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Effect of norfenfluramine and two structural analogues on brain 5-hydroxyindoles and serum prolactin in rats

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Fenfluramine and norfenfluramine elevate serum prolactin in rats (Fuller et al 1976), apparently by a mechanism involving the release of 5-hydroxytryptamine (Quattrone et al 1978). Here we describe studies with two structural analogues of norfenfluramine, one of which depletes 5-hydroxytryptamine as do norfenfluramine and fenfluramine, the other of which does not. The effects of these analogues on serum prolactin are consistent with the idea that the elevation of prolactin is mediated by 5-hydroxytryptamine release.

Male Wistar rats, 190–210 g, obtained from Harlan Industries, Cumberland, Indiana, had free access to food and water. The compounds were synthesized in the Lilly Research Laboratories as hydrochloride salts and were injected i.p. in aqueous solutions (0·05 mmol kg^{-1}). Rats were decapitated 1 or 6 h after injection. Whole brains were rapidly excised, frozen on dry ice, and stored frozen before analysis. 5-Hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations were determined spectrofluorometrically after condensation with *o*-phthalaldehyde (Miller et al 1970). Blood collected from the cervical wound was allowed to clot, then serum obtained after centrifugation was stored frozen. Prolactin concentration in serum was measured by radioimmunoassay using the NIAMDD kit and is expressed as ng of NIAMDD rat prolactin RP-1 ml^{-1}.

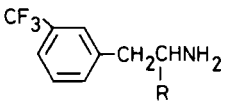

Table 1 shows the effect of norfenfluramine (α-methyl-m-trifluoromethyl-phenylethylamine) on 5-HT and 5-HIAA concentrations in brain and on prolactin concentrations in the serum of male rats. Norfenfluramine was injected at 12 mg kg^{-1}, and the analogues were injected at equimolar doses (0·05 mmol kg^{-1}). Norfenfluramine depleted 5-HT and 5-HIAA in brain and elevated serum prolactin, effects that have been reported earlier (Fuller et al 1976, 1978). The α-ethyl analogue of norfenfluramine likewise depleted the 5-hydroxyindoles and elevated serum prolactin. In contrast, the α-n-propyl analogue had no effect either on brain 5-hydroxyindoles or on serum prolactin.

Fenfluramine is thought to cause a rapid release of 5-HT (Trulson & Jacobs 1976; Kannengiesser et al 1976; Clineschmidt & McGuffin 1978), and this release of 5-HT not only may produce the elevation of serum prolactin observed with fenfluramine and norfenfluramine but also results in depletion of 5-hydroxyindole levels. Our data showing that the α-ethyl analogue of norfenfluramine depletes 5-hydroxyindoles and elevates serum prolactin, whereas the α-propyl analogue, which does not deplete 5-hydroxyindoles, does not elevate serum prolactin, are compatible with the idea (Quattrone et al 1978) that the release of 5-HT mediates the increase in serum prolactin.

The compounds used were racemates, and the stereoisomers of norfenfluramine and fenfluramine have been shown to differ in their potency in affecting brain 5-hydroxytryptaminergic and dopaminergic systems (Crunelli et al 1980; Jori et al 1973; Bendotti et al 1980). For instance, the (−)-isomer of fenfluramine has been suggested to influence dopamine neurons directly, whereas the

Table 1. Structure-activity relationships in the lowering of brain 5-hydroxyindoles and elevation of serum prolactin in rats by alkyl-substituted m-trifluoromethyl-phenylethylamines. All compounds were injected i.p. at 0·05 mmol kg^{-1}. Brain concentrations of 5-hydroxytryptamine (5-HT) and of 5-hydroxyindoleacetic acid (5-HIAA) were measured at 6 h, serum prolactin at 1 h after injection. Mean values ± standard errors for 5 rats per group are shown.

