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Stimulation of hepatic inositol 1,4,5-trisphosphate kinase activity by Ca^{2+}-dependent and -independent mechanisms

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A cytosolic fraction derived from rat hepatocytes was used to investigate the regulation of inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] kinase, the enzyme which converts Ins(1,4,5)P_3 to inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P_4]. The activity was doubled by raising the free Ca^{2+} concentration of the assay medium from 0.1 μM to 1.0 μM. A 5 min preincubation of the hepatocytes with 100 μM-dibutyryl cyclic AMP (db.cAMP) plus 100 nM-tetradecanoylphorbol acetate (TPA) resulted in a 40% increase in Ins(1,4,5)P_3 kinase activity when subsequently assayed at 0.1 μM-Ca^{2+}. This effect was smaller at $[Ca^{2+}] > 0.5$ μM, and absent at 1.0 μM-Ca^{2+}. Similar results were obtained after preincubation with 100 μM-db.cAMP plus 300 nM-vasopressin (20% increase at 0.1 μM-Ca^{2+}; no effect at 1.0 μM-Ca^{2+}). Preincubation with vasopressin, db.cAMP or TPA alone did not alter Ins(1,4,5)P_3 kinase activity. It is proposed that these results, together with recent evidence implicating Ins(1,3,4,5)P_4 in the control of Ca^{2+} influx, could be relevant to earlier findings that hepatic Ca^{2+} uptake is synergistically stimulated by cyclic AMP analogues and vasopressin.

INTRODUCTION

A rise in the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) is a key event associated with the occupation of many classes of external receptor. The Ca^{2+} is usually derived both from intracellular stores and from the extracellular space [1–5]. Although the molecular details remain unclear, recent evidence suggests that both phases of this Ca^{2+} response are controlled by second messengers derived ultimately from PtdInsP_2. The hydrolysis of this membrane phospholipid by phospholipase C is enhanced following agonist binding and results in the formation of diacylglycerol an activator of protein kinase C, and Ins(1,4,5)P_3, the molecule responsible for release of Ca^{2+} from intracellular stores [6,7]. More recently, it has been proposed that Ins(1,3,4,5)P_4 [8], which is produced from Ins(1,4,5)P_3 by the enzyme Ins(1,4,5)P_3 kinase [9], might serve to promote Ca^{2+} influx from the extracellular space [10–12]. According to this hypothesis, regulation of Ins(1,4,5)P_3 kinase activity should therefore influence Ca^{2+} influx; at present the best-documented regulator of this enzyme is Ca^{2+} itself [13,14].

In liver, agonists such as vasopressin, which hydrolyse PtdInsP_2 [15–17], also increase $[Ca^{2+}]_i$ [17–19]. This is associated with enhanced Ca^{2+} influx [1–3,20–22], an increased rate of Ca^{2+} cycling across the plasma membrane, and a small gain in total intracellular Ca^{2+} [1–3]. Although glucagon itself also raises $[Ca^{2+}]_i$ [19,22,23], apparently via a stimulation of PtdInsP_2 hydrolysis by cyclic AMP [23,24; but see also 25,26], this is not accompanied by any major change in total intracellular Ca^{2+} [27]. However, when present together, glucagon and Ca^{2+}-mobilizing agonists have been shown to stimulate markedly hepatic Ca^{2+} accumulation [27], net $^{45}Ca^{2+}$ uptake [28] and net uptake of Ca^{2+} from the extracellular medium [29,30]. Measurements of unidirectional $^{45}Ca^{2+}$ influx have confirmed that the underlying mechanism is a synergistic activation of inward Ca^{2+} transport [31]. The mechanism by which cyclic AMP exerts this effect is unclear. One hypothesis is that cyclic AMP might stabilize the Ca^{2+} channel once it has been opened by some other signal [31]. However, no direct evidence for this hypothesis has been forthcoming. Another postulated mechanism, that the primary effect of cyclic AMP is at the level of mitochondrial Ca^{2+} influx [27], appears both indirect and difficult to reconcile with the currently accepted view that non-mitochondrial stores normally play the more important role in intracellular Ca^{2+} homeostasis [2–5]. A third suggestion is that cyclic AMP acts by increasing the concentration of PtdInsP_2 [32]; yet the potentiation of Ca^{2+} influx is seen with agonists which are already potent generators of Ins(1,4,5)P_3 and diacylglycerol. Moreover, it is by no means certain that increased flux through phospholipase C would in itself alter the balance between net Ca^{2+} influx and efflux which, as shown in dynamic studies [29,30], seems to occur in the presence of cyclic AMP. The aim of the present study was to investigate whether this alteration could be accounted for by a stimulation of Ins(1,4,5)P_3 kinase activity.

