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# Fenfluramine Blockade of CNS Stimulant Effects of Amphetamines

HARVEY J. BERGER▲, CLINTON C. BROWN, and JOHN C. KRANTZ, Jr.

**Abstract** □ Fenfluramine, 15 mg./kg. s.c., produced a slight but significant decrease in spontaneous motor activity in male Swiss mice as measured in a circular photocell activity cage. Treatment with fenfluramine 15 min. prior to either dextroamphetamine or methamphetamine, 2.5 mg./kg. i.p., significantly diminished the hyperactivity caused by these compounds. In a second experiment in mice, fenfluramine prolonged pentobarbital sleeping time, whereas dextroamphetamine and methamphetamine shortened barbiturate narcosis. Pretreatment with fenfluramine reversed the reduction of pentobarbital sleeping time induced by amphetamine. In a final experiment, it was found that fenfluramine did not produce amphetamine stereotypy in male Wistar rats. Fenfluramine administered prior to dextroamphetamine or methamphetamine, however, prevented the appearance of typical compulsive gnawing behavior in 80% of the animals. These results, using three different indexes of CNS excitation, demonstrate that fenfluramine reliably antagonizes amphetamine-induced stimulation in mice and rats. The observed reduction of the CNS activity of amphetamines by fenfluramine points to a possible clinical application of this agent in the prophylactic management of amphetamine abuse. Preliminary clinical trials using various psychological tests tend to confirm that fenfluramine decreases the effects of dextroamphetamine in man.

**Keyphrases** □ Amphetamine-induced CNS stimulation—blockade effect of fenfluramine, rats, mice □ Fenfluramine blockade—amphetamine-induced CNS stimulation, rats, mice □ CNS stimulation, amphetamine induced—blockade effect of fenfluramine, rats, mice

The anorexigenic agent fenfluramine, *N*-ethyl-α-methyl-*m*-(trifluoromethyl)phenethylamine hydrochloride, differs from its structural analog amphetamine (1–3) in that it does not produce CNS stimulation yet may produce a sedative action (4). Furthermore, fenfluramine reduction of amphetamine toxicity in grouped mice has been demonstrated (5).

Recently, Jonsson *et al.* (6) showed the blockade of intravenous amphetamine euphoria in man by oral pretreatment with α-methyl-*p*-tyrosine, an inhibitor of

