Hormonal Stimulation of Cyclic AMP Accumulation and Glycogen Phosphorylase Activity in Calcium-Depleted Hepatocytes from Euthyroid and Hypothyroid Rats

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Activation of glycogen phosphorylase by hormones was examined in hepatocytes isolated from euthyroid and hypothyroid female rats and incubated in Ca^{2+} -free buffer containing 1 mM-EGTA. Basal glycogen phosphorylase activity was decreased in Ca^{2+} -free buffer. However, the activation of hepatocyte glycogen phosphorylase, in the absence of extracellular Ca^{2+} , in response to adrenaline, glucagon or phenylephrine was slightly lower, whereas that by vasopressin was abolished. The activation of glycogen phosphorylase by phenylephrine, adrenaline or isoproterenol (isoprenaline) in hepatocytes from euthyroid rats incubated in the absence of Ca^{2+} was not accompanied by any detectable increase in total cyclic AMP. The log-dose/response curves for activation of phosphorylase by phenylephrine or low concentrations of adrenaline were the same in hepatocytes from hypothyroid as compared with euthyroid rats, whereas the response to isoproterenol was greater in hepatocytes from hypothyroid rats. However, the increases in total cyclic AMP accumulation caused by adrenaline or isoproterenol were greater in hepatocytes from hypothyroid rats than in hepatocytes from euthyroid rats. The increases in cyclic AMP accumulation caused by adrenaline or isoproterenol in Ca^{2+} -depleted hepatocytes from hypothyroid rats were blocked by propranolol, a β -adrenergic antagonist. In contrast, propranolol was only partially effective as an inhibitor of the activation of glycogen phosphorylase by phenylephrine or adrenaline in hepatocytes from hypothyroid rats and ineffective on phosphorylase activation in cells from euthyroid rats. These data indicate that the α -adrenergic activation of glycogen phosphorylase is not affected by the absence of extracellular Ca^{2+} , and the extent to which total cyclic AMP was increased by adrenergic amines did not correlate with glycogen phosphorylase activation.

The activation of glycogen breakdown and gluconeogenesis by catecholamines in the rat liver is a response which displays both α - and β -adrenergic components (Sherline *et al.*, 1972; Tolbert *et al.*, 1973; Exton & Harper, 1975). The increase in hepatic cyclic AMP resulting from catecholamine stimulation is blocked by propranolol, a β -adrenergic antagonist, but not by α -adrenergic blockers such as phentolamine or phenoxybenzamine. However, β -adrenergic-receptor-mediated activation of phosphorylase (presumably via elevation of cyclic AMP concentration) plays a minor role in adrena-

line activation of hepatic phosphorylase (Sherline *et al.*, 1972; Saitoh & Ui, 1976; Hutson *et al.*, 1976; Birnbaum & Fain, 1977). The α -adrenergic-receptor-mediated pathway is thus the major component of hepatic glycogenolysis in rats. Unlike the β -adrenergic response, α -adrenergic activation of phosphorylase occurs without detectable increases in intracellular cyclic AMP or increases in protein kinase activity (Sherline *et al.*, 1972; Exton & Harper, 1975; Hutson *et al.*, 1976; Saitoh & Ui, 1976; Cherrington *et al.*, 1977; Keppens *et al.*, 1977; Birnbaum & Fain, 1977). This α -adrenergic activation of phosphorylase is blocked by phentolamine, phenoxybenzamine or ergot derivatives and is unaffected by propranolol.

