The mechanism of tetrahydroaminoacridine-evoked release of endogenous 5-hydroxytryptamine and dopamine from rat brain tissue prisms

T.N. Robinson, R.J. De Souza, A.J. Cross & A.R. Green

Astra Neuroscience Research Unit, 1 Wakefield Street, London WC1N 1PJ

1 Tetrahydroaminoacridine (THA) is an acetylcholinesterase (AChE) inhibitor which may have a greater therapeutic effect in Alzheimer-type dementia (ATD) than other cholinergic agents. This suggests possible non-cholinergic properties. We have therefore studied the effects of THA on the release of endogenous 5-hydroxytryptamine (5-HT) from rat cortical prisms and dopamine from striatal prisms.

2 In the presence of K^+ (1 mM), THA stimulated release of both 5-HT and dopamine. THA ($100\ \mu\text{M}$)-evoked monoamine release was comparable, but not additive with the release produced by K^+ (35 mM). The effect was not maximal at 1 mM THA. THA-evoked release of 5-HT was independent of the presence of Ca^{2+} in the external medium.

3 Drugs acting on the cholinergic system, nicotine, mecamylamine, atropine, oxotremorine, physostigmine and neostigmine (all $10\ \mu\text{M}$) had no effect on 5-HT and dopamine release. 4-Aminopyridine (4-AP), a potent acetylcholine-releasing agent, had no effect on 5-HT release and was approximately 100 fold less active than THA on dopamine release.

4 Both THA and reserpine enhanced the release of 5-HT in the presence of the monoamine oxidase inhibitor, pargyline. Reserpine- but not THA-evoked release was abolished in the absence of pargyline. Reserpine ($5\ \text{mg kg}^{-1}$, i.p.) markedly depleted brain monoamine concentrations 3 h after injection, while THA ($15\ \text{mg kg}^{-1}$, i.p.) had no effect.

5 Chloroamphetamine and fenfluramine both released 5-HT in a Ca^{2+} -independent manner and with a similar potency to THA, while (+)-amphetamine released dopamine with a similar potency to THA. The effects of the amphetamines were not maximal at 1 mM. However, unlike THA, chloroamphetamine-evoked release of 5-HT was additive with release evoked by K^+ (35 mM).

6 Clomipramine ($IC_{50} = 0.036\ \mu\text{M}$), imipramine ($IC_{50} = 0.20\ \mu\text{M}$) and THA ($IC_{50} = 19.9\ \mu\text{M}$) all inhibited the uptake of [^3H]-5-HT into a P_2 membrane preparation. However, none of these compounds inhibited [^3H]-5-HT uptake into tissue prisms during the release experiments in which the reuptake inhibitor fluoxetine ($5\ \mu\text{M}$) was present.

7 We conclude that THA does not release endogenous 5-HT through a cholinergic, reserpine- or amphetamine-like mechanism or through inhibition of reuptake. The possibility exists that the release may occur via blockade of 4-AP-insensitive K^+ channels.

Introduction

Tetrahydroaminoacridine (THA) is a potent, reversible inhibitor of acetylcholinesterase which has been reported to be of therapeutic potential in some patients with Alzheimer-type dementia (ATD) (Summers *et al.*, 1986). The effectiveness of THA in comparison with other agents acting on the cholinergic system has led to the suggestion that THA may exert at least part of its action through some as yet undefined mechanism. The structural similarity

between THA and 4-aminopyridine led Summers *et al.* (1986) to suggest that THA may block neuronal potassium channels in a manner similar to 4-aminopyridine and thus enhance acetylcholine release. Subsequent electrophysiological experiments (Drukarch *et al.*, 1987; Osterrieder, 1987; Rogawski, 1987; Schauf & Sattin, 1987; Stevens & Cotman, 1987) have confirmed that THA blocks potassium channels in a variety of preparations, although, in

general, high concentrations of THA (0.1–1 mM) are required.

