Biochem. J. (1981) **200**, 109–114
Printed in Great Britain

Evidence that lanthanum ions stimulate calcium inflow to isolated hepatocytes

Janice C. PARKER and Gregory J. BARRITT
Clinical Biochemistry Unit, Flinders University School of Medicine, Flinders Medical Centre, Bedford Park, South Australia 5042, Australia

(*Received 24 April 1981/Accepted 15 June 1981*)

$LaCl_3$ stimulated the initial rate of $^{45}Ca^{2+}$ exchange measured under steady-state conditions in isolated liver cells. $Cu^{2+} > La^{3+} = Fe^{3+} > Fe^{2+} = Zn^{2+} > Ni^{2+} > Mn^{2+}$ also stimulated $^{45}Ca^{2+}$ exchange. Compartmental analysis of $^{45}Ca^{2+}$-exchange curves obtained in the presence or absence of La^{3+}, and in the presence or absence of adrenaline, showed that the predominant effect of La^{3+} is to stimulate the inflow of Ca^{2+} to the cell from the medium. No evidence for an inhibition of Ca^{2+} outflow from the cell was obtained. In the presence of La^{3+}, adrenaline caused no further stimulation of Ca^{2+} inflow to the cell. In the absence of adrenaline, La^{3+} increased the uptake of Ca^{2+} (measured by atomic-absorption spectroscopy) by isolated hepatocytes incubated at 1°C. The proposal that La^{3+} stimulates Ca^{2+} inflow to the liver cell by inducing a conformational change in the Ca^{2+}-inflow transporter of the plasma membrane is briefly discussed.

The distribution of exchangeable Ca^{2+} in isolated hepatocytes has been studied in this laboratory by using $^{45}Ca^{2+}$-exchange techniques (Barritt & Parker, 1980; Barritt *et al.*, 1981). Two kinetically distinct compartments of exchangeable cellular Ca^{2+} were detected. These were identified as a slowly exchangeable compartment, which includes Ca^{2+} present in the mitochondria and endoplasmic reticulum (cf. Claret-Berthon *et al.*, 1977), and a rapidly exchangeable compartment, tentatively identified as exchangeable Ca^{2+} in the cytoplasm. Analysis of data obtained in the presence of adrenaline or phenylephrine showed that each of these agents stimulates both Ca^{2+} inflow to the cell from the extracellular medium (cf. Assimacopoulos-Jeannet *et al.*, 1977; Keppens *et al.*, 1977; Foden & Randle, 1978) and Ca^{2+} outflow from the mitochondria and (possibly) other intracellular organelles (Barritt *et al.*, 1981).

The aim of the present experiments was to use La^{3+} ions to investigate the transport of Ca^{2+} across the plasma membrane of liver cells and the nature of the effect of adrenaline on this process. It has been shown that La^{3+} ions displace Ca^{2+} bound to superficial sites on the plasma membranes of a large number of cells (reviewed by Weiss, 1974), inhibit the activity of the $(Ca^{2+} + Mg^{2+})$-dependent ATPase enzymes in plasma-membrane preparations from erythrocytes (Schatzmann & Bürgin, 1978) and adipocytes (Pershadsingh & McDonald, 1980), and at high concentrations inhibit Ca^{2+} outflow from cells catalysed by the Na^+/Ca^{2+} exchange transporter (Katzung *et al.*, 1973; Baker, 1978). However, in the present investigation no evidence for an inhibition by La^{3+} of the outward movement of Ca^{2+} across the liver cell plasma membrane was obtained. Instead, the unexpected observation that La^{3+} stimulates the inflow of Ca^{2+} to the liver cell was made. This appears to be the first time that a stimulation by La^{3+} of Ca^{2+} inflow to any cell type has been documented. Moreover, the observation that $^{45}Ca^{2+}$ exchange is also stimulated by Cu^{2+}, Ni^{2+} and Mn^{2+} as well as by La^{3+} may be of considerable importance in connection with the ability of these ions to mimic some of the actions of insulin on cells (Saggerson *et al.*, 1976; Schimmel, 1978). In the present paper, evidence indicating that La^{3+} stimulates Ca^{2+} inflow to the liver cell is presented, and the implications of this observation are briefly discussed.

