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Pharmacological Properties of 403U76, a New Chemical Class of 5-Hydroxytryptamine- and Noradrenaline-reuptake Inhibitor

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Abstract

403U76 (5-chloro-[[2-[(dimethylamino)methyl]phenyl]thio]benzene-methanol hydrochloride) is a potent, competitive, inhibitor of 5-hydroxytryptamine (5-HT) and noradenaline reuptake into rat brain synaptosomes. Inhibition of 5-HT uptake in-vivo by 403U76 was demonstrated by potentiation of the behavioural effects of 5-hydroxytryptophan in rats and mice and blockade of *p*-induced depletion of 5-HT in rats.

The firing of 5-HT-ergic dorsal raphe neurons in rats was decreased after intravenous administration of low doses of 403U76 as would be predicted for a 5-HT uptake inhibitor. 403U76 antagonized tetrabenazine-induced sedation, an effect associated with inhibitors of noradrenaline uptake, but not with inhibitors of 5-HT uptake. Thus 403U76 affects noradrenergic as well as 5-HT-ergic neurotransmission in-vivo. Potential anxiolytic activity was indicated by reductions in isolation-induced vocalizations in neonates after 403U76 treatment.

Low intravenous doses of 403U76 were well tolerated and had no sustained cardiovascular effects. There were no deleterious behavioural side-effects at active doses. Effects observed on isolated tissues or transmitter receptors occurred only at very high concentrations and were pharmacologically unimportant. Thus 403U76 can be considered a potential antidepressant/anxiolytic agent that is a potent, selective inhibitor of 5-HT and noradrenaline reuptake.

The biogenic amine hypothesis of endogenous depression prevalent in the mid to late 1960s suggested that depression was due to a deficiency of monoamines, noradenaline and 5-hydroxytryptamine (5-HT) at postsynaptic receptors (Schildkraut 1965; Coppen 1967; Schildkraut & Kety 1967; Lapin 1969; Bunney 1975). Clinical studies suggested that there are two subtypes of depression: one defined by abnormalities in noradrenaline function, the other by an abnormality in 5-HT function (Maas 1975).

The tricyclic antidepressants are believed to be primarily beneficial for depression associated with a deficiency of noradrenaline. Their mechanism of antidepressant action is believed to be due to their ability to block the reuptake of noradrenaline at the transporter in neuronal membranes. These agents produce anticholinergic or cardiovascular side-effects that are undesirable. More recently, a new class of antidepressants, the selective 5-HT reuptake inhibitors (SSRI) have been found to alleviate depression without producing the undesirable side-effects produced by tricyclics. These agents block the reuptake of 5-HT at the transporter in neuronal membranes. In this paper, we describe the pharmacological properties of a new chemical class of reuptake inhibitor, a diphenyl sulphide, which evolved from our attempts to find a novel antidepressant agent.
This compound, 403U76, is shown in Fig. 1.

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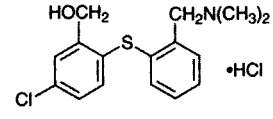

FIG. 1. 5-Chloro-2-[[2-[(dimethylamino)methyl]phenyl]thio]benzene-methanol hydrochloride.

Materials and Methods

Uptake studies

Crude synaptosomal preparations and uptake studies were conducted according to the procedure of Patrick et al (1987).

Inhibition of p-*chloroamphetamine (PCLA) depletion of 5-HT in-vivo*

The ability of 2, 5, 10, 15, or 20 mg kg^{-1} of 403U76 to antagonize PCLA-induced depletions of 5-HT in brain was studied by giving the drug 30 min before an injection of PCLA (10 mg kg^{-1}, i.p.). The rats were killed by decapitation 1·5 h after the administration of 403U76 and each whole brain was assayed for biogenic amine content according to the procedure of Jones-Humble et al (1992).

Binding assays

In-vitro binding assays were conducted according to the procedure and assay conditions listed in Table 1.

Electrophysiological studies

Firing rates of dorsal raphe 5-HT-ergic neurons were

Table 1. Conditions of receptor binding assays.

