Effect of salicylate on perfused rat hearts. The percent of phosphorylase a was calculated after 10 min of perfusion. Glycogen content measured as a sum of glucose (after hydrolysis) per g and lactate production was expressed as μmoles of lactate per h per g. The results are expressed on a fresh weight basis.

<table>
<thead>
<tr>
<th>Determinations*</th>
<th>Control</th>
<th>Salicylate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph. a (per cent)</td>
<td>7.2 ± 3**</td>
<td>23 ± 7</td>
</tr>
<tr>
<td>Glycogen content</td>
<td>83.3 ± 8</td>
<td>10.1 ± 4</td>
</tr>
<tr>
<td>Lactate production</td>
<td>25 ± 3.2</td>
<td>112.5 ± 14</td>
</tr>
</tbody>
</table>

*6 hearts were used as control and 6 for treatment with sodium salicylate. **S. E. M.

Our finding that salicylate increases phosphorylase a activity might be explained through a mechanism involving the uncoupling of oxidative phosphorylation. For example, previous work from our laboratory has shown that DNP results in elevated levels of phosphorylase a of heart and skeletal muscle. This could be explained by a decrease in the ATP/ADP ratio which would increase phosphofructokinase activity and thereby maintain glucose-6-phosphate at a low level in the cell. Since glucose-6-phosphate is known to inhibit phosphorylase b to a conversion, this inhibition would be reduced in the presence of the uncoupling agent resulting in higher phosphorylase a levels. In support of this idea, other experiments with salicylic acid have shown that in the isolated rat diaphragm the content of inorganic phosphate is increased, whereas creatine phosphate and ATP are severely reduced in the presence of the drug.

The results of Segal and Blair of phosphorylase a inhibition in isolated hemidiaphragms is found only 30 min after salicylate administration, a time which, according to the authors, glycogenolysis proceeds at a much slower rate even though glycogen is still present in relatively high amounts.

Interaction of N-(DL-seryl)N'-(2,3,4-trihydroxybenzyl)-hydrazine with L-Dopa decarboxylase from pig kidney

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Institute of Biological Chemistry, Faculty of Pharmacy, Institute of Nervous and Mental Diseases, University of Perugia, I-06100 Perugia (Italy), 19 July 1976

Summary. Interaction of seryl trihydroxy-benzyl-hydrazine with a highly purified preparation of Dopa decarboxylase from pig kidney has been studied. This compound was found not to be a powerful inhibitor in vitro. Kinetic and spectral data suggest some possibilities on the binding nature of the inhibitor and substrates.

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The N-(DL-seryl)N'-(2,3,4-trihydroxybenzyl)-hydrazine (Ro 4-4602) is a product used, in addition to L-Dopa, in the treatment of Parkinson's disease. This compound is an inhibitor of peripheral Dopa decarboxylase and does not appear to pass the blood-brain barrier at therapeutic doses in Parkinsonism. Its effect on cellular metabolism lies in potentiating the biochemical behaviour of peripherally administrated L-Dopa. This pharmacological action, largely investigated from a clinical point of view, reflects the interaction of this compound at the molecular level with peripheral L-Dopa decarboxylase. Such interaction has been studied in vitro measuring the inhibition on crude homogenate of hog or rat kidney, 1-06700 Perugia (Italy), 19 July 1976.

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Materials and methods. The enzyme, purified according to Borri Voltattorni et al, appeared to be homogenous in polyacrylamide gel electrophoresis and in the ultra-centrifuge. Its coenzyme content was in good agreement with that reported by Christenson et al. L-m-tyrosine and L-0-tyrosine were synthetized from corresponding racemic forms by the action of D-amino acid oxidase and catalase in a 0.05 M pyrophosphate buffer, pH 8.3 under O2 stream. The enzymatic degradation of the D-amino acid form was followed measuring at time intervals the formation of the keto acid form with 2,4-dinitrophenyl hydrazine. The end-point of the reaction was achieved when, even after further additions of enzymes, no more keto acid was detected. Then the reaction mixture, brought to pH 3, was poured on a 50 x 8 Dowex column. After elution of the keto acid with 0.2 M pyridine buffer, pH 3.1, the L-amino acid was eluted with 2 M pyridine buffer, pH 5. Ether was added

Michaelis-Menten constants and inhibition constants of Ro 4-4602 for different substrates for Dopa-decarboxylase

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$K_m \times 10^4$</th>
<th>$K_i \times 10^4$</th>
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<tbody>
<tr>
<td>L-Dopa</td>
<td>0.68</td>
<td>3.40</td>
</tr>
<tr>
<td>L-5HTP</td>
<td>1.64</td>
<td>18.00</td>
</tr>
<tr>
<td>L-m-tyrosine</td>
<td>2.10</td>
<td>3.65</td>
</tr>
<tr>
<td>L-o-tyrosine</td>
<td>5.70</td>
<td>3.65</td>
</tr>
</tbody>
</table>

The addition of the inhibitor to an enzyme solution at pH 6.8 changed the spectral properties by decreasing the intensity of the 420 nm and the 335 nm peaks (figure 2). The absorbance at 420 nm of the enzyme plus increasing amounts of Ro 4-4602 and the concentration of this compound in a reciprocal plot are reported in the inset of figure 2. The dissociation constant measured in this way was $3.2 \times 10^{-4}$ M. This value was in line with $K_i$ for 3 substrates but not for L-5HTP. The lower affinity of Ro 4-4602 observed only in presence of L-5HTP is not easy to explain and might be related to the indolic ring of this substrate.

The different types of inhibition observed with the various aromatic amino acids discloses some possibilities about the binding nature of the inhibitor and substrates. The modified absorption peaks induced by the compound on the enzyme-bound pyridoxal-P suggests its binding to the active site.

On the other hand, the observed 'pure' non-competitive inhibition towards 3 substrates may indicate that the inhibitor and these substrates do not compete for the same site on the enzyme surface, but that they are able to become simultaneously attached to the enzyme. The disagreement between these data can be interpreted in at least two ways. 2 substrate binding sites could exist on the enzyme molecule, one for L-Dopa and the other one common for the other three substrates. The Ro 4-4602 could bind to the enzyme at the same site as L-Dopa. At present, this is just a hypothesis which must be confirmed by further investigations; moreover, it would