ELSEVIER

CYTOKINE

www.elsevier.com/locate/jnlabr/ycyto

Cytokine 26 (2004) 1–8

Interleukin-1 β inhibits Na^+-K^+ATPase activity and protein expression in cardiac myocytes

Sawsan I. Kreydiyyeh[a,*], Christina Abou-Chahine[a], Randa Hilal-Dandan[b]

[a]*Department of Biology, Faculty of Arts & Sciences, American University of Beirut, Beirut, Lebanon*
[b]*Department of Pharmacology, University of California, San Diego, CA, USA*

Received 7 September 2003; received in revised form 11 November 2003; accepted 26 November 2003

Abstract

Recent studies have shown that heart diseases are always accompanied with high levels of IL-1β and a decrease in Na^+-K^+ ATPase concentrations. This work studies the involvement of the cytokine in the observed changes in the pump. Rats were injected intraperitoneally with 400 mg of IL-1β and 4 h later, the heart was isolated and a crude homogenate of the right and left ventricles was prepared and tested for Na^+-K^+ATPase activity and protein expression. IL-1β inhibited by around 70% the activity of the ATPase in the left and right ventricles. This inhibition of the pump was ascribed to a decrease in its protein expression as demonstrated by western blot analysis. A dose and time response study conducted on isolated cardiac myocytes confirmed the inhibitory role of the cytokine on the ATPase and showed that IL-1β exerts its maximal down-regulatory effect at 2 h and at a dose of 20 ng/ml. The cytokine caused also an up-regulation of the $NaKCl_2$ cotransporter. Both MEK and p38MAPK were shown to be involved in the signaling pathway activated by the cytokine. It can be concluded that the decrease in the Na^+-K^+ATPase concentration observed in heart diseases is a consequence of the accompanying high levels of IL-1β, and may be responsible for the different symptoms that accompany cardiac ischemia.
© 2004 Elsevier Ltd. All rights reserved.

Keywords: IL-1β; Cardiomyocytes; Na^+-K^+ATPase; $NaKCl_2$; MAPK

1. Introduction

There has been an increasing body of evidence supporting the existence of an inflammatory component in both the development and progression of heart failure [1,2]. Cytokines, in particular TNF-alpha, IL-1 and IL-6, have been identified as major players in the pathogenesis of the disease, and in the failing heart, an increase in their circulating levels has been noted that correlates with the severity of the symptoms and a worse prognosis [3,4]. These inflammatory mediators [5] have been shown, in addition, to induce cardiac hypertrophy and exert a negative inotropic effect on the perfused heart [6,7].

Heart failure coupled with ischemia and myocardial hypertrophy were associated in turn with a decrease in the concentration of myocardial Na^+-K^+ATPase [8] an enzyme located in the T tubules and peripheral sarcolemma [9] and responsible for the generation and maintenance of the electrochemical gradient for Na^+ and K^+.

Cardiac glycosides, which have a positive inotropic [10] effect, have been used for around a century in the treatment of patients with heart failure and target the Na^+-K^+ATPase. The glycosides inhibit the Na^+-K^+ pump, increase intracellular sodium, and reduce the sodium electrochemical gradient that constitutes the driving force for calcium extrusion through the Na^+/Ca^{++} exchanger, leading thus to an increase in intracellular calcium content, and consequently enhanced contractility.

The Na^+-K^+ pump is a heterodimer composed of an α catalytic subunit and a β glycoprotein subunit. Many isoforms of each subunit have been detected and are expressed in a tissue-specific manner [11]. Rat cardiomyocytes express the alpha 1 and alpha 2 isoforms while

* Corresponding author. Fax: +961-1-744461.
E-mail address: sawkreyd@aub.edu.lb (S.I. Kreydiyyeh).

1043-4666/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cyto.2003.11.014

in humans all three isoforms 1, 2 and 3 are present [9]. Schwinger et al. [12] demonstrated a decrease in the protein expression of the α1 and α3 subunits by, respectively, 38 and 30% in human failing hearts while the activity decreased by 42%.

In addition to glycosides, loop-diuretics are also used in the treatment of heart failure [13]. They are thought to alleviate pulmonary edema, congestive heart failure and hypertension by inhibiting the renal $NaKCl_2$ cotransporter and promoting diuresis and natriuresis. Many other reports showed, however, that the clinical relief of symptoms preceded in many instances the onset of diuresis [14,15] and occurred even in presence of marked impairment of renal function [16,17] suggesting a direct effect of the loop-diuretics on the cardiomyocytes $NaKCl_2$ cotransporter [18]. It is well known that the activity of this transporter is geared by the sodium gradient established by the Na^+-K^+ATPase.

Regulation of Na^+-K^+ATPase seems thus to be critical for a proper functioning of the myocardium and any impairment in its activity is expected to result in changes in the electrochemical gradient that drives the sodium influx and calcium extrusion leading to alterations in the contraction/relaxation processes.

Although in heart failure, changes in the Na^+-K^+ATPase activity and expression were noted to accompany increases in circulating levels of IL-1β, the presence of a cause–effect relationship between the cytokine and the pump has not been properly investigated.

