The action of α -adrenergic agonists on plasma-membrane calcium fluxes in perfused rat liver

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The effect of α -adrenergic agonists on Ca^{2+} fluxes was examined in the perfused rat liver by using a combination of Ca^{2+} -electrode and $^{45}\text{Ca}^{2+}$ -uptake techniques. We showed that net Ca^{2+} fluxes can be described by the activities of separate Ca^{2+} -uptake and Ca^{2+} -efflux components, and that α -adrenergic agonists modulate the activity of both components in a time-dependent manner. Under resting conditions, Ca^{2+} -uptake and -efflux activities are balanced, resulting in Ca^{2+} cycling across the plasma membrane. The α -adrenergic agonists vasopressin and angiotensin, but not glucagon, stimulate the rate of both Ca^{2+} efflux and Ca^{2+} uptake. During the first 2–3 min of α -agonist administration the effect on the efflux component is the greater, the net effect being efflux of Ca^{2+} from the cell. After 3–4 min of phenylephrine treatment, net Ca^{2+} movements are essentially complete, however, the rate of Ca^{2+} cycling is significantly increased. After removal of the α -agonist a large stimulation of the rate of Ca^{2+} uptake leads to the net accumulation of Ca^{2+} by the cell. The potential role of these Ca^{2+} flux changes in the expression of α -adrenergic-agonist-mediated effects is discussed.

A redistribution of cellular Ca^{2+} plays a vital role in the mechanism whereby α -adrenergic agonists induce many hepatic responses (reviewed by Exton, 1981; Williamson *et al.*, 1981; Taylor *et al.*, 1983a). Aspects of this redistribution which so far have been described are the mobilization of two intracellular pools of Ca^{2+} (Reinhart *et al.*, 1982a), a rise in the cytoplasmic free Ca^{2+} concentration (Murphy *et al.*, 1980; Barritt *et al.*, 1981) and a transient stimulation of the rate of net Ca^{2+} efflux from the cell, even at an extracellular Ca^{2+} concentration of 1.3 mM (Blackmore *et al.*, 1982; Reinhart *et al.*, 1982a; Sies *et al.*, 1983).

A further feature, revealed in the preceding paper (Reinhart *et al.*, 1984), is that both intracellular and extracellular Ca^{2+} are necessary for the full expression of α -adrenergic-agonist-induced responses in perfused rat liver. This feature carries with it the implication that Ca^{2+} movements across the plasma membrane are modulated by α -adrenergic agonists. Consequently we considered it important to attempt to define these movements and the nature of the control imposed by these agonists. To achieve this we have used a technique

that allows the simultaneous determination both of net Ca^{2+} movements and of the Ca^{2+} uptake rate in intact perfused rat liver.

We report here that the movement of Ca^{2+} across the plasma membrane is regulated by the activity of a Ca^{2+} -translocation cycle that consists of Ca^{2+} -efflux and Ca^{2+} -influx components. α -Adrenergic agonists modulate the activity of both components. The net flow of Ca^{2+} being determined by the time course and magnitude of stimulation of each. The possibility is discussed that this cycle contributes, among other things, to the regulation of the cytoplasmic free Ca^{2+} concentration.

Experimental

Animals and perfusions

Male Wistar-strain albino rats weighing between 230 and 270 g and either having free access to food, or being starved for 48 h (Taylor *et al.*, 1983a) were used (see the legends to the Figures). Rats were anaesthetized with sodium pentobarbitone (50 mg/kg body wt.) and the livers perfused with Krebs–Henseleit (1932) bicarbonate medium equilibrated with O_2/CO_2 (19:1) using a non-recir-

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culating mode as described previously (Reinhart *et al.*, 1982b).

Perfusate calcium-concentration determination

This was continuously monitored with a Radiometer F2112 Ca^{2+} -selective electrode placed in a flow-through chamber. Signal amplification was achieved with an Orion model 901 microprocessor ion analyser coupled to a Spectra-Physics SP4100 computing integrator. Further details are described in Reinhart *et al.* (1982a, 1983). Free Ca^{2+} concentrations were calculated from total concentrations by using an algorithm of the program 'Comics' (Perrin & Sayce, 1967) as described previously (Reinhart *et al.*, 1983).

