Antagonistic effects of neuroleptics and apomorphine on synaptosomal tyrosine hydroxylase *in vitro*

We have previously shown (Christiansen & Squires, 1974) that low concentrations of haloperidol can partially reverse the apomorphine-induced inhibition of synaptosomal tyrosine hydroxylase from rat striatum. Since it is known that haloperidol and apomorphine have opposite and antagonistic effects on the turnover of dopamine *in vivo* (Andén, Rubenson & others, 1967; Kehr, Carlsson & others, 1972; Lahti, McAllister & Wozniak, 1972), it was concluded that apomorphine and haloperidol compete for the same presynaptic dopamine receptor on the synaptosomal membrane. To exclude the possibility that haloperidol might antagonize the inhibitory effect of apomorphine by some other mechanism, we have tested nine other neuroleptics, which differ greatly with respect to dopamine receptor blockade *in vivo*.

In contrast to the ability of most neuroleptics to accelerate dopamine turnover *in vivo*, we have seldom observed stimulation of synaptosomal tyrosine hydroxylase by neuroleptics *in vitro*, but often some inhibition at concentrations above 1 μM. In the concentrations used here (2 to 6 $\times$ 10^{-7}M) the neuroleptics alone did not greatly affect synaptosomal tyrosine hydroxylase activity.

Tyrosine hydroxylase activity was determined by measuring both [^{3}H]water (^{3}H–H_2O) and [^{3}H]catecholamines formed from 3,5-L-[^{3}H]tyrosine as previously described (Christiansen & Squires, 1974). We have recently found, by re-chromatography of the boric acid eluate on Dowex-50 according to Stolk (1973), that dopamine is the only catecholamine formed from L-tyrosine in striatal synaptosomes.

In the experiments with α-and β-flupenthixol the ^{3}H-H_2O assay was modified to remove interfering acidic metabolites by passing the Dowex-50 eluate through a Dowex-1 anion exchange column (Karobath, 1971). This procedure reduces the ^{3}H-H_2O blank relative to the control.

In Table 1 the nine test substances are listed in order of decreasing anti-apomorphine potency using the ^{3}H-H_2O assay. This order is similar to that obtained with the [^{3}H]dopamine assay, as well as in tests for apomorphine and amphetamine antagonism *in vivo* (Janssen, Niemegeers & Schellekens, 1965; Møller Nielsen, Pedersen & others, 1973). Clozapine and thioridazine, the weakest apomorphine antagonists in the present system, are clinically active antipsychotics which are weak antagonists of apomorphine- and amphetamine-induced stereotyped behaviour and which do not

Table 1. *The effects of several neuroleptics on the inhibition of synaptosomal tyrosine hydroxylase by apomorphine* ($6{\cdot}7 \times 10^{-7}$M).

Neuroleptic tested and concn ($\times 10^{-7}$)	[^{3}H]Dopamine assay				^{3}H-H_2O assay			
	Neuroleptic alone % of control	Neuroleptic + apomorphine % of control	Apomorphine alone % of control	% reversal†	Neuroleptic alone % of control	Neuroleptic + apomorphine % of control	Apomorphine alone % of control	% reversal†
Spiramide 1·6	89 ± 3	69 ± 3**	39 ± 5	60	91 ± 6	73 ± 13	28 ± 6	72
Thioproperazine 3·1	79 ± 4	66 ± 3**	38 ± 1	68	86 ± 1*	70 ± 5**	40 ± 1	65
Perphenazine 4·2	89 ± 4	64 ± 5**	32 ± 3	56	107 ± 1*	66 ± 6**	28 ± 3	48
Fluphenazine 3·8	100 ± 3	58 ± 2***	31 ± 3	39	108 ± 14	65 ± 5*	30 ± 8	45
α-Flupenthixol 4·0	108 ± 4	73 ± 3**	48 ± 2	42	122 ± 3*	81 ± 2***	51 ± 1	42
Chlorpromazine 4·7	94 ± 1	72 ± 1***	51 ± 2	49	103 ± 4	71 ± 1*	52 ± 4	37
Thioridazine 5·4	72 ± 3	42 ± 4	32 ± 3	25	89 ± 5	43 ± 8	28 ± 3	25
Clozapine 6·1	87 ± 7	52 ± 4	48 ± 4	14	107 ± 4	49 ± 5	38 ± 3	16
β-Flupenthixol 3·8	100 ± 4	55 ± 2*	48 ± 2	13	108 ± 7	52 ± 2	51 ± 1	2

All results are the mean values ± s.d. of 2 determinations.
* $0{\cdot}05 > P > 0{\cdot}01$; ** $0{\cdot}01 > P > 0{\cdot}005$; *** $0{\cdot}005 > P > 0{\cdot}001$. The superscripts in neuroleptic column indicate *P*-values compared with the control group, while those in the neuroleptic + apomorphine column indicate *P*-values compared with the apomorphine group (Student's *t*-test).