Experimental

Chemicals

$LaCl_3$ was obtained from E. Merck, Darmstadt, Federal Republic of Germany. Analytical-grade $CuCl_2$, $ZnCl_2$, $NiCl_2$, $CoCl_2$, $SrCl_2$, $FeCl_3$, $FeSO_4$ and $MnCl_2$ were purchased from Ajax Chemicals, Auburn, N.S.W., Australia. All other reagents were

0306-3283/81/100109-06$01.50/1

purchased from the sources described previously (Barritt *et al.*, 1981).

$^{45}Ca^{2+}$ exchange

The isolation of parenchymal cells from the livers of fed rats, assessment of their integrity, including measurement of the amount of lactate dehydrogenase released from the cells, and the measurement of $^{45}Ca^{2+}$ exchange by isolated hepatocytes under steady-state conditions were performed as described previously (Barritt *et al.*, 1981). The incubation medium contained, in a final volume of 6.0ml, 117mM-NaCl, 4.7mM-KCl, 1.2mM-KH_2PO_4, 1.2mM-$MgSO_4$, 24mM-$NaHCO_3$ and 20mM-Tes (2-{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino}-ethanesulphonic acid)/KOH, 1.3mM-$CaCl_2$, other additions as indicated, and hepatocytes [30mg wet wt. (2×10^6 cells)/ml]. The final pH of the medium was 7.4. Before initiation of $^{45}Ca^{2+}$ exchange, the cells were preincubated in this incubation medium (in the presence of 1.3mM-$CaCl_2$, metal salts and/or adrenaline, when present) for 15min before the addition of tracer quantities of $^{45}CaCl_2$ (0.3MBq, 16nmol of $^{45}Ca^{2+}$ per 6ml). The amount of $^{45}Ca^{2+}$ associated with the cells was measured after centrifugation through $LaCl_3$ and silicone oil, as described previously (Barritt *et al.*, 1981).

$LaCl_3$ (2.3mM) was included in the wash medium [150mM-NaCl/7% (w/v) bovine serum albumin] to which the cells were briefly exposed before centrifugation through silicone oil in order to displace $^{45}Ca^{2+}$ from superficial binding sites (Assimacopoulos-Jeannet *et al.*, 1977; Barritt *et al.*, 1981). However, in other experiments in which $^{45}Ca^{2+}$ exchange was measured (1.3mM extracellular Ca^{2+}) at either 2°C or 37°C with La^{3+} present in the NaCl/bovine serum albumin wash medium, the amounts of $^{45}Ca^{2+}$ associated with the cells at times between 0.5 and 4min after the initiation of $^{45}Ca^{2+}$ exchange were found to be the same as those observed when La^{3+} was omitted from the wash medium (G. J. Barritt, unpublished work).

Fits of the exponential eqn. (1):

$$q_4 = q_{41} - q_{42} \cdot e^{-\lambda_2 t} - q_{43} e^{-\lambda_3 t} \qquad (1)$$

where q_4 is the quantity of $^{45}Ca^{2+}$ associated with the cells and q_{41}, q_{42}, q_{43}, λ_2 and λ_3 are constants, and the series

$$C_1 \underset{k_{12}}{\overset{k_{21}}{\rightleftharpoons}} C_2 \underset{k_{23}}{\overset{k_{32}}{\rightleftharpoons}} C_3$$

and parallel

$$C_2 \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} C_1 \underset{k_{13}}{\overset{k_{31}}{\rightleftharpoons}} C_3$$

configurations of a three-compartment closed system to the data were performed by using a non-linear iterative curve-fitting procedure as described previously (Barritt *et al.*, 1981). Definitions of the symbols and kinetic parameters employed are as follows. C_1 represents exchangeable Ca^{2+} in the extracellular medium, and C_2 and C_3 the two compartments of exchangeable Ca^{2+} associated with

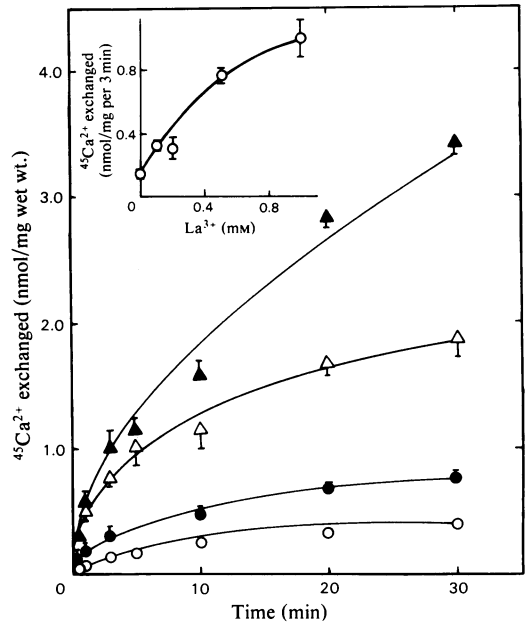

Fig. 1. *Effects of increasing concentrations of La^{3+} on $^{45}Ca^{2+}$ exchange by isolated hepatocytes*

