The Ca^{2+} -mobilizing actions of vasopressin and angiotensin differ from those of the α -adrenergic agonist phenylephrine in the perfused rat liver

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A Ca^{2+} -sensitive electrode was used to study net Ca^{2+} -flux changes induced by the administration of phenylephrine, vasopressin and angiotensin to the perfused rat liver. The studies reveal that, although the Ca^{2+} responses induced by vasopressin and angiotensin are similar, they are quite different from the Ca^{2+} fluxes induced by phenylephrine. The administration of phenylephrine is accompanied by a stimulation of a net amount of Ca^{2+} efflux (140 nmol/g of liver). A re-uptake of a similar amount of Ca^{2+} occurs only after the hormone is removed. In contrast, the administration of vasopressin or angiotensin to livers perfused with 1.3 mM- Ca^{2+} induces the release of a relatively small amount of Ca^{2+} (approx. 40 nmol/g of liver) during the first 60 s. This is followed by a much larger amount of Ca^{2+} uptake (70–140 nmol/g of liver) after 1–2.5 min of hormone administration, and a slow efflux or loss of a similar amount of Ca^{2+} over a period of 6–8 min. At lower concentrations of perfusate Ca^{2+} (less than 600 μM) these hormones induce only a net efflux of the ion. These results suggest that at physiological concentrations of extracellular Ca^{2+} the mechanism by which α -adrenergic agonists mobilize cellular Ca^{2+} is different from that involving vasopressin and angiotensin. It seems that the hormones may have quite diverse effects on Ca^{2+} transport across the plasma membrane and perhaps organellar membranes in liver.

INTRODUCTION

It is well established that the interaction of a number of hormones, neurotransmitters and other external stimuli with appropriate cell-surface receptors in many tissues rapidly mobilize Ca^{2+} so as to increase the cytoplasmic Ca^{2+} concentration and thereby stimulate numerous Ca^{2+} -sensitive physiological responses. Such mobilization of Ca^{2+} appears to involve the generation of one or more intracellular signals, among which inositol 1,4,5-trisphosphate (reviewed by Berridge, 1984; Berridge & Irvine, 1984) and possibly polyamines (Koenig *et al.*, 1983) appear to be candidates.

In the liver tissue, the above-mentioned phenomena have been examined in the main with the Ca^{2+} -mobilizing agents, α -adrenergic agonists, vasopressin and angiotensin. In this regard, it seems to be assumed that each of the receptors for these agents is multi-functional, in that they form part of a common general transducing mechanism for the mobilization of intracellular Ca^{2+} (see, e.g., Exton, 1981; Morgan *et al.*, 1983; Berridge, 1984; Reinhart *et al.*, 1984*b,c*). To our knowledge, however, no systemic study has been undertaken to compare in any detail the Ca^{2+} -flux response induced by α -adrenergic agonists, vasopressin and angiotensin. Furthermore, it appears that progress towards a better understanding of the mechanism of action of these hormones is at present hindered by conflicting evidence in at least two areas: namely, the source or subcellular compartmentation of the intracellular calcium that is mobilized, and the extent to which extracellular Ca^{2+} is involved in triggering the effects of the hormones under physiological conditions (Reinhart *et al.*, 1982, 1984*b*; Mauger *et al.*, 1984; Shears

& Kirk, 1984*a,b*). Also, evidence is appearing to suggest that the mechanism of signal transduction for α -adrenergic agonists is not the same as that of the vasopressin peptides (see, e.g., Dehaye *et al.*, 1981; Corvea *et al.*, 1984; Huerta-Bahena & Garcia-Sainz, 1985). If the assumption is correct that all three receptor types form part of a general transducing mechanism, it is reasonable to predict that the Ca^{2+} responses induced by each would indeed be similar.

We have examined this assumption in the present paper. Specifically, we have used the sensitive Ca^{2+} -selective-electrode technique, previously employed in this laboratory (Reinhart *et al.*, 1982), to compare the abilities of phenylephrine (an α_1 -adrenergic agonist), vasopressin and angiotensin to induce changes in Ca^{2+} fluxes in perfused rat liver over a range of perfusate Ca^{2+} concentrations. Our data provide clear evidence that, although the pattern of Ca^{2+} mobilization induced by vasopressin is similar to but not identical with that by angiotensin, it is quite different from that induced by phenylephrine. The implications this could have for the commonality of the mechanism by which these hormones lead to a rise in the cytosolic Ca^{2+} concentration, and hence to the triggering of physiological responses induced by the hormones in the perfused rat liver (and probably other tissues), are discussed.

