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An *in vivo* dialysis and behavioural study of the release of 5-HT by *p*-chloroamphetamine in reserpine-treated rats

[1]A. Adell, G.S. Sarna, [2]P.H. Hutson & [3]G. Curzon

Department of Neurochemistry, Institute of Neurology, 1 Wakefield Street, London, WC1N 1PJ

1 Reserpine (2.5 mg kg^{-1} i.p.) decreased rat brain 5-hydroxytryptamine (5-HT) by 86% 24 h later but most components of the 5-HT-dependent behavioural syndrome induced by *p*-chloroamphetamine (PCA, 5 mg kg^{-1} i.p.) or 5-methoxy-N,N-dimethyltryptamine (5-MeODMT, 5 mg kg^{-1} i.p.) over 1 h after administration were unaffected. However, Straub tail was increased after giving PCA or 5-MeODMT and head weaving was decreased after giving 5-MeODMT.

2 Frontal cortex extracellular 5-HT concentrations of vehicle pretreated rats before injection of PCA, as calculated from dialysate 5-HT concentrations, were about 1/1000th of corresponding brain values. Extracellular 5-hydroxyindoleacetic acid (5-HIAA) and brain values were comparable with each other. Dialysate 5-HT increased after PCA with peak values at 20–40 min.

3 Reserpine pretreatment reduced dialysate 5-HT concentration before PCA was given but the net increase (AUC) over the 1 h after PCA did not differ significantly from that seen in animals pretreated with vehicle. Dialysate 5-HIAA values slowly decreased after PCA injection in both reserpine and vehicle pretreated groups.

4 The results suggest that PCA causes the 5-HT syndrome by releasing 5-HT from the neuronal cytoplasm but that physiological release of 5-HT occurs from vesicular stores.

Introduction

The activation of brain 5-hydroxytryptamine (5-HT) receptors induces the 5-HT syndrome in rats, the main components of which are head weaving, forepaw treading, hind limb abduction, flat body posture, Straub tail and wet-dog shakes (Grahame-Smith, 1971; Jacobs, 1976; Sloviter *et al.*, 1978). Some or all of these components can be elicited by drugs which selectively release neuronal 5-HT, such as *p*-chloroamphetamine (PCA; Trulson & Jacobs, 1976; Kuhn *et al.*, 1985), as well as by 5-HT receptor agonists (Hjorth *et al.*, 1982; Tricklebank *et al.*, 1985a,b; Smith & Peroutka, 1986).

The PCA-induced 5-HT behavioural syndrome has been suggested to result from the release of neuronal 5-HT from a small non-vesicular compartment, since it still occurs after pretreatment with reserpine (Kuhn *et al.*, 1985) which disrupts vesicular storage (Shore & Giachetti, 1978). It has also been shown that PCA releases [^{3}H]-5-HT from synaptosomes prepared from the brains of reserpine-treated rats (Ross & Kelder, 1977). More detailed studies of components of the 5-HT syndrome showed that reserpine did not prevent the induction of forepaw treading or flat body posture by the 5-HT agonists 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) and 5-methoxy-N,N-dimethyltryptamine (5-MeODMT), but drastically reduced the head weavings elicited by these drugs (Tricklebank *et al.*, 1985a,b).

The 5-HT behavioural syndrome has usually been scored by methods based on rating scales for its components (Andrews *et al.*, 1982; Tricklebank *et al.*, 1985a,b; Smith & Peroutka, 1986) or by 'all or none' procedures (Trulson & Jacobs, 1976; Sloviter *et al.*, 1978) which do not quantify either the intensity or the duration of each component. As Kuhn *et al.* (1985) investigated the effect of reserpine pretreatment on the 5-HT syndrome elicited by PCA by an 'all or none' method, the interpretation of their findings is uncertain. Also, while *in vivo* intracerebral dialysis shows that PCA releases 5-HT (Sharp *et al.*, 1986) and that the time course of release and the induced 5-HT syndrome parallel each other (Hutson & Curzon, 1989), the effect of reserpine pretreatment NaCl containing BRIJ 35/polyoxyethylene lauryl

[1] Present address: Department of Neurochemistry, Centro de Investigacion y Desarrollo, CSIC, Jorge Girona Salgado 18-26, 08034, Barcelona, Spain.
[2] Present address: Merck, Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex, CM20 2QR.
[3] Author for correspondence.

on release is unknown. It is conceivable that reserpine both reduces the release of 5-HT by PCA and increases the response of receptors to it so that the behavioural effect is unaltered.

