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The substituted amphetamines 3,4-methylenedioxymethamphetamine, methamphetamine, p-chloroamphetamine and fenfluramine induce 5-hydroxytryptamine release via a common mechanism blocked by fluoxetine and cocaine

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The abilities of the substituted amphetamines 3,4-methylenedioxymethamphetamine (MDMA), methamphetamine, p-chloroamphetamine (PCA) and fenfluramine to induce synaptosomal [^{3}H]serotonin (5-HT) release were compared using a novel microassay system. The rank order of release potencies was found to be (±)PCA ≈ (+)-fenfluramine > (+)-MDMA ≫ (+)-methamphetamine. Combination of two drugs at their EC_{50} did not cause more release than either drug alone at an equivalent concentration. In addition, the 5-HT uptake blockers fluoxetine and cocaine inhibited the release induced by MDMA, methamphetamine, PCA and fenfluramine to the same percentage. However, threshold concentrations of the substituted amphetamines known to inhibit uptake did not attenuate the release caused by higher concentrations of these compounds. These results suggests that MDMA, methamphetamine, PCA and fenfluramine cause 5-HT release via a common mechanism. Furthermore, these results indicate that the 5-HT uptake blockade induced by these substituted amphetamines in vitro is different from that induced by either fluoxetine or cocaine.

Amphetamine analogues; Monoamine release; Synaptosomes; 5-HT (5-hydroxytryptamine, serotonin)

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA), a substituted amphetamine derivative, is a drug of abuse that has been subject of recent investigation because of its neurotoxic effects in experimental animals (Stone et al., 1986; Schmidt, 1987; O'Hearn et al., 1988) and possibly in humans (Price et al., 1989). The neurotoxicity of MDMA consists of a long-lasting degeneration of serotonin (5-hydroxytryptamine, 5-HT) axon terminals in forebrain regions, as evidenced by decreases in levels of 5-HT and its principal metabolite 5-hydroxyindoleacetic acid (Stone et al., 1986; Schmidt et al., 1987), decreases in 5-HT synthesis (Stone et al., 1986) and 5-HT uptake activity (Battaglia et al., 1987), and decreases in immunocytochemical staining of 5-HT axons (O'Hearn et al., 1988; Scallet et al., 1988). The neurotoxic effects of MDMA in animals are similar to those of a number of closely related substituted amphetamines, such as methamphetamine (Ricaurte et al., 1980; Axt et al., 1990), another drug of abuse; p-chloroamphetamine (PCA), a very potent serotonergic neurotoxin (Fuller, 1978; Sanders-Bush and Steranka, 1978), and the anorectic compound fenfluramine (Molliver and Molliver, 1988; Appel et al., 1989). The chemical structures of MDMA, PCA, fenfluramine and methamphetamine are shown in fig. 1. Because of their similar long-term effects on 5-HT axons, these four substituted amphetamines may produce neurotoxicity via the same mechanism.

A common feature of MDMA, methamphetamine, PCA and fenfluramine is that they cause the release of 5-HT from presynaptic terminals (Wong et al., 1973; Schmidt and Gibb, 1985; Nichols et al., 1982; Buczko et al., 1975). This release is blocked by 5-HT uptake inhibitors (Schmidt and Gibb, 1985; Ross, 1976; Hekmatpanah and Peroutka, 1990; Maura et al., 1982; Schmidt et al., 1987), suggesting that it is mediated by the 5-HT uptake carrier. Since the long-term neurotoxic effects of the substituted amphetamines are also blocked by 5-HT uptake inhibitors (Azmitia et al., 1990; Fuller et al., 1975; Schmidt, 1987; Hotchkiss and Gibb, 1980), or by other treatments that prevent 5-HT

