COMMUNICATIONS

Blockade by pimozide of a noradrenaline sensitive adenylate cyclase in the limbic forebrain: possible role of limbic noradrenergic mechanisms in the mode of action of antipsychotics

Blockade of catecholaminergic and particularly of dopaminergic receptor sites has been widely implicated in the mechanism of action of various antipsychotic agents, and neuroleptics such as the diphenylbutylpiperidine derivative pimozide appear to be selective blockers of dopamine receptors (Janssen, 1967; Janssen, Niemegeers & others, 1968; Andén, Butcher & others, 1970; Andén, 1974). A number of recent studies have suggested that the blockade by antipsychotics of dopamine receptors in the mesolimbic system may be more closely correlated with their antipsychotic activity than the action of these drugs on striatal dopamine receptors (van Rossum, Janssen & others 1970; Andén, 1972; Andén & Stock, 1973). Recent results from our laboratories indicate that the occurrence of extrapyramidal side effects in man and the degree of amphetamine antagonism in animals with unilateral lesions of the substantia nigra are positively correlated with the potency of neuroleptics in blocking striatal dopamine receptors, but suggest a dissociation between dopaminergic blockade in striatum and limbic forebrain and antipsychotic efficacy (Sulser, Stawarz & Blumberg, 1974). In this regard, it is pertinent that clozapine, an effective antipsychotic that does not cause extrapyramidal side effects (Berzewski, Helmchen & others, 1969; Angst, Bente & others, 1971a; Angst, Jaenicke & others, 1971b) in man, is not cataleptic and is ineffecive in antagonizing the action of the dopamine agonist apomorphine (Stille, Lauener & Eichenberger, 1971).

At the molecular level, adenylate cyclase receptors have been proposed for dopamine in both the caudate nucleus and the limbic system (Kebabian, Petzold & Greengard, 1972; Clement-Cormier, Kebabian & others, 1974) and for catecholamines in the cerebral cortex (Von Hungen & Roberts, 1973; Perkins & Moore, 1973). Moreover, under a variety of test situations, antipsychotic drugs have been shown to interfere with the cyclic AMP generating system in various cortical and subcortical areas of the brain (Uzunov & Weiss, 1971; Palmer, Robinson & others, 1972; Kebabian & others, 1972; Von Hungen & Roberts, 1973; Horn, Cuello & Miller, 1974; Clement-Cormier, & others, 1974).

To test the hypothesis whether or not the antipsychotic pimozide is indeed a selective dopamine blocker, we studied its ability to block the stimulation of the formation of cyclic AMP caused by various neurotransmitters which are present in tissues from limbic forebrain.

Male Sprague-Dawley rats (180–200 g) were decapitated and the limbic forebrain (amygdala, preoptic area, olfactory tubercle, part of the nucleus accumbens, nucleus interstitialis, stria terminalis) was rapidly removed. The methods employed for the preparation and incubation of tissue slices were essentially those of Kakiuchi & Rall (1968) with minor modifications. The tissue samples were placed in ice cold Krebs-Ringer bicarbonate buffer (pH 7·4), previously saturated with 5% CO_2 in oxygen. The tissue was chopped into blocks of approximately 0·3 mm³ on a chilled glass plate resting on ice. The chopped tissue was pooled in cold Krebs-Ringer buffer and washed three times. Fresh Krebs-Ringer buffer was then added to the washed slices

