Effect of Norepinephrine and Heart Rate on Intracellular Sodium Activity and Membrane Potential in Beating Guinea Pig Ventricular Muscle

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The effect of 3 μM norepinephrine (NE) on intracellular sodium activity (a\textsubscript{Na}\textsuperscript{i}) and resting membrane potential was studied by continuous intracellular recordings with a conventional and an ion-selective microelectrode. The electrodes were impaled simultaneously in small (diameter, 0.3 mm) superfused trabeculae of the beating guinea pig ventricle at 37°C. In the absence of NE, changes of the beating rate produced an increase of a\textsubscript{Na}\textsuperscript{i} by 1.5±0.17 mM (from 0 to 1 Hz) and 1.9±0.47 mM (from 0 to 2 Hz). In the presence of NE, there was a very small significant increase of a\textsubscript{Na}\textsuperscript{i} during constant stimulation (1 Hz) and at \([K^+]_o\) of 4.7 and 11.5 mM. After 7 minutes of exposure, a\textsubscript{Na}\textsuperscript{i} increased by 0.5±0.19 mM (mean±SEM, n=4) at \([K^+]_o\) of 4.7 mM and by 0.5±0.22 (n=6) at \([K^+]_o\) of 11.5 mM. Resting membrane potential became more positive by 1 mV at both levels of \([K^+]_o\). The effect of NE became also clearly manifest from the configurational changes of action potentials (profound increase in plateau height and duration). Stimulation of the Na\textsuperscript{+}-K\textsuperscript{+} pump by NE became manifest from the changes of resting membrane potential and a\textsubscript{Na}\textsuperscript{i} after abrupt cessation of stimulation. The magnitude and the rate of the decrease in a\textsubscript{Na}\textsuperscript{i} and the initial rate of hyperpolarization were significantly greater in the presence of NE than in its absence. Comparison of the effect of NE on the changes of a\textsubscript{Na}\textsuperscript{i} during constant rate and after the transition from stimulation to quiescence suggests that the increase of Na\textsuperscript{+} influx after administration of NE is counterbalanced by an increased rate of Na\textsuperscript{+}-K\textsuperscript{+} pumping in the beating guinea pig ventricle. (Circulation Research 1991;68:1482–1489)

In cardiac tissue, direct measurements of the effect of catecholamines on intracellular sodium activity (a\textsubscript{Na}\textsuperscript{i}) have been made in quiescent and active Purkinje fibers,\textsuperscript{1–5} in quiescent ventricular tissue,\textsuperscript{1} and in quiescent isolated myocytes.\textsuperscript{6} Most of these studies suggest that catecholamines stimulate the Na\textsuperscript{+}-K\textsuperscript{+} pump directly. In quiescent cardiac tissue and in active canine Purkinje fibers, this effect leads to a decrease of intracellular sodium.\textsuperscript{1,2,4,6}

The change from a quiescent to a beating state will affect transmembrane ionic fluxes and intracellular activities. Compared with quiescence, active ventricular muscle is exposed to rapid sodium influx during the action potential, which has been shown to change substantially the transmembrane sodium gradient.\textsuperscript{7–10} In addition, exchange processes that are linked to the sodium gradient (Na\textsuperscript{+}-Ca\textsuperscript{2+} and Na\textsuperscript{+}-H\textsuperscript{+} exchange) may also increase the intracellular Na\textsuperscript{+} load.\textsuperscript{9}

The present investigation was carried out to measure the effect of norepinephrine (NE) on a\textsubscript{Na}\textsuperscript{i} in beating ventricular muscle (physiological sodium load). Because catecholamines increase Ca\textsuperscript{2+} influx and efflux only in beating preparations (use dependence\textsuperscript{11,12}), the effect of NE on a\textsubscript{Na}\textsuperscript{i} is expected to depend on heart rate. Our results are in agreement with a direct stimulating effect of NE on Na\textsuperscript{+}-K\textsuperscript{+} pumping. However, the steady-state intracellular Na\textsuperscript{+} in fibers beating at 1 Hz changed very little. This suggests that, in beating myocardium, the effect of NE on Na\textsuperscript{+}-K\textsuperscript{+} pumping is synergistic to the increased Na\textsuperscript{+} influx via the Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange and Na\textsuperscript{+} channels in that it maintains normal intracellular Na\textsuperscript{+}.