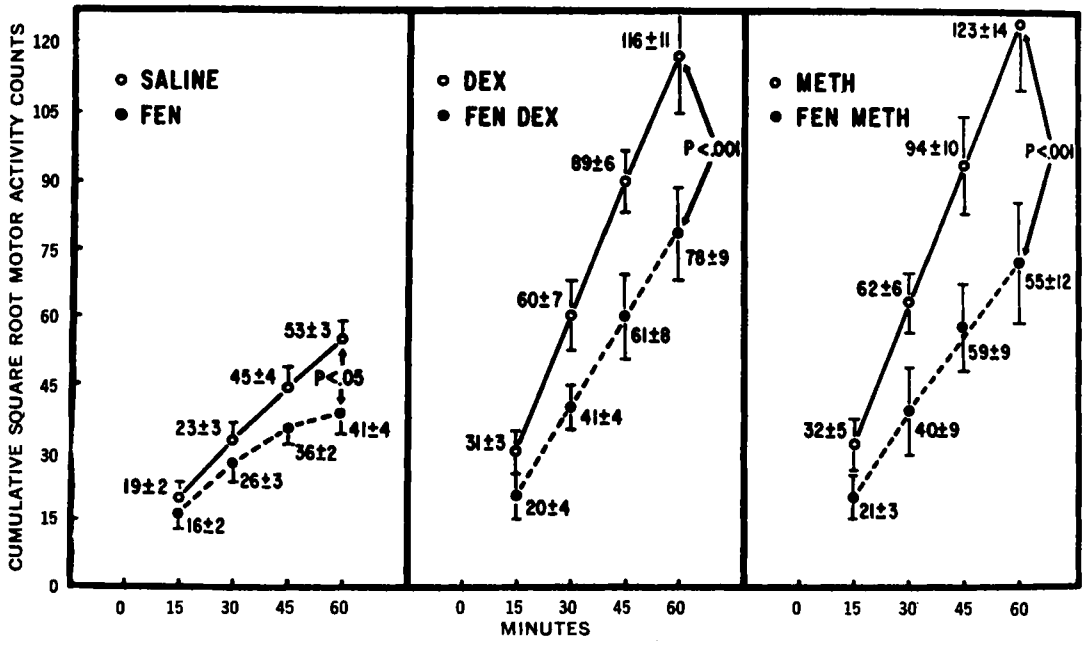


**Figure 1**—*Effect of fenfluramine (FEN) on dextroamphetamine- (DEX) and methamphetamine- (METH) induced increase in spontaneous motor activity in mice. Spontaneous motor activity counts are presented in a cumulative fashion and expressed in square roots as a function of time. Each value represents the mean ± standard deviation for 20 mice. Fenfluramine was administered at 15 mg./kg. s.c., with dextroamphetamine and methamphetamine at 2.5 mg./kg. i.p. Fenfluramine was injected 15 min. prior to dextroamphetamine or methamphetamine.*

tyrosine hydroxylase. This finding suggests the use of α-methyl-*p*-tyrosine as a drug-blocking agent in amphetamine abuse.

The results of four separate experiments in the present study show that fenfluramine pretreatment antagonizes the CNS stimulant properties of dextroamphetamine sulfate and methamphetamine in mice, rats, and man. The effects of fenfluramine on three amphetamine-induced responses were studied: (*a*) increased spontaneous motor activity in mice, (*b*) decreased pentobarbital sleeping time in mice, and (*c*) typical stereotyped behavior in rats.

## EXPERIMENTAL

Male Swiss white CD-1 mice[1], 32–38 g., and male Wistar rats[1], 250–300 g., were used. Mice were caged in groups of eight 4 weeks prior to testing. Rats were isolated in individual cages in a room separate from mice. Animals were maintained at 23–26° on laboratory food[2] and water *ad libitum*. Housing and experimentation were performed in the same room with regulated 12-hr. light and dark cycles.

Fenfluramine was administered at 15 mg./kg. s.c.; dextroamphetamine and methamphetamine were administered at 2.5 mg./kg. i.p. Controls received 0.2 ml. of the vehicle, physiologic saline. The routes of administration were chosen to ensure that fenfluramine would not merely interfere with the uptake and absorption of the amphetamines from the peritoneal cavity.

The experimental design called for the random selection of 20 animals for each of six treatment groups as outlined in Figs. 1–3. In all three experiments, fenfluramine was injected 15 min. prior to either dextroamphetamine or methamphetamine.

**Spontaneous Motor Activity**—Three circular photocell activity cages with six beams[3], 33 cm. in diameter, were utilized simultaneously. The circuits were wired so that activation of any photocell was independent of the other beams. Spontaneous motor activity counts were registered on inaudible digital counters during four 15-min. trial periods, totaling 1 hr. Individual mice were placed in the activity cages immediately following the second drug injection, approximately 3 hr. after the start of the light cycle.

**Pentobarbital Sleeping Time**—The time from the loss to the regaining of the righting reflex was recorded as the "sleeping time." Whenever one animal was checked for the return of the righting reflex, animals still asleep were also checked to standardize external stimulation. Equal numbers of mice in the compared drug treatments were injected simultaneously.

**Amphetamine Stereotypy**—One hour following the first drug administration, a trained observer, unfamiliar with the agents injected, recorded upon observation for 5 min. the presence or absence of typical amphetamine-induced gnawing behavior in naive rats (7). Continuous sniffing and small head movements with periodic exploratory activity were chosen as indicative of standard stereotypy.

