Short communication

Inhibition of (Na/K)-ATPase by NFE Induces an Increase in Mechanical Activity of Perfused Guinea-Pig Heart

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Abstract. The effect of 1-(5-nitro-2-furyl)-2-(phenylsulfonyl)-2-(furylcarbonyl)-ethylene (NFE) on stimulation of (Na/K)-ATPase by sodium and potassium ions was tested in isolated, partially purified sarcolemmal preparation from guinea-pig hearts. NFE inhibited competitively the stimulation of the enzyme by increasing concentrations of potassium. This inhibition was characterized by a significant ($p < 0.001$) increase of the $K_{0.5}$ value, a considerable decrease of the Hill's cooperativity constant n as well as by an insignificant diminution of the V_{max} value. Contrary to the effect on stimulation by potassium, NFE inhibited non-competitively the stimulation of the ATPase by sodium ions with a significant ($p < 0.001$) depression of V_{max} but without any considerable effect on the $K_{0\,5}$ and n values. These results indicated that NFE may interact with the molecule of (Na/K)-ATPase in a locus close to or identical with the potassium binding site of the enzyme, i.e., in a similar mode as it was well documented for ouabain. This possibility was strongly supported by the finding that NFE administered at the concentration of 0.1 μmol/l in the perfusion medium increased significantly ($p < 0.01$) the mechanical activity of isolated perfused guinea-pig heart (Langendorff preparation). Nevertheless, it also caused some adverse effects such as a slight increase in coronary flow resistance and in heart rate as well as in the left ventricular end-diastolic pressure. In spite of these differences in the character of cardiostimulatory effects of ouabain and NFE, our results revealed that the latter substance seems to interact with

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the sarcolemma similarly to ouabain, i.e., by inhibiting the stimulation of heart sarcolemmal (Na/K)-ATPase by potassium ions.

Key words: Heart sarcolemmal (Na/K)-ATPase — 1-(5-nitro-2-furyl)-2-(phenylsulfonyl)-2-(furylcarbonyl)-ethylene (NFE) — Ouabain like action — Perfused guinea-pig heart

Introduction

Heart sarcolemmal (Na/K)-ATPase is generally recognized as the target molecule responsible for the binding and inotropic action of cardiac glycosides, e.g., ouabain (for review see Skou and Esmann 1992). The latter effect involves an accumulation of intracellular sodium occurring upon cardiac glycosides-induced inhibition of the enzyme. This is in turn enhancing the influx of calcium and/or decreasing its efflux via the (Na/Ca)- exchange mechanism operating in cardiac muscle cell membranes. The finally resulting increase of intracellular calcium concentration yields an increase of contractions of the heart muscle. Nevertheless, not only xenobiotica are capable of modulating the sarcolemmal (Na/K)-ATPase activity (Kölbel et al. 1994). Inhibition of (Na/K)-ATPase by D,L-aspartic acid which is interacting also in the ouabain binding site of the enzyme (Fedelešová et al. 1973; Fedelešová et al. 1975a) produced several ouabain-like effects in dog heart (Fedelešová et al. 1975b).

The nucleophilic amino-acid residues believed to be responsible for the selectivity of binding sites for monovalent cations on the molecule of (Na/K)-ATPase have already been described by Breier et al. (1988). It may be assumed that agents which can attack selectively these residues may induce inhibition of (Na/K)-ATPase activity.

Furylethylenes represent a group of biologically active compounds whith a broad spectrum of antimicrobial (Kellová et al. 1984), antitumour (Drobnica et al. 1981), mutagenic (Šturdík et al. 1985; Šturdík et al. 1986) and also oncogenic (Hei et al. 1992) effects. In clinical and veterinary medicine, the antimicrobial treatment with nitrofurans was, however, often accompanied by side-effects including certain cardiotoxicity (Biel et al. 1993). Nevertheless, the molecular mechanism of these side-effects still has to be elucidated. The recently synthesized furylethylene derivative – 1-(5-nitro-2-furyl)-2-(phenylsulfonyl)-2-(furylcarbonyl)-ethylene (NFE) is a typical representative of electrophilic agents able to attack SH, OH and NH_2 groups as well (Špirková et al. 1991). Hence it is highly possible that the effect of substance on the heart may involve an interaction of NFE with some SH and NH_2 groups in sodium- or potassium-binding sites of the sarcolemmal (Na/K)-ATPase. The aim of the present study was to investigate the effect of NFE on the (Na/K)-ATPase and mechanical activity of the isolated perfused heart.