Materials and Methods

Preparation and Solutions

Guinea pigs weighing 250–350 g were killed by a blow on the head. The heart was rapidly removed and

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placed in a preparation chamber. Small trabeculae (250 μm in diameter and 1 mm in length) were obtained from either ventricle. Use of larger preparations rendered stable, simultaneous impalments of two microelectrodes impossible in the presence of NE. The excised trabeculae were fixed with small insect pins on the silicon floor of a small tissue bath (volume, 0.67 ml). The preparation was excited at different rates by field stimulation (rectangular pulses of 2 msec, double threshold strength). Simultaneously active preparations were discarded. Temperature was kept at 36–37°C by heating the chamber with a Peltier element that was fixed to the bottom of the chamber. Temperature was constantly monitored throughout the experiment.

The preparation was allowed to equilibrate in the recording chamber for 1 hour. The composition of Tyrode’s solution was as follows (mM): Na+ 153.5, K+ 4.7, Ca2+ 1.3, Mg2+ 0.6, Cl− 136.5, HCO3− 25, H2PO4− 0.5, and glucose 20. In some experiments, the solution contained 11.5 mM K+ and Na+ was reduced by 6.8 mM. This small reduction in [Na+]o will cause a decrease of the [Na+]i, by approximately 5%,13 an effect that was neglected in our experiments. The solutions were equilibrated with a mixture of 95% O2−5% CO2 to obtain normoxic conditions at pH 7.4. The effect of catecholamines was studied by exposure of the preparation to NE in a concentration of 3 × 10−5 M. NE was added just before the test, and 5 × 10−5 M sodium EDTA was included in the Tyrode’s solution to prevent autoxidation. EDTA in this concentration did not exert any electrophysiological effect in preliminary experiments.

Measurement of Membrane Potential and Intracellular Sodium Activity

Conventional 3M KCl-filled glass microelectrodes, pulled from borosilicate glass (Clark Medical Instruments, Pangborne, England), were used to record transmembrane potentials. Tip resistances were 15–20 MΩ. a’Na was measured by microelectrodes filled with the neutral Na+ carrier ETH 227.14 Fabrication and calibration of these electrodes has been described in detail elsewhere.13 The DC resistances of the Na+-sensitive electrodes varied between 60 and 100 GΩ. Calibration curves were obtained before and after each experiment (at 34–36°C) using the calibration chamber described by Weingart and Hess.15 For calculation of a’Na, an activity coefficient of 0.764 was used. The calibration response of the Na+-sensitive electrodes was not sensitive to the addition of NE and/or EDTA.

Recording Technique

Each muscle was impaled by a conventional microelectrode and a Na+-sensitive microelectrode (inter-electrode distance <0.5 mm). The intracellular potential recorded by the conventional microelectrode (resting membrane potential [Vr]) and the intracellular potential recorded by the ion-selective microelectrode (VNa) were referred to the potential of a third microelectrode placed in the superfusing solution close to the impaling sites. The potential sensitive to intracellular sodium (VNa−Vr) was converted to α’Na using the individual calibration curve for each electrode. The selectivity of the neutral Na+ carrier ETH 227, with respect to K+ and Ca2+, has been discussed extensively in previous work from others16 and this laboratory.17 The small but significant changes of VNa−Vr observed in the present experiments (Figures 1 and 2) may have indeed been influenced by interference with other ions (see “Discussion”).

The ion-selective electrodes were connected to high-input impedance preamplifiers (Analog Devices 515), and the signals were amplified by a differential instrumentation amplifier. A pen recorder (mark VII, Watanabe) served for recording of Vr, VNa (before filtering), and VNa−Vr. The differential voltage VNa−Vr was obtained after filtering Vr, VNa and Vr by low-pass filters (corner frequency, 0.1 Hz) before subtraction.16,18,19

Homogeneity of the resting potential was tested in the following way: Each preparation was impaled at three different sites before the experiment started. Homogeneity of the preparation was regarded as sufficient if the maximal difference in resting potential between the three impaled sites did not exceed 1.5 mV. Experiments were only accepted and continued if the impalement of the reference and the ion-selective electrodes remained stable throughout the control and the test period (mechanical displacement of the sodium-sensitive electrode usually produced a small stepwise increase in a’Na). This strict criterion was necessary to monitor changes of VNa−Vr corresponding to a change of a’Na<1 mM. It limited markedly the number of experiments, because adding NE and/or changing heart rate was leading to an increase in developed tension associated with mechanical instability of the impalments in the majority of the experimental attempts.

