Effects of fasting on hepatic catecholamine receptors

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1. INTRODUCTION

Catecholamines through both α- and β-adrenergic receptors activate glycogenolysis and gluconeogenesis in the rat liver [1–4]. There is considerable evidence that epinephrine activates glycogenolysis through interaction with the α_1-adrenoceptors in livers of adult rats. Glycogenolytic activation via the β-receptor is negligible in these animals. The characteristics of catecholamine activation of these processes have been reported to be altered in adrenalectomized [5,6] and hypothyroid rats [7–9]. In these animals, a decrease in α-adrenergic action concomitant with an increase in β-adrenergic action was observed [5,6,8,9]. It was further reported that the number of α-adrenergic receptors either remained unaltered [5,6], or decreased [9] whereas β-adrenergic receptor number was shown to increase [6–8]. The increase in β-receptor numbers was also associated with increases in the catecholamine-sensitive adenylate cyclase activity [5,7,8]. Adrenalectomy as well as abnormal thyroid states often affect animals' food intake and normal weight-gain. We have reported that decreases in hepatic glycogen phosphorylase activities (*a* form and total) as well as cAMP-dependent protein kinase elution pattern in adrenalectomized rats which lost weight after surgery, resembled those in briefly-fasted rats [5]. These observations prompted us to investigate the effects of fasting on the catecholamine-mediated regulation of hepatic glucose production. Here, we report that 20–24 h fasting resulted in no significant change in the number of binding sites for the mixed α_1-,α_2-adrenergic antagonist [^{3}H]dihydroergo-

cryptine ([^{3}H]DHE). However, there was a 25% decrease in the density of α_1-adrenergic binding sites labeled with [^{3}H]prazosin, and a 50% reduction in the number of binding sites for the selective α_2-adrenergic antagonist [^{3}H]yohimbine. Reciprocally, there was a marked increase (100%) in the density of the binding sites for the β-antagonist [^{125}I]iodocyanopindolol ([^{125}I]CYP). In isolated hepatocytes, 20–24 h fasting caused an increase in the catecholamine-sensitive accumulation of cyclic AMP (cAMP) and in gluconeogenesis.

2. EXPERIMENTAL

[^{3}H]DHE (21.9 Ci/mmol), [^{3}H]prazosin (18.0 Ci/mmol), [^{3}H]yohimbine (35 Ci/mmol), and [^{125}I]CYP (2200 Ci/mmol) were from New England Nuclear. The sources of other chemicals have been reported [3,10,11]. Methods previously described in detail were used with minor modifications to prepare plasma membrane from rat liver [11], to perform radioligand binding assays [3,11], to prepare hepatocytes, to determine cyclic AMP contents and to estimate gluconeogenesis [10]. Plasma membranes were isolated from livers of 220–270 g fed or 20–24 h fasted male Sprague-Dawley rats. 5'-Nucleotidase assay [12] showed similar specific activities of this enzyme in membranes prepared from fed or fasted rats. This indicates that fasting does not affect the constitution of the plasma membrane fraction used in radioligand binding experiments. Incubation media and washing buffers used in the vacuum filtration procedure for the binding of [^{3}H]DHE, [^{3}H]prazosin, and [^{3}H]yohimbine

contained 50 mM Tris (pH 7.5), 0.8 mM ascorbic acid, and 3 mM catechol. ($\pm$)Propranol was present at 10 μM in the incubation medium. Binding of the β-receptor ligand [125I]CYP was performed in [13] with some modifications. Incubations were performed at 25°C for 60 min in 50 mM Tris buffer (pH 7.6), containing 150 mM NaCl, and 7–15 μg plasma membrane protein. The filters were washed with 10 mM Tris buffer (pH 7.5), containing 150 mM NaCl. For α-adrenergic ligand binding, specific binding is defined as the difference between binding in the absence and presence of 10 μM phentolamine. Specific binding of [125I]CYP is defined as the difference in binding in the absence and presence of 1 μM ($\pm$) propranolol. Throughout this report, 'binding' means specific binding of the radioligand as described above. Values for binding were averages of 4 determinations. Experiments were performed using at least two different plasma membranes preparations.

Protein was determined as in [14] with bovine serum albumin as standard.

