natural ability of the vitreous to inhibit new blood vessel growth.

1 Institute of Ophthalmology, London.
2 Reprint request to J.B.W., Departments of Biochemistry and Rheumatology, University of Manchester Medical School, Stopford Building, Oxford Road, Manchester, M13 9PT, England.

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Hepatic proline after bile duct ligation in rats

T.S. Chen, C.L. Boesch and C.M. Leevy

V.A. Medical Center, East Orange (New Jersey 07019, USA), July 27, 1982

Summary. Since biliary hyperplasia of fascioliasis correlated with hepatic proline level, we examined the occurrence of a similar chemical stimulus during bile obstruction. Uptake of tritiated proline and glycine rose in both hepatocytes and a bile duct enriched cell fraction, following duct ligation in rats. The increased hepatic content of proline but not glycine suggests that proline has a role in post-obstructive biliary proliferation.

Proline infused i.p. or into the common bile duct of rats induces hyperplasia of biliary ducts. Both models were suggested by experiments with *Fasciola hepatica* infestation[1,2]. The parasite secretes proline at a high rate into the bile fluid. When *F. hepatica* is placed within the bile duct, the rat liver shows early biliary hyperplasia prior to the development of inflammation and fibrosis. Similar duct excess appears after adult liver flukes, encased in mesh, are implanted into the abdominal cavity. The hyperplastic response may be inhibited by the concurrent administration of L-azetidine-2-carboxylic acid, a proline analog[3]. These observations indicate that a chemical stimulus induces biliary proliferation. We wondered whether a similar signal may also have a role in the biliary hyperplasia after obstruction of the common bile duct. Hence we undertook to measure the hepatic uptake and level of proline after ligating the duct in rats. For comparison glycine was also measured.

Methods. Male Sprague-Dawley rats, 250–300 g each, underwent under light ether anesthesia ligation of the distal common bile duct with double suture ligatures. Sham operated rats had their abdominal cavities opened and the bile duct gently touched.

1, 3 and 7 days after surgery the animals were sacrificed after perfusion and removal of their livers. 2 h prior to perfusion each rat received i.p. 250 μc of ^{3}H proline or ^{3}H glycine (each sp. act. 20 Ci/mmole). The liver was perfused in situ with 100 ml Krebs-Ringer buffer containing 0.1% collagenase and 0.05% hyaluronidase. The fluid was delivered by a constant infusion pump, retrograde into the hepatic vein, via the inferior vena cava after tying off the tributaries and the supradiaphragmatic segment. The excised liver was further digested with 1% collagenase for 10 min to prepare a suspension of single cells. The hepatocyte fraction was removed by centrifugation at 50 × g for 1 min. This fraction contained 95% hepatocytes. The bile duct enriched fraction in the supernatant was collected at 300 g for 5 min[4]. The hepatocytes, after 3 washes with Eagle's minimal essential medium (MEM, from GIBCO), were further purified by layering over lymphocyte separation medium (Histopaque, Sigma), and after centrifugation at 400 × g for 30 min collecting the pellet. The duct cell enriched fraction was suspended in 10 ml MEM and underlayered with 10 ml of 15% metrizamide (Winthrop Labs)[4]. Following spinning at 300 g for 8 min, the duct cells were removed from the interface. This fraction, observed under the light microscope, contained 40–60% duct cells, the remainder were Kupffer (20–30%), sinusoidal and other small oval cells (20–30%). Cells of the 2 fractions were solubilized in 1 N NaOH, and radioactivity counted in Aquasol II (New England Nuclear). Protein, proline and

Table 1. Hepatic proline content and uptake after duct ligation[a]

		Proline content (μmole/μg liver)	^{3}H Proline uptake[b] Hepatocyte	Duct cell enriched fraction
Control		0.22 ± 0.05	610 ± 60	610 ± 150
Sham operated day	1	0.31 ± 0.06	–	–
	3	0.43 ± 0.05	530 ± 150	590 ± 210
Post ligation day	1	0.71 ± 0.15*	1500 ± 470*	1100 ± 440*
	3	0.73 ± 0.16*	1500 ± 380*	2400 ± 550*
	7	0.63 ± 0.01*	2200 ± 450*	980 ± 380

Table 2. Hepatic glycine content and uptake after duct ligation[a]

		Glycine content (μg/mg liver)	^{3}H Glycine uptake[b] Hepatocyte	Duct cell enriched fraction
Control		1.7 ± 0.44	60 ± 10	80 ± 20
Sham operated day	1	2.2 ± 1.8	–	–
	3	1.5 ± 0.45	–	–
Post ligation day	1	1.7 ± 0.73	300 ± 80*	180 ± 10*
	3	2.6 ± 1.6	250 ± 10*	1200 ± 380*
	7	2.8 ± 1.8	230 ± 20	800 ± 100*

[a]Values in the table represent the mean ± SD, the result of 6 rats in each group. [b]cpm × 10^{-3}/μg liver protein. *$p < 0.05$ compared to controls.

glycine were measured by the method of Lowry et al., of Rojkind and Gonzalez and of Ohmori et al., respectively[5-7]. All 3 constituents were determined in samples of livers excised before hepatic perfusion.

