J. Pharm. Pharmac., **24**, 1972, 886–893 *Received April* 25, 1972

Effects of fenfluramine on the adrenergic system

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The influence of fenfluramine on sympathetic transmission has been examined in various intact and isolated preparations. Fenfluramine both facilitated and inhibited the responses elicited by the sympathetic stimulation according to the dose administered and the preparation used. Fenfluramine,to a lesser extent than amphetamine, antagonized, in some preparations, the neuron blocking action of guanethidine. Fenfluramine did not antagonize the sympathomimetic effects elicited by amphetamine in the isolated atria and the isolated vas deferens unless very high concentrations of both drugs were used.

The pharmacology of fenfluramine has been investigated by Le Douarec, Schmitt & Laubie (1966) and by Bizzi, Bonaccorsi & others (1970) and conclusions from these and other studies point to its poor central stimulating properties in comparison with amphetamine (Alphin, Funderburk & Ward, 1964; Le Douarec & Schmitt, 1964). Fenfluramine has even been reported to possess a central depressant action (Munro, Seaton & Duncan, 1966; Ziance & Kinnard, 1967). The cardiovascular effects of fenfluramine are similar to those of amphetamine although much less pronounced. Phenoxybenzamine, reserpine and guanethidine antagonize the fenfluramine-induced hypertensive response (Le Douarec & others, 1966; Franko, Houkomp & Ward, 1965) suggesting that fenfluramine induces its cardiovascular effects by an indirect sympathomimetic action. Recently, Glenn Sipes, Ziance & Buckley (1971) studied the involvement of noradrenaline release in the cardiovascular actions of fenfluramine, and their data support previous conclusions that suggested the drug induced peripheral sympathomimetic responses by releasing noradrenaline. We have compared the influence of fenfluramine on the sympathetic transmission in isolated and intact preparations with that of amphetamine, and determined whether the blockade of the sympathetic transmission induced by guanethidine is antagonized by fenfluramine.

As there is evidence that fenfluramine inhibits the effects of amphetamine (Jespersen & Bonaccorsi, 1969; Berry, Poyser & Robertson, 1971) we examined the interaction of the two phenethylamines in some isolated preparations.

METHODS

Contraction of the inferior eyelid

Sprague Dawley rats, 300 g, were anaesthetized with urethane (1·25 g/kg). Retraction of the inferior eyelid elicited by the preganglionic stimulation of the cervical

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sympathetic chain (Gertner, 1956) was recorded by means of a thread tied through the eyelid and attached to an isometric transducer (Grass FT 03) fed into a Grass 5P5 Polygraph. Supramaximal electrical stimulation with varying frequencies and a pulse width of 1 ms was applied to the cervical sympathetic trunk through bipolar platinum ring electrodes with a Grass S4 stimulator for 15 s, every 5 min. Drugs were administered intravenously after obtaining the first frequency response curve.

Blood pressure

The carotid artery and the jugular vein of rats, 200 g, anaesthetized with urethane were cannulated. The head of each animal was fixed in a stereotaxic apparatus and a bipolar stainless steel electrode (0·25 mm diameter, 0·5 mm uninsulated tip) implanted at the coordinates corresponding to the posterior hypothalamus, according to the König & Klippel atlas (1963). Carotid blood pressure was recorded via a Statham P 23 AB pressure transducer fed into a Grass polygraph. Electrical stimulation of the posterior hypothalamus was made through a Grass model S4 stimulator, with a stimulus isolation unit. Square wave pulses of 1 ms duration, 5–10 V intensity and 70 Hz were applied for 15 s.

Rat isolated atria

Rats were killed by cutting the throat. The atria were rapidly removed and suspended in Krebs bicarbonate at 32° which was vigorously aerated with 5% carbon dioxide in oxygen. Contractile force was recorded via a Grass strain gauge attached to a Grass polygraph recorder. Rate of contraction was determined by counting beats in a 6 s interval. Atria beating above 240 or below 180 were discarded. The preparations were left 1 h, to allow the beat rate to attain a steady level before any drug was added. Chronotropic responses to cumulative doses of fenfluramine and amphetamine were obtained according to van Rossum (1963).