EXPERIMENTAL PROCEDURES

Hepatocytes were isolated by collagenase perfusion of rat livers as recently described [33]. All experimental incubations were performed in a shaking water bath at 37 °C under an atmosphere of O_2/CO_2 (19:1) and using a cellular concentration of 50 mg/ml. The basic incubation

Abbreviations used: InsP, inositol monophosphate; InsP_2 inositol bisphosphate; InsP_3 inositol trisphospate; InsP_4, inositol tetrakisphosphate (with positional determinants of the phosphate groups as indicated); PtdInsP_2, phosphatidylinositol 4,5-bisphosphate; TPA, tetradecanoylphorbol acetate; $[Ca^{2+}]_i$, cytosolic free Ca^{2+} concentration; db.cAMP dibutyryl cyclic AMP.

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medium was Krebs–Henseleit bicarbonate buffer (pH 7.4) containing physiological amino acids, 5 mM-glucose and 0.5% defatted bovine serum albumin. All experimental additions were present for 5 min, following an initial equilibration of the cells for 10 min at 37 °C. When present, tetradecanoylphorbol acetate (TPA) was added 15–30 s after the other agents. For enzyme studies, a 4 ml aliquot of the cell suspension (from a total of 5 ml) was withdrawn after incubation and immediately centrifuged (30 s at 100 ***g***). Each cell pellet was reconstituted to 1 ml with a hypo-osmolar medium consisting of 10 mM-KCl/10 mM-Hepes (pH 7.0). After 10 min at 4 °C, iso-osmolarity was restored by the addition of saturated KCl, and the cells homogenized by 20 strokes in a loose-fitting (Teflon/glass) homogenizer. Unbroken cells were removed by centrifugation (10 min at 200 ***g***) and a 175 μl aliquot of the supernatant further centrifuged for 90 min at 8 °C (90000 ***g***) in a Beckman Airfuge.

Enzyme assays were performed at 37 °C in a volume of 50 μl and were started by the addition of 5 μl of the final supernatant. Reaction rates were linear for at least 5 min. The assay medium for Ins(1,4,5)P_3 kinase measurements was exactly as described previously [13,14], except that the concentration of Ins(1,4,5)P_3 was 100 nM. The lower concentration was necessary to minimize the dephosphorylation of Ins(1,4,5)P_3 by cytosolic Ins-(1,4,5)P_3 phosphomonoesterase activity, which is relatively pronounced in hepatoycytes [34]. Under these conditions, the mean ratio of $InsP_4$ to $InsP_2$ formed at 0.1 μM-Ca^{2+} was $2.7 \pm 0.2:1$ ($n = 7$), with a correspondingly greater ratio at higher Ca^{2+} concentrations. Ins-(1,4,5)P_3 phosphomonoesterase activity was measured as the total InsP plus $InsP_2$ formed from [2-^{3}H]Ins(1,4,5)P_3 [13]. Assays were terminated after 5 min by the addition of 10% (w/v) trichloroacetic acid and the water-soluble extract was washed twice with 5 ml of diethyl ether. The tritiated inositol phosphates were separated by anion-exchange chromatography on 0.6 ml Dowex AG 1 × 8 columns. These were sequentially eluted with 0.1 M-formic acid washes containing increasing strengths of ammonium formate: 2 × 5 ml of 0.4 M for $InsP_2$ plus InsP (or 2 × 3 ml for assays of phosphomonoesterase activity); 3 × 5 ml of 0.8 M for $InsP_3$; and 1 × 5 ml of 1.5 M for $InsP_4$. The eluted fractions were mixed with 10 ml of xylene/Triton X-100 (2:1) scintillant containing 0.8% (w/v) 2,5-diphenyloxazole and 0.02% (w/v) 1,4-bis-(5-phenyloxazol-2-yl) benzene and quantified by liquid-scintillation spectrometry. Cross-contamination of [^{3}H]-Ins(1,4,5)P_3 in the other fractions was corrected for but never amounted to more than 10% of the total $InsP_4$ c.p.m. or more than 5% of the total $InsP_2$.