$^{45}\text{Ca}^{2+}$ -uptake assay

A trace amount of $^{45}\text{CaCl}_2$ (approx. 3×10^7 d.p.m./ml) was infused by means of a glass/glass infusion syringe (EVA Superglass μ -Micromatic) mounted on a Braun Perfusor IV, into either the inflow cannula or the outflow cannula of the perfused liver system. Plastic or metal/glass syringes resulted in uneven infusion rates. Samples of perfusate (about 1 ml) were collected every 5 s between 10 s and 4.0 min after the infusion of $^{45}\text{CaCl}_2$. For each time point, two 200 μl samples were added to scintillation vials containing 10 ml of scintillant {6 g of butyl-PBD [5-(biphenyl-4-yl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole] in 400 ml of 2-methoxyethanol and 600 ml of toluene}, and the radioactivity determined to within 0.1% error in a Beckman LS-330 liquid-scintillation counter. All radioactivity counts obtained were corrected for the background radioactivity in the vials plus the scintillant. For the purposes of calculating the rate of $^{45}\text{Ca}^{2+}$ removal by the liver, the average of radioactivities for samples collected between 3.5 and 4 min only was calculated. This short sampling period (30 s) was chosen as representing a compromise between the error due to single-point determinations on the one hand, and error due to changes in the specific radioactivity of intracellular $^{45}\text{Ca}^{2+}$ during prolonged sampling periods on the other. The difference between the radioactivity in the perfusate after infusion into the inflow and outflow cannulae, respectively, was taken to represent the $^{45}\text{Ca}^{2+}$ removed by the liver. During the course of these experiments the extent of net Ca^{2+} movement was continuously monitored with the Ca^{2+} -Selectrode.

Materials

$^{45}\text{CaCl}_2$ was obtained from The Radiochemical Centre Amersham, Bucks., U.K. Ca^{2+} -electrode membranes (F2002) and filling solution S43316 were obtained from Radiometer, Copenhagen, Denmark. Phenylephrine, glucagon, [8-

arginine]vasopressin and [5-valine]angiotensin were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Other chemicals used were of analytical grade.

Expression of data

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

Results

Basal $^{45}\text{Ca}^{2+}$ removal

An examination of Ca^{2+} movements in the perfused rat liver by the simultaneous analysis of $^{45}\text{Ca}^{2+}$ -uptake rates and Ca^{2+} -selective electrode recordings is shown in Fig. 1. Data in Fig. 1(a) show that a significant proportion of the $^{45}\text{Ca}^{2+}$ infused into the inflow perfusion medium is removed by the liver, a relative plateau of radioactivity usually being observed within 3–4 min of infusing the $^{45}\text{Ca}^{2+}$. The rate of $^{45}\text{Ca}^{2+}$ uptake corresponds to 47 ± 9 nmol of $\text{Ca}^{2+} \cdot \text{min}^{-1} \cdot (\text{g of liver})^{-1}$ ($n = 5$) and is represented by Δ_b in Fig. 1(a).

Fig. 1(b) shows the Ca^{2+} -selectrode recording for the same experiment. It can be seen that, during $^{45}\text{Ca}^{2+}$ administration, the perfusate total Ca^{2+} concentration remains constant, indicating that the $^{45}\text{Ca}^{2+}$ removal observed in Fig. 1(a) represents the exchange of $^{40}\text{Ca}^{2+}$ for $^{45}\text{Ca}^{2+}$.

Because liver contains large extracellular as well as intracellular pools of Ca^{2+} (Claret-Berthon *et al.*, 1977), it was important to attempt to differentiate between that proportion of $^{45}\text{Ca}^{2+}$ exchanging with extracellular Ca^{2+} from that exchanging with intracellular $^{40}\text{Ca}^{2+}$.

