

THE SYMPATHOMIMETIC ACTIVITY OF FENFLURAMINE HYDROCHLORIDE ON RAT VAS DEFERENS

D.R. MOTTRAM & D. WADHWANI

Department of Pharmacology, School of Pharmacy, Liverpool Polytechnic, Liverpool L3 3AF

- 1 The peripheral, pharmacological effects of the anorexigenic agent, fenfluramine hydrochloride, have been investigated on rat isolated vas deferens.
- 2 Characteristic spiked contractions were observed within 2 to 3 min after exposure to fenfluramine; these contractions reached a rate of around 13 per min and were of variable height.
- 3 Pre-treatment of vasa with the indirectly acting sympathomimetic amine, tyramine, greatly reduced both the height and rate of contraction induced by fenfluramine.
- 4 The uptake inhibitor, desipramine, required a concentration in excess of $10\text{ }\mu\text{M}$ to affect fenfluramine-induced contractions. Effects of desipramine on fenfluramine contractions were of equal magnitude whether desipramine was administered before fenfluramine or at the height of the fenfluramine-induced contractions.
- 5 Pre-treatment with debrisoquine (0.5 mM), reduced the contractions in response to fenfluramine over a period of time.
- 6 Fenfluramine, added to vasa from rats which had been injected intraperitoneally with 5 mg/kg reserpine 24 h and 48 h previously, failed to induce its characteristic contractions.
- 7 It is concluded that fenfluramine can be classed as an indirectly acting sympathomimetic amine on peripheral adrenergic nerve terminals.

Introduction

Amphetamine and fenfluramine have both been shown to produce an anorectic effect in animals and man. However, they differ pharmacologically in a number of respects, the most noticeable being the marked central locomotor stimulating properties of amphetamine (Le Douarec & Neveu, 1970).

The comparative biochemical modes of action of amphetamine and fenfluramine, in producing anorectic effects in animals and man have been well documented. Both noradrenaline (Weissman, Koe & Tenen, 1966) and dopamine (Kruk, 1973) have been suggested as intermediates in the anorectic effects of amphetamine. On the other hand, it has been suggested that the mediator for fenfluramine is 5-hydroxytryptamine (Jespersen & Scheel-Krüger, 1970; Funderburk, Hazelwood, Ruckart & Ward, 1971; Samanin, Ghezzi, Valzelli & Garattini, 1972; Clineschmidt, 1973; Ghezzi, Samanin, Bernasconi, Tognoni, Gerna & Garattini, 1973). The biochemical action of fenfluramine is not, however, confined to the serotonergic nerve terminal, since fenfluramine has also been shown to affect noradrenaline concentrations, both centrally and peripherally (Costa,

Groppetti & Revuelta, 1971; Sipes, Ziance & Buckley, 1971; Ziance, Sipes, Kinnard & Buckley, 1972; Mitchell & Mottram, 1976).

The purpose of the present study was to investigate, more fully, the *in vitro* peripheral effects of fenfluramine in an attempt to characterize the mode of action of the drug on noradrenergic nerve terminals.

Methods

Experiments were carried out on the vasa deferentia of male Wistar rats. The rats, weighing 200–275 g, were killed by a blow to the head. The vasa were excised, stripped of extraneous material and suspended in 10 ml organ baths containing Tyrode solution (composition (g/l): NaCl 8.0, KCl 0.2, MgCl_2 0.2, CaCl_2 0.2, NaH_2PO_4 0.05, NaHCO_3 1.0 and glucose 1.0) maintained at 37°C and aerated with a mixture of 95% O_2 and 5% CO_2 . Isometric contractions were recorded with Devices 2 oz strain gauge transducers and two channel recorder. All drugs were freshly prepared and administered in Tyrode solution.

