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Mg2+. The enzyme assays were accompanied by measurements of the phosphorylation with [γ-32P]ATP. After incubation of intact rabbit erythrocytes with adrenaline (Fig. 1), maximal activation of carbonic anhydrase occurred within 5 min.

In chicken erythrocytes, one of the two isolated isoenzymes could be activated and phosphorylated (for conditions, see the legend of Fig. 2). The carbonic anhydrase from rabbit erythrocytes was separated into three isoenzymes. One of them is activated in the presence of cyclic AMP, ATP and Mg2+ by a protein kinase prepared from rabbit erythrocytes.

From these results we conclude that the observed mechanism might be of physiological significance.


The Effect of Age on the Number of Monoamine Oxidase Active Centres in the Rat Heart

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The inhibition of monoamine oxidase (MAO, EC 1.4.3.4) activity by the acetylenic inhibitor clorgyline (M & B 9302) is thought to proceed by a reversible association with the active centre of the enzyme after a ‘suicide’ reaction to form a covalent bond with the flavin prosthetic group (see Rando, 1974; Fowler & Callingham, 1978). Clorgyline has been used to distinguish between two forms of monoamine oxidase, termed monoamine oxidases-A and -B, where form A is more sensitive to the inhibitor (Johnston, 1968). In the rat liver, clorgyline is such a potent inhibitor of monoamine oxidase-A that the concentrations of enzyme and inhibitor are of the same order (Fowler & Callingham, 1978).

Hearts from male Wistar rats were homogenized in 0.25M sucrose, buffered with 10 mm potassium phosphate, pH 7.8. Crude homogenates and mitochondrial fractions were prepared, and assayed for monoamine oxidase activity by a radiochemical method with 5-[^3H]hydroxytryptamine and[^3H]tyramine as substrates.

With tyramine as substrate, it was found that there was an initial competitive inhibition of monoamine oxidase activity by clorgyline, which became non-competitive when enzyme and inhibitor were preincubated together for 1 h, consistent with a ‘suicide’ reaction. Time courses of inhibition were obtained for the two substrates, both of which are deaminated by monoamine oxidase-A only in this tissue (see Fowler et al., 1978). With each substrate, the inhibition of monoamine oxidase activity reached a plateau within 10 min of preincubation, which did not increase even when the period of preincubation was lengthened to 4 h. This would suggest that, in this preparation the concentrations of enzyme and inhibitor are of the same order, and that the observed inhibition is due to a stoichiometric reaction between the available inhibitor and enzyme (see Fowler & Callingham, 1978). Furthermore, preincubation of submaximal concentrations of clorgyline with rat heart monoamine oxidase produced, on a plot of percentage inhibition against clorgyline concentration, straight lines that passed through the origin. The concentration of clorgyline that causes exactly 100% inhibition of monoamine oxidase activity is that which is equal to the concentration of enzyme (Fowler & Callingham, 1978), and thus the molar concentration of monoamine oxidase can be found.

Dilution of the crude mitochondrial fractions to decrease the enzyme concentration resulted in an almost identical decrease in the amount of clorgyline required to produce 100% inhibition. Addition of bovine serum albumin to increase the concentration of non-enzyme protein had no effect. The amount of clorgyline needed per unit of enzyme
Michaelis constants were determined from initial velocities of the reaction at five substrate concentrations. The number of clorgyline-binding sites was determined from the concentration of clorgyline needed to give 100% inhibition of monoamine oxidase activity divided by the total protein concentration. Figures in brackets represent the absolute values expressed as percentages of the values for mitochondria from rats weighing 140g. Significance levels were determined by Student's t test.

<table>
<thead>
<tr>
<th>Rat weight (g)</th>
<th>Substrate</th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ (nmol/h per mg of protein)</th>
<th>Clorgyline binding (fmol/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>140</td>
<td>5-Hydroxytryptamine</td>
<td>68 ± 11</td>
<td>425 ± 22 (100)</td>
<td>654 ± 38 (100)</td>
</tr>
<tr>
<td>304</td>
<td></td>
<td>69 ± 10</td>
<td>618 ± 26** (145)</td>
<td>820 ± 58* (126)</td>
</tr>
<tr>
<td>487</td>
<td></td>
<td>100 ± 20</td>
<td>1818 ± 129** (428)</td>
<td>3302 ± 202** (505)</td>
</tr>
<tr>
<td>140</td>
<td>Tyramine</td>
<td>44 ± 8</td>
<td>830 ± 49 (100)</td>
<td>666 ± 39 (100)</td>
</tr>
<tr>
<td>304</td>
<td></td>
<td>47 ± 17</td>
<td>1254 ± 139* (151)</td>
<td>808 ± 46* (126)</td>
</tr>
<tr>
<td>487</td>
<td></td>
<td>68 ± 13</td>
<td>3718 ± 267** (448)</td>
<td>3164 ± 146** (475)</td>
</tr>
</tbody>
</table>

* $P<0.05$.
** $P<0.01$.

activity was the same for both crude homogenates and mitochondrial fractions. Thus there appears to be very little binding of clorgyline to sites other than the active centres of monoamine oxidase.

In earlier work, it was found that there was a selective increase in the specific activity of monoamine oxidase-A in the rat heart as the animal grew older (Callingham & Lyles, 1975). To determine the effects of age on clorgyline binding, three groups, each of 24 rats, of mean body weight 140, 304 and 487g were killed, and heart homogenates and mitochondrial fractions prepared.

With 5-hydroxytryptamine and tyramine as substrates, there was a corresponding increase in both specific activity and specific binding of clorgyline as the weights of the animals increased with age without any change in $K_m$. The results from mitochondrial fractions are summarized in Table 1. Similar results were also obtained when crude homogenates were used.

It would appear that the increase in the specific activity of rat heart monoamine oxidase towards substrates for monoamine oxidase-A is due to an increase in the availability of enzyme active centres, rather than an increase in the molecular-turnover number of the enzyme. This approach has been made possible by the remarkably low amount of non-specific binding of clorgyline in these crude preparations of rat heart, which is in contrast with that in the rat liver (Fowler & Callingham, 1978).

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