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# 5-Hydroxytryptamine release *in vivo* from a cytoplasmic pool: studies on the 5-HT behavioural syndrome in reserpinized rats

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**1** Treatment of rats with reserpine in order to disrupt vesicular amine storage reduces 5-hydroxytryptamine (5-HT) levels throughout brain by 90–95%.

**2** Despite the drastic reduction in brain 5-HT content by reserpine, the 5-HT releasing drug *p*-chloroamphetamine (PCA) produces a behavioural syndrome in reserpine-treated rats which is not different from that observed in normal animals given PCA.

**3** Prior treatment of reserpinized rats with *p*-chlorophenylalanine (PCPA), the irreversible tryptophan hydroxylase inhibitor which inhibits the synthesis of new 5-HT, prevents the PCA-induced behavioural syndrome.

**4** The 5-HT receptor antagonist methergoline, blocks the PCA effect in reserpine-treated rats.

**5** Treatment of reserpinized rats with pargyline, a non-selective inhibitor of monoamine oxidase, in order to increase cerebral 5-HT levels, shifts the PCA dose-response curve for inducing the 5-HT behavioural syndrome to the left.

**6** The specific 5-HT uptake blocker, fluoxetine, protects normal and reserpine-treated rats from the 5-HT depleting effects of PCA but does not always prevent the PCA-induced 5-HT behavioural syndrome.

**7** These results indicate that PCA releases 5-HT into the synapse from a small cytoplasmic pool which is resistant to reserpine and suggest that this newly synthesized compartment of 5-HT represents the 'functional' transmitter pool.

## Introduction

Treatment of rats with an irreversible inhibitor of monoamine oxidase (MAOI) followed by L-tryptophan produces a characteristic behavioural-neurological syndrome which is apparently mediated by 5-hydroxytryptamine (5-HT; Grahame-Smith, 1971; Green & Grahame-Smith, 1976). The newly formed 5-HT 'spills over' into the synapse after its levels exceed the capacity of the presynaptic neurone to store or metabolize it (Green & Youdim, 1975; Ashkenazi *et al.*, 1983). The behavioural syndrome can also be produced by direct 5-HT receptor agonists such as 5-methoxy-*N,N*-dimethyltryptamine (Grahame-Smith, 1971) or by drugs that release 5-HT from the presynaptic neurone including *p*-chloroamphetamine (PCA; Trulson & Jacobs, 1976). This syndrome has been used extensively to study drugs which act on the brain 5-HT neuronal system by altering synthesis and release, or by interacting with 5-HT receptors directly.

It has been proposed that 5-HT is stored in the presynaptic neurone in different pools or compartments (Shields & Eccleston, 1973; Glowinski, 1975; Lane & Aprison, 1978; Morot-Gaudry, Bourgoin & Hamon, 1981; Sanders-Bush, 1982; Tracqui *et al.* 1983). In general, one pool is quite large, comprising approximately 80–90% of total brain 5-HT, while the other pool is much smaller, containing 10–20% of total brain 5-HT. The large compartment is thought to be a storage or reserve pool for the transmitter and the small pool is often referred to as the 'functional' pool, by inference, since it is generally assumed to contain the newly-synthesized 5-HT which is preferentially released. However, most research on the various comparments of 5-HT has been somewhat indirect (neurochemical) and lacking in physiological correlates. In the present experiments we sought to identify the functional pool of 5-HT by linking release from this pool to the occurrence of the

characteristic 5-HT behavioural syndrome. In order to do so, we chose to study the 5-HT syndrome in reserpine-treated rats. The use of such animals for investigating the functional pool of 5-HT *in vivo* seemed ideal for several reasons. First, the vesicular content of 5-HT is largely eliminated (Shore & Giachetti, 1978) leaving only the small, newly synthesized pool intact. Second, tryptophan hydroxylation and hence 5-HT synthesis remains normal (Morot-Gaudry *et al.*, 1981; Long *et al.*, 1983) and third, postsynaptic 5-HT receptors as judged by [$^3$H]-5-HT binding remain normal (Bennet & Snyder, 1976).

