in presynaptically located serotonergic reuptake transporter protein. This long-term effect may represent an altered mechanism of synthesis of the transporter (downregulation). Alternatively, fenfluramine may destroy the serotonergic terminals on which 5-HT transporters are located. To distinguish between these two alternatives, we applied an assay of neurotransmitter-specific nerve endings (α) to brain tissue from two animal models of reduced 5-HT transporter density. In Model 1, serotonergic nerve terminals were destroyed (rats received 5,7-dihydroxytryptamine [5,7-DHT] intracisternally); in Model 2, there was a loss of 5-HT transporters *per se* on otherwise intact serotonergic nerve terminals. The manner in which α declined as transporter density was decreased (reducing V_{max} values) in animal Models 1 and 2 was found to be significantly different. In rats treated with fenfluramine, the association between 5-HT transporter density and α was the same as in the neurotoxic model.

Index Entries: Fenfluramine; 5-hydroxytryptamine (5-HT, serotonin); nerve terminal; synaptosomes; neuronal uptake; neurotoxicity; transporters.

Introduction

Fenfluramine (Ponderax®) is used in the treatment of obesity because of its ability to nonselectively increase serotonergic neurotransmission to hypothalamic appetite control centers (Pinder, 1975). Serotonergic neurotransmission is enhanced because fenfluramine releases 5-HT from serotonergic nerve terminals (Mennini et al., 1991; Fuller et al., 1988; Sarkissian et al., 1990) and blocks its reuptake (Borroni et al., 1983; Hekmatpanah and Peroutka, 1990) by competitively competing with 5-HT for the transporter site (Kouyoumdjian et al., 1979). Unlike its archetype, this *m*-trifluoromethyl-*N*-ethyl derivative of amphetamine does not induce central stimulation or develop dependency, therefore it is

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not a substance of abuse in homo sapiens. However, in character with other substituted amphetamines (e.g., 3,4-methylenedioxyamphetamine [MDA]; 3,4-methylenedioxymethamphetamine [MDMA]; *p*-chloroamphetamine [PCA]) and classical serotonergic neurotoxins (e.g., 5,7-dihydroxytryptamine [5,7-DHT]), fenfluramine administration produces long-lasting depletions of brain serotonin (Duhault and Verdavainne, 1967; Opitz, 1967; Harvey and McMaster, 1975; Clinschmidt et al., 1976; Steranka and Sanders-Bush, 1979; Kleven et al., 1988; Colado et al., 1993) and 5-HIAA (Steranka and Sanders-Bush, 1979; Fuller et al., 1988; Kleven et al., 1988; Colado et al., 1993) in laboratory animals. In addition, diminished densities of 5-HT transporters, as shown by decreased 5-HT uptake site binding (Schuster et al., 1986; Appel et al., 1990; Johnson and Nichols, 1990; Wagner and Peroutka, 1990) or by reduced rates at which 5-HT is taken up by synaptosomes (Steranka and Sanders-Bush, 1979; Schuster et al., 1986), have been reported. These reductions in markers of 5-HT-containing nerve endings are taken to demonstrate the loss of serotonergic neurons, but do they?

Evidence of Serotonergic Neurotoxicity by Fenfluramine

Classical indices of serotonergic neurotoxicity are: long-term reductions in total brain serotonin (5-HT); vesicular 5-HT and 5-HT reuptake sites, which are believed to represent the loss of central 5-HT-containing cells; loss of neurotransmitter stores; and loss of serotonergic nerve terminals. All these long-term alterations are observed after the administration of known serotonergic neurotoxins, such as 5,7-DHT and PCA. Fenfluramine also produces these effects, suggesting that this compound acts as a serotonergic neurotoxin. However, not all indices of neurotoxicity are a part of fenfluramine's long-term effects. Behavioral changes that can be detected in rats given 5,7-DHT are not found in rats that received fenfluramine (Lorens et al., 1990). Baumgarten et al. (1992) list several other tests of neurotoxicity that fail to detect fenfluramine-induced neuropathology. These include astrogliosis, neuronal sprouting, and reduced mRNA for tryptophan hydroxylase. Sotelo and Zamora (1978) found no effect on the cell bodies in the B9 group of neurons (an area rich in serotonergic cell somas) in rats given fenfluramine. Other studies also show that serotonergic cell bodies and axons are spared after fenfluramine treatment (Appel et al., 1989; Molliver and Molliver, 1990; Kalia, 1991; Sotelo, 1991). This information indicates that fenfluramine has a confined effect on serotonergic neurons.

