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Adrenergic Stimulation of Na/K Pump Current in Adult Rat Cardiac Myocytes in Short-term Culture

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Abstract. Passive membrane properties, steady-state Na/K pump current (I_p) and modulation of I_p by adrenergic agonists were studied with patch-clamp techniques in adult rat ventricular myocytes that were freshly isolated or maintained in culture for 1–4 days. Freshly isolated (day 0) myocytes had a 1.7–1.8 times smaller specific membrane resistance compared with that of cells on any day in culture. From day 0 to 4 there was a progressive decrease in cell capacitance (−17.6 ± 0.8 pF/day) without a parallel decline in cell dimensions. The pump current density (1.55 pA/pF) was stable over the 0–4 days in culture. In rod-shaped myocytes norepinephrine (NE) and isoproterenol (ISO) stimulated I_p in a dose-dependent manner, with an apparent affinity of 36 ± 8 and 1.5 ± 0.4 nM, and maximum stimulation of 0.65 ± 0.02 and 0.57 ± 0.02 pA/pF, respectively. Nadolol suppressed this effect, suggesting that it was mediated by β-adrenergic receptor activation. An inverse relationship was found between steady-state I_p and the stimulation of I_p by NE. In contrast to what was shown in guinea pig cardiac myocytes, in rat myocytes isoproterenol stimulation of I_p was not increased by intracellular [Ca] and it did not change the I_p-membrane potential relation. These results show that in adult rat cardiac myocytes NE and ISO are potent stimulators of Na/K pump activity, and this effect may be studied using rat myocytes maintained in short-term culture.

Key words: Na/K pump — β-adrenergic receptor — Cell culture — Patch clamp — Adult rat cardiac myocyte — Norepinephrine — Isoproterenol

Introduction

Catecholamines increase heart rate and contractility. This well-known outcome of adrenergic receptor (AR) stimulation is based on coordinated modulation by catecholamines of a number of ion fluxes and metabolic processes in the heart (Hartzell, 1988). Ion transport mediated by the Na/K pump has been suggested to be one of the processes activated by norepinephrine (NE) through the β-adrenergic receptors (β-AR) (Hougen, Spicer & Smith, 1981; Wasserstrom, Schwartz & Fozzard, 1982). Those original experiments were done with multicellular preparations where the direct influence of NE on the Na/K pump could not be separated from the indirect effects of changes in ion concentrations in interstitial clefts and intracellularly (Gadsby, 1983; Desilets & Baumgarten, 1986). In isolated cardiac myocytes isoproterenol (ISO), a β-AR agonist, has been shown to stimulate the Na/K pump by some authors (Desilets & Baumgarten, 1986; Gao, Mathias, Cohen & Baldo, 1991; Stimers & Liu, 1994), but not by others (Glitsch et al., 1989; Bahinski & Gadsby, 1990; Bielen, Glitsch & Verdonck, 1991; Ishizuka & Berlin, 1993). Some reasons for this controversy may reside in the complexity of adrenergic regulation of Na/K pump, in particular the species dependence of this regulation or its dependence on intracellular Ca concentration ($[Ca]_i$) (Gao et al., 1991; Charpentier et al., 1996). Another possibility is that freshly isolated adult cardiac myocytes (cells in which most previous data were obtained) have not been systematically tested for their appropriateness as a model for studying adrenergic regulation of the Na/K pump. For example, freshly isolated myocytes may have been injured by the isolation procedure and so might not be stable with respect to intracellular mechanisms linking adrenergic receptors to the Na/K pump. If this is true, then some recovery of the mechanism could occur in cardiac cells maintained in culture; however, activity of the Na/K pump and its stimulation by adrenergic agonists in freshly isolated and cultured adult myocytes has not been previously compared.

In this study we have investigated the possibility of

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using rat cardiac myocytes in short-term culture as a model system for investigations on the Na/K pump and its regulation by adrenergic agonists. Data are presented showing that rat myocytes in short-term culture are suitable for patch clamp studies of Na/K pump function and adrenergic regulation. Additional evidence is presented showing that the mechanism for adrenergic regulation of the Na/K pump in rat appears to be qualitatively different from that reported for guinea pig ventricular myocytes. Some of these results have been presented in abstract form (Dobretsov & Stimers, 1996; Stimers & Dobretsov, 1996).

Materials and Methods

Cell Isolation and Culture

Ventricular myocytes were isolated from adult male Sprague-Dawley rats (250–350 g) by methods previously described (Stimers, 1992) and modified according to procedures of Isenberg & Klockner (1982). The animal treatment conformed with the ''Guide for the Care and Use of Laboratory Animals'' (National Institutes of Health Publication No. 85-23, revised 1985). The buffered salt solution (BSS) used was a phosphate/bicarbonate buffered solution that contained (in mM): 110 NaCl, 3.8 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25 $NaHCO_3$, 11 glucose, 0.2 $CaCl_2$ equilibrated with 95% O_2/5% CO_2 (pH = 7.4). Hearts were removed from rats and perfused at 37°C via the aorta on a Langendorff apparatus with solutions as follows: (i) BSS for 5 min to clear blood from the heart; (ii) nominally Ca-free BSS for 5 min; (iii) 0.05% collagenase (type II, Worthington, Freehold, NJ) in Ca-free BSS for 10–20 min. The ventricles were then minced, washed twice briefly in BSS containing 0.2 mM $CaCl_2$ and 0.4% bovine serum albumin (BSA, fraction V). Minced pieces of cardiac tissue were then placed in a 50-ml centrifuge tube containing 10 ml of the same solution without the BSA. The tube was then put into a shaking water bath for 10 min. The supernatant was then aspirated off the tissue pieces and replaced with an equal volume of fresh solution. The shaking was repeated five times. The supernatant collected from the second through fifth shaking periods was set aside to allow the isolated myocytes to settle to the tube bottom. The solution was aspirated and replaced with ice-cold ''KB-solution'' containing (in mM): 85 KCl, 5 N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), 5 tris-hydroxymethyl-aminomethane (TRIS), 25 KH_2PO_4, 0.5 ethyleneglicol-bis-N,N,N′,N′-tetraacetic acid (EGTA), 5 $MgSO_4$, 5 K-pyruvate, 5 Na_2-creatine phosphate, 20 taurine, 0.1 $CaCl_2$, 20 dextrose (*see* Isenberg & Klockner, 1982) and allowed to stand for 30 min. Myocytes were then placed into 60-mm tissue culture dishes (Falcon 3002, Becton Dickinson, Lincoln Park, NJ) with 3 ml per dish of either KB solution or culture medium composed of: 60% M199 (Gibco, Grand Island, NY), 4% fetal bovine serum (Gibco) and a balance of the following (in mM): 116.4 NaCl, 4.7 KCl, 0.94 NaH_2PO_4, 0.83 $MgSO_4$, 25 $NaHCO_3$, 5.55 glucose. Dishes with KB solution were refrigerated and used the same day in experiments (day 0 cells). Cells in culture medium were maintained in an incubator at 36°C under 5% CO_2/95% air for 1–4 days until used for experiments.