## Methods

### *Behavioural experiments*

Male Sprague-Dawley rats weighing between 250–350 g were housed in group cages with food and water available *ad libitum* and maintained on a 12 h light–12 h dark cycle. Unless stated otherwise, rats were treated with reserpine 24 h before behavioural testing. Control animals received 0.9% saline. Only those rats displaying the characteristic reserpine posture (ptosis, hunch) and loss of body weight in excess of 5% (Halaris & Freedman, 1975) were selected for testing since these signs are reliable indicators of complete reserpinization. Subsequently, rats chosen by these criteria did not show post-decapitational convulsions. After test injections, animals were placed in clear plexiglas cages in groups of four and observed for 5 min at 15 min intervals for up to 2 h for expression of the 5-HT behavioural syndrome. Symptoms including head weaving, reciprocal forepaw treading, hindlimb abduction, salivation, wet-dog shakes and hyperactivity were noted. Individual rats were scored by observers (blind to the treatment) as either displaying the syndrome or not. This syndrome has been carefully and extensively characterized and the concurrent appearance of three or more of the indicated symptoms (primarily head weaving, forepaw treading, and hindlimb abduction) indicates positive expression of the 5-HT syndrome (Jacobs, 1976; Green and Grahame-Smith, 1976; Sloviter *et al.*, 1978). We chose an all-or-none method of scoring the behavioural syndrome for the reasons given by Sloviter *et al.* (1978): this method represents the most conservative and least subjective assessment method. Furthermore, doses of drugs were selected from previous results which demonstrated that they produced a behavioural syndrome of maximal intensity (Trulson & Jacobs, 1976; Deakin & Green, 1978).

### *Biochemical procedures*

All biochemical determinations were performed at the appropriate times (see below) after behavioural testing. Briefly, rats were killed by decapitation and various brain regions were dissected and frozen on dry ice. Tissue was stored in liquid nitrogen until assayed (usually within 1 week). The levels of 5-HT and its major metabolite 5-hydroxyindoleacetic acid (5-HIAA) were determined in the hypothalamus, raphe nuclei, thoracic spinal cord, and striatum by h.p.l.c. with fluorescence detection as described by Wolf & Kuhn (1983). The data were analysed by an analysis of variance and individual group effects were compared *a posteriori* with the Neuman-Keuls test.

### *Materials*

*p*-Chloroamphetamine (PCA), *p*-chlorophenylalanine (PCPA) and pargyline were purchased from Sigma. Fluoxetine was gift from Dr R. Fuller of Eli Lilly (Indianapolis, IN) and methergoline was donated by Farmitalia (Milano). Reserpine (Serpasil) was purchased from Ciba-Geigy.

All drugs were prepared fresh and injected intraperitoneally in volumes of 2 ml kg$^{-1}$ body weight. Fluoxetine was dissolved in sterile water and methergoline was prepared for injection in 0.01 N HCl. Reserpine was provided as an injectable solution. All other drugs were dissolved in 0.9% NaCl.

## Results

### *Behavioural effects of PCA in reserpine-treated rats: pharmacological characterization*

Reserpine itself did not produce any symptoms of the 5-HT behavioural syndrome. Injection of PCA at a dose of 5.0 mg kg$^{-1}$ elicited a very intense behavioural syndrome in all reserpinized rats. The PCA effect was rapid in onset (5–10 min) and reached maximal intensity within 30–45 min. All symptoms of the syndrome were observed during the most intense period of the PCA effect. These data are included in Table 1. Pretreatment of reserpinized rats with PCPA (250 mg kg$^{-1}$, 24 h after reserpine and 72 h before PCA), an irreversible inhibitor of tryptophan hydroxylase (Koe & Weissman, 1966), in order to abolish the 5-HT synthetic capacity, completely blocked the effects of PCA. Similarly, the 5-HT receptor antagonist methergoline (5 mg kg$^{-1}$) effectively prevented the PCA-induced syndrome in reserpinized rats when injected 60 min before PCA. In agreement with Deakin & Green (1978), the methergoline-treated rats did demonstrate episodes of elevated, intense locomotor activity after PCA. If

