REVIEW ARTICLE

Calcium: its modulation in liver by cross-talk between the actions of glucagon and calcium-mobilizing agonists

Fyfe L. BYGRAVE*† and Angelo BENEDETTI†

*Division of Biochemistry and Molecular Biology, Faculty of Science, Australian National University, Canberra, ACT 2601, Australia, and †Istituto di Patologia Generale, Università di Siena, 53100 Siena, Italy

INTRODUCTION

Few would dispute the necessity to study cellular Ca^{2+} movements – indeed, in higher organisms, changes in cytosolic free $[\text{Ca}^{2+}]$ are a vital physiological event from the very beginning of life at the moment of fertilization, to its very end at the final beat of the heart. These changes are brought about either as a result of mobilizing intracellular Ca^{2+} or as a result of inflow of the ion from the extracellular medium. The means by which cells control Ca^{2+} fluxes, and particularly Ca^{2+} inflow, is increasingly becoming a focus of attention. Changes in intracellular $[\text{Ca}^{2+}]$, especially that following Ca^{2+} inflow across the cell membrane induced by a range of different stimuli, are the trigger not only in the short-term but also in the long-term of many vital physiological responses in cells in a range of tissues and species. Moreover, aberrant control of Ca^{2+} fluxes appears to be associated with many pathological situations.

During the past decade in particular, a large number of articles have been published about many aspects of cellular Ca^{2+} fluxes: about the discovery and generation of the new second messenger $\text{Ins}(1,4,5)\text{P}_3$ and of the related enzymic reactions that generate and dispose of it; about the range of agonists able to induce changes in cellular Ca^{2+} fluxes; about the cellular compartments that appear to contain agonist-sensitive Ca^{2+} pools; about the continual development of new techniques to measure and locate the ion within cells; and about how ‘cross-talk’ between second messenger systems, such as those generating cyclic AMP and $\text{Ins}(1,4,5)\text{P}_3$ for instance, are able to induce/modulate Ca^{2+} fluxes. Numerous offerings and ideas have in turn evolved from these studies as to the nature of the mechanisms involved in the control of cell Ca^{2+} . The aim of this article is to contribute further to this knowledge by integrating a number of relevant observations and reports obtained over the years by numerous investigators and to synthesize a general scheme that we hope will be useful in the current debates about the control of cellular Ca^{2+} fluxes. We focus on liver tissue, drawing upon the large body of information available about such fluxes derived from studies from both single cells (hepatocytes) and the intact organ (perfused liver). We also focus particularly on the issue of how glucagon (and cyclic AMP) might regulate/modulate Ca^{2+} fluxes induced by calcium-mobilizing agonists. This is an aspect of the control of Ca^{2+} fluxes in liver that is not only important in many aspects of liver function, but also one that we believe holds clues as to the mechanism of hormone-induced Ca^{2+} inflow and outflow in this tissue.

BRIEF HISTORY OF RELEVANT EVENTS

Ringer (1883) and Heilbrunn (1952) were among the first to recognize the ubiquitous role of Ca^{2+} in physiological events. Features of their work were later extended by others who

envisaged the ion as having a regulatory role in cell metabolism (Lehninger, 1950; Bygrave, 1966, 1967; Gevers and Krebs, 1966; Rasmussen, 1970). During the 1970s, the numerous studies on cellular Ca^{2+} fluxes were confined largely to movements across the membranes of isolated organelles, in particular those of the mitochondria and endoplasmic reticulum (e.g. Lehninger et al., 1967; Bygrave, 1978a; Williamson et al., 1981; Åkerman and Nicholls, 1983). Initially, little attention was paid to the possible role of hormones in the control of these intracellular events, but towards the end of that decade a realization emerged that certain hormones and also cyclic AMP might play a role in the control of intracellular Ca^{2+} (Bygrave, 1978a; Rasmussen, 1981; Borle, 1981; Exton, 1981). Others were advocating a possible controlling role of cyclic AMP in Ca^{2+} fluxes across the cell (plasma) membrane (Friedmann and Park, 1968; Borle, 1972) and that Ca^{2+} and cyclic AMP could have an interactive role in the control of cell metabolism (Rasmussen and Barrett, 1984).

A further significant landmark occurred towards the end of the 1970s. This was the realization that cyclic AMP generation and action (Robison et al., 1971) on their own could not account for the mechanism by which α -adrenergic agonists, vasopressin and angiotensin induce metabolic events such as glycogenolysis; the evidence was mounting that Ca^{2+} was involved in some (at that time unknown) way (Van de Werve et al., 1977; Assimacopoulos-Jeanett et al., 1977; Keppens et al., 1977; Keppens and de Wulfe, 1977; reviewed by Exton, 1981, 1985, 1988). In studies quite separate, but which were later to be very relevant to this subject, knowledge was being obtained about the role of Ca^{2+} in the hydrolysis of phosphatidylinositol (Michell, 1975). The role of $\text{Ins}(1,4,5)\text{P}_3$ as second messenger in the action of Ca^{2+} -mobilizing agonists was reviewed by Berridge (1987), and the fascinating story of the concepts leading up to it is outlined by Michell (1992).

During the 1980s a number of reports appeared in which the general question was raised as to whether glucagon (or cyclic AMP) was itself able to induce cellular Ca^{2+} fluxes in isolated organelles, in intact cells or in the perfused rat liver. In many instances the responses induced by glucagon were compared with those induced by Ca^{2+} -mobilizing hormones which, it was realized early on, are far more potent than glucagon in their ability to mobilize Ca^{2+} . However, what a number of those reports revealed, and others continue to reveal, is that glucagon is able to exert effects in liver such that the Ca^{2+} flux-inducing action of Ca^{2+} -mobilizing agonists is greatly modified. It is this aspect, the means by which glucagon is able to modify the action of Ca^{2+} -mobilizing agonists, that this review primarily addresses.

First, we briefly review the patterns of Ca^{2+} inflow and outflow induced by Ca^{2+} -mobilizing agonists that occur in the perfused rat liver and in hepatocytes, and where possible we integrate information gained from each of the two experimental systems.

We show how these patterns are altered by prior administration or co-administration of glucagon with these agonists. Bearing in mind current views about how the inflow of Ca^{2+} might take place, we attempt to identify cellular sites at which glucagon might be acting so as to modify cellular Ca^{2+} fluxes induced in turn by the action of Ca^{2+} -mobilizing agonists. Finally, we attempt to draw this information together in a form that will permit further experimentation both in respect of the basic mechanisms involved and in respect of some of the physiological events affected by the combined action of glucagon and Ca^{2+} -mobilizing agonists.

FEATURES OF Ca^{2+} FLUXES INDUCED BY Ca^{2+} -MOBILIZING AGONISTS

Prior to 1980, the majority of studies on Ca^{2+} flux changes in hepatocytes and intact perfused liver employed ^{45}Ca . This radionuclide served as a tracer for Ca^{2+} movements and distribution and provided information about the rates of movement of the ion across cell and intracellular membranes, about the nature of the intracellular Ca^{2+} pools and, more particularly, about how hormones effect these events (see Berry et al., 1991). From the earliest studies it was soon apparent that the hormones mobilizing Ca^{2+} induced multiple effects. For example it was shown that α -adrenergic agonists could stimulate the rate of Ca^{2+} inflow (e.g. Assimacopoulos-Jeanett et al., 1977) as well as Ca^{2+} outflow (e.g. Blackmore et al., 1978; Poggioli et al., 1980) in hepatocytes. Around this time an extensive analysis of hormone-induced Ca^{2+} mobilization in the perfused rat liver, also employing ^{45}Ca , appeared (Claret-Berthon et al., 1977). Later, Barritt et al. (1981) showed that under steady-state conditions adrenaline is able to stimulate the rate of inflow, the exchange and the loss of ^{45}Ca from hepatocytes. An important technical advance was the synthesis and development of fluorescent indicators that could be loaded into hepatocytes and used to report on the changes in intracellular $[\text{Ca}^{2+}]$ following administration to cells of an appropriate agonist (see, e.g., Tsien et al., 1982). Although other intracellular probes are available (see, e.g., Borle and Snowdowne, 1986), the fluorescent indicators quin2 and more recently fura2 have been used by most researchers to measure intracellular free Ca^{2+} in hepatocytes, largely on account of their ease of handling and non-invasive properties. The application of these indicators has provided an important step forward in enabling a further understanding of the effects of α -agonists and glucagon on Ca^{2+} fluxes in cells.

As implied above, the two principal experimental systems that have contributed to our current understanding of cellular Ca^{2+} fluxes in liver are the perfused rat liver and single cells (hepatocytes) prepared therefrom. While each system inherently has particular advantages and disadvantages (see Exton, 1975; Berry et al., 1991), information gained from both, described below, is necessary in order to fully appreciate how such Ca^{2+} fluxes operate under the influence of the various agonists in question.

STUDIES ON Ca^{2+} FLUXES IN THE PERFUSED RAT LIVER INDUCED BY Ca^{2+} -MOBILIZING AGONISTS

Early studies on Ca^{2+} movements in the perfused rat liver relied on measurements of perfusate Ca^{2+} by using ^{45}Ca , atomic absorption spectroscopy or a Ca^{2+} -selective electrode. The latter two sets of measurements were often restricted to experimental situations where the perfusate Ca^{2+} needed to be maintained at a relatively low concentration so as to increase the sensitivity of measurement (see, e.g., Althaus-Salzmann et al., 1980; Sies et al.,

1981; Morgan et al., 1983). As is now known and shown below, such a situation can have a large bearing on the nature of the responses observed. Many of these problems were overcome by the application of a sensitive Ca^{2+} -selective electrode to the outflow medium (detailed in Reinhart et al., 1982; see also Sies et al., 1983) that, together with appropriate backoff instrumentation, enabled the detection of a $+1 \mu\text{M}$ Ca^{2+} change in extracellular $[\text{Ca}^{2+}]$ when the extracellular $[\text{Ca}^{2+}]$ was varied from approx. zero to 1.3 mM. This has provided a sensitive system through which much information has been gained about a number of aspects of the hormonal control of cellular Ca^{2+} fluxes in liver from studies of the perfused rat liver system alone (reviewed in Reinhart et al., 1984d). Other relevant features of this system are that the extracellular $[\text{Ca}^{2+}]$ is able to be rapidly altered and (in the flowthrough, non-recirculating set-up) any agent or agonist can be rapidly administered and, most importantly, rapidly removed. It should be appreciated that in the description which follows, it is the information gained at higher extracellular $[\text{Ca}^{2+}]$ that is physiologically relevant. Work with lower extracellular $[\text{Ca}^{2+}]$, on the other hand, can be useful experimentally.

In the perfused liver, the α_1 -adrenergic agonist phenylephrine induces a sizable efflux of Ca^{2+} within 3–4 s, the amount extruded depending on the concentration and the time for which the hormone is administered. Details about the properties of the hormone-sensitive Ca^{2+} pools in rat liver are reviewed in Reinhart et al. (1984d). The amount of Ca^{2+} efflux caused by phenylephrine, vasopressin and angiotensin II is less at higher extracellular $[\text{Ca}^{2+}]$. Conversely, the amount of Ca^{2+} taken up increases as the extracellular $[\text{Ca}^{2+}]$ is increased. Influx of Ca^{2+} is induced only after removal of the agonist. This latter feature, which clearly distinguishes the actions of phenylephrine from those of other Ca^{2+} -mobilizing agonists (Altin and Bygrave, 1985), cannot be seen in experiments with hepatocytes unless a perfusion system is used.

Ca^{2+} influx promoted by vasopressin exhibits a pattern similar to that with phenylephrine only when the extracellular $[\text{Ca}^{2+}]$ is relatively low, i.e. approx. 500 μM or less (see also Benedetti et al., 1989). At higher concentrations of Ca^{2+} , vasopressin induces a small efflux of the ion but, and while still being infused, spontaneously induces a large influx followed by a second small efflux. A second pulse of vasopressin administered later, even when the previously depleted intracellular pools have refilled (see below), induces only Ca^{2+} efflux. When the length of the time of administration of the (first) vasopressin pulse is varied between 15 and 45 s, the extent of efflux is the same but the extent of influx is more pronounced as the period of administration is increased. Responses to angiotensin are essentially similar to those induced by vasopressin. A number of these general features of agonist-induced Ca^{2+} fluxes in the perfused rat liver are illustrated in Figure 1. The oscillatory phenomena observed following the administration of a Ca^{2+} -mobilizing agonist to the perfused rat liver seen at low $[\text{Ca}^{2+}]$ in the Figure have been studied in some detail by Graf et al. (1987).

