Limiting factors in the antagonism of neuroleptics on dopamine-sensitive adenylate cyclase

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Since the original finding that dopamine-stimulated adenylate cyclase can be antagonized by neuroleptic drugs (Kebabian, Petzold & Greengard, 1972) several attempts have been made to correlate the potency of these drugs in that enzyme system with their potency in pharmacological or clinical studies (Clement-Cormier, Kebabian & others, 1974; Kabobath & Leitich, 1974; Miller, Horn & Iversen, 1974). Although a good correlation was found for the phenothiazine and thioxanthene group, it became evident that the inhibitory effects of haloperidol and pimozide observed *in vitro* were too low compared with their high potency *in vivo*. To explain this discrepancy, it has been suggested that the low aqueous solubility of the butyrophenones and the diphenylbutylpiperidines might have contributed to their low potency *in vitro* (Clement-Cormier & others, 1974). The present investigations were undertaken in order to try to solve this problem.

Dopamine-sensitive adenylate cyclase was measured in rat brain striatum homogenates as previously reported (Kebabian & others, 1972). The addition of dopamine (10^{-4}M) produced a two- or three-fold increase in the enzyme activity. The IC50 values represent the drug concentrations producing 50% inhibition of increase of the cyclic AMP production measured in the presence of dopamine. The butyrophenones were dissolved in water by adding one or two drops of glacial acetic acid while the diphenylbutylpiperidines were dissolved in ethanol to reach a concentration of 5% in the incubation mixtures. Owing to the slight stimulatory effect of ethanol, the same amount was added to the incubation mixtures without neuroleptic drugs.

Table 1 gives the IC50 values for three butyrophenones and three diphenylbutylpiperidines in the dopamine-sensitive adenylate cyclase test. In each separate group of drugs a relatively good correlation was obtained when compared with the values in the apomorphine test in dogs. Nevertheless, in spite of their practically equal potency in certain pharmacological tests, haloperidol and pimozide differed markedly in their ability to inhibit the dopamine-stimulated adenylate cyclase. Therefore a correlation between the *in vitro* and *in vivo* test is only valid within a given group of neuroleptic drugs. A very good parallel between both activities was recently found for 18 different butyrophenones (Iversen, Rogawski & Miller, 1975) and for the two isomers of butaclamol one of which is active, the other inactive (Miller, Horn & Iversen, 1975). Table 1 also shows certain physicochemical properties of the tested neuroleptics. Here the butyrophenones and diphenylbutylpiperidines differed markedly as shown by a 1000 or 10 000-fold difference in the partition coefficient and a more than 1000-fold difference in solubility in water. This may possibly explain the relatively low potency of pimozide and its congeners as inhibitors of the dopamine-sensitive adenylate cyclase.

To test this hypothesis I have examined to what extent the neuroleptic drug is really dissolved or aspecifically bound to different structures in the test tube. For this purpose, labelled neuroleptics ([^{3}H]haloperidol spec. act. 10·5 Ci mM^{-1}; [^{3}H]pimozide spec.act. 14 Ci mM^{-1}; [^{3}H]clopimozide spec.act. 496 mCi mM^{-1}) were added at different concentrations to incubation mixtures in the presence or absence of rat striatum homogenate. After centrifugation, the radioactivity was

Table 1. *Drug concentrations* (IC50) *causing 50% inhibition in the dopamine-sensitive adenylate cyclase test and ED50 values in the apomorphine test in dogs.* The partition coefficient (log P = (drug concn in octanol)/(drug concn in water)) and the solubility of the drugs (mg in 100 ml 0·05M citrate phosphate buffer pH 7·4) are given.