Some suggest the biochemical changes that indicate fenfluramine may be neurotoxic merely reflect the drug's pharmacology. Fenfluramine selectively stimulates serotonergic neurons to release 5-HT, consequently reducing vesicular 5-HT, which affects measures of total brain serotonin and 5-HT-like immunoreactivity. Also, the administration of 5-HT reuptake blockers has been shown to reduce 5-HT transporter protein expression (Kovachich et al., 1992; Lesch et al., 1993). It is difficult to attribute the depletion of these markers of serotonergic nerve terminals to any pharmacological action when significant reductions are found up to 8 mo after fenfluramine administration (Zaczek et al., 1990), well after the drug is cleared from the body. However, apart from the loss of serotonergic nerve terminals, down-regulated transporter protein expression could explain a reduced transporter density in the absence of pharmacological activity.

Decreased Reuptake Transporter Density

It is well established that the administration of fenfluramine to laboratory animals results in a dose-dependent reduction of 5-HT reuptake transporter density. However, the nature of this effect remains a contentious issue. Decreased reuptake transporter density has been used to quantify neuronal loss for many years. Neurotransmitter specificity in the assessment of neuropathology is easily achieved with the use of radioligand binding to reuptake sites (B_{max}) or synaptosomal uptake rate of radiola-

beled neurotransmitter (V_{max}). These assays, however, are inappropriate to determine whether a reduction in transporter density represents a loss of transporters *per se* or the loss of the nerve terminal on which the transporters are located. Indeed, the reduction of transporters on otherwise intact terminals is usual. It has been shown that the administration of reuptake blockers suppresses the expression of the transporter protein, resulting in decreased transporter densities (Kovachich et al., 1992; Lesch et al., 1993). Fenfluramine is a 5-HT reuptake blocker and may downregulate 5-HT transporter expression, effectively creating a state of reduced transporter numbers on otherwise intact terminals, in the absence of pharmacological activity. However, apart from the loss of serotonergic nerve terminals, downregulated transporter protein expression could explain a reduced transporter density in the absence of pharmacological activity.

The present study was undertaken to develop a method of quantifying neurotransmitter-specific nerve terminals. It was envisaged that this method would generate a tool that could distinguish between a loss of transporters *per se* and a loss of nerve terminals.

Maximal Synaptosomal Loading of Neurotransmitter (α)

The design of such a tool centered on the phenomenon of forming synaptosomes from nerve endings or synaptic boutons. Each synaptosome contains transporters for specific neurotransmitters, thereby identifying the neuron of origin. By recognizing the synaptosome as a finite reservoir of neurotransmitter, a measure of maximal loading of synaptosomes with neurotransmitter would be directly related to, or linearly associated with, the number of synaptosomes in the preparation that employ that particular neurotransmitter. Since the amount of nerve endings available determines the number of synaptosomes that can be formed, maximal synaptosomal loading of neurotransmitter may provide a reuptake-transporter-density-independent measure of nerve ending content.

Na^+-dependent uptake of radiolabeled neurotransmitter into synaptosomes follows an exponential time-course (Fig. 1) (which can be fitted to the equation $y = \delta - \alpha\beta^x$), where the maximum amount of neurotransmitter that can be taken up by synaptosomes is denoted by α (*see* Methods).

