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THE HEPATIC ALPHA$_1$-ADRENERGIC RECEPTOR

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Abstract—Since the relatively recent advent of radioligand binding techniques, it has been possible to directly identify and characterize hepatic adrenergic receptors as well as study their physiological regulation. While it is now clear that alpha$_1$-adrenergic receptors constitute the major population of hepatic adrenergic receptors and are primarily responsible for the actions of catecholamines in liver, relatively little is known about the molecular mechanisms underlying alpha$_1$-responses. Recent results suggest that guanine nucleotides may be implicated in the transmission of the hormonal signal from the hepatic alpha$_1$-receptor to its effectors in a manner analogous to that described for adenylate cyclase-linked receptors. The lack of an easily measurable proximal membrane response for the alpha$_1$-receptor has been a severe handicap in our understanding of the mechanism of transmission of the hormonal signal. It is likely that until such a response is defined, alpha$_1$-adrenergic research will continue to lag behind research on the beta-adrenergic receptor.

Catecholamines exert their effects through interaction with specific adrenoreceptors located on the external surface of the plasma membrane of target cells. These receptors are divided into two main classes, alpha and beta, based on differences in their sensitivity to various adrenergic agonists and antagonists (Table 1). Alpha-adrenergic receptors have been further subdivided into alpha$_1$ and alpha$_2$-receptors. Historically this subdivision was made on anatomical grounds, alpha$_2$-receptors being located pre-synaptically and alpha$_1$-receptors corresponding to post-synaptic sites [1]. However, this distinction no longer appears to hold true especially outside the central nervous system. In such tissues, alpha-adrenergic receptors may be more usefully classified on the basis of their pharmacological characteristics [2]. To date the most useful drugs for discriminating alpha$_1$- and alpha$_2$-adrenergic receptors have been the antagonists prazosin and yohimbine. Prazosin is a much more potent antagonist at alpha$_1$- than alpha$_2$-sites, whereas yohimbine is a highly selective alpha$_2$-adrenergic antagonist.

Alpha-adrenergic receptors of the alpha$_1$-type are associated with mobilization of intracellular calcium. These receptors mediate the majority of classical alpha-adrenergic responses, such as smooth muscle contraction and stimulation of glycogenolysis in liver of certain species. In general, it appears that alpha$_1$-receptors are not coupled to adenylate cyclase. In contrast, alpha$_2$-adrenergic receptors mediate catecholamine inhibition of adenylate cyclase activity.

The present review will be devoted to recent progress made in our laboratory on hepatic alpha-adrenergic receptors, focusing on the identification, pharmacological and physiological characterization and regulation of alpha$_1$-adrenergic receptors.

CHARACTERIZATION OF HEPATIC ADRENERGIC RECEPTORS

Hepatic adrenergic receptors have been directly identified and characterized in isolated plasma membranes using specific radio-labelled agonists and antagonists [3–7]. These studies have shown that three types of adrenergic receptors are present in liver plasma membranes; beta$_2$, alpha$_1$ and alpha$_2$. As indicated in Table 2, the majority of hepatic adrenergic receptors are of the alpha$_1$-subtype.

To date, it has not been possible to measure a physiological response following binding of an agonist to the alpha$_1$-adrenergic receptor in purified liver plasma membranes. With the possible exception of phosphatidylinositol turnover, cellular integrity seems to be required. In order to be able to correlate more closely binding and physiological studies, we undertook to characterize alpha$_1$-receptors in isolated hepatocytes using the specific alpha$_1$-adrenergic radioligand, (^{3}H)prazosin.

As shown in Fig. 1, the specific binding of (^{3}H) prazosin to isolated hepatocytes is a saturable process, a plateau being obtained at a radioligand concentration of 2 nM. Scatchard analysis revealed

Table 1. Pharmacological characteristics of alpha- and beta-adrenergic receptors

Characteristics	Alpha-receptor	Beta-receptor
Agonist potency order	Epi ≥ Nor > Phenyl > Iso	Iso > Epi ≥ Nor > Phenyl
Typical antagonists	Phentolamine Phenoxybenzamine Dihydroergocryptine Yohimbine	Propranolol Alprenolol Pindolol

Abbreviations used are: Nor, norepinephrine; Epi, epinephrine; Phenyl, phenylephrine.