Hepatocytes were incubated in the presence of 1.3mM-extracellular Ca^{2+} in the absence (○) or presence of (●) 0.2mM-, (△) 0.5mM- or (▲) 1.0mM-$LaCl_3$ for 15min before initiation of $^{45}Ca^{2+}$ exchange by the addition of tracer amounts of $^{45}CaCl_2$. The composition of the incubation medium and measurement of the amount of $^{45}Ca^{2+}$ associated with the cells were as described in the Experimental section. The values are the means ± S.E.M. for six (no $LaCl_3$) and four (0.2mM- and 0.5mM-$LaCl_3$) separate determinations for each time point, or the means of two separate determinations (1.0mM-$LaCl_3$). The lines were drawn from a fit of the data by eye. The inset shows the quantity of $^{45}Ca^{2+}$ exchanged at 3min as a function of the La^{3+} concentration. The observed amount of $^{45}Ca^{2+}$ exchanged at 3min in the presence of 0.1mM-La^{3+} is taken from the data of Fig. 2. The curve was drawn from a fit of the data to the Michaelis–Menten equation in which the values for the rate of $^{45}Ca^{2+}$ exchange in the absence of La^{3+}, the maximum rate of $^{45}Ca^{2+}$ exchange in the presence of saturating concentrations of La^{3+}, and K_m (the concentration of La^{3+} that gives half-maximal stimulation of the rate of $^{45}Ca^{2+}$ exchange) are 0.15 and 1.43nmol/3min per mg wet wt. and 0.7mM, respectively.

the cell. Q_1, Q_2 and Q_3 (nmol/mg wet wt.) represent the quantities of exchangeable Ca^{2+} in these three compartments respectively. The fractional transfer rate (rate constant) for the transfer of Ca^{2+} from compartment j to compartment i is represented by k_{ij} (min^{-1}) and the flux of Ca^{2+} between these compartments by R_{ij} (nmol/min per mg wet wt.).

Ca^{2+} uptake at 1°C

The composition of the incubation medium (12ml final volume) was the same as that described above. Hepatocytes isolated as described previously (Barritt *et al.*, 1981), i.e. in the absence of added Ca^{2+}, were equilibrated with the incubation medium in the absence of added Ca^{2+} and in the presence or absence of $LaCl_3$ for 3min at 1°C. Ca^{2+} uptake was initiated by the addition of $CaCl_2$ (1.3mM final concn.) at $t = 0$min (cf. Hinnen *et al.*, 1979). At the times indicated in Fig. 3, samples (2.0ml) of the incubation medium were removed, added to 11ml of 150mM-NaCl/7% (w/v) bovine serum albumin/2.3mM-$LaCl_3$ (Assimacopoulos-Jeannet *et al.*, 1977) at 0°C, centrifuged at 500*g* for 1min, the supernatant was removed by aspiration, and the tubes were inverted and allowed to drain for 2h. The amount of Ca^{2+} present in the cell pellet was determined by atomic-absorption spectroscopy as described previously (Barritt *et al.*, 1981). The amount of Ca^{2+} present in cells before exposure to 1.3mM extracellular Ca^{2+} was also measured and subtracted from that observed after initiation of Ca^{2+} uptake, in order to determine the net amount of Ca^{2+} accumulated by the cells.

Results

Concentrations of $LaCl_3$ within the range 0.2–1.0mM stimulated $^{45}Ca^{2+}$ exchange in isolated liver cells (Fig. 1). Half-maximal stimulation was observed at 0.7mM-La^{3+} (Fig. 1, inset). Cu^{2+}, Fe^{3+}, Fe^{2+}, Zn^{2+}, Ni^{2+} and Mn^{2+} also stimulated $^{45}Ca^{2+}$ exchange (Table 1). Moreover, 75μM-Cu^{2+} gave a greater degree of stimulation than that observed in the presence of 250μM-La^{3+} (Table 1).

