Calcium-mobilizing hormones and phorbol myristate acetate mediate heterologous desensitization of the hormone-sensitive hepatic Na⁺/K⁺ pump

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The Na⁺/K⁺ pump in rat hepatocytes is stimulated in response to Ca²⁺-mobilizing hormones such as [arginine]vasopressin (AVP), angiotensin II and adrenaline, as well as tumour promoters such as 4β-phorbol 12β-myristate 13α-acetate (PMA). The ability of these agents to increase cellular contents of diacylglycerol and activate protein kinase C may be necessary to observe this response. In the present work, ouabain-sensitive ⁸⁶Rb⁺ uptake was studied in isolated rat hepatocytes to help to explain why stimulation of the Na⁺/K⁺ pump by Ca²⁺-mobilizing hormones and tumour promoters is not temporally sustained relative to other hormone responses. A transient stimulation (3–4 min) of the Na⁺/K⁺ pump was observed in hepatocytes exposed to high (10 nM), but not low (0.1 nM), concentrations of AVP. Experiments with the Ca²⁺ chelator EGTA and the Na⁺ ionophore monensin indicate that the rapid secondary decrease in Na⁺/K⁺-pump activity which occurs after AVP stimulation is not due to changes in cytosolic Ca²⁺ and Na⁺ concentrations. When added after the stimulation and rapid decrease in Na⁺/K⁺-pump activity induced in hepatocytes by a high concentration of AVP, a second challenge with AVP or PMA failed to stimulate the pump. Similarly, previous exposure of hepatocytes to angiotensin, adrenaline or PMA attenuated the subsequent Na⁺/K⁺-pump responses to AVP and PMA. In contrast, previous exposure to AVP had no significant effect on subsequent stimulation of the Na⁺/K⁺-pump by monensin, glucagon, forskolin or 8-*p*-chlorophenylthio cyclic AMP. In addition, exposure to monensin had no effect on subsequent responses to AVP and PMA. These data indicate that high concentrations of Ca²⁺-mobilizing hormones and PMA result in heterologous desensitization of the hepatic Na⁺/K⁺ pump to subsequent stimulation by Ca²⁺-mobilizing hormones and PMA, but not by cyclic-AMP-dependent agonists or monensin.

INTRODUCTION

Most agents which alter metabolic activity of the rat liver also stimulate the hepatic plasma-membrane Na⁺/K⁺ pump. For example, insulin and epidermal growth factor both increase Na⁺/K⁺-pump activity. This increase is secondary to hormonal stimulation of Na⁺ influx through the amiloride-sensitive Na⁺/H⁺ exchanger (Fehlmann & Freychet, 1981; Fehlmann *et al.*, 1981; for review see Pouyssegur, 1985). In contrast, glucagon stimulates the hepatic Na⁺/K⁺ pump by a different, presumably cyclic-AMP-dependent, mechanism which is not secondary to Na⁺ influx (Fehlmann & Freychet, 1981; Radomska-Pyrek *et al.*, 1982; Ihlenfeldt, 1981).

Ca²⁺-mobilizing hormones such as adrenaline, angiotensin II and [arginine]vasopressin (AVP) have been shown to activate the Na⁺/K⁺ pump in liver cells (Burgess *et al.*, 1981; Capiod *et al.*, 1982; Radomska-Pyrek *et al.*, 1982; Berthon *et al.*, 1983, 1985; Lynch *et al.*, 1984, 1986). This occurs through an unknown cyclic-AMP-independent mechanism that does not rely on Na⁺ influx. Exogenous diacylglycerols (Acyl₂Gro) and phospholipase C (from *Clostridium perfringens*) also stimulate the Na⁺/K⁺ pump in liver cells (Lynch *et al.*, 1986). These and other findings have implicated the

