Effects of adrenalectomy on binding to and actions of adrenergic receptors

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Adrenalectomy results in significant changes in the mechanism of adrenergic activation of hepatic glycogenolysis. In adrenalectomized rats a greater role for the β -adrenergic receptor is observed, whereas the α_1 -adrenergic-mediated phosphorylase activation declines. Our present findings document that adrenalectomy causes a significant decrease in the high-affinity population of the α_1 -adrenergic receptor labelled with [3 H]adrenaline. Our data indicate a large increase in the number of β -adrenergic binding sites after adrenalectomy. This increase was not consistent with the observed modest increase in the β -adrenergic-mediated activation of cyclic AMP accumulation and glycogen phosphorylase. When α -adrenergic antagonists are present along with the catecholamine, a 100% increase in the adrenaline-mediated accumulation of cyclic AMP in hepatocytes from adrenalectomized rats was observed. Adrenalectomy was also shown to cause a significant increase in the hepatic α_2 -adrenergic binding sites. These data are consistent with an inhibitory role on the β -adrenergic-mediated activation of glycogenolysis by the hepatic α_2 -adrenergic receptor in adrenalectomy.

INTRODUCTION

Catecholamines through both α - and β -adrenergic receptors, activate glycogenolysis and gluconeogenesis in the rat liver (Tolbert *et al.*, 1973; Exton & Harper, 1975; Hutson *et al.*, 1976; El-Refai & Exton, 1980a). In adult normal rats, adrenaline activates glycogenolysis primarily through interaction with the α_1 -adrenergic receptors, and this effect is believed to be mediated by *myo*-inositol 1,4,5-trisphosphate production and elevation of free cytosolic [Ca^{2+}] (Blackmore *et al.*, 1978; Berridge, 1984). Activation of glycogenolysis by an increase in intracellular cyclic AMP resulting from adrenaline interaction with the β -adrenergic receptor is minor in these animals. In adrenalectomized rats, however, greater role for the β -adrenergic cyclic AMP-dependent mechanism has been observed (Chan *et al.*, 1979; Guellaen *et al.*, 1978; Wolfe *et al.*, 1976). In contrast, α_1 -adrenergic-mediated phosphorylase activation and Ca^{2+} mobilization decline in hepatocytes from adrenalectomized rats (Guellaen *et al.*, 1978; Chan *et al.*, 1979).

It has been reported that adrenalectomy resulted in a small but non-significant decrease in the number of α_1 -adrenergic binding sites as measured with [3 H]dihydroergocryptine (Guellaen *et al.*, 1978) and [3 H]noradrenaline (Chan *et al.*, 1979). Hanoune and co-workers subsequently presented data which indicated that the binding of agonists to α_1 -adrenergic binding sites is modulated by guanine nucleotides and that adrenalectomy abolished that mechanism (Goodhardt *et al.*, 1982). The involvement of this mechanism in α_1 -adrenergic modulation of glycogenolysis is yet to be demonstrated. A parallel between the enhancement in adenylate cyclase activation and an increase in the number of β -adrenergic receptors in rat liver plasma membranes of adrenalectomized rats has been reported (Wolfe *et al.*, 1976; Guellaen *et al.*, 1978). The modest increase in adenylate cyclase activation, however, could

not be directly correlated with the much bigger increase in the number of β -adrenergic binding sites (Wolfe *et al.*, 1976; Chan *et al.*, 1979). This consistently observed apparent disparity between β -adrenergic receptor density and their action in adrenalectomy has not been addressed. One may envisage an effect of adrenalectomy on the β -adrenergic-receptor–adenylate cyclase coupling mechanism. Alternatively, an effect on the interaction between the β -adrenergic receptors and α_2 -adrenergic receptors, which has been shown to exert an inhibitory action on adenylate cyclase (Jard *et al.*, 1981; Smith & Limbird, 1981; Kather & Simon, 1981; Atlas & Sabol, 1981), may be considered.

In the present study we have used both rat liver plasma membranes and isolated rat hepatocytes to explore the changes in the relationships between α_1 -, α_2 - and β -adrenergic receptors in catecholamine activation of glycogen phosphorylase as a result of adrenalectomy.

MATERIALS AND METHODS

Materials

[3 H]Prazosin (18.0 Ci/mmol), [3 H]yohimbine (35 Ci/mmol) and [125 I]iodocyanopindolol (2200 Ci/mmol) were from New England Nuclear. The sources of other chemicals have been reported previously (Chan *et al.*, 1979; El-Refai *et al.*, 1979; El-Refai & Chan, 1982, 1984). Male Sprague–Dawley rats (220–270 g) were obtained from Simonson and fed *ad libitum* on Purina Lab Chow. Bilateral adrenalectomy was performed by a dorsal approach as described previously (Chan *et al.*, 1979). Adrenalectomized rats were given 0.5% NaCl to drink and were used 3–5 days after operation.

