Cyanocyproheptadine: role of cholinolytic properties in modulating neuroleptic-induced elevation of striatal homovanillic acid (HVA)

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The weak potency of clozapine in blocking amphetamine-induced rotational behaviour in animals with unilateral lesions of the substantia nigra (Stawarz, Hill & others, 1975) and its failure both to antagonize the agonist action of apomorphine (Stille, Lauener & Eichenberger, 1971) and to elicit extrapyramidal side effects in man (Angst, Bente & others, 1971a; Angst, Jaenicke & others, 1971b) correlate with its weak potency in elevating the concentrations of striatal HVA (Bürki, Ruch & others, 1973; Stawarz & others, 1975). Since clozapine exerts considerable cholinolytic properties (Stille & others, 1971; Miller & Hiley, 1974; Snyder, Greenberg & Yamamura, 1974) and because cholinolytic drugs have been shown to reduce the neuroleptic-induced elevation of HVA in the striatum (O'Keefe, Sharman & Vogt, 1970; Andén, 1972; Bowers & Roth, 1972), it is conceivable that the cholinolytic properties of clozapine-like drugs are responsible for their weak effect on striatal HVA *in vivo*. However, a dose of clozapine which is more than twice the ED50 for cholinolytic activity, failed to interfere with the increase in striatal HVA caused by haloperidol or loxapine (Bürki, Ruch & others, 1974). Moreover, unlike central cholinolytic drugs, clozapine also failed to reduce the chlorpromazine-induced elevation of striatal HVA (unpublished results from this laboratory).

The availability of 3-cyanocyproheptadine and its enantiomers provided an opportunity to further explore the role of the cholinolytic properties of a drug in modifying the elevation of striatal HVA as the neuroleptic and cholinolytic properties of this drug reside in different enantiomers.

Central oxotremorine antagonism. Mice treated with oxotremorine were tested for their ability to maintain balance for 15 s on a wooden bar (rotarod) 1 inch in diameter rotating at 6 rev min^{-1}. Oxotremorine, 0·3 mg kg^{-1} was given intravenously 55 min after the oral administration of the test compounds and the rotarod test was performed 5 min later. The dose of the test compounds required to protect 40% of the animals (ED40) was determined according to Finney (1964).

Peripheral mydriatic potency. The diameter of the pupil was measured with the aid of an ocular micrometer 60 min after treatment with a test compound as described by Stone, Meckelnburg & Torchiana (1958). The dose required to dilate the pupil to 1·5 micrometer units (ED 1·5) was calculated according to Finney (1964).

Antiavoidance activity. Operant avoidance procedures provide a sensitive test for screening potential neuroleptics in primates (Fielding & Lal, 1974). In the present experiments, squirrel monkeys were trained to lever press to avoid electric shock on a modified Sidman avoidance schedule. The apparatus employed was identical to that described by Hanson, Witoslawski & others (1966). Each lever press postponed a 1 s electric shock to the feet via a grid floor for 36 s. If a response was not made during a shock, which would terminate it, additional shocks were programmed at 36 s intervals. No animal was shocked more than 50 times in any one half-hour period. Cumulative oral doses of the test compounds were administered during a 4 h test session. The first dose was given 30 min pre-session and the remaining doses were administered at 90 min intervals. The data collected during the 30 min immediately following each dose were discarded. The dose of the test compounds necessary to produce a shock incidence of 1/2 the maximal number of shocks (25) possible in ½ h (ED25) was estimated by regression analysis according to Finney (1964). Drug and control sessions were alternated and at least one week was allowed to lapse between sessions.

Biochemical determinations. Male Sprague-Dawley rats (180–220 g) were decapitated 4 h after the administration of drugs. This time was chosen because we have established in previous experiments that the concentration of HVA reached its maximum at approximately 3 to 4 h following blockade of striatal dopamine receptors by neuroleptic drugs. The brains were rapidly removed and the striata were dissected free of residual cortex. The tissues were immediately frozen on dry ice and stored at —20° until ready for assay. Striatal HVA was extracted as described in detail by Stawarz & others (1975) and determined fluorometrically according to Andén, Roos & Werdinius (1963).

The data in Table 1 demonstrate that the classical neuroleptic and cholinolytic properties of (±)-3-cyanocyproheptadine reside in the two enantiomers, the (—)-enantiomer being a potent neuroleptic (3 times more potent than chlorpromazine in the antiavoidance test) with weak or no cholinolytic properties and the (+)-enantiomer having significant cholinolytic activity (3–6 times that of clozapine) but no neuroleptic properties. The finding that the (+)-enantiomer of 3-cyanocyproheptadine is less potent than the racemic mixture as an oxotremorine antagonist is most likely the result of the neuroleptic (depressant) properties of the racemate contributing to its apparent cholinolytic activity in antagonizing oxotremorine tremor.

