Volume 244, number 1, 89-92 FEB 06788 February 1989

Evidence for a sodium/calcium exchanger and voltage-dependent calcium channels in adipocytes

Harrihar A. Pershadsingh*, L.-Y. Lee and Kenneth W. Snowdowne

*Department of Laboratory Medicine, University of California, San Francisco, CA 94143-0808 and Department of Biochemistry, University of the Pacific School of Dentistry, 2155 Webster Street, San Francisco, CA 94115, USA

Received 12 December 1988

The objective of these studies is to identify and characterize Ca^{2+}-transport systems that may be of potential importance in the action of Ca^{2+}-mobilizing hormones in the adipocyte. Using the Ca^{2+}-sensitive photoprotein, aequorin, $[Ca^{2+}]_i$ was estimated to be 0.15 μM, assuming an intracellular $[Mg^{2+}]$ of 1 mM. Substitution of Na^+ with choline$^+$ caused a transient increase in $[Ca^{2+}]_i$ which was inversely related to extracellular $[Na^+]$, consistent with operation of a mediated Na^+-Ca^{2+} exchange system. The stoichiometry was $3Na^+$:Ca^{2+}. Elevation of extracellular K^+ caused an increase in $[Ca^{2+}]_i$ that was blocked by the Ca^{2+} channel antagonist, diltiazem, by omitting extracellular Ca^{2+}, or by substituting Sr^{2+} for Ca^{2+}. These findings indicate the presence of an Na^+-Ca^{2+} exchanger and voltage-sensitive Ca^{2+} channels in adipocytes.

Na^+-Ca^{2+} exchange; Ca^{2+}, intracellular; Ca^{2+} channel; Aequorin; (Adipocyte)

1. INTRODUCTION

Interest in Ca^{2+} homeostasis in the adipocyte derives from abundant data implying that Ca^{2+} serves as a mediator of hormone action [1,2]. These studies were based primarily on radioisotopic studies using intact cells and purified subcellular fractions (review [1]). Measurements of free intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) in rat adipocytes using the fluorescent intracellular Ca^{2+} indicator, fura-2, have been reported [3,4]. In view of the potential pitfalls of fura-2 [5,6], we sought to measure $[Ca^{2+}]_i$ in adipocytes using the Ca^{2+}-sensitive protein, aequorin [7,8]. We provide direct evidence for the existence of an Na^+-Ca^{2+} exchanger and voltage-dependent Ca^{2+} channels in the surface membrane of adipocytes.

Correspondence address: H.A. Pershadsingh, Department of Laboratory Medicine, CED-230, Box 0808, University of California, San Francisco, CA 94143-0808, USA

2. EXPERIMENTAL

Adipocytes isolated from epididymal fat pads of 250 g Sprague-Dawley rats [9] were loaded with aequorin [7,8] and analyzed at 22°C. Aequorin luminescence was measued in a specially constructed photometer [10], modified to immobilize mammalian cells in a flow-through cuvette [7]. Cells ($\sim 2 \times 10^5$) in 1 ml Krebs-Ringer phosphate (KRP) containing (in mM) 128 NaCl, 5.2 KCl, 10 Na_2HPO_4, 1.4 $MgSO_4$, 1.4 $CaCl_2$, 5.6 dextrose, 3% bovine serum albumin (BSA) (pH 7.4) were placed in the photometer cuvette. $[Ca^{2+}]_i$ was quantified by injecting 10 μl of the Ca^{2+} ionophore, A23187 (0.01 M in butanol), into the cuvette through a light-tight port with a spring-loaded Hamilton syringe. The light generated was electronically integrated. Background luminescence (L_0) of unloaded cells (0.1% of the A23187 signal) and the A23187-evoked integral (L_{max}) were used to calculate the fractional luminescence (L_0/L_{max}) and $[Ca^{2+}]_i$ interpolated on calibration curves supplied with the aequorin sample, relating L_0/L_{max} to free $[Ca^{2+}]$ [10,11]. Investigations of Na^+-Ca^{2+} exchanger and Ca^{2+} channel activities required rapid changes in the extracellular medium. This was done by perifusing cells entrapped in the cuvette with glass wool with a Krebs-Henseleit bicarbonate (KHB) buffer containing (in mM) 120 NaCl, 4 KCl, 1.0 KH_2PO_4, 1.4 $MgSO_4$, 24 $NaHCO_3$, 5.6 dextrose, 1.4 $CaCl_2$, 1% BSA (pH 7.4) at 37°C. The composition was varied by iso-osmotic substitution of NaCl with choline chloride or KCl.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