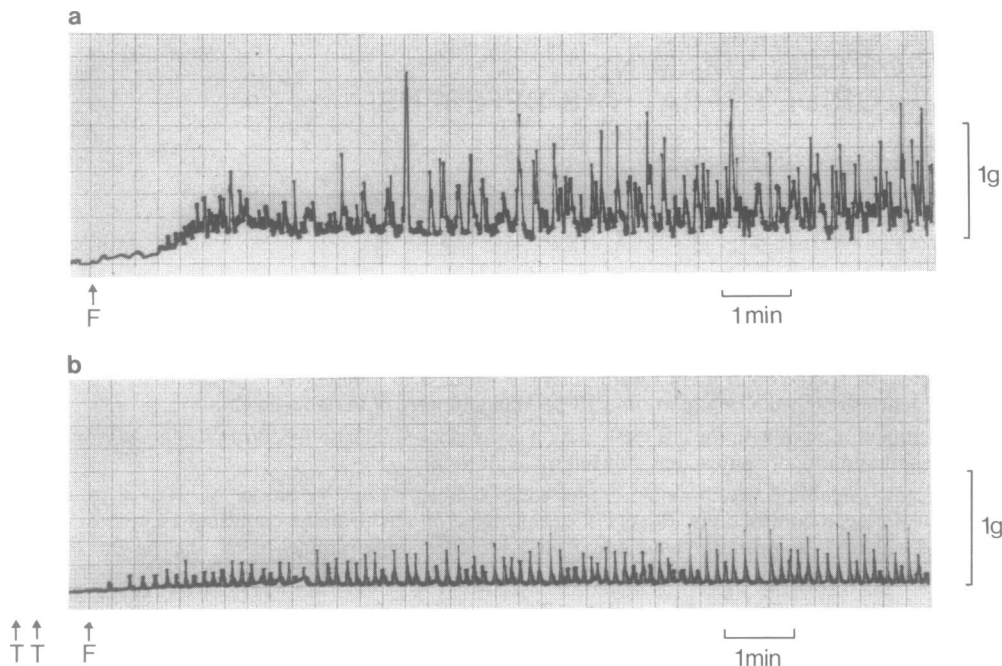


Figure 1 Tracings from one of five experiments, showing the effects of pre-treatment with 0.4 mM tyramine (T) before the addition of 0.2 mM fenfluramine (F) to both test and control vasa deferentia of the rat. (a) Control; (b) tyramine pre-treated. The bathing fluid was changed at the end of each 3 min exposure to tyramine. Vertical calibration 1 g, horizontal calibration 1 minute.

Pre-treatment with tyramine

Paired vasa, from the same animal, were set up in organ baths. The test vas was exposed to 0.4 mM tyramine, administered twice at 3 min intervals, fresh Tyrode solution being added at the end of each 3 min period; 0.2 mM fenfluramine was then added to both test and control vasa.

Desipramine pre- and post-treatment

Various concentrations of desipramine (0.1 μ M to 0.1 mM) were added to one of a pair of vasa, 2 min before the addition of 0.2 mM fenfluramine to both paired vasa. The same range of concentrations of desipramine was also added to vasa at the height of the contractions produced by 0.2 mM fenfluramine.

Pre-treatment with debrisoquine

Debrisoquine sulphate (0.5 mM) was added to one of a pair of vasa 5 min before the addition of fenfluramine (0.2 mM). The same dose of fenfluramine was added to the control vas. Contractions were recorded over a 30 min period.

Effects of reserpine-treatment on fenfluramine responses

Reserpine (5 mg/kg, i.p.) was injected into rats on two consecutive days; 24 h after the second injection, animals were killed in the usual manner and their vasa suspended in organ baths. Control rats were treated similarly, but an equal volume of vehicle was injected. Fenfluramine (0.2 mM) was then added to the vasa from both test and control rats.

The following drugs were used: fenfluramine hydrochloride (Ponderax), tyramine hydrochloride, desipramine hydrochloride, debrisoquine sulphate, reserpine, noradrenaline acid tartrate.

Results

Pre-treatment with tyramine

Contractions of control vasa defentia were recorded after the addition of 0.2 mM fenfluramine hydrochloride (Figure 1). Contractions began within 2 min and reached a rate of around 13 contractions per minute. The heights of contraction varied, but reached maximum 20 to 30 min after the addition of fenfluramine.

