The activity of the Na^+/H^+ antiporter in cultured cardiac cells is dependent on the culture conditions used

Richard D. Green*, Christian Frelin, Paul Vigne and Michel Lazdunski

Centre de Biochimie, Centre National de la Recherche Scientifique, Parc Valrose, 06034 Nice Cedex, France and **Department of Pharmacology, University of Illinois College of Medicine, Chicago, IL 60612, USA*

Received 2 December 1985

When chick cardiac cells are maintained in serum-free NCTC-135 medium, the pH_i dependence of the Na^+/H^+ exchanger is much more acidic ($pK = 7.0$) than when a serum-free M199 medium is used ($pK > 7.5$). Phorbol esters activate the Na^+/H^+ exchanger and produce a cell alkalinization in cells maintained in NCTC-135 medium by shifting the pH_i dependence of the system to more alkaline values. They have no action on cells maintained in M199 medium. The alkaline pH_i dependence of the Na^+/H^+ exchanger and the loss of responsiveness of phorbol ester action in M199 medium correlate with increased rates of phosphatidylinositol turnover.

(Cardiac cell) *Na^+/H^+ antiport* *Amiloride* *Phorbol ester* *Phosphatidylinositol* *Cell culture*

1. INTRODUCTION

Regulation of the intracellular pH (pH_i) in cardiac cells involves a Na^+/H^+ antiport [1,2]. Previous studies have defined the biochemical and pharmacological properties of the Na^+/H^+ antiport in cardiac cells [3,4] and in non-cardiac cells [5,6]. One of the interesting properties of the Na^+/H^+ antiport in non-cardiac cells is its ability to be activated by a variety of stimuli [6–14]. Activation occurs via a shift in the pH_i dependence of the antiport system [9–14]. Here we report that, in cultured chick cardiac cells, the pH_i dependence of the Na^+/H^+ antiport depends on the culture conditions used and can be modified by activators of protein kinase C.

Abbreviations: EIPA, ethylisopropylamiloride; TPA, phorbol-12-acetate-13-myristate; BCECF, 2,7-biscarboxyethyl-5(6)-carboxyfluorescein; PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate

2. MATERIALS AND METHODS

Culture media and foetal calf serum were purchased from Gibco. $^{22}NaCl$ (0.5 Ci/mg) and *myo*-[2-^{3}H]inositol (17 Ci/mmol) were from Amersham. Amiloride (*N*-amidino-3,5-diamino-6-chloropyrazine carboxamide) and EIPA (*N*-amidino-3-amino-5-ethylisopropylamino-6-chloropyrazine carboxamide) were kindly provided by Dr E.J. Cragoe jr.

Cardiac cells were isolated as described [3] and seeded into a medium consisting of Eagle's minimal essential medium (3 parts), NCTC-135 medium (1 part), 5% charcoal-treated calf serum. After 2–3 days in culture, cells were rinsed with serum-free medium and further incubated overnight in either serum-free NCTC-135 medium, serum-free M199 medium or the same medium as used for seeding the cells.

$^{22}Na^+$ uptake experiments on cardiac cells were performed as described in [3]. The internal pH of nigericin-treated cells was determined from the K^+ distribution ratio [10].

pH_i measurements using the fluorescence of

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

BCECF were conducted as described [15] and calibrated according to Moolenaar et al. [9].

Analysis of inositol phospholipids was performed using previously published methods [16,17].

3. RESULTS AND DISCUSSION

When chick cardiac cells were maintained under normal culture conditions, the pH_i for half-maximum activation of the amiloride-sensitive $^{22}Na^+$ uptake was 7.3 ± 0.1 (mean and range from 5 expts). Full activation of the Na^+/H^+ exchanger was observed between pH_i 6.9 and 7.6 indicating a cooperative interaction of internal H^+ with the Na^+/H^+ exchanger. When chick cardiac cells were shifted for 18 h in a serum-free NCTC-135 culture medium, the internal pH dependence of the Na^+/H^+ exchange system changed. The pH_i for half-maximum activation of the antiporter decreased to 7.0 ± 0.05 (4 expts). When chick cardiac cells were similarly shifted into serum-free M199 culture medium, the internal pH dependence of the Na^+/H^+ exchanger shifted in the opposite direction. Maximal activity of the exchanger was then observed between pH_i 6.2 and 7.25, then, for higher pH_i values, it declined.

The main panel of fig.1 presents 3 representative pH_i dependences of the activity of the Na^+/H^+ exchange system in cardiac cells cultured in normal culture medium, in NCTC-135 medium and in M199 medium. The inset of fig.1 shows that the maximum activity of the Na^+/H^+ exchanger, measured at a pH_i value of 6.2 was about the same in all 3 culture conditions.