One serious limitation to the study of Ca^{2+} fluxes in the perfused rat liver has been the inability to date to directly monitor changes in intracellular $[\text{Ca}^{2+}]$. Some success in perfused heart has been achieved, however, using intracellular indicators (Levine et al., 1990). On the other hand, qualitative information about the extent to which these changes occur can be gauged from the appearance of glucose (from glycogen breakdown) in the medium, particularly following alterations to the extracellular $[\text{Ca}^{2+}]$ (see Reinhart et al., 1984b) and by assessing the degree to which oxygen uptake is stimulated. This point is amplified in the data illustrated in Figure 2. Here, changes in perfusate Ca^{2+} ,

Figure 1 Extracellular $[\text{Ca}^{2+}]$ influences the Ca^{2+} flux responses in perfused rat liver induced by phenylephrine or vasopressin

The data, obtained with a Ca^{2+} -selective electrode and perfused rat liver system, show that Ca^{2+} inflow (downwards deflection) is minimal for both agonists at low extracellular $[\text{Ca}^{2+}]$. They also show that at extracellular $[\text{Ca}^{2+}] > 500 \mu\text{M}$, repeated pulses of phenylephrine ($2 \mu\text{M}$) are able to induce repeated outflow/inflow cycles; by contrast, vasopressin (10 nM) will induce only one 'typical' cycle and thereafter only Ca^{2+} outflow. The curves shown in red illustrate the cycles that occur at a physiological Ca^{2+} concentration (1.3 mM). For further general details see the text and Reinhart et al. (1982), Altin and Bygrave (1986) and Benedetti et al. (1989). See Graf et al. (1987) for further information concerning the oscillatory behaviour observed at low perfusate $[\text{Ca}^{2+}]$.

oxygen uptake and glucose output following administration of phenylephrine were assessed in situations where the extracellular $[\text{Ca}^{2+}]$ was either maintained at 1.3 mM or reduced to nominally zero in the presence of EGTA. The data reveal two basic types of response; one transient and the other sustained.

Transient responses are characterized as being rapid in onset, occurring within seconds, and once the maximum response is attained they decay quite quickly; in addition, in the case of perfusate Ca^{2+} measurements (Figure 2a), no inflow is observed when the agonist phenylephrine is withdrawn (see above). The responses occur independently of extracellular $[\text{Ca}^{2+}]$ and hence are dependent on the mobilization of intracellular Ca^{2+} . While not shown here (but see Reinhart et al., 1984b), the response can be rapidly re-activated by providing an excess of Ca^{2+} to the perfusate medium.

Sustained responses are equally rapid in onset but once the maximum is attained, little or no decay is observed. Clearly these responses are dependent on Ca^{2+} inflow from the extracellular medium. As will be shown later in this review, much of this information is able to be obtained also with hepatocytes. Stimulation of oxygen uptake by Ca^{2+} -mobilizing agonists (see, e.g., Figure 2c) most probably reflects movement of Ca^{2+} into the mitochondria which then stimulates enzymes of the citrate cycle (see, e.g., Denton and McCormack, 1985; Assimacopoulos-Jeanett et al., 1986). These aspects will not be discussed further here. As a final point at this juncture, it needs to be appreciated

that changes in glucose output and oxygen consumption are a good index of changes in intracellular $[\text{Ca}^{2+}]$ for studies when only one of the glycogenolytic inducing agonists is used. Their use in examining synergism (see below), like that of phosphorylase activity, is somewhat limited.

EFFECTS OF GLUCAGON ON Ca^{2+} FLUXES INDUCED BY Ca^{2+} -MOBILIZING AGONISTS IN THE PERFUSED RAT LIVER

Administration of glucagon alone to the perfused rat liver induces a reproducibly significant efflux of Ca^{2+} . This was shown in early studies by Friedmann and Park (1968), Friedmann (1972), Friedmann and Dambach (1973) and Kraus-Friedmann (1986), and has since been confirmed in other laboratories (e.g. Morgan et al., 1985; Altin and Bygrave, 1986; Rashed and Patel, 1987; Benedetti et al., 1989). Where examined, the effects of glucagon could be reproduced when dibutyryl cyclic AMP was the agonist. The amount of Ca^{2+} released, however, and the rate of release are much less than that mobilized by phenylephrine, vasopressin or angiotensin; these glucagon-induced responses appear to be increased substantially when the extracellular $[\text{Ca}^{2+}]$ is lowered (Benedetti et al., 1989). On the other hand, when pre-administered, glucagon greatly alters the characteristics of Ca^{2+} mobilization induced by all three of the above-mentioned Ca^{2+} -mobilizing agonists (Morgan et al., 1985; Altin and Bygrave, 1986) as well as by ADP, ATP and epidermal growth factor

Figure 2 Extracellular $[Ca^{2+}]$ sustains agonist-stimulated, Ca^{2+} -sensitive, intracellular responses in the perfused rat liver

Phenylephrine ($2 \mu M$) was administered for the time indicated by the bar in the presence of $1.3 \text{ mM } Ca^{2+}$ (red line) or nominally zero Ca^{2+} by the addition of EGTA. It is seen (1) that Ca^{2+} outflow (upwards deflection), in terms of both magnitude and rapidity of response, is unaffected by variations in extracellular $[Ca^{2+}]$; (2) that phenylephrine-induced inflow will not occur following removal of the agonist when extracellular $[Ca^{2+}]$ is approx. zero; and (3) that both glucose output and oxygen uptake induced by the action of phenylephrine require high extracellular $[Ca^{2+}]$ and therefore Ca^{2+} inflow in order to exhibit a sustained response to the hormone. For further details see the text and Reinhart et al. (1984b,d). Similar information can be gleaned with hepatocytes, for example from the studies of Charest et al. (1985a) and Combettes et al. (1986).

(EGF) (Altin and Bygrave, 1987a) and by phosphatidic acid and arachidonic acid (Altin and Bygrave, 1987b). Preadministration of glucagon to the perfused rat liver was shown to also suppress the oscillations induced by vasopressin alluded to above (Graf et al., 1987). Earlier Jenkinson and Koller (1977) had shown that the combined addition of α_1 - and β -agonists increased synergistically the efflux of ^{45}Ca from liver slices of the guinea pig. Similar findings were made by Cocks et al. (1984).

Figure 3 Glucagon modulates vasopressin-induced Ca^{2+} fluxes in the perfused rat liver

The data show that, in the presence of 1.3 mM extracellular Ca^{2+} , whereas glucagon (Gluc.; 10 nM) alone (a) induces a slight outflow of the ion, its co-administration with vasopressin (Vaso.; 10 nM) (c) results in little effect on Ca^{2+} outflow induced by vasopressin (cf. b) but significant (synergistic) increases in Ca^{2+} inflow. A second co-administration of the two hormones also induces Ca^{2+} inflow. Note that the scale of perfusate $[Ca^{2+}]$ is different to that shown in Figure 1. For further details see the text and Altin and Bygrave (1986)

A major effect of pre-administered glucagon is to significantly attenuate the initial Ca^{2+} efflux induced by these agonists. Its other major effect is to greatly accelerate Ca^{2+} inflow. The magnitude of this synergistic effect on Ca^{2+} inflow is dependent on the period of preincubation; maximal effects in the perfused rat liver system are seen after approx. 4 min of preincubation. An important observation relating to the issue of the possible mechanisms involved, however, is that while co-administration of glucagon with the Ca^{2+} -mobilizing agonists does not influence the extent of efflux induced by the agonists alone, glucagon still enhances Ca^{2+} inflow to a considerable extent (Altin and Bygrave, 1986). This indicates that the effects of glucagon in the perfused rat liver are virtually immediate and that its earliest effects are on Ca^{2+} inflow and later, either directly or indirectly as a consequence of some presently unknown action, on Ca^{2+} outflow. As mentioned above, the effects of glucagon on agonist-induced inflow are not observed when the extracellular $[Ca^{2+}]$ is relatively low (Benedetti et al., 1989), similar to the action of vasopressin alone on Ca^{2+} inflow also mentioned above. Glucagon will, however, promote Ca^{2+} inflow induced by a second pulse of vasopressin if the extracellular $[Ca^{2+}]$ is above approx. $500 \mu M$. These experiments thus reveal that, despite the hormone regime under study, the Ca^{2+} gradient across the plasma membrane is a significant factor determining whether the synergistic effects of glucagon with Ca^{2+} -mobilizing agonists on Ca^{2+} inflow will (or will not) be manifest. In the perfused liver the acceleration of Ca^{2+} inflow seen with vasopressin and angiotensin in these conditions is much greater than that seen with phenylephrine. A number of the features described above of the effect of glucagon on vasopressin-induced Ca^{2+} fluxes in the perfused rat liver are illustrated in Figure 3.

The information in Figure 3 reveals another important aspect about the massive Ca^{2+} inflow induced by the synergistic effects of the two hormones. That is, despite the very large amount of

the ion taken up by the liver, there is no sign of damage having occurred, at least over the time course of the experiment. This is reflected in the fact that a second administration of the hormones at 74 min into the perfusion will produce a second response only slightly less than the first.

In summarizing to this point, several features are evident. The first is that glucagon alone is able to induce a small but significant outflow of Ca^{2+} from the perfused rat liver. Secondly, the onset of this outflow follows a short lag period, and the magnitude of it is less than that resulting from the action of Ca^{2+} -mobilizing agonists. Thirdly, glucagon-induced Ca^{2+} outflow appears to increase as the extracellular $[\text{Ca}^{2+}]$ is decreased. Finally, the major effect of glucagon in the perfused rat liver is (a) when co-administered with Ca^{2+} -mobilizing agonists, to immediately promote Ca^{2+} inflow without altering Ca^{2+} outflow, (b) when administered before Ca^{2+} -mobilizing agonists, to attenuate the ability of α -agonists to promote Ca^{2+} outflow, and (c) to greatly augment the ability of these agonists to promote Ca^{2+} inflow.

STUDIES ON Ca^{2+} FLUXES IN HEPATOCYTES INDUCED BY Ca^{2+} -MOBILIZING AGONISTS

As pointed out by Crofts and Barritt (1989) and Berry et al. (1991), accurate measurement of rates of hormone-stimulated Ca^{2+} inflow in hepatocytes has proved difficult. The main methods employed to measure rates of Ca^{2+} inflow are the use of ^{45}Ca under steady-state conditions (see Barritt et al., 1981), (indirect) measurement of increases in intracellular Ca^{2+} using fluorescent indicators following the addition of Ca^{2+} to a medium containing negligible Ca^{2+} , measurement of the rates of quenching of fluorescence of intracellular quin2 following Mn^{2+} addition (see, e.g., Crofts and Barritt, 1990), and measurement of the initial rate of activation of glycogen phosphorylase following the addition of Ca^{2+} to a medium containing negligible Ca^{2+} . A further complication to studies of Ca^{2+} inflow is that there is mounting evidence that more than one system facilitates Ca^{2+} entry in the plasma membrane of liver cells (Barritt et al., 1981; Hughes et al., 1986; Altin and Bygrave, 1987a; Barritt and Hughes, 1991; Llopis et al., 1992). The potentially useful technique of patch-clamping appears to be technically difficult in liver plasma membranes (see, e.g., Sawanobori et al., 1989; Barritt and Hughes, 1991).

These potential complications may in large part account for some of the apparent discrepancies between groups on the characteristics of Ca^{2+} inflow in hepatocytes. For instance, Crofts and Barritt (1989) found that vasopressin-induced Ca^{2+} inflow measured in quin2-loaded cells exhibits a biphasic curve as the extracellular $[\text{Ca}^{2+}]$ is increased; the second phase showed no signs of saturation even at 5 mM Ca^{2+} . By contrast, the data of Mauger et al. (1984) and Joseph et al. (1985) showed Ca^{2+} inflow induced by noradrenaline, vasopressin and angiotensin to be saturated at 1–2 mM external Ca^{2+} . Mauger et al. (1985) also showed that glucagon alone can stimulate the initial rate of Ca^{2+} inflow.

Both the basal and hormone-stimulated Ca^{2+} inflow systems exhibit a broad specificity for bivalent metal ions (Crofts and Barritt, 1990), with Mn^{2+} , Co^{2+} , Ni^{2+} and Zn^{2+} all able to quench the fluorescence of Ca-quin2 loaded into hepatocytes.