Radioligand binding

Preparation of plasma membranes from control and adrenalectomized rats and radioligand binding to those

membranes were carried out as described previously (El-Refai *et al.*, 1979; El-Refai & Chan, 1982, 1984). It has been observed that results obtained for sham-operated rats are not different from those obtained for intact rats. Incubation media and washing buffers used in the vacuum-filtration procedure for the binding of [3 H]prazosin and [3 H]yohimbine contained 50 mM-Tris/HCl (pH 7.6), 0.8 mM-ascorbic acid and 3 mM-catechol. ($\pm$)-Propranolol was present at 10 μ M in the incubation medium. The [3 H]adrenaline binding system was similar to that described above except that Tris buffer at pH 8.0 was used. Binding of the β -adrenergic-receptor ligand [125 I]iodocyanopindolol was performed as reported previously (El-Refai & Chan, 1982, 1984). Incubations were performed at 25 $^{\circ}$ C for 60 min in 50 mM-Tris buffer (pH 7.6), containing 150 mM-NaCl and 7–15 μ g of plasma-membrane protein. The filters were washed with 10 mM-Tris buffer (pH 7.5) containing 150 mM-NaCl. For α -adrenergic binding, specific binding is defined as the difference between ligand binding in the absence and presence of 10 μ M-phenolamine. Specific binding of [125 I]iodocyanopindolol is defined as the difference in the radioligand binding in the absence and presence of 1 μ M-($\pm$)propranolol. Throughout this paper, the term 'binding' means specific binding of the radioligand as described above. Values for binding were averages of four determinations. Experiments were performed with at least two different plasma-membrane preparations. Binding analysis was carried out by the data-fitting routine 'ABDA' described by McPherson (1983) and an adaptation of the 'LIGAND' program (Munson & Rodbard, 1980) to an IBM personal computer (McPherson, 1983).

Hepatocyte isolation and incubation

Hepatocytes were isolated by a modification (Exton & Harper, 1975) of the method of Berry & Friend (1969). They were incubated in the medium and under the conditions described previously (Chan *et al.*, 1979). Viability of hepatocytes was checked routinely by the Trypan Blue exclusion method. Only preparations in which viability exceeded 90% were used.

Glycogen phosphorylase *a* and cyclic AMP determinations

Glycogen phosphorylase *a* activity was assayed (Stalmans & Hers, 1975) in liver-cell homogenates prepared as described previously (Chan *et al.*, 1979). Total cyclic AMP in hepatocyte suspensions was determined by the binding assay (Gilman, 1970) after purification of the nucleotide by ion-exchange chromatography (Hardman & Sutherland, 1969). Unless otherwise stated, cell samples were taken for determination of phosphorylase *a* activity and cyclic AMP 2 min after hormone additions. Units of phosphorylase activity are μ mol of [14 C]glucose 1-phosphate incorporated into glycogen/min per g wet wt. of cells. Data from a representative experiment are shown, with means $\pm$ S.E.M. from triplicate or quadruplicate incubations. All experiments were repeated at least three times to ensure reproducibility.

Protein determination

Protein was determined as described by Lowry *et al.* (1951), with bovine serum albumin as standard.

Fig. 1. Scatchard plots for the binding of [3 H]adrenaline to liver plasma membranes from control and adrenalectomized (ADX) rats

Values shown are averages of data obtained from three different experiments using different preparations of plasma membranes. Average protein concentration in these experiments was 2.0 mg/ml.

