Biochimica et Biophysica Acta 860 (1986) 429–433
Elsevier

BBA 70335

BBA Report

Effect of changes in the rate of ionophore A23187-induced calcium influx on the pump-leak steady-state distribution of calcium in inosine-fed human red cells

Teresa Tiffert [b,*] and Virgilio L. Lew [a]

[a] *Physiological Laboratory, Cambridge University, Cambridge (U.K.) and* [b] *Division of Hematology, Department of Medicine, The Johns Hopkins University, Baltimore, MD 21205 (U.S.A.)*

(Received March 7th, 1986)

Key words: Ca^{2+} transport; Ionophore A23187; Inosine; Erythrocyte membrane

We studied the effect of varying the rate of ionophore A23187-induced calcium influx on the mean calcium content of inosine-fed human red cells in pump-leak steady state. Slow calcium infusion caused only a marginal reduction in the mean calcium content of cells in the steady state relative to their content after sudden calcium addition.

The divalent cation ionophore A23187 has been extensively used to identify and characterize calcium effects and calcium transport in a variety of cells. In red cells, the ionophore-permeabilization method, alone or in conjunction with non-disruptively incorporated calcium chelators, has enabled measurement of cytoplasmic Ca and Mg buffering, physiological Ca^{2+} levels, the characterization of active and passive calcium transport, and the effects of intracellular Ca^{2+} on other ion transport systems and on cell metabolism [1–20]. A common experimental feature in all these previous studies was that the increase in calcium influx mediated by the ionophore was implemented suddenly, either by addition of calcium in the presence of ionophore or by addition of ionophore in the presence of Ca. When the ionophore-induced calcium influx was below about 30 mmol/l cells per h, the mean calcium content of inosine-fed red cells was found to reach steady levels which were lower than those in ATP-depleted cells [1,7,18,19]. Such steady states were interpreted as representing the balance between ionophore-induced passive calcium influx and active calcium extrusion by the powerful red cell calcium pump [1,18]. Recent results [21], however, suggest that despite the well documented uniformity of ionophore-induced calcium permeability [16], the distribution of calcium in those steady-states is extremely heterogeneous. Most of the cell-associated calcium was found to be contained near equilibrium within a fraction of cells whose ATP production failed to match consumption by the pump and thus became ATP depleted. Brown and Johnston [17] found that a rise in internal calcium stimulated lactic acid production but with delays of up to 20 min and with a biphasic Ca^{2+}-concentration dependence. This suggests that the rate at which ionophore-induced calcium influx is raised, or that at which Ca^{2+} changes within the cells, may critically influence their ability to sustain ATP synthesis rates that would match ATP breakdown by the pump. It has therefore become necessary to investigate whether more gradual increases in ionophore-induced calcium influx would allow a larger proportion of cells to balance the metabolic demand of the calcium pump, thus reducing the fraction of

* Present address: Physiological Laboratory, Cambridge University, Cambridge, CB2 3EG, U.K.

TABLE I

EFFECT OF THE RATE OF CALCIUM INFUSION AT CONSTANT IONOPHORE CONCENTRATION ON THE MEAN CALCIUM CONTENT OF INOSINE-FED HUMAN RED CELLS

$[Ca_i^T]$ represents the total cell calcium content and $[Ca_o^T]$ the external calcium concentration in steady state or at equilibrium, as estimated from the redistribution of ^{45}Ca-tracer in the cell suspensions [8]. The value of $[Ca_i^T]_{eq}$ (penultimate column), which represents the expected cell calcium content at equilibrium with the measured external calcium concentration in steady state ($[Ca_o^T]_{st.st.}$), was calculated from $[Ca_i^T]_{eq} = [Ca_o^T]_{st.st.} (r^2/\alpha)$, [8,18]. The last column reports $([Ca_i^T]_{st.st.}/[Ca_i^T]_{eq}) \cdot 100$, where $[Ca_i^T]_{st.st.}$ represents the mean total calcium content of the cells in steady state. If we assume that the mean total calcium content of the cells in steady state is mainly contained within calcium-equilibrated cells [21], the last column simply represents the fraction of calcium-equilibrated cells in the steady state.