Because we have shown previously that IL-1-β decreases the protein expression of the Na^+-K^+ATPase in the rat kidney and intestine [19,20] and up-regulates the $NaKCl_2$ cotransporter, we speculate that this pro-inflammatory cytokine may exert a similar effect in cardiac myocytes.

The aim of this work was to study the effect of IL-1β on the activity and protein expression of the Na^+-K^+ ATPase and $NaKCl_2$ symporter in the rat heart using two different approaches: treating the whole animal with the cytokine or isolating myocytes and then treating them with IL-1β.

2. Results

2.1. IL-1β reduces the activity and protein expression of the Na^+-K^+ ATPase in the right and left ventricles

IL-1β reduced protein expression of the Na^+-K^+ ATPase in right and left ventricular homogenates prepared from animals treated with the cytokine by 45 and 40%, respectively (Fig. 1a,b).

Interleukin-1 β inhibited also the activity of the ATPase in both ventricles by around 70% (Fig. 2a,b).

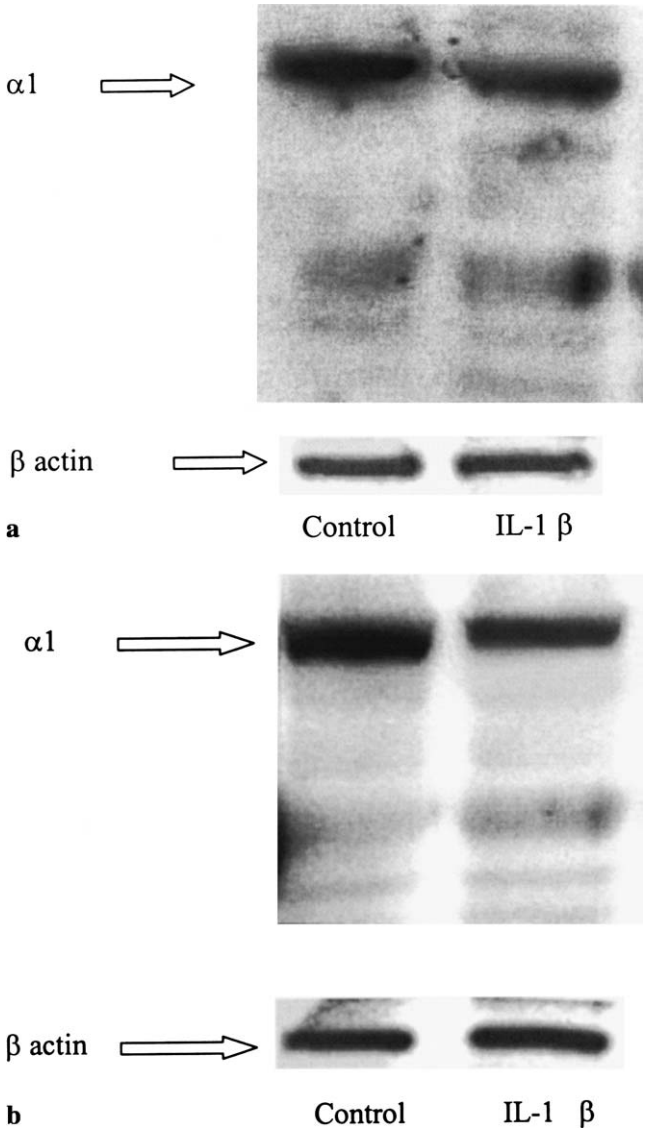
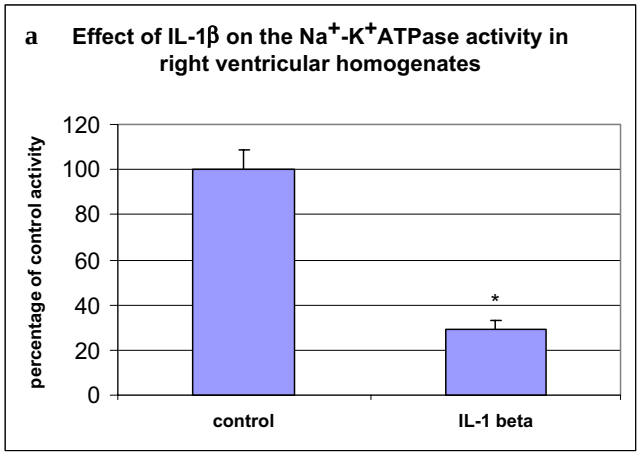


Fig. 1. Western blot showing the effect of IL-1β on the protein expression of the Na^+-K^+ATPase in the (a) right and (b) left ventricular homogenates. The blot is representative of an experiment that was repeated three times. The amount of protein loaded was 40 μg. The protein expression of β actin was used to check for equal loading.

2.2. Effect of IL-1β on the Na^+-K^+ ATPase of myocytes isolated from treated animals

Because the ventricular homogenate is a mixture of different types of cells, the observed change in the ATPase activity and expression may not reflect necessarily changes in cardiomyocytes. To clarify this point cardiomyocytes were isolated from animals treated with IL-1β, and the activity and protein expression of the ATPase was studied in a homogenate of these isolated cells rather than in a homogenate of the whole ventricle. IL-1β caused down-regulation of the pump in isolated cardiomyocytes (Fig. 3) and inhibited its activity (Fig. 4) by around 85%.



