TIME COURSE OBSERVATION ON PROTOKYLOL DISTRIBUTION IN RATS FOLLOWING PROTOKYLOL ADMINISTRATION

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Protokylol, a sympathomimetic drug has been utilized in general clinical practice as a bronchodilator in recent years. To date no reports concerning the actual drug metabolism are available. As a result of our present research, it was found that protokylol in a manner similar to catecholamine, changes into a substance with a high fluorescence intensity by trihydroxyindole reaction. Using this method it has become possible to measure minute amounts of protokylol (1–3).

The present paper outlines a time course observation on protokylol distribution in rats following an intravenous injection of protokylol.

METHODS

1. Method for determination of protokylol in biological material

Wistar strain rats of both sexes weighing from 200 to 300 g were used, and anesthetized by an intraperitoneal injection of 2 g/kg of urethan.

Protokylol was administered intravenously into the carotid vein to rats at a dose of 50 mg/kg. The rats were then sacrificed at regular intervals of 10 min, 1 hr, 2 hr, 5 hr and 24 hr. Samples from various organs were excised and weighed. 0.4% perchloric acid was added to the samples, a homogenate prepared and left standing for 30 min at 2°C. Next, centrifugation (8,500 x g 2°C, 10 min) was conducted and the protein free filtrate was collected.

Prior to alumina column chromatography the protein free filtrate was neutralized to pH 4 with 5 N K₂CO₃ using pH paper at room temperature. The neutralizer with potassium perchlorate precipitate was transferred to a polyethylene centrifuge tube and spun down at 8,500 x g for 10 min. The supernatant was decanted into a 50 ml Erlemeyer flask containing 1.0 g of acid washed alumina (Merck 71695 acid washed), 1 ml of 0.2 M EDTA solution and two drops of 1% phenolphthalein solution in ethyl alcohol. Purification of acid washed aluminum oxide was made (1).

The pH of the slurry was brought up to 8.3 ± 0.3 with 1 N NH₄OH and shaken on an automatic shaker for 5 min in such a way as to maintain the light pink color of the content throughout the shaking time by an addition of 1 N NH₄OH if the mixture became decolorized. After shaking, the slurry was transferred into a glass column with a glass
wool plug. The Erlenmeyer flask was rinsed with 0.2 M sodium acetate solution and the liquid forced through the alumina column under an air pressure of 200 mmHg. The column was washed with two portions of 5 ml of 0.2 M sodium acetate solution and 10 ml of H2O. Elution was performed by two successive portions of 10.0 ml of 0.5 M acetic acid into a collecting tube. Two milliliters of 0.2 M EDTA solution were added to each eluate. The procedure of trihydroxyindole reaction was adopted from Saito’s method (1). Fluorescence intensity of samples were read on an Aminco-Bowman Spectrophotofluorometer at two activation wave lengths: 400 m\textmu and 430 m\textmu (uncorrected instrumental values). The fluorescence was read at 500 m\textmu (uncorrected instrumental value). Recording of the spectrums of excitation and emission of each sample was performed on a Shimazu Unicorder 100 M recorder. The native fluorescence of protokylol has a maximum activation wave length of 300 m\textmu and a remarkable maximum fluorescence wave length of 330 m\textmu. We applied the trihydroxyindole reaction used in the measurement of catecholamine and discovered that the fluorescent substance of protokylol, as in the case of catecholamine, showed a shift in the direction of long wave lengths with a maximum activation wave length of 430 m\textmu, and a maximum fluorescence wave length of 500 m\textmu. Further, this fluorescent substance of protokylol showed an approx. 3 fold stronger fluorescent substance as compared to the native fluorescence of protokylol. The next point arising in measurement of protokylol is the relationship with total catecholamine. In the present experiment, it was considered that actual measured values of protokylol contain endogenous catecholamine. The total catecholamine in the animals prior to protokylol administration was measured, and from the measured values of protokylol (protokylol +endogenous catecholamine) the endogenous total catecholamine was deducted.