For measurements of Ca^{2+} influx, hepatocytes were incubated exactly as described above. After 10 min preincubation, EGTA (1.3 mM final) was added, followed after 1 min by $^{45}Ca^{2+}$ (0.63 μCi) and other additions as indicated. After a further 5 min 100 μl aliquots were removed and placed immediately on to Millipore membrane filters (5 μm pore size) where non-cellular material was removed by filtration. The hepatocytes were then washed with approx. 4 ml of a cold solution containing 135 mM-NaCl/12 mM-Hepes/2 mM-EGTA (pH 7.4). The filters were dissolved in 10 ml of scintillant consisting of toluene/2-methoxy-ethanol (2:1, v/v) containing 6% (w/v) 5-(biphenyl-4-yl)-2-(4-*t*-butylphenyl)-1-oxa-3,4-diazole and 1% (v/v) Triton X-100. Radioactivity was determined by liquid-scintillation spectrometry. Results are presented as means ± S.E.M. and statistical analysis was by Student's *t* test for unpaired data.

The sources of materials were as described previously [13,14,29,30,33], except for Dowex AG 1 × 8 200–400 mesh, which was from BioRad Laboratories Pty Ltd (Australia). Ins(1,4,5)P_3 was a generous gift from Dr. R. F. Irvine, Cambridge, U.K. Other chemicals were of analytical grade.

RESULTS

Before investigating other potential regulatory factors it was important to establish the effect of Ca^{2+} on hepatic Ins(1,4,5)P_3 kinase activity. The results presented in Fig. 1 show that raising the Ca^{2+} concentration in the assay medium from 0.1 to 1.0 μM more than doubled the Ins(1,4,5)P_3 kinase activity present in a cytosolic fraction derived from hepatocytes. When calculated from four independent experiments, the half-maximal concentration of Ca^{2+} required for this effect was 0.38 ± 0.04 μM. A possible role for protein kinase A (the target enzyme for cAMP) was next investigated. However, when hepatocytes were incubated in the presence of glucagon, or db.cAMP directly, there was no alteration in the activity of Ins(1,4,5)P_3 kinase as subsequently assayed in the high-speed supernatant (Table 1). This was the case at both 0.1 and 1.0 μM-Ca^{2+}.

In the next series of experiments db.cAMP was used in conjunction with the receptor agonist vasopressin, since it is under these conditions that cyclic AMP most markedly stimulates Ca^{2+} influx. Incubation of the

