An *In Vitro* Study into the Mechanisms of Lidocaine and Befol Actions on Sodium Exchange in Normal and Hypoxia-Exposed Rat Cardiomyocytes

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Using benzofuran isophthalate, a fluorescent probe for sodium ions, intracellular (sarcoplasmic) Na^+ concentrations ($[Na^+]_i$) were estimated in cardiomyocytes isolated from the left ventricle of rats. Lidocaine (1-100 μM) had little effect on $[Na^+]_i$ in resting (unstimulated) cardiocytes, while befol lowered it by virtue of its inhibitory effect on Na/H exchange. In cardiomyocytes exposed to "chemical" hypoxia (produced by 5 mM KCN+30 mM 2-deoxyglucose), $[Na^+]_i$ were three times higher than in resting cells, and the Na-blocking effects of both lidocaine and befol were much stronger. When these two drugs were used together, potentiation of these effects was observed, which may be accounted for by their action on different Na-transporting systems.

Key Words: *intracellular sodium; befol; lidocaine; cardiomyocytes; hypoxia*

Increases in the cytoplasmic concentration of free sodium ions ($[Na^+]_i$) are among signals activating the cell. In cardiomyocytes and vascular smooth muscle cells, a rapid Na^+ flux via voltage-dependent Na channels stimulates the entry of extracellular Ca^{2+} into the cells and muscular contraction. Linkage between $[Na^+]_i$ and the intracellular concentration of free Ca^{2+} and H^+ ions is also effected through the systems of Na^+/H^+ and Na^+/Ca^{2+} exchanges located in the sarcolemma [4]. The activity of these transport systems depends on the maintenance of an electrochemical Na^+ gradient by Na,K-ATPase. The concordant functioning of ion carriers, voltage-sensitive Na and Ca channels, and ATP-dependent pumps is impaired in various disease states such as ischemia, poisoning with cardiac glycosides, and cardiomyopathy [10]. One manifestation of ionic imbalance is an increase diastolic Na^+ level, which is regarded as a potentially arrhythmogenic factor [6]. Drugs that influence Na exchange have been used as antiarrhythic agents; particularly popular among them are those that block the "fast" Na channels (e.g., novocainamide, ethmosine, and lidocaine).

An important aspect of research on the pharmacological regulation of Na exchange concerns the altered drug sensitivity of Na channels and Na-dependent transport systems under conditions of myocardial ischemia. However, this aspect still remains largely unexplored, as most studies of molecular mechanisms by which cardioselective drugs act have been conducted on intact myocytes.

The present study was designed to compare the mechanisms responsible for the maintenance of Na homeostasis in well-oxygenated cardiomyocytes and those exposed to "chemical" hypoxia.

MATERIALS AND METHODS

The main drugs use were lidocaine and the new antidepressant befol with characteristic antiarrhythmic properties [1].

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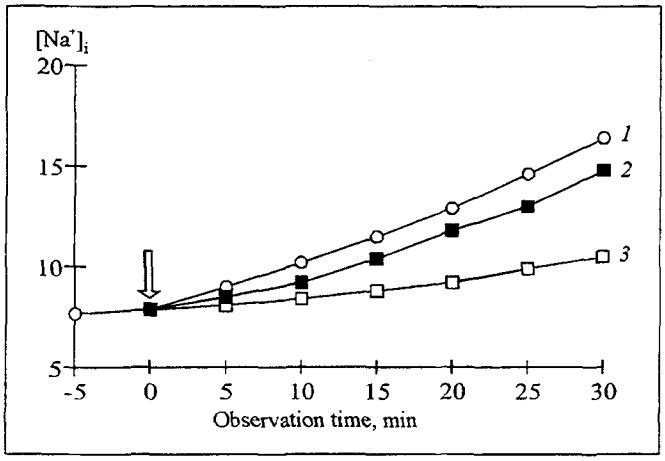

Fig. 1. Effects of ethyl isopropyl amiloride (10 μM; 2) and befol (80 μM; 3) on the ouabain-mediated elevation of Na^+ concentration in intact cardiomyocytes; 1) effect of ouabain (10 μM) in the absence of other agents. The arrow marks the time when the compounds were added.