**Table 1** Behavioural effects of *p*-chloroamphetamine (PCA) in reserpinized rats

| *Treatment* | *Behavioural score* |
|---|---|
| PCA (5 mg kg$^{-1}$) | 35/35 |
| PCPA (250 mg kg$^{-1}$) + PCA (5 mg kg$^{-1}$) | 0/19* |
| Methergoline (5 mg kg$^{-1}$) + PCA (5 mg kg$^{-1}$) | 0/8* |
| Reserpine (2.5 mg kg$^{-1}$) + PCA (5 mg kg$^{-1}$) | 4/4 |

Rats were injected with the indicated drug doses 24 h after receiving 5 mg kg$^{-1}$ reserpine. *p*-Chlorophenylalanine (PCPA) was administered 72 h before PCA. Methergoline was administered 60 min before PCA. The second reserpine dose was given 6 h before PCA. The behavioural score represents the number of rats displaying the syndrome/number of treated rats. Asterisk indicates that these values are statistically, significantly different ($p < 0.05$) from PCA according to a contingency table amwlysis.

**Table 2** Effect of pargyline on the behavioural response to *p*-chloroamphetamine (PCA) in reserpinized rats

| | *Pretreatment* | |
|---|---|---|
| *Dose of PCA* (mg kg$^{-1}$) | *Control* (Behavioural score) | *Pargyline* |
| 0.10 | – | 2/5 |
| 0.25 | 0/5 | 5/5* |
| 0.50 | 0/5 | 5/5* |
| 2.50 | 4/5 | – |
| 5.00 | 5/5 | 5/5 |

Reserpinized rats were pretreated with 75.0 mg kg$^{-1}$ of pargyline 30 min before PCA. The behavioural score was derived as described in Table 1. Asterisk indicates that these values are statistically, significantly different ($P < 0.05$) from control animals according to a contingency table analysis.

rats were given a second injection of reserpine (2.5 mg kg$^{-1}$) 24 h after the first dose and tested with PCA 6 h later, PCA still elicited an intense syndrome (Table 1).

In some experiments reserpinized rats were treated with 75 mg kg$^{-1}$ pargyline 30 min before PCA in order to increase brain 5-HT concentrations. Apart from reversing some of the overt symptoms of reserpinization (e.g., ptosis), pargyline was largely without behavioural effects at the time of PCA injection. However, pargyline treatment markedly enhanced the effects of PCA in reserpinized rats. The data in Table 2 indicate that doses of PCA as low as 0.1 mg kg$^{-1}$ produced the 5-HT behavioural syndrome in some pargyline-treated rats whereas doses of 0.25–0.50 mg kg$^{-1}$ produced an intense behavioural syndrome in all MAO-inhibited animals. In contrast, these very low doses of PCA were without effect in rats treated only with reserpine.

The 5-HT uptake inhibitor fluoxetine protects the brain from the 5-HT depleting effects of PCA, presumably by inhibiting the uptake of PCA into 5-HT neurones (Fuller *et al.*, 1975). However, we observed that fluoxetine did not always prevent the PCA-induced behavioural syndrome. While fluoxetine protected all animals against the behavioural effects of 2.5 mg kg$^{-1}$ PCA, approximately 50% of the reserpinized rats given 5.0 mg kg$^{-1}$ of PCA after fluoxetine (10 mg kg$^{-1}$) displayed the syndrome. However, if the PCA dose was increased to 10.0 mg kg$^{-1}$ 100% of the animals showed a rather intense syndrome. These data are also presented in Table 3. In addition, non-reserpinized rats were tested for protection by fluoxetine against PCA behavioural effects