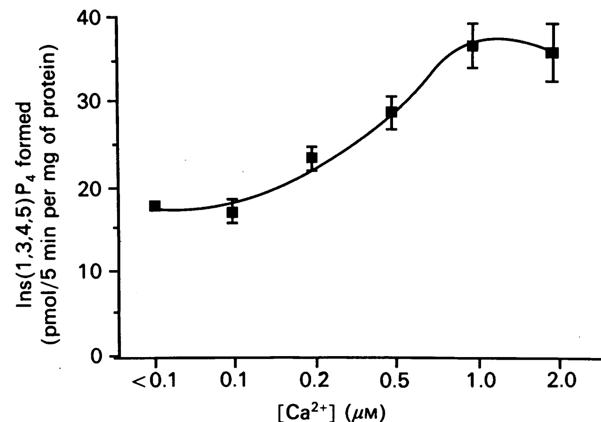

Fig. 1. Ca^{2+} dependence of hepatic Ins(1,4,5)P_3 kinase activity

A cytosolic fraction was obtained from hepatocytes following homogenization and high-speed centrifugation. Assays were performed at 37 °C for 5 min in 50 μl of a medium (pH 7.0) containing 110 mM-KCl, 10 mM-NaCl, 10 mM-Hepes, 7 mM-$MgSO_4$ 5 mM-cysteine, 5 mM-K-ATP, 5 mM-2,3-bisphosphoglycerate, 5 mM-EGTA, 100 nM-Ins-(1,4,5)P_3 and [2-^{3}H]Ins(1,4,5)P_3 (50 nCi/ml). $CaCl_2$ (0.5 mM) was added to give the range of Ca^{2+} concentrations indicated. The assay was started by addition of 5 μl of cytosolic extract and terminated with 0.5 ml of 10% trichloroacetic acid (w/v). After extraction with diethyl ether, inositol phosphates in the aqueous fraction were separated by anion-exchange chromatography and quantified by liquid-scintillation spectrometry. Each point represents the mean of six independent observations. Further details are given under 'Experimental procedures'.

Table 1. Effect of pretreatment with glucagon or db.cAMP on hepatic Ins(1,4,5)P_3 kinase activity

Hepatocytes were pretreated for 5 min in Krebs–Henseleit medium with the additions as described. Assays were performed as outlined in the legend to Fig. 1 at either 0.1 or 1.0 μM-Ca^{2+}. Results are the means of nine independent observations.

Condition	Ca^{2+} (μM)...	Ins(1,3,4,5)P_4 formed (pmol/5 min per mg of cell protein) 0.1	1.0
Control		15.0±1.0	25.9±1.3
Glucagon (10 nM)		15.0±1.4	26.8±2.1
db.cAMP (100 μM)		14.0±1.1	26.9±2.0

Table 2. Effect of pretreatment with db.cAMP and vasopressin on hepatic Ins(1,4,5)P_3 kinase activity

Hepatocytes were pretreated for 5 min with the additions as described. Assays were performed as outlined in the legend to Fig. 1 at either 0.1 or 1.0 μM-Ca^{2+}. Results are the means of nine independent observations. *$P < 0.05$ versus unpretreated control.

Condition	Ca^{2+} (μM)...	Ins(1,3,4,5)P_4 formed (pmol/5 min per mg of cell protein) 0.1	1.0
Control		13.3±0.8	24.8±0.9
db.cAMP (100 μM)		11.5±0.8	23.4±1.6
Vasopressin (300 nM)		14.9±0.7	23.9±1.0
Vasopressin (300 nM)+ db.cAMP (100 μM)		15.9±0.6*	26.0±1.6

hepatocytes with either vasopressin or db.cAMP alone had no affect on Ins(1,4,5)P_3 kinase activity (Table 2). However, in the presence of both agents there was a 20% increase in activity, as measured at 0.1 μM-Ca^{2+}; no change was seen at 1.0 μM-Ca^{2+}. This response was further investigated by the use of TPA, an exogenous activator of protein kinase C (Table 3). Incubation of the cells with db.cAMP or TPA alone did not alter Ins(1,4,5)P_3 kinase activity. However, in combination these agents evoked a 40% increase in Ins(1,3,4,5)P_4 production when subsequently assayed at 0.1 μM-Ca^{2+}. As was the case for vasopressin (Table 2), when TPA was used in conjunction with db.cAMP at 1.0 μM-Ca^{2+}, there was no further increase in the activity of Ins(1,4,5)P_3 kinase. This is shown in more detail in Fig. 2. The stimulatory effect of TPA plus db.cAMP is largely independent of that seen with Ca^{2+} alone, since it was still apparent at Ca^{2+} concentration below 0.1 μM, and since the two responses were never more than additive. This Ca^{2+}-independent effect was masked at Ca^{2+} concentrations > 0.5 μM.