Table 1

Estimates of adipocyte intracellular free calcium concentration using aequorin

Expt	Fractional luminescence ($-\log L_o/L_{max}$)	Estimated $[Ca^{2+}]_i$ for $[Mg^{2+}]$ of:		
		0.0 mM	1.0 mM	2.0 mM
1	6.18	20	140	200
2	6.18	20	140	200
3	6.23	10	130	180
4	6.18	20	140	200
5	5.97	40	190	280
6	6.14	20	150	220
Mean ± SE	6.15 ± 0.04	22 ± 4	148 ± 8	213 ± 13

Estimations of $[Ca^{2+}]_i$ (nM) were derived from calibration curves supplied by J.R. Blinks for the allotment of aequorin used (see section 2), and were determined for $[Mg^{2+}]$ 0.0, 1.0 and 3.0 mM, respectively

A23187 and BSA were purchased from Sigma (St. Louis, MO), collagenase from Cappel Labs (West Chester, PA), and aequorin from Dr J.R. Blinks (Mayo Foundation, Rochester, MN). Diltiazem was a gift from Marion Labs (Kansas City, MO).

3. RESULTS AND DISCUSSION

Estimates of $[Ca^{2+}]_i$ from 6 separate adipocyte preparations are shown in table 1. Because Mg^{2+} influences the interaction between Ca^{2+} and aequorin [10], estimates of $[Ca^{2+}]_i$ for $[Mg^{2+}]$ of 0, 1 and 3 mM are given. Intracellular $[Mg^{2+}]$ ($[Mg^{2+}]_i$) in rat tissues ranges from 0.5 to 1.0 mM [12]. Assuming $[Mg^{2+}]_i$ to be 1.0 mM, the average $[Ca^{2+}]_i$ in adipocytes was estimated at approx. 0.15 μM. Estimates of $[Ca^{2+}]_i$ in adipocytes obtained using fura-2 ranged from 45 to 60 nM [3,4], 2–3-fold less than ours. Given the uncertainties in the intracellular distribution of fura-2 and the possible interferences of as yet uncharacterized fluorescent metabolic intermediates of fura-2 [5,6], and the lack of a precise estimate of $[Mg^{2+}]_i$, we regard the estimates as reasonably close. Refinements of both techniques are clearly desirable.

Reversal of the transmembrane Na^+ gradient by iso-osmotic replacement of Na^+ with choline$^+$ caused a transient increase in $[Ca^{2+}]_i$ (fig.1A), ris-