**Table 3** Effects of fluoxetine on the *p*-chloroamphetamine (PCA)-induced behavioural syndrome in reserpinized and control rats

| | *Behavioural score* | |
|---|---|---|
| *Treatment* | *Reserpinized* | *Control* |
| PCA (10 mg kg$^{-1}$) | 10/10 | 10/10 |
| Fluoxetine (10 mg kg$^{-1}$) + PCA (2.5 mg kg$^{-1}$) | 0/7* | |
| Fluoxetine (10 mg kg$^{-1}$) + PCA (5 mg kg$^{-1}$) | 4/8* | – |
| Fluoxetine (10 mg kg$^{-1}$) + PCA (10 mg kg$^{-1}$) | 8/8 | 9/11 |
| Fluoxetine (10 mg kg$^{-1}$) | 0/7 | 0/8 |

Normal or reserpinized rats were treated with the indicated drugs and observed for expression of the behavioural syndrome. Reserpine was injected 24 h before testing and fluoxetine was injected 30 min before PCA. The behaviour score was derived as described in Table 1. Asterisk indicates a statistically, significantly different value ($P < 0.05$) from PCA (5.0 mg kg$^{-1}$) alone according to a contingency table analysis.

for comparison. Fluoxetine was ineffective in protecting against the behavioural effects of 10 mg kg$^{-1}$ of PCA in approximately 80% of these rats. Although most of the symptoms could be observed in normal rats treated with fluoxetine plus PCA, their appearance was sporadic and of much lower intensity than observed in reserpinized rats.

*Biochemical changes in reserpinized rats; effects of pharmacological manipulations on 5-HT neurochemistry*

The results of neurochemical studies are presented in Figures 1–3. It can be seen in Figure 1 that reserpine reduced brain 5-HT by more than 90% in the hypothalamus, striatum, and spinal cord whereas the mesencephalic raphe nuclei were depleted by only 75%. Reserpine also produced its characteristic increase in 5-HIAA in all brain areas assayed. Injections of PCA significantly reduced the remaining 5-HT stores in reserpinized rats in the hypothalamus, striatum, and spinal cord while raphe 5-HT increased slightly after PCA. The levels of 5-HIAA in reserpinized rats were slightly lowered by PCA. These effects reached statistical significance in the hypothalamus and raphe. Treatment of reserpinized rats with PCPA almost completely depleted the brain areas of 5-HT and 5-HIAA with the exception of the raphe which was somewhat more resistant to the effects of PCPA.

The effects of fluoxetine and PCA (alone and in combination) on 5-HT neurochemistry in reserpinized rats are shown in Figure 2. Fluoxetine (10 mg kg$^{-1}$) alone produced a slight but significant increase in 5-HT in the hypothalamus and raphe and reduced 5-HIAA in all brain areas. The effects of the low dose of PCA were similar to those shown in Figure 1 in that 5-HT and 5-HIAA levels were slightly decreased. The higher dose of PCA (10 mg kg$^{-1}$) lowered 5-HIAA to a greater extent in each brain area while the levels of 5-HT were slightly elevated in the hypothalamus and raphe. If fluoxetine was injected prior to either dose of PCA, the 5-HT depleting effects of PCA were prevented and the levels were actually increased above control 5-HT values. In fact, groups of animals treated with fluoxetine plus PCA were not different from groups treated only with fluoxetine. In contrast to results with 5-HT, fluoxetine did not protect against the PCA-induced lowering of 5-HIAA, although fluoxetine itself lowers 5-HIAA.

Treatment of non-reserpinized rats with fluoxetine and PCA produced effects which were similar to those oberved in reserpinized rats. Although fluoxetine itself increased 5-HT levels only in the raphe as compared to control, it effectively prevented the PCA-induced reductions in 5-HT. Once again, both fluoxetine and PCA lowered 5-HIAA in normal animals and combined treatment with these drugs did not return 5-HIAA to control values (i.e., the neurochemical profile resembled that of animals treated only with fluoxetine).