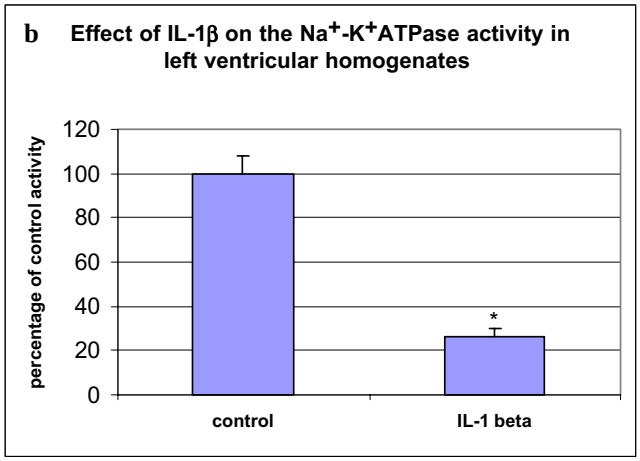


Fig. 2. Percentage of control activity of the Na^+-K^+ATPase activity in (a) right and (b) left ventricular homogenates prepared from animals treated with 400 ng of IL-1β or TNF-α. Values are means ± SEM of six observations. *$P < 0.01$.

2.3. Effect of the cytokine on the Na^+-K^+ ATPase of treated isolated myocytes

To know whether IL-1β exerts its effect directly on cardiomyocytes or through the release of other factors by other cells, myocytes were isolated and treated with different doses of the cytokine for different time periods. IL-1β reduced in a dose and time-dependent manner the protein expression (Fig. 5) and activity (Fig. 6) of the Na^+-K^+ATPase in isolated myocytes. The highest inhibitory effect was observed at 2 h and a dose of 20 ng/ml. The results suggest that the cytokine acts directly on the cardiomyocytes.

2.4. Involvement of mitogen-activated protein kinases in the down-regulatory effect of IL-1β

To know whether the effect of IL-1β is mediated through mitogen-activated protein kinases (MAPK), isolated myocytes were incubated with the cytokine in the simultaneous presence of PD98059 or SB202190, respective inhibitors of MEK and p38 kinase (Fig. 7). The down-regulatory effect on the Na^+-K^+ATPase did not appear in this case suggesting an involvement of the MAP kinases in the signaling pathway that mediates the effect of IL-1β.

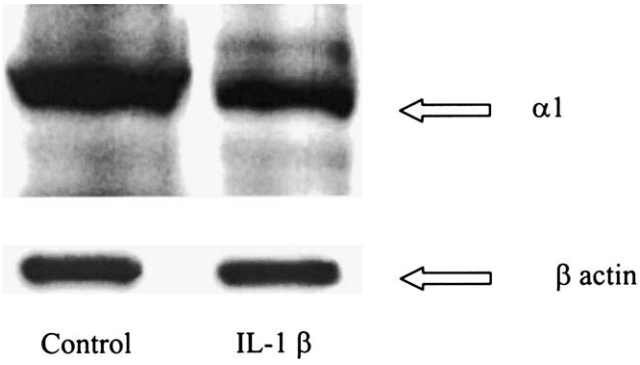


Fig. 3. Effect of IL-1β on the protein expression of the α1 subunit of the Na^+-K^+ATPase in cardiac myocytes isolated from treated animals. The blot is representative of an experiment that was repeated three times. Protein expression of β actin was used to check for equal loading.

2.5. IL-1β up-regulates the $NaKCl_2$ cotransporter

Our previous work on the effect of IL-1β on colonocytes [20] demonstrated that in addition to the Na^+-K^+ATPase, the cytokine affects the protein expression of the $NaKCl_2$ cotransporter. To see whether a similar effect could be exerted in cardiomyocytes, we studied the protein expression of the $NaKCl_2$ cotransporter in

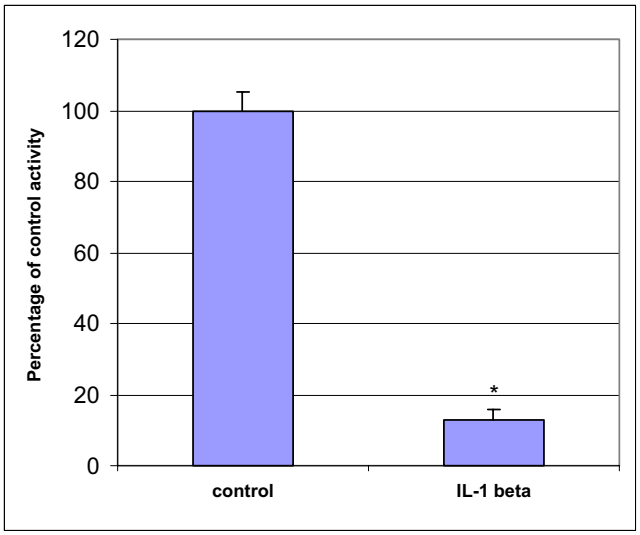


Fig. 4. Percentage of control activity of the Na^+-K^+ATPase in myocytes isolated from treated animals. Values are means ± SEM of six observations. *$P < 0.01$.

