Effect of Extracellular ATP on the Na⁺ Current in Rat Ventricular Myocytes

Frédérique Scamps, Guy Vassort

Abstract
Extracellular ATP concentration can rise because of its release by nerve terminals and by damaged cells during ischemia. After the activation of P₂-purinergic receptors, ATP induces a positive inotropic effect and increases the L-type Ca²⁺ current via activation of a G protein but without cAMP production. In addition, ATP shifts the voltage characteristics of Ca²⁺ current toward hyperpolarized potentials. If ATP produced similar effects on the Na⁺ current (I_na), this compound should also affect cardiac excitability and conduction. Using the whole-cell patch clamp to record I_na in rat ventricular cells, we show that extracellular application of ATP induced hyperpolarizing shifts in the current-voltage relation and the availability of I_na. The ED₅₀ for the shifts in both conductance and availability was obtained with 0.7 μmol/L ATP. Maximal shifts in conductance and availability were respectively 9.7±0.6 and 10.6±0.7 mV. The leftward shift of the availability curve is responsible for the decrease of I_na amplitude at less polarized holding potentials. These effects were not cholina toxin sensitive and thus cannot be attributed to activation of the Gₐ protein. At 100 μmol/L, ATP⁺ and α,β-methylene ATP could induce shift, whereas UTP and β,γ-methylene ATP as well as ADP and adenosine were without effect. Thus, depending on the resting membrane potential, ATP should either enhance excitability or favor slow conduction and weaken cardiac electrical homogeneity and consequently favor arrhythmia. (Circ Res. 1994;74:710-717.)

Key Words • Na⁺ current • P₂-purinergic receptors • ATP

The Na⁺ current (I_na) gives rise to the fast initial depolarization of the cardiac action potential and is responsible for conduction in healthy cardiac tissues. For many years, pharmacologic studies of this current have focused on the effects of the so-called "class I" antiarrhythmic agents, since I_na is responsible in large part for rhythm disturbance.¹ More recently, it has emerged that this current, like the Ca²⁺ current (I_Ca), could be hormonally regulated, since putative sites for phosphorylation by protein kinase A and C are present on the α subunit, the pore-forming subunit.² In agreement with this concept, several laboratories have reported that β-adrenergic agonists modulate I_na amplitude through protein kinase A-dependent phosphorylation and through a direct coupling with a G protein³ that might also govern a leftward shift of I_na kinetics. Depending on the value of holding potential, this leads to either an increase or a decrease in I_na amplitude.⁴⁻⁶ It was also shown that angiotensin II, probably through protein kinase C-induced phosphorylation, enhanced the open probability of Na⁺ channels.⁷⁻⁸ ATP is released during stimulation of both sympathetic and parasympathetic nerves and is an active neurotransmitter. Besides, the local extracellular purine nucleotide concentration can temporarily exceed 100 μmol/L after local trauma, including ischemia, as a consequence of release from various cell types.⁹ Since the classical observations of Drury and Szent-Gyorgyi,¹⁰ it is known that adenosine and ATP exert electrophysiologic effects on the mammalian heart. Recently, it has been shown that activation of P₂-purinergic receptors by external application of ATP in the micromolar range increases the I_Ca amplitude, which leads to a positive inotropism in rat ventricle.¹¹⁻¹³ We subsequently demonstrated that the P₂-purinergic receptors are linked to a G protein, which most probably directly activates the Ca²⁺ channel without variation in cAMP levels.¹⁴ The increase in I_Ca was more marked at weak depolarizations because of a shift toward hyperpolarized potentials of the current-voltage and the availability relations.¹⁵ If similar effects of ATP occurred in the Na⁺ channel, they would markedly affect cardiac excitability. Thus, this prompted us to investigate the effect of the P₂-purinergic receptors on I_na. A particular attention was focused on the time-dependent shift of I_na kinetics. Our results show that ATP induces a significant shift toward hyperpolarized potentials of both the Na⁺ conductance and the availability curves in rat cardiac myocytes.

