EFFECT OF DIPIN ON INDUCTIVE SYNTHESIS OF CYTOCHROME P_{450} IN THE ENDOPLASMIC RETICULUM OF RAT LIVER CELLS

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After a single injection of the alkylating agent dipin into rats in a dose of 15 mg/kg, followed by delayed intensive induction of cytochrome P_{450} in the endoplasmic reticulum of rat hepatocytes produced by phenobarbital, the level of inductive synthesis of the enzyme was reduced. The effect of the chemical mutagen on inductive synthesis of cytochrome could be demonstrated for seven days.

Sufficient facts have now been accumulated to show the inducing action of phenobarbital (PB) on the synthesis of cytochrome P_{450} [13], a key enzyme of the NADP.H_2-dependent detoxicating system of the endoplasmic reticulum (ER) of rat hepatocytes [2]. It has also been shown that the induction of enzymes by the action of PB is connected basically with their synthesis de novo [12]. Some workers hold the view that PB is a combined inducer and depressor of the genome [11, 14].

It was therefore interesting to investigate the action of a chemical mutagen on the inductive synthesis of cytochrome P_{450} in the ER with the aid of PB.

Despite many investigations of the effects of ionizing radiation on inductive enzyme synthesis [5, 6, 7, 15], the present investigation is of great theoretical importance because the biological effect of radiation is not identical with the action of chemical mutagens, including the so-called radiomimetics. In the present case the late effects of a small dose of chemical mutagen were studied, unlike in most other investigations when the action of relatively large doses of radiation on enzyme synthesis was examined [3, 16].

EXPERIMENTAL METHOD

Noninbred male rats weighing 150-170 g were used. Dipin (tetraethylenimide of piperazinediphosphoric acid) [4], in a dose of 15 mg/kg, was injected once into the animals, while PB, as the soluble sodium salt (Merck) was injected four times in doses of 60 mg/kg at intervals of 24 h. The substances were dissolved in distilled water and injected intraperitoneally.

The rats were killed by decapitation. The liver was perfused with 0.25 M sucrose solution through the inferior vena cava and then extirpated, weighed, and homogenized in 0.25 M sucrose solution in the ratio of 1:3. The homogenate was centrifuged at 9,000 g for 15 min on a high speed 18 MSE centrifuge at 4°C to sediment mitochondria and nuclei. The content of cytochrome in the microsomal faction of the homogenate was determined with the SF-10 spectrophotometer [1]. The difference between the optical density at the maximum at 450 nm and the minimum at 490 nm, calculated per microgram phosphorus of DNA per gram tissue, was used as the index of the cytochrome P_{450} content, expressed in conventional units:

$$\frac{\Delta \text{ OD } 450\text{—}490}{g \cdot P_{DNA}}.$$

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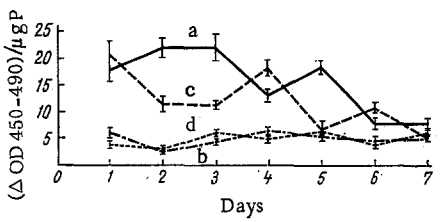

Fig. 1. Concentration of cytochrome P_{450} under different experimental conditions: a) phenobarbital-Na; b) dipin; c) dipin + phenobarbital-Na; d) control.

EXPERIMENTAL RESULTS

After four injections of PB into the animals there was a marked increase in the cytochrome P_{450} level, and 24 h after the last injection of the inducer the cytochrome concentration in ER of the hepatocytes was 4.6 times higher than in the control. During the next two days the cytochrome concentration reached its maximum. The dynamics of the cytochrome concentration followed a gradually falling fluctuating curve. On the sixth day after injection of PB the cytochrome concentration ER of the hepatocytes was indistinguishable from the control (Fig. 1a).

During investigation of the effect of dipin on adaptive synthesis of cytochrome P_{450} in the ER of the hepatocytes for seven days, the cytochrome level was found to be indistinguishable from that of the corresponding control (Fig. 1b). Considering that practically all of the dipin and its metabolites is excreted in about three days [10], the combined action of dipin and PB was tested after an interval of seven days in order to prevent any possible chemical interaction between them.

After a single injection of dipin into the animals followed by injection of PB after seven days, a sharp decrease in the cytochrome P_{450} concentration in the ER membranes was observed compared with its level after PB alone (Fig. 1c). On the second and third days after the last injection of PB the cytochrome concentration of the liver was practically halved. On the whole, the curve of inductive cytochrome synthesis in animals receiving the mutagen was almost the mirror image of the curve of inductive synthesis of the enzyme in the intact animals. The curves of inductive synthesis of cytochrome, the cytochrome level in the animals receiving dipin, and the cytochrome concentration in the rats receiving dipin and PB were indistinguishable from the control on the sixth day of investigation.

The late aftereffect of a small dose of a chemical mutagen can thus lead to a reduction in the inducibility of an enzyme despite its intensive stimulation. These facts concur with others obtained by one of the writers [9] in relation to inhibition of regenerative proliferation in the rat liver after partial hepatectomy if the animal, treated with a noncytostatic dose of the mutagen, was exposed to stimulation with PB. An unequivocal effect also was observed when the time interval between the mutagenic and inducing action was 90 days [8].

The decrease in the inductive synthesis of cytochrome P_{450} observed in this investigation in animals receiving a single therapeutic dose of dipin seven days before induction was probably connected with changes in the genetic apparatus of the liver cells. The existence of genetic injuries induced by mutagens and functional in character can be postulated.

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