Inhibition of cardiac Na^+ currents by isoproterenol

BERND SCHUBERT, ANTONIUS M. J. VANDONGEN,
GLENN E. KIRSCH, AND ARTHUR M. BROWN
Department of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, Texas 77030

SCHUBERT, BERND, ANTONIUS M. J. VANDONGEN, GLENN E. KIRSCH, AND ARTHUR M. BROWN. *Inhibition of cardiac Na^+ currents by isoproterenol.* Am. J. Physiol. 258 (Heart Circ. Physiol. 27): H977–H982, 1990.—The mechanism by which the β-adrenergic agonist isoproterenol (ISO) modulates voltage-dependent cardiac Na^+ currents (I_{Na}) was studied in single ventricular myocytes of neonatal rat using the gigaseal patch-clamp technique. ISO inhibited I_{Na} reversibly, making the effect readily distinguishable from the monotonic decrease of I_{Na} caused by the shift in gating that customarily occurs during whole cell patch-clamp experiments (E. Fenwick, A. Marty, and E. Neher, *J. Physiol. Lond.* 331: 599–635, 1982; and J. M. Fernandez, A. P. Fox, and S. Krasne, *J. Physiol. Lond.* 356: 565–585, 1984). The inhibition was biphasic, having fast and slow components, and was voltage-dependent, being more pronounced at depolarized potentials. In whole cell experiments the membrane-permeable adenosine 3′,5′-cyclic monophosphate (cAMP) congener 8-bromo-cAMP reduced I_{Na}. In cell-free inside-out patches with ISO present in the pipette, guanosine 5′-triphosphate (GTP) applied to the inner side of the membrane patch inhibited single Na^+ channel activity. This inhibition could be partly reversed by hyperpolarizing prepulses. The nonhydrolyzable GTP analogue guanosine-5′-*O*-(3-thiotriphosphate) greatly reduced the probability of single Na^+ channel currents in a Mg^{2+}-dependent manner. We propose that ISO inhibits cardiac Na^+ channels via the guanine nucleotide binding, signal-transducing G protein that acts through both direct (membrane delimited) and indirect (cytoplasmic) pathways.

patch-clamp technique; β-adrenergic agonist; G proteins

NEUROTRANSMITTERS MODULATE heart rate, force of contraction, and spread of the cardiac impulse, but how this is accomplished is incompletely understood. Our ignorance is particularly marked in the case of fast cardiac Na^+ channels that underlie the upstroke of the action potential and the rate at which it is propagated through the heart. For example, the β-adrenergic agonist isoproterenol (ISO) is thought to decrease the maximum velocity of the upstroke in depolarized ventricular myocytes presumably by acting on the Na^+ current, but there are few convincing demonstrations that indicate this (1, 2, 16, 32). Furthermore, should this be the case, the underlying mechanism remains to be adduced. To study this process and its mechanism directly, we investigated the action of ISO on Na^+ currents in cultured heart muscle cells using the patch-clamp method (12). However, such an approach is complicated by the fact that in patch-clamp experiments the control Na^+ current (I_{Na}) decreases because of a shift of the steady-state inactivation curve h_∞-voltage (V) toward more negative potentials (6, 7, 25). Therefore it was necessary to first evaluate the rate at which the h_∞-V shift occurs under control conditions. After doing this we found that ISO inhibited I_{Na} in partially depolarized heart cell membranes with a biphasic time course. Direct and indirect G signaling pathways appear to mediate the two components.

METHODS

Preparation of cells. Primary cardiac cell cultures were prepared from hearts of 1- to 3-day-old neonatal rats (25, 28). Hearts were removed under sterile conditions, and the ventricles were cut into small pieces. The tissue pieces were incubated at 37°C for 5 min in Ca^{2+}-free Hanks' solution containing 0.5% trypsin (Sigma T 0134). The supernatant was removed and the pelleted cells were added to Dulbecco's modified Eagle's medium (DMEM-10 FCS) to stop enzyme action. The cell suspensions were seeded on glass cover slips in 35-mm Falcon dishes containing the culture medium. The cultures were incubated at 37°C in an H_2O-saturated, 5% CO_2-95% O_2 air atmosphere. Cells were used within 24–48 h.

Electrophysiological recording and data analysis. Whole cell and single-channel recordings were made using patch-clamp techniques (12). In all experiments commercial patch-clamp amplifiers were used. For whole cell current clamp we selected small spherical cells (10 μm in diam), the membranes of which behaved as a simple resistance-capacitance circuit with a time constant <100 μs (25). The whole cell and single-channel currents described here have the gating, conductance, and pharmacological properties of cardiac Na^+ channels (3, 8, 11, 18, 25, 37, 38). Test potentials were always on the positive limb of the I-V curve, the currents had none of the features of inadequate space clamp and were similar to currents obtained at half extracellular Na^+ concentration ($[Na^+]_o$) (37, 38). Patch pipettes had tip resistances of 1.5–5.0 MΩ unless otherwise mentioned, and the input resistance of the cells was ~1.0 GΩ. Capacitive transient cancellation and series resistance adjustments were made to provide optimum settling and attenuation of the capacitive current transient. To correct for liquid junction potentials between the bath solution and the pipette solution, the current was zeroed when the bare pipette was placed into the bath solution.