Solutions

The techniques used for isolating and measuring I_p were similar to those previously described (Stimers, Liu & Kinard, 1993). Extracellular solutions contained (in mM): 145 NaCl; 5.4 KCl; 1.0 $MgCl_2$; 2.0 $BaCl_2$; 0.2 $CdCl_2$; 10 HEPES; 5.5 dextrose (pH = 7.4), and intracellular solutions (patch pipette) contained: 50 Na; 100 Cs; 40 Cl; 120 aspartate; 0.1 Ca; 10 Mg; 1.1 EGTA; 10 HEPES; 5 ATP; 0.5 GTP; 10 dextrose (pH = 7.2–7.3). In some experiments N-methyl-D-glucamine (NMG) or K were used to replace Cs as the major intracellular cation. Free-Ca concentration in this solution was ≤20 nM as calculated with ''Calcium'' a program in BASIC (Chang, Hsieh & Dawson, 1988). In some experiments we also have used internal solutions with estimated free [Ca] of 100, 500 and 1000 nM buffered with 1.1 mM EGTA or 20 nM buffered with 11 mM EGTA. Osmolality of all solutions was in the range of 290–310 mOsm. Ca was omitted from and Cd and Ba were added to external solutions in order to suppress K and Ca currents, and Na/Ca exchange. K was omitted from and Na was raised up to 50 mM and ATP added to internal solutions in order to suppress K currents and activate the Na/K pump in the forward mode. Na currents were suppressed by voltage clamping myocytes at a membrane potential of −40 mV.

Patch Clamp

Myocytes were patch clamped in the whole-cell mode. Tissue culture dishes containing myocytes were placed on the stage of an inverted microscope (Diaphot, Nikon) and perfused with prewarmed solutions (31–33°C) at a rate of 5 ml/min. When switching solutions there was a lag of less than 1 sec or 5–7 sec depending on the system used. In either case, once the solution entered the culture dish it quickly (<10 sec) engulfed the myocyte from which currents were being recorded, since the inlet tubing was placed close to and directed at that myocyte. Fire-polished patch electrodes (Corning 7052) had tip diameters in the range of 1–4 μm and resistance of 1–2 MΩ when filled with Cs aspartate intracellular solution (mean = 1.4 ± 0.02 MΩ, n = 167). Voltage commands were generated and data recorded using PClamp software and Axolab 1100 computer acquisition system (Axon Instruments, Burlingame, CA). Holding current (I_h; at holding potential of −40 mV) was continuously recorded on a chart recorder. Using conventional techniques (Lindau & Neher, 1988) series resistance (R_s) and membrane capacitance (C_m) were estimated from the peak amplitude and time constant of the decay of the average of 20 current transients elicited by a −10 mV pulse from the holding potential of −40 mV for 10 msec. Time constant and peak capacitive current were determined by fitting a single exponential function to the decay of the OFF current transient (from 90% to 10% of amplitude) and extrapolation of the fitted curve back to the first sample point after the voltage step. The change in steady-state I_h caused by these hyperpolarizing voltage steps was used to estimate myocyte input resistance (R_{in}). These measurements were repeated several times in every experiment to ensure the stability of the leak, electrical and diffusional access to the cell interior and to ensure that recorded currents were correctly normalized to C_m at any time point of an experiment. Records of capacitive transients were filtered at 5 kHz and digitized at a sampling rate of 10 kHz. Chart records of I_h were digitized after experiments using Un-Scan-It software (Silk Scientific, Orem, UT). To measure the voltage dependence of I_p a voltage-ramp protocol going from +60 to −100 mV at 40 mV/s was used in some experiments. This protocol was applied to every cell at least three times: under control conditions (#1), during the activation of I_p by NE (#2) and after the Na/K pump was blocked with ouabain (#3). The I_p values over the range of V_m of the ramp protocol were then obtained by point by point subtraction of currents elicited by voltage ramp #3 and those elicited by ramps #1 or #2. There was no R_s compensation applied in these experiments, because the R_s values were typically small (4.3 ± 0.1 MΩ, 164 cells), the input resistance was relatively high (310 ± 10 MΩ, 164 cells), and the I_h did not exceed 500

pA. We have previously published experiments showing that the error in a voltage command (−100–40 mV) under these conditions is insignificant (about 1.5%) (Dobretsov & Stimers, 1997).

Materials

Unless otherwise indicated all compounds used in this study were purchased from Sigma Chemical (St. Louis, MO). Norepinephrine (DL-Noradrenaline) was obtained from Fluka (Switzerland). Stock solutions of 10 mM NE, ISO and nadolol were prepared daily in Milli-Q UF Plus water (Millipore, Bedford, MA) and kept refrigerated before experiments. Stock solution of 1 mM prazosin HCl contained 85% of 0.1 N HCl and 15% methyl alcohol. The final V/V concentration of methyl alcohol used in experiments with 10 μM prazosin was 0.15%, which did not influence the I_p in rat ventricular myocytes in control experiments.

Data Analysis

Data were collected from 18 separate cell cultures. In 13 cultures myocytes were studied on several (at least 2 and up to 5) different days after isolation. Data were excluded from analysis when any of the following occurred: (i) seal resistance, membrane resistance or series resistance spontaneously and irreversibly changed; or (ii) there was a rapid, irreversible rundown of I_p. Data were subjected to statistical analysis using a *t*-test and nonlinear least-squares regression using Origin (MicroCal, Northampton, MA). NE and ISO dose dependencies of I_p stimulation were fit by the Michaelis-Menten equation:

$$I_p = (I_{max} \cdot [X])/(K_{0.5} + [X]), \quad (1)$$

where I_{max} is the maximum effect, $K_{0.5}$ is the apparent affinity and [X] is the concentration of NE or ISO. I_{max} and $K_{0.5}$ were allowed to vary to obtain the best fit to the data. Average values are expressed as mean ± SEM. Significant differences were defined as having a *P* value less than 0.05.

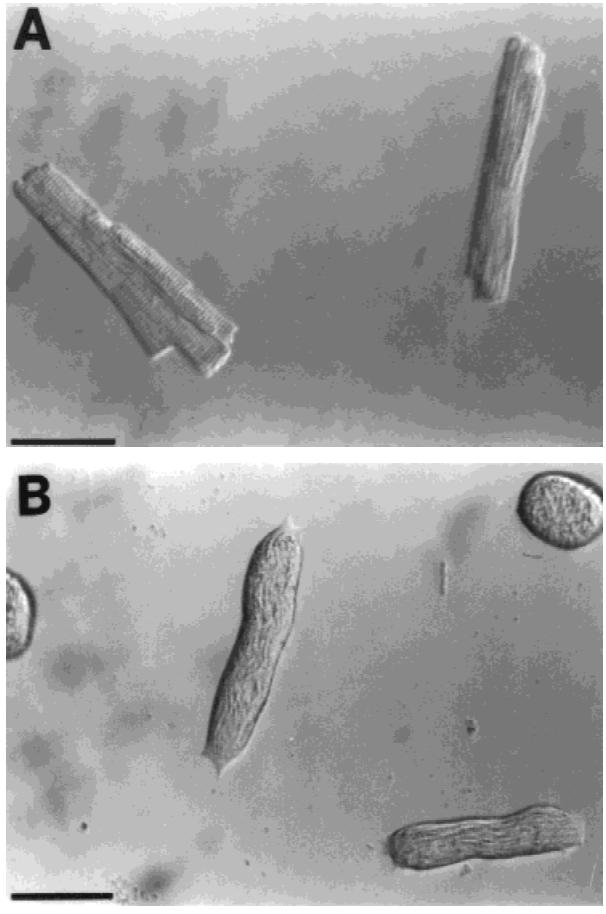

Fig. 1. Morphological appearance of freshly isolated (*A*) and cultured (*B*) myocytes. Adult rat ventricular myocytes 1 hr after isolation (*A*) and after 3 days in culture (*B*). Rounded cells in *B* are cardiac myocytes that have undergone dedifferentiation. Scale bar = 50 μM.

