April 26, 1974

Dr. O. K. Behrens
Dr. M. D. Cise
Dr. L. Lemberger
Dr. B. B. Molloy
Dr. J. Parli
Dr. J. S. Wold
Dr. D. T. Wong

Copy to:

Dr. I. H. Slater
Dr. I. F. Bennett
Dr. J. A. Clemens
Mr. R. D. Dillard
Dr. R. Frederickson
Mr. R. W. Kattau
Dr. W. B. Lacefield
Dr. D. B. Meyers
Mr. J. E. Owen
Dr. R. P. Pioch
Dr. R. C. Rathbun
Dr. M. J. Schmidt
Dr. P. Stark
Dr. M. Steinberg
Mr. R. M. Van Frank
Mr. J. S. Ward

Dr. E. F. Alder
Dr. M. D. Bray
Dr. D. M. Brennan
Dr. H. Campbell
Dr. C. N. Christensen
Dr. J. R. Easton
Dr. W. H. Flynn
Dr. R. H. Furman
Mr. W. R. Gibson
Mr. C. P. Gorman
Dr. R. S. Griffith
Dr. W. W. Hargrove
Dr. E. B. Herr, Jr.
Mr. P. D. James
Dr. R. S. Johnson
Dr. G. F. Kiplinger
Dr. W. R. Kirtley
Dr. R. J. Kraay
Dr. A. L. Kranzfelder
Dr. J. H. Marsden
Dr. Jack Mills
Dr. F. B. Peck, Jr.
Dr. A. Pohland
Dr. Ian Shedden
Dr. E. M. Van Heyningen (2)

COMPOUND 110170 PROJECT TEAM MEETING

MARCH 29, 1974

The major purpose of this meeting was to complete a pre-IND critical path scheme recently devised by Dr. [redacted] and others. A copy of the completed form is attached. The target date for having the IND completed and ready for submission was set at October 8, 1974.

The 90 day toxicity study in rats is into the 9th week. All of the rats at the high dose (.09% in the diet) have died. The rats at the next lower dose (.03% in the diet) are eating well and gaining weight. A pronounced hyperirritability in these rats was observed during the second to the fourth weeks and has now disappeared from some of the rats. The .03% group and also the lower dose (.01%) group are expected to survive until the end of the 90 days, and Dr. Wold felt that the study would be adequate without starting any more animals. Pathology data will be available on about one-third of the rats that died in the high dose group.

EXHIBIT

— FULLER 9 —

The 90 day toxicity study in dogs is in the fourth week and is going well. Daily doses are 5, 10, and 20 mg/kg. In addition, a group of dogs is receiving 20 mg/kg every other day--this dosage schedule was adopted because of the unusually long half-life of 110140 and its active primary amine metabolite. Mydriasis in the dogs is diminishing, and overall their activity seems fairly normal.

A short acute toxicity study in guinea pigs has indicated that 110140 is more toxic in guinea pigs than in rats (as had been observed in a non-systematic way at McCarty Street). Also the toxic effect seems to be different than in rats (the guinea pigs die sooner).

The consideration of a pair of generic names for compound 110140 and for compound [redacted] (which has an o-methoxy instead of the p-trifluoromethyl on the phenoxy ring) was resumed. There was agreement to recommend flufenpromin and mefenpromin as possible generic names for 110140 and [redacted] respectively, with trifluprominе-methopromine and fluphenamine-mephenamine as alternates.

Ray W. Fuller
Project Team Chairman

as

attachment

ENHANCEMENT OF AMPHETAMINE LEVELS IN RAT BRAIN BY 82816* IN COMPARISON WITH CHLORIMIPRAMINE

* The oxalate salt corresponding to compound 110140

Studies Completed by

Ray W. Fuller

(Curriculum Vitae in Master File [redacted]

With the Technical Assistance of

Harold D. Snoddy

Division of Biochemical and Physiological Research

The Lilly Research Laboratories

Eli Lilly and Company

Indianapolis, Indiana 46206

August 1974

SUMMARY

The levels of amphetamine in rat brain 2 hours after i.p. injection of tritium-labeled amphetamine (10 mg/kg) was significantly higher in rats that had been pretreated with either Compound [redacted] or with chlorimipramine.

INTRODUCTION

The ability of compounds to potentiate the pharmacologic effects of amphetamine was first used to screen for potential antidepressant drugs. Tricyclic antidepressant drugs like imipramine, desmethylimipramine, nortriptyline and protriptyline potentiate amphetamine effects and enhance amphetamine levels in brain by inhibiting the para-hydroxylation of amphetamine (Lewander, 1969). The ability to inhibit amphetamine metabolism is shared by other antidepressant drugs, including iprindole (Miller et al., 1970; Freeman and Sulser, 1972)--which does not appear to be an amine uptake inhibitor in the usual sense. We, therefore, wanted to know if 110140 would affect levels of amphetamine in rat brain, and did this comparative study with chlorimipramine.

METHODS

Male Wistar-derived rats (Harlan Industries, Cumberland, Indiana) weighing about 125-150 g were housed singly in hanging wire cages in a 22-24° room with food and water available ad libitum. The rats were given an i.p. injection of ^{3}H-d-amphetamine sulfate (10 mg/kg; 1 μC_i per 100 g). Some rats had been pretreated 1 hr previously with chlorimipramine (Geigy) or with

Compound [redacted] dl-N-methyl-3-phenyl-3-[(α,α,α-trifluoro-p-tolyl)oxy]propylamine oxalate. Rats were killed by decapitation 2 hrs after the dose of d-amphetamine, and amphetamine levels in brain were measured by extraction from brain homogenates into benzene at pH 10 followed by liquid scintillation counting of the benzene extract. Metabolites of amphetamine are not extracted by this procedure.

RESULTS

Table 1 shows the levels of amphetamine in rat brain 2 hrs after the drug was injected. Chlorimipramine at doses of 1 or 3 mg/kg had no effect, but at a dose of 10 mg/kg it significantly increased amphetamine levels. Compound [redacted] significantly elevated amphetamine levels at doses of 3 and 10 mg/kg but not at 1 mg/kg.

These results show that 110140 shares with chlorimipramine and with other antidepressant drugs (such as desmethylimipramine and iprindole) the ability to enhance amphetamine levels in rat brain.

REFERENCES

Freeman, J. J. and Sulser, F. (1972). Iprindole-amphetamine interactions in the rat: The role of aromatic hydroxylation of amphetamine on its mode of action. J. Pharmacol. Exptl. Therap. 183, 307-315.

Lewander, T. (1969). Influence of various psychoactive drugs on the in vivo metabolism of d-amphetamine in the rat. Eur. J. Pharmacol. 6, 38-44.

Miller, K. W., Freeman, J. J., Dingell, J. V. and Sulser, F. (1970). On the mechanism of amphetamine potentiation by iprindole. Experientia 26, 863-864.

156

Table 1: Effect of [redacted] and Chloroimipramine on Amphetamine Levels in Rat Brain.

Group		d-Amphetamine Levels, µg/g
Control		2.51 ± 0.26
CMI	1 mg/kg	3.24 ± 0.54
	3	3.41 ± 0.38
	10	4.49 ± 0.58*
110140	1	2.[illegible] ± 0.43
	3	5.97 ± 0.31*
	10	6.24 ± 0.86*

*Significantly different from control $P < .025$.
Mean values ± standard errors for 5 rats per group are shown.

Rats were given chloroimipramine or [redacted] at various doses i.p. 1 hr prior to injection of tritrated d-amphetamine. Two hours later, rats were killed and amphetamine levels in brain were measured.

C O N F I D E N T I A L

May 18, 1978

Members:

Dr. H. A. Barnett
Mr. R. H. Carmichael
Dr. M. D. Cise
Dr. R. W. Fuller
Dr. G. E. Gutowski
Dr. B. G. Jackson
Dr. L. Lemberger
Dr. B. B. Molloy
Dr. J. Parli
Mr. G. L. Schmitz
Dr. R. Shulman
Dr. J. S. Wold
Dr. D. T. Wong

cc:

Dr. E. F. Alder
Ms. J. A. Allan
Dr. M. D. Bray
Dr. D. M. Brennan
Dr. H. Campbell
Dr. C. N. Christensen
Dr. P. V. Demarco
Dr. N. R. Easton
Dr. E. H. Flynn
Dr. R.C.A. Frederickson
Dr. R. H. Furman
Dr. W. W. Hargrove
Dr. E. B. Herr
Mr. P. D. James
Dr. I. S. Johnson
Dr. G. F. Kiplinger (Erl Wood)
Dr. W. R. Kirtley
Dr. J. H. Marsden
Dr. T. W. McIntyre
Dr. R. E. McMahon
Dr. J. Mills
Dr. D. M. Morton
Dr. A. Pohland
Dr. P. Roffey
Dr. I. Shedden
Dr. M. R. Schinitsky
Dr. I. H. Slater
Dr. E. M. Van Heynin
Mr. E. E. Van Meter
Dr. J. G. Whitney
Dr. R. H. Williams

Minutes No. 78-1

FLUOXETINE PROJECT TEAM MEETING

May 15, 1978

Phase I clinical studies. The treatment phase of a metaboli
study with radiocarbon-labeled fluoxetine has been completed at t
Lilly Clinic. The complete identification and analysis of urinar
metabolites have not been completed, but the results so far avail
able indicate that radioactivity is excreted into the urine over
very long time following fluoxetine administration. Urine sample

were collected for 33 days after a single dose of ^{14}C-fluoxetine, and some ^{14}C was excreted even in the last samples collected. Fluoxetine and/or some of its metabolites are avidly retained in tissues.

A protocol for a dose-ranging study of fluoxetine given in combination with L-5-hydroxytryptophan (L-5-HTP) has been submitted to the FDA. In order to obtain L-5-HTP from Sigma, a letter from the FDA stating their approval of its use in the study was required and was provided. Dr. Lemberger has the L-5-HTP, which has been found to have acceptable purity. This study will begin shortly.

Phase II clinical studies. Two clinical studies evaluating th antidepressant activity of fluoxetine have gotten underway. was the first investigator to treat patients. Within a few days after fluoxetine treatment was started, the first patient showed symptoms resembling an extrapyramidal reaction typically produced by neuroleptic drugs. The symptoms responded to Cogentin, and the patient continued on th 4 week course of therapy with fluoxetine. Neither this patient nor another in his study who has completed the treatment regimen showed significant improvement in depressive symptoms.

The second study that is underway is by at One patient completed the treatment regimen and showed no significant improvement. A third study has been at the approved, and the drug has been shipped; however, the study is not yet underway.

Additional studies that are planned to evaluate fluoxetine as an antidepressant agent are by at the will participate in this study) and by in Dr. William Potter at the NIMH has indicated plans to study fluoxetine in depression but has not yet submitted a protocol.

will study fluoxetine in dystonia musculorum deformans, a condition that he ha treated previously with L-tryptophan. His study is not yet started

of the plans to evaluate fluoxetine in postanoxic intention myoclonus but he has not yet obtained clinical protocol approval from his institutional review committee.

and his colleagues at hope to have a protocol prepared by this summer for the study of fluoxetine in narcolepsy/cataplexy. They have reported favorable responses to fluoxetine in an animal model of this disease.

Some other potential areas in which fluoxetine might be evaluated were discussed briefly. in New York has postulated an involvement of serotonin in obsessive-compulsiv behavioral disorders and has reported that chlorimipramine, a les specific inhibitor of serotonin uptake than fluoxetine, was effective in treating this condition. He has asked to study fluoxetine, but this study will be held up until additional safet experience is available from studies with depressed inpatients.

Work originally reported from M.I.T. and later extended elsewhere has indicated that fluoxetine can produce analgesic activity in certain types of animal experiments. Though fluoxeti was not active in animal tests here thought to be the most reliab predictors of clinical analgesic potential, the possibility of evaluating analgesic effects of fluoxetine clinically will be considered if investigators want to use it to elucidate a role of brain serotonin in pain perception.

A marked reduction in food intake has been reported by Goudi et al. in rats treated with fluoxetine combined with L-5-HTP. In addition, Wurtman and Wurtman have reported that fluoxetine selectively reduces total caloric intake while sparing protein consumption in rats given a choice of foods. Since some marketed (fenfluramine) and experimental (MK-212) anorectic drugs are thou to act by stimulating serotonin receptors either directly or indirectly, there is a theoretical basis for anticipating an anorexic action of fluoxetine, especially if combined with L-5-HT There are no plans at present to study fluoxetine as an appetite suppressant drug, but Dr. Lemberger will give special attention t possible effects on appetite in his studies of fluoxetine given i combination with L-5-HTP.

Dr. Schinitsky has some experimental data in rats showing th fluoxetine alone or in combination with L-5-HTP can reduce alcoho consumption. There are also reports in the literature implicatin role of brain serotonin in alcohol preference in rats. Consideri the difficulties inherent in evaluating the effect of a drug on alcohol consumption in humans, the team does not plan at this tim to do clinical studies with fluoxetine in this area.

Plans for international trials. At the joint U.S.-European Clinical Research Planning Committee meeting in March, the decisi was made to give priority to fluoxetine over nisoxetine in prepar for clinical studies in European countries. The details of the synthetic method have been supplied to the U.K. so that plans can made for synthesizing material there to be used in the clinical studies overseas. Fluoxetine has been submitted to the bacterial mutagen screen, the results of which are required for clinical trials in Italy. Teratology studies required for CSM approval of studies in the U.K. have been completed, and a written report is being prepared. As soon as the exact nature of the additional studies required by the CSM is clarified, they will be started as well.

Toxicology. Some acute toxicity studies and preliminary pil studies for 90 day subacute studies in rats and dogs with a combi tion of fluoxetine and L-5-HTP have been done. These studies are planned in preparation for the use of the fluoxetine/L-5-HTP combination in clinical studies involving fairly long-term treatm

Supply. The supply of capsules is adequate for the clinical studies already arranged for, and about 5 kg of bulk material is hand.

Ray W. Fuller
Project Team Chairman

C O N F I D E N T I A L

August 2, 1978

Members:

Dr. H. A. Barnett
Mr. R. H. Carmichael
Dr. M. D. Cise
Dr. R. W. Fuller
Dr. G. E. Gutowski
Dr. B. G. Jackson
Dr. L. Lemberger
Dr. B. B. Molloy
Dr. J. Parli
Mr. G. L. Schmitz
Dr. R. Shulman
Dr. J. S. Wold
Dr. D. T. Wong

cc:

Dr. E. F. Alder
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Dr. H. Campbell
Dr. C. N. Christensen
Dr. P. V. Demarco
Dr. N. R. Easton
Dr. E. H. Flynn
Dr. R.C.A. Frederickson
Dr. R. H. Furman
Dr. W. W. Hargrove
Dr. E. B. Herr
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Dr. R. E. McMahon
Dr. J. Mills
Dr. D. M. Morton
Dr. A. Pohland
Dr. P. Roffey
Dr. I. Shedden
Dr. M. R. Schinitsky
Dr. I. H. Slater
Dr. E. M. Van Heyningen
Mr. E. E. Van Meter
Dr. J. G. Whitney
Dr. R. H. Williams

Minutes No. 78-2

FLUOXETINE PROJECT TEAM MEETING

July 31, 1978

Phase II clinical studies. Three trials in mental depression have started and a fourth is about to start. Dr. has given the four week course of fluoxetine treatment to three patients, and five at the patients have completed the treatment course in Dr. Two patients have been entered in a study by study at Dr. at the study in : has been delayed because the FDA thought the protocol did not exclude women of child-bearing age, despite the fact that the protocol statement is the same as in the previous protocols they had approved.