Expt. No.	Donor	[A23187] in suspension (μmol/l susp)	Final [Ca] in suspension (μmol/l susp)	[EGTA] (μM)	Rate of Ca infusion (μmol/l susp per min)	$[Ca_i^T]$ (μmol/l cells)		$[Ca_o^T]$ (μmol/l medium)		α	Estimated $[Ca_i^T]_{eq}$ at $[Ca_o^T]$ in steady state (μmol/l cells)	Percent of equilibrium
						in steady state	at equilibrium	in steady state	at equilibrium			
1	VLL	1.0	A. 70	20	sudden	2.2						
			B. 70	20	5.6	1.6						
			C. 70	20	6.2	2.4						
2	VLL	1.0	A. 125	0	sudden	5.8	591	138	73		1104	0.53
			B. 125	0	12.3	49	620	134	70	0.25	1072	3.7
			C. 125	0	12.3	8.0	628	138	69		1104	0.72
3	VLL	1.0	A. 225	0	sudden	4.0	979	250	141		1850	
			B. 225	0	22.5	4.0	1032	250	135	0.27	1850	0.22
			C. 225	0	22.5	4.4	1012	250	138		1850	
4	VLL	1.3	A. 225	0	sudden	521	1034	192	135	0.26	1480	35
			B. 219	0	26.0	360	1034	203	128	0.24	1690	21
			C. 215	0	26.0	207	1037	216	124	0.24	1800	12
5	JG	1.3	A. 256	20	sudden	822	952	193	179	0.33	1048	78
			B. 256	20	sudden	821	947	193	179	0.34	1018	81
			C. 226	20	11.0	577	847	187	157	0.32	1045	55
			D. 222	20	11.0	515	834	189	154	0.32	1056	49
6	VLL	1.3	A. 225	20	sudden	393	1072	206	131	0.21	1771	22
			B. 225	20	sudden	463	1051	199	133	0.22	1627	28
			C. 231	20	13.4	189	1129	214	131	0.20	1940	10
			D. 232	20	13.4	383	1097	215	136	0.21	1857	21

ATP-depleted cells and consequently the mean calcium content of the cells in steady state. In the experiments reported here, we investigate this point by comparing the steady-state mean calcium content of ionophore-treated, inosine-fed red cells, suspended in media to which calcium was infused at different rates, but to the same final concentration.

Red cells from fresh, heparinized blood were washed twice with a medium containing, in mM: KCl, 80; NaCl, 70; Hepes-Na (pH 7.55), 10; $MgCl_2$, 0.2; EGTA, 0.1, and twice more with the same medium but without EGTA. After these washes the cells were suspended at 10% hematocrit in the same medium, containing in addition 10 mM inosine and the concentrations of EGTA shown in Table I for each of the experiments performed. This suspension was preincubated for about 15 min at 37°C before addition of ionophore A23187 (2 mM stock solution in DMSO), under vigorous magnetic stirring, and then divided into three or four equal parts for addition of calcium in the amounts and at the rates indicated in Table I. The same concentrated ^{45}Ca stock solution, with a specific activity of about 10^7 cpm/μmol, was used in all experiments. Sudden addition of calcium was done by means of a one-step dispensing pipette. Continuous infusion was performed by a pump-driven syringe at a constant rate. The concentration of calcium in the infusion solution varied from 2.5 to 50 mM and the volumes delivered to 2 ml of cell suspension from 20 to 50 μl. The rate of calcium infusion was monitored in 10 μl samples taken at regular intervals during the infusion. The measured rate of change in calcium concentration in the suspension is reported in Table I for all the experiments. The mean calcium content of the cells was measured between 15 and 40 min after the onset of calcium infusion or sudden calcium addition in 0.05 ml