This assay was then applied to preparations of brain tissue from three experimental groups of rats. One group received the serotonergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT). This generated brain tissue exhibiting reduced 5-HT reuptake transporter densities as a consequence of the abolition of 5-HT-containing nerve terminals (Model 1). Another group was treated with the irreversibly binding agent N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). Although not well established (*see* Discussion), EEDQ administration effectively ablates 5-HT transporters, whereas the serotonergic nerve terminals remain otherwise intact (Model 2). The manner in which α varied as the density of the 5-HT transporter was decreased by the two different procedures (Models 1 and 2) was compared. Determining which model best represented the α/transporter density association found in fenfluramine-treated rats may illustrate the nature of fenfluramine-induced 5-HT transporter loss.

Methods

Drugs and Treatment

Ethical clearance was obtained through the University of Queensland Animal Experimentation Ethics Committee (approval nos. clin/res/11/91/sand and bioc/225/93/phd). All rats used in this study were males of weight range 250–350 g; 2.5–60 mg/kg of *d,l*-fenfluramine were delivered as single ip injections or 20 mg/kg ip daily for 4 d to male Wistar rats. In addition, fenfluramine was administered in 4 × 0.5–10 mg/kg doses at 12-h intervals to Sprague-Dawley rats.

A second group of Wistar rats received ip injections containing 2–4 mg/kg of EEDQ.

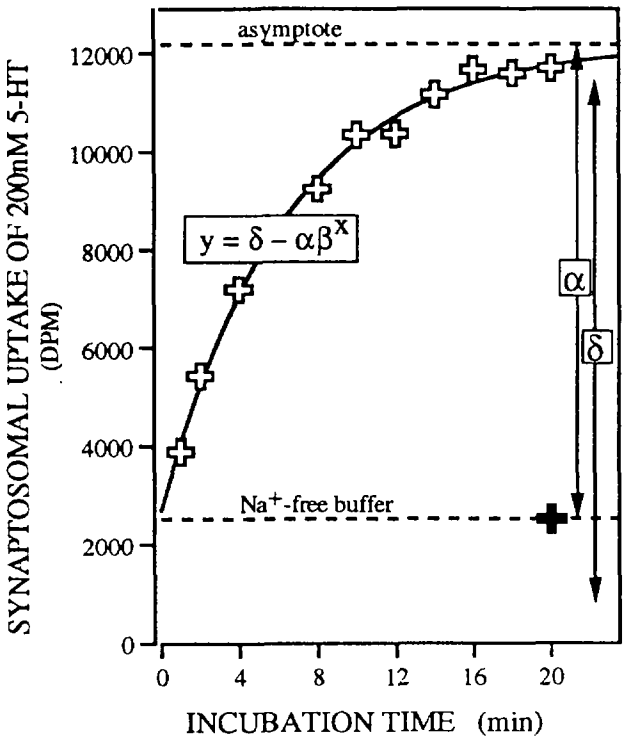

Fig. 1. Na^+-dependent synaptosomal 5-HT uptake time-course data (✙) fitted to an exponential equation. δ represents total synaptosomal loading (asymptote), α represents specific maximal synaptosomal loading, and β denotes the declining ratio of the increase in synaptosomal content of radiolabeled neurotransmitter (curve factor). The *y*-intercept represents the level of nonspecific synaptosomal uptake, the level of which is equated by an incubation in Na^+-free buffer for 20 min (✚).

Higher doses of EEDQ proved lethal to Wistar rats. To gain a complete range of 5-HT transporter densities, experimental Model 2 was expanded with an in vitro procedure. Synaptosomal preparations from control animals were incubated in the presence of 0.1–200 μ*M* of the alkylating agent phenoxybenzamine (PBA) (Frenken and Kaumann, 1987) for 30 min at 4°C, and then processed as below.

A third group of Sprague-Dawley rats were xylazine/ketamine-anesthetized before receiving an intracisternal injection of 8–100 μg 5,7-dihydroxytryptamine in 20 μL of 0.5% ascorbic acid. Some animals were pretreated with 20 mg/kg of desipramine (delivered ip, 45 min prior) to protect noradrenergic neurons from 5,7-DHT neurotoxicity (Bjorklund et al., 1975).

