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Sex specific differences in noradrenaline uptake and its inhibition by maprotiline

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Presynaptic uptake inhibition of monoamines by tricyclic drugs (with the exception of iprindole) is considered to be the main characteristic of their mode of action relevant to antidepressant activity. The extent of uptake inhibition of noradrenaline and/or 5-hydroxytryptamine (5-HT) varies with each drug and its metabolites. Maprotiline (Ludiomil, CIBA-Geigy) is a new tetracyclic antidepressant (Kielholz, 1972) which inhibits only noradrenaline uptake, not affecting 5-HT uptake even at high concentrations (Maître, Waldmeier & others, 1974). It thus provides a tool for investigating the role of selective noradrenaline uptake inhibition in biochemical subgroups of endogenous depression (Pühringer, Wirz-Justice & Hole, 1975).

Although extensively studied pharmacologically, *in vitro* monoamine uptake has not been systematically studied to elucidate naturally occurring changes: circadian (Wirz-Justice, 1974; Endersby & Wilson, 1974) and possible seasonal (Wirz-Justice, 1974) variations have been observed, but the influence of sex and endocrinological state is not known. This may be an important factor, particularly since application of steroid hormones has been found to influence uptake (Endersby & Wilson, 1974; Wirz-Justice, Hackmann & Lichtsteiner, 1974). Therefore it seemed useful to investigate *in vitro* noradrenaline uptake to determine the extent, if any, of sex-specific differences in uptake and its inhibition by maprotiline.

Male (350 g) and female (250 g) rats were kept at least one month in a controlled environment (lights on from 05.00 to 19.00 h; temp. 24 $\pm$ 1·5°), and had free access to food. Only females having at least two consecutive regular four-day oestrus cycles (controlled by daily vaginal smears before 08.30 h) were used on the morning of pro-oestrus. Both male and female rats were killed at 10 h, and the brain regions

dissected out were: parietal cortex (without white matter), medulla, anterior hypothalamus and the medio-ventral hypothalamus including the median eminence. Slices from each region (0·2 × 0·2 mm) were preincubated for 10 min in Krebs-Ringer bicarbonate buffer with maprotiline (1×10^{-6}M), and further incubated for 10 min with [^{14}C]noradrenaline (1×10^{-7}M) as described by Wirz-Justice & others (1974).

The mean protein (mg) for each region dissected was similar for both male and female rats. Statistical analysis of the uptake data was with a non-parametric test, as tissue : medium ratios for uptake have been found not to be normally distributed (Wirz-Justice & others, 1974). The White test is suitable for detecting population differences from two independent populations (Siegel, 1956).

Noradrenaline uptake was significantly higher in pro-oestrus females than in males in all regions investigated (Table 1). At pro-oestrus (a time of maximum plasma oestradiol concentrations (Butcher, Collins & Fugo, 1974), uptake was higher than in males by 63% in anterior, and 41% in medio-ventral hypothalamic slices. This can be compared with results showing that oestradiol increases noradrenaline uptake in ovariectomized rat hypothalamic slices by 84% (Endersby & Wilson, 1974). However the uptake studies in males were made during November–December 1974, in females during March–April 1975. We have previously found that monoamines may show a seasonal variation in uptake, dopamine uptake being higher in spring than in winter. Therefore the higher noradrenaline uptake in females in spring may be partially dependent on the time of year the investigations were made. That a sex specific difference is still important, is supported by the influence of sex steroids not

Table 1. *Noradrenaline uptake in male and female rat brain slices**.

	Cortex	Medio-ventral hypothalamus	Anterior hypothalamus	Medulla
Male 10h	87·3 s.d. 19·2	140·0 s.d. 34·6	144·2 s.d. 28·3	52·7 s.d. 22·1
n	(10)	(10)	(10)	(9)
Female pro-oestrus 10h	118·9 s.d. 16·7	197·8 s.d. 49·3	232·6 s.d. 52·7	119·6 s.d. 25·4
n	(9)	(9)	(9)	(9)
P§	<0·01	<0·01	<0·01	<0·01

* Expressed as mean with s.d. of the tissue: medium ratio (count min^{-1} mg^{-1} protein after 10 min uptake/count min^{-1} μl^{-1} incubation medium).
§ Significance male *vs* female calculated with the White test.

only on monoamine uptake (Endersby & Wilson, 1974; Wirz-Justice & others, 1974), but also on tyrosine hydroxylase (Beattie, Rodgers & Soyka, 1972), monoamineoxidase (Luine, Khylchevskaya & McEwen, 1975), and the higher noradrenaline turnover in female rat brain (Gordon & Shellenberger, 1974).

Further differences were found in the extent to which maprotiline (at 1×10^{-6}M) inhibited noradrenaline uptake (Table 2), being less efficient in pro-oestrus female rats than in males in the cortex and in the medio-ventral hypothalamus, the region involved in neuroendocrinological control mechanisms. These investigations were only carried out during the morning of pro-oestrus, when the cns monoamine changes that trigger off the luteinizing hormone surge leading to ovulation take place. It may be that a pattern of uptake and uptake-inhibition exists, varying during the oestrus cycle.