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None of the eight patients who completed the four-week treatment showed distinct drug-induced improvement. One patient improved, but the improvement started during the placebo period before drug treatment and the continued improvement may not have been due to fluoxetine. The most encouraging finding has been in one patient still being treated at This patient had been hospitalized previously and treated unsuccessfully with conventional drug therapy, with psychosurgery, and with electroconvulsive therapy. She is now on fluoxetine and is doing well, though one must be cautious about attributing this to fluoxetine.

There have been a fairly large number of reports of adverse reactions. These have been varied, and their relationship to fluoxetine is not clearly established. The first depressed patient to receive fluoxetine showed dystonia resembling an extrapyramidal reaction; this was treated with Cogentin for a few days and the patient continued on fluoxetine without further problems. Another report mentioned enlarged thyroid and liver in a patient on fluoxetine; there was no change in liver function tests, and thyroid function was not evaluated. One patient showed ocular changes in the ophthalmologic examination following fluoxetine treatment. The changes were described as an epithelial corneal defect in one eye, iritis in the other eye. Dr. , a local ophthalmologist, felt these changes were not likely to be caused by a drug. Dr. , a researcher at interested in eye pathology, has been consulted and will advise on the possible utility of animal toxicity studies in regard to these ocular changes. One patient with a history of alcoholism and cirrhosis consumed alcohol while taking fluoxetine and showed abnormal blood chemistry and, abnormal EEG. The blood chemistry changes included elevation in glucose, SGOT, inorganic phosphate, SGPT and uric acid and were thought to most likely be due to alcohol. Another depressed patient developed psychosis manifested by paranoid delusions while taking fluoxetine. Akathisia and restlessness were reported in some patients.

Cerebrospinal fluid samples have been obtained before and during treatment from all patients in the and studies, but not all of these samples have been analyzed. One patient in showed a decrease in 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid from 39 to 31 ng/ml and another from 27 to 15 ng/ml comparing placebo period to fluoxetine period. This decrease in 5-HIAA concentration is an indicator that fluoxetine was effective in blocking serotonin uptake. At not only basal 5-HIAA concentration but also 5-HIAA accumulation after the administration of probenecid to block its efflux from the cerebrospinal fluid were measured. One patient showed a

basal level of 5-HIAA of 18 ng/ml and an accumulation after probenecid to 127 ng/ml during the placebo period. During fluoxetine treatment the basal 5-HIAA concentration was 15 ng/ml and the accumulation after probenecid was to only 68 ng/ml. However the probenecid concentration measured in the cerebrospinal fluid was lower during the fluoxetine treatment period. Additional 5-HIAA data should indicate clearly if the dosage regimen of fluoxetine is adequate to inhibit serotonin uptake in brain.

in California has fluoxetine effects in dystonia musculorum deformans, disorder that he claims to have treated previously. The first patient response on 30 mg daily of the dose was lowered to 20 has not responded.

One current school of thought among psychiatric researchers is that a subgroup of depressed patients are deficient in serotoninergic function and would be helped by a drug like fluoxetine. These patients possibly can be identified by measurement of 5-HIAA concentration in the cerebrospinal fluid. The possibility that preselection of patients in this way might be necessary in order to expect favorable responses to fluoxetine in a reasonable percentage of the patients tried has been considered. However, zimelidine and fluvoxamine, two other specific inhibitors of serotonin uptake, have been claimed to work as antidepressant agents in a high percentage of non-selected depressed patients. Another means of selecting patients would be to choose those who have failed to respond to marketed tricyclic drugs, most of which affect norepinephrine primarily and serotonin only slightly or not at all. This strategy has been discussed with the investigators who are considering the possibility of doing that in the VA hospital there.

<u>Phase I clinical studies.</u> Dr. Lemberger has given L-5-hydroxytryptophan (5HTP) in combination with fluoxetine to human volunteers. This dose-ranging protocol was designed in anticipation of efficacy studies with this combination. For example, Dr. has previously reported that 5HTP alone

is useful in treating If that effect is, as Dr. believes, due to enhanced function of central serotonin neurons, then a combination with fluoxetine should act synergistically. Dr. Lemberger obtained 5HTP prepared for clinical use from Sigma and gave it along with 30 mg fluoxetine at doses from 50 mg up to 1000 mg. These total amounts were given in divided doses. Most subjects showed some diarrhea after the first dose of 5HTP but not after subsequent doses. If the 5HTP was not given until one hour or more after the fluoxetine dose that problem was alleviated. Some nausea was encountered at doses of 400 mg 5HTP per day and higher. One individual who was to receive 1000 mg total of 5HTP showed a change in mood (euphoria) after the first 200 mg dose. This work was completed before the FDA questioned the protocol, and some further clinical work may be done when their questions are answered.

Toxicology. A toxicity study of the combination of fluoxetine and 5HTP will probably be re-started sometime in December. Three other studies in toxicology are anticipated. The first is a fertility study necessary for CSM submission in the U.K. This will probably start in December and will last eleven months. Clinical trials in the U.K. apparently cannot be done until this report is available. The second study is a comparison of fluoxetine with fluvoxamine and zimelidine--two other specific inhibitors of serotonin uptake being evaluated clinically in Europe and the U.S.--in terms of their ability to cause phospholipidosis in rats. That study should begin in August and will involve only one week of treatment. The third study will be done with and fluoxetine in monkeys to determine if a decreased white blood count can be produced by and if the earlier small changes observed with fluoxetine were real.

Ray W. Fuller
Project Team Chairman

FLUOXETINE PROJECT TEAM MEETING

Minutes No. 79-1

July 23, 1979

Phase II Clinical Studies

Mental depression. Clinical studies in mental depression are proceeding under the modified protocols to use higher doses (up to 60 mg daily) of fluoxetine. There have been some encouraging reports of rapid and fairly dramatic improvement. Some patients have converted from severe depression to agitation within a few days; in one case the agitation was marked and the patient had to be taken off drug. In future studies the use of benzodiazepines to control the agitation will be permitted.

Recently the FDA instructed us to discontinue all studies in women of childbearing potential until segment I fertility studies in animals were completed. Investigators have been notified of this restriction, which represents a significant impediment to the progress of the clinical trials.

All of the studies up to now have been open-label studies, and plans are in progress for double-blind controlled studies comparing fluoxetine to placebo, imipramine, or amitriptyline. Protocols are generally written, but firm arrangements have not yet been made with the investigators. Dr. [redacted] in [redacted] has agreed to do a double-blind study but first will do some dose-ranging studies in which fixed doses at three different dose levels are given to a few patients. He proposed doing only 4 patients per dose level, but the team felt this number should be increased at least to 10 in order to yield meaningful data.

[redacted]

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EXHIBIT

FULLER 11

[redacted]

One patient in Dr. [redacted]'s study has shown leukopenia while taking fluoxetine. The patient may have taken two other drugs, Macrodantin and baclofen, as well. Drug treatment was stopped, and the white cell counts slowly rose. Bone marrow biopsy showed that there were cells representing precursors of all series in various stages of maturation including neutrophils and latent normoblasts, consistent with a recovering marrow. Plasma samples collected and sent here for analysis showed relatively high levels of fluoxetine and desmethylfluoxetine. There is no way to be certain whether the leukopenia was related to fluoxetine.

[redacted]

<u>Decisions related to the occurrence of leukopenia</u>. A report of the occurrence of leukopenia in one patient during fluoxetine treatment is being submitted to the FDA, and the data will be supplied to all clinical investigators. So far fluoxetine has been given to [illegible] volunteer subjects and 63 patients. All investigators who are currently treating patients with fluoxetine have been informed by telephone of the leukopenia, and none of them has been alarmed. The protocol requirements for regular white blood counts and differential counts will be adhered to strictly in the future. The project team agreed to continue clinical studies in depression, [redacted] according to the present schedule, but to delay further studies in [redacted] and to postpone the initiation of any studies in [redacted] and [redacted] until more data are available.

Toxicology

Plans for combination toxicity studies of fluoxetine given with L-5-hydroxytryptophan/carbidopa are being made final, and the materials for this study have been ordered.

The fertility study will start next week, and the earliest date by which a report could be issued would be December 1. This report will then have to be reviewed by the FDA before studies in women of childbearing potential can be resumed.

The earlier data on possible effects of fluoxetine on white blood counts in monkeys will be reviewed and additional studies with fluoxetine and nisoxetine in monkeys have been planned.

[redacted]

Ray W. Fuller

Dr. R. W. Fuller
Dr. L. Lemberger
Dr. I. H. Slater

July 23, 1979

Food and Drug Administration
Bureau of Drugs, HFD 120
Attention: Document Control Room 10B-34
5600 Fishers Lane
Rockville, Maryland 20857

Gentlemen:

Re: IND 12274 - Compound LY110140 - (Fluoxetine Hydrochloride (Psychotropic Agent)

IND Protocol No. 13, which was submitted August 7, 1978, outlined a study by [redacted] in patients with primary major depressive disorders. The dosage regimen was revised in accordance with our letter of December 11, 1978. It is again being revised, as indicated below.

During the first week of the study, each patient will be given one placebo capsule each morning. If at the end of the week the Hamilton score shows a decrease of 20% or falls below 20, placebo will be continued for another week. If the Hamilton score at the end of the second week again shows a 20% decrease or falls below 20, the patient will not continue in the study. This revision necessitates a change in Section 2.f.2. regarding severity of depression from "at least 18" to "at least 20."

The initial dose of fluoxetine will be one 20-mg capsule given in the morning of the first day. On days 2 and 3, a 20-mg capsule will be given both in the morning and at noon. On day 4, two 20-mg capsules will be given in the morning and one 20-mg capsule at noon. At the investigator's discretion, this dose may be continued for five weeks. It may be reduced if clinically indicated, and, in instances where the dose is reduced because of agitation, diazepam may be administered as needed.

The protocol was amended March 16, 1979, to include patients with severe or disabling compulsive or obsessive

EXHIBIT
SLATER 8

Pz 221 2274

symptoms. If such patients are enrolled in the future, the dosage regimen outlined above will be used.

The administration of chloral hydrate for sleep will not be restricted to only once a week as indicated in the protocol.

Very truly yours,

ELI LILLY AND COMPANY

H. A. Barnett, M.D.
Medical Advisor
Regulatory Affairs

HAB:bs

May 12, 1981

Dr. C. N. Christenson
cc: Archives
Dr. M. E. Amundson
Dr. J. L. Emmerson
Dr. R. W. Fuller
Dr. D. G. Hoffman
Dr. S. G. Lake
Dr. D. M. Morton
Dr. P. Stark

MORTALITY DATA ON ONGOING FLUOXETINE (LY110140) ONE-YEAR DOG STUDY (D-3760)

As delineated in our phone conversation of May 11, two deaths have recently occurred in the ongoing one-year fluoxetine (LY110140) dog study, D-3760, which is currently at six months. These deaths occurred in females of the high dose (20 mg/kg) group on April 23 and May 1. One additional high dose female died earlier in the study, January 18. Thus to date, three of ten dogs (three of five females) in the high dose group have died. No mortality has been seen at the lower two doses of 4.5 and 10 mg/kg. Through consultation with Drs. Amundson, Emmerson, and Hoffman it was suggested that the FDA pharmacologist responsible for fluoxetine should be notified of our data establishing 20 mg/kg as a toxic dose in dogs and advising him that the high dose was decreased to 10 mg/kg (effective May 12) for the remainder of the study. The middle and low doses remain unchanged.

LY110140 has produced a heterogeneous response among dogs, especially within the high dose group. However, the toxic signs do show dose-dependent incidence and/or severity. These include fine tremors, mydriasis, slow pupil response, anorexia, and occasional emesis. Three high dose dogs have shown transient increases in SGPT and CPK; other clinical chemistry and hematology values have been essentially normal. Electrocardiograms taken at two weeks and three months into the study have only shown an apparent dose-related slowing of the basal heart rate; however, it was not severe enough to be labeled a clinically significant bradycardia. There is no evidence of any LY110140-related interference with conduction in the heart. Blood levels of fluoxetine and norfluoxetine taken at two weeks and one month of the study were not elevated in the dogs that died; blood samples taken at three and five months have not yet been analyzed. A total of six dogs (two males and four females) from the high dose group were removed from treatment for periods of 1-17 days due to severe occurrences of either aggressive behavior, ataxia, or anorexia. In the preceeding 90-day dog study (D-3304) at 5, 10, and 20 mg/kg, no deaths occurred although similar CNS toxic signs were evident.

Mortality Data on Ongoing Fluoxetine (LY110140) One-Year Dog Study
(D-3760)

The following is a description of the three female dogs which died at the high dose level. The toxicity seen with LY110140 in dogs appears to be an extension of its pharmacologic effects.

Dog 1

Time of Death:	2 months
Observations:	Severe anorexia, weight loss, ataxia, hypoactive, fine tremors, mydriasis, slow pupil response.
Removal from Treatment:	Off 6 days when became recumbent and could not stand, recovered while off treatment; less severe signs after resumption of treatment.
Death:	25 days after resumption of treatment, unobserved death.
Gross Necropsy:	Mild diffuse reddening of lungs. Pancreas contained multifocal areas of hemorrhage.
Histopathology (preliminary):	Vessels were congested with erythrocytes in the lung and pancreas. Interlobular hemorrhage was present in the pancreas.

Dog 2

Time of Death:	5 months
Observations:	Marked agressive behavior (technician bit attempting dosing), fine tremors, mydriasis, slow pupil response, anorexia.
Removal from Treatment:	Off 6 days, on 4 days, and back off 17 days due to marked aggressive behavior, recovered while off treatment, less severe signs after resumption.
Death:	22 days after resumption of treatment, unobserved death, appeared healthy 15 hours prior to death.
Gross Necropsy:	Lung and liver were congested with erythrocytes.
Histopathology (preliminary):	Vessels were congested with erythrocytes in the kidney, liver, lung, adrenal, and lymph node. Villi tips were congested with erythrocytes in the duodenum.

Mortality Data on Ongoing Fluoxetine (LY110140) One-Year Dog Study (D-3760)

Dog 3

Time of Death:	5-1/2 months
Observations:	Severe anorexia, marked weight loss, severe ataxia, fine tremors, SGPT elevation, slow pupil response, mydriasis.
Removal from Treatment:	Off 6 days due to severe anorexia and ataxia, improved somewhat while off treatment and after resumption (maintenance of weight and SGPT decline).
Death:	Euthanized in moribund condition 51 days after resumption of treatment, after tonic convulsive episode prior to euthanasia.
Gross Necropsy:	No observations.
Histopathology (preliminary):	Slides not yet available.

G. T. Brophy
Project Leader
811-8605

db

April 4, 1991

Dr. C. M. Beasley
Dr. D. J. Goldstein
Dr. J. H. Heiligenstein
Ms. M. M. Huff
Dr. D. N. Masica
Dr. M. Perelman
Dr. D. W. Robertson
Dr. W. L. Thompson
Mr. E. A. West
Dr. D. T. Wong

FLUOXETINE (PROZAC) AND SEROTONIN PRODUCTION IN BRAIN

The Newsweek reporter that Ed West arranged for me to talk with earlier said Leonard Finz claims that Lilly had data from animal experiments done in 1973 and 1974 showing that fluoxetine caused "downregulation of serotonin production" and that these data were never published. The data should have caused us to realize that fluoxetine would make some depressed patients worse and make some patients manic, Finz is alleged to be saying. I was pleased that this issue was not mentioned in the Newsweek article. But, if the Newsweek reporter had understood correctly the charges Finz had made, I anticipate we will here them again. Thus I am supplying the following clarification.