Table 2. Hepatic adrenergic receptors

Ligand	Receptor	B_{max} (fmoles/mg protein)	K_D (nM)
(^{3}H Dihydroalprenolol	$Beta_2$	70	2.2
(^{3}H) Prazosin	$Alpha_1$	750	0.1
(^{3}H) Yohimbine	$Alpha_2$	120	5

a single class of receptors, with a maximum number of (^{3}H)prazosin binding sites of 60 ± 9 fmoles per mg of total protein. Since 1 mg of total protein represents about 0.56×10^6 cells, this corresponds to approximately 70,000 receptors per hepatocyte. The dissociation constant of (^{3}H)prazosin for its binding site was 0.21 ± 0.01 nM, which is very similar to the value obtained in isolated liver plasma membrane [6]. The association of (^{3}H)prazosin to hepatocytes at 37° was rapid, reaching equilibrium within 10 min, and specific binding remained stable for at least 15 min. Dissociation experiments showed that 75% of (^{3}H) prazosin specifically bound dissociated in 15 min.

The pharmacological nature of the binding sites for (^{3}H)prazosin present on hepatocytes was assessed by competition between the radioligand and various adrenergic agonists and antagonists. The values obtained denoted an order of potency typical of alpha receptors: (−)norepinephrine (K_D = 1.8 μM) ≥ (−)epinephrine (K_D = 2.1 μM) ≫(−)isoproterenol (K_D = 227 μM). The sites studied also manifested stereospecificity in favor of laevo-isomers as shown by the relative K_D of the leavo and dextro-isomers of norepinephrine (K_D = 1.8 and 250 μM respectively). As expected, the site labelled with

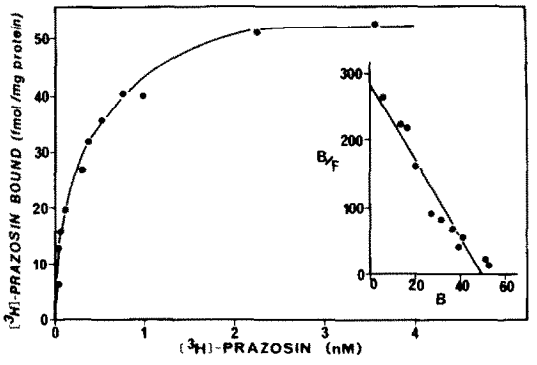

Fig. 1. Specific binding of (^{3}H)prazosin to isolated hepatocytes as a function of radioligand concentration. Isolated hepatocytes (0.82 mg protein/ml) were incubated for 10 min at 37° with increasing concentrations of (^{3}H)prazosin in a final volume of 1 ml of Krebs–Ringer bicarbonate buffer (pH 7.4) containing 1 mM ascorbate, 1 mM catechol and 1 mM tropolone. Non-specific binding, determined in the presence of 10 μM phentolamine, represented 35–60% of total binding. Each value is the mean of triplicate determinations.
Inset: Scatchard plot of (^{3}H)prazosin binding to rat hepatocytes. The ratio B/F of bound (^{3}H)prazosin (fmoles/mg protein) to free (^{3}H)prazosin (nM) is plotted as a function of bound (^{3}H)prazosin (fmoles/mg protein). The slope of the plot was determined by linear regression analysis (r = 0.95, $P < 0.001$).

(^{3}H)prazosin belongs to the $alpha_1$ subclass inasmuch as prazosin (K_D = 0.2 nM) was 7500 times more potent than the $alpha_2$ antagonist yohimbine (K_D = 1500 nM) in competing for the binding site. The beta antagonist (−)propranolol exhibited a weak affinity as compared to the classical alpha drug, phentolamine. Taken together, these data demonstrate the $alpha_1$-adrenergic nature of the site characterized with prazosin in isolated hepatocytes.

