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Biochimica et Biophysica Acta 1192 (1994) 107–111

BBA Biochimica et Biophysica Acta

Intracellular pH, cytosolic calcium concentration and electrical activity in RINm5F insulinoma cells

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(Received 27 September 1993)

Abstract

The addition of L-lactate or acetate to RINm5F cells caused a transient intracellular acidification, an increase in $[Ca^{2+}]_i$ and induced electrical activity. The subsequent withdrawal of lactate or acetate resulted in an intracellular alkalinization with no apparent changes in $[Ca^{2+}]_i$ nor electrical activity. Intracellular alkalinization and acidification by application and withdrawal of NH_4Cl were both accompanied by transient increases in $[Ca^{2+}]_i$ in the absence of electrical activity. The induction of electrical activity by lactate was associated with the appearence of inward whole cell currents. Changes in intracellular pH may affect $[Ca^{2+}]_i$ though not necessarily by altering plasma membrane potential. The inward currents associated with lactate application may represent an organic anion conductance contributing towards the stimulation of electrical activity by organic acids.

Key words: Beta cell; RINm5F cell; pH, intracellular; Cytosolic calcium ion; Membrane potential

1. Introduction

It has been suggested that intracellular pH (pH_i) may be a factor involved in the modulation of electrical and hence secretory activity in the pancreatic β-cell [1–3]. This proposal was based upon the observations that agents, such as weak acids and amiloride, known to cause intracellular acidification potentiate electrical activity and insulin release [1,2,4–6] whilst manipulations designed to alkalinize the cell interior inhibited electrical and secretory activity [1,3,4–6]. The demonstration that both Ca^{2+}- and nucleotide-sensitive K^+ channels (K_{Ca} and K_{ATP}) in β-cells can be modulated by $[H^+]$ [3,7,8] provides a mechanism coupling pH_i with plasma membrane potential and hence insulin release. In direct contrast, other studies have shown that intracellular alkalinization can also be associated with stimulation and inhibition of insulin secretion respectively [9,10]. Thus, changes in pH_i may exert multiple effects upon the secretory apparatus of the β-cell. The role of pH_i as a modulator in the β-cell is further obscured by the extremely poor correlation between changes in pH_i and other aspects of β-cell function, such as secretory activity, in response to various types of stimulus [11,12]. The present study has investigated the effects of three agents which alter pH_i on cytosolic $[Ca^{2+}]$ and on electrical activity associated with the plasma membrane in the insulin-secreting cell line RINm5F. Whilst this cell line is known to be poorly responsive, in terms of insulin release, to nutrient secretagogues such as glucose, tumoural cell lines have been valuable in the study of certain aspects of β-cell physiology including intracellular pH and cytosolic $[Ca^{2+}]$ regulation [12–14] and electrophysiology [15]. In particular, changes in the above parameters can be elicited in tumoural β-cells such as RINm5F and HIT-T15 by lactate, possibly because these cell types, unlike native β-cells, have a carboxylic acid carrier system which transports lactate into the cell [16,17].

2. Materials and methods

The RINm5F insulinoma cell line was maintained in culture in RPMI 1640 medium supplemented with 10% foetal calf serum, penecillin (100 U/ml) and streptomycin (100 μg/ml) (Flow Laboratories, UK). 2′,7′-