Ion-selective electrodes and nuclear magnetic resonance, which were employed until recently to record $[Na^+]_i$, are not devoid of serious drawbacks [11]. Japanese investigators [7,10] have demonstrated the feasibility of utilizing the fluorescent probe benzofuran isophthalate for quantitative estimation of $[Na^+]_i$ in rat cardiomyocytes. In the form of acetoxymethyl ester, designated as SBFI-AM), this fluorescent indicator crosses, by virtue of its hydrophobicity, the plasma membrane to reach the sarcoplasm where it is rapidly hydrolyzed to an acid (SBFI) owing to the high activity of nonspecific intracellular esterases, without being able to penetrate into subcellular organelles or into the intravesicular space of the reticulum. The binding to Na^+ strongly alters the conformation of the latter compound, which is reflected in altered fluorescence parameters whose recording can provide an estimate of the intracellular Na^+ concentration ($[Na^+]_i$).

Cardiomyocytes, isolated from the left ventricle of rats as previously described [9], were perfused with a modified Krebs solution equilibrated with 95% O_2 and 5% CO_2 (pH 7.4). The isolation medium was of the following composition (mM): 113 NaCl, 4.6 KCl, 1.2 $MgCl_2$, 3.5 NaH_2PO_4, 21.9 $NaHCO_3$, 5 glucose. After the final resuspension of cardiomyocytes, $CaCl_2$ (2.45 mM) was added to the suspension ($10-15\times10^6$ cells), followed by the addition of SBFI-AM to a final concentration of 5 μM and by incubation with the probe for 30 min at room temperature. The cells were then washed twice with a fresh Krebs buffer to remove the free (unbound) indicator.

One-milliliter samples were placed in cells of an MPF-3 spectrofluorimeter (Hitachi) and their fluorescence (500 nm) was recorded at the excitation wavelengths of 340 nm and 380 nm corresponding to the absorption maxima of the Na^+-bound and free forms of the SBFI probe, respectively [10]. To convert fluorescence intensity values into Na^+ concentrations, a calibration procedure was performed as previously described [7].

Sarcoplasmic Na^+ concentrations were calculated by the formula: $[Na^+]_i=K_d\times\beta\times(R-R_{min})/(R_{max}-R)$, where R is the ratio of fluorescence intensities at the excitation wavelengths 340 nm (F_{340}) and 380 nm (F_{380}); R_{min} and R_{max} are as above but at zero $[Na^+]_i$ and saturating (150 mM) $[Na^+]_i$; β is the ratio of fluorescence intensities (2.1±0.1) at 380 nm for the free and bound forms of the probe; and K_d is the equilibrium dissociation constant for the probe-Na^+ complex equal to 20.8±1.4 mM [7].

The data obtained were statistically analyzed by Student's t test.

RESULTS

$[Na^+]_i$ measurements in resting cardiomyocytes showed that the intracellular Na^+ concentration virtually did not change with time (at least during the 30-min observation period), remaining at the level of 7.8±0.9 mM. $[Na^+]_i$ is determined by the activity of the mechanisms transporting Na^+ into the cell, and by the Na,K-ATPase-mediated Na^+ exit from the cell. These two processes are balanced in unstimulated (resting) cells.

The Na,K-ATPase inhibitor ouabain added to cardiomyocytes at 10 μM caused a slow but steady rise of $[Na^+]_i$, so that the latter rose to 16.4±1.5 mM after 30 min of incubation (Fig. 1). Lidocaine in the concentration range used (10-100 μM) did not influence the time-course of ouabain-mediated rise in $[Na^+]_i$. Nor was this rise inhibited by tetrodotoxin (10 μM) used as a reference compound, indicating that no Na^+ entered the cell via sarcolemman Na channels.

