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Anorectic activity of fluoxetine and norfluoxetine in mice, rats and guinea-pigs

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Abstract—The present study aimed to establish the role of the metabolite norfluoxetine in the anorectic activity of fluoxetine, and to relate the anorectic doses (ED50) to the brain concentrations of the parent drug and its metabolite. Fluoxetine showed anorectic activity at increasing intraperitoneal doses (ED50 = 39·1, 34·7 and 21·7 μmol kg^{-1} in mouse, rat and guinea-pig, respectively) and norfluoxetine was slightly more active (24·3, 22·9 and 19·1 μmol kg^{-1}, respectively) in all three species. In terms of maximum concentration (C_{max}) and area under the curve (AUC) within the experimental period (0–90 min), brain concentrations varied widely and were poorly related to the dose; guinea-pig appeared to be much more sensitive to fluoxetine than was mouse or rat. Administered norfluoxetine was present in the brain of the three species in approximately the same order as fluoxetine, i.e. lower in guinea-pig than in mouse or rat. The C_{max} and AUC of norfluoxetine after fluoxetine administration was 50–60% of the values after an equiactive dose of norfluoxetine in mouse and guinea-pig, and more than 80% in rat.

Fluoxetine is an antidepressant agent (Benfield et al 1986; Bergstrom et al 1988), which has recently been proposed for the treatment of obesity (Ferguson & Feighner 1987; Freeman 1988). Its anorectic effect is believed to result from enhanced 5-HT-ergic transmission, through inhibition of 5-HT presynaptic reuptake (Goudie et al 1976; Wong & Fuller 1987; Wong et al 1988). Recent studies (Gobbi et al 1992) have shown that fluoxetine can enhance 5-HT release, in-vitro, through a mechanism different from that of (+)-fenfluramine, an established anorectic agent (Nathan & Rolland 1987; Samanin & Garattini 1990). However, doubt is cast on the importance of 5-HT in the action of fluoxetine by the observation that 5-HT antagonists do not affect the anorexia induced by fluoxetine in food-deprived rats (Wong et al 1988; Garattini et al 1991).

Fluoxetine is extensively dealkylated to norfluoxetine in-vivo in man and animals (Parli & Hicks 1974; Bergstrom et al 1988; Caccia et al 1990); this metabolite also inhibits 5-HT uptake although to a lesser extent than the parent compound. However, in rats, norfluoxetine is slightly more active than fluoxetine in reducing food intake and probably plays a role in the parent drug's anorectic activity (Caccia et al 1992).

Information on the pharmacological activity of norfluoxetine in other animal species is still lacking, hence our interest in comparative studies in various species to determine the differences and similarities in the pharmacological behaviour of the drug and its active metabolite.

Materials and methods

Animals and drug administration. Male CD1 albino mice, 20–25 g, male CD-COBS rats, 175–200 g, and male albino guinea-pigs, 250–300 g (Charles River, Italy), were housed at constant temperature (20–24°C) and relative humidity (60 ± 5% for mice and rats; 50 ± 5% for guinea-pigs) with a 12-h light/dark cycle, and acclimatized to the research facility for one week before the study.

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Animals were trained to eat their daily ration in 4 h (1000–1400 h). On the day of the experiment drug or metabolite was injected intraperitoneally and after 30 min food was made available. The amount of food eaten during the next 60 min was measured. Fluoxetine hydrochloride and norfluoxetine maleate (E. Lilly, Florence, Italy) were dissolved in saline and gum arabic, respectively, for injection.

The ED50 was calculated from five dose levels, with five animals per group at each dose, according to the method of De Lean et al (1978). In a second experiment, fluoxetine and norfluoxetine were given to groups of animals at a dose corresponding to the anorectic ED50 and animals were killed by decapitation at various times thereafter for measurements of drug and metabolite concentrations in brain.

Fluoxetine and norfluoxetine were extracted from brain homogenates with benzene, after adding nomifensine as internal standard, derivatized with heptafluorobutyric anhydride solution and analysed by electron capture gas-liquid chromatography (Caccia et al 1990).

Over the sampling interval the area under the concentration-time curve (AUC) was determined by the trapezoidal rule. The maximum concentration (C_{max}) and the time of its occurrence (t_{max}) were read directly from the concentration-time data for both compounds.

Results

The results of these experiments are summarized in Fig. 1 and Table 1. Fig. 2 illustrates the time course of fluoxetine and norfluoxetine in the brain of animals following administration of drug.