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SSDI 0005-2736(94)00042-N

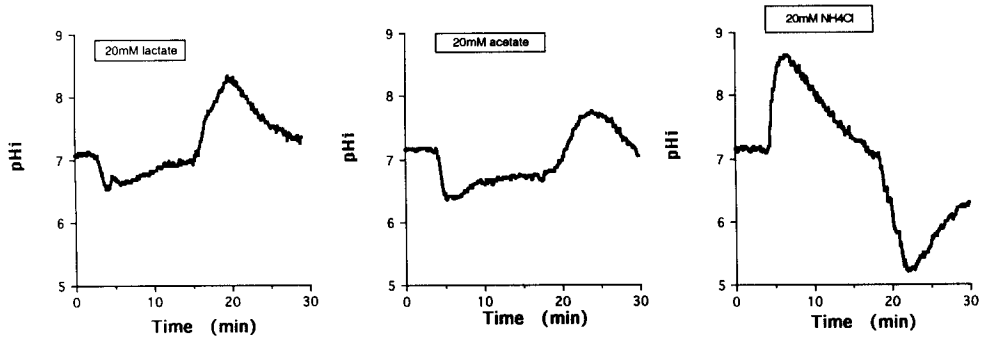

Fig. 1. Effects of L-lactate, acetate and NH_4Cl (all 20 mM) on intracellular pH (pH_i) in RINm5F cells loaded with BCECF. Each trace is representative of at least three similar experiments; the degree of variation was small and consisted of minor quantitative differences.

bis(carboxyethyl)-5',6'-carboxyfluorescein (BCECF) AM ester and fura-2 AM ester were purchased from Molecular Probes (Eugene, OR, USA). L-Lactate, amphotericin B and other biochemicals were obtained from Sigma (Poole, Dorset, UK).

For the fluorescence studies, cells were detached from culture flasks by treatment with trypsin-EDTA, washed once in RPMI and resuspended in medium consisting of (in mM) NaCl (135), KCl (5.0), $MgCl_2$ (1.2), NaH_2PO_4 (1.0), $CaCl_2$ (1.2) and Hepes (10) (pH 7.4). Cells ($0.5–2 \cdot 10^6$) were loaded either with BCECF (10 μM, 20 min incubation) or with fura-2 (3 μM, 50 min incubation), resuspended in fresh medium and allowed to adhere to 10-mm glass coverslips pre-coated with Cell-Tak (Collaborative Research Bedford, MA, USA). The coverslip was then mounted in a cuvette adapted for continuous perifusion in a Perkin-Elmer LS5 Fluorescence spectrophotometer. The cells were perifused at a rate of 6 ml/min. at 37°C. pH_i was assessed from the 500:450 nm excitation ratio at 530 nm emission using FCCP to calibrate the signals [18]. Cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_i$) was assessed from the 340:380 nm excitation ratio at 510 nm emission. No attempt was made to calibrate these recordings since such calibration is known to be extremely unreliable, as discussed previously [19].

Recordings of membrane potential and whole cell currents in RINm5F cells were performed using the