EXPERIMENTAL

Animals and perfusions

Male Wistar-strain albino rats weighing 230–270 g and having free access to food were used for all experiments.

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Rats were anaesthetized with sodium pentobarbitone (50 mg/kg body wt.) and the livers perfused with Krebs-Henseleit (1932) bicarbonate buffer equilibrated with O_2/CO_2 (19:1) as described previously (Reinhart *et al.*, 1982). All livers were pre-perfused for 15 min with media containing 1.3 mM added $CaCl_2$ at 35 °C and at a flow rate of 3.5 ml/min per g wet wt. of liver. In many experiments Ca^{2+} was infused by means of an infusor pump directly into the cannula feeding the portal vein to facilitate rapid change of perfusate Ca^{2+} .

Perfusate Ca^{2+} determinations

For most experiments the perfusate Ca^{2+} concentration was continuously monitored exactly as described previously (Reinhart *et al.*, 1982). A Radiometer F2112 Ca^{2+} -selective electrode was placed into a small flow-through chamber, close to the liver, and coupled to a Radiometer K801 (Ag/AgCl) reference electrode via an agarose/KCl salt bridge. Signal amplification was achieved by connecting both electrodes to an Orion model 901 microprocessor ion analyser set to the grounded-solution mode. Output was channelled through a bucking-voltage device (Madeira, 1975) to a Spectra-Physics SP4100 computing integrator which was programmed to display changes in the perfusate Ca^{2+} concentration (peak height) and the total amounts of Ca^{2+} taken up or extruded by the liver (integration mode). Further details are described by Reinhart *et al.* (1982). In the present work the electrical background noise was decreased even further than was accomplished previously (Reinhart *et al.*, 1982) by increasing the conductivity of the salt bridge connecting the electrodes, more extensive shielding of all signal leads and the cabinet surrounding the perfusion system, and by using an R-C filter to by-pass extraneous electrical noise pick-up by the electrode system.

Perfusate glucose and oxygen determinations

Glucose release by the liver was determined by the glucose oxidase/peroxidase method as previously described (Reinhart *et al.*, 1982). Oxygen consumption by the liver was calculated from the difference between influent and effluent oxygen concentrations measured with a Clark-type oxygen electrode modified for a flow-through mode of operation.

Chemicals and materials

Phenylephrine, [Arg⁸]vasopressin, [Val⁵]angiotensin and the glucose assay kit were obtained from Sigma. Ca^{2+} -selective electrode membranes (F2112) and filling solution S43316 were obtained from Radiometer, Copenhagen, Denmark. Other chemicals used were of analytical grade.

Expression of data

All experiments were performed at least three times. Where indicated, data are expressed as means $\pm$ S.E.M. for the numbers of independent experiments described.

RESULTS

Further characterization of Ca^{2+} -flux changes associated with phenylephrine action in the perfused rat liver

Studies of the action of Ca^{2+} -mobilizing hormones can be exploited to considerable advantage in the perfused rat liver, especially since the induced Ca^{2+} -flux changes can

Fig. 1. Effect of varying the time of phenylephrine administration on Ca^{2+} fluxes in perfused rat liver

Livers were perfused with Krebs-Henseleit buffer containing 1.3 mM added Ca^{2+} as described in the Experimental section. After a 15 min equilibration period, livers were then infused with phenylephrine (2.0 μ M final concn.) for various times between 15 s and 10 min (arrowed). Sufficient time (approx. 5 min) was allowed between pulses to let Ca^{2+} influx proceed to completion. A representative trace of only the first four pulses is shown for clarity in (a). The data in each curve of (b) were obtained from experiments similar to those shown in (a). The amount of Ca^{2+} efflux that occurred with each pulse of phenylephrine is expressed as a function of the duration of each pulse. The data were obtained from experiments conducted at 1.3 mM ($\bullet$), 300 μ M ($\circ$) and 50 μ M ($\blacklozenge$) perfusate Ca^{2+} respectively. For experiments conducted at 50 μ M- and 300 μ M- Ca^{2+} , 1.3 mM- Ca^{2+} was infused only during the first 10 min of the pre-perfusion. The liver was then perfused for 5 min at the lower Ca^{2+} concentration before the administration of phenylephrine. In these experiments, each pulse of phenylephrine was followed by a 5 min infusion of 1.3 mM- Ca^{2+} (and another 5 min perfusion at the lower Ca^{2+} concentration) to replenish the hormone-sensitive pool of Ca^{2+} . Data are means $\pm$ S.E.M. for three to five independent experiments.