When fluoxetine (or any other serotonin uptake inhibitor) is given, serotonin concentration builds up in the synaptic cleft and synaptic receptors for serotonin are activated to a greater extent. The most important consequence is an amplification of serotonergic signals to other neurons. Another consequence, probably because presynaptic autoreceptors are activated, is that the serotonin neurons decrease their firing and their synthesis of serotonin. Almost certainly serotonin release into the synaptic cleft is reduced, although that is harder to measure directly. These rapid adaptive responses, which occur within minutes, limit the degree of serotonin accumulation in the synaptic cleft and keep it within physiological bounds. The responses place a "ceiling" on the degree to which serotonergic function can be increased by uptake inhibition, which would be expected to limit side effects that might otherwise result.

Our findings that fluoxetine <u>decreases</u> serotonin production (downregulation is not a term generally used for this rapid type of effect) have been published and agree with findings of other scientists who studied fluoxetine or other serotonin uptake inhibitors.

The first publications on fluoxetine were at the FASEB meeting in April, 1974. Among the 6 abstracts presented there, one was by K. W. Perry and R. W. Fuller, entitled "Effect of 3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine HCl (Lilly 110140), a specific inhibitor of serotonin uptake, on 5-hydroxyindole levels and turnover in rats." The abstract described a decrease in brain 5-hydroxyindoleacetic acid (5HIAA) levels after fluoxetine administration to rats. 5HIAA is the major metabolite of serotonin in brain, and a decrease in its level without a decrease in serotonin (5HT) level indicates a reduction in serotonin turnover. The abstract stated "The reduction of 5HIAA levels appears to result from reduced turnover of 5HT secondary to inhibition of 5HT reuptake from the synaptic cleft and may be due to increased stimulation of a presynaptic receptor or to a trans-synaptic feedback mechanism."

EXHIBIT
FULLER 6

The same observations were mentioned in an abstract at the Association for the Psychophysiological Study of Sleep, 14th annual meeting, June 6-9, 1974.

The following publications describe the decrease in serotonin synthesis (Finz called it "downregulation of serotonin production") that occurs following fluoxetine administration and discuss its significance:

1. R. W. Fuller, K. W. Perry and B. B. Molloy. Effect of an uptake inhibitor on serotonin metabolism in rat brain: Studies with 3-(α-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine (Lilly 110140). Life Sci. 15, 1161-1171 (1974).
2. F. P. Bymaster and D. T. Wong. Effect of Lilly 110140, 3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine, on synthesis of 3H-serotonin from 3H-tryptophan in rat brain. Pharmacologist 16, 244 (1974).
3. R. W. Fuller and M. Steinberg. Regulation of enzymes that synthesize neurotransmitter monoamines. Adv. Enz. Regul. 14, 347-390 (1976).
4. R. W. Fuller and D. T. Wong. Inhibition of serotonin reuptake. Fed. Proc. 36, 2154-2158 (1977).
5. R. W. Fuller and D. T. Wong. Serotonin reuptake blockers in vitro and in vivo. J. Clin. Psychopharmacol. 7, 6 Suppl. 26S-43S (1987).
6. M. J. Schmidt, R. W. Fuller and D. T. Wong. Fluoxetine, a highly selective serotonin reuptake inhibitor: a review of preclinical studies. Brit. J. Psychiat. 153, Suppl. 3, 40-46 (1988).
7. R. W. Fuller and D. T. Wong. Fluoxetine: A serotonergic appetite suppressant drug. Drug Develop. Res. 17, 1-15 (1989).
8. R. W. Fuller, D. T. Wong and D. W. Robertson. Fluoxetine, a selective inhibitor of serotonin uptake. Med. Res. Rev. 11, 17-34 (1991).

Although serotonin production is decreased acutely by uptake inhibition, there is an increased amount of serotonin in the synaptic cleft, the site where it has access to synaptic receptors for serotonin. An increase in extracellular serotonin concentration acutely after fluoxetine administration to rats has been shown by cytofluorimetric, in vivo voltammetric, push-pull cannula and brain microdialysis techniques. References can be found in the 1991 review article above. Accompanying the increase in extracellular serotonin concentrations are neurochemical, neuroendocrine, behavioral and other changes indicative of increased serotonergic function after fluoxetine.

Data similar to these with fluoxetine have been reported in the scientific literature for various other selective inhibitors of serotonin uptake. Among neuropharmacologists, there is no lack of appreciation that serotonin uptake inhibitors cause a decrease in serotonin production and an increase in serotonergic transmission, both consequences of the increased synaptic concentrations of serotonin resulting from uptake inhibition.

Ray W. Fuller

Here, my reply

From: MCVAX0::FULLER
To: THOMPSON ROBERT 6, CC: WONG, ROBERTSON, MASICA, WHEADON, WEINSTEIN, ZERB E, THOMPSON, FULLER, WEBER
CC:
Subj: AMK/86A

I find it surprising that anyone would consider the chemical structures of fluoxetine and amphetamine to be similar. Fluoxetine has much more structural similarity to drugs such as chlorpheniramine, orphenadrine, diphenhydramine, chlorpheno[illegible]amine, and the like. Amphetamine is simply alpha-methyl-phenylethylamine. Amphetamine and fluoxetine share a phenyl group and an amine group but there any similarity ends. Amphetamine is a primary amine. Fluoxetine is a secondary amine. Fluoxetine could not be converted metabolically to amphetamine, which has a phenyl-carbon-carbon-nitrogen skeleton lacking in fluoxetine.

Pharmacologically, fluoxetine is dissimilar to all of the abovementioned drugs. Some of the above are antihistamines, which fluoxetine is not. The multiple behavioral effects of amphetamine are all thought to result from interactions with catecholamines, mainly release of dopamine and norepinephrine. Fluoxetine has no

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#207 10-FEB-1989 [illegible] MAIL

direct effects on catecholamine neurons at relevant doses, but selectively inhibits serotonin uptake.

I trust the information Dr. Wong has supplied will clarify some of the differences in pharmacologic effects of fluoxetine and amphetamine. In November 1985 Martin Hynes, David Wong and myself prepared a report on Fluoxetine's preclinical pharmacology profile: No evidence for abuse liability and bound that with several reprints and other reports. Copies were sent to the project team chairman (then David Brennan) and to the clinical monitor (then Joe Warnicke) as well as to others in the medical/regulatory division. If that material is no longer available there, I have a copy in my office.
Ray W. Fuller

From: FULLER RAY W (MCVAX0::FULLER)

To: THOMPSON ROBERT 6 (INDYVM1::RM62385)
cc: WONG DAVID T (MCVAX0::WONG)
ROBERTSON DAVID W (MCVAX0::RX31579)
MASICA DANIEL N (INDYVM1::RM62930)

Press RETURN for more...

LMail>

86

Dave will reply

#206 7-FEB-1991 11:44:24.67

From: MCVAX0::WONG MAIL
To: WEBER,THOMPSON, CC:ROBERTSON,FULLER,BOUCHY,MAYR,NICKELSEN,WONG
CC:
Subj: RE: FLUOXETINE VS AMPHETAMINE

HANS:

GREETINGS!
LET ME APPROACH YOUR QUESTIONS AS FOLLOWS:

1. CHEMICAL STRUCTURE OF FLUOXETINE IS VERY DIFFERENT FROM THAT OF AMPHETAMINE. FLUOXETINE HAS TWO AROMATIC RINGS AND A PROPYLAMINE SIDE CHAIN BELONGING TO PHENOXYPHENYPROPYLAMINE SERIES. WHERAS AMPHETAMINE IS A METHYL-PHENETHYLAMINE. FLUOXETINE IS NOT METABOLIZED TO AMPHETAMINE.

2. AS YOU KNOW, FLUOXETINE AT DOSES UP TO 10 MG/KG P.O. DID NOT CHANGE THE MOUSE LOCOMOTOR ACTIVITY. TABLE 9. STARK,FULLER, & WONG, J. CLIN. PSYCHIATRY 46, 7-13, 1985.

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ON THE OTHER HAND, IT IS WELL RECOGNIZED THAT AMPHETAMINE INCREASES LOCOMOTOR ACTIVITY AND AS ILLUSTRATED BY A RECENT STUDY(JONES, MARSDEN AND ROBBINS, PSYCHOPHARMACOLOGY 102, 364-372, 1990).

3. AS SUMMARIZED IN TWO REVIEW PAPERS(WONG & FULLER, INT. J OBESITY(1), SUPPL. 2, 125-135, 1987; FULLER & WONG, DRUG DEV. RES. 17, 1-15, 1989), THESE ARE THE DIFFERENCES IN THE SUPPRESSION OF FOOD INTAKE IN RODENTS BY FLUOXETINE AND AMPHETAMINE:

A. STRESS INDUCED EATING:
FLUOXETINE AND OTHER SEROTONERGIC DRUGS SUPPRESS EATING INDUCED BY TAIL PINCH IN RATS, WHERAS AMPHETAMINE DID NOT(ANTELMAN ET AL., 1981)
B. INSULIN INDUCED EATING:
FLUOXETINE AND OTHER SEROTONERGIC DRUGS SUPPRESS INSULIN INDUCED HYPERPHAGIA, BUT AMPHETAMINE AND OTHER DOPAMINERGIC DRUGS DID NOT.
C. CARBOHYDRATE SELECTION:
FLUOXETINE AND OTHER SEROTONERGIC DRUGS SELECTIVELY SUPPRESSED CARBOHYDRATE INTAKE, WHERAS AMPHETAMINE REDUCED PROTEIN AND CARBOHYDRATE INTAKE(WURTMAN & WURTMAN, 1977; 1979).

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D. TOLERANCE:
FLUOXETINE SUPPRESSION OF EATING WAS MAINTAINED THROUGHOUT 8 OR 21 DAYS, BUT SUPPRESSION BY AMPHETAMINE WAS NOT MAINTAINED. (ROWLAND ET AL., 1982; WONG & FULLER, 1987).

REFERENCE FOR B:CARRUBA ET AL., 1985.

4. FLUOXETINE DECREASED SELF-ADMINISTRATION OF AMPHETAMINE IN RATS HAVE BEEN DEMONSTRATED(YU ET AL., LIFE SCIENCES 39, 1383-1388, 1986; PORRINO ET. AL., LIFE SCIENCES 45, 1529-1538, 1989). ON THE OHTER HAND, NEUROCHEMICAL LESIONS OF CENTRAL SEROTONERGIOS SYSTEMS INCREASED SELF-ADMINISTRATION OF AMPHETAMINE. THESE OBSERVATIONS AS WELL AS THOSE OF PRECURSOR STUDIES LED THE AUTHOR TO SUGGEST THAT INCREASE OF BRAIN SEROTONIN LEVELS TENDS TO DECREASE AMPHETAMINE SELF-ADMINISTRATION.

I AM SENDING YOU THESE REFERENCES.

BEST REGARDS.

Press RETURN for more...

LMail>

#206 7-FEB-1991 11:44:24 MAIL

DAVID

From: WONG DAVID T (MCVAX0::WONG)

To: WEBER HANS P (INDYVM1::XG01198)
THOMPSON ROBERT G (INDYVM1::RM02985)
cc: ROBERTSON DAVID W (MCVAX0::RX31579)
FULLER ROY W (MCVAX0::FULLER)
BOUCHE CLAUDE (INDYVM1::XG01621)
MAYR GERHARD (INDYVM1::YH07513)
NICKELSEN THOMAS N (INDYVM1::XG02233)
WONG DAVID T (WONG)

LMail>

The inquiry

#202 6-FEB-1991 11:08:54.35 MAIL

From: MCVAX0::RZ32329 "THOMPSON, LEIGH (XJIS4)"
To: FULLER R,ROBERTSON DAVID,MASICA D,WHEADON D,WEINSTEIN,ZERBE
CC:
Subj: PLEASE HELP. I think we have abuse studied well in animals and the

pharmacology should be clear.
From: MCVAX0::INDY::"WEBER HANS J " 6-FEB-1991 [illegible]
To: THOMPSON,
CC:
Subj: AMK/BGA

Date sent: 6 February 1991 [illegible]
AMK/BGA

I just received important information that fluoxetine has been discussed extensively at a BGA/AMK meeting. It seems that in agreement with the experts safety concerns incl. suicidality have been significantly reduced. The idea of restricted indication (patients with suicide attempts in history) was discussed but not supported by the experts.

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#202 6-FEB-1991 [illegible] MAIL

The following remains to be seen as critical:
- the authorities are afraid about an explosion of fluoxetine use, esp. if it comes to new indications such as obesity.
- they feel uncomfortable with treatment of depression by practicioners, esp. if a new drug has an unclean profile. Education, e.g. promotional material, may help to find the right patients. (Specifically, it was mentioned that fluoxetine should not be first choice in all depression, e.g. agitated pts., one of the previous Hoechst mistakes).
- also, the question was raised whether fluoxetine would be an amphetamine-like drug which may explain stimulating and anorectic effects. It turned out that not enough was known about the pharmacology in this respect.

Reg. the last point, I ask for urgent support. Prof. Mueller-Oerlinghausen can take this point back to the next meeting if armed with specific information. I'm aware of little literature which may not be sufficient. The chemical structure indeed has some similarities. His questions are such as: is the drug metabolized to amphetamine, does the high dose for anorectic effects suggests amphetamine-like action, how did the drugs compare in screening tests??

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#202 6-FEB-1991 11:08:54.35 MAIL

Detailed information would be most appreciated. Also, M-O knows Ray Fuller and a direct telephone contact might help.

Thank you and Regards., Hans

From:	WEBER HANS J	IVM1 7	XG01198
Subject:	AMK/BGA		
To:	THOMPSON ROBERT G	IVM1	
cc:	BOUCHY CLAUDE	IVM1	
	MAYR GERHARD	IVM1	
	NICKELSEN THOMAS N	IVM1	
	OESTERREICH SABINE	IVM1	
	THOMPSON LEIGH	RVAX	
	WEINSTEIN ALLAN J	IVM1	

From: THOMPSON LEIGH (MCVAX0::RZ32329)

To: FULLER RAY W (MCVAX0::FULLER R)
ROBERTSON DAVID W (MCVAX0::RX31579)
MASICA DANIEL N (BADYVM1::BMK5930)

Press RETURN for more...

LMail>

Bob Thompson's memo

#203 6-FEB-1991 11:22:46.84
From: MCVAX0::INDY::"THOMPSON ROBERT G " MAIL
To: FULLER, WONG, THOMPSON,
CC:
Subj: AMK/BGA

Date sent: 6 February 1991, 11:24:09
Comments:
Gentlemen,
Please see forwarded note from Hans Weber regarding ongoing discussions within BGA on fluoxetine.
The primary need for information/data now related to the BGA question/concern that fluoxetine may have amphetamine-like activity. I sense that this concern is heightened by the wt reduction with fluoxetine as compared to the wt gain with TCAs.

