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Sertraline, 1S,4S-N-Methyl-4-(3,4-Dichlorophenyl)-1,2,3,4-Tetrahydro-1-Naphthylamine, a New Uptake Inhibitor with Selectivity for Serotonin

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ABSTRACT

Sertraline [1S,4S-N-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthylamine] was found to be a highly selective and potent competitive inhibitor of synaptosomal serotonin uptake. Sertraline also selectively reduced *ex vivo* uptake of serotonin and strongly antagonized the serotonin-depleting action of *p*-chloroamphetamine, indicating potent blockade of serotonin uptake *in vivo*. Acute and repeated dosing of sertraline decreased serotonin content of whole blood. Sertraline only weakly inhibited rat heart uptake of i.v. [^{3}H]norepinephrine. In substantiation of selective blockade of serotonin uptake, sertraline potentiated various symptoms of 5-hydroxytryptophan but did not reverse reserpine-induced hypothermia. Sertraline was a very weak inhibitor of [^{3}H]quinuclidinyl benzilate binding to rat brain membranes *in vitro* and did not produce anticholinergic effects in mice *in vivo*. Sertraline was well tolerated in mice, rats and dogs, with no locomotor stimulant effects in rats or untoward cardiovascular effects in dogs. The major metabolite, N-demethylsertraline, was also a selective serotonin uptake blocker. Sertraline strongly reduced immobility of mice in the Porsolt swim test for antidepressants. After repeated dosing in rats, sertraline diminished the cyclic AMP response of limbic forebrain adenylate cyclase to norepinephrine, as well as the binding of [^{3}H]dihydroalprenolol to cortical membranes. It is proposed that selective blockade of serotonin reuptake can induce activation of norepinephrine neurons and subsequent down-regulation of norepinephrine receptors and that sertraline, a highly selective inhibitor of serotonin uptake, may be an efficacious antidepressant without anticholinergic or cardiovascular side-effects.

The need for drugs which lack the obtrusive and limiting side effects of the tricyclic antidepressants has prompted the search for agents with greatly enhanced selectivity for specific mechanisms believed to be essential for antidepressant efficacy. The potential role of derangements of 5-HT pathways in the etiology of depression has long been suspected. Low levels of the 5-HT metabolite, 5-HIAA, have been found in the cerebrospinal fluid of depressed patients as have abnormal serum levels of 5-HT and its precursor, tryptophan (for reviews, see Saletu *et al.*, 1977; Garver and Davis, 1979; Cooper, 1979; Sugrue, 1981). Also, there are some groups of depressed patients who show a particularly good response to tricyclic antidepressants with a strong component of 5-HT uptake blockade (Maas, 1975). This has given impetus to the development of newer compounds which accentuate inhibition of 5-HT reuptake, *e.g.*, zimelidine, fluoxetine and fluvoxamine (Shopsin *et al.*, 1981). Experience with these agents has suggested that even more selective 5-HT reuptake inhibitors may possess considerable therapeutic value in terms of the ratio of efficacy to side effects.

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In addition to a role for 5-HT in depression, the involvement of NE has been inferred from observations that drugs effective in affective disorders ostensibly facilitate NE pathways in brain, such as tricyclic NE uptake blockers and MAO inhibitors (Koe, 1975; Garver and Davis, 1979; Sugrue, 1981). Recently, the role of NE has been given a new perspective by the findings that down-regulation of a NE receptor-coupled adenylate cyclase as well as *beta* adrenergic receptors is induced by chronic but not acute administration of antidepressant drugs (Vetulani *et al.*, 1976; Banerjee *et al.*, 1977). The relatively slow appearance of therapeutic effects of antidepressant drugs could coincide with this delayed onset of down-regulation of postsynaptic NE receptors in rats. A hypothesis is proposed which combines both 5-HT and NE involvement and suggests that 5-HT uptake blockers, by suppressing the firing of raphe 5-HT neurons (Sheard *et al.*, 1972), would relieve the tonic inhibition on NE neurons of the locus ceruleus. Although the reported studies on inhibition of firing of 5-HT neurons by 5-HT uptake blockers are acute experiments, an important characteristic of these agents is the reduction of 5-HT synthesis and turnover after

ABBREVIATIONS: 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; NE, norepinephrine; MAO, monoamine oxidase; DA, dopamine; PCA, *p*-chloroamphetamine; QNB, quinuclidinyl benzilate; DHA, dihydroalprenolol; 5-HTP, 5-hydroxytryptophan; PCPA, *p*-chlorophenylalanine.

chronic as well as acute administration (Sugrue, 1981). This property, suggestive of decreased functional activity of 5-HT neurons (Sugrue, 1981; Ögren *et al.*, 1979), would lead to activation of NE neurons and subsequently to down-regulation of postsynaptic NE receptors. Thus, selective 5-HT uptake blockers should be efficacious antidepressants, unencumbered by direct cardiovascular and anticholinergic side effects.

Our laboratories have been interested in the stereochemical and conformational determinants of monoamine uptake blocking activity and reported previously on the stereoselective blockade of NE, DA and 5-HT uptake by tametraline, (+)-*trans*-(1R,4S)-N-methyl-4-phenyl-1,2,3,4-tetrahydro-1-naphthylamine (Sarges *et al.*, 1974; Koe, 1976). Of particular interest is the present finding that the new compound depicted in figure 1, (+)-*cis*-(1S,4S)-N-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthylamine (sertraline), possesses potent and selective 5-HT uptake blocking activity. This communication describes the neurochemical, behavioral and pharmacological characterization of sertraline as a well-tolerated, highly selective 5-HT uptake inhibitor devoid of anticholinergic activity. Especially noteworthy is our finding that sertraline on repeated administration causes a down-regulation of NE receptor-coupled adenylate cyclase of limbic forebrain and *beta* adrenoceptors of cerebral cortex in rats, an effect which may be predictive of antidepressant efficacy (Mobley and Sulser, 1981).

Methods

Materials. The four isomers of N-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthylamine, CP-51,973 HCl, sertraline (CP-51,974) HCl, CP-52,002 HCl and CP-52,003 HCl (MW 343) and (+)-*cis*-(1S,4S)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthylamine (CP-62,508 HCl hemihydrate) were synthesized at Pfizer Central Research (Groton, CT).

Standard drugs used in this study were gifts: EXP-561 (4-phenylbicyclo[2.2.2]octan-1-amine) HCl·H_2O from E. I. duPont de Nemours & Co. (Wilmington, DE); fluvoxamine maleate from Phillips-Duphar B.V. (Weesp, The Netherlands); zimelidine HCl and norzimelidine HCl from Astra Läkemedel AB (Södertälje, Sweden); fluoxetine HCl from Eli Lilly and Co. (Indianapolis, IN); chlorimipramine HCl, imipramine HCl and desipramine HCl from Ciba-Geigy Corp. (Summit, NJ); benztropine mesylate, amitriptyline HCl and nortriptyline HCl from Merck, Sharp & Dohme (West Point, PA); doxepin HCl, tametraline (CP-24,441) HCl from Pfizer Inc.; Org 6582 (*dl*-8-chloro-11-anti-aminobenzo(*b*)-bicyclo[3.3.1]nona-3,6a(10a)-diene) HCl from Organon Inc. (West Orange, NJ); and tetrabenazine from Hoffmann-La Roche Inc. (Nutley, NJ).

Other compounds were obtained from commercial sources: PCA HCl from Regis Chemical Company (Chicago, IL); pargyline HCl from Saber Laboratories, Inc. (Morton Grove, IL); *d*-amphetamine sulfate from Mann Research Laboratories (New York, NY); atropine sulfate from Merck & Co., Inc. (Rahway, NJ); oxotremorine (base and sesquifumarate) and reserpine from Aldrich Chemical Co. Inc. (Milwaukee, WI); and scopolamine HBr, ICN Pharmaceuticals, Inc. (Plainview, NY). Labeled monoamines were: 5-[1,2-^{3}H(N)]-hydroxytryptamine binoxalate, 32 Ci/mmol; 3,4-[7-^{3}H(N)]-dihydroxyphenylethylamine, 27 Ci/mmol; DL-[7-^{3}H(N)]NE HCl, 13 Ci/mmol; L-[benzilic-4,4′-^{3}H]QNB, 33 Ci/mmol; and [side chain-2-^{14}C]-tryptamine bisuccinate, 59 mCi/mmol from New England Nuclear (Boston, MA); and *l*-[7,8-^{3}H]noradrenaline, 40 Ci/mmol from Amersham Corp. (Arlington Heights, IL).

Drug doses reported as milligrams per kilogram were based on the weight of the salt supplied, except as indicated. Compounds were administered as solutions in a vehicle consisting of either water or the mixture (% by volume), 5% ethanol + 5% Emulphor-EL-620 + 90% normal saline (injection volume, 10 ml/kg of mice and 5 ml/kg of rats). Emulphor EL-620, a polyoxyethylated vegetable oil, specific gravity 1.04 to 1.05, was purchased from GAF Corp., Graselli, NJ.

Mice were Swiss-Webster CD males weighing 17 to 25 g from Charles River Laboratories, Inc. (Wilmington, MA). Rats were Charles River Sprague-Dawley CD males weighing 180 to 220 g. Dogs were female beagles (symptom studies) or mongrels of either sex weighing about 10 kg (cardiovascular studies).

Biochemical Studies

Synaptosomal uptake of 5-HT, DA and NE. Monoamine uptake studies were conducted with crude synaptosomal fractions of rat corpus striatum (for 5-HT and DA uptake) or hypothalamus (for NE uptake) as described previously (Koe, 1976). The monoamines, [^{3}H]-5-HT, [^{3}H]DA and [^{3}H]NE were diluted with the respective unlabeled amine to achieve a final concentration of 0.1 μM in the synaptosomal mixtures, which were incubated for 10 min at 37°C. After incubation, uptake was determined as described in the following paragraph.