A central role for Ca^{2+} in the α -adrenergic

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activation of hepatic phosphorylase has been proposed (Pointer *et al.*, 1976; Van de Werve *et al.*, 1977; Keppens *et al.*, 1977; Assimacopoulos-Jeannet *et al.*, 1977; Blackmore *et al.*, 1978). According to this scheme, catecholamine action mediated via α -receptors results in increased cytosolic Ca^{2+} , which in turn stimulates phosphorylase kinase activity and thereby activates phosphorylase. One approach to the study of the role of Ca^{2+} in hormonal activation of phosphorylase has been to examine the effects of hormones on phosphorylase activity in the absence of extracellular Ca^{2+} (Pointer *et al.*, 1976; Stubbs *et al.*, 1976; Keppens *et al.*, 1977; Van de Werve *et al.*, 1977; Assimacopoulos-Jeannet *et al.*, 1977). Chan & Exton (1977) reported that α -agonists stimulate cyclic AMP accumulation in the absence but not in the presence of extracellular Ca^{2+} , and concluded that activation of phosphorylase by α -agonists in these cells is largely linked to this increase in cyclic AMP.

We reported that hypothyroidism selectively enhances activation of phosphorylase and stimulation of cyclic AMP accumulation by β -adrenergic agonists in isolated rat hepatocytes (Malbon *et al.*, 1978). The present paper extends these studies and investigates hormonal activation of phosphorylase and stimulation of cyclic AMP accumulation by hepatocytes from both euthyroid and hypothyroid rats in the absence of extracellular Ca^{2+} .

Materials and Methods

Most reagents were obtained from sources listed previously (Malbon *et al.*, 1978). [8-Arginine]-vasopressin (grade VI, 367 i.u./mg) was obtained from Sigma Chemical Co.

Rats were rendered hypothyroid by maintaining them on an iodine-deficient diet (U.S. Biochemical Corp., Cleveland, OH, U.S.A.) and drinking water containing 0.00625% propylthiouracil for 18–25 days. The euthyroid control rats were fed on the same iodine-deficient test diet in which the normal amount of iodine had been replaced by the com-

mercial supplier. Hepatocytes were isolated from 175–225 g fed female Sprague-Dawley rats (Charles River, CD strain) by the procedure of Berry & Friend (1969) as modified by Malbon *et al.* (1978). The digestion of liver was performed in Ca^{2+} -free buffer. Ca^{2+} depletion was produced by the following procedure. The isolated liver cells were resuspended and washed twice with Krebs-Ringer bicarbonate Ca^{2+} -free buffer (120 mM-NaCl, 4.8 mM-KCl, 1.2 mM- KH_2PO_4 , 1.2 mM- MgSO_4 , 24 mM- NaHCO_3 , pH 7.4 at 37°C) containing EGTA (1 mM). Portions (approx. 4 ml) of packed cells were incubated for 30 min with 8 ml of Ca^{2+} -free buffer containing 1 mM-EGTA. The hepatocytes were centrifuged in an IEC clinical centrifuge operating at setting no. 2 for 2 min, resuspended in fresh Ca^{2+} -free buffer containing 1 mM-EGTA, added to no. 2017 Falcon plastic tubes (approx. 7×10^6 cells/ml) and incubated for 20 min before addition of hormones. The tubes were constantly shaken in an orbital water bath operating at 130–200 rev./min. Cyclic AMP accumulation and the activity of glycogen phosphorylase were measured as described previously (Malbon *et al.*, 1978). The activity of glycogen phosphorylase is expressed in arbitrary units which represent μmol of glucose 1-phosphate incorporated into glycogen over a 20 min incubation per mg of homogenate protein. Protein was assayed by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard.

Results

In the absence of extracellular Ca^{2+} (hepatocytes incubated in Ca^{2+} -free buffer containing 1 mM-EGTA), basal glycogen phosphorylase activity of hepatocytes from euthyroid rats was slightly decreased (Table 1). The activation of phosphorylase by vasopressin was abolished in the absence of Ca^{2+} , whereas the activation in response to the α -adrenergic agonist phenylephrine, adrenaline or glucagon was only moderately decreased.

The time course for activation of glycogen

Table 1. *Effect of Ca^{2+} on activation of glycogen phosphorylase by hormones*

Liver cells (3.8×10^6 – 5.3×10^6 /tube) were incubated for 20 min in Krebs-Ringer bicarbonate buffer with the indicated concentrations of Ca^{2+} . The hormones were added 1 min before the end of the incubation. The values are the means of two paired experiments.

