DIURNAL CHANGE IN ORNITHINE DECARBOXYLASE ACTIVITY OF RAT LIVER

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SUMMARY

Liver ornithine decarboxylase activity was found to undergo a remarkable diurnal change with a peak at 20:00 in rats receiving laboratory chow ad libitum. The enzyme was almost undetectable in starved rats but increased rapidly on refeeding a protein-rich diet, indicating that the diurnal change in the enzyme level was due to cyclic ingestion of dietary protein. The dietary induction of ornithine decarboxylase appeared to be mediated by growth hormone and glucocorticoids, acting synergistically.

Polyamines have long been known to be widely distributed in mammalian tissues, plants and microorganisms (1), but their exact physiological role is unknown. During the past few years increasing attention has been paid to ornithine decarboxylase, the enzyme for the first step of polyamine synthesis in mammalian tissues, since its activity was found to increase remarkably during the early stage of tissue growth. Namely, it increased in liver after partial hepatectomy (2,3) or after administration of growth hormone (4) or thioacetamide (5), in kidney after folate administration (6), in the ventral prostate after androgen treatment (7), in the oviduct and uterus by estrogen treatment (8) and in epidermis cell culture on addition of the epidermis growth factor (9).

The present investigation demonstrates that rat liver ornithine decarboxylase undergoes a profound diurnal change in activity due to cyclic variation in the amount of protein intake, indicating the possible role of

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polyamines in the diurnal change in liver growth.

MATERIALS AND METHODS

Male Spraque-Dawley rats weighing 100 to 120 g were maintained under natural light from a window at a constant temperature of 23°. Hypophysectomized rats (external auditory canal method) were purchased from Nihon Hypox Laboratories. Laboratory chow containing 25 % protein was obtained from Oriental Yeast Co., Ltd. Bovine growth hormone (NIH-GH-B13) was supplied by the Endocrinology Study Section of the National Institute of Health, U.S.A.

Ornithine decarboxylase activity was assayed using the 80,000 g supernatant of a liver homogenate in 0.25 M sucrose - 1 mM mercaptoethanol. The reaction mixture contained 1 ml of liver supernatant, 125 mμCi of DL-ornithine -1-^{14}C, 0.1 mM of L-ornithine, 2 μM of pyridoxal phosphate and 40 mM of Tris-HCl buffer, pH 7.4, in a final volume of 2.5 ml. Incubation was carried out at 37° for 1 hour with shaking in an air-tight, 25 ml conical flask, with a center well containing a piece of filter paper immersed in 0.05 ml of 10 % KOH. The reaction was stopped by injection of 0.5 ml of 2 N HCl and the mixture was shaken for an additional 20 minutes. The filter paper was then transferred to a vial and radioactivity was determined after addition of scintillation fluid. Values were corrected for the counts in blanks without enzyme. Liver polyamines were measured by the fluorescence method of Herbst and Dion (10) after butanol extraction using the method of Raina (11). The rate of DNA synthesis in liver was determined by measuring thymidine-T incorporation. Thymidine-5-T (15 μCi) was injected into rats intraperitoneally. Two hours later animals were killed. A liver homogenate was made with 0.25 M sucrose - 1 mM mercaptoethanol and the nuclear fraction was obtained by centrifugation at 600 g. After removal of acid soluble materials by extraction with cold 5 % trichloroacetic acid nucleic acids were extracted with hot 5 % trichloroacetic acid and their radioactivity was measured.