Results

Morphological Characteristics

Freshly isolated adult rat cardiac myocytes (also referred to as day 0 cells) and myocytes from short-term cultures were studied in these experiments. As in other serum-supplemented cultures of adult cardiac cells (*see* Piper, Volz & Schwartz, 1990) myocytes in our cultures were morphologically ''dedifferentiating'' with time, becoming rounded, flattened over the substratum, and losing striations. Myocytes that had an ''adult'' morphological appearance (rodlike shape and visible striations; Fig. 1) comprised about 80% of myocytes on day 0 (with rounded or visibly unhealthy cells comprising the rest of the cell population) and up to 40–50% of myocytes in day 4 cultures. Compared to freshly isolated cells (Fig. 1*A*) cultured myocytes with adult appearance had smoother surfaces and edges; some of these myocytes developed lammeliopodia and irregularity in the pattern of striation (Fig. 1*B*). After 6–7 days all cultured myocytes were dedifferentiated (myocytes at this time could be distinguished from fibroblasts and other contaminating cells by the presence of two nuclei and spontaneous contractions).

Data presented below provide characterization of only rod-shaped freshly isolated or cultured myocytes (cells in which adrenergic activation of I_p was observed consistently from culture to culture and on different days in culture). In several experiments with dedifferentiated cardiac myocytes we found that NE or ISO (1–50 μM) activated I_p in only a fraction of the cells tested (3 out of 5 dedifferentiated myocytes studied on day 4 in culture) or did not influence I_p at all (5 cells, days 7–10; *data not shown*). If dedifferentiation of myocytes leads to uncoupling of adrenergic receptors from the Na/K pump, then future studies of these cells may help to understand the signal transduction mechanisms involved with adrenergic regulation of the pump in rat ventricular myocytes. In the present investigation, however, we are considering only rod-shaped myocytes with adrenergic receptor coupling to the Na/K pump preserved.

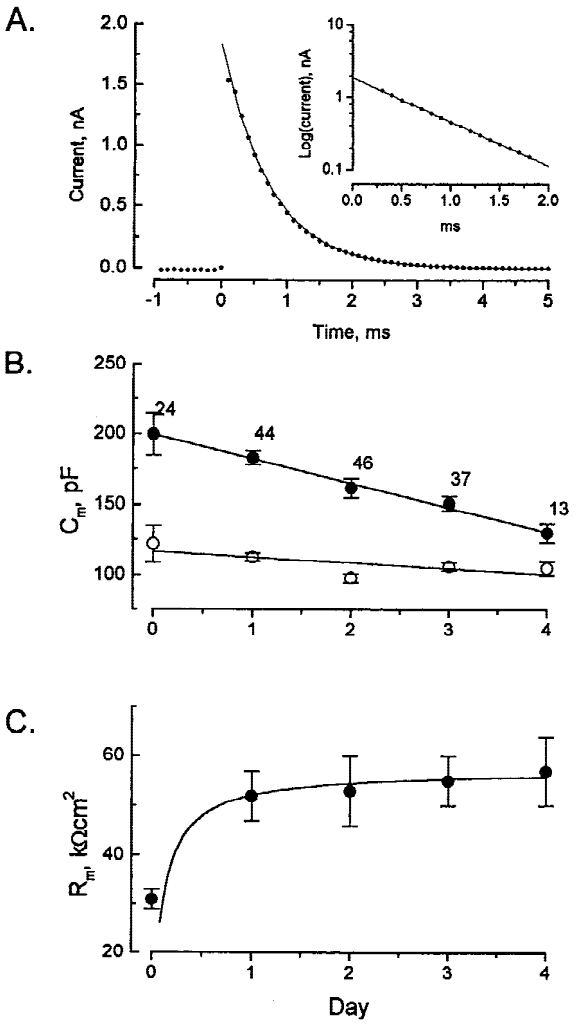

Fig. 2. Passive properties of freshly isolated and cultured rod-shaped myocytes. (*A*) Typical record of a capacity transient recorded on turning off a 10 mV hyperpolarizing pulse. This transient is the average of 20 consecutive pulses recorded at a sampling speed of 0.1 msec/point (10 kHz). Inset shows the same data plotted on a semilogarithmic scale. The transient current was fit by a single exponential function (solid line) with a time constant of 0.72 msec. Analysis of these data estimates R_s of 5.5 MΩ, C_m of 132 pF, and R_m of 76.6 $k\Omega \cdot cm^2$ for this cell. (*B*) C_m measured (●) and estimated from length and width (○) of freshly isolated cells or cells maintained in short-term culture for 1–4 days. Symbols represent average data collected from at least 3 independent cultures for each time period (number of cells studied are shown on the plot). Lines are linear regressions of the data and have slopes of −17.6 ± 0.8 pF/day (●; $P < 0.05$) and −3.6 ± 1.9 pF/day (○). (*C*) Average R_m calculated for the same cells shown in (*B*). R_m values were calculated using the measurements of R_{in} and C_m. Curved line was drawn by eye.

Passive Membrane Properties

Figure 2*A* illustrates how C_m and R_m were determined for each cell. Shown is the off transient elicited by a 10-mV hyperpolarizing pulse. From these data a single exponential function was fit to the decay phase between 90 and 10% of the maximum amplitude of the transient (solid line). The inset in this figure shows the same data on a semilogarithmic scale to indicate that the data are well fit by a straight line, confirming that there was a single exponential decay of the current transient. From the fit we obtain estimates for R_s, R_{in} and C_m as described in Methods. Rod-shaped myocytes in culture were found to have a progressively declining C_m (Fig. 2*B*, filled circles). While the average C_m for freshly isolated cells was 200 ± 15 pF ($n = 24$), cells studied on day 4 had a significantly reduced C_m of 130 ± 7 pF or about 65% of that on day 0 ($n = 13$; Fig. 2*B*). This reduction in C_m occurred without a corresponding change in visual measurements of myocyte length and width. The average length of myocytes cultured for 4 days was about 10% less than that of cells on day 0 (115 ± 5 μm and 131 ± 3 μm, 13 and 24 cells, respectively) and the cell width was not different (26 ± 1 μm in both groups). To compare measured myocyte C_m and cell dimensions we used length and width measurements to calculate cell surface area (assuming a cylindrical shape; Claycomb & Palazzo, 1980), and then converted it to capacitance assuming 1 μF/cm^2 (Fig. 2*B*, open circles). Comparison of changes in these values (measured and calculated C_m) over time in culture suggests that some folding of the myocyte surface membrane, such as the T-tubular system, may be decreasing with time in culture. This finding could be an advantage for patch-clamp studies by reducing geometrical complexity of the cell.

Cultured myocytes had progressively increasing R_{in} (*data not shown*). The mean value of R_{in} in freshly isolated cells was 170 ± 13 MΩ ($n = 24$) and on day 4 in culture it was 437 ± 49 MΩ ($n = 13$). This increase in R_{in} could be due to the above described progressive decrease in the cell surface area. Therefore, using the measured C_m and 1 μF/cm^2 as a value of specific C_m, we have converted R_{in} to specific membrane resistance (R_m, Fig. 2*C*), a parameter that unlike R_{in} does not depend on cell surface area. The R_m calculated for cells on day 0 was about 60% of that in cultured myocytes (31 ± 2 $k\Omega \cdot cm^2$ in 24 cells on day 0 and 52 ± 5 $k\Omega \cdot cm^2$ in 44 cells on day 1, significantly different). There were no statistically significant changes in R_m between cells on any of the days (1–4) in culture (Fig. 2*C*).

Protocol and Control Experiments

The raw-record in Fig. 3*A* illustrates the protocol used in most of our experiments to measure I_p and the changes caused by NE. Unless otherwise stated pipette solutions contained 50 mM Na and V_h was clamped at −40 mV. After the cell membrane was ruptured (time 0 in all figures) and whole-cell configuration established, it typically took 3–5 min for I_h to reach a steady-state (Fig. 3*A*). Then myocytes were exposed sequentially to the following solutions: (i) 1 mM ouabain solution to briefly block I_p; (ii) NE was applied and washed out; and (iii) ouabain containing solution was reapplied. Ouabain, a specific

inhibitor of the Na/K pump, was used in these experiments to identify the Na/K pump current (I_p). The value of I_p was calculated by subtraction of the steady-state level of I_h recorded when the Na/K pump was active and the level of I_h during the Na/K pump blockade (arrow labeled I_p in Fig. 3*A*). The effect of NE on I_p was estimated by measuring the peak change of I_h caused by its application (arrow labeled I_{NE} in Fig. 3*A*). This measurement will be discussed in more detail below.