Rat isolated tail artery

Rats, 300 g, were anaesthetized with urethane and the central artery of the tail was dissected free and cannulated. The preparation was mounted in a 50 ml bath containing Krebs bicarbonate at 37° and aerated with 5% carbon dioxide in oxygen. Arteries were perfused by means of a constant output pump at a flow rate of 8–9 ml/min. Intraluminal injections were given through a rubber valve close to the artery. Increase in perfusion pressure was recorded by means of a mercury manometer. Postganglionic transmural stimulation was elicited with a pair of 4 cm platinum electrodes incorporated in plexiglass, placed along both sides of the artery. Twenty bursts of square wave pulses of 1 ms duration and supramaximal voltage were delivered every 4 min at 10 Hz from a S4 Grass stimulator.

Rat isolated vas deferens

Vasa deferentia dissected from rats, 200 g, were suspended in 20 ml of Krebs bicarbonate. Isotonic contraction were recorded through a frontal writing lever. Cumulative dose response curves to amphetamine and fenfluramine were obtained according to van Rossum (1963).

Isolated vas deferens of the guinea-pig

The left vas deferens from a guinea-pig, 500 g, was isolated together with the hypogastric nerve and immersed in a 50 ml organ bath containing Krebs bicarbonate aerated with 5% carbon dioxide in oxygen. The preganglionic portion of the hypogastric nerve was stimulated through a bipolar ring electrode with square wave pulses, at supramaximal voltage with 0·3 ms duration delivered every 2 min at 10 Hz for 5s. Contractions were recorded with an isotonic frontal lever.

Drugs. (+)-Amphetamine sulphate (Recordati, Milan); (±)-fenfluramine hydrochloride (Servier Lab. Paris); guanethidine sulphate (Ciba, Basel).

RESULTS

Eyelid of the rat

Stimulation of the cervical sympathetic trunk elicits a contraction of the inferior eyelid (Gertner, 1956, Spriggs, 1966; Morpurgo, 1968).

Amphetamine is known to potentiate the contraction to sympathetic nerve stimulation and to reverse the blockade induced by guanethidine (Obianwu, 1969). The effects of fenfluramine and amphetamine on the frequency response curve induced by the electrical stimulation of the cervical sympathetic trunk were therefore compared. The sensitization induced by 3×10^{-6} mol/kg of amphetamine was more pronounced on the duration of the contraction, which was markedly prolonged, than on the height of the contraction. Fenfluramine in 7 out of 9 experiments, induced a small increase in duration at 2×10^{-5} mol/kg and a small decrease of contraction height lasting not more than 15 min at 4×10^{-5} mol/kg. However, when the adrenergic transmission