Materials and Methods

Cell Isolation
Single ventricular cells were isolated from Wistar rat hearts according to Reference 16. Briefly, a rat weighing 160 to 250 g was anesthetized with ethyl carbamate; the heart was removed and cannulated through the aorta on a Langendorff apparatus warmed at 37°C. The heart was perfused for 5 minutes with a Ca²⁺-free Tyrode's solution, and then perfusion was switched in a Ca²⁺-free Tyrode's solution containing collagenase (1.2 mg/mL, Boehringer type A) for 1 hour. Cells were stored in Petri dishes in a Ca²⁺-containing Tyrode's solution at 37°C. Composition of the Tyrode's solution was (mmol/L) NaCl 117, KCl 5.7, NaHCO₃ 4.4, KH₂PO₄ 1.5, MgCl₂ 1.7, CaCl₂ 1, HEPES 21.1, glucose 11.7, creatine 10, and taurine 20. pH was adjusted at 7.6 at room temperature.

Solutions and Drugs
The cells were superfused with a low Na⁺-HEPES solution of the following composition (mmol/L): NaCl 10, N-methyl-D-
glucose 127, CaCl2 20, CaCl2 1.8, MgCl2, 1.8, NaHCO3 10, and HEPES 10 (pH 7.4 with HCl). In most of the experiments, 100 μmol/L Cd2+ was added to block IK and further reduce INa (see below). In some experiments, the Na+ concentration was reduced to 3 mmol/L, and then no Cd2+ was added. Experiments were performed at room temperature (21±1°C). The internal solution in the patch electrode (soft glass, 0.5 to 0.8 MΩ) contained (mmol/L) CsCl 120, MgCl2 6, Na2-ATP 5, Tris-creatine phosphate 5, Na2-GTP 0.4, Cs2-EGTA 5, CaCl2 0.062 (free Ca2+, 2×10−3 mol/L), and HEPES 20 (pH 7.2 with CsOH).

α,β-Methyleneadenosine 5‘-triphosphate (αβ-metATP), β,γ-methyleneadenosine 5‘-triphosphate (βγ-metATP), adenosine, and ADP were from Sigma Chemical, France; ATP, ATP-γ-S, GTP, GTP-γ-S, ADP-β-S, and UTP were from Boehringer Mannheim.

**INa Measurements and Data Analysis**

Voltage-clamp experiments were performed using the whole-cell recording method. As a routine protocol for INa recordings, the holding potential was maintained at −80 mV, a prepulse to −130 mV for 150 milliseconds was applied before depolarization to −40 or −55 mV for 50 milliseconds, and then the cell was repolarized to −80 mV. The frequency was 0.5 Hz. For current-voltage and inactivation curves, the values of the test pulse and conditioning pulse were varied, respectively. The amplitude of INa was measured as the difference between peak inward current and current at the end of the 50-millisecond pulse and recorded on-line. Currents were filtered at 10 kHz, digitized at 15 kHz, and analyzed by a Tandon computer 486/33. The leakage current was small, and in most experiments, no leakage correction was necessary. Determination of membrane capacitance (Cm) was calculated according to the following equation: Cm=Ct·Io/ΔEm[1−(Ia/Io)], where Ct is the time constant of Cm, Io is maximum capacitance current value, ΔEm is the amplitude of voltage step (2 mV), and Ia is the amplitude of steady-state current (negligible in our conditions because of K+ current blockade by Cs+). Mean Cm was 95±19 pF, and mean Ct was 554±176 microseconds (n=49). Series resistance (Rs) was calculated as follows: Rs=ΔEm/Io. Mean Rs was 5.5±2.0 MΩ (n=49). In 10 mmol/L extracellular Na+ (n=10) or 10 μmol/L Cd2+ (n=10), a conditioning prepulse of 5 minutes was applied to −60 mV and totally suppressed by 6 μmol/L tetrodotoxin; no T-type IC could be observed as previously reported on this preparation. The experimental conditions (10 mmol/L extracellular Na+ plus 100 μmol/L Cd2+, or 3 mmol/L extracellular Na+) were designed to allow a reasonable voltage control by reducing INa amplitude. Addition of 100 μmol/L Cd2+ induced a 31±5% decrease of INa amplitude measured at −40 mV, which, besides inhibiting IK, enabled us to get a smooth voltage dependence of the current-voltage relation, a criterion of reasonable membrane voltage control, and demonstrated the stability of the inactivation curve (Fig 1). Voltage giving half inactivation (V1/2) was −92.6±2.8 mV in control (n=11) and −93.5±2.2 mV in the presence of Cd2+ (n=13); the values were not significantly different. It must be pointed out that Na+ outward currents could not be detected with accuracy because of a too fast activation compared with capacity transient under our experimental conditions.