be continuously and precisely monitored over a range of extracellular (perfusate) Ca^{2+} concentrations from zero to 1.3 mM (Reinhart *et al.*, 1982, 1984b). Also, it is possible with this system to infuse the hormone or agent for any specific time and thereby assess not only the Ca^{2+} -flux

changes associated with the triggering 'on' of the system but also those that occur when infusion of the agent is terminated.

In the first part of this work and as a necessary prelude to the data to be presented below, we have used this system to examine further two features of phenylephrine-induced Ca^{2+} fluxes not previously characterized in perfused rat liver, namely the time of phenylephrine administration required to induce maximal responses at different Ca^{2+} concentrations, and the consequence of varying the time between repetitive administrations of the α -agonist.

Duration of phenylephrine administration required to induce maximal Ca^{2+} efflux

Fig. 1(a) shows a representative Ca^{2+} -selective-electrode trace of the Ca^{2+} response obtained when phenylephrine was administered to the liver for specific times of between 15 s and 10 min duration. Clearly, the longer the duration of the phenylephrine pulse, the greater the amount of Ca^{2+} effluxed. The maximum rate of Ca^{2+} efflux, 70–100 nmol of Ca^{2+} /min per g of liver, is achieved with a phenylephrine pulse of 40–50 s duration. The relation between the duration of phenylephrine administration and the amount of Ca^{2+} effluxed is shown in Fig. 1(b): at 1.3 mM extracellular Ca^{2+} the amount of Ca^{2+} effluxed reaches a maximum (140 nmol of Ca^{2+} /g of liver) after 3 min of phenylephrine stimulation. By this time, all net Ca^{2+} movement out of the liver is complete, and prolonging the hormone stimulation does not result in any further Ca^{2+} efflux.

When similar experiments were carried out but with 300 μM and 50 μM perfusate Ca^{2+} , efflux of the ion was not complete until 4–5 min of phenylephrine infusion at 300 μM extracellular Ca^{2+} , and 6–8 min of phenylephrine infusion at 50 μM extracellular Ca^{2+} (Fig. 1b). In conducting these experiments and those in Fig. 2 (see below), it was necessary to infuse 1.3 mM- Ca^{2+} for approx. 5 min after each hormone challenge. This was done to accelerate the refilling of the hormone-sensitive intracellular Ca^{2+} pool. The data in Fig. 1(b) also suggest that, when the duration of phenylephrine stimulation exceeds about 3 min, the amount of Ca^{2+} effluxed from the liver perfused with media containing low Ca^{2+} (50 μM) is 10–15% greater than the amount of Ca^{2+} effluxed from the liver perfused with media containing 1.3 mM- Ca^{2+} under otherwise similar experimental conditions.

Variation of the time between consecutive administrations of phenylephrine

The representative Ca^{2+} -selective-electrode trace shown in Fig. 2(a) provides an indication of two things. First it shows that a longer interval between consecutive pulses of phenylephrine allows more Ca^{2+} to be taken up. Secondly it shows that, if the second pulse is administered early enough, it interferes with the Ca^{2+} -inflow response associated with the first pulse. More specifically, the data in Fig. 2(a) indicate that, with 1.3 mM extracellular Ca^{2+} , the maximum rate of Ca^{2+} influx that occurs between two consecutive 5-min pulses of phenylephrine is 40–50 nmol of Ca^{2+} /min per g of liver, and that this is achieved when the second pulse of the agonist is administered at least 40–50 s after the first.

In Fig. 2(b) the amount of Ca^{2+} taken up by the liver is expressed as a function of the time interval between the administration of two consecutive phenylephrine pulses.