We have now reinvestigated the behavioural effect of PCA in rats pretreated with reserpine by scoring the frequency or duration of each behavioural component (Dourish *et al.*, 1985). This procedure avoids the disadvantages of previous scoring methods. Also, the release of brain 5-HT was monitored by *in vivo* dialysis and the responsiveness of the 5-HT receptors mediating the syndrome was studied by measuring its components when induced by the 5-HT agonist 5-MeODMT.

Methods

Male Sprague-Dawley rats (Charles River, U.K.) weighing 250–350 g were used in all experiments. They were housed individually under a 12 h light-dark cycle with food and water available at all times.

Behavioural experiments

Rats were given reserpine ($2.5\,mg\,kg^{-1}$, i.p.) or vehicle 24 h before the administration of either PCA ($5\,mg\,kg^{-1}$, i.p.) or 5-MeODMT ($5\,mg\,kg^{-1}$, i.p.). All animals pretreated with reserpine showed ptosis at the time of injection. The dose of PCA was chosen because it caused submaximal expression of the major components of the 5-HT syndrome in normal rats (Fernando & Curzon, 1981; Kennett *et al.*, 1985). After PCA or 5-MeODMT injection, rats were placed in individual perspex cages ($25 \times 25 \times 21$ cm) with grid floors and behaviours videotaped for 1 h. The frequency of head weaving, forepaw treading, wet-dog shakes and rearing, and the duration of hind limb abduction, flat body posture, Straub tail, locomotion and grooming were scored over 1 h using a microcomputer (Donohoe *et al.*, 1987).

Intracerebral dialysis

Concentric dialysis probes made essentially as described by Hutson *et al.* (1985) were fitted with 36G steel microcannulae (Brain Research Instruments Co, Princeton, U.S.A.). Cuprophan hollow fibres (Enka AG, Wuppertal, F.R.G.) were used for the dialysis membranes. Before implantation, the recoveries of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) by the probes were determined as follows. Probes were perfused with artificial cerebrospinal fluid (CSF) (composition in mM; NaCl 125, KCl 2.5, $MgCl_2$ 1.18, $CaCl_2$ 1.26) at $0.25\,\mu l\,min^{-1}$ using a microlitre syringe pump (Harvard Apparatus Ltd, South Natick, U.S.A.) and immersed in a beaker containing $10\,ng\,ml^{-1}$ of each indole compound. Successive 20 min (5 μl) samples of perfusate were collected in tubes containing 10 μl of mobile phase (see below) and analysed by high-performance liquid chromatography (h.p.l.c.). Percentage recoveries were 57 ± 1 for 5-HT and 51 ± 2 for 5-HIAA ($n = 10$, mean $\pm$ s.e.mean).

Rats were given reserpine ($2.5\,mg\,kg^{-1}$, i.p.) or vehicle 24 h before injecting PCA ($5\,mg\,kg^{-1}$, i.p.) as in the behavioural experiments. Following anaesthesia with sodium pentobarbitone ($60\,mg\,kg^{-1}$, i.p.), dialysis probes were implanted in frontal cortex (3.2 mm anterior to bregma, 6.0 mm ventral to it and 2.5 mm lateral to the midline) according to Paxinos & Watson (1982). Animals were left to recover for approximately 18 h and artificial CSF then perfused through the probe as described. Dialysis samples were collected for 1 h before giving PCA and for 4 h 40 min subsequently.

Biochemical measurements

Immediately after collection, each dialysis sample was assayed for 5-HT and 5-HIAA by h.p.l.c. with electrochemical detection. The mobile phase consisted of 0.15 M NaH_2PO_4, 0.01 mM octyl sodium sulphate, 0.5 mM EDTA (pH 3.8 adjusted with phosphoric acid) and 12.5% methanol. Indoles were separated on an Altex ultrasphere 3 μm ODS column (4.6 mm × 7.5 cm) (Beckman Ltd, U.K.). The electrochemical detector was an ESA Coulochem model 5100A (Severn Analytical Ltd) with a dual electrode analytical cell (model 5011). The conditioning cell was set at −0.04 V, electrode 1 at +0.10 V and electrode 2 at +0.30 V with respect to palladium reference electrodes.