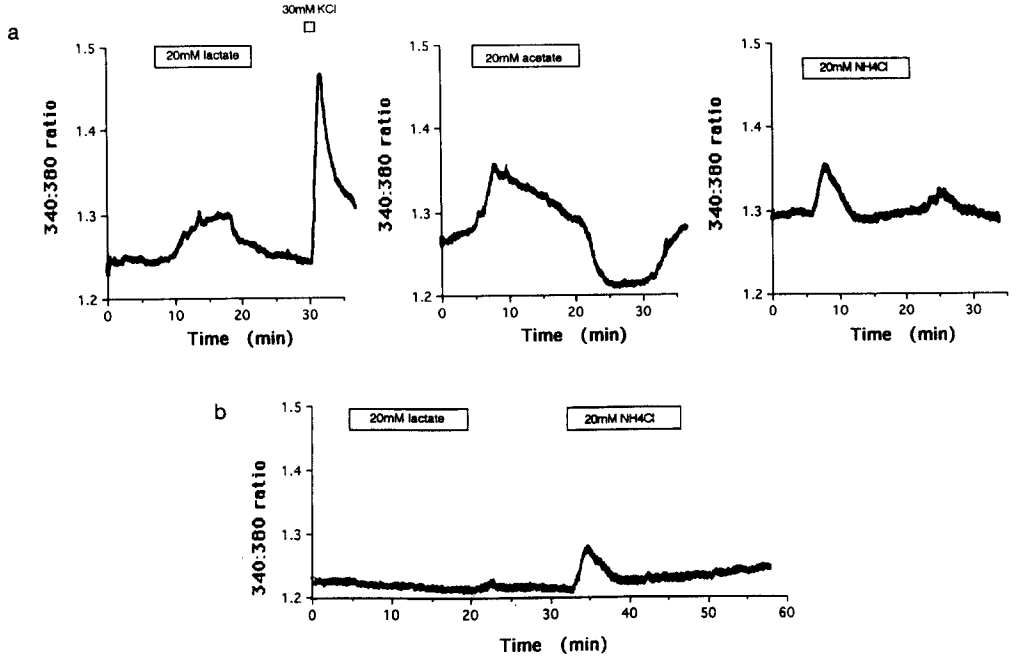

Fig. 2. (a) Effects of L-lactate, acetate and NH_4Cl (all 20 mM) on cytosolic $[Ca^{2+}]$, as assessed by 340:380 fluorescence ratio, in RINm5F cells loaded with fura-2. Each trace is representative of at least three similar experiments; the degree of variation was small and consisted of minor quantitative differences. (b) Effects of L-lactate and NH_4Cl (both 20 mM) on cytosolic $[Ca^{2+}]$, as assessed by 340:380 ratio, in RINm5F cells loaded with fura-2 and incubated in the presence of 5 μM verapamil. The trace is representative of two similar experiments which gave essentially similar results.

'perforated patch' configuration of the patch-clamp technique [20]. The electrode solution contained (in mM) KCl (10), K_2SO_4 (76), NaCl (10), $MgCl_2$(1), Hepes (10) (pH 7.1) supplemented with amphotericin B (240 μg/ml). Voltage and current signals were recorded using a List EPC-7 patch-clamp amplifier and filtered at 100 Hz.

3. Results

The addition of 20 mM L-lactate or acetate to RINm5F cells resulted in a rapid intracellular acidification as assessed by BCECF fluorescence (Fig. 1). In the case of lactate, this effect was probably the result of lactate$^-$/H^+ co-transport into the cell via a cinnamate-sensitive carrier [21]. The weak acid acetate, which diffuses into the cell in its undissociated form, caused a more rapid and pronounced acidification. In both cases, a gradual recovery occurred. The subsequent withdrawal of lactate or acetate from the perifusate produced a large intracellular alkalinization, presumably via a reversal of the above processes. Addition and withdrawal of the weak base ammonium caused a predicted rise and fall, respectively, in pH_i (Fig. 1).

The effects of these manipulations on $[Ca^{2+}]_i$, as assessed by 340:380 fluorescence ratio in fura-2-loaded RIN cells, is shown in Fig. 2a. The addition of lactate caused, following a 2–3 min delay, a gradual increase in $[Ca^{2+}]_i$. The maximum amplitude of this effect was approximately 25–30% of that evoked by a brief depolarization of the cells with 30 mM K^+ and returned to basal levels within the 15 min period of exposure to lactate. The subsequent withdrawal of lactate from the perifusate was not accompanied by any further change in $[Ca^{2+}]_i$. Application of acetate to RINm5F cells produced a similar transient increase in $[Ca^{2+}]_i$. In this case, however, withdrawal of acetate from the medium lead to an apparent transient reduction in $[Ca^{2+}]_i$. Alkalinization of the cell interior by addition of NH_4Cl was also found to cause a transient increase in intracellular calcium concentration, whilst a smaller rise in $[Ca^{2+}]_i$ followed the removal of NH_4Cl from the cell perifusate. The effects of NH_4Cl, but not of lactate, on $[Ca^{2+}]_i$ persisted in the presence of 5 μM verapamil, a blocker of voltage-sensitive calcium channels (Fig. 2b).

In order to investigate whether the above changes in intracellular pH and $[Ca^{2+}]$ were associated with electrical events at the level of the plasma membrane, current-clamp and voltage-clamp recordings were made in RINm5F cells using the patch-clamp technique. The perforated patch configuration was used for these studies since it retains the major intracellular constituents and thereby allows recording to be made from metabolically viable cells. Under current clamp conditions, the addition of lactate was found to cause a depolarization of the cells followed by intense electrical activity (Fig. 3). This pattern of electrical activity is characteristic of the stimulated β-cell and represents the generation of Ca^{2+}-dependent action potentials via the opening of voltage-sensitive calcium channels [22]. Addition of acetate was found to have a similar effect (Fig. 3). In both cases, the evoked electrical activity