Fig. 2. Phenylephrine-induced Ca^{2+} response obtained when the time between two consecutive pulses of hormone is varied

Livers were perfused with Krebs–Henseleit buffer containing 1.3 mM- Ca^{2+} as described in the Experimental section. After a 15 min equilibration period, phenylephrine (2 μM) was infused for 5 min (arrowed). The Ca^{2+} -selective electrode trace (a) shows the changes in perfusate Ca^{2+} obtained when the time interval between two consecutive 5 min pulses of phenylephrine was varied between 30 s and 10 min. Each pair of pulses was followed by a 'rest period' of approx. 5 min to permit refilling of the hormone-sensitive Ca^{2+} pool. Only the response corresponding to the first two pairs of pulses is shown for clarity. (b) shows the amount of Ca^{2+} influx that occurs as a function of the time between the two consecutive phenylephrine administrations indicated on the axis. These data were derived from experiments of the type shown in (a), conducted at (●) 1.3 mM-, (○) 300 μM - and (◆) 50 μM - Ca^{2+} respectively. Details for the perfusions carried out at 50 μM and 300 μM extracellular Ca^{2+} are exactly as described in Fig. 1(b). The data are means $\pm$ S.E.M. for three to five independent experiments.

It can be deduced that at 1.3 mM extracellular Ca^{2+} the uptake response is essentially complete within 4–5 min of hormone removal. Similarly it can be deduced that at 50 μM extracellular Ca^{2+} less than 50% of the hormone-sensitive pool is refilled by 5 min of phenylephrine removal; in this instance the maximum rate of Ca^{2+} influx was estimated to be 10–15 nmol of Ca^{2+} /min per g of liver. For 300 μM extracellular Ca^{2+} , the values are intermediate. These data thus indicate that at sub-

Fig. 3. Comparison of the Ca^{2+} -flux response in perfused rat liver induced by phenylephrine, vasopressin and angiotensin at different concentrations of extracellular Ca^{2+}

Livers were perfused with Krebs-Henseleit buffer containing (a) 50 μM -, (b) 300 μM -, (c) 650 μM - and (d) 1.3 mM- Ca^{2+} respectively. For the experiments conducted at 50 μM -, 300 μM - and 650 μM - Ca^{2+} , the extracellular Ca^{2+} concentration was maintained at 1.3 mM for the first 10 min of the 15 min equilibration period by infusion from a stock solution; this was followed by a 5 min perfusion at the lower Ca^{2+} concentration. In each experiment phenylephrine (2 μM , A), vasopressin (10 nM, B) or angiotensin II (10 nM, C) was infused for 6 min as indicated by the arrows. Each trace is a representative result from four to six independent experiments.

physiological concentrations of extracellular Ca^{2+} the removal of phenylephrine is followed by a lower maximum rate of Ca^{2+} uptake by the liver compared with that which occurs at 1.3 mM extracellular Ca^{2+} , and hence that under these conditions a much longer time is required to refill the hormone-sensitive pool of Ca^{2+} .

Comparison of the Ca^{2+} fluxes induced by phenylephrine, vasopressin and angiotensin

The Ca^{2+} response obtained when maximally effective doses of phenylephrine, vasopressin or angiotensin are infused to livers at perfusate Ca^{2+} concentrations of 50 μM , 300 μM , 650 μM or 1.3 mM is shown in Fig. 3. In each situation the agent was infused twice, each for 6 min, with an intervening recovery interval of 10 min. These conditions differ from those used in an earlier study (Reinhart *et al.*, 1982) in that they allow for the Ca^{2+} efflux and influx responses to be near-fully expressed at both high and low extracellular Ca^{2+} concentrations and allow time for the depleted agonist-sensitive pools of Ca^{2+} to be refilled (see Fig. 2).

The traces in Fig. 3(A) outline the apparent trend by which infusing the first pulse of phenylephrine induces a smaller net amount of Ca^{2+} efflux as the extracellular Ca^{2+} concentration is increased, i.e. although the peak height is approximately the same for all four responses, the area under the curve diminishes. A second trend is evident in Fig. 3(A); as the perfusate Ca^{2+} concentration is increased, so do the rate and extent of Ca^{2+} inflow after

removal of the hormone. In these experiments, and consistent with the information in Fig. 2, recovery is near-maximal even when the external Ca^{2+} concentration is only 600 μM and occurs within approx. 6 min of terminating the administration of the agonist. Administration of a second pulse of the agonist produces a similar response to the first at perfusate Ca^{2+} concentrations of 600 μM and 1.3 mM. At 50 μM perfusate Ca^{2+} , however, a different response is consistently observed. It exhibits a characteristic multi-peaked shape that is less evident at 300 μM extracellular Ca^{2+} . Approximately the same amount of Ca^{2+} is effluxed as in the first Ca^{2+} response. Attempts to link individual peaks with individual intracellular Ca^{2+} pools by using inhibitors of mitochondrial respiration were attempted, but proved unsuccessful.