Materials and Methods

Materials

1-(5-nitro-2-furyl)-2-(phenylsulfonyl)-2-(furylcarbonyl)-ethylene (NFE) was synthesized at the Department of Organic Chemistry, Slovak Technical University, Bratislava, by means of the Lehnert condensation method. The corresponding analytical data about the substance obtained by spectroscopic techniques were described by Špirková et al. (1991). All other chemicals used in experiments were of analytical grade and were purchased from Sigma (USA) and Lachema (Czech Republic).

Preparation of sarcolemmal membranes

Sarcolemmal membrane fraction from guinea-pig heart was isolated using the method of hypotonic shock combined with NaCl treatment (Kostka et al. 1981).

Estimation of (Na/K)-ATPase activity

(Na/K)-ATPase activity in partially purified membrane fraction was determined as the difference in the amounts of phosphate liberated by spilitting of ATP in the presence of 1.25–100.00 mmol/l NaCl, 0.125–10.000 mmol/l KCl and 4 mmol/l $MgCl_2$ (if not stated otherwise) or in the presence of 4 mmol/l $MgCl_2$ only. The enzyme reaction was run in 0.5 ml of incubation medium containing 50 mmol/l imidazole-HCl buffer, pH 7.0, and 20–40 μg of membrane proteins at 37 °C for 10 min; it was started by simultaneous addition of ATP (final concentration 4 mmol/l) and NFE dissolved in dimethyl sulfoxide (DMSO) and stopped by ice-cold trichloroacetic acid (0.75 mol/l). The final concentration of DMSO brought with NFE did not exceed 0.5% v/v in incubation medium. Orthophosphate was estimated using the method of Taussky and Shorr (1953). Protein content in membrane fractions was determined according to Markwell et al. (1978). Kinetic parameters of activation of (Na/K)-ATPase by potassium and sodium ions were determined by non-linear regression of the experimental data obtained from the Michaelis-Menten relationship equipped with the Hill's cooperativity constant n (equation 1) using the PC software Sigma Plot 5.0.

$$V = \frac{V_{\max}\, c^n}{K_{0.5}^n + c^n} \tag{1}$$

where: V and $V_{\max}$ represent the velocities of enzyme reaction at concentrations of sodium or potassium equal to c and $c \to \infty$; $K_{0.5}$ represents the affinity constant for stimulation of ATPase activity by sodium or potassium.

Perfusion protocol

Heparinized (200 IU i.v.) male quinea-pigs weighing 270–330 g were anaesthetized with diethyl ether. After cannulation of the aorta, the heart was excised and perfused at constant flow rate (10 ml/min) and 37 °C using the Langendorff technique. Krebs-Henseleit (KH) solution containing in mmol/l: 118 NaCl, 15 $NaHCO_3$, 2.68 KCl, 1.66 $MgSO_4$, 2.0 $CaCl_2$, 1.18 KH_2PO_4 and 11 glucose, pH 7.40 ± 0.05, filtered through a 5 mm millipore filter and gassed with a mixture of 95% O_2 + 5% CO_2 served as perfusion medium. Left ventricular pressure, measured by means of an intraventricular balloon, as well as perfusion pressure were linked to pressure transducers (Gould P23, USA), monitored by LDP 186 (Tesla, Czech Republic) and registered by Chiracard 600 T (Chirana, Slovakia).

Epicardial electrogram was recorded with the aid of a pair of platinum electrodes. After 15 minutes of stabilization perfusion with KH-medium in non-recirculating regime, hearts were switched over to 20 min perfusion with KH solutions containing in addition either dimethyl sulfoxide (DMSO, 0.1 mmol/l) or NFE (0.1 μmol/l) plus DMSO. Myocardial function was evaluated by monitoring the following variables: Left ventricular systolic pressure (LVP_S), left ventricular diastolic pressure (LVP_D), the difference between LVP_S and LVP_D (LVP_{S-D}) as a measure of mechanical activity, and the electrical activity of the heart (beats/min). Coronary flow resistance was calculated as LVP_D/coronary flow (provided by the pump). Six consecutive measurements of the above variables were made during each perfusion, the total time interval being of 20 min. The effect of NFE was evaluated as a difference between data obtained in the presence of NFE plus DMSO and those in the presence of DMSO alone. Results were expressed as means ± S.E.M. Statistical significance between consecutive measurements in each type of experiment was checked by means of the F-test and between different experimental groups by Student's t-test.