In a separate experiment, rats were given either reserpine or vehicle as before, killed 24 h later and their brains removed for determination of 5-HT, 5-HIAA and also dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). The brains were homogenized in 0.4 M perchloric acid containing 0.1% sodium metabisulphite, 0.01% EDTA, 0.1% cysteine and centrifuged at $3000 \times g$ for 20 min. Aliquots of supernatants were then filtered through 0.45 μm filters (Gelman) and analysed by h.p.l.c. as described, except for the mobile phase which was made up of 0.1 M KH_2PO_4, 1 mM octyl sodium sulphate, 0.1 mM EDTA (pH 2.75) and 18% methanol.

Drugs

Reserpine, PCA and 5-MeODMT were purchased from Sigma. DL-PCA hydrochloride was dissolved in 0.9% NaCl and 5-MeODMT suspended in 0.9%

ether (0.005%). Reserpine was dissolved in a few drops of glacial acetic acid and diluted in distilled water. Drugs were injected in volumes of 1 ml kg^{-1} body weight.

Statistics

Differences between the behavioural effects of drugs on vehicle and reserpine pretreated rats were assessed using the two-tailed Mann-Whitney U-test or Duncan's multiple range test. The effects of reserpine on whole brain levels of indoles and catechols were analysed by Student's *t* test. In the *in vivo* intracerebral dialysis study the mean of the four values immediately before PCA injection was taken as the basal concentration for each indole compound. The effects of PCA and reserpine on extraneuronal levels of 5-HT and 5-HIAA were assessed by two-way analysis of variance followed by Newman-Keuls' multiple range test.

Results

As shown in Table 1 reserpine depleted whole brain levels of 5-HT and dopamine very substantially and increased 5-HIAA and HVA.

Results in Table 2 and Figure 1 show that the overall patterns of the behavioural effects of PCA and 5-MeODMT were largely unaffected by pretreatment with reserpine, major components of the behaviour not being significantly altered. However, head weaving frequency was significantly decreased (after 5-MeODMT) and Straub tail behaviour was significantly increased after giving either drug.

Table 1 Effect of reserpine (2.5 mg kg^{-1}, i.p.) on whole brain levels of 5-hydroxytryptamine (5-HT), 5-hydroxyindole-acetic acid (5-HIAA), dopamine (DA), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)

	Vehicle ($n = 6$)	*Reserpine* ($n = 6$)
5-HT	5.04 ± 0.49	0.69 ± 0.27**
5-HIAA	2.03 ± 0.10	2.62 ± 0.29*
DA	8.88 ± 1.09	0.44 ± 0.12**
DOPAC	0.76 ± 0.08	0.68 ± 0.09
HVA	0.45 ± 0.03	0.73 ± 0.14**

Reserpine or vehicle was injected 24 h before killing. Values (mean ± s.e.mean) are expressed as pmol mg^{-1} wet wt tissue. *$P < 0.01$, **$P < 0.001$ compared with vehicle-injected rats (Student's *t* test).

In the absence of PCA or 5-MeODMT, the above components of the 5-HT syndrome were, at the most, marginally present in the vehicle and reserpine-treated groups, but appreciable amounts of normal locomotor, rearing and grooming behaviour occurred and was comparable in both groups (Figure 1). PCA significantly increased locomotion and almost totally inhibited rearing and grooming. These effects of PCA were unaltered by reserpine pretreatment. 5-MeODMT had a negligible effect on locomotion in both vehicle and reserpine-treated rats, decreased rearing significantly and comparably in both of these groups but significantly decreased grooming only in reserpine pretreated rats.

Before injection of PCA, extraneuronal 5-HT was measurable in dialysates from rats pretreated with

Table 2 Effects of reserpine on stereotyped behaviour and motor activity induced by *p*-chloroamphetamine (PCA 5 mg kg^{-1}, i.p.) or 5-methoxy-N,N-dimethyltryptamine (5-MeODMT 5 mg kg^{-1}, i.p.)