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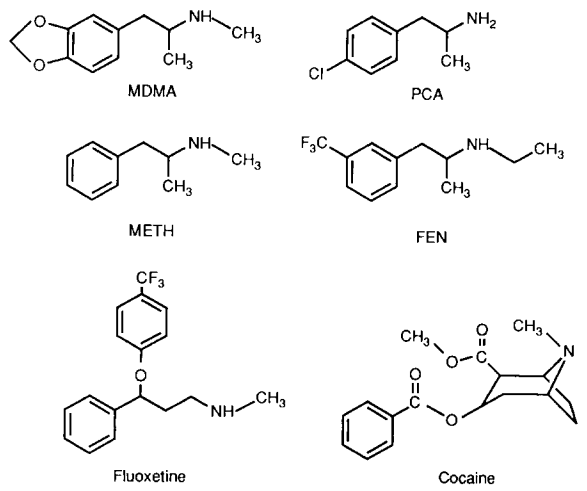

Fig. 1. Chemical structures of MDMA, methamphetamine (METH), PCA, fenfluramine (FEN), fluoxetine and cocaine.

release (Berger et al., 1989), it appears that 5-HT release plays an important role in the sequence of events that lead to neurotoxicity.

Apart from the involvement of the uptake carrier, the mechanism by which the substituted amphetamines cause 5-HT release is not entirely clear. These drugs may enter the serotonergic terminal and displace 5-HT from its intraneuronal binding sites which may be followed by transport of the displaced 5-HT out of the terminal by the uptake carrier acting in reverse (Sanders-Bush and Martin, 1982). However, it is not known how, or in fact whether, the substituted amphetamines enter the terminal. They may be taken up by the uptake carrier and/or they may passively diffuse through the membrane due to their high lipophilicity. The release-inhibiting effect of uptake blockers could thus be due to either preventing the entry of the releasing drug into the terminal or blocking the transport of 5-HT out of the terminal (Fuller, 1980). The substituted amphetamines may have some interaction with the uptake carrier because (1) PCA, MDMA and fenfluramine also inhibit the accumulation of 5-HT into synaptosomes or brain slices (Wong et al., 1973; Steele et al., 1987; Belin et al., 1976; Kannengiesser et al., 1976; Knapp and Mandell, 1976) and (2) this uptake inhibiting effect, in particular that of fenfluramine, is observed at lower concentrations than the releasing effect (Carruba et al., 1977; Borroni et al., 1983; Ross et al., 1977; Kannengiesser et al., 1976; Knapp and Mandell, 1976). Several studies have however been unable to demonstrate directly that PCA, fenfluramine or MDMA are substrates for uptake (Ross and Ask, 1980; Ross, 1976; Belin et al., 1976; Sanders-Bush and Steranka, 1978; Azmitia, unpublished observations).

MDMA, methamphetamine, PCA and fenfluramine all appear to cause carrier-mediated 5-HT release. However, it is unclear whether the mechanism for this release is the same for each compound. There are several reports describing differences among the actions of these substituted amphetamines on serotonin neurons. For example, fenfluramine, but not PCA, is able to inhibit the 5-HT depletion induced by H75/12, an agent whose 5-HT-depleting action requires uptake into 5-HT terminals (Fattacini et al., 1991). This finding suggests that fenfluramine, but not PCA, blocks the neuronal uptake of H75/12 into serotonin neurons in vivo. Further differences exist between PCA and fenfluramine in two pharmacological effects that are regarded to result from serotonergic stimulation, possibly through 5-HT release (Pawlowski et al., 1980). In the flexor reflex test in the spinal cord-transsected rat, the 5-HT uptake blocker zimelidine prevents potentiation by PCA but not that induced by fenfluramine. Similarly, zimelidine inhibits the hyperthermia in rats induced by PCA but not that induced by fenfluramine. Differences between PCA- and fenfluramine-induced hyperthermia in rats have also been described by Frey (1975).

In the present study, we used a novel microassay system to investigate whether MDMA, methamphetamine, PCA and fenfluramine cause 5-HT release via the same mechanism. We first characterized this system, then we compared the 5-HT-releasing action of these drugs using three approaches. In the first series of experiments, we determined whether a combination of two drugs releases more 5-HT than does either drug alone at an equivalent concentration. We hypothesized that if these drugs caused release via different mechanisms, then a combination of two of them may cause a significant higher percentage of release than each one alone at an equivalent concentration. In a second set of experiments, we determined whether 5-HT uptake blockade inhibits the 5-HT-releasing action of the four substituted amphetamines to the same extent. 5-HT uptake was inhibited using fluoxetine, a specific 5-HT uptake blocker (Wong et al., 1983), and cocaine, a more general monoamine uptake blocker (Richelson and Pfenning, 1984). Both fluoxetine and cocaine do not contain the amphetamine structure and are not considered substituted amphetamines (fig. 1). Finally, in a third series of experiments, we tested whether fenfluramine or one of the other substituted amphetamines could act as an uptake blocker and attenuate the releasing effect of another substituted amphetamine.

