THE EFFECTS OF COMMON BILE DUCT LIGATION UPON THE RAT LIVER β-ADRENERGIC RECEPTOR–ADENYLATE CYCLASE SYSTEM

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1. Introduction

Among the alterations of the physiological functions of the liver in abnormal states, some result from modulations of the adenylate cyclase-coupled β-adrenergic receptors: lack of insulin [1], hypothyroidism [2] and adrenalectomy [3–6] lead to an enhanced catecholamine sensitivity of cyclase. This is due in the latter case to a parallel increase in the β-adrenergic binding sites [3,7]. Further, the activity of hepatic hormone-sensitive adenylate cyclase is influenced during development [8,9] and carcinogenesis [10] in rats, as well as in cirrhotic states in man [11]. It is known that cholestasis modifies the ultrastructural and histochemical appearances of the bile canalicular membranes, as well as their composition, enzyme content and protein turnover [12–14]. We were thus prompted to assess the possible alterations of adenylate cyclase, a major membrane-bound enzyme, during cholestasis. This report provides the first demonstration of a specific alteration in the hepatic adenylate cyclase-coupled β-adrenergic receptor system after common bile duct ligation in the rat.

2. Materials and methods

(–)[^{3}H]Dihydroalprenolol (51 Ci/mmol) was supplied by the New England Nuclear Corp. and [α-^{32}P]ATP (20–30 Ci/mmol) was obtained from Amersham Centre. Sodium taurochenodeoxycholate was kindly donated by Dr R. Infante (Hôpital Saint-Antoine, Paris) and sodium taurocholate was obtained from Maybridge. Sources of other materials have been described [15]. Female, albino, Wistar rats (~150–200 g body wt) were anesthetized with ether. Through a short midline incision, the common bile duct was isolated and ligated by double ligation just below the hilum. After the operation (44–48 h) the rats were decapitated and the liver rapidly removed for membrane preparation. Normal and cholestatic rat liver plasma membranes were prepared according to the procedure in [16] up to step 11, and were stored in liquid nitrogen. Several batches of membranes from 6–8 pooled livers were used in the experiments reported here and each gave similar results. Protein was measured according to Lowry's procedure using bovine serum albumin as standard. Binding of tritiated dihydroalprenolol to rat liver plasma membranes was carried out as in [7]. Assays were performed at equilibrium in the presence of 0.25–0.35 mg membrane protein and 0.1 mM phentolamine. Nonspecific binding was measured in the presence of 5 μM (±)alprenolol. Results are expressed as fmol [^{3}H]dihydroalprenolol specifically bound/mg membrane protein. Adenylate cyclase (EC 4.6.1.1) activity was measured as in [15,17] and expressed as nmol cyclic AMP formed in 10 min/mg protein. ATPase (EC 3.6.1.4), 5′-nucleotidase (EC 3.1.3.5), leucine aminopeptidase (EC 3.4.11.1) and γ-glutamyltransferase (EC 2.3.2.2) were assayed as in [11]. Alkaline phosphatase (EC 3.1.3.1) and alkaline phosphodiesterase I (EC 3.1.4.1) were assayed as in [18,19], respectively. Enzyme activities were expressed as nmol product formed . min^{-1} . mg protein^{-1}. P_i was estimated by the method in [20].

3. Results

Bile duct ligation for 2 days resulted in a cholestasis as assessed by an 8-fold increase in the serum concentration of total bilirubin. Although not altering the recovery of protein in the purified plasma membranes from control and cholestatic rats, respectively, 1.3 and 1.6 mg membrane protein/g liver, bile duct ligation affected several plasma membrane bound enzymatic activities: alkaline phosphatase activity was increased 1.6-fold, whereas γ-glutamyltransferase, 5′-nucleotidase, leucine aminopeptidase, ATPase and alkaline phosphodiesterase I activities were reduced by 20–73% (table 1).

The adenylate cyclase activity was also influenced by cholestasis, as shown in table 2. Basal activity was increased 1.7-fold. NaF-, GTP- and glucagon-stimulated activities were not significantly modified, although the activation factor over basal activity was slightly reduced. The main changes occurred in the catecholamine-sensitive adenylate cyclase:

(i) Adenylate cyclase responded to (−)isoproterenol, while it was relatively insensitive to catecholamines in normal rats (table 2) [6];

(ii) Both the activation factor over basal (8.8- versus 5.8-fold) and the maximal level of enzyme activity (2.72 versus 1.04 nmol cyclic AMP formed in 10 min/mg protein) were enhanced under stimulation of the enzyme by (−)isoproterenol + GTP (table 2).