**Statistics**

Results are given as mean±SD. A paired t test was used for significance, and a value of P<0.05 was considered statistically significant.

**Results**

**Effect of ATP on INa Amplitude**

In a first series of experiments, after the patch was broken, a cell was repeatedly depolarized for 50 milli-seconds every 2 seconds to −40 mV from a −90-mV holding potential during a 5-minute period to estimate current rundown attributable to the spontaneous shift in voltage dependence of INa recorded in 10 mmol/L extracellular Na+ in the presence of 100 μmol/L Cd2+. At this value of holding potential, which gave roughly 30% to 40% of available Na+ channels, a slight decrease in INa amplitude was usually observed as shown in Fig 2A. This reduction in INa was a result of the background shift in kinetics usually observed for this current. The application of 10 μmol/L ATP induced a much faster decrease to less than half peak value. A steady state was reached after 1 minute. On removal of ATP, INa did not recover its initial amplitude.

Fig 2B shows, on a different cell, the effect of external application of 0.3 and 1 μmol/L ATP on INa amplitude recorded every 2 seconds in the same solution as in Fig 2A. A conditioning prepulse to −130 mV for 150 milliseconds was first applied to ensure full availability of INa. INa was elicited by depolarizations to −40 mV (largest amplitude on the record) or to −55 mV (small-
Fig 2. Plots showing the effect of external application of ATP on Na⁺ current amplitude. A, Effect of ATP on Na⁺ current amplitude is shown at a depolarized holding potential. Holding potential was maintained at −90 mV without prepulse to −130 mV. The cell was depolarized for 50 milliseconds to −40 mV every 2 seconds. As seen from Figs 1B and 4B, roughly 30% to 40% of the Na⁺ current was available at a holding potential of −90 mV. Membrane capacitance was 90 pF. B, Effect of ATP on Na⁺ current amplitude is shown at a hyperpolarized holding potential. At the top of panel B, the same voltage-clamp protocol as in Fig 1A is shown, except that the test pulse was set at −55 mV to estimate the evolution of the spontaneous shift in kinetics for 14 minutes. Membrane capacitance was 85 pF. At the bottom of panel B, the test pulse was set at −40 or −55 mV as indicated. Dashed lines give the control amplitude level at −55 or −40 mV. See the text for further details. Membrane capacitance was 95 pF.

et amplitude on the record). Depolarizations to −55 mV were chosen to better evaluate the possible spontaneous shift in the voltage dependence of Iₙa. Indeed, because of the slope of the current-voltage curve, this potential range is a very sensitive shift index. The top of Fig 2B is an example of Iₙa amplitude recordings at −55 mV for 14 minutes in a control cell, which allows for comparison on the same time scale, but on a different cell, of the effect of ATP application (bottom of Fig 2B). Usually, when, after 2 to 3 minutes of recordings, the amplitude of Iₙa was nearly stable at −55 mV, ATP was applied. As seen in Fig 2B, bottom, 0.3 and 1 μmol/L ATP induced a progressive increase in the amplitude of Iₙa, measured at −55 mV. On coming back to −40-mV test depolarizations, Iₙa was also increased under ATP but to a lesser extent. The time course of increase in Iₙa amplitude under ATP corresponded to the time over which the effect occurred, since solution changes were performed in <2 seconds. The effect was dependent on the ATP concentration; it was faster with higher ATP concentrations, with the half-maximal effect being obtained after 90 seconds with 1 μmol/L and 15 seconds with 10 μmol/L.

Effect of ATP on the Iₙa-Voltage Relation, Conductance, and Availability

The current-voltage relations obtained from the above experiment are shown in Fig 3. Under control conditions, the threshold for Iₙa activation was −55 mV, the maximal peak current was −35 mV, and the Na⁺ reversal potential (Eₜ), extrapolated from the ascending branch of the current-voltage curve, was between −5 and 0 mV (expected from 11 mmol/L intracellular Na⁺). In the presence of 1 μmol/L ATP, the Iₙa-voltage relation was shifted to the left: the threshold for activation was between −65 and −60 mV, the maximal peak current was −40 mV, and Eₜ was not changed. As expected from a shift of conductance toward hyperpolarized potentials, the maximal amplitude of Iₙa was increased. The shift and the increase in maximal Iₙa amplitude were not reversible. The shift was not a “spontaneous” shift of the current-voltage curve since, as stated above, experiments were performed only when current measured at −55 mV was stable or increased.
very slowly, i.e., when shift was negligible. As shown in Fig 2 (see also Fig 7), there is a fast and clear change in the slope of the $I_{Na}$ amplitude time course linked to ATP application.