More recently Drukarch *et al.* (1988) have found that THA releases radiolabelled noradrenaline, 5-hydroxytryptamine (5-HT) and dopamine from pre-loaded slices, and they suggested that this activity was through a reserpine-like action by interfering with the uptake mechanism of these transmitters into storage vesicles. We describe here the effects of THA on the release of endogenous 5-HT from cortical prisms and dopamine from striatal prisms. The mechanism of action of THA is compared with cholinergic ligands, K⁺-channel blockers, and compounds known to influence monoamine release.

A preliminary account of some of this work has been presented to the British Pharmacological Society (De Souza *et al.*, 1989).

Methods

Animals

Adult male Lister Hooded rats (Harlan Olac, Bicester) were used for all experiments. Animals weighed 200–300 g and were housed in groups of 6 in conditions of constant temperature (21°C) and controlled lighting (light period 07 h 00 min–19 h 00 min) and fed an *ad libitum* diet of RMI expanded pellets and tap water.

Monoamine release from tissue prisms

The release of endogenous 5-HT and dopamine from tissue prisms was measured by a modification of the method of Auerbach & Lipton (1985).

Rats were stunned and killed by decapitation and the brains were rapidly removed. The olfactory tracts were separated from the inferior surface of the frontal cortex and a single coronal cut was made to separate the frontal cortex from the rest of the brain. Frontal cortex was used to measure endogenous 5-HT release. Striata were removed for the measurement of endogenous dopamine release. Prisms of tissue (300 × 300 µm, 45°) were prepared with a McIlwain tissue chopper. The slices were suspended in 30 ml Krebs-Ringer bicarbonate buffer (pH 7.4, containing (mM): NaCl 118.5, KH₂PO₄ 1.18 mM, MgSO₄ 1.18 mM, NaHCO₃ 25 mM, CaCl₂ 2.54 mM and glucose 10). For the measurement of 5-HT release the 5-HT uptake inhibitor, fluoxetine (5 µM) was present and for the measurement of dopamine release, nomifensine (10 µM), a dopamine uptake inhibitor, was present in the buffer throughout unless otherwise stated. Both 5-HT and dopamine release were routinely measured in the presence of 10 µM pargyline.

Tissue prisms were preincubated at 37°C for 1 h, the buffer being changed every 15 min. The buffer was discarded leaving a packed slice suspension. Aliquots of the slice suspension (60 µl, 1 mg protein) were distributed evenly into baskets made from plastic test tubes with nylon mesh bases. The baskets of slices were preincubated in 1 ml of buffer for 10 min at 37°C. The baskets were blotted and immersed in a second set of vials containing 1 ml fresh, pregassed buffer (containing drug where appropriate) and incubated for 5 min (basal release). The baskets were blotted and transferred into vials containing buffer plus 35 mM KCl (containing drug where appropriate) for a further 5 min (K⁺-evoked release). The baskets were then blotted and placed in vials containing 1 ml of 0.1 M NaOH solution containing sodium dodecyl sulphate (0.7% w/v) and NaCO₃ (2% w/v) to solubilize the slices prior to analysis of the protein content by a phenol reagent technique (Dawson & Heatlie, 1984). Supernatants (800 µl) from the basal and K⁺-evoked incubations were mixed with perchloric acid (2 M, containing cysteine, 1% w/v; sodium metabisulphite, 1% w/v; EDTA, 0.1% w/v), centrifuged at 1000 g for 5 min and the supernatants analysed for their 5-HT or dopamine content.

[³H]-5-HT uptake under basal release conditions

Tissue prisms were prepared from rat frontal cortex and incubated as described for the basal release of 5-HT, except that [³H]-5-HT (10 nCi) was present during the 5 min incubation period. The baskets were then rapidly washed twice in ice-cold Krebs buffer before being immersed in solubilising solution as described above. Duplicate aliquots of the solubilised slices were taken for liquid scintillation counting, and protein was assayed in the remainder as described above.

Analysis of 5-HT, dopamine and their metabolites by h.p.l.c.