PHYSIOLOGICAL RESPONSE

The major physiological action of epinephrine in the liver is to increase glucose production due to a stimulation of hepatic glycogenolysis and gluconeogenesis (Fig. 2). Catecholamine stimulation of glycogen phosphorylase, the enzyme which catalyses the breakdown of glycogen to glucose-1-phosphate, can occur either via a beta-adrenergic cAMP-dependent pathway, or by a calcium dependent pathway mediated by $alpha_1$-receptors. In isolated hepatocytes from adult rat liver, glycogen phosphorylase activation by adrenergic agonists displays an order of potency characteristic of alpha-adrenergic effects [8–11] (Table 3). Alpha-adrenergic antagonists such as phentolamine are much more potent in inhibiting epinephrine stimulated glycogen phosphorylase activity than beta-antagonists. Furthermore, the $alpha_1$-selective antagonist prazosin is over 10,000-fold more potent than the $alpha_2$-antagonist, yohimbine (Table 3). From these results it is clear that catecholamine stimulation of glycogen phosphorylase activity is mediated primarily via $alpha_1$-adrenergic receptors, at least in the case of adult male rat liver.

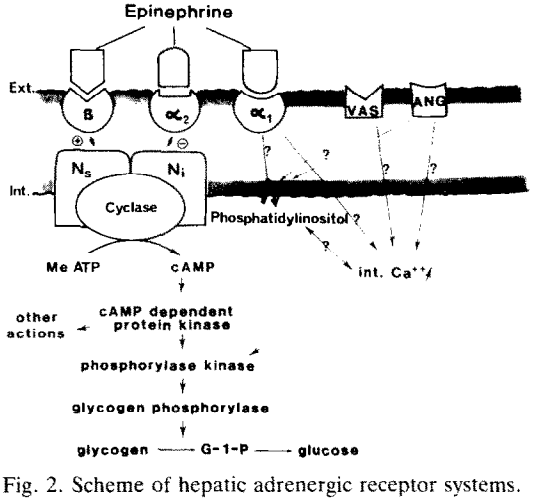

Fig. 2. Scheme of hepatic adrenergic receptor systems.

Table 3. Effect of adrenergic agonists and antagonists on glycogen phosphorylase activity in isolated hepatocytes

Agonists	K_a (μM)	Maximal activation (% over basal level)	Antagonists	$K_{i\,app}$(μM)
(−) Norepinephrine	0.31	100–120	Prazosin	0.00085
(−) Epinephrine	0.76	100–160	Dihydroergocryptine	0.25
(−) Phenylephrine	1.00	80	Phenoxybenzamine	0.30
(−) Nordefrine	1.47	100	2-Bromo-alpha-ergocryptine	0.43
(+) Norepinephrine	3.30	85–95	Phentolamine	1.71
(+) Epinephrine	5.20	160	Labetalol	3.30
Dopamine	12.50	55	Hydroxybenzylpindolol	6.20
(−) Isoproterenol	24.00	65	(±) Alprenolol	8.00
(±) Metanephrine	25.00	50	Yohimbine	11.00
Metaraminol	27.50	25	Azapetine	11.70
			Pindolol	75.00
			(±) Propranolol	113.00

Data taken from Ref. 9.

PHYSIOLOGICAL REGULATION OF HEPATIC ADRENERGIC RECEPTORS

Both adrenergic control of hepatic glucose metabolism and the adrenergic receptors themselves are subject to regulation by a wide variety of physiological and pathological states. Table 4 lists some of the factors which have been found to regulate hepatic adrenergic receptors. Among the most important are thyroid and glucocorticoid hormones. In addition, a change in the balance between alpha$_1$- and beta$_2$-receptors, as well as adrenergic control of glycogen phosphorylase activity, occurs during development, regeneration and malignant transformation and is also sex dependent [26].