Acyl₂Gro–protein kinase C pathway in the mechanism by which Ca²⁺-mobilizing hormones alter Na⁺/K⁺-pump activity in rat tissues (Lynch *et al.*, 1986; Greene & Lattimer, 1986). One problem with this hypothesis, at least in the hepatocyte system, is that hormonally mediated Acyl₂Gro formation is a sustained event (Bockkino *et al.*, 1985), whereas stimulation of the Na⁺/K⁺ pump by Ca²⁺-mobilizing hormones is transient (Burgess *et al.*, 1981; Capiod *et al.*, 1982; Berthon *et al.*, 1983, 1985; Lynch *et al.*, 1986). This would suggest that the two events are not causally related. However, other support for the Acyl₂Gro mechanism of Na⁺/K⁺-pump activation comes from the finding that the transient time course exhibited by Ca²⁺-mobilizing hormones is mimicked by tumour promoters such as PMA (Lynch *et al.*, 1986). This is remarkable, since other events mediated by these agents in liver are temporally sustained. For example, Ca²⁺-mobilizing hormone-mediated changes in InsP₃ (Charest *et al.*, 1985; Blackmore *et al.*, 1987), Acyl₂Gro (Bockkino *et al.*, 1985), phosphorylase activity and glycogen synthase activity (Blackmore *et al.*, 1986) are sustained long after the Na⁺/K⁺ pump has returned to or approached basal activity. PMA-mediated changes in glycogen synthase activity (Roach & Goldman, 1983; Blackmore *et al.*, 1986), acetyl-CoA carboxylase activity (Vaartjes *et al.*, 1987) and inhibition of α₁-adrenergic and

Abbreviations used: AVP, [8-arginine]vasopressin; InsP₃, *myo*-inositol 1,4,5-trisphosphate; Acyl₂Gro, *sn*-1,2-diacylglycerols; PMA, 4β-phorbol 12β-myristate 13α-acetate; Na⁺/K⁺ pump, cation transport presumably mediated by Na⁺/K⁺-dependent ATPase.

Fig. 1. Time course of maximal and supramaximal concentrations of AVP to activate the hepatic Na⁺/K⁺ pump

(a) Na⁺/K⁺-pump activity was measured in 5 ml liver cell suspensions for 1 min periods between 0–1, 1–2, 2–3, 5–6 and 10–11 min after the addition of albumin/saline (○), 0.1 nM-AVP (◆) or 10 nM-AVP (●). The data are means from triplicate determinations from a single experiment which is representative of two such studies. (b) Ouabain-sensitive ⁸⁶Rb⁺ uptake was measured for 1 min intervals between 0–1, 1–2, 2–3, 5–6, 9–10, 10–11, 11–12, 12–13 and 20–21 min after the addition of 10 nM-AVP (●) or albumin/saline (○). At 10 min, an additional 10 nM-AVP was added to the cells represented by the ● symbol only. The data are means ± S.E.M. for triplicate determinations from a single experiment which is representative of two such studies.

fluoroaluminate effects (Lynch *et al.*, 1985a; Blackmore & Exton, 1986) are also temporally sustained, in contrast with the Na⁺/K⁺-pump response. This indicates that some secondary change at or very proximal to the Na⁺/K⁺ pump is responsible for the transient response to AVP and PMA.

In the present paper, ouabain-sensitive ⁸⁶Rb⁺ uptake was studied in isolated hepatocytes to determine why stimulation of the Na⁺/K⁺ pump by Ca²⁺-mobilizing hormones and tumour promoters is transient relative to other physiological responses. The rapid decrease in Na⁺/K⁺-ATPase-mediated cation-transport activity that occurs after stimulation does not appear to be secondary to decreases in intracellular Na⁺ or increases in receptor-mediated cytosolic Ca²⁺, but is rather due to a rapid heterologous desensitization of the response.

EXPERIMENTAL

Hepatocytes prepared from 180–220 g male rats were isolated as previously described (Blackmore & Exton, 1985), washed three times by centrifugation and equilibrated for 15 min before uptake studies in a buffer containing 120 mM-NaCl, 4.5 mM-KCl, 1.5 mM-CaCl₂, 1.2 mM-MgSO₄, 1.2 mM-NaH₂PO₄, 25 mM-NaHCO₃ [pH 7.4 maintained by constant gassing with O₂/CO₂ (19:1)], 11 mM-glucose, 5 mM-glutamic acid and 1.5% (w/v) gelatin.