## RESULTS

The effect of fenfluramine on amphetamine-induced increase in spontaneous motor activity in mice is shown in Fig. 1. Results are expressed as mean cumulative square root counts with standard deviations. The data were transformed because the frequency distribution of raw scores is usually skewed in the positive direction, with higher scores being farther from the mean than the lower scores. Thus, skewness of the distribution is minimized by taking square root scores (8, 9).

Fenfluramine-treated mice produced a mean of 40.9 ± 8.6 motor activity counts in 60 min. as compared to 52.7 ± 9.8 for saline controls. This significant difference ($p < 0.05$) reflects the sedative action of fenfluramine in contrast to the stimulant effect of its structural analog, dextroamphetamine. Such a finding is in agreement with that of Ziance *et al.* (4), indicating that fenfluramine showed no signs of CNS stimulation. However, both dextroamphetamine and methamphetamine significantly increased ($p < 0.001$) spontaneous motor activity to 116.4 ± 16.2 and 123.3 ± 8.9 counts, respectively, in the hour. Fenfluramine treatment prior to either dextroamphetamine and methamphetamine significantly lowered ($p < 0.001$) the amphetamine-induced increase in random motor activity to 77.9 ± 12.7 counts. While either dextroamphetamine or methamphetamine caused a 130% increase above saline control, fenfluramine limited this increment to only 46%, indicating that fenfluramine is able to block partially the hyperactivity produced by amphetamines.

[1] Charles River Breeders.
[2] Purina.
[3] Actophotometers, Metro Industries.

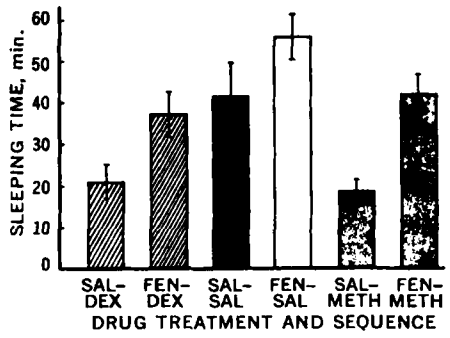


**Figure 2**—*Effect of fenfluramine (FEN) on dextroamphetamine- (DEX) and methamphetamine- (METH) induced antagonism of pentobarbital sleeping time in mice. Pentobarbital sleeping times are expressed as time in minutes from loss to regaining of the righting reflex. Each value represents the mean ± standard deviation for at least 20 mice. Fenfluramine was administered at 15 mg./kg. s.c., with dextroamphetamine and methamphetamine at 2.5 mg./kg. i.p. Fenfluramine was injected 15 min. prior to dextroamphetamine or methamphetamine. All mice received pentobarbital (40 mg./kg. i.p.) as the third injection. The dashed bars represent animals that received dextroamphetamine, while the shaded bars represent animals that received methamphetamine. The solid and open bars are both controls. SAL = saline.*

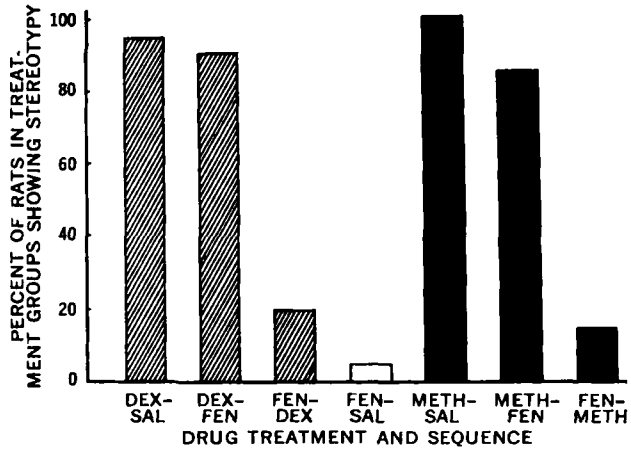


**Figure 3**—*Effect of fenfluramine (FEN) on amphetamine stereotypy in rats. The presence of compulsive gnawing behavior in rats following drug treatments is expressed as percent of the total number of animals in each group. Drug treatments consisted of 20 rats. Fenfluramine was administered at 15 mg./kg. s.c., with dextroamphetamine (DEX) and methamphetamine (METH) at 2.5 mg./kg. i.p. Fenfluramine was injected 15 min. prior to dextroamphetamine or methamphetamine. Observations were made 1 hr. following the first drug administration. SAL = saline.*