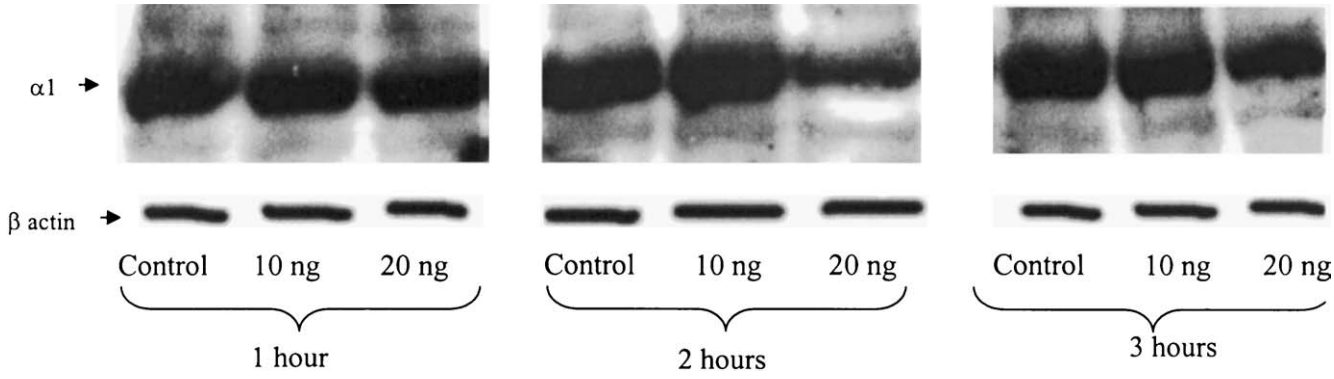


Fig. 5. Time and dose response study on the effect of IL-1β on the protein expression of the Na^+-K^+ATPase in treated isolated myocytes. Loaded protein: 70 μg. The blot is representative of an experiment repeated six times. Protein expression of β actin was used to check for equal loading.

ventricular homogenates prepared from animals treated with the cytokine and in isolated myocytes treated with IL-1β (Figs. 8 and 9). IL-1β up-regulated the cotransporter in both cases as it did in colon cells.

3. Discussion

This work has used three different approaches to study the effect of IL-1β on the rat cardiac Na^+-K^+ATPase. The first involved treating the animals with the cytokine and then studying ATPase activity and protein expression in homogenates of the right and left ventricles. In the second approach, animals were treated with the cytokine and then cardiomyocytes were isolated and a cell lysate prepared and used in the study of the ATPase. The third approach consisted of isolating the cardiomyocytes and then treating them with the cytokine.

IL-1β reduced the protein expression of the α1 subunit of the Na^+-K^+ATPase in right and left ventricular homogenates and exerted a significant inhibition of the ATPase activity which correlated with its down-regulatory effect and could thus be ascribed to the decrease in the number of the catalytic α1 subunits. Since the Na^+-K^+ATPase in the ventricular homogenates is derived from a mixture of different cells (endothelial cells, macrophages, fibroblasts), the observed changes in the protein expression and activity of the pump are due to the net effect of the cytokine on the cell mixture and not on the myocytes in particular.

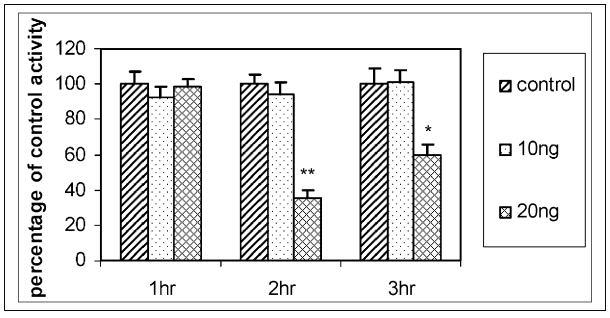


Fig. 6. Dose and time response study on the effect of IL-1β on the Na^+-K^+ATPase activity in isolated myocytes treated with the cytokine. Values are means ± SEM of six observations. $^*P < 0.05$; $^{**}P < 0.01$.

To know the exact effect of IL-1β on cardiac myocytes, a pure population of cardiomyocytes was prepared from rats treated with IL-1β, and was used to study changes in Na^+-K^+ATPase activity and protein expression. The results reconfirmed the down-regulatory effect of the cytokine on the ATPase as well as the inhibitory effect on the enzymatic activity.

An inflammatory response is the result of a cytokine cascade, where one cytokine leads to the release of another until the final response is elicited. Thus IL-1β, a pro-inflammatory cytokine, when injected to rats, may have exerted its effect directly on myocytes or indirectly by acting on other target cells and causing the release of other cytokines or inflammatory mediators. To elucidate this point, cardiomyocytes were isolated and then treated in vitro with IL-1β. The inhibitory effect of the cytokine appeared in this case also, demonstrating that IL-1β exerts its effect on the myocyte ATPase independent of any influence from neighboring tissues as may occur in vivo in the whole animal.

Because IL-1β is known to signal through the MAPK cascade [21,22] we investigated the involvement of these kinases in the signal transduction pathway activated by IL-1β. PD98059 and SB202190, respective inhibitors of MEK and p38 MAPK, abrogated the effect of IL-1β on the protein expression of the α1 subunit, inferring that both MAPKs are involved in the signaling pathway. The results are in agreement with those reported by Ng et al. [23] who showed an implication of ERK and p38 MAPKs in the IL-1β-induced delayed phosphorylation of STAT3 in cardiac myocytes.

