Three Na^+,K^+-ATPase forms in rat heart as revealed by K^+/ouabain antagonism

P. Mansier, P.S. Cassidy†, D. Charlemagne, M. Preteseille and L.G. Lelievre*

INSERM U 127, Hôpital Lariboisière Université Paris 7, 41, Bd de la Chapelle 75010 Paris, France

Received 23 December 1982

Abstract and keywords not received

1. INTRODUCTION

The involvement of Na^+,K^+-ATPase in the inotropic effect of ouabain in rat heart remains controversial ([1–6]. A sustained positive inotropic effect has been observed in vivo with 10^{-8}–10^{-7} M ouabain [1–5], whereas inhibition of the enzyme in vitro was detectable only at 10^{-5}–10^{-4} M [1,2]. In Ca^{2+}-free perfused rat heart, Na^+,K^+-ATPase activity was reported inhibited by therapeutic doses of ouabain (10^{-8} M) [6]; this enzyme form was undetectable in non-perfused heart [6]. Thus, it appears that there is a close parallel at similar drug doses between the positive inotropic effect and Na^+,K^+-ATPase inhibition. In vivo, K^+ interfered with the effects of ouabain. In the rat, K^+ did not affect the development of the inotropic response, but markedly lowered its amplitude [4]. In an attempt to correlate the enzyme form highly sensitive to ouabain with the physiological site responsible for the inotropic effect, we investigated the role(s) of K^+ on the enzyme activity. We report here that potassium (10 mM) does not affect the low sensitivity form (LS1) present in hearts maintained at a physiological calcium level. In Ca^{2+}-free perfused hearts, part of the high sensitivity form (HS_2) is converted into a low sensitivity form (LS_2) by K^+. These data are consistent with the hypothesis that the HS_2 form is the in vitro representation of the inotropic site for ouabain in rat heart.

* To whom correspondence should be addressed

† Present address: Cardiac Muscle Research, Laboratory R-415, Boston University Medical Center, School of Medicine, 80 East Concord Street, Boston, MA 02118 USA

2. EXPERIMENTAL

2.1. *Heart perfusions and isolation of highly-enriched sarcolemmal fractions*

After killing the rats by cervical dislocation, the heart was removed and perfused with either a 2 mM Ca^{2+}-containing solution or a Ca^{2+}-free medium [6]. We utilized the technique in [7]. The left ventricle balloon volume was adjusted to produce a normal diastolic pressure (~18–22 mm Hg). Hearts perfused with an ice-cold 2m mM Ca^{2+}-containing solution exhibited a maximum pressure of 74 mm Hg compared to 100 mm Hg in control hearts at 37°C (table 1). Hearts perfused with the ice-cold Ca^{2+}-free relaxing buffer rapidly stopped in diastole. ($p = 25$ mm Hg). From each type of heart, microsomes highly enriched in sarcolemmal vesicles were isolated as in [8]. To reveal latent enzyme activities, the vesicles were treated by repetitive freezings and thawings [6].

2.2. *Estimation of Na^+,K^+-ATPase activity*

The Na^+,K^+-ATPase activity was assayed as in [6] using 10 μg protein/ml incubation medium [9]. The sarcolemmal vesicles were preincubated with ouabain (Boehringer) for 30 min at 37°C with or without 10 mM KCl in the assay medium containing 4 mM $MgCl_2$, 100 mM NaCl and 40 mM Imidazole–HCl (pH 7.4). The reaction was then initiated with the addition of either ATP or ATP + KCl (final con.: 4 or 4 and 10 mM, respectively) and carried out over 6 time points to assess linearity. Every minute, aliquots were taken and the released orthophosphate was measured [10]. The Na^+,K^+-ATPase activities represented 50 ± 10% of

Published by Elsevier Biomedical Press
00145793/83/0000–0000/$3.00

the total ATPase activities. These assays in the presence of ATP, which favours ouabain binding [11], were linear with respect to time, suggesting that the enzyme–ouabain equilibrium was reached in <1 min (the shortest time used). This is in full agreement with literature data [12–14].

3. RESULTS

The preparation we used were highly enriched in sarcolemmal vesicles, with specific activities of Na^+,K^+-ATPase varying between 86 ± 15 and 100 ± 18 μmol phosphate.h^{-1}.mg^{-1} for hearts maintained at 2 mM Ca^{2+} and for Ca^{2+}-free perfused hearts, respectively.

When native and opened vesicles from hearts maintained at a physiological calcium level were preincubated with ouabain in a K^+-free medium, they exhibited a monophasic dose–response curve (fig. 1A). The ouabain concentration needed to half-maximally inhibit the activity (IC_{50}) was $3 \pm 0.7 \times 10^{-5}$ M (table 2), a value consistent with earlier reports [1–3,6,12,15–17]. This value was not significantly modified by the addition of 10 mM K^+ to the preincubation medium, confirming [17].