	Vehicle + PCA	*Reserpine + PCA*	*Vehicle + 5-MeODMT*	*Reserpine + 5-MeODMT*
Head weaving (frequency)	209.5 ± 24.3	150.2 ± 24.1	26.7 ± 6.3	9.2 ± 1.2*
Forepaw treading (frequency)	305.5 ± 39.4	322.0 ± 52.6	859.3 ± 75.0	802.0 ± 112.2
Hind limb abduction (min)	53.6 ± 1.2	54.5 ± 1.4	29.6 ± 2.6	31.2 ± 5.6
Flat body posture (min)	51.3 ± 4.4	55.0 ± 1.6	28.0 ± 3.5	30.2 ± 6.0
Straub tail (min)	0 ± 0	0.2 ± 0.1**	1.0 ± 0.2	2.2 ± 0.4*
Wet-dog shakes (frequency)	8.7 ± 2.8	16.2 ± 3.5	0.7 ± 0.2	1.0 ± 0.3

Reserpine or vehicle was injected 24 h before PCA or 5-MeODMT. Values are means ± s.e.mean of 6 rats per group. *$P < 0.05$, **$P < 0.02$ with respect to corresponding vehicle-injected rats (Mann-Whitney U test).

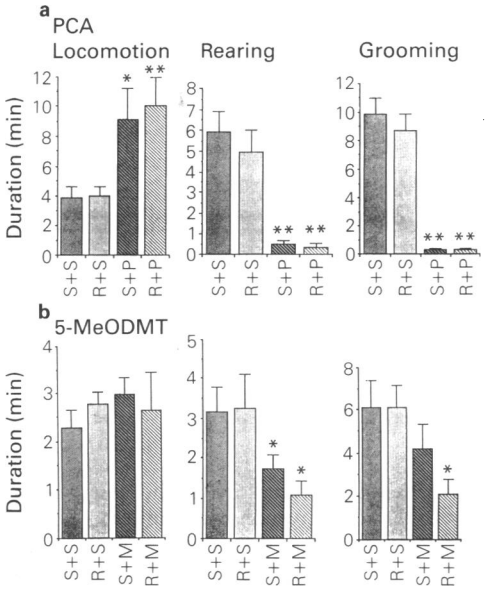

Figure 1 Locomotion, rearing and grooming in rats pretreated with vehicle or reserpine (2.5 mg kg^{-1}, i.p.) and given 0.9% NaCl, *p*-chloroamphetamine (PCA, 5 mg kg^{-1}, i.p.) (a) or 5-methoxy-N,N-dimethyltryptamine (5-MeODMT, 5 mg kg^{-1}, i.p.) (b) 24 h later. S = vehicles, R = reserpine, P = PCA, M = 5-MeODMT. Values are mean durations (min) of each behavioural component for 6 rats; vertical lines indicate s.e.mean. $*P < 0.05$, $**P < 0.01$ with respect to values for appropriate control rats not given PCA or 5-MeODMT (Duncan's multiple range test).

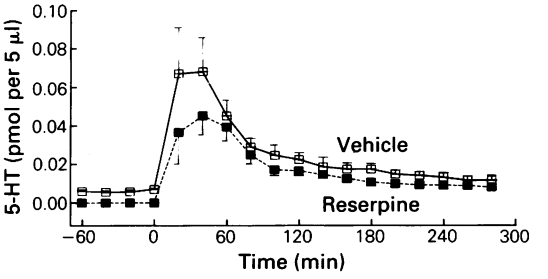

Figure 2 Effect of reserpine (2.5 mg kg^{-1}, i.p.) on the dialysate 5-hydroxytryptamine (5-HT) concentration in frontal cortex before and after *p*-chloroamphetamine (PCA, 5 mg kg^{-1}, i.p. at zero time). Values are uncorrected for recovery. Two way ANOVA showed significant effects of time ($F [14, 209] = 16.22$ and reserpine $F [1,209] = 22.71$; both $P < 0.001$). Basal 5-HT levels were taken as the mean of the four values before PCA. The mean ± s.e.mean was 0.0059 ± 0.0007 pmol per 5 µl ($n = 8$) for vehicle-injected rats and < 0.002 pmol per 5 µl (detection limit of 5-HT) for all reserpine-treated rats ($n = 10$). These values differ significantly ($P < 0.05$). Increases after PCA were significant ($P < 0.05$) over 1 and 4 h in the vehicle and reserpine pretreated groups. Net 5-HT release (increase of area under the curve) over 1 h after giving PCA (i.e. the period over which behaviour was measured) was 0.164 ± 0.047 and 0.122 ± 0.028 pmol ml^{-1} h^{-1} for the vehicle and reserpine pretreated groups, respectively (difference NS). Vertical lines indicate s.e.mean.

