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Short communication

Influence of fenfluramine and norfenfluramine stereoisomers on the firing rate of central monoaminergic neurons in the rat

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The influence of acute administration of stereoisomers of fenfluramine and norfenfluramine on the firing rate of central monoaminergic neurons was investigated in rats anaesthetized with chloral hydrate. The firing rate of dorsal raphe (DR) and locus coeruleus (LC) neurons was inhibited. The parent drugs were more active on DR neurons than on LC neurons, and the converse was true for the demethylated metabolites. In both cases the d isomers were more active than the l isomers. No effect was observed on the electrical activity of A10 dopaminergic neurons. These differences in potency and selectivity could have therapeutic implications.

Fenfluramine; Dorsal raphe; Locus coeruleus; A10 neurons

1. Introduction

The anorectic drug, fenfluramine (N-ethyl-α-methyl-m-(trifluoromethyl)phenethylamine), has been used for many years in the treatment of obesity and recent studies have shown that it could also have beneficial effects on infantile autism (Campbell, 1987). The mechanism of action of fenfluramine is currently the subject of discussion.

Monoaminergic neurons seem to play an important role not only in the control of food intake but also in the physiopathology of autism (Young et al., 1987) and fenfluramine has multiple effects on the function of these neurons. Fenfluramine is a racemic mixture of two isomers, d- and l-fenfluramine, which act differently on central serotonergic and catecholaminergic systems.

d-Fenfluramine is more potent than l-fenfluramine in increasing serotonin (5-HT) release and inhibiting its re-uptake, two effects which are important for its anorectic activity (Garattini and Samanin, 1976; Garattini et al., 1979).

Conversely, l-fenfluramine is reported to be more potent than d-fenfluramine in modifying the levels, metabolism and synthesis of catecholamines in the brain (Invernizzi et al., 1986). However, the functional significance of the effects of fenfluramine on noradrenaline (NA) and dopamine (DA) turnover is not clear and may be complicated by the drugs transformation into the active metabolite, norfenfluramine, which is also a racemic mixture of two isomers.

In order to better understand the influence of fenfluramine and its metabolite on monoaminergic neurons, we studied the effect of these drugs on the firing rate of these neurons. We therefore used electrophysiological techniques to investigate the influence of fenfluramine and norfenfluramine stereoisomers on the electrical activity of dorsal raphe (DR) serotonergic neurons,

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locus coeruleus (LC) noradrenergic neurons and A10 dopaminergic neurons in vivo.

2. Materials and methods

2.1. Electrode implantation and recording

Male Wistar rats (200-300 g) were anaesthetized with chloral hydrate (400 mg/kg i.p.) and mounted in a stereotaxic apparatus. Their temperature was maintained at 36-37 °C by means of a heating pad. A small bone flap was removed above the implantation point and the venous sinus was tied and cut. Electrical activity was recorded by means of extracellular glass micropipettes filled with NaCl (2 M) solution saturated with fast green. The electrodes were implanted at the following coordinates: DR 0.0-0.5 mm anterior, 0.0 mm lateral to lambda, 4.5-5.5 ventral to cortex; LC 1.7-2.2 mm posterior, 1.1-1.3 mm lateral to lambda, 5.5-6.5 mm ventral to cortex; A10 1.8-2.2 mm anterior, 0.4-0.6 mm lateral to lambda, 7.5-8.5 mm ventral to cortex. Spikes were passed through an impedance adapter and an amplifier into a Tektronix oscilloscope (5103N). Signals were also passed into an amplitude discriminator and a digital counter. At the end on the experiment, a spot of fast green was deposited at the tip of the electrode. The animal was perfused with a solution of 4% formaldehyde and the brain was removed for histological control of the position of the electrode.

2.2. Identification of the cells

DR neurons were characterized by a regular firing rate and a low frequency of discharge of 0.2-2 spikes/s (Aghajanian et al., 1978). LC neurons were characterized by a stable firing rate of 0.5-5 spikes/s and a typical response to noxious stimuli (Korf et al., 1974). A10 neurons were characterized by bursts of spikes of decreasing amplitude and a frequency of discharge of 1-9 spikes/s (Bunney et al., 1973).

