Effects of Sematilide, a Novel Class III Antiarrhythmic Agent, on Delayed Rectifier K⁺ Current in Guinea Pig Atrial Myocytes

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Received December 15, 1995   Accepted June 20, 1996

ABSTRACT—Effects of sematilide, a novel class III antiarrhythmic agent, on the delayed rectifier K⁺ current (IK) were examined in guinea pig atrial myocytes using a voltage clamp technique. Sematilide inhibited both time-dependent outward current upon depolarization and tail currents (IK-tail) at -40 mV. The concentration of sematilide required for a 50% decrease in IK-tail was approximately 50 μM. The sematilide-sensitive current obtained using a triangular voltage command exhibited marked inward rectification and had the maximum amplitude at -30 mV. These results suggest that sematilide inhibits rapidly activating IK in guinea pig atrial myocytes, resulting in the prolongation of action potential duration and refractoriness.

Keywords: Sematilide, Delayed rectifier K⁺ current, Guinea pig atrium

Sematilide hydrochloride (N-[2-(diethylamino)ethyl]-4-[(methylsulfonyl)-amino]benzamidate HCl) is a structural analog to sotalol and being evaluated as a class III antiarrhythmic agent in undergoing clinical trials. We have demonstrated that sematilide prolongs the action potential duration and effective refractory period in a concentration-dependent manner without affecting other action potential parameters in guinea pig atrium (1). Similar observations have been reported in the ventricle and atrium of several species: rabbit atrium (2) and ventricle (3), guinea pig ventricle (4) and canine ventricle (5). Recently, it was shown that sematilide preferentially inhibits a rapidly activating delayed rectifier K⁺ current (IKr) in guinea pig ventricular myocytes (4). These findings strongly suggest that the target of sematilide like other class III antiarrhythmic agents is IKr in the ventricle. Effects of sematilide on delayed rectifier K⁺ current (IK) in atrial myocytes is, however, not clear yet. The present study was undertaken to elucidate how sematilide affects IK in guinea pig atrial myocytes.

Guinea pig atrial myocytes were enzymatically dissociated as previously described (6, 7). The methods used to record transmembrane currents under whole-cell voltage clamp were similar to those originally developed by Hamill et al. (8). The resistance of microelectrodes filled with internal solution was approximately 2–3 MΩ. A single atrial myocyte was voltage-clamped using an amplifier (CEZ-2200; Nihon Kohden, Co., Ltd., Tokyo). In some of the experiments, triangular shaped pulses were applied as a voltage-clamp command using a multi-pulse generator (FS-1915; NF Electronics, Tokyo). All experiments were carried out at 36±1°C. Membrane current signals were acquired and analyzed using programs for IBM-AT compatible computers: AQ and Cellsoft that were supplied by Dr. Wayne Giles (Univ. Calgary, Canada). The HEPES-buffered salts solution (external solution) contained 137 mM NaCl, 5.9 mM KCl, 2.2 mM CaCl₂, 1.2 mM MgCl₂, 14 mM glucose and 10 mM HEPES (pH 7.2 by NaOH). The pipette filling solution (internal solution) contained 100 mM K-aspartate, 50 mM KCl, 1 mM MgCl₂, 0.85 mM CaCl₂, 5 mM EGTA, 5 mM Na₂-ATP and 5 mM HEPES (pH 7.2 by KOH). The pCa of the internal solution was maintained at 7.5 with a Ca-EGTA buffer. The following drugs were used in the present study: sematilide (Lot No. 11053791; produced by Berlex Laboratories, Cedar Knolls, NJ, USA and supplied to Nippon Roussel Co., Ltd., Tokyo); nicardipine (Sigma, St. Louis, MO, USA). Data are expressed as the mean ±S.E. in the text and figures.

Figure 1A shows the effect of sematilide on IK. A myocyte was depolarized from a holding potential of -40 to -10 mV for 200 msec at 0.2 Hz. To abolish the voltage-
dependent Ca²⁺ current, 0.3 μM nicardipine was added to the external solution. Application of 30 μM sematilide reduced \( I_K \) at −10 mV and the tail current at −40 mV (\( I_{K-tail} \)). Further addition of sematilide (100 μM) abolished \( I_K \) and \( I_{K-tail} \). Figure 1B (a and b) shows the current sensitive to 30 and 100 μM sematilide (I_{sema}), respectively, which were obtained by subtraction of the current in the presence of sematilide from the control on the computer. The concentration-dependent effect of sematilide on \( I_{K-tail} \) was summarized in Fig. 1C. \( I_{K-tail} \) upon repolarization to −40 mV after the depolarization for 200 msec from −40 to 0 (open circles) and +40 mV (closed circles) in the presence
of sematilide was normalized by that in the absence and plotted against the concentration of sematilide. According to the relationships, the inhibition of IK by sematilide was not affected by depolarizing potentials of 0 and +40 mV, and the concentration of sematilide required for a 50% decrease in IK-tail (IC50) was approximately 50 μM at both potentials.