Receptor	^{3}H-ligand (concn)	Species	Tissue	Displacing agent for nonspecific binding	Reference
Sigma	[^{3}H](+)-SKF 10,047 (3 nM)	Guinea-pig	Whole brain	10^{-5} M (+)-Ethylketocyclozaine	Ferris et al (1986a, b)
α_1-Adrenergic	[^{3}H]WB4101 (0·2 nM)	Rat	Whole brain	10^{-4} M Noradrenaline	Greenburg et al (1976)
α_2-Adrenergic	[^{3}H]Clonidine (4 nM)	Rat	Whole brain	10^{-5} M Noradrenaline	Greenburg et al (1976)
β-Adrenergic	[^{3}H]Dihydroalprenolol (5 nM)	Rat	Cortex	10^{-5} M Propranolol	Ferris & Beaman (1983)
5-HT$_{1a}$	[^{3}H]8-OH-DPAT (0·2 nM)	Rat	Hippocampus	10^{-5} M 5-HT	Peroutka (1986)
5-HT$_2$	[^{3}H]Ketanserin (0·5 nM)	Rat	Frontal cortex	10^{-6} M Ketanserin	Leysen et al (1982)
Adenosine1	[^{3}H]Cyclohexyladenosine (3 nM)	Rat	Whole brain	10^{-5} M 2-Chloroadenosine	Burns et al (1980)
Adenosine2	[^{3}H]Ethylcarboxamide adenosine (4 nM)	Rat	Striatum	10^{-4} M N^6-Cyclopentyl adenosine	Burns et al (1986)
Benzodiazepine	[^{3}H]Diazepam (1·5 nM)	Rat	Forebrain	3×10^{-6} M Diazepam	Marangos & Martino (1981)
Central benzodiazepine	[^{3}H]Zolpidem (1 nM)	Rat	Cerebellum	2×10^{-6} M Ro 15-1788	Langer & Arbilla (1988)
Peripheral benzodiazepine	[^{3}H]Ro5-4864 (0·5 nM)	Rat	Kidney	1×10^{-6} M Ro 5-4864	Langer & Arbilla (1988)
Cholecystokinin-8	[^{3}H]CCK8 (0·2 nM)	Mouse	Cerebral cortex	10^{-6} M CCK4	Saito et al (1981)
Cholinergic M_1	[^{3}H]Pirenzepine (1 nM)	Rat	Forebrain	10^{-6} M Atropine	Watson et al (1983)
Cholinergic M_2	[^{3}H]QNB (0·05 nM)	Rat	Heart	10^{-6} M Atropine	Watson et al (1983)
Dopamine (D_1)	[^{3}H]SCH23390 (0·25 nM)	Rat	Striatum	10^{-6} M (+)-Butaclamol	Hess et al (1986)
Dopamine (D_2)	[^{3}H]Raclopride (1 nM)	Rat	Striatum	10^{-6} M (+)-Butaclamol	Kohler et al (1985)
Leukotriene D_4	[^{3}H]Leukotriene D4 (0·4 nM)	Guinea-pig	Lung	2×10^{-7} M Leukotriene D_4	Cheng & Townley (1984)
Substance P	[^{3}H]Substance P (1 nM)	Rat	Forebrain minus cortex	10^{-5} M Substance P	Viger et al (1983)

5-HT, 5-hydroxytryptamine or serotonin; 8-OH-DAPT, 8-hydroxydipropylaminotetralin; CCK, cholecystokinin, QNB, quinuclidinyl benzilate; GABA, gamma-aminobutyric acid; PAF, platelet-activating factor; ET, endothelin; TBPS, *t*-butylbicyclophosphorothionate.

measured according to the technique of Aghajanian et al (1972). Male Sprague-Dawley rats, 220–280 g, were anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.) and restrained in a stereotactic apparatus. Body temperature was maintained at 36–37°C with a thermostatically controlled heating pad. Most of the spontaneously active 5-HT-containing neurons were located within the co-ordinates of 0·5–1·0 mm anterior from lambda suture, 0–0·2 mm lateral to midline and 5·5–6·5 mm below the dura. Extracellular action potentials were passed through a high impedance amplifier, displayed on a storage oscilloscope and monitored on a window discriminator and audiomonitor. The signal from the window discriminator integrated into a 10-s period was plotted on a chart recorder as rate histograms.

At the end of each experiment, the position of the electrode tip was marked by passing a 25-mA negative current through the recording barrel for 15 min which deposited a discrete spot of fast green dye. The rats were then perfused with 10% formalin solution, the brains removed and sliced in 50 serial sections, stained with cresyl violet and counter-stained with neutral red for histological verification.

The 5-HT-ergic nature of recorded cells was also confirmed pharmacologically by the administration of the 5-HT agonist *N,N*-dimethyltryptamine. 403U76 and other uptake blockers were dissolved in 0.9% NaCl solution and administered through a cannulated lateral tail vein in a volume of 0·05–0·25 mL per injection. Only one cell was recorded per rat.