Rav, David, Lou, Rich - what package of information can we gather for Prof Mueller-Oerlenhausen? I was extremely impressed with him when Charles and I met him in Boston last year to discuss the suicide data. He is very objective and will play a key role with the BGA through the AMK. Rav, because you know

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#203 6-FEB-1990 11:22:46 MAIL
him, can I ask that anyone with data contact you directly. It will be important for us to proxy documentation to Hans as soon as possible after sharing with Allan and Leigh.

Rav, I will be available to help as needed on this important request.
Regards
Bob
---------- Forwarded Message ----------
February 6, 1991 09:18

To: THOMPSON ROBERT G IVM1
cc: BOUCHY CLAUDE IVM1
MAYR GERHARD IVM1
NICKELSEN THOMAS N IVM1
OESTERREICH SABINE IVM1
THOMPSON LEIGH RVAX
WEINSTEIN ALLAN J IVM1

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LMail>

Drugs affecting serotonin neurons

By Ray W. Fuller
Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, USA

1 Introduction

Most of the currently identified neurons that make and release serotonin as their neurotransmitter are in the central nervous system (CNS), and those will be the focus in this chapter. Serotonin in the gastrointestinal tract is mainly in enterochromaffin cells of the mucosal epithelium, but there are serotonin neurons in the enteric nervous system [1]. Serotonin neurons in the CNS have their cell bodies mainly clustered along the midline (raphe areas) of the midbrain and brain stem, and they send widespread projections to many regions of the forebrain as well as downward to the spinal cord. Their projection to many parts of the CNS involved in many different physiologic functions [2, 3] allows serotonin neurons to influence many brain functions, and they may play some part in the pathologic state in diverse diseases. Also, drugs that modify the function of serotonin neurons have various therapeutic uses or potential therapeutic uses as well as being valuable pharmacologic tools in helping to understand physiologic roles of serotonin neurons.

2 Sites of drug action

Serotonin neurons synthesize serotonin from the amino acid tryptophan via the intermediate 5-hydroxytryptophan. The two enzymes involved are tryptophan 5-hydroxylase, a highly specific enzyme thought to be present only in serotonin-forming cells, and L-aromatic amino acid decarboxylase, a relatively nonspecific and ubiquitous enzyme. Serotonin is stored in granules or vesicles, from which it is released at nerve impulse into the synaptic cleft. After acting on the postsynaptic receptor to complete the process of neurotransmission across the serotonergic synaptic cleft, the serotonin is inactivated by being removed from the synaptic cleft via specific membrane uptake carriers, which transport it back into serotonergic nerve terminals. There it may be re-used in storage granules or degraded enzymatically by monoamine oxidase, the major product being 5-hydroxyindoleacetic acid. Serotonin receptors occur not only postsynaptically but also presynaptically on cell bodies or axon terminals of serotonin neurons. These receptors on serotonin neurons are called autoreceptors; the terminal autoreceptors have the physiologic role of sensing the concentration of serotonin in the synaptic

cleft and modulating the further release and synthesis of serotonin. Drugs can intervene at several sites to affect serotonergic function. For instance, inhibitors of tryptophan 5-hydroxylase or L-aromatic amino acid decarboxylase reduce serotonin synthesis and decrease neuronal serotonin content. Inhibitors of monoamine oxidase inhibit serotonin metabolism and increase serotonin content, as well as the content of other monoamines. Drugs can act directly at postsynaptic or presynaptic serotonin receptors to mimic or to antagonize the action of serotonin. Drugs can deplete serotonin stores by releasing serotonin from storage granules. Inhibitors of the serotonin uptake carrier increase serotonin concentration in the synaptic cleft, thereby enhancing serotonergic function, as do serotonin-releasing drugs. Drugs can destroy serotonin neurons or terminals, producing long-lasting deficits in serotonergic function. These various classes of drugs have been highly useful to elucidating functional roles of serotonin neurons, and some have therapeutic applications.

3 Serotonin uptake inhibitors

During the past 15 years, many compounds have been identified and described that are selective inhibitors of the serotonin uptake carrier. These include alaproclate [4], CGP 6085A [5], citalopram [6], cyanoimipramine [7], femoxetine [8], fluoxetine [9], fluvoxamine [10], indalpine [11], Org 6582 [12], panuramine [13], paroxetine [14], pirandamine [15], RU 25591 [16], sertraline [17], SL 81.0385 [18], zimelidine [19] and others, and have been the subject of previous review articles [20, 21]. Drugs that inhibit the serotonin uptake carrier enhance serotonin function by causing serotonin molecules to remain in the synaptic cleft longer and to activate the postsynaptic receptors to a greater extent. Inhibition of the serotonin uptake carrier in brain slices or brain synaptosomes can be demonstrated *in vitro* through the use of radioactive serotonin. Selectivity can be demonstrated by showing that the compounds do not inhibit the uptake of other brain monoamines such as norepinephrine or dopamine and do not interfere with binding of radioligands to neurotransmitter receptors such as muscarinic cholinergic, histaminergic and alpha adrenergic receptors *in vitro* [22, 23]. Inhibition of the serotonin uptake carrier *in vivo* has been demonstrated by *ex vivo* techniques of showing inhibited serotonin uptake *in vitro* by synaptosomal or slice preparations from

animals treated with the uptake inhibitor [9, 24], or by showing that the carrier-dependent depletion of brain serotonin by agents such as p-chloromethamphetamine [25], p-chloroamphetamine [26], fenfluramine [27, 28] and H75/12 [29] is antagonized or prevented. Increased extraneuronal concentrations of brain serotonin produced by uptake inhibitors have been demonstrated by cytofluorometric [30], *in vitro* voltammetric [31] and push-pull cannulae [32] techniques. Several functional consequences of uptake inhibitors apparently result from increased serotonergic activation of synaptic receptors. These consequences include a decrease in serotonin synthesis and in the firing of serotonin neurons [26, 33, 34], probably mainly as a result of increased activation of autoreceptors but perhaps due in part to trans-synaptic feedback loops.

Despite the decreased activity of serotonin neurons, serotonergic neurotransmission is enhanced after uptake inhibition, leading to behavioral and other changes, especially when uptake inhibitors are combined with serotonin precursors (tryptophan or 5-hydroxytryptophan). For example, serotonin uptake inhibitors increase serum corticosterone concentration in rats [35] by increasing corticotropin-releasing hormone secretion from the hypothalamus and adrenocorticotrophin release from the anterior pituitary [36]. Serotonin uptake inhibitors inhibit muricidal behavior in rats [37] and potentiate behavioral effects of 5-hydroxytryptophan such as head twitch in mice [38] and the discriminative cue stimulus property of 5-hydroxytryptophan in rats [39]. Serotonin uptake inhibitors have analgesic effects in some experimental paradigms and potentiate analgesic effects of opioid drugs [40, 41]. Serotonin uptake inhibitors decrease total food intake in rats [42], selectively suppress carbohydrate consumption [43] and suppress stress-induced [44] or insulin-induced [45] hyperphagia in rats.

4 Serotonin releasers

Drugs such as reserpine, tetrabenazine and Ro 4–1284 release serotonin from granular stores in nerve terminals, but they also release other brain monoamines, e. g., dopamine, norepinephrine and epinephrine. They are not useful as tools for specifically manipulating serotonergic function. p-Chloroamphetamine and fenfluramine are halogenated analogs of amphetamine that release serotonin selec-

tively. Most of their acute effects result from release of serotonin into the synaptic cleft and activation of postsynaptic serotonin receptors [46–49].

p-Chloroamphetamine was developed as an anorectic drug [50] and was also tested as an antidepressant drug [51] but was never marketed. Fenfluramine has been marketed for many years in the racemic form as an anorectic drug, and recently d-fenfluramine has been introduced as a more specifically acting serotonergic anorectic drug.

Other substituted amphetamines have similar serotonin-releasing actions, e. g., H75/12 (4-methyl-α-ethyl-methylamine) and 3,4-methylenedioxymethamphetamine (MDMA) [52]. As mentioned above, the serotonin release these agents induce is carrier-dependent and can lead to depletion of brain serotonin. Antagonism of that depletion is a useful marker of inhibition of the serotonin uptake carrier. The acute functional effects of the drugs are also antagonized by pretreatment with uptake inhibitors, because the uptake inhibitor prevents the acute release of serotonin. As examples, pretreatment with fluoxetine blocks the discriminative stimulus properties of fenfluramine [53], hyperthermia caused by fenfluramine in rats kept at warm ambient temperature [54], elevation of serum corticosterone [55] and serum prolactin concentration [56] by fenfluramine, the initial elevation of plasma renin activity by fenfluramine [57], the increase in twitch frequency of the suprahyoideal muscles by fenfluramine in anesthetized cats [58] and the fenfluramine-induced increase in striatal acetylcholine concentrations in rats [59].

Serotonin-releasing drugs and serotonin uptake-inhibiting drugs have many pharmacologic similarities, because both increase synaptic concentrations of serotonin and enhance activation of postsynaptic serotonin receptors. Both classes of drugs decrease food intake and increase serum corticosterone concentrations, for example. There are subtle differences between the two classes of drugs, however. For instance, enhancement of serotonergic function by uptake inhibitors requires that the serotonin neurons be firing and releasing serotonin. The degree of enhancement of serotonergic function by uptake inhibitors may be limited by compensatory mechanisms that quickly come into play to dampen serotonin release as synaptic concentrations of serotonin build up. Thus the diminished firing of serotonin neurons limits the degree to which synaptic concentrations of

serotonin are increased after uptake inhibition. In contrast, serotonin-releasing drugs increase synaptic concentrations of serotonin independently of firing of the serotonin neuron, the degree of increase in synaptic concentrations of serotonin being limited only by the availability of presynaptic stores of serotonin. As a result of these differences, serotonin-releasing drugs produce some effects that uptake inhibitors do not produce unless 5-hydroxytryptophan (or tryptophan) is combined with the uptake inhibitor to increase the influx of serotonin into the synaptic cleft. Examples of such effects are increases in serum prolactin concentration in male rats [34, 56, 60–62] and reduction of blood pressure in spontaneously hypertensive rats [63, 64].

5 Direct-acting serotonin receptor agonists

5.1 Indoles

Several indoleethylamines structurally related to serotonin can interact with serotonin receptors. These include N,N-dimethylserotonin [65], N,N-dipropylserotonin [66], tryptamine and its N-alkyl derivatives [67], bufotenin [68], 5-carboxamidotryptamine and its N,N-dipropyl derivative [69], α-methyl and 2-methylserotonin [70], and 5-methoxytryptamine and its N-alkylated derivatives [68]. Some of the indolealkylamines are naturally occurring compounds, whereas RU24969 [70] and related compounds and also indorenate (TR3369) [71] are indole-containing compounds created by medicinal chemists. Some of these indolealkylamines vary in their selective affinity for the various serotonin receptor subtypes (see below). N,N-Dimethyl-5-methoxytryptamine has been a useful indole substitute for serotonin in whole-animal studies because it crosses the blood brain barrier and is somewhat protected against metabolism.

5.2 Piperazines

Numerous 1-phenylpiperazines and related compounds are centrally acting serotonin receptor agonists [see 72]. Quipazine [73, 74] and MK-212 [75] were the first 1-arylpiperazines described to be serotonin agonists. 1-(m-Trifluoromethylphenyl) piperazine (TFMPP) [76] and 1-(m-chlorophenyl-) piperazine (mCPP) [77] were the most-studied members of a larger series of substituted 1-arylpiperazines

that were described subsequently. mCPP had been patented as an anorectic drug before its mechanism of action was known [78]. Urapidil is a substituted o-methoxyphenylpiperazine whose antihypertensive effects may be mediated by activation of brain serotonin receptors [79]. Buspirone [80], ipsapirone (TVXQ 7821) [81] and gepirone [82] are di-N-substituted piperazines with serotonin agonist activity.

5.3 Serotonin receptor heterogeneity

As early as the 1950's, Gaddum and Picarelli [83] presented pharmacologic evidence for serotonin receptor heterogeneity, defining "M" and "D" receptors in peripheral tissues that responded to serotonin. In 1979, Peroutka and Snyder [84] used radioligand-binding methods to define two distinct types of serotonin receptors in brain, the 5HT-1 and 5HT-2 receptors. Soon thereafter, it was recognized that the 5HT-1 binding site was not homogeneous [85], and now 5HT-1A, 5HT-1B, 5HT-1C and 5HT-1D receptors have been described and differentiated in brain. In addition, the 5HT-3 receptor has been found to be similar or identical to the "M" receptor of Gaddum, and the 5HT-2 receptor now seems to be pharmacologically identical to the "D" receptor of Gaddum [86].

There is no reason to believe that our understanding of serotonin receptor subtypes is complete at this time, and already evidence for additional subtypes of serotonin receptors is building [see 87]. The 5HT-1A [88], 5HT-2 [89] and 5HT-1C [90, 91] receptors have been cloned, and eventually the most complete and informative classification of serotonin receptors may be on the basis of protein structure [87, 92]. These different receptor subtypes are distributed differently among anatomic regions in brain, and different receptor subtypes presumably receive serotonergic signals in various neuronal pathways in brain. The multiple receptors offer a tantalizing opportunity for drug development, and the currently intense search for agonists and antagonists that act selectively on these receptor subtypes will provide important pharmacologic tools for elucidating the physiologic functions of these receptors [93].

Uptake inhibitors and releasers, by increasing synaptic concentrations of serotonin, probably increase activation of all of these receptor subtypes located synaptically. Direct-acting agonists, in contrast,

may have selectivity and thus activate only one or certain ones of the receptor subtypes. Consequently, different direct agonists may share some specific actions but not all actions of indirect agonists (uptake inhibitors and releasers).

5.4 5HT-1A-Receptor-selective agonists

Although many of the previously known serotonin receptor agonists have relatively nonselective affinity for multiple serotonin receptor subtypes, there are some highly selective agonists of 5HT-1A receptors available currently. Perhaps the most selective of these is 8-hydroxy-2-(di-n-propylamino) tetralin [68, 94], which has stereoselective affinity for 5HT-1A receptors [95] and has been widely used as a prototypic 5HT-1A receptor agonist in exploring functions of 5HT-1A receptors [93]. Buspirone is an anxiolytic drug whose mechanism of action was unknown for many years and was at one time thought to involve dopamine [96]; in the past few years, buspirone was found to have high affinity for the 5HT-1A receptor [97, 98], and now it is generally believed that 5HT-1A receptor activation accounts for the anxiolytic effects of buspirone [99]. Several structural analogs of buspirone, including gepirone [82], ipsapirone [100] and SM-3997 [101] also are selective 5HT-1A receptor agonists and have anxiolytic effects in humans and/or animals [102]. Some other direct-acting serotonin agonists also have higher affinity for 5HT-1A receptors than for other serotonin receptor subtypes, including LY165163 [103, 104] and MDL 72832 [105].

