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REFERENCES

BOGDANSKI, D. F. & BRODIE, B. B. (1969). *J. Pharmac. exp. Ther.*, **165**, 181–189.

GRAEFE, K. H., BÖNISCH, H. & TRENDELENBURG, U. (1971). *Arch. exp. Path. Pharmak.*, **271**, 1–28.

IVERSEN, L. L. (1967). *The uptake and storage of noradrenaline in sympathetic nerves*, p. 151. Cambridge: Cambridge Univ. Press.

LINDMAR, R. & LÖFFELHOLZ, K. (1972). *Arch. exp. Path. Pharmak.*, **274**, 410–414.

LÖFFELHOLZ, K., LINDMAR, R. & MUSCHOLL, E. (1971). *Ibid.*, **270**, R87.

PATON, D. M. (1972). *Pharmacology*, **7**, 78–88.

STEIN, W. D. (1967). *The movement of molecules across cell membranes.* p. 126. New York: Academic Press.

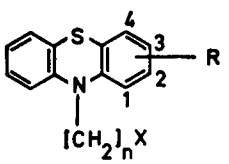
WHITE, T. D. & PATON, D. M. (1972). *Biochim. biophys. Acta*, **266**, 116–117.

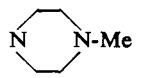
Differential effects on mouse brain catecholamine turnover of chlorpromazine, trifluoperazine and closely-related non-tranquillizing analogues

There is convincing evidence that the major tranquillizing drugs accelerate the turnover of brain dopamine. Although they do not greatly change the brain level of dopamine itself, they cause a marked rise in the brain levels of dopamine metabolites (Carlsson & Lindqvist, 1963; Andén, Roos & Werdinius, 1964; Laverty & Sharman, 1965), they increase the rate at which dopamine disappears from the brain when synthesis is blocked by α-methyltyrosine (Sharman, 1966; Corrodi, Fuxe & Hökfelt, 1967), and they accelerate the turnover of [^{14}C] labelled dopamine formed in the brain from [^{14}C] tyrosine (Nybäck, Sedvall & Kopin, 1967; Gey & Pletscher, 1968). Their effects on brain noradrenaline turnover are less consistent; nevertheless, many tranquillizers raise the normetanephrine level in the brains of mice pretreated with monoamine oxidase inhibitors (Scheel-Krüger, 1972) and accelerate the disappearance of noradrenaline from the brains of rats when either tyrosine hydroxylase or dopamine-β-hydroxylase is inhibited (Andén, Corrodi & Fuxe, 1972).

The strongest reason for believing that these effects are causally related to the tranquillizing action is the correlation that exists between the effect of tranquillizers on catecholamine turnover in animals and their tranquillizing potency in man (Roos, 1965; Nybäck & Sedvall, 1970). However, other correlations have been observed between the tranquillizing potency of aminoalkylphenothiazines and some of their

physicochemical properties (Seeman & Bialy, 1963; Green, 1967a) and biochemical actions *in vitro* (Davis & Brody, 1966; Gabay & Harris, 1967), but none of these correlations has yet been shown to successfully predict that aminoalkylphenothiazines in which the ring substituent is elsewhere than in the 2-position (e.g. I), or in which the phenothiazine ring and terminal amino-group are separated by a tetramethylene instead of a trimethylene chain (e.g. II) would be largely devoid of tranquillizing activity (Green, 1967b). To test whether these two types of non-tranquillizing



	R	n	X
(I)	4-Cl	3	NMe_2
(II)	2-Cl	4	NMe_2
(III)	2-Cl	3	NMe_2
(IV)	$2-CF_3$	3	

phenothiazines can be differentiated from active tranquillizers, such as chlorpromazine (III) or trifluoperazine (IV), by their influence on brain catecholamine turnover, compounds (I) and (II) have been compared with (III) and (IV) for their effects on α-methyltyrosine-induced depletion of mouse brain dopamine and noradrenaline.

Adult male mice (CFLP strain, Carworth Europe, Huntingdon) were kept at an environmental temperature of 30–32° to prevent hypothermia (O'Keefe, Sharman & Vogt, 1970). All drugs were dissolved in 0·9% NaCl and injected subcutaneously in a volume of 10 ml kg^{-1}. The phenothiazines (20 μmol kg^{-1} of I, II and III, 10 μmol kg^{-1} of IV) were given 30 min before 250 mg kg^{-1} of α-methyltyrosine methyl ester hydrochloride. Two h later, the mice were killed by cervical dislocation. The brains were removed, rinsed in ice-water, blotted, placed in small polythene bags, frozen in acetone and solid CO_2 and stored in solid CO_2 overnight. The frozen brains from pairs of identically treated mice were weighed and homogenized in 3 ml of 0·4 N perchloric acid containing 0·05% sodium metabisulphite. After centrifugation, 2 ml of the clear homogenate was added to 6 ml of 0·5M tris buffer (pH 9). The catecholamines were then extracted onto 300 mg of activated alumina, eluted from the alumina with 0·05 N perchloric acid, and assayed fluorimetrically in the same sample after iodine oxidation (Shellenberger & Gordon, 1971). Mice given 0·9% NaCl in place of the phenothiazine or the α-methyltyrosine were included as controls.