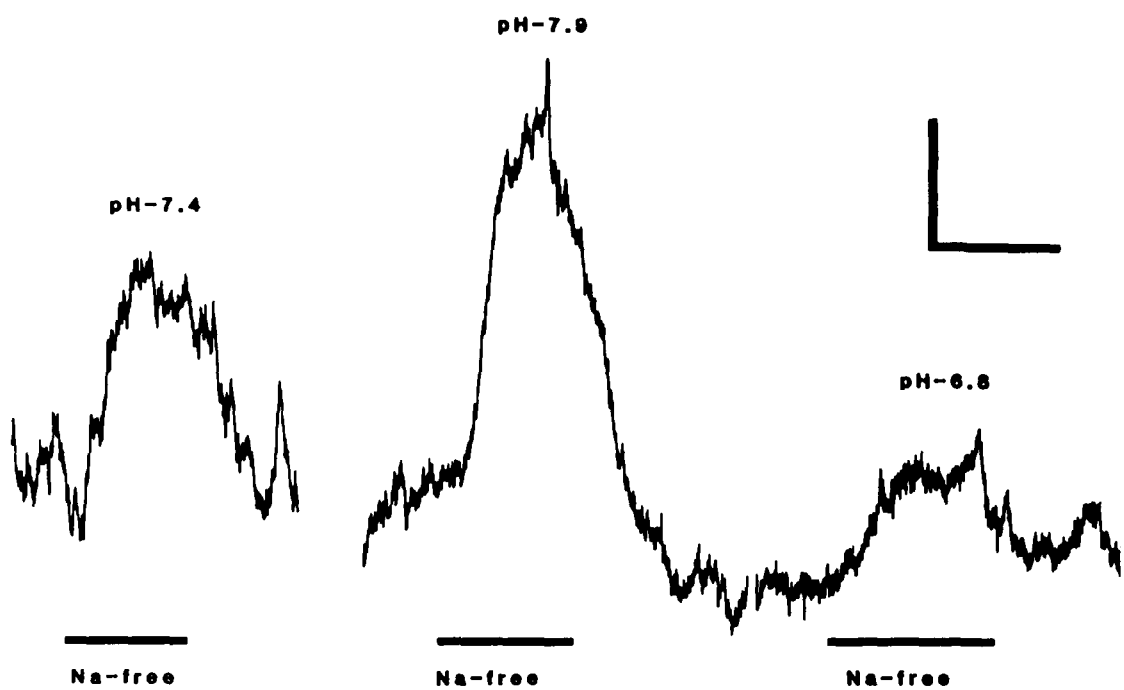

Fig.1. Evidence for the presence of an Na^+-Ca^{2+} exchanger. Exposure of aequorin-loaded adipocytes to Na^+-free KHB, pH 7.4, caused a transient increase in $[Ca^{2+}]_i$ (left trace). Na^+-free KHB, pH 7.9, had a greater (middle trace) while Na^+-free KHB, pH 6.8, had a diminished (right trace) effect on $[Ca^{2+}]_i$. Calibration bars: vertical, 50 nA; horizontal, 5 min.

ing sharply to approx. 2-fold above the basal level and returning within 2–7 min. We interpret this rise in $[Ca^{2+}]_i$ as increased Ca^{2+} influx via an Na^+-Ca^{2+} exchange carrier that continues as long as intracellular $[Na^+]$ ($[Na^+]_i$) is available to exchange with extracellular Ca^{2+}. When $[Na^+]_i$ falls to a sufficiently low level, the exchanger transports Ca^{2+} in both directions and $[Ca^{2+}]_i$ falls as other Ca^{2+}-transporting systems remove Ca^{2+} from the cytoplasm. The activity of the Na^+-Ca^{2+} exchanger was sensitive to changes in the extracellular pH. Perifusion of cells with Na^+-free KHB at pH 7.9 caused a greater increase in $[Ca^{2+}]_i$ than at pH 7.4 whereas at pH 6.8 a diminished response was noted (fig.1B,C). Similar results have been obtained in other cell systems [13]. These observations provide direct evidence for the presence of an Na^+-Ca^{2+} exchanger in the adipocyte, supporting the indirect radioisotopic findings of Clausen et al. [14].