Currents were digitized and recorded at 44 kHz on a pulse-code modulated videocassette recorder (PCM VCR) for off-line analysis. Before digitization currents were filtered at 5 kHz (−3dB) using a four-pole Bessel

0363-6135/90 $1.50 Copyright © 1990 the American Physiological Society

filter. The data were then transferred to a MicroVax II computer for further analyses. The single-channel records were filtered before analysis using a digital Gaussian finite impulse response filter.

The experimental chamber (volume 200–500 μl) was placed on an inverted microscope stage. For some experiments fast exchange of external solutions was accomplished by directing flow from a glass pipette to the cell under investigation. Each change to ISO-containing solution was preceded by a change to Na^+-free solution (pipette solution, vide infra) to calibrate the solution exchange rate. In other experiments the chamber was perfused at rates of up to 3 ml/min.

To suppress outward currents in whole cell experiments, the pipettes were filled with Cs^+-rich solution of the following composition (in mM): 118 CsOH, 118 aspartic acid, 6.4 $MgCl_2$, 5 ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA), 0.5 guanosine 5′-triphosphate (GTP), 4.2 ATP, 2.7 $CaCl_2$, 5 *N*-2-hydroxyethylpiperazine-*N′*-2-ethanesulfonic acid (HEPES), pH 7.3; 290 mosM. Free concentrations of Mg^{2+} and Ca^{2+} were calculated (5) to be 2 mM Mg^{2+} and 0.1 μM Ca^{2+}. External solution contained (in mM) 137 NaCl, 5.4 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 5 $CoCl_2$, 10 glucose, 10 HEPES, pH 7.4; 290 mosM. Ca^{2+} currents were suppressed by addition of Co^{2+} in the presence of Mg^{2+}.

For recording single Na^+ channel currents from excised inside-out membrane patches the pipette solution had the following composition (in mM): 137 NaCl, 5.4 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 glucose, 10 HEPES, pH 7.4; 290 mosM. The inner surface of the membrane patch faced the following bathing solution (in mM): 110 CsOH, 110 aspartic acid, 20 $CsCl_2$, 2 $MgCl_2$, 5 EGTA, 5 HEPES, pH 7.3; 290 mosM. When indicated $MgCl_2$ was substituted with cesium aspartate to maintain an osmolarity of 290 mosM. EGTA was substituted with EDTA to make the solution Mg^{2+} free.

All experiments were performed at room temperature (20–22°C). Results are given as means ± SD. Unless indicated otherwise results are significant at the 2.5% level.

Drugs. (*l*)-Isoproterenol as a (*d*)-bitartrate salt and 8-bromoadenosine 3′,5′-cyclic monophosphate (8-bromo-cAMP) were purchased from Sigma Chemical (St. Louis, MO). GTP and guanosine-5′-*O*-(3-thiotriphosphate) (GTPγS) were obtained from Boehringer Mannheim, FRG.

RESULTS

In control solutions when the steady-state inactivation parameter for I_{Na}, h_∞, (17) was adjusted to a value close to 1 using 200-ms prepulses to −120 mV, peak I_{Na} increased initially during the first few minutes, probably because of removal of slow inactivation, then decreased slowly (Fig. 1*A*). At a holding potential (HP) of −60 mV without conditioning prepulses and h_∞ at ~0.5, peak I_{Na} decreased more quickly (Fig. 1*B*). The decrease was especially fast when large patch pipettes (0.5–1 MΩ) were used and was caused by a shift of the steady-state inactivation h_∞-voltage (V) relationship as shown in Fig. 2. In six experiments performed at a HP of −60 mV the midpoint of h_∞-*V* shifted at an average rate of 0.9 ± 0.6 mV/min (Fig. 2*B*), which would have produced on the average a 4% reduction of I_{Na} per minute. Under these experimental conditions inactivation of I_{Na} during the test pulse became progressively slower as has been reported previously (4, 23). To minimize the spontaneous decline and kinetic changes of I_{Na} at a HP equal to −60 mV, smaller pipettes (1.5–5 MΩ) were used in the remaining experiments.

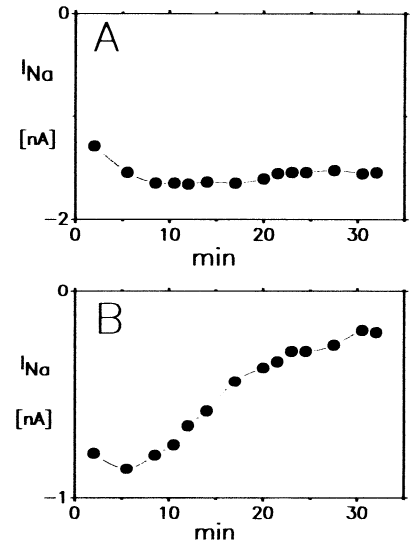

FIG. 1. Spontaneous reduction of I_{Na} amplitude at 2 different steady-state inactivation levels. Depolarizing test pulses to 0 mV were applied for 10 ms at 0.5 Hz. Amplitudes of whole cell Na^+ currents (I_{Na}) are plotted against time. *A*: holding potential (HP) was −60 mV. Test pulses were preceded by 200-ms-long hyperpolarizing prepulses to −120 mV, where h_∞ was found to be close to 1 in whole cell experiments. *B*: same cell as in *A*. No prepulses were applied. At a HP of −60 mV h_∞ is close to 0.5.