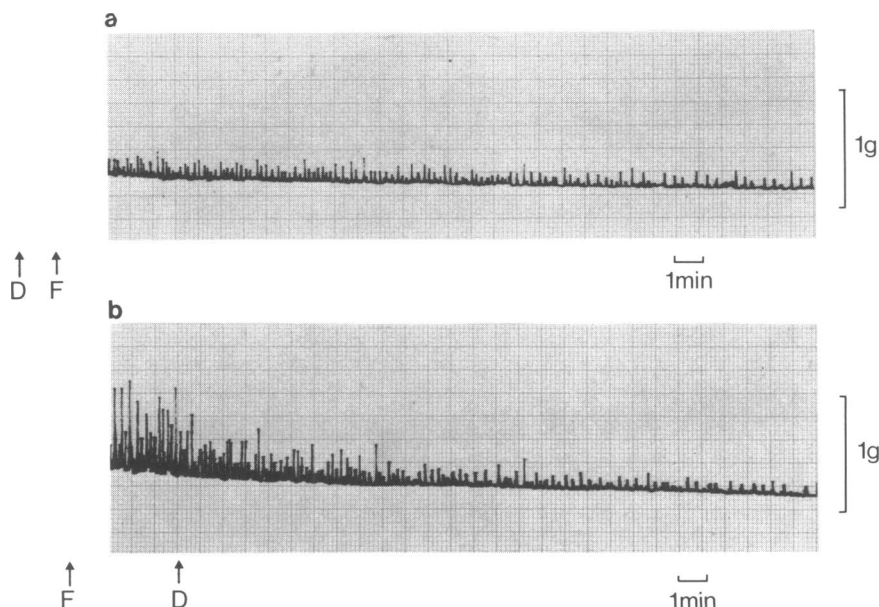


Figure 2 Tracings from the results of experiments ($n=22$) on the effect of pre- and post-treatment with desipramine, on the contractile response of rat vas deferens to fenfluramine. (a) Contractions of a vas deferens that had received $50\text{ }\mu\text{M}$ desipramine (D) 2 min before the addition of 0.2 mM fenfluramine (F). (b) Effects of $50\text{ }\mu\text{M}$ desipramine (D) added to the vas deferens at the height of the contractions produced by 0.2 mM fenfluramine (F). Vertical calibration 1 g , horizontal calibration 1 minute .

Pre-treatment with the indirectly acting sympathomimetic amine, tyramine, before the addition of fenfluramine, caused a reduction in both the rate and height of the contractions produced by fenfluramine (Figure 1).

Desipramine pre- and post-treatment

Concentrations of desipramine between $0.1\text{ }\mu\text{M}$ and $10\text{ }\mu\text{M}$, administered to vasa before the addition of fenfluramine, did not affect the subsequent rate or height of contraction produced by fenfluramine. Concentrations of desipramine in excess of this ($20\text{ }\mu\text{M}$, $50\text{ }\mu\text{M}$ and 0.1 mM) reduced the height, and subsequently the rate of contraction produced by fenfluramine (Figure 2).

In addition to this, the same range of concentrations of desipramine were added to vasa at the height of their contractions to fenfluramine, and a parallel effect was seen. No reduction in rate or height of contraction was produced until a dose of $10\text{ }\mu\text{M}$ desipramine was exceeded, after which a similar effect to that produced by pre-treatment of desipramine was observed (Figure 2).

Pre-treatment with debrisoquine

Vasa which had been treated with debrisoquine (0.5 mM), before the addition of fenfluramine, exhibited a reduced response to fenfluramine, compared with control vasa that had received fenfluramine alone (Figure 3). It was noted that debrisoquine itself, when administered alone to the vas deferens, produced fenfluramine-like contractions, although these were soon reduced and ultimately abolished.

