The Influence of Propranolol and Dibenzyline on Glycogenolytic Effects of Some Biogenic Amines in Rat Brain Slices

The control of glycogen concentration in the brain tissue is a suitable model for the biochemical action and activity of cyclic 3′, 5′-AMP (CAMP) in the brain tissue. CAMP is now known to exert control over the activities of both the phosphorylase and glycogen synthethase systems.

The regulation of CAMP levels in cerebral cortical slices at present has been found to be influenced by biogenic amines such as noradrenaline, dopamine, histamine and serotonin[1-8]. In view of published data in the cerebral cortex, the existence of 2 interacting regulatory units governing adenyl cyclase activity was suggested; 1 for adenosine and 1 for biogenic amines. Evidence for separate regulatory receptors for histamine and noradrenaline in rabbit cerebellum and cerebellar cortex has also been reported[6,7]. The data recently obtained suggested the existence of compartmentalized pools of adenine nucleotides serving as precursors for CAMP, and suggest that these pools are regulated in a synergistic manner by separate 'receptors' for adenosine, histamine and either noradrenaline or serotonin[9].

We recently reported the glycogenolytic effects of adrenaline, noradrenaline, dopamine, histamine, serotonin, as well as of CAMP and cyclic N-2-O-dibutyryl-3′, 5′-AMP (db-CAMP) in rat brain slices[10-13]. The greatest glycogenolytic effect was obtained with db-CAMP, histamine, noradrenaline and serotonin, while the effects of dopamine and CAMP were not so expressive[13].

As the possibility was suggested that both α- and β-adrenergic blocking drugs could interact with regulatory unit of adenyl cyclase[14], it seemed to be of interest to test whether either β-adrenergic blocking drug propranolol or α-adrenergic-blocking drug dibenzyline would be able to prevent the glycogenolytic influence of some biogenic amines, as well as of CAMP and db-CAMP, indicating the existence of one or more regulatory units of adenyl cyclase for biogenic amines in the brain tissue.

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Table I. In vitro effect of noradrenaline, dopamine, histamine, serotonin, CAMP and db-CAMP on glycogen concentration in brain of rat treated before with propranolol (10 mg/kg)

Treatment of the tissue	Brain slices		
	Cortex	Caudate	Thalamus
Controls	31.4 ± 1.3	48.5 ± 1.2	13.4 ± 1.0
Noradrenaline ($10^{-4}\mu M$/ml)	28.5 ± 1.4	46.5 ± 1.4	14.5 ± 1.5
Dopamine ($10^{-4}\mu M$/ml)	30.0 ± 1.0	44.5 ± 1.3	13.8 ± 1.2
Histamine ($10^{-4}\mu M$/ml)	18.4 ± 1.0[a]	22.5 ± 1.4[a]	7.0 ± 1.0[a]
Serotonin ($10^{-4}\mu M$/ml)	17.5 ± 1.3[a]	24.1 ± 1.2[a]	8.1 ± 1.1[b]
CAMP ($10^{-3}\mu M$/ml)	21.4 ± 1.4[b]	33.5 ± 1.6[b]	8.0 ± 1.2[a]
db-CAMP ($10^{-3}\mu M$/ml)	14.5 ± 1.4[a]	23.0 ± 1.4[a]	4.5 ± 1.5[a]

[a] $p < 0.01$ in comparison with the controls. [b] $p < 0.01$ in comparison with the controls. The amount of glycogen is expressed in mg /100ml of tissue. The numbers indicate the mean value (M) of 5 experiments ± S.E.M.

Table II. In vitro effect of noradrenaline, dopamine, histamine, serotonin, CAMP and db-CAMP on glycogen concentration in brain of rat treated before with dibenzyline (10 mg/kg)

Treatment of the tissue	Brain slices		
	Cortex	Caudate	Thalamus
Controls	29.4 ± 1.3	48.5 ± 1.1	16.8 ± 1.0
Noradrenaline ($10^{-4}\mu M$/ml)	17.7 ± 1.2[a]	20.4 ± 1.1[a]	7.1 ± 1.0[a]
Dopamine ($10^{-4}\mu M$/ml)	19.4 ± 1.1[b]	22.0 ± 1.5[b]	10.5 ± 0.9[b]
Histamine ($10^{-4}\mu M$/ml)	16.5 ± 1.0[a]	24.1 ± 1.2[a]	7.0 ± 1.0[a]
Serotonin ($10^{-4}\mu M$/ml)	15.1 ± 1.3[a]	25.1 ± 1.2[a]	8.5 ± 0.9[a]
CAMP ($10^{-3}\mu M$/ml)	19.8 ± 1.0[b]	30.5 ± 1.0[a]	9.7 ± 1.0[b]
db-CAMP ($10^{-3}\mu M$/ml)	10.1 ± 1.0[a]	19.8 ± 1.0[a]	6.1 ± 0.9[a]

[a] $p < 0.01$ in comparison with the controls. [b] $p < 0.05$ in comparison with the controls. The amount of glycogen is expressed in mg/100 ml of tissue. The numbers indicate the mean value (M) of 5 experiments ± S.E.M.