2. A study on protokylol distribution in subcellular fractions of rat lung after administration of protokylol

Rat lungs were homogenized, the homogenate was centrifuged at 500 \times g for 5 min, and the sediment (debris fraction) was collected. The supernatant solution was centrifuged at 10,000 \times g for 60 min and the sediment (mitochondria fraction) was collected. Finally, the supernatant solution was centrifuged at 100,000 \times g for 1 hr and the sediment (microsome fraction) collected. In this experiment, "supernatant" refers to a supernatant fraction obtained from the centrifugation of 100,000 \times g for 1 hr. Protein was measured by the method of Lowry et al. (5).

RESULTS

1) The protokylol level in the rat plasma showed a high value 60 min after intravenous injection of protokylol at a dose of 50 mg/kg. Two hr after injection, the plasma protokylol level lowered remarkably but a small amount was measurable. A slight trace of protokylol was detectable in the rat plasma 5 to 24 hr after injection (Fig. 1).

2) Rat urine collected for 24 hr after protokylol was administered at dose of 50 mg/kg and the total amount of protokylol excreted in the urine was measured. Twenty-four hr urine specimens of non-treated rats were used to determine the values of endogenous cate-
cholamines. Recordings of the spectrums of excitation and fluorescence of urinary samples and external standard of protokylol was done.

The same fluorescence peaks at the activation wave length 300 m\(\mu\) and fluorescence wave length 330 m\(\mu\) were recorded as both external standards of protokylol and urinary sample.
eluate obtained from alumina column chromatography (Fig. 2). This fact suggests that a part of essentially unchanged protokylol is excreted into urine.

3) When the protokylol distribution in rats was investigated 10 min after intravenous injection of protokylol at a dose of 50 mg/kg, a high concentration was seen in the kidney, heart, lung, spleen, liver and striated muscle. Protokylol however showed poor distribution in the intestine. Only a slight trace was seen in the brain (Table 1).

4) When the protokylol distribution in the rat was observed 60 min after injection, it was noted that large amounts of protokylol were still seen in the kidney, heart and lung. A rapid decrease was seen in the liver with a complete disappearance in the brain. A still more remarkable lowering of protokylol concentration was seen in various organs 5–24 hr after protokylol administration (Table 1).

5) A detailed observation of protokylol distribution was made in the subcellular fractions of rat lung 10 min after administration of protokylol at a dose of 50 mg/kg. Protoky-

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**TABLE 1. Tissue distribution of protokylol in rats following protokylol administration (Protokylol 50 mg/kg i.v.).**

<table>
<thead>
<tr>
<th>Time</th>
<th>tissue</th>
<th>*Plasma</th>
<th>Kidney</th>
<th>Heart</th>
<th>Lung</th>
<th>Brain</th>
<th>Liver</th>
<th>Spleen</th>
<th>Muscle</th>
<th>Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4.22±0.32</td>
<td>0.262±0.081</td>
<td>0.67±0.081</td>
<td>0.228±0.081</td>
<td>0.172±0.081</td>
<td>0.162±0.081</td>
<td>0.851±0.081</td>
<td>1.952±0.081</td>
<td>0.272±0.081</td>
</tr>
<tr>
<td>10 min</td>
<td></td>
<td>487.38±20.03</td>
<td>101.378±20.03</td>
<td>45.02±12.18</td>
<td>34.192±7.08</td>
<td>0.645±0.06</td>
<td>2.798±0.59</td>
<td>4.795±0.78</td>
<td>1.788±0.87</td>
<td>1.183±0.27</td>
</tr>
<tr>
<td>1 hr</td>
<td></td>
<td>216.88±3.36</td>
<td>12.618±8.79</td>
<td>23.13±2.89</td>
<td>21.872±0.14</td>
<td>0.278±0.14</td>
<td>0.470±0.11</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2 hr</td>
<td></td>
<td>5.685±0.134</td>
<td>0.134±0.071</td>
<td>0.071±0.071</td>
<td>0.195±0.019</td>
<td>N.D.</td>
<td>0.700±0.071</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5 hr</td>
<td></td>
<td>23.78±0.378</td>
<td>0.378±0.650</td>
<td>0.502±0.502</td>
<td>N.D.</td>
<td>0.248±0.023</td>
<td>N.D.</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>24 hr</td>
<td></td>
<td>14.78±0.23</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>0.032±0.032</td>
<td>N.D.</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* *µg/L plasma (µg/g wet weight)*