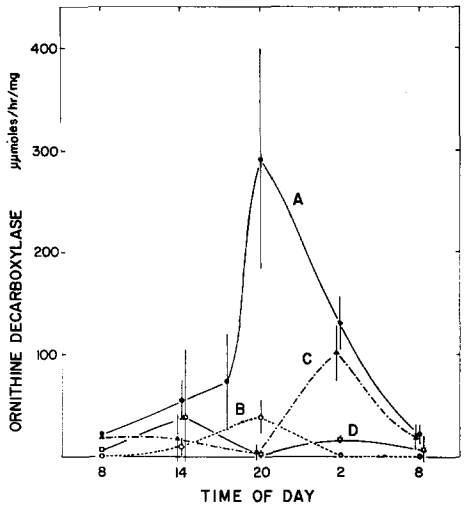

Fig. 1. Effects of starvation, hypophysectomy and adrenalectomy on the diurnal change in liver ornithine decarboxylase. A, Control. B, Starved from 10:00 of the day of experiment. C, Hypophysectomized 7 days before. D, Adrenalectomized 3 days before. For all but B, rats were fed laboratory chow ad libitum. Results are the mean and standard deviation of 4 rats.

RESULTS AND DISCUSSION

As shown in Fig. 1, liver ornithine decarboxylase activity exhibits a diurnal change with a peak at 20:00 in rats with free access to laboratory chow (Curve A). The cyclic variation in enzyme activity is dependent on food intake since the enzyme activity is almost undetectable in starved rats (Curve B). The enzyme was nearly completely lost in hypophysectomized rats although animals received laboratory chow ad libitum (Curve C). In adrenalectomized rats, the phase of the rhythm changed and the peak was lower (Curve D). The latter results suggest that hormones from the pituitary and adrenals may be involved in the dietary induction of the decarboxylase.

Next, the effect of the composition of the food was examined. Synthetic diet containing either 50 % or 0 % casein as the protein component was given to rats which had been starved for 26 hours. Fig. 2 shows that liver ornithine decarboxylase was only induced in animals on the protein-rich diet, indicating that protein induces the enzyme. Some gluconeogenic enzymes in liver, such as serine dehydratase and phosphoenolpyruvate carboxykinase, are known to be induced by a protein-rich diet (12,13), but they are also induced by starvation (13,14). Therefore, the dietary inductions of the decarboxylase and of gluconeogenic enzymes in liver must have different mechanisms.

Fausto reported recently that ornithine decarboxylase was induced by casein hydrolyzate in rat liver (15). This induction was observed to take place even in hypophysectomized rats. The reason of the discrepancy between his results and ours is not clear exactly.

It has been reported that growth hormone induces a marked increase in rat liver ornithine decarboxylase (4). As already mentioned, our results suggested that pituitary and adrenal hormones participate in the dietary induction of the enzyme. To confirm this, the effect of administration of

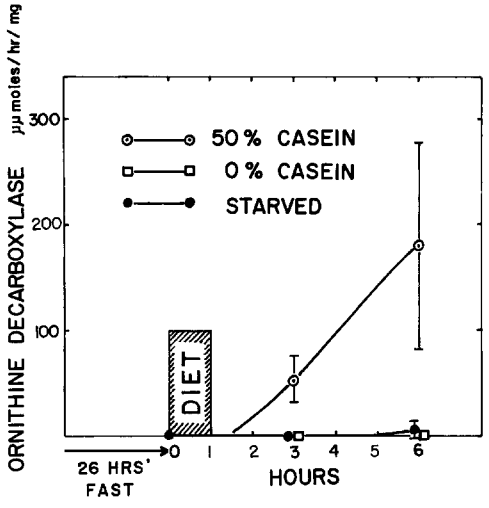

Fig. 2. Effect of dietary protein content on induction of ornithine decarboxylase. Results are the mean and standard deviation of 3 to 4 rats.

these hormones was studied. Starved rats were employed to avoid the complication of the effect of diet. As shown in Fig. 3, the liver ornithine decarboxylase was induced in normal rats by injection of either growth hormone or prednisolone. The effects of these hormones were not so great in adrenalectomized or hypophysectomized rats as in normal rats. These hormones caused the most induction when administered together. The synergistic effects of the two hormones suggest that they act by different mechanisms. Panko and Kenney reported recently that rat liver ornithine decarboxylase was induced by several hormones, including hydrocortisone, insulin and glucagon (16) but further studies are needed on the mechanism of enzyme induction.

