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PERIPHERAL EFFECTS OF FENFLURAMINE

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1 The peripheral cardiovascular effects of the centrally acting anorexigenic agent, fenfluramine hydrochloride, have been investigated in the rat.

2 After intravenous administration of fenfluramine, an immediate hypotensive response, followed by a reflex rise in blood pressure was recorded. This was followed by a prolonged fall in blood pressure which frequently failed to return to pre-drug levels.

3 The antagonists, propranolol and atropine, failed to inhibit this hypotensive effect of fenfluramine.

4 The effects of 5 mg/kg fenfluramine for 1 h on the blood pressure responses to the sympathomimetic amines, tyramine, methoxamine and metaraminol were studied.

5 The responses to the indirectly acting tyramine were reduced by 50% following fenfluramine, while those to the directly acting methoxamine remained unaffected by the drug. Responses to metaraminol, an amine with both direct and indirect actions, were also unaffected to a significant degree by fenfluramine.

6 Studies on rat isolated vas deferens again showed that responses to tyramine are greatly reduced following fenfluramine.

7 In addition fenfluramine itself produced spontaneous contractions of the vas deferens. These contractions were blocked by the α-adrenoceptor blocking agents phentolamine and thymoxamine.

8 It is suggested that fenfluramine exerts an effect at the adrenergic nerve terminal, either by displacing noradrenaline stores or by inhibition of the amine uptake process.

Introduction

Fenfluramine hydrochloride has been shown to be an anorexigenic agent in animals and man (Le Douarec, Schmitt & Laubie, 1966; Munro, Seaton & Duncan, 1966). The chemical structure of fenfluramine is similar to that of amphetamine, another drug capable of suppressing food intake; however, the introduction of a trifluoromethyl group in the *meta* position on the benzene ring, and an ethyl group on the nitrogen atom of the amphetamine molecule abolishes central stimulant activity (Alphin, Funderburk & Ward, 1964).

Two preliminary investigations into the peripheral effects of fenfluramine have been reported (Franko, Honkomp & Ward, 1965; Sipes, Ziance & Buckley, 1971) and in both cases a hypertensive response to the drug was recorded.

In the brain, fenfluramine produces a long-lasting dose-dependent lowering of 5-hydroxytryptamine concentrations (Duhault & Verdavainne, 1967; Opitz, 1967; Costa, Groppetti & Revuelta, 1971), which, it has been suggested, accounts for the anorexigenic effect of this drug (Jespersen & Scheel-Kruger, 1970; Samanin, Ghezzi, Valzelli & Garattini, 1972). However, a reduction in noradrenaline concentrations following fenfluramine has also been observed both in the brain (Duhault & Verdavainne, 1967) and peripherally (Sipes *et al.*, 1971).

The present study was undertaken to investigate more fully the peripheral effects of fenfluramine. In this context both the direct effects of the drug and the possibility of an indirect response, mediated through sympathetic nerve terminals, were studied.

Methods

Rat blood pressure

Rats, weighing 175–225 g, of either sex, were anaesthetized with urethane (0.75 g/kg i.p. and 0.75 g/kg s.c.). A polythene cannula, inserted into the right carotid artery, was used to measure blood pressure via a Devices transducer and recorder. Drugs were administered through a polythene cannula inserted in the left external jugular vein.

Blood pressure responses to various doses of fenfluramine were recorded for periods up to one hour. Dose–response relationships to various sympathomimetic amines, chosen for their different modes of action, were recorded before and after

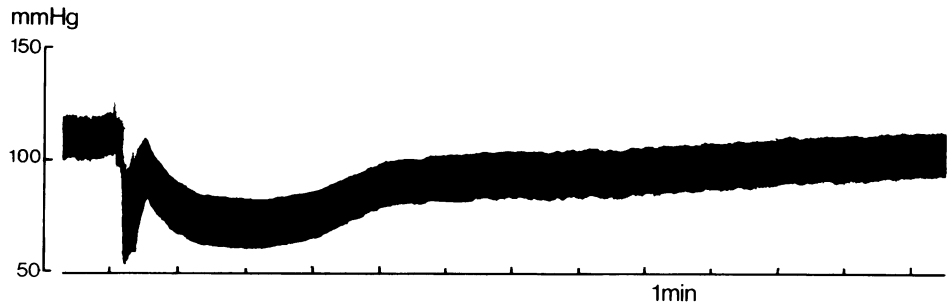

Figure 1 Blood pressure response to fenfluramine hydrochloride 5 mg/kg in the anaesthetized rat.

exposure to 5 mg/kg fenfluramine for one hour.

Drugs were administered in solution in 0.9% w/v NaCl (saline) and volumes administered at any one time did not exceed 0.2 ml.

Rat isolated vas deferens

Male rats, weighing 200–250 g, were killed by a blow on the head. The vasa deferentia 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.

