incubation with adenosine, depicted in Fig. 1, the total amount of urate produced after 1 h was 56 nmol/ml. This total is comprised of urate synthesized from adenosine and from intermediary metabolites. Thus over the 1 h incubation 40 nmol of adenosine/ml was metabolized to urate. The total amount of adenosine utilized was 80 nmol/ml. Therefore only 50% of the adenosine initially added was metabolized to urate. Since no other purine base or riboside accumulated apart from 5 nmol of inosine/h per ml, it is suggested that at least part of the remaining adenosine was phosphorylated to adenine nucleotides in the hepatocytes. In rat hepatocytes such a phosphorylation of adenosine to adenine nucleotides has been observed (Lund et al., 1975).

At present it is not clear whether the inhibition of urate synthesis de novo is caused by adenosine or by its conversion into other compounds, tentatively identified as adenine nucleotides. Further experiments are required to determine directly the

metabolic fate of adenosine in chick hepatocytes by using the labelled compound.

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Adenosine-binding to cerebral preparations in interpretation of adenosine activation of adenosine 3′:5′-cyclic monophosphate formation

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Adenosine increases cyclic AMP formation in guinea-pig cerebral-cortical tissue (Sattin & Rall, 1970) and mediates effects of electrical stimulation on cyclic AMP accumulation (Newman & McIlwain, 1977). Potentiation of adenosine actions by inhibitors of adenosine uptake including dipryridamole (Huang & Daly, 1974), and a decrease in both basal and stimulated cyclic AMP values by added extracellular adenosine deaminase (Huang et al., 1973; Newman & McIlwain, 1977), suggest that the actions involve extracellular adenosine receptors. In rat fat-cell membranes two classes of adenosine-receptor sites with affinity constants of 9.5 μM and 0.95 μM have been demonstrated (Malbon et al., 1978). In brain tissue there has not been demonstration of specific binding sites on particulate fractions, although (NH4)2SO4 fractionation of supernatant from rat brain homogenate yielded a peak of [3H]adenosine-binding activity (Ko, 80 nM) on DEAE-cellulose column chromatography (Shimizu, 1979). This material, which also bound cyclic [3H]AMP, probably corresponded to the adenosine-analogue-binding protein found in liver supernatants by Sugden & Corbin (1976) and Ueland & Doskeland (1977), and was also distinguished from the membrane receptor in not being affected by the adenosine agonist theophylline.

In the present work a ligand-binding assay with [3H]-adenosine and glass microfibre filters has been employed to determine the distribution and properties of adenosine-binding sites in mammalian brain. Binding assays were conducted in 10 mM-Tris/HCl (pH 7.4)/5 mM-MgCl2 at protein concentrations of 0.5 mg/ml, for 90 min at 0°C, and in all cases non-specific binding was accounted for by subtraction of values for [3H]adenosine bound in the presence of a 500–1000-fold excess of unlabelled adenosine. Crude mitochondrial fractions prepared from guinea-pig, rat or beef neocortex all showed high-affinity binding with affinity constants of 0.59 ± 0.17 μM and 1.28 ± 0.18 μM respectively (means ± S.D. for three observations in each case). The binding was significantly greater in guinea-pig tissue (9.6 ± 0.5 pmol/mg of protein) than
Table 1. Effects of theophylline and 2-chloroadenosine on the binding of [3H]adenosine to crude mitochondrial and to synaptosomal fractions of guinea-pig cerebral cortex

The fractions were assayed as described in the text in a final volume of 200μl with 0.1μM [3H]adenosine, with and without unlabelled 0.1 mM adenosine to correct for non-specific binding and with the agents indicated below. Bound adenosine was measured by retention on Whatman GF/B glass microfibre filters after washing with 15-20ml of assay buffer, and results are expressed as percentages of the activity in the absence of added agents. Results are means± range or S.D. for the numbers of observations shown in parentheses.