5-Hydroxytryptophan (5-HTP) test in mice

The method used was a modification of that by Corne et al (1963). Mice were randomly assigned to treatment groups of six per cage. The test substance was administered intraperitoneally or orally at various doses. Ten (i.p.) or thirty-eight minutes (p.o.) later the mice were administered 5-HTP (50 mg kg^{-1}, i.p.) and transferred to individual plexiglass observation cages. Twenty-two minutes following the 5-HTP injection the mice were evaluated for the number of head twitches displayed in a 2-min period. Mice were observed in pairs and head twitches were tabulated by an observer using a key tab lab counter and a timer.

ED50 values were calculated using the method of Corne et al (1963) based on the maximal number of head twitches observed after 800 mg kg^{-1} 5-HTP alone.

5-HTP test in rats

The method used was a modification of that previously described (Smith & Peroutka 1986). Rats were randomly assigned to treatment groups of six per cage. For potency determination, the test substance was administered orally over a range of doses 135 min before intraperitoneal administration of 75 mg kg^{-1} 5-HTP. The rats were scored 45 min later, 3 h after the initial dosing. Scoring consisted of a 5-min observation period, during which the number of wet-dog shakes and presence of the serotonin syndrome (forepaw treading, tremor, hind limb abduction, Straub tail, and rigidity) for each rat was recorded. Each wet-dog shake resulted in a score of 1. The presence of three or more of the serotonin syndrome symptoms resulted in an automatic score of 10, the maximal score for any individual rat. A score of 60 for any given group was equal to 100% potentiation and ED50 values were calculated according to the percent maximal response using the method of Litchfield & Wilcoxon (1949).

Tetrabenazine test in rats

Groups of six rats were given either vehicle, 403U76 or fluoxetine at 12·5, 25, and 50 mg kg^{-1} intraperitoneally and desmethylimipramine (5 mg kg^{-1}, i.p.) as the positive control. Compounds or vehicle were given 30 min before tetrabenazine (20 mg kg^{-1}, i.p.). Thirty minutes after tetrabenazine administration, the following symptoms were scored by a blind observer: blepharoptosis (0–4 points), muscle rigidity (0–4 points) and sedation as reflected in decreased exploratory activity (0–8). The sum of the scores on the rating scale were converted to a percent of the rating for the desmethylimipramine positive controls and were corrected for the ratings, if any, received by rats given vehicle before tetrabenazine.

Anxiolytic test in rats

Neonates and their mothers were housed together throughout the experiment except when neonates were marked, weighed, and tested. Thirty minutes after entering the lab, each mother was removed from her litter and her neonates were individually placed in a 35 cm × 35 cm arena. The number of ultrasonic vocalizations was measured for a 1-min session using a QMC Mini Bat Detector suspended 20 cm above the arena floor. The vocalizations were measured using 42 kHz as the center of a 10-kHz recording range. The neonate's activity for each session was measured by the number of photocell crossings using a Columbus instruments Opto-Varimax activity meter.

The mother was returned to her neonates after the entire litter had been tested. Three to four hours after the first session, neonates were injected intraperitoneally with vehicle or test compound. After injection, neonates were exposed to a second 1-min test session where ultrasonic vocalizations and activity were recorded. The response to the drugs was not considered a clearly identifiable anxiolytic effect when a significant change in vocalization was accompanied by a significant change in activity. Drugs causing both activity and vocalization changes at the same doses were arbitrarily considered as nonselective and not anxiolytic.

Cardiovascular studies in dogs

The acute haemodynamic profile of 403U76 was assessed according to the procedures of Steffen & Wastila (1992). 403U76 was administered to two groups of adult male beagle dogs weighing 10–12 kg. The dogs were anaesthetized with pentobarbitone sodium (30 mg kg^{-1}, i.v.) and intubated and ventilated with room air via a Harvard Apparatus respirator adjusted to deliver a volume of 13 mL kg^{-1} at a rate of 20 strokes min^{-1}. A high-dose group (n = 6) and a low-dose group (n = 3) of dogs were studied. In the high-dose group, 403U76 was administered via a cannula in the left femoral vein as five consecutive doses of 0·1, 0·3, 1·0, 3·0, and 10·0 mg kg^{-1} at 30-min intervals. All doses were administered as a bolus except the 10·0 mg kg^{-1} dose which was infused over a 10-min period.

In the low-dose group, 403U76 was administered as three consecutive bolus doses of 0·01, 0·03, and 0·1 mg kg^{-1} at 30-min intervals. Vehicle was administered in an identical manner at the equivalent volumes of 0·1, 0·3, 1·0, 3·0, and 10·0 mL at the respective time intervals.

Data analysis

Data are expressed as mean percent change from pretreatment control values. Intragroup analysis was performed using paired Student's *t*-test. Intergroup analysis was performed using unpaired Student's *t*-test.

