PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 150, 380–384 (1975)

Lack of Effect of Portacaval Shunt on Ornithine Decarboxylase Activity in Regenerating Rat Liver (39040)

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(Introduced by M. Lipkin)

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Ornithine decarboxylase catalyzes the decarboxylation of ornithine to putrescine which is then a precursor to the polyamines, spermine and spermidine. This enzyme is of interest, because, at least in mammals, it is perhaps the key regulatory step in the metabolic production of polyamines (23). The role of polyamines has been the subject of much investigation and a number of reviews have discussed in detail their occurrence, synthesis, degradation and possible function (1, 4, 15, 21, 23).

In animals, several lines of evidence have indicated a relationship between polyamine synthesis and accumulation and rapid cell growth. In regenerating rat liver and in developing rat, chick, and amphibian embryos the polyamine levels parallel rapid cell growth (18, 22). In regenerating rat liver the spermidine level also parallels increases in the level of RNA synthesis (12, 16, 17).

As with the polyamine and RNA levels, there seems to be a relationship between ornithine decarboxylase activity and rapid cell growth. High levels of ornithine decarboxylase in rat liver are found as soon as four hours after partial hepatectomy, and also in the early stages of the development of chick, rat, and amphibian embryos (22). The level of ornithine decarboxylase can also be enhanced by hormonal stimulation, such as that caused in the liver by growth hormone (13) or in the ventral prostate by androgen treatment (15). In addition, certain rat sarcomas and cultured rat hepatoma lines have also been shown to have very high ornithine decarboxylase activity (10, 22). Fast growing Morris hepatomas have been found to have very high ornithine decarboxylase activity while a number of slower growing and more differentiated hepatomas have considerably less elevated levels (24).

Though there is a wealth of evidence suggesting a link between high ornithine decar boxylase activity and rapid cell growth it has been very difficult to show any firm direct relationship between high enzyme levels and RNA synthesis and cellular growth.

The report by Fischer *et al.* (6) that the increase in ornithine decarboxylase, which normally follows partial hepatectomy, did not occur in rats which had end to side portacaval shunts is in apparent conflict with the postulated relationship between ornithine decarboxylase activity and rapid cell growth. Despite the portacaval shunt it appears that relatively normal liver regeneration does occur since it has been shown that the rate of DNA synthesis and the number of dividing cells in the regenerating liver of the portacaval shunted and control animals do not differ (5, 21). In addition, the ligation and sectioning of the portal vein before partial hepatectomy has been shown to delay liver regeneration only slightly (3).

The work of Fischer *et al.* (6) would show that in the case of liver regeneration a rise in the ornithine decarboxylase level is not essential for renewed cellular growth. This would make much of the conjecture about the role of this enzyme incorrect. We have attempted to repeat their experiment but were unable to confirm this finding. The well established pattern of an increased ornithine decarboxylase activity in the liver following partial hepatectomy has been observed. Animals with the portacaval shunt also showed a sharp rise in the ornithine decarboxylase activity following partial hepatectomy. The enzyme level was, in fact, found to reach a peak value several hours faster in those animals with the diverted portal blood flow than in the control animals.

Materials. L-ornithine 1-^{14}C monohydrochloride (58 mCi/mmole) and NCS tissue

solubilizer were obtained from Amersham/Searle Corporation. Pyridoxal phosphate was obtained from Sigma, and (Tris) Tris (hydroxymethyl) amonomethane from Fisher Scientific Company. Dithiothreitol and *N*-2-hydroxy ethylpiperazine-*N*-2-ethanesulfonic acid (Hepes) were obtained from Calbiochem.

Methods. Assay of ornithine decarboxylase. The ornithine decarboxylase activity was determined by measuring the production of $^{14}CO_2$ from the carboxyl labeled ornithine using a modification of the method of Russell and Snyder (19). The standard ornithine decarboxylase assay mixture consisted of 50 m*M* Hepes buffer, pH 7.4, 1 m*M* EDTA, 1 m*M* dithiothreitol, 0.05 m*M* pyridoxal-5′-phosphate, 0.06 m*M* L-ornithine (L-ornithine 1-^{14}C 1 μCi/1 μMole), and an enzyme preparation containing 0.5–3.5 mg protein. The reaction volume was 2.0 ml. After preincubation of the enzyme, the reaction mixtures were shaken for 50 min at 37° and the reaction stopped by injecting 1 ml of 2 *M* citric acid into the flask. After the reaction was stopped the flasks were allowed to stand 60 min at room temperature before the center wells were removed and placed in vials containing 15 ml of a diotol phosphor. The $^{14}CO_2$ trapping solution in the center well was 0.20 ml of NCS solubilizer. All determinations were performed in triplicate and control incubations without enzyme or with acid inactivated enzyme were both done routinely with each experiment.

The enzyme activity was expressed as p-moles or nmole of product formed during the 50 min incubation period per mg protein in reaction flask. The substrate concentration was not a saturating level, but rather a level near the K_m concentration which did not excessively dilute the labeled substrate.

