J. Pharm. Pharmac., 1975 **27**, 359

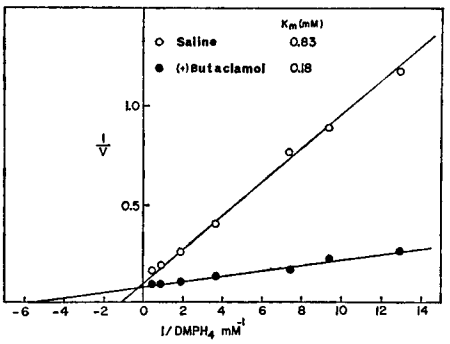
COMMUNICATIONS

Stereospecificity of dopamine receptors involved in the regulation of the kinetic state of tyrosine hydroxylase in striatum and nucleus accumbens

We have recently demonstrated that antipsychotic drugs of various chemical classes when injected into rats increase the affinity of tyrosine hydroxylase (TH) for pteridine cofactor in striatum and nucleus accumbens (Zivkovic, Guidotti & Costa, 1974; Zivkovic, Guidotti & others, 1975). Since this effect of antipsychotics can be readily blocked by apomorphine we have inferred that the regulation of the kinetic state of striatal TH might be mediated by postsynaptic dopamine receptors (Zivkovic & Guidotti, 1974; Zivkovic, Guidotti & Costa, 1975). Recently, a strict relation between the postsynaptic dopamine receptor and the adenylyl cyclase system sensitive to dopamine has been demonstrated (Kebabian, Petzold & Greengard, 1972). This system has been widely used in testing the ability of drugs to affect the activity of dopamine receptors (Clement-Cormier, Kebabian & others, 1974). Moreover, it has been shown that only the (+)-enantiomer of a new antipsychotic drug butaclamol [(±)-(4a,13b-*trans*)-(3-hydroxy,13b-*trans*)-3-tetra-butyl-2,3,4,4a,8,9,13b,14-octohydro-1-H-benzo[6,7] cyclohepta[1,2,3,-de]pyrido[2,1-a]isoquinolin-3-ol] is able to block the stimulation by dopamine of adenylyl cyclase in cell-free homogenates of striatum and olfactory tubercle (Miller, Horn & Iversen, 1975; Lippmann, Pugsley & Merker, 1975). This suggests a high stereospecificity of the dopamine receptor for its antagonist. The results of the present report demonstrate that the (+)-, but not the (−)-, enantiomer of butaclamol decreases the apparent Km of TH for pteridine cofactor.

Experiments were on male Sprague Dawley rats, 150 g, (Zivic-Miller, Allison Park, Pa.). Butaclamol was injected intraperitoneally and rats were decapitated 30 min after the injection. The nucleus accumbens and striatum were punched out from the transversal brain slices as described by Zivkovic & others (1975a). TH activity was measured by a modification (Zivkovic, & others, 1974) of the method of Waymire, Bjur & Weiner (1971). Protein concentration was measured according to Lowry, Rosebrough & others (1951).

Fig. 1 shows that (+)-butaclamol, injected into rats at the dose of 5 mg kg^{-1} (i.p.) 30 min before decapitation, decreased the apparent Km of striatal TH for 2-amino-4-hydroxy-6, 7-dimethyltetrahydropterine ($DMPH_4$) by about 4·5-fold. The Km of the enzyme for tyrosine (0·88 mM) was not affected. (−)-Butaclamol, injected in 10 times higher doses, did not change either the Km of TH for tyrosine or that for $DMPH_4$. The changes of TH activity in striatum and nucleus accumbens after (injections of various doses of (+)- or (−)-butaclamol are shown in Fig. 2. (+)-Butaclamol was almost equipotent in increasing TH activity in striatum and nucleus accumbens: the ED50 calculated from Fig. 2, was 0·25 and 0·4 mg kg^{-1} respectively. In contrast, 400 times higher dose of (−)-butaclamol failed to produce significant change in TH activity in both regions investigated. Thus, the dopamine receptor that is involved in the regulation of the kinetic state of TH appears to be highly stereospecific. Similar stereospecificity for the butaclamol enantiomers is exhibited by adenylyl cyclase sensitive to dopamine (Miller, & others, 1975; Lippmann & others, 1975) as well as by the receptor involved in the regulation of some behavioural patterns



