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SHORT COMMUNICATIONS

Effect of phentolamine on the increase in brain glycolysis following the intraventricular administration of dibutyryl-3,5-cyclic adenosine monophosphate and sodium fluoride to mice

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IN PREVIOUS studies, it was shown that the β-receptor blocking drug, *dl*-propranolol, reduced brain glycolysis and increased glycogenesis by decreasing the activity of the adenyl cyclase system in the mouse brain.[1,2] Evidence for this conclusion came from the finding that not only did this drug reduce brain glycolysis *in vivo*, but that it also prevented the increase in brain glycolysis caused by the intraventricular administration of noradrenaline, isoprenaline, dibutyryl-3,5 cyclic adenosine monophosphate (DB-AMP) or sodium fluoride to conscious mice. These substances have been shown by other investigators either to stimulate the membrane bound adenyl cyclase system to synthesize 3,5-cyclic adenosine monophosphate (cyclic AMP) from ATP[3,4] or to simulate the effect of cyclic AMP.[5] The present study was, therefore, undertaken to determine whether the α-adrenaline receptor blocking drug, phentolamine, had any effect on the increase in glycolysis caused by the intraventricular administration of DB-AMP or sodium fluoride. It has been shown previously that phentolamine has a different effect to *dl*-propranolol on mouse brain glycolysis *in vivo*.[6]

Specific pathogen free albino mice of the Alderley Park strain (18–22 g) of either sex were used. The drug or physiological saline (control group) was injected intraperitoneally into groups of at least five animals. The mice were killed by immersion in liquid nitrogen at the times shown in the Results. The method of Brittain[7] was used for the intraventricular administration of DB-AMP or sodium fluoride to the conscious animals. To study the effects of phentolamine on the increase in glycolysis caused by DB-AMP or sodium fluoride groups of mice were treated for different periods with phentolamine (30 mg/kg i.p.). One or 2 min before the mice were killed, DB-AMP or the halide was injected into the lateral ventricles. After thorough freezing, the brains were rapidly removed, weighed and triturated with cold 10% (w/v) trichloracetic acid. Following centrifugation, aliquots of the supernatant solution were taken for the determination of "free" glycogen, glucose and lactate by the enzymatic methods described in detail in a previous publication.[1] The acid insoluble precipitate was solubilized with

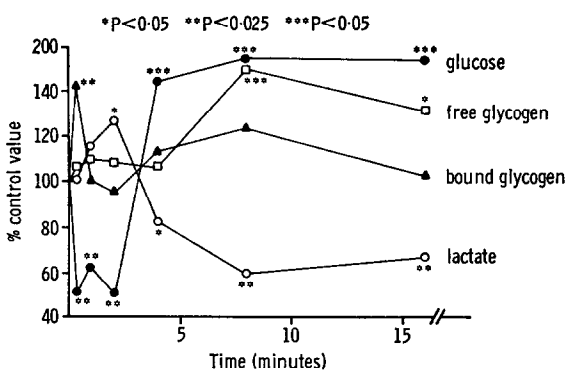

FIG. 1. Effect of DB-AMP on mouse brain glycolysis. DB-AMP injected into lateral ventricles (50 μg in 10 μl) and killed by immersion into liquid nitrogen at the times shown. Each point represents the mean of at least five animals. Significance of the difference from control values given by
* P < 0·01; † P < 0·001.
Control values (±S.E.M.) for
"Free" glycogen —0·665 ± 0·063 μmoles/g wet wt. (as glucose).
"Bound" glycogen—1·155 ± 0·141 μmoles/g wet wt. (as glucose).
glucose —0·637 ± 0·074 μmoles/g wet wt.
lactate —1·970 ± 0·170 μmoles/g wet wt.

alcoholic potassium hydroxide, according to the method of Russell and Bloom,[8] and after acid hydrolysis the glucose formed estimated as described previously.[1]

The results were calculated as μmoles/g wet weight of brain. However, to compare the effects of several substances on the same biochemical parameter, the results are expressed as percentage change relative to the control. The statistical significance was assessed using Students *t*-test.

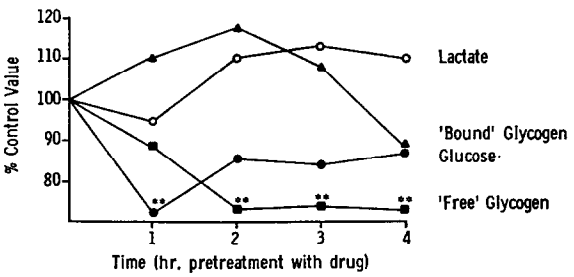

FIG. 2. Effect of DB-AMP and phentolamine on mouse brain glycolysis. Groups of mice pretreated for 60, 120, 180 and 240 min with phentolamine (30 mg/kg i.p.). Two min before being killed, DB-AMP injected into the lateral ventricles (10 μg in 10 μl). Details otherwise as shown in Fig. 1.