The experiments were carried out on adult male wistar rats. Brain slices were prepared according to the method already described[15] and were allowed 10 min in saline[16] at 37 °C. Propranolol (10 mg/kg) or dibenzyline (10 mg/kg) were injected intraperitoneally 30 min before the animals were sacrificed. The brain slices were incubated in the presence of noradrenaline (10^{-4} μM/ml), dopamine (10^{-4} μM/ml), histamine (10^{-4} μM/ml), serotonin (10^{-4} μM/ml), CAMP (10^{-3} μM/ml) and db-CAMP (10^{-3} μM/ml) and after 10 min glycogen was extracted[16] and estimated[17] from the brain tissue.

The results obtained show that propranolol prevented the glycogenolytic effects of noradrenaline and dopamine in vitro, but not that of histamine and serotonin, neither that of CAMP and db-CAMP (Table I). On the other hand, dibenzyline did not block the glycogenolytic effects, either of noradrenaline or dopamine, or histamine and serotonin, as well as of CAMP and db-CAMP (Table II).

CHASIN et al.[18] were the first to show that, in 2 areas of guinea-pig brain, cerebellum and cerebrum plus brain stem, there is a type of receptor shown to be a classical β-adrenergic receptor for the control of CAMP levels. Our data indicate that, in cortex, caudate and thalamus of rat brain, there exists β-adrenergic regulatory unit of adenyl cyclase responsible for the level of CAMP and activity of glycogen phoyphorylase. On the other hand, there must be another type of regulatory unit for histamine, as was suggested[9,18] and perhaps for serotonin. An α-adrenergic regulatory unit most probably would not be involved in the process of glycogenolysis in the brain tissue of rat.

Résumé. On montre que le propranolol empêche les effets glycogénolytiques de la noradrénaline et de la dopamine, bien qu'il n'ait pas d'effet sur les actions glycogénolytiques de l'histamine, de la sérotonine, du 3′, 5′-AMP cyclique et de son dérivé dibutyrique. On en conclu qu'au niveau du cervau des rats l'effet glycogénolytique des catécholamines résulte de l'excitation des récepteurs adrénergiques et que les autres unités régulatrices les adénylcyclases sont responsables des effets de l'histamine et de la sérotonine.

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Effect of Adrenergic Amines on the Membrane Potential of Guinea-Pig Liver Parenchymal Cells in Short Term Tissue Culture

In recent years the effects of catecholamines on the membrane potential of liver cells of several species have been studied in situ[1] in perfused liver[2,3] and in tissue slices[4]. The present experiments show that it is possible to apply the iontophoretic method of drug application[5,6] to examine the effects of adrenergic agonists on isolated parenchymal cells maintained under tissue culture conditions. This approach has two main advantages, 1. it is possible to see individual cells and select the appropriate cell from which to record and 2. it allows a greater resolution of the time course of the responses.

Materials and methods. Guinea-pig liver parenchymal cells were isolated by the enzymic procedure of BERRY and FRIEND[7] using collagenase, 0.025%, and hyaluronidase, 0.05% (both Sigma Type 1). The isolated cells were incubated in plastic petri dishes containing Eagle's medium (Dulbecco's modification), 10% tryptose phosphate broth, 10% foetal calf serum, antibiotics and a fungicide (Nystatin).

For the electrophysiological measurements a Hepes buffered Eagle's medium was used in which the calcium concentration had been increased to 3 mM. Recording was done at room temperature (21–23 °C). Membrane potentials were measured using glass micro-electrodes filled with 2 M potassium citrate (resistance 35–100 $M\Omega$). Micro-pipettes for iontophoresis were filled with 0.5 M solutions of (−)-noradrenaline, (±)-amidephrine or (−)-isoprenaline.

Results and discussion. The cultures contained single cells and small groups of cells. Many were binucleate (Figure 1). Stable membrane potentials ranging from −25 to −40 mV could be recorded from both mononucleate and binucleate cells whether single or in clumps. These values are similar to those reported[4] for cells in slices of guinea-pig liver. Noradrenaline invariably hyperpolarized the cells[4], as shown by A in Figure 2. A puzzling feature was the rather long latency (1–8 sec) of the response, which often could not be appreciably reduced by altering the position of the drug pipette. This may mean that it is necessary to 'flood' the cell with noradrenaline to produce a response, perhaps because the receptor density is relatively low. This interpretation was supported by the finding that the sensitivity of the tissue (expressed in terms of the potential change (mV) produced per nano-coulomb releasing the drug) was low (rarely more than 0.3 mV/nC).

Records B and C in Figure 2 show responses to iontophoretic application of (±)-amidephrine, a sympathomimetic amine which has a selective action on the α adrenoceptors[8,9]. Hyperpolarizations were again observed, although larger pulses were required, and the responses seemed to be more 'spiky' (for an extreme example, see C) than observed with noradrenaline. On the other hand, the strong β-agonist (−)-isoprenaline caused hyperpolarizations only if even larger pulses ($1–6 \times 10^{-7}$ A for 200–500 msec) were applied. The potency difference between noradrenaline and isoprenaline, together with

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