A quantitative assessment was made using two separate experimental approaches. The first procedure adopted aimed to minimize the exchange of $^{45}\text{Ca}^{2+}$ for intracellular $^{40}\text{Ca}^{2+}$ during the 3–4 min sampling time normally used as detailed in the Experimental section. This was achieved by treating perfused livers with digitonin, a detergent that allows equilibration of extracellular $^{45}\text{Ca}^{2+}$ and intracellular $^{40}\text{Ca}^{2+}$ to occur within several minutes (Dubinsky & Cockrell, 1975). In addition, these experiments were performed with livers obtained from rats starved for 48 h when glycogen levels were essentially depleted and glycolytic rates were low (Taylor *et al.*, 1983b). Pretreatment of these livers with antimycin A should then severely impair the energetics of the tissue and further minimize intracellular energy-dependent Ca^{2+} fluxes.

Indeed, as shown in Fig. 1(c) and represented by Δ_c , rates of $^{45}\text{Ca}^{2+}$ removal from the perfusion medium under these conditions were only

Fig. 1. Removal of $^{45}\text{Ca}^{2+}$ by perfused rat liver in the absence of net Ca^{2+} movements

Livers of fed (a, b) or starved (c) rats were perfused for 10 min in media containing 1.3 mM-Ca^{2+} as described in the Experimental section. At 10 min the Ca^{2+} concentration was reduced to $600\text{ }\mu\text{M-Ca}^{2+}$, and 5 min later a trace amount of $^{45}\text{CaCl}_2$ was infused into the outflow cannula for 4 min (as shown by the open arrows). The radioactivity in the perfusate was determined by assaying $200\text{ }\mu\text{l}$ samples, in duplicate, every 5 s. Within 4 min of terminating the $^{45}\text{CaCl}_2$ infusion, perfusate radioactivity returns to basal levels (not shown). At 30 min of perfusion, trace amounts of $^{45}\text{CaCl}_2$ were infused into the inflow cannula. In (b), net changes in the perfusate Ca^{2+} concentration were determined by using a Ca^{2+} -specific electrode as described in the Experimental section. In (c), livers were treated with digitonin ($15\text{ }\mu\text{l/ml}$) and antimycin A ($5\text{ }\mu\text{M}$) at 24 min and $^{45}\text{Ca}^{2+}$ infused as described for (a). Results shown are typical experiments chosen from either four (a, b) or three (c) independent experiments performed.

$15 \pm 4\text{ nmol} \cdot \text{min}^{-1} \cdot (\text{g of liver})^{-1}$ ($n = 3$), i.e. approx. 30% of the rate observed with livers from fed rats. Presumably this rate reflects the exchange of extracellular $^{40}\text{Ca}^{2+}$ for perfusate $^{45}\text{Ca}^{2+}$, al-

though some contribution from residual intracellular exchange processes cannot be ruled out.

In the second set of experiments, the amount of $^{45}\text{Ca}^{2+}$ removed from the perfusate was compared

with the amount of $^{45}\text{Ca}^{2+}$ incorporated into the intracellular compartment(s) of the tissue. Livers from fed animals were treated with $^{45}\text{Ca}^{2+}$ essentially as described in Fig. 1, with the exception that perfusate $^{45}\text{Ca}^{2+}$ contents were monitored over the entire period of $^{45}\text{Ca}^{2+}$ infusion (10 min). On termination of $^{45}\text{Ca}^{2+}$ infusion,

livers were perfused for a further 2 min with $50\text{ }\mu\text{M}$ -EGTA in the absence of added Ca^{2+} . Under these conditions the extracellular Ca^{2+} pool is known to become depleted rapidly (Claret-Berthon *et al.*, 1977; the preceding paper, Reinhart *et al.*, 1984). The tissue was then homogenized and the $^{45}\text{Ca}^{2+}$ content determined. It was found that

Fig. 2. Phenylephrine-induced removal of $^{45}\text{Ca}^{2+}$ by perfused rat liver. Livers of either fed (a, b) or starved (c) rats were perfused as described in the legend to Fig. 1. Livers received a continuous infusion of phenylephrine ($2\text{ }\mu\text{M}$) at 25 min of perfusion (shown by the bold arrows). All other additions are as described in the legend to Fig. 1.