In order to characterize the affinity of the Na^+-Ca^{2+} exchanger for extracellular $[Ca^{2+}]$ ($[Ca^{2+}]_o$), cells were perifused with Na^+-free KHB containing $[Ca^{2+}]_o$ from 10 μM to 1.3 mM. After achieving steady-state luminescence, the cells were perifused with Na^+-free KHB containing 1.3 mM $CaCl_2$ until $[Ca^{2+}]_i$ increased to a new steady state which occurred within 10 min, during a period of constant luminescence. The increase in $[Ca^{2+}]_i$ observed in Na^+-free solution containing 10 μM Ca^{2+} was consistently less than 50% of those in Na^+-free solution containing 1.3 mM Ca^{2+}, while responses obtained with Na^+-free solutions containing 0.13 mM Ca^{2+} or greater were consistently greater than 60%. The apparent dissociation constant (K_d) of the Na^+-Ca^{2+} exchanger for $[Ca^{2+}]_o$ was estimated to be approx. 90 μM. In analogous experiments in which cells were perifused with buffer containing varying $[Na^+]$, the K_d of the Na^+-Ca^{2+} exchanger for extracellular Na^+ $[Na^+]_o$ was estimated to be approx. 40 mM. Using the following values: $[Na^+]_o$ = 140 mM; $[Na^+]_i$ = 14 mM; $[Ca^{2+}]_o$ = 1.2 mM; $[Ca^{2+}]_i$ = 0.15 μM; one can calculate equilibrium potentials for Na^+ and Ca^{2+} electrochemical gradients to be 60 and 118 mV, respectively. Assuming a membrane potential of −58 mV for the adipocyte, as obtained by others [15,16], one can determine that the electrochemical gradient for Ca^{2+} (34.4 kJ/mol) is close to 3-times that for Na^+ (11.6 kJ/mol), suggesting that the Na^+-Ca^{2+} exchanger has no net flux if its stoichiometry is $3Na^+ : Ca^{2+}$, and that it would transport Ca^{2+} inwardly during depolarization and outwardly during repolarization.

Exposure of adipocytes to KHB medium containing 60 mM K^+ by isotonic substitution of KCl for NaCl, a condition that depolarizes the membrane potential by approx. 50 mV [15], caused a rapid and sustained increase in $[Ca^{2+}]_i$ (fig.2A). Reperifusion with normal KHB reversed the effect. The K^+-evoked increase in $[Ca^{2+}]_i$ was completely blocked with the Ca^{2+} channel antagonist, diltiazem (10 μM). Depolarization of cells with

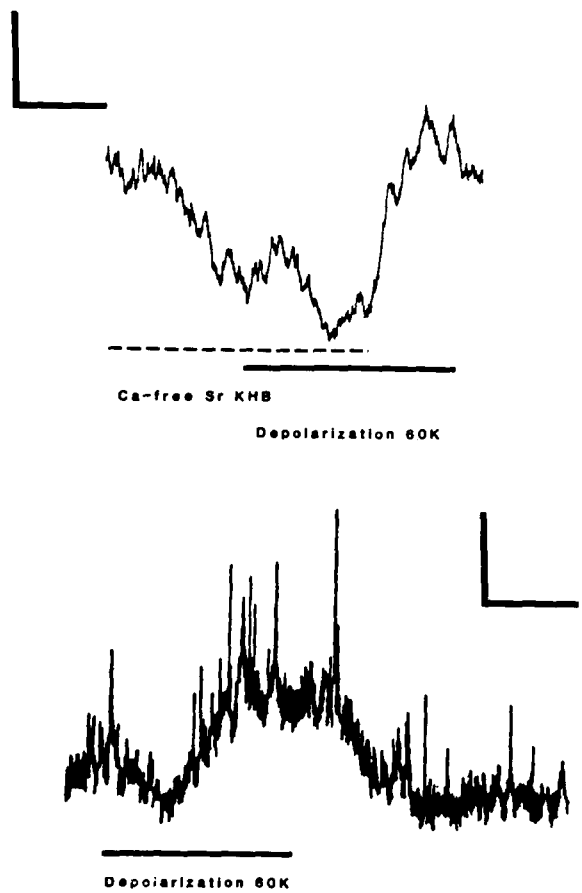

Fig.2. Evidence for the presence of voltage-dependent Ca^{2+} channels. (Lower trace) Exposure of aequorin-loaded adipocytes to buffer containing 60 mM KCl; (upper trace) exposure of cells to the same KHB buffer, in which Sr^{2+} was substituted for Ca^{2+}, decreased the basal $[Ca^{2+}]_i$ and blocked the rise in $[Ca^{2+}]_i$ evoked by 60 mM KCl. Removal of Sr^{2+} and reexposure to 1.4 mM Ca^{2+} caused an abrupt increase in $[Ca^{2+}]_i$. Calibration bars (upper): vertical, 10 nA; horizontal, 8 min; (lower): vertical 10 nA; horizontal, 2 min.

