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SHORT COMMUNICATION

Inhibition of the metabolism of hexobarbital *in vitro*

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In a previous report by Fujimoto *et al*[1] it was suggested, as a result of experiments *in vivo* with certain agents which prolong hexobarbital narcosis, that the metabolism of the barbiturate is inhibited by these agents. Experiments involving the perfusion of the isolated rat liver provided further evidence in support of this conclusion. The agents studied were of current pharmacologic interest; these included β-phenylisopropylhydrazine ((PIH) α-methylphenethylhydrazine)*, N-ethyl-3-piperidyl benzilate (EPB) and N-methyl-3-piperidyl-(N′, N′)-diphenylcarbamate (MPDC).† PIH is a strong monoamine oxidase inhibitor.[2] EPB is a hallucinogenic agent described by Abood *et al*.[3]

Since it is known that the liver microsomal system is responsible for oxidation of hexobarbital,[4] it was of interest to determine whether the inhibition of hexobarbital metabolism, as suggested by the *in vivo*-findings mentioned above, could be demonstrated *in vitro*.

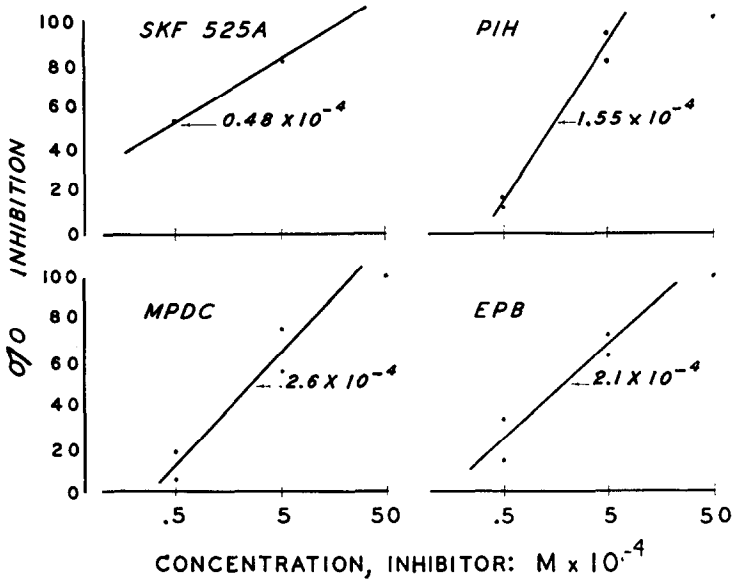

Fig. 1. Inhibition of the metabolism of hexobarbital *in vitro* in a rat liver homogenate supernatant fraction. Concentration of each drug producing 50 per cent inhibition is indicated.

For each drug tested, two adult Sprague–Dawley rats (210–350 g) were stunned and decapitated; the livers were removed immediately, pooled, and homogenized with two volumes of phosphate buffer in a Waring blendor. The supernatant fraction containing the microsomal enzyme system responsible for the enzymic alteration of hexobarbital was then prepared according to the method of Fouts and Brodie[5]. The drug was added directly to the prepared reaction vessels and the degree of

* PIH is also known as JB–516 and is marketed as "Catron".

† EPB is JB–318. MPDC is JB–371. These compounds were obtained through the courtesy of Lakeside Laboratories, Milwaukee, Wisconsin.

inhibition of hexobarbital metabolism was measured by determining the amount of the added hexobarbital remaining in the flasks containing the inhibitor, as compared to that found in the control flasks. Each flask contained 2 μmoles of hexobarbital. Control flasks metabolized hexobarbital at a rate of 1·33 $\pm$ 0·15 (S.D.) μmoles/g/2 hr. The hexobarbital concentrations were determined by the method of Axelrod *et al.*[6] The concentration ranges of drugs were 5×10^{-5} to 5×10^{-3} M. At each level of drug tested, incubations were carried out in duplicate, except for SKF 525-A, with which only single samples were run. This drug had been shown to inhibit this system;[5] thus, it served as a reference drug with which the drugs presently under study could be compared.

The results in Fig. 1 show that the agents PIH, MPDC and EPB, in concentrations slightly larger than that of SKF 525-A, inhibit the liver microsomal system which metabolizes hexobarbital. This inhibition of hexobarbital *in vitro* supports the earlier conclusion that these agents interfere with the inactivation of hexobarbital. Thus the prolongation of sleeping time, higher body concentrations and delay in disappearance from liver perfusate of hexobarbital[1] can be explained by the inhibition of an enzyme system responsible for the metabolism of hexobarbital.

Although the data do indicate the relative potencies of these agents as inhibitors of metabolism of hexobarbital, the present experiments give no information about the specificity of these agents. However, SKF 525-A is known to affect many enzyme systems in the liver, as pointed out by Neubert and Timmler[7]. PIH inhibits monoaminoxidase activity.[2] Our previous work[1] showed that PIH affected not only hexobarbital narcosis but bromsulfalein clearance and thiopental narcosis. EPB affected hexobarbital and thiopenal narcosis but not bromsulfalien clearance. MPDC seemed to be relatively the most specific in inhibiting hexobarbital metabolism in that only hexobarbital narcosis was affected; bromsulfalein clearance and thiopental narcosis were unaffected. Thus it would seem that the least potent compound *in vitro*, MPDC, may on the other hand possess the highest degree of specificity for inhibiting the metabolism of hexobarbital.

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