Results and discussion. The hepatic content of proline increased significantly over control and sham values following duct ligation (table 1). The glycine content, in contrast, was not affected by the ligation procedure (table 2). Uptake of both labeled proline and glycine by hepatocytes and by duct cell enriched fractions was significantly higher than in controls. The proline incorporation in the duct cell fraction was not higher than that in hepatocytes except on day 3 after ligation (table 1). Although a previous study showed that ^{3}H proline appeared to be more concentrated in bile ducts and fibroblast-like cells than in parenchymal cells[8], we did not find a consistent comparative increase in uptake by ductular cells. This may have been due to the heterogeneity of the isolated cell fraction. The radioactive counts of proline and glycine in the 2 cell fractions are extremely low, representing on the average less than a millionth part of the injected doses. This may be attributed in part to the loss of the water soluble amino acids during the isolation procedures and in part to the active secretion of the labels out of the liver. The accelerated metabolism of both proline and glycine in experimental compared to control rats is to be expected with the striking proliferation of hepatocytes and ductular cells that occurs 1 to 2 days after extrahepatic cholestasis[9]. Our results suggest that the increased hepatic content of proline following obstruction of the bile duct is a mechanism for the duct hyperplasia. The mediator role of the amino acid is analogous to that shown for the hyperplastic response in experimental fascioliasis. As proline also regulates collagen production in both human and experimental cirrhosis[10,11], it accounts for the concurrent development of fibrosis and duct hyperplasia that occur in cirrhosis.

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Extraction of two different protein kinase activities from bovine rod outer segments

M. Feraudi*

Klinikum der Universität Heidelberg, Medizinische Poliklinik, Abteilung für Pathophysiologie und Sportmedizin, Hospitalstrasse 3, D-6900 Heidelberg 1 (Federal Republic of Germany), November 16, 1981

Summary. An optimization of the rod outer segment (ROS) preparation technique is described. The protein responsible for ATP-γ ^{32}P binding to bovine ROS was separated from the protein active with protamine on a DEAE Sephadex column. Molecular weight evaluation on a G 100 Sephadex column gave a value of 75,000 for the protein active with ROS, and 42,000 for that active with protamine. 1.25 mM c-AMP or c-GMP reduced the activity to 0.7 or 0.8 of the control respectively. 10 mM c-GMP doubled the yield of the active protein extracted from ROS.

More than 10 years ago, it was observed that incubation of prepared rod outer segment (ROS) membranes of the vertebrate retina with adenosine 5'-[γ-^{32}P]triphosphate (γ-^{32}P-ATP) in light resulted in labeling of the ROS with ^{32}P. Many authors who have studied this phenomenon have assumed that ATP phosphorylates rhodopsin on the ROS membrane after light activation of the substrate and that a protein, possibly weakly bound to ROS, is active in this reaction: the 'rhodopsin kinase'[1-7]. Attempts to isolate and characterize this active protein have also been made. Kühn postulated a mol. wt of 67,000-69,000 while Shichi and Somers[8] estimated it to be 50,000-53,000. To clarify this discrepancy, we publish some of our experimental observations on this active protein, made 3 years ago. In this study we discuss the preparation of bovine ROS with a maximal content of active protein, optimal extraction of the active protein from prepared ROS, its separation on a DEAE Sephadex column, molecular weight determination, and the effect of several ions and cyclic nucleotides on the protein activity and extraction.

Materials and methods. The technique for preparing bovine outer segments from freshly dissected retinae was a modification of a preceding technique[9] with the aim of increasing the yield (cf. results). Flotation and sucrose density gradient centrifugation steps in the dark remained unaltered, but the p[H^+] value was established at 5.9 and the concentration of phosphate buffer was increased to 0.143 M. Dithiothreitol (DTT, 20711, Serva, Heidelberg, FRG) was omitted because it could inhibit the active protein; 0.1 mM phenylmethylsulfonylfluoride (PMSF, Serva, 32395) and 0.31 M K Cl were present in every solution.

Extraction of water-soluble proteins from ROS. ROS from 30 to 35 eyes were gently homogenized with 1.0 ml 0.1 mM PMSF, added with 0.125 ml 140 mM phosphate elution buffer (see below), and centrifuged at $15.10^3 \times$ g. This procedure was repeated 3 times on the same pellet. The 4 supernatants were collected concentrated and equilibrated on an Amicon PM 10 membrane in a microultrafiltration cell; the final volume was less than 1 ml.

Capacity of soluble ROS protein to bind ATP-γ^{32}P to inactivated ROS or protamine was tested in a mixture of the following composition. In a polypropylene 1.5 ml micro test tube, 0.050 ml 3.6 mM γ-^{32}P-ATP (6000 dpm/nmole) (Amersham Buchler Braunschweig, FRG), 0.200 ml 132 mM KH_2PO_4 adjusted with KOH to p[H^+] = 7.89 and containing 8 mM $MgCl_2$; 0.100 ml of the solution to be tested and 0.050 ml alum-ROS suspension (see below) containing 50-60 nmoles rhodopsin in 1.00 ml or 0.050 ml