For *ex vivo* uptake studies, rats received 3.2, 10, 32 and 100 μmol/kg i.p. of test drug or water (controls) ($N = 5$). One hour later rats were sacrificed by decapitation and brains were rapidly dissected to recover corpus striatum or hypothalamus according to Glowinski and Iversen (1966). Tissue from each rat was homogenized in 10 volumes of ice-cold 0.32 M sucrose [containing 1 mg/ml of glucose, 1 μl/ml of 0.1 M EDTA and tris(hydroxymethyl)aminomethane to achieve pH 7.4]. The homogenate was centrifuged in 15-ml Corex centrifuge tubes at 1000 × *g* for 10 min at 0–4°C. Aliquots (0.1 ml) of the supernatant fraction (1 mg of protein) were incubated in duplicate at 37°C with 1.0-ml portions of pH 7.4 incubation buffer (Koe, 1976) containing 0.032 μM [^{3}H] monoamine as follows: [^{3}H]-5-HT, 5 min; [^{3}H]DA, 2 min; and [^{3}H]NE, 10 min (Mireylees *et al.*, 1978). After incubation, the mixtures were filtered through 0.45-μ Millipore filters. The latter were washed with fresh incubation buffer and dissolved in 1.0 ml of 2-methoxyethanol. Radioactivity was determined by liquid scintillation spectrometry. Up-

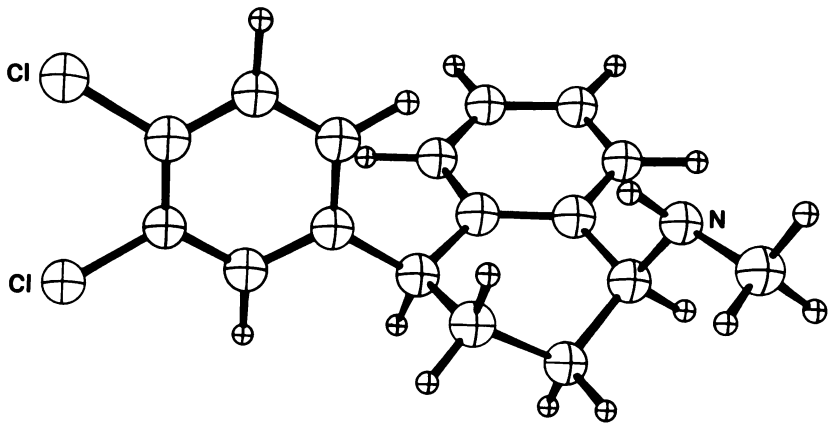


Fig. 1. Lowest energy conformation calculated for sertraline.

take at 0°C was taken as blank. Protein was determined by the method of Lowry *et al.* (1951). Uptake was calculated as picomoles per milligram of protein. The dose blocking uptake by 50% (ED_{50}) was estimated graphically on semilog paper.

PCA-induced depletion of 5-HT from rat brain. Rats received two simultaneous s.c. injections: test drug + PCA, 32 μmol/kg; water + PCA; or water + water (controls) (Buus Lassen *et al.*, 1975). Four hours later rats were sacrificed by decapitation, and each whole brain was assayed for 5-HT content by the method of Bogdanski (Lovenberg and Engelman, 1971). Homogenates of brain in 0.1 N HCl were made alkaline with 0.5 M borate buffer (pH 10) and extracted with butanol. 5-HT in the solvent phase was extracted into 0.1 N HCl. The aqueous extracts (1.0 ml) were acidified with concentrated HCl and the intrinsic fluorescence of 5-HT was determined in the Aminco spectrophotofluorometer. The dose of test drug giving 50% reversal of the PCA-induced 5-HT depletion (ED_{50}) was estimated graphically on semilog paper.

Heart uptake of [^{3}H]NE in rats. Rats received three doses of test drug or vehicle i.p. 80 min and DL[^{3}H]NE, 5 μCi/kg i.v., 60 min before sacrifice. The specific activity of the [^{3}H]NE accumulated in the heart was determined as described previously (Koe and Constantine, 1972; Sarges *et al.*, 1974).

Concentrations of 5-HT, 5-HIAA, DA and NE. Rats received sertraline, 32 μmol/kg, or water (controls) i.p. 1 and 4 hr before sacrifice ($N = 5$). Whole brain was rapidly removed and stored frozen. 5-HT levels were determined as described above. DA and NE levels were determined as follows: the catecholamines were isolated *via* AG50WX4 Na^+ columns, purified on acid-washed Woelm alumina and assayed by the iodine oxidation method in a Technicon AutoAnalyzer (Koe, 1974). The 5-HT metabolite, 5-HIAA, was recovered by ether extraction from the deproteinized brain homogenate and assayed by the intrinsic 5-hydroxyindole fluorescence in 3 N HCl. The monoamine or metabolite content was expressed as a percentage of control levels.

In a separate study, rats received daily doses of sertraline, 32 μmol/kg, or water i.p. for up to 11 days. Seventeen hours after the last injection, rats were anesthetized with sodium pentobarbital and blood was withdrawn from the aorta. 5-HT in whole blood was determined by the modification of the Bogdanski method described by Halevy *et al.* (1965).

Inhibition of MAO *in vitro*. MAO activity in aqueous homogenates of rat brain (167 mg wet weight/ml) and liver (33 mg wet weight/ml) was measured by the method of Wurtman and Axelrod (1963). Duplicate aliquots (0.03 ml) of tissue homogenate were mixed with [^{14}C]tryptamine (18 μM, containing 0.33 μCi/ml of radioactivity) in the absence (control) or presence of test compound in a total volume of 0.3 ml. After incubation at 37°C for 20 min, the mixture was acidified with 2N HCl (0.2 ml) and the ^{14}C-products of MAO action were extracted into toluene (6.0 ml). Radioactivity of the toluene extracts (4.0 ml) was determined by liquid scintillation counting. Incubations with boiled tissue served as blank, which was also equal to MAO activity in the presence of 100 μM pargyline. MAO activity was expressed as a percentage of control.

[^{3}H]QNB binding to rat brain membranes. Binding of [^{3}H]QNB was measured by the method of Yamamura and Snyder (1974). A P2 pellet suspension from whole brain (minus cerebellum) was prepared as described for the uptake studies (Koe, 1976). Aliquots (0.1 ml) of this preparation were incubated in triplicate for 1 hr at 25°C with 1.0 ml of 0.05 M Na-K phosphate buffer (pH 7.4) containing 2 nM DL-[^{3}H]QNB or 1 nM L-[^{3}H]QNB and 0.02 ml of test compound. After incubation, the tissue was collected on 0.45-μ Millipore filters. The latter were washed twice with 5 ml of the phosphate buffer and dissolved in 1.0 ml of 2-methoxyethanol. Radioactivity was determined by liquid scintillation counting. Binding in the presence of 100 μM oxotremorine sesquifumarate was used for determining nonspecific binding. The concentration inhibiting binding by 50% (IC_{50}) was estimated graphically on semilog paper.

NE-sensitive adenylate cyclase of rat limbic forebrain. Rats received sertraline (56 μmol/kg i.p.) or water (controls) b.i.d. (at 8:30 to 9:00 A.M. and 4:30 to 5:00 P.M.) for 4 days ($N = 10$). Another group of rats received imipramine (75 μmol/kg i.p.) or water (controls) b.i.d. for 4 days ($N = 10$). Twenty hours after the last injection, rats were decapitated and limbic forebrains were dissected as described by Blumberg *et al.* (1976). One-half of the limbic forebrain was used for determining basal accumulation of cyclic AMP, whereas the contralateral half was used for measuring the cyclic AMP response to a NE concentration (100 μM) used in similar down-regulation studies (Mishra *et al.*, 1980, 1981; Janowsky *et al.*, 1982). Tissues were sliced using a McIlwain tissue chopper and incubated under the conditions described by Mobley and Sulser (1979). After incubation, slices were frozen in liquid nitrogen, then homogenized in 0.3 N $HClO_4$ using a Polytron PT-10 homogenizer. The protein was separated *via* centrifugation and the supernatant fluids were stored frozen until assay for cyclic AMP. After thawing, the 0.3 N $HClO_4$ extracts were neutralized by addition of excess $CaCO_3$. After centrifugation, the cyclic AMP content of the extracts was determined by radioimmunoassay (New England Nuclear Cyclic AMP, ^{125}I-radioimmunoassay kit).

In a separate study, rats received sertraline (100 μmol/kg s.c.) or water (controls) b.i.d. for 4 days ($N = 20$). On the next day, rats were decapitated. The limbic forebrain from all rats and the cerebral cortex from one-half of the rats in each group were dissected for determination of adenylate cyclase activity (above) and [^{3}H]DHA binding (below), respectively.

[^{3}H]DHA binding to rat cortical membranes. This binding was conducted by the method of Crews *et al.* (1981). Frozen cerebral cortices from the preceding experiment were homogenized in 20 volumes of ice-cold 50 mM tris [hydroxymethyl]aminomethane HCl (pH 8.0) buffer with a Polytron PT 10 homogenizer. The homogenate was centrifuged at 20,000 × *g* for 20 min. The pellet was washed twice with fresh buffer by centrifugation and resuspended in the same volume of buffer. Triplicate mixtures, consisting of 0.1-ml membranes, 0.4 ml of buffer, 0.05 ml of [^{3}H]DHA (2 nM) and 0.01 ml of 50 μM (±)-propanolol or vehicle were incubated at 23°C for 20 min and filtered through Whatman GF/B glass-fiber filters under vacuum. The filters were washed with 5 ml of cold buffer 5 times and kept in Aquasol-2 overnight before liquid scintillation counting.

Behavioral Studies

Interactions with 5-HTP in mice. To study the potentiation of 5-HTP, fasted male mice were dosed orally with 5-HT uptake blockers 1 hr before 5-HTP, 100 mg/kg i.p. ($N = 10$). This dose of 5-HTP by itself causes no clear behavioral effects, but in mice treated with MAO inhibitors (*cf.*, Bogdanski *et al.*, 1958) or 5-HT uptake blockers it causes a syndrome including tremors, head twitches, hind-limb abduction and backwards locomotion. The mice were rated for the presence of each symptom by a "blinded" observer at 10 to 20 min after 5-HTP treatment. The ED_{50} values of 5-HT uptake inhibitors for eliciting each symptom in 5-HTP-treated mice were ascertained by the method of Weil (1952).

Effects on locomotor activity in rats. Rats (250–300 g) were housed under standard laboratory conditions for at least 1 week before experimentation. The animals were then placed individually into 48 locomotor activity chambers (30 cm × 30 cm), enclosed in sound attenuating cabinets equipped with lights and fans, for at least 3 hr of habituation before administration of drugs. Rats received vehicle (5% ethanol-5% Emulphor-90% saline) or ascending log doses of sertraline, tametraline or amphetamine s.c. ($N = 8$ per dose). Locomotor activity, measured as crossovers from one quadrant of the chamber to another, was monitored continuously for at least 6 hr by means of a PDP 11/34 computer. In addition, regular observations were made through a viewing lens located in the door of the cabinet.

Symptomatic effects and drug interactions. Mice were treated s.c. and p.o. with sertraline at doses ranging from 32 to 1000 mg/kg ($N = 5$). At 0.5, 1, 2 and 4 hr after treatment, the animals were inspected for autonomic, skeletal muscular and reflex abnormalities. These mice were also retained for 3 days to ascertain any incidence of delayed mortality. In separate experiments, fasted mice (or rats where noted) were treated with sertraline (32 mg/kg s.c.) or vehicle 1 hr before a

challenge with central nervous system active agents designed to produce a variety of characteristic symptoms ($N = 5$). These challenges, administered i.p. except as indicated, included: bicuculline (10 mg/kg), nicotine (20 mg/kg), picrotoxin (25 mg/kg), pentylenetetrazol (120 mg/kg), strychnine sulfate (2.5 mg/kg), amphetamine sulfate (20 mg/kg), apomorphine HCl (5 mg/kg), levorphanol tartrate (32 mg/kg) or morphine sulfate (100 mg/kg), methacholine chloride (400 mg/kg), neostigmine methyl sulfate (2 mg/kg), tryptamine HCl (640 mg/kg) and NE bitartrate (5 mg/kg i.v. in rats).