2. Materials and methods

2.1. Synaptosomal [^{3}H]5-HT release

Male Sprague–Dawley rats (200–300 g, Taconic Farms, NY), maintained on a 12-h light/dark cycle

with free access to food and water, were used for the studies. Crude whole brain (excluding cerebellum) synaptosomes were prepared by homogenization in 10 volumes per brain weight of 0.32 M ice-cold sucrose, followed by a first sedimentation at $350 \times g$, and a second sedimentation of the S1 supernatant at 12500 $\times g$. The P2 pellet was resuspended in 10 volumes/g brain weight of Krebs–Ringer buffer containing (mM): NaCl 120, KCl 1.86, $CaCl_2$ 1.2, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11.1 and pargyline 0.1. The synaptosomes were then loaded by incubation for 25 min at 37°C with [^{3}H]5-HT (final concentration 100 nM). Non-specific uptake, which accounted for 10% of total uptake, was determined in presence of 10^{-4} M fluoxetine. To terminate the uptake, synaptosomes were spun down for 5 min at $2400 \times g$, resuspended in buffer and spun again for 5 min at $2400 \times g$. The final pellet was resuspended in 12.5 volumes/g brain weight of Krebs buffer. In initial experiments to characterize our release system, we determined the Ca^{2+} dependency of the release induced by 15 mM KCl. In these experiments, the final synaptosomal pellet was resuspended in a modified Krebs–Buffer in which $CaCl_2$ was either replaced with the same amount of $MgSO_4$, or in which calcium was chelated by the addition of 1.5 mM ethyleneglycoltetraacetate (EGTA). The release induced by KCl was determined as described for the first set of experiments (see below).

The release assay was performed in a final volume of 200 μl in 96-well cell culture plates (Nunc, Denmark) that had been coated with poly-D-lysine. Typically, drug effects were studied in quadruplicates and two plates were processed simultaneously.

In the first set of experiments, plates were filled with 150 μl per well of Krebs buffer, with or without the releasing drugs, and were equilibrated at 37°C for 20 min in a Titertek microplate incubator. The release was started by adding 50 μl of the loaded synaptosomes (approximately 80 μg of protein). In the second and third set of experiments, plates were filled with 130 μl of Krebs buffer, with or without the 5-HT uptake inhibitors, and again equilibrated at 37°C. Then 50 μl of synaptosomes were added and the trays were incubated further in order to allow association of the uptake blockers to the transporter protein. After 10 min, the release was started by adding 20 μl of the releasing drugs.

After 20 min at 37°C, release was terminated by spinning the plates at 6°C for 10 min at $2500 \times g$. Supernatants were carefully aspirated and the synaptosomal pellet blow-dried. Twohundred microliters of 100% ethanol were added to each well to extract the radioactivity retained in synaptosomes. After 1 h, 150 μl of ethanol extract were added to 3 ml scintillation fluid and radioactivity was counted for 1 min (Beckman liquid scintillation counter).

2.2. Statistical analysis

Results are expressed as percent of control of the radioactivity remaining in the synaptosomes. Each experiment was repeated at least twice. The Sigmaplot 4.0 curve-fitting program and the logistic function of De Lean et al. (1978) were used to fit concentration-response curves and calculate EC_{50} values. Statistical significance of the release-inhibiting effects of fluoxetine and cocaine was determined on the raw data using the two-tailed Student's t-test. Differences between the means for the percent inhibition caused by the uptake blockers were tested using one-way analysis of variance.