When native or opened vesicles from hearts perfused with a Ca^{2+}-free buffer were preincubated in the presence of ouabain without K^+, a 'low concentration inhibitory effect' was observed between 10^{-9}–10^{-7} M ouabain ($76 \pm 11\%$ inhibition). A residual 'high concentration effect' was completely inhibited with 10^{-4} M ouabain (fig. 1B). The apparent IC_{50} value could only be calculated for the highly sensitive form (HS_2): $1.5 \pm 0.3 \times 10^{-8}$ M (table 2). This concentration–response curve may represent two or more enzyme forms with very close apparent sensitivities.

When K^+ was present during preincubation, these vesicles clearly exhibited a biphasic dose–response curve with the high sensitivity form HS_2 associated with $63\% \pm 13\%$ of the total activity (fig. 1C). A progressive decline in residual activity

Table 1

Effects of perfusion of low or physiological calcium levels on maximum pressure[a] exhibited by isolated rat heart

Perfusion	0 s	5 s	10 s	20 s	60 s	300 s[b]
Ca^{2+} free	100	44	22	25	25	25
2 mM	96	38	44	62	64	74

Prior to perfusion at 4°C, the heart was equilibrated for 10 min as in [7] with Krebs-Ringer bicarbonate buffer at 37°C. The control diastolic and systolic pressures were 18–22 and 96–100 mm Hg, respectively. Similar results were obtained with 6 hearts, the left ventricle diastolic pressure of which varying from 12–22 mm Hg: (a) maximum pressure, mm Hg at 4°C; (b) microsome preparation was initiated after 5 min perfusion.

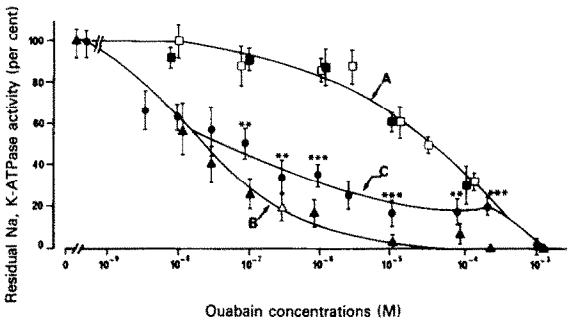

Fig. 1. Dose–response curves of Na^+,K^+-ATPase activity versus ouabain concentrations in microsomes: (A) hearts maintained at 2 mM Ca^{2+}, microsomes preincubated without K^+ (□) or with 10 mM K^+ (■); (B,C) Ca^{2+}-free perfused hearts, microsomes preincubated without K^+ (▲) (B) or with 10 mM K^+ (●) (C) each experiment was performed ≥4 times with ≥4 different preparations. Each point represents the mean of 8–18 obs., with bars denoting SED computed from percentage of inhibition in each experiment: (**) $p<0.01$; (***) $p<0.001$).

Table 2

Effects of heart pretreatment and addition of K^+ (10 mM) on the expression of the Na^+,K^+-ATPase forms.

Preincubations	Heart maintained at 2 mM Ca^{2+}	Ca^{2+}-free perfused heart
Ouabain	$LS_1(3 \pm 0.7 \times 10^{-5}$ M)	$HS_2(1.5 \pm 0.3)$
Ouabain + K^+	$LS_1(3 \pm 0.7 \times 10^{-5}$ M)	$HS_2(1.5 \pm 0.3)$ + LS_2[a]

[a] The progressive decline in residual activity did not allow IC_{50} determination

In parentheses: IC_{50} values ($\times 10^{-8}$ M), ouabain concentrations to half-maximally inhibit the Na^+,K^+-ATPase form

was found between 3×10^{-7} M and 3×10^{-4} M ouabain (LS_2). Since the total Na^+,K^+-ATPase activity remained constant whether or not K^+ was present during preincubation, it may be assumed that this latter enzyme form (LS_2) revealed by K^+ was converted into the high sensitivity form (HS_2) in the absence of K^+.