Drugs were freshly prepared and administered in Tyrode solution. The following drugs were used: fenfluramine hydrochloride (Ponderax), metaraminol tartrate, methoxamine hydrochloride, tyramine hydrochloride, propranolol hydrochloride, atropine sulphate, phentolamine mesylate and thymoxamine hydrochloride.

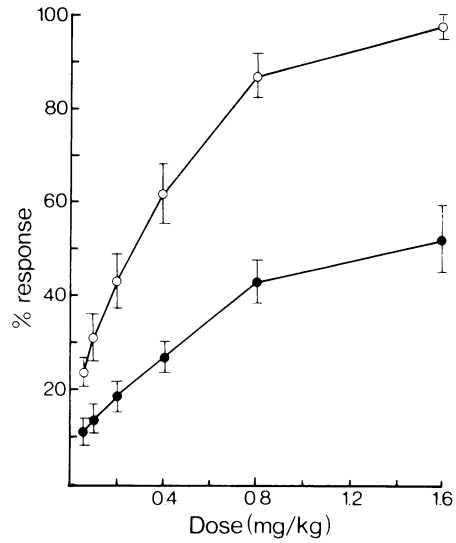

Figure 2 Dose-percentage response relationships for tyramine before (○) and after (●) fenfluramine hydrochloride 5 mg/kg. Responses are measured as the percentage of the maximum control response for each experiment. Each point represents the mean response (± s.e. of the ratio) from 12 experiments.

Results

Direct effects of fenfluramine on rat blood pressure

The recording of a typical blood pressure response to fenfluramine is shown in Figure 1. Fenfluramine 5 mg/kg produced an immediate sharp fall in blood pressure (44 ± 3.6 mmHg, $n = 10$) which was accompanied by respiratory depression. Animals frequently failed to recover following doses of fenfluramine in excess of 10 mg/kg. This hypotensive crisis was followed by a recovery phase, and the blood pressure returned to pre-drug levels. A shallower, but more prolonged phase of hypotension ensued and the blood pressure remained depressed for periods up to 30 minutes. Frequently the blood pressure responses did not revert to pre-drug levels.

Pre-administration of propranolol (up to 1 mg/kg) failed to abolish or reduce the hypotensive effects of fenfluramine. Likewise, atropine (0.5 mg/kg) had no effect on fenfluramine hypotensive responses. Doses of theophylline, up to 5 mg/kg, failed to potentiate the depressor effects of fenfluramine.

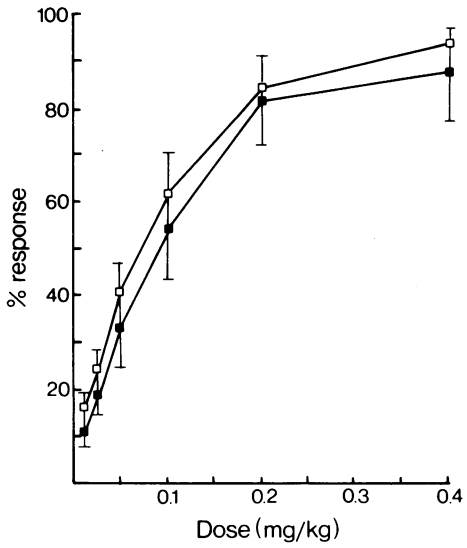

Figure 3 Dose-percentage response relationships for methoxamine before (□) and after (■) fenfluramine hydrochloride 5 mg/kg. Responses are measured as the percentage of the maximum control response for each experiment. Each point represents the mean response (± s.e. of the ratio) from 8 experiments. Differences in pressor responses before and after fenfluramine are not significant ($P > 0.5$, Student's t test).

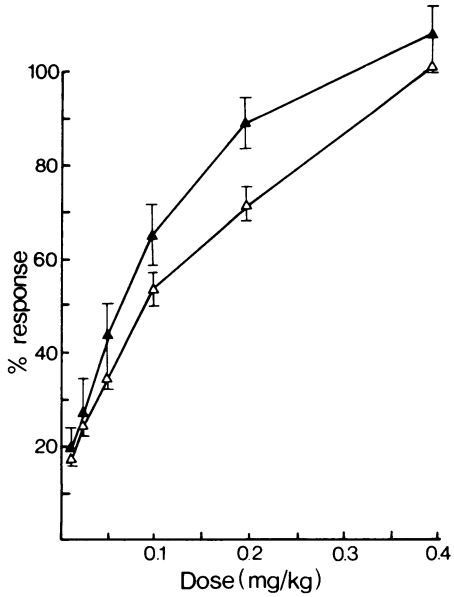

Figure 4 Dose-percentage response relationsips for metaraminol before (△) and after (▲) fenfluramine hydrochloride 5 mg/kg. Responses are measured as the percentage of the maximum control response for each experiment. Each point represents the mean response (± s.e. of the ratio) from 7 experiments. Differences in responses before and after fenfluramine are not significant ($P > 0.05$, Student's t test).