Concn. of added Ca^{2+} (mM)	Basal value (units)	Activation of glycogen phosphorylase by hormone (change in activity, % of basal)			
		Phenylephrine ($2 \mu\text{M}$)	Adrenaline ($1 \mu\text{M}$)	Glucagon (1 nM)	Vasopressin (2 munits/ml)
0 (+1 mM-EGTA)	1.09	+44	+83	+85	+2
0.14	1.29	+54	+86	+95	+36
1.4	1.34	+57	+71	+88	+36
4.2	1.23	+76	+99	+111	+91

phosphorylase by adrenaline was maximal at 1 min and declined over the next 7 min in hepatocytes incubated in Ca^{2+} -free buffer (results not shown). Similar data were obtained for hepatocytes incubated in the presence of Ca^{2+} .

The next series of studies compared the response of hepatocytes from euthyroid and hypothyroid rats incubated in the absence of extracellular Ca^{2+} to catecholamines. Previously we found an enhanced β -adrenergic activation of phosphorylase *a* in hepatocytes from hypothyroid rats (Malbon *et al.*, 1978). Basal glycogen phosphorylase activities were equivalent in Ca^{2+} -depleted hepatocytes from euthyroid and hypothyroid rats [0.9 ± 0.2 and 1.0 ± 0.3 units (means $\pm$ S.E.M., $n = 4$), respectively] and were approx. 10% lower than the values reported previously for hepatocytes incubated in buffer containing Ca^{2+} (Malbon *et al.*, 1978). The dose-response relationships for activation of phosphorylase by phenylephrine, adrenaline and isoproterenol (isoprenaline) are shown in Fig. 1(a). Hypothyroidism resulted in an enhanced activation of phosphorylase by isoproterenol, but not by phenylephrine or adrenaline (Fig. 1a). In the presence of $50 \mu\text{M}$ -3-isobutyl-1-methylxanthine, an inhibitor of cyclic AMP phosphodiesterase, basal phosphorylase activities in Ca^{2+} -depleted hepatocytes from both euthyroid and hypothyroid rats were not appreciably changed (1.1 ± 0.3 and 0.9 ± 0.3 units respectively). However, $50 \mu\text{M}$ -isobutylmethylxanthine markedly potentiated the activation of phosphorylase by isoproterenol, adrenaline or phenylephrine in Ca^{2+} -depleted hepatocytes from hypothyroid rats (Fig. 1b). In contrast, little change in hormonal activation of phosphorylase was obtained in hepatocytes from the euthyroid rat in the presence of $50 \mu\text{M}$ -isobutylmethylxanthine (Fig. 1b). The enhanced activation of phosphorylase in response to phenylephrine or adrenaline by the methylxanthine in hepatocytes from hypothyroid rats incubated in the absence of Ca^{2+} (Fig. 1b) was not seen in hepatocytes incubated in the presence of Ca^{2+} (Malbon *et al.*, 1978).

In order to evaluate the possible role of cyclic AMP in the activation of phosphorylase by hormones in Ca^{2+} -depleted hepatocytes, cyclic AMP accumulation was measured under the same conditions. Basal cyclic AMP accumulation was 2.1 and 2.0 pmol/ 10^6 cells in Ca^{2+} -depleted hepatocytes from euthyroid and hypothyroid rats, respectively, values somewhat higher than those reported in their counterparts incubated in the presence of normal Ca^{2+} (Malbon *et al.*, 1978). As shown in Fig. 1(c), no detectable increases in cyclic AMP accumulation were seen in hepatocytes from euthyroid rats incubated in the absence of Ca^{2+} in response to stimulation by phenylephrine, adrenaline or isoproterenol, although the activation of phosphoryl-

ase by each of these agents was considerable under the same conditions (Fig. 1a). Hypothyroidism potentiated cyclic AMP accumulation by these hepatocytes in response to adrenaline and to isoproterenol (Fig. 1c), whereas hypothyroidism potentiated the activation of phosphorylase in response to isoproterenol but not that in response to adrenaline (Fig. 1a).