83.6 ± 7.4% (*n* = 4) of the total radioactivity removed by the liver over a 10 min period was recovered within the liver. Therefore both procedures adopted indicate that most of the ⁴⁵Ca²⁺ removed from the perfusate reflects exchange for intracellular ⁴⁰Ca²⁺. We thus conclude from these data that the observed removal of ⁴⁵Ca²⁺ in the absence of net Ca²⁺ fluxes (Fig. 1b) represents cycling of Ca²⁺ across the plasma membrane.

Effect of hormones on Ca²⁺ removal by the liver

The effect of the α-adrenergic agonist phenylephrine on liver Ca²⁺ fluxes is shown in Fig. 2. As we reported previously (Reinhart *et al.*, 1982a), phenylephrine induces the net efflux of approx. 120 nmol of Ca²⁺ · (g of liver)⁻¹ within 3–4 min of agonist administration. However, by 5 min after agonist administration, the net movement of Ca²⁺ is complete (Fig. 2b). The rate of ⁴⁵Ca²⁺ removal by liver after its infusion at this time has increased to almost twice basal values (referred to as Δ_h in Fig. 2a). Exchange of ⁴⁵Ca²⁺ for extracellular ⁴⁰Ca²⁺ is affected only slightly by phenylephrine (Δ_e in Figs. 1c and 2c).

As described above, additional experiments were carried out to estimate the amount of ⁴⁵Ca²⁺ incorporated into intracellular compartment(s) during the course of a 10 min infusion of ⁴⁵Ca²⁺ in the presence or absence of phenylephrine. A determination was made of the ⁴⁵Ca²⁺ content of liver homogenates prepared 2 min after cessation of ⁴⁵Ca²⁺ infusion when the extracellular exchangeable ⁴⁵Ca²⁺ was depleted by treatment for 2 min with 50 μM-EGTA in the absence of added ⁴⁰Ca²⁺ as described above. It was found that phenylephrine induced a 57% increase in ⁴⁵Ca²⁺ uptake into the intracellular compartment as compared

with control values. These data then suggest that the stimulation of ⁴⁵Ca²⁺ removal from the perfusate induced by phenylephrine represents an increased exchange of ⁴⁵Ca²⁺ for intracellular ⁴⁰Ca²⁺ (see also Barritt *et al.*, 1981). Since this increase occurs in the absence of net Ca²⁺ fluxes (Fig. 2a), then this exchange again represents the cycling of extracellular for intracellular Ca²⁺.

The effect of several other hormones on the rate of ⁴⁵Ca²⁺ removal by the perfused rat liver also was examined. Table 1 shows that vasopressin (3 m-i.u./ml) and angiotensin (100 μM) stimulate the rate of ⁴⁵Ca²⁺ uptake to an extent similar to that induced by phenylephrine. By contrast, glucagon has only a very small effect on ⁴⁵Ca²⁺ uptake, consistent with the minimal effect of this agent on stimulating the rate of net Ca²⁺ efflux from liver (Reinhart *et al.*, 1982a).

Role of extracellular Ca²⁺ in ⁴⁵Ca²⁺ removal

Fig. 3 shows that both basal (Δ_b, ○) and hormone-stimulated (Δ_h, ●) rates of ⁴⁵Ca²⁺ respond to extracellular Ca²⁺ over a similar concentration range. ⁴⁵Ca²⁺ removal is not significant below 50 μM-Ca²⁺, is half-maximal at approx. 250 μM-Ca²⁺, and is near-maximal at 600 μM-Ca²⁺. At all concentrations of Ca²⁺ above 50 μM the proportion of phenylephrine-induced to basal ⁴⁵Ca²⁺ removal is similar.

Table 1. Ability of various hormones to induce removal of ⁴⁵Ca²⁺ by perfused rat liver

Livers of fed rats were perfused and removal of ⁴⁵Ca²⁺ measured as described in the Experimental section. For each rat, ⁴⁵Ca²⁺-uptake rates were determined by analysing the averages for samples taken every 5 s between 3.5 and 4 min of ⁴⁵CaCl₂ administration. Concentrations of hormones used were 2 μM for phenylephrine, 100 μM for glucagon, 3 m-i.u./ml for vasopressin and 100 μM for angiotensin. Results are means ± s.d. for the number of independent experiments given in parentheses.