	Dopamine-sensitive adenylate cyclase IC50 (M)	Apomorphine test in dog ED50 (mg kg^{-1})	Partition coefficient log P	Solubility mg per 100 ml
Butyrophenones				
Haloperidol	$7·5 \times 10^{-7}$	0·018	4·3	1·1
Pipamperone	$2·8 \times 10^{-6}$	0·9	2·4	290
Azaperone	$7·2 \times 10^{-6}$	1·0	3·3	3·4
Diphenylbutylpiperidines				
Clopimozide	6×10^{-6}	0·006	7·1	< 0·1
Penfluridol	8×10^{-6}	0·014	7·6	< 0·1
Pimozide	$1·5 \times 10^{-5}$	0·011	6·3	< 0·1

measured in the supernatant. As shown in Table 2 the amount of labelled neuroleptic in the sediment depends on the type and the concentration of drugs and on the presence or absence of enzyme. For instance, at 10^{-6}M 86% of clopimozide, 70% of pimozide and 29% of haloperidol were recovered in the pellet. Without enzyme, the percentages were much lower, but for clopimozide they remained at a relatively high level indicating that the drug had precipitated in the test tubes. Since the *in vitro* antagonism of neuroleptics occurred competitively (Clement-Cormier & others, 1974; Miller & others, 1974) the inhibitory compound had to remain in solution. Therefore the amount of labelled neuroleptics in the pellet may be considered as bound aspecifically. From these experiments, it is obvious that a comparison between the *in vitro* or *in vivo* potency of different neuroleptic drugs needs a correction factor owing to the low solubility of certain drugs. For instance, taking into account the results of Table 2, the corrected IC50 values would be approximately $5{\cdot}3 \times 10^{-7}$, $9{\cdot}7 \times 10^{-7}$ and 5×10^{-6}M for haloperidol, clopimozide and pimozide respectively. Nevertheless, these different IC50 values cannot explain the fact that clopimozide has a duration of action 10 times longer than pimozide (Janssen, Niemegeers & others, 1975). Furthermore, despite this correction factor, the inhibiting concentration of pimozide is still much higher than that of flupenthixol or fluphenazine. Therefore, besides the problem of solubility which may be relevant for certain drugs, other properties such as the capacity to cross the blood-brain barrier or to be taken up preferentially in some areas of the brain require to be considered in the interpretation of *in vivo* results. It was found that pimozide and clopimozide are present in much higher concentrations in the striatum than in the other areas of rat brain (Heykants & Laduron, unpublished results).

Table 2. *Percentage of labelled neuroleptics in the pellet after centrifuging incubation mixtures as described for measuring dopamine-sensitive adenylate cyclase.*

	with (+) and without (−) Striatum homogenate after					
	Haloperidol		Pimozide		Clopimozide	
M	+	−	+	−	+	−
10^{-8}	41	8	58	31	59	47
10^{-7}	37	6	68	28	82	66
10^{-6}	29	2	70	33	86	69

In conclusion, a correlation between the antagonism on the dopamine-sensitive adenylate cyclase and the clinical or pharmacological potency of neuroleptic drugs, only seems possible with a given class of compounds or to compounds having similar physicochemical properties. This *in vitro* test although useful in studying the mechanism of action of neuroleptics and the dopamine receptors, is probably unsuitable as a routine test for screening new drugs, since it does not allow for the duration of action and pharmacokinetic properties of the neuroleptic drugs.

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REFERENCES

Clement-Cormier, Y. C., Kebabian, J. W., Petzold, G. L. & Greengard, P. (1974). *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1113–1117.

Janssen, P. A. J., Niemegeers, C. J. E., Schellekens, K. H. L., Lenaerts, F. M. & Wauquier, A. (1975). *Arzneimmittel-Forsch.*, **25**, 1287–1294.

Iversen, L. L., Rogawski, M. & Miller, R. J. (1975). *Molec. Pharmac.*, in the press.

Kabobath, M. & Leitich, H. (1974). *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2915–2918.

Kebabian, J. W., Petzold, G. L. & Greengard, O. (1972). *Ibid.*, **69**, 145–2149.

Miller, R. J., Horn, A. S. & Iversen, L. L. (1974). *Molec. Pharmac.*, **10**, 759–766.

Miller, R. J., Horn, A. S. & Iversen, L. L. (1975). *J. Pharm. Pharmac.*, **27**, 212–213.