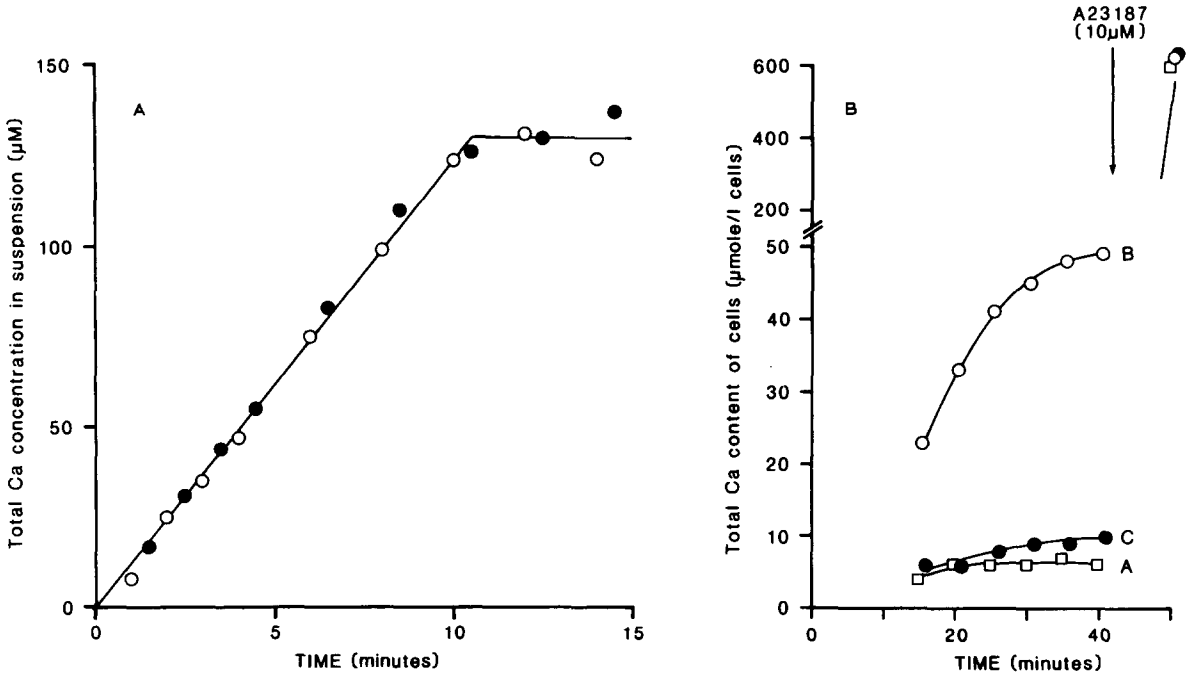

Fig. 1. Effect of the rate of calcium infusion on the mean cell calcium level at low calcium influx. The experiment corresponds to that reported second in Table I. Panel A shows the measured change with time in the total calcium level of suspensions (B) (○) and C (●). In suspension A (□), calcium was added at zero-time in one step, to the same final level as in suspensions B and C. Panel B shows the change with time in the mean total calcium content of the cells in the three suspensions. In all instances the first samples were taken after calcium infusion was completed. Note the break in the ordinate scale to accomodate the points after extra ionophore addition.