In the presence of $50 \mu\text{M}$ -isobutylmethylxanthine, basal cyclic AMP accumulations were 1.8 ± 0.2 and 2.5 ± 0.5 pmol/ 10^6 cells for Ca^{2+} -depleted hepatocytes from euthyroid and hypothyroid rats respectively. The methylxanthine potentiated the cyclic AMP response of Ca^{2+} -depleted hepatocytes from both euthyroid and hypothyroid rats to phenylephrine, adrenaline or isoproterenol (Fig. 1d). The additional accumulation of cyclic AMP in euthyroid rat hepatocytes stimulated by adrenaline was apparently of little consequence in the activation of phosphorylase by this agent under these same conditions (Fig. 1b). Furthermore, half-maximal activation of phosphorylase was achieved at $1 \mu\text{M}$ -phenylephrine in Ca^{2+} -depleted hepatocytes from euthyroid rats, although at this concentration of phenylephrine no significant increase in cyclic AMP accumulation was detected (Fig. 1d). In the hypothyroid rat hepatocytes the presence of isobutylmethylxanthine potentiates both the increase in cyclic AMP and the activation of phosphorylase by phenylephrine or adrenaline (Figs. 1b and 1d). However, closer examination of the data in the Ca^{2+} -depleted hepatocytes from hypothyroid rats reveals the following: (i) that half-maximal activation of phosphorylase occurs at concentrations of phenylephrine or adrenaline which fail to produce a significant rise in cyclic AMP accumulation (Figs. 1b and 1d), and (ii) although isoproterenol stimulated a much greater cyclic AMP response than did phenylephrine (Fig. 1d), it could not stimulate phosphorylase to the extent obtained with phenylephrine (Fig. 1b). Though both responses are potentiated by isobutylmethylxanthine, the correlation between the accumulation of cyclic AMP and the activation of phosphorylase is quite poor.

Blocking studies using the β -antagonist propranolol ($10 \mu\text{M}$) and the α -antagonist phenoxybenzamine ($10 \mu\text{M}$) were conducted in an attempt to define the contribution of β -adrenergic receptors to cyclic AMP accumulation in Ca^{2+} -depleted hepatocytes from hypothyroid rats (Table 2). There was not enough cyclic AMP accumulation in euthyroid rat hepatocytes under the conditions used in Table 2 to see any inhibition caused by blockers. The Ca^{2+} -depleted hepatocytes from hypothyroid rats accumulated cyclic AMP in response to adrenaline, and this response was decreased by $10 \mu\text{M}$ -propranolol. Phenoxybenzamine likewise blocked the increase in cyclic AMP stimulated by $0.1 \mu\text{M}$ -adrena-

line, but had little effect on the response stimulated by isoproterenol.

In Ca^{2+} -depleted hepatocytes obtained from hypothyroid rats hormonal stimulation of cyclic AMP accumulation in the presence of $50\mu\text{M}$ -isobutylmethylxanthine was also evaluated in the presence of α - and β -adrenergic blocking drugs (Table 2). There was a small increase in cyclic AMP accumulation in response to phenylephrine, which was blocked by either propranolol or phenoxybenzamine. Propranolol blocked the rise in cyclic AMP stimulated by $0.1\mu\text{M}$ -adrenaline or $0.1\mu\text{M}$ -isoproterenol. Phenoxybenzamine decreased cyclic AMP accumulation in response to $0.1\mu\text{M}$ -adrenaline or -isoproterenol by approximately half. These data demonstrate that the cyclic AMP response of hypothyroid rat hepatocytes (after Ca^{2+} depletion) to low concentrations of adrenaline or isoproterenol is mediated through a process that can be completely blocked by propranolol. This suggests that the increase in cyclic AMP caused by phenylephrine and adrenaline is largely mediated through a