In adult rat myocytes there are known to exist two isoforms of the α subunit (α_1 and α_2) of the Na/K pump that have different affinities for ouabain (K_d of 50–100 μM and 30–300 nM, respectively (Sweadner, 1989). The use of 1 mM ouabain to measure I_p in this study characterized the combined activity of both isoforms of the Na/K pump. Given this large difference in affinity it would be expected that the reactivation of I_p following washout of ouabain might proceed along a bi-exponential time course. However, previous studies suggest that the high affinity α_2 isoform comprises only about 10% of the total Na/K pumps in adult cardiac cells (Sweadner & Farshi, 1987; Lucchesi & Sweadner, 1991; Dobretsov & Stimers, 1997). Given this small contribution of α_2 to the total current it would be difficult to record a second component to the reactivation currents and so no attempt was made at kinetic analysis of current trace following washout of ouabain.

As in previous studies, control experiments verified that the ouabain-sensitive change in I_h was I_p by showing that there was no ouabain-sensitive current when K_o was 0 or when all Na was removed intracellularly and extracellularly (Stimers et al., 1993; Dobretsov & Stimers, 1997). Also, control experiments were done to test if under the conditions of these experiments NE activated currents other than I_p that could interfere with I_p analysis. Figure 3*B* shows that when ouabain was applied simultaneously with NE removal, so that the Na/K pump was still stimulated by NE, the level of I_h was identical to that obtained by ouabain inhibition without NE stimulation. This shows that the current activated by NE was ouabain sensitive. Conversely, when ouabain was applied first, NE application in the presence of ouabain had no effect on I_h (Fig. 3*C*). Also when experiments were done with Na-free solutions to inhibit I_p, NE had no effect on I_h (*data not shown*). An additional control is described below (*see* Fig. 4*B*). These results confirm that under the conditions of our experiments NE did not stimulate or inhibit any currents other than I_p.

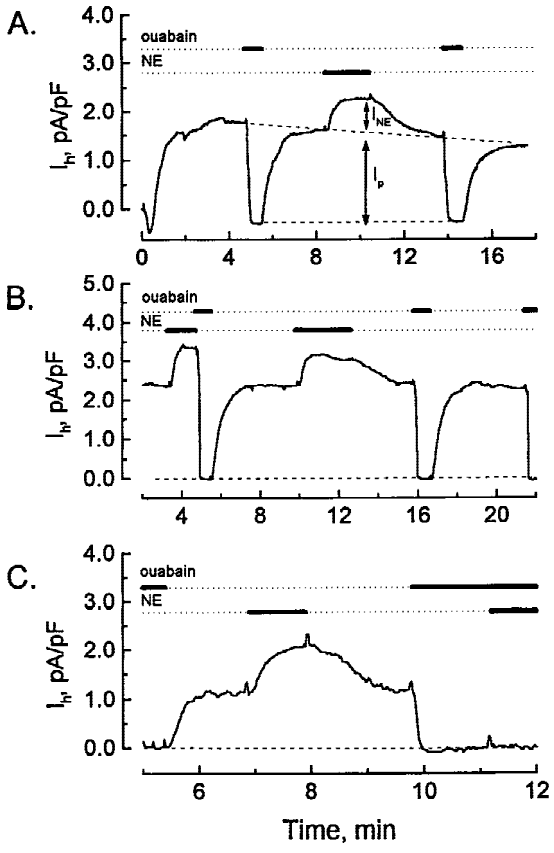

Fig. 3. Protocol for measuring I_p and norepinephrine influence. Records of I_h made using pipettes loaded with 50 mM Na and 5 mM ATP Cs-aspartate solution in rod-shaped myocytes from 1–3 day cultures. In each trace time 0 was set as the time of rupture of the membrane patch to establish the whole cell configuration. (*A*) Panel *A* illustrates that the I_p amplitude was measured as the difference between I_h levels recorded during and between periods of the Na/K pump blockade (dotted lines and double arrow labeled I_p). The NE or ISO response was measured as the maximum increase in I_h above the level of I_p. (*B*) In this cell, the protocol was modified so that ouabain was applied during the maximum response to NE applied initially followed by the standard protocol as in (*A*). This shows that whether or not the myocyte was stimulated with NE, inhibition of I_p brought I_h to the same level. (*C*) In this myocyte the standard protocol was followed by an application of NE in the continued presence of ouabain. This shows that if I_p is inhibited, NE has no effect on I_h under these conditions. In all panels, currents were normalized to cell C_m (150, 186 and 110 pF for cells in *A, B* and *C*, respectively) to express I_p as current density. Bars above each trace labeled NE or ouabain indicate the time of application of NE (1 μM in *A*, 10 μM in *B* and *C*) and 1 mM ouabain. Dotted lines connecting the heavy bars are only a guide.

Steady-State Na/K Pump Current

In contrast to C_m and R_m, the steady-state I_p was relatively stable in myocytes studied on different days in culture. The mean I_p value calculated for all studied cells was 1.55 ± 0.03 pA/pF (n = 163; solid line in Fig. 4*A*). Data show a small decrease in I_p on day 1 (10% relative to the mean for all cells, $P < 0.05$). Differences between the average and I_p measured on days 0, 2, 3 and 4 in culture were not statistically significant.

Activation of I_p by Norepinephrine

Examples of individual rod-shaped myocyte responses to application of NE are shown in Figs. 3*A* (1 μM), *B* and *C*

