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Metabolism of fenfluramine to norfenfluramine in guinea-pigs

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Abstract—After injection of fenfluramine into guinea-pigs, the *N*-dealkylated metabolite norfenfluramine was present in brain at higher concentrations and persisted longer than the parent drug, fenfluramine. Contrary to a claim in previous literature, the guinea-pig does metabolize fenfluramine to norfenfluramine, hence the ability of fenfluramine to cause acute and long-term depletion of brain 5-hydroxytryptamine in this species does not prove that fenfluramine, instead of nonfenfluramine, can produce these effects.

Fenfluramine is an appetite suppressant drug with chemical and pharmacological similarities to *p*-chloroamphetamine. Both are halogenated analogues of amphetamine, and both suppress food intake by releasing 5-hydroxytryptamine (5-HT) (Clineschmidt et al 1974; Garattini et al 1975). In rats, fenfluramine and *p*-chloroamphetamine not only deplete 5-HT acutely due to release of intraneuronal stores, but they also have long-term neurotoxic effects on 5-HT neurons leading to persistent loss of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), tryptophan hydroxylase, and 5-HT uptake capacity, all parameters associated specifically with 5-HT neurons in brain (Clineschmidt et al 1978; Sanders-Bush & Steranka 1978; Harvey et al 1977). *p*-Chloroamphetamine (an *N*-alkyl analogue of *p*-chloroamphetamine) and norfenfluramine (the *N*-desalkyl analogue of fenfluramine), produce the same effects on brain 5-HT neurons, and it is not established to what extent the primary amine metabolites versus the *N*-alkyl amines are responsible for these effects when the latter drugs are administered.

Schuster et al (1986) stated that fenfluramine "is not changed into an active metabolite" in guinea-pigs and concluded after showing that fenfluramine caused long-term depletion of brain 5-HT in guinea-pigs that metabolism to norfenfluramine is not required in order for fenfluramine to produce long-term depletion of brain 5-HT. Their belief that fenfluramine was not metabolized to norfenfluramine in guinea-pigs was based on a paper by Beckett et al (1973), who reported that the nitrone was the major metabolite when fenfluramine was incubated with guinea-pig liver microsomes in-vitro but did not study fenfluramine metabolism in-vivo. No data on whether fenfluramine is metabolized to norfenfluramine in guinea-pigs in-vivo appear to have been published. However, a related compound, methamphetamine, is extensively metabolized by *N*-dealkylation in guinea-pigs (Caldwell et al 1972), so it would be surprising if fenfluramine were not *N*-dealkylated in this species as it is in others. To investigate this issue, we treated guinea-pigs with fenfluramine and measured brain concentrations of parent drug and of norfenfluramine along with 5-hydroxyindoles, whose concentrations are influenced by fenfluramine.

Methods

Male Hartley guinea-pigs, 155–190 g, from Charles River, Portage, MI, were given (±)-fenfluramine hydrochloride (A. H. Robins, Richmond, VA), 12·5 mg kg^{-1} i.p. Groups of 5 guinea-pigs were killed at 1, 2 or 4 h after fenfluramine injection. Whole brains from treated and untreated guinea-pigs were quickly removed and frozen on dry ice, then stored at −15°C until analysis. (±)-Norfenfluramine hydrochloride was synthesized in the Lilly Research Laboratories, Indianapolis, IN. Fenfluramine and norfenfluramine concentrations in brain were measured by high performance liquid chromatography with ultraviolet detection. Tissue was homogenized in 5 volumes of 0·1 M trichloroacetic acid. The supernatant fluid after centrifugation was passed through a Bond-Elute C18 column to concentrate the amines, which were eluted by the mobile phase buffer used in the subsequent chromatographic step; the buffer was 0·01 M Na_2HPO_4 adjusted to pH 3 with H_3PO_3 containing 30% acetonitrile and 1·5 mL triethylamine per litre. A Rainin Microsorb 3 μ C18 column (4·6 × 100 mm) was used with a flow rate of 1·5 mL min^{-1} at 40°C. An ISCO absorbance monitor Model 1840 was used to measure absorbance at 263 mm with 0·01 absorbance units full scale. In these chromatographic conditions, the retention times were 163 s for norfenfluramine and 249 s for fenfluramine. Norfenfluramine concentration in brain was also measured by reaction with fluorescamine, a reagent which forms a fluorophore with primary amines but does not react with fenfluramine (Udenfriend et al 1972; Fuller et al 1974). 5-HT and 5-HIAA were measured by liquid chromatography with electrochemical detection (Fuller et al 1987).

