# The noradrenergic cyclic AMP generating system in the rat limbic forebrain and its stereospecificity for butaclamol

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Butaclamol, a benzocycloheptapyridoisoquinolinol derivative, is a new neuroleptic agent that can be separated into two optical isomers. The pharmacological activity of the drug resides entirely in the (+)-enantiomer, the (—)-enantiomer being inactive (Bruderlein, Humber & Voith, 1975; Voith & Cummings, 1975). The (+)-enantiomer has been found to block the stimulation of a dopamine sensitive adenylate cyclase in cell-free homogenates of the corpus striatum (Miller, Horn & Iversen, 1975) and olfactory tubercle (Lippmann, Pugsley & Merker, 1975), whereas the (—)-enantiomer lacks activity. Moreover, the (+)-enantiomer, but not the (—)-enantiomer of butaclamol decreases the apparent $K_m$ of tyrosine hydroxylase for the pteridine cofactor $DMPH_4$ in striatum and nucleus accumbens (Zivkovic, Guidotti & Costa, 1975). Recently, stereospecificity has also been demonstrated for the blockade of a noradrenaline sensitive adenylate cyclase in homogenates in the rat limbic forebrain (Horn & Phillipson, 1975).

Studies from our laboratory have previously demonstrated a specific noradrenergic cyclic AMP generating system in slices of the rat limbic forebrain that can be antagonized by various neuroleptic agents (Blumberg, Taylor & Sulser, 1975; Blumberg, Vetulani & others, 1976). In contrast to equivocal results obtained with adenylate cyclase systems in cell free preparations, the noradrenergic cyclic AMP generating system in slices displays unequivocal characteristics of a central noradrenaline receptor that adapts its sensitivity to noradrenaline in a manner inversely related to the degree of its stimulation by the catecholamine (Blumberg & others, 1976; Vetulani, Stawarz & Sulser, 1976). In studying receptor characteristics, it thus appears that physical damage to the cyclase system is one important factor, since the magnitude of the hormonal response is known to vary inversely with the degree of homogenization (Oye & Sutherland, 1966). In the present studies, we describe the action of the two enantiomers of butaclamol on the specific noradrenaline-sensitive cyclic AMP system in slices of the limbic forebrain.

Male Sprague-Dawley rats (180 to 200 g) were decapitated and the limbic forebrain area was rapidly dissected and removed as described by Blumberg & others (1976). The tissue slices were prepared and incubated in Krebs-Ringer bicarbonate buffer (pH 7·4) essentially according to Kakiuchi & Rall (1968) as modified in our laboratory and described by Blumberg & others (1975). The cyclic AMP was isolated by ion exchange chromatography (Dowex AG 50-W-X8; $H^+$ form) and assayed by the protein binding assay of Gilman (1970). Proteins were determined according to Lowry, Rosebrough & others (1951).

In the first series of experiments, (+)-and (—)-butaclamol (50 μM) were added to the incubation medium 14 min before an equimolar concentration of noradrenaline. Table 1 shows that the (+)-enantiomer blocks the noradrenaline-stimulated increase in cyclic AMP by 60% while the (—)-enantiomer does not exert a significant effect. In the next series of experiments, we studied the effect of various concentrations

Table 1. *Effect of equimolar concentrations of the* (+)- *and* (—)-*enantiomers of butaclamol on the cyclic AMP response to noradrenaline* (*NA*) *in limbic forebrain slices.*

| | Cyclic AMP response† pmol $mg^{-1}$ protein ± s.e.m. NA | Drug + NA | % control response |
|---|---|---|---|
| (+)-Butaclamol | 203·4 ± 17·9 (5) | 80·4 ± 12·4 (9)* | 40* |
| (—)-Butaclamol | 125·3 ± 24·6 (5) | 103·9 ± 31·8 (8) | 83 |

The enantiomers of butaclamol ($5 \times 10^{-5}$M) were added to the incubation medium 14 min before NA ($5 \times 10^{-5}$M). The reaction was terminated 10 min following the addition of NA and cyclic AMP isolated and assayed as described. Number in parentheses indicates the number of samples. Basal control concentrations were in pmol $mg^{-1}$ protein ± s.e.m.: 28·9 ± 2·3.

†Difference in the concentration of cyclic AMP beteen the p paration exposed to $5 \times 10^{-5}$M NA (with or without butaclam and that of the control preparation.

*$P<0.01$ (calculated by two-tailed *t*-test).