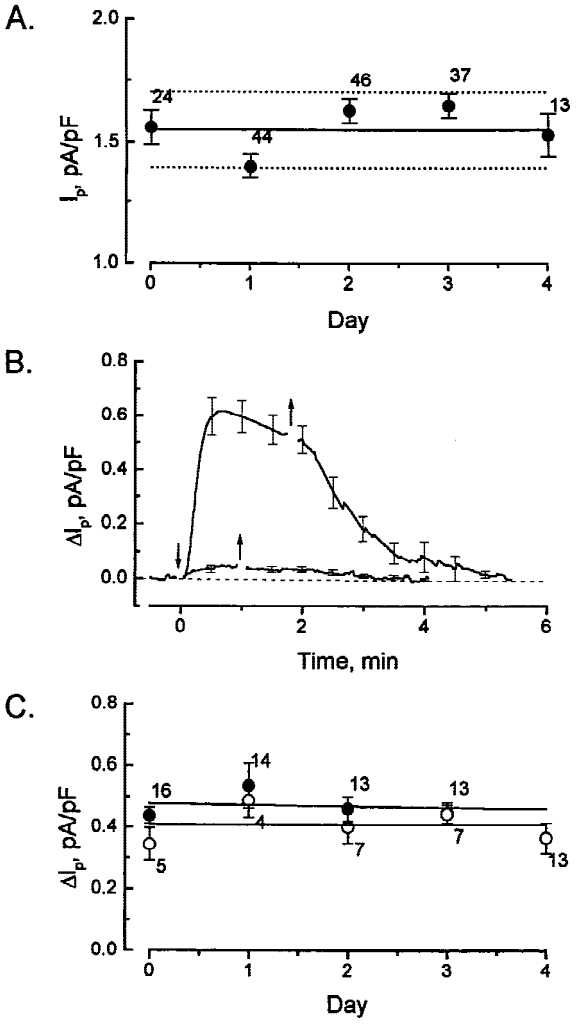

Fig. 4. Na/K pump current and its stimulation by NE over time in culture. (*A*) I_p was measured in cultured myocytes on different days after isolation. Each symbol represents the average of data collected in at least 3 different cultures (number of cells studied is shown next to symbols). Solid line represents the average I_p calculated for all studied cells (1.55 ± 0.03 pA/pF; $n = 164$). Dotted lines show ± 10% of average limits. (*B*) Upper trace represents the average response of I_p to application of 1–50 μM NE for 2 min (12 myocytes) recorded under standard conditions ($[Na]_{pip}$ = 50 mM; time of application indicated by arrows). The lower trace is the average of 3 control records made with addition of 10 mM vanadate to the standard pipette solution to block the Na/K pump. All traces were offset to 0 pA/pF by subtracting the average value of I_h for the 10 sec just prior to application of NE in each cell which was set to time 0. (*C*) I_p measured at the time of its maximum response to 50 μM NE (●) or 10 μM NE (○) and normalized to steady state I_p. Number of myocytes studied on each day is shown in the plot. Regression lines with slopes of −0.01 ± 0.02 pA/pF/day (50 μM NE) and 0.00 ± 0.02 pA/pF/day (10 μM NE) were fit to the data. Note that on day 3 the symbols overlap and there is no point for 50 μM NE on day 4.

(10 μM) and 5 (up to 1 μm). In all rod-shaped myocytes studied, NE consistently evoked a rapid, outward increase in I_h, typically reaching a maximum in 30–60 sec. Following removal of NE, I_h returned, with some delay, to the pre-drug level. Figure 4*B* shows the average response of I_h to saturating doses (1–50 μM) of NE where the drug was applied for about 2 min (upper trace). Most studied myocytes (>90%) responded to NE with a relatively steady increase of I_h; however, in a few cells the effect of NE was transient, decaying after the peak to almost pre-drug level within 1–2 min (*not shown*). The nature of these transient responses was not studied in the present investigation. However, if this declining response to NE effected our measurement of the maximum effect of NE on I_p by blunting the observed maximum response, then we would expect to see an inverse correlation between the maximum response and the rate of decline in the NE response. No relationship was found between the decay rate and the peak amplitude of the current responses to NE or ISO (correlation coefficient = −0.1; $P > 0.05$). Therefore the peak amplitude of I_p change was used to characterize the adrenergic activation of I_p in this study.

As discussed above, these experiments were conducted under conditions that suppressed all membrane currents other than I_p; thus changes in recorded I_h should directly correspond to changes in I_p. Nevertheless, control experiments were done to test if NE activated currents other than I_p. The lower trace in Fig. 4*B* illustrates the average of three records made under conditions that suppressed >95% of I_p by 10 mM vanadate in the patch pipette ($[Na]_{pip}$ = 50 mM). In these experiments 10 μM NE caused virtually no change in I_h. Similarly, NE did not affect I_h in experiments that suppressed I_p by omitting Na from the pipette solution by K-free or ouabain containing extracellular solutions (3, 2 and 3 experiments, respectively).

To determine if the influence of NE on I_p depends on time in culture we measured the effect of saturating NE concentrations (10 and 50 μM) on myocytes in culture for 0–4 days. Figure 4*C* summarizes these data and shows that there was no significant change in the magnitude of the NE-induced increase in I_p for either 50 μM NE (filled circles) or 10 μM NE (open circles). For all days 50 μM NE stimulated I_p on average 1.43-fold. Because we found no evidence for any time-dependent changes in NE stimulation of I_p, in subsequent figures and in the discussion, data from all days (0–4) have been pooled.

To address the physiological relevance of these results, experiments were performed with pipette solutions containing a more closely physiological composition with 10 mM Na and 140 mM K. In these experiments the steady state I_p was much smaller as expected (0.27 ± 0.05 pA/pF, $n = 6$) compared to the above results with 50 mM Na_i; however, there was still substantial stimulation of ouabain-sensitive I_p by 10 μM NE by 0.16 ± 0.08 pA/pF ($n = 6$). Thus under physiological activation of the Na/K pump, NE stimulates I_p by 59%.

Comparison of Norepinephrine and Isoproterenol Effects on I_p

It has been suggested that adrenergic activation of the Na/K pump in cardiac myocytes is mediated by β-AR (Desilets & Baumgarten, 1986; Hougen et al., 1981; Wasserstrom et al., 1982). Furthermore, in previous studies on AR regulation of the Na/K pump in cardiac myocytes, the β-AR agonist, ISO, was used to selectively stimulate those receptors (Desilets & Baumgarten, 1986; Glitsch et al., 1989; Bahinski & Gadsby, 1990; Bielen et al., 1991; Gao et al., 1991; Ishizuka & Berlin, 1993). Therefore, to facilitate comparison of our results to those of previous reports, the effect of NE was compared with that of ISO. This was done in experiments where various concentrations of each drug (1 nM–50 μM) were applied to myocytes either singly or sequentially. Figure 5 shows records with sequential application of four doses of NE and ISO (Fig. 5*A* and *B*, respectively). Data from this type of experiment along with data from experiments where only a single dose of the drug (1, 10 or 50 μM) was applied to each cell were combined and fitted with Eq. 1. Results from these experiments are summarized in Fig. 5*C* where the data have been normalized to the maximum response for each drug. Saturating concentrations (above 1 μM) of NE and ISO stimulated I_p by 0.65 ± 0.02 and 0.57 ± 0.02 pA/pF, respectively. The stimulation of I_p was found to be more sensitive to ISO than to NE. The apparent affinity for NE obtained from this fit was 36 ± 8 nM while that for ISO was 1.5 ± 0.4 nM. The similarity of the I_p stimulation caused by saturating concentrations of NE and the selective β-AR agonist ISO suggests that NE is activating I_p via the β-AR. In support of this suggestion we have found that 10 μM nadolol, a selective β-AR antagonist, completely suppressed the effects of 10 nM − 1 μM NE (Fig. 5*C*). In accord with this, the α-AR selective antagonist, prazosin (10 μM) tested in 2 cells did not influence the effect of 10 nM and 100 nM NE on I_p (*data not shown*).