Na⁺/K⁺-pump activity was evaluated by measuring ouabain-sensitive ⁸⁶Rb⁺ uptake during 1 or 5 min intervals as previously described (Lynch *et al.*, 1986). None of the agents employed in the present study had any significant effect on the ouabain-insensitive portion of the ⁸⁶Rb⁺ uptake, which represented approx. 10–15%

of the total uptake in 5 min measurements and 15–20% in 1 min measurements. In liver cells, monensin causes an increase in cellular Na⁺ (Hughes *et al.*, 1980) and a decrease in cellular potassium (P. F. Blackmore & J. H. Exton, unpublished work) as measured by atomic-absorption spectrophotometry. The increase in Na⁺ is due to monensin's action as a Na⁺ ionophore, and the decrease in K⁺ is probably secondary to stimulation of the Na⁺ pump. In ⁸⁶Rb⁺-uptake studies, monensin, as well as the other compounds used in this study, had no significant effect on the ouabain-insensitive portion (i.e. non-Na⁺/K⁺-pump mediated) of the ⁸⁶Rb⁺ uptake. In other words, monensin is not a ⁸⁶Rb⁺ or K⁺ ionophore.

Acyl₂Gro contents were measured with a differential-refractometer detector after h.p.l.c. of neutral-lipid extracts from hepatocytes as described by Bocckino *et al.* (1985). Similar results (not shown) were obtained by t.l.c. followed by charring or Coomassie Blue staining of lipids.

RESULTS

Relationship between AVP-mediated changes in Acyl₂Gro, Ca²⁺ and Na⁺-pump activity

As demonstrated for AVP in Fig. 1, the Na⁺/K⁺-pump stimulation by Ca²⁺-mobilizing hormones reached a peak at 1–2 min and returned to or approached basal values after 4–10 min (Burgess *et al.*, 1981; Berthon *et al.*, 1983, 1985; Lynch *et al.*, 1986). In contrast, the cellular contents of Acyl₂Gro, the proposed second messenger for Na⁺/K⁺-pump stimulation, increased during 10 min (Bocckino *et al.*, 1985). It has been proposed that the decreases in Na⁺/K⁺-pump activity

Fig. 2. Effect of prolonged stimulation by a supramaximal concentration of AVP on the ability of forskolin, 8-*p*-chlorophenylthio cyclic AMP and glucagon to activate the Na⁺/K⁺ pump

Cells were incubated for 15 min with 10 nM-AVP (right bars) or with saline (left bars). Na⁺/K⁺-pump activity was then measured in these cells in response to albumin/saline (5 μl; □), forskolin (100 μM; ▨), 8-*p*-chlorophenylthio cyclic AMP (1 μM; ▧) or glucagon (0.01 μM; ▤). The data are means ± S.E.M. from triplicate determinations from a single experiment, which is representative of two such studies.

that occur after stimulation by Ca²⁺-mobilizing agents are a consequence of increases in intracellular Ca²⁺ (Capiod *et al.*, 1982; Berthon *et al.*, 1983, 1985). To test this hypothesis, hepatocytes were incubated with the Ca²⁺-chelator EGTA for 15 min before the measurement of Na⁺/K⁺-pump activity. This Ca²⁺-chelation treatment, the effectiveness of which has previously been shown (Lynch *et al.*, 1986; Blackmore *et al.*, 1986), did not significantly effect the time course (results not shown) or concentration-response (Lynch *et al.*, 1986) of AVP-mediated changes in Na⁺/K⁺-pump activity.