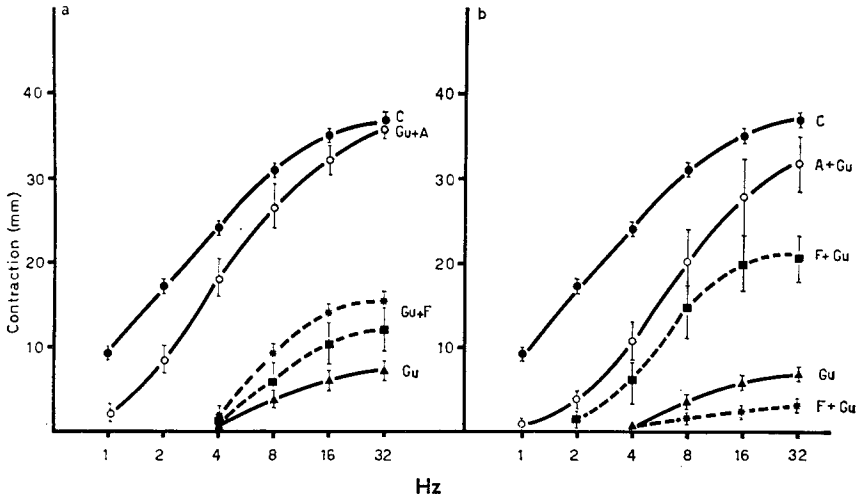

FIG. 1. Eyelid of the rat. Antagonism of guanethidine adrenergic nerve blockade by amphetamine and fenfluramine. Eyelid contraction of anaesthetized rats to preganglionic stimulation of the superior cervical sympathetic trunk. Trains of pulses of 1 ms duration and 5–10 V were applied for 15 s every 5 min at various rate as indicated in the abscissae.

a. The initial frequency response curve (C) which is decreased after an intravenous injection of 2 mg/kg of guanethidine (Gu) is repeated after 20 min of infusion with amphetamine, 3×10^{-6} mol/kg (Gu + A), or fenfluramine 2×10^{-5} mol/kg (*) (Gu + F), or fenfluramine 4×10^{-5} mol/kg (■).

b. Symbols as in b. Amphetamine and fenfluramine were infused before inducing guanethidine blockade. Vertical lines show s.e. of the means.

was impaired by guanethidine, fenfluramine, 4×10^{-5}mol/kg, was able to partly reverse the block but only if administered before guanethidine. Amphetamine (3×10^{-6} mol/kg), on the contrary, caused a strong reversal of the guanethidine-induced adrenergic nerve blockade whatever the sequence of the drugs (Fig. 1).

Blood pressure

Stimulation of the posterior hypothalamus induces an increase in blood pressure in anaesthetized rats (Folkow & Rubinstein, 1966; Morpurgo, 1968). Table 1 shows

Table 1. *Modifications induced by fenfluramine and amphetamine of centrally evoked sympathetic responses in anaesthetized rats.*

Drug	Dose* (mg/kg, i.v.)	Pressor responses (mm Hg ± s.e.) after hypothalamic stimulation
Saline		67·8 ± 3·3 (16)†
Fenfluramine	9×10^{-6}	62·5 ± 2·5 (3)
Fenfluramine	4×10^{-5}	48·7 ± 4·2 (4)
Amphetamine	$5{\cdot}4 \times 10^{-6}$	90·0 ± 10·0 (3)
Amphetamine	$1{\cdot}3 \times 10^{-5}$	76·0 ± 10·0 (3)
Amphetamine	$5{\cdot}4 \times 10^{-5}$	75·0 ± 7·6 (3)

* Drugs were injected slowly enough to produce the minimal change in the basal blood pressure. Usually fenfluramine at the dose of 4×10^{-5} caused a slight fall in the blood pressure. Responses were recorded 10 min after the administration of the drugs.

† Figures in parentheses refer to the number of animals.

that fenfluramine at doses higher than 10^{-5} mol/kg reduced the centrally evoked hypertensive response. The reduction was 30% 5 min after administration of 4×10^{-5} mol/kg (i.v.) and the effect decreased in the following hour. Amphetamine, 5×10^{-6} mol/kg, elicited a weak potentiation of the hypertensive response which was not dose-related and not significantly different from control responses.

Rat isolated atria

Spontaneously beating isolated atria exposed to cumulative doses of amphetamine displayed a dose dependent increase in rate, fenfluramine had much lower intrinsic activity and affinity (Fig. 2). Since fenfluramine showed poor sympathomimetic effects we examined its interaction with amphetamine. Two doses of fenfluramine were chosen to give a medium (30%) or maximum (100%) effect. The response to amphetamine in the presence of the medium dose of fenfluramine (3×10^{-6}M) was not modified, but was reduced in combination with the high dose (3×10^{-5}) when amphetamine was added to the bath before fenfluramine; there was always an additive interaction between the drugs (Fig. 3).

Isolated arteries

Amphetamine ($2{\cdot}7 \times 10^{-6}$M) potentiated the nerve mediated response of the isolated tail artery of rat (Fig. 4). Fenfluramine had a similar action but at higher concentrations (5×10^{-6}M) and the potentiation obtained was always smaller than that with amphetamine.