4. DISCUSSION

Using rat hearts perfused with either physiological Ca^{2+} or Ca^{2+}-free buffers, we detected 3 forms of Na^+,K^+-ATPase activities, two in hearts relaxed by Ca^{2+}-free perfusion ($HS_2 + LS_2$) and one in hearts maintained at a physiological level of calcium (LS_1). The LS_2 form was K^+-dependent, whereas LS_1 was not. These 3 forms were related to the specific binding of ouabain during preincubation, since they were detected under identical assay conditions. The effects of K^+ on the high sensitivity Na^+,K^+-ATPase activity were consistent with the effects of K^+ on the inotropic action of ouabain [15]; i.e., no detectable change in the onset of inhibition induced by ouabain, but a decrease in the amplitude of the inhibition. The role of K^+ on the relative proportion of HS_2/LS_2 enzyme forms reported here agrees with the differential effects of K^+ on the two [^{3}H]ouabain receptor found in canine and guinea pig cardiac sarcolemma preparations [18,19]. Nevertheless, at low doses of ouabain (10^{-8}–10^{-6} M), the degree of inhibition found here (63–76%) was disproportionate to both the magnitude of the inotropic effect (up to 15%) [4,15] and to the low percentage (10%) of high affinity [^{3}H]ouabain-binding sites found in rat cardiac sarcolemma preparations ([1–3] unpublished). It is possible that our isolation procedure changed the properties of the enzyme by increasing the proportion of highly sensitive forms. Consistent with [20], this hypothesis was reinforced by our observation [6] that a different membrane preparation procedure led to 50% of the HS_2 form (instead of 63–76%). The Ca^{2+}-depletion, which in rat hepatocytes was found to stimulate the Na^+,K^+-pump [21] 2-fold may account for the high proportion of the HS_2 form. Due to the flexiblity of $Na^+/K^+/ATP$ stoichiometry in the transport phenomenon at the cardiac level [5], the rate of ATP hydrolysis as an index of Na^+, K^+-ATPase activity may overestimate the exact proportion of HS_2 forms [18]. This supposes that it is the amount of ouabain–enzyme complex formed rather than the degree of enzyme inhibition which is the determining factor. The convergency of the two criteria, ouabain sensitivity (this work, [6]) and K^+-dependency, both in vitro and in vivo, strengthens the hypothesis that part of the Na^+,K^+-ATPase activity in rat heart may also be the digitalis receptor in vivo, as it is in other species.

ACKNOWLEDGEMENTS

This work was made possible by the financial contribution of the 'Société Française de Cardiologie' and by a grant from the Institut National de la Santé et de la Recherche Médicale (PRC N° 131030), as well as a fellowship to P.M. from the DGRST.

REFERENCES

[1] Erdmann, E., Philipp, G. and Scholz, H. (1980) Biochem. Pharmacol. 29, 3219–3229.
[2] Adams, R.J., Schwartz, A., Grupp, G.I., Lee, S.W., Wallick, E.T., Powell, T., Twist, V.W. and Gathiram, P. (1982) Nature 296, 167–169.
[3] Finet, M., Noel, F. and Godfraind, T. (1982) Arch. Intern. Pharmacodyn. Ther. 256, 168–170.
[4] Akera, T. and Brody, T.M. (1978) Pharmacol. Rev. 29, 187–220.
[5] Akera, T. and Brody, T.M. (1982) Annu. Rev. Physiol. 44, 375–388.
[6] Mansier, P. and Lelievre, L.G. (1982) Nature 300, 535–537.
[7] Apstein, C. (1977) Circ. Res. 41, 206–217.
[8] Jørgensen, P.L. and Skou, J.C. (1969) Biochem. Biophys. Res. Commun. 37, 39–46.
[9] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[10] Anner, B. and Mossmayer, M. (1975) Anal. Biochem. 65, 305–309.
[11] Schwartz, A., Matsui, H. and Laughter, A.H. (1968) Science 159, 323–325.
[12] Schwartz, A., Lindenmayer, G.E. and Allen, J.C. (1975) Pharmacol. Rev. 27, 3–134.
[13] Akera, T., Choi, Y.R. and Yamamoto, M. (1979) in: Na^+,K^+-ATPase: Structure and kinetics (Skou, J.C. and Nørby, J.G. eds) pp. 405–421, Academic Press, New York.
[14] Wallick, E.T., Dowd, F.J., Allen, J.C. and Schwartz, A. (1974) J. Pharmacol. Exp. Ther. 189, 434–444.

[15] Akera, T., Yamamoto, S. Chubb, J. McNish, R. and Brody, T. (1979) Pfuger's Arch. 308, 81–88.
[16] Allen, J.C. and Schwartz, A. (1969) J. Pharmacol. Exp. Ther. 168, 42–46.
[17] Urayama, O., Nakao, M. (1979) J. Biochem. 86, 1371–1381.
[18] Wellsmith, N.V. and Lindenmayer, G.E. (1980) Circ. Res. 47, 710–720.
[19] Godfraind, T. de Pover, A. and Tona-Lutete, D.N. (1980) Biochem. Pharmacol. 29, 1195–1199.
[20] Choi, Y.R. and Aker, T. (1978) Biochim. Biophys. Acta 508, 313–327.
[21] Capiod, T., Berthon, B., Poggioli, J., Burgess, G.M. and Claret, M. (1982) FEBS Lett. 141, 49–52.