The protein concentration determinations were done spectrophotometrically by using the 280 and 260 nm absorbance of the protein solutions and also by using the Lowry protein determination method (14).

Portacaval shunt. End to side portacaval shunts were performed on mature Charles Rivers female rats weighing 250–300 g. After the operation the rats were maintained on Purina Chow and Mouse Chow, and water, *ad libitum*. The animals were not used for the partial hepatectomies until after they had recovered from the operation and had gained weight above that of their preoperation weight. The partial hepatectomy usually took place 1–3 months after the shunt.

Partial hepatectomy. Partial hepatectomies were carried out on portacaval shunt animals and also on normal controls. The normal control animals from Carsworth and Charles Rivers were matched for age with the portacaval shunt animals rather than weight, since the shunted animals lost weight initially after the operation. The 270–400 g rats were anesthetized using ether and the partial hepatectomy was performed with 60–70% of the liver being removed by the method of Higgins and Anderson (9). All operations were done between 10 and 12 AM since there is a circadian periodicity of many cellular functions of the liver (2, 8). The animals were killed by decapitation. The liver tissue was homogenized in 2 vol of a 100 m*M* Tris–HCl, 1 m*M* dithiothreitol solution, pH 7.4. The liver homogenate was centrifuged for 1 h at 38,000*g*. and the supernatant fraction was then immediately used for the enzyme assay. Enzyme fractions were not stored before using. The level of enzyme activity was found to be strongly dependent upon the extent of the partial hepatectomy, and 20–50% partial hepatectomy values were discarded.

Mitotic counts. A small sample of liver tissue was removed during the partial hepatectomy and then again after the animal was killed, these samples were stained using Bouin's fixative so that mitotic counts could be made.

Results. Enzyme stability and assay conditions. The homogenate obtained from the livers of 200 g female rats 18 hr after partial hepatectomy was used to determine some of the physical properties of the enzyme. As reported by others (7), the stability of the enzyme was shown to be dependent upon the presence of dithiothreitol in the homogenate solution, and also in the reaction mixture. The release of CO_2 in the reaction flask was found to be linear for the 50-min incubation when 1 m*M* dithiothreitol was present. The reaction rate was proportional to the protein concentration up to 3.5 mg per reaction flask.

Dialysis of liver homogenate did not affect the enzyme activity, but the reaction rate was

linear to somewhat higher protein concentrations after dialysis. The enzyme solutions with 1 m*M* dithiothreitol could be kept at 0° for several hours without loss of enzyme activity or somewhat less reliably at −90° for several days. The enzyme was also found to be stable for more than 2 hr in the excised liver tissue kept on ice, but tissues were not kept on ice for more than 20 min before homogenization and centrifugation were begun. The enzyme was found to be unstable in frozen liver tissue.

Several different buffers were used in the reaction mixture and Hepes buffer was found to give the highest enzyme activity. The buffers were used at 50 m*M* and are listed according to decreasing enzyme activity, Hepes, Tris, glycylglycine, 25 m*M* sucrose–Tris, and potassium phosphate. The enzyme activity in the phosphate buffer was only 36% of that in Hepes buffer.

The effects of portacaval shunt. The total liver weight of 21 end to side portacaval shunt animals after recovery from the operation, expressed as percent animal weight was 2.6 ± 0.3. In 17 normal animals the average liver weight was 4.2 ± 0.5%. These liver weights are presumed to reflect the hepatic blood flow and nutrient supply. Therefore, the large difference in liver weight between the two animal groups should indicate the extent to which the operations were successful. These weights are very similar to those reported by Fischer *et al.* (6).

In several animals portal venous angiograms were made by injecting dyes in the explanted spleen. The portal venous blood flow of portacaval operated animals was shown to bypass the liver. This was found to be the case in animals recently operated on and in animals 12 months after the operation.

The pattern of the ornithine decarboxylase activity increase in rat liver following partial hepatectomy is shown in Fig. 1. The normal animals reached a peak enzyme level of 1900 pmoles/mg protein/50 min about 18 hr after surgery. This is 280-fold that of the initial level of 7 ± 7 pmoles. This result is similar to other reported values. The portacaval shunt animals in contrast reached a maximal enzyme activity within 8 hr after partial hepatectomy. The peak value of enzymatic activity of 2000 pmoles/mg protein/50 min is essentially that seen with the control animals, while the preoperative value of 6 ± 7 pmoles is also nearly identical. The increase in the ornithine decarboxylase activity following partial hepatectomy has not been altered quantitatively, although there is a slight change in chronology.