Results

Neurochemical studies

Inhibition of biogenic amine uptake. The ability of 403U76 to inhibit uptake of biogenic amines was tested in synaptosomal preparations from rat hypothalamus and striatum. 403U76 inhibited neuronal uptake of 5-HT into hypothalamic synaptosomes (Table 2) with an IC50 value of 2·1 ± 0·4 × 10^{-9} M. It was a potent, but weaker (one twenty-sixth) inhibitor of neuronal uptake of noradrenaline into striatal synaptosomes with an IC50 value of 5·5 ± 1·0 × 10^{-8} M. Dopamine uptake into striatal synaptosomes was not affected at concentrations as high as 10^{-5} M. Fluoxetine, a SSRI, inhibited 5-HT and noradrenaline uptake with IÇ50 values of 1·2 ± 0·4 × 10^{-7} and 1·1 ± 0·1 × 10^{-6} M, respectively. Fluvoxamine, another SSRI, and imipramine, a tricyclic antidepressant which inhibits 5-HT and noradrenaline uptake, were also less potent than 403U76 as inhibitors of 5-HT uptake (Table 2).

Inhibition of PCLA depletion of 5-HT in-vivo. Systemic administration of PCLA depletes brain 5-HT, an action requiring entry into the neuron via the 5-HT uptake site. Blockade of PCLA-induced 5-HT depletion is evidence of inhibition of 5-HT uptake in-vivo. Pretreatment of rats with 403U76 blocked PCLA depletion of 5-HT in a dose-dependent manner, with an oral ED50 of 5·5 mg kg^{-1}. These results confirm the inhibition of 5-HT uptake seen in-vitro.

In vitro binding assays. 403U76 had little or no pharmacologically significant binding affinity in-vitro at the adrenergic (α_1, β), 5-HT-ergic (5-HT_{1a}, 5-HT_2), dopaminergic (D_1, D_2), benzodiazepine, substance P, leukotriene D4, adenosine (A1, A2), GABA or cholecystokinin receptors. It bound weakly to muscarinic (M_1 and M_2) receptors with IC50 values of 1 × 10^{-6} M, to the α_2-receptor with an IC50 of 8·8 × 10^{-7} M, and to the sigma receptor with an IC50 of 2 × 10^{-6} M. None of these binding affinities are potent enough to suggest an alternate mechanism of action for 403U76 (Table 3).

Electrophysiological studies

Effects on firing of dorsal raphe 5-HT-ergic neurons. 5-HT-reuptake inhibitors decrease the firing rates of 5-HT-ergic neurons in the dorsal raphe nucleus by enhancing feedback inhibition. Uptake blockade increases 5-HT concentrations at synapses within the raphe nucleus, which stimulates 5-HT_{1a} autoreceptors on 5-HT neurons. Stimulation of 5-HT_{1a} receptors results in neuronal hyperpolarization and reduced neuronal excitability. A rate histogram demonstrating the effect of 403U76 on the firing rate of a single raphe neuron is shown in Fig. 2. In chloral hydrate-anaesthetized

Table 2. Effects of 403U76, fluoxetine and imipramine on radiolabelled neurotransmitter uptake into synaptosomes prepared from rat brain.

	IC50 (M) for inhibition of biogenic amine uptake[a]		
	Noradrenaline	Dopamine	5-HT
403U76	5·5 ± 1·0×10^{-8}	14·3 ± 3·8%	2·1 ± 0·4×10^{-9}
Fluoxetine	1·1 ± 0·1×10^{-6}	22·6 ± 1·6%	1·2 ± 0·4×10^{-7}
Fluvoxamine	1·6 ± 1·1×10^{-6}	1·0 ± 1·3%	2·7 ± 0·7×10^{-8}
Imipramine	1·5 ± 0·8×10^{-7}	7·0 ± 1·7×10^{-5}	5·0 ± 0·4×10^{-7}

[a]The concentration of compound which inhibits noradrenaline, dopamine or 5-HT uptake by 50% (IC50, M) was determined in rat brain synaptosomes. IC50 values or percent inhibition > 10^{-5} M are means ± s.e.m. of 3-17 determinations.

Table 3. Effect of 403U76 on the specific binding of various ligands to membrane fragments obtained from different brain areas.