Experiments like those described for phenylephrine (Fig. 3A) were carried out also with vasopressin being administered in place of the α -agonist. Data in Fig. 3(B) show that the Ca^{2+} responses differ in several key respects. The first major difference is that, after the initial pulse of vasopressin, increases in the external net Ca^{2+} concentration diminish far more significantly as the perfusate Ca^{2+} concentration increases. Together with this, a spontaneous influx of the ion occurs at 1.3 mM perfusate Ca^{2+} , after some 40 s, when only approx. 40 nmol of Ca^{2+} /g of liver has effluxed. This is complete by approx. 4 min; a trend towards this situation is apparent even at 650 μM perfusate Ca^{2+} . The second major

Fig. 4. Effect of varying the time of vasopressin and angiotensin administration on the Ca^{2+} response

Livers were perfused with Krebs–Henseleit buffer containing 1.3 mM- Ca^{2+} . After a 15 min pre-perfusion period, vasopressin (10 nM) infusion was begun as indicated by the arrow. The traces shown indicate the Ca^{2+} response obtained from separate experiments in which vasopressin was administered for 15 s (·····), 30 s (----) and 45 s (—) respectively. Each trace is representative of three independent experiments conducted for each duration of hormone administration. Changes in perfusate Ca^{2+} were measured with the Ca^{2+} -selective electrode. An essentially identical result was obtained with angiotensin (10 nM) instead of vasopressin.

difference of note is that, consequent to termination of vasopressin infusion, no clear evidence of Ca^{2+} influx (rate < 15 nmol/min per g of liver) is observed for any concentration of perfusate Ca^{2+} . The third major difference is that the Ca^{2+} response to the second pulse of vasopressin administration is much less than the first in all instances except at 1.3 mM perfusate Ca^{2+} . At 50 μM perfusate Ca^{2+} , vestiges of the multi-peaked trace seen after phenylephrine infusion are apparent. In other experiments (results not shown) it was found that at 1.3 mM perfusate Ca^{2+} the spontaneous Ca^{2+} -flux changes seen with the pulse of vasopressin could be induced a second time only when the recovery period between consecutive pulses was about 40 min or longer.

The above sequence of experiments was repeated, this time with angiotensin in place of phenylephrine (Fig. 3C). In general the Ca^{2+} responses are similar to those induced by vasopressin. The major points of differences between angiotensin and vasopressin are the sharper Ca^{2+} -efflux peaks at all concentrations of perfusate Ca^{2+} , and the occurrence of the spontaneous re-influx of Ca^{2+} at lower perfusate Ca^{2+} concentrations.

Other features of the Ca^{2+} response induced by vasopressin and by angiotensin

At both 50 μM and 1.3 mM extracellular Ca^{2+} , the onset of the Ca^{2+} -flux changes induced by vasopressin occurs after 10 ± 1 s and 11 ± 1 s, respectively, of administration (results not shown). The onset of these responses is thus

Fig. 5. Effect of vasopressin on perfusate Ca^{2+} concentration, oxygen uptake and glucose release in the perfused rat liver

Livers were perfused with Krebs–Henseleit buffer containing 1.2 mM- Ca^{2+} . After the first 15 min of pre-perfusion, vasopressin (10 nM) was infused as indicated (arrowed). The Ca^{2+} -selective electrode trace of the perfusate Ca^{2+} concentration is shown in (a) and the concurrent changes in the perfusate oxygen and glucose concentration are shown in (b) and (c) respectively. Oxygen was measured with a Clark-type oxygen electrode; glucose was measured in samples of perfusate collected at 15 s and 30 s intervals. Other details are as stated in the Experimental section. Each trace in (a) and (b) is representative of six to eight independent experiments. The data in (c) are the means of three independent experiments; S.E.M. values were always less than 0.03 mM and have been omitted for clarity.