At an h_∞ of 1.0, ISO at 40 μM decreased the I_{Na} amplitude by 3.6 ± 1.4% (n = 5 cells). At a more positive potential (HP = −60 mV), however, ISO at 40 μM reduced whole cell I_{Na} by 51 ± 22% (n = 5 cells). In four additional experiments at the same HP (HP = −60 mV), ISO at 1 μM inhibited I_{Na} in <1 min after application by 40.8 ± 17.5% (Fig. 3). The inhibition after ISO application occurred rapidly and was much larger than the I_{Na} reduction that might be expected to result from the spontaneous h_∞-*V* shift at this HP. Furthermore, the ISO effect was partially reversible (Fig. 3*A*). After washout I_{Na} amplitude was restored to 86.2 ± 8.5% (n = 4 cells).

To further evaluate the onset and reversibility of the ISO response, experiments were performed using a rapid perfusion system. Brief (5–10 s) application of ISO at 1 μM produced an inhibition that occurred quickly initially and more slowly subsequently (Fig. 4). The rapid phase occurred immediately after ISO application without measurable delay. The reduction produced during this fast component of inhibition was 25 ± 11% in a potential range from −60 mV to −90 mV (n = 7 cells). Larger inhibitions of up to 38% were observed at −60 mV, and smaller ones of ~10% occurred at −90 mV indicating a voltage dependence of the effect (Table 1). The reduction was followed by a transient recovery during which the inhibition decreased to 13 ± 9% (n = 7 cells). This was

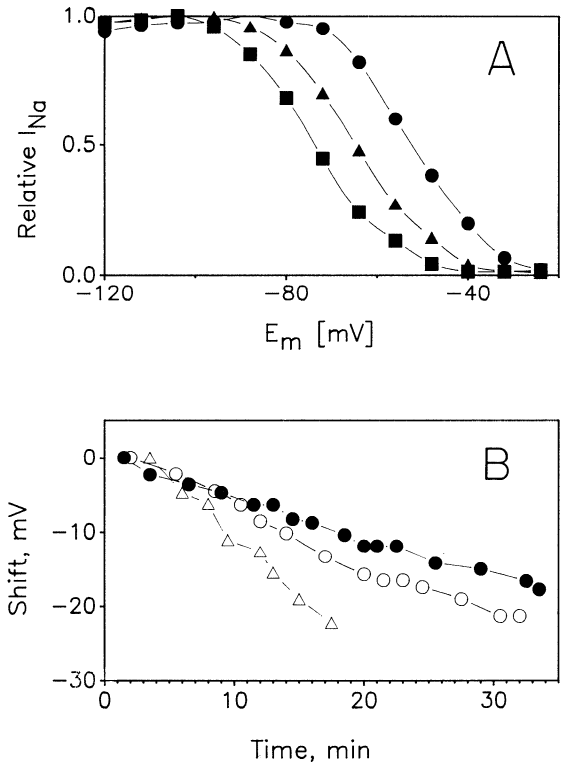

FIG. 2. Spontaneous shift of the h_∞-V curve during whole cell experiments. HP was −60 mV as in Fig. 1, *A* and *B*. Test pulses applied for 10 ms to 0 mV were preceded by 200-ms-long prepulses of different amplitudes. *A*: I_{Na} amplitude resulting from test pulse was plotted against prepulse potential. Potential at which I_{Na} amplitude reached 0.5 ($V_{0.5}$) was 52 mV at 2 min after establishing the whole cell configuration (●). Curve was shifted to left by 13 mV after 17 min (▲) and by 21 mV after 32 min (■). *B*: shift in midpoints of h_∞-V curves [(E(h_∞ = 0.5)] are plotted as a function of time during time course of a whole cell experiment. Data from 3 different representative experiments are shown. First points in plots correspond to following values for midpoints of the h_∞-V curves of −57.9 mV (●), −51.7 mV (○), and −60.3 mV (△).