In mice treated with saline or fenfluramine, a large proportion of the counts was recorded within the initial 15-min. period, suggesting that much of the spontaneous activity was exploratory in nature. However, mice receiving dextroamphetamine or methamphetamine showed a more uniform distribution of activity counts over the four 15-min. periods. Thus, the spontaneous motor activity measured by the photocell cages reflects increased stimulation and not merely increased general exploration.

As shown in Fig. 2, control mice receiving pentobarbital alone slept for 42 ± 8.1 min. Pretreatment with fenfluramine produced a significant increase ($p < 0.01$) in pentobarbital sleeping time to 56 ± 6.1 min. Conversely, dextroamphetamine and methamphetamine each decreased pentobarbital sleeping time to 21 ± 3.5 and 19 ± 2.7 min., respectively. When fenfluramine was administered prior to dextroamphetamine or methamphetamine, the sleeping time induced by pentobarbital was essentially the same as that evoked by the barbiturate alone (37 ± 5.2 or 43 ± 3.9 min., respectively). Because of the significant increase in pentobarbital sleeping time following fenfluramine (from 42 to 56 min.), it would appear that the depressant properties of fenfluramine were principally responsible for this antagonism.

The experiments on amphetamine stereotypy (Fig. 3) yielded further evidence of blockade by fenfluramine of the CNS activity of amphetamines. Fenfluramine was observed to induce gnawing behavior in only one of 20 rats, while dextroamphetamine and methamphetamine produced typical stereotypy in almost all animals observed (19/20 and 20/20, respectively). When administered prior to amphetamines, fenfluramine prevented the appearance of the stereotyped behavior in 80% of the rats. However, when fenfluramine was given after dextroamphetamine or methamphetamine, no antagonism was evident, and the animals exhibited standard signs of amphetamine stereotypy. This would suggest that the blockade produced by fenfluramine might be due to interference with receptors specific to amphetamine activity.

## DISCUSSION

The precise mechanism by which fenfluramine produces the observed blockade of dextroamphetamine and methamphetamine needs further elucidation. It has been reported (5) that fenfluramine does not antagonize all of the effects of amphetamines. For example, fenfluramine neither depletes brain norepinephrine nor does it alter elevated levels of free fatty acids evoked by amphetamines. Whether the antagonism by fenfluramine of dextroamphetamine and methamphetamine is due to changes in the metabolism of amphetamine leading to the formation of a physiologically active metabolite or to interaction with the adrenergic receptor mechanism is not yet known.

In the motor activity study, individual mice were used in the photocell cages, instead of the more common groups of three to five, because of the effects of group size on amphetamine toxicity and activity (10, 11). All experiments were carried out at the same time of day, because circadian rhythm and the time of testing relative to the length of the light and dark cycles have been shown to affect amphetamine responses (12).

Amphetamines administered orally (10–30 mg.) in man evoke stimulation of the sensory cortex, which is manifested in increased alertness, motor and speech activity, and varying degrees of euphoria (13). In addition, characteristic stereotyped behavior shown by repetitious ponderous movements is common. Each of the three experimental parameters in mice and rats was chosen to parallel a clinical expression of amphetamine use.

The observed acute blockade of the psychoactivity of amphetamines by fenfluramine might suggest an application in man in the prophylactic blockade of the euphoric effects of high doses of amphetamines. To determine whether fenfluramine is actually valuable as a drug-blocking agent in amphetamine abuse, long-term administration of fenfluramine is required; such studies are now planned in both experimental animals and man.