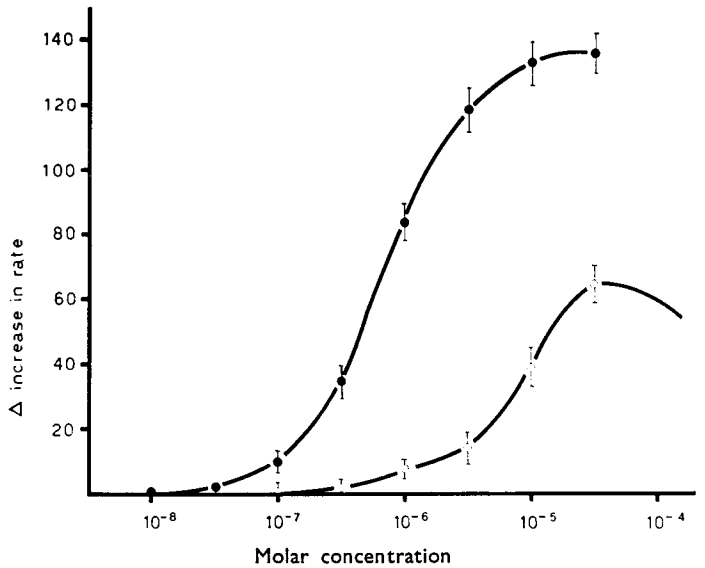

FIG. 2. Rat isolated atria. The dose response curves from spontaneously beating isolated atria using amphetamine (●) and fenfluramine(○). Vertical lines show s.e. of the means.

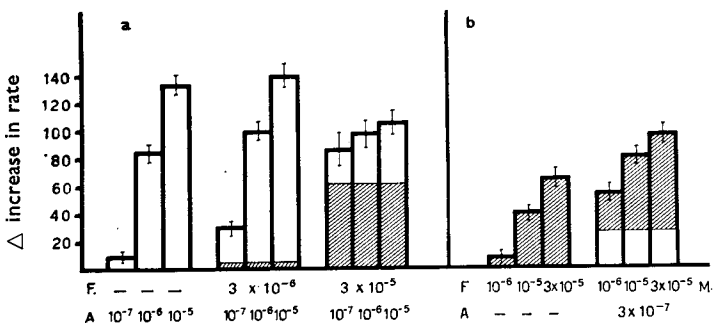

FIG. 3. Rat isolated atria. Increase in atrial rate induced by amphetamine (open bars) or fenfluramine (hatched bars), given alone or in association. In a, amphetamine was given after fenfluramine. In b, fenfluramine was given after amphetamine.

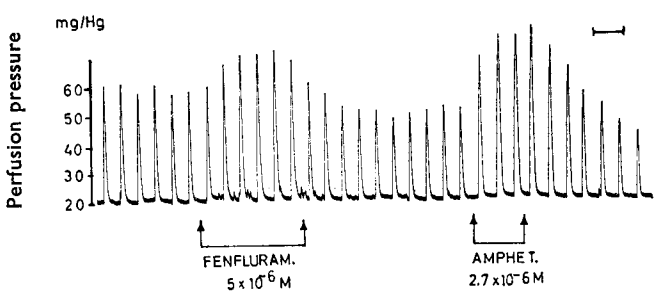

FIG. 4. Rat isolated tail artery. Effect of amphetamine and fenfluramine on the increase in perfusion pressure induced by 20 s period of stimulation delivered every 4 min at 10 Hz. Time=4 min.