2.3. Drugs

Drugs were obtained from the following sources: (±)-PCA · HCl from Sigma Chemical Company (St. Louis, MO); (+)-fenfluramine · HCl from A.H. Robins Company (Richmond, VA); (+)-MDMA · HCl, (+)-methamphetamine · HCl and (−)-cocaine · HCl from the National Institute of Drug Abuse; fluoxetine · HCl from Eli Lilly Company (Indianapolis, IN), and [^{3}H]5-HT (specific activity 25.2 Ci/mmol) from New England Nuclear (Boston, MA).

3. Results

3.1. Calcium dependency of KCl-induced release

A 20-min incubation with 15 mM KCl caused 20–25% [^{3}H]5-HT release in our system. This potassium-stimulated release was Ca^{2+}-dependent. In Ca^{2+}-free buffer ($CaCl_2$ replaced by $MgSO_4$), 15 mM KCl caused a very small release (93.1 ± 2.4 versus 76.4 ± 0.8% in normal buffer, % of corresponding controls ± S.E.M.). Similarly, in presence of 1.5 mM EGTA, 15 mM KCl caused no release (97.6 ± 3.5 versus 80.6 ± 1.2% in normal buffer).

3.2. Release potencies and effects of drug combinations

To determine the potencies of MDMA, methamphetamine, PCA and fenfluramine to cause 5-HT release in our system, concentration–response curves were established that consisted of seven points in the range of 10^{-7}–10^{-3} M. Representative curves from one experiment are shown in fig. 2. Table 1 shows mean values, obtained from three to four different experiments, of the drug concentrations that caused 50% release (EC_{50}). (±)-PCA and (+)-fenfluramine were very similar in their potency to cause [^{3}H]5-HT release (EC_{50} around 3 μM), while (+)-MDMA was slightly less (8 μM), and (+)-methamphetamine con-

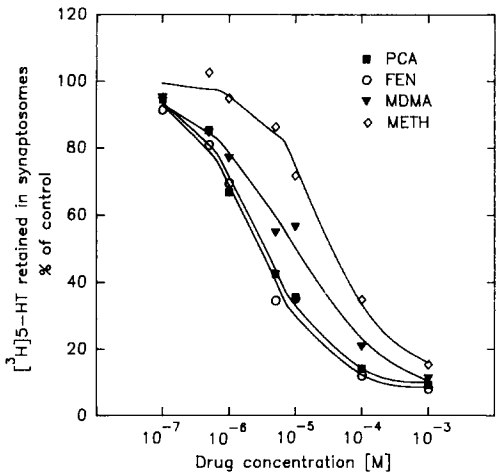

Fig. 2. Concentration–response curves for the synaptosomal [^{3}H]5-HT release induced by MDMA (filled triangles), methamphetamine (open diamonds), PCA (filled squares) and fenfluramine (open circles). The amount of [^{3}H]5-HT release (ordinate) is expressed in % of controls of the amount of radioactivity remaining in the synaptosomes after a 20-min incubation at 37°C with the releasing drug at the approximate concentration (abscissa). Control values were in the range of 20000–25000 counts per min/well.

siderably less (23 μM) potent. Each drug reduced the synaptosomal counts to approximately 10% of control within two orders of magnitude of its EC_{50}.

To test whether the [^{3}H]5-HT-releasing effects were additive, two drugs were combined at their approximate EC_{50} concentration (3 μM for PCA and fenfluramine, 10 μM for MDMA and methamphetamine) and compared to the effects of each of these drugs alone at EC_{50} and at $2 \times EC_{50}$. The results are shown in four separate graphs in fig. 3. Each graph shows the [^{3}H]5-HT-releasing effect of the tested substituted amphetamine either alone at its EC_{50} and at $2 \times EC_{50}$, or in combination with one of the other drugs. In no case did the combination of two drugs at the EC_{50} exceed the releasing effect of the single drugs at $2 \times EC_{50}$.