Fenfluramine-, EEDQ-, and 5,7-dihydroxytryptamine-treated animals were sacrificed by cervical dislocation 10 d to 7 wk, 48 h, and 8 d, respectively, after receiving their injection(s). Whole brains, including cerebellum, were rapidly removed, divided along the midline, and slowly frozen in 0.32*M* sucrose to –20°C, and then stored at –70°C until use. Controls were generated for all experimental groups by injecting rats with the vehicle used to deliver each drug.

Synaptosome Preparation

One half-brain from each group was rapidly thawed in 0.32*M* sucrose at 37°C. Immediately on thawing, the tissue was placed in ice-cold 0.32*M* sucrose (10% w/v) and homogenized with a motor-driven (500 rpm) Teflon™-glass homogenizer for synaptosome preparation by the method of Dodd et al. (1981). The final pellet was resuspended in ice-cold 0.32*M* glucose and stored on ice until use (within 2 h).

Synaptosomal Uptake Assay

Na^+-dependent 5-[^{14}C]HT or 5-[^{3}H]HT (NEN-Du Pont [Sydney, Australia]; SA 54.5 mCi/mmol and 25.7 Ci/mmol) uptake assays were performed in a total volume of 0.5 mL containing 25 m*M* HEPES, 4.75 m*M* KCl, 1.2 m*M* $MgSO_4$, 1.2 m*M* K_2HPO_4, 1.25 m*M* $CaCl_2$, 10 μ*M* pargyline, and either 120 m*M* NaCl (total uptake) or 120 m*M* Tris base (background), both adjusted to pH 7.4. Incubations were performed at 37°C, initiated by the addition of 100 μL of synaptosome suspension (150–550 μg protein/tube). The assay was terminated 3 min later by filtration using a cell harvester (Brandel, Gaithersburg, MD) followed immediately by 2 × 1 mL washes with ice-cold 0.9% NaCl onto prewetted Whatman GF/B filters. Filters were equilibrated with 4.5 mL of scintillation fluid (Emulsifier-Safe,

Packard, Canberra, Australia) and counted at approx 92 and 48% efficiency for 5-[^{14}C]HT and 5-[^{3}H]HT, respectively, on a Beckman LS–3801 scintillation counter with on board quench correction.

Uptake time-course assays (to determine α) were performed by incubating synaptosomes with 200 n*M* 5-[^{14}C]HT or 5-[^{3}H]HT in a total volume of 0.5 mL of HEPES buffer (as above), for varying times (8–12 time-points; 0–20 min). Data gained from these assays were fitted to the modified exponential equation ($y = \delta - \alpha\beta^x$), where α represents the difference between the *y*-intercept (equivalent to nonspecific uptake) and the asymptote (δ: total synaptosomal loading), and β denotes the exponential curve factor as previously described (Westphalen and Dodd, 1993a). Protein concentration was determined by the method of Markwell et al. (1978) against bovine serum albumin standards.

Statistical Analysis

Concentration dependence data from uptake rate assays were Eadie-Hofstee transformed and fitted by linear regression analysis. The *y*-intercept corresponds to maximal uptake rate (V_{max}) and the slope signifies the affinity (K_m).

Parameter estimates and product-moment correlations were obtained from fitting data to equations using the NONLIN program (SYSTAT Inc., Evanston, IL). Statistical comparisons between mean K_m values was performed using paired *t*-tests. To determine a statistical deviation of a straight line from a slope of 1, a *t* value was obtained using the equation:

$$t = \{(\text{slope} - 1)/[(\text{Sy}\cdot x)^2(1/n + x^2/\text{E}x^2)]^{1/2}\}$$
$$\text{df} = n - 2 \qquad (1)$$

A *t* value to test the significant departure of a straight line intercepting the *y* axis at the point $y = 0$ was obtained using the formula:

$$t = \{(y\text{-intercept} - 0)/[(\text{Sy}\cdot x)^2/\text{E}x^2)]^{1/2}\}$$
$$\text{df} = n - 2 \qquad (2)$$

This denotes the residual mean squares derived from the deviation from regression analysis of data fitted to a straight line.

