THE EFFECT OF DIHYDROERGOTOXIN, PHENTOLAMINE AND PINDOLOL ON CATECHOLAMINE-STIMULATED ADENYLCYCLASE IN RAT CEREBRAL CORTEX

R. MARKSTEIN and H. WAGNER

Biological and Medical Research Division, SANDOZ Ltd., Basel Switzerland

Received 19 April 1975

1. Introduction

There is now considerable evidence that in certain regions of the central nervous system neurons exist which bear adrenoceptors, activation of which alters the cyclic AMP level within the cell [1–3]. Recently, a relationship between noradrenaline-induced formation of cyclic AMP in rat brain cortex and behavior has been reported [4]. Since dihydroergotoxin (Hydergine ®) is effective in the treatment of disturbances in behavior occurring in elderly people, the effect of the drug on central catecholamine activity has been investigated and compared with the effects of phentolamine and pindolol. Experiments were performed in the rat; rat cerebral cortex, like human cortex, is more sensitive than that of many other species to the stimulant effects of noradrenaline [5–7].

2. Materials and methods

2.1. *Chemicals*

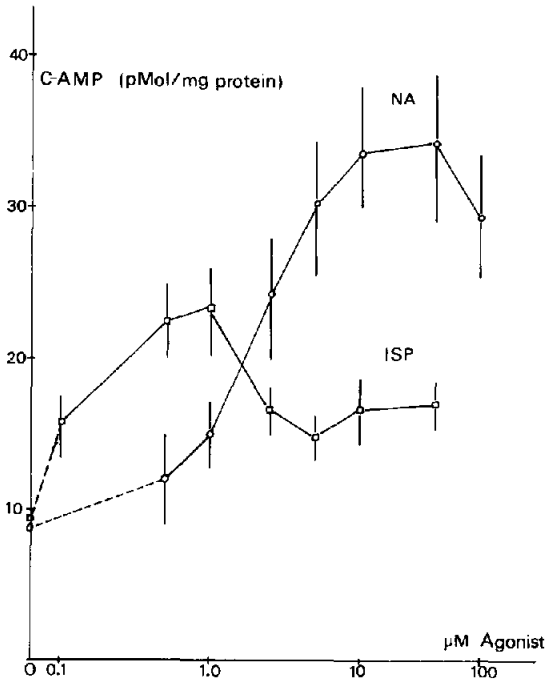
L-noradrenaline-bitartrate was obtained from Hoechst and 1-isoproterenol HCl from Sigma. Phentolamine HCl was a gift from Ciba-Geigy. D,L-Pindolol and dihydroergotoxin mesilate came from Sandoz.

2.2. *Tissue preparation and incubation procedure*

Young male rats (Sandoz-Wistar) weighting approx. 100 g were sacrificed by decapitation. In each experiment, cortical tissue, obtained from 4 rats, was chopped into pieces of approx. 0.34 mm × 0.34 mm × 1 mm and suspended in Krebs-Ringer bicarbonate buffer at 30°C (50 mg tissue/ml). The suspension was gently stirred and gassed with a mixture of 95% O_2–5% CO_2 and incubated for a period of 35 min in order to stabilize intracellular cyclic AMP levels. Aliquots of suspension were transferred into incubation vessels containing fresh medium and incubated for further 15 min. Noradrenaline or isoproterenol in concentrations between 10^{-7}M and 10^{-4} M was added and the reaction stopped 8 min later by homogenizing aliquots from each vessel with cold 0.4 N $HClO_4$. The protein was separated from the homogenate by centrifugation. The supernatant was neutralized with $KHCO_3$ and the cyclic AMP content determined by a solid phase protein binding assay [8]. The measured cyclic AMP values were correlated with the protein content which was determined by the biuret method. No cyclic AMP could be detected in samples treated with cyclic AMP-phosphodiesterase, providing evidence for the identity of the measured values of cyclic AMP.

3. Results

Both noradrenaline and isoproterenol caused dose-dependent increases in the cyclic AMP content (fig.1). The maximum increase obtained with noradrenaline (10^{-5} M) was between 20 and 30 pmol/mg protein, whereas isoproterenol increased the level of cyclic AMP by a maximum of only 10–15 pmol/mg protein at 10^{-6} M. Higher concentrations or incubation periods in excess of 8 min caused decreases in cyclic AMP content. A second series of experiments were performed in which noradrenaline 5×10^{-6} M or isoproterenol 5×10^{-7} M was added to the incubation mixture in the presence of varying concen-



trations of dihydroergotoxin, phentolamine and pindolol.