In order to determine which cellular Ca^{2+}-transport processes were affected by La^{3+}, $^{45}Ca^{2+}$-exchange data for cells incubated with 1.3mM extracellular Ca^{2+} in the presence or absence of 0.1mM-La^{3+} were obtained and subjected to kinetic analysis. Similar experiments and analysis were also performed for cells treated with adrenaline. It was first established that incubation of the cells in the presence of 0.1mM-La^{3+} for the duration of the $^{45}Ca^{2+}$-exchange experiments (60min) caused no damage to the cells as assessed by light microscopy, the proportion of cells that excluded Trypan Blue, the intracellular ATP concentrations and the quantity of lactate dehydrogenase released during the incubation (results not shown).

La^{3+} increased both the initial rate and the plateau of the $^{45}Ca^{2+}$-exchange curve (Fig. 2). In the presence of 0.1mM-La^{3+}, adrenaline caused no additional stimulation of the initial rate of $^{45}Ca^{2+}$ exchange (Fig. 2). A fit of exponential equations to each of the four sets of data (Fig. 2) established that eqn. (1), which contains two exponential terms, is the simplest equation that is consistent with the data. This indicates that a minimum of two compartments of exchangeable Ca^{2+} are associated with the cells (Barritt *et al.*, 1981). Both the series and the parallel configurations of these cellular compartments of exchangeable Ca^{2+} (see the Experimental section) were found to be consistent with each set of

Table 1. *Effects of various metal ions on $^{45}Ca^{2+}$ exchange by isolated hepatocytes measured under steady-state conditions*
The composition of the incubation medium and measurement of the quantity of $^{45}Ca^{2+}$ exchanged at 3min were as described in the legend of Fig. 1. The metal ions were added as the chloride salt, with one exception, $FeSO_4$. The values are the means ± S.E.M. for the numbers of separate determinations indicated in parentheses. Degrees of significance were determined by using Student's *t* test for unpaired samples: N.S., not significant ($P > 0.05$).

Metal ion	Concn. (μM)	$^{45}Ca^{2+}$ exchanged at 3min (nmol/mg wet wt. of cells)	Significance
None		0.16 ± 0.01 (16)	—
La^{3+}	250	0.59 ± 0.04 (12)	$P < 0.001$
Cu^{2+}	25	0.21 ± 0.02 (2)	N.S.
	50	0.38 ± 0.02 (4)	$P < 0.02$
	75	1.08 ± 0.05 (2)	$P < 0.001$
	100	2.69 ± 0.39 (4)	$P < 0.001$
Fe^{3+}	250	0.54 ± 0.02 (6)	$P < 0.001$
Fe^{2+}	250	0.32 ± 0.02 (4)	$P < 0.001$
Zn^{2+}	250	0.31 ± 0.01 (6)	$P < 0.001$
Ni^{2+}	250	0.21 ± 0.01 (6)	$P < 0.001$
Mn^{2+}	250	0.21 ± 0.01 (4)	$P < 0.001$
Co^{2+}	250	0.18 ± 0.01 (4)	N.S.
Sr^{2+}	250	0.15 ± 0.01 (2)	N.S.

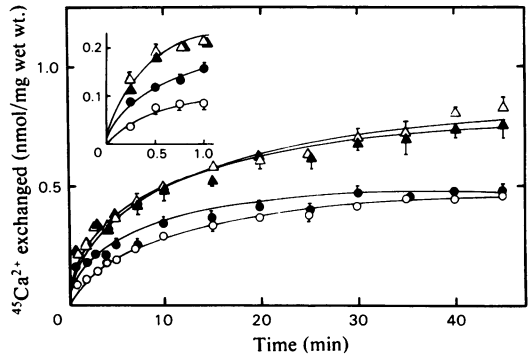

Fig. 2. *Effect of 0.1 mM-La^{3+} on $^{45}Ca^{2+}$-exchange curves obtained under steady-state conditions in the presence or absence of adrenaline*