Since the observed inhibition of the Na^+-K^+ATPase by IL-1β was accompanied by down-regulation of its

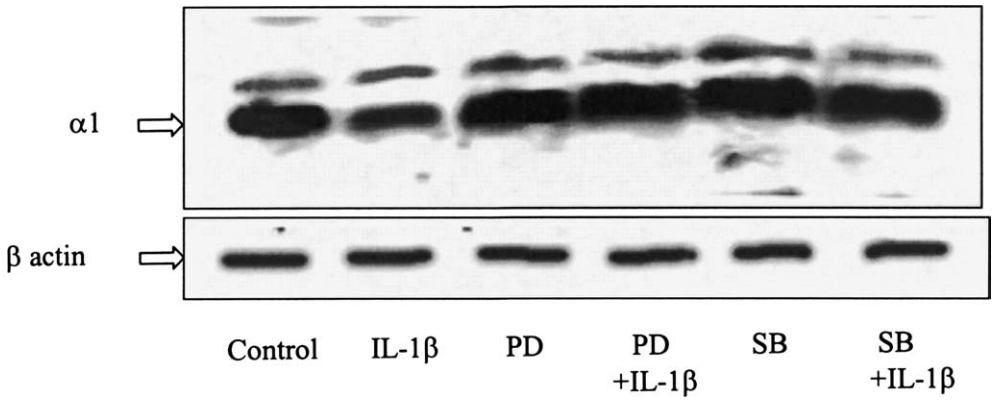


Fig. 7. Effect of IL-1β added to isolated myocytes in presence of MAPK inhibitors on the protein expression of the Na^+-K^+ATPase. Protein loaded: 70 μg. The blot is representative of an experiment repeated three times. Protein expression of β actin was used to check for equal loading.

catalytic α1 subunit, it may be attributed, at least partially, to a decrease in the number of enzyme molecules. Such an inhibition is expected to decrease the sodium gradient and sodium-dependent electrolyte movements that regulate excitation–contraction coupling in cardiac myocytes especially calcium movements. Cytosolic calcium increases during contractions and is restored back to normal levels upon relaxation via the Na^+-Ca^{++} exchanger whose activity is dependent on the sodium gradient established by the Na^+-K^+ pump. An inhibition of the pump is thus expected to dissipate the sodium gradient, inhibit the Na^+-Ca^{++} antiporter, increase cytosolic calcium and by so doing alter the systolic–diastolic cycle and delay relaxation. In fact, inhibition of the Na^+-K^+ATPase has been reported in some cases of heart failure [24,25] and was associated with increases in the levels of pro-inflammatory cytokines. Similarly increases in cytosolic calcium were reported to occur in association with increases in the levels of IL-1β and TNF-α [26]. The demonstrated inhibitory effect of IL-1β on the pump may thus provide a possible explanation of the previously reported increase in cytosolic calcium levels [27,28] that occur in myocytes following IL-1β treatment and whose cause remained controversial. We provide here a plausible explanation by attributing it indirectly to an inhibition of the Na^+-K^+ pump and consequently to the Na^+/Ca^{++} exchanger. Although our data lend support to this hypothesis, it still needs to be confirmed by further experimental work.

The inhibition of the pump is expected also to alter the function of the Na^+/H^+ exchanger which regulates intracellular pH and the activity of which is dependent on the sodium gradient generated by the Na^+-K^+ATPase. IL-1β is thus expected to increase intracellular acidity, a process that has already been reported in the ischemic heart where pH_i and pH_o were noted to reach values as low as 6.0 and 6.5, respectively [29,30].

This work showed that IL-1β reduced the activity of the Na^+-K^+ATPase but up-regulated the $NaKCl_2$ cotransporter. A change in the Na^+-K^+ATPase activity is known to elicit always a change of an opposite nature in the activity of the $NaKCl_2$ cotransporter [31]. This is consistent with our findings which are also in line with

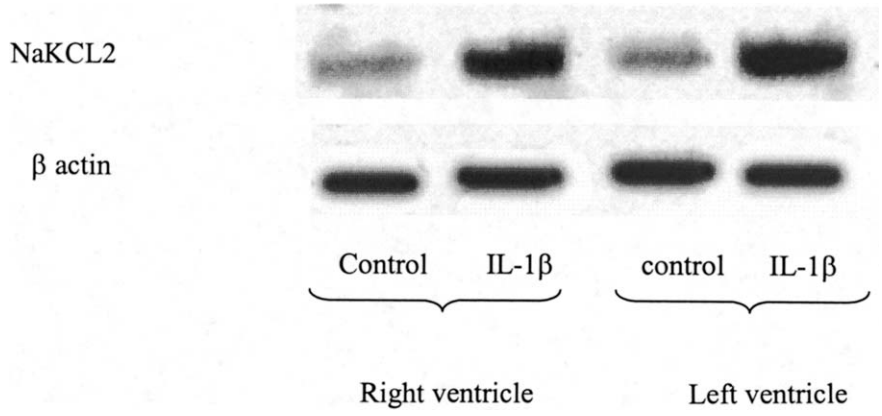


Fig. 8. Effect of IL-1β on the protein expression of the $NaKCl_2$ cotransporter in ventricular homogenates prepared from treated animals with the cytokine. Protein loaded: 150 μg. The blot is representative of an experiment repeated three times. Protein expression of β actin was used to check for equal loading.