in such a way that 1 ml aliquots contained 75–100 mg tissue.   At 2 min intervals 1 ml
of the tissue suspension was removed with an Eppendorf pipette and placed in beakers
containing 30 ml Krebs-Ringer buffer.   The tissue samples were allowed to incubate
in a Dubnoff incubator at 37° for 30 min with 5 % CO_2 in oxygen being bubbled into
each beaker.   The medium was then aspirated and 30 ml fresh Krebs-Ringer buffer
(37°) were added to the beaker.   At this time either drugs or solvents in a volume of
100 μl were added.   The sample was allowed to incubate for 14 min.   At this time
either putative neurotransmitters or the solvent were added to the media in a volume
of 100 μl.   The incubation continued for another 10 min at which time all but 3 ml
of the buffer was aspirated from the beaker.   The remaining buffer and tissue slices
were quickly transferred with a Pasteur pipette to a test tube containing 0·5 ml 2·1N
perchloric acid and homogenized for 30 s using a Polytron (Brinkman) homogenizer.
The perchloric acid contained a standard amount of ^{3}H-cyclic AMP (1000 counts
min^{-1} 2·7 ml; specific activity 24·1 ci mmol^{-1}) for recovery.   The samples were centri-
fuged at 3700 rev min^{-1} for 30 min and the supernatant was applied to a column of
Dowex (AG50-W-X8, H$^+$ form, 100–200 mesh).   The nucleotide was then eluted
with 0·1N HCl, the eluate lyophilized and assayed according to Gilman (1970).
Proteins were determined according to Lowry, Rosebrough & others (1951).
Pimozide was generously supplied by McNeil Laboratories, Fort Washington,
Pennsylvania.   The drug was dissolved in 1·5 % tartaric acid (final concentration).

Because the limbic forebrain receives ascending noradrenergic fibres originating
in cell bodies of the pons and medulla oblongata, dopaminergic fibres from the A10
region and serotoninergic fibres from the anterior raphé complex (Andén, Dahlström
& others, 1966; Dahlström & Fuxe, 1964), it was of interest to know first whether or
not an adenylate cyclase sensitive to one, two or all three of the putative neuro-
transmitters (noradrenaline, dopamine, 5-HT) was present in slices of the limbic
forebrain.

Fig. 1A shows the effects of all three neurohormones on the accumulation of cyclic
AMP in slices of the limbic forebrain.   Noradrenaline produced a marked increase in
the level of cyclic AMP.   The concentration necessary for half-maximum stimulation
(Ka) was about 2·7 × 10^{-6}M and that for maximum stimulation (450 % of control)
was about 10^{-5}M noradrenaline.   The time course of the effect of 5 × 10^{-5}M nor-
adrenaline on the level of cyclic AMP is depicted in Fig. 1B.   The maximum rise in
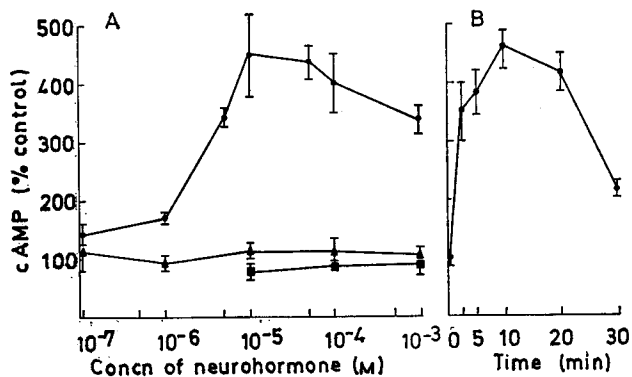

FIG. 1.   (A) Effect of various concentrations of (−)-noradrenaline (●——●), dopamine (▲——▲)
and 5-HT (■——■) on the accumulation of cyclic AMP in slices from the limbic forebrain of
rats.   Basal control values were 26·0 ± 2·0 p mol cyclic AMP per mg protein.   (B) Time course
of the effect of (−)-noradrenaline (5 × 10^{-5}M) on the accumulation of cyclic AMP in tissue slices
from the limbic forebrain.   Control values were 46·0 ± 4·9 p mol cyclic AMP per mg protein.
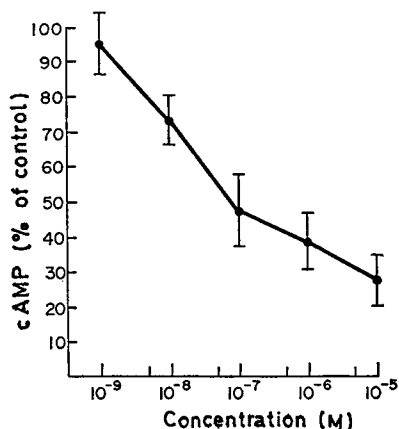All values are expressed as the mean percentage of control values ± s.e.m.   N = 4.