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Table 1. *Potency of racemic (±)-3-cyanocyproheptadine and its enantiomers in test procedures for neuroleptic and cholinolytic activity.*

Compounds	Test procedure: Antiavoidance activity (ED25)*	Mydriatic activity (ED1·5)*	Oxotremorine antagonism (ED40)*
(±)-3-Cyano-cyproheptadine	0·9 (0·3–3·0)	8·1 (6·4–10·6)	0·8 (0·6–1·0)
(−)-3-Cyano-cyproheptadine	0·7 (0·5–1·0)	>20	>20
(+)-3-Cyano-cyproheptadine	>27	4·0 (3·2–4·8)	1·5 (0·7–3·7)
Cyproheptadine	>27	3·9 (3·3–4·5)	0·7 (0·3–1·6)
Clozapine	32·9 (18·9–144·9)	26·7 (22·8–32·5)	4·0
Chlorpromazine	2·2 (1·9–2·7)	—	—

* Values refer to mg kg^{-1}, orally; 95% confidence intervals are given in parentheses.

A dose response curve was established on the effect of the two enantiomers of 3-cyanocyproheptadine on the concentration of HVA in the striatum (Fig. 1). As expected, (—)-3-cyanocyproheptadine caused a significant dose dependent increase in the concentration of HVA. However, the (+)-enantiomer which possessed cholinolytic but no neuroleptic properties had no effect on the striatal concentration of HVA. In the next series of experiments we established a dose response curve for the racemic mixture of 3-cyanocyproheptadine so that the effect of the (—)-enantiomer alone could be directly compared with that of the (—)-enantiomer in the racemic mixture. If cholinolytic properties themselves were capable of reducing the neuroleptic-induced rise in HVA in the striatum, the dose-response curve would be expected to shift to the right. However, analysis of variance showed that there is no significant difference between the two dose response plots in Fig. 1.

The present studies with 3-cyanocyproheptadine thus demonstrate that the presence of potent cholinolytic properties in a neuroleptic drug does not necessarily shift the dose-response curve for elevation of striatal HVA to the right. The results suggest that

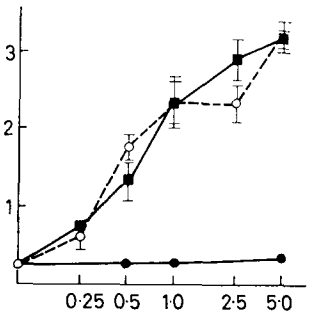

FIG. 1. Effect of 3-cyanocyproheptadine on the concentration of HVA in the striatum of male Sprague-Dawley rats. The values represent the mean content of HVA in $\mu g\ g^{-1}$ tissue ± s.e.m. 4 h after the intraperitoneal administration of the enantiomers or the racemic mixture. The control value in $\mu g\ g^{-1}$ ± s.e.m. is 0·24 ± 0·05 (n = 13). The values plotted on the abscissa are given as the concentration of the (+)-enantiomer the concentration of the (−)-enantiomer or the concentration of the (−)-enantiomer as administered in a racemic mixture. of 3-cyanocyproheptadine (mg kg^{-1}). ● (+)-enantiomer, ○ (−)-enantiomer, ■ racemic mixture. Ordinate: HVA($\mu g\ g^{-1}$ tissue). Each value represents the mean of 4 determinations (striata of 2 rats pooled for each determination).

other factors may be responsible for the observed reduction in the concentration of HVA following the combined administration of certain cholinolytic compounds with a neuroleptic drug, e.g. blockade of dopamine uptake by synthetic cholinolytic drugs followed by a feedback mediated decrease in the turnover of dopamine. The pharmacology of the racemic mixture of 3-cyanocyproheptadine also reveals that strong cholinolytic properties do not necessarily preclude a neuroleptic profile of a drug.

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REFERENCES

ANGST, J., BENTE, D., BERNER, P., HEIMANN, H., HELMCHEN, H. & HIPPIUS, H. (1971a). *Pharmakopsych. Neuropsychopharmak*, **4**, 201–211.

ANGST, J., JAENICKE, U., PADRUITT, A. & SCHARFETTER, CH. (1971b). *Ibid.*, **4**, 192–200.

ANDÉN, N. E. (1972). *J. Pharm. Pharmac.*, **25**, 905–906.

ANDÉN, N. E., ROOS, B. E. & WERDINIUS, B. (1963). *Life Sci.*, **7**, 448–458.

BOWERS, M. B. & ROTH, R. M. (1972). *Br. J. Pharmac.*, **44**, 301–306.

BÜRKI, H. R., RUCH, W., ASPER, H., BAGGIOLINI, M. & STILLE, G. (1973). *Schweiz. med. Wschr.*, **103**, 1716–1724.

BÜRKI, H. R., RUCH, W., ASPER, H., BAGGIOLINI, M. & STILLE, G. (1974). *Eur. J. Pharmac.*, **27**, 180–190.

FIELDING, S. & LAL, H. (1974). *Industrial Pharmacology*, Volume I: Neuroleptics, p. 53. New York: Futura.