The chromatographic system comprised a Spectroflow SF 400 pump and a Promis automatic sample injector (Spark-Holland). Residual microparticulate material was removed by a guard column (30 × 4.6 mm, Spheri-5-RP-18). A reversed phase analytical cartridge column (100 × 4.6, Spheri-5-OD-MP, Anachem) was used at a constant temperature of 30°C. Analytes were detected with a model 5010 electrochemical cell (ESA) and the potential across the electrodes (+0.35 V) was applied from an ESA Coulochem controller (model 5100A). The mobile phase (flow rate 1 ml min⁻¹) comprised KH₂PO₄ (0.1 M), EDTA (0.0035% w/v), sodium octane sulphonate (0.016% w/v) and methanol (10%

v/v) and was adjusted to pH 3.0 with concentrated phosphoric acid and degassed before use. Chromatograms were analysed using the peak area ratio of standards to analyte.

Animal treatment

Groups of rats were pretreated with either vehicle (0.9% w/v NaCl), THA (15 mg kg⁻¹), reserpine (5 mg kg⁻¹), fenfluramine (10 mg kg⁻¹) or amphetamine (3 mg kg⁻¹) and the animals killed 3 h later. All drugs were injected i.p. The brains were dissected, and the frontal cortices, striata and hippocampi were removed onto solid CO₂. Monoamines were extracted from these samples and estimated by h.p.l.c. Drugs were dissolved in saline with the exception of reserpine which was dissolved in an acetic acid/water mixture adjusted to pH 5 with 1 M NaOH.

Tissue extraction

Brain regions were homogenized in ice-cold 0.2 M perchloric acid containing 0.1% (w/v) cysteine, 0.1% (w/v) sodium metabisulphite and 0.01% (w/v) EDTA using a 2 ml glass/glass Potter-Elvehjem homogenizer. The homogenates were centrifuged at 15,000 *g* for 5 min and the supernatants analysed for 5-HT, dopamine, and their metabolites by h.p.l.c.

Uptake of [³H]-5-HT by a washed P₂ preparation

Fresh whole rat cortex was homogenized in 10 volumes of 50 mM Tris-HCl, pH 7.4 containing 0.32 M sucrose with a Potter-Elvehjem homogenizer, and the homogenate centrifuged at 1000 *g* for 5 min at 4°C. The supernatant was removed, centrifuged at 20,000 *g* for 20 min at 4°C and the resulting pellet resuspended, by use of a glass/teflon homogenizer in 20 volumes oxygenated Krebs-Ringer phosphate-buffered saline (KRS (mM): NaCl 118, NaH₂PO₄ 32, KCl 4.7, CaCl₂ 1.8, MgSO₄ 1.2, EDTA 1.3, glucose 5.6 and ascorbic acid 1.7; pargyline 100 μM, pH 7.4 with 2 M NaOH). The resuspended preparation was further diluted with KRS to 40 volumes and incubated at 37°C for 15 min before use at this dilution in the [³H]-5-HT uptake assay.

P₂ preparations were incubated in 96-well microtitre plates in the presence of [³H]-5-HT (0.1 μCi per well) and drugs where appropriate. Following 5 min incubation at 37°C the membranes were filtered through GF/B glassfibre filters using a Skatron semi-automatic cell-harvester and the filters washed with 0.9% NaCl (2.5 ml) in 4 s at room temperature. The filters were dried and the radioactivity counted by liquid scintillation counting. Specific [³H]-5-HT uptake was defined as the difference in uptake in the absence and presence of 100 μM imipramine.

Statistics

Where appropriate, data were analysed by a one-way analysis of variance followed by Dunnett's test for differences from control.