Receptor	Drug concn (M)	Inhibition of specific binding (%)
Sigma	$2{\cdot}3 \pm 0{\cdot}7 \times 10^{-6}$	50
Muscarinic (M_1)	$1{\cdot}5 \pm 0{\cdot}3 \times 10^{-6}$	50
Muscarinic (M_2)	$1{\cdot}3 \pm 0{\cdot}4 \times 10^{-6}$	50
5-HT_{1A}	1×10^{-5}	37 ± 11
5-HT_2	1×10^{-5}	65 ± 3
Dopamine D_1	1×10^{-5}	15 ± 4
Dopamine D_2	1×10^{-5}	46 ± 13
α_1-Adrenergic	1×10^{-5}	0
α_2-Adrenergic	$8{\cdot}8 \pm 0{\cdot}8 \times 10^{-7}$	50
β-Adrenergic	1×10^{-5}	0
Adenosine A1	1×10^{-5}	0
Adenosine A2	1×10^{-5}	0
GABA	1×10^{-5}	0
Cholecystokinin	1×10^{-5}	0
Benzodiazepine	1×10^{-5}	0
Substance P	1×10^{-5}	0
Leukotriene D_4	1×10^{-5}	0

Receptor binding studies were performed under the conditions listed in Table 1. Values given are concentrations of 403U76 which inhibit total binding by 50% (IC50, M) or percent inhibition at 1×10^{-5} M. Mean ± s.e.m. of 3–4 experiments performed in duplicate are reported.

rats, 403U76 decreased neuronal firing with an intravenous of 0·0088 mg kg^{-1} dose. By comparison, fluoxetine, fluvoxamine, and imipramine had ED50 values of 0·96, 0·23 and 0·78 mg kg^{-1}, respectively. Of the 5-HT-uptake inhibitors tested, 403U76 was found to be the most potent inhibitor of raphe firing by the intravenous route. These data are further evidence that 403U76 is a potent 5-HT-uptake inhibitor.

Behavioural studies

Potentiation of 5-HTP in-vivo. Administration of high doses of the 5-HT precursor 5-HTP to rats or mice induces symptoms collectively known as the serotonin syndrome, which result from increased 5-HT synthesis and subsequent stimulation of 5-HT receptors. Selective 5-HT uptake inhibitors potentiate the behavioural effects of a threshold dose of 5-HTP by preventing inactivation of 5-HT via reuptake systems present in neuronal membranes. Two symptoms of the serotonin syndrome, head twitch in mice and whole body wet-dog shakes in rats, can be easily quantified and were used to evaluate 5-HTP potentiation.

In mice, 403U76 potentiated the effects of 5-HTP (Fig. 3A), with ED50 values of 1·2 mg kg^{-1}, intraperitoneally, and 8·4 mg kg^{-1} orally vs ED50 values of 4·3 and 13·1 mg kg^{-1} for fluoxetine. In rats, (Fig. 3B) the ED50 values for 5-HTP potentiation were 1·4 mg kg^{-1} intraperitoneally and 9·6 mg kg^{-1} orally for 403U76, and 22·0 and 17·0 mg kg^{-1} for fluoxetine. Fluvoxamine was less potent than 403U76 in the 5-HTP potentiation test in mice, and imipramine was inactive. The duration of action for 403U76 in the 5-HTP potentiation test following oral administration was approximately 10 h in mice and 6 h in rats.

Antagonism of tetrabenazine-induced sedation in-vivo. Antagonism of tetrabenazine-induced sedation is commonly used to screen for potential antidepressants. Most drugs which antagonize tetrabenazine-induced sedation, potently and selectively block noradrenaline reuptake. Rats were intraperitoneally administered 403U76 30 min before an intraperitoneal injection of tetrabenazine (20 mg kg^{-1}), and then scored for sedation 30 min later. 403U76 antagonized tetrabenazine-induced sedation with an ED50 value of 29·0 mg kg^{-1}. The selective 5-HT uptake inhibitor, fluoxetine, was inactive in this test at doses up to 50 mg kg^{-1}, whereas the tricyclic antidepressant desmethylimipramine, which selectively inhibits noradrenaline reuptake, was active with an ED50 of 1·8 mg kg^{-1} i.p. It is unusual for a compound (even dual inhibitors of 5-HT and noradrenaline uptake) to both potentiate 5-HTP and to antagonize tetrabenazine-induced sedation. These results suggest that 403U76 may exhibit antidepressant properties associated with 5-HT uptake inhibitors and noradrenaline uptake inhibitors in man.

Effects of 403U76 on isolation-induced ultrasonic vocalizations in rat pups. Rat pups emit species typical 42 kHz ultrasonic vocalizations when separated from their mothers and litter mates and placed in a novel environment. Anxiolytic drugs and 5-HT uptake inhibitors (which are effective in some anxiety related disorders) reduce the rate of vocalization without a change in the pups' overall motor activity. In this test 7 to 12-day-old pups were separated from their mothers and ultrasonic vocalizations and motor activity monitored.