Table 1
Hepatic plasma membrane enzyme activities in normal and cholestatic rats

Enzyme activities	Spec. act. (nmol . min^{-1} . mg protein^{-1})	
	Normal rat plasma membranes	Cholestatic rat plasma membranes
Alkaline phosphatase	28.3	44.6 (+57)[a]
5′-Nucleotidase	450	330 (−27)
Leucine aminopeptidase	17.4	5.3 (−70)
γ-Glutamyltransferase	78	21 (−73)
Alkaline phosphodiesterase I	910	530 (−42)
ATPase: Total	630	480 (−24)
Mg^{2+}	500	400 (−20)
Na^+, K^+	130	80 (−38)

[a] Values in parenthesis indicate the % change as compared to membranes from normal rats

Table 2
Hepatic plasma membrane adenylate cyclase activities in normal and cholestatic rats

Adenylate cyclase activity	Spec. act. (nmol . mg protein^{-1} . 10 min^{-1})	
	Normal rat plasma membrane	Cholestatic rat plasma membrane
Basal	0.18	0.31
+ 10 mM NaF	1.50 (8.3)[a]	1.80 (5.8)
+ 10 μM GTP	0.90 (5)	1.12 (3.6)
+ 1 μM glucagon	1.78 (9.9)	1.93 (6.2)
+ 10 μM (−)isoproterenol	0.18 (1)	0.49 (1.6)
+ 10 μM (−)isoproterenol + 10 μM GTP	1.04 (5.8)	2.72 (8.8)

[a] Values in parenthesis indicate the activation factors over basal activity

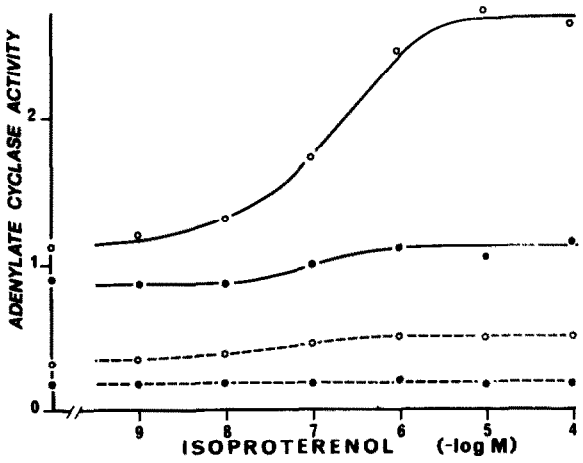

Fig.1. (−)Isoproterenol-stimulated adenylate cyclase activity in liver plasma membranes from control (•) and bile duct ligated (○) rats. Rat liver plasma membranes (~1 mg protein/ml) were incubated for 10 min at 30°C in the presence of the indicated concentrations of (−)isoproterenol. Isoproterenol-sensitive adenylate cyclase activities were assayed in the absence (dotted lines) or in the presence (solid lines) of 10 μM GTP. Adenylate cyclase was measured as in [15,17]. Each value, expressed as nmol cyclic AMP formed in 10 min/mg protein, represents the mean of 3 determinations, agreeing in ⩽ ±5%.

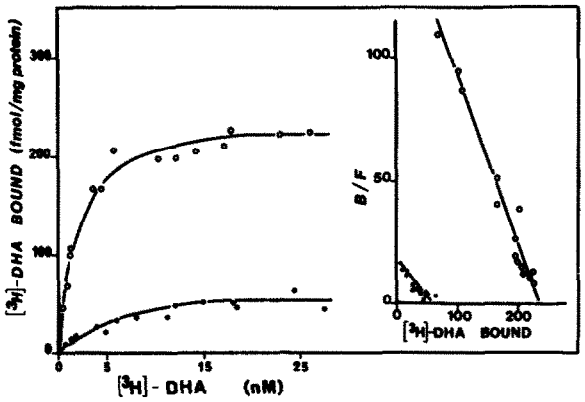

Fig.2. Specific binding of tritiated dihydroalprenolol to rat liver plasma membranes as a function of dihydroalprenolol concentration. Rat liver plasma membranes (1.2–1.6 mg protein/ml) obtained from control (•) and cholestatic (○) rats were incubated with various concentrations of [^{3}H]dihydroalprenolol (0.5–30 nM) and specific binding was determined as in section 2. Each value is the mean of 2 determinations. Results are expressed as fmol [^{3}H]dihydroalprenolol bound/mg protein and are representative of 2 such experiments. Inset: Scatchard plots of dihydroalprenolol binding to control (•) and cholestatic (○) rat liver plasma membranes. The ratio B/F of bound dihydroalprenolol (fmol/mg protein) to free dihydroalprenolol (nM) is plotted as a function of bound dihydroalprenolol (fmol/mg protein). The slopes of the plots yield K_d values of 1.5 and 3.5 nM in cholestatic and control rat membranes, respectively.