Vr and a’Na during control superfusion were compared with the values obtained during the test period using the paired Student’s t test (all measurements during control and test conditions were performed during single stable impalments of the reference and the ion-selective microelectrodes).

Results

Effect of a Change in Heart Rate on a’Na and Membrane Potential in Absence of Norepinephrine

An abrupt change of heart rate is associated with a transient change in transmembrane potential and with a change of a’Na.8 Analysis of the time course and the magnitude of these changes can be used to study Na+-K+ pumping and its modification by extracellular K+ and intracellular Na+8,10,20 or NE. In preliminary experiments, the rate-related changes in a’Na and Vr were measured during superfusion with normal Tyrode’s solution. This was necessary to compare the
rate-related changes in \( a'_{\text{Na}} \) and \( V_M \) (and to estimate the change in Na\(^+\)-K\(^+\) pumping, Figure 5) after administration of NE. The effects of heart rate on \( a'_{\text{Na}} \) and \( V_M \) have been studied before in guinea pig ventricular muscle,\(^{21,22}\) in sheep Purkinje fibers,\(^8\) and in rat atrial muscle (in low Ca\(^{2+}\) bath medium).\(^{19}\)

The effect of sudden cessation of different stimulation rates on \( a'_{\text{Na}} \) and \( V_M \) in the absence of NE is shown in Figure 1, upper and middle panels, and Table 1. The results observed were similar to those described by Cohen et al\(^8\) for sheep Purkinje fibers and by Wang et al\(^{22}\) for guinea pig ventricular muscle. The initial rate of hyperpolarization (\(-dV_M/dt\)) depended on heart rate; it increased 3.4-fold from the transition from 0.5 to 0 Hz to the transition from 2 to 0 Hz. The time course of the change in \( a'_{\text{Na}} \) was similar at the different rate changes. The relative change in steady-state \( a'_{\text{Na}} \) is shown in Figure 1, lower panel, and Table 1. The transition from 0 to 2 Hz caused \( a'_{\text{Na}} \) to increase by \(-2 \text{mM},^{21,22}\)

**Effect of Norepinephrine on \( a'_{\text{Na}} \) and Membrane Potential at Constant Heart Rate**

The effect of NE on \( V_M \) and \( a'_{\text{Na}} \) in a single experiment is shown in Figure 2. All the muscle bundles were paced at 1 Hz. There was a considerable increase in the height of the plateau and in the duration of the action potential. Resting potential \( V_M \) shifted temporarily by 1.5 mV to a more positive value (steady-state change of +0.5 mV). \( a'_{\text{Na}} \) as derived from the change in \( V_{NaE} - V_M \) and the individual calibration curve of the ion-sensitive electrode, increased slightly from 4.6 to 5.0 mM. Figure 3 shows the mean changes in \( V_M \) and \( a'_{\text{Na}} \) after addition of 3 \( \mu \text{M} \) NE to the superfuse at two levels of \([K^+]_o\) 4.7 mM (\(n=6\)) and 11.5 mM (\(n=6\)). In all experiments, the presence of NE became clearly manifest from the configurational changes of action potentials (profound increase in plateau height and action potential amplitude). At both levels, it was possible to maintain five or six stable ion-selective electrode impalements for 6–8 minutes after the application of NE. \( a'_{\text{Na}} \) increased significantly by \(-0.5 \text{mM} \) after 6–8 minutes of exposure at both levels of \([K^+]_o\). The changes of mean \( a'_{\text{Na}} \) at 6 minutes (decrease at \([K^+]_o\) of 4.5 mM) and 8 minutes (increase at \([K^+]_o\) of 11.5 mM) after application of NE probably were due to