Statistical comparison of differing equations fitted to the same data was performed using an *F* value calculated from the sums of squares determined by NONLIN, according to the equation (Munson and Rodbard, 1980):

$$F = [(\text{SS}_{\text{fit \#1}} - \text{SS}_{\text{fit \#2}})/(\text{df}_{\text{fit \#1}} - \text{df}_{\text{fit \#2}})/\text{SS}_{\text{fit \#2}}/\text{df}_{\text{fit \#2}}]$$
$$\text{df} = \text{df}_{\text{fit \#1}} - \text{df}_{\text{fit \#2}}, \text{df}_{\text{fit \#2}} \qquad (3)$$

Significance levels were assessed according to a two-tailed test design. All variances of means are reported as the standard error of the mean (SEM).

Results

Synaptosomes prepared from 5,7-DHT-, EEDQ-, and *d,l*-Fenfluramine-treated rats all exhibited various levels of 5-HT reuptake transporter density, as measured by reduced maximal radiolabeled 5-HT uptake rates (V_{max}) compared with control. Although V_{max} decreased, the (K_m) remained unchanged in each of the experimental groups compared with control (5,7-DHT: K_m = 104 ± 4.9% of control; EEDQ: K_m = 95 ± 9.4%; PBA: K_m = 107 ± 24%; Fenfluramine: K_m = 102 ± 8.4%; Control: K_m = 90.4 ± 7.9 n*M*). This demonstrates that 5-HT reuptake transporters were abolished in each experimental group without altering the affinity of 5-HT for the reuptake transporter.

Maximal 5-[^{14}C]HT or 5-[^{3}H]HT loading of synaptosomes (α) prepared from 5,7-DHT-treated animals showed significant decreases compared with control that paralleled the degree of transporter loss as measured by V_{max} (Fig. 2A). Preventing the neurotoxic effects of 5,7-DHT on noradrenergic neurons without affecting its serotonergic neurotoxicity was achieved by pretreating animals with 20 mg/kg desipramine 45 min before 5,7-DHT administration (Bjorklund et al., 1975). This noradrenergic protection did not significantly alter the relationship between α and V_{max} gained from 5-HT uptake assays. These variables were