Isolated hepatocytes were incubated for 15 min in the presence of 1.3 mM-$CaCl_2$ and in the presence (●, ▲) or absence (○, △) of 0.1 μM-adrenaline and in the presence (△, ▲) or absence (○, ●) of 0.1 mM-$LaCl_3$ before initiation of $^{45}Ca^{2+}$ exchange. The composition of the incubation medium and measurement of the amounts of $^{45}Ca^{2+}$ associated with the cells were as described in the Experimental section. Each point is the mean ± S.E.M. for 7–12 separate determinations. The inset shows the quantities of $^{45}Ca^{2+}$ exchanged during the first 1 min of the exchange curve. The lines were drawn by using the values of the constants (Table 2) obtained for a fit of the series configuration of three compartments of exchangeable Ca^{2+} to the data. Similar lines of best fit were obtained for a fit of the parallel configuration to the data.

data (Fig. 2, Table 2). The numerical values obtained for a fit of the series configuration are shown in Table 2. The fractional standard deviations of the constants obtained for a fit of the parallel configuration to the data were similar in magnitude to those for the series configuration (results not shown).

Consideration of the results obtained for a fit of the series configuration to the data of Fig. 2 shows that La^{3+} caused an increase in the size of both compartments of exchangeable Ca^{2+} (Table 2). This was associated with an increase of almost 300% in the flux (R_{21}) between the small compartment and the medium. The flux between the large compartment and the small compartment (R_{32}) was only slightly increased. In the presence of La^{3+}, the fractional transfer rate, k_{21}, for the transfer of Ca^{2+} from the medium to the small compartment was increased by about 250%. By comparison, changes in the other fractional transfer rates were much smaller (<70% of the control value). In the presence of La^{3+}, adrenaline caused no additional increase in the flux R_{21} or fractional transfer rate k_{21}. Similar conclusions about the observed changes in kinetic parameters are reached when the results obtained for a fit of the parallel configuration to the data of Fig. 2 are considered (results not shown).

La^{3+} stimulated both the initial rate and extent of Ca^{2+} uptake at 1°C by cells which had previously been maintained in a medium nominally free of Ca^{2+} (Fig. 3). The initial rate of Ca^{2+} uptake was increased by about 3.6- and 6-fold in the presence of

Table 2. *Effect of La^{3+} on the kinetic parameters obtained for a fit of the series configuration of three compartments of exchangeable Ca^{2+} in the absence and presence of adrenaline*

The series configuration (see the Experimental section) was fitted to the data of Fig. 2 (7–12 separate determinations at each time point), obtained at 1.3 mM extracellular Ca^{2+}, as described in the Experimental section. The values ± S.D. of each parameter are shown. The quantity of exchangeable Ca^{2+} in compartment 1 (Q_1), the medium, was 42 nmol/mg wet wt. The fractional transfer rate (rate constant) for the transfer of Ca^{2+} from compartment j ($j = 1$, 2, or 3) to compartment i ($i = 1$, 2, or 3) is represented by k_{ij} (min^{-1}), and the flux of Ca^{2+} between these compartments by R_{ij} (nmol/min per mg wet wt.). The degrees of significance for comparison of the value under test with the control (no La^{3+} or adrenaline), assessed by the t test described by Boxenbaum *et al.* (1974), were: *$P < 0.05$; **$P < 0.001$.

	No adrenaline		Adrenaline present	
	No La^{3+}	La^{3+} present	No La^{3+}	La^{3+} present
Fluxes (nmol/min per mg)				
R_{21}	0.25 ± 0.10	0.89 ± 0.25*	0.58 ± 0.12*	0.57 ± 0.07
R_{32}	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.004	0.03 ± 0.01
Fractional transfer rates (min^{-1})				
k_{21}	0.59 ± 0.23 (×10^{-2})	2.1 ± 0.6 (×10^{-2})*	1.4 ± 0.3 (×10^{-2})*	1.4 ± 0.2 (×10^{-2})
k_{12}	3.0 ± 1.8	4.3 ± 1.6	4.2 ± 1.2	2.3 ± 0.4
k_{23}	8.3 ± 1.3 (×10^{-2})	6.4 ± 1.2 (×10^{-2})	7.8 ± 1.3 (×10^{-2})	5.9 ± 1.7 (×10^{-2})
k_{32}	0.40 ± 0.14	0.20 ± 0.05	0.21 ± 0.05	0.13 ± 0.04
Compartment sizes (nmol/mg)				
Q_2	0.08 ± 0.02	0.20 ± 0.02**	0.14 ± 0.02*	0.25 ± 0.02
Q_3	0.39 ± 0.02	0.65 ± 0.04**	0.36 ± 0.02	0.56 ± 0.06
Weighted sums of squares [(nmol/mg)^2]	1.63 × 10^{-3}	2.94 × 10^{-3}	7.74 × 10^{-3}	3.31 × 10^{-3}