<table>
<thead>
<tr>
<th>Concentration of agent (μM)</th>
<th>Binding (%)</th>
<th>Theophylline</th>
<th>2-Chloroadenosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>50.5 ± 2.8</td>
<td>33.3 ± 3.7</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>49.7 ± 6.3</td>
<td>22.2 ± 3.5</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>37.8 ± 15.9</td>
<td>12.0 ± 9.0</td>
<td></td>
</tr>
</tbody>
</table>

...in rat or bovine (4.2 ± 0.8 pmol/mg of protein) tissue. Comparable binding was shown by the synaptosomal (P,B) fraction prepared from guinea-pig cerebral or cerebellar cortex (Kd 1.39 ± 0.28 μM and 0.44 μM respectively). No inhibitor of adenosine deaminase activity was necessary to demonstrate this high-affinity binding, in contrast with the findings in fat-cell membranes (Malbon et al., 1978). The Kd is similar to the value of 0.5 μM found by Premont et al. (1979) for the concentration of adenosine giving half-maximal stimulation of adenylate cyclase in rat striatal homogenates, the lower affinity of 30 μM found in intact tissue (Sattin & Rall, 1970) probably being accounted for by restricted access of adenosine to its receptor. The greater number of binding sites in guinea-pig brain may account for the greater sensitivity of its cyclic AMP-generating system to adenosine, when compared with rat (see Traversa & Newman, 1979).

Addition of the adenosine deaminase inhibitor erythros-9-[1-(1-hydroxyethyl)heptyl]adenine (Skolnick et al., 1978) to the binding-assay medium revealed a second lower-affinity binding site with Kd values of 20.2 μM and 26.3 μM in guinea-pig cortical and cerebellar synaptosomes respectively. This may correspond to adenosine kinase, for which a Kd of 20 μM for adenosine has been calculated (Shimizu et al., 1972). Myelin and microsomal fractions from guinea-pig cerebral cortex each showed binding with apparent affinity constants of about 2–3 μM, unaffected by the presence of the adenosine deaminase inhibitor. Table 1 shows that the high-affinity binding to crude mitochondrial and to synaptosomal fractions was opposed by theophylline and by 2-chloroadenosine, which are respectively antagonist and agonist for the adenosine-activated adenylyl cyclase.

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A kinetic study on phosphofructokinase isolated from the muscle of Carcinus maenas (the common shore crab)

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The requirement for free Mg2+ in the reaction catalysed by phosphofructokinase was first suggested by Paetkau & Lardy (1967), who showed that the rate of the reaction for the rabbit muscle enzyme was negligible until the concentration of Mg2+ was in excess of that for ATP. Mavis & Stellwagen (1970), using the yeast enzyme, demonstrated that the rate of reaction was dependent on the concentrations of MgATP and free Mg2+, but not that of free ATP. Direct evidence for the existence of two distinct types of binding sites for Mn2+ on phosphofructokinase, one of which was probably associated with the active site, was obtained by electron paramagnetic resonance (Cottam & Uyeda, 1973).

The results of kinetic studies on the mechanism of phosphofructokinase have not been in general agreement. A sequential mechanism with the substrates, fructose 6-phosphate and MgATP2-, adding in random order was proposed by several authors (Kee & Griffin, 1972; Hanson et al., 1973; Bar-Tana & Cieland, 1974a, b). More recently, an Ordered Bi Bi mechanism has been suggested for the enzymes isolated from erythrocytes (Etiemble et al., 1977) and Lactobacillus plantarum (Simon & Hofer, 1978).

In the present study the mechanism and the role of Mg2+ in the phosphofructokinase reaction were studied by using steady-state kinetics at eight different fixed concentrations of the free metal ion.

Phosphofructokinase was isolated from the pincer and leg muscles of the common shore crab, Carcinus maenas. It had a specific activity of 19 units/mg of protein at 25°C (for all enzymes in this paper, 1 unit = 1 μmol/min). Polyacrylamide-gel electrophoresis of the purified enzyme showed that over 70% of the protein was located in three separate bands, each of which was associated with enzyme activity, suggesting the occurrence of different polymeric forms of the active enzyme.

1980