Befol (final concentrations 1-100 μM in th incubation medium) inhibited the rise in $[Na^+]_i$, though relatively slightly; thus, it failed to elicit a 50% inhibition even at 100 μM, i.e., its $IC_{50}>100$ μM. The activity of befol could be determined by its influence on the Na/H and/or Na/Ca transfer systems which transport Na^+ into cells in exchange for H^+ and Ca^{2+}, respectively. To check this, we used the compound ethyl isopropyl amiloride (EIPA) which selectively inhibits Na/H exchange at 10 μM [2] and which caused in this concentration a 80% inhibition of the rise in $[Na^+]_i$ under the action of ouabain (Fig. 1), but failed to cause a significant change in the Na response of the cells even at 50 μM — a concentration that is inhibitory to Na/Ca exchange as well. These result indicate that Na^+ entry into resting,

normally oxygenated cardiomyocytes is mediated by the Na/H antiport, and that the Na,K-ATPase works to prevent elevation of $[Na^+]_i$ in such cells. In these circumstances, the Na-blocking effect of befol is therefore determined by its selective action on Na/H exchange.

To produce hypoxic conditions, cardiomyocytes were incubated in a medium containing the inhibitor of oxidation reactions KCN (5 mM) and the D-glucose antimetabolite 2-deoxyglucose (30 mM), i.e., compounds in whose presence cardiomyocytes are incapable of utilizing oxygen [5,8]. After 20 min of their exposure to this combination, a marked increase in the fluorescence intensity at 340 nm was recorded, indicating a higher Na^+ level in the cells. The reasons for the observed increase in $[Na^+]_i$ may be the following: 1) spontaneous activation of cardiomyocytes, accompanied by opening of the Na channels and Na^+ entry into the cell [10]; 2) stimulation of Na/H exchange, mediated by elevation of proton concentration in the cytoplasm (in the presence of normal oxygenation, accumulation of acid equivalents is hindered by the metabolic utilization of acid products [4]; and 3) reduced activity of the energy-dependent Na-K pump.

To assess the impact of lidocaine and befol on Na exchange, these drugs were added to cardiomyocyte suspensions 5 min before the addition of KCN and 2-deoxyglucose. Neither lidocaine nor befol showed a substantial increase in their Na-blocking activity. Lidocaide decreased the $[Na^+]_i$ in a dose-dependent manner, its inhibitory effect at 100 μM (the highest concentration used) being 40% on average. The IC_{50} of befol was 23±4 μM. When lidocaine and befol were added together, potentiation of their inhibitory effects was observed (Fig. 2), which may be accounted for by the action of these drugs on different Na-transporting systems.

The cardiomyocyte membrane is known to have Na channels of at least three types whose activity is determined by the cardiac contraction phase and by their sensitivity to variations in the action potential and to biologically active substances [3]. The basis of the enhanced Na-blocking effect exerted by lidocaine on cardiomyocytes exposed to "chemical" hypoxia appears to be its activation predominantly of the voltage-dependent Na channels in these cells,

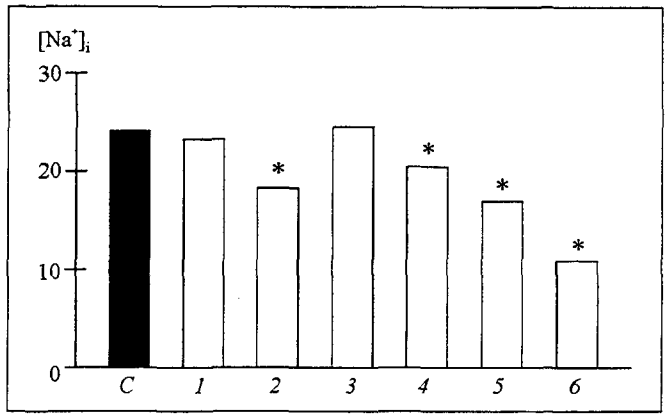

Fig. 2. Effects of lidocaine, befol, and their combination on the Na^+ concentration in hypoxia-exposed cardiomyocytes. *C*) control; *1*) 10 μM lidocaine; *2*) 30 μM lidocaine; *3*) 2 μM befol; *4*) 10 μM befol; *5*) 10 μM lidocaine+2 μM befol; *6*) 30 μM lidocaine+10 μM befol. *Significant difference from the control ($p<0.05$).

which are the target for antiarrhythmic agents with the membrane-stabilizing type of action. The chain of events described by the scheme metabolic inhibition → elevation of intracellular pH → stimulation of Na/H exchange explains why the activity of befol was increased in our experimental model of hypoxia, if Na/H exchange is regarded as its target.

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