The enzyme activity was assayed both with and without dithiothreitol in the reaction mixture. This was done because Fischer *et al.* had not used any reducing agent in their assay system, and some particular problem due to poor enzyme stability might have explained the disparity between the results of the two laboratories. The enzyme activity

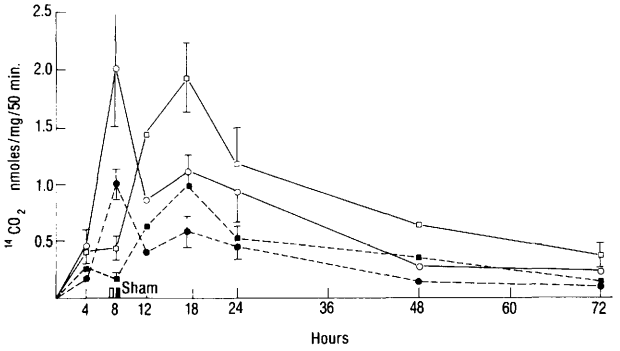

FIG. 1. Ornithine decarboxylase activity as a function of time, following partial hepatectomy. Each point represents the average activity obtained from the liver homogenate from two to four animals, each assayed separately. The error is ±SD. Points without error bars are average values of two experimental points. The solid lines represent the enzyme activity level as assayed in the assay mixture containing dithiothreitol, the dotted lines in the absence of dithiothreitol. Circle marked lines are portacaval shunt operated animals, the square marked lines are the control animals. The enzyme activity of the sham operated animals after 8 hr is shown. The open bar is the value for normal controls and the solid bar is the value for shunted animals.

was routinely 50% lower in the absence of dithiothreitol in the reaction mixture, but the relative patterns of the enzyme activity over the 72-hr period were not affected.

Sham operations. Both normal and portacaval shunt operated animals were subjected to sham partial hepatectomy operations in order to determine the effect of the operation stress on the animals. The ornithine decarboxylase activity did increase as a result of the sham operation, but this increase was considerably less than that caused by partial hepatectomy. Both groups of animals had nearly the same increase in enzyme activity 8 hr after the sham operation (Fig. 1).

Rate of liver regeneration. To help determine whether there were differences in the rate of liver regeneration in the portacaval shunt operated animals and the controls, the increase in mitotic figures was determined. During the first 24 hr after partial hepatectomy, neither group of animals showed any increase in cell division. At 48 and 72 hr they had nearly identical cellular division rates. The control animals had an average of 24 mitotic figures per 1000 cells counted at 48 hr and 15 after 72 hr. The portacaval animals had an average of 25 and 14 mitotic figures per 1000 cells at 48 and 72 hr. Unfortunately, the peak time of mitotic activity was probably missed. In addition, the relative rate at which the liver regained weight after the operation was the same in the groups of animals.

Discussion. The enzyme ornithine decarboxylase can have wide fluctuations in its concentration. Apparently its activity is controlled by its concentration rather than by product or other metabolite molecule regulation. The liver enzyme level is influenced by specific factors such as growth hormone, partial hepatectomy, and also by a variety of other stimuli. The infusion of large amounts of glucose and mannitol and even the intraperitoneal injection of celite can stimulate the enzyme level (20). In addition, carbon tetrachloride, a hepatoxic compound which is known to cause liver necrosis followed by liver regeneration, has been shown to stimulate ornithine decarboxylase in rat liver to about the same extent as that caused by surgical partial hepatectomy (11). The ornithine decarboxylase level also increases following surgical partial hepatectomy in animals that have cirrhotic livers as the result of prior carbon tetrachloride injections (6).

In view of the variety of conditions under which ornithine decarboxylase levels in the liver can be stimulated, the report that the portacaval shunt can abolish the rise in enzyme activity, which normally follows partial hepatectomy, is surprising.

Our findings show no suppression by the portacaval shunt of the increase in ornithine decarboxylase activity in regenerating rat liver. The disagreement between these results and those of Fischer *et al.* are not explainable in terms of any obvious errors or fundamental differences in the surgical or assay procedure. The enzyme does have a rapid turnover rate and its level has been shown to be influenced by a number of factors. This poses a number of problems in insuring that the enzyme activity is maintained during the handling of the animals and the preparation of the enzyme for the assay. It may be that the reported lack of enzyme activity in portacaval shunt operated animals is due to difficulties in preserving the activity in the absence of a reducing agent present during the isolation or assay procedure.

Summary. The influence of the end to side portacaval shunt on the level of activity of the enzyme ornithine decarboxylase following partial hepatectomy was investigated. A sharp rise in the enzyme level was found in both the shunted animals and in the normal controls. The chronology of the increase in enzymic activity was somewhat altered in the portacaval shunted animals, but both groups had similar peak values. The rate of cell division was also found to be unaffected. This report finds no evidence of support for the reported inhibition by the portacaval shunt of the increase in ornithine decarboxylase following partial hepatectomy.

The authors thank George F. Brown for his technical assistance. This work was supported in part by USPHS Grant No. CA-08748 and USPHS Fellowship No. CA-01332-01.

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Received May 21, 1975. P.S.E.B.M. 1975, Vol. 150.