403U76 reduced the vocalization rates of separated pups

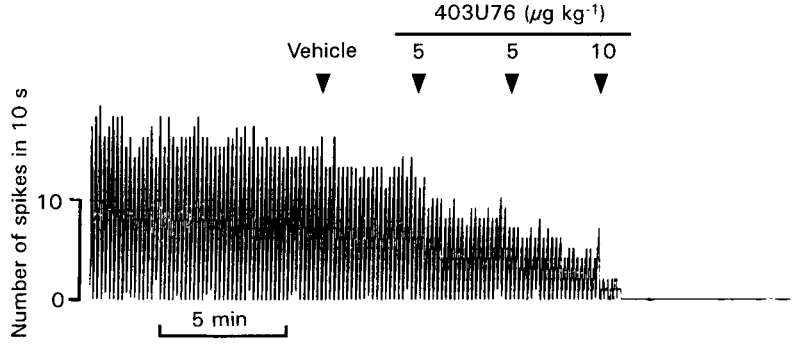

FIG. 2. Effects of cumulative intravenous doses of 403U76 on the firing rate of a 5-HT neuron in the dorsal raphe. The spontaneous firing was reduced in a dose-dependent manner and firing was totally blocked after a cumulative dose of 20 mg kg^{-1}.

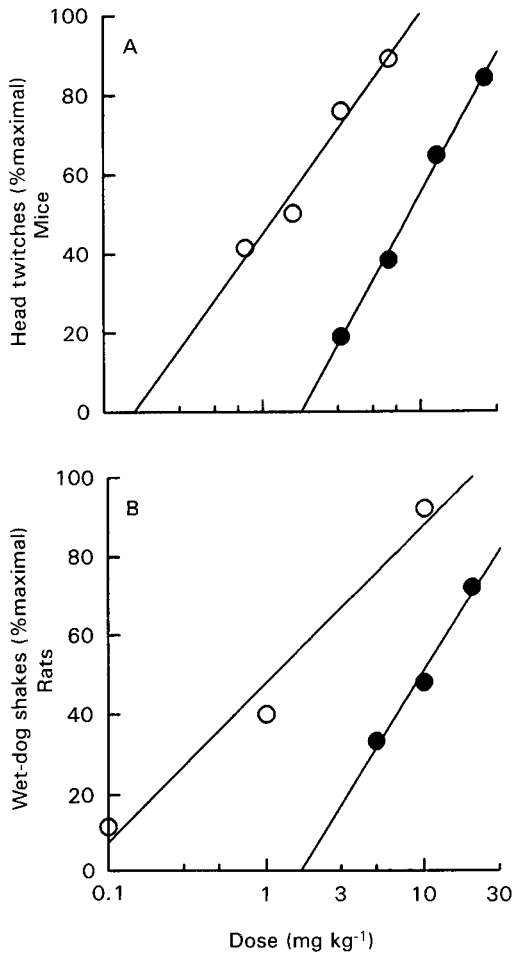

FIG. 3. Potentiation of the behavioural effects of 5-hydroxytryptophan in mouse (A) and rat (B) by 403U76. Number of head twitches were counted in mouse (n = 18) and number of whole body wet-dog shakes were counted in rat (n = 18). ED50 values were calculated using method of Corne et al (1963). Administration: ○ intraperitoneal, ● oral.

without affecting motor activity, with an ED50 of 0.15 mg kg^{-1} (Table 4). Of all the 5-HT uptake inhibitors tested, 403U76 was the most potent in reducing vocalizations. In fact, the compound was more potent than buspirone, a clinically effective anxiolytic drug with 5-HT_{1a}-receptor agonist activity. These results suggest that 403U76 may have anxiolytic as well as antidepressant activity.