Effects of fenfluramine on blood pressure responses to sympathomimetic amines

The sympathomimetic amines, tyramine (indirectly acting), methoxamine (direct) and metaraminol (direct and indirect), were chosen for their different modes of action at adrenergic nerve terminals.

Dose-response relationships were measured for all three amines, before and after exposure of the animals to fenfluramine 5 mg/kg for one hour. Mean results for each drug were plotted graphically. Figure 2 shows that fenfluramine produced a marked reduction in the pressor responses to tyramine. At the dose level of tyramine which produced a maximum control response, fenfluramine produced a reduction in response of approximately 50%. Figure 3 shows the effect of fenfluramine on responses to methoxamine. In this case there was a slight, though not significant reduction in pressor responses following fenfluramine. The effects of fenfluramine on responses to metaraminol are shown in Figure 4. There was a slight increase in the response to the amine following fenfluramine administration. However, the difference in response is not significant for any dose level.

Effects of fenfluramine on tyramine responses in rat isolated vas deferens

Cumulative dose–response relationships to tyramine were obtained in the rat vas deferens before and after exposure to 0.2 mM fenfluramine for one hour. Fenfluramine produced an almost total abolition of tyramine responses (Table 1).

Table 1 Effects of fenfluramine (0.2 mM), for 1 h, on the cumulative dose–response relationship to tyramine in rat isolated vas deferens

Dose of tyramine (μM)	*Control response*	*Response after fenfluramine*
5.0	5.5 ± 0.4	0.0
10	13.0 ± 0.6	0.0
20	25.3 ± 1.6	1.4 ± 0.2
50	53.9 ± 5.0	3.0 ± 0.1
100	88.0 ± 3.8	3.5 ± 0.3
200	99.4 ± 0.7	4.2 ± 0.2

Results are the mean of 6 experiments, and are expressed as mean percentage response ± s.e. of the ratio for each dose of tyramine.

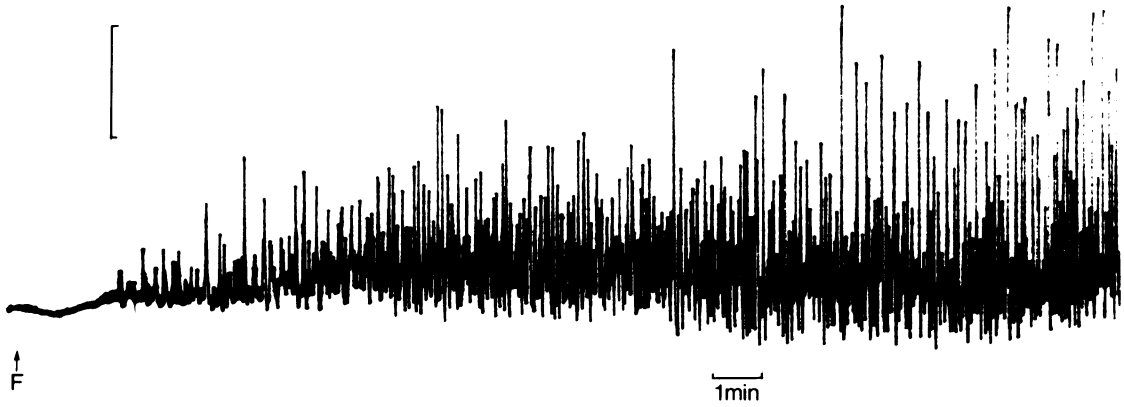

Figure 5 The response of rat isolated vas deferens to 0.2 mM fenfluramine (F). Vertical calibration 2 g, horizontal calibration 1 minute.

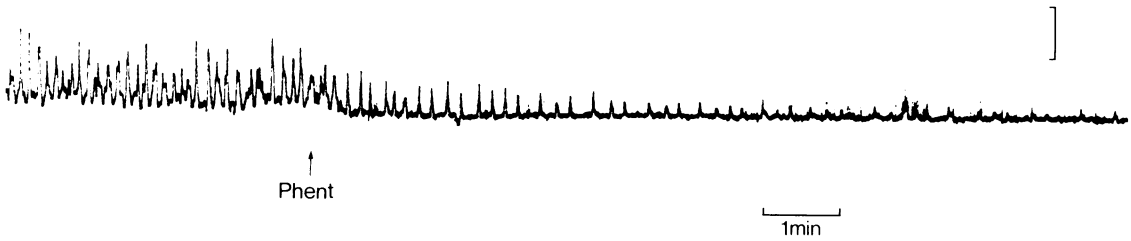

Figure 6 The reduction caused by 50 μM phentolamine (Phent), of contractions of the rat isolated vas deferens produced by 0.2 mM fenfluramine. Vertical calibration 2 g, horizontal calibration 1 minute.