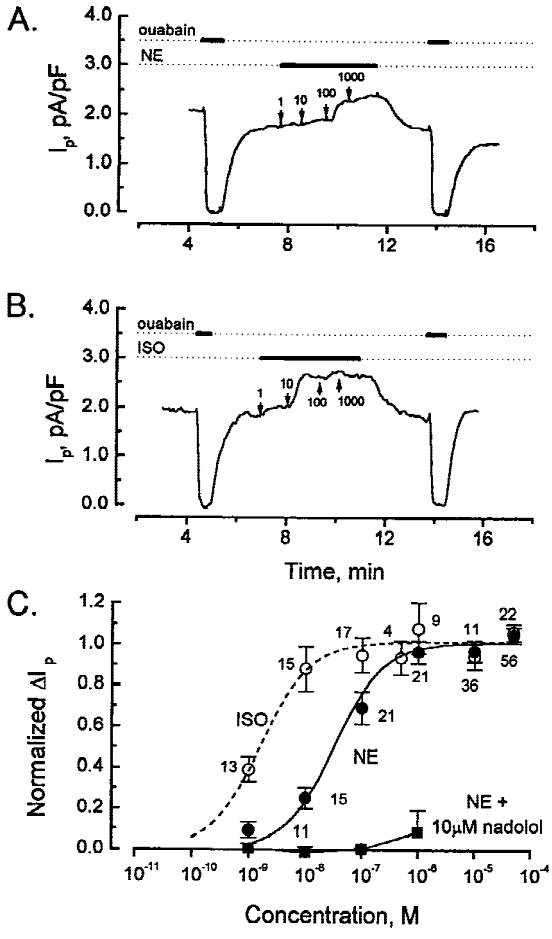

Fig. 5. Dose dependence of activation of I_p by NE and ISO. (*A* and *B*) Records of I_h from cells sequentially exposed to 1–1000 nM NE (*A*) or ISO (*B*). Bars above the traces indicate the times of application of 1 mM ouabain and NE. Arrows and corresponding numbers indicate time of application of the indicated concentration of the drug (in nM). Records were obtained in cells cultured for 2 days ((*A*) C_m = 151 pF) and 1 day ((*B*) C_m 117 pF). (*C*) Each point represents the average increase of I_p caused by each concentration of NE (●) or ISO (○) measured in several cells from at least three different cell cultures normalized to the maximum response obtained with each drug. Smooth curves represent the best-fit of equation 1 to each data set. The fit parameters for NE were: I_{max} = 0.65 ± 0.02 pA/pF and $K_{0.5}$ = 36 ± 8 nM (solid curve); and for ISO were: I_{max} = 0.57 ± 0.02 pA/pF and $K_{0.5}$ = 1.5 ± 0.4 nM (dashed curve). Filled squares represent average increase of I_p caused by the indicated dose of NE in the presence of 10 μM nadolol (7 cells) normalized to the maximum response obtained with NE alone. Numbers next to symbols indicate the number of cells studied.

Ca and Voltage Dependence of Adrenergic Activation of I_p

The activation of I_p mediated by β-AR agonists has been previously demonstrated in patch-clamp experiments in guinia pig ventricular myocytes, but was reported to depend on intracellular Ca (Gao et al., 1991; Gao et al., 1994; Gao et al., 1996). At low Ca_i, ISO inhibited the Na/K pump; however at >150 nM Ca_i I_p was stimulated by ISO (Gao et al., 1991). In the results reported above in this study, rat ventricular myocytes differ from guinea pig myocytes in that a large stimulation of I_p by ISO and NE was routinely observed with $[Ca]_i$ buffered with 1.1 mM EGTA to about 20 nM. Moreover, in some experiments, like that shown in Fig. 3*B*, $[Ca]_i$ was buffered to about 20 nM with 11 mM EGTA to increase the buffer capacity for $[Ca]_i$. Under these conditions NE and ISO still caused a large activation of I_p.

To better understand this difference in β-AR stimulation of I_p between rat and guinea pig a wider range of $[Ca]_i$ was explored. Effects of ISO on I_p were studied in experiments with free [Ca] buffered to 100, 500 and 1000 nM in patch pipette solution. A summary of these results together with the observations at 20 nM $[Ca]_i$ is

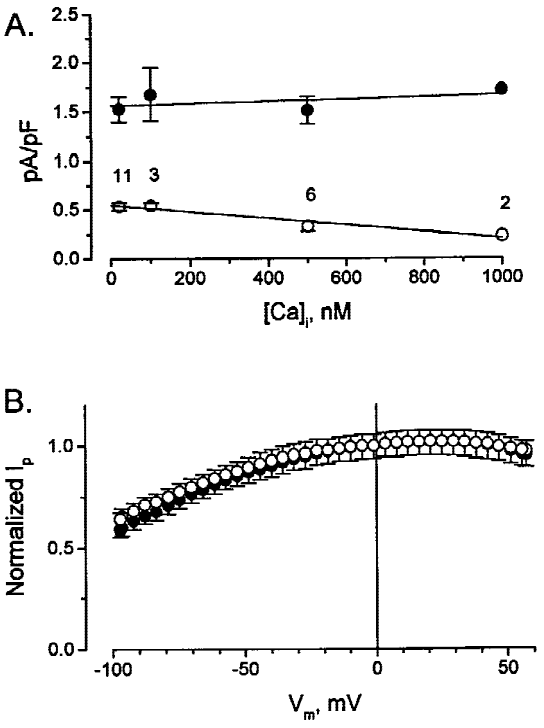

Fig. 6. Influence of $[Ca]_i$ and V_m on I_p and adrenergic stimulation of I_p. (*A*) Symbols represent average I_p (●) and I_p increase caused in the same myocytes by 10 μM ISO (○). Cells were studied with 50 mM Na, 5 mM ATP Cs-aspartate patch-pipette solution with [Ca] buffered by 1.1 mM EGTA at 20, 100, 500 and 1000 nM. Number of cells studied at each $[Ca]_i$ is indicated by number next to symbol. Lines are linear regressions to each data set and have slopes of 0.1 ± 0.1 pA/pF/μM (I_p) and −0.33 ± 0.05 pA/pF/μM (I_p increase in ISO). (*B*) Average data on the I_p-V_m relationship studied under conditions of standard protocol in 11 cells before application of NE (●) and at the peak influence of 10–50 μM NE (○) normalized to I_p measured at $V_m = 0$ mV.

shown in Fig. 6*A*. The upper set of points (filled circles) represents I_p measured under these conditions while the lower set of data (open circles) represents the magnitude of I_p increase above the resting level caused by ISO. These data indicate no Ca-dependent inhibition of steady-state I_p (the slope of the regression analysis is not significantly different from zero) and no increase in adrenergic stimulation of I_p with a rise in $[Ca]_i$. In fact, at 20 nM $[Ca]_i$ ISO increased I_p by 0.54 ± 0.03 pA/pF; however, its effect at 500 nM $[Ca]_i$ was to increase I_p by only 0.33 ± 0.05 pA/pF ($P < 0.05$), significantly less than at 20 nM Ca_i. In two experiments at 1 μM $[Ca]_i$, ISO increased I_p by 0.30 and 0.17 pA/pF; however, under these conditions myocytes were spontaneously contracting which caused concern about the quality of those measurements. Taken together these data suggest that the Ca_i-dependent mechanism proposed for β-adrenergic stimulation of I_p in guinea pig myocytes (Gao et al., 1991; Gao et al., 1994; Gao et al., 1996) is not a mechanism of β-adrenergic activation of I_p in rat myocytes. Rather, in rat myocytes increasing $[Ca]_i$ caused a significant reduction in the stimulation of I_p induced by ISO.

It has also been reported that the stimulatory effect of ISO on I_p in guinea pig ventricular myocytes is voltage dependent. The I_p-V_m relation during adrenergic stimulation was found to be shifted by about 30 mV in the negative direction on the voltage axis (Gao et al., 1996). To measure the I_p-V_m relationship in rat ventricular myocytes voltage ramps were applied as previously described (Stimers et al., 1993; Dobretsov & Stimers, 1997). Voltage ramps were applied before and during stimulation of myocytes with 10 or 50 μM NE (11 cells). Average results of these experiments are plotted in Fig. 6*B* with the data normalized to the value measured at 0 mV. In these experiments NE caused a scaled increase in the I_p-V_m curve by 0.5–0.7 pA/pF (Fig. 6*B*); however, as revealed by normalizing the data, NE did not change the shape of the I_p-V_m relationship or shift it along the voltage axis. Similarly, we did not find changes in the I_p-V_m relationships during ISO induced activation of I_p (*data not shown*).