4–5 s slower than the onset of those induced by phenylephrine under identical experimental conditions (Reinhart *et al.*, 1982). Similar conclusions had been reached previously by Charest *et al.* (1983), studying the ability of vasopressin and α -adrenergic agonists to induce increases in cytoplasmic Ca^{2+} in rat hepatocytes.

The effect of varying the time of vasopressin administration to the liver perfused with media containing 1.3 mM extracellular Ca^{2+} is shown in Fig. 4. The data show that a maximal change in the Ca^{2+} response is achieved when vasopressin is administered for only 45 s. Shorter times of hormone administration, however, result in a decrease in the amount of spontaneous Ca^{2+} uptake that occurs after the administration of the first pulse and results also in a decrease in the total net amount of Ca^{2+} efflux associated with subsequent short vasopressin pulses (results not shown). Essentially similar results were obtained with angiotensin.

Correlation with glucose output and oxygen uptake

Because the Ca^{2+} response induced by vasopressin at 1.3 mM extracellular Ca^{2+} was different from that induced by phenylephrine, it seemed important to examine the effects of vasopressin on other physiological responses, such as, oxygen uptake and glucose output. The data in Figs. 5(a), 5(b) and 5(c) show the vasopressin-induced Ca^{2+} , oxygen-uptake and glucose-output responses respectively. The oxygen-uptake response induced by vasopressin (Fig. 5b) returned slowly to the pre-stimulated value, even while the hormone was still being infused. This result is quite unlike the stimulation of oxygen uptake induced by phenylephrine at 1.3 mM extracellular Ca^{2+} , which is sustained for the duration of hormone administration (see Reinhart *et al.*, 1982). In contrast with the transient nature of the Ca^{2+} and oxygen responses induced by vasopressin, the stimulation of glucose output by vasopressin (Fig. 5c) appeared sustained for the duration of vasopressin stimulation. On removal of the hormone, however, the return of glucose output to the basal value was not as rapid as observed with phenylephrine (see Reinhart *et al.*, 1982). Again the pattern of responses induced by angiotensin was similar to that of vasopressin (results not shown).

DISCUSSION

In the light of the findings reported in this paper, we suggest that certain aspects of the mechanism of action of Ca^{2+} -mobilizing hormones may need to be revised, at least as they relate to liver tissue. The Ca^{2+} -selective electrode used in the present study revealed differences in the action of phenylephrine, vasopressin and angiotensin in inducing Ca^{2+} fluxes in the perfused rat liver over a range of perfusate Ca^{2+} concentrations from 50 μM to 1.3 mM.

The data on Ca^{2+} -flux changes in the intact liver obtained with phenylephrine as stimulus (Fig. 3A) extend that obtained previously (Reinhart *et al.*, 1982). Clearly, in the course of a prolonged pulse of the agonist, a precise amount of Ca^{2+} is released from the liver. As long as the agonist continues to be infused, the liver remains depleted of this Ca^{2+} . Removal of the agonist then triggers the re-uptake of the same amount of Ca^{2+} that previously had been released. This sequence can be repeated as many as 25 times (Reinhart *et al.*, 1982) provided that the perfusate Ca^{2+} concentration is greater than approx. 50 μM and sufficient time is allowed for the depleted intracellular Ca^{2+} pool(s) to refill (the present work; and see Morgan *et al.*, 1982). These features have been observed in qualitative terms also by Sies *et al.* (1983) and by Morgan *et al.* (1982); unfortunately, though, and as recognized by Sies *et al.* (1983), the large electrical noise background in their traces did not permit a quantitative analysis of the Ca^{2+} -flux changes. A similarly large scatter exists in the data presented by Morgan *et al.* (1982), who measured changes in extracellular Ca^{2+} by atomic absorption spectroscopy in a perfusate Ca^{2+} concentration of 1 mM. The accumulated errors involved in measuring changes of several μM Ca^{2+} when the perfusate Ca^{2+} concentration is greater than about 300 μM previously led us to abandon this particular experimental approach (J. G. Altin & F. L. Bygrave, unpublished work).