![Graph showing the effect of sudden cessation of stimulation at different rates on the change in resting membrane potential (\(\Delta V_M\), upper panel) and on the change of intracellular sodium activity (\(\Delta a'_{\text{Na}}\), middle panel) in control conditions. The average values of \(\Delta a'_{\text{Na}}\) and \(\Delta V_M\) are relative to the 5-minute poststimulation steady-state value. Transition from 0.5 to 0 Hz is indicated by open squares (mean±SEM; for \(\Delta V_M\), \(n=6\); for \(\Delta a'_{\text{Na}}\), \(n=5\)). Transition from 1 to 0 Hz is indicated by closed diamonds (for \(\Delta V_M\), \(n=21\); for \(\Delta a'_{\text{Na}}\), \(n=16\)), and transition from 2 to 0 Hz is indicated by closed squares (for \(\Delta V_M\) and \(\Delta a'_{\text{Na}}\), \(n=3\)). The differences in the number of experiments between the \(\Delta V_M\) and the \(\Delta a'_{\text{Na}}\) measurements are due to the fact that the impalements of ion-sensitive electrodes became unstable in some of the experiments. The lower panel shows the change (mean±SEM) in steady-state \(a'_{\text{Na}}\) after a change in stimulation rate (for 0–0.5 Hz, \(n=13\); for 0–1 Hz, \(n=29\); for 0–2 Hz, \(n=3\)).](http://circres.ahajournals.org/)

**Table 1. Effect of Transition From Activity (Different Rates) to Quiescence on Membrane Potential and Intracellular Sodium Activity**

<table>
<thead>
<tr>
<th>Transition</th>
<th>( V_{M\text{max}} ) (mV)</th>
<th>(-dV_M/dt) (mV/sec)</th>
<th>( a'_{\text{Na}}(C) ) (mM)</th>
<th>( a'_{\text{Na}}(PD) ) (mM)</th>
<th>( a'_{\text{Na}}(\text{max}) ) (mM)</th>
<th>( \tau ) (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>From 2 to 0 Hz</td>
<td>3</td>
<td>4.9±0.36</td>
<td>-0.15±0.033</td>
<td>8.3±1.4</td>
<td>6.0±1.1</td>
<td>-2.3±0.4</td>
</tr>
<tr>
<td>From 1 to 0 Hz</td>
<td>21</td>
<td>2.7±0.20</td>
<td>-0.10±0.009</td>
<td>6.7±0.4</td>
<td>4.8±0.5</td>
<td>-2.0±0.2</td>
</tr>
<tr>
<td>From 0.5 to 0 Hz</td>
<td>6</td>
<td>1.2±0.31</td>
<td>-0.04±0.004</td>
<td>5.4±0.7</td>
<td>4.8±0.8</td>
<td>-1.1±0.2</td>
</tr>
</tbody>
</table>

Values are mean±SEM. \( V_M \), resting membrane potential; \( a'_{\text{Na}} \), intracellular sodium activity; \( V_{M\text{max}} \), maximal postdrive hyperpolarization; \(-dV_M/dt\), initial rate of hyperpolarization after the frequency change (obtained by differentiating the initial portion of \( V_M \) during postdrive hyperpolarization); \( a'_{\text{Na}}(C) \), steady-state \( a'_{\text{Na}} \); \( a'_{\text{Na}}(PD) \), 5-minute postdrive \( a'_{\text{Na}} \); \( a'_{\text{Na}}(\text{max}) \), difference in \( a'_{\text{Na}} \) after the frequency change; \( \tau \), exponential time constant of decrease in \( a'_{\text{Na}} \) after the frequency change. For the differences in number of experiments between \(\Delta V_M\) and \(\Delta a'_{\text{Na}}\) measurements see legend to Figure 1.
FIGURE 2. Effect of norepinephrine (3 μM) in a single experiment on the action potential amplitude (panel A), resting membrane potential (V<sub>M</sub>, panel B), and intracellular sodium activity (a<sub>Na</sub>, panel C) at [K+]₀ of 4.7 mM. Two action potential tracings obtained at high speed with a pen recorder are superimposed on panel A. Two minutes after administration of norepinephrine, a marked increase in plateau height and duration of the action potential is observed. Panel B shows a temporary shift of the resting potential by 1.5 mV (the upper part of the pen recorder tracings obtained at low recording speed is cut off by hand). Panel C shows a slight increase (from 4.6 to 5.0 mM) throughout the 6-minute exposure period.