KHB containing 60 mM K^+, in which $[Ca^{2+}]_o$ was replaced with Sr^{2+} or in which Ca^{2+} was omitted, had no effect on aequorin luminescence (fig.2B). Although Ca^{2+} channels also conduct Sr^{2+}, the latter has a much lower affinity for aequorin, and therefore was not expected to evoke the luminescence reaction [8,13]. These observations are consistent with the existence of voltage-dependent Ca^{2+} channels in the adipocyte plasma membrane.

The control of intracellular Ca^{2+} homeostasis has an important role in the transduction of physiological signals. An Na^+-Ca^{2+} exchange with a stoichiometry of $3Na^+ : Ca^{2+}$ at zero net flux suggests that the system is electrogenic and active under normal resting conditions, and may therefore regulate transmembrane Ca^{2+} flux during activation by hormones that affect the adipocyte membrane potential. Also, both Na^+-Ca^{2+} exchange carriers and voltage-sensitive Ca^{2+}channels are potential routes of agonist-induced regulation of $[Ca^{2+}]_i$ and have important implications for activation of adipocytes by Ca^{2+}-mobilizing hormones.

Acknowledgements: This work was supported by a Research and Development Award from the American Diabetes Association, grants from the UCSF Academic Senate and REAC Committees on Research and BSRG grant S07 RR05355 (H.A.P.) and USPHS grant DK37729 (K.W.S.).

REFERENCES

[1] McDonald, J.M., Graves, C.B. and Christensen, L.C. (1984) in: Calcium and Cell Function (Cheung, W.Y. ed.) vol. 5, pp. 207–209, Academic Press, New York.
[2] Cryer, A. and Van, R.L.R. (1984) New Perspectives in Adipose Tissue: Structure and Function and Development, Butterworths, London.
[3] Draznin, B., Kao, M. and Sussman, K.E. (1987) Diabetes 36, 174–178.
[4] Draznin, B., Sussman, K.E., Kao, M., Lewis, D. and Sherman, N. (1987) J. Biol. Chem. 262, 14385–14388.
[5] Highsmith, S., Bloebaum, P. and Snowdowne, K.W. (1986) Biochem. Biophys. Res. Commun. 138, 1153–1162.
[6] Scanlon, M., Williams, D.A. and Fay, F.S. (1987) J. Biol. Chem. 262, 6308–6312.
[7] Borle, A.B. and Snowdowne, K.W. (1986) Methods Enzymol. 124, 90–116.
[8] Borle, A.B., Freudeurich, C.C. and Snowdowne, K.W. (1986) Am. J. Physiol. 251, C323–C326.
[9] Rodbell, M.J. (1961) J. Biol. Chem. 239, 375–380.
[10] Blinks, J.R. (1982) in: Techniques in Cellular Physiology (Baker, P.F. ed.) vol. P1, part II, pp. 1–38, Elsevier, Amsterdam.
[11] Allen, D.G. and Blinks, J.R. (1979) in: Detection and Measurement of Free Calcium in Cells (Ashley, C.C. and Campbell, A.K. eds) pp. 159–174, Elsevier, Amsterdam.
[12] Veloso, D., Guynn, R.W., Oskarsson, M. and Veech, R.L. (1973) J. Biol. Chem. 248, 4811–4819.
[13] Snowdowne, K.W. and Borle, A.B. (1985) J. Biol. Chem. 260, 14998–15007.
[14] Clausen, T., Elbrink, J. and Martin, B.R. (1974) Acta Endocrinol. 77, suppl. 191, 137–143.
[15] Cheng, K., Haspel, H.C., Vallano, M.L., Osotimehin, B. and Sonnenberg, M. (1980) J. Membrane Biol. 56, 191–201.
[16] Stark, A., Read, P.D. and O'Doherty, J. (1980) Diabetes 29, 1040–1043.