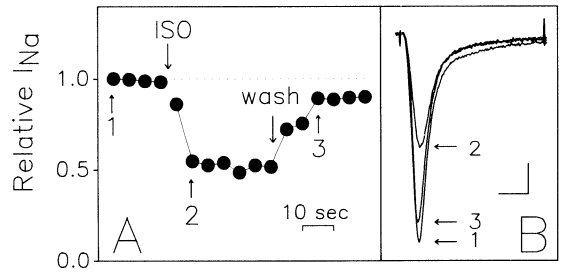

FIG. 3. Isoproterenol (ISO) inhibits I_{Na} reversibly. *A*: normalized amplitudes of whole cell Na^+ currents are plotted against time. HP was set to −60 mV. Test pulses of 10 ms to 0 mV were applied at a frequency of 0.2 Hz. Application and washout of ISO started as indicated by arrows. *B*: superimposed individual current traces from *A* are shown. Calibration bars correspond to 200 pA vertically and 2 ms horizontally. There is a slight partly reversible change in I_{Na} kinetics that is typical for this type of experiment. Occurrence of this change would be in agreement with idea that ISO induces a shift in the voltage dependence of inactivation.

then followed in all cases by a delayed inhibition after ISO had been washed out of the bath, which reduced I_{Na} amplitude within 1 min to a level of 43 ± 30% of control (n = 7 cells).

This biphasic response is reminiscent of the two different pathways by which the guanine nucleotide binding, signal-transducing G protein (G_s), the stimulatory regulator of adenylate cyclase, couples the β-adrenoreceptor to voltage-dependent cardiac calcium channels. One pathway is cytoplasmic and indirect via activation of adenylate cyclase, production of cAMP, and activation of protein kinase *a* (21, 29). The other is membrane delimited and assumed to be direct with G_s acting independently of cytoplasmic mediators (35, 36). Because of the rapid and slow components of the I_{Na} inhibition by ISO, we wanted to test whether similar mechanisms might be operative in the present circumstances as well. To determine which mechanism mediates the ISO effect on cardiac I_{Na}, we used conditions in which only one would be expected to predominate. To test for the indirect pathway the membrane permeable cAMP analogue, 8-bromo-cAMP, was added to the bath. In six experiments, 8-bromo-cAMP at 10 μM reduced whole cell I_{Na} by 29 ± 13% in <1 min when applied at a HP of −60 mV. After application of 8-bromo-cAMP, ISO at 1 μM decreased I_{Na} amplitude further by 14 ± 4% (Fig. 5; n = 3 cells) suggesting that ISO may act through different pathways on the I_{Na}. However, in this type of experiment the I_{Na} reduction was less reversible during the observed time interval as compared with the reversibility after application of ISO alone (cf. Fig. 3*A*).

To test for the direct pathway, we measured single-

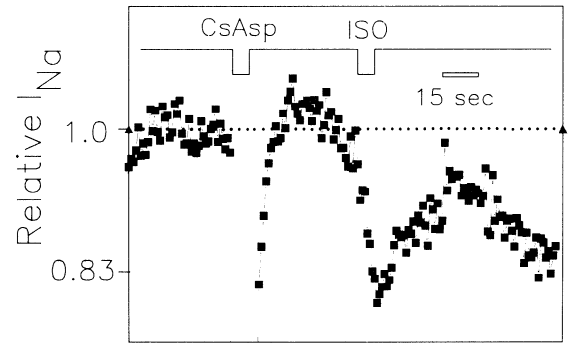

FIG. 4. Two components in the inhibition of the I_{Na} by ISO. HP was set to −70 mV. Test pulses to 0 mV were applied for 55 ms at a frequency of 1 Hz. Normalized amplitudes of whole cell Na^+ currents are plotted against time. A system for fast application of solution was used. Cesium aspartate (CsAsp) was applied for 8 s just before application of ISO. CsAsp application resulted in a rapid and fully reversible decrease of the I_{Na} amplitude. ISO at a concentration of 1 μM when applied during time interval as indicated by bar induced a biphasic inhibition.

TABLE 1. *Sodium current inhibition by ISO depends on voltage*

HP	Amount of Inhibition, %		
	Rapid phase	Partial recovery	Delayed phase
−90	11	6	17
−80	14	4	12
−75	32	10	65
−70	38	25	65
−70	18	15	33
−70	24	24	88
−60	39	8	20
Mean±SD	25±11	13±9	43±30

Means ± SD were calculated separately for each phase of I_{Na} reduction or recovery from all 7 different cells (n). Each entry in the table represents data from one cell. ISO, isoproterenol; HP, holding potential.

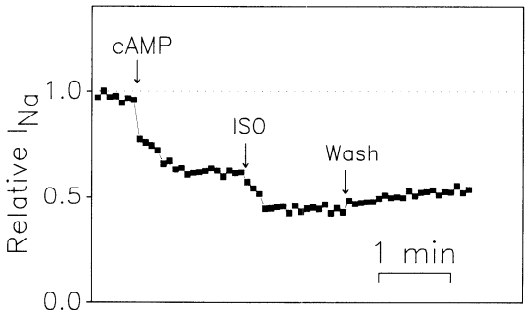

FIG. 5. 8-bromo-cAMP inhibits cardiac I_{Na}. HP was −60 mV. Test pulses applied for 10 ms to −10 mV were preceded by a 10-mV hyperpolarizing prepulse to −70 mV of 200 ms in duration to a potential where h_∞ was close to 0.5. Amplitude of prepulse remained constant throughout experiment. Normalized amplitudes of whole cell Na^+ currents are plotted against time. Application of 10 μM 8-bromo-cAMP started as marked by arrow and was followed by addition of 1 μM ISO. Washing resulted in a moderate recovery.