Effects of continued exposure to AVP

We observed that stimulation of the Na⁺/K⁺ pump was more transient with higher concentrations of AVP than with lower concentrations (Fig. 1a). For example, 10 nM-AVP (Fig. 1b) stimulated the Na⁺/K⁺ pump by 60–150% in different experiments. This stimulation decreased to 0–20% over 4–10 min (Fig. 1). In contrast, a 100-fold lower concentration of AVP (0.1 nM) continued to stimulate the pump for 10–11 min, and decreased by only 20% compared with 0–1 min (Fig. 1a). Fig. 1(b) shows that, 10 min after exposure to a supramaximal concentration of AVP (10 nM; see Lynch

et al., 1986), the addition of a second bolus of 10 nM-AVP (i.e. final concn. = 20 nM) failed to increase the pump activity. These findings indicate that tachyphalaxis or desensitization of this response occurs as a consequence of exposure to a high concentration of AVP.

Fig. 2 shows that, when liver cells were exposed to a desensitizing concentration of AVP for 10 min, the Na⁺/K⁺ pump was still responsive to cyclic-AMP-mediated agonists such as glucagon, 8-*p*-chlorophenylthio cyclic AMP and forskolin. The data of Fig. 2 suggest that there is no change in the Na⁺ pump itself in AVP-desensitized cells (since it is still capable of responding to increases in cyclic AMP).

The effect of an initial 15 min incubation with AVP on the subsequent response to PMA is depicted in Fig. 3(a). This panel shows that, after a 15 min incubation in the presence of 10 nM-AVP, further addition of AVP (black bars) or PMA (cross-hatched bars) was without effect on Na⁺/K⁺-pump activity, although both were effective when added after a 15 min incubation with saline.

Effects of continued exposure to angiotensin II, adrenaline and PMA

Previous studies have shown that exposure of liver cells to high concentrations of angiotensin (100 nM), adrenaline (1 μM) and PMA (2 μM) results in a stimulation of the Na⁺/K⁺ pump which is similar to that observed with AVP in magnitude and time course (Burgess *et al.*, 1981; Radominska-Pyrek *et al.*, 1982; Berthon *et al.*, 1983, 1985; Lynch *et al.*, 1986). The protocol of Fig. 3(a) was employed in order to determine if the decreases in Na⁺/K⁺-pump activity which occurred after stimulation by AVP, angiotensin II, adrenaline and PMA were also due to desensitization. Pretreatment of liver cell suspensions for 15 min with angiotensin II, adrenaline or PMA attenuated subsequent responses to AVP and PMA (Figs. 3b–3d), but not to monensin (Table 1). These results support the hypothesis that a heterologous desensitization of the Na⁺/K⁺-pump response occurs as a result of exposure to Ca²⁺-mobilizing hormones and PMA.

Effects of continued exposure to glucagon and monensin

Fig. 3(e) shows the changes in Na⁺/K⁺-pump activity in liver cells exposed to glucagon. In contrast with AVP, high concentrations of glucagon caused a continuing stimulation of the pump (results not shown), in agreement with previous studies (Ihlenfeldt, 1981; Fehlmann & Freychet, 1981). In Fig. 3(e), the protocol from Fig. 3(a) was employed to test the effects of PMA and AVP in cells previously incubated with glucagon. After incubation for 15 min with glucagon, Na⁺/K⁺-pump activity remained elevated, and neither AVP nor PMA produced a further stimulation. In contrast, as reported by Ihlenfeldt (1981), increasing the intracellular Na⁺ with monensin stimulated Na⁺/K⁺-pump activity in both control and glucagon-stimulated cells (Table 1).

Stimulation of the Na⁺/K⁺ pump by monensin reached a maximum over 2–6 min in different experiments. Subsequently, the extent of stimulation decreased slowly (Fig. 4). Addition of a second bolus of monensin re-stimulated the Na⁺/K⁺ pump once the activity had been allowed to decrease over a 15 min period (Table 1). Thus the decrease in Na⁺/K⁺-pump activity with monensin does not appear to be due to desensitization, but rather to inactivation of the ionophore. In agreement with this

Fig. 3. Effect of prolonged exposure to various agents on subsequent responses to AVP and PMA