Effects of pretreatment with reserpine on responses to fenfluramine

Fenfluramine failed to produce its characteristic contractions on vasa from animals which had received reserpine (Figure 4). These tissues were then challenged with a submaximal dose of noradrenaline, the contractions produced indicating that the tissues had maintained their sensitivity towards the neurotransmitter (Figure 4). Following the washout of noradrenaline from the organ bath containing these reserpinized tissues, which had been exposed to fenfluramine, the characteristic contractions induced

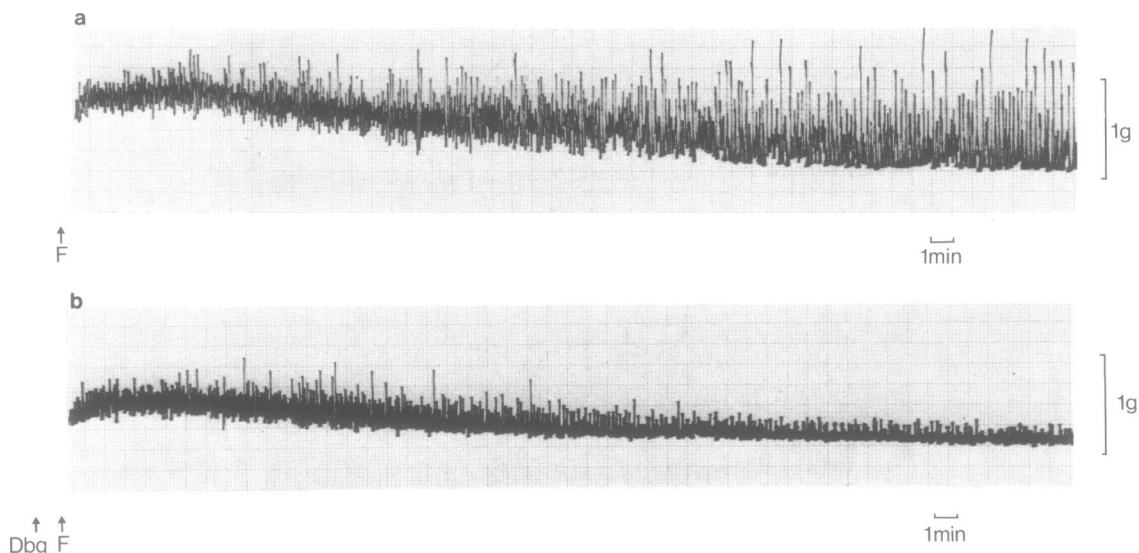


Figure 3 Tracings from one of five experiments in which the effects of pre-treatment with 0.5 mM debrisoquine (Dbq) on contractions produced by 0.2 mM fenfluramine (F) in rat vas deferens were observed. (a) Control; (b) debrisoquine pre-treated vas deferens. Vertical calibration 1 g, horizontal calibration 1 minute.

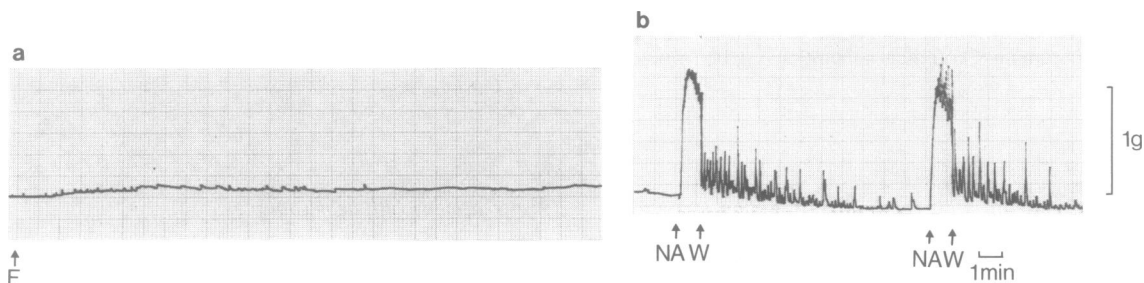


Figure 4 (a) Tracing from one of six experiments in which 0.2 mM fenfluramine (F) was added to isolated vasa from rats injected 24 h and 48 h previously with reserpine 5 mg/kg. (b) Tissues were subsequently challenged with 5 μ M noradrenaline (NA) which was washed out (W) after 1 min exposure. Vertical calibration 1 g, horizontal calibration 1 minute.

by fenfluramine were observed for a short period of time (Figure 4).

Discussion

Fenfluramine has been shown to reduce noradrenaline concentrations in the brain (Duhault & Verdavainne, 1967; Ziance *et al.*, 1972) and peripherally (Sipes *et al.*, 1971). Mitchell & Mottram (1976) suggested that fenfluramine exerts an effect at adrenergic nerve terminals either by depleting stores of noradrenaline or by inhibiting the amine uptake process, or by a combination of the two.