Porsolt mouse "behavioral despair" test. Mice were housed under standard laboratory conditions for 1 week before experimentation. Drugs dissolved in the ethanol-Emulphor-saline mixture were administered s.c. One hour later mice were placed individually into 1 of 2 Plexiglas cylinders (20 cm high × 10 cm diameter) containing 6 cm of water at 21–23°C. The animals were then observed for the presence of immobility during a 6-min test. A mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water (Porsolt *et al.*, 1977). Because little immobility was observed during the first 2 min, only ratings during the last 4 min were recorded using electromechanical equipment located in an adjacent room (Browne, 1979). The length of time (maximum 240 sec) that a mouse remained immobile was then converted to a percentage of the mean immobility time exhibited by the corresponding vehicle control animals.

Pharmacological Studies

Counteraction of reserpine hypothermia in mice. In accordance with the antidepressant screening method of Askew (1963), mice were placed in a room with an ambient temperature of 20°C. The mice were individually housed in plastic chambers with a cardboard bottom. All mice were injected with reserpine (2 mg/kg s.c.) and retained at 20°C for 18 hr. Their rectal temperatures were then ascertained, immediately after which they received saline or drug treatment, and rectal temperatures were measured at 1, 2 and 3 hr after this second injection.

***In vivo* estimates of anticholinergic activity mice.** Fasted male mice were dosed s.c. with sertraline, standard anticholinergic agents or uptake blockers 1 hr before receiving oxotremorine, 0.32 mg/kg i.p. ($N = 5$). At 0.5 hr after oxotremorine, mice were evaluated on an all or none basis for blockade of the tremors, salivation and diarrhea that were seen in concomitantly treated vehicle control mice. In a separate study, mice treated with sertraline or the same standard drugs were challenged 1 hr later with tetrabenazine (32 mg/kg i.p.) and pupil sizes were measured 1 hr after that challenge.

Preliminary cardiovascular evaluation. Mongrel dogs of either sex were anesthetized with sodium pentobarbital (35 mg/kg i.v.). Femoral artery blood pressure was measured with a transducer (Narco-Biosystems, model RP-1500), the electrical output of which operated a biotachometer (Narco-Biosystems, Type 7302) for determination of heart rate. Both variables were recorded continuously with a physiograph (Narco-Biosystems, model 4CPM). A cannula was inserted in a femoral vein for injection of drugs. Sertraline, dissolved in water at a concentration of 5 mg/ml, was administered i.v. to dogs at 0.1, 0.4 and 1.0 mg/kg ($N = 2$ per dose). Blood pressure responses were elicited at 5-min intervals with epinephrine, 1 or 2 μg/kg i.v.; NE, 1 μg/kg i.v.; bilateral carotid artery occlusion for 30 sec; and 5-HT, 1, 4 and 10 μg/kg i.v., each challenge twice before and then once after administration of sertraline. In a separate experiment, in which sertraline was injected i.v. at 1.0 mg/kg to each of four dogs, needle electrodes were located s.c. in the right foreleg and left hindleg for lead II electrocardiogram recording on an electrocardiograph (Cambridge model VS-III); blood pressure and heart rate were also measured as above.

Results

Biochemical Studies

***In vitro* uptake of serotonin, DA and NE in rat brain synaptosomal preparations.** Introduction of chlorine atoms at positions 3,4 of the 4-phenyl ring of tametraline to give (+)-*trans*-(1R,4S)-N-methyl-4-(3,4-dichlorophenyl)-1-aminotetralin (CP-52,003) increased potencies for blocking monoamine uptake as follows: 25-fold for 5-HT uptake, 5-fold for DA uptake and 2-fold for NE uptake (table 1). In contrast to CP-52,003, the (+)-*cis*-(1S,4S)-isomer, sertraline, exhibited marked potency and selectivity as an inhibitor of 5-HT uptake. The inhibition of 5-HT uptake by sertraline was competitive (K_i of 0.013 μM; fig. 2) and comparable in potency with the structurally rigid prototype uptake blocker, EXP-561 (K_i 0.019 μM) (Koe, 1976). Sertraline exhibited better selectivity for inhibiting 5-HT uptake relative to NE uptake than fluvoxamine, zimelidine, norzimelidine, fluoxetine or chlorimipramine (table 2). However, it was somewhat less selective for blocking 5-HT uptake relative to DA uptake than these agents.

The corresponding enantiomers of CP-52,003 and sertraline, (−)-*trans*-(1S,4R) [CP-52,002] and (−)-*cis*-(1R,4R) [CP-51,973], respectively, were also strong blockers of monoamine uptake. Both were equally potent in inhibiting 5-HT and DA uptake, but CP-52,002 was 8 times more active against NE uptake than CP-51,973 (table 1). The demethyl metabolite (CP-62,508) of sertraline was also a strong, selective blocker of 5-HT uptake. Compared with the parent compound, it was about 8 times less active against 5-HT uptake and about 4 times less active against NE and DA uptake (table 2). *In vitro*, CP-62,508 and norzimelidine were equipotent against 5-HT uptake. However, the sertraline metabolite was more selective, as it was 12 times less active than the zimelidine metabolite against NE uptake.

***Ex vivo* uptake of serotonin, DA and NE.** Synaptosomal preparations from rats injected with 5-HT uptake blockers exhibited a dose-related decrease in ability to accumulate [^{3}H]-5-HT. The dose which results in a 50% reduction of 5-HT uptake (ED_{50}) reflects intrinsic activity of the drug and its penetration into brain. Sertraline was more active in blocking synaptosomal uptake of 5-HT *ex vivo* than several other 5-HT uptake inhibitors (table 3). The ED_{50} values determined for fluoxetine, chlorimipramine and Org 6582 were about the same as those reported by Mireylees *et al.* (1978). The selectivity of sertraline was also shown by the much weaker decreases in *ex vivo* uptake of DA and NE at doses which elicited strong blockade of 5-HT uptake (table 4). Serotonin uptake into striatal synaptosomes from rats treated with sertraline (32 μmol/kg i.p.) was more than 50% inhibited for at least 8 hr after administration of the drug (fig. 3). Thereafter, 5-HT uptake activity *ex vivo* gradually increased and returned to that shown by control rats by 24 hr.

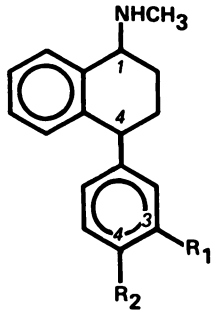
Antagonism of PCA-induced depletion of serotonin from rat brain (*in vivo* uptake). Serotonin uptake blockers show a dose-dependent antagonism of the 5-HT-depleting action of PCA, a drug which requires uptake into 5-HT neurons to exert its effect. Sertraline was 6 times more potent than chlorimipramine and 60 times more potent than amitriptyline in reversing the 5-HT depletion elicited by PCA (table 5). The ED_{50} values found for chlorimipramine and amitriptyline were in good agreement with those described by Buus Lassen *et al.* (1975). When administered simultaneously with PCA, sertraline protected rats from the PCA effect for 17 hr (table 6).

Heart uptake of [^{3}H]NE *in vivo*. Rats pretreated with sertraline showed a slight decrease in heart uptake of i.v. [^{3}H]NE, but only after high doses of the drug ($ED_{50} > 100$ μmol/kg i.p.). In contrast, the corresponding ED_{50} values for chlorimi-

TABLE 1

Inhibition of monoamine uptake by N-methyl-4-phenyl-1,2,3,4-tetrahydro-1-naphthylamines in synaptosomal preparations of rat brain

Uptake of 0.1 μM [^{3}H]-5-HT, [^{3}H]DA or [^{14}C]DA and [^{3}H]NE was run as previously described (Koe, 1976). Crude synaptosomes were prepared from corpus striatum for 5-HT and DA uptake and hypothalamus for NE uptake. IC_{50} values are means of two to eight determinations.



Compound	Conformation	R_1	R_2	IC_{50}			IC_{50} Ratio	
				5-HT	DA	NE	DA/5-HT	NE/5-HT
					×10^{-8}M			
Tametraline[a]	(+)-*trans*-1R,4S	H	H	0.84	0.15	0.018	0.19	0.021
CP-52,003	(+)-*trans*-1R,4S	Cl	Cl	0.033	0.033	0.011	1.0	0.30
Sertraline	(+)-*cis*-1S,4S	Cl	Cl	0.058	1.1	1.2	19	21
CP-52,002	(−)-*trans*-1S,4R	Cl	Cl	0.45	0.23	0.050	0.51	0.11
CP-51,973	(−)-*cis*-1R,4R	Cl	Cl	0.46	0.29	0.38	0.63	0.83

[a] CP-24,441; IC_{50} values from Koe (1976).

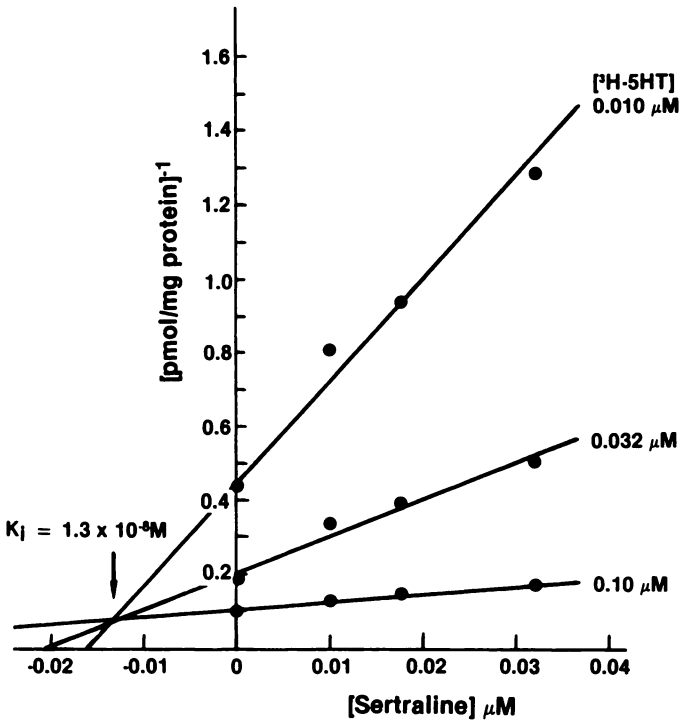


Fig. 2. Dixon plot of the inhibition of [^{3}H]-5-HT uptake by sertraline. Rat striatal synaptosomes were incubated with 0.01, 0.032 and 0.10 μM [^{3}H] 5-HT and varying concentrations of inhibitors for 5 min at 37°C.

TABLE 2

Inhibition of monoamine uptake in synaptosomal preparations of rat brain

Uptake of 0.1 μM [^{3}H]5-HT, [^{3}H]DA or [^{14}C]DA and [^{3}H]NE was run as previously described (Koe, 1976). IC_{50} values are means of 2 to 12 determinations.