2.3. Study of drug effect

The electrical activity of the cell was recorded during a control period of a few minutes and the mean number of spikes/10 s was calculated. The drug studied was then perfused into the jugular vein by means of a perfusion pump (flow 6 ml/h). When inhibition was observed, the mean total dose required to produce a 50% effect (ID_{50}) was calculated by multiplying the dose perfused (in mg kg^{-1} s^{-1}) by the time (in s) required to obtain 50% inhibition. In view of the rapid start of the drug effect, the lag between the beginning of the perfusion and the beginning of drug effect was not taken into consideration. At least six experiments were performed for every drug and every region studied. Only one cell was studied in each animal. Fenfluramine and norfenfluramine isomers (Servier) were dissolved in saline. Doses refer to the bases. Statistical analysis of the results was done with Student's t-test.

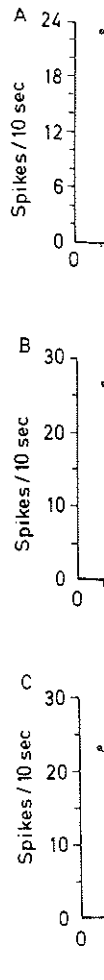
3. Results

3.1. d-Fenfluramine

The i.v. perfusion of d-fenfluramine at doses of 0.2-0.5 mg kg^{-1} min^{-1} decreased the electrical activity of DR neurons in a dose-dependent manner (fig. 1A). Similarly, d-fenfluramine (0.5 mg kg^{-1} min^{-1}) decreased the frequency of discharge of LC neurons (fig. 1B) but did not modify the activity of A10 neurons (highest cumulative dose tested: 5 mg kg^{-1}) (fig. 1C). Apomorphine was used in some experiments to pharmacologically test the dopaminergic nature of the recorded cell. d-Fenfluramine did not modify the inhibitory effect of apomorphine. The inhibitory effect of d-fenfluramine on DR and LC neurons appeared to be reversible as partial recovery of the activity was observed in the minutes following the end of the perfusion.

3.2. d-Norfenfluramine

d-Norfenfluramine (0.1 mg kg^{-1} min^{-1}) reduced the electrical activity of 6 out of 8 DR



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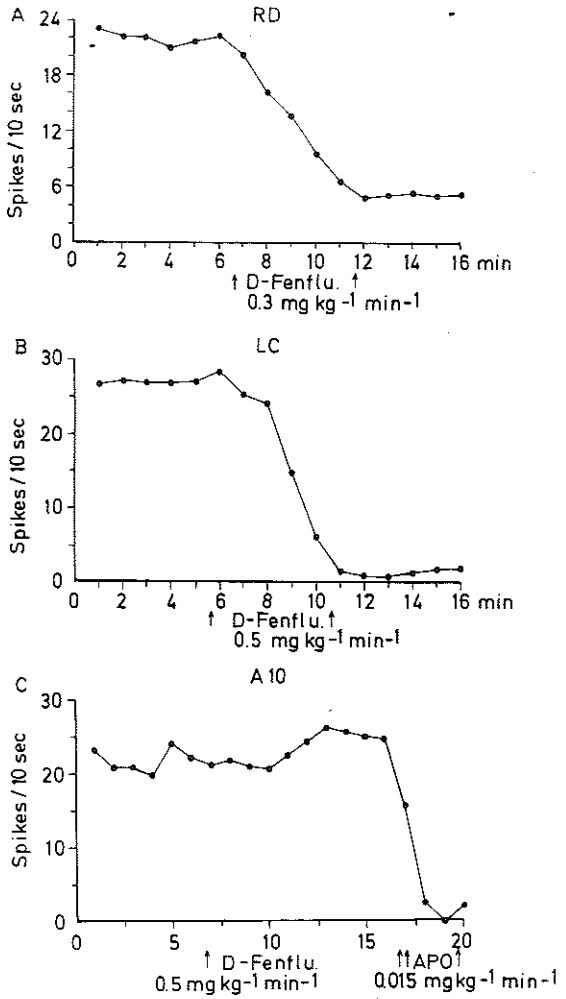

Fig. 1. Effect of an i.v. perfusion of d-fenfluramine (D-fenflu.) on the electrical activity of (A) a dorsal raphe (DR) serotonergic neuron, (B) a locus coeruleus (LC) noradrenergic neuron, (C) an A10 dopaminergic neuron, the dopaminergic nature of which was confirmed by the inhibitory effect of apomorphine (APO). The mean number of spikes/10 s calculated every minute is represented against time.

neurons. It also decreased the frequency of discharge of LC neurons but did not modify the activity of A10 neurons. The highest cumulative dose which could be tested was 1.1 mg kg^{-1} because the animals had difficulties with respiration when perfused with higher doses. d-Norfenfluramine did not modify the inhibitory effect of apomorphine used as pharmacological control. The inhibitory effect of d-norfenfluramine on DR and LC neurons also appeared to be reversible.