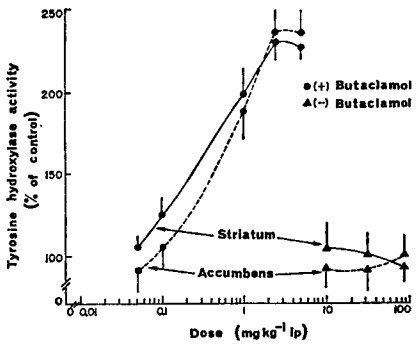


FIG. 1. Double reciprocal plots of the velocity of striatal tyrosine hydroxylase versus various concentrations of $DMPH_4$. Rats received intraperitoneal injection of saline (5 ml kg^{-1}) or (+)-butaclamol (5 mg kg^{-1}) 30 min before decapitation. Velocity is expressed as nmol of CO_2 formed h^{-1} mg^{-1} protein.

FIG. 2. Effect of intraperitoneal injections of various doses of (+)- or (−)-butaclamol on tyrosine hydroxylase activity in striatum and nucleus accumbens. Rats were killed 30 min after treatment. Tyrosine hydroxylase activity was assayed in presence of 0·4 mM $DMPH_4$ (Km 0·8 mM). Each point is the mean of 5 experiments. Vertical bars represent s.e.m.

(Humber, Bruderlein & Voight, 1974). This good correlation implies that the receptor which mediates the regulation of the kinetic state of TH present in dopaminergic neurons and the receptor which is involved in the regulation of some behavioural patterns have similar, if not identical, steric configuration. Moreover, from our experiments it appears that the receptors in striatum and in nucleus accumbens have similar affinity for the enantiomers of butaclamol.

We are grateful to Dr. W. Lippmann of Ayerst Research Laboratories, Montreal, Canada for the supply of (+)- and (−)-butaclamol.

Laboratory of Preclinical Pharmacology,
National Institute of Mental Health,
Saint Elizabeths Hospital, Washington, D.C. 20032 U.S.A.

B. ZIVKOVIC
A. GUIDOTTI
E. COSTA

February 7, 1975

REFERENCES

CLEMENT-CORMIER, Y. C., KEBABIAN, J. W., PETZOLD, G. L. & GREENGARD, P. (1974). *Proc. Nat. Acad. Sci. USA*, **71**, 1113–1117.

HUMBER, L., BRUDERLEIN, F. & VOIGHT, K. (1974). *4th Int. Sypm. Med. Chem.*, Noordwijkerhout, Netherlands (Abstr.).

KEBABIAN, J. W., PETZOLD, G. L. & GREENGARD, P. (1972). *Proc. Nat. Acad. Sci. USA*, **69**, 2145–2149.

LIPPMANN, W., PUGSLEY, T. & MERKER, J. (1975). *Life Sci.*, **16**, 213–224.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). *J. biol. Chem.*, **193**, 265–275.

MILLER, R. J., HORN, A. S. & IVERSEN, L. L. (1975). *J. Pharm. Pharmac.*, **27**, 212–213.

WAYMIRE, J. C., BJUR, R. & WEINER, N. (1971). *Analyt. Biochem.*, **43**, 588–600.

ZIVKOVIC, B. & GUIDOTTI, A. (1974). *Brain Res.*, **79**, 505–509.

ZIVKOVIC, B., GUIDOTTI, A. & COSTA, E. (1974). *Molec. Pharmac.*, **10**, 727–735.

ZIVKOVIC, B., GUIDOTTI, A. & COSTA, E. (1975). *Brain Res.*, in the press.

ZIVKOVIC, B., GUIDOTTI, A., REVUELTA, A. & COSTA, E. (1975a). *J. Pharmac. exp. Ther.*, in the press.