These results suggest that this aspect of monoamine metabolism, *in vitro* uptake and its inhibition, is dependent on the sex (and endocrinological state) of the animal used. Such differences in biochemical mechanisms and pharmacological response have been relatively neglected, but if confirmed have certain implications.

Table 2. % *Control noradrenaline uptake after preincubation with maprotiline* (1×10^{-6}M)*.

	Cortex	Medio-ventral hypothalamus	Anterior hypothalamus	Medulla
Male 10h	37 s.d. 7	32 s.d. 7	32 s.d. 9	38 s.d. 8
n	(9)	(10)	(10)	(9)
Female pro-oestrus 10h	50 s.d. 6	43 s.d. 8	35 s.d. 8	43 s.d. 10
n	(8)	(9)	(9)	(8)
P§	<0·05	<0·01	n.s.	n.s.

* Expressed as mean with s.d. of count min^{-1} mg^{-1} after maprotiline preincubation $\times 100$ count min^{-1} mg^{-1} control incubation.
§ Significance male *vs* female calculated with the White test.

Firstly, the observation of a differential distribution of depression (unipolar depressions being more common in women than in men, Angst & Scharfetter, 1972), together with the high incidence of psychic disturbances linked with the premenstrual phase, post-partum, and the menopause, may have a biochemical correlate. Female sex hormones can modify the neurochemical substrates postulated to be involved in depressive states (Janowsky, Fann & Davis, 1971). Thus research strategies investigating biochemical changes in affective disorders must modify a general hypothesis (Akiskal & McKinney, 1973) to allow for the reality of apparent differences in depressive phenomena in male and female patients.

Secondly, a given depressive symptomatology may be the functional effect of different mechanisms. Noradrenaline turnover, noradrenaline uptake are higher in female rats than in males. Platelet MAO activity in women is higher than in men and varies during the menstrual cycle and pregnancy (Wirz-Justice, Pühringer & others, 1975). Erythrocyte COMT activity is reduced in depressive women but not in men (Dunner, Cohn & others, 1971). There is a tendency for women to have higher 5-hydroxyindole acetic acid in csf than men (Åsberg, Bertilsson & others, 1973). Analogous to these results, platelet 5-HT uptake inhibition by chlorimipramine appears to be less efficient in women than in men (in preparation). The identification of one or several steps in monoamine metabolism which may be disturbed in a given patient could provide a rationale for more specific therapeutic measures, i.e. whether an uptake inhibitor (and which one), or a MAO inhibitor, and/or a given hormone should be used.

Thirdly, whether a depressive patient is male or female must be considered in evaluating a given pharmacological response and in determining, for example, which steady state plasma concentration of maprotiline is sufficient for clinical efficacy.

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Impairment of salicylate uptake from rat small intestine following pretreatment with a folic acid antagonist

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Amethopterin, a folic acid antagonist, has been shown by Bognel (1965) to inhibit active transport of glucose in the intestine, and Robinson, Antonioli & Vanotti (1966) found that *in vitro* it reduced uptake of L-phenylalanine by rat intestine. Its effect on salicylate which is not absorbed from the intestine by an active process, and its uptake *in vivo* have been examined.

Male Wistar rats, 220–230 g, were treated with amethopterin (Lederle) 20 and 40 mg kg^{-1}, 48 h previously; the subcutaneous route was chosen to obviate any effects of a high local concentration in the gut. The animals were fasted overnight anaesthetized with sodium pentobarbitone, 60 mg kg^{-1} (s.c.) and the whole length of the small intestine was perfused according to Schanker, Tocco & others (1958). Sodium salicylate 50 $\mu g\ ml^{-1}$ in Krebs solution at 37° was passed through the gut at 2 ml min^{-1} by peristaltic pump and blood samples (0·2 ml) were taken at 30 min intervals and assayed fluorometrically for total unchanged salicylate (Davison, Guy & others, 1961).

Robinson & others (1966) found marked changes in the intestinal mucosa 48 h after treatment with amethopterin (40 mg kg^{-1}, oral), so representative sections were taken from gut and were stained with haematoxylin and eosin, or in some cases by the P.A.S. technique, and micrometer readings were made at four opposite points on each section to give a mean value for total depth of tissue (from villus tip to serosal surface) and for mucosal depth (from villus tip to muscularis mucosae).

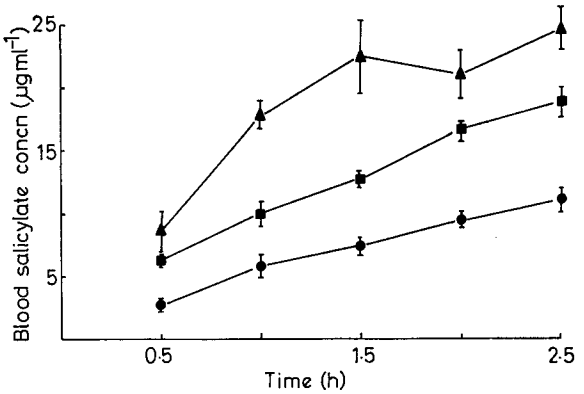

FIG. 1. Salicylate uptake from small intestine of rats pre-treated with amethopterin. ▲ Control, 0·9% saline; ■ Amethopterin 20 mg kg^{-1}; ● Amethopterin 40 mg kg^{-1}, (n = 5).