Discussion

In this study in mouse, rat and guinea-pig, we have established the relative potencies of fluoxetine and norfluoxetine on food intake of food-deprived animals and related the anorectic doses (ED50) to the brain concentrations of the parent drug and its active metabolite. The ED50 calculated for intraperitoneal doses of fluoxetine given to overnight-fasted animals trained to take their food during a period of 4 h, and measured food consumption within 1 h indicated that, within the limits of the experimental procedure, mice, rats and guinea-pigs are almost equally sensitive to fluoxetine. The same was true for norfluoxetine.

The anorectic effect of fluoxetine may be related to the brain availability of the unchanged compound or its active metabolite, taking into account the values within the interval over which the anorectic activity was measured. Previous studies have established that, at least in the rat, fluoxetine and norfluoxetine are distributed almost evenly in discrete brain areas (Benfield et al 1986); similarly the subcellular distribution of the two compounds is not different, about 40% of both compounds being available in synaptosomes and mitochondria and in the nuclei fractions, and 10% in microsomes and soluble fractions (Caccia et al 1990).

The brain concentrations required to obtain an anorectic

Table 1. Effects of fluoxetine and *norfluoxetine* on food intake, maximum brain concentrations and area under the concentration-time curve.

Species	Compound	Anorectic ED50 (μmol kg^{-1} i.p. ± 95% fiducial limits)	Brain C_{max} (nmol g^{-1})[a]		AUC (nmol g^{-1} h)[b]	
			Fluoxetine	Norfluoxetine	Fluoxetine	Norfluoxetine
Mouse	Fluoxetine	39·1 (±8·4)	34·5 (6·7)	21·8 (1·8)	37·7	14·6
	Norfluoxetine	24·3 (±12·9)	—	30·8 (1·0)	—	30·9
	% contribution[c]			70%		47%
Rat	Fluoxetine	34·7 (±14·9)	48·7 (6·9)	27·3 (4·7)	50·3	19·7
	Norfluoxetine	22·9 (±6·3)	—	21·7 (6·5)	—	24·1
	% contribution[c]			125%		82%
Guinea-pig	Fluoxetine	21·7 (±17·3)	5·0 (1·5)	4·0 (1·9)	4·8	3·4
	Norfluoxetine	19·1 (±12·9)	—	7·0 (2·6)	—	5·6
	% contribution[c]			53%		61%

[a] Observed values (mean with s.d.; n = 5); [b] mean area under the curve (calculated only up to 90 min); [c] value obtained for administration as a percentage of the same parameter following administration of an equiactive dose of fluoxetine.

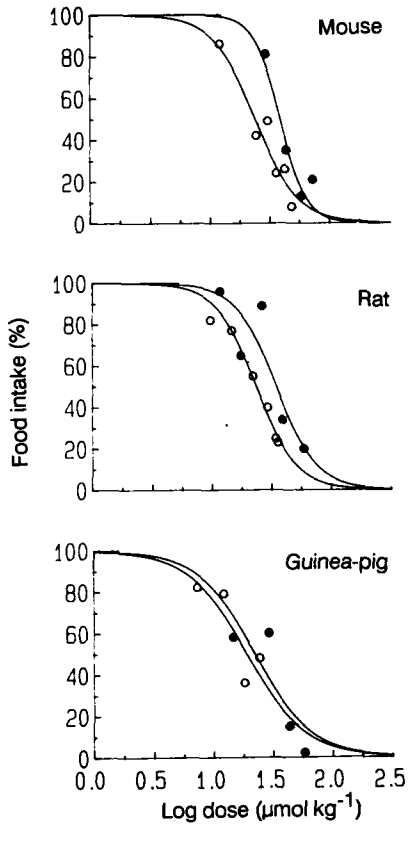

FIG. 1. Food intake (for 1 h), 30 min following intraperitoneal injection of fluoxetine (●) or norfluoxetine (○) to mice, rats and guinea-pigs.