As explained in the Experimental procedures section, the hepatic cytosolic fraction also contained some residual Ins(1,4,5)P_3 phosphomonoesterase activity, which

Table 3. Effect of pretreatment with db.cAMP and TPA on hepatic Ins(1,4,5)P_3 kinase activity

Hepatocytes were pretreated for 5 min with the additions as indicated. Assays were performed as outlined in the legend to Fig. 1 at either 0.1 or 1.0 μM-Ca^{2+}. Results are the mean of six to nine independent observations. *$P < 0.001$ versus unpretreated control.

Condition	n	Ca^{2+} (μM)...	Ins(1,3,4,5)P_4 formed (pmol/5 min per mg of cell protein) 0.1	1.0
Control	9		15.0±0.9	28.5±1.7
db.cAMP (100 μM)	6		16.8±3.1	32.1±4.6
TPA (100 nM)	6		17.9±2.2	31.5±4.5
db.cAMP (100 μM)+ TPA (100 nM)	9		20.9±1.1*	30.6±2.0

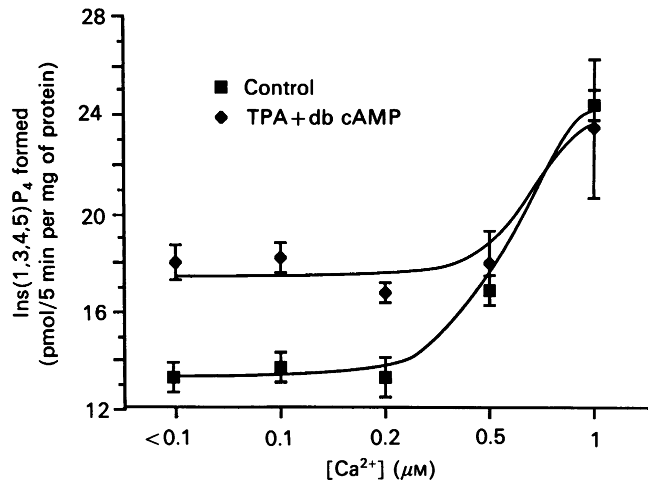

Fig. 2. Ca^{2+} dependence of hepatic Ins(1,4,5)P_3 kinase activity in the presence or absence of TPA plus db.cAMP

Hepatocytes were preincubated for 5 min in Krebs–Henseleit medium with either no further addition, or in the presence of TPA (100 nM) plus db.cAMP (100 μM). Assays were performed as described in the legend to Fig. 1 and each point represents the mean of six independent observations.

under the assay conditions employed, amounted to no more than 30% of the Ins(1,4,5)P_3 kinase activity. To test whether alterations in phosphomonoesterase could account for the changes described above, this enzyme was assayed under optimal conditions (Table 4). Neither raising the Ca^{2+} concentration from 0.1 to 1.0 μM, nor adding TPA and db.cAMP (either alone or in combination) altered the rate of dephosphorylation of Ins(1,4,5)-P_3. Therefore, the changes in Ins(1,3,4,5)P_4 previously described in response to these manipulations are best explained in terms of stimulated Ins(1,4,5)P_3 kinase activity.

Although the effects of vasopressin and db.cAMP on Ins(1,4,5)P_3 kinase are highly reminiscent of those previously described for Ca^{2+} influx, the finding that TPA could substitute for vasopressin raised a further possi-

Table 4. Effect of different pretreatment conditions and Ca^{2+} concentrations on hepatic Ins(1,4,5)P_3 phosphomonoesterase activity

Cells were incubated, homogenized and certrifuged as described in the legends to Fig. 1 and Table 1. Phosphomonoesterase activity was assayed for 5 min by addition of 5 μl of supernatant to 50 μl of a medium containing 110 mM-KCl, 10 mM-NaCl, 10 mM-LiCl, 10 mM-Hepes, 5 mM-EGTA, 2 mM-$MgSO_4$, 10 nM-Ins(1,4,5)P_3 and [2-^{3}H]-Ins(1,4,5)P_3 (50 nCi/ml); $CaCl_2$ was added to adjust the free Ca^{2+} concentration to either 0.1 or 1.0 μM. Results are the means of six independent observations.