Drug	IC_{50}			IC_{50} Ratio	
	Corpus Striatum		Hypothalamus	DA/5-HT	NE/5-HT
	5-HT	DA	NE		
		×10^{-8} M			
Sertraline[a]	0.058	1.1	1.2	19	21
CP-62,508[b]	0.45	3.8	4.6	8.4	10
Fluvoxamine	0.54	45	1.9	83	3.5
Zimelidine	4.5	43	12	9.6	2.7
Norzimelidine	0.45	21	0.36	47	0.80
Fluoxetine[c]	0.27	12	0.74	44	2.7
Chlorimipramine[c]	0.099	8.1	0.11	82	1.1
Imipramine[c]	0.81	20	0.066	25	0.081
Desipramine[c]	3.4	21	0.0056	6.2	0.0016
Amitriptyline[c]	1.2	13	0.13	11	0.11
Nortriptyline[c]	1.7	11	0.025	6.5	0.0014

[a] Sertraline is (+)-*cis*-(1S,4S)-N-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthylamine.

[b] CP-62,508 is (+)-*cis*-(1S,4S)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthylamine.

[c] IC_{50} values from Koe (1976).

pramine and imipramine were 18 and 7.0 μmol/kg, respectively (table 7).

Effects on monoamine metabolism. One and 4 hr after the administration of sertraline (32 μmol/kg s.c.), brain content of 5-HT, DA or NE in rats was unaltered (table 8). The slight decrease in 5-HIAA levels 1 hr after sertraline was indicative of the decrease in 5-HT turnover associated with blockade of 5-HT uptake. Sertraline under several treatment protocols lowered 5-HT concentrations in whole blood of rats to 50% of control levels (table 9). This effect probably reflects the blockade of 5-HT uptake in platelets by sertraline (Ross *et al.*, 1976a). Sertraline was ineffective as an inhibitor of MAO activity in rat brain and liver homogenates (table 10). Compared with pargyline, sertraline was inert against brain MAO and ~100 times less potent against liver MAO. This lack of inhibitory activity *in vitro* is consistent with the unchanged brain levels of 5-HT, DA and NE in rats treated with sertraline (table 8).

Effects on NE receptor-coupled adenylate cyclase of rat limbic forebrain. Chronic administration of antidepressant drugs to rats, in contrast to acute injection, induces a decrease in responsiveness of the cyclic AMP generating system

TABLE 3

5-HT uptake in striatal synaptosomes of rats pretreated for 1 hr with drugs (*ex vivo* uptake)

Ex vivo uptake of [^{3}H]5-HT (0.032 μM) was conducted by the method of Mireylees *et al.* (1978). ED_{50} values were estimated from semilog plots of tabular data (mean % ± S.E.). Mean [^{3}H]5-HT uptake in corpus striatum of control rats was 3.67 ± 0.15 pmol/mg of protein ± S.E. (N = 30).

Compound	Doses in μmol/kg i.p.				ED_{50}
	3.2	10	32	100	
	% of control uptake				μmol/kg
Sertraline	65.8 ± 4.2[a]	47.9 ± 2.2[a]	29.9 ± 4.7[a]	17.8 ± 1.5[a]	9
Fluvoxamine	78.3 ± 4.7[a]	66.9 ± 9.6[a]	43.9 ± 2.0[a]	33.6 ± 1.4[a]	24
Zimelidine	77.0 ± 3.7[a]	64.5 ± 3.0[a]	44.3 ± 2.1[a]	34.7 ± 1.4[a]	23
Fluoxetine	111.3 ± 4.3	90.7 ± 4.5	49.5 ± 1.6[a]	40.3 ± 1.0[a]	32
Chlorimipramine	94.3 ± 3.5	86.1 ± 5.0	56.1 ± 3.6[a]	42.1 ± 2.1[a]	54
Org 6582	92.3 ± 1.7	68.9 ± 4.5[a]	55.2 ± 2.0[a]	35.6 ± 1.2[a]	43

[a] $P < .05$ or less for comparison with contemporary controls (N = 5).

TABLE 4

5-HT, NE and DA uptake in synaptosomes of rats pretreated for 1 hr with sertraline (*ex vivo* uptake)

Ex vivo uptake of ^{3}H-monoamines (0.032 μM) was conducted by the method of Mireylees *et al.* (1978). ED_{50} values were estimated from semilog plots of tabular data (mean % ± S.E.). Mean uptakes in picomoles per milligram of protein ± S.E. (N = 5) were: hypothalamic [^{3}H]5-HT, 2.42 ± 0.18; hypothalamic [^{3}H]NE, 1.05 ± 0.07; and striatal [^{3}H]DA, 11.7 ± 1.0.

Monoamine	Tissue	Doses in μmol/kg i.p.				ED_{50}
		3.2	10	32	100	
		% of control uptake				μmol/kg
5-HT	Hypothalamus	84.1 ± 11.1	46.9 ± 5.0[a]	22.5 ± 2.2[a]	20.2 ± 1.9[a]	9
NE	Hypothalamus	112.4 ± 14.7	104.6 ± 16.3	95.3 ± 9.9	73.3 ± 14.7	>100
DA	Corpus striatum	91.3 ± 5.6	87.9 ± 3.7	86.3 ± 8.6	47.7 ± 7.7[a]	94

[a] $P < .05$ or less for comparison with controls (N = 5).

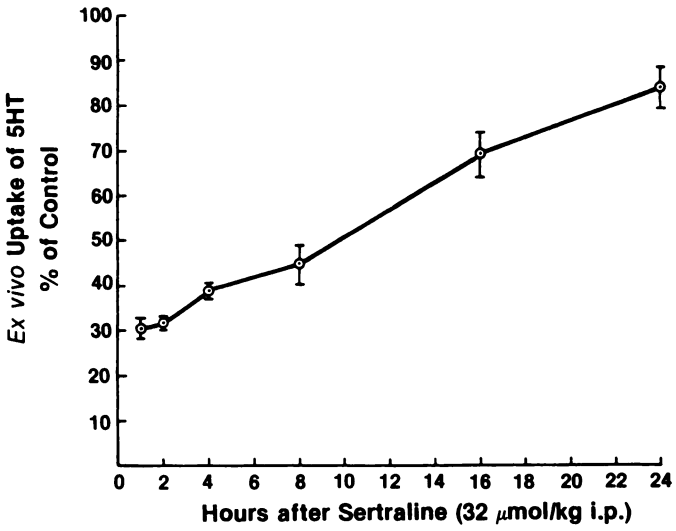


Fig. 3. [^{3}H]-5HT uptake in striatal synaptosomes of rats treated with sertraline as a function of time after dosing (*ex vivo* uptake). Separate groups of 30 rats received sertraline (32 μmol/kg i.p.) or water (controls). Five sertraline treated and five control rats were sacrificed at 1, 2, 4, 8, 16 and 24 hr after treatment. Serotonin uptake *ex vivo* was ascertained as described under "Methods" and calculated as a percentage of control uptake. For all time periods except 24 hr, $P < .001$ to .01 *vs.* contemporary controls.

of limbic forebrain to NE. The delayed onset of this effect is reminiscent of the slow onset of therapeutic action of antidepressants, suggesting that down-regulation of this NE receptor coupled system may play a role in the mechanism of action of these agents (Mobley and Sulser, 1981). Injecting rats with sertraline (56 μmol/kg i.p., b.i.d.) for 4 days induced a 30% decrease ($P < .05$) in the accumulation of cyclic AMP in limbic forebrain slices stimulated by 100 μM NE without affecting basal activity (fig. 4A). Similarly, administration of imipramine at 75 μmol/kg i.p., b.i.d. for 4 days caused a 25% decrease ($P < .05$) in the cyclic AMP response to NE without affecting basal activity (fig. 4B).

Effects on NE receptor-coupled adenylate cyclase and *beta* adrenoceptors. In a similar study demonstrating down-regulation, s.c. administration of sertraline (100 μmol/kg b.i.d.

TABLE 5

Effect of drugs on the 5-HT content of rat brain 4 hr after PCA treatment (*in vivo* "5-HT" uptake)

This assessment of *in vivo* uptake in 5-HT neurons was conducted by the method of Buus Lassen *et al.* (1975). All treatment groups (vehicle or drug) in table 5 also received PCA (32 μmol/kg s.c.). ED_{50} values (dose to block 5-HT depletion by 50%) were estimated from semilog plots of tabular data (mean % ± S.E.). Mean 5-HT levels (micrograms per gram ± S.E.) of the control groups (*i.e.*, rats receiving only vehicle) in the four experiments were: 0.517 ± 0.033 (N = 12), 0.390 ± 0.012 (N = 5), 0.447 ± 0.003 (N = 10) and 0.522 ± 0.015 (N = 5), respectively.

Treatment	Dose	5-HT Content of Whole Brain	ED_{50}
	μmol/kg	% of control (N)	μmol/kg
Vehicle		50.4 ±1.8 (12)	
Sertraline	0.32 s.c.	59.7 ± 2.9 (12)[a]	
	1.0 s.c.	83.2 ± 1.8 (12)[b]	0.68 s.c.
	3.2 s.c.	113.4 ± 4.3 (12)[b]	
Vehicle		40.3 ± 4.5 (5)	
Sertraline	0.32 i.p.	41.6 ± 5.2 (5)	
	1.0 i.p.	83.3 ± 5.1 (5)[b]	0.69 i.p.
	3.2 i.p.	119.8 ± 5.9 (5)[b]	
Vehicle		47.1 ± 2.4 (10)	
Chlorimipramine	1.0 s.c.	60.3 ± 5.5 (5)[c]	
	3.2 s.c.	65.2 ± 3.7 (10)[b]	4.4 s.c.
	10 s.c.	92.2 ± 1.8 (10)[b]	
Vehicle		53.0 ± 2.6 (5)	
Amitriptyline	10 s.c.	63.9 ± 4.2 (5)	
	32 s.c.	74.0 ± 5.1 (5)[d]	43 s.c.
	100 s.c.	89.0 ± 4.5 (5)[b]	

[a] $P < .02$ *vs.* vehicle group.
[b] $P < .001$ *vs.* vehicle group.
[c] $P < .05$ *vs.* vehicle group.
[d] $P < .01$ *vs.* vehicle group.

TABLE 6

Effect of sertraline on the 5-HT content of rat brain 17 hr after PCA treatment

Rats received the two injections s.c. simultaneously 17 hr before sacrifice. Mean 5-HT levels (micrograms per gram ± S.E.) of controls (vehicle + vehicle group) was 0.439 ± 0.021 (N = 15).

Injections		Doses	5-HT Content of Whole Brain
First	Second		
		μmol/kg s.c.	% of control (N)
Vehicle	Vehicle		100.0 ± 3.6 (15)
Vehicle	PCA	32	56.3 ± 1.9 (15)[a]
Sertraline	Vehicle	32	100.4 ± 2.1 (5)
Sertraline	PCA	32	112.2 ± 8.3 (15)

[a] $P < .001$ vs. controls.