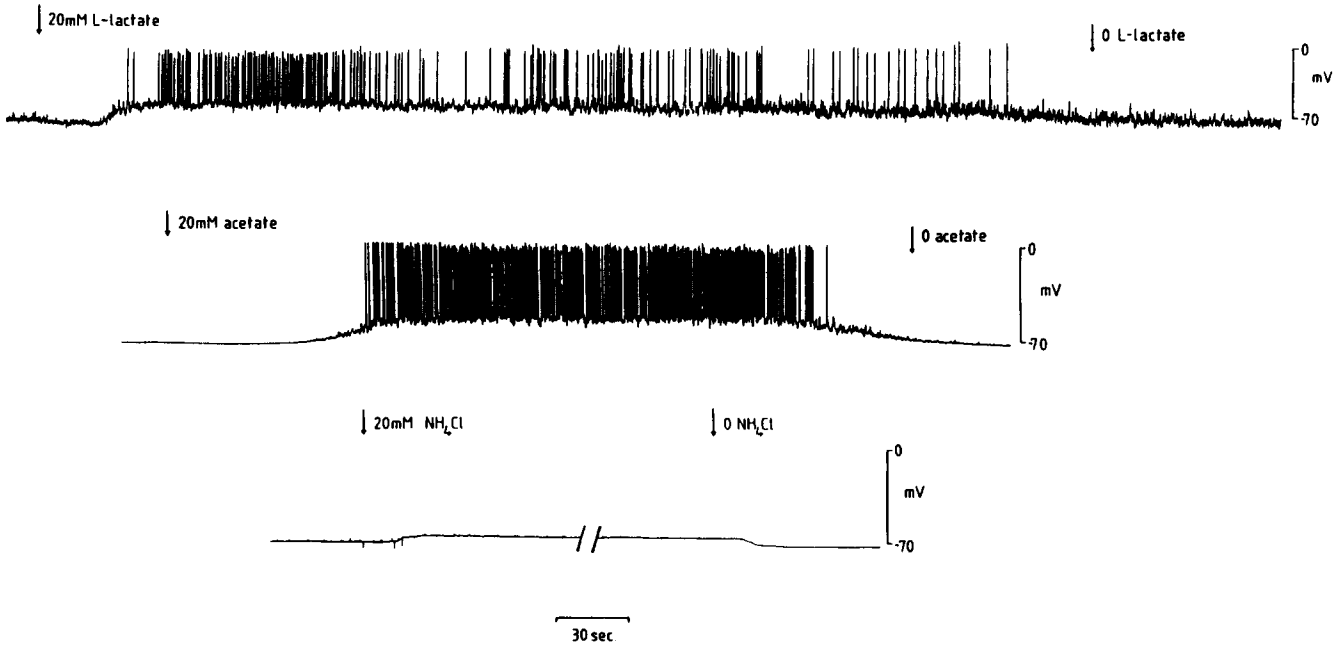

Fig. 3. Perforated-patch recordings of membrane potential in RINm5F cells; effects of addition and withdrawal of L-lactate, acetate and NH_4Cl. NHCl was applied for a period of 10 min. Each recording is representative of 2–5 similar experiments which gave essentially similar results.

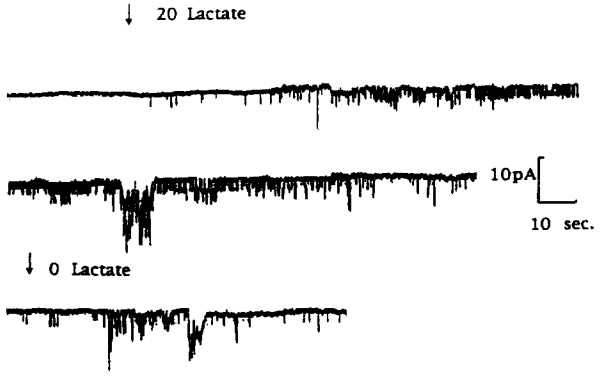

Fig. 4. Perforated patch recording of whole cell current in a RINm5F cell. The holding potential was −60 mV. This recording is representative of three similar experiments which gave essentially similar results.