3. RESULTS AND DISCUSSION

3.1. Effects of fasting on the binding of α-adrenergic ligands and activation of hepatic glycogen phosphorylase

The effect of fasting on phenylephrine, an α-adrenergic agonist, activation of glycogen phosphorylase in isolated hepatocytes is shown in fig.1. It is
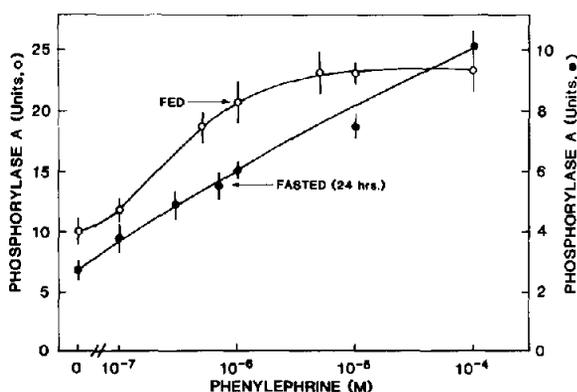

Fig.1. Effects of increasing concentrations of phenylephrine on phosphorylase a activity in hepatocytes from fed (o) and 24 h fasted (•) rats. Data points are averages of 3 expt $\pm$ SEM.
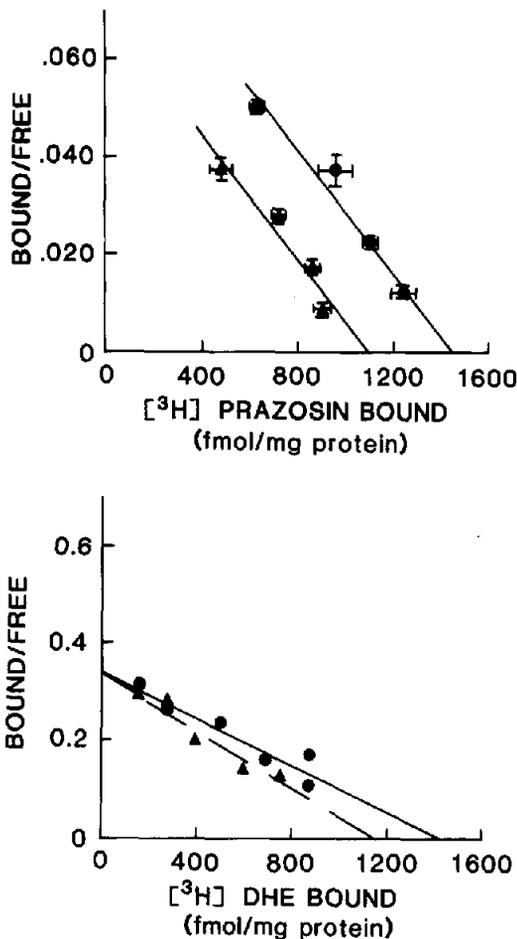

Fig.2. Scatchard plots for the binding of [3H]prazosin (top), and [3H]DHE (bottom), to plasma membranes from fed (•), and fasted (▲) rats. Data were calculated by normalizing protein concentrations to 20 μg/ml for [3H]prazosin binding, and 2 mg/ml for [3H]DHE binding.

seen that fasting caused about a 60% decrease in the maximally-stimulated phosphorylase activity, as well as a marked reduction in the sensitivity towards the α-agonist as shown by a rightward shift in the dose–response curve. Fig.2A shows Scatchard plots [15] for the binding of [3H]prazosin, a selective α_1-adrenergic antagonist, to liver plasma membranes from fed and fasted rats. Values for dissociation contents (K_D) and the maximal number of binding sites (B_{max}) derived from these plots are given in table 1. The binding parameters obtained for membranes from fed rats are comparable to values in [9,16]. Fasting resulted in a

Table 1

Values for K_D and B_{max} derived from Scatchard plots for the binding of α- and β-adrenergic radioligands to liver plasma membranes isolated from fed and 24 h fasted rats