Drugs

Sodium-1-octane sulphonic acid was obtained from Eastman-Kodak (Liverpool) and 'h.p.l.c. grade' methanol and H₂O from Rathburn Chemicals (Walkerburn, Scotland). [³H]-5-HT (27.8 Ci mmol⁻¹) was supplied by New England Nuclear Du Pont (U.K.) and fluoxetine and nomifensine were gifts from Eli Lilly Co. (Indiana, U.S.A.) and Hoechst Pharmaceuticals (Hounslow), respectively. 9-Amino-1,2,3,4-tetrahydroacridine HCl hydrate (THA) was supplied by Aldrich Chemical Co. (Dorset) and all other chemicals were obtained from Sigma Chemical Co. (Poole).

Results

Effects of drugs on the release of endogenous 5-HT

The release of endogenous 5-HT from tissue prisms of rat frontal cortex was dependent on the K⁺ ion concentration with a plateau at 50–70 mM K⁺ (data not shown). A K⁺ concentration of 35 mM was used for the standard K⁺-evoked release in all subsequent experiments. THA stimulated the basal release of 5-HT and the release produced by THA (100 μM) was comparable to that evoked by K⁺ (35 mM) (Figure 1). Chloroamphetamine and fenfluramine (100 μM) also caused release of 5-HT (78% and 68% of the K⁺-evoked signal respectively). Reserpine caused less release of 5-HT than the above compounds (33% of the K⁺-evoked release) while 4-aminopyridine (4-AP) did not produce any significant release of 5-HT (Figure 1).

In the presence of THA (100 μM) no additional release of 5-HT was observed in the presence of 35 mM K⁺. However, in the presence of chloroamphetamine (100 μM) or fenfluramine (100 μM) additional 5-HT release was observed with K⁺ (35 mM) (Figure 1).

When 5-HT release was investigated in the absence of added Ca²⁺, the K⁺-evoked signal was abolished (Figure 1), but the release caused by THA was unaffected. K⁺ did not evoke any further release when combined with the drugs in the absence of Ca²⁺ (Figure 1).

THA was more potent than fenfluramine in stimulating basal 5-HT release, but neither of these compounds had produced a maximal effect by 1 mM, at which concentration the release of 5-HT by THA

Figure 1 Effects of drugs on the release of endogenous 5-HT in the presence and absence of Ca^{2+} . Drugs were tested at $10\ \mu\text{M}$ and $100\ \mu\text{M}$ as indicated. Results are the mean of 3–27 experiments, each conducted in triplicate; vertical bars show s.e.mean. In the presence of Ca^{2+} , basal release (open columns) in the presence of drug was tested for significant difference from control basal release. K^{+} -evoked release ($35\ \text{mM}$, hatched column) in the presence of drug was tested against control K^{+} -evoked release. $*P < 0.01$. Experiments conducted in the absence of Ca^{2+} lacked $2.5\ \text{mM}\ \text{CaCl}_2$ in the buffers throughout the experiment. In the absence of Ca^{2+} there was no significant difference between basal and K^{+} -evoked release in each condition ($P < 0.05$).

was comparable to that evoked by K^{+} at $70\ \text{mM}$ (Figure 2).

Effects of drugs on the release of endogenous dopamine

THA and (+)-amphetamine stimulated the basal release of dopamine and the release produced by these compounds at $100\ \mu\text{M}$ was comparable to that evoked by K^{+} ($35\ \text{mM}$) (Figure 3). Reserpine and 4-AP ($100\ \mu\text{M}$) caused less release than THA (22% and 25% of the K^{+} -evoked signal respectively). THA and (+)-amphetamine were of similar potency in releasing dopamine (Figure 4) and these compounds

Figure 2 Concentration-dependency of drug evoked 5-HT release. Results are mean of 3 experiments each conducted in triplicate; vertical bars show s.e.mean. Lack of error bar means it is within the point. Data are expressed as a percentage of K^{+} -evoked release ($35\ \text{mM}$) with basal release subtracted. Tetrahydroaminoacridine ($\square$); fenfluramine ($\blacksquare$).

