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.

Research Laboratories,
A/S Ferrosan,
Sydmarken 5, 2860 Soeborg,
Denmark.

JAN CHRISTIANSEN
RICHARD F. SQUIRES

April 29, 1974

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 &

others, 1966; Ungerstedt, 1971). Andén (1972) has shown that clozapine, an antipsychotic drug which rarely produces EPS, increases HVA in the limbic system somewhat more than in the caudate nucleus of rabbits. In the present study we have administered acute or chronic doses of chlorpromazine, thioridazine, clozapine, or fluphenazine enanthate to rabbits and have examined the effects of these treatments upon HVA levels in three brain regions which contain dopamine.

Male white rabbits (approximately 2·0 kg) were injected with chlorpromazine (10 mg kg^{-1}, s.c.), thioridazine (10 mg kg^{-1}, s.c.), clozapine (10 mg kg^{-1}, s.c.), fluphenazine enanthate (1 mg kg^{-1}, i.m.) or saline. For acute experiments animals were kept at a constant temperature of 32° for 6 h and then killed after light ether anaesthesia. For chronic experiments animals were injected with either chlorpromazine (10 mg kg^{-1}) or thioridazine (10 mg kg^{-1}) daily for 3 weeks. On the final day animals were again kept at 32° for 6 h after the injection and then killed under light ether. Animals given fluphenazine chronically were injected intramuscularly once a week (four injections) and were killed one week after the last injection. These animals were kept at 32° for 6 h before death. Brains were rapidly removed and the following three regions were dissected. Both caudate nuclei were dissected from the walls of the lateral ventricles. For the limbic region, the following dissection was made. From the ventral surface just anterior to the optic chiasm a horizontal incision was made down to the anterior commissure and was extended anteriorly at the same depth along the medial edge of both olfactory tracts. From the dorsal surface, septum was dissected anteriorly until it was contiguous with the ventral dissection. A triangle of forebrain tissue was thus removed. Sections for light microscopy indicated that the limbic dissection included nucleus accumbens, olfactory tubercle, and septum. The hypothalamic section consisted of a cube of tissue approximately 0·5 cm on a side lying above the pituitary stalk. For 30 dissections of each region, mean net weights (mg $\pm$ standard deviation) were: caudate 116 $\pm$ 2, limbic 175 $\pm$ 3, hypothalamic 728 $\pm$ 17. Both caudate nuclei were pooled for assay; otherwise regional samples from each animal were individually assayed for HVA.

Table 1. *Homovanillic acid concentration* ($\mu g\ g^{-1}$) *in three regions of rabbit brain following acute and chronic administration of antipsychotic drugs.*

Condition	Caudate	Limbic	Hypothalamic
Control	4·10 $\pm$ 0·23(12)[a]	2·67 $\pm$ 0·18(12)	0·90 $\pm$ 0·04(12)
Thioridazine			
acute (10 mg kg^{-1}, s.c.—6 h)	7·79 $\pm$ 0·64(8)[b,c]	4·08 $\pm$ 0·49(8)[b]	1·35 $\pm$ 0·16(8)[b]
chronic (10 mg kg^{-1} day^{-1} for 3 wk)	5·35 $\pm$ 0·38(5)[d]	3·96 $\pm$ 0·62(5)[d]	1·54 $\pm$ 0·26(5)[d]
Chlorpromazine			
acute (10 mg kg^{-1}, s.c.—6 h)	7·57 $\pm$ 0·49(9)[b,c]	3·69 $\pm$ 0·27(9)[b]	1·74 $\pm$ 0·16(9)[b]
chronic (10 mg kg^{-1} day^{-1} for 3 wk)	5·55 $\pm$ 0·25(8)[d]	3·71 $\pm$ 0·21(8)[d]	1·28 $\pm$ 0·12(8)[d]
Clozapine			
acute (10 mg kg^{-1}, s.c.—6 h)	5·54 $\pm$ 0·39(8)[b]	3·87 $\pm$ 0·21(8)[b]	1·22 $\pm$ 0·05(7)[b]
Fluphenazine enanthate			
acute (1 mg kg^{-1} i.m.—6 h)	12·94 $\pm$ 0·69(6)[b,c]	6·29 $\pm$ 0·59(6)[b]	1·84 $\pm$ 0·07(6)[b]
chronic (1 mg kg^{-1} wk^{-1}— 4 injections)	8·67 $\pm$ 0·63(7)[d]	5·73 $\pm$ 0·47(7)[d]	2·07 $\pm$ 0·22(7)[d]

a = Values as $\mu g\ g^{-1}$ $\pm$ s.e. (Number of animals).
b = $P < 0·005$ acute *vs* control.
c = $P < 0·005$ acute *vs* chronic.
d = $P < 0·005$ chronic *vs* control.