Figure 1 further shows that the affinity of (−)isoproterenol for the system was not significantly altered after common bile duct ligation (EC_{50} = 0.22 and 0.1 μM in cholestatic and normal rat liver membranes, respectively).

The results depicted in fig.2 show that the number of [^{3}H]dihydroalprenolol binding sites was 4-times higher 2 days after bile duct ligation (240 fmol dihydroalprenolol bound/mg protein versus 60 in controls). The K_d of the tritiated ligand for its binding sites remained unchanged (1.5 versus 3.5 nM).

It was important to decide whether the observed changes could be due to the enhanced local concentration of bile salts after biliary obstruction. The in vitro effects of two bile salts were tested: sodium taurocholate, which is the major bile salt in rat [21] and sodium taurochenodeoxycholate which is one of the dihydroxylated bile salts whose concentration is greatly increased 3 days after bile duct ligation [21]. Liver membranes derived from normal rats were incubated 30 min at 25°C with increasing concentrations of either bile salt, then washed 3-times in 10 vol. ice-cold buffer. No increase in the number of binding sites was observed, but a fall of 37% and 65% in the binding site density, at the highest concentration (3 mM) of taurocholate and taurochenodeoxycholate, respectively (data not shown).

4. Discussion

As reported [12,13,22,23], extrahepatic cholestasis alters membrane-bound enzyme activities in rat liver: the alkaline phosphatase activity is enhanced while other enzymatic activities are decreased (table 1). We observe a fall in the γ-glutamyltransferase activity 2 days after ligation. Such a decrease is not surprising since it has already been observed, in rat [22] and guinea pig [24] liver fractions, as an early consequence (1 day) of bile duct ligation.

The present work provides the first direct demonstration that common bile duct ligation, in rat, results in a specific increase in both hepatic catecholamine-sensitive adenylate cyclase activity (fig.1) and β-adrenergic receptor number (fig.2). The maximal isoproterenol-induced activity of the enzyme was 2.6-times higher in cholestatic rat liver membranes than in normal membranes (table 2) but the affintiy of the enzyme system for the agonist was unchanged (fig.1). In [7] we have demonstrated that tritiated dihydroalprenolol, a potent β-adrenergic antagonist, binds to rat liver plasma membranes with the characteristics expected of β-adrenergic receptors. Scatchard analysis of this binding to cholestatic rat liver membrane revealed a 4-fold enhancement in the β-adrenergic binding site density, with no change in the K_d of the radiolabeled ligand (fig.2). Since the amount of protein recovered at the end of the membrane preparation is similar in normal and cholestatic animals, the increase in the number of binding sites does not appear to reflect a difference in the 'purification' of the membranes. It is difficult to decide whether this increase is due to a true increase in the synthesis of the β-adrenergic receptors or to their unmasking. Nevertheless, a direct effect of bile salt on some essential membrane-bound component is unlikely.

Two days after bile duct ligation, basal adenylate cyclase activity was slightly enhanced, but not sensitive to 10 μM (−)propranolol in vitro (data not shown). This contrasts with the results reported for the bile salt-stimulated adenylate cyclase activity in colonic mucosa [25,26]. NaF, GTP and glucagon-stimulated cyclase to the same extent in normal and cholestatic rat liver membranes (table 2), suggesting that the number of catalytic sites is not modified during extrahepatic cholestasis. It has been reported in individuals with jaundice [27,28] that the liver may be the major source of the glucagon-stimulated increase in plasma cyclic AMP concentration. However, we did not observe a significant increase in the glucagon-sensitive adenylate cyclase activity in hepatic membranes derived from cholestatic rats (table 2).

In conclusion, extrahepatic cholestasis results in a specific increase in the β-adrenergic receptor number, which is accompanied by an increase in catecholamine-stimulated adenylate cyclase. The enhancements in both parameters were not identical, probably due to the absence of a one-to-one relationship between β-adrenergic receptors and adenylate cyclase (in preparation).

This represents a novel example of a β-adrenergic receptor modulation in response to a situation where a physiological process (bile flow) is suppressed. This preliminary observation should lead to further investigation of other hormone-sensitive systems (e.g., the α-adrenergic receptor), during experimental cholestasis.

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