DB-AMP (10 μg in a total volume of 10 μl) when injected into the ventricles of conscious mice caused the animals to show reduced locomotor activity for up to 10 min. Intraventricular injection of the same volume of physiological saline resulted in slight loss of activity for 1–2 min after injection. DB-AMP produced a rapid increase in glycolysis, as shown by the increase in brain lactate and decrease in glucose, followed by a compensatory decrease in glycolysis and increase in glucogenesis (Fig. 1). These effects were counteracted to some extent by pretreating the mice with phentolamine (Fig. 2). It is noticeable, however, that in those mice pretreated with phentolamine and DB-AMP a marked glycogenolysis occurred for most of the experimental period.

Sodium fluoride (50 μg in 10 μl) caused marked hyperexcitability within 1–2 sec of administration. In some cases, clonic-tonic convulsions occurred during this period. All the animals recovered, but their locomotor activity was reduced for the duration of the experiments. The maximal changes in brain glycolysis occurred 1 min after the intraventricular injection of the halide and coincided with the peak behavioural effects (Fig. 3). Phentolamine (30 mg/kg i.p.) did not appreciably affect the increase in glycolysis caused by sodium fluoride (Fig. 4).

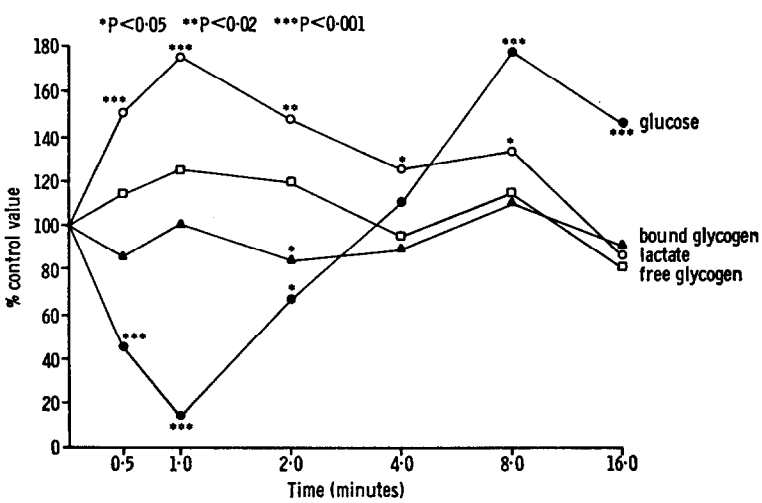

FIG. 3. Effect of sodium fluoride on mouse brain glycolysis. Sodium fluoride injected into latera ventricles of conscious mice (50 μg in 10 μl). Details otherwise as shown in Fig. 1.

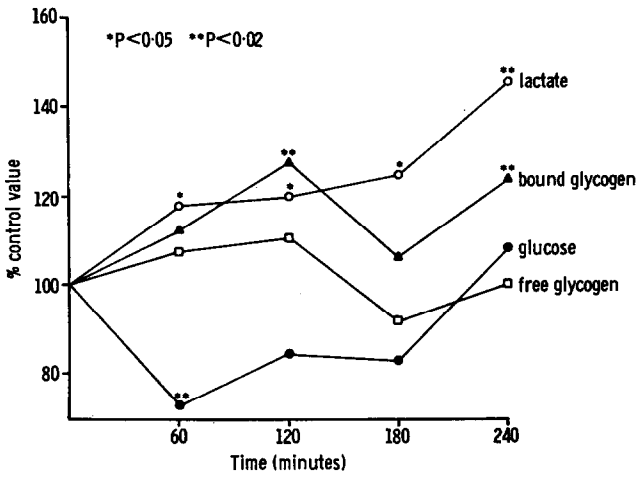

FIG. 4. Effect of sodium fluoride and phentolamine on mouse brain glycolysis. Groups of mice pretreated for 60, 120, 180 and 240 min with phentolamine (30 mg/kg i.p.). One min before being killed, sodium fluoride was injected into the lateral ventricles (50 μg in 10 μl). Details otherwise as shown in Fig. 1.

The results of this study clearly distinguish the α-adrenoceptive blocking drug phentolamine, from the β-blocker *dl*-propranolol, which has been shown to antagonize the effects of both DB-AMP and sodium fluoride on mouse brain glycolysis.[2] Weiss[9] and Weiss and Costa[10] have shown that in the rat pineal gland, the α-blocking drugs phentolamine and phenoxybenzamine are less effective than *dl*-propranolol and dichloroisoprenaline in antagonizing the effect of noradrenaline. The effect of sodium fluoride on pineal gland cyclase activity could not be blocked by propranolol[10] even though the effects of its injection into the lateral ventricles of mice can be blocked by this drug.

From the present study, it appears that phentolamine has a different mode of action from that of propranolol on brain glycolysis. This result does not, however, justify the conclusion that the adrenergic receptors in the brain are analogous to the α- and β-receptors that occur in peripheral tissues.

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