TABLE 7

Effect of drugs on heart uptake of [^{3}H]NE in rats

Heart uptake of [^{3}H]NE (5 μCi/kg i.v.) *in vivo* was conducted as described previously (Koe and Constantine, 1972). For effects of other antidepressants or uptake blockers, see Koe and Constantine (1972), Koe (1975, 1976). ED_{50} values were estimated from semilog plots of tabular data (mean % ± S.E.). Mean specific activities of heart [^{3}H]NE (microcuries per nanomole ± S.E.) for the control groups were: Sertraline run, 6.04 ± 0.28 (N = 15); chlorimipramine run, 6.59 ± 0.27 (N = 5); imipramine run, 7.27 ± 0.42 (N = 10).

Compound	Dose	[^{3}H]NE Accumulation in Rat Heart	ED_{50}
	μmol/kg i.p.	% of control (N)	μmol/kg
Sertraline	10	98.9 ± 3.9 (9)	
	32	87.9 ± 3.1 (9)[a]	>100
	100	77.2 ± 5.7 (12)[b]	
Chlorimipramine	3.2	116.8 ± 7.9 (5)	
	10	76.9 ± 8.0 (5)[a]	18
	32	24.4 ± 1.6 (5)[c]	
Imipramine	1.8	94.7 ± 5.1 (10)	
	5.6	58.0 ± 3.6 (10)[c]	7.0
	17.8	14.6 ± 1.4 (10)[c]	

[a] $P < .05$ vs. controls.
[b] $P < .01$ vs. controls.
[c] $P < .001$ vs. controls.

for 4 days) caused a 19% decrease ($P < .05$) in the cyclic AMP response of limbic forebrain slices to 100 μM NE. At the same time, cortical membranes from the sertraline-treated rats showed a 10% decrease ($P < .01$) in the binding of 2 nM [^{3}H] DHA (table 11). As in the preceding study (fig. 4), basal adenylate cyclase activity was not altered by sertraline *ex vivo* (table 11). To ascertain that these *ex vivo* results originated from *in vivo* effects, sertraline (100 μM) added to normal limbic forebrain slices was found to have no effect on cyclic AMP accumulation without or with 100 μM NE. [^{3}H]DHA binding was only very weakly inhibited by sertraline *in vitro* (IC_{50}, 38 μM).

Behavioral Studies

Interactions with 5-HTP in mice. In the present study, 5-HTP-elicited tremors were the symptom most sensitive to potentiation by 5-HT uptake blockers, contrary to past reports (*e.g.*, Ross *et al.*, 1976b). Nevertheless, the relative drug potencies on each of the four symptoms evaluated were roughly parallel (table 12). Depending on endpoint, sertraline was some 3 to 10 times more potent than fluvoxamine, zimelidine and fluoxetine in potentiating the behavioral effects of 5-HTP. Chlorimipramine was far less potent still and desipramine was without potentiating effect at a dose of 178 mg/kg p.o. At a dose of 5.6 mg/kg p.o., sertraline was moderately active in potentiating 5-HTP when given as a 0.5-hr pretreatment, probably more active when given as a 1-, 2- or 3-hr pretreatment, but virtually inactive when given as a 4-hr pretreatment (table 13).

Effects on locomotor activity in rats. Sertraline at doses ranging from 1.0 to 320 mg/kg s.c. did not cause any increase in locomotor activity over the entire 6-hr monitoring period (fig. 5). In contrast, tametraline and amphetamine elicited a dose-related elevation of motor activity with threshold doses of 1.78 and 0.56 mg/kg s.c., respectively.

Symptomatic effects after acute treatment. In a systematic examination (22 signs), sertraline produced no symptoms in mice at 32 mg/kg p.o. or s.c. As doses were increased to 100, 320 and 1000 mg/kg, irritability, stimulant effects, tremors, sporadic convulsions, motor deficits, diarrhea, flushing and mydriasis were seen in increasing intensity. Peak action was generally between 2 and 4 hr after treatment. Based on a 3-day mortality observation period, the LD_{50} of sertraline in mice by either route was between 320 and 1000 mg/kg. Sertraline was also given at single doses of 10, 20, 30 and 50 mg/kg p.o. (in capsules) to two female beagle dogs at each dose. At the lowest level, dogs were mydriatic and anorectic, but otherwise asymptomatic. At higher doses, increased salivation, tremors and twitches were observed, along with the mydriasis and anorexia at 10 mg/kg. None of the dogs at any dose level exhibited motor stimulation, circling or stereotypy. The duration of the anorexia was 12 to 15 hr, but eating resumed late in the day after treatment and the dogs recovered uneventfully.

Drug interaction studies in mice and rats. As a 1-hr pretreatment, sertraline at 32 mg/kg s.c. failed to modify the convulsant or lethal effects of high doses of bicuculline, nicotine, picrotoxin, pentylenetetrazol or strychnine sulfate in mice. It significantly prolonged by about 100% the latency to tonic convulsions after electroconvulsive shock (50 mA; 0.2 sec; transcorneally), but none of the mice were totally protected against the tonic seizure. At this same dose, sertraline did not alter amphetamine- or apomorphine-elicited stereotypy, or le-

TABLE 8

Content of 5-HT, 5-HIAA, DA and NE in rat brain after administration of sertraline

Treatment	Time	Whole Brain			
		5-HT	5-HIAA	DA	NE
	hr	μg/g			
Vehicle	1	0.62 ± 0.01	0.66 ± 0.02	0.83 ± 0.03	0.31 ± 0.01
Sertraline[a]	1	0.66 ± 0.03	0.56 ± 0.02[b]	0.80 ± 0.02	0.29 ± 0.02
Vehicle	4	0.59 ± 0.04	0.54 ± 0.01	0.85 ± 0.02	0.30 ± 0.01
Sertraline[a]	4	0.64 ± 0.04	0.52 ± 0.01	0.86 ± 0.02	0.33 ± 0.01

[a] Thirty two micromoles per kilogram s.c.
[b] $P < .02$ vs. vehicle group (N = 5).

TABLE 9

5-HT content of rat blood after administration of sertraline

Mean 5-HT concentration (micrograms per milliliter ± S.E.) of whole blood from control rats was 1.267 ± 0.055 (N = 20).

Sertraline (32 μmol/kg i.p.)	Interval Between Doses	Whole Blood Content of 5-HT 17 hr after Final Injection
	hr	*% of control (N)*
Single dose		79.7 ± 7.3 (5)[a]
Three doses	4	50.0 ± 6.7 (5)[b]
Four doses	24	48.0 ± 4.6 (5)[b]
Eleven doses	24	53.7 ± 4.9 (5)[b]

[a] $P < .05$ *vs.* contemporary controls.
[b] $P < .01$ *vs.* contemporary controls.

TABLE 10

Inhibition of rat brain and liver MAO *in vitro*

MAO activity was determined by incubating brain and liver homogenates with [^{14}C] tryptamine (18 μM). Percentages are calculated from the mean radioactivity of extracted products of duplicate incubations. Average MAO activity of control brain and liver were 1110 cpm/16.7 mg wet weight and 1249 cpm/3.3 mg wet weight, respectively.

Compound	Conc.	MAO Activity	
		Brain	Liver
	M	*% of control*	
Sertraline	10^{-4}	102	62
	10^{-5}	93	90
	10^{-6}	100	102
Pargyline	10^{-4}	0	2
	10^{-5}	26	34
	10^{-6}	56	57

vorphanol- or morphine-elicited Straub tail or hyperactivity. Consistent with other evidence that sertraline is not anticholinergic, there was no effect on time to death after the cholinergic challenges, methacholine chloride or neostigmine. Nor did sertraline alter time to death after tryptamine (mice) or NE (rats).

Effects in Porsolt mouse behavioral despair test. In this model mice placed in an inescapable swim-tank remain motionless, whereas animals treated with antidepressants exhibit a dose-related reduction in this immobility, reflecting more persistent attempts to escape from the swim-tank (Porsolt *et al.*, 1977; Browne, 1979). Vehicle-treated mice were immobile for a mean of 180 of the 240-sec test period. In contrast, sertraline elicited a marked decrease in immobility, a behavioral profile characteristic of that shown by antidepressant drugs (fig. 6). Zimelidine and fluoxetine also caused a decrease in immobility, but the effect showed an inverted "U" dose-response. Fluvoxamine was inactive at all doses tested.

Pharmacological Studies

Counteraction of reserpine hypothermia in mice. Whereas desipramine, at 10 mg/kg p.o., significantly elevated rectal temperatures of mice made hypothermic by reserpine pretreatment, neither sertraline nor the other selective 5-HT uptake blockers exerted this effect (table 14). These findings are consistent with prior indications that reversal of reserpine hypothermia correlates with inhibition of NE, but not 5-HT uptake.

Assessment of anticholinergic activity. At a dose of 32 mg/kg s.c., sertraline completely failed to prevent oxotremorine-elicited parasympathetic symptoms in mice (table 15). Comparable inactivity was also found after desipramine, chlorimipramine, fluoxetine and tametraline. On the other hand, the oxotremorine-elicited tremor, salivation and diarrhea were blocked by atropine and benztropine at doses in the 1 mg/kg range and by the anticholinergic tricyclics, amitriptyline and doxepin, in the 5 to 20 mg/kg range. In confirmation of the oxotremorine results, mice treated with sertraline (32 mg/kg) and then with tetrabenazine were not mydriatic when pupil sizes were examined 1 hr later (table 16). Fluoxetine and tametraline also failed to produce mydriasis in this study. On the other hand, extreme mydriasis was seen after low doses of atropine and scopolamine. Dose-related mydriasis was also

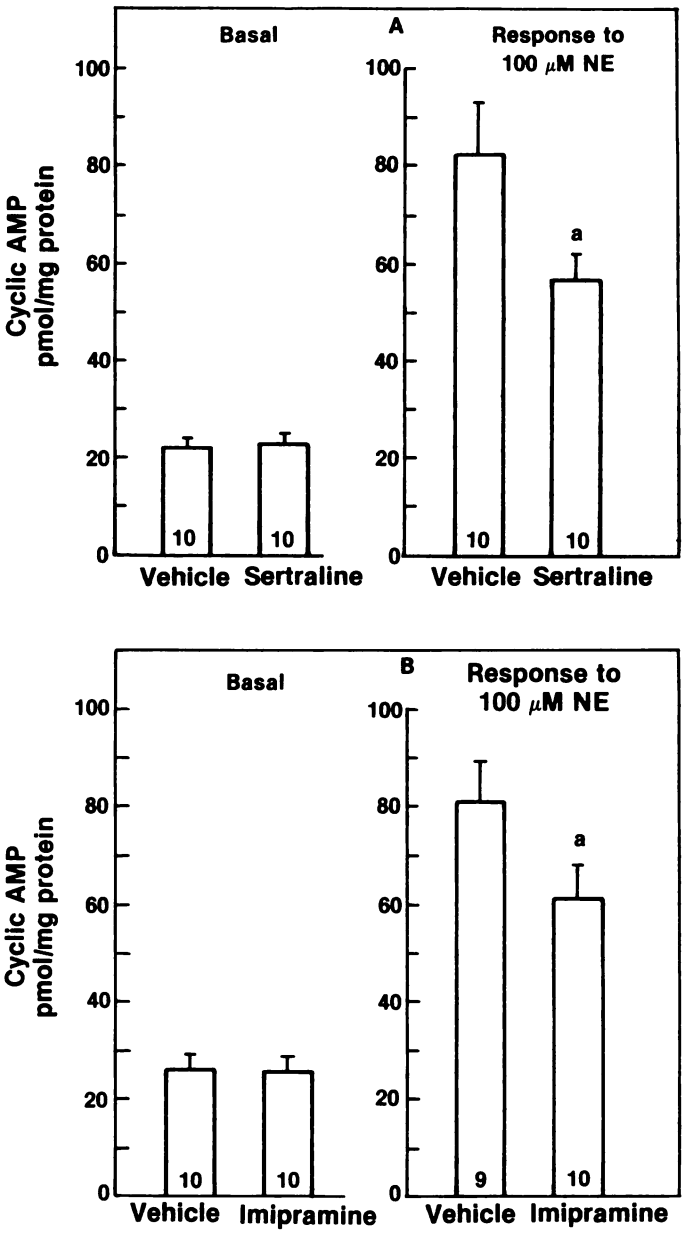


Fig. 4. Effect of sertraline (A) and imipramine (B) on the generation of cyclic AMP by 100 μM NE in slices of rat limbic forebrain. In A, rats received sertraline (56 μmol/kg i.p.) or vehicle b.i.d. for 4 days. In B, rats received imipramine (75 μmol/kg i.p.) or vehicle b.i.d. for 4 days. Rats were sacrificed 20 hr after the last injection. Bars represent mean ± S.E. of picomoles of cyclic AMP per milligram of protein. Number is given inside bar. Response = cyclic AMP accumulation with 100 μM NE minus accumulation without NE (basal). [a] $P < .05$ *vs.* response for vehicle group (rank-sum test; Dixon and Massey, 1969).