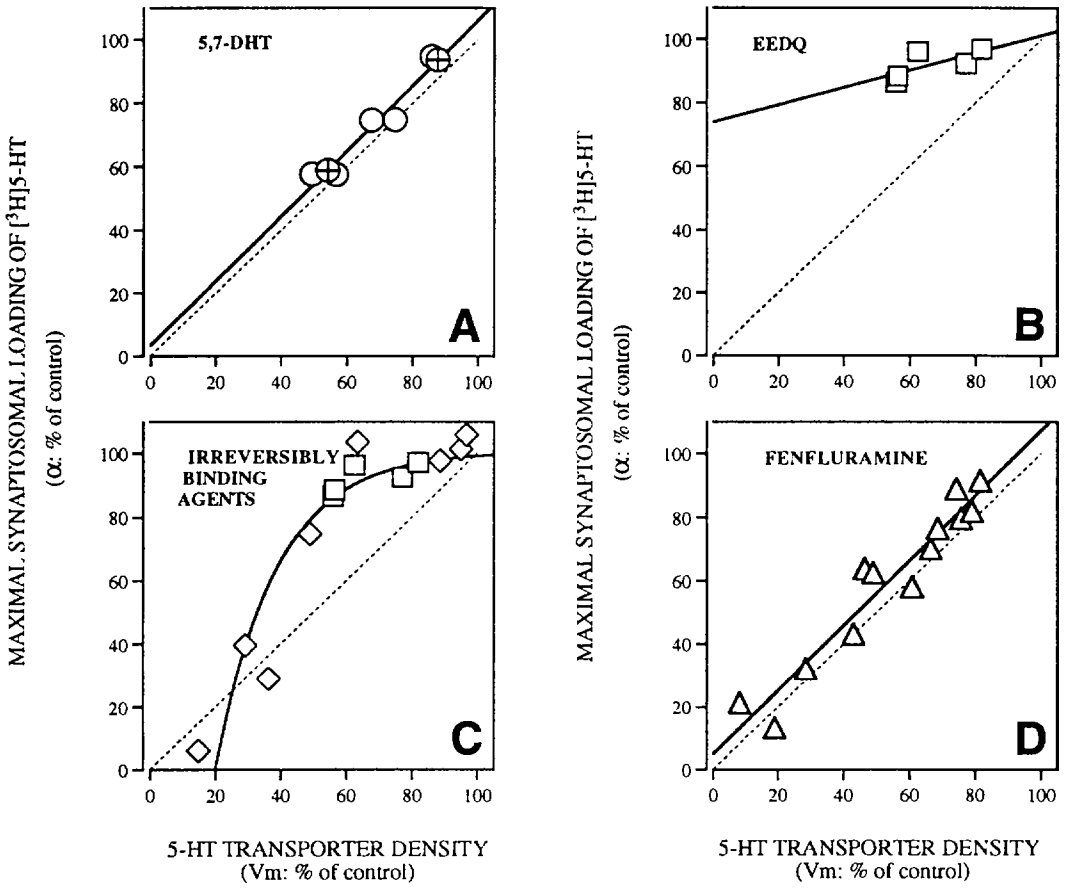

Fig. 2. Three techniques in generating various levels of 5-HT reuptake transporter loss (expressed as V_{max}) plotted against maximal radiolabeled 5-HT synaptosomal loading (expressed as α). **(A)** 5-HT transporter loss as a consequence of 5-HT nerve terminal loss, produced by intracisternal injections of 8–100 μg 5,7-dihydroxytryptamine (5,7-DHT) in the absence (○) or presence (⊕) of 20 mg/kg desipramine. 5,7-DHT decreases serotonergic and noradrenergic nerve terminal content by destroying their respective neurons. In this group, the relationship between V_{max} and α was found to be linear (r^2 = 0.95) with no significant difference from a slope of 1 (slope = 1.03; p = 0.20). This straight line intercepts the y-intercept at a point not significantly removed from y = 0 (y-intercept = 3.4%; p = 0.15). Protecting noradrenergic neuron destruction with desipramine does not alter this relationship. **(B)** 5-HT transporter loss as a consequence of their removal by ip injections of 2–4 mg/kg EEDQ (□). The relationship between V_{max} and α was different from that produced by 5,7-DHT. This association was not determined as linear (r^2 = 0.51). **(C)** When combined with the data gained from the in vitro application of 0.1–200 μ*M* phenoxybenzamine to preparations of control synaptosomes (◇), a nonlinear correlation was demonstrated. A significant improvement of fit was gained when modeling the data to an exponential equation (p = 0.004) rather than a straight line. **(D)** *d,l*-Fenfluramine (△) administration induced a decrease in 5-HT transporters that was associated with α in a linear manner (r^2 = 0.92). This linear regression was not significantly unlike a straight line of a slope 1 (slope = 1.028; p = 0.44) that passes through the origin (y-intercept = 4.9%; p = 0.10).

found to be linearly correlated (r^2 = 0.95, $p < 10^{-3}$). The slope of this straight line did not differ significantly from 1 (t = 1.46, df = 5, p = 0.20). In addition, the y-intercept was not significantly removed from zero (t = 1.70, df = 5, p = 0.15). Combined, the statistics suggest that the α/V_{max} correlation in Model 1 did not differ from an association of $\alpha = V_{max}$.

Plotting maximal 5-HT loading of synaptosomes prepared from EEDQ-treated animals against α gained from the same preparation (Fig. 2B) produced a differing profile to that exhibited by the serotonergic neurotoxin. When combined with the data gained from the in vitro administration of PBA, the alkylated group showed an exponential trend in the association of α to V_{max} (Fig. 2C). Although a linear relationship was found ($r^2 = 0.78, p < 10^{-4}$), a significant improvement in fit was obtained when the data were modeled to the exponential equation as compared with the equation of a straight line ($F = 14.2$, df = 1,10, $p = 0.004$).