Fig 4 shows the availability and conductance curves from the same experiment. In this cell, 1 μmol/L ATP induced an 8.8-mV shift of the availability curve and a 6.3-mV shift of conductance toward hyperpolarized potentials. Table 1 gives the mean parameters of $I_{Na}$ conductance and availability recorded in 10 mmol/L extracellular Na$^+$ containing 100 μmol/L Cd$^{2+}$. On three cells, it was found that a $-130$-mV prepulse was enough to ensure full availability of $I_{Na}$ by comparing the availability curves performed with a $-130$- or a $-150$-mV prepulse in the control condition and in the presence of ATP. No differences were observed between these two protocols, nor was the effect of ATP modified. The dose-response curves for the shift in $V_{1/2}$ of $I_{Na}$ conductance and availability under ATP application are shown in Fig 5. ATP did not significantly change the slope factors or the maximal conductance. From the fit of the data according to $y = B_{max} \times [\text{ATP}]^n/(\text{ATP} + K_{1/2})^n$, the ATP concentrations for half-maximal effects ($K_{1/2}$) were 0.63±0.1 and 0.79±0.1 μmol/L for $I_{Na}$ availability and conductance shifts, respectively. These values of $K_{1/2}$ were not significantly different. The Hill slopes ($n$) were 1.3 and 1.2 for availability and conductance shifts, respectively. The maximal shift ($B_{max}$) in $I_{Na}$ availability was 10.6±0.7 mV and in conductance was 9.7±0.6 mV.

**ATP Effects in the Absence of Cd$^{2+}$**

ATP is known to bind divalent cations. To check whether the presence of Cd$^{2+}$ ions in the bath solution could interfere with ATP effects, experiments were conducted in the absence of Cd$^{2+}$. To be confident of voltage control during the maximal surge of $I_{Na}$, the experiments were performed in a 3 mmol/L external Na$^+$ solution. Table 1 gives the mean characteristics of $I_{Na}$ conductance and availability in 3 mmol/L external Na$^+$. Under this experimental condition, 1 μmol/L ATP induced a similar shift in the $I_{Na}$ conductance ($7.4±1.8$ mV, $n=4$) and consequently a similar increase in maximal $I_{Na}$ amplitude (Fig 6). The second hump in the current-voltage relation was due to $I_{Ca}$, which was increased by ATP mainly via activation of a $G_o$ pro-

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**Fig 4.** Effect of ATP on Na$^+$ current ($I_{Na}$) availability and conductance. A, Current tracings corresponding to the availability curve at indicated conditioning voltages are shown. B, Availability curve ($l_{max}$) was fitted according to the Boltzmann equation: $l_{max} = 1/(1+\exp[(V-V_{1/2})/s])$, where $V$ is the test potential, $l_{max}$ is the potential giving half-maximal effect, and $s$ is the slope factor. $V_{1/2}$ was $-91.7$ and $-100.5$ mV and $s$ was 6 and 5.6 mV in the control condition (a) and in the presence of ATP (A), respectively. Conductance $[G/G_{max} = l_{max}/(V-V_{m})]$, where $V_{m}$ is reversal potential) was fitted according to the Boltzmann equation: $G/G_{max} = 1/(1+\exp[-(V-V_{1/2})/s])$. $V_{1/2}$ was $-42.6$ and $-48.9$ mV and $s$ was 5 and 4.9 mV in the control condition (a) and in the presence of ATP (A), respectively. Data are for the same cell as in Fig 2B, bottom, and Fig 3.

**Fig 5.** Dose-response curves of ATP for conductance and availability shifts. Curves were fitted according to a sigmoid (see text). Shifts of conductance (c) and availability (m) are shown. Values are mean±SD; $P<.05$ for all values. The number of experiments is in parentheses.