Ligand	Fed rats			Fasted rats		
	K_D (nM)	B_{max}	n	K_D (nM)	B_{max}	n
[³H]prazosin	0.32 ± 0.02	1468 ± 63	3	0.32 ± 0.07	1080 ± 39	3
[³H]DHE	9.0 ± 1.0	1640 ± 204	3	10 ± 3.0	1409 ± 261	3
[¹²⁵I]CYP	0.09 ± 0.02	15.6 ± 2.2	4	0.10 ± 0.02	29.1 ± 3.7	4
[³H]Yohimbine	21.0 ± 4.0	110 ± 17	4	21.1 ± 1.9	65 ± 3	4

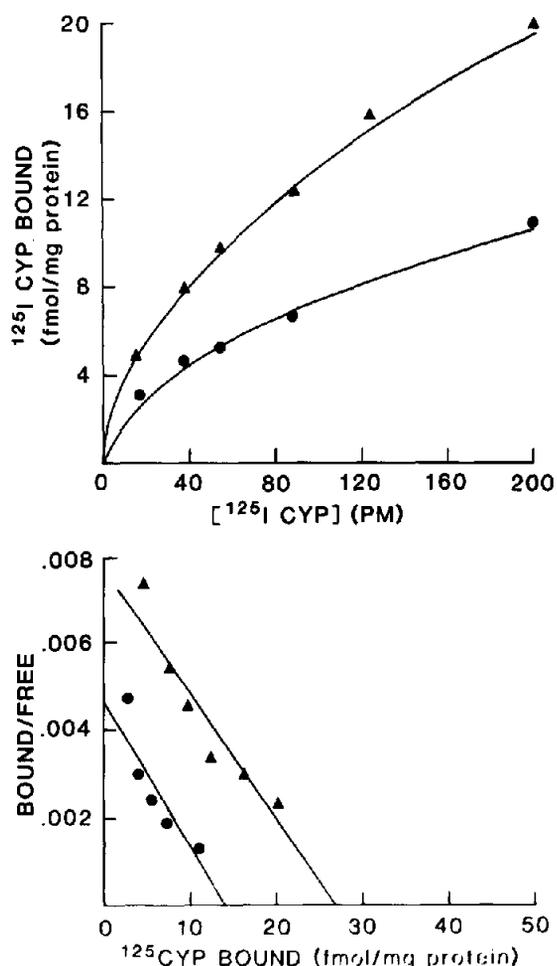B_{max}-values are given in fmol/mg protein; n is the number of experiments

Fig.3. Scatchard plots for the binding of [¹²⁵I]CYP to plasma membranes from fed (●), and fasted (▲) rats. Data were calculated by normalizing protein concentrations to 25 µg/ml.

25% reduction in the B_{max} for the α_1-adrenergic antagonist prazosin. Scatchard plots derived from binding data of [³H]DHE are shown in fig.2B. The values for K_D and B_{max} are given in table 1. For this mixed α-receptor ligand, fasting caused about a 15% decrease in the maximal number of binding sites. This change however, is not statistically significant. These results are similar to those obtained for adrenalectomized and hypothyroid rats in which no change in B_{max} for [³H]DHE [6], and a 30% decrease in B_{max} for [³H]prazosin [9] have been observed.

Since catecholamine activation of rat hepatic glycogen phosphorylase is primarily α_1-receptor mediated [3,4,16], the reduced α_1-adrenergic ligand binding could at last in part be responsible for the decrease phenylephrine sensitivity in hepatocytes from fasted rats.

3.2. Effects of fasting on the β-adrenergic system

The new β-adrenergic antagonist [¹²⁵I]CYP was used for the identification and characterization of β-adrenergic binding sites in the rat liver. This radioligand has been used successfully [13] to identify β-adrenergic receptors in guinea pig lung and left ventricular membranes. We found [¹²⁵I]CYP to bind to rat liver plasma membranes with great affinity. Specific binding was >70% of total binding. Binding of this radioligand was rapid and reached equilibrium within 15 min at 25°C. It was possible to dissociate 80% of the bound [¹²⁵I]CYP from plasma membranes using propranolol within 40 min. β-Adrenergic agonists and antagonists competed with [¹²⁵I]CYP binding in a manner consistent with its binding to β-adrenergic receptors. Binding of CYP was also stereospecific, with

Table 2

Effect of epinephrine on epinephrine stimulation of cAMP accumulation in hepatocytes from fed and 24 h fasted rats