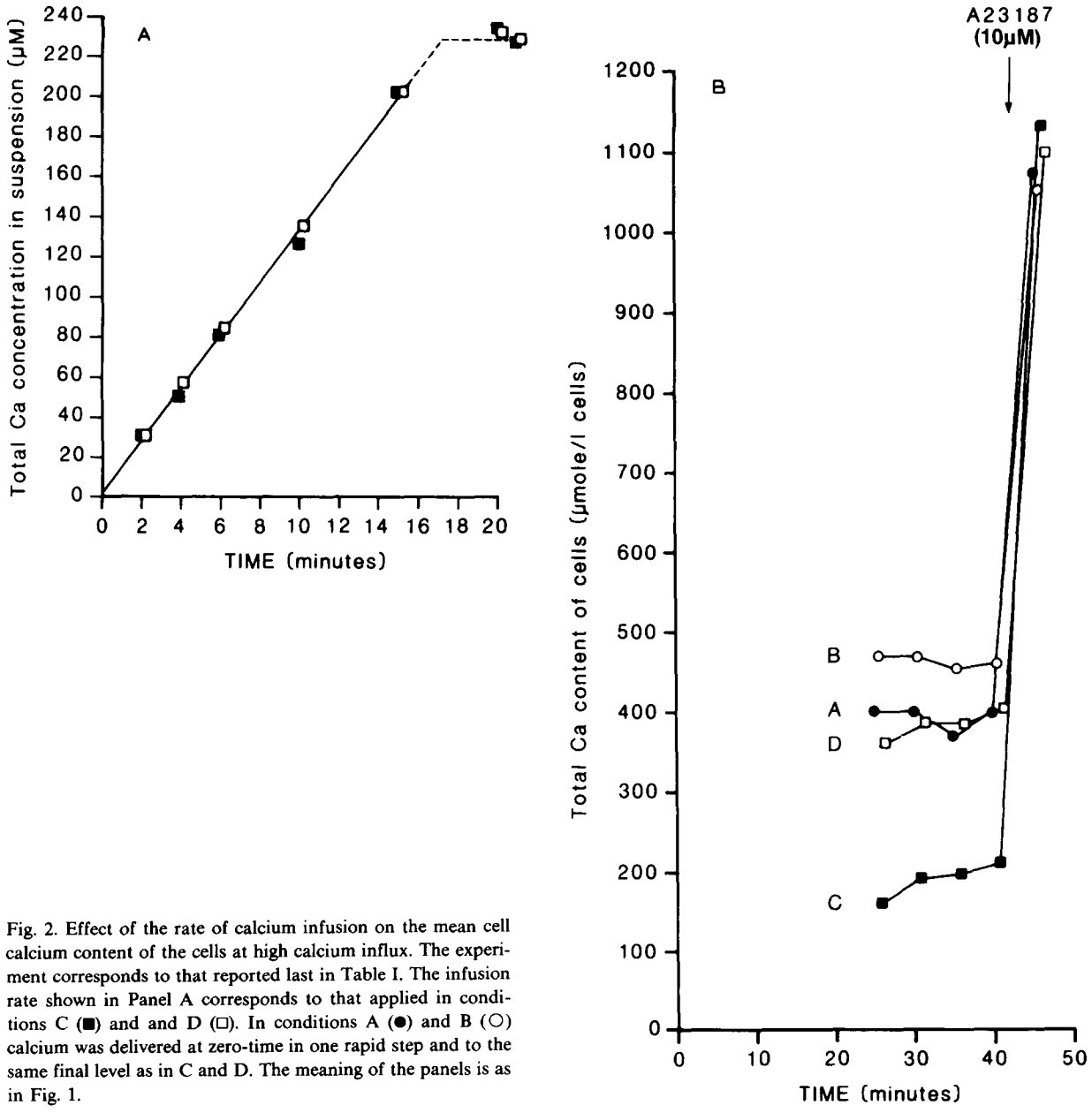

Fig. 2. Effect of the rate of calcium infusion on the mean cell calcium content of the cells at high calcium influx. The experiment corresponds to that reported last in Table I. The infusion rate shown in Panel A corresponds to that applied in conditions C (■) and and D (□). In conditions A (●) and B (○) calcium was delivered at zero-time in one rapid step and to the same final level as in C and D. The meaning of the panels is as in Fig. 1.

samples of the cell suspension, after separating the cells by centrifugation through dibutylphthalate oil as described before [1,18]. At 40 min, additional ionophore was added to the suspension to increase its concentration by 10 μM in order to induce calcium equilibration in all the cells. The condition with high ionophore yields α, the fraction of ionized calcium within the cells, from

$$\alpha = r^2([Ca_o^T]/[Ca_i^T])_{eq}$$

where r is the ratio of internal to external proton concentrations, and the second factor represents the ratio of external to total cell calcium con-

centrations at equilibrium [1,8]. With α, the expected $[Ca_i^T]_{eq}$ may be computed for any $[Ca_o^T]$ (penultimate column, Table I). In the first three experiments of Table I, the combination of ionophore and calcium concentrations was set to provide calcium influxes from 2 to 8 mmol/l cells per h, a range well below the maximal metabolic and calcium extrusion capacity of most cells. Except for one condition (curve B in the experiment of Fig. 1), the rate of change in calcium influx had no meaningful effect on the mean calcium content of the cells in the steady-state.