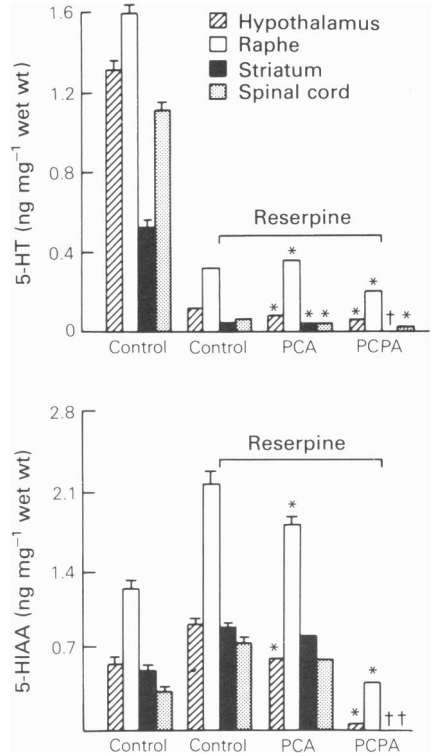


**Figure 1** Effects of reserpine alone or in combination with *p*-chloroamphetamine (PCA) or *p*-chlorophenylalanine (PCPA) on 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels in various brain regions. Rats were injected with reserpine 5 mg kg$^{-1}$ or 0.9% saline (controls) and killed 24 h later. PCA (5 mg kg$^{-1}$) was injected into reserpinized rats 60 min before they were killed. PCPA (250 mg kg$^{-1}$) was injected 72 h before rats were killed. The levels of 5-HT and 5-HIAA were determined by h.p.l.c. with fluorescence detection. $n = 6$ for controls, 14 for reserpine controls, 8 for PCA group and 5 for PCPA group. $^*P < 0.05$ compared to reserpine. † not detectable (less than 0.01 ng mg$^{-1}$ wet weight for 5-HT and less than 0.025 ng mg$^{-1}$ wet weight for 5-HIAA).

## Discussion

It is significant that the entire 5-HT behavioural syndrome can be elicited in rats after disruption of vesicular 5-HT storage with reserpine. Although reserpine abolishes 5-HT storage and reduces brain

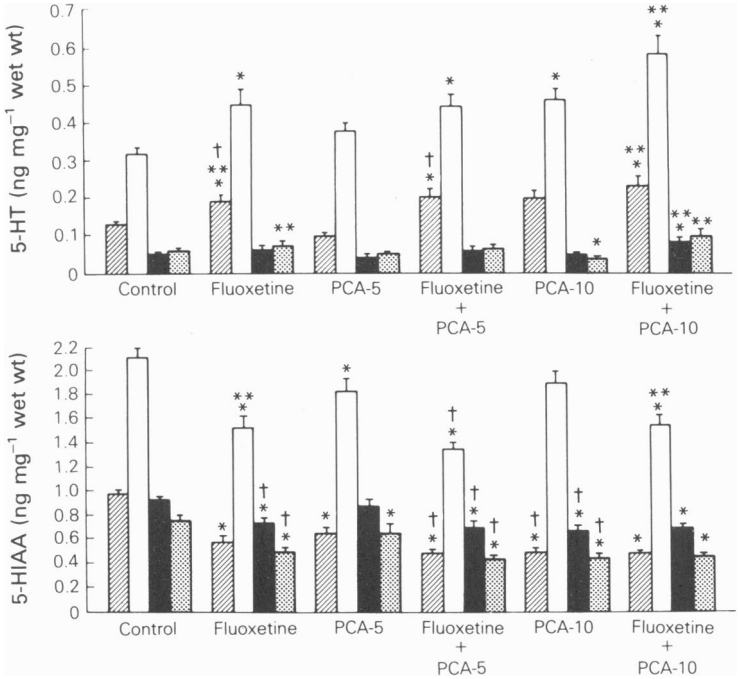


**Figure 2** Effects of fluoxetine and *p*-chloroamphetamine (PCA) on 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels in reserpinized rats. Fluoxetine was administred 30 min before either dose of PCA (5 mg kg$^{-1}$ or 10 mg kg$^{-1}$) and 90 min before rats were killed. Rats were killed 60 min after either dose of PCA. The levels of 5-HT and 5-HIAA were determined by h.p.l.c. with fluorescence detection. $n=7$ for each treatment. The symbols are: $^{**}P<0.025$ compared to PCA-10; $^{*}P<0.05$ compared to control; $\dagger P<0.01$ compared to PCA-5. The key for the brain regions is the same as in Figure 1.