Additions (μM)	Fed	Fasted
	(nmol cAMP/g cell)	
Saline	557 ± 14	781 ± 20
Epinephrine (5)	664 ± 20	1006 ± 24
Epi (5) + yohimbine (0.1)	655 ± 20	972 ± 40
Epi (5) + yohimbine (10)	646 ± 10	938 ± 20
Epi (5) + propanolol (50)	588 ± 14	816 ± 12

Hepatocytes were incubated in the presence of blockers,, where designated, for 10 min. Epinephrine was then added and aliquots were taken 2 min later for cAMP determinations. Values taken from a typical experiment, are averages of 5 incubations each ± SEM
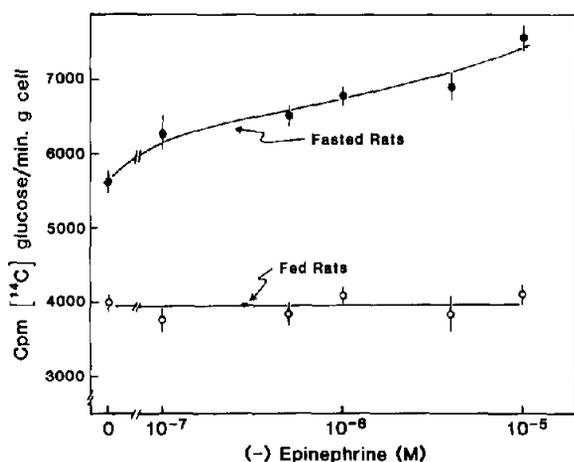

Fig.4. [^{14}C]Glucose production from [1-^{14}C]lactate in hepatocytes isolated from fed (○) and 24 h fasted (●) rats. Aliquots (1 ml) of hepatocyte suspension (40 mg wet wt) were incubated with 5 μM phenoxybenzamine for 10 min. Epinephrine at concentrations indicated and [1-^{14}C]lactate (5 mM, final conc.) were then added to each flask and incubation was carried out for another 30 min. Specific radioactivity of [1-^{12}C]lactate was 20 uCi/mmol. Data presented are averages of 3 incubations ± SEM.

(−)-propranolol 2 orders of magnitude more potent that (+)propranolol in displacing the radioligand. Unlike the binding of [^{3}H]dihydroalprenolol and [^{125}I]iodohydroxybenzylpindolol [6,7,17], binding of [^{125}I]CYP was not affected by the α-adrenergic antagonist phentolamine at up to 0.1 mM.

Fig.3 shows the binding of [^{125}I]CYP to liver plasma membranes from fed and fasted rats. The binding parameters obtained are presented in table 1. Fasting for 24 h resulted in 100% increase in the number of β-adrenergic binding sites labeled with [^{125}I]CYP. Interestingly, this rate of increase in the β-adrenergic binding sites is similar to the synthesis rate of β-receptors in cultured human lung cells [18]. This increase in β-receptor sites represents a functional increase with respect to β-adrenergic action as shown by an enhanced epinephrine-sensitive cyclic AMP accumulation in hepatocytes isolated from fasted rats (table 2). Epinephrine-mediated elevation of cAMP contents, in either fed or fasted rat hepatocytes, was completely blocked by the addition of propranolol, consistent with a β-adrenoreceptor mediated action.

The increase in β-receptor action induced by fasting was further demonstrated by its effect on epinephrine stimulation of gluconeogenesis in isolated hepatocytes, in the presence of a maximally effective concentration of an α-antagonist, phenoxybenzamine. In the presence of the α-blocker, epinephrine, at a wide range of concentrations, exerted no effect on the conversion of [1-^{14}C]lactate to glucose in hepatocytes from fed rats (fig.4). In contrast, there was a clear dose-dependent stimulation of [^{14}C]glucose production by epinephrine (plus phenoxybenzamine) in cells from fasted rats. The addition of 10 μM (±)-propranolol completely inhibited the epinephrine action, at all concentrations tested (not shown). These observations are consistent with an increase in β-adrenergic action on gluconeogenesis in the livers of fasted rats.