In cardiac cells cultured in NCTC-135 medium, TPA (0.1 μM), a potent activator of protein kinase C [18], produced a mean 2.6-fold activation of the initial rate of amiloride-sensitive $^{22}Na^+$ uptake (range 1.6–4.3-fold in 7 expts). Half-maximum activation by TPA was observed at 25 nM (fig.2). In cells cultured in normal culture medium, TPA (0.1 μM) produced a mean 2.2-fold activation of the Na^+/H^+ exchanger (range 2–2.5-fold). In contrast TPA did not produce any activation of the Na^+/H^+ exchange system in cells cultured in M199 medium (not shown). The inset of fig.2 shows that TPA in NCTC-135 medium produced a shift in the pH_i dependence of the system by about 0.4 pH unit in the alkaline range. TPA did not change the maximal activity of the Na^+/H^+ exchange system measured at a pH_i value of 6.20.

Intracellularly trapped BCECF is a useful probe for fluorescence monitoring pH_i variations in cardiac cells [15]. Fig.3A shows that when NH_4^+-loaded cardiac cells were shifted to a NH_4^+-free and Na^+-free medium, a large cell acidification was observed. Upon the addition of Na^+ to the external medium,

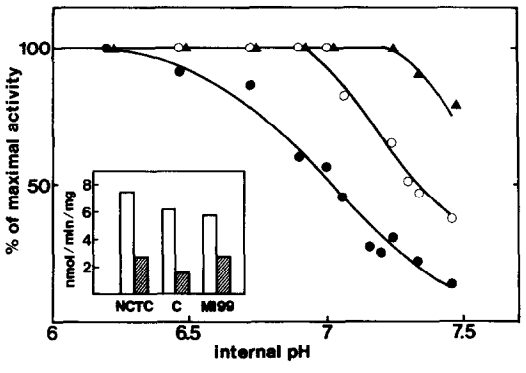

Fig.1. Internal pH dependence of the Na^+/H^+ exchanger of chick cardiac cells maintained in different culture media: serum-free M199 medium (▲), serum-free NCTC-135 medium (●), normal growth medium (○). Inset: initial rates of $^{22}Na^+$ uptake measured at a pH_i value of 6.2 in the absence (open bars) or the presence (shaded bars) of 0.1 mM amiloride in the 3 culture conditions.

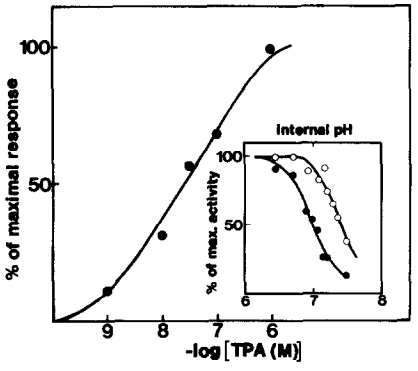

Fig.2. The effect of TPA on the activity of the cardiac Na^+/H^+ exchanger. Dose-response curve for TPA activation of the initial rate of Na^+ uptake by cardiac cells maintained in NCTC-135 medium. Cells were pre-equilibrated for 15 min in Na^+-free, 70 mM K^+ medium in the presence of 1 μM nigericin. Inset: the internal pH dependence of the Na^+/H^+ exchanger in cardiac cells maintained in NCTC-135 medium in the absence (●) or presence (○) of 0.1 μM TPA.