At 15 min before the addition of $^{86}\text{RbCl}$ and measurement of Na^+/K^+ -pump activity, 5 ml samples of cells were incubated with albumin/saline or dimethyl sulphoxide solvent (5 μl ; all panels, left three bars), 10 nM-AVP (panel *a*, right three bars), 100 nM-angiotensin II (panel *b*, right three bars), 1 μM -adrenaline (panel *c*, right three bars), 2 μM -PMA (panel *d*, right three bars), 10 nM-glucagon (panel *e*, right three bars) or 7.5 μM -monensin (panel *f*, right three bars). During this 15 min period Na^+/K^+ -pump activity remains relatively constant in response to albumin/saline and dimethyl sulphoxide, but increases in response to AVP, angiotensin II, adrenaline, PMA, glucagon and monensin. With the exception of glucagon, the increases are followed by rapid decreases in activity (see the text). Na^+/K^+ -pump activity was measured after these 15 min preincubations in the absence (□) or presence of 10 nM-AVP (■) or 2 μM -PMA (▤). Since each panel represents a different experiment, control responses (left bars) are shown for each experiment. Bars represent the averages from triplicate determinations, and error bars indicate the S.E.M.

idea, AVP and PMA were capable of stimulating Na^+/K^+ -pump activity to the same extent in control and monensin-treated hepatocytes (Fig. 3*f*).

The activity of the Na^+/K^+ pump in both control and hormone-stimulated liver cells may be limited by the

internal Na^+ concentration (Ihlenfeldt, 1981). Since hormone treatment decreases intracellular Na^+ , it is possible that this might explain the apparent desensitization caused by AVP. In order to test this hypothesis, the time course of AVP stimulation of the Na^+/K^+ pump

Table 1. Effect of prolonged stimulation by various agents on the ability of monensin to activate the Na⁺/K⁺ pump

The protocol used in Fig. 3 (left panel) was employed to test the effect of prolonged incubation with various agents on monensin stimulation of the Na⁺/K⁺ pump. Hepatocyte suspensions (5 ml) were exposed to the indicated agent 15 min before the measurement of Na⁺/K⁺-pump activity. The data are means $\pm$ S.E.M. of triplicate determinations and are representative of two such studies.

Preincubation condition	Na ⁺ /K ⁺ -pump activity (nmol of K ⁺ /5 min per mg dry wt.)	
	No monensin	+ 7.5 μ M-Monensin
Albumin/saline (5 μ l)	14.6 $\pm$ 0.6	30.0 $\pm$ 1.1
Dimethyl sulphoxide (5 μ l)	14.2 $\pm$ 2.0	25.4 $\pm$ 1.6
AVP (10 nM)	14.1 $\pm$ 0.9	29.4 $\pm$ 0.8
Angiotensin II (10 nM)	18.0 $\pm$ 1.8	32.5 $\pm$ 0.9
Adrenaline (1 μ M)	20.0 $\pm$ 0.8	32.1 $\pm$ 1.0
PMA (2 μ M)	19.9 $\pm$ 0.4	30.1 $\pm$ 1.1
Glucagon (10 nM)	24.3 $\pm$ 0.5	37.0 $\pm$ 2.0
Monensin (7.5 μ M)	17.1 $\pm$ 0.4	25.7 $\pm$ 1.0

was measured in the absence or presence of monensin (which increases intracellular Na⁺). This manipulation did not alter the time course of AVP-mediated Na⁺/K⁺-pump stimulation (Fig. 4), suggesting that the apparent desensitization caused by Ca²⁺-mobilizing hormones and PMA is not secondary to changes in intracellular Na⁺.

DISCUSSION

Relationship between AVP-mediated changes in cellular Ca²⁺ and Na⁺, and the decrease in Na⁺-pump activity that occurs after stimulation

As demonstrated in Fig. 1 for AVP, transient stimulation of Na⁺/K⁺-pump activity and net K⁺ uptake occurs in rat hepatocytes in response to Ca²⁺-mobilizing hormones such as adrenaline, angiotensin II and AVP (Burgess *et al.*, 1981; Radomska-Pyrek *et al.*, 1982; Capiod *et al.*, 1982; Berthon *et al.*, 1983, 1985; Lynch *et al.*, 1986).