The association between 5-HT transporter density and α in rats treated with fenfluramine (Fig. 2D) was linear ($r^2 = 0.92$, $p < 10^{-6}$). The slope and *y*-intercept of this straight line did not differ significantly from 1 ($t = 0.80$, df = 11, $p = 0.44$) and zero ($t = 1.78$, df = 11, $p = 0.10$), respectively. These statistical tests suggest that the fenfluramine data are not significantly removed from a relationship of $\alpha = V_{max}$, analogous to that observed with the serotonergic neurotoxin 5,7-DHT.

Discussion

The quantitation of neurotransmitter-specific nerve terminal loss is well achieved by measuring radioligand binding to presynaptically located neurotransmitter reuptake sites. However, when the neuropathological process is thought to affect this transporter-protein marker of nerve terminals *per se*, the assessment becomes questionable. Such an example is the controversial interpretation of the well-established finding of long-term decreases in 5-HT reuptake transporter densities induced by the administration of fenfluramine. The analysis presented in this article suggests that fenfluramine-induced reduction in the number of 5-HT reuptake transporters is a consequence of the loss of serotonergic nerve terminals. Two animal models of potential scenarios of fenfluramine-induced 5-HT transporter loss were established. Model 1 displayed decreased 5-HT transporter protein densities owing to the abolition of the terminals on which the they are located. This was accomplished by destroying the entire serotonergic neuron using the selective neurotoxin 5,7-DHT. Model 2 displayed decreased 5-HT transporter densities on otherwise intact serotonergic nerve terminals. In this case, EEDQ was used to bind to the transporter protein irreversibly.

The binding of EEDQ to centrally located 5-HT transporter protein is not well established. EEDQ is known to bind to 5-HT transporters located on platelets (Biessen et al., 1988), which are reported to be identical to brain 5-HT transporters (Langer et al., 1981). In addition, Wistar rats that received 4 mg/kg EEDQ exhibited reduced V_{max} for 5-HT uptake and decreased [^{3}H]paroxetine binding in brain preparations (Westphalen and Dodd, 1993a). However, Pinto et al. (1993) found no change in the density of [^{3}H]paroxetine-labeled 5-HT uptake sites in Sprague-Dawley rats that received 12 mg/kg of EEDQ. This discrepancy may be the result of the different strain of rats used. We have found that EEDQ administered to Sprague-Dawley rats had a different effect on the kinetics of synaptosomal uptake of 5-HT, as compared with its effect in Wistar rats (Westphalen et al., 1994). Although it remains possible that EEDQ may be acting indirectly to reduce 5-HT transporter density, EEDQ administration to Wistar rats served the purpose of generating Model 2.

Each model presented varying degrees of transporter loss (as portrayed by decreased V_{max} values without change in K_m) generated by the administration of a range of doses of their particular transporter-eliminating agent. This parameter was then compared with maximal synaptosomal loading (α) of radiolabeled 5-HT, an assay argued to measure the number of neurotransmitter-specific synaptosomes, thereby quantifying the number of neurotransmitter-specific nerve endings available to form synaptosomes (Westphalen and Dodd, 1993a).

α was obtained by fitting an exponential equation (Eq. [1]) to data gained from uptake time-course assays performed on synapto-

somes prepared from the brains of rats belonging to Models 1 and 2. In Model 1, it was found that the change in a paralleled the decrease in V_{max} (Fig. 2A), illustrated by the data not significantly deviating from a linear regression of slope 1 that passed through the origin. That is, as increasing doses of toxin were applied, more serotonergic neurons were being destroyed, eliminating both the transporters and the terminals on which they are located. However, in Model 2, although α did correlate with V_{max}, this was not in a fashion that was linearly related to the transporter density. This was demonstrated by a significant deviation of the data from an equation of a straight line with a slope of 1. The dissimilarity between the experimental models was made more distinct when a significant improvement of fit was gained after fitting the Model 2 data to an exponential rather than straight line equation. Whatever the mechanism by which EEDQ and phenoxybenzamine decreased 5-HT transporter number, the profile of V_{max} to α was found to be different from that where serotonergic neurons are destroyed by 5,7-DHT.