Figure 3 Effects of drugs on the release of endogenous dopamine. All drugs were tested at $100\ \mu\text{M}$ except neostigmine ($10\ \mu\text{M}$). The release in the presence of $35\ \text{mM}\ \text{K}^{+}$ (hatched column) is also shown. 4-AP, 4-aminopyridine; 3,4-diAP, 3,4-diaminopyridine. Results are the mean of 3–8 experiments, each conducted in triplicate; vertical bars show s.e.mean. Release in the presence of drug was tested for significant difference from control basal release. $*P < 0.01$.

Figure 4 Concentration-dependency of drug evoked dopamine release. Results are mean of 3 experiments, each conducted in triplicate; vertical bars show s.e.mean. Lack of error bar indicates that it is within the point. Data are expressed as a percentage of K^+ -evoked release (35 mM) with basal release subtracted. Tetrahydroaminoacridine ($\square$); (+)-amphetamine, ($\blacksquare$); 4-aminopyridine ($\triangle$).

had produced a near maximal effect by 1 mM. 4-AP was nearly 100 fold less potent than THA in releasing dopamine (Figure 4).

Omission of pargyline from the medium

When the 5-HT release experiments were repeated in the absence of pargyline (Figure 5; compare with Figure 1) the observed 5-HT release was significantly decreased compared with experiments conducted in the presence of pargyline. The basal release of 5-HT produced by THA (100 μ M) was still comparable, but not additive, with the K^+ -evoked release. In contrast, chloroamphetamine-evoked and K^+ -evoked release of 5-HT were additive. The reserpine-evoked release was abolished in the absence of pargyline and the K^+ -evoked signal was significantly reduced ($P < 0.01$) in the presence of reserpine. In the absence of pargyline, fenfluramine did not significantly stimulate basal 5-HT release but the compound did reduce the K^+ -evoked signal ($P < 0.05$).

The effect of drugs acting on the cholinergic system on THA- and 4-AP-induced release of 5-HT and dopamine

Carbachol, nicotine, oxotremorine, physostigmine, atropine and mecamylamine (all at 10 μ M) were without effect on basal 5-HT release, and atropine and mecamylamine had no effect on THA (10 μ M)-evoked 5-HT release (data not shown). Neostigmine

Figure 5 Effect of drugs on basal (open columns) and K^+ -evoked (35 mM, hatched columns) 5-HT release in the absence of pargyline. Release was measured as described in the methods except that buffers lacked 50 μ M pargyline. All drugs were test at 100 μ M. Results are the mean of 3 experiments each conducted in triplicate; vertical bars show s.e.mean. Basal release in the presence of drug was tested for significant difference from control basal release. K^+ -evoked release in the presence of drug was tested for significant difference from control K^+ -evoked release. ** $P < 0.01$; * $P < 0.05$.

(10 μ M) and carbachol (100 μ M) had no effect on basal dopamine release (Figure 3). 4-AP and 3,4-diaminopyridine, stimulated basal dopamine release (Figure 3), an effect not increased in the presence of neostigmine (10 μ M).

Effect of THA on 5-HT reuptake

To examine whether any of the apparent effects on 5-HT release by THA were due to an alteration of reuptake several experiments were performed.

Figure 6 Uptake of [^3H]-5-HT by frontal cortical prisms under basal release conditions. Tissue prisms were incubated with [^3H]-5-HT under release conditions as described in the methods. Slices were prepared and incubated in the absence and presence of $5\text{ }\mu\text{M}$ fluoxetine as indicated. Results are the mean of 3 experiments, each conducted in triplicate; vertical bars show s.e.mean. Control (open columns); tetrahydroaminoacridine ($100\text{ }\mu\text{M}$, hatched columns); imipramine ($100\text{ }\mu\text{M}$, solid columns).

In the absence of fluoxetine, THA ($100\text{ }\mu\text{M}$) produced the same degree of uptake inhibition of 5-HT into cortical prisms as that seen with imipramine ($100\text{ }\mu\text{M}$). However in the presence of fluoxetine ($5\text{ }\mu\text{M}$), a condition under which all earlier release studies were performed, no further inhibition of uptake was observed following addition of THA or imipramine (Figure 6). Furthermore, when inhibition of [^3H]-5-HT uptake was measured in a washed P_2 preparation, THA was over 200 fold less potent than imipramine or clomipramine (Table 1).