TABLE 11

Decrease in the response of NE receptor-coupled adenylate cyclase and [^{3}H]DHA binding of *beta* adrenoceptors after subacute administration of sertraline

Rats received the treatments indicated and were sacrificed 18 to 20 hr after the last injection. Limbic forebrain was dissected for determination of cyclic AMP generation. Response = accumulation of cyclic AMP with 100 μM NE minus accumulation without NE (basal). Cerebral cortex was dissected from half of the rats in each group for measurement of [^{3}H]DHA binding. Entries are mean ± S.E. values (*N* in parenthesis).

Treatment (b.i.d. for 4 days)	NE Receptor-Coupled Adenylate Cyclase		[^{3}H]DHA Binding (2 nM)
	Basal activity	Response to 100 μM NE	
	pmol/mg protein (N)		*fmol/mg protein (N)*
Vehicle, water s.c.	20.4 ± 1.4 (20)	98.4 ± 7.3 (20)	71.9 ± 1.2 (10)
Sertraline, 100 μmol/kg s.c.	21.5 ± 1.2 (20)	76.7 ± 4.0 (20)[a]	64.7 ± 1.5 (10)[b]

[a] $P < .05$ *vs.* vehicle group; 81% control.
[b] $P < .01$ *vs.* vehicle group; 90% control.

noted after amitriptyline, doxepin and desipramine; chlorimipramine was modestly active, but less potent than the other tricyclics tested. In consonance with its lack of anticholinergic activity in these *in vivo* tests, sertraline was a very poor inhibitor of [^{3}H]QNB binding to muscarinic receptors of rat brain membranes compared with amitriptyline, doxepin and anticholinergic agents (table 17).

Cardiovascular effects in dogs. Sertraline, administered at 0.1, 0.4 or 1.0 mg/kg i.v. ($N = 2$ per dose), either had no effect or caused only slight, transient changes in blood pressure and heart rate in anesthetized dogs. Electrocardiograms were unaffected, except for rate changes by sertraline at 1.0 mg/kg i.v. Pressor responses to epinephrine and NE and to bilateral carotid occlusion were not altered. Control blood pressure responses to 5-HT (1, 4 and 10 μg/kg i.v.) were variable. As previously described by other investigators, 5-HT was found to cause either increases, decreases or biphasic changes in blood pressure in dogs, none of which were dose-related (see reviews by Erspamer, 1954; Page, 1954). Sertraline (0.1 mg/kg) had no effect on responses to 5-HT; at 0.4 mg/kg i.v., it enhanced responses to the highest dose of 5-HT and, at 1.0 mg/kg, it enhanced responses to the two highest doses of 5-HT.

Discussion

The results of the present study show that sertraline is a selective inhibitor of synaptosomal 5-HT uptake, more potent than several other 5-HT uptake blockers (zimelidine, fluvoxamine or fluoxetine) and only weakly active against NE uptake (200 times less active than desipramine) and DA uptake. Sertraline is considerably more selective than zimelidine, fluvoxamine, fluoxetine and chlorimipramine in blocking 5-HT uptake relative to NE uptake. Amitriptyline, considered to exert antidepressant effects *via* blockade of 5-HT uptake (Maas, 1975; Garver and Davis, 1979), is only one-twentieth as active as sertraline against 5-HT uptake. The potent and selective 5-HT uptake blockade observed *in vitro* for sertraline is also seen in *ex vivo* and *in vivo* experiments. At doses of sertraline which markedly depress *ex vivo* uptake of 5-HT, the uptake of DA or NE is much less inhibited or not affected, respectively. Sertraline is also very effective in reversing PCA-induced depletion of brain 5-HT, a finding again indicative of potent blockade of 5-HT uptake *in vivo* as PCA presumably must enter 5-HT neurons to effect depletion. Functional evidence of potent 5-HT uptake blockade is provided by the strong potentiation of 5-HTP-induced behavioral effects in mice. In this test, sertraline again is much more active than fluvoxamine, zimelidine, fluoxetine or chlorimipramine. The lack of NE uptake blocking activity is further substantiated by the finding that sertraline only weakly inhibits heart uptake of i.v. [^{3}H]NE in rats and the observation that sertraline (and other 5-HT uptake blockers) do not reverse reserpine-induced hypothermia in mice. In rats, *ex vivo* uptake of 5-HT is depressed by sertraline for at least 8 hr with uptake activity slowly returning to control values by 24 hr. In mice, potentiation of 5-HTP symptoms by sertraline is obtained for pretreatment times of 0.5 to 3 hr, with the 4-hr pretreatment being much less effective.

Although several-fold less active than sertraline, the N-demethyl metabolite (CP-62,508) is still a selective 5-HT uptake blocker. This suggests that selective inhibition of 5-HT uptake after sertraline is probably maintained *in vivo*. N-demethylsertraline is about 4 times less potent than sertraline in blocking NE uptake. In contrast, norzimelidine, the major metabolite and presumed active entity of zimelidine (Ross *et al.*, 1981; Brown *et al.*, 1980), is about equally effective in blocking NE and 5-HT uptake. The equally potent effect of norzimelidine on NE pathways could also contribute to the pharmacological action of zimelidine. The *in vitro* uptake data in the present study for zimelidine and norzimelidine are somewhat different from those reported by Ross and Renyi (1977). Although we also find that norzimelidine is 10 times more active than zimelidine in inhibiting 5-HT uptake, in agreement with these authors, our results indicate that NE uptake blocking potency increases 30-fold on demethylation of zimelidine, compared with the 3- to 4-fold increase found by Ross and Renyi (1977). The reason for this difference is unclear, but dissimilarities in the methods of measuring uptake may contribute to the disparate potencies.

TABLE 12

Potentiation of 5-HTP symptoms by 5-HT uptake inhibitors in mice

DL-5-HTP was administered at a dose of 100 mg/kg i.p. 1 hr subsequent to the uptake blockers. Symptoms were evaluated 10 to 20 min later in groups of 10 mice at four dosage levels for each compound.

Compound	ED_{50} (mg/kg p.o.) and 95% CL for Causing the Following Symptoms in 5-HTP-Treated Mice			
	Tremors	Head twitch	Limb abduction	Backward locomotion
Sertraline	1.0 (0.7–1.5)	2.6 (1.9–5.5)	3.2 (2.3–4.3)	3.8 (2.9–4.9)
Fluvoxamine	12.9 (10.6–15.7)	25.2 (19.9–31.8)	14.7 (12.3–17.6)	14.2 (11.7–17.2)
Zimelidine	11.2 (7.2–17.5)	31.2 (4.5–219)	9.9 (6.8–14.6)	12.6 (8.1–19.4)
Fluoxetine	9.9 (6.5–15.5)	31.2 (4.5–219)	9.9 (6.5–15.5)	22.2 (16.4–30.1)
Chlorimipramine	47.3 (35.8–62.5)	87.6 (70.5–108.9)	32.0 (25.6–40.1)	54.3 (44.8–65.7)
Desipramine	>178	>178	>178	>178

TABLE 13

Potentiation of 5-HTP symptoms by sertraline in mice at various pretreatment times

Treatment	Interval until 5-HTP	Incidence of Mice Showing Various Symptoms 10–20 min After 5-HTP (100 mg/kg i.p.)		
		Tremor	Head twitch	Limb abduction
	hr			
Vehicle	1	0/10	1/10	1/10
Sertraline				
5.6 mg/kg p.o.	0.5	4/10	5/10	1/10
5.6 mg/kg p.o.	1	7/10	6/10	7/10
5.6 mg/kg p.o.	2	10/10	9/10	5/10
5.6 mg/kg p.o.	3	8/10	10/10	6/10
5.6 mg/kg p.o.	4	1/10	2/10	0/10

Unlike tricyclic antidepressants, sertraline has a low affinity for muscarinic receptors (60-fold less than amitriptyline) and lacks significant anticholinergic activity in mice. Thus, sertraline would not be expected to show anticholinergic effects, making it particularly suitable for use in geriatric patients. Steady-state concentrations of rat brain 5-HT, DA and NE are not altered by sertraline, which is also devoid of inhibitory activity toward MAO. Sertraline, like other 5-HT uptake blockers, lowers 5-HIAA levels in brain and decreases the 5-HT content of blood by inhibiting platelet uptake of 5-HT (Ross *et al.*, 1976a). Sertraline is well tolerated in mice and rats at single doses up to 100 mg/kg p.o. or s.c. At doses which result in marked blockade of 5-HT uptake *in vivo*, significant psychomotor stimulation is not observed. In rats, sertraline fails to increase locomotor activity even at doses that are more than 100 times higher than the threshold dose of tametraline and amphetamine for producing stimulation. In dogs, sertraline is well tolerated orally (50 mg/kg); among the symptoms elicited are anorexia and mydriasis, but no stimulation. No significant effects on blood pressure, heart rate or electrocardiogram of anesthetized dogs are elicited by sertraline. Pressor responses to epinephrine, NE or bilateral carotid occlusion are not affected by sertraline. On the other hand, enhancement of responses to 5-HT occurs, a result consistent with selective blockade of 5-HT uptake by this compound.

Mouse behavioral despair is an animal test considered to be predictive of the efficacy of antidepressant drugs. In this model, antidepressants reduce the immobility ("despair") of mice placed into an inescapable swimming cylinder (Porsolt *et al.*, 1977; Browne, 1979). Sertraline elicits a consistent decrease in immobility over a wide-dose range (threshold dose, 3.2 mg/kg s.c.) in contrast to fluvoxamine (inactive), zimelidine or fluoxetine.