5.5 Other selective agonists

Highly selective agonists for receptor subtypes other than 5HT-1A receptors are not yet available, and these are needed as pharmacologic tools. Although m-chlorophenylpiperazine and m-trifluoromethylphenylpiperazine have sometimes been referred to as selective 5HT-1B receptor agonists [106, 107], insufficient selectivity between 5HT-1B and other serotonin receptors such as 5HT-1A [108, 109], 5HT-1C [110] and 5HT-2 [68] receptors prevent these agents from being very useful in discriminating among serotonin receptor subtypes [111]. Perhaps the most selective 5HT-1B receptor agonist available to date is CGS 12066B, 7-trifluoromethyl-4(4-methyl-1-piperazinyl-)-

pyrrolo [1, 2a] quinoxaline, although the compound does not have high potency [109]. RU 24969 is sometimes referred to as a selective 5HT-1B agonist [112], but radioligand-binding studies show it has similar affinity for 5HT-1A and 5HT-1B receptors [68]. Agonists with some selectivity toward 5HT-2 receptors include 4-bromo-2,5-dimethoxyamphetamine [113, 114] and its iodo analog [115]. Indolealkylamines that are hallucinogenic have particular affinity for the 5HT-2 receptor [65]. 2-Methylserotonin is used as a relatively selective agonist at 5HT-3 receptors [69]. GR43175, 3-(2-dimethylamino)ethyl-N-methyl-1H-indole-5-methanesulfonamide or sumatriptan [116], and 5-carboxamidotryptamine [117] are used as selective agonists for 5HT-1 receptor subtypes.

6 Serotonin receptor antagonists

Antagonists of serotonin receptor-mediated functions have been known almost as long as serotonin itself. Antagonists led to the first recognition of serotonin receptor heterogeneity in the mid-1950's, when Gaddum and his colleagues defined "M" and "D" receptors in peripheral tissues based on differential antagonism of indoleamine-mediated effects [83]. In recent years the focus has been on identifying antagonists with high selective affinity toward specific serotonin receptor subtypes.

Serotonin antagonists that have been considered to be selective for the 5HT2 receptor include ketanserin [118], ritanserin [119, 120], altanserin [119], LY53857 [121], LY281067 [122], ICI 169, 369 [123], ICI 170, 809 [124], setoperone [119, 120], MDL 11, 939 [125] and irindalone [126]. However, because the 5HT-1C receptor has high homology and pharmacologic similarity to the 5HT-2 receptor [87, 92], many of these antagonists also are highly potent blockers of 5HT-1C receptors [127], and it is now important to find antagonists that discriminate better between these two receptor subtypes.

Various other serotonin antagonists that block 5HT2 receptors but have less selectivity among serotonin receptor subtypes include metergoline [128], methysergide [68, 129], cyproheptadine [68, 129], pizotifen [130], methiothepin [131], danitracen [132], mianserin [133], cinanserin [134] and benzoctamine [135]. Trazodone is an interesting example of a drug that is a potent 5HT-2 receptor antagonist [136], but is metabolized [137] to m-chlorophenylpiperazine, a serotonin re-

ceptor agonist [77]. Thus a mixture of pharmacologic effects, including serotonergic influences in opposite directions, has been demonstrated over a range of trazodone doses in animals [138]. The molecular basis for the therapeutic actions of trazodone in depression [139] remains unknown.

Selective antagonists of 5HT-3 receptors have been developed in recent years, including MDL 72222 [140], ICS 205-930 [69], GR 38032F [141], BRL 24924 and BRL 43694 [142–144], zacopride [144] and LY278584 [145, 146]. The use of some of these compounds as radioligands has made it possible to demonstrate the presence of 5HT-3 receptors in brain [146, 147].

Several α and β adrenoreceptor-blocking drugs have high affinity for 5HT-1A and 5HT-1B receptors [148–150], and some such as pindolol and penbutolol have been useful experimentally as antagonists of these receptor subtypes [151–153]. Some of these compounds are partial agonists at 5HT-1A receptors and may show properties of agonists or antagonists in different experimental paradigms [154, 155]. There have been attempts to synthesize analogs of these drugs as more selective 5HT-1A receptor ligands [156–158].

2.3 Serotonin-depleting drugs

The short-term depletion of brain serotonin can be produced by drugs such as reserpine that impair granular storage or by inhibitors of serotonin synthesis. Since reserpine, tetrabenazine and related compounds deplete other brain monoamines in addition to serotonin, specific inhibitors of serotonin synthesis are generally used for short-term depletion of serotonin. Tryptophan 5-hydroxylase, the initial enzyme in serotonin biosynthesis localized only in serotonin-forming cells, is the preferred target of synthesis inhibitors. The most widely used inhibitor of tryptophan 5-hydroxylase is p-chlorophenylalanine [159]. Other inhibitors of tryptophan 5-hydroxylase include 6-fluorotryptophan [160].

p-Chlorophenylalanine has been used for more than two decades to produce long-lasting but reversible depletion of brain serotonin as a means of investigating a role of serotonin in physiologic functions [161], in pathologic changes [162] or in drug actions [163]. Often serotonin depletion by p-chlorophenylalanine produces similar functional effects as serotonin depletion by other classes of agents such

as reserpine, fenfluramine and 5,7-dihydroxytryptamine [e. g., 164], but sometimes differences are noted among classes of serotonin-depleting drugs [e. g., 165, 166]. Among several possible explanations for these differences, including different degrees of serotonin depletion in various brain and spinal cord serotonergic pathways, one is that lesions in serotonin neurons are expected to reduce cotransmitters as well as serotonin, whereas p-chlorophenylalanine would reduce serotonin concentration by inhibiting serotonin synthesis but would not reduce intraneuronal concentrations of cotransmitters in serotonin neurons [167].

Recently evidence has been presented that some serotonin axons in rat brain were not sensitive to p-chlorophenylalanine [168]. The insensitive axons were especially prominent in the limbic system, in cranial motor and parasympathetic nuclei, and some had common morphological features such as large varicosities and location adjacent to neural somata suggestive of axosomatic synapses.

8 Drugs that are neurotoxic to serotonin neurons

5,6- [169] and 5,7-dihydroxytryptamine [170] are neurotoxic to serotonin neurons in an analogous way to the neurotoxic effect of 6-hydroxydopamine on catecholamine neurons [171]. These hydroxylated analogs of the natural transmitters have affinity for the membrane uptake carriers on serotonin and catecholamine neurons, respectively. They are easily autoxidized molecules that give rise to hydrogen peroxide, free radicals and other reactive species that lead to cytotoxicity [172]. 5,6- and 5,7-Dihydroxytryptamines are accumulated via the membrane uptake carrier into serotonin neurons and have destructive effects on those neurons, just as 6-hydroxydopamine is accumulated via the membrane uptake carrier into catecholamine neurons and destroys those neurons.

For nearly 20 years, 5,6- and more recently 5,7-dihydroxytryptamine have been used to lesion serotonin neurons in brain. They have been valuable tools in elucidating functional roles of serotonin neurons, especially particular neuronal tracts. Prior lesioning of serotonin neurons with these neurotoxins attenuates or abolishes many functional effects of indirect-acting serotonin agonists (uptake inhibitors or releasers) while often enhancing effects of direct-acting agonists by causing supersensitivity of postsynaptic receptors [see 173].

p-Chloroamphetamine is not only a serotonin-releasing drug whose acute effects are mediated by enhanced activation of postsynaptic serotonin receptors, but p-chloroamphetamine at higher doses can actually be neurotoxic. The first clue to this came in 1970 when Frey [174] reported that brain serotonin in rats was depleted rapidly and remained depleted for as long as 4 weeks after only 5 days of treatment with p-chloroamphetamine. Sanders-Bush et al. [175] extended these findings and focused on the long duration of p-chloroamphetamine's actions. Now it is documented that many immunofluorescent serotonin-containing fibers are eliminated by p-chloroamphetamine and that all parameters specifically associated with serotonin neurons that have been measured are reduced for weeks or months after a few doses or even a single dose of p-chloroamphetamine. Parameters shown to remain decreased for long periods after administration of p-chloroamphetamine or its structural congeners include, in addition to serotonin content, the content of 5-hydroxyindoleacetic acid (the metabolite of serotonin), serotonin turnover, tryptophan hydroxylase, serotonin uptake capacity and serotonin uptake carriers labeled with radioligands [175–178]. Thus it seems clear that p-chloroamphetamine can be neurotoxic to brain serotonergic nerve axon terminals, especially to the fine projections with minute varicosities arising from dorsal raphe serotonin neurons that can be distinguished from coarse-beaded fibers arising from medial raphe nuclei [179].

In contrast to 5,6- and 5,7-dihydroxytryptamine, which do not penetrate the blood-brain barrier and must be applied intraventricularly or directly into brain tissue, p-chloroamphetamine can be given systemically to deplete serotonin in the CNS [180]. The neuroanatomic specificity of p-chloroamphetamine effects differs from that of the dihydroxytryptamines [180, 181].

In addition to p-chloroamphetamine, some other substituted amphetamines that release serotonin acutely produce long-lasting effects on brain serotonin neurons similar to those of p-chloroamphetamine, apparently reflecting neurotoxicity. These analogs include fenfluramine [182, 183] and 3,4-methylenedioxymethamphetamine (MDMA) [178, 184]. Because fenfluramine is used therapeutically in humans and MDMA is used recreationally in humans, the possible hazards of these compounds have received attention recently [185–187], but no direct evidence of any sort exists to support the idea that neuro-

toxic effects occur in humans as they do in rodents [185–187] and in non-human primates [188].

The type of adverse consequences that might be expected if brain serotonergic neurons or terminals were destroyed is not certain. The degree to which serotonergic function is impaired by p-chloroamphetamine and related drugs when they deplete brain serotonin in laboratory animals has not been studied extensively. Rats treated with p-chloroamphetamine a week or more earlier such that brain serotonin is depleted by half or more are relatively normal in appearance. There have been some demonstrations of impaired or altered cerebral function in these animals, however. For instance, Vorhees et al. [189] reported that rats treated with a 5-mg/kg i. p. dose of p-chloroamphetamine showed hypoactivity and increased defecation in open-field testing for as long as 30 days. They showed facilitated acquisition in a shock avoidance Y-maze task for up to 15 days. Brain serotonin was still reduced by 40 % after 38 days.

Grabowska and Michaluk [190] reported that p-chloroamphetamine injected at 2 mg/kg daily for 5 days did not affect locomotion measured three days after the last dose in rats but it intensified the stimulation caused by apomorphine. Those findings and other data in the paper were taken by the authors as confirming "our hypothesis about the possible inhibitory role of serotonin in the apomorphine-induced locomotor stimulation in rats".

Ogren et al. [191] reported perhaps the most convincing evidence to date of impaired serotonergic function after p-chloroamphetamine-induced depletion of brain serotonin. They gave two doses of p-chloroamphetamine hydrochloride (10 mg/kg), which resulted in 63 % depletion of serotonin concentration in whole brain. Pretreatment with zimelidine completely prevented the depletion of serotonin. Eight days after p-chloroamphetamine treatment, rats failed in the acquisition of a two-way conditioned avoidance response. Rats that had been pretreated with zimelidine and then given p-chloroamphetamine acquired the avoidance response at least as well as control rats; zimelidine completely prevented the depletion of brain serotonin by p-chloroamphetamine.

Yamaguchi et al. [192] reported potentiation of apomorphine-, methamphetamine- and phencyclidine-induced dopaminergic behaviors (sniffing, licking, gnawing, biting) in rats after p-chloroamphetamine (20 mg/kg i. p.) given 72 and 48 hours before the drug challenge.

Kollner et al. [193] gave one i. p. injection of p-chloroamphetamine or of fenfluramine to rats on day 8 of postnatal life, which they had earlier found to produce a depletion of brain serotonin for several months, and observed that the rats were unable to learn a self-shaped avoidance behavior in a peripheral field avoidance test. The investigators concluded that serotoninergic neuron groups participate in the inhibition of incorrect responses and differentiation learning.

Nabeshima et al. [194] gave one dose of p-chloroamphetamine (10 mg/kg i. p.) 13 days before drug challenge and observed that the intensity of head-weaving, turning, forepaw treading, hind-limb abduction and Straub tail induced by 5-methoxy-dimethyltryptamine and the intensity of head-twitch, turning and backpedalling induced by phencyclidine were markedly increased. By contrast, serotonin-mediated behaviors induced by an acute dose of p-chloroamphetamine were attenuated. Striatal serotonin and ketanserin binding sites were increased.

9 Therapeutic uses of drugs affecting serotonin neurons

Because serotonin neurons project to many parts of the brain and spinal cord and can influence these regions involved in CNS regulation of many bodily functions, drugs that alter serotonergic function have current or potential uses in many diseases.

Uptake inhibitors are antidepressant drugs and are useful in treating other psychiatric diseases such as obsessive-compulsive disorder [195–196], panic disorder [197, 198], body-dysmorphic disorder [199] and alcoholism [200, 201] and in treating obesity [202, 203] and bulimia [204]. Releasers are useful in treating some of these same disorders, especially obesity (fenfluramine). p-Chloroamphetamine has also been reported to be effective in treating depression [51].

5HT-1A receptor selective agonists are useful in treating anxiety [99, 205] and apparently also depression [206] but not obsessive-compulsive disorder [207].

5HT-2 receptor antagonists may be useful in treating cardiovascular diseases based on their block of vascular and perhaps platelet 5HT2 receptors [118, 208–211] and may be useful in psychiatric disorders such as schizophrenia [212], anxiety [213, 214] and depression [215] based on their central effects.

5HT-3 receptor antagonists have shown efficacy in clinical trials as anti-emetic agents in patients undergoing cancer chemotherapy [216, 217] and may also be useful in treating anxiety [218] and schizophrenia [219].

Different classes of serotonergic drugs may be useful in treating migraine [220]. Serotonin uptake inhibitors have been evaluated, based on the idea that their depletion of platelet serotonin may prevent triggering of migraine attack by serotonin coming from blood platelets [221]. Several antimigraine drugs, e. g., dihydroergotamine, methysergide and pizotifen, are 5HT-2 receptor antagonists, and that may be involved in their mechanism of therapeutic efficacy, although since they are partial agonists their agonist activity has also been suggested as relevant to their antimigraine use [222, 223]. Many 5HT-2 receptor antagonists are also antagonists at 5HT-1C receptors, and that may account for their effectiveness in migraine. Recently 5HT-3 antagonists, thought to act by inhibiting sensory nerve transmission of pain messages [224], have been reported to have antimigraine efficacy [225]. Two new indole agonists at 5HT-1 receptors, GR-43175 and AH-25086, are under development as antimigraine drugs acting directly on cerebral blood vessels [226–228].

Clinical nontherapeutic uses of drugs affecting serotonin neurons

Some serotonergic drugs are being used in clinical studies, not for treatment of a disease, but as pharmacologic probes. The drug is given to elicit a measurable response, the magnitude of which is taken as a marker of central serotonergic function. Agents being used in this way include direct agonists – m-chlorophenylpiperazine [229] and MK-212 [230], a serotonin releaser – fenfluramine [231], and the serotonin precursors – tryptophan [232] and 5-hydroxytryptophan [233]. Responses being measured include increases in serum hormone concentrations, notably cortisol, growth hormone and prolactin, and body temperature or behavioral changes [229]. Studies have been done to probe central serotonergic function in various disease states, e. g., depression [233], agoraphobia and panic disorder [234] and obsessive-compulsive disorder [235]; after substance abuse [236, 237]; and after acute [238] or chronic [239] treatment with drugs.

11 Summary and conclusions

Advances in serotonin pharmacology, the development of drugs that intervene at specific sites to modify serotonergic function, have accompanied advances in the understanding of physiologic roles of serotonin present in neurons and elsewhere and of serotonin receptors that are widely distributed in brain and many peripheral tissues. The pharmacologic advances have sometimes been stimulated by developments in serotonin physiology, such as the recognition of multiple serotonin receptor subtypes, and in other cases have been a major factor in providing new insights into physiologic roles of serotonin. Drugs that modify serotonin function have a variety of therapeutic applications currently and many more potential therapeutic uses to be explored in the future. Having drugs that act with high specificity or selectivity on particular enzymes in serotonin biosynthesis, on particular serotonin receptors, or at other sites such as uptake carriers for serotonin not only offers the hope of improved clinical therapy in diseases caused by abnormal serotonergic function or in which alteration of serotonergic function can alleviate symptoms, but also provides valuable pharmacologic tools for learning more about serotonin physiology and probing the functional status of serotonergic systems. The next few years promise to yield important new serotonergic drugs.