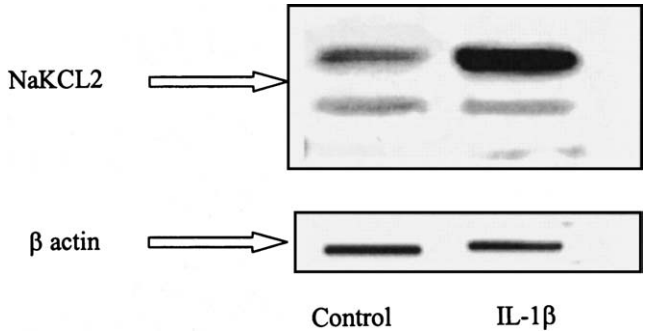


Fig. 9. Effect of IL-1β on the protein expression of the $NaKCl_2$ cotransporter in a homogenate of isolated myocytes treated with the cytokine. The blot is representative of an experiment repeated three times. Protein expression of β actin was used to check for equal loading.

those of Liu et al. [31] who reported an induced stimulation of the $NaKCl_2$ transporter by ouabain, an inhibitor of the pump.

A stimulation of the $NaKCl_2$ cotransport is known to induce cell swelling [32,33] and Clemo and Baumgarten [34] ascribed the observed decrease in the volume of atrial myocytes by ANP to an inhibition of the cotransporter. On the other hand, cell swelling and high levels of IL-1β were reported to accompany myocardial ischemia [35], but the causes of the changes in cell volume remained unidentified. Our findings may provide an explanation, by attributing it to an up-regulation of the $NaKCl_2$ cotransporter.

It can be concluded that many of the symptoms of heart failure may be ascribed to the effect of IL-1β on cardiomyocytes. By inhibiting the Na^+-K^+ATPase, the cytokine increases intracellular sodium and inhibits the Na^+-H^+ and Na^+-Ca^{++} exchangers resulting in a decrease in pH_i and an increase in cytosolic calcium that may lead to alterations in the systolic–diastolic cycle. The inhibition of the pump would lead also to up-regulation of the $NaKCl_2$ cotransporter resulting in cell swelling, a prominent feature of ischemic cardiomyocytes.

The Na^+-K^+ pump constitutes thus an important target site of action for IL-1β, and many of the reported cytokine actions are mediated through an effect on this ATPase.

It should be noted, however, that although this work provides some possible explanations to the symptoms that accompany heart failure, still more experimental work needs to be conducted to confirm them and establish the direct or indirect implication of IL-1 β in the disease. The long term effect of the cytokine on heart functions and Na^+-K^+ATPase activity would also need to be investigated.

4. Materials and methods

Male Sprague–Dawley rats (*Rattus norvegicus*) weighing 150–200 g were handled all through in accordance with the Guide for Laboratory Animal Facilities and Care, US Department of Health, Education and Welfare.

4.1. Animal treatment

Animals were injected i.p. with 400 ng IL-1β and 4 h later, the heart was isolated. These doses were chosen based on a previous study on the effect of this cytokine on the Na^+-K^+ pump in intestines and kidneys. Control animals received instead, an equal volume of physiological saline (200 μl).

Four hours after the injection of the cytokine, the heart was excised and placed in ice cold Ca^{++}-free perfusion buffer containing: 113 mM NaCl, 4.7 mM KCl, 0.6 mM KH_2PO_4, 0.6 mM Na_2HPO_4, 1.2 mM $MgSO_4{\cdot}7H_2O$, 12 mM $NaHCO_3$, 12 mM $KHCO_3$, 20 mM D-glucose, 10 mM Na^+-Hepes, 2 mM creatine, 2 mM carnitine, non-essential amino acids, pH = 7.2 at 37 °C, cannulated via the aorta and perfused with the same buffer for 4 min at 37 °C. The buffer was oxygenated with 95%O_2, 5%CO_2 throughout the perfusion step. The left and right ventricles were then isolated and homogenized in Tris buffer containing: 200 mM NaCl; 5 mM $MgCl_2{\cdot}6H_2O$; 2 mM EGTA; 5 mM KCl; 200 mM Tris–HCl, pH 7.4. Aliquots of the homogenate were withdrawn for western blot analysis or to assay for the Na^+-K^+ATPase activity.

4.2. Myocyte isolation and treatment

Myocytes were isolated as described by Hilal-Dandan et al. [36], suspended in oxygenated M 119 medium (pH 7.2) (0.5–1 million cells per ml) supplemented with penicillin (100 μg/ml), streptomycin (100 μg/ml), 1 μl/ml leupeptin and 0.1% BSA and incubated with different concentrations of IL-1β for different time periods at 37 °C.

When the involvement of MAP kinases was tested, PD98059 and SB202190, respective inhibitors of MEK and p38 MAP kinase were added to the cells at a final

concentration of 50 μM, 15 min prior to the addition of IL-1β. At the end of the incubation period, cells were collected by a 5 min centrifugation at 1500*g* and 4 °C.