As intimated above, a serious drawback to studies with the perfused rat liver has been the inability to gain quantitative information about changes in intracellular $[\text{Ca}^{2+}]$ following agonist presentation. Now, using hepatocytes loaded with appropriate fluorescent indicators that monitor changes in intracellular $[\text{Ca}^{2+}]$ (Tsien et al., 1982), it is possible to gauge with

a reasonable degree of accuracy the magnitude of and the rapidity with which intracellular $[\text{Ca}^{2+}]$ is altered under a variety of experimental conditions, for example. Such measurements in turn provide information about the changes occurring to Ca^{2+} inflow (Berry et al., 1991). Because of the lack of appropriate inhibitory agents, however, it is not always possible at the present time to strictly distinguish between contributions to raising the cytoplasmic $[\text{Ca}^{2+}]$ from Ca^{2+} inflow on the one hand, and intracellular Ca^{2+} mobilization on the other.

The following observations most pertinent to this review have been made in studies of this nature. (1) The Ca^{2+} -mobilizing agonists vasopressin, angiotensin, phenylephrine, adrenaline and noradrenaline all rapidly increase intracellular $[\text{Ca}^{2+}]$ (Charest et al., 1983, 1985a; Sistare et al., 1985; Combettes et al., 1986). The onset of the increase occurs at approx. 2–3 s, with maximal changes attained by approx. 10 s. The order of potency is adrenaline, phenylephrine, vasopressin. A similar order of potency was observed from measurements of ^{45}Ca inflow (Mauger et al., 1984) and this in turn is similar to what is observed with activation of phosphorylase *a* (Charest et al., 1985a). (2) Glucagon alone is also able to induce an increase in intracellular $[\text{Ca}^{2+}]$. However, the time of onset lags by some 10 s and the rapidity and magnitude of the change are not quite as great (Charest et al., 1983; see also Mauger and Claret, 1986). (3) Reducing the extracellular $[\text{Ca}^{2+}]$ does not affect the time of onset nor the magnitude of the change in intracellular $[\text{Ca}^{2+}]$, but does reduce the time for which the response is sustained (Charest et al., 1985a). This group showed that varying the extracellular $[\text{Ca}^{2+}]$ between 30 and 500 μM had little effect on either the initial rate or the maximal extent of the intracellular Ca^{2+} increase. However, whereas 500 μM extracellular $[\text{Ca}^{2+}]$ prolonged the rise in intracellular $[\text{Ca}^{2+}]$, 30 μM extracellular $[\text{Ca}^{2+}]$ allowed intracellular $[\text{Ca}^{2+}]$ to return to basal levels by 4 min following vasopressin addition to the cells. These experiments again illustrate the importance of extracellular Ca^{2+} in maintaining the sustained responses alluded to in an earlier section of this review (see text and Figure 2). (4) Parallel with rapid increases in intracellular $[\text{Ca}^{2+}]$ and other metabolic responses following vasopressin addition to hepatocytes, Charest et al. (1983, 1985b) demonstrated that $\text{Ins}(1,4,5)\text{P}_3$ plus $\text{Ins}(1,3,4)\text{P}_3$ production caused by adrenaline, vasopressin and angiotensin occurred very rapidly (within seconds), attaining a maximum by 5 s and remaining maximal for at least 10 min. Of interest is that by comparison with their abilities to induce Ca^{2+} inflow, vasopressin and angiotensin were better able to induce $\text{Ins}(1,4,5)\text{P}_3$ plus $\text{Ins}(1,3,4)\text{P}_3$ formation. Seemingly at odds with these findings, Hansen et al. (1991) have shown that phenylephrine-induced accumulation of $\text{Ins}(1,4,5)\text{P}_3$ in rat hepatocytes, while attaining a maximum also by 5 s, rapidly decreased thereafter; by 30 s a new steady-state level was reached that was 30 % of the maximal level. The apparent discrepancy between these two groups, however, is possibly explained by the fact that the former group were examining both isoforms of *myo*-inositol trisphosphate, i.e. $\text{Ins}(1,4,5)\text{P}_3$ and $\text{Ins}(1,3,4)\text{P}_3$, whereas the latter group examined the turnover of $\text{Ins}(1,4,5)\text{P}_3$. Studies by Tennes et al. (1987) have shown that guinea pig hepatocytes stimulated with angiotensin produce $\text{Ins}(1,4,5)\text{P}_3$ maximally by 10 s and that this declines rapidly during the next 10 min. By contrast, $\text{Ins}(1,3,4)\text{P}_3$ gradually rises over 10 min.

Mauger et al. (1984) examined the initial rate of Ca^{2+} inflow into hepatocytes and found that Ca^{2+} inflow was activated for as long as the hormones occupied their receptors. Increasing the extracellular $[\text{Ca}^{2+}]$ led, in contrast with the report of Barritt et al. (1981), to a saturation of Ca^{2+} inflow; but see Barritt and Hughes (1991) who elaborate on this point.

Studies on single hepatocytes (see, e.g., Woods et al., 1986, 1987) have shown that Ca^{2+} -mobilizing hormones induce oscillations in intracellular Ca^{2+} , the frequency of which depends on the agonist concentration. It is of interest that in this single-cell system the responses to vasopressin are different from those to phenylephrine (Sanchez-Bueno and Cobbald, 1993), as in the intact perfused rat liver (Altin and Bygrave, 1985; see Figure 1).

EFFECTS OF GLUCAGON OR OF DIBUTYRYL CYCLIC AMP ON Ca^{2+} FLUXES IN HEPATOCYTES INDUCED BY Ca^{2+} -MOBILIZING AGONISTS

As intimated above, glucagon alone appears able to induce an increase in intracellular $[\text{Ca}^{2+}]$ as well as having a number of other Ca^{2+} -flux-related effects in hepatocytes. It is able to increase ^{45}Ca movement into hepatocytes (Keppens et al., 1977), but Studer et al. (1984) report that glucagon at physiological concentrations has no effect on intracellular $[\text{Ca}^{2+}]$. The interesting observation of Assimacopoulos-Jeanett et al. (1982) and Morgan et al. (1983) that increases in dibutyryl cyclic AMP are able to reverse vasopressin-induced Ca^{2+} mobilization (see also Crane et al., 1982) appears to be consistent with the finding (Keppens and De Wulf, 1984) that vasopressin inhibits the maintenance of cyclic AMP levels in hepatocytes by activating phosphodiesterase. Charest et al. (1985b) have shown that glucagon is able to potentiate Ca^{2+} inflow induced by extracellular ATP.

In kinetic studies of the influence of glucagon on ^{45}Ca inflow induced by Ca^{2+} -mobilizing agonists, Mauger et al. (1985) and Poggioli et al. (1986) reported that co-addition of glucagon with vasopressin greatly stimulated Ca^{2+} inflow. The dose-response curves for either glucagon or vasopressin were unaffected by the other agent, i.e. the EC_{50} did not change but the maximal effects of each were enhanced. The effects of glucagon were seen also with dibutyryl cyclic AMP. These workers concluded that both hormones were activating the same gating mechanism.

Burgess et al. (1986), using guinea pig hepatocytes, showed that the β -adrenoceptor agonist isoprenaline alone induced Ca^{2+} efflux when the extracellular $[\text{Ca}^{2+}]$ was low but not when it was high. They also observed a significant potentiation by this agent of angiotensin-induced Ca^{2+} mobilization. They suggested that the interacting effects did not involve steps subsequent to the generation of signals by angiotensin. Because there appeared to be no apparent increase in the total intracellular pool size induced by isoprenaline, it was concluded that the agonist may have increased the sensitivity of the endoplasmic reticular pool to $\text{Ins}(1,4,5)\text{P}_3$.

Studies by Kass et al. (1990) on Mn^{2+} inflow showed that glucagon alone had no effect on Mn^{2+} inflow but that its prior administration to hepatocytes enhanced vasopressin-induced Mn^{2+} inflow and abolished the latency period observed between vasopressin addition and the onset of Mn^{2+} uptake. The effects of glucagon could be mimicked by dibutyryl cyclic AMP. Crofts and Barritt (1990), however, provided evidence that the effects of co-addition of glucagon and vasopressin on Mn^{2+} -induced quenching were the same as the sum of their individual effects.

The oscillations in intracellular $[\text{Ca}^{2+}]$ induced by vasopressin or phenylephrine mentioned earlier are also able to be modulated by elevated levels of intracellular cyclic AMP induced by glucagon, dibutyryl cyclic AMP or forskolin (Somogyi et al., 1992; Sanchez-Bueno et al., 1993; see also Capiod et al., 1991). While the elevated cyclic AMP does not itself induce the oscillations, it enhances both the peak free Ca^{2+} and the frequency of 'spikes' induced by phenylephrine. Again, and consistent once

more with differences seen between the effects of elevated cyclic AMP levels on Ca^{2+} fluxes in the perfused rat liver (Altin and Bygrave, 1986; Figure 3), the effects on vasopressin-induced spikes are different to those induced by phenylephrine; they do not affect peak free Ca^{2+} but induce an eventual fall in spiking frequency (Sanchez-Bueno et al., 1993). Of additional interest is the finding of Somogyi et al. (1992) that protein kinase C activation by diacylglycerol, generated together with $\text{Ins}(1,4,5)\text{P}_3$ during phosphatidylinositol breakdown, inhibits the cytoplasmic Ca^{2+} oscillations.

There is a feature of the synergism between glucagon and Ca^{2+} -mobilizing hormones that appears to have been neglected from the experimental viewpoint, yet may hold clues to the mechanism(s) involved. Some years ago, Blackmore et al. (1984) reported that when the pH of the incubation is increased to values near 8, the ability of glucagon and Ca^{2+} -mobilizing agonists to increase hepatocyte cell Ca^{2+} is greatly enhanced. Indeed, at this pH, glucagon appeared to be a more potent Ca^{2+} -mobilizing agonist than vasopressin, adrenaline or angiotensin. These findings were observed also in the perfused rat liver (Altin and Bygrave, 1987a), where direct measurements of the synergism (as described in Figure 3) showed little glucagon-induced influx at pH 7.7 but a large influx at higher pH values. Ca^{2+} influx was virtually immediate upon hormonal stimulation. Of some interest also was the finding that, at pH 8, vasopressin alone induced no Ca^{2+} efflux, but rather a much greater (than at pH 7.4) influx of the ion. It would appear that further exploration of this system should provide useful information in the future.

EFFECTS OF GLUCAGON AND Ca^{2+} -MOBILIZING AGONISTS ON THE Ca^{2+} EXTRUDING PUMP, THE Ca^{2+} -ATPase

It will be evident that the plasma membrane, like the mitochondrial and endoplasmic reticular membranes, possesses separate Ca^{2+} transporter systems that permit routes of Ca^{2+} inflow which are different from those of Ca^{2+} outflow (see Barritt, 1992). This enables a 'cycling' of the ion between the two compartments into and out of which it is moving. It has been suggested, following the principles of substrate cycling laid down earlier by Newsholme and Crabtree (1976), that this form of control may be important in the regulation of Ca^{2+} fluxes induced by hormones as discussed here (Bygrave, 1978a,b). This has obvious implications for the regulation of metabolic processes in different cell compartments that are sensitive to Ca^{2+} (Bygrave, 1967; Rasmussen, 1981). In this context it is relevant to consider whether Ca^{2+} cycling across the plasma membrane might be altered by the action of hormones, not only on Ca^{2+} inflow mechanisms but also on the Ca^{2+} -ATPase.

It has been reported that the purified Ca^{2+} -ATPase is inhibited by the action *in vitro* of glucagon (Lotersztajn et al., 1981, 1985; Lin et al., 1983) and more recently it has been suggested that the Ca^{2+} -ATPase is coupled to a G_s -like protein (Lotersztajn et al., 1992; Jouneaux et al., 1993). In examining the influence of glucagon and Ca^{2+} -mobilizing agonists in an enriched plasma membrane fraction obtained from a low-speed centrifugation of the homogenate, Prpic et al. (1982) observed a degree of inhibition (some 30% after 3 min) of Ca^{2+} -ATPase by vasopressin, adrenaline and angiotensin; glucagon was without effect. In these experiments, the agonist was first administered to the perfused rat liver for a short time; the plasma membrane was subsequently prepared and Ca^{2+} -ATPase activity determined. The effects of vasopressin, the most potent of the agonists studied in these experiments, did not appear to be significant after 1 min of administration. Epping and Bygrave (1984), using a plasma membrane-enriched liver cell fraction preparation derived from

an initial centrifugation at medium to high speed, could not detect any hormone sensitivity of Ca^{2+} -ATPase or Ca^{2+} uptake when the perfused liver was first administered with phenylephrine.