Agent	Concn. (μM)	Rate of ⁴⁵ Ca ²⁺ removal by the liver (nmol/min per g wet wt.)
Phenylephrine	15.8 ± 1.2	43.8 ± 3.3 (5)
Glucagon	1.9 ± 2.5	5.3 ± 6.9 (3)
Vasopressin	14.1 ± 3.2	39.2 ± 8.9 (4)
Angiotensin	12.7 ± 1.1	35.3 ± 3.1 (3)

Fig. 3. Effect of extracellular Ca²⁺ concentration on the rate of ⁴⁵Ca²⁺ removal by perfused rat liver

Livers of fed rats were perfused as described in the Experimental section. At 10 min of perfusion of Ca²⁺ concentration of the perfusate was adjusted to the values indicated. Rates of ⁴⁵Ca²⁺ removal were measured as described in Experimental section. Each data point shown is the result from one independent experiment. For the sake of clarity, s.e.m. values have been omitted; however, ± 4.28 μM was never exceeded. At Ca²⁺ concentrations of 200 μM and higher, values for hormone-treated livers were significantly different from control values (*P* < 0.05).

Fig. 4. Temporal progression of the onset and decay of phenylephrine-stimulated rates of $^{45}\text{Ca}^{2+}$ removal by perfused rat liver

Livers of fed rats were perfused as described in the legend to Fig. 1, with the exception that for each time point the infusion of $^{45}\text{CaCl}_2$ through the liver was initiated 3.5 min before the administration of phenylephrine. Hence for each time point the rates of $^{45}\text{Ca}^{2+}$ uptake between 3.5 and 4 min of $^{45}\text{CaCl}_2$ infusion were determined as described in the Experimental section. The data represent means $\pm$ S.E.M. for between three and six independent experiments performed for each time point.

Time course of phenylephrine-stimulated $^{45}\text{Ca}^{2+}$ removal

The time course of phenylephrine-stimulated $^{45}\text{Ca}^{2+}$ removal is depicted in Fig. 4. Between 30 s and 60 s after α -agonist administration (i.e. the earliest measurements possible), the rate of $^{45}\text{Ca}^{2+}$ removal into livers is significantly stimulated. Half-maximal effects are observed at between 60 s and 90 s and near-maximal effects at 2.5 min. The effect on $^{45}\text{Ca}^{2+}$ removal is sustained for the duration of phenylephrine administration, in contrast with the numerous transient responses elicited by the agonist (the preceding paper, Reinhart *et al.*, 1984). After withdrawal of the α -agonist, a further stimulation of the rate of $^{45}\text{Ca}^{2+}$ removal is observed, coinciding with a net accumulation of Ca^{2+} by the liver (Reinhart *et al.*, 1982a). Maximal effects are observed by approx. 90 s after hormone removal, and thereafter the rate of Ca^{2+} removal slowly declines to reach basal values after 5–10 min.

It is important to recall that, during the first 3–4 min after either the addition of phenylephrine or its removal, there is significant net movement of Ca^{2+} out of or into the liver (Reinhart *et al.*, 1982a;

Fig. 2). Under these circumstances, therefore, liver Ca^{2+} fluxes are not in a steady state, i.e. the rate of Ca^{2+} uptake is not equal to the rate of Ca^{2+} efflux.

Discussion

The data presented have revealed the effects of α -adrenergic agonists on the complex interplay between rates of $^{45}\text{Ca}^{2+}$ removal and rates of net Ca^{2+} movements in perfused rat liver. $^{45}\text{Ca}^{2+}$ removal was defined in terms of rates, requirement for extracellular Ca^{2+} , hormone specificity, and time-dependent changes after either the administration or withdrawal of the hormone.