The major point arising out of the present work is that administration of vasopressin and angiotensin to livers perfused with 1.3 mM- Ca^{2+} induces Ca^{2+} -flux changes

which, in several respects, are quite different from those observed after phenylephrine administration (Figs. 3B and 3C). First, a much smaller quantity of Ca^{2+} is released from the liver; this is soon followed by a spontaneous uptake of the ion even while the hormone is still being infused. A similar difference between vasopressin and phenylephrine in inducing a Ca^{2+} response is present in the data of Sies *et al.* (1983). In their experiments, Morgan *et al.* (1982) observed no difference between the effects of the two hormones on Ca^{2+} fluxes in the perfused rat liver and concluded that they have similar mechanisms of action. A second important difference between the actions of phenylephrine and those of vasopressin and angiotensin in the rat liver perfused with 1.3 mM- Ca^{2+} is that, within the time course of one experiment (< 60 min), a second pulse of the hormone fails to elicit the same response as observed with the initial pulse (see Fig. 3).

This work raises a number of questions concerning the mechanism of action of Ca^{2+} -mobilizing hormones in liver. Firstly, the spontaneity of the Ca^{2+} influx observed when vasopressin or angiotensin is infused into the intact liver, and the subsequent inability of these hormones to induce a second response (see also Breant *et al.*, 1981), is of interest. In this regard, there now is good evidence that a further action of Ca^{2+} -mobilizing hormones, in addition to mobilizing intracellular Ca^{2+} (reviewed by Reinhart *et al.*, 1984b; Williamson *et al.*, 1985) is to induce inward movement of the ion (Barritt *et al.*, 1981; Blackmore *et al.*, 1984; Mauger *et al.*, 1984) and an increase in the rate of plasma-membrane Ca^{2+} cycling (Reinhart *et al.*, 1984a). The present work in fact shows that phenylephrine, vasopressin and angiotensin differ in their ability to stimulate a net inward movement of Ca^{2+} . Whether Ca^{2+} channels (see, e.g., Taylor *et al.*, 1985) and/or specific interactions between these and the various hormone receptors are involved remains to be clarified. Furthermore, the possible effects of these hormones on the plasma-membrane Ca^{2+} -activated ATPase (Lin *et al.*, 1983; Prpić *et al.*, 1984) also requires clarification (Epping & Bygrave, 1984).

Another question that arises out of this work is the relation between the observed Ca^{2+} -flux changes and the physiological responses as reflected in the increased glucose production and oxygen uptake (Fig. 5). Although we did not measure changes in cytoplasmic Ca^{2+} in this work, Charest *et al.* (1983) have shown with rat hepatocytes that vasopressin induces an increase in such Ca^{2+} . Clearly it will be important to attempt to measure changes in cytoplasmic Ca^{2+} in the intact perfused rat liver.

The third question relates to the nature of the signal(s) generated by these hormones at the plasma membrane. It has been reported by several laboratories (Burgess *et al.*, 1984; Thomas *et al.*, 1984; Berridge, 1984; Berridge & Irvine, 1984) that the purported (common) signal generated by each of these hormones is inositol 1,4,5-trisphosphate; this appears to induce a release of Ca^{2+} from only non-mitochondrial sources (see, e.g., Shears & Kirk, 1984b), and thus presumably from endoplasmic reticulum (Burgess *et al.*, 1984; Prentki *et al.*, 1984; Dawson & Irvine, 1984; Hirata *et al.*, 1984; Joseph *et al.*, 1984). However, the present work, showing that the pattern of Ca^{2+} mobilization induced by phenylephrine is different from that induced by vasopressin and angiotensin, supports the idea (Dehaye *et al.*,

1981) detailed by Huerta-Bahena & Garcia-Sainz (1985) that the nature of the signals generated by these two sets of hormones is different. Whether the signal(s) generated by these hormones have different effects on Ca²⁺ transport across the plasma membrane and/or organellar membranes, or whether different hormone-operated Ca²⁺ channels are involved at the plasma membrane, requires investigation. In this regard the intracellular fate of the Ca²⁺ spontaneously taken up by the liver (Figs. 3B and 3C) is of especial interest.

In conclusion it would appear that the mechanism by which vasopressin and angiotensin mobilize Ca²⁺ in the perfused rat liver is not the same as that for α -adrenergic agonists, a feature that may be relevant also to other issues.

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