Res Exp Med (1992) 192:131–136

Research in
Experimental Medicine

Liver regeneration following portacaval shunt in rats: 3′, 5′-cyclic AMP changes in plasma and liver tissue

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Received July 31, 1991 / accepted December 4, 1991

Summary. Hepatic regeneration following portacaval shunt was evaluated in the light of the changes of 3′, 5′-cyclic AMP (cAMP) levels in plasma and liver tissue, and also plasma cAMP levels after glucagon administration, using a 70% hepatectomy and portacaval shunt model in rats. Ratios of liver to body weight recovered to 60% of pre-operative ratios 2 weeks after the operation. Plasma cAMP level decreased slightly, but insignificantly, 1 day and 3 days after the operation, then recovered to the pre-operative level. Hepatic cAMP concentration increased for 2 weeks after the operation. Plasma cAMP levels induced after glucagon administration did not increase until 3 days after the operation and were only 50% of pre-operative levels 4 weeks after the operation. From these results, liver regeneration following the portacaval shunt was remarkably delayed, but continued until at least 2 weeks after the operation.

Key words: Liver regeneration – Portacaval shunt – 3′, 5′-cyclic AMP

Introduction

The effect of the portacaval shunt upon liver regeneration remains controversial. It is generally recognized that considerable atrophy results from the restriction of portal blood flow, but even such a liver is capable of restoring its hepatic mass [3]. However, not all investigators agree with such a conclusion.

The cyclic nucleotide 3′, 5′-cyclic AMP (cAMP) contributes to the liver regeneration process, and the onset of DNA synthesis and mitosis seem to be governed by early cAMP-dependent events [12]. On the other hand, it is considered that the plasma cAMP level after stimulation by glucagon is an accurate index of the hepatic cAMP response [5]. However, such evidence of liver regeneration following the portacaval shunt has not yet been investigated.

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Thus, the purpose of this paper is to reassess liver regeneration following the portacaval shunt in the light of the changes in plasma and hepatic cAMP as well as the hepatic cAMP response to glucagon.

Materials and methods

Wistar male rats weighing 300 to 350 g were used. All animals were housed in individual cages, permitted water and standard diet ad libitum, and were examined after a 24-h fast.

Seventy-eight rats were divided into two groups: Group 1 – portacaval shunt alone (PCS group); Group 2 – simultaneous portacaval shunt and 70% hepatectomy (PCS + HX group).

Ether anesthesia was employed for all operative procedures. Techniques used in the production of end-to-side portacaval shunt in these rats were accomplished by means of Funovics' cuff technique [6]. Hepatectomy was performed using the technique of Higgins and Anderson [7].

Twelve rats (six from each group) were used to observe the plasma cAMP level after stimulation by glucagon. Under the anesthesia using intraperitoneal administration, 50 mg/kg of thiopental sodium and 50 μg/kg of glucagon (Novo, Denmark) were given intravenously through the dorsal vein of the penis. Blood samples (50 μl) were taken from the tail vein at 0, 10, and 20 min and immediately diluted with 0.2 ml of m*M* EDTA solution in saline. The livers were excised from the other 66 rats and were quickly frozen, using liquid nitrogen. This procedure was performed before the operation, after 1, 3, 7, 14, and 28 days (each time using six rats from each group), using intraperitoneal administration of 50 mg/kg of thiopen-tal sodium.

To ascertain the plasma and tissue cAMP levels, samples were treated by means of the method described by Honma et al. [8], and the cAMP was measured using the cyclic AMP kit (Yamasa, Japan).

A frozen block of liver was sliced at −20°C by a microtome to prepare thin (10 μm) frozen sections, which were then lyophilized in vacuo. Lyophilized sections, after being accurately weighed on a microbalance, were put into 0.1 ml of the 0.1 N HCl-10 m*M* EDTA solution, which was then immersed in boiling water for 3 min to provide an acid extract of the tissue suitable for the assay.

To 100 μl of a sample (a standard or test solution containing cyclic AMP), 100 μl of the dioxanetriethylamine mixture containing succinic anhydride was added. After 10 min at room temperature, the reaction mixture was added to 0.5 to 1.0 ml of 0.5 *M* imidazole buffer containing 0.5% bovine serum albumin, 8 m*M* theophylline, and 0.01% streptomycin. To 100 μl of the above mixture, 100 μl of [^{125}I] SCAMPTME (15,000–20,000 cpm in an amount less than 10^{-14} mole) and 100 μl of the diluted antisera were added: the mixture was kept at 4°C overnight (about 15 h). A cold solution of dextran-coated charcoal (0.5 ml) was added to the above mixture, which was cooled in an ice-cold water bath. The charcoal was then spun down, and 0.5 ml of the supernatant was counted for radioactivity in a gamma spectrometer.