Results and Discussion

Similarly to other electrophilic reagents such as fluorescein isothiocyanate (Carilli et al. 1982; Farley et al. 1984), p-bromophenyl isothiocyanate (Ziegelhöffer et al. 1983) or trinitrobenzene sulfonic acid (De Pont et al. 1984), NFE also depressed the activity of (Na/K)-ATPase with an IC_{50} value of approximately 15 μmol/l (data not shown). As concerns the specific sites of interaction with the enzyme molecule, isothiocyanates were described to react (namely at pH values lower than 8) predominantly, if not exclusively with SH groups in the ATP binding sites of the enzyme (Ziegelhöffer et al. 1983; Breier et al. 1989). Nevertheless, at pH values exceeding 8 the interaction with NH_2 groups in the same active sites cannot be excluded (Carilli et al. 1982). In contrary to isothiocyanates, trinitrobenzene sulfonic acid as a selective amino reagent was found to modulate the stimulation by potassium of the enzyme. This indicates that the reagent may interact in or at least close to the potassium binding site of the (Na/K)-ATPase (Breier et al. 1986; Breier et al. 1987). It was also reported that NFE may interact with both SH and NH_2 groups, respectively (Špirková et al. 1991). However, the mechanism of inhibition of (Na/K)-ATPase with NFE did not involve any competition between ATP and the latter substance (Štefko 1991; Breier et al. 1994). It was characterized by an NFE-induced depression in the affinity of (Na/K)-ATPase to potassium ions, manifested in the significant increase of the respective $K_{0.5}$ value but an unchanged V_{max} value for activation of the enzyme with potassium (Fig. 1). In addition, the accompanying decrease in cooperativity constant n indicates that NFE depresses the cooperativity between potassium binding sites of the (Na/K)-ATPase. The changes observed in $K_{0.5}$ and n values may be explained by direct interaction of NFE with those structures on the enzyme molecule which are involved in binding of potassium.