Table 2. *Effect of various concentrations of* (+)- *and* (—)-*butaclamol on the cyclic AMP response to 5* μM *noradrenaline* (*NA*) *in limbic forebrain slices.*

| | Cyclic AMP response† pmol $mg^{-1}$ protein ± s.e.m. | % Control response |
|---|---|---|
| 5 μM NA (Control) | 73·9 ± 8·8 (25) | |
| 5 μM NA + 0·1 μM (+)-butaclamol | 39·3 ± 4·8* (18) | 53* |
| 5 μM NA + 1·0 μM (+)-butaclamol | 35·3 ± 8·3* (12) | 48* |
| 5 μM NA + 10 μM (+)-butaclamol | 28·3 ± 6·2* (13) | 38 |
| 5 μM NA (Control) | 68·4 ± 5·3 (25) | |
| 5 μM NA + 0·1 μM (—)-butaclamol | 95·2 ± 12·7 (11) | 139 |
| 5 μM NA + 1·0 μM (—)-butaclamol | 71·4 ± 8·2 (13) | 104 |
| 5 μM NA + 10 μM (—)-butaclamol | 55·3 ± 5·5 (14) | 81 |

The enantiomers of butaclamol were added to the incubation medium 14 min before NA. The reaction was terminated 10 min after the addition of NA and cyclic AMP isolated and assayed as described in the text. Numbers in parentheses indicate the number of samples. The basal concentrations of cyclic AMP were in p mol $mg^{-1}$ protein ± s.e.m.: 33·4 ± 2·0.

† Difference in the concentration of cyclic AMP between the preparation exposed to 5 μM NA (with or without butaclamol) and that of the control preparation.

* $P<0.01$ (calculated by two-tailed *t*-test).

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of the (+)- and (—)-enantiomers of butaclamol on the increase in cyclic AMP elicited by 5 $\mu$M noradrenaline (approximate Ka value). As shown in Table 2, (+)-butaclamol inhibits the increase in cyclic AMP in a dose-dependent manner, having an IC50 of approximately 0·5 $\mu$M. (—)-Butaclamol was found to be a weak inhibitor of the noradrenaline stimulated rise in cyclic AMP, having an IC50 $>>10$ $\mu$M. Neither (+)- nor (—)-butaclamol, in concentrations from 0·1 to 50 $\mu$M, changed the basal concentration of the nucleotide.

The present results indicate that the blocking effect of butaclamol on the specific noradrenergic cyclic AMP generating system in slices of the limbic forebrain also resides in the (+)-enantiomer thus also demonstrating sterospecificity for central noradrenaline receptor blockade. The availabilty of a stereochemically specific antagonist of a central noradrenaline adenylate cyclase receptor should provide an important tool to further elucidate this system. Although the stereospecific blockade by butaclamol of limbic dopamine receptors is quantitatively more pronounced (Lippmann & others, 1975), the present results do nevertheless further support the view that blockade of noradrenergic receptors in the limbic system may also contribute to the pharmacologic and perhaps therapeutic action of antipsychotic drugs.

We are grateful to Dr D. J. Marshall of Ayerst Research Laboratories Montreal, Canada for the generous supply of the (+)- and (—)-enantiomers of butaclamol. The research was supported by USPHS Grants MH-11468 and 5-TO1-GM0058.

February 2, 1976

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# A pharmacologic model of Huntington's chorea

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There are few animal models for Huntington's chorea, a hereditary disorder characterized by involuntary choreic movements and psychotic behavior (Bruyn, 1968). Most models for this disease are produced by the systemic administration of L-dopa to normal animals (Mones, 1973) or L-dopa in animals with lesions in the striatum (Sax, Butters & others 1973) or dopaminergic tracts (Ng, Gelhard & others, 1973). However, recent studies on the involuntary movements produced in animals by the intrastriatal (i.s.) injection of 3-methoxytyramine (3-MT) may lead to a useful model of the disease (Cools, 1972; Dill & Campbell, 1973; Furgeson, Dill & Dorris, 1976). The i.s. injection of 3-MT, a normal brain metabolite of dopamine, and mescaline both produced identical movement disorders in rats and squirrel monkeys (Dill & Campbell, 1973) leading the authors to speculate that 3-MT could be a factor in psychosis. Thus, the proposed 3-MT model could reflect both motor and behavioural aspects of the disease.

If 3-MT-induced movements can serve as a model for Huntington's chorea, they should respond to other drugs in a manner similar to the responses of the disease to these drugs. For example, L-dopa therapy is known to exacerbate the symptoms of Huntington's chorea (Klawans, 1970), while neuroleptic drugs are known to be beneficial (Whittier, 1973). Monoamine oxidase inhibitors (MAOIs) plus methionine loading, factors, which theoretically should increase brain concentrations of 3-MT, are known to exacerbate the symptoms of some psychotic states (Pollin, Cardon & Kety, 1961; Brune & Himwich, 1962; Park, Baldessarini & Kety, 1965). Thus, a study was made of the effects of L-dopa, a MAOI and three neuroleptic drugs on 3-MT-induced dyskinesias in rats.

Male albino rats (250–300 g) were permanently cannulated bilaterally with stainless steel cannulae in the neostriatum at stereotaxic co-ordinates A +7·8,

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