By combining the Ca^{2+} -electrode and ^{45}Ca techniques in the perfused rat liver, Reinhart et al. (1984c) were able to show that net Ca^{2+} fluxes across the plasma membrane could be described in terms of the activities of a Ca^{2+} cycle comprising separate Ca^{2+} inflow and outflow components. The α_1 -adrenergic agonists phenylephrine, vasopressin and angiotensin were found to stimulate both inflow and outflow in a time-dependent manner; the latter occurring before the former. By comparison, glucagon had a relatively small but significant effect in this study. As mentioned above, Altin and Bygrave (1986) observed that following co-administration of glucagon and vasopressin to perfused rat livers, Ca^{2+} outflow was unaffected but Ca^{2+} inflow was greatly enhanced. Finally, and again using the Ca^{2+} -electrode and ^{45}Ca techniques, Altin and Bygrave (1987c) were able to demonstrate in the perfused rat liver that the main effect of these hormones was to activate a Ca^{2+} inflow pathway rather than to inhibit Ca^{2+} outflow via the Ca^{2+} -ATPase. On the other hand, a recent study has concluded that dibutyryl cyclic AMP stimulates Ca^{2+} efflux in rat hepatocytes (F. L. Bygrave, A. Gamberucci, R. Fulceri, R. Giunti and A. Benedetti, unpublished work).

Thus the extent to which hormones, particularly glucagon, activate the plasma membrane Ca^{2+} -ATPase appears to remain a debatable issue. Clarification of the possible role of G proteins in Ca^{2+} -ATPase activity is needed (see Jouneaux et al., 1993). We also should not lose sight of the fact that the high sensitivity of the Ca^{2+} -ATPase to intracellular $[\text{Ca}^{2+}]$ (see Lotersztajn et al., 1981), independent of any hormone action, is likely to be a prevailing factor determining Ca^{2+} outflow in liver.

DOES GLUCAGON EMPTY THE (Ca^{2+} -MOBILIZING) AGONIST-SENSITIVE INTRACELLULAR Ca^{2+} STORES?

Mechanisms by which Ca^{2+} entry might be evoked in non-excitable cells in general have been considered recently by Irvine (1992) [see also Putney (1990) and Neher (1992)]; the general issue will not be elaborated upon here. What is clear from these considerations, however, is that Ca^{2+} entry appears to be able to be controlled to some (possibly quite large) degree by the extent to which intracellular Ca^{2+} stores themselves are empty or full. Presumably the greater the extent of emptying of these stores, the greater the potential ability to exert a positive effect on plasma membrane Ca^{2+} inflow. In the present context, the question arises as to whether evidence exists linking glucagon action to the emptying (or refilling) of these stores and whether this purported action has any bearing on altering intracellular $[\text{Ca}^{2+}]$.

Studies with both hepatocytes and the perfused rat liver have provided evidence that glucagon releases Ca^{2+} from the same intracellular stores as does vasopressin. Combettes et al. (1986), studying the role of agonists in altering intracellular $[\text{Ca}^{2+}]$, observed that when the extracellular $[\text{Ca}^{2+}]$ is high, low glucagon concentrations stimulate both vasopressin-induced Ca^{2+} inflow and the final intracellular $[\text{Ca}^{2+}]$ reached. When the extracellular $[\text{Ca}^{2+}]$ is low, on the other hand, glucagon and vasopressin both mobilize Ca^{2+} from a common pool. Of further interest, and similar to results in experiments with perfused rat liver (see above), was the finding that under conditions where the extracellular $[\text{Ca}^{2+}]$ is low, glucagon was unable to potentiate the effect of vasopressin in inducing an increase in intracellular $[\text{Ca}^{2+}]$. Combettes et al. (1986) concluded that glucagon accelerates the fast phase of vasopressin-induced inflow and elevates the final intracellular $[\text{Ca}^{2+}]$ attained, by (a) releasing Ca^{2+} from the same

internal store as that affected by vasopressin, and (b) potentiating the inflow of external Ca^{2+} induced by vasopressin. In a related study, Mauger and Claret (1986) showed that glucagon mobilizes Ca^{2+} from a pool that is also sensitive to the action of Ca^{2+} -mobilizing agonists.

Kass et al. (1990), examining Mn^{2+} inflow into hepatocytes, showed that prior emptying of the endoplasmic reticular store with the inhibitor of endoplasmic reticular Ca^{2+} inflow 2,5-di-(tert-butyl)-1,4-benzohydroquinone enhanced the action of glucagon or of dibutyryl cyclic AMP on vasopressin-induced Mn^{2+} inflow. Of additional interest was their finding that the effects of glucagon on vasopressin-induced Mn^{2+} inflow were unaffected by membrane depolarization and by pertussis toxin treatment.

Several reports from studies with the perfused rat liver following the sequential administration of the agonists provide evidence that glucagon mobilizes Ca^{2+} from the same store as that mobilized by phenylephrine (Reinhart et al., 1982) or by vasopressin (Kimura et al., 1982; Kraus-Friedmann, 1986; Mine et al., 1988a; Benedetti et al., 1989).

Related to these issues is the question of which intracellular store the Ca^{2+} enters as a result of the synergistic action of glucagon and Ca^{2+} -mobilizing agonists on hepatocytes and the perfused rat liver as discussed above. The studies of Morgan et al. (1985) provided evidence that mitochondria were the intracellular site into which this Ca^{2+} enters. The evidence was based on both invasive analyses of the mitochondrial Ca^{2+} content following hormonal treatment of hepatocytes and non-invasive analyses of Ca^{2+} movements following treatment of intact hepatocytes with specific inhibitors of mitochondrial energy transduction.

In a study examining the influence of vasopressin and/or glucagon on mitochondrial Ca^{2+} and Ca^{2+} -sensitive oxidative enzymes in the perfused rat liver, Assimacopoulos-Jeannet et al. (1986) also showed that these hormones additively induce increases in intramitochondrial $[\text{Ca}^{2+}]$. This was reflected also in the stimulation of the activities of pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase.

In agreement with these conclusions, Altin and Bygrave (1986) rapidly isolated mitochondria and endoplasmic reticulum from the perfused rat liver following co-administration of glucagon with Ca^{2+} -mobilizing agonists. Their data revealed an accumulation of Ca^{2+} into the subsequently isolated organelles, particularly mitochondria, although significant increases in the endoplasmic reticulum-enriched fraction also were evident. Although considerable care was taken in those studies to minimize post-isolation Ca^{2+} redistribution (see Reinhart et al., 1984a), it is possible that some redistribution did take place especially involving Ca^{2+} release from the microsomes and its reuptake into the mitochondria. Benedetti et al. (1989), for example, have found that lowering the temperature to approx. 4 °C, as occurs in the course of the invasive organellar isolation technique, induces a rapid efflux of Ca^{2+} from the microsomes that could move into mitochondria which readily take up Ca^{2+} at these temperatures (Fulceri et al., 1991; Banhegyi et al., 1991). Banhegyi et al. (1991) have shown that a non-invasive technique using hepatocytes provided evidence that it is a non-mitochondrial pool into which the bulk of the Ca^{2+} enters following co-administration of glucagon and vasopressin. It is significant however that, at low temperatures, the majority of the Ca^{2+} entered the mitochondria (Banhegyi et al., 1991). In this regard Assimacopoulos-Jeannet et al. (1986) reported that, provided Na^+ is kept to a minimum and the mitochondria are maintained in ice-cold media, the (mitochondrial) preparation remains stable for the duration of the isolation procedure.

Kleineke and Soling (1985) concluded from a non-invasive study of perfused rat liver that the endoplasmic reticulum and not the mitochondria is the hormone-sensitive intracellular store. Further evidence for a reticular component for Ca^{2+} deposition is the observation that destruction of the endoplasmic reticulum with bromotrichloromethane prevented the synergistic accumulation of Ca^{2+} by perfused rat liver (Benedetti et al., 1989). Bond et al. (1987) had previously examined this issue by employing electron probe analyses of the intact liver and also had concluded that the Ca^{2+} had entered the endoplasmic reticulum (but see Altin and Bygrave, 1988).

Mine et al. (1988a) examined Ca^{2+} mobilization in rat hepatocytes loaded with aequorin and concluded that the intracellular Ca^{2+} pool mobilized by glucagon is different from that mobilized by the vasoactive agents phenylephrine and angiotensin II. This conclusion was based on the observation that brief prior treatment of hepatocytes with dinitrophenol prevented glucagon but not angiotensin II from inducing an increase in cytoplasmic Ca^{2+} . Kraus-Friedmann (1986) had previously shown that glucagon releases Ca^{2+} from a carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP)-releasable pool.

As implied above, it seems that the experimental conditions can influence the extent to which mitochondria or the endoplasmic reticulum (or both) provide the agonist-sensitive pool of Ca^{2+} . Most workers seem to be of the view, however, that the bulk of this pool is non-mitochondrial.

OTHER Ca^{2+} FLUX-RELATED SIGNALS GENERATED BY GLUCAGON

During the 1970s a number of reports appeared indicating that administration of glucagon to the intact rat, or to the perfused liver, or to hepatocytes, induced changes in the ability of the subsequently isolated organelle to transport Ca^{2+} (for reviews see Bygrave, 1978a; Soboll and Sies, 1989). A large body of work showed, for instance, that administration of glucagon to intact rats will enhance the ability of the subsequently isolated microsomes to accumulate Ca^{2+} *in vitro* (e.g. Bygrave and Tranter, 1978; Reinhart and Bygrave, 1981; Andia-Waltenbaugh et al., 1981). With mitochondria too, it was established that prior glucagon administration to the intact rat led to an increased ability of the subsequently isolated organelle to retain Ca^{2+} (e.g. Prpic et al., 1978). While it is somewhat unclear how and if these phenomena relate to the control by glucagon of hepatic Ca^{2+} fluxes, they provide a framework for a more recent set of observations indicating that Ca^{2+} uptake and release can be modulated by metabolites whose cytosolic concentrations can increase very rapidly following glucagon action.

EFFECTORS GENERATED BY GLUCAGON ACTION THAT MIGHT SERVE AS MODULATORS OF Ca^{2+} FLUXES

There is increasing evidence that intracellular Ca^{2+} fluxes can be affected not only by intracellular second messengers like $\text{Ins}(1,4,5)\text{P}_3$ and cyclic AMP but also by variations in the (cytosolic) concentration of metabolites resulting from the action of glucagon. For instance, it is well known that glucagon rapidly increases the flux into and the rate of hydrolysis of glucose 6-phosphate within the endoplasmic reticulum since this hormone stimulates glucose production. This event could promote Ca^{2+} inflow and storage in these organelles; physiological concentrations of glucose 6-phosphate have been observed to stimulate Ca^{2+} uptake by isolated liver microsomes and by digitonin-permeabilized hepatocytes (Benedetti et al., 1985, 1987). In this

respect, a recent study by Banhegyi et al. (1991) showed that the extent of Ca^{2+} inflow into a reticular pool(s) of isolated hepatocytes caused by co-administration of glucagon and vasopressin (see above) can be correlated with the extent of glucose production. It seems possible, therefore, that increased fluxes of glucose 6-phosphate and concomitant stimulation of reticular active Ca^{2+} translocation (see above) could be involved in the synergistic Ca^{2+} inflow induced by glucagon and Ca^{2+} -dependent agonists. It is notable that glucose 6-phosphate appeared to promote Ca^{2+} storage into both $\text{Ins}(1,4,5)\text{P}_3$ -sensitive and -insensitive endoplasmic reticular pools (Benedetti et al., 1988), and therefore a sensitization by glucagon to $\text{Ins}(1,4,5)\text{P}_3$ -dependent agonists can be envisaged. The effect on the $\text{Ins}(1,4,5)\text{P}_3$ -insensitive Ca^{2+} pool also could contribute to the termination of the effect of $\text{Ins}(1,4,5)\text{P}_3$ -dependent agonists by reaccumulating released Ca^{2+} (Benedetti et al., 1986). However, the relative roles of the activation of these two pools need further clarification.

Inorganic P_i is another metabolite able to induce changes in Ca^{2+} fluxes. The ability of P_i to promote mitochondrial Ca^{2+} inflow and storage *in vitro* under conditions of high Ca^{2+} loading is well known. At concentrations that exist in the cytosol, P_i also has been shown to promote Ca^{2+} inflow into (smooth) liver microsomal vesicles and the reticular pool(s) of permeabilized hepatocytes (Fulceri et al., 1990). Glucagon stimulates P_i uptake into perfused livers and increases the cytosolic $[\text{P}_i]$ (e.g. Bygrave et al., 1990). Hence, variations in cytosolic $[\text{P}_i]$ may also play a role in the modulation of intracellular reticular Ca^{2+} fluxes by glucagon.

Other metabolic effects induced by glucagon that are potentially relevant to the regulation of intracellular Ca^{2+} fluxes in liver are the variation in redox state (e.g. Rusinko and Lee, 1989) and the increase in acyl-CoA cellular content (Comerford and Dawson, 1993). Both events have been shown to affect cellular Ca^{2+} fluxes.