In light of a recent suggesting that α_2-adrenergic receptor action could inhibit β-adrenergic activation of adenylate cyclase in the rat liver [19], the effect of fasting on epinephrine-mediated cAMP increases may not therefore be viewed as changes in β-receptor action alone. We therefore investigated the changes in α_2-adrenergic receptors in response to fasting, using [^{3}H]yohimbine, a selective α_2-antagonist [20,21]. Fig.5 shows Scatchard plots for the binding of [^{3}H]yohimbine. Binding para-
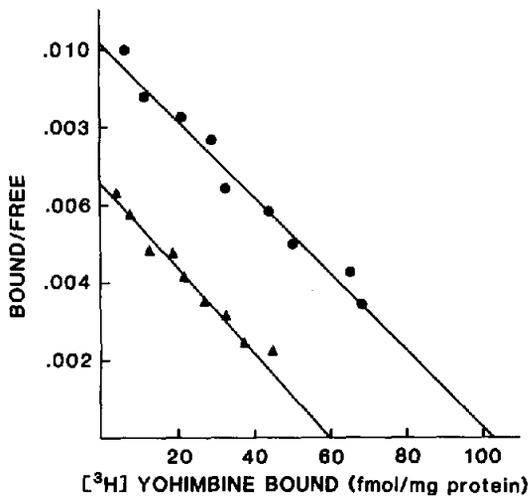
Fig.5. Scatchard plots for the binding of [³H]yohimbine to plasma membranes from fed (●) and fasted (▲) rats. Data were calculated by normalizing protein concentrations to 2 mg/ml.

meters are shown in table 1. In comparison to the α_1-receptor subtype, fasting caused a more dramatic reduction in this α_2-ligand binding. A 50% reduction in the number of binding sites for [³H]-yohimbine was observed, after a 24 h fast. This declined further to 20% to the fed value after fasting for 72 h (not shown). In contrast to the effects of yohimbine described for other systems [22–24], addition of a wide range of concentrations of yohimbine had no effect on epinephrine stimulation of cAMP accumulation in hepatocytes from either fed or fasted rats. Typical results are shown in table 2. These data indicate that in spite of the marked difference in yohimbine binding between fed and fasted rats, the α_2-antagonist was equally ineffective in influencing β-adrenergic action in either preparations.

The changes in α- and β-adrenergic binding site densities reported here are consistent with a coordinated shift in the mechanism of hepatic glucose production from predominantly glycogenolytic in the fed state to gluconeogenic in the fasted condition. The action of catecholamines in adult rat liver glycogenolysis is clearly α_1-receptor mediated. Even in the fasted state when hepatic glycogen store is depleted, activation of glycogen

phosphorylase, although with reduced sensitivity, is still largely via the α_1-receptor. The type of adrenergic action on hepatic gluconeogenesis is, however, less clear. Mechanisms from solely α-adrenergic [1,25] to a mixed α- and β-adrenergic [2,26] have been reported. It is rather clear, however, that regulations exerted by epinephrine on key regulatory enzymes in the gluconeogenic pathway, e.g., pyruvate kinase [27], and those regulating fructose-2, 6-bisphosphate levels [27] are predominantly β-adrenergic. Although controls of pyruvate kinase [11,28,29] and pyruvate carboxylation [29] via an α-adrenergic mechanism have been observed, the potencies of epinephrine on these enzyme systems are far less than those described for more typical hepatic α-adrenergic actions, namely, phosphorylase activation [2,30] and calcium mobilization [30]. Our results are consistent with an increased role for the β-receptor in regulating hepatic gluconeogenesis in the fasted state.

Fasting selectively reduces α-adrenergic receptor densities in the rat liver. This coincides with a decreased role of hepatic glycogenolysis in glucose production, a pathway normally activated predominantly by the α_1-adrenergic receptor. The marked increase in β-adrenergic binding site density correlates well with increases in the β-receptor-mediated stimulation of cAMP accumulation and gluconeogenesis in livers of fasted rats. In view of the uncertain function of the hepatic α_2-adrenergic receptors, the physiological significance of the fasting-induced loss in this adrenergic receptor subtype remains unclear. The resemblance among some of the effects of brief fasting, adrenalectomy [5], and abnormal thyroid states [7–9], on hepatic catecholamine receptors and actions may be fortuitous. However, reduced food intake, for animals whose weight gains deviated markedly from normal after treatment in particular, as part contributor to these changes cannot be ruled out.

ACKNOWLEDGEMENTS

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