FIG. 2. Inhibiting effect of pimozide on the accumulation of cyclic AMP elicited by (−)-noradrenaline in slices from the limbic forebrain of rats.   The data are expressed as a percentage of control stimulation elicited by 5×10^{-6}M (−)-noradrenaline (mean values $\pm$ s.e.m.) which was $62\cdot23 \pm 5\cdot9$ p mol cyclic AMP per mg protein.   Basal control values: $26\cdot1 \pm 2\cdot1$ p mol cyclic AMP per mg protein.   N = 6.

the level of the nucleotide occurred after 10 min of incubation.   At 30 min, its level was still significantly elevated.   There is evidence that the noradrenaline sensitive adenylate cyclase in limbic forebrain structures is localized postsynaptically as the sensitivity to noradrenaline increased markedly following destruction of presynaptic noradrenergic nerve terminals with 6-hydroxydopamine (Blumberg & Sulser, 1974). Interestingly, dopamine and 5-HT were without effect at all concentrations tested. It is noteworthy that the presumptive selective dopamine blocker pimozide, when added to the incubation medium for 14 min before the addition of noradrenaline, caused a dose-dependent inhibition of this specific noradrenergic cyclic AMP response (Fig. 2) while not changing the basal level of the cyclic nucleotide.   The increase in cyclic AMP caused by 5×10^{-6}M noradrenaline was reduced by 50% in the presence of $7\cdot5 \times 10^{-8}$M pimozide.   In separate experiments it was found that pimozide (10^{-3} to 10^{-6}M) did not significantly change the activity of cyclic AMP phosphodiesterase in *in vitro* preparations from the limbic forebrain (unpublished observations from this laboratory).   It is concluded, therefore, that the drug's effect on the noradrenaline-induced accumulation of cyclic AMP is the consequence of an inhibition of the noradrenaline specific adenylate cyclase.

The present results demonstrate the occurrence in slices of the limbic forebrain of a noradrenaline but not a dopamine or 5-HT-sensitive adenylate cyclase, and show that the noradrenaline-induced increase in cyclic AMP in this area of the brain is blocked by low concentrations of pimozide.   These results thus indicate that pimozide is not a selective dopamine blocking agent.   Although dopamine did not alter the accumulation of cyclic AMP in slices from limbic forebrain, a dopamine-sensitive adenylate cyclase exists in homogenates of mesolimbic structures and is inhibited by neuroleptics (Clement-Cormier & others, 1974; Horn & others, 1974).   The reasons for the differences in hormonal sensitivity to this catecholamine in slices and homogenates are unknown.

Since the limbic forebrain receives ascending noradrenergic fibres through the medial forebrain bundle and receives and integrates sensory inputs from all the sensory systems and refluxes information into the entire hypothalamic and lower reticular fields (Gloor, 1955; MacLean, 1958; Nauta, 1958), it is tempting to speculate that the noradrenaline sensitive cylic AMP system in this area of the brain may be implicated in the mode of action of antipsychotics.   This hypothesis is strengthened

by our finding that clozapine, an antipsychotic with pharmacologically weak or lacking antidopaminergic properties, is as effective as pimozide in blocking the cyclic AMP response to noradrenaline in the limbic forebrain (unpublished observations). The elucidation of the role of noradreneric mechanisms and of cyclic AMP as a second intracellular messenger in the limbic forebrain and its modification by antipsychotics is obviously an important area for future investigation.

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