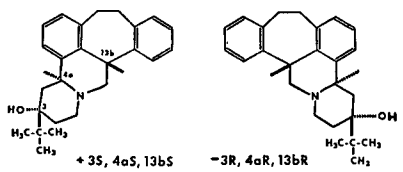
Effect of butaclamol on dopamine-sensitive adenylate cyclase in the rat striatum

Neuroleptic drugs exhibit a wide spectrum of pharmacological effects (Matthysse, 1973; Keller, Bartholini & Pletscher, 1973; Miller & Hiley, 1974). Of these actions dopamine receptor blockade is widely thought to be responsible for the parkinsonian side effects seen with these drugs and in addition correlates well with the antipsychotic effects of neuroleptics. Evaluation of the topography of the receptor at which these drugs act has been facilitated recently by the use of an *in vitro* system—the dopamine-stimulated adenylate cyclase present in dopamine-rich regions of the mammalian cns (Kebabian, Petzold & Greengard, 1972; Horn, Cuello & Miller, 1974). Previous results have illustrated the close correlation between blockade of the stimulating effects of dopamine on this adenylate cyclase and neuroleptic potency (Miller, Horn & Iversen, 1974). We now describe the action of butaclamol, (I) a neuroleptic of novel structure, (Humber, Bruderlein & Voigt, 1974; Bruderlein, Humber & Voigt, 1975) in this system. Among other features butaclamol (a racemate) exhibits optical isomerism.



The experimental procedure is described in the legend to Fig. 1 and is the same as previously reported (Miller & others, 1974). The addition of dopamine (100 μM) to homogenates of rat striatum produced an approximately two-fold increase in adenylate cyclase activity. Fig. 1 shows the effects of increasing concentrations of the (+)- and (−)-enantiomers of butaclamol on the stimulation of adenylate cyclase by 100 μM dopamine. The (+)-enantiomer was a potent inhibitor whereas the (−)-enantiomer was completely without effect at concentrations up to 10 μM, Fig. 2 shows the effect of the addition of various concentrations of the (+)- or (−)-enantiomers of butaclamol on the dose-response curve for dopamine. At lower concentrations of the (+)-enantiomer the dose-response curve was shifted to the right indicating that the inhibition was competitive with dopamine. At higher concentrations of the (+)-enantiomer the inhibition became partly non-competitive. Neither the (+)- nor the (−)-enantiomer inhibited basal adenylate cyclase activity in concentrations up to 10 μM. The molecular basis of the non-competitive interaction seen at higher concentrations is not known and is under investigation.

Butaclamol is interesting from a structural point of view for several reasons. The "tricyclic" skeleton containing the two benzene rings and the central seven membered ring has more in common with the tricyclic antidepressants than neuroleptics of the phenothiazines or thioxanthene class. Unlike the members of the other two classes of tricyclic neuroleptics, the nitrogen containing portion of the molecule is part of a fairly rigid ring system. The presence of the three assymetric carbon atoms gives rise to optical isomerism. It is of interest, therefore, that, as in the thioxanthenes, which exhibit *cis-trans* isomerism and stereo-selective neuroleptic activity (Miller & others, 1974), most of the activity of butaclamol is confined to one isomer. It is of note that as in the more potent neuroleptics such as fluphenazine and α-flupenthixol there is a hydroxyl "tail" to the molecule which may add in some way to its potency.

The neuroleptic activity of butaclamol resides solely in the (+)- enantiomer (Humber, & others, 1974; Bruderlein, & others, 1975). The experiments

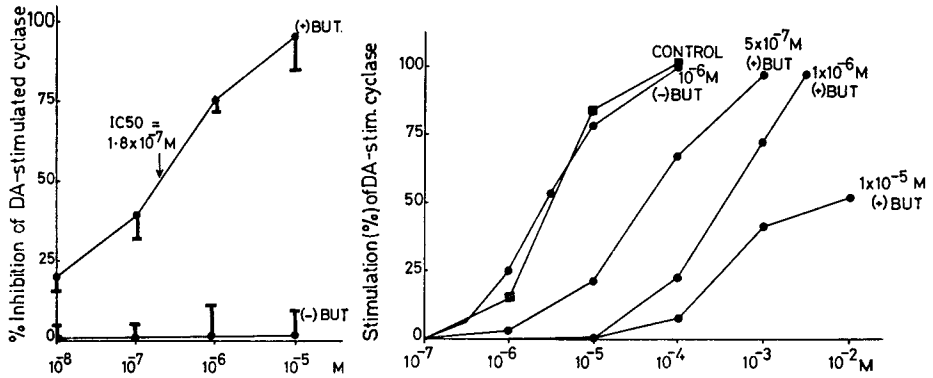


FIG. 1. Effect of varying concentrations (+)- or (—)-butaclamol on stimulation of adenylate cyclase activity in rat striatal homogenates by 100 μm dopamine. Striata were removed and homogenized in 25 volumes of 2mM maleate buffer pH7·4 containing 2mM EGTA. 50μl aliquots of this homogenate were added to 250μl of incubation buffer containing 0·5mM ADP, 2mM $MgSO_4$, 10mM theophyline, 80mM tris maleate pH 7·4 and 0·2mM EGTA. Incubations were carried out at 30° for 2·5 min. The reaction was terminated by boiling for 2·5 min. The contents of each tube were centrifuged to remove denatured protein and aliquots of the supernatant were assayed for cyclic AMP by the method of Brown & others (1972). Mean basal cyclic AMP levels were 34·3 $\pm$ 3·3 picomoles per assay tube (2mg wet weight) and in the presence of 100μM dopamine 69·0 $\pm$ 4·1 picomoles per assay tube (2mg wet weight). Points are means of at least 5 incubations.

FIG. 2. Effect of various concentrations of (+)- or (—)-butaclamol on the dose response curve for stimulation of adenylate cyclase activity by dopamine. Experimental details are given in Fig. 1. Basal cyclic AMP levels were 31·1 $\pm$ 3·6 picomoles per assay tube (2 mg wet weight). Points are means of at least 5 incubations.

reported here thus produce further evidence for the correlation between neuroleptic activity and dopamine receptor blockade. It is anticipated that the isomers of butaclamol will be valuable as pharmacological tools for investigating of central dopaminergic phenomena.

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Note in proof: We have been informed that the dopamine stimulated adenylate cyclase of the olfactory tubercle shows similar stereospecificity for butaclamol (Lippmann, Pugsley & Merker, *Life Sci.*, in press).

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