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**TABLE 1. Na$^+$ Current Conductance and Availability Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>10 mmol/L Na$^+$</th>
<th>3 mmol/L Na$^+$</th>
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<tbody>
<tr>
<td>Conductance</td>
<td></td>
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</tr>
<tr>
<td>$V_{1/2}$, mV</td>
<td>$-42.4±4.3$</td>
<td>$-46.9±2.5$</td>
</tr>
<tr>
<td>$s$, mV</td>
<td>$4.8±0.6$</td>
<td>$3.7±0.3$</td>
</tr>
<tr>
<td>$n$</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>Availability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{1/2}$, mV</td>
<td>$-93.5±2.2$</td>
<td>$-93.2±3.6$</td>
</tr>
<tr>
<td>$s$, mV</td>
<td>$6.0±0.3$</td>
<td>$6.6±0.9$</td>
</tr>
<tr>
<td>$n$</td>
<td>13</td>
<td>6</td>
</tr>
</tbody>
</table>

$Na_{in}$ indicates extracellular Na$^+$; $V_{1/2}$, potential giving half-maximal conductance or availability; $s$, slope factor; and $n$, the number of experiments. Values are mean±SD. Parameters were obtained from fits to the Boltzmann equation.
Cd\(^{2+}\), but they did not shift kinetic parameters. Thus, it appeared that the thio-containing compounds could induce an extra effect on \(I_{\text{Na}}\) amplitude when recorded in the presence of Cd\(^{2+}\). On the one hand, in the present study Cd\(^{2+}\) was used for its blocking properties on \(I_{\text{Na}}\) and \(I_{\text{Ca}}\); on the other hand, Cd\(^{2+}\) appears to have a high affinity for sulfur and easily forms disulfur bridges. In agreement with these properties, the dose-response curve of the Cd\(^{2+}\)-blocking effect on \(I_{\text{Na}}\) amplitude was shifted to the right in the presence of 100 \(\mu\)mol/L ATP\textsubscript{S} (half-block occurred at 138 instead of 77 \(\mu\)mol/L Cd\(^{2+}\)). Direct measurements of free Cd\(^{2+}\) using a polarographic method\(^{20}\) show that ATP\textsubscript{S} effectively chelated Cd\(^{2+}\) in solution, which accounted for the decrease of the blocking effect of Cd\(^{2+}\) on \(I_{\text{Na}}\). It was also shown that Cd\(^{2+}\) binding to ATP was negligible in the presence of millimolar Mg\(^{2+}\) and Ca\(^{2+}\) ions. In the absence of Cd\(^{2+}\), the effects of ATP\textsubscript{S} were similar to those of ATP: 100 \(\mu\)mol/L ATP\textsubscript{S} induced a shift in availability (6.6±1.8 mV) and a slight increase in the maximal peak \(I_{\text{Na}}\) related to the shift in conductance (18.2±8.5% increase) (\(n=7\)). At 100 \(\mu\)mol/L, \(\alpha\beta\)-metATP induced a 4.6±0.8 mV shift in availability (\(n=6\)).

**Effect of Cholera Toxin Treatment**

The P\(_2\)-purinergic receptors have been shown to increase the L-type \(I_{\text{Ca}}\) via activation of a G protein that is sensitive to cholera toxin.\(^{15}\) To know whether the effects of external ATP on \(I_{\text{Na}}\) were also mediated through activation of a G protein, the cells were incubated with 10 \(\mu\)g/mL cholera toxin for at least 3 hours at 37°C. For each cell, the efficiency of cholera toxin treatment was evaluated by measuring \(I_{\text{Na}}\) amplitude before the experiment. Treatment was considered efficient when \(I_{\text{Ca}}\) density was \(\approx\)20 pA/pF, i.e., threefold the basal density.\(^{15}\) In every case, it was also checked that nontreated cells isolated from the same heart were responsive to ATP. Fig 7 shows that, as for control cells, application of 1 \(\mu\)mol/L ATP induced a progressive increase in \(I_{\text{Na}}\) amplitude measured at \(-55\) mV in 10 mmol/L extracellular Na\(^{+}\) in the presence of 100 \(\mu\)mol/L Cd\(^{2+}\). Fig 8 shows the corresponding current-voltage relations, availability, and conductance curves and confirms that cholera toxin treatment did not prevent the shifts in these curves. However, a leftward shift in \(E_{\text{rev}}\) was always observed under ATP application, which counteracted the driving force effect of conductance shift on maximal \(I_{\text{Na}}\) amplitude. Table 2 summarizes the effects of different concentrations of ATP on the shifts of availability, conductance, and \(E_{\text{rev}}\) observed on cholera toxin–treated cells. The shifts of the activation and inactivation parameters were of similar magnitude as in control cells at all ATP concentrations.