the interruption of two or three experiments, respectively (due to mechanical displacement of one of the electrodes). After washout of NE (6 minutes) a small nonsignificant decrease of a<sub>Na</sub>/mM was measured (−0.4 mM) at [K+]₀ of 4.5 mM, whereas a<sub>Na</sub>/mM had returned close to control at [K+]₀ of 11.5 mM. Resting membrane potential became more positive by ∼1 mV at both levels of [K+]₀ and returned to control level after removal of NE.

In one experiment, V<sub>M</sub> and a<sub>Na</sub> were measured on administration of NE in a quiescent fiber. In this experiment, no significant changes of V<sub>M</sub> and a<sub>Na</sub> were observed.

Effect of Norepinephrine on a<sub>Na</sub> and Membrane Potential With Changes in Heart Rate

Controversy has existed whether the stimulating effect of catecholamines on the Na⁻⁺-K⁺ pump is direct or secondary to changes in extracellular K⁺. Therefore, NE was added to our preparations at two different extracellular K⁺ levels (4.7 and 11.5 mM). The higher K⁺ level was introduced to minimize the interference of changes in [K⁺]. Combination of NE with an abrupt change in rate (from 1 to 0 Hz) made the continuous stable impalements of two microelectrodes particularly difficult. We succeeded in obtaining three successful experiments at each K⁺ level (stable continuous impalments during a change from 1 to 0 Hz in the absence and presence of NE). The original tracings of V<sub>M</sub> and a<sub>Na</sub> are shown in Figure 4 for [K⁺]. of 4.7 mM (upper tracings) and for [K⁺]. of 11.5 mM (lower tracings). At both K⁺ levels, measurements are shown in the presence and absence of NE (obtained during a single impalement of both electrodes). The data of all six experiments are summarized in Table 2. In essence, the effect of NE was the same at both levels of [K⁺]. The (exponential) time constant τ of the decrease in a<sub>Na</sub> was shortened markedly by NE. Moreover, the difference between the steady state a<sub>Na</sub> levels at 1 Hz and 0 Hz increased, with the exception

FIGURE 3. Graphs showing changes in intracellular sodium activity (Δa<sub>Na</sub>, upper panel) and resting membrane potential (ΔV<sub>M</sub>, lower panel) after exposure to norepinephrine (3 μM) in normoxic Tyrode’s solution at [K⁺]₀ of 4.7 mM (mean±SEM, filled circles) and at [K⁺]₀ of 11.5 mM (mean±SEM, filled squares). The preparations were continuously stimulated at 1 Hz. At higher [K⁺]₀, measurements were obtained in all six experiments up to 8 minutes, until the ion-sensitive electrodes were displaced in three experiments. At normal [K⁺]₀ (with stronger contractions than at [K⁺]₀ of 11.5 mM), unstable impalments (decreasing n) occurred earlier during the test period. *p<0.05 and †p<0.01 vs. control.
There is a large amount of evidence for a catecholamine-induced stimulation of the Na⁺-K⁺ pump in cardiac tissue and in various other excitable cells (for review see Phillis and Wu). Some controversy exists whether pump stimulation is a direct effect of catecholamine administration or occurs secondary to changes in extracellular K⁺. Several reports are in favor of a directly mediated effect: 1) Isoproterenol decreased intracellular Na⁺ in unstimulated isolated cardiac myocytes, where major extracellular K⁺ accumulation is unlikely. 2) At elevated extracellular K⁺ (when the Na⁺-K⁺ pump is saturated by K⁺), adrenergic stimulation still causes intracellular Na⁺ to decrease. In addition, a catecholamine-induced increase in pump current after rapid stimulation was still observed in high extracellular K⁺ and active transport of potassium was still stimulated. The present results, which were obtained in beating ventricular myocardium, are in agreement with a direct, stimulating effect of norepinephrine on Na⁺-K⁺ pumping. In all experiments (and at both levels of [K⁺]o) in which the effect of abrupt changes in heart rate was studied, the time constant of the changes in aNa and the changes of VM were consistently modified by NE. The smaller transient hyperpolarization after cessation of stimulation observed at 11.5 mM [K⁺], may have two causes: 1) The higher membrane conductance at elevated [K⁺]o will deliver less VM change for the same amount of pump current. 2) A given accumulation or depletion of [K⁺]o will have less effect on VM at elevated [K⁺].