Few reports appear to have addressed the issue of whether Ca^{2+} fluxes induced by glucagon, with or without vasopressin, are influenced by the nutritional status of the animal. The work of Sistare et al. (1985) examining glucagon-induced Ca^{2+} fluxes in rat hepatocytes was carried out with 24-h-starved animals and the data appear qualitatively similar to those obtained by others using hepatocytes from fed animals. On the other hand, Rashed and Patel (1987) found that in 24-h-fasted animals, glucagon failed to induce ^{45}Ca efflux, but that induced by phenylephrine was unaffected. They suggested that the cellular redox potential regulates the ability of glucagon to promote Ca^{2+} efflux, probably through altering the cyclic AMP concentration. It was also reported (Banhegyi et al., 1991) that the synergistic inflow of Ca^{2+} induced by glucagon and Ca^{2+} -mobilizing agonists was reduced in hepatocytes from fasted rats (as compared to those from fed rats). As in the study of Rashed and Patel (1987), Ca^{2+} inflow was restored in fasted hepatocytes in the presence of gluconeogenic substrates. The extent of Ca^{2+} inflow appeared to correlate with the extent of glucose production; see also Taylor et al. (1983).

OTHER ASPECTS OF GLUCAGON-STIMULATED MODULATION OF (Ca^{2+} -MOBILIZING) AGONIST-INDUCED Ca^{2+} FLUXES: HORMONE SENSITIVITY AND RAPIDITY OF ONSET

An important observation by two groups in studies with hepatocytes and with the perfused rat liver is that glucagon, besides potentiating the changes in Ca^{2+} fluxes, appears also to increase the sensitivity of the cells to the agonists in question. Thus Morgan et al. (1985) noted that while very low doses of noradrenaline would not increase intracellular $[\text{Ca}^{2+}]$, pre-

incubation of the cells with glucagon allowed noradrenaline to induce a large increase. This change in sensitivity was interpreted to be correlatable with large increases in binding of noradrenaline to sites on the hepatocyte surface. These workers suggested that a modification by cyclic AMP at the level of the α_1 -adrenergic receptor mediated this effect. Altin and Bygrave (1986), making dose-response measurements with the perfused rat liver, observed that the sensitivity of vasopressin and angiotensin (but not so much that of phenylephrine) was apparently increased by the presence of a low glucagon concentration. It should be noted that Poggioli et al. (1986) did not observe an alteration in the sensitivity to the agonists. As found by Morgan et al. (1985), the increase in sensitivity is such that the concentrations of hormones that are inducing the effects on Ca^{2+} fluxes is well within the physiological range. Buhler et al. (1978), for example, have analysed the circulating glucagon and noradrenaline concentrations, finding these to be 0.1–0.01 nM. Conditions of severe stress, like some other conditions *in vivo*, cause a concomitant increase in plasma adrenaline and glucagon (Eigler et al., 1979). Elevated levels of glucagon in the blood appear to accompany hyperglycaemia in diabetes mellitus (Unson et al., 1989).

Studer et al. (1984) have called into question the physiological relevance of a number of reported effects of glucagon both *in vivo* and *in vitro*, on account of the potentially apparently high (unphysiological) concentrations of glucagon employed in the experiments. It is difficult to argue against this point in many instances; the message is clear.

Several reports have now appeared indicating that an early action of glucagon is on Ca^{2+} inflow. For instance, Mauger et al. (1985) showed that the initial rate of ^{45}Ca uptake by hepatocytes was stimulated by glucagon, and Staddon and Hansford (1986) using the Ca^{2+} -free/ Ca^{2+} re-admission technique with fura2-loaded hepatocytes also found that glucagon could stimulate Ca^{2+} inflow. More recently, Bygrave et al. (1993a), using the same technique, have confirmed and extended the finding of Staddon and Hansford (1986) to show that the effects of glucagon on Ca^{2+} inflow are already maximal by 5–10 s after its addition to hepatocytes and therefore are far more rapid in onset than those on mobilization of intracellular Ca^{2+} pools. In this latter study, dibutyl cyclic AMP could reproduce the effects on Ca^{2+} inflow seen with glucagon.

POSSIBLE LOCI IN THE SIGNALLING TRANSDUCTION PATHWAYS WHERE CROSS-TALK MIGHT TAKE PLACE AND THE RELATED ISSUE OF WHETHER GLUCAGON IS ABLE TO GENERATE $\text{Ins}(1,4,5)\text{P}_3$ IN THE ABSENCE OR PRESENCE OF Ca^{2+} -MOBILIZING AGONISTS

As we have seen above, there is mounting evidence that practically from the moment of its administration to a suspension of hepatocytes, glucagon itself is able to rapidly induce the mobilization of Ca^{2+} . Such rapidity of action raises the issue of the extent to which cross-talk might be taking place at or near the level of the G proteins that couple receptors to various effector molecules (see Figure 4), or at points distal to the site of the plasma membrane-located agonist-receptor interactions (Figure 5). It will also have become apparent that one potential mechanism whereby glucagon could influence the action of Ca^{2+} -mobilizing agonists is through altering the ability of the cell to generate $\text{Ins}(1,4,5)\text{P}_3$.

Earlier studies from a number of laboratories indicated that glucagon alone has no significant effect on $\text{Ins}(1,4,5)\text{P}_3$ generation in hepatocytes (Prpic et al., 1982; Kaibuchi et al., 1982; Charest et al., 1985b) and fails to increase $\text{Ins}(1,4,5)\text{P}_3$ breakdown (Creba et al., 1983). Moreover, Poggioli et al. (1986) and Williamson

and Hansen (1987) showed that glucagon, while enhancing adrenaline-induced Ca^{2+} inflow in hepatocytes, had no effect on the generation of $\text{Ins}(1,4,5)\text{P}_3$. Burgess et al. (1986, 1991) found (like Cocks et al., 1984) that isoprenaline administration to guinea pig hepatocytes caused little, if any, increase in $\text{Ins}(1,4,5)\text{P}_3$ formation in the absence or presence of angiotensin II.

As will now be discussed, this issue has been re-opened by several sets of data. First, Wakelam et al. (1986) have provided evidence that glucagon is able to activate two signal-transduction systems in hepatocytes depending on the agonist concentrations employed. At low (physiological; K_a approx. 0.25 nM) concentrations, glucagon activates a system that leads to the generation of inositol phosphates and little, if any, cyclic AMP. Adenylate cyclase is activated only at higher concentrations ($> 10^{-9}$ M) with concomitant production of cyclic AMP. Wakelam et al. (1986) also showed that by 5 s after administration of glucagon to hepatocytes, $\text{Ins}(1,4,5)\text{P}_3$ is already increased by some 15%, by 10 s it is maximally generated (by some 35% over basal) and by 30 s it has returned to near-basal levels. The amount of $\text{Ins}(1,4,5)\text{P}_3$ generated was, however, considerably less than that generated by the action of α_1 -adrenoceptors or by vasopressin.

Secondly, vom Dahl et al. (1988), working with the perfused rat liver, showed that while vasopressin and glucagon each were able to promote $[\text{H}^3]\text{inositol}$ release from the liver of a rat previously administered $[\text{H}^3]\text{inositol}$, with vasopressin the response was Li-sensitive but with glucagon it was Li-insensitive.

Thirdly, and consistent with the conclusions of Wakelam et al. (1986), Mine et al. (1988b) have shown that the cytoplasmic $[\text{Ca}^{2+}]$ can be increased following glucagon administration to aequorin-loaded rat hepatocytes, through an action apparently largely independent of cyclic AMP generation. The action of glucagon was very rapid in onset, the response was transient, and the increase in cytoplasmic $[\text{Ca}^{2+}]$ was accompanied by only a very small elevation of cyclic AMP. Mine et al. (1988b) concluded that at least part of the action of glucagon on Ca^{2+} mobilization, especially stimulation of Ca^{2+} inflow, is a process that occurs independently of cyclic AMP generation.

Fourthly, Unson et al. (1989) have described the biological properties of the glucagon antagonist des-His¹-[Glu²⁷]glucagon amide. This analogue bound to liver membranes but did not stimulate cyclic AMP production. It did, however, activate a pathway that led to the generation of inositol phosphates. The authors concluded that either there are two distinct glucagon receptors on the hepatocyte plasma membrane, or there is one binding site but two G proteins.

Finally, a group of recent reports have appeared that concern the cloning and expression of the glucagon receptor and other closely related receptors. Jelinek et al. (1993) recently described the cloning, expression and signalling properties of the rat glucagon receptor. The cloned receptor was reported to increase intracellular cyclic AMP following the binding of glucagon. Moreover, and most relevant to the present discussion, it transduced a signal that led to a very rapid elevation (i.e. within seconds) of the cytoplasmic $[\text{Ca}^{2+}]$. Thus the cloned glucagon receptor is able to transduce signals that lead to the accumulation of two different second messengers, cyclic AMP and Ca^{2+} . In a related study, Abou-Samra et al. (1992) reported the cloning and expression of the parathyroid hormone receptor. Upon activation, both adenylate cyclase and phospholipase C were stimulated. This led to intracellular accumulation of cyclic AMP and inositol trisphosphates with concomitant rapid increases in the cytoplasmic $[\text{Ca}^{2+}]$ (Abou-Samra et al., 1992). The calcitonin receptor also has been cloned and expressed (Chabre et al., 1992). Upon activation, it too stimulated the generation of cyclic

Figure 4 Possible interactions associated with the hepatocyte plasma membrane that might be involved in the cross-talk between the actions of glucagon and agonists that mobilize Ca^{2+}

The interaction of glucagon or the agonist (both indicated by A) with its receptor (R) activates a G protein (shown in I). This activated G protein, located in the plasma membrane, in turn produces a range of possible effects on the effector molecules shown (see II) that lead directly or indirectly to modulation of Ca^{2+} fluxes (indicated by the broken lines). The membrane environment would provide rapid and sensitive interactions without the relatively slow diffusion of generated messengers through the cytoplasmic medium. α , β and γ are subunits of the G protein located in the plasma membrane. AC, adenylate cyclase; PLC, phospholipase C; cAMP, cyclic AMP. For further explanation, see the text.

AMP and inositol trisphosphates, again with rapid increases in intracellular $[\text{Ca}^{2+}]$.

Thus all three of these closely related receptors are able to be activated in such a way that the individual (single) receptor efficiently couples to multiple effector systems, i.e. those leading to generation of both cyclic AMP and inositol trisphosphates. In the light of this, it is of some interest that Yamaguchi (1991) showed that calcitonin was able to stimulate basal Ca^{2+} inflow in rat hepatocytes as well as potentiate adrenaline-induced Ca^{2+} inflow.

All of these observations thus raise the issue of the nature of the locus/loci at which the signals generated by glucagon and by Ca^{2+} -mobilizing agonists interact so as to permit cross-talk. A number of articles have appeared reviewing the evidence for the role of G proteins in signal transduction (see, e.g., Freissmuth et al., 1989; Taylor, 1990; Simon et al., 1991; Cockcroft and Thomas, 1992; Milligan, 1992) and in coupling to ion channel pathways (Brown, 1991). What is clear is that G proteins allow for great diversity in signal transduction, acting as switches by being able to rapidly activate or desensitize effector molecules. Moreover, a single receptor can in principle activate multiple G protein molecules, thus amplifying the ligand binding event (Simon et al.,

1991). Similar G proteins may also be able to generate signals with different time constants.

Cross-activation, it has been suggested (Taylor, 1990; Simon et al., 1991), can be an essential part of the G-protein-mediated information-transducing circuit, co-ordinating and integrating signal cross-talk. In situations where a G protein is closely linked spatially with an ion channel in a membrane, there is an increased likelihood of an increase in the time constant (Brown, 1991). Thus directly coupled G protein-ion channel pathways have faster time constants and new steady states are reached more quickly.