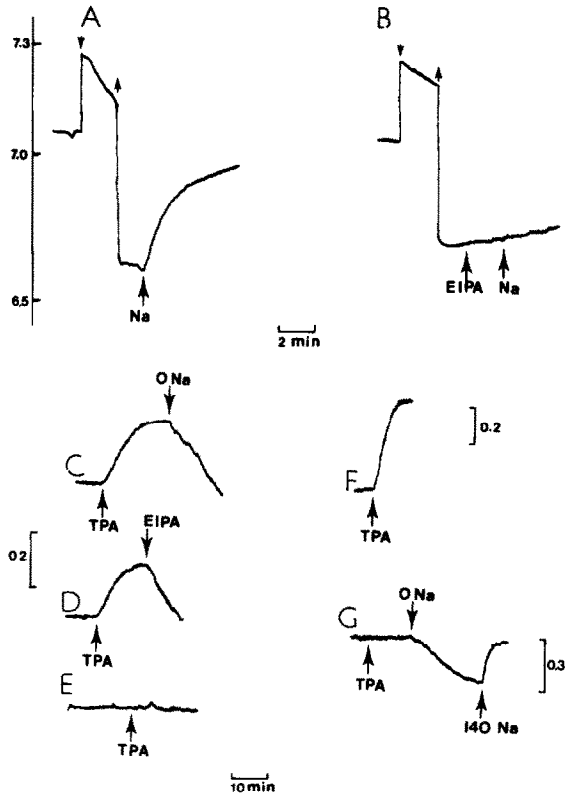

Fig.3. The effect of TPA on the pH_i of cardiac cells. A,B: BCECF-loaded cardiac cells were incubated in a Na^+-free medium. After stabilization of the fluorescence, 30 mM NH_4Cl was added (↓) to the cuvette for 2 min. Cells were then shifted to a Na^+-free and NH_4^+-free medium (↑). At the time indicated Na^+ (140 mM) or EIPA (0.1 mM) were added and pH_i followed. C–G: Influence of TPA on the pH_i of chick cardiac cells maintained in normal culture medium (C–E), NCTC-135 medium (F) and M199 medium (G). Cells were incubated in a 140 mM Na^+ or Na^+-free medium (E). 0.1 μM TPA and 0.1 mM EIPA were added at the times indicated.

pH_i rapidly recovered. EIPA, a blocker of the Na^+/H^+ exchanger [19] prevented the normal pH_i recovery that was induced by Na^+ (fig.3B). Addition of TPA to chick cardiac cells that had been cultured in normal culture medium produced a cell alkalinization of about 0.25 pH unit (fig.3C). TPA action was rapidly reversed when cells were shifted to a Na^+-free medium (fig.3C) or upon addition of EIPA (fig.3D). TPA did not produce any significant cell alkalinization if cardiac cells were pretreated with EIPA (fig.3E). All these experiments taken together suggest that the cell alkalinization produced by TPA is due to an activation of the Na^+/H^+ exchange. Fig.3F shows that TPA produced a more rapid and larger change in pH_i in cardiac cells that were cultured in NCTC-135 medium than in normal culture medium. TPA had no effect on the pH_i of chick cardiac cells that had been maintained in M199 medium although these cells responded to a shift to a Na^+-free medium by a cell acidification which was restored upon readdition of external Na^+ (fig.3G).

In summary: (i) the properties of interaction of the cardiac Na^+/H^+ exchanger with internal H^+ were not the same when different culture conditions were used; (ii) when cardiac cells were maintained in NCTC-135 medium, TPA produced an activation of the Na^+/H^+ exchanger which led to a cell alkalinization. Activation was produced by shifting the pH_i dependence of the system to more alkaline pH_i values; (iii) the effect of TPA also depended on the culture conditions used. It was the most prominent in cells maintained in NCTC-135 medium. TPA had no effect on cells maintained in M199 medium; (iv) TPA action was more pronounced when the internal pH dependence of the Na^+/H^+ exchanger was observed at acidic pH_i values.

In vitro differentiation of skeletal muscle cells has been reported to be accompanied by a shift in the pH_i dependence of the Na^+/H^+ antiport to more alkaline values and by a loss of the responsiveness to TPA action [10]. These results were interpreted to suggest that in differentiated myo-

Table 1

Effect of culture media on the short-term [^{3}H]inositol labelling of inositol phospholipids

Phospholipid	Culture medium	
	NCTC-135	M199
PI	3011 ± 470	6617 ± 319
PIP	114 ± 53	276 ± 29
PIP_2	107 ± 17	566 ± 9

15 h before the experiments, cells were shifted to serum-free NCTC-135 or M199 medium. Cells were then incubated for 1 h with [^{3}H]inositol (15 μCi/200 μl), in a medium consisting of 140 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5 mM glucose buffered with 25 mM Hepes-Tris at pH 7.4. The results shown are dpm/well (mean ± SE, $n=4$)

Table 2

Effect of culture medium on the loss of [^{3}H]inositol from phosphatidylinositol labelled with [^{3}H]inositol

	Radioactivity in cell phosphatidylinositol	Radioactivity released into the medium
Control	22840 ± 672	–
Switch to NCTC medium	18508 ± 169	27346 ± 3415
Switch to M199 medium	14904 ± 1284	44790 ± 2053
Switch to M199 medium + inositol	14400 ± 228	39850 ± 3135