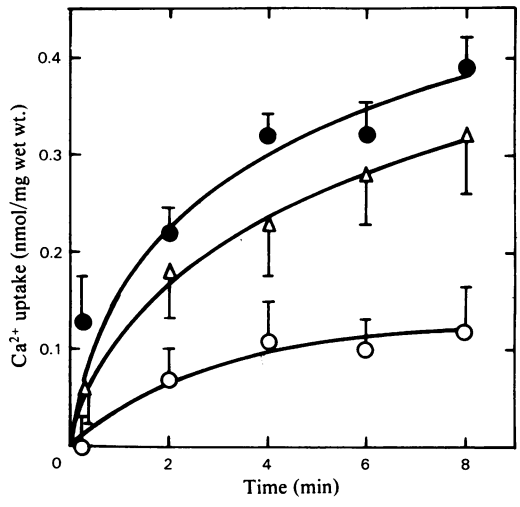

Fig. 3. *Effect of La^{3+} on the uptake of Ca^{2+} by isolated hepatocytes measured at 1°C and 1.3 mM extracellular Ca^{2+}*

The composition of the incubation medium and measurement (by atomic-absorption spectroscopy) of the amounts of Ca^{2+} taken up by the cells in the absence of $LaCl_3$ (○) and in the presence of (△) 0.1 μM- and (●) 0.2 mM-$LaCl_3$ were as described in the Experimental section. Each point represents the mean ± S.E.M. of the values from three to six separate experiments.

0.1 mM- and 0.2 mM-La^{3+} respectively. When experiments were conducted in the presence of antimycin A (5 μg/ml) and oligomycin (10 μg/ml), the amounts of Ca^{2+} taken up in the presence and absence of 0.1 mM-La^{3+} were similar to those shown in Fig. 3 (results not shown), indicating that the uptake of Ca^{2+} by mitochondria is not the rate-limiting step in the entry of Ca^{2+} to the cells (cf. Hinnen *et al.*, 1979).

Discussion

Two kinetically distinct compartments of exchangeable Ca^{2+} associated with the liver cell were observed in both the presence and absence of 0.1 mM-La^{3+}. Furthermore, the quantity of Ca^{2+} in the small compartment was increased in the presence of 0.1 mM-La^{3+}. Since La^{3+} has been shown to displace rapidly exchangeable Ca^{2+} from superficial binding sites on a number of cells (Weiss, 1974; Hellman, 1978; Langer *et al.*, 1979), including liver cells (Claret-Berthon *et al.*, 1977), these observations provide further evidence which is consistent with the conclusion (Barritt *et al.*, 1981) that both kinetically distinct compartments of exchangeable Ca^{2+} associated with isolated hepatocytes are located within the cell.

The results of the $^{45}Ca^{2+}$-exchange experiments, together with the effects of La^{3+} on Ca^{2+} uptake at 1°C, are consistent with the proposal that concentrations of La^{3+} within the range 0.1–0.2 mM increase the catalytic activity of the putative transporter of Ca^{2+} inflow across the plasma membrane. The presence of 0.1 mM-La^{3+} in the extracellular medium did not inhibit the flux of Ca^{2+} across the boundary of the large cellular compartment of exchangeable Ca^{2+}, which includes exchangeable Ca^{2+} in the mitochondria (Barritt *et al.*, 1981). Since low concentrations of La^{3+} (about 0.1 μM) have previously been shown to inhibit Ca^{2+} inflow to isolated mitochondria (Reed & Bygrave, 1974), it is concluded that, under the conditions employed in the present experiments, no significant quantities of La^{3+} enter the liver cell cytoplasm (cf. Weiss, 1974; Szász *et al.*, 1978; Flatt *et al.*, 1980). This may explain why no inhibition of Ca^{2+} outflow was observed, although the activity of the $(Ca^{2+}+Mg^{2+})$-dependent ATPase in plasma-membrane vesicles prepared from some other cells is inhibited by low concentrations of La^{3+} (Schatzmann & Bürgin, 1978; Pershadsingh & McDonald, 1980). In contrast with the effects of La^{3+}, the possibility that stimulation of the initial rate of $^{45}Ca^{2+}$ exchange by Cu^{2+}, Fe^{3+}, Fe^{2+}, Zn^{2+}, Ni^{2+} or Mn^{2+} involves entry of the cation to the cell cannot be excluded.