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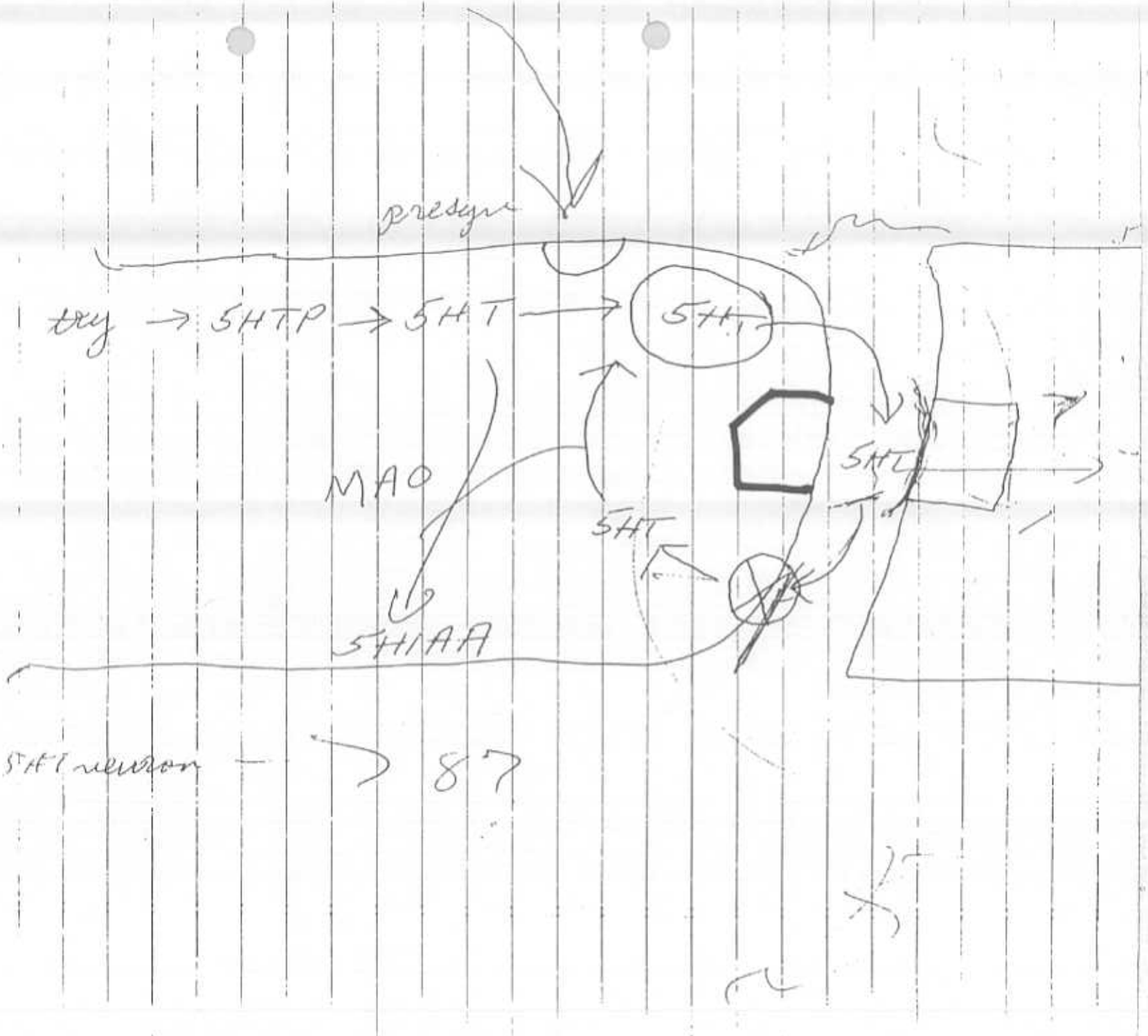
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Fluoxetine, a Selective Inhibitor of Serotonin Uptake

Ray W. Fuller, David T. Wong, and David W. Robertson

Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana 46285

I. INTRODUCTION

During the past 16 years, selective inhibitors of serotonin uptake have been described, used as pharmacologic tools in preclinical research, and developed for therapeutic purposes. Fluoxetine was the first of these to appear in the scientific literature,[1,2] and fluoxetine is now used for treating depressive emotional states in the United States and in many other countries.[3–7] Fluoxetine is derived from a chemical series of phenoxyphenylpropanamines (Fig. 1) that inhibit monoamine uptake with varying degrees of selectivity. Tomoxetine[8] and nisoxetine[9] (Fig. 1) are selective inhibitors of norepinephrine (and epinephrine) uptake from this series, whereas fluoxetine and norfluoxetine are selective inhibitors of serotonin uptake,[9,10] illustrating the importance of substituents on the phenoxy ring as determinants of affinity for monoamine transporters in this chemical class. Development of fluoxetine and the current knowledge of its molecular properties, pharmacologic actions, and therapeutic uses are reviewed here.

II. STRUCTURE–ACTIVITY RELATIONSHIPS

The phenoxyphenylpropanamine skeleton has proved to be a suitable framework for preparation of a variety of serotonin and norepinephrine uptake inhibitors. Relatively subtle molecular modifications can result in dramatic alterations in selectivity of the molecule for either the serotonin or norepinephrine uptake carrier. For example, while fluoxetine inhibits serotonin and norepinephrine reuptake with IC_{50} values of 70 and 10,000 n*M*, respectively, the corresponding values for tomoxetine are 1500 and 4 n*M*.[8,10]

The trifluoromethyl substituent of fluoxetine is a pivotal aspect of the mol-

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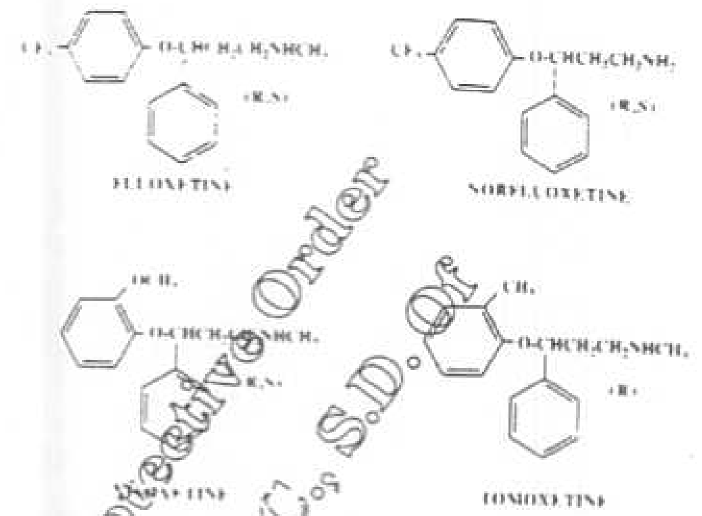

Figure 1. Chemical structures of fluoxetine and some related compounds.

ecule, and is responsible for a great deal of the potency and selectivity of fluoxetine as a serotonin reuptake inhibitor. The parent *N*-methylphenoxyphenylpropanamine, lacking any substituent on the phenoxy ring, is approximately one-seventh as potent as fluoxetine as an inhibitor of serotonin reuptake (IC_{50} = [illegible] nM). The effect of the trifluoromethyl substituent is regiospecific, and the *ortho* and *meta* isomers are more than two orders of magnitude less potent than fluoxetine. Moreover, replacement of this substituent with any of the halogens or electron-donating substituents leads to dramatic decreases in the potency of the molecules as inhibitors of serotonin reuptake.

Hydrophobic, electronegative substituents have increased potency and se-

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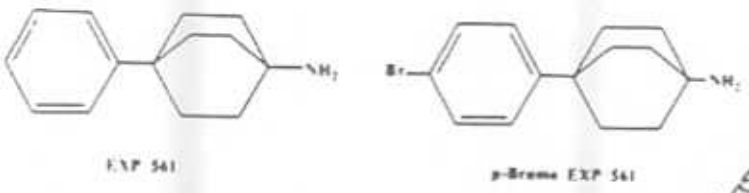
Ray W. Fuller *received the Ph.D. degree in biochemistry from Purdue University in 1961, after earning B.A. and M.A. degrees from Southern Illinois University. After spending two years as director of the biochemistry research laboratory at the Fort Wayne (IN) State Hospital, he joined the Lilly Research Laboratories in 1963 and currently is a Lilly Research Fellow. He is Adjunct Professor of Neurobiology at Indiana University School of Medicine and Visiting Lecturer in the Department of Brain and Cognitive Sciences at the Massachusetts Institute of Technology. His research has focused on biochemical neuropharmacology, especially drug effects on central and peripheral monoaminergic neurons.*

David T. Wong *received the Ph.D. degree in biochemistry from the University of Oregon in [illegible] after earning a B.S. degree in chemistry from Seattle Pacific College and an M.S. degree from Oregon State University. Following postdoctoral training at the University of Pennsylvania, he joined the Lilly Research Laboratories in 1968 and currently is a Lilly Research Advisor. He is Adjunct Professor of Biochemistry at Indiana University School of Medicine. His research interest has been in biochemical neuropharmacology, especially drugs acting on transporters and receptors of neurotransmitters.*

David W. Robertson *received his undergraduate education at Stephen F. Austin State University and then completed his M.S. and Ph.D. degrees in Organic Chemistry at the University of Illinois. He joined the Lilly Research Laboratories in 1981 and currently serves as Director of CNS Research. His research interests include the design of chemical probes of serotonin receptors and phosphodiesterase isozymes.*



EXP 561 p-Bromo EXP 561

Figure 2. Chemical structures of EXP-561 and p-bromo EXP-561

lectivity of molecules from several structurally distinct classes of serotonin reuptake inhibitors. For example, EXP-561 (Fig. 2) is a relatively nonselective inhibitor of serotonin and norepinephrine reuptake (IC_{50} values were 93 and 80 nM, respectively); addition of the *para*-bromo substituent (Fig. 2) increased selectivity for serotonin reuptake by almost 100-fold.[11,12] The work of Koe, Sarges, and Welch[13–15] has demonstrated that the 1-amino-4-phenyltetralin series, by appropriate manipulation of substituents and stereochemistry, can yield either selective serotonin or norepinephrine uptake inhibitors. In the *trans*-1*R*,4*S* series, the unsubstituted compound tametraline is a potent, selective norepinephrine uptake inhibitor (IC_{50} = 18 nM; 28-fold selectivity versus serotonin uptake). In the *cis*-1*S*,4*S* series the unsubstituted compound is relatively impotent as either a serotonin or a norepinephrine uptake inhibitor, but incorporation of 3,4-dichloro substituents produced sertraline, a potent (IC_{50} = 60 nM) and selective (20-fold) inhibitor of serotonin reuptake.[15] Thus, in the phenoxyphenylpropanamine and structurally diverse series of compounds, well defined SAR patterns emerge, and potency and selectivity of compounds for the serotonin uptake carrier can be readily manipulated via substituent changes.

While suitably placed substituents are important in designing serotonin uptake inhibitors, conformational and stereochemical features are also of great consequence. For example, sertraline selectively inhibits serotonin reuptake, but its *trans* stereoisomer is completely nonselective and inhibits serotonin and norepinephrine uptake with similar IC_{50} values.[15] Because of the importance of these stereochemical and conformational features, we probed the three-dimensional structure of fluoxetine using x-ray crystallography and computational techniques.[16,17] Moreover, we have compared the conformation of fluoxetine with those of selective norepinephrine uptake inhibitors, including tomoxetine and nisoxetine.

A computer-generated ORTEP representation of fluoxetine is depicted in Figure 3. In the solid state, the two aromatic rings are skewed, a spatial arrangement which minimizes torsional strain among the propanamine backbone atoms. The methylene units of the propanamine skeleton adopt staggered conformations, and an antiperiplanar relationship exists between N11 and C3 (see Fig. 3 for numbering system). Importantly, the propanamine portion does not adopt a fully extended conformation, but folds toward the trifluoromethylphenoxy ring. This folded conformation, and the proper spatial orientation between the phenoxy ring and the basic amine, appear to be important features of selective serotonin uptake inhibitors.

We and others[18] have used a variety of computational techniques to study

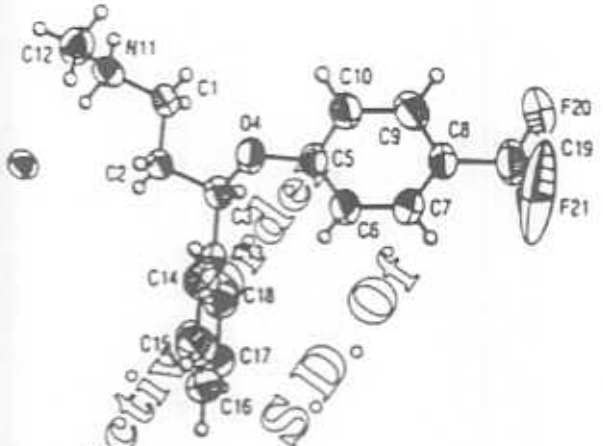


Figure 3. Computer-generated ORTEP plot of fluoxetine hydrochloride derived from x-ray coordinates. The atoms are numbered as discussed in the text.

fluoxetine, and the calculated lowest-energy conformation(s) mimics the x-ray structure of the drug. However, because of the flexibility of the phenoxyphenylpropanamine skeleton, it is difficult to state with certainty whether the conformation from the x-ray study is indeed the conformation which interacts with the serotonin uptake site. Because of these conformational ambiguities, we have compared the x-ray structure of fluoxetine with that of sertraline,[19] a conformationally defined serotonin reuptake inhibitor, and molecular overlap between the two structures is shown in Figure 4.[17] The substituted phenoxy ring and amine of fluoxetine overlap well with the dichlorophenyl ring and amine of sertraline. Moreover, the monosubstituted phenyl ring of fluoxetine lies in the same spatial position as the fused aromatic ring of sertraline, although these two aromatic rings are skewed. The close correspondence of the fluoxetine and sertraline conformations suggests that the low-energy fluoxetine conformation depicted in Figure 3, despite the intrinsic flexibility of the molecule, probably does contribute to its potent interaction with the serotonin uptake carrier.