One potential complication with the experimental approach used in the present study is the possibility that $^{45}\text{Ca}^{2+}$ removal by the liver represents $^{45}\text{Ca}^{2+}$ exchange with extracellular $^{40}\text{Ca}^{2+}$ rather than with intracellular $^{40}\text{Ca}^{2+}$. This problem can be minimized, however, since previous kinetic analyses of cellular Ca^{2+} fluxes have indicated that $^{45}\text{Ca}^{2+}$ exchange with extracellular Ca^{2+} is approximately one order of magnitude faster than with intracellular Ca^{2+} (see Borle, 1975, 1981). Hence within 2–3 min of $^{45}\text{Ca}^{2+}$ infusion, the rapidly exchangeable extracellular Ca^{2+} pool in cell suspensions (Borle, 1975; Martin *et al.*, 1975; Severson *et al.*, 1976; Foden & Randle, 1978), perfused rat liver (Claret-Berthon *et al.*, 1977), or isolated liver plasma-membrane fragments (Shlatz & Marinetti, 1972) is almost totally equilibrated with $^{45}\text{Ca}^{2+}$. The direct examination of this exchange under our experimental conditions (Figs. 1 and 2) is consistent with these reports, indicating that, within 3–4 min after $^{45}\text{Ca}^{2+}$ addition, more than 70% of $^{45}\text{Ca}^{2+}$ removal represents $^{45}\text{Ca}^{2+}$ uptake by the liver.

Thus, after allowing for exchange with extracellular $^{45}\text{Ca}^{2+}$ (Fig. 1c), the analysis of measurements of both $^{45}\text{Ca}^{2+}$ -uptake rates and of the perfusate Ca^{2+} concentration as adopted here represents a powerful procedure whereby (1) net Ca^{2+} movements may be interpreted in terms of changes in the rate of Ca^{2+} -efflux or Ca^{2+} -influx components, and (2) transient changes in the rates of each of these components can be examined, something not possible in kinetic studies, which must be performed under steady-state conditions (Borle, 1975).

An analysis of $^{45}\text{Ca}^{2+}$ -uptake rates under conditions where there are no net Ca^{2+} movements (Figs. 1 and 2) has revealed that Ca^{2+} is continually recycling across the hepatic plasma membrane, indicating that the net movement of Ca^{2+} is the resultant of Ca^{2+} -efflux and Ca^{2+} -uptake activities. This rate of cycling, corresponding to approx. $190 \text{ fmol} \cdot \text{s}^{-1} \cdot \text{cm}^{-2}$ (assuming $2.8 \text{ cm}^2/\text{mg wet wt. of liver}$; Claret & Mazet, 1972) is

very similar to the basal flux of $^{45}\text{Ca}^{2+}$ uptake previously observed in a kinetic analysis of liver Ca^{2+} fluxes (Claret-Berthon *et al.*, 1977), and in a range of other tissues (reviewed by Borle, 1981).

A second point is that α -adrenergic agonists were found to have complex effects on this Ca^{2+} -translocation cycle, since the activities of both Ca^{2+} -influx and Ca^{2+} -efflux components are modulated. We reported previously (Reinhart *et al.*, 1982a) that one of the earliest responses to α -adrenergic agonists is an increase in the rate of net Ca^{2+} efflux from the perfused rat liver occurring within 5 s of agonist administration. However, the rate of $^{45}\text{Ca}^{2+}$ influx is also stimulated by the agonist within 30 s of its administration. This clearly indicates that not only are both components of the Ca^{2+} -translocation cycle activated, but also that the efflux component is stimulated to a far greater extent. This finding may be relevant for the interpretation of data obtained by Assimacopoulos-Jeannet *et al.* (1977) and Foden & Randle (1978), who showed that α -adrenergic agonists stimulate $^{45}\text{Ca}^{2+}$ uptake by rat liver cells. By examining only one component of the translocation cycle, these data have been misinterpreted as being indicative of net Ca^{2+} fluxes.