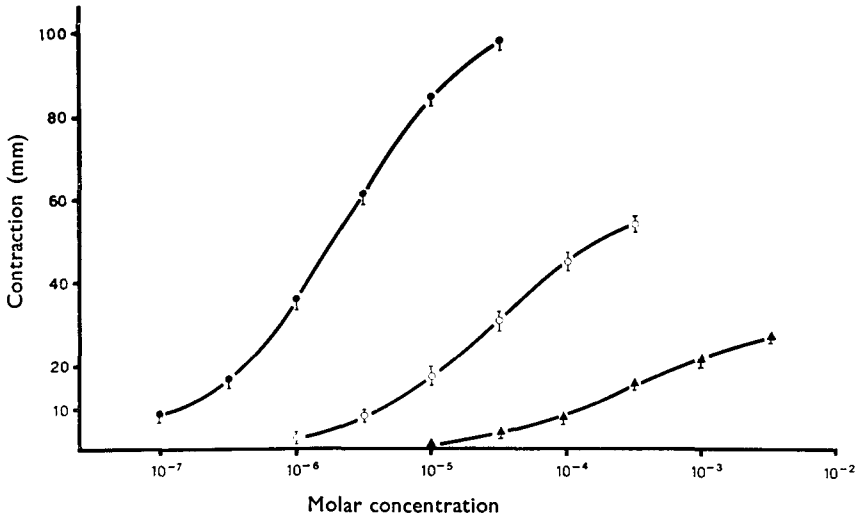

FIG. 5. Rat isolated vas deferens. The dose response curves from the isolated vas deferens using noradrenaline (●), amphetamine (○) and fenfluramine (▲). Vertical lines show s.e. of the means.

Rat isolated vas deferens

Amphetamine caused a poor contraction of this preparation and fenfluramine was even less effective. With the intrinsic activity of noradrenaline at 1, amphetamine only reaches a value of 0·6 and fenfluramine of 0·3 (Fig. 5). Rhythmic contractions were always present when fenfluramine or amphetamine was added to this preparation.

Guinea-pig isolated vas deferens stimulated through the hypogastric nerve

Amphetamine potentiated the contraction of the guinea-pig vas deferens elicited by electrical stimulation of the hypogastric nerve. The potentiation was dose-dependent

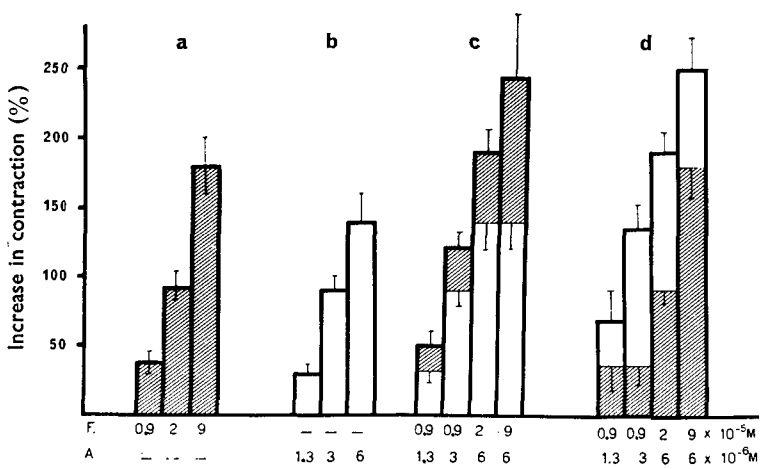

FIG. 6. Guinea-pig vas deferens stimulated through the hypogastric nerve for 5 s every 2 min with pulses of 0·3 ms duration, supramaximal voltage, at 10 Hz. The histograms show % increase in response to electrical stimulation in the presence of amphetamine (open bars) or fenfluramine (hatched bars) or in the presence of both drugs. Fenfluramine was added to the bath alone (a) or after amphetamine (c). Amphetamine was added alone (b) or after fenfluramine (d)

and was seen with a concentration of amphetamine as low as 1×10^{-6}M. Fenfluramine was also active but only at concentrations 9 times higher than amphetamine (Fig. 6a, b).