To enhance our understanding of the effects of conformation on selectivity of the phenoxyphenyl propanamine antidepressants, we have also determined the x-ray structures of tomoxetine and nisoxetine, two selective norepinephrine uptake inhibitors.[20] In all three structures, there is a synclinal orientation about the C2–C3 bond (numbering system is same as depicted in Fig. 3), indicating that the propanamine side chain folds toward the phenoxy moiety; the C1–C2–C3–C4 dihedral angles were 67, 56, and 60.6 degrees for nisoxetine, tomoxetine, and fluoxetine, respectively. The dihedral angle formed by the four atoms of the propanamine backbone (N11–C1–C2–C3) was 83 degrees for nisoxetine. This was in dramatic contrast to the antiperiplanar relationships that exist in the solid-state conformations of fluoxetine and tomoxetine (180 and 162 degrees, respectively), suggesting that the energy barrier for rotation about the N—C bond on this backbone is low; this has

Figure 4. Molecular overlap between fluoxetine and sertraline. The low-energy conformations of both compounds were overlaid using the software program QUANTACHARm (Polygen, Inc.), and displayed using a Silicon Graphics Iris terminal. Fluoxetine and sertraline are represented in green and red, respectively. The trifluoromethylphenoxy and dichlorophenyl rings are on the left, while the amine moieties are on the lower right.

been confirmed using computational techniques. Thus, there appears to be sufficient conformational mobility in this side chain to enable proper orientation of the amine such that affinity for either the serotonin or norepinephrine uptake carrier is maximized. While there may be subtle differences in the conformational populations of fluoxetine and the two *ortho*-substituted norepinephrine uptake inhibitors, their close similarities suggest that the dramatic substituent effects on selectivity result from a localized interaction of the phenoxy region of these molecules with the uptake carriers, rather than a global impact of substituents on molecular conformation. The serotonin uptake carrier seems to have a strong preference for large lipophilic substituents in the para position of the phenoxy ring, while such a substituent is not tolerated by the norepinephrine uptake carrier; moreover, the norepinephrine uptake carrier seems to prefer electron-donating, lipophilic substituents in the ortho position of the phenoxy ring.

III. SPECIFICITY: LACK OF INTERACTIONS WITH NEUROTRANSMITTER RECEPTORS

A variety of antidepressant drugs, including the tricyclics, not only are uptake inhibitors but also are high-affinity antagonists of several neurotransmitter receptors. Antagonism of muscarinic receptors is the cause of anticholinergic side effects that are common in patients taking tricyclic antidepressant drugs, including dry mouth, dizziness, blurred vision, constipation, urinary retention, tachycardia, and memory dysfunction. Antagonism of α_1 adrenergic receptors in brain may be responsible for sedative effects of some antidepressant drugs, and antagonism of vascular α_1 adrenergic receptors may cause side effects such as postural hypotension, which is especially of concern in elderly patients. Antagonism of central histaminergic H1 receptors may cause drowsiness, a side effect that can limit the activities of patients being treated with antidepressant drugs. Fluoxetine has little affinity for these receptors which mediate the side effects of other antidepressant drugs, including muscarinic receptors, histaminergic receptors, or α_1 or α_2 adrenergic receptors, as studied either in rat brain membranes[21] or human brain membranes.[22] There is direct evidence that fluoxetine does not affect vascular α-adrenergic receptors at therapeutic doses in humans.[23] Nor does fluoxetine have affinity for other central neurotransmitter receptors that have been studied (i.e., serotonin $5HT_1$ or $5HT_2$ receptors, GABA receptors, benzodiazepine receptors, dopamine receptors, or opiate receptors.[21,22] The low affinity of fluoxetine for neurotransmitter receptors is consistent with a lower incidence of anticholinergic side effects, sedation, and postural hypotension in the clinical use of fluoxetine compared with tricyclic antidepressant drugs.[7]

Fluoxetine also lacks direct cardiac effects that are common among tricyclic antidepressant drugs.[24,25] Upward et al.[26] compared cardiac effects of fluoxetine and amitriptyline given at therapeutic doses to depressed patients. Amitriptyline altered the electrocardiogram by shortening the sinus cycle length and by prolonging both the PR interval and the QRS duration. In contrast, fluoxetine had no effect on the electrocardiogram or on systolic time intervals. The findings in humans are compatible with earlier data comparing fluoxetine and amitriptyline in dogs. Steinberg et al.[27] confirmed previous reports and

Table I
Serotonin-Depleting Agents Whose Effects are Carrier-Dependent and are Antagonized by Fluoxetine

Agent	Reference(s)
p-Chloroamphetamine	28
p-Bromoamphetamine	29
p-Iodoamphetamine	30
Fenfluramine	31
Norfenfluramine	32
MDMA	33
H75/12	28
H77/77	34

Abbreviations: MDMA = 3,4-methylenedioxymethamphetamine, H75/12 = 4-methyl-α-ethyl-meta-tyramine, and H77/77 = 4,α-dimethyl-meta-tyramine.

observed that amitriptyline decreased mean blood pressure and systemic vascular resistance, increased heart rate, and slowed cardiac conduction, effects due apparently to antagonism of α_1 and muscarinic cholinergic receptors and to direct cardiac effects. In contrast, fluoxetine did not have these effects in dogs.

IV. DEMONSTRATION OF UPTAKE INHIBITION IN VIVO

The potency and specificity of fluoxetine as an inhibitor of serotonin uptake in vivo have been demonstrated in several ways. Brain synaptosomal preparations from rats treated with fluoxetine have shown reduced uptake of radiolabeled serotonin in vitro.[27] This ex vivo demonstration of serotonin uptake inhibition is especially useful in comparing relative potency among serotonin uptake inhibitors and in demonstrating oral efficacy and duration of action.

Another way to show inhibition of the serotonin uptake carrier in brain in vivo is the antagonism of serotonin depletion by drugs whose effects are dependent upon the uptake carrier (Table I). Carlsson and Lindqvist[35] had used H75/12 in this way to study serotonin uptake inhibition by a series of compounds. Meek et al.[36] had shown that *p*-chloromethamphetamine-induced depletion of brain serotonin was antagonized by clomipramine and other uptake inhibitors. We used *p*-chloroamphetamine in initial demonstrations of in vivo uptake inhibition by fluoxetine.[37] Table I lists a number of serotonin-depleting drugs, all substituted amphetamines, whose effects have been blocked by fluoxetine. Not only are the serotonin-depleting effects of these drugs blocked, but their functional effects produced by initial release of serotonin are also blocked by fluoxetine pretreatment. For instance, fluoxetine antagonizes the *p*-chloroamphetamine-induced increase in serum corticosterone concentration in rats,[38] the fenfluramine-induced increase in striatal acetylcholine content in rats,[39] and the MDMA-induced suppression of dorsal raphe firing in a midbrain slice preparation in vitro.[40]

A third means of demonstrating occupancy of serotonin uptake carrier sites in vivo uses a radiolabel for that carrier, tritiated cyanoimipramine. Wolfe et al.[41] showed that tritiated cyanoimipramine given intravenously to rats labeled

serotonin uptake carriers in hypothalamus and cortex, and that fluoxetine was a potent inhibitor (ED_{50} values 0.27 and 0.21 mg/kg i.v., respectively) of tritiated cyanoimipramine binding. Other serotonin uptake inhibitors displaced tritiated cyanoimipramine binding with potencies similar to their blockade of H75/12-induced depletion.[42] This type of approach may eventually be applicable to evaluation of serotonin uptake inhibition in brains of humans, through the use of imaging techniques such as positron emission tomography.

Inhibition of serotonin uptake is expected to result in increased concentrations of serotonin within the synaptic cleft, and certain relatively direct evidence suggests synaptic concentrations of serotonin are increased by fluoxetine. Cytofluorometric[43] and voltammetric[44] techniques suggested increased extraneuronal concentrations of serotonin in rat brain after fluoxetine administration. Guan and McBride[45] measured extracellular concentrations of serotonin via push–pull cannulae in the nucleus accumbens of awake and freely moving rats. Fluoxetine, at doses of 5 and 10 mg/kg i.p., previously shown to be effective in inhibiting serotonin uptake, increased extracellular serotonin concentration 2- and 13-fold, respectively. Auerbach et al.[46] applied fluoxetine via a microdialysis probe in rat hypothalamus and found a 6-fold increase in serotonin concentration in the dialysis fluid.

Blood platelets have a serotonin uptake carrier similar to that on serotonin neurons; therefore fluoxetine inhibits serotonin uptake by these cells as well.[47] In the initial clinical studies with fluoxetine, this property of fluoxetine was used to show that well tolerated doses of fluoxetine were efficacious in blocking serotonin uptake in humans.[48] At doses of 20–30 mg/day, fluoxetine resulted in decreased uptake of radiolabeled serotonin in vitro by blood platelets from individuals receiving the drug. Since platelets derive their serotonin entirely by uptake, fluoxetine administration also led to a decreased content of serotonin in blood platelets.

V. INFLUENCE OF METABOLISM ON SELECTIVITY

Fluoxetine has about the same selectivity as clomipramine in vitro with regard to concentrations needed to inhibit serotonin uptake relative to those that inhibit norepinephrine uptake.[10] Clomipramine loses its selectivity as a serotonin uptake inhibitor in vivo by virtue of its metabolism by *N*-demethylation to chlordesipramine, which is a selective inhibitor of norepinephrine uptake.[49] Other tricyclic antidepressant drugs which are tertiary amines like clomipramine are metabolized by *N*-demethylation to secondary amines; for example, imipramine and amitriptyline are metabolized to desipramine and nortriptyline, respectively. With all of the tricyclic drugs, the secondary amine metabolites are weaker inhibitors of serotonin uptake and more potent inhibitors of norepinephrine uptake than are the parent tertiary amines. In the case of fluoxetine, metabolic *N*-demethylation to the primary amine does not shift the selectivity of amine uptake inhibition. Norfluoxetine is essentially as potent and selective as a serotonin uptake inhibitor as is the parent drug, fluoxetine.[10,50]

When fluoxetine is administered to animals, the serotonin uptake inhibition that results initially is caused by fluoxetine itself. With time, fluoxetine is

Table II
Influence of Metabolic *N*-Demethylation on Some Selective Inhibitors of Serotonin Uptake

Uptake Inhibitor	Metabolite	Selectivity of Metabolite
Clomipramine	Chlordesipramine	A potent and selective inhibitor of norepinephrine uptake, so metabolism destroys selectivity of clomipramine (clomipramine does not inhibit serotonin serotonin uptake selectively in vivo.[49,50,52,53]
Zimelidine	Norzimelidine	A more potent inhibitor of serotonin uptake than parent drug, so metabolism is necessary for maximum efficacy.[49,54,55]
Fluoxetine	Norfluoxetine	Similarly selective and potent as the parent drug.[50] Metabolism does not appreciably alter selectivity or potency.[50,51]
Citalopram	Desmethylcitalopram	Less potent and much less selective than the parent drug, but still a highly selective inhibitor of serotonin uptake.[56] Serotonin uptake is therefore inhibited selectively in vivo after citalopram administration.[56,57]

converted to norfluoxetine, and the long duration of serotonin uptake inhibition following a single dose of fluoxetine is due at later times to the formation and persistence of norfluoxetine.[50,51] The impact of metabolism by *N*-demethylation on the potency and selectivity of fluoxetine in relation to other serotonin uptake inhibitors is shown in Table II. Metabolism has an important influence on zimelidine in yet another way; the *N*-demethylated product, norzimelidine, is more potent than zimelidine as a serotonin uptake inhibitor.[49] Even at early times after zimelidine administration to rats, brain concentrations of norzimelidine are much higher than those of zimelidine,[55] suggesting that norzimelidine accounts for most of the in vivo inhibition of serotonin uptake after zimelidine administration. Inhibition of the metabolic conversion of zimelidine to norzimelidine results in marked loss of serotonin uptake inhibition.[54] Thus metabolism by *N*-demethylation destroys the potency and selectivity of clomipramine but is necessary for maximum efficacy of zimelidine.

Citalopram is probably the most selective agent known relative to its potency for inhibiting serotonin uptake in vitro versus its potency for inhibiting norepinephrine uptake in vitro.[56] Citalopram is a tertiary amine and is metabolized by *N*-demethylation to a secondary amine, desmethylcitalopram.[56] Desmethylcitalopram is in fact only one-fourth as potent as citalopram in inhibiting serotonin uptake in vitro and is 11 times more potent than citalopram in inhibiting norepinephrine uptake in vitro.[56] Therefore, the selectivity for serotonin/norepinephrine uptake inhibition was reduced nearly 50-fold by *N*-demethylation. However, the selectivity of citalopram is so high that even as a 50-fold less selective agent, desmethylcitalopram retains 100-fold selectivity for inhibiting serotonin uptake versus norepinephrine uptake.[56] Therefore, doses of citalopram that are given to inhibit serotonin uptake in vivo do not, in fact, inhibit norepinephrine uptake.[56,57] Citalopram is like fluoxetine

Table III
Comparison of Fluoxetine Enantiomers

Parameter	Potency of Enantiomer	
	R	*S*
Inhibition of [^{3}H]-serotonin uptake by rat brain synaptosomes in vitro	K_i = 33 nM	K_i = 21 nM
Inhibition of [^{3}H]-fluoxetine binding to rat brain membranes in vitro	IC_{50} = 7.7 nM	IC_{50} = 4.1 nM
Inhibition of [^{3}H]-serotonin uptake by human blood platelets in vitro	IC_{50} = 3.8 nM	IC_{50} = 3.6 nM
Ex vivo inhibition of [^{3}H]-serotonin uptake by rat brain stem synaptosomes	ED_{50} (i.p.) = 8.7 mg/kg	ED_{50} (i.p.) = 7.4 mg/kg
Antagonism of *p*-chloroamphetamine-induced depletion of brain serotonin in mice	ED_{50} (i.p.) = 2.1 mg/kg	ED_{50} (i.p.) = 1.2 mg/kg
Potentiation of morphine analgesia in mice	ED_{50} (s.c.) = 3.6 mg/kg	ED_{50} (s.c.) = 5.7 mg/kg
Inhibition of saccharin solution consumption in rats	ED_{50} (i.p.) = 6.1 mg/kg	ED_{50} (i.p.) = 4.9 mg/kg
Inhibition of food consumption in meal-fed rats	ED_{50} (i.p.) = 11 mg/kg	ED_{50} (i.p.) = 9 mg/kg
Inhibition of food consumption in 2-deoxyglucose-hyperphagic rats	ED_{50} (i.p.) = 10 mg/kg	ED_{50} (i.p.) = 7 mg/kg

The above data are based on Wong et al.,[39] Robertson et al.,[60] Wong et al.,[61] and on unpublished data.

but different from clomipramine in being able to produce selective inhibition of serotonin uptake in vivo.

VI. STEREOSELECTIVITY

Both enantiomers of fluoxetine inhibit serotonin uptake. The affinity of the enantiomers for the serotonin uptake carrier, as determined by their ability to inhibit the uptake of radiolabeled serotonin by intact synaptosomes and to inhibit the binding of radiolabeled fluoxetine to synaptosomal membranes, is shown in Table III. Little stereoselectivity was apparent, the enantiomers having similar affinity, so that the eudismic ratio is near unity. The enantiomers are also similarly effective in vivo, the ED_{50} values for inhibition of brain serotonin uptake ex vivo being about the same for the two enantiomers (Table III). Both enantiomers were effective in antagonizing *p*-chloroamphetamine-induced depletion of brain serotonin in mice, in potentiating morphine analgesia or in causing analgesia in mice, and in decreasing ingestive behavior in rats (Table III).