A third point relevant to the action of α -adrenergic agonists is that, after the termination of net Ca^{2+} efflux within 3–4 min of α -agonist administration, the rate of Ca^{2+} cycling across the plasma membrane has almost doubled. This observation is consistent with the data of Foden & Randle (1978) showing that phenylephrine stimulates $^{45}\text{Ca}^{2+}$ uptake in hepatocytes treated with the agonist for longer than 5 min, and with a kinetic analysis of $^{45}\text{Ca}^{2+}$ fluxes showing that 15 min after α -agonist administration the rate of both $^{45}\text{Ca}^{2+}$ -influx and $^{45}\text{Ca}^{2+}$ -efflux exchange has increased (Barritt *et al.*, 1981). The finding that (1) the rate of both hormone-induced and basal cycling is dependent on the extracellular Ca^{2+} concentration, and (2) that maximal rates of cycling are not observed until 2–3 min after α -agonist administration, allows us to suggest that this cycling activity is responsible for the maintenance of the sustained responses described in the preceding paper (Reinhart *et al.*, 1984). These sustained responses were shown also to be dependent on the extracellular Ca^{2+} concentration and to exert their effect 3–4 min after phenylephrine treatment. Thus, if intracellular Ca^{2+} pools are capable of only transiently elevating the cytoplasmic Ca^{2+} concentration, then it is possible that the Ca^{2+} -translocation cycle at the plasma membrane may act to maintain this elevated level of Ca^{2+} . The findings that the addition of extracellular Ca^{2+} to livers perfused with media containing low Ca^{2+} concentrations (less than $1\ \mu\text{M}$) restores the expression of sustained

phenylephrine-induced responses [the preceding paper (Reinhart *et al.*, 1984)] and that phenylephrine induces a transient elevation of the cytoplasmic Ca^{2+} concentration in hepatocytes incubated in low- Ca^{2+} media (Murphy *et al.*, 1980), are consistent with such a proposal. Whether the extracellular Ca^{2+} pool that is mobilized represents bulk-phase Ca^{2+} or extracellular bound Ca^{2+} remains to be established.

The analysis of the temporal progression of α -adrenergic agonist-induced Ca^{2+} fluxes (Fig. 4) has revealed that a further pattern of Ca^{2+} flux changes is observed after removal of the α -agonist from the perfusion medium. Within seconds of withdrawing the agonist there occurs a rapid increase in the rate of both $^{45}\text{Ca}^{2+}$ uptake and net Ca^{2+} influx into the liver. Since the rate of $^{45}\text{Ca}^{2+}$ uptake is greater than the measured rate of net Ca^{2+} influx, it appears that an inhibition of the rate of Ca^{2+} efflux does not contribute to the net inflow of Ca^{2+} , but rather that this is due to the opening of a Ca^{2+} channel or gate. Furthermore, since the sustained effects of phenylephrine on glycogenolysis and respiration are returning towards basal levels during this time, then it appears that the consequence of this Ca^{2+} inflow is not to elevate the cytoplasmic Ca^{2+} concentration, but rather to replete the intracellular hormone-sensitive Ca^{2+} pools (Reinhart *et al.*, 1982a). As this pool is being repleted, the rates both of net Ca^{2+} inflow and of Ca^{2+} cycling are returning towards basal levels. Thus there appears to be some close communication between the hormone-sensitive pool(s) of intracellular Ca^{2+} and the rates of plasma-membrane Ca^{2+} fluxes, both during and after stimulation with α -adrenergic agonists. The hormone specificity of these effects is similar to that found previously for net Ca^{2+} efflux (Reinhart *et al.*, 1982a) and is such as to suggest that these Ca^{2+} movements are part of a more general mechanism whereby hormone-receptor interactions are translated into cellular responses.

Hence we propose that both basal and hormone-stimulated cellular Ca^{2+} movements involve a Ca^{2+} -translocation cycle across the hepatic plasma membrane. Short-term (<2 min) effects of α -agonists are dependent mainly on the mobilization of a small intracellular pool of Ca^{2+} (Reinhart *et al.*, 1982, 1983, 1984), which is, at least in part, extruded from the cell. Sustained effects appear to be dependent on the activity of Ca^{2+} cycling across the plasma membrane and hence the extracellular Ca^{2+} concentration. Under these conditions this cycle may act to keep the cytoplasmic Ca^{2+} concentration elevated (Murphy *et al.*, 1980; Barritt *et al.*, 1981). By analogy to numerous cell types [reviewed by Borle (1981) and Barritt (1982)], such a cycle may consist of an outwardly directed Ca^{2+} -

ATPase/ Ca^{2+} pump (Lotersztajn *et al.*, 1981) and one or more types of Ca^{2+} -uptake channels. Attempts should be made to identify these components in molecular terms, and to relate Ca^{2+} flux alteration to changes in the cytoplasmic free Ca^{2+} concentration.

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