On this preparation, as with isolated atria, an attempt was made to see how fenfluramine could affect the subsequent administration of amphetamine. When the increase of the response to sympathetic stimulation after fenfluramine had reached a steady state, amphetamine was added and the reverse sequence was also tried (Fig. 6c, d). The figure shows that only additive effects occurred between the drugs. Fig. 7 shows

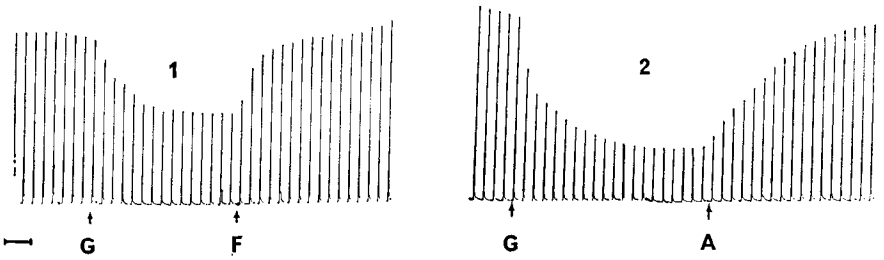

FIG. 7. Guinea-pig vas deferens. The hypogastric nerve was stimulated for 5 s every 2 min with pulses of 0·3 ms duration, supramaximal voltage, at 13 Hz. The records show the effect of fenfluramine (F) (3×10^{-5}) after exposure to 1 μg/ml of guanethidine (G) (record 1) and the effect of amphetamine (A) ($2{\cdot}7 \times 10^{-6}$) after exposure to 2 μg/ml of guanethidine (record 2). Time = 5 min.

the effects of amphetamine and fenfluramine in antagonizing guanethidine neuron blocking action. Amphetamine, $2{\cdot}7 \times 10^{-6}$M added to the bath, caused a complete reversal of the blockade induced by guanethidine while fenfluramine, 3×10^{-5}M, showed a poor antagonism. Higher concentrations did not show a stronger antagonism.

DISCUSSION

From the data presented it may be seen that in our experimental conditions fenfluramine showed sympathomimetic activity similar to that of amphetamine although reduced in potency. Fenfluramine had no vasoconstrictor activity on the rat isolated tail artery, caused a small or no contraction of the vas deferens and it induced a modest rate increase on spontaneously beating isolated atria. Amphetamine was always more active.

In preparations involving sympathetic transmission fenfluramine effects were in some cases qualitatively different from amphetamine. Fenfluramine antagonized the blood pressure increase following hypothalamic stimulation, while amphetamine was without effect. The eyelid contraction induced by the electrical stimulation of the sympathetic trunk was prolonged by 2×10^{-5} mol/kg of fenfluramine and inhibited by 4×10^{-5} mol/kg. However the effect of fenfluramine on isolated preparations stimulated through their sympathetic innervation (isolated artery, isolated vas deferens) was qualitatively similar to that induced by amphetamine with an enhancement of the evoked contraction size at doses 2–10 times higher than amphetamine.

Fenfluramine did not antagonize guanethidine-induced neuron blockade in the isolated vas deferens, but showed some antagonistic effect on the guanethidine blockade of the sympathetic transmission in the eyelid. Amphetamine at lower concentrations reversed the blockade on both preparations.

No evidence of an interaction between fenfluramine and amphetamine could be drawn from the *in vitro* observations, at variance with the results described *in vivo* where fenfluramine was shown to antagonize the amphetamine toxicity in grouped mice (Jespersen & Bonaccorsi, 1969) or the amphetamine induced hypertension (Berry & others, 1971).

In conclusion fenfluramine seems to possess sympathomimetic activities much less marked than amphetamine. In addition, the effect shown by fenfluramine on the responses elicited by the stimulation of the sympathetic pathways seemed to differ from those elicited by amphetamine. Whether this is due to a different mechanism of action remains unclear. The two drugs do not appear to interact *in vitro*, suggesting that the antagonism observed *in vivo* may be not due to a specific antagonism at receptor levels. This concept is further supported by a recent observation by Bernardi & Jori (1972) who could not demonstrate any cross tolerance between the two drugs on the increase of homovanillic acid in the striatum of rat.

Acknowledgement

The technical assistance of Miss Cristina Fossati is gratefully acknowledged.

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