All of these events can be further modulated in turn by the actions of cyclic AMP-dependent protein kinases and by diacylglycerol and protein kinase C. Phosphorylation of both the G protein receptors and the G proteins themselves (see Brown, 1991) will add to the possibilities for amplification, memory, changes in sensitivity and sharing of pathways in signal transduction (Taylor, 1990). In this context, the report of Dasso and Taylor (1992) in which evidence was provided that α_1 -adrenoceptors and V_1 -vasopressin receptors share the same pool of G proteins in rat hepatocytes is of interest. Also relevant to this general issue is the extent to which any cyclic AMP generated

Figure 5 Scheme illustrating current knowledge of the effects of glucagon or elevated cyclic AMP on events affecting Ca^{2+} mobilization in liver cells

Three Ca^{2+} inflow pathways in the plasma membrane are shown: I, the pathway activated as a result of depletion of the intracellular Ca^{2+} stores; II, the pathway activated by direct action of glucagon or cyclic AMP; and III, the putative receptor (vasopressin)-operated pathway (this pathway is shared by Mn^{2+} and Ca^{2+}). Three pools of endoplasmic reticular Ca^{2+} are shown; one discharged by $\text{Ins}(1,4,5)\text{P}_3$, one discharged by cyclic AMP action and a third insensitive to known Ca^{2+} -discharging messengers. Although for clarity separate pumps are shown for each pool, a common active uptake transport system may operate. This system(s) is activated by cyclic AMP. X represents the putative messenger generated by the emptying of intracellular agonist-sensitive stores; + or - represent activation or inhibition respectively by glucagon or elevated intracellular cyclic AMP of the pathway in question; * represents the event in which glucagon sensitizes Ca^{2+} -mobilizing hormones to their receptors.

through glucagon action is metabolized by cyclic AMP phosphodiesterases and the degree to which adenylate cyclase itself is activated/desensitized (Houslay et al., 1992). Another factor having a potentially critical bearing on this is the extent to which intracellular cyclic AMP is free or is bound to intracellular ligands (see, e.g., Sammak et al., 1992). Many of these points are collected together in the scheme shown in Figure 4.

Two further points can now be made concerning the locus/loci at which signalling cross-talk occurs. The first is that it is clear that cyclic AMP can potentiate Ca^{2+} mobilization induced by Ca^{2+} -mobilizing agonists at loci distal to the point at which the second messengers are generated (see Figure 5). It is also clear that in the bulk of the experiments showing this, a relatively long time (> 60 s) was usually allowed to elapse between additions of glucagon/dibutyl cyclic AMP/forskolin and the Ca^{2+} -mobilizing agonist. It is possible that the rapid responses to glucagon action (i.e. those occurring within say 5 s) may result not from the generation of cyclic AMP, but from the generation of $\text{Ins}(1,4,5)\text{P}_3$ or some other, as yet unidentified, factor. Thus not only could the glucagon concentration used be important in determining the second messenger produced, but also the time at which measurements are made may have an effect: longer times of glucagon action may provide information different to that produced at shorter times, especially considering the rapid turnover of $\text{Ins}(1,4,5)\text{P}_3$ generated by low glucagon concentrations (Wakelam et al., 1986). Whether or not this turns out to be a crucial issue remains to be determined.

In principle, cross-talk between the second messenger-transducing systems activated by glucagon and by Ca^{2+} -mobilizing agonists could occur before agonist-induced generation of $\text{Ins}(1,4,5)\text{P}_3$, or following the production of $\text{Ins}(1,4,5)\text{P}_3$, or at both sets of loci. It seems reasonable to suggest that, in those

experimental situations where intracellular cyclic AMP was elevated (by forskolin or via the membrane-permeant dibutyl cyclic AMP) and synergism resulted, the interaction is one that is downstream of cyclic AMP and of $\text{Ins}(1,4,5)\text{P}_3$ formation.

A number of authors (Altin and Bygrave, 1986; Poggioli et al., 1986; Mauger and Claret, 1988; Kass et al., 1990) have expressed the view that the synergistic action of glucagon and Ca^{2+} -mobilizing agonists occurs close to Ca^{2+} channels or on the carriers themselves located at the plasma membrane. The suggestions are that control might be effected through cyclic AMP perhaps phosphorylating Ca^{2+} channels and/or (Poggioli et al., 1986) through some product of $\text{Ins}(1,4,5)\text{P}_3$ metabolism. One other possible area where control by cyclic AMP could be exerted on $\text{Ins}(1,4,5)\text{P}_3$ -mediated Ca^{2+} mobilization is at the level of the $\text{Ins}(1,4,5)\text{P}_3$ receptor. In this respect Mauger et al. (1989) have described an $\text{Ins}(1,4,5)\text{P}_3$ receptor from liver membranes with high and low affinity binding sites. In their view, vasopressin increased the number of high affinity binding sites, and phosphorylation (by dibutyl cyclic AMP) through cyclic AMP-dependent protein kinase inhibited the effects of vasopressin.

Burgess et al. (1991) showed that in permeabilized guinea pig hepatocytes, cyclic AMP-dependent protein kinase can phosphorylate the $\text{Ins}(1,4,5)\text{P}_3$ receptor/channel on the endoplasmic reticulum, although, as the authors point out, the outcome of this phosphorylation is not yet clear. They found the action of cyclic AMP-dependent protein kinase to have at least two consequences on $\text{Ins}(1,4,5)\text{P}_3$ -induced Ca^{2+} release; one to increase the total amount of Ca^{2+} released and the other to reduce the value of the EC_{50} for $\text{Ins}(1,4,5)\text{P}_3$ -induced Ca^{2+} release from approx. 0.7 mM to approx. 0.14 mM. Each of these groups (Mauger et al., 1989; Burgess et al., 1991) thus have

suggested that the cross-talk between glucagon and Ca^{2+} -mobilizing agonists that results in potentiated Ca^{2+} mobilization occurs at the endoplasmic reticulum.

The other possibility, that cross-talk occurs at or before hormone-induced phosphatidylinositol metabolism, has been proposed by Blackmore and Exton (1986). The results of their experiments, in which the effects of 4β -phorbol 12-myristate 13-acetate and aluminium fluoride on Ca^{2+} mobilization in rat hepatocytes were examined, led them to suggest that the mobilization of Ca^{2+} , induced by glucagon, results from cyclic AMP-dependent phosphorylation of Np, phosphatidylinositol bisphosphate, or the event leading to $\text{Ins}(1,4,5)\text{P}_3$ formation.

In contrast to some of the reports mentioned above, Pittner and Fain (1989) observed a synergistic increase of $\text{Ins}(1,4,5)\text{P}_3$ formation when bromocyclic AMP was administered to rat hepatocytes with vasopressin. They suggested, not dissimilar to Blackmore and Exton (1986), sites of action of elevated cyclic AMP as possibly being one or other of the vasopressin receptor, the Gp (Np) protein or the phospholipase C enzyme itself.

While not directly related to the issue of the mechanism of synergism between glucagon and agonist-induced Ca^{2+} mobilization, it is relevant to point out that Hughes et al. (1987) reported that a GTP-binding protein is involved in the process by which vasopressin and EGF stimulate receptor-operated Ca^{2+} inflow transporters (see also Barritt and Hughes, 1991). EGF does not increase $\text{Ins}(1,4,5)\text{P}_3$ formation, and pertussis toxin treatment (which blocks the action of G proteins) of rat hepatocytes abolished EGF- and vasopressin-induced Ca^{2+} inflow. Also relevant are the reports showing that 4β -phorbol 12-myristate 13-acetate treatment of hepatocytes, which leads to activation of protein kinase C, attenuates the Ca^{2+} -mobilizing action of glucagon (Staddon and Hansford, 1986; see also Garcia-Sainz et al., 1985; Staddon and Hansford, 1989; Pittner and Fain, 1991). Whether some of the signals being generated here result from hormone-induced phosphatidylcholine breakdown (Exton, 1990) remains an interesting possibility.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

This review has considered a number of observations, obtained from studies both with the whole organ (perfused rat liver) and with hepatocytes isolated therefrom, concerning the interaction of the second messenger-mediated signalling systems originating at the plasma membrane and for which glucagon and Ca^{2+} -mobilizing agonists are the stimuli. A number of experimental approaches and techniques, combined with the deployment of a range of pharmacological agents, have already been brought into play to examine the signalling cross-talk phenomenon. Apart from the perfused rat liver system, these include the increasingly widespread use of single-cell systems to examine the influence of various hormone regimes on Ca^{2+} mobilization in oscillatory phenomena (e.g. Schoff et al., 1991), in computer-assisted mathematical modelling (e.g. Somogyi and Stucki, 1991) and following the application of microinjection techniques (e.g. Glennon et al., 1992). All of these approaches will continue to provide relevant information and, in combination with the cloning techniques now available, results of some of which were mentioned above, exciting new insights into signalling cross-talk are assured. Two other recent developments with great potential for the present study are noteworthy. One is the ability to express a Ca^{2+} -mobilizing receptor, such as the V_1 -vasopressin receptor cloned from rat liver, in *Xenopus* oocytes (Nathanson et al., 1992) and to then examine the subcellular distribution of Ca^{2+} signals by confocal microscopy following activation of the receptor. A second is the ability to target recombinant aequorin into mito-

chondria and observe the changes specifically in mitochondrial $[\text{Ca}^{2+}]$ in response to various stimuli (Rizzuto et al., 1992). No doubt all of this will be further aided by the development of even more diverse indicators including those designed to measure rapid changes in intracellular cyclic AMP (Tsien, 1992; Sammak et al., 1992).

While each of the experimental systems (whole organ and single cells) offers particular attributes to investigations of cross-talk, there is a remarkable similarity and confluence among the data already obtained. The integration of the information gained from each is providing considerable information in turn about the nature of the synergistic response. The view previously expressed in a number of reports, i.e. that enhanced mobilization and especially Ca^{2+} inflow is a major target for cross-talk between these signalling systems, has been reinforced. An interesting facet of hormone-induced Ca^{2+} inflow, not often considered, is that irrespective of what combination of hormones is present, Ca^{2+} inflow will not take place unless a substantial Ca^{2+} gradient exists across the plasma membrane.

The major features of the synergism so far revealed (see Figure 5) are (1) that it can occur at very low (physiological) concentrations of each of the two sets of hormones, (2) that responses are able to be induced which are very rapid in onset, and (3) that the earliest response, which is practically immediate, appears to be Ca^{2+} inflow; only after some 30 s is glucagon able to mobilize Ca^{2+} apparently from the same internal Ca^{2+} store as that mobilized by vasopressin; this is non-mitochondrial and most probably endoplasmic reticular in origin. Just as there appear to be $\text{Ins}(1,4,5)\text{P}_3$ -sensitive and -insensitive Ca^{2+} pools in the endoplasmic reticulum, so one might also envisage glucagon-sensitive and -insensitive pools in the same organelle, all of which are in some way functionally interactive.

Despite these revelations, the mechanism by which cross-talk between the two second messenger-mediated signalling systems occurs to bring about the potentiation in Ca^{2+} mobilization is still largely unknown. Data from a number of separate reports, all examining different aspects of glucagon action as well the molecular biology of the glucagon and other closely related receptors, provide strong evidence for Ca^{2+} mobilization induced by glucagon alone. This apparently occurs not through the generation of cyclic AMP, but rather through the generation of $\text{Ins}(1,4,5)\text{P}_3$, albeit at lower concentrations than those generated by 'classical' Ca^{2+} -mobilizing agonists such as α_1 -adrenergic agonists, vasopressin and angiotensin. The extent to which this action of glucagon (at low concentrations) contributes to the cross-talk phenomenon remains to be addressed, as does the question of whether this in turn is a component of the mechanism by which glucagon mobilizes Ca^{2+} from intracellular Ca^{2+} stores. Recent findings that activation of the cloned glucagon receptor results in Ca^{2+} mobilization independent of cyclic AMP production but with generation of $\text{Ins}(1,4,5)\text{P}_3$ is particularly intriguing and raises the question of the extent to which G proteins might play a part in cross-talk.

Suggested intracellular sites of action of glucagon are the Ca^{2+} transporters in mitochondria (Morgan et al., 1985), in the endoplasmic reticulum (Burgess et al., 1986; Mauger et al., 1989) and in the plasma membrane (Poggioli et al., 1986; Altin and Bygrave, 1986; Kass et al., 1990). It is possible that all sites are modified but perhaps to different extents and at different times following glucagon administration. Whilst glucagon action is known to be able to lead to the phosphorylation of cytoplasmic proteins (Garrison and Borland, 1977) including the multi-functional Ca^{2+} /calmodulin-dependent protein kinase (Connelly et al., 1987) as well as membrane proteins (Vargas et al., 1982), it is not yet established if any of these are components of, or are

in any way associated with, the above-mentioned Ca^{2+} transport systems. Presumably details about possible phosphorylation events will become clearer once the molecular architecture of these Ca^{2+} transporters/channels becomes known.

Another area of recent study in which the outcome is of potential interest is the hormonal regulation of the $\text{Ins}(1,4,5)\text{P}_3$ receptor. It is known that the receptor can be phosphorylated by a cyclic AMP-dependent protein kinase resulting in a decrease in the ability of $\text{Ins}(1,4,5)\text{P}_3$ to release Ca^{2+} from microsomal stores (Ferris et al., 1991). It has been suggested (Mauger et al., 1989) that in liver such a mechanism could participate in the synergistic action of cyclic AMP and $\text{Ins}(1,4,5)\text{P}_3$ -dependent hormones on Ca^{2+} mobilization. The extent to which this occurs remains to be assessed.