vehicle but not from animals pretreated with reserpine, the difference between the two groups being significant (Figure 2). However, dialysate 5-HT rose substantially on giving PCA to either group, peaking at 20–40 min after injection and then declining, but remaining significantly elevated more than 1 and 4 h later for the vehicle and reserpine pretreated groups, respectively. Mean increases over appropriate base-line values were less for the reserpine pretreated rats than for the vehicle pretreated controls at 20 and 40 min after PCA injection and significantly so at 20 min ($P < 0.05$). However, the area under the curve (AUC) for increase of 5-HT over the 1 h after giving PCA (i.e. the period over which behaviour was assessed) was not significantly different for the reserpine and vehicle-pretreated rats, though it was 26% smaller in the former group. Increases of dialysate 5-HT for both groups were similar at all times greater than 40 min after PCA injection. Figure 3 shows the individual peak values for the two groups. It can be seen that the spread of

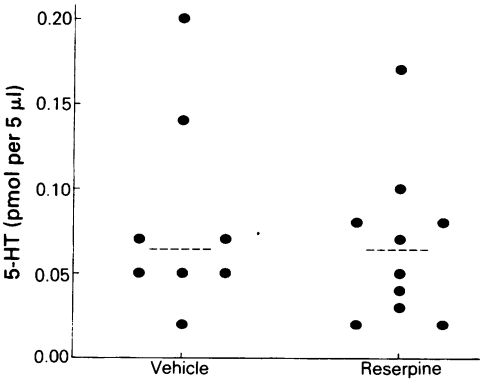

Figure 3 Individual peak values for dialysate 5-hydroxytryptamine (5-HT) after *p*-chloroamphetamine (PCA). General details as described in Figure 1. Values for reserpine and vehicle pretreated groups did not differ significantly. Mean peak times were 30 ± 4 (s.e.mean) and 38 ± 4 min for vehicle and reserpine pretreated groups, respectively (difference NS). Median peak values are shown as broken lines.

values was considerable but the median concentrations were almost identical.

Dialysate 5-HIAA values are shown in Figure 4. After injection of PCA both vehicle and reserpine pretreated groups showed a slow decline in dialysate 5-HIAA concentrations to about 2/3 of base-line values.

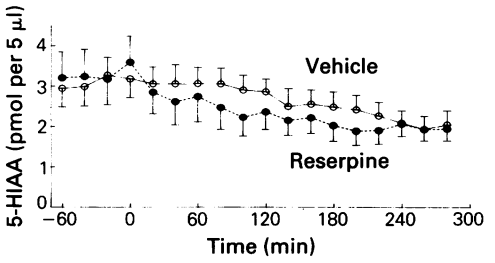

Figure 4 Effect of reserpine (2.5 mg kg^{-1}, i.p.) on dialysate 5-hydroxyindoleacetic acid (5-HIAA) concentration before and after *p*-chloroamphetamine (PCA, 5 mg kg^{-1}, i.p.). General details as described in Figure 1. Overall, reserpine significantly reduced 5-HIAA in dialysates (F [1,213] = 3.96, $P < 0.05$).

Discussion

Base-line dialysate 5-HT levels and increases after giving PCA were much lower than previously found (Sharp *et al.*, 1986; Hutson & Curzon, 1989), possibly because dialysates were previously collected within a few hours of inserting the probe in the earlier studies but a day later in the present experiments. Westerink *et al.* (1987) were only able to detect 5-HT in cortical dialysates on the first day and suggest that this largely came from platelets accumulating around the recently implanted probe. It might also have derived from terminals damaged by insertion of the probe. Our measurements were made at a time which was probably sufficient for healing of damage but not long enough for gliosis to alter the environment of the probe (Benveniste & Diemer, 1987; Benveniste *et al.*, 1987).

The base-line extracellular 5-HT concentration in the region of the dialysis probe as calculated from Figure 2 and *in vitro* 5-HT recovery values was 2.0 pmol ml^{-1}. Using a frontal cortex 5-HT concentration of 1.7 nmol g^{-1} = 2.1 nmol ml^{-1} water (assuming 80% water) previously obtained in our laboratory (Kennett *et al.*, 1986), a tissue/extracellular space ratio for 5-HT of 1050 can be calculated which is comparable with data on other transmitters (Westerink *et al.*, 1987). The corresponding ratio of 1.4 for 5-HIAA is consistent with the ratios of about 1 obtained for transmitter metabolites (Westerink *et al.*, 1987).