We had recently observed that extrahepatic cholestasis in rats causes a marked increase in the response of liver adenylate cyclase to the beta-adrenergic agonist, isoproterenol [27]. We therefore investigated the effect of cholestasis on the ratio of alpha$_1$- and beta$_2$-adrenergic receptors and on the stimulation of phosphorylase activity by catecholamines in liver [15]. As shown in Fig. 3, a 60% decrease in the number of alpha$_1$-adrenergic receptors (285 vs 680 fmoles/mg protein) and a simultaneous 170% increase in the number of beta$_2$-adrenergic receptors (67 vs 25 fmoles/mg protein) occurred two days after bile flow obstruction. The reciprocal modification in the numbers of alpha$_1$- and beta$_2$-adrenergic sites was accompanied by a change in the manner of stimulation of glycogen phosphorylase by catecholamines in hepatocytes (Fig. 4). Originally alpha$_1$ in normal rats (phenylephrine) K_a = 0.9 μM, isoproterenol K_a = 7.1 μM), the stimulation became predominantly beta$_2$ adrenergic in cholestatic animals (phenylephrine K_a = 3.7 μM, isoproterenol K_a = 0.06 μM). Furthermore stimulation of glycogenolysis by epinephrine was blocked by phentolamine but not by propranolol in control rats, whereas propranolol was more effective than phentolamine in cholestatic rodents [15].

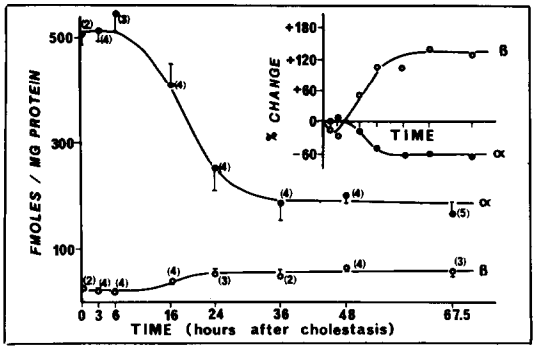

Fig. 3. Time course of the variation in the number of hepatic alpha$_1$- and beta-adrenergic binding sites after bile duct ligature. Binding of (^{3}H)prazosin (●) and (^{3}H)dihydroalprenolol (○) was measured in rat liver plasma membranes prepared at various times after bile duct ligature (from Ref. 15).

Table 4. Changes in the balance between alpha- and beta-adrenergic receptors in rat liver

	Alpha$_1$ sites (fmoles/mg tissue)	Beta$_2$ sites	Phosphorylase activity	References
Normal	750	70	Alpha	[3, 6]
Foetal state		↗	Beta	[12, 13]
Weaning rats	→	↗	Beta	[14]
Regeneration		↗	Beta	[15]
Malignant transformation		↗	Beta	[16]
Adrenalectomy	→	↗	Beta	[6, 17–19]
Hypothyroidism	→	↗	Beta	[20–24]
Hyperthyroidism	↘	↘	Beta	[23, 25]

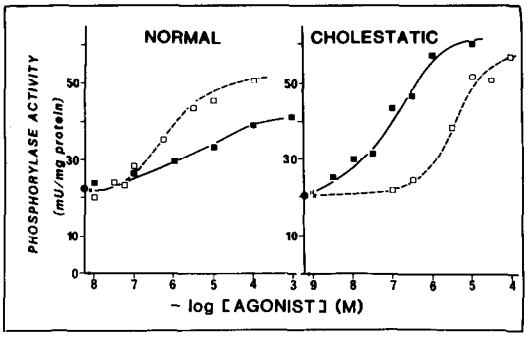

Fig. 4. Activation of glycogen phosphorylase activity by adrenergic agonists in hepatocytes isolated from normal and cholestatic rats. Hepatocytes from normal (52×10^6 cells in 5.8 ml) or cholestatic (45×10^6 cells in 5.8 ml) rats were incubated in the absence or presence of concentrations of (−)phenylephrine (□), (−)isoproterenol (■) (from Ref. 15).

Modification of the regulation of glycogenolysis after cholestasis did not seem secondary to an alteration in the metabolism of thyroid hormones or in the action of glucocorticoids. However, cholestasis provoked a 10-fold increase in the number of hepatic mitoses and in the incorporation of thymidine into liver DNA of cholestatic animals. Similar changes in hepatic adrenergic receptors were observed in regenerating livers, following 2/3 hepatectomy. It would therefore appear that the changes following extrahepatic cholestasis may be linked to a regenerative process.