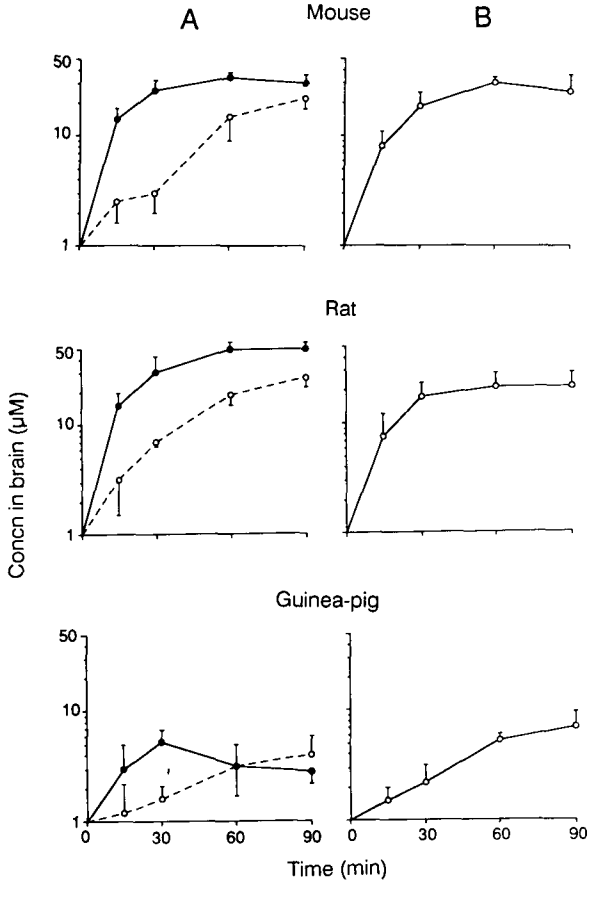

FIG. 2. Mean brain concentration-time curves of fluoxetine (●) and its metabolite norfluoxetine (○) after equiactive anorectic doses of fluoxetine hydrochloride (A) or norfluoxetine maleate (B). Each value is the mean ± s.d., n = 4–5.

ED50 after either drug, as measured by C_{max} and AUC, varied widely and were poorly correlated to the dose; in relation to the brain concentrations of the parent compound the guinea-pig appeared to be much more sensitive to fluoxetine than were either the mouse or the rat. Norfluoxetine, after its administration, was present in the brain of the three species in the same order as fluoxetine. It also appeared that the active metabolite plays an important role in the pharmacological action of the parent drug in all three species, the C_{max} and AUC values for norfluoxetine being comparable following equiactive doses of the two compounds. In all species tested norfluoxetine was present in blood and brain as the main metabolite, albeit in variable amounts and with different anorectic potency in terms of active brain concentrations necessary to reduce food intake, but nevertheless accounting for a substantial part of the parent drug's anorectic action. Norfluoxetine may possibly also contribute significantly to the anorectic activity of fluoxetine in man. In view of the metabolite-to-parent drug ratio approaching unity at steady-state in man (Benfield et al 1986), the clinical

significance of the active metabolite norfluoxetine awaits further investigation.

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Further studies on the anti-nociceptive effect of vasopressin

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Abstract—The possible interactions of pathways which mediate anti-nociception when stimulated by α_2-adrenoceptor agonists and arginine vasopressin (AVP) were investigated. Yohimbine, an α_2-antagonist, failed to modify the anti-nociceptive response of AVP. However, clonidine pretreatment, in sub-effective and effective doses, potentiated the anti-nociceptive response of a sub-effective dose of AVP. This potentiation was attenuated by yohimbine and completely anatagonized by naloxone. These studies suggest that pathways related to the opioidergic system and those stimulated by α_2-agonists may be utilized by AVP in eliciting the anti-nociceptive response.

Attempts have been made in recent years to understand the mechanism responsible for the anti-nociceptive activity produced by arginine vasopressin (AVP) recorded in different animal models. This effect has been reported to be independent of the opioidergic system (Berkowitz & Sherman 1982; Hart & Oluyomi 1990). An important non-opiate pathway for eliciting anti-nociception is one which is stimulated by α_2-adrenoceptor agonists. α_2-Agonists, such as clonidine produced a significant anti-nociceptive response which was antagonized by yohimbine and unaffected by naloxone (Paalzow & Paalzow 1976; Spaulding et al 1979; Chance 1983).

Even though opioidergic and α_2-adrenergic anti-nociception are independent, an interaction between them and a common pathway have been proposed (Spaulding et al 1979; Ramaswamy et al 1981, 1983b). The possible interaction between AVP and α_2-agonists in eliciting anti-nociception has not been examined. In the present study, an effort has been made to analyse such an inter-play.

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Materials and methods

Male Swiss albino mice, 25–30 g, housed in a 12 h dark: 12 h light cycle with free access to food and water were used. Anti-nociception was tested using the acetic acid-induced abdominal constriction assay. The onset and number of abdominal constrictions were noted for a period of 10 min following the injection of acetic acid (0·6%; 10 mL kg^{-1}; i.p.). The anti-nociceptive effect of clonidine (0·1 and 1·0 μg kg^{-1}; i.p.) and AVP (0·8 and 4·0 μg kg^{-1}; i.v.) were recorded by administering them 15 or 10 min, respectively, before acetic acid challenge.