Statistical analysis was carried out according to the Student's *t*-test.

Results

The percentage ratios of the liver weight to body weight in the PCS group decreased to 2.8 ± 0.2% on day 3 after the operation from 3.4 ± 0.2% of the preoperative value and recovered a little to 3.1 ± 0.1% 2 weeks after the operation. Those ratios in the PCS + HX group reached the minimum level (1.3 ± 0.1%) 1 day after the operation and then increased to 2.1 ± 0.1% 2 weeks after the operation, but did not recover to the level of the values in the PCS group (Fig. 1).

The plasma cAMP level before the operation was 73.6 ± 30.4 pmol/ml, and showed no significant difference after the operation in the PCS group. This level slightly decreased 1 day and 3 days after the operation in the PCS + HX group,

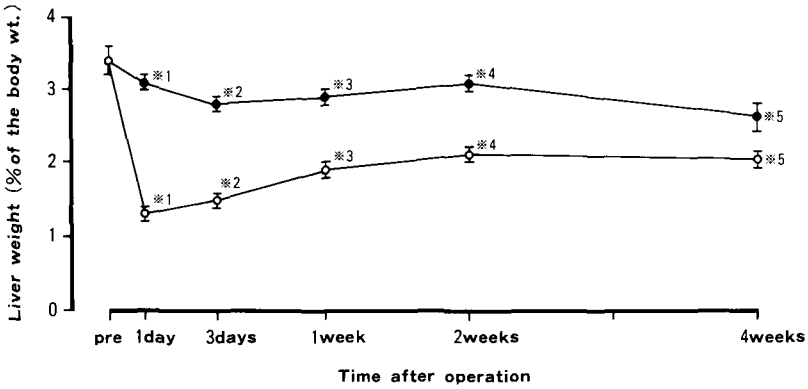

Fig. 1. Liver weight (% b. wt; mean ± SEM) following protacaval shunt with 70% hepatectomy (○—○) and portacaval shunt alone (●—●); * $P < 0.05$

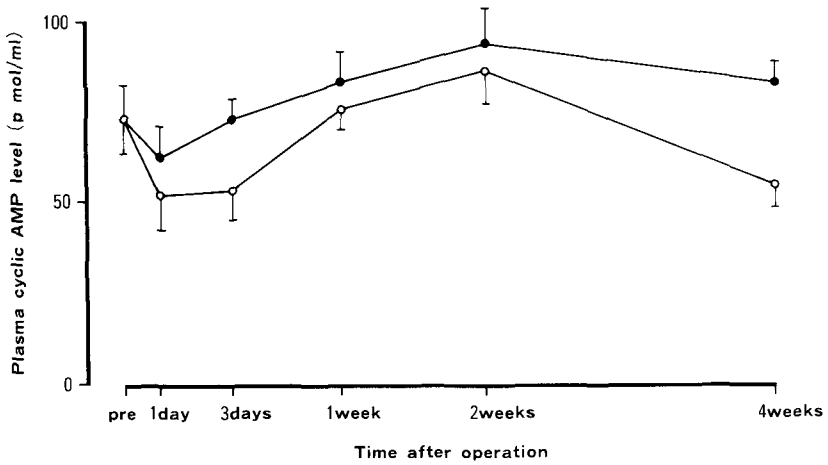

Fig. 2. Plasma cyclic AMP level (mean ± SEM) following portacaval shunt with 70% hepatectomy (○—○) and portacaval shunt alone (●—●)

then recovered to the pre-operative value. However, there was no significant difference between the two groups (Fig. 2).

The tissue cAMP content in the liver was 2.1 ± 0.1 nmol/g of wet weight, showing no significant difference after the operation in the PCS group. In the PCS + HX group, this content increased significantly with a maximum level of 4.6 ± 0.1 nmol/g wet weight. 1 week after the operation, then decreased to the level in the PCS group during the 4 weeks following the operation (Fig. 3). The tissue cAMP content in the liver after glucagon administration was not measured because a direct correlation between cAMP levels in plasma and liver tissue after glucagon administration had already been established [9].

The plasma cAMP level after glucagon stimulation reached the maximum level 10 min later. This 10-minute rate, indicating the value of the level 10 min after stimulation, was 1051.2 ± 184.3 pmol/ml, showing a marked decrease in