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Results

Fig. 1 shows that norfenfluramine was present in guinea-pig brain at concentrations already higher than those of parent drug at 1 h after fenfluramine injection and that norfenfluramine persisted after 4 h when fenfluramine levels had declined to approximately the limits of detection of the method. The

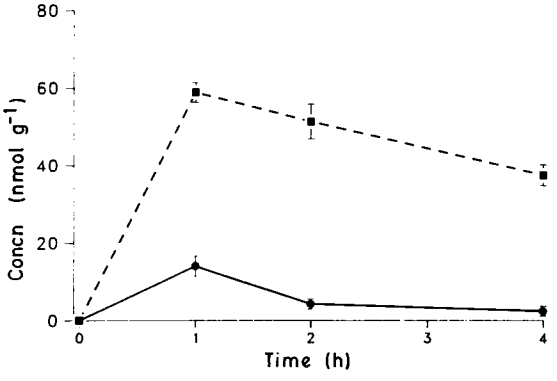

FIG. 1. Norfenfluramine (■) and fenfluramine (●) concentrations in guinea-pig brain after injection of fenfluramine HCl (12·5 mg kg^{-1} i.p.). Mean values ± standard errors for 5 guinea-pigs per group are shown.

concentrations of norfenfluramine and fenfluramine shown in Fig. 1 were measured by liquid chromatography. Norfenfluramine concentrations measured by the fluorescamine method agreed well with those shown in Fig. 1. The fluorescamine method gave values of 53·6 ± 3·7, 52·0 ± 3·4 and 32·0 ± 2·2 nmol of norfenfluramine per g (wet weight) of brain tissue at 1, 2 and

4 h after fenfluramine injection. Fig. 2 shows that 5-HT and 5-HIAA concentrations were decreased by this dose of fenfluramine during this time period.

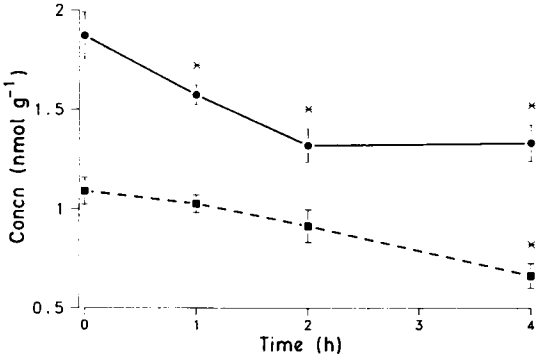

FIG. 2. 5-HT (●) and 5-HIAA (■) concentrations in guinea-pig brain after injection of fenfluramine HCl (12·5 mg kg^{-1} i.p.). Mean values ± standard errors for 5 guinea-pigs per group are shown. Asterisks indicate significant differences from control group (Student *t*-test, $P < 0.05$).