In the present experiments, we observed a very small increase in steady-state aNa in ventricular fibers beating at 1 Hz. Whether this change per se, although statistically significant, represented a real increase in intracellular Na⁺ cannot be decided with certainty. This is because the Na⁺-sensitive resin has no absolute specificity and because changes of other ion species may contribute in part to these very small changes of Na⁺-sensitive voltage, which certainly represent the lower limit for detectable changes in aNa. However, our results exclude a decrease of aNa. They are in accordance with measurements of aNa in quiescent sheep Purkinje fibers but are in contrast with the results in quiescent dog Purkinje fibers or quiescent isolated rabbit myocytes, in which a decrease of aNa was measured after administration of NE. In the present study, aNa was not measured systematically at 0 Hz; therefore, it cannot be decided with certainty whether this discrepancy is due to species difference or to a rate-related effect. In a single control experiment, aNa did not decrease in a quiescent fiber; this finding suggests that species differences might be the most likely explanation. In addition, the metabolic state of the fiber should be taken into account, as discussed below.

A potential difference in the effect of NE on aNa between beating and resting muscle is related to the

Discussion

Stimulation of sympathetic receptors modifies cardiac function in a complex way. Contraction, heart rate, and impulse conduction are affected by catecholamines. Underlying mechanisms of these effects may include alterations of ionic currents, the activity of ionic pumps, and intercellular communication. Among the important electrophysiological effects of adrenergic agonists are 1) the modulation of Ca²⁺ channel kinetics resulting in an increase of intracellular Ca²⁺ (for review see Reuter), 2) the modulation of the Na⁺ channel function, 3) the increase in the hyperpolarization-activated inward current (iNa1 in Reference 3), and 4) the increase in K⁺ conductance (iXNa in Reference 27).

Figure 4. Original recordings of membrane potential (VM) and intracellular sodium activity (aNa) after sudden cessation of stimulation (1 Hz) in the absence (left side) and presence (right side) of 3 mM norepinephrine. Upper tracings of VM and aNa were obtained at [K⁺]o of 4.7 mM and lower tracings at [K⁺]o of 11.5 mM (stable continuous impalements at both K⁺ levels with >30 minutes of experimental time between the left and the right panels).

of one experiment at [K⁺]o of 11.5 mM. The rate of change of initial hyperpolarization (−dVM/dt) after the transition from 1 to 0 Hz was increased by NE. As expected, the magnitude −dVM/dt was smaller at the higher [K⁺]o in the presence and absence of NE (see “Discussion”) than at normal [K⁺]o. The time constant τ and decrease of τ after the administration of NE was similar at both levels of extracellular potassium.
TABLE 2.  Effect of Norepinephrine on Membrane Potential and Intracellular Sodium Activity During the Transition From Activity (1 Hz) to Quiescence at Two Different Levels of Extracellular Potassium