4.3. Western blot analysis

Crude membrane homogenates were prepared by lysis and homogenization of the myocytes followed by two consecutive 4 min spins at 1000*g* and 3300*g* and 4 °C. Membrane proteins were determined using the Bio-Rad protein assay (Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA 94547 USA), and equal loads were (30 μg) resolved on 8% polyacrylamide gels using a Mini-Protean 3 System (Bio-Rad, CA, USA) and transferred to a PVDF membrane (Bio-Rad, CA, USA). The PVDF membrane was then washed, blocked and incubated overnight at 4 °C with a rabbit polyclonal anti-Na^+/K^+ATPase α-1 IgG (Upstate biotechnology, Lake Placid, NY 12946), or with an anti-rat sodium potassium chloride cotransporter antibody (Alpha Diagnostic International, TX, USA). Detection of the signal was by enhanced chemiluminescence using luminol reagent (Santa Cruiz Biotechnology Inc., CA, USA)

4.4. Na^+-K^+ ATPase assay

Crude myocyte membrane homogenates and ventricular homogenates were diluted in Tris buffer (200 mM NaCl; 5 mM $MgCl_2 \cdot 6H_2O$; 2 mM EGTA; 5 mM KCl; 200 mM Tris–HCl, pH 7.4) to a respective concentration of 0.1 mg and 1 mg protein/ml. Homogenates were incubated with saponin (0.02%) for 30 min at room temperature, then 50 μl aliquots were re-incubated at 37 °C for 10 min in the presence or absence of ouabain (4 mM final concentration). The reaction was then initiated by the addition of ATP to a final concentration of 1.25 mM and terminated after a 1 h incubation at 37 °C by the addition of trichloroacetic acid (200 μl, 11%). The samples were centrifuged at 3000*g* for 5 min and the amount of inorganic phosphate liberated in the supernatant was measured colorimetrically according to the method of Taussky and Shorr [37]. The activity of the enzyme was determined by measuring the ouabain-inhibitable inorganic phosphate liberated and percent inhibition was calculated as follows:

$$1 - \frac{\mathrm{Pi(IL1\beta) - Pi(IL1\beta + ouabain)}}{\mathrm{Pi(control) - Pi(control + ouabain)}} \times 100.$$

4.5. Statistical analysis

Results of the enzymatic assay were reported as means ± SEM. Statistical significance was tested by a Student *t*-test or a one-way analysis of variance followed by a Tukey–Kramer multiple comparisons test.

Changes in the protein expression were reported as percentages of the control values using the gel-Pro analyzer(2) software.

Acknowledgements

This work was supported by a grant from the University Research Board.

References

[1] Mann DL. Inflammatory mediators and the failing heart: past, present and the foreseeable future. Circ Res 2002;91:988–98.
[2] Niebauer J. Inflammatory mediators in heart failure. Int J Cardiol 2000;72:209–13.
[3] Seta Y, Shan K, Bozkurt B, Oral H, Mann DL. Basic mechanisms in heart failure: the cytokine hypothesis. J Card Fail 1996;2:243–9.
[4] Torre-Amione G, Kapadia S, Lee J, Durand JB, Bies RD, Young JB, et al. Tumor necrosis factor-alpha and tumor necrosis factor receptors in the failing human heart. Circulation 1996;93:704–11.
[5] Shamraj OI, Lingrel JB. A putative fourth Na+,K+-ATPase alpha-subunit gene is expressed in testis. Proc Natl Acad Sci U S A 1994;91:12952–6.
[6] Yokoyama T, Vaca L, Rossen RD, Durante W, Hazarika P, Mann DL. Cellular basis for the negative inotropic effects of tumor necrosis factor-alpha in the adult mammalian heart. J Clin Invest 1993;92:2303–12.
[7] Yokoyama T, Nakano M, Bednarczyk JL, McIntyre BW, Entman ML, Mann DL. Tumor necrosis factor-α provokes a hypertrophic growth response in adult cardiac myocytes. Circulation 1997;95:1247–52.
[8] Bundgaard H, Kjeldsen K. Human myocardial Na,K-ATPase concentration in heart failure. Mol Cell Biochem 1996;163–164:277–83.
[9] McDonough AA, Zhang Y, Shin V, Frank JS. Subcellular distribution of sodium pump isoform subunits in mammalian cardiac myocytes. Am J Physiol 1996;270:C1221–7.
[10] Shamraj OI, Grupp IL, Melvin D, Gradoux N, Kremers W, Lingrel JB, et al. Characterisation of Na.K-ATPase, its isoforms, and the ionotropic response to ouabain in isolated failing human hearts. Cardiovasc Res 1993;27:2229–37.
[11] Blanco MP, Lederer WJ. Isozymes of the Na+/K+ ATPase: heterogeneity in structure, diversity in function. Am J Physiol Renal Physiol 1999;275:F633–50.
[12] Schwinger RHG, Wang J, Frank K, Muller-Ehmsen J, Brixius K, McDonough A, et al. Reduced sodium pump α1, α2, and β1-isoform protein levels and Na+/K+ ATPase activity but unchanged Na+–Ca++ exchanger protein levels in human heart failure. Circulation 1999;99:2105–12.
[13] Taylor SH. Diuretics in heart failure: some knowns and unknowns. J Cardiovasc Pharmacol 1993;22(Suppl 3):S40–50.
[14] Dikshit K, Vyden JK, Forrester JS, Chetterjee K, Prakash R, Swan HJC. Renal and external hemodynamic effects of furosemide in congestive heart failure after acute myocardial infarction. N Engl J Med 1973;288:1087–90.
[15] Biagi RW, Bapat BN. Furosemide in acute pulmonary oedema. Lancet 1967;1:849–50.
[16] Andreasen F, Eriksen UH, Guul SJ, Nielsen LP, Bech OM, Daimant B, et al. A comparison of three diuretic regimens in heart failure. Eur J Clin Invest 1993;23:234–9.