Fig. 4 shows the diurnal change in the ornithine decarboxylase activity,

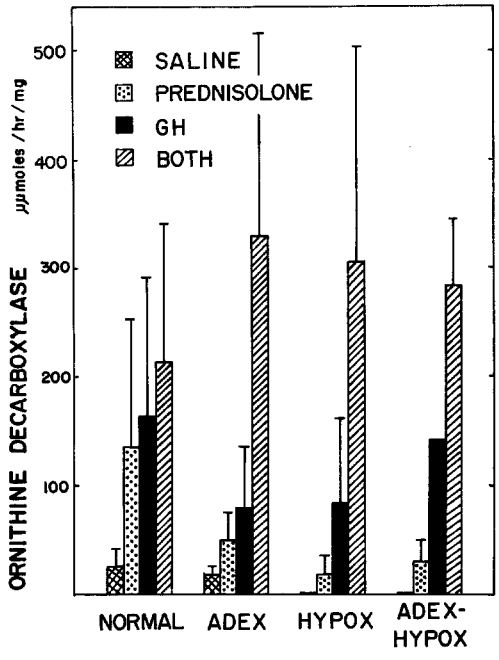

Fig. 3. Synergistic effects of growth hormone and glucocorticoid on ornithine decarboxylase. Rats were injected intraperitoneally with 0.2 mg of bovine growth hormone or 2 mg of prednisolone hemisuccinate or both 4 hours before sacrifice. Food was removed at the time of injection (9:00). Results are the mean and standard deviation of 3 to 5 rats except the second column from the right which represents only one rat.

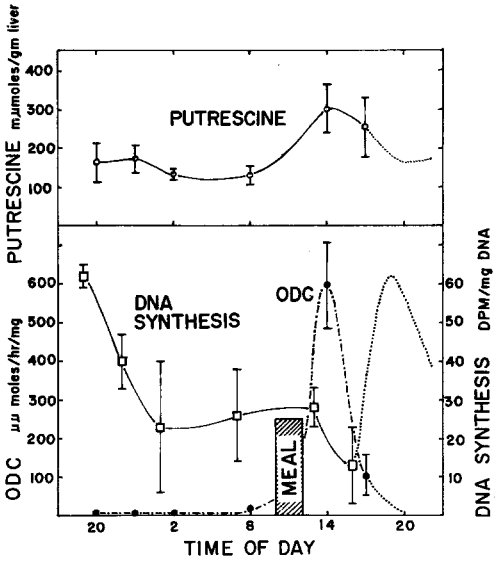

Fig. 4. Diurnal changes in the putrescine content, DNA synthesis and ornithine decarboxylase in the liver of meal-fed rats. Results are the mean and standard deviation of 3 rats. ODC, Ornithine decarboxylase.

putrescine content and rate of DNA synthesis in the liver of meal-fed rats. Rats were given laboratory chow from 10:00 to 12:00 every day for 3 weeks. The decarboxylase activity rose sharply after the feeding period, attained a peak at 14:00, that is 4 hours after the start of feeding, and then decreased rapidly to zero. The result clearly confirms that the diurnal change in the enzyme activity is due to cyclic variation in food intake. The peak enzyme activity was about the same as that in regenerating liver. Slight induction of the decarboxylase in starved animals on refeeding has been reported by Schrock et al. (17). The liver putrescine content was found to change diurnally, the peak coinciding with that of decarboxylase, while no significant change was observed in the content of spermidine or spermine. DNA synthesis also exhibited a cyclic variation with a peak at 19:00, 5 hours later than that of decarboxylase. Other experiments showed that the rate of DNA synthesis was highest at 2:00 in rats fed ad libitum, the peak being 6 hours later than that of the decarboxylase. The close parallel between the syntheses of putrescine and DNA suggests that putrescine may play a role in liver growth, which has been shown to undergo a diurnal change due to variation in food intake (18).

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