3.3. Effects of 5-HT uptake blockade using fluoxetine and cocaine

At high concentrations, both fluoxetine and cocaine cause a decrease in the [^{3}H]5-HT content of the synaptosomes in our system (data not shown). Thus, we first determined those uptake blocker concentrations which would attenuate the release induced by one of the substituted amphetamines (i.e. PCA), but which would not cause an extensive decrease in counts on their own. These concentrations were found to be around 80 nM for fluoxetine and 5 μM for cocaine, causing a small decrease in synaptosomal counts of 15–25% compared to control. Subsequently, we determined whether fluoxetine or cocaine at these concentrations would attenuate the [^{3}H]5-HT-releasing action of all four substituted amphetamines. We tested different concentrations of each substituted amphetamine that produced 5-HT release in the range of 30–60%. In fig. 4, results of typical experiments are shown for those concentrations which produced a comparable amount of release

TABLE 1

Mean EC_{50} values (±S.E.M.) for the synaptosomal [^{3}H]5-HT release induced by the four substituted amphetamines. The data from n = 3–4 experiments were combined and the EC_{50} values were calculated using Sigmaplot 4.0 and the logistic function of De Lean et al. (1978).

	Isomer	EC_{50} (μM)	n
Fenfluramine	+	2.92±0.35	3
PCA	±	3.43±0.45	4
MDMA	+	7.96±1.77	4
Methamphetamine	+	23.63±6.76	3

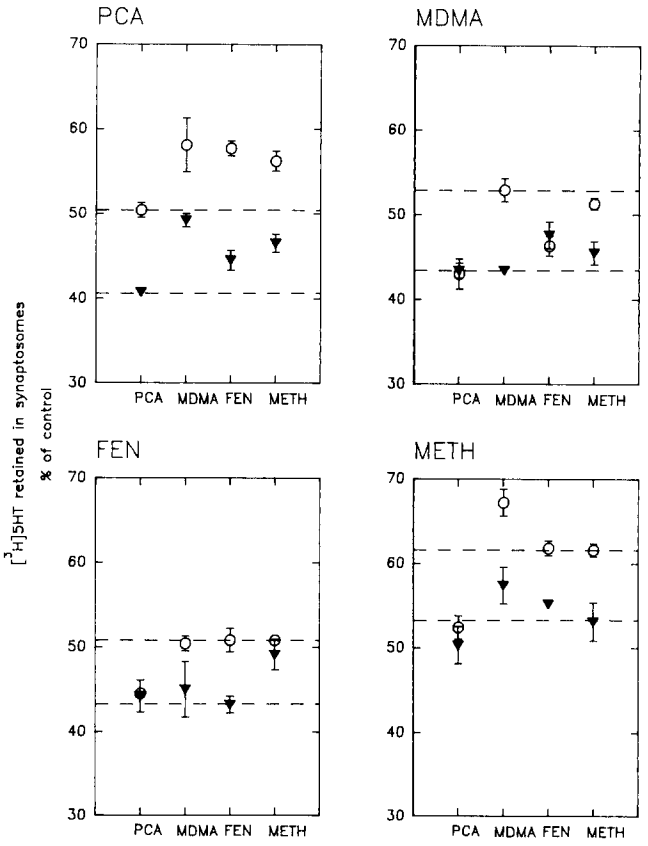

Fig. 3. Release of [^{3}H]5-HT from preloaded synaptosomes induced by the substituted amphetamines alone or in combinations of two; expressed in % of controls of the radioactivity remaining in synaptosomes (ordinate) after a 20-min incubation period. Each drug was used at the approximate EC_{50} concentration (3 μM for PCA and fenfluramine, 10 μM for MDMA and methamphetamine). Open circles show the effects of the drugs alone at their EC_{50}, while solid triangles show their effects in combination with the test drug that is indicated in the upper left corner. The two dashed lines indicate the values for the test drugs at EC_{50} and at $2 \times EC_{50}$. The values for all drug combinations fall either in between the two dashed lines, or are not significantly different from them, indicating that the combination of two drugs does not release more 5-HT than each of the drugs alone at an equivalent concentration.