	Brain 5-hydroxyindoles, μg g^{-1}		Serum prolactin
	5-HT	5-HIAA	ng ml^{-1}
Vehicle-treated control	0·57 ± 0·02	0·57 ± 0·02	8·1 ± 1·8
R = Methyl (norfenfluramine)	0·22 ± 0·02*	0·33 ± 0·04*	74·2 = 11·2*
R = Ethyl	0·40 ± 0·01*	0·37 ± 0·02*	85·9 ± 6·0*
R = n-Propyl	0·57 ± 0·01	0·53 ± 0·02	9·3 ± 3·0

* Significant difference from control group ($P < 0·01$).

* Correspondence.

(+)-isomer preferentially affects 5-HT (Crunelli et al 1980). It is conceivable that prolactin could be elevated through more than one mechanism by these compounds, either enhancement of 5-hydroxytryptaminergic function or inhibition of dopaminergic function. However, the evidence favours the idea that enhancement of 5-hydroxytryptaminergic function is the mechanism by which fenfluramine increases serum prolactin concentration in rats (Quattrone et al 1978). The current findings are consistent with that interpretation, but a differing involvement of 5-HT and dopamine in the actions of the individual stereoisomers of the compounds studied cannot be ruled out.

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Evidence implicating a prostaglandin as the mediator of intestinal fluid secretion induced by pithing: inhibition by indomethacin, morphine and pentobarbitone

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There is evidence that the fluid transport mechanism of the small intestine is influenced by the autonomic nervous system. For example, noradrenaline stimulates the absorption of sodium and chloride by activating α-adrenoceptors (McColl et al 1968), while cholinergic stimulation results in the net secretion of fluid (Hubel 1976). However, Beubler et al (1978) and Lembeck & Beubler (1979) have shown, using the 'enteropooling' assay, that destruction of the extrinsic nerve supply of the rat small intestine by pithing does not alter the fluid volume within the lumen.

The aim of the present study was to re-examine these findings using a more accurate recirculation method, so that further experiments could be undertaken to investigate the effects of drugs on fluid transport in vivo without the complications of extrinsic nerve activity and the use of an anaesthetic. This latter point was prompted by the suggestion of Tothill (1976) that the anaesthetics pentobarbitone and urethane may interfere with the release of PGE_2 from the mucosa of the rat small intestine. This may be of some importance since prostaglandins have been implicated as physiological modulators of intestinal fluid transport (Beubler & Juan 1977).

Materials and methods

Male and female hooded rats (190–290 g) were kept in wire-bottomed cages and were deprived of food for 24 h but were given free access to drinking water. Anaesthesia was produced using halothane inhalation, the trachea cannulated and the brain and spinal cord destroyed by a 2 mm diameter needle introduced through the eye orbit. The method of pithing used was the same as the initial procedure described by Shipley & Tilden (1947) except that the vagi were not cut, the carotid arteries and jugular veins were not tied off and the animals were not pre-treated with atropine as in the preparation described by Gillespie & Muir (1967). The animal were then placed on an electrically heated pad (approximately 35 °C) and artificially respired using an air pump at the rate of 53 strokes min^{-1} and a volume of 1 ml/100 g of body weight.

A recirculation technique was used to measure the net fluid transported by the jejunum over 20 min, as has been described previously (Coupar 1978). This involved using an isosmotic solution containing (g $litre^{-1}$): NaCl 8·57, KCl 0·37, dextrose 1·0 and phenolsulphonphthalein (PSP) 0·02, to act as a non-absorbable marker for water transport. Recirculation of this solution through the lumen of the jejunum by gas lift was initiated 10 min after completing the pithing procedure. Results are expressed as the net amount of water absorbed (+) or secreted (−) per g wet weight of intestinal tissue during the 20 min perfusion.

Samples of recirculation fluid were also extracted for stable prostaglandins by an extraction procedure similar to that described by Unger et al (1971). The samples were acidified to pH 3·5 with 1% v/v formic acid and extracted with two equal volumes of chloroform, which were evaporated at 35 °C by the use of a rotary evaporator. The dry residue was blown in a stream of N_2 until all traces of formic acid were removed.

The dry extract was dissolved in 5 ml of Krebs-Henseleit solution and bioassayed against authentic PGE_2 using superfused rat fundus strips by the method similar to that of Ferreira & De Souza Costa (1976). Fundus strips were

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