We previously presented evidence that suggests that Ca²⁺-mobilizing hormones activate the Na⁺/K⁺-pump through an InsP₃-independent mechanism which relies on the ability of these agents to increase cellular Acyl₂Gro (Lynch *et al.*, 1986). This theory predicts that the resulting activation of the Na⁺/K⁺ pump is due either to Acyl₂Gro-dependent activation of protein kinase C (Nishizuka, 1984) or to physicochemical changes in the plasma membrane brought about by the increases in Acyl₂Gro (which are large relative to total phosphatidylinositol 4,5-bisphosphate) occurring in response to these agents (Bocckino *et al.*, 1985). The fact that PMA also activates the pump does not discriminate between these two possible mechanisms, since the phorbol ester also produces large increases in Acyl₂Gro in hepatocytes (Bocckino *et al.*, 1985). Although the Acyl₂Gro mechanism of activation of the Na⁺/K⁺ pump is supported by some experimental evidence, it does not account for the transient nature of the stimulation, since Acyl₂Gro

Fig. 4. Effect of increasing intracellular Na⁺ by monensin on the activity of the Na⁺/K⁺ pump in response to a supra-maximal concentration of AVP

Na⁺/K⁺ pump activity was measured for 1 min periods as described in Fig. 1 legend. Albumin/saline (○), 10 nM-AVP (●), 20 μ M-monensin (□) or a combination of AVP and monensin (■) was added at zero time. Each point represents the average of triplicate determinations.

continues to increase with time in response to AVP exposure (Bocckino *et al.*, 1985). However, the finding that the time course with which PMA alters Na⁺/K⁺-pump activity is similar to that of the Ca²⁺-mobilizing hormones (Lynch *et al.*, 1986) supports the mechanism and suggests that some secondary change is involved in the decrease.

A possible explanation for the decrease in Na⁺/K⁺-pump activity that occurs after stimulation is that the increase in cytosolic Ca²⁺ induced by Ca²⁺-mobilizing hormones is inhibitory to the Na⁺/K⁺ pump (Capiod *et al.*, 1982; Berthon *et al.*, 1983, 1985; cf. Lynch *et al.*, 1986). In order to test this hypothesis we performed time-course studies in cells pretreated with EGTA (results not shown). We have previously characterized the Ca²⁺-chelating effectiveness of this EGTA pretreatment in liver cells (Lynch *et al.*, 1986; Blackmore *et al.*, 1986). The finding that transient stimulation of the Na⁺/K⁺ pump in response to AVP still occurs in cells depleted of Ca²⁺ argues against the possibility that hormone-mediated increases in cytosolic Ca²⁺ inhibit stimulation of the pump. Additional support for this hypothesis comes from the finding that PMA does not alter cytosolic Ca²⁺ or InsP₃ (Lynch *et al.*, 1985a), but has a similar action to AVP on the pump (Lynch *et al.*, 1986).

Since the intracellular Na⁺ concentration is an important controller of Na⁺/K⁺-pump activity, it seemed possible that the decrease in Na⁺ concentration which occurs in liver cells after exposure to Ca²⁺-mobilizing hormones (Berthon *et al.*, 1983, 1985) might subsequently prevent further stimulation of the Na⁺/K⁺ pump. To test

this hypothesis, intracellular Na^+ concentrations were artificially elevated with the Na^+ ionophore monensin (Hughes *et al.*, 1980). This gave the expected increase in Na^+/K^+ -pump activity (Figs. 3f and 4, and Table 1). However, as shown in Fig. 4, this did not alter the transient nature of the response to AVP. This finding argues against the possibility that hormone-mediated decreases in cellular Na^+ concentration inhibit further stimulation of the Na^+/K^+ pump in cells exposed to AVP. If this were a possibility, it would also be expected that glucagon would alter Na^+/K^+ -pump activity in a biphasic manner. This is not the case. Glucagon responses remain elevated for up to 20 min (Fig. 3e, and results not shown), and indeed for hours (Ihlenfeldt, 1981; Fehlmann & Freychet, 1981).