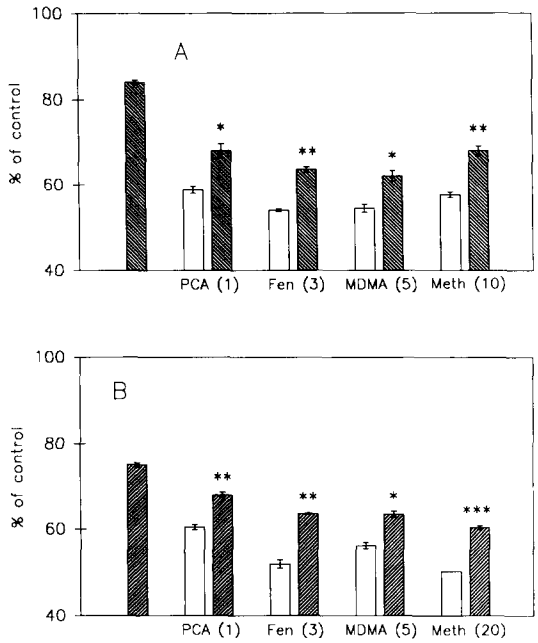

Fig. 4. Attenuation of substituted amphetamine-induced [^{3}H]5-HT release by 80 nM fluoxetine (A) or 5 μM cocaine (B). Open bars show the effects of the releasing drugs on control synaptosomes; hatched bars show their effects on synaptosomes that were preincubated for 10 min with the uptake blocker. The release is expressed in % of controls of the radioactivity remaining in the synaptosomes after drug treatment. The numbers in parentheses indicate drug concentrations in μM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Student's t-test, performed on the raw data.

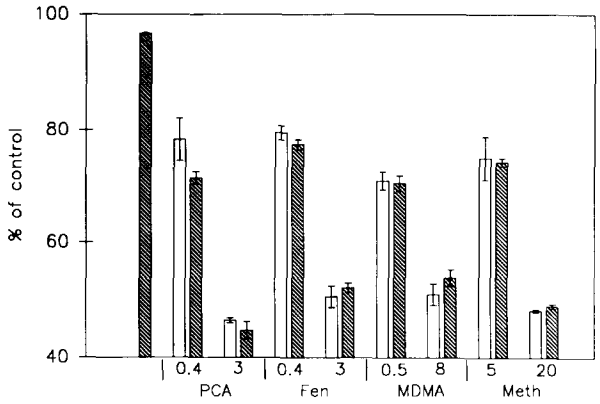

Fig. 5. Inability of prior exposure to a threshold concentration of fenfluramine (Fen, 0.1 μM) to block 5-HT release induced by PCA, MDMA or methamphetamine (Meth) at the indicated concentrations (μM). Ordinate: % of controls of radioactivity remaining in synaptosomes after drug treatment. Open bars show the effects of the releasing drugs on control synaptosomes; hatched bars show their effects on synaptosomes that were preincubated for 10 min with 0.1 μM fenfluramine.

(about 40–50%). Fluoxetine, 80 nM, and 5 μM cocaine each blocked significantly the releasing action of all four substituted amphetamines.

To determine whether the blocking effect of fluoxetine and cocaine is the same for each substituted amphetamine, we calculated the percentage of inhibition for each tested substituted amphetamine concentration and determined the means over all experiments (seven experiments for fluoxetine and three for cocaine, table 2). The blocking effects of both 5 μM cocaine and 80 nM fluoxetine were between 9–12%. One-way analysis of these values did not reveal any significant differences between the four substituted amphetamines.

TABLE 2

Mean values (±S.E.M.) for the percent inhibition of substituted amphetamine-induced release caused by fluoxetine and cocaine. One-way analysis of variance revealed no significant differences between these values.

	Fluoxetine [a]	n [b]	Cocaine [a]	n [b]
MDMA	10.05 ± 1.51	7	9.06 ± 1.13	8
Methamphetamine	9.93 ± 1.53	7	11.78 ± 1.13	5
PCA	11.46 ± 1.22	12	11.09 ± 1.59	9
Fenfluramine	10.23 ± 0.86	11	11.39 ± 2.15	8

[a] % inhibition of release, [b] number of data points tested for each substituted amphetamine.