Having established two distinct outcomes of α/V_{max} correlations, depending on whether reuptake transporter numbers are reduced or whether the terminals on which they are located are lost, α and V_{max} data gained from fenfluramine-treated rats were analyzed. When a graph of α and V_{max} was plotted (Fig. 2D), the resulting association was not significantly different from a straight line of slope 1 that passed through the origin. This suggests that fenfluramine-induced 5-HT transporter loss was the result of the loss of serotonergic nerve endings.

A suggested pathology of 5-HT-containing terminals that spares the cell soma and axons may explain why the long-term reduction in 5-HT transporter numbers return toward normal levels (Zaczek et al., 1990). Having the cell body and axonal processes still intact leads to the potential for the regeneration of nerve endings. Although this concept is not well established, we have reported that 5-HT transporter densities, initially reduced to 20% of control by high-dose fenfluramine treatment, returned to 72% of control 25 wk later. This recovery was paralleled by the measure α (to 79% of control), reflecting an increased number of serotonergic nerve endings (Westphalen and Dodd, 1993b). The rate at which this reversibility occurs is markedly different from the time taken for uptake binding agent-altered reuptake transporter densities to recover (Stadlin et al., 1993). In addition, transporter density reduction caused by reuptake blocker-induced down-regulation never produces levels of approx 20% of control (Kovachich et al., 1992), as does fenfluramine. Also, chronic treatment of reuptake blockers is necessary to produce down-regulation of reuptake transporters (Kovachich et al., 1992; Lesch et al., 1993), whereas significant transporter reductions occur after a single administration of fenfluramine (Steranka and Sanders-Bush, 1979; Wagner and Peroutka, 1990).

A conclusion of fenfluramine-induce serotonergic nerve terminal loss is in conflict with a recent study that shows no change in 15 m*M* K^+-induced 5-HT released from serotonergic synaptosomes prepared from fenfluramine-treated rats when compared with control (Gobbi et al., 1993). This result demonstrates an unaltered content of 5-HT-containing vesicles and structures that initiate the release of their contents, items that are located in the serotonergic terminal. However, when 65 m*M* K^+ was used, control animals exhibited 5-HT release, but fenfluramine-treated animals did not (Puig de Parada et al., 1993). This study also showed no change in the basal release of 5-HT after 5,7-DHT treatment as compared with vehicle administration. Kalia and O'Malley (1993) found that animals exhibiting long-term *d,l*-fenfluramine-induced reductions in 5-HT did not differ from control in their ability to take up retrograde label. However, caution must be taken when interpreting axonal transport data, because only completely destroyed neurons fail to transport compounds from terminals to cell bodies. Receptor-mediated retrograde axonal transport is known to occur in damaged neurons (Curtis et al., 1993). In fact, damaged neurons have been shown to display increased

axonal retrograde transport (Curtis et al., 1993). If fenfluramine was to produce cell soma and axon-sparing 5-HT nerve terminal pathology, a notion not without evidence, it is plausible to find increases in retrograde transport in fenfluramine-treated animals.

Summary

This study focused on the finding of long-term 5-HT transporter loss after high-dose fenfluramine administration to laboratory animals. This effect is commonly used to determine the loss of serotonergic nerve terminals, an accurate assessment of the degree of serotonergic neuron destruction by neurotoxins. However, when the toxicity of the transporter-ablating agent used is unknown, it must be remembered that only the transporter is being measured, not the neuron as a whole. Fenfluramine-induced 5-HT transporter loss is such a case. This study presents data gained from an assay that is suggested to measure synaptosome number, reflecting the quantity of nerve endings available to form synaptosomes. This assay was able to distinguish between two models of 5-HT transporter loss: (1) a loss of transporters *per se* and (2) the loss of the nerve terminals on which the transporters are located. It was found that rats possessing reduced 5-HT transporter densities by way of fenfluramine administration exhibited assay data that were not significantly different from Model 2.

Acknowledgments

This work was supported by the Sandoz Fund for Gerontological Research and the National Health and Medical Research Council of Australia.

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