Glial glycogen stores are regulated by α-adrenergic receptors

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The turnover of glial glycogen stores may be important in providing metabolic support for neuronal activity (Wolfe & Nicholls, 1967; Pentreath & Kai-Kai, 1982) and is important that we understand the mechanisms by which this process takes place. NA is known to promote glycogen breakdown in glial cell lines and it is assumed that this is the result of a β-adrenergic receptor-evoked increase in intracellular cyclic AMP (Passonneau & Crites, 1976). However, the evidence for this is somewhat circumstantial and requires further investigation. Here we have attempted to clarify this point by employing a technique which relies on the ability of radiolabelled 2DG to be partially incorporated into glial glycogen (Pentreath et al., 1982) and we show that NA increases [14C]2DG incorporation into glycogen in primary astrocyte cultures. Although increased intracellular cyclic AMP may affect [14C]2DG incorporation into glycogen, it may not be the only route by which NA exerts its effect. Our results suggest that NA acts via both α- and β-adrenergic receptors and that the α-adrenergic, cyclic AMP-independent receptors may predominate in primary astrocyte cultures.

Astrocyte-enriched cultures were prepared (Cambray-Deakin et al., 1985) and grown on 60mm diameter plastic dishes until they were confluent (18-22 days in vitro) at which stage they were used in experiments. Cultures were preincubated for 60min at 37°C in glucose-containing (20mM) medium to ensure maximal glycogen content (Cummins et al., 1983), washed, then incubated for a further 60min at 37°C in medium (pH7.4) of the following composition: NaCl (120mM), NaHCO3 (10mM), Na2HPO4 (1mM), KCl (2.5mM), MgSO4 (1mM), CaCl2 (2.5mM), glucose (20mM); Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 20mM], ascorbic acid (1mM), 1895

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
<th>Kd (nm)</th>
<th>Bmax (pmol/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Adrenergic</td>
<td>[3H]DHA</td>
<td>0.088 ± 0.003</td>
<td>0.051 ± 0.007</td>
</tr>
<tr>
<td>Muscarinic</td>
<td>[3H]QNB</td>
<td>0.079 ± 0.014</td>
<td>0.044 ± 0.003</td>
</tr>
<tr>
<td>Serotinergic</td>
<td>[3H]Serotonin</td>
<td>70.0 ± 13.9</td>
<td>4.4 ± 0.6</td>
</tr>
</tbody>
</table>

Table 1. Kinetic parameters for three neurotransmitter-binding sites on astrocyte membranes

Cultures at 18-22 days in vitro were scraped into ice-cold distilled water and disrupted with a sonicator. Crude membranes were prepared by centrifugation at 50000g for 30min. The binding of [3H]DHA, [3H]QNB and [3H]serotonin was determined as in Bylund (1978), Gross et al. (1981)Ubzov et al. (1979) respectively. Kinetic parameters were determined by Scatchard analysis and S.E.M.'s indicate the variation consequent upon linear regression.

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McHardy, K. & Fajzsi, C. (1983) Brain Res. 6, 251-257
Uzbekov, M., Murphy, S. & Rose, S. (1979) Brain Res. 168, 195-199
tropolone (0.1 mM), pargyline (0.1 mM), [14C]2DG (6mCi/mmol, Amersham, U.K.), final concentration 2.5 x 10^-5 M in the presence or absence of one or more of the agents indicated in Table 1. After incubation, cultures were washed, the material from two cultures pooled and the [14C]2DG-labelled glycogen extracted according to Pentreath et al. (1982).

Table 1 shows that in control cultures some 9% of the total [14C]2DG was incorporated into glycogen. The extent to which [14C]2DG was incorporated into glycogen was increased by approx. 29% after incubation with NA. The increase would appear to reflect a decrease in the glycogen content of the cultures leading to an increase in the specific activity of the radio-labelled glycogen as our unpublished observations have shown that NA reduces the glycogen content of these cultures by some 34% when compared with control. Should NA induce changes in the glycogen content of these cultures by stimulating intracellular cyclic AMP levels, one would expect that its effect would be enhanced by other agents which also increase cyclic AMP levels. However, the phosphodiesterase inhibitor isobutylmethylxanthine produced only a slight (11%) augmentation of the response evoked by NA. Forskolin stimulates adenylate cyclase activity by its action at a site quite separate from adrenergic receptors (Seamon et al., 1981) and was found to cause a marked increase in [14C]2DG incorporation into glycogen in these cultures. However, there was no enhancement of this effect when NA was also included in the incubation medium. Isoproterenol is a supposedly specific β-adrenergic receptor agonist and was found to be more potent than NA in increasing [14C]2DG incorporation into glycogen. However, its effect was only partially blocked (30% inhibition) by the β-adrenergic receptor antagonist propranolol. Curiously, the α-adrenergic receptor antagonist phentolamine produced a greater inhibition (70%) of the effect of isoproterenol and the ability of isoproterenol to increase [14C]2DG incorporation into glycogen was abolished by a combination of the two antagonists. These results suggest that isoproterenol was acting as an α-adrenergic agonist on these cultures. Such an action of isoproterenol has been observed in studies on glycogenolysis in the mammalian liver (Hems & Whitton, 1980) and isoproterenol, at the concentration used here, does exhibit high affinity for glial α-adrenergic receptors (Ebersolt et al., 1981).

In summary, our results show that adrenergic receptor activation elicits a change in the extent to which [14C]2DG is incorporated into glial glycogen stores. This effect appears to be predominantly under the control of α-adrenergic, cyclic AMP-independent receptors although a smaller β-adrenergic, cyclic AMP-dependent process may also exist.

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Some unusual kinetic properties of human platelet lipoxygenase

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A study of the kinetic properties of soya bean lipoxygenase (EC 1.13.11.12) led Funk et al. (1981) to revive the idea that product activation may play a fundamental role in lipoxygenase catalysis.

Human platelet lipoxygenase catalyses the specific hydroperoxidation of arachidonic acid. Activation of this enzyme

Abreviation used: 12-HPTE, 12-hydroperoxyeicosatetraenoic acid

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