Effects of Penfluridol on Dopamine-Sensitive Adenylate Cyclase in Corpus Striatum and Substantia Nigra of Rats

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Summary. Penfluridol, a neuroleptic with diphenylbutyl piperidine structure, blocked the dopamine-sensitive adenylate cyclase in homogenates of corpus striatum and substantia nigra of rats, probably by a competitive antagonism versus dopamine.

Since the discovery of COURVOISIER et al.[2] that chlorpromazine blocks the effects of adrenaline on blood pressure, many studies were performed to evaluate the molecular mechanism of the action of neuroleptics. Probably a blockade of central dopamine receptors seems to be of particular relevance for their neuroleptic and antipsychotic effects[3]. This blockade seems to occur by a competitive mechanism against dopamine[4], not only induced by phenothiazines, but also by butyrophenones and the diphenylbutyl piperidine derivative pimozide. However, all these neuroleptics have other effects in addition to the blockade of dopamine receptors. Even pimozide, which for some time was regarded as the 'purest' dopaminergic blocker, has effects on noradrenergic[5] and serotoninergic[6] mechanisms. On the other hand, NOSE and TAKEMOTO[6], basing on a series of experiments, suggested that penfluridol, a compound of the diphenylbutyl piperidine series, might block dopamine receptors selectively. It seemed, therefore, of interest to study the interactions of this drug with dopamine on the adenylte cyclase in two important areas of the extrapyramidal system, namely in the corpus striatum and the substantia nigra. The dopamine-sensitive adenylate cyclase seems to be the in vitro system most appropriate for studying subcellular mechanisms of neuroleptics.

Materials and methods. Male albino Wistar rats (TNO/W70, F. Winkelmann, D-4791 Borchen) of 150–200 g were used. The rats were decapitated and the striata or substantiae nigrae were rapidly prepared, weighed and gently homogenized by hand in a glass homogenizer with a teflon pestle in ice-cooled 50 volumes (w/v) (striata) or 20 volumes (subst. nigrae) of 2 m*M* *tris*-(hydroxymethyl)-aminoethane maleate buffer (pH = 7.4). The activity of the dopamine-sensitive adenylate cyclase was estimated by a slight modification of the method of CLEMENT-CORMIER et al.[4], as described elsewhere[7], the concentration of cycl. AMP by GILMAN's protein binding assay[8]. It was necessary to pool the subst. nigrae of at least 3 animals for one estimation.

Results and discussion. In homogenates of rat striata, dopamine, as expected, stimulated the synthesis of cycl. AMP in a dose-dependent manner (figure, *a*). A very similar dose-relationship of dopamine occurred in homogenates of the substantia nigra (figure, *b*), indicating a similar potency of dopamine in striatal and nigral tissue.

[1] Acknowledgment. We thank Dr P. Janssen (Beerse, Belgium) for a generous gift of penfluridol.

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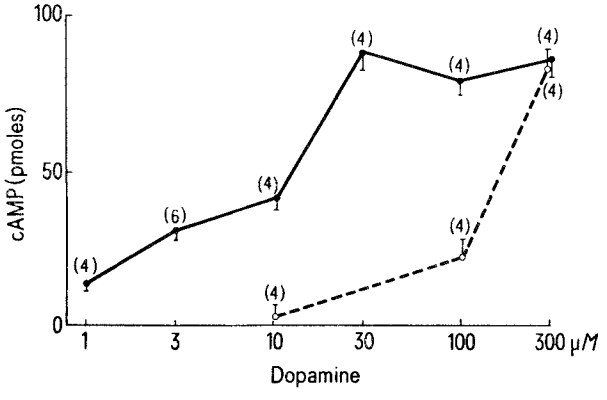
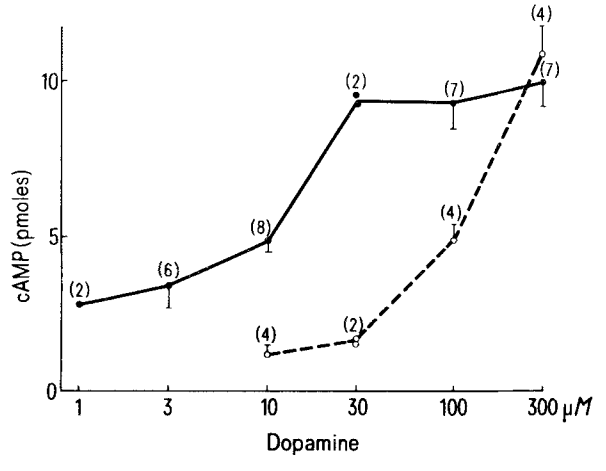


a) Stimulation by dopamine of the adenylate cyclase in homogenates of rat striata in control tissue (continuous line) or in presence of 3 µ*M* penfluridol (dotted line). Abscissa: dopamine concentration «µ*M*», ordinate: stimulation of cycl. AMP synthesis during 5 min by dopamine «pmoles cycl. AMP/mg tissue ± S. E. M.». N is indicated by the numbers in brackets. Basal activities in controls: 74.0 ± 1.8, in penfluridol-treated tissue: 74.0 ± 1.9 pmoles cycl. AMP/mg tissue. Significances: $p < 0.001$ at 10 and 100 µ*M* dopamine, n. s. at 300 µ*M* dopamine (Student's *t*-test).