<table>
<thead>
<tr>
<th>Experiment</th>
<th>$V_{m(\text{max})}$ (mV)</th>
<th>$-dV_{m(\text{dt})}$ (mV/sec)</th>
<th>$a_{Na}^{\text{max}}$ (mM)</th>
<th>$a_{Na}(\text{max})$ (mM)</th>
<th>$\tau$ (sec)</th>
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<tbody>
<tr>
<td>[K$^+$]$_o$=4.7 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−NE</td>
<td>2.8</td>
<td>−0.08</td>
<td>6.2</td>
<td>−1.8</td>
<td>92</td>
</tr>
<tr>
<td>+NE</td>
<td>3.0</td>
<td>−0.12</td>
<td>6.4</td>
<td>−2.1</td>
<td>76</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−NE</td>
<td>3.0</td>
<td>−0.10</td>
<td>5.8</td>
<td>−1.0</td>
<td>109</td>
</tr>
<tr>
<td>+NE</td>
<td>4.0</td>
<td>−0.17</td>
<td>5.9</td>
<td>−1.9</td>
<td>87</td>
</tr>
<tr>
<td>3</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>−NE</td>
<td>2.5</td>
<td>−0.21</td>
<td>6.4</td>
<td>−1.3</td>
<td>82</td>
</tr>
<tr>
<td>+NE</td>
<td>4.5</td>
<td>−0.38</td>
<td>6.5</td>
<td>−1.7</td>
<td>51</td>
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<tr>
<td>Mean (±SEM)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>−NE</td>
<td>2.8 (0.14)</td>
<td>−0.13 (0.04)</td>
<td>6.1 (0.17)</td>
<td>−1.4 (0.24)</td>
<td>94 (7.8)</td>
</tr>
<tr>
<td>+NE</td>
<td>3.8 (0.44)</td>
<td>−0.22 (0.08)</td>
<td>6.3 (0.20)</td>
<td>−1.9 (0.12)</td>
<td>71 (10.4)</td>
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<td>[K$^+$]$_o$=11.5 mM</td>
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<td>1</td>
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<tr>
<td>−NE</td>
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<td>5.5</td>
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</tr>
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<td>4.1</td>
<td>−0.9</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
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</tr>
<tr>
<td>−NE</td>
<td>1.0</td>
<td>−0.04</td>
<td>3.7</td>
<td>−0.7</td>
<td>85</td>
</tr>
<tr>
<td>+NE</td>
<td>1.8</td>
<td>−0.14</td>
<td>4.2</td>
<td>−1.1</td>
<td>75</td>
</tr>
<tr>
<td>Mean (±SEM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−NE</td>
<td>1.3 (0.14)</td>
<td>−0.05 (0.01)</td>
<td>4.6 (0.53)</td>
<td>−0.8 (0.09)</td>
<td>95 (4.6)</td>
</tr>
<tr>
<td>+NE</td>
<td>1.9 (0.30)</td>
<td>−0.11 (0.02)</td>
<td>4.7 (0.57)</td>
<td>−1.1 (0.12)</td>
<td>60 (7.9)</td>
</tr>
</tbody>
</table>

$V_{m(\text{max})}$, maximal postdrive hyperpolarization; $-dV_{m(\text{dt})}$, initial rate of hyperpolarization at transition from 1 to 0 Hz; $a_{Na}^{\text{max}}$, intracellular sodium activity at 1 Hz; $a_{Na}(\text{max})$, difference in intracellular steady-state sodium activity after transition from 1 to 0 Hz; $\tau$, exponential time constant of decrease in intracellular sodium activity after transition from 1 to 0 Hz.

effect of NE on Na$^+$ influx. In resting ventricular fibers, the cellular Na$^+$ load consists of a background Na$^+$ current (including a steady-state component of the fast Na$^+$ inward current), and Na$^+$ entry through Na$^+$-Ca$^{2+}$ exchange is presumably small in the resting state. During activity, Na$^+$ influx through the fast Na$^+$ channel and through the Na$^+$-Ca$^{2+}$ exchange will contribute to an increased cellular Na$^+$ load (for discussion see Boyett et al). Even in quiescent isolated myocytes, in which $a_{Na}^{\text{max}}$ decreased after administration of NE, evidence for an increased Na$^+$ influx was found. It has been shown that the rapid Na$^+$ inward current is increased in the presence of isoproterenol, an effect that is probably mediated by cAMP-dependent phosphorylation of the Na$^+$ channel. In addition, a decrease in cellular Na$^+$ load may exist between Purkinje and ventricular cells: The Na$^+$ current $I_{Na}$, which substantially contributes to background $a_{Na}^{\text{max}}$ in Purkinje fibers, is not likely to exist in ventricular fibers.