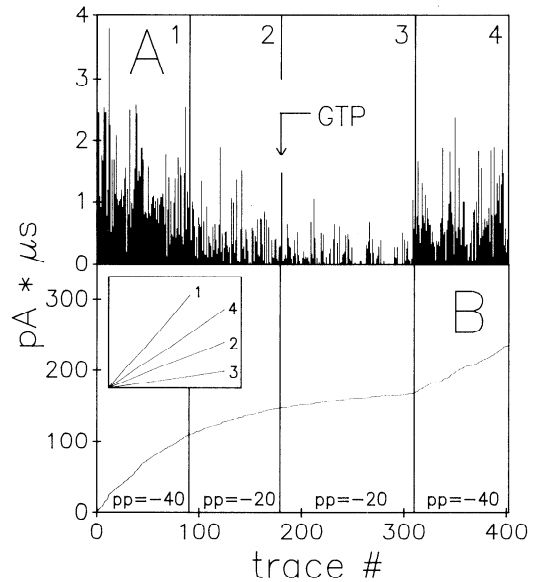

FIG. 6. ISO inhibition of single-channel Na^+ currents by a direct pathway. Single-channel Na^+ currents were recorded from excised inside-out membrane patches of neonatal rat ventricle cells. Depolarizing voltage-clamp pulses of 55 ms to −60 mV were applied at 0.5 Hz from a HP of −105 mV. These pulses were preceded by hyperpolarizing prepulses (pp) of different amplitudes. During *periods 1* and *4* the prepulse amplitude was equal to −145 mV and during *periods 2* and *3* was equal to −125 mV. Throughout the experiment ISO was present in the pipette solution at a concentration of 10 μM. Guanosine 5′-triphosphate (GTP) at 500 μM was applied to inner side of membrane patch as indicated by arrow. *A*: single-channel traces were integrated, and integral was plotted against trace number. *B*: cumulative plot of integrated currents. Straight lines were fitted to data points for each time period separately. In inset the time origin of these lines was aligned to facilitate comparison. Normalized values for slopes have been determined as follows: 1, 0.36, 0.13, 0.6 for *periods 1–4*, respectively.

channel Na^+ currents from excised inside-out membrane patches using solutions from which phosphorylating substrates were absent. With ISO present at the extracellular surface and GTP absent intracellularly (Fig. 6*A*, 1 and 2), single-channel activity was partially inactivated on switching from a prepulse of −145 to −125 mV. Subsequent addition of GTP to the intracellular surface (Fig. 6*A*, 3) further reduced channel activity. Switching the prepulse back to −145 mV partially restored activity

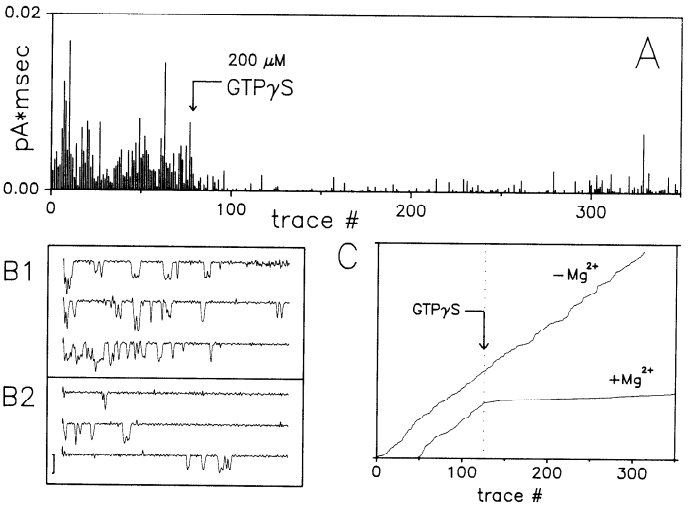

FIG. 7. Guanosine-5′-*O*-(3-thiotriphosphate) (GTPγS) inhibition of single Na^+ channel currents is Mg^{2+}-dependent. Single Na^+ channel currents were recorded from excised inside-out membrane patches. Depolarizing steps of 55 ms to −50 mV were applied at 0.5 Hz from a holding potential of −90 mV. Test pulses were preceded by 200-ms duration prepulses to −140 mV. *A*: as in Fig. 6, charge movement through Na^+ channels as a measure of channel activity was determined by integrating individual current traces, and integral is plotted against trace number. Nonhydrolyzable GTP analogue GTPγS at 200 μM was applied at arrow to intracellular surface of membrane. *B*: representative records from membrane patch in *A* are shown before (*B1*) and after (*B2*) application of GTPγS. Calibration bar corresponds to 2.0 pA. Only the first 30 ms of each trace are shown. In *B2*, only nonempty sweeps were selected. *C*: cumulative plot of single Na^+ channel currents against trace number. Mg^{2+}-free conditions are labeled as $-Mg^{2+}$. GTPγS at 400 μM was added to bathing solution at arrow. Time origin of plot for Mg^{2+}-containing solutions (same patch as in *A*) was shifted to align time of GTPγS application. Slopes of both curves before GTPγS application have been normalized to facilitate comparison.