Figure 2 shows Isema elicited upon depolarization to various potentials between −30 and +30 mV. IK and IK-tail were recorded under the same experimental conditions as those in Fig. 1 and Isema was obtained by subtraction. The current-voltage (I-V) relationship of peak Isema during depolarization had a bell-shape and shows the maximum at around −10 mV (Fig. 2B, 59±5 pA at −10 mV, n=6). In contrast, as shown in Fig. 2C, the amplitude of IK-tail at −40 mV was similar in the range of depolarizing potential between +10 and +30 mV (71±18, 76±19 and 73±19 pA at +10, +20 and +30 mV, respectively, n=10). According to the I-V relationship of IK-tail, the half activation voltage for Isema was approximately −15 mV (n=10).

To estimate the contribution of Isema to repolarization in an action potential (AP), a triangular command pulse that has a similar shape to AP in guinea pig atrium was applied in Fig. 3. Cells were depolarized to +35 mV from the holding potential of −80 mV and then repolarized to −80 mV at the rate of 1.1 V/sec. As shown in Fig. 3A, exposure to 100 μM sematilide reduced a part of the outward current. The Isema was obtained by subtraction of the current in the presence of 100 μM sematilide from that in the absence in five separate cells and the summarized data were plotted against time and membrane potential in the lower panel of Fig. 3B. In the upper panel, the triangular command pulse and AP recorded from guinea pig atrium using a conventional microelectrode in the absence and presence of 100 μM sematilide are superimposed. Isema exhibited a marked inward rectification and reached a maximum around −30 mV.

In the present study, sematilide caused an inhibition of IK in guinea pig atrial myocytes. Sematilide-sensitive current (Isema) is considered to be a IKr because of the following results: i) Isema shown in Fig. 3 had a marked inward rectification that is characteristic of IKr; ii) the half activation voltage of Isema (Fig. 2B, −15 mV) was almost
identical to that of $I_{Kr}$ reported in guinea pig atrium (9); iii) the concentration-inhibition relationships for sematilide (Fig. 1C) were not affected by the activation potential of $I_{K}$ (0 and +40 mV), whereas more slowly activating delayed rectifier $K^+$ current ($I_{KS}$) may be activated at +40 than 0 mV (9); and iv) in the presence of 10 iM E4031, a potent class III antiarrhythmic agent which is a selective blocker of $I_{Kr}$, sematilide had no additional decrease in $I_{K}$ (n=4, no detectable change in all of them). The IC50 of sematilide to inhibit $I_{K\text{tail}}$ was approximately 50 pM and voltage-independent between 0 and +40 mV. In the previous study, it was demonstrated that the action potential duration (APD) at 50% repolarization and effective refractory period were significantly increased in the presence of 10 $\mu$M sematilide. Sematilide, therefore, may affect the APD and refractory period in a multi-cellular preparation at slightly lower concentrations than that for $I_{K\text{tail}}$ inhibition in single myocytes. Although the reason for this is not completely clear, the relation between the inhibition of $I_{Kr}$ and the prolongation of AP may not be linear.

Class III antiarrhythmic agents are characterized by a reverse frequency dependence of their effect (4, 10, 11). Sematilide was also reported to have the reverse frequency dependent effect on APD in guinea pig (4) and rabbit ventricular muscle (3), whereas the reverse frequency dependence was not observed in guinea pig atrium (1). The mechanism of the difference was not determined in the present study. In guinea pig ventricle, it has been postulated that sematilide interacts with the channel in the resting state (4). E4031, however, inhibits cloned HERG $K^+$ channels, which may be responsible for $I_{Kr}$, as an open channel blocker (12). Open channel blocking does not fit the model of reverse frequency dependence (12) but rather the opposite as shown for almokalant (13).

The I-V relationship of $I_{\text{sema}}$ shown in Fig. 3 indicates that the activation of $I_{K\text{r}}$ offsets membrane potential
toward hyperpolarization between $-70$ and $+20$ mV. In guinea pig ventricular myocytes, sematilide markedly slows the AP repolarization in the voltage range of $+20$ and $-20$ mV but does not affect the further repolarization, since the inward rectifier K$^+$ current ($I_{Kr}$) is not affected by sematilide (4). The AP repolarization in atrial myocytes was also slowed by sematilide in the potential range of $-30$ and $-70$ mV (1), probably because $I_{K1}$ is much smaller in the atrium than in the ventricle (14); and thereby, the decrease in $I_{K1}$ may result in a significant decrease in total repolarizing current in the potential range.

In conclusion, sematilide, like other class III antiarrhythmic agents, effectively inhibits a $I_{Kr}$ in guinea pig atrium. Similar voltage dependence between $I_{sema}$ ($=I_{Kr}$) and prolongation of APD strongly suggests that inhibition of $I_{sema}$ by sematilide can contribute to prolongation of APD and refractoriness.

Acknowledgments
The authors thank Dr. Wayne Giles (University of Calgary) for providing the data acquisition and analysis programs for IBM-AT.

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