Effects of drugs on monoamine and metabolite concentrations in rat brain

Administration to rats of THA (15 mg kg^{-1}) or (+)-amphetamine (3 mg kg^{-1}), did not alter the concen-

Table 1 Inhibition of [^3H]-5-hydroxytryptamine ([^3H]-5-H) uptake in a rat brain P_2 preparation

Compound	IC_{50} (nM)
Clomipramine	36 ± 11
Imipramine	200 ± 9
THA	$19,900 \pm 7200$

The inhibition of [^3H]-5-HT uptake by tetrahydroaminoacridine (THA), clomipramine and imipramine was measured as described in the methods. IC_{50} values (nM) are the mean $\pm$ s.e.mean of three to six experiments.

tration of 5-HT, dopamine or their respective metabolites in the striatum or frontal cortex 3 h later (Table 2). However reserpine (5 mg kg^{-1}) caused pronounced depletion of dopamine and 5-HT with concomitant increases in the concentration of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA); the fall in dihydroxyphenylacetic acid (DOPAC) was modest but statistically significant. A similar pattern of change was seen for 5-HT and 5-HIAA in the striatum and hippocampus (data not shown). Injection of fenfluramine (10 mg kg^{-1}) caused depletion of 5-HT, but not dopamine, in all three brain areas studied. However fenfluramine differed from reserpine in that there was not a concomitant rise in 5-HIAA concentration.

Discussion

THA stimulated both basal 5-HT and dopamine release from brain slices in a concentration-dependent way, the stimulation of 5-HT release being calcium-independent. This agrees with a recent report that THA releases prelabelled [^3H]-5-HT, [^3H]-dopamine and [^3H]-noradrenaline from brain tissue prisms (Drukarch *et al.*, 1988).

The effect was clearly seen at a THA concentration of 10^{-5} M . It is however unlikely that the concentration of THA when given therapeutically would rise above 10^{-6} M (Hsieh *et al.*, 1983). While it is difficult to extrapolate from concentrations required *in vitro* to those necessary *in vivo*, the potency of THA was similar to that of the amphetamines, so an effect of THA *in vivo* cannot be ruled out. The effect of THA on monoamine release could occur through a variety of mechanisms, including blockade of reuptake, depletion of monoamine stores or through a cholinergic mechanism. It is well established that both nicotinic and muscarinic presynaptic heteroreceptors can regulate the release of [^3H]-dopamine from striatal slices (Giorgiueff *et al.*, 1977;

Table 2 Concentrations of monoamines in rat striatum and frontal cortex 3 h following the i.p. injection of saline, tetrahydroaminoacridine (THA, 15 mg kg⁻¹), (+)-amphetamine (3 mg kg⁻¹), fenfluramine (10 mg kg⁻¹) and reserpine (5 mg kg⁻¹)

	DA	Striatum DOPAC	HVA	5-HT	Frontal cortex 5-HIAA	n
Control	4645 ± 230	3086 ± 124	683 ± 47	705 ± 52	347 ± 52	5
THA	3934 ± 480	2958 ± 394	707 ± 76	819 ± 54	419 ± 20	3
(+)-Amphetamine	5012 ± 138	2864 ± 178	930 ± 33	665 ± 24	470 ± 41	6
Fenfluramine	4717 ± 212	2730 ± 196	868 ± 45	**204 ± 26	305 ± 12	6
Reserpine	**259 ± 28	*2183 ± 120	**2432 ± 148	**159 ± 16	**679 ± 40	6

Results are the mean ± s.e.mean from 3–6 animals as indicated (n), and are expressed as ng g⁻¹ wet weight. Drug treatments are compared with control for each monoamine to test for significant differences in each brain region.

**P < 0.01; *P < 0.05.

DA, dopamine; DOPAC, dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid.