(Fig. 6*A*, 4). Fig. 6*B* quantitatively illustrates the changes in channel activity determined by calculating the slopes of the cumulative integral plots. Qualitatively the same results were obtained in four more experiments.

In the same patch-clamp configuration, the nonhydrolyzable GTP analogue GTPγS at 200 μM added to the static bath in the absence of ISO reduced single Na^+ channel currents by 95.7 ± 2.6% (n = 6 cells, Fig. 7). The response to GTPγS was poorly reversible. Washout during periods longer than 30 min sometimes resulted in a slight restoration of single-channel currents (not shown). It was not studied systematically to see whether single-channel parameters are changed after application of GTPγS. To test whether the inhibition by GTPγS was caused by activation of an endogenous G protein rather than a direct effect of GTPγS on the Na^+ channel, we examined the requirement for Mg^{2+}. In a series of experiments, Mg^{2+} was either present at a constant concentration at any time during an experiment or it was omitted from all solutions (see METHODS). In contrast to the large inhibition observed in the presence of Mg^{2+}, GTPγS decreased the slope of the accumulated currents in its absence by only 14.3 ± 9.5% (n = 4 cells, NS) (Fig. 7*C*).

DISCUSSION

We confirmed that during whole cell patch clamp measurements of I_{Na} using neonatal heart muscle cells,

a shift of the h_∞-V curve occurs and with large patch pipettes (0.5–1.0 MΩ), the customary shift was ~1 mV/min. In our experiments smaller patch pipettes were used, and we determined that ISO inhibited I_{Na} more quickly and more extensively than could be accounted for by the shift in h_∞-V that occurs in control solutions. Furthermore, the ISO inhibition was partially reversible. Previous reports (2, 16, 32) that ISO reduced V_{max} in heart cells did not clearly establish that I_{Na} is involved. A more recent report (1) in which the patch-clamp technique was used did not evaluate the shift in h_∞-V, and the interpretation was, therefore, inconclusive.

The present results show that *1*) ISO inhibited whole cell Na^+ currents in a voltage-dependent manner; *2*) the timecourse of ISO inhibition is biphasic; *3*) 8-bromo-cAMP reduced I_{Na} in whole cell experiments; *4*) in inside-out patches GTP reduced Na^+ channel opening probability in the presence of ISO; and *5*) GTPγS had very similar effects in the absence of ISO. This is strong evidence for involvement of G_s, and in other experiments we have demonstrated that G_s can mimic the direct effects of GTPγS (30). Furthermore, G_s appears to produce its effects on cardiac Na^+ channels via direct and indirect signaling pathways (30) in a manner similar to that which has been proposed for cardiac Ca^{2+} channels (21, 29, 34–36).

The inhibition of cardiac I_{Na} by ISO is voltage dependent, being more pronounced at depolarized conditioning potentials. In this respect the effect of ISO on I_{Na} is similar to the effect of local anesthetics (14, 22). One might speculate that behind the voltage dependence of the ISO effect is, just as in the case of local anesthetics, a shift of the h_∞-V curve. The finding that in the presence of ISO in the pipette solution, the GTP-induced reduction of single-channel current could be partly reversed by hyperpolarizing prepulses (Fig. 6) strongly supports the idea that ISO shifts the h_∞-V curve.

There are several answers to the question of what might cause the h_∞-V curve to shift. A direct interaction of an endogenous G_s protein activated by β-adrenoreceptor activation might be responsible as well as the phosphorylation caused by the indirect pathway. Our data are consistent with the idea that both mechanisms are involved in mediating the ISO effect on the I_{Na}.

It remains to be determined whether regulation by the ISO or other catecholamines is an exclusive feature of cardiac I_{Na} or if this type of regulation is also characteristic of Na^+ channels of neuronal and/or skeletal muscle origin. Dopamine, another catecholamine, has been reported to reduce Na^+ current in dorsal rat ganglion cells (27).

Catecholamines may inhibit cardiac I_{Na} and, consequently, conduction velocity and excitability in circumstances that are likely to be of great relevance clinically. During the acute phase of myocardial ischemia external K^+ concentrations of ~15 mM have been reported in the ischemic zone (13, 15, 24). The resulting depolarization of heart cells to between −60 and −50 mV partly inactivates cardiac Na^+ channels, and the residual Na^+ channels that are still available play an essential role in the generation of cardiac impulses and conduction (2, 9). The norepinephrine that is released immediately after myocardial ischemia from sympathetic nerve terminals (10, 26, 33) might further depress the residual Na^+ current. Furthermore, local differences in catecholamine concentrations within the ischemic zone combined with the fast-acting direct pathway might result in additional inhomogeneities causing excitability to become nonuniform, which is an important prerequisite for the initiation and maintenance of ventricular tachyrhythmia (19). Our hypothesis would help explain the correlation between a high level of catecholamines and a greater risk of severe arrhythmias (20, 31) during acute myocardial infarction.