β -adrenergic mechanism. However, in Ca^{2+} -depleted hepatocytes the effects of phenylephrine and adrenaline were partially inhibited by phenoxybenzamine, an α -adrenergic antagonist (Table 2). There was also some slight inhibitory effect of phenoxybenzamine on the increase in cyclic AMP stimulated by isoproterenol. Whether these data indicate some α -adrenergic component of cyclic AMP accumulation in Ca^{2+} -depleted hepatocytes from hypothyroid rats or some non-specific effect of phenoxybenzamine remains to be demonstrated.

Discussion

The present studies on hormonal regulation of phosphorylase in Ca^{2+} -depleted hepatocytes from euthyroid and hypothyroid rats provide several new insights into α - and β -adrenergic-receptor-mediated activation of this enzyme. Incubation of hepatocytes from euthyroid rats in the absence of extracellular Ca^{2+} only moderately decreased the ability of phenylephrine to activate phosphorylase.

Table 2. Effects of α - and β -adrenergic blocking agents on cyclic AMP accumulation by Ca^{2+} -depleted hepatocytes from hypothyroid rats in response to phenylephrine, adrenaline or isoproterenol

Hepatocytes were incubated for 20 min in Ca^{2+} -free buffer containing 1 mM-EGTA in the presence or absence of either propranolol or phenoxybenzamine. At 1 min after the addition of hormones the incubation was terminated and the cyclic AMP accumulation measured. In series B, isobutylmethylxanthine (IBMX) was added to each tube at the start of the 1 minute incubation to give a final concentration of $50\mu\text{M}$. The data provided in series A are from four separate experiments and are expressed as means $\pm$ S.E.M.; the data provided in series B are from three separate experiments.

Additions	Cyclic AMP accumulation (pmol/ 10^6 cells)			
	Basal	Change caused by adrenergic agent		
		Phenylephrine ($1\mu\text{M}$)	Adrenaline ($0.1\mu\text{M}$)	Isoproterenol ($0.1\mu\text{M}$)
Series A				
None	2.0 ± 0.5	-0.1 ± 0.3	$+2.8 \pm 1.2$	$+3.2 \pm 0.8$
$10\mu\text{M}$ -propranolol	1.8 ± 0.4	-0.7 ± 0.2	$+1.3 \pm 0.3$	$+0.2 \pm 0.5$
$10\mu\text{M}$ -phenoxybenzamine	1.7 ± 0.5	-0.6 ± 0.4	$+1.1 \pm 0.6$	$+2.6 \pm 1.1$
Series B				
IBMX	1.7 ± 0.2	$+0.8 \pm 0.3$	$+5.9 \pm 1.4$	$+8.4 \pm 1.7$
IBMX + $10\mu\text{M}$ -propranolol	1.9 ± 0.4	0 ± 0.3	0 ± 0.4	-0.2 ± 0.3
IBMX + $10\mu\text{M}$ -phenoxybenzamine	2.4 ± 0.4	$+0.1 \pm 0.4$	$+3.7 \pm 0.6$	$+4.3 \pm 0.9$

Fig. 1. Dose-response curves for hormone-stimulated phosphorylase activation and cyclic AMP accumulation in Ca^{2+} -depleted hepatocytes from euthyroid (○) and hypothyroid (●) rats

The hepatocytes were incubated as described in the Materials and Methods section before the addition of $50\mu\text{M}$ -isobutylmethylxanthine (IBMX) and/or hormones. Activation of glycogen phosphorylase activity was measured in Ca^{2+} -depleted hepatocytes isolated from euthyroid (○) and hypothyroid (●) rats in response to various concentrations of phenylephrine, adrenaline or isoproterenol in the absence (a) or presence (b) of $50\mu\text{M}$ -isobutylmethylxanthine. Cyclic AMP accumulation was measured in Ca^{2+} -depleted hepatocytes isolated from euthyroid (○) and hypothyroid (●) rats in response to various concentrations of phenylephrine, adrenaline or isoproterenol in the absence (c) or presence (d) of $50\mu\text{M}$ -isobutylmethylxanthine. The data are means $\pm$ S.E.M. of four separate experiments. Phosphorylase activity and cyclic AMP accumulation were assayed in separate sets of tubes in duplicate. Basal cyclic AMP accumulations and phosphorylase activities are given in the text.