Effect of Alternate Intracellular Cation Substitution

In the experiments described so far our standard internal solution contained 50 mM Na and 100 mM Cs. Previous studies have shown that the choice of alternate cations making up the balance of the intracellular solution along with Na can influence the magnitude of the steady-state I_p (Kinard & Stimers, 1992). To explore the effects of the cation substitute we also performed some experiments using 100 mM K or 100 mM NMG in place of the 100 mM Cs. Using K in the pipette solution resulted in a slightly smaller steady state I_p of 1.2 ± 0.1 pA/pF with a 0.7 ± 0.1 pA/pF stimulation by NE. Substituting with NMG caused steady-state I_p to be larger (2.2 ± 0.4 pA/pF) and a smaller stimulation of I_p (0.4 ± 0.1 pA/pF). These results along with those for NE and ISO stimulation with Cs in the pipette solution are plotted in Fig. 7. Results from two additional experiments are also plotted where I_p was measured not by inhibition with ouabain, but rather as the current activated by brief (about 60 sec) increases in $[K]_o$ from 0 to 5.4 mM to elicit a nonsteady-state reactivation pump current. This procedure was similar to that used by Ishizuka and Berlin (1993) in their experiments which failed to find any influence of ISO on I_p in adult rat cardiac myocytes (open circle in Fig. 7). Taking all these data together a pattern emerges suggesting that there is an inverse relationship between the level of I_p measured without and with stimulation by β-AR agonists.

Discussion

In the present study we have investigated the β-adrenergic-mediated regulation of the Na/K pump in adult rat cardiac myocytes in short term culture. In this study it was shown that rod-shaped myocytes over 0–4 days in culture provide a stable preparation for measuring Na/K pump current. To our knowledge this is the first study evaluating the stability of I_p in any adult cardiac myocytes maintained in short term culture. We have found that the steady-state I_p in rod-shaped adult rat myocytes cultured 1–4 days varies < 10% from its average value of 1.55 pA/pF. Furthermore, under the conditions of these experiments, it was shown that NE and ISO both produce a substantial stimulation of I_p (40–45%) in adult rat cardiac myocytes, that is also stable over 4 days in culture. However, it appears that the mechanism for this stimulation in the rat is qualitatively different from that reported in guinea pig myocytes (Gao et al., 1991).

Properties of Rod-Shaped Myocytes in Culture

Before discussing our findings regarding the adrenergic stimulation of the Na/K pump it is first necessary to discuss the effect of culturing rat myocytes for 0–4 days to demonstrate that this is an appropriate and stable model for these studies. During the 0–4 days in culture 30–50% of the cardiac myocytes maintained visible striations, rodlike shapes, a relatively constant apparent cell size and steady-state I_p. These rod-shaped myocytes appeared normal in that C_m was within the range of 140–210 pF reported for freshly isolated rat ventricular cells (Isenberg & Klockner, 1982; Ellingsen et al., 1993). Consistent with our finding of a 27% decrease in C_m it was previously reported that C_m of cardiac myocytes decreased by 26% over 3 days in culture (Ellingsen et al., 1993). Since over this same time period there was not a comparable decrease in cell size, it is likely that the change in C_m in culture was due to a disintegration and/or uncoupling from the surface membrane of the T-tubules. This suggestion is supported by similar findings in guinea pig ventricular myocytes where the T-tubular system was lost in 1–2 days in culture (Lipp et al., 1995). From the difference in cell capacitance calculated from the myocyte length and width (120 pF) and that measured in myocytes on day 0 (200 pF; Fig. 3*A*) we estimate that the cell surface membrane residing in the T-tubules is about 40% of total surface area of freshly isolated myocytes, which is only slightly higher than previous estimates (27–36%, Page, 1978). Despite the apparent loss of the T-tubules, the I_p density remained constant on all days in culture, suggesting that the Na/K pump density is uniform in the surface membrane and the t-tubules.

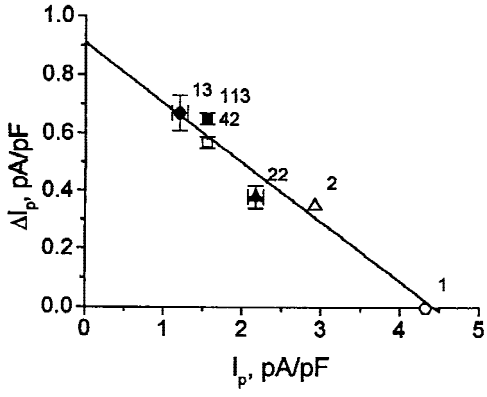

Fig. 7. Dependence of β-AR stimulation of I_p on steady state I_p. This figure shows steady state I_p and its stimulation by saturating concentrations of NE or ISO under different conditions. The conditions used were 50 mM Na_i in all cases and 100 mM K (●); 100 mM Cs (■ and □); and 100 NMG (▲). The empty triangle (△) represents the average of two measurements where 100 mM Cs was used internally but I_p was measured as the current activated by 5.4 mM K following a prolonged period of exposure to 0 mM K. This procedure is similar to the technique used previously by Ishizuka and Berlin (1993) in their study on rat myocytes. Using the data obtained in our experiments, a linear regression reveals a significant negative correlation between I_p and the ability of β-AR agonists to stimulate I_p (slope = −0.2; correlation coefficient = −0.93). This regression analysis predicts that if I_p is very large (similar to that recorded by Ishizuka and Berlin (1993); ❍), then adrenergic stimulation will fail to further increase I_p.

R_m was estimated from our data to be about 30 $k\Omega \cdot cm^2$ in freshly isolated cells and 50 $k\Omega \cdot cm^2$ in cultured myocytes. The lower R_m of freshly isolated myocytes compared to that of cultured cells may have several possible explanations. Myocyte membranes may become nonselectively conductive to ions during the isolation procedure that is typically carried out at low $[Ca]_o$ (Piper et al., 1990). Enzymatic treatment and mechanical dissociation of myocytes may also cause damage to the sarcolemma. Another possible source of the nonselective ion conductance is that myocytes need at least 4 hr after isolation to start sealing areas of former intercalated disks (Claycomb & Palazzo, 1980). Our data suggest that it takes about 1 day in culture for myocytes to recover from membrane damage caused by the isolation procedure. This suggests that myocytes in short-term culture may have some advantages over freshly isolated myocytes in that they have a simpler geometry (loss of T-tubules) which will provide better voltage control and they have recovered from the isolation procedure. Of course there are disadvantages to using cultured cells. They are more difficult to maintain alive and in a differentiated state and the use of serum potentially could introduce factors into the system that might interfere with the properties to be studied.

Despite these apparent changes in cell morphology and membrane capacitance and resistance, I_p and its

regulation by NE were found to be preserved in adult rod-shaped cultured myocytes, proving the suitability of this preparation for this investigation.

Adrenergic Stimulation of Na/K Pump in Rat Cardiac Myocytes

The presence and extent of adrenergic regulation of the Na/K pump in cardiac myocytes is a matter of current controversy. In this study, saturating concentrations of NE and ISO caused a similar peak increase in I_p by 0.55–0.65 pA/pF, which did not vary with time in culture. The concentration of NE and ISO needed for half-activation of I_p in our experiments was 36 and 1.5 nM, respectively. Thus activation of the Na/K pump in rat cardiac myocytes occurs at both physiologically and pharmacologically relevant concentrations of these two agonists (*see* Horackova & Wilkinson, 1992). The difference in drug concentration between NE and ISO that causes half-activation of I_p corresponds to well-known differences in affinities of NE and ISO for β-AR (*see* Stiles, Caron & Lefkowitz, 1984). The NE-induced stimulation of I_p was suppressed by nadolol (β-AR antagonist) but not by prazosin (α-AR antagonist), suggesting that the effect of physiological concentrations of NE on the Na/K pump is mediated primarily by β-ARs in rat myocytes (*see* also Desilets & Baumgarten, 1986; Wasserstrom et al., 1982).

Thus, our data support the results of previous reports that either NE or ISO stimulates the Na/K pump in cardiac muscle cells (Vassalle & Lin, 1979; Wasserstrom et al., 1982; Desilets & Baumgarten, 1986). However, several groups using isolated sheep Purkinje fibers (Glitsch et al., 1989), guinea pig myocytes (Bahinski & Gadsby, 1990; Gao et al., 1991; Gao et al., 1994; Gao et al., 1996) or rat myocytes (Ishizuka & Berlin, 1993) have found little or no effect of ISO on I_p. It is still to be resolved if cell differences (ventricular myocytes *vs.* Purkinje fibers), species differences (guinea pig *vs.* rat) or experimental conditions (ion composition of solutions, method of measuring I_p, etc.) may account for differences in adrenergic regulation of the Na/K pump activity observed in experiments with cardiac muscle. Our present results may shed some light on some of these difficult questions as discussed below.