b) Stimulation by dopamine of the adenylate cyclase in homogenates of the substantia nigra of rats in control tissue (continuous line) or in presence of 3 µ*M* penfluridol (dotted line). Basal activities in controls: 10.28 ± 0.40, in penfluridol-treated tissue: 9.40 ± 0.34 pmoles cycl. AMP/mg tissue. Significances: $p < 0.001$ at 10 and $p < 0.005$ at 100 µ*M* dopamine, n. s. at 300 µ*M* dopamine (Student's *t*-test).

However, the maximal stimulation in nigral tissue was only about one-tenth of that in striatal tissue. Penfluridol inhibited the dopamine-sensitive adenylate cyclase in both brain areas in a similar way, inducing a shift of the dose-response-curve to the right.

These results clearly indicate the occurrence of a dopamine-stimulated adenylate cyclase in the substantia nigra of rats. This observation is in good agreement with that published by PHILLIPSON and HORN[9], just when our manuscript was in preparation. The dopamine receptors in both brain regions seem to have a similar affinity for dopamine. The reason for the difference in the efficacy of dopamine might be either a difference in the density of dopamine receptors in both regions or a difference in the transmission from the receptors to the enzyme. If the theory is correct that dopamine, released from dendrites of dopaminergic neurones in the substantia nigra, again reacts with dopaminergic neurones[10-12] (in contrast to the nerve endings, where dopamine-sensitive adenylate cyclase probably reflects the reaction of dopamine with receptors at non-dopaminergic neurones), then the dopamine-sensitive adenylate cyclase seems to play a role in auto-inhibitory actions of dopamine, released from the dendrites and acting on dopaminergic neurones, originating in the substantia nigra. It is tempting to speculate that an adenylate cyclase system might also play a role in dopamine receptors, located presynaptically at the nerve endings in dopaminergic neurones ('autoreceptors'), although the bulk of cycl. AMP, formed after stimulation by dopamine, seems to be located postsynaptically[13] and hence might mask the small amount of cycl. AMP, formed presynaptically.

Penfluridol is apparently a competitive inhibitor of dopamine in both brain regions, since it induces a parallel-shift of the dose-response-curves of dopamine to the right. A Lineweaver-Burk-plot of our results (which is not shown here) clearly supports these conclusions.

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Potentiation by Taurine of the Inotropic Effect of Ouabain and the Content of Intracellular Ca^{++} and Taurine in the Heart

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Summary. Under certain conditions, taurine (3.0 m*M*) potentiated cardiac contractile response to ouabain in the normal medium. The potentiation by taurine was also observed in the low K^+ medium, in which the positive inotropic effect of ouabain increased. The potentiation as seen in both media was, at least in part, due to the increase by taurine of Ca^{++} content in the heart. Taurine in the heart was not directly related to this potentiation.

Taurine is present in mammalian hearts in large quantities[2-4], and the concentration of myocardial taurine can be altered by some diseases[5-7] and drugs[7]; its function in the heart, however, has long been unknown. At present, taurine is known to possess an antiarrhythmic effect. For instance, READ and WELTY[8] reported that i.v. taurine depressed the development of epinephrine-induced ventricular premature contraction and stopped digoxin-induced arrhythmias in vagotomized dogs. In addition, the present authors showed that taurine prevented the prolongation of P–R intervals caused by acutely-infused ouabain in rats[9], and the T wave-disappearance in the guinea-pig treated chronically with digitoxin[10]. Unlike the effect of taurine in whole animals, in isolated hearts taurine increased contractile response to strophanthin-K[11] and inhibited a decrease of contractile force by Ca^{++}-free media[12].

Ca^{++}, an important ion in an excitation-contraction coupling, was shown to be taken up more extensively in the taurine-treated heart than in the control[12] and with the sarcoplasmic reticulum isolated from rat skeletal muscle, taurine slowed a rate of loss of Ca^{++} transport caused by phospholipase C[13], suggesting that taurine was acting as a membrane stabilizer.

We therefore attempted to find whether or not the potentiation by taurine of the positive inotropic effect of ouabain paralleled changes in intracellular Ca^{++} and taurine contents in isolated heart preparations. In addition, since the inotropic response to ouabain was increased in a low K^+ medium[14], it was also examined whether or not taurine could potentiate further the ouabain-induced inotropism in the medium.

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