**Discussion**

In the present study, we report that external application of ATP in the micromolar range alters \(I_{\text{Na}}\) because of a leftward shift in both activation and availability characteristics. Consequently, the effect of ATP on \(I_{\text{Na}}\) amplitude depends on the holding potential; at hyperpolarized potentials, an increase in \(I_{\text{Na}}\) was observed that was due to the shift in activation, whereas \(I_{\text{Na}}\) was reduced at depolarized potentials consequent to reduced availability. The shift in availability was not due
to a "spontaneous" shift, which under our experimental conditions was <0.7 mV/min or often absent after a short period of stabilization after patch breaking. Such a slow spontaneous shift has been reported. ATP also induced a shift in the current-voltage relation, which had a kinetic of onset clearly faster than the spontaneous shift occurring in this parameter (see Figs 2 and 7).

We already reported several effects of ATP on myocardial cells. Among them, it was described that P2-purinoceptor stimulation increases L-type I_{Ca} that involves a G_{i} protein without cAMP production. The increase in I_{Ca} was due to both a shift in conductance and to an increase in the maximal opening probability of the Ca^{2+} channel. However, the shift accounted for a small amount of the observed increase in I_{Ca}, with the major part of the increase being attributed to the activation of a G_{i} protein by ATP. Furthermore, this major part of I_{Ca} increase was reversible, whereas the shift in kinetics was not. It should be noticed that, after cholera toxin treatment to fully activate G_{i} protein, the addition of ATP still induced a minute increase in I_{Ca}, which, under this experimental condition, could be solely attributed to the leftward shift in the Ca^{2+} conductance. Unlike I_{Ca}, the increase in maximal I_{Na} under ATP is entirely attributable to the shift in conductance. Maximal current thus occurs at more negative membrane potential, and the consequent increase in the electrochemical driving force (up to 10 mV) is sufficient to account for the maximal 25% increase in peak current with 10 mmol/L extracellular Na^{+} and E_{rev} of 0 mV. We propose that ATP-induced hyperpolarizing shifts in I_{Na} and I_{Ca} conductance and availability rely on a common mechanism of action. Because I_{Ca} kinetics do not shift during the course of an experiment under patch-clamp conditions, we are confident that this effect of ATP on kinetics is representative of a physiological process. The understanding of this process should help to clarify its apparent irreversibility in isolated cells.

Rather similar effects to those of ATP on I_{Na} conductance and availability were reported with &-adrenergic stimulation. However, the shifts we observed under external ATP application were not cholera toxin sensitive, which excludes the involvement of cAMP and of a G_{i} protein. In these experiments, ATP also induced a shift in E_{rev} of I_{Na} toward negative potentials, which produced a decrease in the driving force for Na^{+} ions, an effect that counteracted the conductance shift effect on maximal I_{Na} amplitude. The shift in E_{rev} is probably related to a change in internal Na^{+}, with a 3- to 4-mmol/L increase in internal Na^{+} accounting for the 6- to 8-mV shift we obtained. Such an increase in internal Na^{+} could be localized at the membrane after a hypothetical ATP-induced negative charge increase.

The increase in internal Na^{+} could also be due to activation of the Na-H antiport reported under P2-purinoceptor stimulation. Such a shift in E_{rev} was also observed in two control cells, with the consequent lack of I_{Na} increase. The reason that this effect is better seen on cholera toxin-treated cells is unknown. However, cholera toxin leads to an increased level of cAMP through the activation of G_{i} proteins; thus, one can assume cross talk of P2-purinoceptors with several cAMP effectors.

