Induction of microsomal drug-metabolizing enzymes caused by hexobarbital

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Summary. Hexobarbital was given to anaesthetized mice for a period of 7 h by repeated i. p. injection, first of 100 mg/kg, then several times of 50 mg/kg. A high level of hexobarbital was maintained in the liver. The activity of microsomal drug-metabolizing enzymes was induced by this treatment with hexobarbital. 30 min after a single i. p. injection of 100 mg/kg of hexobarbital, there was a significant inhibition of aminopyrine N-demethylase but none of cytochrome c and neotetrazolium reductases. – Hexobarbital in vitro inhibits aminopyrine N-demethylase but not cytochrome c reductase.

It is well-known that barbiturates induce the biosynthesis of drug-metabolizing liver enzymes[1-4]. Especially phenobarbital is often used in order to increase the activities of these enzymes in experimental animals. Hexobarbital, on the other hand, does not induce these enzymes in rodents[3] but does so in dog[5]. In dog, hexobarbital is degraded slowly and thus remains in the liver for long enough to achieve induction; but in rat and mouse, it is metabolized so rapidly that the time required for induction is not reached. By application of hexobarbital and diethylaminoethyl phenyldiallylacetate (CFT 1201) at the same time, Remmer was able to induce the biosynthesis of microsomal drug-metabolizing enzymes in rodents, too[3]. But because CFT 1201 inhibits these enzymes[6,7] the duration of action of hexobarbital is increased so that this substance causes induction as supposed by Remmer[3]. But it cannot be excluded that CFT 1201, besides being an enzyme inhibitor of these enzymes, has an inducing effect, too, as do some substances of a similar structure[8-13]. So enzyme induction achieved in Remmer's experiment may be due to CFT 1201 action or to the common action of both substances.

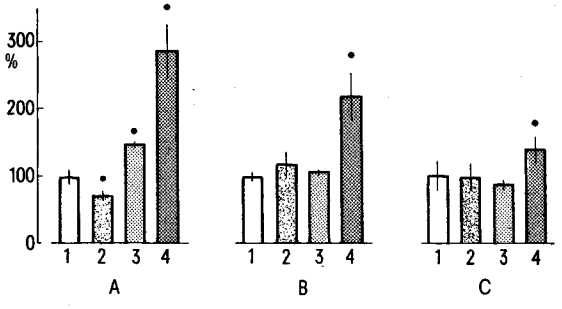

Fig. 1. Liver enzymes under hexobarbital treatment. *A* Aminopyrine N-demethylase, *B* cytochrome c reductase, *C* neotetrazolium reductase. The columns denote: *1* control group, *2* 30 min after a single hexobarbital dose, *3* after a 7-h-treatment with hexobarbital, *4* after a 2 × 7-h-treatment with hexobarbital. The points above the columns denote a difference from the control group of $p = 0.5$. Means ± SD are given.

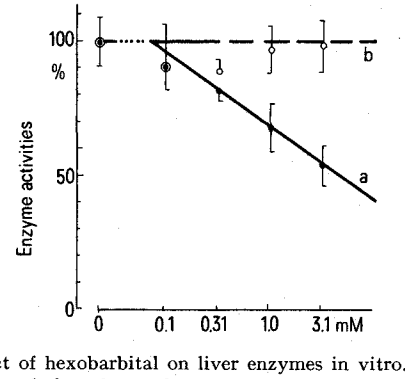

Fig. 2. Effect of hexobarbital on liver enzymes in vitro. *a* Aminopyrine N-demethylase, *b* cytochrome c reductase.
Abscissa: hexobarbital concentrations in a log scale, ordinates: enzyme activities in percent of control. Means ± SD are given.

In our study, we prolonged the action time of hexobarbital by repeated injections during a period of 7 hours at the moment when the mice showed signs of awaking from the preceding injection.

Methods. We used male mice of the NMRI strain (b.wt between 18 and 22 g) which had free access to drinking water and Altromin® chow. Starting at 10 h a.m., 10 mice were injected 100 mg/kg sodium hexobarbital (Evipan-Natrium®) i.p. When they began to awake, they were injected 50 mg/kg sodium hexobarbital i.p., and this treatment with 50 mg/kg sodium hexobarbital was continued until the animals had slept continuously for 7 h. The next day, 17 h after the last application of the narcotic, the animals were killed by decapitation. A second group of 5 mice were injected a single 100 mg/kg i.p. dose of sodium hexobarbital 30 min before being killed, and a third group of 5 mice were used as a control group. Besides these mice, 10 mice were treated for 2 days with sodium hexobarbital in the same way as in the first experiment so that they received the barbiturate for 7 h each day and were killed on the third day, together with the other mice. The livers of all animals were removed immediately after killing and frozen rapidly in liquid nitrogen. The liver specimens were stored at −20 °C, then thawed and homogenized in ice-cold isotonic KCl solution with a Potter-Elvehjem type homogenizer fitted with a teflon pestle. From these crude homogenates, 12,000 × g-supernatants were prepared by centrifugation in which the activities of aminopyrine N-demethylase, NADPH : cytochrome c oxidoreductase

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('cytochrome c reductase') and neotetrazolium reductase were measured as described earlier in this journal[12]. Significance was calculated by Student's t-test[14].