Furthermore, the activation of phosphorylase by phenylephrine in the absence of Ca^{2+} was not accompanied by a significant increase in total cyclic AMP. In the presence of isobutylmethylxanthine the activation of phosphorylase by phenylephrine in euthyroid-rat hepatocytes incubated in the absence of Ca^{2+} was essentially the same as noted in hepatocytes incubated in normal Ca^{2+} (Malbon *et al.*, 1978). Hepatocytes from the hypothyroid rat which were incubated in the absence of Ca^{2+} , in contrast, displayed an enhanced response to phenylephrine in the presence of isobutylmethylxanthine which was not seen in hypothyroid-rat hepatocytes incubated in the presence of normal Ca^{2+} (Malbon *et al.*, 1978).

Chan & Exton (1977) reported that the activation by phenylephrine of glycogen phosphorylase in hepatocytes incubated in Ca^{2+} -free buffer was due to an increase in cyclic AMP. However, the same laboratory later suggested that the primary effect of α -adrenergic catecholamines was to stimulate the release of intracellular Ca^{2+} rather than to increase cyclic AMP (Blackmore *et al.*, 1978). This was based on the finding that in hepatocytes incubated for shorter periods of time in Ca^{2+} -free buffer there was an α -adrenergic activation of glycogen phosphorylase which was not accompanied by an increase in cyclic AMP accumulation. Tolbert *et al.* (1980) compared the effect of varying degrees of calcium depletion on the activation of glycogen phosphorylase in hepatocytes by vasopressin and adrenaline. In hepatocytes incubated for 20 min in buffer containing 1.4 mM- Ca^{2+} and 2.8 mM-EGTA the subsequent activation of glycogen phosphorylase by vasopressin was completely abolished, whereas that by adrenaline was decreased by only 20%. In hepatocytes incubated for 20 min in Ca^{2+} -free buffer without or with added EGTA (1.4 mM), there was still a good increase in phosphorylase in response to adrenaline (Tolbert *et al.*, 1980). However, in the present studies, as in those of Chan & Exton (1977), hepatocytes were incubated for 30 min in Ca^{2+} -free buffer containing 1 mM-EGTA, washed, resuspended and then incubated for an additional 20 min before addition of hormones in the same buffer. The differences between our data and those of Chan & Exton (1977) might be due to variations in viability of hepatocyte preparations which may affect their ability to retain intracellular Ca^{2+} . Possibly our hepatocytes were able to retain their intracellular stores of Ca^{2+} to a greater extent than those used by Chan & Exton (1977). This might be due to variations in commercially available preparations of collagenase or to the fact that Chan & Exton (1977) incubated hepatocytes obtained from large male rats in buffer containing gelatin. All our studies were done with hepatocytes from female rats incubated in the absence of any added protein.

