THE EFFECT OF TRANQUILLIZING DRUGS ON THE CONCENTRATION OF THE SULPHATE ESTER OF 4-HYDROXY-3-METHOXYPHENYLETHANE-1,2-DIOL IN RAT BRAIN

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A total of 17 butyrophenone, phenothiazine, benzodiazepine and imidazoline tranquillizing drugs were examined for their ability to increase the cerebral concentration of the sulphate ester of 1(4-hydroxy-3-methoxyphenyl)ethane-1,2-diol (MHPG-SO_3H), a metabolite of noradrenaline in the rat brain. Of these drugs, when given in a dose of 10 mg/kg i.p., only trifluperidol, haloanisone, azaperone, clozapine and haloperidol were found to increase the cerebral concentration of MHPG-SO_3H. This effect is unrelated to the ability of such drugs to antagonize the lethal effects of an intravenous injection of noradrenaline and suggests that the properties of the central noradrenaline receptors differ from those of the peripheral noradrenaline receptors.

Major tranquillizing drugs increase the concentrations of dopamine metabolites in the brain (Carlsson & Lindqvist, 1963; Andén, Roos & Werdinius, 1964; Laverty & Sharman, 1965) and apomorphine reduces the cerebral concentration of the dopamine metabolite homovanillic acid (HVA) (Roos, 1969). A compensatory increase, or decrease, in the activity of dopaminergic neurones in response to dopamine receptor blockade, or activation, is thought to be responsible for these effects. Some major tranquillizing drugs have been shown to accelerate the turnover of cerebral noradrenaline (Andén, Butcher, Corrodi & Ungerstedt, 1970; Andén, Corrodi & Fuxe, 1972). Meek & Neff (1973) have suggested that the rate of formation of the ethereal sulphate of 1(4-hydroxy-3-methoxyphenyl)ethane-1,2-diol (MHPG-SO_3H) could serve as an index of the rate of formation of noradrenaline in the rat brain. The present study was carried out to find out if major tranquillizing drugs could change the concentration of MHPG-SO_3H in the rat brain and whether such changes, if any, were related to the peripheral anti-noradrenaline activity of such drugs.

Methods Male, albino rats (200-400 g) were used. The drugs injected were made up as follows. Aceperone, haloperidol, benperidol, droperidol, moperone, haloanisone and pimozide were dissolved in the minimum concentration of tartaric acid (0.01-0.1 M) required to keep them in solution. Xylazine was dissolved in 0.1 N HCl and the solution neutralized with 0.1 N NaOH. Trifluperidol, seperidol and oxypertine were made up in mixtures of ethanol and 0.9% w/v NaCl solution (saline). 2-[*N*-cyclopentyl-*N*-(2,6-dichlorophenyl)-amino]-2-imidazoline HBr (St 797), 2-[*N*-allyl-*N*-(2,6-dichlorophenyl)amino]-2-imidazoline HBr (St 567), chlorpromazine, trifluperazine and clozapine, were dissolved in saline. Azaperone was prepared from the commercially available solution (Stresnil) diluted with saline, and spiperone was dissolved in 0.01 N HCl. Phenoxybenzamine was dissolved in ethanol acidified with HCl and the solution diluted with saline. Probenecid was dissolved in the minimum volume of 0.1 N NaOH, the pH was then adjusted to near neutrality with 0.1 N HCl and the solution diluted with water. Control animals were injected with the corresponding solvent.

The rats were killed by decapitation 1 h after the intraperitoneal administration of the drugs or the vehicle solution, the brains were dissected out and the tissue anterior to the pons, including the whole midbrain, was used. The brain was divided along the central fissure and each half was assayed separately for MHPG-SO_3H. MHPG-SO_3H was extracted and estimated fluorimetrically by the method of Meek & Neff (1972). Recovery of authentic MHPG-SO_3H added to brain tissue was 77 ± 3% (mean ± s.e.; $n = 30$). Results are given uncorrected for recoveries.

Results Only five of the major tranquillizing drugs listed under **Methods** caused an increase in the concentration of MHPG-SO_3H in the rat brain. These results are given in Table 1, which also shows their relative potencies in a test of peripheral anti-noradrenaline activity, reported by Janssen, Niemegeers & Schellekens (1965) and Janssen, Niemegeers, Schellekens & Lenaerts (1967). None of the other major tranquillizing drugs showed any significant effect on the concentration of MHPG-SO_3H at a dose of 10 mg/kg. We have been able to confirm the results of Meek &

Table 1 The effect of some major tranquillizing drugs on the concentration of the sulphate ester of 1(4-hydroxy-3-methoxyphenyl)ethane-1,2-diol (MHPG-SO_3H) in the brain of the rat.