Although the ability of La^{3+} to inhibit many Ca^{2+}-transport systems has been clearly documented (reviewed by Weiss, 1974), there have been few reports of a stimulation of Ca^{2+} transport by this cation. Hinnen *et al.* (1979) have shown that La^{3+} and Tb^{3+} increase the rate of Ca^{2+} uptake by Ehrlich ascites-tumour cells. La^{3+} may stimulate Ca^{2+} inflow across the plasma membrane by inducing a conformational change in the Ca^{2+} inflow carrier through its combination with this transport protein or with phospholipids of the plasma membrane (cf. the ability of La^{3+} to stimulate some Ca^{2+}-dependent processes; reviewed by Weiss, 1974).

The present results are consistent with the proposal (Barritt *et al.*, 1981) that one of the actions of adrenaline is to stimulate the inflow of Ca^{2+} from the extracellular medium to the cytoplasm. Thus the changes induced by 0.1 mM-La^{3+} in the flux of Ca^{2+} between the medium and the small compartment of cellular exchangeable Ca^{2+}, the fractional transfer rate k_{21}, and the quantity of exchangeable Ca^{2+} present in the small compartment were found to be similar to those observed in the presence of adrenaline. However, in contrast with adrenaline, La^{3+} does not cause a release of Ca^{2+} from intracellular stores, and hence the plateau of the exchange curve is increased in the presence of La^{3+}.

We gratefully acknowledge the skilled technical assistance provided by Miss Kay Dalton, and the

provision of various metal chlorides by Mr. K. D. Wicks of the School of Physical Sciences, Flinders University of South Australia. This work was supported by a grant from the National Health and Medical Research Council of Australia.

References

Assimacopoulos-Jeannet, F. D., Blackmore, P. F. & Exton, J. H. (1977) *J. Biol. Chem.* **252**, 2662–2669
Baker, P. F. (1978) *Ann. N.Y. Acad. Sci.* **307**, 250–268
Barritt, G. J. & Parker, J. C. (1980) *J. Physiol. (London)* **301**, 45*P*
Barritt, G. J., Parker, J. C. & Wadsworth, J. C. (1981) *J. Physiol. (London)* **312**, 29–55
Boxenbaum, H. G., Riegelman, S. & Elashoff, R. M. (1974) *J. Pharmacokin. Biopharm.* **2**, 123–148
Claret-Berthon, B., Claret, M. & Mazet, J. L. (1977) *J. Physiol. (London)* **272**, 529–552
Flatt, P. R., Boquist, L. & Hellman, B. (1980) *Biochem. J.* **190**, 361–372
Foden, S. & Randle, P. J. (1978) *Biochem. J.* **170**, 615–625
Hellman, B. (1978) *Biochim. Biophys. Acta* **540**, 534–542
Hinnen, R., Miyamoto, H. & Racker, E. (1979) *J. Membr. Biol.* **49**, 309–324
Katzung, B. G., Reuter, H. & Porzig, H. (1973) *Experientia* **29**, 1073–1075
Keppens, S., Vandenheede, J. R. & De Wulf, H. (1977) *Biochim. Biophys. Acta* **496**, 448–457
Langer, G. A., Frank, J. S. & Nudd, L. M. (1979) *Am. J. Physiol.* **237**, H239–H246
Pershadsingh, H. A. & McDonald, J. M. (1980) *J. Biol. Chem.* **255**, 4087–4093
Reed, K. C. & Bygrave, F. L. (1974) *Biochem. J.* **138**, 239–252
Saggerson, E. D., Sooranna, S. R. & Evans, C. J. (1976) *Biochem. J.* **154**, 349–357
Schatzmann, H. J. & Bürgin, H. (1978) *Ann. N.Y. Acad. Sci.* **307**, 125–147
Schimmel, R. J. (1978) *Horm. Metab. Res.* **10**, 128–134
Szász, I., Sarkadi, B., Schubert, A. & Gárdos, G. (1978) *Biochim. Biophys. Acta* **512**, 331–340
Weiss, G. B. (1974) *Annu. Rev. Pharmacol.* **14**, 343–354