Dihydroergotoxin inhibited the stimulant effect of noradrenaline by approximately 50% at a concentration of 10^{-8} M and by approximately 80% at a concentration at 10^{-6} M, but had no significant effect on the increases in cyclic AMP induced by isoproterenol (table 1). With the α-adrenoceptor blocking agent, phentolamine, 50% inhibition of the response to noradrenaline was obtained at 10^{-7} M and approximately 80% inhibition at 10^{-6} M; a weak inhibitory effect on responses to isoproterenol occured at the highest concentration. In contrast, the β-adrenoceptor blocking agent, pindolol, inhibited responses to both noradrenaline and isoproterenol dose-dependently,

Fig. 1. The effect of various concentrations of noradrenaline (NA) and isoproterenol (ISP) on cyclic AMP content (pmol/mg protein) in slices of rat brain cortex. Each value is the mean ± s.e. of three experiments.

Table 1
Cyclic AMP content (pmol/mg protein) in slices of rat brain cortex after stimulation by noradrenaline (NA) and isoproterenol (ISP) in the presence of various concentrations of dihydroergotoxin, phentolamine and pindolol.

Concentration (NA)		Dihydro-ergotoxin	Phentolamine	Pindolol
0	0	9.9 ± 1.0	9.3 ± 0.8	11.0 ± 0.7
$5 \cdot 10^{-6}$ M	0	32.0 ± 2.3	30.0 ± 1.3	31.0 ± 2.0
$5 \cdot 10^{-6}$ M	10^{-9} M	30.5 ± 3.0	28.8 ± 1.3	23.7 ± 1.0
$5 \cdot 10^{-6}$ M	10^{-8} M	21.7 ± 1.0	22.2 ± 0.7	18.8 ± 1.0
$5 \cdot 10^{-6}$ M	10^{-7} M	17.6 ± 0.8	19.5 ± 0.8	12.0 ± 0.7
$5 \cdot 10^{-6}$ M	10^{-6} M	13.0 ± 1.1	13.4 ± 0.6	10.0 ± 0.7

Concentration (ISP)	Antag.	Dihydro-ergotoxin	Phentolamine	Pindolol
0	0	10.9 ± 1.3	1.9 ± 1.3	11.4 ± 1.7
$5 \cdot 10^{-7}$ M	0	24.1 ± 0.7	24.1 ± 0.7	28.8 ± 1.0
$5 \cdot 10^{-7}$ M	10^{-8} M	24.0 ± 2.7	20.2 ± 2.8	21.6 ± 2.3
$5 \cdot 10^{-7}$ M	10^{-7} M	22.7 ± 1.5	20.1 ± 2.0	12.0 ± 1.7
$5 \cdot 10^{-7}$ M	10^{-7} M	20.8 ± 2.6	19.7 ± 0.4	10.9 ± 3.4

Each result with NA is the mean ± s.e. of 9 experiments and each result with ISP the mean ± s.e. of 3 experiments.

complete blockade of the effects of both agonists occurred at a concentration of 10^{-7} M.

4. Disucssion

These experiments show that β-adrenoceptors in rat cortical tissue have similar characteristics to those present in the periphery. They can be stimulated by a selective β-adrenoceptor agonist, isoproterenol, and the effect can be antagonised only with a selective β-adrenoceptor blocking agent. On the other hand the noradrenaline receptors appear to differ from α-receptors in the periphery in that the increase in cyclic AMP induced by noradrenaline can be antagonised both by α- and β-adrenoceptor antagonists at similar concentrations. The results show in addition that dihydroergotoxin has a high affinity for central noradrenaline receptors. It is conceivable that this effect may be related to its therapeutic action in man.

References

[1] Perkins, J. P. and Moore, M. M. (1973) J. Pharmacol. Exp. Ther. 185, 371–378.
[2] Palmer, G. C., Sulser, F. and Robison, G. A. (1973) Neuropharmacology 13, 327–337.
[3] Kalisker, A., Rutledge, C. O. and Perkins, J. P. (1973) Mol. Pharmacol. 9, 619–629.
[4] Skolnick, P. and Daly, J. W. (1974) Science 184, 175–177.
[5] Forn, J. and Krishna, G. (1971) Pharmacology 5, 193–204.
[6] Shimizu, H., Tanaka, S., Suzuki, T. and Matsukado, Y. (1971) J. Neurochem 18, 1157–1161.
[7] Berti, F., Trabucchi, M., Bernareggi, V. and Fumagalli, R. (1973) Advan. Biosci. 9, 475–480.
[8] Fisch, H. U., Pliska, V. and Schwyzer, R. (1972) Eur. J. Biochem. 30, 1–6.