When the concentrations of ionophore and final calcium in the suspension were at levels which produced more than 20% of Ca-equilibrated cells after sudden addition of Ca, as in the last three experiments of Table I, slow calcium influx reduced the fraction of Ca-equilibrated cells in all but one of the six conditions investigated (curve D of Fig. 2). This exception, as well as the noticed differences between carefully duplicated conditions at low (first three experiments) and high (last three experiments) Ca influx values, seem to indicate the operation of subtle, highly unstable factors determining the fraction of Ca^{2+}-equilibrated cells.

The results reported here show that large changes in the rate at which ionophore-mediated calcium influx is increased have relatively little predictive effect on the steady-state calcium content of inosine-fed human red cells. It would seem that when the final calcium influx exceeds 10 mmol/l cells per h, a slow increase in calcium influx tends to improve, if only marginally, the ability of a larger proportion of cells to sustain higher metabolic rates of ATP synthesis. The absolute value of the final calcium influx within each experiment still appears to be the main factor which determines the fraction of cells able to match the inosine-sustained metabolic production of ATP to the rate of ATP breakdown by the calcium pump.

We thank the Burroughs Wellcome Fund for a Wellcome Research Travel Grant to T.T., the Wellcome Trust and the MRC(UK) for funds, J. Gray for technical assistance and R.M. Bookchin for helpful comments on the manuscript.

References

1 Ferreira, H.G. and Lew, V.L. (1976) Nature 259, 47–49
2 Pfeiffer, D.R. and Lardy, H.A. (1976) Biochemistry 15, 935–943
3 Pressman, B.C. (1976) Annu. Rev. Biochem. 45, 501–530
4 Sarkadi, B., Szasz, I. and Gardos, G. (1976) J. Membrane Biol. 26, 357–370
5 Sarkadi, B., Szasz, I., Gerloczy, A. and Gardos, G. (1977) Biochim. Biophys. Acta 464, 93–107
6 Lew, V.L. and Ferreira, H.G. (1978) in Current Topics in Membranes and Transport (Kleinzeller, A. and Bronner, F., eds.), Vol. 10, pp. 217–277, Academic Press, New York
7 Brown, A.M. (1979) Biochim. Biophys. Acta 554, 195–203
8 Lew, V.L. and Brown, A.M. (1979) in Detection and Measurement of Free Ca^{2+} in Cells (Ashley, C.C. and Campbell, A.K., eds.), pp. 423–432, Elsevier/North-Holland, Amsterdam
9 Bookchin, R.M. and Lew, V.L. (1980) Nature 284, 561–563
10 Flatman, P.W. and Lew, V.L. (1980) J. Physiol. (Lond) 305, 13–30
11 Lew, V.L., Bookchin, R.M., Brown, A.M. and Ferreira, H.G. (1980) in Membrane Transport in Erythrocytes (Lassen, U.V., Ussing, H.H. and Wieth, J.O., eds.), pp. 196–207, Munksgaard, Copenhagen
12 Sanchez, A., García-Sancho, J. and Herreros, B. (1980) FEBS Lett. 110, 65–68
13 Flatman, P.W. and Lew, V.L. (1981) J. Physiol. (Lond.) 315, 421–446
14 Till, U., Petermann, H., Wenz, I. and Arese, P. (1981) Cell Calcium 2, 495–507
15 Lew, V.L., Tsien, R.Y., Miner, C. and Bookchin, R.M. (1982) Nature 298, 478–481
16 Simonsen, L.O., Gomme, J. and Lew, V.L. (1982) Biochim. Biophys. Acta 692, 431–440
17 Brown, A.M. and Johnston, M.J. (1983) J. Physiol. (Lond.) 341, 63P
18 Brown, A.M. and Lew, V.L. (1983) J. Physiol. (Lond.) 343, 455–493
19 Scharf, O., Foder, B. and Skibsted, U. (1983) Biochim. Biophys. Acta 730, 295–305
20 Tiffert, T., García-Sancho, J. and Lew, V.L. (1984) Biochim. Biophys. Acta 773, 143–156
21 Lew, V.L. and García-Sancho, J. (1985) Cell Calcium 6, 15–23