Giorguieff-Chesselet *et al.*, 1979; Lehman & Langer, 1982) and synaptosomes (Sakurai *et al.*, 1982; Rapier *et al.*, 1988). It has also been reported that nicotine will stimulate striatal 5-HT release (Westfall *et al.*, 1983).

In addition to its inhibitory activity against AChE (Shaw & Bentley, 1953; Steinberg *et al.*, 1975) THA is centrally active at both muscarinic and, more weakly, at nicotinic receptors (Nilsson *et al.*, 1987; Hunter *et al.*, 1989; Perry *et al.*, 1988; Pearce & Potter, 1988). It was feasible therefore that THA might modulate monoamine release via ACh receptors, either through a direct action or by increasing the concentration of ACh. However, the cholinergic agonist carbachol had no effect on 5-HT or dopamine release (Figure 3) and the selective nicotinic and muscarinic drugs, nicotine, mecamylamine, atropine and oxotremorine, were without effect on 5-HT release under our experimental conditions. Therefore it is unlikely that THA is causing monoamine release through a direct interaction with ACh receptors. Similarly, inhibition of AChE by THA is unlikely to explain the stimulation of monoamine release, as the AChE inhibitors neostigmine and physostigmine were without effect. An alternative explanation might be that THA stimulates the release of ACh and simultaneously potentiates its action on monoamine release through inhibition of AChE (Summers *et al.*, 1986). This is also unlikely however as 4-AP and 3,4-diaminopyridine (3,4-diAP), which are both potent ACh releasing agents (Dolezal & Tucek, 1983; Jones *et al.*, 1988) had markedly less effect than THA on dopamine release (Figure 3) and their action was not potentiated by neostigmine. Furthermore, 4AP did not stimulate 5-HT release. We conclude therefore that THA does not stimulate monoamine release through a cholinergic mechanism.

Reserpine depletes brain monoamine levels through interference with their storage vesicles (Carlsson, 1965) and under our experimental conditions this drug increased basal 5-HT release (Figure 1). THA was more potent than reserpine. However in the absence of pargyline (Figure 5) there was a marked reduction of K⁺-evoked release by reserpine, and no stimulation of basal release. In contrast, in the absence of pargyline THA stimulated basal 5-HT release. Following administration to rats, reserpine depleted 5-HT and dopamine in the striatum with a concomitant rise in their respective metabolites, 5-HIAA and HVA, as previously reported (Kuhn *et al.*, 1985), while THA had no significant effect on the tissue concentrations of monoamines (see Table 2). We conclude therefore that THA does not cause monoamine release by a mechanism analogous to that of reserpine. This conclusion conflicts with that of Drukarch *et al.* (1988) who suggested that THA induced monoamine release through interference with the accumulation of monoamines in storage vesicles. In the study of Drukarch *et al.* (1988) the uptake of radiolabelled monoamines was examined and may therefore have involved a different transmitter pool.

Amphetamine releases a reserpine-resistant pool of endogenous dopamine from slices of rat striatum (Niddam *et al.*, 1985). In our experiments THA and amphetamines caused comparable release of dopamine and 5-HT (Figures 2 and 4). This effect was not maximal at 100 µM THA or 100 µM amphetamine and both drugs at a concentration of 1 mM produced markedly greater release than that caused by 35 mM K⁺. The non-saturating effect of amphetamine on dopamine release is in agreement with previous observations (Parker & Cubeddu, 1988). The similarities between THA- and amphetamine-evoked monoamine release continue when one looks at the

Ca²⁺-dependency of their effects (Figure 1) as the release of 5-HT caused by THA (this paper), fenfluramine and chloroamphetamine (Robinson, unpublished observations) was Ca²⁺-independent. However, the effects of THA and chloroamphetamine on K⁺-evoked 5-HT release as well as basal release, either in the presence (Figure 1) or absence (Figure 5) of pargyline were different. THA (100 µM) released a comparable amount of 5-HT to 35 mM K⁺, but no additional release was achieved when the drug was combined with 35 mM K⁺ while the effects of chloroamphetamine and K⁺ (35 mM) were additive implying that amphetamines cause release from a different pool from K⁺. As the effects of THA are not additive with K⁺, it would seem likely that THA releases 5-HT from a similar pool to K⁺. We therefore conclude that THA does not release monoamines through an amphetamine-like mechanism although, *in vitro*, THA is as potent a releaser of monoamines as amphetamines.