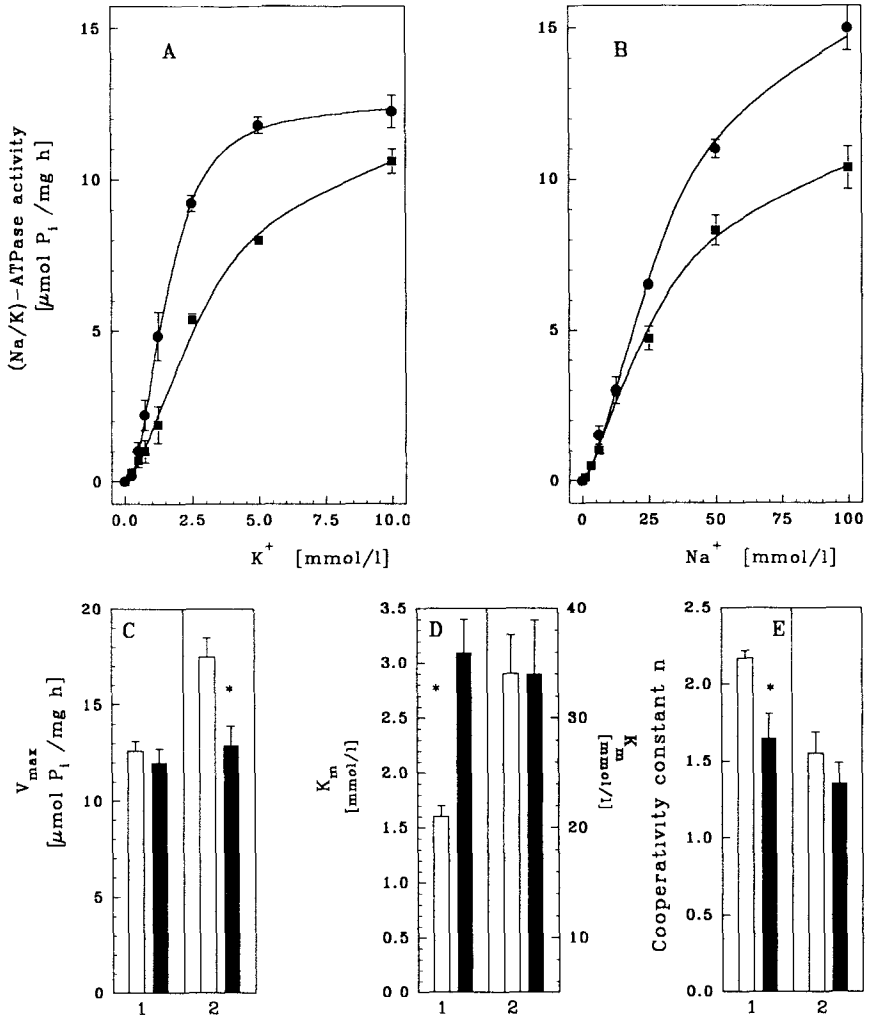

Figure 1. Inhibition of heart sarcolemmal (Na/K)-ATPase activity by NFE. Panel *A*: Effect of NFE on stimulation of ATPase activity by potassium. Panel *B*: Effect of NFE on stimulation of ATPase activity by sodium. Enzyme reaction was run in 0.5 ml of incubation medium containing 50 mmol/l imidazole-HCl buffer, pH 7.0, and 20–40 μg of membrane proteins at 37 °C for 10 min; it was started by simultaneous addition of ATP (final concentration 4 mmol/l) and NFE dissolved in dimethyl sulfoxide (DMSO) and stopped by ice-cold trichloracetic acid (0.75 mol/l). Symbols: ● – control without NFE; ■ – effect of NFE (10 μmol/l). Data represent means ± S_D from 6 independent measurements. Parameters of enzyme kinetics (V_{max} – panel *C*, K_m – panel *D* and cooperativity constant n – panel *E*) were obtained by nonlinear regression applying equation 1 (see Materials and Methods). Parts 1 and 2 represent data obtained from stimulation of (Na/K)-ATPase activity by potassium and sodium in the absence (empty colon) or presence (full colon) of NFE (10 μmol/l). Data represent computed values ± S.E.M. (degree of freedom ~ 45). Significance: * – ($p < 0.001$).