Finally, one might well question the physiological significance of the cross-talk phenomenon in liver. Clearly much of the current research in liver is focused on mechanisms at the cellular and subcellular level. A role for cross-talk between second messenger-generated signalling systems leading to modulated Ca^{2+} fluxes is seen to be crucial, for instance in excitation-contraction coupling in cardiac (see, e.g., Cohen, 1992) and smooth muscle (see, e.g., Abdel-Latif, 1991) cells, and in the rapid secretion of insulin (e.g. Holz and Habener, 1992) and saliva (e.g. McKinney et al., 1989), all of which are well established Ca^{2+} -dependent phenomena. We envisage that one of the roles of cross-talk between the two signalling systems in liver, described in this review, is to amplify and/or sensitize signals of which Ca^{2+} is a vital component. One physiological function in liver recognized only very recently as being subject to rapid, short-term modulation by glucagon and Ca^{2+} -mobilizing hormones is bile secretion (Bygrave et al., 1993b). This group has found that the ability of vasopressin to rapidly (in a time-frame of seconds) alter bile flow in the perfused rat liver is greatly modified when glucagon is preadministered also for only a brief time (Hamada et al., 1992a). This synergistic response, which is induced by physiological concentrations of the agonists, is modulated in turn by the action of cholestatic and choleretic bile salts administered *in vitro* (Hamada et al., 1992b) and by agents that induce cholestasis *in vivo* (Bygrave et al., 1993b). All of these features should provide a useful set of probes with which one can gain further insights in liver, into a genuine physiological system modulated by cross-talk between the actions of glucagon and Ca^{2+} -mobilizing agonists.

F. L. B. is grateful to Dr. Alan Dawson (University of East Anglia), Drs Juan Llopis and Georges Kass (Karolinska Institutet) and to the editorial referees of the Biochemical Journal for helpful comments and suggestions.

REFERENCES

- Abdel-Latif, A. A. (1991) *Cell. Signalling* **3**, 371–385
- Abou-Samra, A.-B., Juppner, H., Force, T., Freeman, M. W., Kong, X.-F., Schipani, E., Urena, P., Richards, J., Bonventre, J. V., Potts, J. T., Jr., Kronenberg, H. M. and Segre, G. V. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 2732–2736
- Åkerman, K. E. O. and Nicholls, D. G. (1983) *Rev. Physiol. Biochem. Pharmacol.* **95**, 149–201
- Althaus-Salzmann, M., Carafoli, E. and Jacob, A. (1980) *Eur. J. Biochem.* **106**, 241–248
- Altin, J. G. and Bygrave, F. L. (1985) *Biochem. J.* **232**, 911–917
- Altin, J. G. and Bygrave, F. L. (1986) *Biochem. J.* **238**, 653–661
- Altin, J. G. and Bygrave, F. L. (1987a) *Biochem. J.* **242**, 43–50
- Altin, J. G. and Bygrave, F. L. (1987b) *Biochem. J.* **247**, 613–619
- Altin, J. G. and Bygrave, F. L. (1987c) *Biochem. Biophys. Res. Commun.* **142**, 745–753
- Altin, J. G. and Bygrave, F. L. (1988) *Biol. Rev.* **63**, 551–611
- Andia Waltenbaugh, A.-M., Tate, C. and Friedmann, N. (1981) *Mol. Cell Biochem.* **36**, 177–184
- Assimakopoulos-Jeannot, F. D., Blackmore, P. F. and Exton, J. H. (1977) *J. Biol. Chem.* **252**, 2662–2669
- Assimakopoulos-Jeannot, F. D., Blackmore, P. F. and Exton, J. H. (1982) *J. Biol. Chem.* **257**, 3759–3765
- Assimakopoulos-Jeannot, F. D., McCormack, J. G. and Jeanrenaud, B. (1986) *J. Biol. Chem.* **261**, 8799–8804
- Banhegyi, G., Fulceri, R., Bellomo, G., Romani, A., Pompella, A. and Benedetti, A. (1991) *Arch. Biochem. Biophys.* **287**, 320–328
- Barritt, G. J. (1992) *Communication Within Animal Cells*, Oxford University Press, Oxford
- Barritt, G. J. and Hughes, B. P. (1991) *Cell. Signalling* **3**, 283–292
- Barritt, G. J., Parker, J. C. and Wadsworth, J. C. (1981) *J. Physiol. (London)* **312**, 29–55
- Benedetti, A., Fulceri, R. and Comporti, M. (1985) *Biochim. Biophys. Acta* **816**, 267–277
- Benedetti, A., Fulceri, R., Ferro, M. and Comporti, M. (1986) *Trends Biochem. Sci.* **11**, 284–285
- Benedetti, A., Fulceri, R., Romani, A. and Comporti, M. (1987) *Biochim. Biophys. Acta* **928**, 282–286
- Benedetti, A., Fulceri, R., Romani, A. and Comporti, M. (1988) *J. Biol. Chem.* **263**, 3466–3473
- Benedetti, A., Graf, P., Fulceri, R., Romani, A. and Sies, H. (1989) *Biochem. Pharmacol.* **38**, 1799–1805
- Berridge, M. J. (1987) *Annu. Rev. Biochem.* **56**, 159–193
- Berry, M. N., Edwards, A. M. and Barritt, G. J. (1991) in *Isolated Hepatocytes: Preparation, Properties and Applications*, pp. 201–236, Elsevier, Amsterdam
- Blackmore, P. F. and Exton, J. H. (1986) *J. Biol. Chem.* **261**, 11056–11063
- Blackmore, P. F., Brumley, F. T., Marks, J. L. and Exton, J. H. (1978) *J. Biol. Chem.* **253**, 4851–4858
- Blackmore, P. F., Waynick, L. E., Blackman, G. E., Graham, C. W. and Sherry, R. S. (1984) *J. Biol. Chem.* **259**, 12322–12325
- Bond, M., Vadasz, G., Somlyo, A. V. and Somlyo, A. P. (1987) *J. Biol. Chem.* **262**, 15630–15636
- Borle, A. B. (1972) *J. Membr. Biol.* **10**, 45–66
- Borle, A. B. (1981) *Rev. Physiol. Biochem. Pharmacol.* **90**, 13–153
- Borle, A. B. and Snowdowne, K. W. (1986) *Methods Enzymol.* **124**, 90–116
- Brown, A. M. (1991) *FASEB J.* **5**, 2175–2179
- Buhler, H. U., DaPrada, M., Haefely, W. and Picotti, G. B. (1978) *J. Physiol. (London)* **276**, 311–320
- Burgess, G. M., Dooley, R. K., McKinney, J. S., Nanberg, E. and Putney, J. W., Jr. (1986) *Mol. Pharmacol.* **30**, 315–320
- Burgess, G. M., Bird, G. S. J., Obie, J. F. and Putney, J. W., Jr. (1991) *J. Biol. Chem.* **266**, 4772–4781
- Bygrave, F. L. (1966) *Biochem. J.* **101**, 488–494
- Bygrave, F. L. (1967) *Nature (London)* **214**, 667–671
- Bygrave, F. L. (1978a) *Biol. Rev.* **53**, 43–79
- Bygrave, F. L. (1978b) *Trends Biochem. Sci.* **3**, 175–178
- Bygrave, F. L. and Tranter, C. J. (1978) *Biochem. J.* **174**, 1021–1030
- Bygrave, F. L., Lenton, L., Altin, J. G., Setchell, B. and Karjalainen, A. (1990) *Biochem. J.* **267**, 69–73
- Bygrave, F. L., Gamberucci, A., Fulceri, R. and Benedetti, A. (1993a) *Biochem. J.* **292**, 19–22
- Bygrave, F. L., Karjalainen, A. and Hamada, Y. (1993b) *Cell. Signalling*, in the press
- Capiod, T., Noel, J., Combettes, L. and Claret, M. (1991) *Biochem. J.* **275**, 277–280
- Chabre, O., Conklin, B. R., Lin, H. Y., Lodish, H. F., Wilson, E., Ives, H. E., Catanzariti, L., Hemmings, B. A. and Bourne, H. R. (1992) *Mol. Endocrinol.* **6**, 551–556
- Charest, R., Blackmore, P. F., Berthon, B. and Exton, J. H. (1983) *J. Biol. Chem.* **258**, 8769–8773
- Charest, R., Blackmore, P. F. and Exton, J. H. (1985a) *J. Biol. Chem.* **260**, 15789–15794
- Charest, R., Prpic, V., Blackmore, P. F. and Exton, J. H. (1985b) *Biochem. J.* **227**, 79–90
- Claret-Berthon, B., Claret, M. and Mazet, J. L. (1977) *J. Physiol. (London)* **272**, 529–552
- Cockcroft, S. and Thomas, G. M. H. (1992) *Biochem. J.* **288**, 1–14
- Cocks, T. M., Jenkinson, D. H. and Koller, K. (1984) *Br. J. Pharmacol.* **83**, 281–291
- Cohen, P. (1992) *Trends Biochem. Sci.* **17**, 408–413
- Combettes, L., Berthon, B., Binet, A. and Claret, M. (1986) *Biochem. J.* **237**, 675–683
- Comerford, J. G. and Dawson, A. P. (1993) *Biochem. J.* **289**, 561–567
- Connelly, P. A., Sisk, R. B., Schulman, H. and Garrison, J. C. (1987) *J. Biol. Chem.* **262**, 10154–10163
- Crane, J. K., Campanile, C. P. and Garrison, J. C. (1982) *J. Biol. Chem.* **257**, 4959–4965
- Creba, J. A., Downes, C. P., Hawkins, P. T., Brewster, G., Michell, R. H. and Kirk, C. J. (1983) *Biochem. J.* **212**, 733–747
- Crofts, J. N. and Barritt, G. J. (1989) *Biochem. J.* **264**, 61–70
- Crofts, J. N. and Barritt, G. J. (1990) *Biochem. J.* **269**, 579–587
- Dasso, L. L. T. and Taylor, C. W. (1992) *Mol. Pharmacol.* **42**, 453–457
- Denton, R. M. and McCormack, J. G. (1985) *Am. J. Physiol.* **249**, E543–E554
- Eigler, W., Sacca, L. and Sherwin, R. (1979) *J. Clin. Invest.* **63**, 114–123
- Epping, R. J. and Bygrave, F. L. (1984) *Biochem. J.* **223**, 735–745
- Exton, J. H. (1975) *Methods Enzymol.* **39**, 25–36
- Exton, J. H. (1981) *Mol. Cell. Endocrinol.* **23**, 233–264
- Exton, J. H. (1985) *Am. J. Physiol.* **248**, E633–E647