RESULTS

Binding of [3 H]adrenaline to membranes from control and adrenalectomized rats

The dose (5–120 nM)-dependent binding of [3 H]-adrenaline was investigated with rat liver plasma membranes isolated from control and adrenalectomized animals. In a pH 8.0 assay system, [3 H]adrenaline labelled a larger number of binding sites than at pH 7.6 (El-Refai *et al.*, 1979). Trypsin treatment, which has been shown to increase the number of binding sites for adrenaline (El-Refai & Exton, 1980b), has no effects at the higher pH. Therefore this increase in adrenaline binding may be a result of conformational changes in the binding protein. Fig. 1 shows the Scatchard (1949) plots derived from these binding data; the binding of this radioligand can be resolved into two binding components. Computer-assisted analysis of the data (McPherson, 1983; Munson & Rodbard, 1980) indicated that adrenalectomy caused no significant change in the dissociation constant of the high-affinity binding component, whereas it caused a significant decrease in the number of binding sites for this component. The K_D for this component was 44 and 50 nM for membranes from control and adrenalectomized rats respectively. The low-affinity-component binding parameters were not changed by adrenalectomy. Previous studies (Chan *et al.*, 1979) using [3 H]noradrenaline binding at different conditions (pH 7.5) showed small but non-significant decreases in the binding of this adrenergic agonist in adrenalectomized rats. Since it has been shown that the binding of adrenaline in this concentration range is to α_1 -adrenergic-type binding sites (El-Refai & Exton, 1980a; Geynet *et al.*, 1981), these data indicate that adrenalectomy causes a decrease in the high-affinity α_1 -adrenergic binding sites for adrenaline. Consequently this decrease in α_1 -adrenergic binding may explain, at least in part, the decrease in the α_1 -adrenergic-mediated

Table 1. Kinetic parameters for the binding of various adrenergic radioligands to liver membranes from control and adrenalectomized rats

Data shown are averages of experiments which were repeated at least three times using two different membrane preparations. Values in parentheses represent the ranges of the parameter values obtained from individual experiments.

Ligand	Binding parameters			
	Control rats		Adrenalectomized rats	
	K_D (nM)	n (fmol/mg of protein)	K_D (nM)	n (fmol/mg of protein)
[³ H]Prazosin	0.3 (0.29–0.35)	1460 (1405–1530)	0.3 (0.22–0.37)	1200 (1100–1250)
[³ H]Adrenaline	44 (43–45)	340 (325–350)	50 (45–67)	190 (170–214)
High-affinity				
Low-affinity	150 (140–160)	290 (270–300)	140 (120–150)	320 (290–350)
[¹²⁵ I]Iodocyanopindolol	0.091 (0.080–0.100)	22 (18–23)	0.146 (0.120–0.150)	102 (90–110)
[³ H]Yohimbine	20 (15–22)	104 (90–120)	26 (17–25)	175 (160–180)

Table 2. Cyclic AMP accumulation in hepatocytes isolated from rats in response to increasing concentrations of adrenaline in the presence or absence of adrenergic antagonists

Both control (CON) and adrenalectomized (ADX) rats were used. Adrenergic antagonists were used at a concentration of 10 μ M. Results are means $\pm$ S.E.M.

[Adrenaline] (μ M)	Cyclic AMP accumulation (pmol/g of cells)					
	Saline		Propanolol		Phenoxybenzamine	
	CON	ADX	CON	ADX	CON	ADX
0	938 $\pm$ 98	678 $\pm$ 26				
0.01		737 $\pm$ 20		712 $\pm$ 34		893 $\pm$ 60
0.1		1035 $\pm$ 43		544 $\pm$ 30		1439 $\pm$ 57
0.5	1073 $\pm$ 72	1479 $\pm$ 41	911 $\pm$ 99	450 $\pm$ 20	1145 $\pm$ 77	2137 $\pm$ 32
1		1878 $\pm$ 84		472 $\pm$ 21		2822 $\pm$ 128
10		2493 $\pm$ 139		539 $\pm$ 42		4759 $\pm$ 257

activation of glycogen phosphorylase in adrenalectomy (Chan *et al.*, 1979; Guellaen *et al.*, 1978).

Binding of adrenergic radioligands to liver plasma membranes from control and adrenalectomized rats

Concentration-dependent binding of the α_1 -adrenergic antagonist [³H]prazosin, the α_2 -adrenergic antagonist [³H]yohimbine and the β -adrenergic antagonist [¹²⁵I]iodocyanopindolol to rat liver plasma membranes isolated from control and adrenalectomized animals was carried out. Table 1 shows the binding parameters derived from the binding data. Adrenalectomy causes a small but reproducible decrease in the number of binding sites for prazosin and a 5-fold increase in the number of binding sites (n) for [¹²⁵I]iodocyanopindolol. There was also a small increase in the dissociation constant for the latter, owing to adrenalectomy. These results are consistent with other published reports (Wolfe *et al.*,

1976; Guellaen *et al.*, 1978; Preksaitis *et al.*, 1982) in which the β -adrenergic antagonists [³H]dihydroalprenolol and [¹²⁵I]iodohydroxybenzylpindolol were used as radioligands.

Adrenalectomy also resulted in an increase in the total number of α_2 -adrenergic binding sites. This increase and the observed inhibitory effects of the α -adrenergic system on the β -adrenergic activation of cyclic AMP accumulation may explain the lack of proportionality between the increase in the β -adrenergic binding sites and the β -adrenergic stimulation of cyclic AMP accumulation in adrenalectomized rats.