The present study indicates that, peripherally, fenfluramine exerts its effects by an indirect sympathomimetic action. This conclusion was arrived at by investigating the effect of fenfluramine on isolated vas deferens following, or in the presence of, various pharmacological agents which themselves affect the uptake, storage and release of noradrenaline in sympathetic nerve terminals.

Tyramine is an indirectly acting sympathomimetic amine, which, it has been suggested, releases vesicle-bound noradrenaline (Muscholl, 1966). It has already been shown (Mitchell & Mottram, 1976) that fenfluramine greatly reduces the response to tyramine both in cardiovascular studies and also in isolated

tissue. The present study shows that this reduction in response is mutual, in that tyramine pre-treatment of the vas deferens greatly reduces the fenfluramine contractions which are characteristically observed on this tissue. This mutual antagonism by these two drugs may be due either to competition for uptake sites on the nerve terminal membrane, or to depletion of noradrenaline levels from its terminal storage sites. However, it would appear that their respective modes of action on the nerve terminal are not identical, since the response of the vas deferens to each drug, individually, differs in a number of respects. Whilst tyramine-induced contractions in the vas are immediate and short-lived, those produced by fenfluramine require some minutes to appear and are then seen as spiked contractions which require up to 30 min to reach their maximum height.

The effect of neuronal uptake inhibition on fenfluramine responses in the vas deferens was investigated using the potent uptake inhibitor, desipramine (Iversen, 1967). Doses of desipramine (up to $1\text{ }\mu\text{M}$) which produce an almost complete inhibition of noradrenaline uptake, failed to affect the responses to fenfluramine. The substituent groups on the catechol hydroxyl and nitrogen moieties of fenfluramine indicate that this drug may be unable to utilize the desipramine-sensitive uptake process for noradrenaline. However, at higher concentrations (greater than $0.1\text{ }\mu\text{M}$) the fenfluramine-induced contractions of the vas were reduced. Westfall (1973), has shown that this concentration of desipramine itself antagonizes the response to noradrenaline on rat vas deferens. This was confirmed in the present study, in that, addition of desipramine (in doses greater than $10\text{ }\mu\text{M}$) to vasa at the height of their contraction to fenfluramine, produced an immediate reduction in these contractions.

Debrisoquine exerts its neurone blocking effect by inhibiting the release of noradrenaline from the nerve terminals. In the present study, debrisoquine was

found to inhibit the fenfluramine-induced contractions of the vas deferens. This inhibition required some time to become established, reflecting the initial, short-acting, noradrenaline releasing property of this group of drugs.

Finally the effect of reserpine treatment on the responses produced by fenfluramine, was investigated. The potent noradrenaline depletion produced by reserpine, resulted in a total blockade of fenfluramine-induced contractions in the vas deferens. The tissues were shown to have maintained their responsiveness to noradrenaline by the exogenous addition of the transmitter which resulted in an immediate contraction of the vasa. Following these additions of noradrenaline and their subsequent washing out from the organ bath, it was noted that fenfluramine-like contractions briefly appeared. It is therefore suggested that these contractions are produced by fenfluramine, which had previously been administered to the tissues and accumulated in the sympathetic nerve terminals, causing a release of exogenous noradrenaline which had been taken up into the nerve terminals during its addition to the bathing fluid.

The results of the present study throw some light on the mode of action by which fenfluramine depletes noradrenaline from tissues both centrally and peripherally. It is suggested that fenfluramine is taken up into adrenergic nerve terminals by a transport process which is not interfered with by the noradrenaline uptake inhibitor, desipramine. Within the nerve terminal, fenfluramine causes the release of the transmitter, noradrenaline, an effect which can be reduced by the acute administration of tyramine, abolished by the chronic administration of reserpine and interfered with by pre-treatment with debrisoquine.

It is concluded that fenfluramine, amongst its other properties, can be classed as an indirectly acting sympathomimetic amine on peripheral adrenergic nerve terminals.

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