Analyses of brain for HVA were by the method of Roth & Surh (1970). Recoveries were determined for each sample by the addition of ^{14}C-HVA and values were corrected. Statistical comparisons were made using unpaired *t*-tests between means for the three regions and treatments. The results are in Table 1.

All acute treatments significantly increased HVA levels in each of the three regions compared to control values. Chronic treatments with each drug also significantly increased HVA levels in each region. However, only the caudate nucleus showed a significant reduction in HVA following each of the three drugs when acute and chronic treatments were compared. The limbic and hypothalamic areas maintained their increased levels of HVA during chronic administration of the three antipsychotic compounds. Chronic treatment with chlorpromazine and thioridazine resulted in regional values for HVA very similar to those obtained after acute treatment with clozapine.

These data suggest that the caudate nucleus of rabbits responds to chronic antipsychotic drug administration differently from limbic forebrain and hypothalamus. Recent studies have shown that adenyl cyclase, the enzyme thought to be closely associated with the dopamine receptor, appears to have identical properties in caudate and limbic tissue (Snyder, 1973). However, Andén & Stock (1973) have shown that the relative increases in HVA in caudate and limbic regions produced by clozapine and haloperidol are different. The former drug, which produces very few EPS in clinical studies, produced greater increases in HVA (expressed as % of control) in limbic compared to caudate regions. Haloperidol, an antipsychotic compound which is sometimes associated with a high incidence of EPS, produced equal increases in HVA in both regions. Further, Andén (1972) found some evidence that anti-acetylcholine drugs reduce the increase in HVA following haloperidol in the caudate nucleus of rabbits but not in the limbic forebrain. Our results add another treatment parameter whereby regional differences in effects upon dopamine metabolism after antipsychotic drugs may be observed and are consistent with the possibility that the clinical effects of antipsychotic drugs may be manifestations of differential regional effects of these compounds upon dopamine receptors.

We thank Dr. Carl Chi for assistance with the dissections and Faye Gomes for preparation of sections for light microscopy.

This work was supported by The Scottish Rite Schizophrenia Research Program and the State of Connecticut. For generously supplying drugs we thank Sandoz Pharmaceuticals for clozapine, and E. R. Squibb and Sons for fluphenazine.

Department of Psychiatry,
Yale University School of Medicine,
333 Cedar Street,
New Haven, Connecticut 06510, U.S.A.

MALCOLM B. BOWERS, Jr.
ANGELICA ROZITIS

April 1, 1974

REFERENCES

ANDÉN, N.-E. (1972). *J. Pharm. Pharmac.*, **24**, 905–906.

ANDÉN, N.-E. & STOCK, G. (1973). *Ibid.*, **25**, 346–348.

ANDÉN, N.-E., ROOS, B.-E. & WERDINIUS, B. (1964). *Life Sci.*, **3**, 149–158.

ANDÉN, N.-E., DAHLSTRÖM, A., FUXE, K., LARSSON, K., OLSON, L. & UNGERSTEDT, U. (1966). *Acta physiol. scand.*, **67**, 313–326.

CARLSSON, A. & LINDQVIST, M. (1963). *Acta pharmac. tox.*, **20**, 140–144.

O'KEEFFE, R., SHARMAN, D. F. & VOGT, M. (1970). *Br. J. Pharmac.*, **38**, 287–304.

ROTH, R. H. & SURH, Y. (1970). *Biochem. Pharmac.*, **19**, 3001–3012.

SNYDER, S. (1973). Paper presented to the American College of Neuropsycho-pharmacology, Palm Springs, Florida.

UNGERSTEDT, U. (1971). *Acta physiol. scand., Suppl.*, 367, 1–48.