Cardiac cells in normal growth medium were labelled with [^{3}H]inositol (1.5 μCi/ml) for 30 h and then switched to unlabelled serum-free NCTC-135 or M199 or M199 + cold inositol medium. 15 h thereafter the radioactivity released in the culture media and contained in cell PI was determined. Data are compared to cells left in normal growth medium supplemented with [^{3}H]-inositol. The inositol in the M199 + inositol group was equal in concentration to that of NCTC-135 medium (the inositol concentrations in NCTC-135 and M199 media are 0.125 and 0.05 mg/l, respectively). Results shown are dpm/well ± SE ($n=3$)

tubes, the Na^+/H^+ exchanger exists in a state that is already activated by protein kinase C [10]. Similarly the results presented here could mean that when cardiac cells are cultured in a M199 medium, the Na^+/H^+ antiport is already phosphorylated by protein kinase C. It would follow that one might expect to find a correlation between the rate of phosphatidylinositol turnover and the state of activation of the Na^+/H^+ exchanger. Experiments were performed to compare PI turnover in cardiac cells grown in NCTC-135 and in M199 medium. Table 1 shows that incorporation of [^{3}H]inositol into both phosphatidylinositol and polyphosphoinositides was greater in cells maintained in M199 compared to those maintained in NCTC-135 medium. Table 2 shows that the loss of ^{3}H from phosphatidylinositol in cells switched to M199 (or M199 + inositol) was approximately twice that of the cells switched to NCTC-135 medium. While neither measurement proves differences in phosphatidylinositol turnover, the combined measurements are strongly suggestive that inositol phospholipids were turning over more rapidly in the cells maintained in M199 medium.

ACKNOWLEDGEMENTS

This work was supported by grants from CNRS, the 'Fondation sur les Maladies Vasculaires', the 'Fondation pour la Recherche Médicale' and INSERM (grant no.80.50.09). We are grateful to M.T. Ravier and C. Roulinat-Bettelheim for expert technical assistance.

REFERENCES

[1] Ellis, D. and MacLeod, K.T. (1985) J. Physiol. 359, 81–105.
[2] Frelin, C., Vigne, P. and Lazdunski, M. (1985) Eur. J. Biochem. 149, 1–4.
[3] Frelin, C., Vigne, P. and Lazdunski, M. (1984) J. Biol. Chem. 259, 8880–8885.
[4] Lazdunski, M., Frelin, C. and Vigne, P. (1985) J. Mol. Cell. Cardiol., in press.
[5] Aronson, P.S. (1985) Annu. Rev. Physiol. 47, 545–560.
[6] Frelin, C., Vigne, P. and Lazdunski, M. (1985) in: Hormones and Cell Regulation (Dumont, J.E. et al. eds) vol. 9, pp. 259–269, Elsevier, Amsterdam, New York.
[7] Frelin, C., Vigne, P. and Lazdunski, M. (1983) J. Biol. Chem. 258, 6272–6276.
[8] Pouysségur, J., Franchi, A., Kohno, M., L'Allemain, G. and Paris, S. (1985) in: Current Topic in Membrane Transport (Aronson, P.S. and Boron, W. eds) Academic Press, in press.
[9] Moolenaar, W.H., Tsien, R.Y., Van der Saag, P.T. and De Laat, S.W. (1983) Nature 304, 645–648.
[10] Vigne, P., Frelin, C. and Lazdunski, M. (1985) J. Biol. Chem. 260, 8008–8013.
[11] Grinstein, S., Rothstein, A. and Cohen, S. (1985) J. Gen. Physiol. 85, 765–787.
[12] Moolenaar, W.H., Tertoolen, L.G.J. and De Laat, S.W. (1984) Nature 312, 371–374.
[13] Grinstein, S., Cohen, S., Goetz, J.D., Rothstein, A. and Gelfand, E.W. (1985) Proc. Natl. Acad. Sci. USA 82, 1429–1433.
[14] Paris, S. and Pouysségur, J. (1984) J. Biol. Chem. 259, 10989–10994.
[15] Frelin, C., Vigne, P., Barbry, P. and Lazdunski, M. (1985) Eur. J. Biochem., in press.
[16] Imai, A., Nakashima, S. and Nozawa, K. (1983) Biochem. Biophys. Res. Commun. 110, 108–115.
[17] Jolles, J., Schrama, L.H. and Gispen, W.H. (1981) Biochim. Biophys. Acta 666, 90–98.
[18] Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U. and Nishizuka, Y. (1982) J. Biol. Chem. 257, 7847–7851.
[19] Vigne, P., Frelin, C., Cragoe, E.J. and Lazdunski, M. (1983) Biochem. Biophys. Res. Commun. 116, 86–90.