The *S* enantiomer of fluoxetine has a longer duration of action than does the *R* enantiomer in rats.[39,62] Therefore, in experiments in which duration of uptake inhibition is a factor, such as antagonism of *p*-chloroamphetamine-induced depletion of brain serotonin in rats, *S*-fluoxetine is more potent than

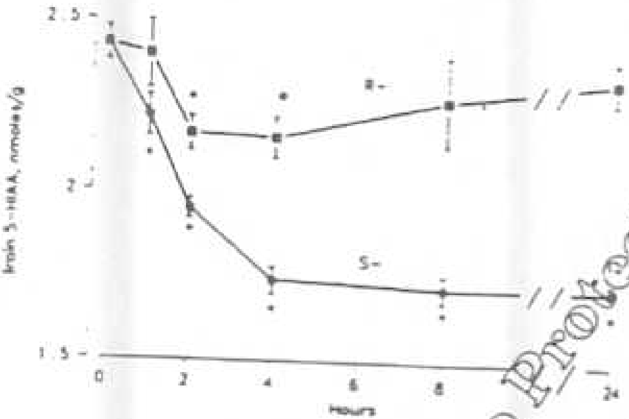


Figure 5. Duration of brain 5HIAA lowering by *R*- and *S*-fluoxetine in rats. The enantiomers were injected at 10 mg/kg i.p. at zero time. Mean values ± standard errors for 5 rats per group are shown. Asterisks indicate significant difference ($p < .05$) from the zero-time control group.

R-fluoxetine. The protective effect of *S*-fluoxetine against *p*-chloroamphetamine-induced depletion of brain serotonin in rats is demonstrably longer than that of *R*-fluoxetine. Antagonism of the serotonin uptake carrier throughout the time interval between *p*-chloroamphetamine injection and measurement of serotonin depletion is necessary for antagonism of the serotonin depletion, at least during the first 24 hours.[50]

Figure 5 shows that *S*-fluoxetine caused a more pronounced and prolonged reduction in brain 5HIAA concentration in rats than did *R*-fluoxetine. These data compare favorably to the shorter ex vivo inhibition of serotonin uptake by *R*-fluoxetine than for *S*-fluoxetine.[59] Brain concentrations of fluoxetine and norfluoxetine were measured after administration of homochiral enantiomers of fluoxetine to rats (Fig. 6); both enantiomers were rapidly converted to norfluoxetine, and at later times the brain concentrations of norfluoxetine were greater than those of parent drug. Potts et al.[63] measured individual enantiomers of fluoxetine by liquid chromatographic enantioseparation after administering racemic fluoxetine orally to rats. They reported similar peak plasma concentrations of the two enantiomers, the *R* enantiomer disappearing from plasma only slightly more rapidly than the *S* enantiomer. Our data in Figure 6 shows only slightly lower levels of *R*-fluoxetine in brain compared with *S*-fluoxetine at times of 4 hours and longer. Brain levels of *R*-norfluoxetine were slightly higher than those of *S*-norfluoxetine after administration of the corresponding fluoxetine enantiomers. Because norfluoxetine was known to be an effective inhibitor of serotonin uptake,[10,50] no explanation for the shorter duration of the pharmacologic effects of *R*-fluoxetine than of *S*-fluoxetine was obvious.

Recently, the homochiral enantiomers of norfluoxetine have been prepared and studied as serotonin uptake inhibitors. Surprisingly, the *R* enantiomer of norfluoxetine was relatively inert as a serotonin uptake inhibitor. Although *R*-fluoxetine is almost as potent as *S*-fluoxetine in inhibiting serotonin uptake (Table III), *R*-norfluoxetine is much less potent than *S*-norfluoxetine. The IC_{50}

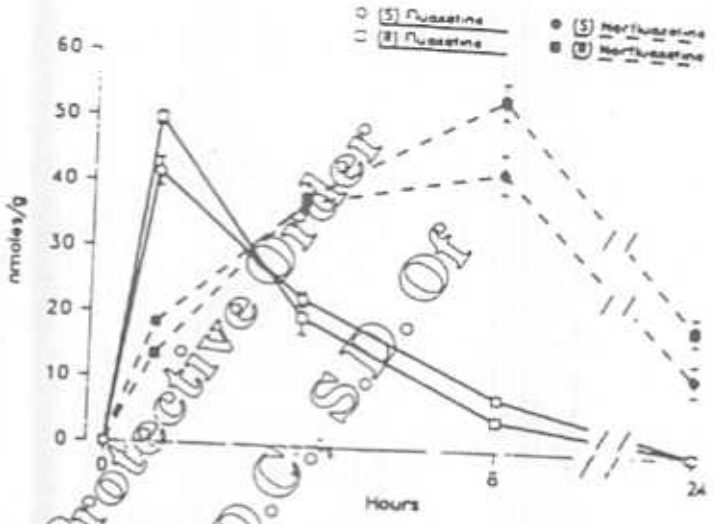


Figure 6. Concentrations of fluoxetine and norfluoxetine in rat brain after injection of *R*-fluoxetine or *S*-fluoxetine.[2] Rats were killed 1, 4, 8 or 24 h after the i.p. injection of *R*-fluoxetine or *S*-fluoxetine (10 mg/kg i.p.). Mean values ± standard errors for 5 rats per group are shown.

of *S*-norfluoxetine as an inhibitor of serotonin uptake by rat brain synaptosomes in vitro was 29.8 n*M* whereas that of *R*-norfluoxetine was 484 n*M*. *S*-norfluoxetine antagonized *p*-chloroamphetamine-induced depletion of brain serotonin in rats with an ED_{50} of about 3 mg/kg, whereas *R*-norfluoxetine did not cause 50% antagonism even at the highest dose tested (20 mg/kg i.p). *S*-norfluoxetine decreased brain 5-hydroxyindoleacetic acid (5HIAA) concentrations at all doses tested in the 2.5–20 mg/kg i.p. range, but *R*-norfluoxetine had no significant effect at these same doses. Thus the shorter duration of serotonin uptake inhibition by *R*-fluoxetine in vivo results from its conversion to a relatively inactive metabolite, *R*-norfluoxetine. The longer duration of serotonin uptake inhibition by *S*-fluoxetine in vivo results from the formation and persistence of a metabolite, *S*-norfluoxetine, as potent as the parent drug in inhibiting serotonin uptake.

VII. FUNCTIONAL EFFECTS OF FLUOXETINE IN VIVO

As a result of the increased concentrations of serotonin in the synaptic cleft following serotonin uptake inhibition by fluoxetine, serotonergic neurotransmission is enhanced. Several functional changes produced by fluoxetine support that interpretation. One neurochemical effect of fluoxetine administration is a decrease in serotonin turnover, which has been demonstrated by a decrease in steady-state concentrations of the serotonin metabolite, 5HIAA,[2] by a diminished accumulation of 5HIAA after probenecid is given to block its efflux from brain,[2] by a diminished rate of disappearance of serotonin when *p*-chlorophenylalanine is given to inhibit its synthesis,[2] and by decreased incorporation of radioactive tryptophan into 5-hydroxyindoles.[84] The de-

creased turnover of brain serotonin after fluoxetine administration is thought to be due to increased activation of autoreceptors on serotonin neurons whose physiologic role is sensing the extraneuronal concentration of serotonin and modulating the further release and synthesis of serotonin.

The increased concentrations of serotonin in the synaptic cleft activate not only autoreceptors but also postsynaptic receptors; also, some neurochemical effects after fluoxetine administration are thought to result from this increased activation of postsynaptic serotonin receptors. One is an increase in norepinephrine turnover that occurs after administration of fluoxetine alone or with 5-hydroxytryptophan, resulting in increased concentrations of the norepinephrine metabolites, DHPG (3,4-dihydroxyphenylethyleneglycol) and MHPG sulfate (3-methoxy-4-hydroxyphenylethyleneglycol sulfate) (H. W. Perry and R. W. Fuller, unpublished data).

Some behavioral and neuroendocrine changes also result from fluoxetine enhancement of brain serotonergic function. In support of a possible role of serotonin in memory processing, fluoxetine has been reported to enhance memory in mice.[66] Brain serotonin neurons can influence aggressive behavior, and fluoxetine (like other serotonin uptake inhibitors) inhibits muricidal (mouse-killing) behavior in rats.[67] Fluoxetine reduces rapid eye movement sleep in rats and cats, and acts synergistically with 5-hydroxytryptophan.[68,69] Fluoxetine increases ACTH[70] and corticosterone[71] concentrations in rat blood, and potentiates the 5-hydroxytryptophan-induced increases in corticosterone[72] and prolactin concentrations.[73,74]

Fluoxetine decreases food intake, an effect also produced by direct-acting serotonin agonists, serotonin precursors, and serotonin-releasing drugs.[75] Goudie et al.[76] first reported that fluoxetine decreased food intake in meal-fed rats. More recently, fluoxetine has been shown to decrease stress-induced,[77] insulin-induced,[78] and 2-deoxyglucose-induced[78,79] eating in rats and to decrease food intake in genetically obese as well as in normal mice.[80] Fluoxetine also decreased saccharin-induced excessive fluid consumption in rats.[81] In rats given a choice of diet low or high in protein, fluoxetine and other serotonergic drugs selectively decreased ingestion of the low protein diet, resulting in diminished carbohydrate intake but not diminished protein intake.[82] Repeated administration of fluoxetine to laboratory animals results in continued depression of food intake[83,84] and decreased weight gain or loss of body weight in normal and in obese animals.[80]

Fluoxetine also selectively decreases alcohol intake in rats with a choice between alcohol solutions and water,[85] even in genetic strains of alcohol-preferring rats.[86] This effect of fluoxetine is not due to effects on taste or smell, since intragastric self-administration of ethanol is also reduced.[87] Apparently fluoxetine and other serotonin uptake inhibitors interfere with the reinforcing effects of alcohol.

Fluoxetine differs from some less selective uptake inhibitors, whose earlier mentioned interactions with neurotransmitter receptors can sometimes antagonize functional effects of serotonin uptake inhibition in vivo. For instance, some tricyclic drugs are potent antagonists of $5HT_2$ receptors,[88,89] an effect that can counteract the enhanced serotonin function ordinarily resulting from uptake inhibition. Becker and Pleece[90] recently compared fluoxetine and clomipramine, both of which are potent inhibitors of serotonin uptake in vitro.

Fluoxetine potentiated the 5-hydroxytryptophan-induced head twitches in mice by blocking the neuronal uptake of serotonin formed in the brain from the administered 5-hydroxytryptophan. Clomipramine, in contrast, antagonized the 5HTP-induced head twitches, apparently by blocking central $5HT_2$ receptors.[91] Trazodone is another antidepressant drug that can inhibit serotonin uptake in the test tube and is sometimes referred to as a "serotonin reuptake blocker."[92] The most prominent action of trazodone on serotonergic systems, however, is as a $5HT_2$ receptor antagonist,[93] and trazodone generally impairs rather than enhances serotonergic function in vivo.[93–95] Any enhancement of serotonergic function by trazodone is probably via its metabolite, *m*-chlorophenylpiperazine, which is a direct-acting serotonin receptor agonist, instead of by uptake inhibition (see Ref. 83).

VIII. THERAPEUTIC EFFECTS OF FLUOXETINE IN HUMANS

Fluoxetine is effective in the treatment of depressive states and is currently marketed in the United States and in several other countries for this indication.[4–7] Several other selective inhibitors of serotonin uptake are also effective in treating depression.[96,97] Since the compounds are structurally diverse and share no known pharmacologic action other than serotonin uptake inhibition, it seems likely that serotonin uptake inhibition is the primary effect that leads to alleviation of depressive symptoms. Uptake inhibition would be expected to enhance serotonergic input to the postsynaptic neurons in many brain regions to which serotonergic terminals project, and it is not possible to know at present which brain regions or which postsynaptic neurons are most important in leading to therapeutic effects in depression.

The possibility that adaptive changes in receptor number or sensitivity is a key intermediate step in the action of antidepressant drugs is being studied.[98,99] Although downregulation of β-adrenergic receptors, the earliest discovered receptor adaptation with tricyclic antidepressant drugs (see Ref. 98), generally does not occur with fluoxetine,[84,100] decreases in β-adrenergic receptors after high doses of fluoxetine have been reported in a few discrete regions of rat brain.[101] Desensitization of presynaptic serotonergic receptors has also been suggested to be important in the antidepressant actions of fluoxetine[102] and other antidepressant drugs.[103] Whatever intervening steps may eventually be established, it now appears that fluoxetine and other serotonin uptake inhibitors are effective in depression but do not have a faster onset of action than other antidepressant drugs, their major advantages being reduced side effects.

In addition to depression, fluoxetine has also been shown to be effective in treating obesity.[104–106] Serotonin is thought to be an important neurotransmitter involved in hypothalamic control of food intake and metabolic economy.[107–109] Enhancement of serotonergic function by inhibiting serotonin uptake, by releasing serotonin, by loading with serotonin precursors, or by direct activation of serotonin receptors has been shown to decrease food intake in laboratory animals.[73] The reduction of body weight in obese patients treated with fluoxetine is thought to relate primarily from decreased food intake, although food intake data have not yet been reported in humans. There is

lation of energy utilization,[108,110] and it is possible that mechanisms in addition to decreased food intake are involved in the antiobesity effects of fluoxetine.

Bulimia, an eating disorder most common among young women, is characterized by binges of grossly excessive intake of food, followed by purges (induced vomiting) to prevent absorption of excess calories which would cause unwanted weight gain. Fluoxetine is reported to decrease the binges and purges in bulimic patients.[111,112]

Besides depression, fluoxetine is reported to be effective in other psychiatric disorders. An open trial in 61 patients with obsessive–compulsive disorder showed significant improvement with fluoxetine treatment.[113] Levine et al.[114] reported significant improvement during fluoxetine treatment in 75 patients with obsessive-compulsive disorder studied over a 5-month or longer treatment period. There have been isolated reports of the efficacy of fluoxetine in treating panic disorder.[115,116] Recently, Hollander et al.[117] have reported the successful use of fluoxetine in treating patients with body-dysmorphic disorder, a disorder of preoccupation with some imagined defect in appearance (such as facial flaws) in normal-appearing individuals.

In combination with the serotonin precursor, 5-hydroxytryptophan, fluoxetine may also be useful in the treatment of a neurologic disorder, postanoxic intention myoclonus.[118] Fluoxetine has been reported effective in the treatment of cataplexy, a sudden brief paralysis of voluntary movement and loss of muscle tone following any momentary decrease in alertness; cataplexy almost always occurs in association with narcolepsy.[119] In one patient with advanced diabetes mellitus and secondary autonomic and peripheral neuropathy, fluoxetine being used to treat major depression was reported to relieve diabetic neuropathy pain as well.[120] Fluoxetine was also reported to cause dramatic improvement in the behavioral syndrome associated with pseudobulbar palsy.[121]

IX. SUMMARY

In summary, fluoxetine is a highly selective serotonin uptake inhibitor in vitro and in vivo. The conformation of fluoxetine, which resembles that of sertraline and other serotonin uptake inhibitors, appears to be a key feature that enables its high affinity and selective interaction with the serotonin transporter. The *para*-trifluoromethyl substituent, however, is also a pivotal structural element. The molecular pharmacology of fluoxetine has been well-defined, and its in vivo pharmacological effects appear to be mediated almost exclusively by serotonin uptake inhibition. Its selectivity for the serotonin transporter, lack of affinity for neurotransmitter receptors, and retention of selectivity following metabolism to norfluoxetine make fluoxetine a useful tool to explore pharmacologically induced increases in serotonin neurotransmission. Fluoxetine has found a variety of therapeutic applications. Its use in treating depression has been most extensively studied, but controlled clinical studies also suggest the drug may have a role in treating obesity and bulimia. Moreover, a variety of other psychiatric disorders may be treatable with this drug. Regardless of the outcome of these clinical trials, it is apparent that fluoxetine has found a useful niche in therapy, and can be used as a probe to determine the role of serotonin in modulating human pathophysiologies.

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