† % reversal $= \dfrac{\text{Neuroleptic + apomorphine} - \text{apomorphine.}}{\text{Neuroleptic} - \text{apomorphine}} \times 100$

greatly accelerate dopamine turnover *in vivo* (Stille, Lauener & Eichenberger, 1971). The α-isomer of flupenthixol, but not the β-isomer, effectively antagonizes the inhibitory action of apomorphine on synaptosomal tyrosine hydroxylase. *In vivo*, α-flupenthixol is a potent inhibitor of the behavioural effects produced by apomorphine and amphetamine in several mammalian species while β-flupenthixol has little or no effect (Møller Nielsen & others, 1973).

The correlation between the *in vitro* activity of the neuroleptics reported here, and their potency *in vivo* suggests that the postulated presynaptic dopamine receptors on synaptosomes are similar to postsynaptic dopamine receptors.

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REFERENCES

ANDÉN, N.-E., RUBENSON, A., FUXE, K. & HÖKFELT, T. (1967). *J. Pharm. Pharmac.*, **19**, 627–29.

CHRISTIANSEN, J. & SQUIRES, R. F. (1974). *Ibid.*, **26**, 367–369.

JANSSEN, P. A. J., NIEMEGEERS, C. J. E. & SCHELLEKENS, K. H. L. (1965). *Arzneimittel-Forsch.*, **15**, 104–125.

KAROBATH, M. (1971). *Proc. natn. Acad. Sci.*, **68**, 2370–2373.

KEHR, W., CARLSSON, A., LINDQVIST, M., MAGNUSSON, T. & ATACK, C. (1972). *J. Pharm. Pharmac.*, **24**, 744–747.

LAHTI, R. A., MCALLISTER, B. & WOZNIAK, J. (1972). *Life Sci.*, **11**, 605–613.

MØLLER NIELSEN, I., PEDERSEN, V., NYMARK, M., FRANCK, K. F., BOECK, V., FJALLAND, B. & CHRISTENSEN, A. V. (1973). *Acta pharmac. tox.*, **33**, 353–362.

STILLE, G., LAUENER, H. & EICHENBERGER, E. (1971). *Il farmaco (ed prat.)*, **26**, 603–625.

STOLK, J. M. (1973). *J. Pharmac. exp. Ther.*, **186**, 230–240.

Regional differences in homovanillic acid concentrations after acute and chronic administration of antipsychotic drugs

The clinical effects of antipsychotic drugs may be related to their effects upon dopamine metabolism. Extrapyramidal side-effects (EPS) have been attributed to alterations in dopamine metabolism in the nigrostriatal system. These side-effects, in contrast to antipsychotic effects, show a tendency to diminish during prolonged drug administration. Thus, in clinical settings it may be unnecessary to administer anti-acetylcholine drugs to prevent EPS after a month or so of antipsychotic drug treatment. Furthermore, acute administration of antipsychotic drugs increases dopamine turnover in animal brain (Carlsson & Lindqvist, 1963; Andén, Roos & Werdinius, 1964). O'Keeffe, Sharman & Vogt (1970) showed that chronic administration of these drugs results in tolerance to the effect on dopamine metabolism. These authors found that homovanillic acid (HVA) was no longer increased in cat or monkey caudate nucleus following prolonged antipsychotic drug administration. It appears, therefore, that there are biochemical and clinical correlates of tolerance to EPS produced by antipsychotic drugs. If changes in dopamine metabolism are also related to antipsychotic effects, it will be necessary to demonstrate regional brain effects upon dopamine metabolism which do not show tolerance, since tolerance does not develop to the antipsychotic properties in man. Recently another dopamine system has come under scrutiny in this regard. The so-called limbic dopamine system has been recognized as another forebrain terminus for mesencephalic neurons (Andén, Dahlström &