Effect of adrenalectomy on adrenaline-mediated cyclic AMP accumulation in rat hepatocytes

Table 2 shows the accumulation of cyclic AMP in rat hepatocytes isolated from control and adrenalectomized animals. The effect of adrenaline on cyclic AMP

accumulation in rat hepatocytes was determined in the presence or absence of the β -adrenergic antagonist propranolol and the α -adrenergic antagonist phenoxybenzamine; $0.5 \mu\text{M}$ -adrenaline caused a modest increase in cyclic AMP accumulation in control hepatocytes and larger increase in cyclic AMP accumulation in hepatocytes from adrenalectomized rats. The β -adrenergic antagonist propranolol at $10 \mu\text{M}$ completely abolished the adrenaline-mediated increase in cyclic AMP accumulation in both control and adrenalectomized-rat liver cells. On the other hand, the α -adrenergic antagonist phenoxybenzamine at $10 \mu\text{M}$ in the presence of adrenaline caused an increase in cyclic AMP accumulation over that caused by the adrenergic agonist alone. This phenoxybenzamine-mediated increase in adrenaline effect on cyclic AMP accumulation was much larger in hepatocytes from adrenalectomized rats. Both yohimbine and prazosin were not as effective as phenoxybenzamine in increasing adrenaline-mediated accumulation of cyclic AMP in hepatocytes (results not shown). This may be due to these agents' properties as selective but not specific antagonists and their different relative ability to compete with adrenaline for the inhibitory site.

Table 2 also shows the stimulation of cyclic AMP accumulation in hepatocytes from adrenalectomized rats by increasing concentrations of adrenaline in the presence of propranolol or phenoxybenzamine. The increase in β -adrenergic activation of cyclic AMP accumulation in adrenalectomy is not proportional to the 5-fold increase in the β -adrenergic binding sites for [^{125}I]iodocyanopindolol. Also, the adrenaline-mediated increase in cyclic AMP accumulation was almost doubled in the presence of phenoxybenzamine. These observations may be explained by a blockade of an α -adrenergic action by phenoxybenzamine.

Inhibition of adrenaline-mediated glycogen phosphorylase activation by adrenergic antagonists

Fig. 2 shows the activity of glycogen phosphorylase *a* in hepatocytes from control (a) and adrenalectomized (b) rats by increasing concentrations of adrenaline in the presence of various adrenergic antagonists. The β -adrenergic antagonist propranolol was not effective in inhibiting adrenaline-mediated activation of phosphorylase in hepatocytes from control rats ($P > 0.5$). Adrenalectomy, however, caused a significant increase in the ability of propranolol to inhibit the effect of adrenaline on phosphorylase ($P < 0.05$). This is in accord with the increased β -receptor-mediated action on cyclic AMP accumulation. Yohimbine, although a selective α_2 -antagonist, inhibited activation of phosphorylase at low concentrations, presumably by competition at the α_1 -adrenergic sites (El-Refai & Exton, 1980a). Also, adrenalectomy resulted in a decrease in the ability of yohimbine, an α_2 -adrenergic antagonist, to inhibit the enzyme at high concentrations of adrenaline. The change is marginally significant ($P < 0.1$) when the parameters of the fitted curve are compared (see legend of Fig. 2 for explanation). However, phosphorylase *a* activity changes are significantly lower in adrenalectomized rats ($P < 0.02$) at adrenaline concentrations of 0.5 and $5.0 \mu\text{M}$. These data suggest a yohimbine-mediated relief of adrenaline inhibition of phosphorylase by competition at the α_2 -receptor. Interestingly, although the combined presence of propranolol and yohimbine resulted in additive effects in cells from control rats, their combined

Fig. 2. Adrenergic-antagonist-mediated inhibition of activation of glycogen phosphorylase in hepatocytes from control and adrenalectomized rats by increasing concentrations of adrenaline

Experiments were carried out as explained in the Materials and methods section. Results are means $\pm$ S.E.M. Statistical significance was determined by fitting the data points to a two-parameter model [$y = P_1/(1 + P_2/x)$], where P_1 and P_2 are the two parameters] by using a non-linear regression routine (Dr. M. Bolger, USC School of Pharmacy). A significant difference between the parameters obtained may constitute a significant difference in the inhibition of isotherm. Statistical significance was also established for individual points by Student's *t* test. Symbols: $\circ$, saline (control); $\square$, + propranolol ($10 \mu\text{M}$); $\bullet$, + yohimbine ($10 \mu\text{M}$); $\triangle$, + propranolol + yohimbine.

effects produced greater than additive effects at high concentrations of adrenaline in cells from adrenalectomized rats. This may be a result of increased influence of the β -adrenergic receptor and/or propranolol on the α_1 -adrenergic receptor.