5-HT content by more than 90% (Sanders-Bush & Massari, 1977; Shore & Giachetti, 1978) 5-HT synthetic capacity remains intact (Morot-Gaudry *et al.*, 1981; Long *et al.*, 1983). The ability of PCA to induce the complete behavioural syndrome in reserpinized rats suggests that this drug acts primarily on the small newly synthesized pool of 5-HT. These results extend previous findings that the 5-HT behavioural syndrome can be induced in reserpinized rats (Grahame-Smith, 1971, Ogren & Ross, 1976). If the 5-HT synthetic capacity is blocked by treatment with PCPA, the effect of PCA is completely prevented, indicating that the reserpine-resistant pool of 5-HT, despite its small size, is sufficient to mediate the effects seen. This result also indicates that PCA is not having a direct receptor effect. Furthermore, prevention of the PCA effect in reserpinized rats by methergoline indicates that PCA releases 5-HT onto the appropriate synaptic receptors. Both PCPA and methergoline also prevent the PCA behavioural syndrome in non-reserpinized rats (Green, 1981).

In addition to its ability to deplete the brain of 5-HT, reserpine also disrupts catecholamine storage and it is possible that the effects of PCA are modified in reserpinized rats because of catecholamine depletion. However, several observations argue against a role for catecholamines in the behavioural effects of PCA. First, the 5-HT behavioural syndrome induced by PCA is not modified by prior depletion of catecholamines with α-methyl-*p*-tyrosine (Trulson & Jacobs, 1976). Second, the symptoms scored-here (head weaving, forepaw treading, and hindlimb abduction) are mediated selectively by 5-HT receptors (Jacobs, 1976; Sloviter *et al.*, 1978; Ortmann, Waldmeier, Radeke, Felner & Delini-Stula, 1980; Green *et al.*, 1984) while the hyperactivity component of the 5-HT syndrome is mediated by dopamine receptors (see Green *et al.*, 1984).

Although it has not been possible to determine whether the large and small 5-HT compartments correspond to vesicular and non-vesicular compartments (Tracqui *et al.*, 1983) respectively, several lines of evidence suggest that the reserpine-resistant fraction of 5-HT (i.e., the small compartment), which