In order to study the effects of hexobarbital on aminopyrine N-demethylase and cytochrome c reductase in vitro, we measured the activities of these enzymes in a 12,000 × g-supernatant prepared from the liver of an untreated rat, in the presence of varying concentrations of hexobarbital.

Results and discussion. In the livers of the mice treated for 2 days with hexobarbital, all enzyme activities were increased significantly due to enzyme induction, while in the mice treated for 1 day, only aminopyrine N-demethylase was elevated (see figure 1). In the 2-day-experiment, sleeping times were shortened at the second day as compared to those of the first day of treatment due to accelerated metabolism of hexobarbital brought about by enzyme induction. So we had to administer the barbiturate more often on the second day (namely 1 × 100 mg/kg plus 16 × 50 mg/kg, as an average) than on the first day (namely 1 × 100 mg/kg plus 11 × 50 mg/kg, as an average) so that the animals received 139% more sodium hexobarbital on the second day than the first day. Relative liver weights increased significantly after only the 1-day-treatment, namely from 3.85 ± 0.20% of b.wt with the control group to 6.33 ± 0.71% with the treated group, in all other cases they did not differ significantly from the control group liver weights.

So it is quite possible to induce the biosynthesis of drug-metabolizing enzymes by hexobarbital in the mouse, without any other interfering substance.

30 min after the hexobarbital administration, demethylase is inhibited but the 2 reductases are constant (see figure 1). This corresponds well with the in vitro measurement of enzyme activities where hexobarbital inhibits only the demethylase but not the reductase (see figure 2). The inductive effect of hexobarbital is a typical diphasic one[8–13]. This seems to be an argument in favor of Goldstein and Goldstein's hypothesis[15] that induction of enzyme biosynthesis is a direct consequence of enzyme inhibition caused by the inducing agent. At least in the case of hexobarbital and the substance HOE 17 879[12], it seems as if it were necessary to maintain, for a minimum time, a certain concentration of the inducing substance in liver until this enzyme inhibition results in an induction of enzyme biosynthesis. If a gene codes more than one enzyme, not only the enzyme inhibited by the inducer is induced but the others too, which may be the case with the 2 reductases which are induced but not inhibited by hexobarbital besides the demethylase.

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Effect of desipramine on the contents of some free amino acids of mouse brain[1]

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Summary. I.p. injections of desipramine-HCl (100 mg/kg) produced decreases in the contents of several amino acids of mouse brain after 1 h. Using a 10–100 mg/kg range of doses, these effects appeared to be dose-dependent for α-alanine and aspartate. These changes may be due, in part, to a decrease in cerebral oxidative metabolism (Krebs cycle activity) which occurs secondarily to desipramine-induced hypothermia.

The mechanism of action of tricyclic antidepressants on the mammalian CNS is not well understood. Relationships of their antidepressant actions with availability of norepinephrine at central synaptic sites[2–4] and with central cholinergic mechanisms[5–7] have been suggested. More recently, Patel et al.[8] showed that injections of desipramine produced elevations in the GABA content of mouse brain and that this effect was related to the hypothermia produced by the drug. In contrast, hypothermia produced by administration of allylglycine (a blocker of glutamate-α-decarboxylase (GAD) activity) was associated with a decrease in hypothalamic GABA content in rats[9]. In the latter case, GAD-blockade apparently outstripped the hypothermic effect on brain GABA.

The present study was undertaken to determine the extents to which various doses of desipramine can influence some other cerebral amino acids, some of which, like GABA, are candidate-transmitters.

Material and methods. Male Swiss-Webster mice, weighing 20–30 g, were injected i.p. with 10–100 mg/kg desipramine-HCl (Lakeside Labs, Inc.) dissolved in 0.154 M NaCl, and were decapitated 1 h later. All animals had food and water ad libitum before experiments. 'Acidic and neutral' amino acids were determined, using a Beckman Model 121C amino acid analyzer after deproteinization and preparation of free amino acid extracts[10, 11].

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