Cardiovascular and autonomic effects of 403U76

Acute haemodynamic and ECG effects in pentobarbitone anaesthetized dogs. 403U76 was administered as bolus injections (0·01–3·0 mg kg^{-1}) and as a 10-min infusion at a dose of 10·0 mg kg^{-1} in open-chested dogs. Low doses (0·01–0·1 mg kg^{-1}) of 403U76 (Fig. 4) produced transient dose-dependent increases in mean arterial pressure (MAP) and total peripheral resistance (TPR) and transient dose-dependent decreases in heart rate (HR), cardiac index (CI) and left ventricular dP/dt ($LV_{dP/dt}$). These effects were not statistically significant and each parameter returned to pretreatment values within 80 min after the final dose. Bolus intravenous doses of 1·0 and 3·0 mg kg^{-1} produced variable (±10%) effects on HR and CI, whereas MAP and TPR decreased (10–60%) in a dose-dependent manner. Following the 3·0-mg kg^{-1} dose, MAP and $LV_{dP/dt}$ remained decreased 25% below pretreatment values. A subsequent dose of 10 mg kg^{-1} infused over a period of 10 min had minimal additional effects. The observed effects were not long-lasting and 1 h after the administration of a cumulative dose of 14·4 mg kg^{-1}, MAP, HR, CI, TPR, and $LV_{dP/dt}$ had returned to 80, 95, 80, 108 and 72 of pretreatment values, respectively. The observation that the cardiovascular effects of bolus injections of 403U76 were markedly attenuated following the 10·0-mg kg^{-1} infusion suggests that the haemodynamic effects observed after bolus administration of high doses of 403U76 were most likely due to very high, transient plasma levels. The overall haemodynamic profile observed after the highest doses of 403U76 were very similar to the profile observed after the rapid intravenous administration of tricyclic antidepressants.

General pharmacology over extended dose range in mice and rats

Mice and rats were treated with various doses of 403U76 and observed for changes in respiratory rate, behavioural reflexes, rectal temperature and analgesia. The animals were observed for up to 4 h and then followed for 7 days for delayed signs, including death.

In both species muscle twitches, vasodilation and slight to moderate hypothermia were observed (40 mg kg^{-1} and above in mice, 100 mg kg^{-1} and above in rats). The compound affected the traction reflex and coordination reflex in mice with oral ED50 values of 190 and 190 mg kg^{-1}, respectively, and in rats with oral ED50 values of 250 and 72·5 mg kg^{-1}, respectively.

There were no delayed signs or deaths at doses up to 250 mg kg^{-1}, orally. By the oral route the LD50 for 403U76 was > 250 mg kg^{-1} in both mice and rats. By the intraperitoneal route of administration, 403U76 produced minimal signs of toxicity in both species. Moderate hypothermia, tremors, Straub tail, opisthotonos and clonic convulsions were central nervous system signs observed at doses of 100 mg kg^{-1} and above. Autonomic and respiratory effects included exophthalmos, salivation, gasping and slight respiratory rate increase (100 mg kg^{-1} and above). In addition, rolling, marked cyanosis, spastic movement and abdominal constriction were noted (100 mg kg^{-1} and above). Behavioural reflexes, with the exception of co-ordination (rats: ED50 > 40 mg kg^{-1}, i.p.), were unaffected at doses up to 40 mg kg^{-1}. The only delayed sign in rats was hunched posture.

Table 4. ED50 values (mg kg^{-1}, i.p.) and 95% confidence limits for compounds active in the ultrasonic vocalization test in rats.

	ED50	Confidence limits
5-HT-uptake inhibitors		
403U76	0·15	0·09–0·25
Fluvoxamine	0·76	0·48–1·21
Sertraline	0·82	0·52–1·30
Chlorimipramine	1·67	0·96–2·91
Fluoxetine	2·25	1·48–3·40
5-HT_{1a} agonists		
Buspirone	0·48	0·29–0·80

