POTENT INHIBITORY ACTION OF PILOCARPINE ON HEPATIC DRUG METABOLISM

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It has been reported that pilocarpine inhibited the activity of glucose-6-phosphate dehydrogenase in the calf eye (1) and in pure yeast enzyme (2). In vivo studies showed that pilocarpine decreased the latency period and prolonged the narcosis produced by the injection of hexobarbital (3). Whether or not pilocarpine affects the hepatic drug metabolism was investigated herein.

Male rabbits, weighing 2.5-3.5 kg, were fasted approx. 12 hr with water available ad libitum, then sacrificed. The liver was homogenized with 3 volumes of 1.15 % KCl solution in a Waring blender and followed in a Potter-Elvehjem's teflon-glass homogenizer. A liver supernatant fraction was prepared by centrifugation of the homogenate at 10,700 x g for 40 min. This supernatant fraction was centrifuged at 105,000 x g for 1 hr and the firmly packed pellet of microsomes was resuspended in 1.15 % KCl solution containing 50 mM Tris-HCl buffer, pH 7.4 and diluted to a concentration of 2 mg protein per ml. This suspension was utilized for recording difference spectra. The supernatant fraction, 2.5 ml, was mixed with a solution containing 1.5 pmole NADP, 25 pmole G-6-P, 25 pmole MgCl₂, and 3 pmole substrates. The final volume made a total of 5.0 ml with 0.1 M sodium, potassium phosphate buffer, pH 7.4. Aqueous solutions of inhibitors were added in a final concentration to the mixture with volume of 0.2 ml.

Recently, inhibitory effects of nicotinamide on aminopyrine demethylase activities and aniline hydroxylase activities have been reported (4, 5). In preliminary experiments, the addition of 50 μmole of nicotinamide (final concentration: 10⁻²M) resulted in reduced activity by approx. 10 per cent in nicotine metabolism. All experiments described in this report were performed with nicotinamide-free media. Incubation was carried out at 37°C for 30 min under air in a Dubnoff metabolic shaker.

The rate of hydroxylation of hexobarbital was determined by the method of Cooper and Brodie (6). Metabolism of nicotine was determined by measuring the radioactivity of ³H-nicotine remaining, in a liquid scintillation spectrometer (Aloka LSC-502) by the method reported by Hug (7) and described in details by Tsujimoto et al. (8). Aniline hydroxylation
was determined by a modification of the method of Brodie and Axelrod (9) as reported by Kato and Gillette (10). Protein concentrations were estimated by the method of Lowry et al. (11) using bovine serum albumin as the standard.

The activity of glucose-6-phosphate (G-6-P) dehydrogenase was determined according to the method of Glock and Mclean (12) by following the rate of reduction of NADP at 340 nm in a 1 cm cells at 25 °C using a two-wavelength double beam spectrophotometer (Hitachi model 356). The reaction mixture consisted of 50 μl of liver supernatant, 15 μmole of MgCl₂, 15 μmole of G-6-P, 900 μmole of NADP and 0.1 ml of inhibitor. The final volume made up 3.0 ml with 0.2 M Tris-HCl buffer pH 7.4. G-6-P dehydrogenase activity was corrected for 6-phosphogluconate dehydrogenase activity present in the same supernatant. Difference spectra were recorded according to the method of Schenkman et al. (4) in a Hitachi model 356 spectrophotometer.

As shown in Table 1, pilocarpine was found to inhibit the metabolism of hexobarbital although concentration causing a 50 per cent inhibition (I₅₀) was higher than that obtained with SKF 525-A (2-diethylaminomethyl-2, 2-diphenylvalerate hydrochloride) and DPEA (2, 4-dichloro-6-phenylphenoxyethylamine hydrobromide), the most potent inhibitor of drug metabolism (13). Consequently, it is suggested that the potentiating effect of pilocarpine on the narcosis produced by hexobarbital (3) may result in part from its inhibitory action on the drug metabolism in liver. The inhibitory action of pilocarpine on the metabolism of nicotine was markedly more potent than that of hexobarbital. The extent of pilocarpine inhibition of aniline-hydroxylation was shown to be almost equal to that of hexobarbital-hydroxylation. DPEA showed potent inhibitory action of nicotine metabolism but the reaction of aniline-hydroxylation showed a marked resistance to DPEA. The inhibition produced by pilocarpine was approx. 7 times greater than that obtained with DPEA and proved to be the most potent inhibitor of aniline metabolism among the tested compounds. Imipramine, an antidepressant, bearing the inhibitory action of barbiturate metabolism (14) and SKF 525-A were shown to be less potent inhibitors of nicotine and aniline metabolism as compared to pilocarpine. It has been reported that various substances which were supplied as substrates and inhibitors of hepatic drug metabolism caused two