3.4. *Effects of 5-HT uptake blockade using substituted amphetamines*

In this experiment, we tested whether concentrations of substituted amphetamines that blocked uptake would be able to attenuate the releasing effects of one of these drugs. The synaptosomes were first preincubated with one of the four substituted amphetamines at a concentration on the threshold of causing release but within the range for producing uptake inhibition: 0.1 μM for PCA, fenfluramine and MDMA, and 1 μm for methamphetamine. After 10 min, the same drug or one of the other substituted amphetamines was added at a concentration causing 25 or 50% release (0.4 and 3 μM for PCA and fenfluramine, 0.5 and 8 μM for MDMA, and 5 and 20 μM for methamphetamine). Figure 5 shows the results for fenfluramine. Prior exposure to a threshold concentration of fenfluramine did not cause attenuation of release caused by subsequent administration of fenfluramine at a higher concentration. Likewise, combination of low fenfluramine with high PCA, MDMA or methamphetamine also did not cause attenuation of release (fig. 5). MDMA, methamphetamine and PCA were similarly unable to block the releasing effect of itself or the other substituted amphetamines (data not shown). We then repeated these experiments using slightly higher pre-incubation concentrations of the drugs: 0.3 μM for PCA and fenfluramine, 0.5 μM for MDMA, and 5 μM for methamphetamine. These concentrations, which caused 15–25% release on their own, were equally unable to

attenuate the release induced by subsequent administration of higher concentrations of the substituted amphetamines (data not shown).

4. Discussion

In this study, we compared directly the release of [^{3}H]5-HT from preloaded synaptosomes induced by the substituted amphetamines MDMA, methamphetamine, PCA and fenfluramine. For this purpose, we used a novel microassay system in which the release was determined in 96-well tissue culture plates in a total volume of 200 μl. In agreement with earlier studies, we could demonstrate that all four drugs cause [^{3}H]5-HT release and that the release is blocked by 5-HT uptake inhibitors. Also, we found EC_{50} values for the release that are in the same range or lower than those reported in earlier studies (Ross, 1976; Kannengiesser et al., 1976; Borroni et al., 1983; Ross et al., 1977; Knapp and Mandell, 1976; Schmidt et al., 1987; Tessel and Rutledge, 1976). Only two recent studies (McKenna et al., 1991; Hekmatpanah and Peroutka, 1990) have described EC_{50} values that are about one-tenth of ours. This discrepancy may be due to differences in assay protocols. A very low concentration of [^{3}H]5-HT was used (9 nM) in these studies, and the synaptosomes were not washed after the loading step. Furthermore, the KCl-induced [^{3}H]5-HT release in these two studies was not Ca^{2+}-dependent (see McKenna et al., 1991), whereas in our system the KCl-induced release showed Ca^{2+} dependency. The validity of our assay system to study 5-HT release is further indicated by the finding that the rank order of release potencies found in the present study, i.e. (±)-PCA ≃ (+)-fenfluramine > (+)-MDMA ≫ (+)-methamphetamine (table 1), is very similar to the one found by McKenna et al. (1991). Additional confirmation is that in our system a decreased content in serotonergic synaptosomes (obtained by in vivo treatment with PCA) reduces the PCA-induced [^{3}H]5-HT release (data not shown).

Three sets of experiments were performed in order to determine whether differences exist in the way the four substituted amphetamines cause 5-HT release from presynaptic terminals. In the first set of experiments, we found that the combination of two of the substituted amphetamines at their approximate EC_{50} did not cause more 5-HT release than either drug alone at the equivalent concentration, i.e. at $2 \times EC_{50}$ (fig. 3). This result suggests that all of these substituted amphetamines are interchangeable with one another when causing release. In other words, these drugs may compete for the same site on the serotonergic terminal. This site may in fact be the 5-HT uptake carrier itself, since we have found that MDMA, methamphetamine, PCA and fenfluramine display the same rank order in their affinity to the 5-HT uptake carrier (Poblete et al., 1989, and unpublished observation) as in their potencies for release.