- Exton, J. H. (1988) *Rev. Physiol. Biochem. Pharmacol.* **111**, 117–224
- Exton, J. H. (1990) *J. Biol. Chem.* **265**, 1–4
- Ferris, C. D., Cameron, A. M., Bredt, D. S., Haganir, R. L. and Snyder, S. H. (1991) *Biochem. Biophys. Res. Commun.* **175**, 192–198
- Freissmuth, M., Casey, P. J. and Gilman A. G. (1989) *FASEB J.* **3**, 2125–2131
- Friedmann, N. (1972) *Biochim. Biophys. Acta* **274**, 215–225
- Friedmann, N. and Dambach, G. (1973) *Biochim. Biophys. Acta* **307**, 339–403
- Friedmann, N. and Park, C. R. (1968) *Proc. Nat. Acad. Sci. U.S.A.* **61**, 504–508
- Fulceri, R., Bellomo, G., Gamberucci, A. and Benedetti, A. (1990) *Biochem. J.* **272**, 549–552
- Fulceri, R., Bellomo, G., Mirabelli, F., Gamberucci, A. and Benedetti, A. (1991) *Cell Calcium* **12**, 431–439
- Garcia-Sainz, J. A., Mendlovic, F. and Martínez-Olmedo, M. A. (1985) *Biochem. J.* **228**, 277–280
- Garrison, J. C. and Borland, M. K. (1977) *J. Biol. Chem.* **253**, 7091–7100
- Gevers, W. and Krebs, H. A. (1966) *Biochem. J.* **98**, 720–735
- Glennon, M. C., Bird, G. S. J., Kwan, C.-Y. and Putney, J. W., Jr. (1992) *J. Biol. Chem.* **267**, 8230–8233
- Graf, P., vom Dahl, S. and Sies, H. (1987) *Biochem. J.* **241**, 933–936
- Hamada, Y., Karjalainen, A., Setchell, B. A., Millard, J. E. and Bygrave, F. L. (1992a) *Biochem. J.* **281**, 387–392
- Hamada, Y., Karjalainen, A., Setchell, B. A., Millard, J. E. and Bygrave, F. L. (1992b) *Biochem. J.* **283**, 575–581
- Hansen, C. A., Yang, L. and Williamson, J. R. (1991) *J. Biol. Chem.* **266**, 18573–18579
- Heilbrunn, L. V. (1952) *An Outline of General Physiology*, 3rd edn., Saunders, Philadelphia
- Holz, G. G. and Habener, J. F. (1992) *Trends Biochem. Sci.* **17**, 388–393
- Houslay, M. D., Griffiths, S. L., Horton, Y. M., Livingstone, C., Lobban, M., Macdonald, F., Morris, N., Pryde, J., Scotland, G., Shakur, Y., Sweeney, G. and Tang, E. K. Y. (1992) *Biochem. Soc. Trans.* **20**, 140–146
- Hughes, B. P., Milton, S. E. and Barritt, G. J. (1986) *Biochem. J.* **238**, 793–800
- Hughes, B. P., Crofts, J. N., Auld, A. M., Read, L. C. and Barritt, G. J. (1987) *Biochem. J.* **248**, 911–918
- Irvine, R. F. (1992) *FASEB J.* **6**, 3085–3091
- Jelinek, L. J., Lok, S., Rosenberg, G. B., Smith, R. A., Grant, F. J., Biggs, S., Bensch, P. A., Kuijper, J. L., Sheppard, P. O., Sprecher, C. A., O'Hara, P. J., Foster, D., Walker, K. M., Chen, L. H. J., McKernan, P. A. and Kindsvogel, W. (1993) *Science* **259**, 1614–1616
- Jenkinson, D. H. and Koller, K. (1977) *Br. J. Pharmacol.* **59**, 163–175
- Joseph, S. K., Coll, K. E., Thomas, A. P., Rubin, R. and Williamson, J. R. (1985) *J. Biol. Chem.* **260**, 12508–12515
- Jouneaux, C., Audiger, Y., Goldsmith, P., Pecker, F. and Lotersztajn, S. (1993) *J. Biol. Chem.* **268**, 2368–2372
- Kaibuchi, K., Takai, Y., Ogawa, Y., Kimura, S., Nishizuka, Y., Nakamura, T., Tonomaru, A. and Ichihara, A. (1982) *Biochem. Biophys. Res. Commun.* **104**, 105–112
- Kass, G. E. N., Llopis, J., Chow, S. C., Duddy, S. K. and Orrenius, S. (1990) *J. Biol. Chem.* **265**, 17486–17492
- Keppens, S. and de Wulf, H. (1977) *Biochim. Biophys. Acta* **588**, 63–69
- Keppens, S. and de Wulf, H. (1984) *Biochem. J.* **222**, 277–280
- Keppens, S., Vandenheede, J. R. and de Wulf, H. (1977) *Biochim. Biophys. Acta* **496**, 448–457
- Kimura, S., Kugai, N., Tada, R., Kojima, I., Abe, K. and Ogata, E. (1982) *Horm. Metab. Res.* **14**, 133–138
- Kleineke, J. and Soling, H.-D. (1985) *J. Biol. Chem.* **260**, 1040–1045
- Kraus-Friedmann, N. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8943–8946
- Lehninger, A. L. (1950) *Physiol. Rev.* **30**, 393–429
- Lehninger, A. L., Carafoli, E. and Rossi, C. S. (1967) *Adv. Enzymol.* **29**, 259–320
- Levine, M. J., Meuse, A. J., Watanabe, J., Bentivegna, L. A., Johnson, R. G., Grossman, W. and Morgan, J. P. (1990) *Biophys. J.* **57**, 173
- Lin, S.-H., Wallace, M. A. and Fain, J. N. (1983) *Endocrinology (Baltimore)* **113**, 2268–2275
- Llopis, J., Kass, G. E. N., Gahm, A. and Orrenius, S. (1992) *Biochem. J.* **284**, 243–247
- Lotersztajn, S., Hanoune, J. and Pecker, F. (1981) *J. Biol. Chem.* **256**, 11209–11215
- Lotersztajn, S., Epand, R., Mallat, A., Pavoine, C. and Pecker, F. (1985) *Biochimie* **67**, 1169–1176
- Lotersztajn, S., Pavoine, C., Deterre, P., Capeau, J., Mallat, A., Le Nguyen, D., Dufour, M., Roust, B., Bataille, D. and Pecker, F. (1992) *J. Biol. Chem.* **267**, 2375–2379
- Mauger, J.-P. and Claret, M. (1986) *FEBS Lett.* **195**, 106–110
- Mauger, J.-P. and Claret, M. (1988) *J. Hepatol.* **7**, 278–282
- Mauger, J.-P., Poggioli, J., Guesdon, F. and Claret, M. (1984) *Biochem. J.* **221**, 121–127
- Mauger, J.-P., Poggioli, J. and Claret, M. (1985) *J. Biol. Chem.* **260**, 11635–11642
- Mauger, J.-P., Claret, M., Pietri, F. and Hilly, M. (1989) *J. Biol. Chem.* **264**, 8821–8826
- McKinney, J. S., Desole, M. S. and Rubin, R. P. (1989) *Am. J. Physiol.* **257**, C651–C657
- Michell, R. W. (1975) *Biochim. Biophys. Acta* **415**, 81–147
- Michell, R. W. (1992) *Trends Biochem. Sci.* **17**, 274–276
- Milligan, G. (1992) *Biochem. Soc. Trans.* **20**, 135–140
- Mine, T., Kojima, I. and Ogata, E. (1988a) *Acta Endocrinol.* **119**, 301–306
- Mine, T., Kojima, I. and Ogata, E. (1988b) *Biochim. Biophys. Acta* **970**, 166–171
- Morgan, N. G., Blackmore, P. F. and Exton, J. H. (1983) *J. Biol. Chem.* **258**, 5110–5116
- Morgan, N. G., Charest, R., Blackmore, P. F. and Exton, J. H. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4208–4212
- Morgan, N. G., Charest, R., Blackmore, P. F. and Exton, J. H. (1985) *Biochem. Soc. Trans.* **13**, 217–218
- Nathanson, M. H., Moyer, M. S., Burgstahler, A. D., O'Carroll, A.-M., Brownstein, M. J. and Lolait, S. J. (1992) *J. Biol. Chem.* **267**, 23282–23289
- Neher, E. (1992) *Nature (London)* **355**, 298–299
- Newsholme, E. A. and Crabtree, B. (1976) *Biochem. Soc. Symp.* **41**, 61–109
- Pittner, R. A. and Fain, J. N. (1989) *Biochem. J.* **257**, 455–460
- Pittner, R. A. and Fain, J. N. (1991) *Biochem. J.* **277**, 371–378
- Poggioli, J., Berthon, B. and Claret, M. (1980) *FEBS Lett.* **115**, 243–246
- Poggioli, J., Mauger, J.-P. and Claret, M. (1986) *Biochem. J.* **235**, 663–669
- Prpic, V., Spencer, T. L. and Bygrave, F. L. (1978) *Biochem. J.* **176**, 705–714
- Prpic, V., Blackmore, P. F. and Exton, J. H. (1982) *J. Biol. Chem.* **257**, 11323–11331
- Putney, J. W., Jr. (1990) *Cell Calcium* **11**, 611–624
- Rashed, H. M. and Patel, T. B. (1987) *J. Biol. Chem.* **262**, 15953–15958
- Rasmussen, H. (1970) *Science* **170**, 404–412
- Rasmussen, H. (1981) *Calcium and cAMP as Synaptic Messengers*, Wiley, New York
- Rasmussen, H. and Barrett, P. Q. (1984) *Physiol. Rev.* **64**, 938–984
- Reinhart, P. H. and Bygrave, F. L. (1981) *Biochem. J.* **194**, 541–549
- Reinhart, P. H., Taylor, W. M. and Bygrave, F. L. (1982) *Biochem. J.* **208**, 619–630
- Reinhart, P. H., van de Pol, E., Taylor, W. M. and Bygrave, F. L. (1984a) *Biochem. J.* **218**, 415–420
- Reinhart, P. H., Taylor, W. M. and Bygrave, F. L. (1984b) *Biochem. J.* **220**, 35–42
- Reinhart, P. H., Taylor, W. M. and Bygrave, F. L. (1984c) *Biochem. J.* **220**, 43–50
- Reinhart, P. H., Taylor, W. M. and Bygrave, F. L. (1984d) *Biochem. J.* **223**, 1–13
- Ringer, S. (1983) *J. Physiol. (London)* **4**, 29–42
- Rizzuto, R., Simpson, A. W. M., Brini, M. and Pozzan T. (1992) *Nature (London)* **358**, 325–327
- Robison, G. A., Butcher, R. W. and Sutherland, E. W. (1971) *Cyclic AMP*, Academic Press, New York
- Rusinko, N. and Lee, H. C. (1989) *J. Biol. Chem.* **264**, 11725–11731
- Sammak, P. J., Adams, S. R., Harootyan, A. T., Schliwa, M. and Tsien, R. Y. (1992) *J. Cell Biol.* **117**, 57–72
- Sanchez-Bueno, A. and Cobbold, P. H. (1993) *Biochem. J.* **291**, 169–172
- Sanchez-Bueno, A., Marrero, I. and Cobbold, P. H. (1993) *Biochem. J.* **291**, 163–168
- Sawanobori, T., Takanishi, H., Hiraoka, M., Iida, Y., Kamisaka, K. and Maezawa, H. (1989) *J. Cell. Physiol.* **139**, 580–585
- Schoff, C., Sanchez-Bueno, A., Brabant, G., Cobbold, P. H. and Cuthbertson, K. S. R. (1991) *Biochem. J.* **273**, 799–802
- Sies, H., Graf, P. and Estrela, J. M. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3358–3362
- Sies, H., Graf, P. and Crane, D. (1983) *Biochem. J.* **212**, 271–278
- Simon, M. I., Strathmann, M. P. and Gautam, N. (1991) *Science* **252**, 802–808
- Sistare, F. D., Picking, R. A. and Haynes, R. C., Jr. (1985) *J. Biol. Chem.* **260**, 12744–12747
- Soboll, S. and Sies, H. (1989) *Methods Enzymol.* **174**, 118–130
- Somogyi, R. and Stucki, J. W. (1991) *J. Biol. Chem.* **266**, 11068–11077
- Somogyi, R., Zhao, M. and Stucki, J. W. (1992) *Biochem. J.* **286**, 869–877
- Staddon, J. M. and Hansford, R. G. (1986) *Biochem. J.* **238**, 737–743
- Staddon, J. M. and Hansford, R. G. (1989) *Eur. J. Biochem.* **179**, 47–52
- Studer, R. K., Snowdowne, K. W. and Borle, A. B. (1984) *J. Biol. Chem.* **259**, 3596–3604
- Taylor, C. W. (1990) *Biochem. J.* **272**, 1–13
- Taylor, W. M., Reinhart, P. H. and Bygrave, F. L. (1983) *Biochem. J.* **212**, 555–565
- Tennes, K. A., McKinney, J. S. and Putney, J. W., Jr. (1987) *Biochem. J.* **242**, 797–802
- Tsien, R. Y. (1992) *Am. J. Physiol.* **263**, C723–C728
- Tsien, R. Y., Pozzan, T. and Rink, T. J. (1982) *J. Cell Biol.* **94**, 325–334
- Unson, C. G., Gurzenda, E. M. and Merrifield, R. B. (1989) *Peptides* **10**, 1171–1177
- Van de Werve, G., Hue, L. and Hers, H.-G. (1977) *Biochem. J.* **162**, 135–142
- Vargas, A. M., Halestrap, A. P. and Denton, R. M. (1982) *Biochem. J.* **208**, 221–229
- vom Dahl, S., Graf, P. and Sies, H. (1988) *Biochem. J.* **251**, 843–848
- Wakelam, M. J. O., Murphy, G. J., Hruby, V. J. and Houslay, M. D. (1986) *Nature (London)* **323**, 68–71
- Williamson, J. R. and Hansen, C. A. (1987) *Biochem. Actions Horm.* **14**, 29–71
- Williamson, J. R., Cooper, R. H. and Hoek, J. B. (1981) *Biochim. Biophys. Acta* **639**, 243–295
- Woods, N. M., Cuthbertson, K. S. R. and Cobbold, P. H. (1986) *Nature (London)* **319**, 600–602
- Woods, N. M., Cuthbertson, K. S. R. and Cobbold, P. H. (1987) *Cell Calcium* **8**, 79–100
- Yamaguchi, M. (1991) *Mol. Cell. Endocrinol.* **75**, 65–70