Drug, solvent and dose	Concentration of MHPG-SO_3H (pmol/g brain) Control	Treated	Anti-noradrenaline activity (Janssen et al., 1965, 1967)
0.9% w/v NaCl solution (saline)(2.5 ml/kg)	Untreated 431 ± 20(4)	493 ± 19(26)	
0.01 M tartaric acid (2.5 ml/kg)		561 ± 18(14)†	
Trifluperidol (95% C_2H_5OH : 5% saline (10 mg/kg)	462 ± 37(4)	628 ± 30(8)***	−
Haloanisone (0.02 M tartaric acid) (10 mg/kg)	529 ± 48(4)	812 ± 78(8)*	++
Azaperone (saline) (10 mg/kg)	365; 323	795 ± 80(3)*	less than haloanisone
Clozapine (saline) (10 mg/kg)	560; 541	768 ± 32(4)**	
Haloperidol (0.01 M tartaric acid) (10 mg/kg)	555 ± 11(6)	731 ± 57(6)**	−−
Aceperone (0.01 M tartaric acid) (10 mg/kg)		552 ± 69(6)	+++

Comparison with own control values; Student's t test, $^*P < 0.05$, $^{**}P < 0.02$, $^{***}P < 0.01$. Comparison with animals treated with saline; Student's t test, †$P < 0.05$. Number of observations in parentheses. Each observation is a mean value from the two estimates of the concentration of MHPG-SO_3H obtained with the separate halves of each brain. The rats were killed 1 h after the intraperitoneal administration of the drugs or vehicle solutions.

Neff (1973) in showing that probenecid causes an increase in the cerebral concentration of MHPG-SO_3H which is linear with increasing dose, and that phenoxybenzamine also produces an increased concentration of this metabolite of noradrenaline in the brain.

Discussion The present results confirm and extend those recently reported by Keller, Bartholini & Pletscher (1973) who showed that haloperidol and clozapine were potent in increasing the concentration of MHPG-SO_3H in rat brain. We have examined a larger number of both butyrophenone, phenothiazine and imidazoline tranquillizing drugs. Their effectiveness in increasing the cerebral concentration of MHPG-SO_3H appears to bear no relation to their reported activity in protecting rats against the lethal effects of a large intravenous dose (1.25 mg/kg) of noradrenaline (Janssen *et al.*, 1965), the test used by these authors to define the anti-noradrenaline potency of such drugs. Thus, if the increase in the concentration of MHPG-SO_3H is a result of a compensatory increase in the activity of central noradrenergic neurones in response to noradrenaline receptor blockade, such central receptors have properties which are different from those of the peripheral noradrenaline receptors. The small increase in the concentration of MHPG-SO_3H in the brain, after the injection of tartaric acid, may be a similar effect to that seen in the mouse where the i.p. injection of ascorbic acid or citric acid (Ceasar, Hague, Sharman & Werdinius, unpublished observations) increased the concentration of free MHPG in the hypothalamus.

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References

ANDÉN, N.-E., BUTCHER, S.G., CORRODI, H. & UNGERSTEDT, U. (1970). Receptor activity and turnover of dopamine and noradrenaline after neuroleptics. *Eur. J. Pharmac.*, **11**, 303-314.

ANDÉN, N.-E., CORRODI, H. & FUXE, K. (1972). Effect of neuroleptic drugs on central catecholamine turnover assessed using tyrosine- and dopamine-hydroxylase inhibitors. *J. Pharm. Pharmac.*, **24**, 177-182.

ANDÉN, N.-E., ROOS, B.-E. & WERDINIUS, B. (1964). Effects of chlorpromazine, haloperidol and reserpine on the levels of phenolic acids in rabbit corpus striatum. *Life Sci. Oxford*, **3**, 149-158.

CARLSSON, A. & LINDQVIST, M. (1963). Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. *Acta pharmac. tox.*, **20**, 140-144.

JANSSEN, P.A.J., NIEMEGEERS, C.H.E. & SCHELLEKENS, K.H.L. (1965). Is it possible to predict the clinical effects of neuroleptic drugs (major tranquillizers) from animal data? Part 1. 'Neuroleptic activity spectra' for rats. *Drug Research*, **15**, 104-117.

JANSSEN, P.A.J., NIEMEGEERS, C.J.E., SCHELLEKENS, K.H.L. & LENAERTS, F.M. (1967). Is it possible to predict the clinical effects of neuroleptic drugs (major tranquillizers) from animal data? Part IV. An improved experimental design for measuring the inhibitory effects of neuroleptic drugs on amphetamine- or apomorphine-induced 'chewing' and agitation in rats. *Drug Research*, **17**, 841-854.

KELLER, H.H., BARTHOLINI, G. & PLETSCHER, A. (1973). Increase of 3-methoxy-4-hydroxyphenylethylene glycol in rat brain by neuroleptic drugs. *Eur. J. Pharmac.*, **23**, 183-186.

LAVERTY, R. & SHARMAN, D.F. (1965). Modification by drugs of the metabolism of 3,4-dihydroxyphenylethylamine, noradrenaline and 5-hydroxytryptamine in the brain. *Br. J. Pharmac. Chemother.*, **24**, 759-772.

MEEK, J.L. & NEFF, N.H. (1972). Fluorometric estimation of 4-hydroxy-3-methoxyphenylethyleneglycol sulphate in brain. *Br. J. Pharmac.*, **45**, 435-441.

MEEK, J.L. & NEFF, N.H. (1973). The rate of formation of 3-methoxy-4-hydroxyphenylethyleneglycol sulfate in brain as an estimate of the rate of formation of norepinephrine. *J. Pharmac. exp. Ther.*, **184**, 570-575.

ROOS, B.-E. (1969). Decrease in homovanillic acid as evidence for dopamine receptor stimulation by apomorphine in the neostriatum of the rat. *J. Pharm. Pharmac.*, **21**, 263-264.

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