lasted for 3–6 min whilst no further changes were apparent upon removal of the organic acid from the medium. The application of NH_4Cl was found to cause an extremely small reversible depolarization whereas neither its addition nor withdrawal were accompanied by the generation of action potentials (Fig. 3). Under voltage-clamp conditions, addition of lactate to RINm5F cells was followed by the appearence of inward current activity (Fig. 4). These whole cell currents were estimated to have a conductance of approximately 80pS and appeared to consist of up to six distinct levels of conductance, possibly representing the number of operative units of conductance ('channels') in that cell. The currents could be observed at holding potentials between −20 and −70 mV but not at more positive potentials and were found to persist when Cs^+ was substituted for K^+ in the electrode solution. It is therefore unlikely that these inward currents are carried via Ca^{2+} or K^+ channels, and it is tentatively suggested that they may represent the outward conductance of the $lactate^-$ anion following rapid equilibration of lactate across the plasma membrane via the lactic acid carrier. Preliminary studies of the actions of acetate and NH_4Cl on voltage-clamped RINm5F cells indicated that whilst application of the weak acid could induce similar currents to those seen with lactate, neither addition nor withdrawal of NH_4Cl was associated with the above changes in whole cell current (not shown).

4. Discussion

Previous studies in our laboratory have demonstrated that organic acids, including lactate, propionate and acetate, can activate HIT-T15 insulinoma cells [23,24]. The results of the present study demonstrate that lactate and acetate depolarize RINm5F cells and elicit electrical activity and a rise in $[Ca^{2+}]_i$. As is the case with HIT-T15 cells, addition of lactate or a weak acid to RINm5F also causes a reversible intracellular acidification [24,25], raising the possibility that changes in pH_i may influence both electrical and $[Ca^{2+}]_i$ in the β-cell [1–3]. In fact, the results of this study indicate that both intracellular acidification and alkalinization can, under certain circumstances, cause a rise in $[Ca^{2+}]_i$. Thus, application of both organic acids and NH_4Cl were found to raise $[Ca^{2+}]_i$, whilst acidification due to removal of NH_4Cl was also effective. These findings probably reflect the potentially numerous mechanisms through which changes in pH_i could affect $[Ca^{2+}]_i$, including effects on plasma membrane potential and hence gating of voltage-sensitive Ca^{2+} channels [1–5] and also the mobilization of Ca^{2+} from intracellular stores [26–29]. The observation that NH_4Cl, in contrast to organic acid treatment, increased $[Ca^{2+}]_i$ in the presence of verapamil, a blocker of voltage-sensitive Ca^{2+} channels, suggests that intracellular alkalinization may liberate Ca^{2+} from intracellular stores. The absence of an increase in $[Ca^{2+}]_i$ during alkalinization by withdrawal of lactate or acetate could be the result of depletion of intracellular stores during the intense electrical activity triggered by addition of these agents. The resultant entry of calcium into the cell could exert this secondary effect via Ca^{2+}-induced Ca^{2+} release or as a result of generation of inositol 145-trisphosphate [30,31].

Whilst both organic acids and NH_4Cl were effective in raising $[Ca^{2+}]_i$ in RIN cells, only organic acid treatment provoked a depolarization leading to electrical activity. This observation would be consistent with mobilization of intracellular Ca^{2+} stores by intracellular alkalinization, and also indicates a dissociation between intracellular pH per se and electrical activity. Thus, whilst it is possible that changes in pH_i contribute towards the modulation of membrane potential and hence electrical activity, it appears likely that activation of RIN cells by organic acids also involves (an) additional mechanism(s).

It has previously been proposed that an electrogenic conductance pathway for certain organic anions could, at least in part, underlie the stimulatory actions of such substances on the β-cell [24,32,33]. This study provides the first direct evidence of a conductance for organic anionic species in insulin-secreting cells. The exact nature of this conductance is at present unknown, although it has been previously demonstrated that certain types of anion channel show a considerable lack of selectivity in conducting a range of anionic species [34,35], raising the possibility that lactate and related organic anions might also be conducted via such a channel. Clearly, further work is required to fully investigate this conductance and its role in the regulation of β-cell function.

Acknowledgements

This work was supported by the North-Western Regional Health Authority and by The Royal Society.

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