During the period of exposure of the vas deferens to fenfluramine it was noted that the drug produced intermittent but frequent contractions of the tissue (Figure 5); these contractions usually started within 5 min after addition of the drug and persisted for periods in excess of one hour. The amplitude of contraction steadily increased to a maximum which, on average, was equal to the height of contraction produced by a dose of 5 μM noradrenaline.

The spontaneous contractions of vasa deferentia following fenfluramine were abolished by the addition of the α-adrenoceptor blocking agents phentolamine (0.4 mM) or thymoxamine (0.8 mM). Contractions were reduced immediately after the addition of the antagonists, though abolition of the contractions usually occurred 10–15 min after the addition of the drug (Figure 6).

Discussion

Previous work on the effects of fenfluramine on the cardiovascular system indicated that, following an initial hypotensive response, the drug produced a prolonged hypertension (Franko *et al.*, 1965; Sipes *et al.*, 1971). In the present study the initial hypotensive response was followed by a short period of recovery, but this in turn was followed by a further prolonged hypotensive response. This discrepancy may be due to a species difference as the present results were obtained on anaesthetized rats whereas Franko *et al.* and Sipes *et al.* used anaesthetized dogs and cats. If, as results seem to indicate, the more prolonged pressure changes following fenfluramine are mediated by an indirect effect, then the species difference may be due to different modes of action of fenfluramine at the adrenergic terminals of the different animals.

It seems likely that the prolonged effects of fenfluramine are due to an indirect action, but the rapid onset of the initial hypotensive response would suggest that this is due to a direct effect of the drug on a receptor system within the vascular smooth muscle. The possibility of fenfluramine exerting its effect through stimulation of either muscarinic receptors or β-adrenoceptors was therefore considered. However, both atropine and propranolol failed to reduce the degree of hypotension produced by fenfluramine.

To complete the investigation, potentiation by theophylline of the fenfluramine response was studied to establish whether or not fenfluramine had an effect on cyclic adenosine 3′,5′-monophosphate levels through an effect beyond the β-receptor. No such potentiation was seen. It is therefore concluded that fenfluramine produces a hypotensive response through a direct action which is not mediated by stimulation of known autonomic receptor sites.

The three sympathomimetic amines, methoxamine, tyramine and metaraminol, all produced dose-dependent rises in blood pressure through their respective direct, indirect and mixed sympathomimetic modes of action. These responses were affected to a greater or lesser degree by the exposure of the animals to 5 mg/kg fenfluramine for one hour.

The greatest effect was seen with the indirectly acting tyramine and the least affected amine was the directly acting methoxamine. These results suggest that fenfluramine exerts an effect presynaptically at the adrenergic nerve terminal. Biochemical studies (Sipes *et al.*, 1971) on tissue noradrenaline concentrations, following fenfluramine, indicated that the drug releases or displaces noradrenaline from one or more of its neuronal storage sites. The results of the present study lend weight to this theory, since fenfluramine reduced the responses to tyramine by 50% whilst the responses to methoxamine remained unaltered by the drug. It might have been expected that metaraminol, a sympathomimetic amine with both direct and indirect actions, would have been affected to an intermediate degree by fenfluramine. However, the results showed that responses to metaraminol following fenfluramine were increased, though not significantly, over control levels. The possibility that fenfluramine may act as an amine uptake inhibitor at the adrenergic nerve terminal must therefore not be overlooked. This would account for the marked reduction in responses to tyramine and the slight potentiation of responses to metaraminol, since more amine would be available for its direct sympathomimetic effect.

The suggestion that fenfluramine exerts an effect via the sympathetic nerve terminal was confirmed by results on rat isolated vasa deferentia. Fenfluramine produced contractions in the vas deferens, but it seems unlikely that these responses were mediated via a direct effect on fenfluramine-sensitive receptor sites, for a number of reasons. Contractions were not produced immediately following the addition of the drug, as was seen with most agonist–receptor interactions, but periods of up to 5 min elapsed before an effect was seen. The responses themselves were dissimilar to those normally seen following the addition of an agonist being of a phasic appearance and of irregular height indicative of an indirect, packaged release of a transmitter substance. The prolonged period of contractions produced by fenfluramine again suggests, not a direct effect but either an indirect effect or a sustained release of the drug from a storage site. Finally the contractions produced by fenfluramine were abolished by the addition of an α-adrenoceptor blocking agent (phentolamine or thymoxamine).

In conclusion, the results of the present study indicate that fenfluramine produces a prolonged hypotensive response in the anaesthetized rat and 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. However, the effects of the drug on adrenergic terminals and its cardiovascular effects are not necessarily related.

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