All these studies indicate that adrenergic receptors are not static entities in the plasma membrane, but are subject to dynamic regulation by an ever increasing number of factors. From a physiological point of view, it is surprising that the same final effect (namely glycogenolysis), caused by the same hormonal factors, is mediated by two different intermediary mechanisms depending on the physiological state or on the species. One possible reason could be that insufficient selective pressure was exerted during evolution. Conversely it may be that neither pathway (alpha or beta) offered sufficient advantage to be specifically retained.

EFFECT OF GUANINE NUCLEOTIDES ON HEPATIC ALPHA$_1$-ADRENERGIC RECEPTORS

Rodbell and coworkers were the first to recognize the important regulatory effects of guanine nucleotides on receptor-coupled adenylate cyclase systems [28]. It is now well demonstrated that GTP is a physiological regulator of adenylate cyclase activity and that guanine nucleotides are required for the hormonal stimulation of the enzyme. In addition, they reduce the affinity of the receptor specifically for agonist agents, such as beta-adrenergic agonists, without altering the binding for antagonists [29–31]. The regulatory effects are mediated through specific guanine nucleotide binding proteins, which serve to transmit the hormonal signal from the receptor to the catalytic moiety of the adenylate cyclase system. The role of guanine nucleotides is still unclear in the case of receptors, such as the alpha$_1$-adrenergic receptor, which are not coupled to adenylate cyclase activity.

We therefore investigated the effect of guanine nucleotides on the interaction of adrenergic agents with the alpha$_1$-adrenergic receptor of rat liver membranes by competition studies using the alpha$_1$-specific antagonist, (^{3}H)prazosin [19]. The non-hydrolysable guanine nucleotide analogue, Gpp(NH)p, caused a significant 3–10-fold decrease in the affinity of the alpha$_1$-adrenergic receptor for the agonists phenylephrine, epinephrine and norepinephrine, but had no effect on binding of the alpha-adrenergic antagonists prazosin and phentolamine. A right-shift and a steepening of agonist competition curves, was also observed in membranes prepared from isolated hepatocytes (Fig. 5). Computer modeling of the epinephrine competition curves, indicate that, in the absence of guanine nucleotides, approximately 20% of the receptors are in a high affinity state ($K_D = 0.03\ \mu M$) and 80% in a low affinity state ($K_D = 2.0\ \mu M$). In the presence of guanine nucleotides, the curves can best be described by a single state with a low affinity for epinephrine ($K_D = 3.0\ \mu M$). Gpp(NH)p would therefore appear to induce a transformation of high affinity sites to a low affinity form similar to that found in the absence of nucleotides.

The Gpp(NH)p-mediated reduction in epinephrine binding affinity was still observed when measured in the presence of saturating concentrations of the beta-blocker, propranolol (10 μM) and the alpha$_2$-selective antagonist, yohimbine (100 nM). This indicates that the effect is not due to an action of the guanine nucleotide on epinephrine binding to hepatic beta- or alpha$_2$-adrenergic receptors, but is truly due to binding to the alpha$_1$-receptor. The effect appears to be guanine nucleotide

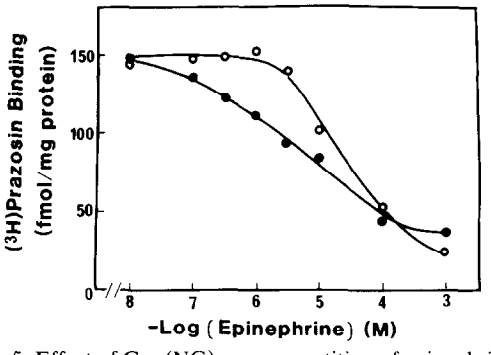

Fig. 5. Effect of Gpp(NG)p on competition of epinephrine for (^{3}H)prazosin binding in hepatocyte membranes. (^{3}H) prazosin (1 nM) was incubated with a washed 1000 g membrane fraction prepared from isolated rat hepatocytes (0.35 mg/ml) either in the absence (●) or presence (○) of 400 μM Gpp(NH)p. Incubations were carried out for 10 min at 37° in a final volume of 1 ml of 50 mM Tris–HCl buffer (pH 7.4) containing 1 mM ascorbate, 1 mM catechol, 1 mM tropolone, 25 μM iproniazide and increasing concentrations of epinephrine. Results represent total (^{3}H)prazosin binding. IC_{50} values for epinephrine are 4.19 ± 0.94 μM (r =6) and 20.88 ± 3.40 μM (r = 6) in the absence and presence of Gpp(NH)p, respectively.