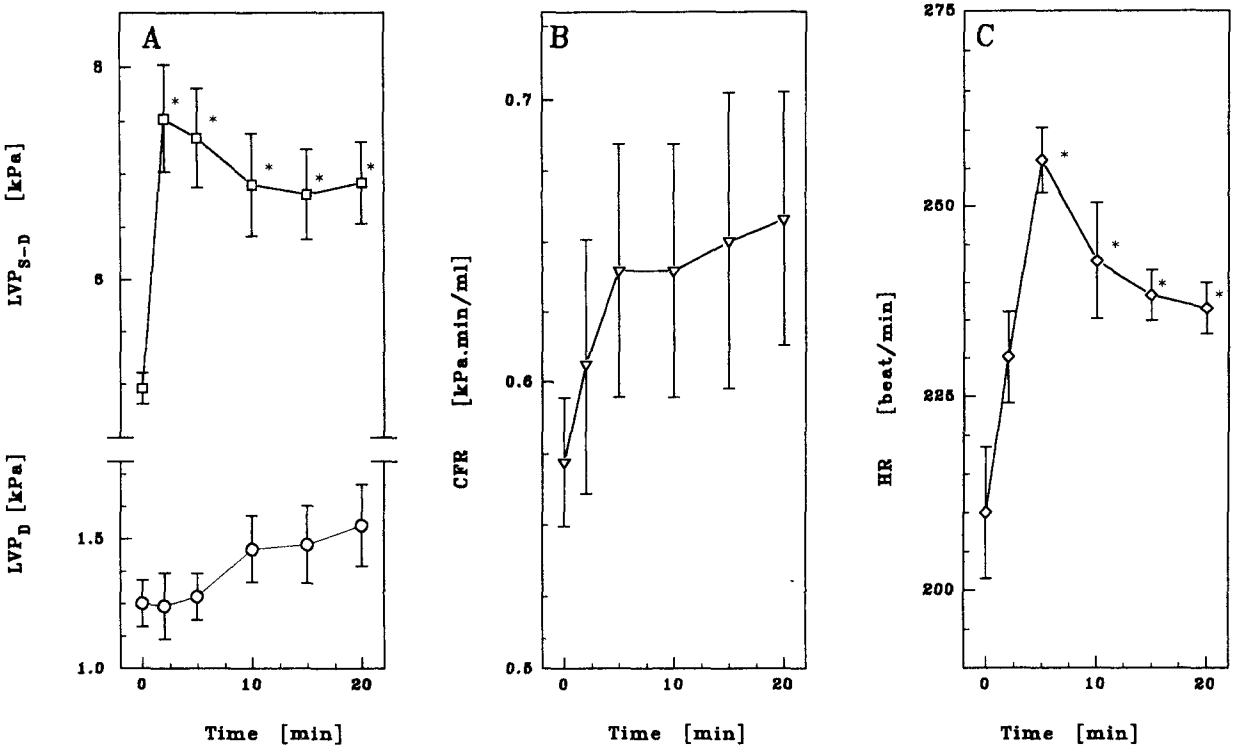

Figure 2. Time dependence of NFE effect on haemodynamic parameters of isolated perfused guinea-pig heart (concentration of NFE 0.1 μmol/l, for perfusion protocol see Materials and Methods). Panel *A*: Effect of NFE on the mechanical activity of the heart expressed as the difference between systolic and diastolic pressure in left ventricle (LVP_{S-D}, □) and left ventricular diastolic pressure (LVP_D, ○). Panel *B*: Effect of NFE on coronary flow resistance expressed as ratio between LVP_D and coronary flow (provided by the pump). Panel *C*: Effect of NFE on heart rate. Data are expressed as mean ± S.E.M. from 8 independent values. Significance: * – ($p < 0.01$) against control (at time 0).

It has been shown that 5-nitro-2-furylethylene derivatives exhibit high reactivity providing a series of addition or substitution reactions with low-molecular weight thiols, amines and alcohols (Rosenberg et al. 1985; Baláž et al.1987). These features led to expect that NFE would react with those among the nucleofilic groups of the (Na/K)-ATPase-lipoprotein complex which are involved in the formation of the proposed coordination bonds with monovalent cationic ligands of the enzyme (Ziegelhöffer et al.1986; Breier et al. 1988). Hence, it is highly probable that NFE will attack the molecule of (Na/K)-ATPase preferentially in a similar place as it does trinitrobenzene sulfonic acid, i.e., in the potassium binding site of the enzyme (Breier et al. 1986; Breier et al. 1987). From the aspect of stimulation of (Na/K)-ATPase with sodium ions, the interaction of NFE with the enzyme molecule may be fully considered as non-competitive with decrease in the V_{max} value only and without any significant change in the $K_{0.5}$ and n values. (Fig. 1). The effects of trinitrobenzene sulfonic acid (Breier et al. 1986) and NFE proved to be very similar also in this respect.

For application in perfusate, hydrophobic NFE was first dissolved in DMSO. A final concentration of 0.1 mmol/l of the solvent was needed to maintain NFE fully dissolved in the KH medium. Same concentration of DMSO added alone to the KH medium affected the functional variables of the isolated perfused heart only slightly. Absence of arrhythmias, non-substantial change in coronary flow resistance and an approximately 10%, but statistically insignificant decrease in mechanical activity of the heart could only be noted (data not shown). Presence of 0.1 μmol/l NFE and 0.1 mmol/l DMSO in perfusate led to significant ($p < 0.01$) increase in mechanical activity of the heart and in heart rate (Fig. 2). The accompanying slight elevations in coronary flow resistance and end-diastolic pressure were not found statistically significant ($p > 0.1$). The observed considerable increase in mechanical activity of the heart induced by NFE resembles to certain extent the effect observed after application of ouabain (Goldberg 1966). Considering that NFE may inhibit the activity of sarcolemmal (Na/K)-ATPase in its potassium binding site and at the same time is also capable of inducing positive inotropy in the guinea-pig heart, it appears there is much similarity between the effect of NFE and that of cardiac glycosides on the heart (Repke 1963). Therefore, whether ouabain and NFE will bind to sarcolemma in the same locus or not, the mechanism of their action may be fairly similar, i.e., inhibition of (Na/K)-ATPase (the sodium pump) with consequent intracellular accumulation of sodium and its fast exchange for calcium (Skou and Esmann 1992).

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