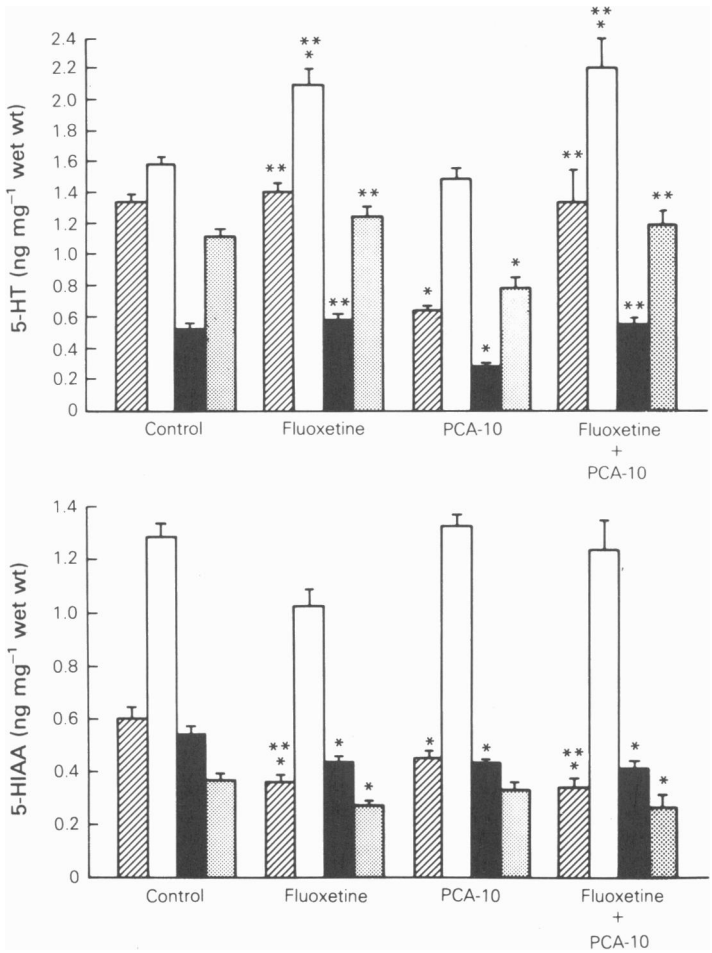


**Figure 3** Effects of fluoxetine and *p*-chloroamphetamine (PCA) on 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels in normal rats. Rats were treated with 10 mg kg$^{-1}$ of fluoxetine or PCA (10 mg kg$^{-1}$) or their combination as described in the legend to Figure 2. The levels of 5-HT and 5-HIAA were determined by h.p.l.c. with fluorescence detection. $n = 5$ for each treatment. The symbols are: $^{**}P < 0.01$ as compared to PCA-10; $^{*}P < 0.025$ as compared to control. The key for brain regions is given in Figure 1.

is released by PCA, is located outside storage vesicles. First, tryptophan hydroxylase, the initial and rate-limiting enzyme in 5-HT biosynthesis (Jequier *et al.*, 1967), is a cytoplasmic enzyme (Joh *et al.*, 1975; Kuhn & Lovenberg, 1984) as is aromatic L-amino acid decarboxylase (Glowinski, 1975), the enzyme which converts 5-hydroxytryptophan to 5-HT. Thus, 5-HT is synthesized outside storage vesicles and this process is not disrupted by reserpine. Second, all 5-HT molecules are not packaged into vesicles after synthesis. In fact, it has been known for some time that 5-HT can exist outside vesicles or storage granules. For example, Fuxe *et al.* (1966) demonstrated that 5-HT accumulating within neurones after treatment with MAOI could not be localized to any subcellular storage organelle. Similarly, Tissari & Raunu (1975) demonstrated that 5-HT could accumulate in MAOI-treated neonatal rats, prior to the completed development of synaptosomes or vesicles. Subcellular fractionation studies also indicate that more 5-HT is found in supernatant and cell debris fractions than in vesicular fractions (Halaris & Freedman, 1977). Third, 5-HT within storage vesicles exchanges with the extra-vesicular space (Sanders-

Bush & Massari, 1977; Tracqui *et al.*, 1983).

If the small reserpine-resistant pool of 5-HT is cytoplasmic and if release occurs from this pool, pharmacological manipulations which alter the size of the cytoplasmic pool should also alter the PCA-induced syndrome. Indeed, treatment of reserpinized rats with pargyline, a non-selective inhibitor of MAO, increases 5-HT concentrations in reserpinized rats by 10 fold (Wolf, Youdim & Kuhn, unpublished observation). After such treatment, the PCA dose-response curve for producing the 5-HT behavioural syndrome is shifted dramatically to the left. Similarly, increasing brain 5-HT concentrations in normal animals by injection of L-tryptophan also enhances the behavioural effects of PCA (Brown & Growdon, 1980). Furthermore, reductions in the size of the cytoplasmic 5-HT pool in reserpinized rats with PCPA prevents the PCA-induced behavioural syndrome (Table 1).