A further mechanism which might result in an apparent increase in release is the blockade of reuptake by THA. While THA at high concentrations can inhibit reuptake (Table 1) this effect would not have been seen in the release studies since the presence of fluoxetine (5 µM) apparently blocks the uptake of [³H]-5-HT (Figure 6). While these data are consistent with the observations of Drukarch *et al.* (1988) that THA can inhibit monoamine uptake, they demonstrate that such inhibition is not responsible for the stimulation of 5-HT release.

Summers *et al.* (1986), when reporting on the use of THA in the long-term treatment of ATD patients, suggested that THA might have pharmacological activity in addition to AChE inhibition, possibly resulting from its structural similarity to 4-AP. The latter blocks K⁺ channels (Llinas *et al.*, 1976; Yeh *et al.*, 1976) and stimulates the basal release of radiolabelled ACh, GABA and glutamate from mouse brain synaptosomes (Tapia & Sitges, 1982) and radiolabelled dopamine from striatal slices (Arzate *et al.*, 1986). THA also blocks K⁺ channels in a number of tissues, although in general high (1–0.1 mM) concentrations are required (Drukarch *et al.*, 1987; Schauf & Sattin, 1987; Rogawski, 1987; Osterrieder, 1987; Stevens & Cotman, 1987) thus raising the possibility that THA might cause mono-

amine release through modulation of K⁺ channels. The K⁺ channel modulators, 4-AP and 3,4-diAP, can stimulate the release of endogenous ACh from slices of rat cortex (Vizi *et al.*, 1977; Dolezal & Tucek, 1983; Jones *et al.*, 1988) but THA was much less potent than 4-AP in this system (unpublished observations). However, 4-AP did not cause release of 5-HT, and although 4-AP and 3,4-diAP released dopamine (Figure 3), 4-AP was almost 100 fold less potent than THA (Figure 4). Additionally, THA-evoked release of 5-HT differs from 4-AP-induced ACh release in being insensitive to the removal of Ca²⁺ from the external medium. Therefore if THA affects monoamine release via K⁺ channels it must be through a different population of channels from the 4-AP-sensitive channels which modulate ACh release and indeed it has recently been postulated that THA acts on different K⁺ currents from 4-AP (Osterrieder, 1987; Stevens & Cotman, 1987). It should be noted that K⁺ channel blockers can cause neurotransmitter release in an apparently Ca²⁺-independent manner (Tapia & Sitges, 1982; Barnes *et al.*, 1987). Recently, Reiner & McGeer (1988) reported that THA increased the action potential duration of central histamine neurones maintained *in vitro* at concentrations as low as 1 µM and this is probably through blockade of K⁺ channels. As the authors point out, the membrane properties of histaminergic neurones are very similar to those of 5-HT neurones. These electrophysiological observations therefore complement our data and taken together, suggest that blockade of K⁺ channels may explain the stimulation of 5-HT release by THA.

When describing the benefits of THA in the treatment of ATD, Summers *et al.* (1986) suggested that it was probable that THA acts like 4-AP in blocking K⁺ channels, and that this was the basis for THA having a more impressive therapeutic activity than classical AChE inhibitors such as physostigmine. We have shown that THA releases endogenous monoamines with an *in vitro* potency similar to that of amphetamine, and that this is not through 4-AP-sensitive K⁺ channels. Bearing in mind the established monoamine deficits in ATD (Cross *et al.*, 1986; Palmer *et al.*, 1988) we consider that the ability of THA to stimulate monoamine release may be of therapeutic value.

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