Hepatocytes isolated from euthyroid rats did not

show an enhanced accumulation of cyclic AMP in the absence of Ca^{2+} after the addition of phenylephrine or adrenaline (compare Fig. 1 with Malbon *et al.*, 1978). However, the effects of these amines on cyclic AMP accumulation were inhibited by 10 μM -phenoxybenzamine (results not shown). In contrast, cyclic AMP increases stimulated by α -adrenergic amines in hepatocytes from hypothyroid rats incubated in the absence of Ca^{2+} were only partially inhibited by phenoxybenzamine (Table 2). In our experiments there was little correlation between measurements of total cyclic AMP accumulation in the presence of α -adrenergic catecholamines and the activation of glycogen phosphorylase. In hepatocytes from euthyroid rats incubated without Ca^{2+} , 1 μM -phenylephrine in the presence of isobutylmethylxanthine stimulated phosphorylase activity more than half-maximally (Fig. 1b), yet no significant rise in cyclic AMP accumulation was obtained under these conditions (Fig. 1d). In hepatocytes from hypothyroid rats this dissociation between phosphorylase activation and cyclic AMP accumulation in response to low concentrations of phenylephrine in the presence of isobutylmethylxanthine was even more pronounced (Figs. 1b and 1d). It is difficult to attribute these changes in phosphorylase activity to small, undetectable changes in cyclic AMP, since isoproterenol stimulated a good cyclic AMP response but had little effect on phosphorylase (Fig. 1b).

These data suggest that if the cyclic AMP accumulated by hepatocytes in response to phenylephrine, adrenaline or isoproterenol is mediating α - or β -adrenergic activation of phosphorylase, this cyclic AMP is not reflected in the total intracellular pool. Alternatively, these cyclic AMP changes may reflect the occupancy of only that portion of the β -adrenergic receptors in normal hepatocytes that are coupled to adenylate cyclase. According to this alternative scheme, activation of phosphorylase would result from agonist occupancy of another portion of the total receptor population, which are perhaps coupled to some other membrane system which generates an unknown 'second messenger'. This possibility has been suggested for the activation of phosphorylase by glucagon (Birnbaum & Fain, 1977; Okajima & Ui, 1976). Although this has been opposed by Cherrington *et al.* (1977), the more recent data of Cote & Epand (1979) support the view that effects of glucagon on total cyclic AMP have little correlation with glycogenolysis. They prepared *N*- α -trinitrophenyl-glucagon and found that this derivative stimulated hepatic glycogenolysis without affecting cyclic AMP accumulation. Thus the available data suggest that either something else besides cyclic AMP is involved in glucagon action or the pool of cyclic AMP involved in glycogenolysis is compartmentalized. The latter

explanation would account for all the observed effects, including α -adrenergic activation of glycogenolysis, but it is difficult to prove or disprove at the moment.

Our data indicate that extracellular Ca^{2+} is not required for the activation of glycogenolysis by α -adrenergic catecholamines. Furthermore, cyclic AMP is no more likely to mediate this effect in Ca^{2+} -depleted than in normal hepatocytes. However, vasopressin action was abolished by Ca^{2+} -depletion under conditions in which catecholamine action was little affected. This might be explained if vasopressin interaction with its receptors is a Ca^{2+} -dependent process or if vasopressin works through a process dependent on the entry of extracellular Ca^{2+} .

Blackmore *et al.* (1978, 1979), Chen *et al.* (1978) and Salzmann *et al.* (1978) have postulated that the primary effect of α -adrenergic catecholamines is to release bound stores of intracellular calcium rather than to promote entry of extracellular calcium. Fain (1978) suggested that α -adrenergic catecholamine effects on phosphatidylinositol turnover are linked to α -adrenergic activation of glycogenolysis. Catecholamines increase the incorporation of labelled phosphate into phosphatidylinositol by isolated rat hepatocytes (Kirk *et al.*, 1977; Billah & Michell, 1979) through a mechanism involving α_1 -adrenergic receptors (Tolbert *et al.*, 1980). With vasopressin the increase in phosphatidylinositol turnover is seen in the absence of extracellular Ca^{2+} despite the finding that glycogenolysis is not affected by vasopressin in the absence of Ca^{2+} (Kirk *et al.*, 1978; Billah & Michell, 1979; Tolbert *et al.*, 1980). These data point to the possibility that increased turnover of hepatic phosphatidylinositol is a primary event which is linked in an unknown fashion to the release of intracellular Ca^{2+} and entry of extracellular Ca^{2+} .

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