Evidence that exposure of liver cells to AVP causes heterologous desensitization of the Na^+ -pump response

Three lines of evidence support the hypothesis that the decrease in Na^+/K^+ -pump activity that occurs after stimulation by AVP is due to a heterologous desensitization. (1) Changes in cytosolic and cellular Ca^{2+} , phosphorylase activity and Na^+/K^+ -pump activity are maximal with 0.1 nM-AVP. However, owing to a large receptor reserve, the parameters that more closely parallel receptor occupancy, such as radioligand binding and second-messenger production (e.g. Acyl_2Gro and InsP_3), are not maximally affected until the AVP concentration reaches 10 nM (Lynch *et al.*, 1985a,b, 1986). Desensitization phenomena are typically associated with exposure of cells to higher rather than to lower concentrations of hormones. Consistent with this hypothesis, the decrease in Na^+/K^+ -pump activity that followed stimulation was more rapid and extensive in cells exposed to a higher (10 nM) rather than a lower (0.1 nM) concentration of AVP (Fig. 1a).

(2) Exposure to high concentrations of AVP resulted in an increase, followed by a decrease, in Na^+/K^+ -pump activity. This prevented subsequent responses to AVP and PMA (Figs. 1b and 3a), but had no significant effect on subsequent responses to monensin or cyclic-AMP-mediated agonists (Fig. 2 and Table 1). These findings further support our conclusion (Lynch *et al.*, 1986) that PMA and AVP stimulate the Na^+/K^+ pump through a common mechanism and indicate that this mechanism is distinct from that utilized by cyclic-AMP-mediated agonists.

(3) Exposure of liver cells to other Ca^{2+} -mobilizing hormones or PMA also attenuated subsequent responses to AVP and PMA (Figs. 3b–3d), but not to monensin (Table 1).

Site of the desensitization caused by Ca^{2+} -mobilizing hormones and PMA

In liver cells, both PMA and Ca^{2+} -mobilizing hormones stimulate the Na^+/K^+ pump. However, in contrast with the Ca^{2+} -mobilizing hormones, PMA does not stimulate phosphatidylinositol 4,5-bisphosphate hydrolysis or activate phosphorylase (Lynch *et al.*, 1985a). This finding, and the observation that Acyl_2Gro formation (Bocckino *et al.*, 1985), as well as other physiological events, are sustained in response to Ca^{2+} -mobilizing hormones lead us to believe that the desensitization effect of PMA and Ca^{2+} -mobilizing hormones on the Na^+/K^+ pump occurs distal to phosphatidylinositol 4,5-bisphosphate hydrolysis and Acyl_2Gro formation.

Assuming that protein kinase C is involved, the next possible site of action is protein kinase C itself. However, several effects of PMA that are believed to be mediated by protein kinase C in liver cells are sustained beyond 10 min, e.g. the inactivation of glycogen synthase (Roach & Goldman, 1983; Blackmore *et al.*, 1986), the activation of acetyl-CoA carboxylase (Vaartjes *et al.*, 1987), and the inhibition of α_1 -adrenergic responses (Corvera *et al.*, 1986; Lynch *et al.*, 1985a). Thus it seems unlikely that desensitization of protein kinase C activity is responsible for the transient nature of the Na^+/K^+ -pump stimulation seen in response to Ca^{2+} -mobilizing hormones and PMA.

We conclude therefore that the apparent desensitizing effect of these agents on the Na^+/K^+ -pump is either distal to or does not involve protein kinase C. As we have pointed out previously (Lynch *et al.*, 1986; Bocckino *et al.*, 1985), the use of PMA as a tool to mimic the effect of Acyl_2Gro on protein kinase C is complicated by the ability of PMA to increase Acyl_2Gro without increasing InsP_3 (Bocckino *et al.*, 1985). Thus we cannot conclude unequivocally that the actions of PMA and Ca^{2+} -mobilizing hormones on the desensitization of the Na^+/K^+ pump are due to activation of protein kinase C, since they could be due to physicochemical changes in the plasma membrane resulting from the generation of Acyl_2Gro . Na^+/K^+ -ATPase has been isolated from rat liver by immunoaffinity chromatography (Hubert *et al.*, 1986). This should permit a demonstration of possible changes in the phosphorylation state of the α - and β -subunits of the enzyme during activation or desensitization of the pump by hormones.