NOTE ADDED IN PROOF

While our paper was being prepared to go to press, Ono et al. (27a) published results that confirm the indirect effect we observed.

We thank Patricia Neal and Theresa Afinni for expert technical assistance with the tissue culture, and J. Breedlove, V. Price, and D. Witham for secretarial assistance.

This work was supported by National Heart, Lung, and Blood Institute Grants HL-36930 and HL-37044 and the American Heart Association (Texas Affiliate).

Permanent address of B. Schubert: Div. of Cellular and Molecular Cardiology, Central Institute for Cardiovascular Research, Academy of Sciences of the GDR, Berlin-Buch, GDR.

Address for reprint requests: A. Brown, Dept. of Physiology and Molecular Biophysics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

Received 19 June 1989; accepted in final form 10 November 1989.

REFERENCES

1. ARITA, M., I. HISATOME, K. ONO, AND T. KIYOUSE. Beta-receptor mediated inhibition of cardiac sodium current and conduction (Abstract). *Proceedings of U.S.-Japan Seminar on Mechanisms Regulating Initiation and Conduction of the Cardiac Impulse.* Okazaki, Japan: National Inst. Physiol. Sci., 1988.
2. ARITA, M., T. KIYOSME, M. AORNINE, AND S. IMANISHI. Nature of "residual fast channel" dependent action potentials and slow conduction in guinea pig ventricular muscle and its modification by isoproterenol. *Am. J. Cardiol.* 51: 1433–1440, 1983.
3. CACHELIN, A., J. DE PEYER, S. KOKUBUN, AND H. REUTER. Sodium channels in cultured cardiac cells. *J. Physiol. Lond.* 340: 389–401, 1983.
4. EBIHARA, L., AND C. JECK. Spontaneous loss of inactivation in rat neonatal heart cells studied using the whole-cell voltage clamp technique (Abstract). *Biophys. J.* 53: 162a, 1988.
5. FABIATO, A., AND F. FABIATO. Calculator programs for computing the composition of the solutions containing multiple metals and ligands used in skinned muscle cells. *J. Physiol. Paris* 75: 463–505, 1979.
6. FENWICK, E., A. MARTY, AND E. NEHER. Sodium and calcium channels in bovine chromaffin cells. *J. Physiol. Lond.* 331: 599–635, 1982.
7. FERNANDEZ, J. M., A. P. FOX, AND S. KRASNE. Membrane patches and whole-cell membranes: a comparison of electrical properties in rat clonal pituitary (GH_3) cells. *J. Physiol. Lond.* 356: 565–585, 1984.
8. FOLLMER, C. H., R. C. TENEICK, AND J. Z. YEH. Sodium current kinetics in cat atrial myocytes. *J. Physiol. Lond.* 384: 169–197, 1987.
9. FOZZARD, H. A., AND J. C. MAKIELSKI. The electrophysiology of acute myocardial ischemia. *Annu. Rev. Med.* 36: 275–284, 1985.
10. GOLDSTEIN, D. S. Plasma norepinephrine as an indication of sympathetic neural activity in clinical cardiology. *Am. J. Cardiol.* 48: 1147–1154, 1981.
11. GRANT, A. O., C. F. STARMER, AND H. C. STRAUSS. Unitary sodium