In the second set of experiments, we determined whether blockade of the 5-HT uptake carrier would block similarly the release caused by all four substituted amphetamines. The results show that 5 μM cocaine and 80 nM fluoxetine block the release induced by each of the four substituted amphetamines to approximately the same extent, i.e. 9–12% (table 2). This capacity of the two uptake blockers to inhibit the release induced by all four drugs to the same percentage suggests that the uptake carrier is involved to the same extent in the 5-HT-releasing action of these drugs. The relatively low values for the percent release inhibition are due to the low concentrations of uptake blockers used. In additional experiments, we observed a more pronounced inhibition with higher uptake blocker concentrations. Yet, at those higher concentrations, cocaine and fluoxetine caused also a greater reduction in synaptosomal [^{3}H]5-HT contents on their own, which obscured the blocking effect on the release induced by low substituted amphetamine concentrations. Whether this release induced by uptake blockers is genuine, as suggested by Peyer et al. (1982), or whether it is the result of inhibiting the reuptake of spontaneously released [^{3}H]5-HT needs to be explored in further studies.

Finally, we investigated whether any of the four substituted amphetamines which are known to act as uptake blocker, could attenuate the releasing effect of the other drugs. The same protocol with a preincubation period was used that could demonstrate the blocking activity of cocaine and fluoxetine. In contrast to cocaine and fluoxetine, the substituted amphetamines themselves, when used at concentrations on the threshold of causing release, could not block the release induced by another substituted amphetamine (fig. 5 and other data not shown). It is feasible that these threshold concentrations used for the preincubation may have been too low to cause a marked inhibition of 5-HT uptake. However, when we used higher concentrations of the substituted amphetamines (that caused about 15–25% release and that should have caused inhibition of uptake), we still could not detect attenuation of release. Thus, in this assay system, we did not observe a protective effect that could be attributed to the uptake inhibitory activity of the substituted amphetamines observed in in vitro studies (Wong et al., 1973; Steele et al., 1987; Belin et al., 1976; Kannengiesser et al., 1976; Knapp and Mandell, 1976). Hence, we could not confirm whether there are differences in the uptake inhibitory effect between PCA and fenfluramine, as has been suggested based on an in vivo study (Fattacini et al., 1991). Our results are,

however, in agreement with another in vivo study that found that pretreatment with fenfluramine could not block the 5-HT-depleting effects of PCA (Fuller et al., 1988).

The finding that the substituted amphetamines, in contrast to fluoxetine and cocaine, did not attenuate the 5-HT release suggests that the 5-HT uptake blocking effects of the substituted amphetamines are different from those of fluoxetine and cocaine. It is possible that the substituted amphetamines are substrates for the 5-HT uptake carrier and cause their uptake inhibitory effects through competition with 5-HT for the uptake carrier. Cocaine and fluoxetine, in contrast, may inhibit the 5-HT uptake by blocking the uptake carrier on the outside of the membrane. In support of this notion is the finding that amphetamine itself is a substrate for the norepinephrine uptake carrier (Bönisch and Trendelenburg, 1988). Furthermore, cocaine and fluoxetine, neither of which contain the amphetamine core structure (fig. 1), appear to be bulkier molecules than the substituted amphetamines and are less likely to be substrates for uptake. However, direct experimental evidence that the substituted amphetamines are indeed substrates of 5-HT uptake is still lacking (Ross and Ask, 1980; Ross, 1976; Sanders-Bush and Steranka, 1978; Belin et al., 1976; Azmitia, unpublished observations). Further studies are therefore required to compare the nature of the uptake inhibition caused by substituted amphetamines, fluoxetine and cocaine in vitro.

In conclusion, in all three experimental paradigms we could not detect any differences in the 5-HT-releasing action of the four substituted amphetamines. We therefore propose that these drugs all cause 5-HT release via the same mechanism. We hypothesize that the reported differences between PCA and fenfluramine in some experimental tests (Pawlowski et al., 1980; Frey, 1975) are not due to a difference in causing 5-HT release but may have been caused by drug actions on 5-HT receptors or on other transmitter systems. Since the toxicity of PCA and MDMA on 5-HT neurons may be related to release of 5-HT (Azmitia et al., 1990; Berger et al., 1989), we speculate that the tested substituted amphetamines share a common mechanism of producing neurotoxicity.

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