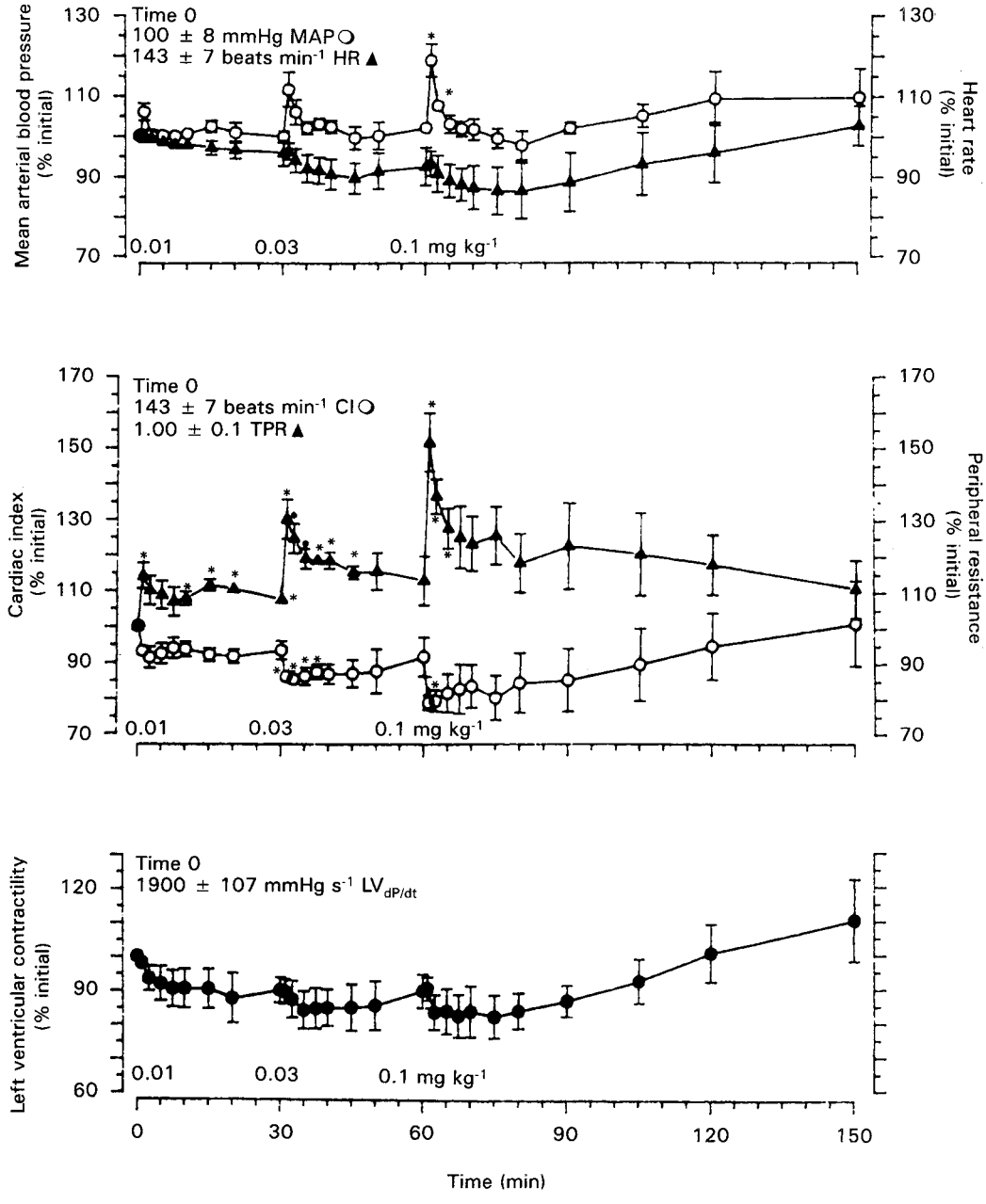

FIG. 4. The haemodynamic effects of 403U76 in anaesthetized beagle dogs (n = 5). Top panel: mean arterial blood pressure (MAP) and heart rate (HR). Middle panel: cardiac index (CI) and calculated total peripheral resistance (TPR). Bottom panel: left ventricular contractility ($LV_{dP/dt}$). 403U76 was administered intravenously at the indicated doses and times. Values in the upper left of each panel indicate the control values at time 0 for each parameter. Values represent the percent change from pretreatment control. Intragroup analysis was performed using paired Student's *t*-test. Intergroup analysis was performed using unpaired Student's *t*-test. $^{*}P$ values less than 0·05 were considered significant.

403U76 showed no delayed mortalities at intraperitoneal doses up to 100 mg kg^{-1}. The approximate LD50 in both species was 160 mg kg^{-1}.

In summary, 403U76 is a potent, competitive inhibitor of 5-HT uptake into rat brain synaptosomes ($IC50 = 2{\cdot}1 \times 10^{-9}$ M). The compound also has potent noradenaline-uptake inhibiting properties ($IC50 = 5{\cdot}5 \times 10^{-8}$ M). Inhibition of 5-HT uptake in-vivo by 403U76 was demonstrated by potentiation of the behavioural effects of 5-hydroxytryptophan (a 5-HT precursor) in rats and mice and blockade of *p*-chloroamphetamine-induced depletion of 5-HT in rats. The firing of 5-HT-ergic dorsal raphe neurons in rats was decreased after intravenous administration of low doses of 403U76, as would be predicted for a 5-HT-uptake inhibitor. 403U76 antagonized tetrabenazine-induced sedation, an effect associated with inhibitors of noradrenaline uptake, but not selective 5-HT uptake inhibitors. These data suggest that the compound affects noradrenergic as well as 5-HT-ergic

neurotransmission in-vivo. Potential anxiolytic activity was indicated by reductions in isolation-induced ultrasonic vocalizations in rat pups.

403U76-induced transient changes in heart rate and blood pressure at low bolus intravenous doses and a cardiovascular depressant effect at high doses in anaesthetized dogs. These cardiovascular depressant effects were most likely due to very high transient plasma levels commonly associated with bolus intravenous administration. Compound 403U76 at doses used to treat depression should be free of most deleterious side-effects commonly associated with antidepressant drugs.

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