3.3. l-Fenfluramine

l-Fenfluramine (0.5 mg kg^{-1} min^{-1}) reduced the frequency of discharge of 6 out of 9 DR neurons and the effect appeared to be reversible. It had no effect on the firing rate of LC neurons (highest cumulative dose studied: 9 mg/kg^{-1}). l-Fenfluramine did not modify the electrical activity of A10 neurons (highest dose studied: 5 mg kg^{-1}) or the inhibitory effect of apomorphine.

3.4. l-Norfenfluramine

l-Norfenfluramine (0.2 mg kg^{-1} min^{-1}) decreased the frequency of discharge of 6 out of 7 DR neurons and the effect appeared to be reversible. At the same dose it also decreased the electrical activity of LC neurons but complete inhibition could not be obtained in the majority of the cells studied (6 out of 7): the firing rate stabilized at a level lower than the control. The firing rate of A10 neurons was not modified (highest cumulative dose tested: 5.5 mg kg^{-1}) nor was the inhibitory effect of apomorphine. The inhibitory potencies of the stereoisomers of fenfluramine and norfenfluramine on the firing rate of DR and LR neurons are shown in table 1.

TABLE 1

Inhibitory potencies of fenfluramine and norfenfluramine stereoisomers on the firing rate of dorsal raphe (DR) and locus coeruleus (LC) neurons. ID_{50}: mean total dose required to produce a 50% inhibition of the frequency of discharge; n: number of experiments.

	ID_{50}, mg/kg (±S.E.)			
	DR	n	LC	n
d-Fenfluramine	0.86 ± 0.12 [a]	9	1.95 ± 0.06	7
d-Norfenfluramine	0.58 ± 0.16	6	0.30 ± 0.06 [c]	6
l-Fenfluramine	2.17 ± 0.36	6	No effect	6
l-Norfenfluramine	1.07 ± 0.16 [b]	8	0.49 ± 0.14	6

Student's t-test: [a] Significantly different from d-fenfluramine on LC ($P < 0.001$), l-fenfluramine on DR ($P < 0.005$). [b] Significantly different from l-fenfluramine on DR ($P < 0.05$), l-norfenfluramine on LC ($P < 0.05$). [c] Significantly different from d-fenfluramine on LC ($P < 0.001$).

4. Discussion

These electrophysiological studies in vivo showed that d- and l-fenfluramine had different effects on the electrical activity of monoaminergic neurons in the rat when administered acutely. Both isomers decreased the firing rate of DR serotonergic neurons but d-fenfluramine was 2.5 times more potent than l-fenfluramine. The electrophysiological data are thus in agreement with behavioural and biochemical studies showing that serotonergic mechanisms are preferentially modified by the d isomer (Invernizzi et al., 1986). It may be assumed that the mechanisms involved in inhibition of the firing are related to the well-known effects of the drug on the uptake and release of 5-HT. d-Fenfluramine also decreased the electrical activity of LC noradrenergic neurons, but it was significantly less potent in C neurons than on DR neurons. This inhibitory effect may be related to enhancement of NA release (Mennini et al., 1981). l-Fenfluramine (even at high doses) did not modify the firing rate of LC neurons.

The two metabolites, d- and l-norfenfluramine, were generally more potent than the parent drugs. Thus d-norfenfluramine was 6.5 times more potent than d-fenfluramine on LC neurons and l-norfenfluramine was about twice as potent as l-fenfluramine in inhibiting DR neurons. Contrary to l-fenfluramine, which did not modify the firing rate of LC neurons, l-norfenfluramine had a partial inhibitory effect on these cells.

Interestingly, none of the compounds tested had any influence on the firing rate of A10 neurons despite the fact that the compounds are reported to modify brain dopamine metabolism (Jori et al., 1973). This shows that a discrepancy may exist between the effect of drugs on transmitter metabolism and on neuronal activity.

In summary, the stereoisomers of fenfluramine and norfenfluramine affected the firing rate of central serotonergic and noradrenergic neurons with different potency and selectivity. Two general conclusions may be drawn: (1) the parent drugs are more active on serotonergic neurons than on noradrenergic neurons and demethylation reverses this characteristic; (2) the d isomers are more potent than the l isomers.

The relative importance of the effects of these isomers in determining their therapeutic properties remains to be elucidated. In view of these results, and taking into account metabolic processes, it may be assumed that the preferential use of d- or l-fenfluramine could modify the therapeutic profile of the drug but a selective action on one type of monoaminergic neuron would not be achieved.

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