Clinically active antidepressants (tricyclics, MAO inhibitors and "atypical" agents) and electroconvulsive shock treatments given chronically to rats down-regulate postsynaptic NE receptors. This effect is detectable as a decrease in the cyclic AMP response to NE of a receptor-coupled adenylate cyclase in limbic forebrain and a reduction in the density of cortical *beta* adrenergic receptors (Vetulani *et al.*, 1976; Banerjee *et al.*, 1977; Wolfe *et al.*, 1978; Mobley and Sulser, 1981). Antidepressant drugs that inhibit neuronal uptake of NE enhance noradrenergic activity at central synapses, thus initiating events which lead to a therapeutic effect over several weeks. This delayed onset of efficacy despite rapid blockade of NE reuptake could coincide with the time needed for down-regulating postsynaptic NE receptors. In the down-regulation process, which could be viewed as an eventual reduction in the sensitivity of transmission in NE pathways, autoreceptors (*alpha*-2) apparently must also become desensitized to overcome the natural negative feedback mechanism regulating NE neurons (Wiech and Ursillo, 1980; Crews *et al.*, 1981).

There is also evidence that facilitation of serotonergic activity may also lead to antidepressant activity: 1) reports of antidepressant activity for L-tryptophan (5-HT precursor) (Coppen *et al.*, 1967, 1972; Van Praag and Korf, 1971); 2) reversal of the therapeutic action of imipramine by the 5-HT synthesis inhibitor, PCPA, and restoration on withdrawal of PCPA (Shopsin *et al.*, 1975); and 3) recognition of subgroups of depressed patients, *i.e.*, those responding to NE uptake blockers ("NE" depressions) and nonresponders who benefit from tricyclics with increased 5-HT uptake blocking activity ("5-HT" depressions) (Maas, 1975; Garver and Davis, 1979). The recent reports of therapeutic activity in depressed patients for several selective 5-HT uptake blockers, such as fluvoxamine (Saletu *et al.*, 1977; Wright and Denber, 1978; Doogan, 1980), citalopram (Gottlieb *et al.*, 1980), zimelidine (Benkert *et al.*, 1977; Cox *et al.*, 1978) and fluoxetine (L. Lemberger, R. W. Fuller and D. T. Wong, private communication) lend strong support for a role of 5-HT in depression.

We have sought a rationale which will accommodate the pronounced selectivity of 5-HT uptake inhibitors like sertraline, as well as the existing evidence for implicating both NE and 5-HT in depression and the action of antidepressant drugs. It may also suggest that division of drug treatments for depression into two types ("noradrenergic" and "serotoninergic") may be unnecessary. Selective 5-HT uptake blockers may be effective antidepressants because of the influence of 5-HT neurons upon NE neurons in brain. Thus, raphe 5-HT neurons may exert inhibitory control over NE neurons of the locus ceruleus (Pujol *et al.*, 1978; McRae-Degueurce *et al.*, 1982). These 5-HT neurons, characterized by an extensive auto- and mutual regulatory network, are inhibited by applied 5-HT and other 5-HT agonists (Wang and Aghajanian, 1982; Aghajanian and Wang, 1978). Serotonin uptaker blockers, MAO inhibitors and 5-HT precursors (tryptophan and 5-HTP) also depress firing of raphe 5-HT neurons, an action that is blocked by PCPA in the case of 5-HT uptake blockers and MAO inhibitors (Aghajanian *et al.*, 1970; Sheard *et al.*, 1972; Trulson and Jacobs, 1975; Aghajanian and Wang, 1978; De Montigny *et al.*, 1981). Consequently, shutdown of firing of these 5-HT neurons by blockers of 5-HT uptake should effect activation of the locus ceruleus and thus elicit 1) a subsequent desensitization of postsynaptic *beta* and presynaptic *alpha*-2 adrenoceptor systems and 2) an ultimate antidepressant response. Consistent with this proposal are the clinical observations of Shopsin and co-workers (1975, 1976) who found that the therapeutic effects of imipramine and tranylcypromine in depressed patients are reversed by coadministration of PCPA but restored upon its withdrawal. Their findings suggest that the antidepressant effects of imipramine and tranylcypromine may involve the suppression of firing of 5-HT neurons caused by inhibition of 5-HT uptake and elevation of 5-HT levels, respectively. Chlorimipramine and zimelidine also suppress firing of raphe 5-HT neurons (Sheard *et al.*, 1972; De Montigny *et al.*, 1981), although their down-regulation of NE receptor systems have been attributed in part to the enhanced ability of the respective demethyl metabolite to block

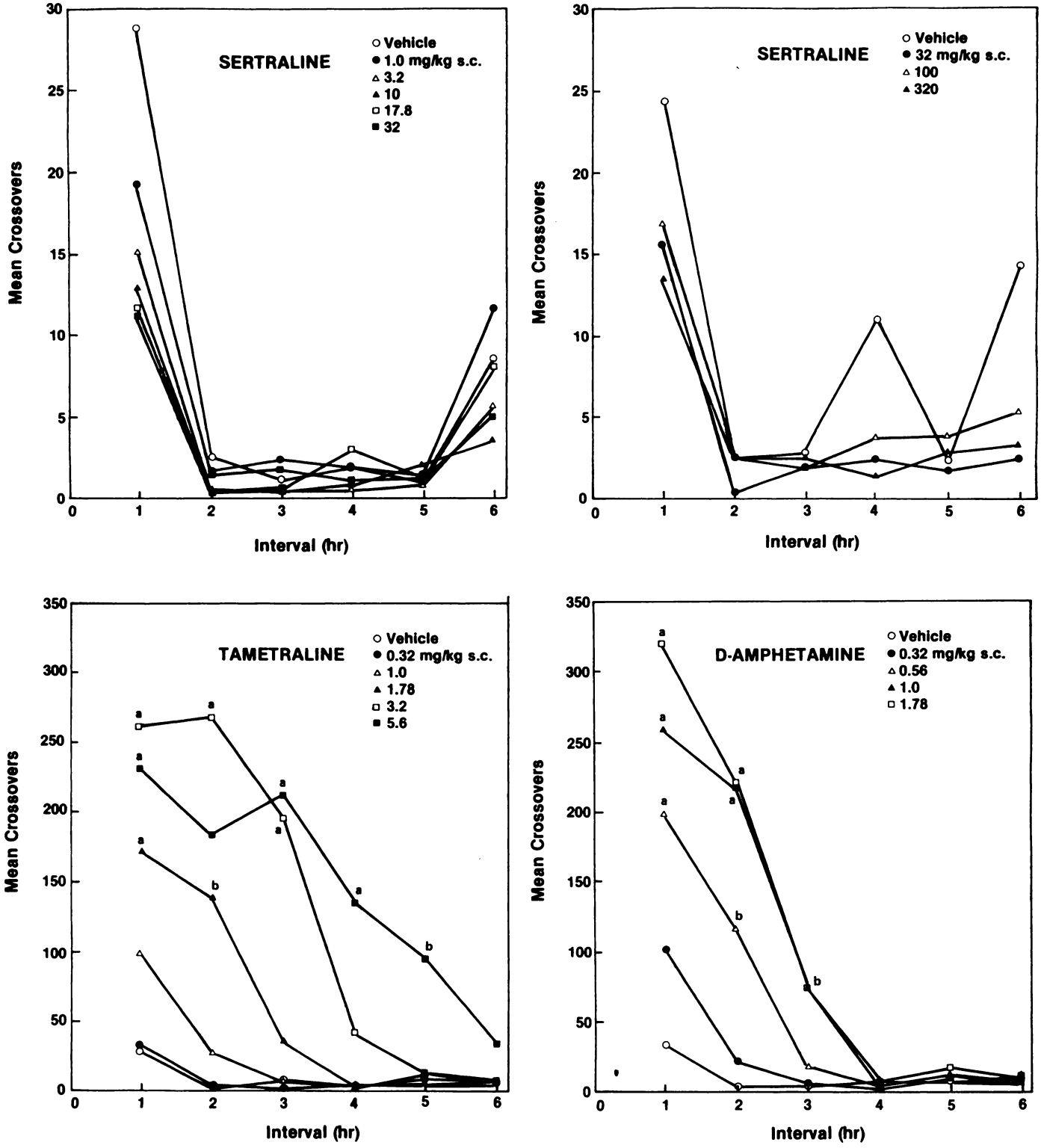


Fig. 5. Locomotor activity of rats as a function of time after administration of sertraline, tametraline and amphetamine. Each point represents mean crossovers for eight rats accumulated for the intervals, 0 to 1, 1 to 2, 2 to 3, 3 to 4, 4 to 5, and 5 to 6 hr. Doses refer to base form of the drug. [a]$P < .001$ *vs.* vehicle controls (Dunnet's t-test). [b]$P < .05$ *vs.* vehicle controls.

NE reuptake (Mishra and Sulser, 1978; Mishra *et al.*, 1980, 1981).

A weak link in our hypothesis that subacute or chronic administration of 5-HT uptake blockers can induce down-regulation (subsensitivity and decreased numbers) of central NE receptors might be that it is based on electrophysiological findings involving acute administration of these drugs. However, it is also known that 5-HT blockers do produce a prolonged decrease in 5-HT synthesis and turnover, which may reduce the functional activity of 5-HT neurons, on chronic