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REFERENCES

- Berthon, B., Burgess, G. M., Capiod, T., Claret, M. & Poggioli, J. (1983) *J. Physiol. (London)* **341**, 25–40.
- Berthon, B., Capiod, T. & Claret, M. (1985) *Br. J. Pharmacol.* **86**, 151–161.
- Blackmore, P. F. & Exton, J. H. (1985) *Methods Enzymol.* **109**, 550–558.
- Blackmore, P. F. & Exton, J. H. (1986) *J. Biol. Chem.* **261**, 11056–11063.
- Blackmore, P. F., Strickland, W. G., Bocckino, S. B. & Exton, J. H. (1986) *Biochem. J.* **237**, 235–242.
- Blackmore, P. F., Lynch, C. J., Uhing, R. J., Fitzgerald, T., Bocckino, S. B. & Exton, J. H. (1987) in *Regulation of Cellular Calcium Homeostasis* (Pfeiffer, D. R., McMillan-Wood, J., Fiskum, G. & Little, S., eds.), Plenum Publishing Corp., New York, in the press.
- Bocckino, S. B., Blackmore, P. F. & Exton, J. H. (1985) *J. Biol. Chem.* **260**, 14201–14207.
- Burgess, G. M., Claret, M. & Jenkinson, D. H. (1981) *J. Physiol. (London)* **305**, 267–277.
- Capiod, T., Berthon, B., Poggioli, J., Burgess, G. M. & Claret, M. (1982) *FEBS Lett.* **141**, 49–52.
- Charest, R., Prpic, V., Exton, J. H. & Blackmore, P. F. (1985) *Biochem. J.* **227**, 79–90.
- Corvera, S., Schwarz, K. R., Graham, R. M. & Garcia-Sainz, J. A. (1986) *J. Biol. Chem.* **261**, 520–526.
- Fehlmann, M. & Freychet, P. (1981) *J. Biol. Chem.* **256**, 7449–7453.

- Fehlmann, M., Canivet, B. & Freychet, P. (1981) *Biochem. Biophys. Res. Commun.* **100**, 254–260
- Greene, D. A. & Lattimer, S. A. (1986) *Diabetes* **35**, 242–245
- Hubert, J. J., Schenk, D. B., Skelly, H. & Leffert, H. L. (1986) *Biochemistry* **25**, 4156–4163
- Hughes, B. P., Blackmore, P. F. & Exton, J. H. (1980) *FEBS Lett.* **121**, 260–264
- Ihlenfeldt, M. J. (1981) *J. Biol. Chem.* **256**, 2213–2218
- Lynch, C. J., Guarino, J. J., Deth, R. C. & Steer, M. L. (1984) *Am. J. Physiol.* **246**, E344–E349
- Lynch, C. J., Charest, R., Bocckino, S. B., Exton, J. H. & Blackmore, P. F. (1985a) *J. Biol. Chem.* **260**, 2844–2851
- Lynch, C. J., Blackmore, P. F., Charest, R. & Exton, J. H. (1985b) *Mol. Pharmacol.* **29**, 196–203
- Lynch, C. J., Wilson, P. B., Blackmore, P. F. & Exton, J. H. (1986) *J. Biol. Chem.* **261**, 14551–14556
- Nishizuka, Y. (1984) *Nature (London)* **308**, 693–698
- Pouyssegur, J. (1985) *Trends Biochem. Sci.* **10**, 453–455
- Radomska-Pyrek, A., Kraus-Friedman, N., Lester, R., Little, J. & Denkins, D. (1982) *FEBS Lett.* **141**, 56–59
- Roach, P. J. & Goldman, M. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 7170–7172
- Vaartjes, W. J., deHaas, C. G. M., Geelen, M. J. H. & Bijleveld, C. (1987) *Biochem. Biophys. Res. Commun.* **142**, 135–140

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