FINNEY, D. J. (1964). *Statistical Methods in Biological Assay*. Chapter 3, p. 58. New York: Hafner.

HANSON, H. M., WITOSLAWSKI, J. J., CAMPBELL, E. H. & ITKIN, A. G. (1966). *Archs int. Pharmacodyn. Thér.*, **161**, 7–16.

MILLER, R. J. & HILEY, C. R. (1974). *Nature*, **248**, 596–597.
O'KEEFE, R., SHARMAN, D. F. & VOGT, M. (1970). *Br. J. Pharmac.*, **38**, 287–304.
SNYDER, S., GREENBERG, D. & YAMAMURA, H. I. (1974). *Archs Gen. Psychiat.*, **31**, 58–61.
STAWARZ, R. J., HILL, H., ROBINSON, S. E., SETLER, P., DINGELL, J. V. & SULSER, F. (1975). *Psychopharmacologia*, **43**, 125–130.
STILLE, G., LAUENER, H. & EICHENBERGER, E. (1971). *Farmaco*, **26**, 603–625.
STONE, C. A., MECKELNBURG, K. L. & TORCHIANA, M. L. (1958). *Archs int. Pharmacodyn. Thér.*, **117**, 419–434.

Modification of the amphetamine-induced stereotypy in rats following inhibition of the noradrenaline release by FLA 136

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Although the stereotyped behaviour induced by amphetamine is very much dependent on dopamine release in the basal ganglia of the mammalian brain (Randrup & Munkvad, 1974) it may also be influenced by central noradrenaline transmission as suggested recently by Mogilnicka & Braestrup (1976). Generally, impairment of transmission in noradrenaline synapses potentiated the amphetamine-induced stereotypy in rats.

FLA 136 alone and in combination with yohimbine was used to test the above hypothesis. FLA 136 resembles clonidine both in structure and in blood pressure lowering effect (Eriksson & Florvall, 1976). The drug also shares with clonidine the ability to decelerate noradrenaline synthesis and utilization (Andén & Grabowska, 1977). These effects result from stimulation of α-adrenoceptors which might occur on the noradrenaline neurons (Andén, Grabowska & Strömbom, 1976). FLA 136 differs from clonidine, however, since it does not stimulate peripheral or central postsynaptic α-adrenoceptors. In the present work, FLA 136 was given at a dose of 15 mg kg^{-1}, i.p., since higher doses can block α-adrenoceptors (Andén & Grabowska, 1977).

The results presented in Table 1 show that under the influence of FLA 136 at a dose of 15 mg kg^{-1}, i.p., the amphetamine-induced stereotypy in rats was significantly potentiated. All items of stereotypy were changed similarly, so that the rats with, for example, score 3 following amphetamine alone were indistinguishable from those with the same score following FLA 136 and amphetamine.

Yohimbine, an α-adrenoceptor blocking agent, shows much greater affinity for the receptors involved in noradrenaline synthesis and utilization than for those mediating functional effects such as stimulation of motor activity and flexor reflex activity (Andén & others, 1976). Under the influence of yohimbine both the synthesis and the utilization of noradrenaline in the rat central nervous system are greatly enhanced. In the experiments presented, the amphetamine-induced stereotypy in rats was inhibited by yohimbine almost in a dose-dependent manner (Table 1). At the same time yohimbine (3·0 mg kg^{-1}, i.p.) abolished the potentiation of the amphetamine-induced stereotypy caused by FLA 136 (Table 1). A similar antagonism between yohimbine and FLA 136 has been found in biochemical experiments where the deceleration of noradrenaline utilization produced by FLA 136 was almost completely inhibited by yohimbine (Andén & Grabowska, 1977).

Table 1. *Influence of FLA 136 (15 mg kg^{-1}, i.p.) and yohimbine on the amphetamine-induced stereotypy in rats.*

Treatment (mg kg^{-1}, i.p.)	Stereotypy score	*P*
Amphetamine (5·0)	28·0 (12)	—
FLA 136 + amphetamine (5·0)	53·5 (12)	<0·001
Yohimbine (3·0) + amphetamine (5·0)	10·0 (6)	<0·001
FLA 136 + yohimbine (3·0) + amphetamine (5·0)	28·0 (6)	n.s.
Amphetamine (10·0)	57·0 (5)	—
Yohimbine (1·0) + amphetamine (10·0)	48·0 (6)	<0·05
Yohimbine (3·0) + amphetamine (10·0)	43·0 (5)	<0·025
Yohimbine (10·0) + amphetamine (10·0)	23·0 (6)	<0·01

FLA 136 and yohimbine were injected 120 and 30 min before the amphetamine injection, respectively. The intensity of stereotypy was evaluated according to the method of Costall, Naylor & Olley (1972) at 10 min intervals up to 3 h following the amphetamine injection. The values given are medians of the sums of the scores during 3 h with the number of animals in parenthesis. The *P* values refer to differences from the stereotypy following amphetamine alone (Mann-Whitney U-test).

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