Results with fluoxetine complement the present findings with PCA. PCA produces a slight but statistically significant reduction in 5-HT in the hypothalamus, striatum, and spinal cord of reserpinized rats. When fluoxetine is injected prior to either $5\,mg\,kg^{-1}$ or $10\,mg\,kg^{-1}$ PCA, the combination actually significantly increases 5-HT concentrations over controls. Fluoxetine protects against the behavioural effects of $2.5\,mg\,kg^{-1}$ of PCA, partially protects against $5.0\,mg\,kg^{-1}$ of PCA and is ineffective in protecting against the largest PCA dose ($10\,mg\,kg^{-1}$). Thus, if PCA-induced reductions in 5-HT are taken as evidence of 5-HT release, prevention of the PCA effect on brain 5-HT levels by fluoxetine does not preclude the occurrence of the behavioural syndrome. On the other hand, the PCA-induced reduction in 5-HT is probably mediated in part by the ability of PCA to inhibit tryptophan hydroxylase (Sanders-Bush *et al.*, 1972). In either case, these data indicate that PCA can still elicit the release of 5-HT in sufficient quantities to produce the syndrome despite 'protection' of the 5-HT system against PCA with fluoxetine. The amount of 5-HT released may be too small for our assay to detect by simply measuring the static concentration of 5-HT. Similar results were observed in non-reserpinized rats where PCA produces a substantially larger reduction in 5-HT levels (Figure 3). Despite protection against PCA-induced 5-HT depletion by fluoxetine, PCA was still effective in producing the 5-HT behavioural syndrome in most rats. These results with fluoxetine and PCA complement Grahame-Smith's (1971) original observation that the absolute level of 5-HT or its synthesis rate in the brain at any time do not predict whether or not the behavioural syndrome will occur. What is most important is the relative amount of release taking place.

We cannot conclude solely from the present results that PCA releases 5-HT exclusively from a cytoplasmic pool under circumstances where vesicle function is normal. Although it is generally concluded that 5-HT release in brain is exocytotic (e.g., see Sanders-Bush, 1982), little direct evidence is available to support this conclusion. This notwithstanding, several observations indicate that the evoked release of 5-HT can occur by a process which is not exocytotic. For example, the 5-HT releasing action of PCA does not mimic exocytosis since it occurs independently of $Ca^{2+}$ (Sanders-Bush, 1982). Furthermore, if reserpinized rats are treated with a second dose of reserpine ($2.5\,mg\,kg^{-1}$) 24 h after the first dose, injection of PCA 6 h later still produces the complete 5-HT syndrome. This result minimizes the possibility that the PCA effect in reserpinized animals is mediated either by a small, surviving population of 5-HT containing vesicles or by newly synthesized vesicles. In related *in vitro* studies, it has also been shown that both PCA (Ross & Kelder, 1971) and fenfluramine (Mennini *et al.*, 1981) can induce 5-HT release from synaptosomes which were prepared from reserpinized animals.

A parallel can be drawn between the present results with the 5-HT neuronal system and the catecholamine neuronal system with respect to transmitter release. Amphetamine can release catecholamines from tissue derived from reserpinized animals (Farnebo, 1971) and *in vivo* reserpine treatment has also been shown to intensify the central stimulating effects of amphetamine (Svensson, 1970). Inhibition of the synthesis of the catecholamines in reserpinized rats also prevents amphetamine effects (Randrup & Munkvad, 1966; Weissman *et al.*, 1966).

Taken together, the present results indicate that 5-HT can be released into the synapse from a very small intraneuronal pool and this small compartment of transmitter is apparently located in the cytoplasm (outside the storage vesicles). In the absence of normal vesicular storage, the amount of 5-HT available for release is determined both by the rate at which the *de novo* synthesis of 5-HT occurs and, more importantly, the rate at which 5-HT is catabolized by MAO. Additional neurochemical and physiological studies in reserpinized rats should provide important information on the regulation of the functional pool of 5-HT.

Finally, a word of caution should be added concerning the method of scoring the 5-HT behavioural syndrome. The all-or-nothing method used here certainly has its advantages; however, certain pitfalls do exist as pointed out by Dickinson *et al.* (1983). Since the method of scoring the intensity of individual components of the syndrome as advocated by Dickinson *et al.* (1983) depends, to some extent, on subjective judgements, no method of scoring seems

ideal. Perhaps future studies on the 5-HT behavioural syndrome should score individual behaviours in addition to a more global score (syndrome present or not).

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