[17] Brater DC. Clinical pharmacology of loop-diuretics in health an disease. Eur Heart J 1992;13:10–4.
[18] Kelso E, McDermott B, Silke B, Spiers P. Positive effect of bumetanide on contractile activity of ventricular cardiomyocytes. Eur J Pharmacol 2000;400:43–50.
[19] Kreydiyyeh SI, Al-Sadi R. Interleukin-1 beta increases urine flow rate and inhibits protein expression of the Na+-K+ ATPase in the rat jejunum and kidney. J Interferon Cytokine Res 2002; 22:1041–8.
[20] Kreydiyyeh SI, Al-Sadi R. The mechanism by which interleukin-1 beta reduces net fluid absorption from the rat colon. Eur Cytokine Netw 2002;13:358–63.
[21] Eder J. Tumour necrosis factor alpha and interleukin 1 signalling: do MAPKK kinases connect it all? Trends Pharmacol Sci 1997;18:319.
[22] Jiang B, Brecher P, Cohen RA. Persistent activation of nuclear factor-kappaB by interleukin-1 beta and subsequent inducible NO synthase expression requires extracellular signal-regulated kinase. Arterioscler Thromb Vasc Biol 2001;21:19257.
[23] Ng DCH, Long CS, Bogoyevitch MA. A role for the extracellular-regulated kinase and p38 mitogen-activated protein kinases in interleukin-1β-stimulated delayed signal transducer and activator of transcription 3 activation atrial natriuretic factor expression, and cardiac myocyte morphology. J Biol Chem 2001; 276:29490–8.
[24] Paganelli F, Mougenot R, Maixxxentt JM. Defective activity and isoform of the Na-K ATPase in the dilated cardiomyopathic hamster. Cell Mol Biol (Noisy-le-grand) 2001;47:255–60.
[25] Long CS. The role of interleukin-1 in the failing heart. Heart Fail Rev 2001;6:81–94.
[26] Kato D, Cragoe EJ, Smith TW. Relations among sodium pump inhibition, Na–Ca and Na–H exchange activities, and Ca–H interaction in cultured chick heart cells. Circ Res 1987;60: 185–93.
[27] Bick RJ, Liao JP, King TW, LeMaistre A, McMillin JB, Buja LM. Temporal effects of cytokines on neonatal cardiac myocyte calcium transients and adenylate cyclase activity. Am J Physiol 1997;272:H1937–44.
[28] Bick RJ, Wood DE, Poindexter B, McMillin JB, Karoly A, Wang D, et al. Cytokines increase neonatal cardiac myocyte calcium concentrations: the involvement of nitric oxide and cyclic nucleotides. J Interferon Cytokine Res 1999;19:645–53.
[29] Karmazym M, Gan XT, Humphreys RA, Yoshida H, Kusumoto K. The myocardial Na+–H+ exchange structure, regulation, and its role in heart disease. Circ Res 1999;85:777–86.
[30] Park CO, Xiao XH, Allen DG. Changes in intracellular sodium and pH in the rat heart during ischaemia: role of the Na+/H+ exchanger. Am J Physiol 1999;276:H1590.
[31] Liu S, Jacob R, Piwnica-Worms D, Lieberman M. Interaction of Na+K+2Cl cotransport and the Na/K pump in cultured chick cardiac myocytes. Mol Cell Biochem 1989;89:147–50.
[32] O'Neil WC, Klein JD. Regulation of vascular endothelial cell volume by NaK2Cl cotransport. Am J Physiol 1992;262(2 pt 1): C436–44.
[33] Hume JR, Duane D, Collier M, Yamazaki J, Horowitz B. Anion transport in heart. Physiol Rev 2000;80:31–81.
[34] Clemo HF, Baumgarten CM. cGMP and atrial natriuretic factor regulate cell volume of rabbit atrial myocytes. Circ Res 1995; 77:741.
[35] Diaz RJ, Armstrong SC, Batthish M, Backx PH, Ganote CE, Wilson GJ. Enhanced cell volume regulation: a key protective mechanism of ischemic preconditioning in rabbit ventricular myocytes. J Mol Cell Cardiol 2003;35(1):45–58.
[36] Hilal-Dandan R, Knter JR, Brunton LL. Characterization of G-protein signaling in ventricular myocytes from the adult mouse heart: differences from rat. Mol Cell Cardiol 2000;32:1211–21.
[37] Taussky HH, Shorr E. Microcolorimetric method for determination of inorganic phosphorous. J Biol Chem 1953;202:675–85.