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Type I Hexobarbital</th>
<th>Type II Nicotine</th>
<th>Aniline</th>
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<tbody>
<tr>
<td>Pilocarpine</td>
<td>1.8 ± 0.4 × 10⁻⁴ (5)†</td>
<td>3.6 ± 1.6 × 10⁻⁸ (6)</td>
<td>1.1 ± 0.13 × 10⁻⁴ (5)</td>
</tr>
<tr>
<td>DPEA</td>
<td>2.6 ± 0.2 × 10⁻⁵ (3)</td>
<td>1.0 ± 0.2 × 10⁻⁸ (5)</td>
<td>7.9 ± 2.3 × 10⁻⁴ (3)</td>
</tr>
<tr>
<td>Imipramine</td>
<td>7.4 ± 1.1 × 10⁻⁵ (4)</td>
<td>6.2 ± 1.0 × 10⁻⁷ (4)</td>
<td>4.7 ± 0.3 × 10⁻⁴ (3)</td>
</tr>
<tr>
<td>SKF 525-A</td>
<td>4.9 ± 0.7 × 10⁻⁵ (4)</td>
<td>&gt;1.0 × 10⁻⁴ (4)</td>
<td>&gt;1.0 × 10⁻² (3)</td>
</tr>
</tbody>
</table>

* All substrates had a concentration of 5.8 × 10⁻⁴ M.
† Values are shown as the mean ± S.E.; numbers in parentheses refer to the number of animals used.
types of spectral changes (type I and type II) when added to nonreduced hepatic microsomes. Hexobarbital is shown to be involved in the class of type I, whereas nicotine and aniline in the class of type II (15, 16). These results suggest that pilocarpine may be a potent inhibitor of the metabolism of the type II substrates mediated by hepatic mixed-function oxidases.

The effect of pilocarpine on G-6-P dehydrogenase was examined. The rate of reduction of NADP was 10.7±2.3 (the mean ± S.E. of NADPH produced in μmole per mg protein per min, n=3). The values in the presence of pilocarpine in concentrations of \(2.5 \times 10^{-4} \text{M}\) and \(2.5 \times 10^{-5} \text{M}\) were 10.5±2.1 and 10.4±2.4 (the mean ± S.E. of NADPH produced in μmole per mg protein per min, n=3) respectively. These data show that pilocarpine was without effect on G-6-P dehydrogenase in liver supernatant.

The type I difference spectrum is characterized by the appearance of a trough at about 420 nm and a peak at about 385 nm, whereas the type II difference spectrum is characterized by a peak at about 430 nm and a trough at about 395 nm. Microsomal cytochrome p-450 plays a role as the oxygen activating enzyme for the oxidation of a variety of drugs (17). The pigment with which these spectral change involve interaction was shown to be cytochrome p-450 and the magnitude of the spectral changes depended on the amount of substrates and of microsomal pigments added, suggesting substrate affinity to the enzyme (18). The difference spectra caused by addition 5 mM of drugs are shown in Fig. 1. Pilocarpine was found to induce a typical type II spectral change. The magnitude at the peak and the trough of spectral changes caused by pilocarpine was much greater than that by nicotine. These results indicate that pilocarpine may interact with the microsomal cytochrome p-450 with a greater affinity than that of nicotine.

![Fig. 1. Pilocarpine-induced spectral changes. The spectra were obtained by the addition of 5 mM of each drug to the rabbit liver microsomal suspension.](image-url)
The present study has revealed the inhibitory action of pilocarpine on hepatic drug metabolism. Nature of the inhibition is now under investigation.

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REFERENCES