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Na^+-overload during ischemia and reperfusion in rat hearts: comparison of the Na^+/H^+-exchange blockers EIPA, HOE642 and EMD96785

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1. Introduction

Intracellular myocardial Na^+ overload during ischemia is an important cause of reperfusion damage via reversed Na^+/Ca^{2+}-exchange. The relative importance of the different influx routes of Na^+ is still a matter of debate. Previously it has been shown that the Na^+-channel plays an important role and its blockade can result in a 60% reduction in Na^+-overload. Another important influx route is via the sarcolemmal Na^+/H^+-exchanger (NHE). In this study the effect of ischemic inhibition of the NHE on intracellular Na^+($[Na^+]_i$), intracellular pH (pH_i), high energetic phosphates (HEPs) and post-ischemic contractile recovery was tested in isolated rat hearts, using three different NHE-blockers: EIPA, HOE642 and EMD96785.

2. Material and methods

Isolated rat hearts were perfused according to Langendorff at a constant pressure of 73.5 mmHg at 37°C with a modified Krebs–Henseleit buffer (pH 7.4) with glucose as substrate and were paced at 5 Hz. Left ventricular developed pressure (LVDP) and end diastolic pressure (EDP) were measured with an intraventricular balloon. $[Na^+]_i$, pH_i and HEPs were measured using ^{23}Na and ^{31}P NMR spectroscopy, respectively. ^{23}Na and ^{31}P were measured simultaneously at frequencies of 105.9 and 162.0 MHz, respectively, on a Bruker Avance DRX400 spectrometer equipped with a dual tuned probe and two digital receivers. ^{23}Na spectra were acquired by adding 288 FIDs using 90° pulses and a 210 ms interpulse delay. ^{31}P spectra were acquired by adding 24 FIDs using 90° pulses and a 2.5 s interpulse delay. ^{31}P and ^{23}Na were both collected with a time resolution of 1 min. To quantify PCr and ATP five ^{31}P spectra were added. To discriminate between intra- and extracellular Na^+, the shift reagent $TmDOTP^{5-}$ (3.5 mM) was added to the perfusate, necessitating a lower free Ca^{2+} concentration (0.85 mM). NHE-blockers were administered in a concentration of 3 μM during 5 min immediately prior to 30 min of global ischemia and 30 min of drug-free reperfusion. Data are presented as mean ± S.E.M.

3. Results

Na^+ overload after 30 min of ischemia was reduced with 30, 58 and 60% using EIPA, HOE642 and EMD96785, respectively. Results are presented in Table 1.

Administration of NHE-blockers did not result in any significant difference in pH_i during ischemia. During reperfusion recovery of pH_i was delayed. Results are presented in Table 2.

During ischemia PCr content decreased to < 5% within 15 min in all groups. After 30 min of reperfusion

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Table 1
$[Na^+]_i$ after 30 min ischemia and after 30 min reperfusion as percent of baseline[a]

	n	30 min I (%)	30 min R (%)
Untreated	7	305 ± 23	162 ± 14
EIPA	6	212 ± 6*	127 ± 7
HOE642	6	157 ± 5*	123 ± 15
EMD96785	6	146 ± 6*	132 ± 5

[a] Data are ± S.E.M.
* $P < 0.001$ vs. untreated.

Table 2
pH_i after 5 min reperfusion[a]

	n	5 min R
Untreated	7	6.90 ± 0.05
EIPA	6	6.78 ± 0.07
HOE642	6	6.79 ± 0.04
EMD96785	6	6.68 ± 0.04

[a] Data are ± S.E.M.

PCr had recovered to 81 ± 6, 95 ± 8, 94 ± 10 and 105 ± 7% in untreated and EIPA, HOE642 and EMD96785 treated hearts, respectively (EMD96785 vs. untreated, $P < 0.05$). ATP content had decreased to < 10% after 30 min of ischemia in all groups. After 30 min of reperfusion ATP had recovered to 29 ± 1, 44 ± 8, 35 ± 3 and 42 ± 4% in untreated and EIPA, HOE642 and EMD96785 treated hearts, respectively (NS).

Administration of HOE642 and of EMD96785 resulted in a better recovery of the rate pressure product (RPP; heart rate × LVDP) after 30 min of reperfusion. Results are presented in Table 3. At the end of reperfusion EDP was 38.0 ± 3.8, 38.3 ± 2.2, 31.9 ± 4.6 and 23.5 ± 4.3 mmHg in untreated and EIPA, HOE642 and EMD96785 treated hearts, respectively (EMD96785 vs. untreated, $P < 0.05$).

Table 3
RPP at start of protocol and after 30 min reperfusion[a]

	n	Start protocol	30 min R
Untreated	7	16.4 ± 1.2	11.5 ± 2.7
EIPA	6	15.8 ± 1.9	12.1 ± 2.1
HOE642	6	16.9 ± 1.3	19.6 ± 2.0*
EMD96785	6	15.6 ± 1.4	20.4 ± 2.3*

[a] Data are 10^3 mmHg/min ± S.E.M.
* $P < 0.05$ vs. untreated.

4. Discussion

The results show that the NHE mediates an important Na^+ influx during ischemia, reflected in a reduced Na^+ overload when the NHE is inhibited. However, EIPA was less effective than the two more specific NHE-blockers, HOE642 and EMD96785, in the concentrations used. Although drugs were not administered during reperfusion, treated hearts showed a slower recovery of pH_i, indicating that the NHE is still (partly) inhibited at that time, probably due to the fact that a complete washout of the drug takes a few minutes. This idea is supported by the finding that EMD96785, which is reported to be the most potent blocker, showed the most pronounced delay. The NHE-inhibition upon reperfusion will reduce reversed Na^+/Ca^{2+}-exchange via decreased NHE-mediated Na^+ influx and, reportedly, via direct inhibition of the Na^+/Ca^{2+}-exchanger protein by the prolonged acidosis. The more pronounced delay in recovery of pH_i in the EMD96785 treated hearts corresponds to the better recovery of the EDP in that group, suggesting that higher concentrations of the two other blockers are required to achieve a similar recovery of the EDP.