DISCUSSION

In adrenalectomy, there is a decrease in the α_1 -adrenergic-mediated activation of hepatic glucose production. The increase in the β -adrenergic binding observed here could be viewed as a compensation for the loss of α_1 -adrenergic effects. However, the process of hepatic glucose production in adrenalectomized animals was shown to be less responsive than normal to stress situations. This was not due to a lack of hepatic glycogen

store (Chan *et al.*, 1979), but rather to a decreased response of the adrenergic system to stimulate hepatic glycogenolysis (Chan *et al.*, 1979; Guellaen *et al.*, 1978). An effect of adrenalectomy on adrenergic action may be exerted either at the level of receptor-effector coupling or at a further step in the regulatory cascade, or both.

It has been previously shown that adrenalectomy decreased α_1 -adrenergic-mediated actions in hepatocytes (Chan *et al.*, 1979; Guellaen *et al.*, 1978). However, the α_1 -adrenergic effects of adrenaline on phosphorylase and Ca^{2+} release in hepatocytes could not be satisfactorily correlated with the changes in the α -adrenergic binding of the α -adrenergic antagonist [^3H]dihydroergocryptine (Guellaen *et al.*, 1978; Preksaitis *et al.*, 1982). Our present finding shows that adrenalectomy causes a 50% decrease in a high-agonist-affinity population of the α_1 -adrenergic receptor which could be labelled with [^3H]adrenaline in hepatic plasma membranes. A decrease in the number of binding sites for the adrenergic radioligand [^3H]noradrenaline has been previously observed in liver plasma membranes isolated from adrenalectomized rats (Chan *et al.*, 1979); however, these changes could not be shown to be statistically significant. The α_1 -adrenergic receptor has been shown to be regulated by guanine nucleotides (El-Refai *et al.*, 1979; El-Refai & Exton, 1980a; Geynet *et al.*, 1981; Goodhardt *et al.*, 1982). Hanoune and co-workers (Goodhardt *et al.*, 1982) have further shown, in agonist competition experiments using the α_1 -adrenergic antagonist [^3H]prazosin, that adrenalectomy results in a decrease in the role of that guanine nucleotide regulation mechanism. This may explain the decrease that we observed in the high-affinity binding of [^3H]adrenaline, which may represent binding to the high-affinity state of the receptor. In this light, we propose that the decrease in the α_1 -adrenergic-mediated activation of glycogen phosphorylase in the liver of adrenalectomized rats is a result of a shift in the balance of the α_1 -adrenergic receptor states towards the low-affinity state.

Our observations provide evidence for an increased involvement of the α_2 -adrenergic receptor in the regulation of glycogen phosphorylase and glycogenolysis in the liver of adrenalectomized rats. As indicated above, as a result of adrenalectomy the β -adrenergic mediation of these processes assumes increasing importance, whereas the α_1 -adrenergic role is decreased (Chan *et al.*, 1979; Guellaen *et al.*, 1978; Wolfe *et al.*, 1976). The observed large increase in the number of β -adrenergic binding sites caused by adrenalectomy reported in the present study, as well as others (Guellaen *et al.*, 1978; Wolfe *et al.*, 1976), however, was not consistent with the observed modest increase in the β -adrenergic-mediated activation of cyclic AMP accumulation and glycogen phosphorylase. We have observed almost 100% increase in the adrenaline-mediated accumulation of cyclic AMP in hepatocytes from adrenalectomized rats in the presence of the α -adrenergic antagonist phenoxybenzamine. A similar but much smaller effect was observed in hepatocytes from normal adult rats. Binding studies further revealed that the number of the α_2 -adrenergic binding sites almost doubled in response to adrenalectomy. These data taken together are consistent with an inhibitory role on the β -adrenergic-mediated activation

of glycogenolysis by the hepatic α_2 -adrenergic receptor in adrenalectomy.

In 24 h-starved rats we have shown an increase in the number of β -adrenergic receptors, with concomitant decrease in the number of α_1 -adrenergic receptors (El-Refai & Chan, 1982). Unlike adrenalectomy, starvation caused a decrease in the α_2 -adrenergic receptors (El-Refai & Chan, 1982). This decrease in the α_2 -adrenergic receptors in starved animals may partly explain their ability to respond to stress and furthermore provides support for the possibility of a regulatory role for the α_2 -adrenergic system in hepatic glycogen metabolism.

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