Table 1. *Brain catecholamine levels in mice given aminoalkylphenothiazines 30 min before α-methyltyrosine methyl ester hydrochloride (250 mg kg^{-1}) and killed 2·5 h later.*

α-Methyltyrosine given after	Dose of drug (mg kg^{-1})	Brain noradrenaline (ng g^{-1} ± s.e.)	% of control	Brain dopamine (ng g^{-1} ± s.e.)	% of control
Saline	—	260 ± 10 (11)	100	371 ± 14 (13)	100
(I)	7·1	237 ± 10 (7)	91	403 ± 22 (7)	109
(II)	7·4	259 ± 17 (4)	100	439 ± 36 (4)	118
(III) (chlorpromazine)	7·1	145 ± 4 (4) **	56	294 ± 17 (8) *	79
(IV) (trifluoperazine)	4·8	210 ± 8 (8) *	81	232 ± 9 (8) **	62

Figures in brackets are the numbers of pairs of mice given each treatment. Difference between drug + α-methyltyrosine and saline + α-methyltyrosine significant according to Student's *t*-test at $*P < 0·01$, $**P < 0·001$

The results of the combined treatment experiments are summarised in Table 1. The noradrenaline and dopamine levels are corrected for a recovery of 50%. Separate experiments showed that at doses up to 20 μmol kg^{-1}, none of the phenothiazines on their own significantly changed the normal brain levels of either noradrenaline (380 ng g^{-1}) or dopamine (700 ng g^{-1}). α-Methyltyrosine methyl ester hydrochloride on its own lowered the brain noradrenaline by over 30% in 2 h and the dopamine by nearly 50%.

Chlorpromazine and trifluoperazine significantly increased the rate of disappearance of both catecholamines after α-methyltyrosine, but whereas chlorpromazine had more effect on noradrenaline than on dopamine, trifluoperazine, which is the stronger tranquillizer in man, had a greater effect on dopamine than on noradrenaline. Both drugs caused sedation and loss of muscle tone, but these effects were more pronounced in the mice treated with chlorpromazine. In contrast, the disappearance of noradrenaline and dopamine was not enhanced by either (I) or (II), nor did these drugs cause any sedation.

These results are consistent with increases in the turnover of noradrenaline and dopamine being causally connected with tranquillizing activity, and also support the suggestion by Nybäck & Sedvall (1970) that the sedation produced by tranquillizers arises from effects on noradrenergic rather than dopaminergic neurons.

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REFERENCES

ANDÉN, N.-E., CORRODI, H. & FUXE, K. (1972). *J. Pharm. Pharmac.*, **24**, 177–182.
ANDÉN, N.-E., ROOS, B.-E. & WERDINIUS, B. (1964). *Life Sci.*, **3**, 149–158.
CARLSSON, A. & LINDQVIST, M. (1963). *Acta pharmac. tox.*, **20**, 140–144.
CORRODI, H., FUXE, K. & HÖKFELT, T. (1967). *Life Sci.*, **6**, 767–774.
DAVIS, P. W. & BRODY, T. M. (1966). *Biochem. Pharmac.*, **15**, 703–710.
GABAY, S. & HARRIS, S. R. (1967). *Ibid.*, **16**, 803–812.
GEY, K. F. & PLETSCHER, A. (1968). *Experientia*, **24**, 335–336.
GREEN, A. L. (1967a). *J. Pharm. Pharmac.*, **19**, 10–16.
GREEN, A. L. (1967b). *Ibid.*, **19**, 207–208.
LAVERTY, R. & SHARMAN, D. F. (1965). *Br. J. Pharmac. Chemother.*, **24**, 759–772.
NYBÄCK, H. & SEDVALL, G. (1970). *Eur. J. Pharmac.*, **10**, 193–205.
NYBÄCK, H. SEDVALL, G. & KOPIN, I. J. (1967). *Life Sci.*, **6**, 2307–2312.
O'KEEFE, R., SHARMAN, D. F. & VOGT, M. (1970). *Br. J. Pharmac.*, **38**, 287–304.
ROOS, B.-E. (1965). *J. Pharm. Pharmac.*, **17**, 820–821.
SCHEEL-KRÜGER, J. (1972). *Archs int. Pharmacodyn. Thér.*, **195**, 372–378.
SEEMAN, P. M. & BIALY, H. S. (1963). *Biochem. Pharmac.*, **12**, 1181–1191.
SHARMAN, D. F. (1966). *Br. J. Pharmac. Chemother.*, **28**, 153–163.
SHELLENBERGER, M. K. & GORDON, J. H. (1971). *Analyt. Biochem.*, **39**, 356–372.