In the present study, we show that in rat ventricular cells Cd^{2+} ions exert an essentially voltage-independent blocking effect of I_{Na}. This result is in agreement with the ones reported in other mammalian cardiomyocytes. A change in the potency of Cd^{2+} to inhibit I_{Na} was observed with thio-containing agents such as ATPγS, GTPγS, and ADPβS. Because the Cd^{2+} block

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**Table 2. Effect of ATP on Cholera Toxin-Treated Cells**

<table>
<thead>
<tr>
<th>ATP, μmol/L</th>
<th>0.3</th>
<th>1</th>
<th>10</th>
<th>100</th>
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<tr>
<td>Availability shift, mV</td>
<td>3.8</td>
<td>6.1±1.2</td>
<td>13.1±3.5</td>
<td>14.9±4.0</td>
</tr>
<tr>
<td>Conductance shift, mV</td>
<td>2</td>
<td>6.5±2.0</td>
<td>9±1</td>
<td>13.9±1.6</td>
</tr>
<tr>
<td>E_{rev} shift, mV</td>
<td>1.5</td>
<td>6.3±1.0</td>
<td>7.6±0.3</td>
<td>7.3±1.4</td>
</tr>
<tr>
<td>n</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

E_{rev} indicates reversal potential; n, the number of experiments. The values of leftward shifts induced by ATP are expressed as mean±SD.

Changes are significant at 1, 10, and 100 μmol/L ATP (P<.05).
eliminate phosphorylation by protein kinase A and the \( G_s \) protein, since similar shifts were obtained after cholera toxin treatment. The Na\(^+\) channel protein has phosphorylation sites for protein kinase C. A P\(_2\)-purinergic stimulation of protein kinase C can be expected, since phosphatidylinositol turnover is accelerated.\(^{13,28}\)

However, phosphorylation by itself on the internal face of the membrane should lead to a rightward shift of the kinetic parameters.\(^{29}\) On the other hand, phosphorylation of sites on the external face of the membrane might lead to a leftward shift. However, the activation of an ectokinase is unlikely, since the lack of Mg\(^{2+}\) ions did not prevent the shifts and since ATP\(\gamma S\) was less effective than ATP. In heart, besides activating the phosphoinositide pathway, P\(_2\)-purinergic receptors induce prostaglandin synthesis.\(^{30}\) In other cell types, external ATP induces the release of prostacyclin.\(^{31}\) It is quite possible that P\(_2\)-purinergic receptors acting on phospholipase C or \( A_1 \) induce a change in the membrane phospholipid composition that leads to the apparent charge effect we report. The sequence of agonist efficacy in inducing shifts in \( I_{Na} \) voltage characteristics is ATP > ATP\(\gamma S\) > \( \alpha \beta\)-metATP > \( \beta \gamma\)-metATP; this does not fit with a P\(_{2\alpha}\) or a P\(_{2\gamma}\)-purinergic receptor.\(^{32}\) The same conclusion was reached concerning the stimulation of phosphoinositide hydrolysis by P\(_2\)-purinergic receptor agonists\(^{28}\) in ventricular myocytes of fetal mice. Another possibility relates to the fact that ATP is a negatively charged molecule. The leftward shifts induced by ATP could thus be a direct charge effect similar to the ones obtained with negatively charged amphiphiles.\(^{33}\) However, other triphosphate compounds, UTP, ITP, and GTP, although similarly charged, did not alter \( I_{Na} \) characteristics. It should be noticed that a leftward shift of the Na\(^+\) availability curve is also obtained with local anesthetics, including quaternary amines, which are positively charged, as well as when adding salicylate ions both externally and internally.\(^{34}\) One can thus propose that the leftward shift is related to the incorporation of ATP in the membrane consequent to its fixation to a recognition site. A change in the surface charges can be expected after perturbation of the phospholipid distribution in the bilayer: by altering the crossbridging of two phospholipid heads by Ca\(^{2+}\) ions due to the interposition of ATP, Ca\(^{2+}\) ions are substituted on the negative binding sites by Na\(^+\), or another monovalent element, with less screening effects.

Significant pathophysiological consequences of the ATP-induced shifts in \( I_{Na} \) characteristics are to be expected. Because of the shift in conductance, ATP should reduce the threshold of excitability that would favor conduction and cardiac homogeneity on highly hyperpolarized cells. However, on cells with a resting membrane potential such that \( I_{Na} \) availability is not maximal, the ATP-induced shifts in availability would further reduce \( I_{Na} \), which should lead to a reduced \( I_{Na} \) and thus to less electrical coupling signal between cells. Furthermore, because similar shifts occur for \( I_{Ks} \), characteristics and because \( I_{Ks} \) amplitude is increased, Ca\(^{2+}\) spiking will be favored. These effects should contribute to the arrhythmic activity already reported in addition to the transient depolarization induced by high ATP concentration.\(^{20}\)
Acknowledgments

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