An estimation of the effect of NE on pump activity can be obtained from the relation of Na$^+$ pump flux ($J_p$) versus $a_{Na}^{\text{max}}$. A calculation of this relation is shown in Figure 5. The underlying model assumes three independent, saturable internal binding sites for Na$^+$, when the $J_p$ is expressed in relative terms (maximal flux in absence of NE=100%), the shape of the curve depends solely on the binding constant for Na$^+$($K_{aNa}$) and the Hill coefficient (n). For the present calculation, $K_{aNa}=10.5\text{ mM}$ and $n=1.94$ were chosen. The fact that the effective n is <3 is probably explained by the interference of internal Na$^+$ binding with K$^+$. In this model, the activation of Na$^+$-K$^+$ pumping can be expressed by the shift of the relation between $J_p$ and $a_{Na}^{\text{max}}$. The calculation of this shift is based on the assumption that NE only affects the pump rate at saturation and leaves $K_{aNa}$ and n unchanged. In such a case, the change in the pump rate at saturation can be calculated from the change in n (Cohen et al, Equation 15). Inserting the values of n in the presence and absence of NE from Table 2 yields the upper curve in Figure 5. This curve corresponds to an approximately 1.3-fold increase in pump rate for a given $a_{Na}^{\text{max}}$. Fitting the value of $a_{Na}^{\text{max}}$ at 1 Hz (Table 2) and the $\Delta a_{Na}^{\text{max}}$ values at different beating rates from
Figure 5. Graph showing calculated relation between intracellular sodium activity (a_{Na}^-) and Na^+ -K^+ pumping in the presence and absence of norepinephrine. The Na^+ efflux (which equals the influx in steady state) is given in relative units. The calculation is based on the model of three internal binding sites for Na^+, a binding constant of 10.5 mM, and a Hill coefficient of 1.94 for internal Na^+. The lower curve indicates the absence of norepinephrine. The filled squares represent the data points obtained from the mean steady-state a_{Na}^- at 1 Hz (Table 2, upper panel) and the changes of a_{Na}^- (∆a_{Na}^-) at different rates obtained from Figure 1, lower panel. In this model, an increase of stimulation rate from 0 to 2 Hz leads to a 1.6-fold increase in Na^+ efflux or Na^+ influx. The upper curve indicates the presence of norepinephrine. The values for the filled circles correspond to the mean values of the three experiments in the upper part of Table 2 (a_{Na}^- and ∆a_{Na}^-). The curve corresponds to a 1.3-fold increase in pump rate for a given a_{Na}^- It is calculated by using the fact that the pump rate at saturation (v_{max}) is a function of the time constant τ (Cohen et al., Equation 15). At rest, norepinephrine causes no decrease of a_{Na}^- at 1 Hz, a_{Na}^- increases only slightly, because the increased Na^+ influx is compensated by the increased pump rate.

Figure 1, lower panel, to the lower curve shows that an increase in rate from 0 to 1 Hz produces a 1.5-fold increase of J_p and a rate change from 0 to 2 Hz, a 1.6-fold increase. Inserting the change of steady-state a_{Na}^- after administration of NE (Figure 3) and the change of a_{Na}^- after the transition from 1 to 0 Hz in presence of NE (Table 2) yields the position of the two points on the upper curve in Figure 5. Figure 5 illustrates, at least semiquantitatively, the importance of the stimulating effect of NE on the Na^+ -K^+ pump in beating muscle, where it counterbalances the increase of Na^+ influx and thus contributes to maintenance of a low intracellular Na^+ level. Theoretically, an eventual decrease or increase of a_{Na}^- at a given heart rate after administration of NE is very sensitive to the shape of the J_p-a_{Na}^- relation. This relation in turn is dynamic and depends, for example, on the interference of internal Na^+ binding with K^+ and on the energetic state of the cell. Thus, the fact that the phosphorylation potential might be influenced by the filling solution of a suction pipette (high [ATP], no ADP, no phosphate) in isolated cells or be different between isolated, quiescent cells and superfused, working cells may in part provide an explanation for the variability of the findings in different experimental settings.

The small depolarization accompanying the effect of NE has been observed before in sheep Purkinje fibers. Its mechanism is not clearly evident. Any type of catecholamine-activated inward movement of positive charge may contribute to this depolarization, such as the isoproterenol-activated Na^-dependent inward current or stimulation of countertransport and cotransport mechanisms involving Na^+ influx (Na^+-Ca^{2+} exchange, Na^+-H^{+} exchange).

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