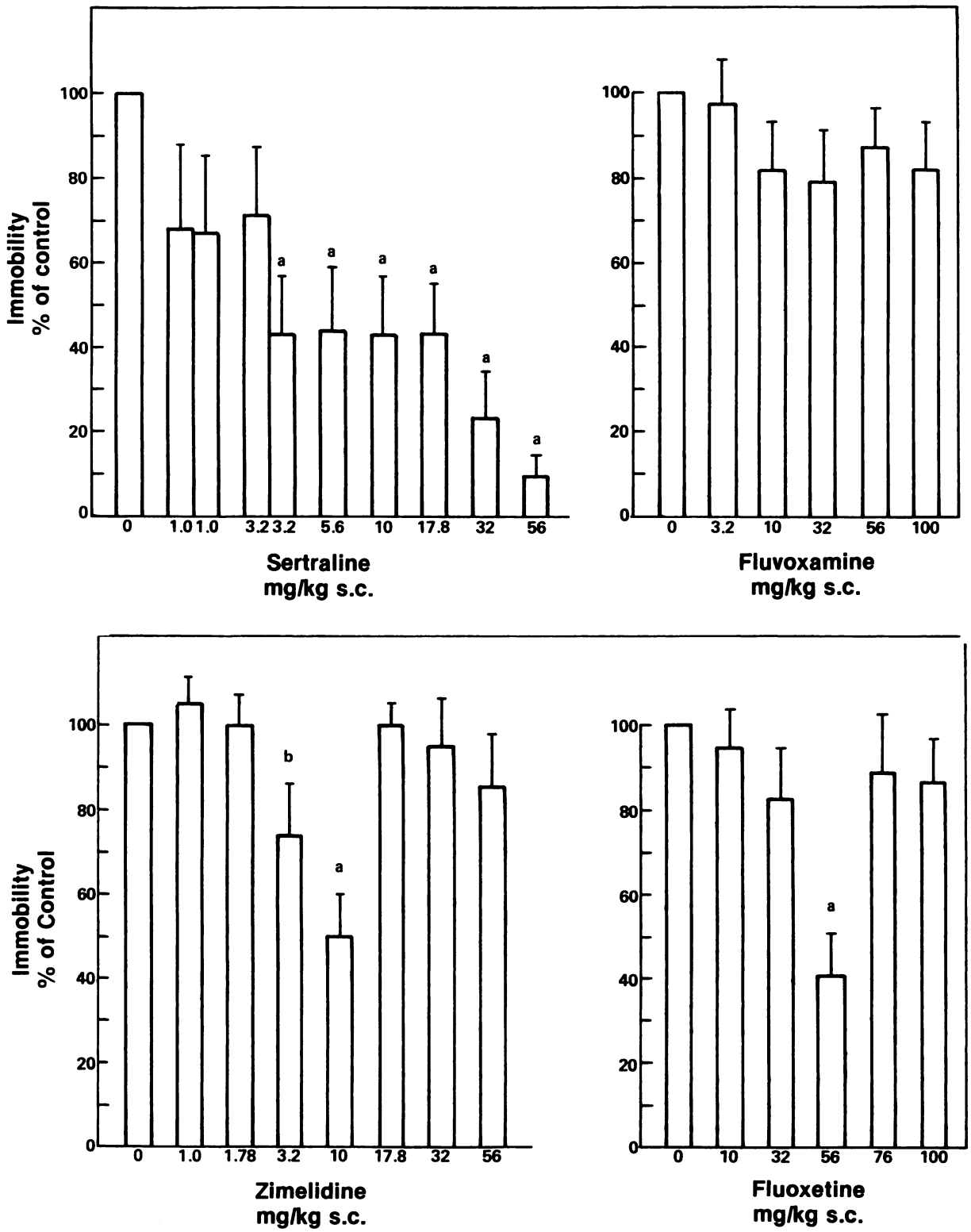


Fig. 6. Immobility (behavioral despair) in the Porsolt mouse forced swimming model for antidepressants as a function of dose of sertraline, fluvoxamine, zimelidine and fluoxetine. The S.E. of the control (0) means ranged from 6.4 to 18.9 in various runs. $N = 6$ mice minimum per group. [a]$P < .01$ contemporary controls (Mann-Whitney U test). [b]$P < .05$ *vs.* contemporary controls.

administration (Sugrue, 1981; Ögren *et al.*, 1979). The end result of this diminished function could be the same as that proposed above, namely, activation of NE neurons followed by desensitization of NE receptors.

In the present study, sertraline is found to induce down-regulation of limbic forebrain NE-stimulated adenylate cyclase and cortical *beta* adrenoceptors after the administration of multiple doses. Thus, sertraline shows efficacy in a test on the molecular level which is predictive of clinical antidepressant activity (Mobley and Sulser, 1981). Although Scatchard anal-

TABLE 14

Reversal of reserpine hypothermia by uptake inhibitors in mice

Combined rectal temperatures ± S.D. for controls (mice receiving reserpine only) ($N = 20$) were: 2 hr, 20.4 ± 1.2; 3 hr, 22.3 ± 4.5.

Compound	Dose	Mean (±S.D.) Rectal Temperature in Groups of Five Mice Previously Treated with Reserpine (2 mg/kg s.c.)	
		2 hr	3 hr
	mg/kg p.o.	°C	
Sertraline	10	20.3 ± 0.3	20.9 ± 0.6
Fluvoxamine	10	20.7 ± 0.5	21.0 ± 0.6
Zimelidine	10	21.0 ± 0.7	21.9 ± 0.9
Fluoxetine	10	21.0 ± 0.3	21.7 ± 0.8
Chlorimipramine	10	19.9 ± 0.7	21.4 ± 0.9
Desipramine	10	23.5 ± 2.5[a]	27.7 ± 5.3[a]

[a] $P < .01$ vs. contemporary controls. At 1 hr, desipramine was also the only uptake blocker reversing reserpine hypothermia ($P < .05$; data not shown).

TABLE 15

Antagonism of oxotremorine-elicited symptoms in mice

Compound	ED_{50} for Blocking Various Symptoms Produced by Oxotremorine (0.56 mg/kg i.p.)		
	Tremor	Salivation	Diarrhea
	mg/kg s.c.		
Sertraline	>32	>32	>32
Atropine	0.9	0.3	0.6
Benztropine	1.8	1.1	0.3
Amitriptyline	5.7	~3.2–10	11.2
Doxepin	11.2	17.7	14.1
Desipramine	>32	>32	~32
Chlorimipramine	>32	>32	>32
Fluoxetine	>32	>32	>32
Tametraline	>32	>32	~32

TABLE 16

Mydriatic effects in mice

Compound	Dose	Median Pupil Sizes in Mice ($N = 5$) Treated with Tetrabenazine (32 mg/kg i.p.)
	mg/kg s.c.	mm × 20
Vehicle		8
Sertraline	32	10
Atropine	1	59
Scopolamine	1	50
Amitriptyline	3.2	16
	10	34
	32	58
Doxepin	3.2	10
	10	15
	32	28
Desipramine	3.2	18
	10	18
	32	29
Chlorimipramine	3.2	6
	10	10
	32	18
Fluoxetine	32	8
Tametraline	3.2	7
	10	11
	32	10

TABLE 17

Inhibition of [^{3}H]QNB binding to rat brain membranes

IC_{50} values are means of two to six determinations. L-[^{3}H]QNB concentration = 1 nM.

Compound	IC_{50}
	μM
Sertraline	19
Atropine	0.021
Benztropine	0.024
Scopolamine	0.007
Amitriptyline	0.32
Doxepin	0.71
Desipramine	3.5
Tametraline	4.7

ysis of the *ex vivo* [^{3}H]DHA binding was not conducted in the present study, antidepressants generally elicit a lowering of receptor density. The greater decrease in adenylate cyclase sensitivity (−19%) compared to the reduction in [^{3}H]DHA binding (−10%) induced by sertraline (table 11) suggest that an attenuated 5-HT neuronal input at cortical NE terminals may have produced "uncoupled" *beta* adrenoceptors (Janowsky *et al.*, 1982; Manier *et al.*, 1983). This down-regulation after sertraline may be attributed to selective blockade of 5-HT uptake, as CP-62,508, the demethyl metabolite of sertraline, still is a selective, although less potent, 5-HT uptake inhibitor. Although a contribution to down-regulation from possible blockade of NE uptake cannot be entirely excluded, sertraline and CP-62,508 are 200 and 800 times less active than desipramine against NE uptake, respectively. It is noteworthy that sertraline only weakly inhibits heart uptake of [^{3}H]NE *in vivo* (23% decrease after 100 μmol/kg i.p.), in marked contrast to chlorimipramine (ED_{50}, 18 μmol/kg i.p.) and other antidepressants with weak to moderate *in vitro* activity against synaptosomal NE uptake, such as amitriptyline, doxepin, mianserin and viloxazine (ED_{50}, 24, 26, 29 and 20 μmol/kg i.p., respectively; Koe, 1975, 1976).

Our demonstration that sertraline, a highly selective 5-HT uptake blocker, can induce down-regulation of NE receptors, in common with clinically active antidepressants, is in contrast to the results of several studies with the 5-HT uptake inhibitor, fluoxetine. Fluoxetine apparently produces neither subsensitivity of NE receptor-coupled adenylate cyclase (Mishra and Sulser, 1978; Mishra *et al.*, 1981) nor a reduction in [^{3}H]DHA binding to *beta* adrenoceptors (Peroutka and Snyder, 1980). The reason for the disparate results is not known. Sertraline and fluoxetine are unique chemical entities, so that as yet undiscovered neurochemical differences may be responsible. On the other hand, it is noteworthy that in the fluoxetine studies cited, the same dose of 10 mg/kg i.p. was used. Because the protocol of administering drug b.i.d. seems to facilitate the down-regulating effects of already potent agents like desipramine (Wolfe *et al.*, 1978), our use of the b.i.d. dosing regimen may have aided in our detection of these effects of sertraline.

From the viewpoint of structure-activity relationships, our studies of the 4-phenyl-1-aminotetralins have shown the dramatic effects of molecular conformation on the selectivity of interaction of these compounds at monoamine uptake sites of neuronal membranes (Sarges *et al.*, 1974; Koe, 1976). A surprisingly substantial enhancement of uptake blocking activity is achieved by introducing chlorine atoms at C-3 and C-4 of tametraline, (+)-*trans*-(1R,4S)-N-methyl-4-phenyl-1-aminotetralin, a potent but nonselective uptake blocker (Koe, 1976).

Unexpectedly, the same substituents confer selective and potent 5-HT uptake inhibiting activity to the (+)-*cis*-(1S,4S)-isomer. Based on the potent inhibition of 5-HT uptake by both the (+)-*cis*-(1S,4S)-3,4-dichloro isomer, sertraline, and the (+)-*trans*-(1R,4S)-3,4-dichloro isomer, CP-52,003, the S conformation of the 4-phenyl ring favors attachment at 5-HT uptake sites. The change in conformation at C-1 from 1R to 1S only slightly decreases 5-HT uptake blocking activity (table 1), suggesting that the 1-N-methylamino group in either conformation and the 4S 3,4-dichlorophenyl ring can approximate the 1-amino-4-phenyl configuration of EXP-561, the structurally rigid prototype inhibitor of 5-HT uptake (Koe, 1976). On the other hand, removing the 3-chloro substituent increases the importance of the conformation at C-1. Thus, (+)-*cis*-(1S,4S)-N-methyl-4-(4-chlorophenyl)-1-aminotetralin, although a strong uptake blocker, is somewhat less inhibitory than the (+)-*trans*-(1R,4S)-isomer toward 5-HT uptake (IC_{50} values, 0.50 μM *vs.* 0.084 μM, respectively) (B. K. Koe and W. M. Welch, unpublished data).

In conclusion, sertraline is a new uptake blocker which potently and selectively inhibits synaptosomal 5-HT uptake *in vitro*. It markedly blocks 5-HT uptake *in vivo* at doses which do not elicit significant psychostimulant or anticholinergic activity in animals. In accord with its selective inhibition of 5-HT uptake, sertraline potentiates pharmacological effects dependent on serotoninergic activity but does not enhance those involving catecholamines. Sertraline does not affect brain monoamine levels, inhibit MAO or block heart uptake of [^{3}H] NE *in vivo*. It is well tolerated in rodents and dogs with no untoward cardiovascular effects in the latter species. The N-demethyl metabolite of sertraline is also a selective 5-HT uptake blocker but somewhat less active than the parent compound. Potential antidepressant activity for sertraline is suggested by its down-regulation of NE receptor-coupled adenylate cyclase (limbic forebrain) and *beta* adrenoceptors (cerebral cortex) in rats after a multiple dosing regimen and its reduction of immobility (despair) in the Porsolt mouse swim test.

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