Discussion

These results reveal that fenfluramine is metabolized by *N*-dealkylation in guinea-pigs just as it is in rats (Morgan et al 1972). In fact, brain concentrations of norfenfluramine were much higher than those of fenfluramine even within 1 h after fenfluramine administration to guinea-pigs. Thus the conclusion by Schuster et al (1986) that norfenfluramine formation is not required for long-term depletion of brain by fenfluramine is unfounded, and the question of whether fenfluramine would deplete 5-HT if it were not metabolized to norfenfluramine remains unanswered. Schuster et al (1986) had cited Garattini et al (1975) as also providing evidence that fenfluramine does not have to be metabolized to norfenfluramine to produce long-term depletion of 5-HT in brain. However, Garattini et al (1975) studied only the acute depletion of brain 5-HT by fenfluramine (4 h), not the long-term neurotoxic effect. Furthermore, they did not provide evidence that SKF 525-A, given to inhibit microsomal *N*-dealkylation, actually prevented norfenfluramine formation in rats. In our own studies on the possible involvement of *N*-dealkylation in the 5-HT-depleting effects of halogenated amphetamines, we were unable to block *N*-dealkylation with microsomal inhibitors to a sufficient extent to ascertain whether *p*-chloroamphetamine formation is necessary for the effect after administration of *N*-alkyl-*p*-chloroamphetamines (Fuller & Baker 1977). There appear to be no data showing 5-HT depletion without the presence of norfenfluramine in brain after fenfluramine administration to any species. Because norfenfluramine levels are so much higher than those of fenfluramine in guinea-pig brain (Fig. 1), the 5-HT depletion probably was caused mainly by norfenfluramine: it is not known whether fenfluramine could produce the same effect if it were not *N*-dealkylated.

Our findings of much higher concentrations of norfenfluramine than of fenfluramine even at early times in guinea-pig brain suggested that *N*-dealkylation of fenfluramine is even more rapid in guinea-pigs than in rats, where brain levels of norfenfluramine at early times after fenfluramine administration were lower than those of fenfluramine (Clineschmidt et al 1978; Mennini et al 1985). In our own experiments, (+)-fenfluramine concentration in brain was 142 ± 11 nmol g^{-1} at 2 h after injection of (+)-fenfluramine (15 mg kg^{-1} i.p.) into rats, compared with a (+)-norfenfluramine concentration of only 30 ± 3 nmol g^{-1}. The faster *N*-dealkylation of fenfluramine in guinea-pigs is not surprising, as Smith & Dring (1970) had reported that *N*-dealkylation of other *N*-alkylamphetamines was more extensive in species such as rabbit, guinea-pig and dog than in man or rat.

(±)-Fenfluramine was used in the present study because Schuster et al (1986) had used the racemic form in their experiments. Our analytical methods do not discriminate enantiomeric forms of norfenfluramine, so our data do not reveal whether the enantiomers of fenfluramine were *N*-dealkylated at different rates. Rats and mice *N*-dealkylate fenfluramine enantiomers at slightly different rates, but humans and dogs metabolize the enantiomers at nearly equal rates (Caccia et al 1982). Since (+)- and (−)-norfenfluramine both are more potent 5-HT releasers than either enantiomer of the parent drug (Mennini et al 1985), the presence of norfenfluramine in either enantiomeric form in guinea-pig precludes the interpretations made by Schuster et al (1986). For investigation of the question they posed ("Would fenfluramine itself cause long-term depletion of brain 5-HT if norfenfluramine formation did not occur?"), the guinea-pig seems to be the least suitable species. Based on the data of Caccia et al (1982) and our current data, the lowest ratios of norfenfluramine: fenfluramine in brain among laboratory animals studied is in the mouse (compared with the rat, dog or guinea-pig). Perhaps the already limited *N*-dealkylation could most readily be inhibited in the mouse.

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Excitatory P_1-purinoceptors on pre- and post-ganglionic cholinergic nerve terminals in the chick oesophagus

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Abstract—The mechanism of action of ATP and the purinoceptors involved have been investigated on the chick oesophagus. The susceptibility of the excitatory responses to ATP/adenosine to tetrodotoxin indicates that their action is neurally mediated. Blockade of ATP/adenosine responses by atropine suggests the involvement of endogenous acetylcholine. ATP action depends on breakdown to AMP/adenosine, since theophylline blocks ATP/adenosine responses. The inhibition of ATP responses by pentolinium implies the involvement of preganglionic fibres.

Adenosine and adenine nucleotides have been reported to inhibit the release of transmitters from both adrenergic and cholinergic nerve fibres (Hayashi et al 1978; Su 1978; Gustafsson et al 1981; Moody & Burnstock 1982). However, ATP, a purine nucleotide, has been observed to produce contraction of the chick oesophagus through a cholinergic mechanism (Bartlet 1974). The present investigation was undertaken to determine the site(s) of action of ATP and the nature of purinoceptors involved in mediating its response in the chick oesophagus.