First, the only previous investigation on isolated rat ventricular myocytes, and therefore directly comparable with our study, is that conducted by Ishizuka and Berlin (1993). These authors did not find any influence of ISO (10 μM) on I_p elicited by brief activation of the Na/K pump by external K (reactivation I_p). Initially, we expected that the use of freshly isolated cells by Ishizuka and Berlin would clarify the reason of failed adrenergic regulation of I_p. From our data, however, it appears that the isolation procedure does not impair mechanisms linking adrenergic receptors with the Na/K pump. Another, obvious difference was that I_p reactivation currents measured by Ishizuka and Berlin were 2–3 times larger than I_p measured under steady-state conditions in the present study. When we analyzed our results obtained by measuring reactivation currents, or using various internal conditions (e.g., using K, Cs or NMG as substitute intracellular cations) we found an inverse correlation between I_p and its stimulation by NE (Fig. 7). Notice that the regression analysis of these data predicts that for very large I_p (such as that measured by Ishizuka and Berlin, open circle in Fig. 7) there should be no further stimulation of I_p by β-AR agonists. Thus, the present results and those of Ishizuka and Berlin, may lie on a continuum of Na/K pump transport that is modulated by experimental conditions.

Second, the other preparation being used to study adrenergic regulation of the Na/K pump is the adult guinea pig cardiac myocyte; however, here the results are more complicated. In guinea pig myocytes the results are also not uniform between laboratories. While Gao et al. (1991) report complex effects of ISO on I_p, others found no effect of ISO (Bahinski & Gadsby, 1990). This conflict has not been addressed by either group of investigators. Similar to our results in rat, in guinea pig myocytes (Gao et al., 1991; Gao et al., 1994; Gao et al., 1996) it was found that β-AR agonists could stimulate I_p; however, the effect seen in the rat cells appeared to involve completely different mechanisms from those in guinea pig heart cells. In guinea pig myocytes it was determined that with low $[Ca]_i$ (<150 nM) ISO inhibited I_p, while high $[Ca]_i$ (>150 nM) stimulated I_p (Gao et al., 1991). It was suggested that I_p had two effects: (i) Ca_i prevented the protein kinase A mediated inhibition of I_p; and (ii) Ca_i caused a protein kinase A induced shift of the voltage dependence of I_p (Charpentier et al., 1996). However, in the rat we found no evidence for inhibition of I_p by NE at any level of $[Ca]_i$ and if anything increasing $[Ca]_i$ resulted in a decrease in the stimulation of I_p by NE. Furthermore, we did not find any shift in the I_p-V_m relationship that could explain the stimulation of I_p by NE. These results suggest that this may represent an interesting example of a species dependence that is expressed as differences in the specific mechanisms mediating the effect of NE with little differences in starting point (receptors) or final outcome of Na/K pump activation. It has been speculated that adrenergic stimulation of the Na/K pump in cardiac myocytes may be a physiologically important mechanism involved in $[Ca]_i$ regulation through the regulation of Na/Ca exchange activity (Wasserstrom et al., 1982; Bers, 1991). Yet it is well known that many mechanisms of $[Ca]_i$ homeostasis in heart muscle cells are markedly species dependent (reviewed by Bers, 1991). To determine if adrenergic stimulation of the Na/K pump is one

of these mechanisms, further evaluation of the effect of β-AR stimulation in rat myocytes and experiments conducted under the same conditions but with cardiac cells isolated from different species need to be done.

Implications about Possible Mechanisms

At this time it is not possible to draw conclusions about the precise mechanisms underlying the stimulation of I_p by NE observed in rat cardiac myocytes. An interesting finding in this study was the tendency for the NE induced stimulation of I_p to decrease with increasing I_p. This suggests that for high turnover rates of the Na/K pump, the kinetics are rate limited by a process that cannot be further stimulated by NE. Furthermore, adrenergic stimuli modulate some kinetic process which was rate limiting for Na/K pump enzymatic turnover under conditions of our experiments, and which could also be modulated by intracellular cations. The concentrations of Na_i and K_o used in this study were at or near saturating levels so that it would be unlikely for their binding to the Na/K pump to represent the rate limiting process under these conditions. As there was no observed change in the I_p voltage dependence, the Na release to the external media also does not appear to be involved in the adrenergic modulation of the Na/K pump cycle. An attractive possibility is that adrenergic stimulation alters the K translocation and intracellular release by the Na/K pump. Previous studies have shown that this step is one of the slowest in the Na/K pump cycle (*see* Glynn, 1993). Moreover, this process should be dependent on intracellular K (or Cs) concentration because of their binding to the intracellular K binding sites of the Na/K pump. It may be speculated that both the changes in steady-state I_p observed in our experiments with different intracellular solutions (K-, Cs-, or NMG-aspartate; Fig. 7), and the effect of NE on I_p involve modulation of the K translocation steps in the Na/K pump cycle. However, further experiments need to be done to explore this possibility.

Physiological Significance

To determine the physiological significance of the stimulation of I_p by NE or ISO, calculations were made to estimate the effect this would have on Na transport. We estimate that in a cell with an accessible volume of about 45 pl and C_m of 200 pF (characteristics of day 0 myocytes in our experiments) the activation of I_p by 0.6 pA/pF is equivalent to a change in Na_i of 5.0 mM/min; however, these measurements were done under conditions of high stimulation of the Na/K pump by 50 mM Na_i and at a membrane potential of −40 mV. Assuming that I_p activation by NE may be scaled according to the known dependencies of I_p on $[Na]_i$ and V_m (Gadsby & Nakao, 1989; Nakao & Gadsby, 1989; Stimers et al., 1993), we estimate that under normal conditions ($[Na]_i$ = 10 mM, V_m = −80 mV) adrenergic stimulation of the Na/K pump will cause a decrease in $[Na]_i$ of about 1.5 mM/min. In our experiments measuring the NE effect with 10 mM Na_i, we calculate that the increased I_p of 0.16 pA/pF corresponds to reduction of $[Na]_i$ of 1.33 mM/min in close agreement with our estimate above. These values estimate the peak rate of Na extrusion by the pump, because under physiological conditions in intact cells activation of the Na/K pump should be self-limiting since its activation will cause a decrease in Na_i that will decrease Na/K pump activity. Thus in previous studies with ion-selective electrodes, where cardiac myocytes had no extrinsic supply of Na_i (e.g., via the patch-pipette in our experiments), the stimulation with NE or ISO (0.1–1 μM) was found to decrease $[Na]_i$ by only 0.53–0.73 mM/min (Desilets & Baumgarten, 1986). While this is a relatively small change in$[Na]_i$, it has been shown that in cardiac myocytes similar changes in Na_i activity cause about a 2-fold change in twitch tension (*see* Bers, 1991).

Conclusion

In conclusion, we have provided evidence for the strong activation of I_p by adrenergic agonists in freshly isolated adult rat cardiac myocytes and in rod-shaped myocytes cultured in serum-supplemented media for up to 4 days. The stability of I_p and its activation by NE in cultured adult rat cardiac myocytes makes these cells a suitable preparation for further detailed studies of adrenergic regulation of the cardiac Na/K pump. Future studies will be directed at determining the mechanism by which β-AR agonists stimulate the Na/K pump. Furthermore, comparative studies are needed to determine if the apparent mechanistic differences between rat and guinea pig can provide insights into other species differences.

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