After reserpine treatment, although brain 5-HT was decreased by 86% and 5-HT was no longer detectable in cortical dialysates, the components of the 5-HT syndrome induced by the 5-HT releaser PCA were not significantly altered (Table 2), apart from the appearance of slight Straub tail behaviour. These results supplement the data of Kuhn *et al.* (1985) that reserpine-treated rats still showed at least three behavioural components of the response to PCA. One possible explanation is that depletion of 5-HT stores by reserpine and resultant decrease of physiological release of 5-HT (as indicated by the inability to detect it in cortical dialysates) increases postsynaptic receptor sensitivity so that the response to PCA is unaltered. However, despite findings that reserpine enhanced responses to agonists acting at 5-HT (Nakamura & Fukushima, 1978) and dopamine (Arnt, 1985) receptors, it did not alter most components of the response to the 5-HT agonist 5-MeODMT. An exception was the Straub tail which (in agreement with findings on PCA treatment) was increased. Also, head weaving frequency was decreased, in agreement with Tricklebank *et al.* (1985a,b).

The above results, as a whole, argue against reserpine increasing the responses of postsynaptic 5-HT receptors. The increased Straub tail behaviour may result from depletion of dopamine by reserpine as dopamine inhibits this behaviour (Bedard & Pycock, 1977; Andrews *et al.*, 1982; Dickinson & Curzon, 1983; Dickinson *et al.*, 1983). Conversely, the decrease of head weaving agrees with its requirement of dopamine as well as 5-HT (Andrews *et al.*, 1982; Tricklebank *et al.*, 1985a,b). However, forepaw treading and hindlimb abduction respectively require and are inhibited by dopamine (Andrews *et al.*, 1982; Dickinson *et al.*, 1983; Tricklebank *et al.*, 1985a,b) but were unaltered by reserpine, which suggests that residual dopamine was sufficient for their expression. Some availability of catecholamines is also consistent with the finding that reserpine did not alter locomotion, rearing or grooming in vehicle, PCA or 5-MeODMT-treated animals, although 50% decreases of locomotion were observed 24 h after giving 2 and 5 mg kg^{-1} of reserpine (Faith *et al.*, 1968; Geyer & Segal, 1973).

The lack of effect of reserpine treatment on most components of the 5-HT syndrome induced by PCA is largely explicable by the finding that release of neuronal 5-HT by PCA in the 1 h period of behavioural assessment did not decrease significantly. As reserpine depletes vesicular 5-HT (Shore & Giachetti, 1978), from which its physiological release probably occurs (Sanders-Bush & Martin, 1982), these results imply that PCA releases 5-HT by a mechanism which is not of physiological importance.

Although reserpine prevents vesicular storage of 5-HT, it does not alter its synthesis under conditions similar to those of the present study (Carlsson & Lindqvist, 1978), but the new 5-HT molecules remain in a small rapidly turning over cytoplasmic pool from which they are presumably released by PCA. Somewhat similar to our findings with PCA, reserpine has been shown to decrease spontaneous dopamine release but not amphetamine-induced release (Niddam *et al.*, 1985; Butcher *et al.*, 1988).

Unlike reserpine, a range of doses of the 5-HT synthesis inhibitor *p*-chlorophenylalanine (PCPA) attenuated forepaw treading and head weaving induced by PCA in parallel with the degree of depletion of brain 5-HT (Dickinson & Curzon, 1983). Under these circumstances newly synthesized 5-HT molecules are probably less available for release by PCA because of avid uptake by the partially emptied vesicles where, as indicated by high 5-HT/5-HIAA ratios (Curzon *et al.*, 1978; Dickinson & Curzon, 1983), they are protected from metabolism. In contrast, reserpine disrupts vesicular storage so that newly synthesized 5-HT remains in the neuronal cytoplasm and is readily metabolised, as indicated by low 5-HT: 5-HIAA ratios (Table 1), but is available for release by PCA.

Kuhn *et al.* (1985) suggest that the cytoplasmic 5-HT is the 'functional' transmitter pool. We agree inasmuch as we find that PCA releases cytoplasmic 5-HT with behavioural consequences. However, as reserpine without PCA markedly reduced dialysate 5-HT, physiological release of 5-HT probably occurs from vesicular stores, perhaps from a specific small pool of recently synthesized 5-HT (Shields & Eccleston, 1972) distinct from the cytoplasmic pool.

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