A double-blind study on a fenfluramine–amphetamine combination in man involving a battery of psychomotor performance tasks was recently reported (14). Preliminary results indicate that: (*a*) fenfluramine alone was insignificantly different from placebo for each of the tasks, (*b*) fenfluramine and dextroamphetamine were significantly different from each other in four of eight tests, and (*c*) the fenfluramine–amphetamine combination produced effects similar to, but *less marked* than, those produced by dextroamphetamine alone.

Fenfluramine had a sedative and dysphoric effect but was not able to suppress completely the amphetamine-induced changes in psychomotor task performance. The possible clinical implications of this work in the blockade of amphetamine abuse are being considered.

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# Dehydration of Crystalline Theophylline Monohydrate and Ampicillin Trihydrate

ELI SHEFTER▲, HO-LEUNG FUNG, and OLIVIA MOK

**Abstract** □ The dehydration kinetics of theophylline monohydrate and ampicillin trihydrate were measured by an X-ray powder diffraction technique in an open system. In the case of theophylline, the hydrate transforms directly to a crystalline anhydrous form with apparent zero-order kinetics. The loss of water from ampicillin trihydrate results in an amorphous state. Commercial micronized ampicillin trihydrate, which contains a small amount of excipients, exhibits a different kinetic order and a faster rate of transformation.

**Keyphrases** □ Theophylline monohydrate—dehydration kinetics measured using X-ray powder diffraction technique □ Ampicillin trihydrate—dehydration kinetics measured using X-ray powder diffraction technique □ Solid-state phase transformations—dehydration of theophylline monohydrate and ampicillin trihydrate studied using X-ray powder diffraction technique □ X-ray powder diffraction technique—used to measure dehydration kinetics for theophylline monohydrate and ampicillin trihydrate □ Phase stability, theophylline monohydrate and ampicillin trihydrate—dehydration kinetics studied using X-ray powder diffraction technique

The solid-state phase transformation of the active ingredient in a dosage form could dramatically alter the pharmaceutical properties of the preparation. The solid phase of the administered drug can influence such important properties as bioavailability (1). Where highly energetic (metastable) forms are incorporated into the formulation, it is exceedingly important that, in addition to chemical stability, the phase integrity of the pharmaceutical be monitored.

Many organic medicinal agents are known to crystallize with solvent molecules as an integral part of their structure. The most widely found group of solvates are the hydrates. The phase stability of these multicomponent solids is governed by temperature, pressure, and the concentration of solvent in the system. However, even though thermodynamics might indicate that a particular solvate is metastable under normal storage conditions, the conversion rate to a desolvated phase could be relatively slow in pharmaceutical terms. The rates at which these desolvation processes take place are exceedingly important to the formulation.

The desolvation process of two crystalline hydrates (theophylline monohydrate and ampicillin trihydrate) was studied using an X-ray powder diffraction method. This investigation is part of a continuing study to delineate the various physical parameters controlling the kinetics of solid-state phase transformations. The principal point of this study is to demonstrate the scope of

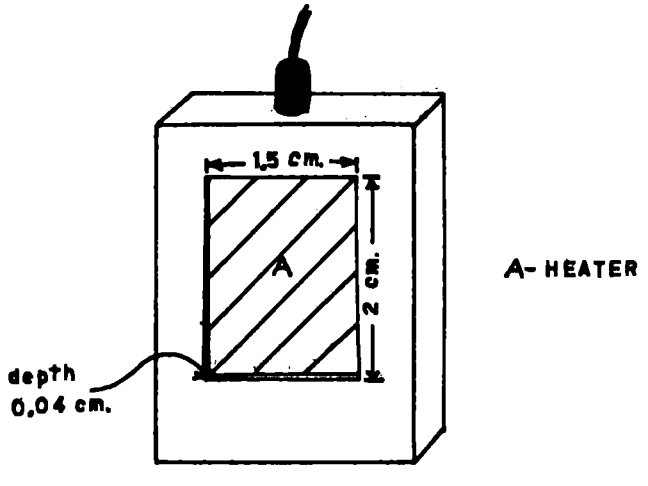


**Figure 1**—*The powder holder.*