Materials and methods

Pre-crop oesophagus, obtained from White Leghorn chicks (1–2 wks) of either sex, was used as described by Mishra & Raviprakash (1980). The oesophagus was stimulated electrically by transmural stimulation (TMS) as described by Paton (1955). The stimulation consisted of monophasic square-wave pulses at a frequency of 1 Hz of 0·5 ms duration delivered at a supramaximal voltage of 5 V for 15 s. The distance between the plantinum wire electrodes placed within and outside the lumen was 8 mm. An interval of 10 min was maintained between two successive doses of drugs/stimuli. The tissues were exposed to an antagonist for 15–30 min before the responses to agonists/stimuli were elicited.

The drugs used were ATP, adenosine, acetylcholine chloride (ACh), theophylline bromide, quinidine sulphate, atropine sulphate, pentolinium tartrate and tetrodotoxin (TTX).

Results

ATP ($3{\cdot}61 \times 10^{-6}$M) and adenosine ($2{\cdot}99 \times 10^{-6}$M) produced contractions of the chick oesophagus and the responses were consistent when the agents were administered at an interval of 10 min. Similarly, ACh ($5{\cdot}5 \times 10^{-7}$M) and TMS induced contractions on this tissue. TTX ($1{\cdot}57 \times 10^{-7}$M) abolished the responses induced by ATP, adenosine and TMS, without significantly affecting those to ACh (Fig 1A). Atropine ($1{\cdot}44 \times 10^{-7}$M) markedly inhibited the responses to ATP, adenosine, ACh and TMS (Figs 1B, 2B). Theophylline ($3{\cdot}86 \times 10^{-5}$M) produced a significant inhibition of the contractions induced by ATP and adenosine without significantly affecting the responses to ACh or TMS (Figs 1C, 2A). Quinidine ($1{\cdot}07 \times 10^{-5}$M) did not alter the responses of ATP, ACh or TMS (not shown). Pentolinium ($7{\cdot}42 \times 10^{-6}$M) significantly inhibited (by about 75 per cent) the ATP response without affecting those of ACh or TMS (Fig. 1D).

Discussion

Since ATP breaks down to AMP/adenosine in the tissues, the present study was conducted using both ATP and adenosine. The ATP/adenosine-induced excitatory responses on the chick oesophagus are purely neurogenic since these responses are abolished by TTX. This, therefore, indicates that purine nucleotides (ATP/adenosine) stimulate impulse propagation in nerves associated with the chick oesophagus. Furthermore, marked inhibition of these responses by atropine implies participation of a cholinergic mechanism. Since the purine agonists are equipotent on a molar basis (adenosine, $1{\cdot}84 \times 10^{-4}$M = AMP, $1{\cdot}84 \times 10^{-4}$M = ADP, $1{\cdot}87 \times 10^{-4}$M and ATP $1{\cdot}97 \times 10^{-4}$M: Bartlet 1974) on the chick oesophagus, it appears that the neuromodulatory role of ATP could be attributed to its hydrolysed products AMP or adenosine which act on the prejunctional P_1-purinoceptors as suggested by De Mey et al (1979) and Moody & Burnstock (1982). This is further substantiated by the fact that theophylline, a selective P_1-purinoceptor antagonist (Burnstock & Meghji 1981) significantly inhibited its responses.

Since both pre- and post-ganglionic cholinergic fibres are located in the myenteric plexus, it was of interest to see whether P_1-purinoceptors are located in preganglionic fibres besides as well as in post-ganglionic cholinergic nerve terminals. Marked inhibition of ATP responses by pentolinium, a ganglionic nicotinic receptor blocker, indicates that the breakdown products of ATP (AMP or adenosine) might be diffusing to the ganglia to interact with P_1-purinoceptors located on the preganglionic cholinergic fibres to release acetylcholine which subsequently activates the post-ganglionic fibres. However, a direct action of these break-down products of ATP on post-ganglionic nerve fibres is still evident since responses appear even

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