N.D.: non detectable
Control: endogenous catecholamine content

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**Fig. 3. Intra-lung protokylol distribution in rats administration. i.v. 50 mg/kg.**
lol was found to be distributed mainly in the microsome and supernatant fractions. It was also noted that the microsome rather than the supernatant fraction showed a higher of value protokylol. No recognizable distribution was seen in the debris and mitochondria fractions (Fig. 3).

DISCUSSION

In the present experiment it was found that the organs showing a high concentration of protokylol distribution were the kidney, heart, lung, spleen, liver and muscle in rats 10 min after protokylol administration, while there was but a small distribution in the intestine and brain. This fact suggests that only small quantities pass the blood-brain barrier. As in most amines, protokylol is concentrated in parenchymatous tissues, such as the kidney, lung, spleen with the exception of the heart. Liver and skeletal muscle showed a somewhat lower level of protokylol, but because of their mass these tissues contain a large amount of protokylol in total when equilibrium is reached. The plasma protokylol disappearance curve suggests that this drug becomes stable within 1 hr after administration. Compared with epinephrine (4), the biological half life of protokylol is longer, but the tissue levels of protokylol are quite low or non-detectable 24 hr after administration. Thus, it is obvious that an accumulation of protokylol in tissues does not occur. Of interest is the extensive distribution of protokylol in the kidney. Our experimental results indicated that a part of protokylol is excreted into the urine essentially unchanged. These facts suggest that the kidney is the major excretory organ of protokylol. Further studies are required concerning the metabolic products of protokylol. Action site of protokylol is in the lung, as the lung revealed extensive distribution 60 min after administration.

Protokylol distribution in subcellular fractions of rat lung 10 min after intravenous administrations at a dose of 50 mg/kg was studied. Distribution in the microsome and supernatant showed high values. No recognizable distribution was seen in the debris and the mitochondria fractions. In this experiment, “debris” includes nucleus and fiber, and the “microsome” are fragments of cell membrane and endoplasmic reticulum. “Supernatant” is the extracellular fluid, intracellular fluid and cytoplasm.

Generally speaking, it can be presumed that protokylol hydrochloride is present in solution in an ionized and water soluble state. It follows that ionized protokylol is often unable to penetrate the lipid membrane, however, a free form of protokylol increases lipid solubility which makes it possible for protokylol to diffuse across the cell membrane. Our experimental results concern the subcellular distribution of protokylol in rat lung and it is suggested that protokylol is present in extracellular fluid, intracellular fluid and cytoplasm as a mobile pool.

SUMMARY

Measurement of protokylol in trace amounts has become possible by use of the trihydroxyindole reaction (2, 3). Utilizing this method, a time course study of protokylol distribution in tissues was made in rats following intravenous injection of protokylol at a
dose of 50 mg/kg.

1. The plasma protokylol level showed a higher value 60 min after administration, but it was markedly lowered after 2 hr. A mere trace of protokylol was detectable 5–24 hr in rat plasma.

2. Protokylol levels in 24 hr urine specimen showed remarkably high values.

3. Protokylol was distributed in relatively high concentrations in the kidney, heart, lung, spleen, liver and muscle 10 min after intravenous injection of protokylol. In the intestine, the distribution was much less with only a slight trace in the brain. Sixty min after protokylol administration, large amounts of protokylol were still detected in the kidney, heart and lung, but the concentration in the liver was markedly decreased. A still more remarkable lowering of protokylol concentration was seen in the various organs 5–24 hr after protokylol administration.

4. A detailed observation of protokylol distribution in the subcellular fraction of rat lung indicated that protokylol was distributed in the microsome and supernatant fraction. No recognizable distribution was seen in the debris and the mitochondria fractions.

REFERENCES

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