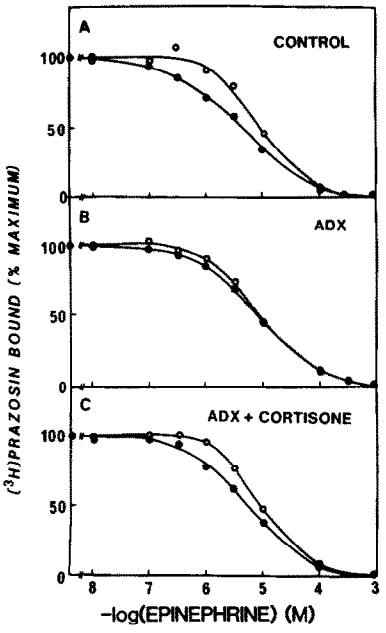

Fig. 6. Competition of epinephrine for (^{3}H)prazosin binding to liver membranes prepared from control (A), adrenalectomized (ADX) (B) and adrenalectomized rats treated with cortisone (C). Rat liver plasma membranes were incubated with 1 nM (^{3}H)prazosin and increasing concentrations of epinephrine in the absence (●) and presence (○) of 100 μM Gpp(NH)p. For details see Ref. 19.

specific since GTP is as effective, and GDP 80% as effective, as Gpp(NH)p whereas CTP, ATP and UTP are less effective.

In order to investigate the possible role of guanine nucleotides in alpha$_1$-responses, binding studies were performed in liver membranes from adrenalectomized rats. As mentioned above, adrenalectomy causes a decrease in alpha-receptor mediated actions in liver as manifested by a decrease in alpha$_1$-stimulation of glycogen phosphorylase activity and calcium mobilization [17]. The quantity of alpha$_1$-adrenergic receptors detected with saturing concentrations of (^{3}H)prazosin, was only slightly decreased in liver membranes prepared from adrenalectomized rats (800 fmoles/mg protein) as compared to control (1000 fmoles/mg protein) [19]. However, adrenalectomy resulted in the loss of the ability of guanine nucleotides to modulate epinephrine binding to hepatic alpha$_1$-adrenergic receptors (Fig. 6). The epinephrine competition curves were steeper and shifted to the right in membranes from adrenalectomized rats and addition of Gpp(NH)p caused no further change. Similar results have been found for beta-adrenergic receptors in systems where the receptor is functionally uncoupled from adenylate cyclase due to a defect in the guanine nucleotide binding protein [32–34]. These results suggested that guanine nucleotides may be implicated in the coupling of the alpha$_1$-receptor to its effectors in a manner analogous to that described for adenylate cyclase-linked receptors. Furthermore, this "coupling" appears to be under the regulation of glucocorticoids since the effects of adrenalectomy both on the nucleotide modulation of epinephrine binding to alpha$_1$-receptors and on alpha$_1$ responses in liver can be reversed by cortisone treatment (Fig. 6).

Support for an agonist induced interaction of the hepatic alpha$_1$-adrenergic receptor with a guanine nucleotide binding protein has also come from *N*-ethylmaleimide (NEM) inactivation studies [35]. Pretreatment of rat liver plasma membranes with this sulfhydryl reagent causes a right shift and a steepening of the epinephrine displacement curves which are best fitted by a single, low affinity state of the receptor (K_D = 1.7 μM). Furthermore, guanine nucleotide regulation of agonists binding to the alpha$_1$-receptor is abolished. Under these conditions (^{3}H)prazosin binding is unaffected, Similar NEM inactivation of guanine nucleotide regulatory function has been observed in other receptor systems where the existence of a specific guanine nucleotide binding protein has been demonstrated [31, 36–39]. This suggests that the effects of guanine nucleotides on agonist-alpha$_1$-receptor interactions may also be mediated via a guanine nucleotide binding protein rather than via a binding site on the receptor *per se*.

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