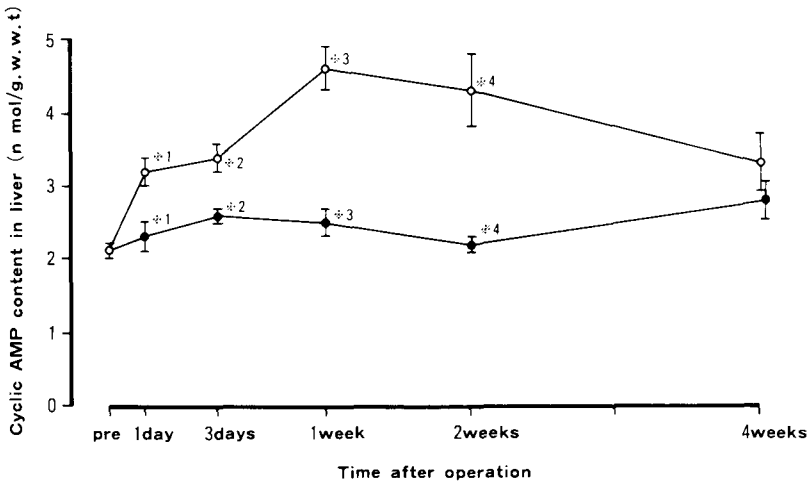

Fig. 3. Cyclic AMP content in liver (mean ± SEM) following portacaval shunt with 70% hepatectomy (○—○) and portacaval shunt alone (●—●): * $P < 0.05$

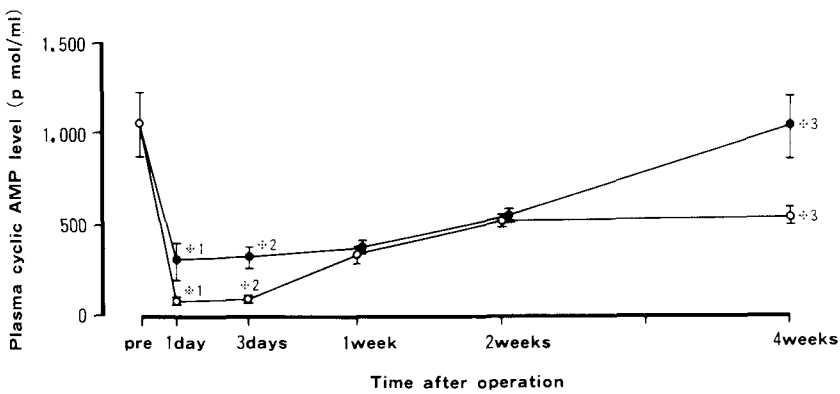

Fig. 4. The 10-min rate (mean ± SEM) indicating the value of the plasma cyclic AMP level 10 min after glucagon stimulation portacaval shunt with 70% hepatectomy (○—○) and portacaval shunt alone (●—●): * $P < 0.05$

both groups, especially in the PCS + HX group, which indicated almost no response over the following 3 days. Both groups went on to gradually recover, but after 4 weeks the PCS + HX group had only one-half the response of the PCS group (Fig. 4).

Discussion

The portacaval shunt operation for portal hypertension has been used as an appropriate model for experimental studies of the significance of portal blood in liver regeneration.

It had previously been held that factors in portal blood play an important role in liver regeneration [11], but Fisher [2] reported in 1962 that portal blood-flow

volume was one of the definitive factors of liver regeneration following a 70% hepatectomy with the partacaval shunt using rats.

However, it has been further suggested that after several studies using liver transplantation models [3] and portacaval transportation models [10, 14] that factors of portal blood, especially pancreatic hormones, are important for liver regeneration. Recently, many investigators have made great efforts to determine the effect of the portacaval shunt upon liver regeneration, but this is as yet unresolved.

On the other hand, the variation in the hepatic cAMP concentration and the relationship between DNA synthesis and the regenerating liver are well documented [4, 13, 15]. It has been suggested that the onset of DNA synthesis and mitosis is governed by cAMP-dependent events [12].

In our study of hepatic regeneration following the portacaval shunt, ratios of liver to body weight recovered to 60% of pre-operative ratios 2 weeks after the operation. Plasma cAMP levels did not vary significantly after the operation, but hepatic cAMP increased for 2 weeks after the operation. From these results, liver regeneration following the portacaval shunt was seen to be remarkably delayed, but continued until at least 2 weeks after the operation.

It is widely recognized that the effects of glucagon are mediated by specific adenylate cyclase systems and that appropriate doses of glucagon may be expected to increase the cAMP levels in glucagon-sensitive tissues. The liver is likely to be the primary target organ and, because of its size and sensitivity to glucagon, may be the prinicipal contributor to any process of transfer of cAMP to plasma [1, 8]. This concept is supported by some observation of hepatectomized animals [5], which showed a lack of effect of glucagon on the plasma cAMP levels, but it is not certain if the plasma cAMP levels after stimulation by glucagon accurately reflect the hepatic cAMP response in liver regeneration following the portacaval shunt.

In our study, the plasma cAMP levels induced after glucagon administration did not increase until 3 days after the operation and were only 50% of the preoperative levels 4 weeks after the operation. From these results, the plasma cAMP response to glucagon stimulation seemed to indicate the functional hepatocytes volume of liver regeneration under the portacaval shunt.

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