Condition	Ca^{2+} (μM)...	Ins(1,4,5)P_3 dephosphorylated (pmoles/min per mg of protein) 0.1	1.0
Control		21.2 ± 1.8	20.2 ± 1.8
db.cAMP (100 μM)		20.7 ± 0.7	–
TPA (100 nM)		22.2 ± 1.3	–
db.cAMP (100 μM) + TPA (100 nM)		22.7 ± 1.9	–

bility for correlating the two responses. We therefore measured $^{45}Ca^{2+}$ uptake in the presence of EGTA to ensure that the peak increases in $[Ca^{2+}]_i$ due to db.cAMP would be short lived, and that the stimulation of Ins-(1,4,5)P_3 kinase due to Ca^{2+} would be kept to a minimum. It was thereby hoped to maximize any stimulatory effect due to the concomitant activation of protein kinases A and C. However, under these conditions Ca^{2+} influx in the presence of db.cAMP, TPA or db.cAMP plus TPA averaged 120 ± 12, 93 ± 9 and 107 ± 10 % respectively of the control value (n = 15). In a single experiment conducted in the absence of EGTA, db.cAMP-stimulated Ca^{2+} influx was inhibited in the presence of TPA (174 ± 4 versus 140 ± 11 % of control respectively; n = 3).

DISCUSSION

Although the liver has been previously shown to possess Ins(1,4,5)P_3 kinase [34], little is known about its regulation. The current study establishes that, as in other tissues [13,14,35–40], the activity of the hepatic enzyme is stimulated by a rise in Ca^{2+} within the physiological range. It was also enhanced following pretreatment of hepatocytes with vasopressin plus db.cAMP. The effect of vasopressin was mimicked by TPA, and was likewise seen only in the presence of db.cAMP and at a low Ca^{2+} concentration (0.1 μM). These results suggest that the Ca^{2+}-independent stimulation is mediated by a concomitant activation of protein kinases A and C. Such a mechanism may be relatively specific to the liver, since TPA alone augmented Ins(1,4,5)P_3 kinase activity in a clonal T-cell line [40], and a cyclic AMP analogue and TPA individually enhanced the production of Ins(1,3,4,5)P_4 [relative to Ins(1,4)P_2] when Ins(1,4,5)P_3 was added to permeabilized insulinoma cells [41].

The underlying aim of the present study was to investigate the possible links between the regulation of Ins(1,4,5)P_3 kinase activity and Ca^{2+} influx. The results will be considered in the light of three possibilities. The first is that cyclic AMP stimulates Ins(1,4,5)P_3 kinase by increasing Ca^{2+} influx (since the enzyme is Ca^{2+}-sensitive). However, this does not apply under the assay conditions employed, since pretreatment with vasopressin alone, which raises $[Ca^{2+}]_i$ [17,22,24], did not enhance Ins-(1,4,5)P_3 kinase activity when it was subsequently measured in the cytosolic extract. Therefore, the stimulation by Ca^{2+}, unlike that due to vasopressin plus db.cAMP, must be readily reversed during cell homogenization. This is consistent with recent evidence, obtained with non-hepatic tissues, that the effect of Ca^{2+} is mediated by a direct interaction between Ins(1,4,5)P_3 kinase and Ca^{2+}-calmodulin [14,37].