channels in isolated cardiac myocytes of rabbit. *Circ. Res.* 53: 823–829, 1983.
12. HAMILL, O. P., A. MARTY, E. NEHER, B. SAKMANN, AND F. J. SIGWORTH. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* 391: 85–100, 1981.
13. HILL, J. L., AND L. S. GETTES. Effects of acute coronary artery occlusion on local myocardial extracellular K^+ activity in swine. *Circulation* 61: 768–778, 1980.
14. HILLE, B. Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. *J. Gen. Physiol.* 69: 497–515, 1977.
15. HIRCHE, H. J., C. H. FRANZ, L. BÖS, R. BISSIG, AND M. SCHARMANN. Myocardial extracellular K^+ and H^+ increase and noradrenaline release as possible cause of early arrhythmias following acute coronary artery occlusion in pigs. *J. Mol. Cell. Cardiol.* 12: 579–593, 1980.
16. HISATOME, I., T. KIYOSME, S. IMANISHI, AND M. ARITA. Isoproterenol inhibits residual fast channel via stimulation of β-adrenoceptors in guinea-pig ventricular muscle. *J. Mol. Cell. Cardiol.* 17: 657–665, 1985.
17. HODGKIN, A. L., AND A. F. HUXLEY. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. Lond.* 117: 500–544, 1952.
18. HUME, J. R., AND W. GILES. Active and passive electrical properties of single bullfrog atrial cells. *J. Gen. Physiol.* 78: 19–42, 1981.
19. JANSE, M. J., F. J. L. VAN CAPELLE, H. MORSINK, A. G. KLEBER, F. WILLMS-SCHOPMAN, R. CARDINAL, C. N. D'ALNONCOURT, AND D. DURRER. Flow of "injury" current and patterns of excitation during early ventricular arrhythmias in acute regional myocardial ischemia in isolated porcine and canine hearts. *Circ. Res.* 47: 151–165, 1980.
20. JEWITT, D. E., C. J. MERCER, D. REID, C. VALORI, M. THOMAS, AND J. P. SCHILLINGFORD. Free noradrenaline and adrenaline secretions in relation to the development of cardiac arrhythmias and heart failure in patients with acute myocardial infarction. *Lancet* 1: 635–641, 1969.
21. KAMEYAMA, M., F. HOFMANN, AND W. TRAUTWEIN. On the mechanism of β-adrenergic regulation of the calcium channel in the guinea pig heart. *Pfluegers Arch.* 405: 285–293, 1985.
22. KHODOROV, B. I. Sodium inactivation and drug-induced immobilization of the gating charge in nerve membrane. *Prog. Biophys. Mol. Biol.* 37: 49–89, 1981.
23. KIRSCH, G. E., AND A. M. BROWN. Kinetic properties of single sodium channels in rat heart and rat brain. *J. Gen. Physiol.* 93: 85–99, 1989.
24. KLEBER, A. G. Resting membrane potential, extracellular potassium activity, and intracellular sodium activity during acute global ischemia in isolated perfused guinea pig hearts. *Circ. Res.* 52: 442–450, 1983.
25. KUNZE, D. L., A. E. LACERDA, D. L. WILSON, AND A. M. BROWN. Cardiac Na currents and the inactivating, reopening and waiting properties of single cardiac Na channels. *J. Gen. Physiol.* 86: 691–719, 1985.
26. LAMMERANT, J., P. DEHERT, AND C. DESCHRYVER. Direct release of catecholamine into the left heart chambers. The enhancing effect of acute coronary occlusion. *Arch. Int. Pharmacodyn. Ther.* 163: 219–229, 1966.
27. MARCHETTI, C., E. CARBONE, AND H. D. LUX. Effects of dopamine and noradrenaline on Ca channels of cultured sensory and sympathetic neurons of chick. *Pfluegers Arch.* 406: 104–111, 1986.
27a. ONO, K., T. KIYOSUE, AND M. ARITA. Isoproterenol, DBcAMP, and forskolin inhibit cardiac sodium current. *Am. J. Physiol.* 256 (*Cell Physiol.* 25): C1131–C1137, 1989.
28. POWELL, T., AND V. W. TWIST. A rapid technique for the isolation and purification of adult cardiac muscle cells having respiratory control and a tolerance to calcium. *Biochem. Biophys. Res. Commun.* 72: 327–333, 1976.
29. REUTER, H. Calcium channel modulation by neurotransmitters, enzymes and drugs. *Nature Lond.* 301: 569–574, 1983.
30. SCHUBERT, B., A. M. J. VANDONGEN, G. E. KIRSCH, AND A. M. BROWN. β-adrenergic inhibition of cardiac sodium channels by dual G protein pathway. *Science Wash. DC* 245: 516–519, 1989.
31. VIDEBAEK, J., N. J. CHRISTENSEN, AND B. STERNDORFF. Serial determination of plasma catecholamines in myocardial infarction. *Circulation* 46: 846–855, 1972.
32. WINDISCH, M., AND H. TRITTHART. Isoproterenol, norepinephrine and phosphodiesterase inhibitors are blockers of the depressed fast Na^+-system in ventricular muscle fibers. *J. Mol. Cell. Cardiol.* 14: 431–434, 1982.
33. WOLLENBERGER, A. M., AND L. SHAHAB. Anoxia-induced release of noradrenaline from the isolated perfused heart. *Nature Lond.* 207: 88–91, 1965.
34. YATANI, A., AND A. M. BROWN. Rapid β-adrenergic modulation of cardiac calcium channel currents by a fast G protein pathway. *Science Wash. DC* 245: 71–74, 1989.
35. YATANI, A., J. CODINA, Y. IMOTO, J. P. REEVES, L. BIRNBAUMER, AND A. M. BROWN. A G protein directly regulates mammalian cardiac calcium channels. *Science Wash. DC* 238: 1288–1292, 1987.
36. YATANI, A., Y. IMOTO, J. CODINA, S. L. HAMILTON, AND A. M. BROWN. The stimulatory G protein of adenylyl cyclase, G_s, also stimulates dihydropyridine-sensitive Ca^{2+} channels. *J. Biol. Chem.* 263: 9885–9895, 1988.
37. YATANI, A., G. E. KIRSCH, L. D. POSSANI, AND A. M. BROWN. Effects of new world scorpion toxins on single-channel and whole cell cardiac sodium currents. *Am. J. Physiol.* 254 (*Heart Circ. Physiol.* 23): H443–H451, 1988.
38. YATANI, A., D. L. KUNZE, AND A. M. BROWN. Effects of dihydropyridine calcium channel modulators on cardiac sodium channels. *Am. J. Physiol.* 254 (*Heart Circ. Physiol.* 23): H140–H147, 1988.