The second possibility is that hepatic Ca^{2+} influx and Ins (1,4,5)P_3 kinase activity are non-related events which are coincidentally stimulated under similar circumstances. This seems unlikely because of the very good correlation between the two responses: the most marked stimulations required both vasopressin plus db.cAMP, with little or no effect in the presence of either agent alone (Table 2 and refs. [27–31]). Moreover, dynamic studies have shown that the stimulation of the rate of net Ca^{2+} accumulation by cyclic AMP is most apparent some minutes after the addition of vasopressin [29,30]. This appears consistent with the current finding (Fig. 2) that the cyclic AMP-dependent activation of Ins(1,4,5)P_3 kinase is less pronounced at those Ca^{2+} concentrations likely to be obtained immediately after agonist stimulation [21]; the effect of cAMP on Ca^{2+} influx would be more significant once $[Ca^{2+}]_i$ had declined from its initial peak. However, it is apparent that this mechanism also functions at early times [31], raising the possibility that a residual Ca^{2+}-independent stimulation of Ins(1,4,5)P_3 kinase might be associated with the initial $[Ca^{2+}]_i$ peak. It should be noted in this context that even a very small stimulation of Ins(1,4,5)P_3 kinase activity might exert profound effects when coupled to a large rate of Ins-(1,4,5)P_3 generation. This is especially true given that a slight rise in Ins(1,3,4,5)P_4 might in turn inhibit the dephosphorylation of its precursor, and accordingly shift metabolic flux even further in favour of Ins(1,4,5)P_3 kinase [42,43].

This leaves the third possibility that regulation of hepatic Ins(1,4,5)P_3 kinase plays an important role in the control of Ca^{2+} influx. Such an interpretation is consistent with recent work showing that, in sea urchin eggs and lacrimal glands, certain physiological responses, which depend upon the influx of Ca^{2+} from the extracellular medium, can be activated in the presence of Ins(1,3,4,5)P_4 [10–12]. Inositol phosphates that are capable of releasing Ca^{2+} from intracellular stores are also needed for the full response, a requirement which is not mimicked by simply raising $[Ca^{2+}]_i$ with a Ca^{2+} ionophore. Although these results are difficult to interpret mechanistically, they do suggest that Ins(1,3,4,5)P_4 plays a role in the control of Ca^{2+} influx. This hypothesis is further strengthened by the very good correlations between the regulation of Ins(1,4,5)P_3 kinase activity (present study) and the earlier investigations of Ca^{2+} influx which have been discussed above.

At this point it should be made clear that protein kinase C probably regulates the metabolism of hepatic Ins(1,4,5)P_3 by multiple means. Stimulation of Ins-(1,4,5)P_3 phosphomonoesterase, a mechanism previously proposed for platelets [44], is apparently ruled out, since the activity of this enzyme was unaffected by prior

incubation of hepatocytes with TPA (Table 4). This is consistent with findings recently obtained with several cell types [41,45,46], including hepatic epithelial cells [47].

Another potential regulatory mechanism is that activation of protein kinase C uncouples PtdInsP_2 hydrolysis from receptor occupation [46]. Indeed there is now good evidence to suggest that this occurs as a normal consequence of agonist binding, but only to a limited degree: the stimulation of Ins(1,4,5)P_3 production is never completely inhibited [47]. In contrast, it has been shown that TPA pretreatment of hepatocytes completely abolishes the very weak activation of PtdInsP_2 hydrolysis normally induced by cyclic AMP analogues [23]. This almost certainly explains our failure to observe any stimulatory effect of TPA on db.cAMP-stimulated Ca^{2+} influx. However, in the presence of physiological agonists, stimulation of Ins(1,4,5)P_3 kinase activity by diacylglycerol and cyclic AMP would occur in conjunction with an elevated (although down-regulated) Ins(1,4,5)P_3 production, thereby satisfying the postulated requirements for increased Ca^{2+} influx.

In conclusion, we have shown that hepatic Ins(1,4,5)-P_3 kinase is stimulated by both Ca^{2+}-dependent and -independent mechanisms. Moreover, it is suggested that, because of the generally good correlation between the two responses, the synergistic stimulation of Ca^{2+} influx previously demonstrated in the presence of glucagon and vasopressin could be explained, at least partially, in terms of increased Ins(1,4,5)P_3 kinase activity.

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