

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

Yasunori Ishii^{1,2}, Katsuhiko Muraki¹, Atsushi Kurihara², Yuji Imaizumi^{1,*} and Minoru Watanabe¹

¹Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabedori, Mizuho-ku, Nagoya 467, Japan

²Biology Section, Shirakawa Laboratory, Nippon Roussel Co., Ltd., 103-1 Ushishimizu, Shirakawa, Fukushima 961, Japan

Received December 15, 1995 Accepted June 20, 1996

ABSTRACT—Effects of sematilide, a novel class III antiarrhythmic agent, on the delayed rectifier K^+ current (I_K) were examined in guinea pig atrial myocytes using a voltage clamp technique. Sematilide inhibited both time-dependent outward current upon depolarization and tail currents (I_{K-tail}) at -40 mV. The concentration of sematilide required for a 50% decrease in I_{K-tail} was approximately $50 \mu\text{M}$. 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 I_K 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]benzamide 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 (I_{Kr}) in guinea pig ventricular myocytes (4). These findings strongly suggest that the target of sematilide like other class III antiarrhythmic agents is I_{Kr} in the ventricle. Effects of sematilide on delayed rectifier K^+ current (I_K) in atrial myocytes is, however, not clear yet. The present study was undertaken to elucidate how sematilide affects I_K 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 $2-3 \text{ M}\Omega$. 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 $36 \pm 1^\circ\text{C}$. 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 CaCl_2 , 1.2 mM MgCl_2 , 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 MgCl_2 , 0.85 mM CaCl_2 , 5 mM EGTA, 5 mM $\text{Na}_2\text{-ATP}$ 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 \pm S.E. in the text and figures.

Figure 1A shows the effect of sematilide on I_K . A myocyte was depolarized from a holding potential of -40 to -10 mV for 200 msec at 0.2 Hz. To abolish the voltage-

* To whom correspondence should be addressed.

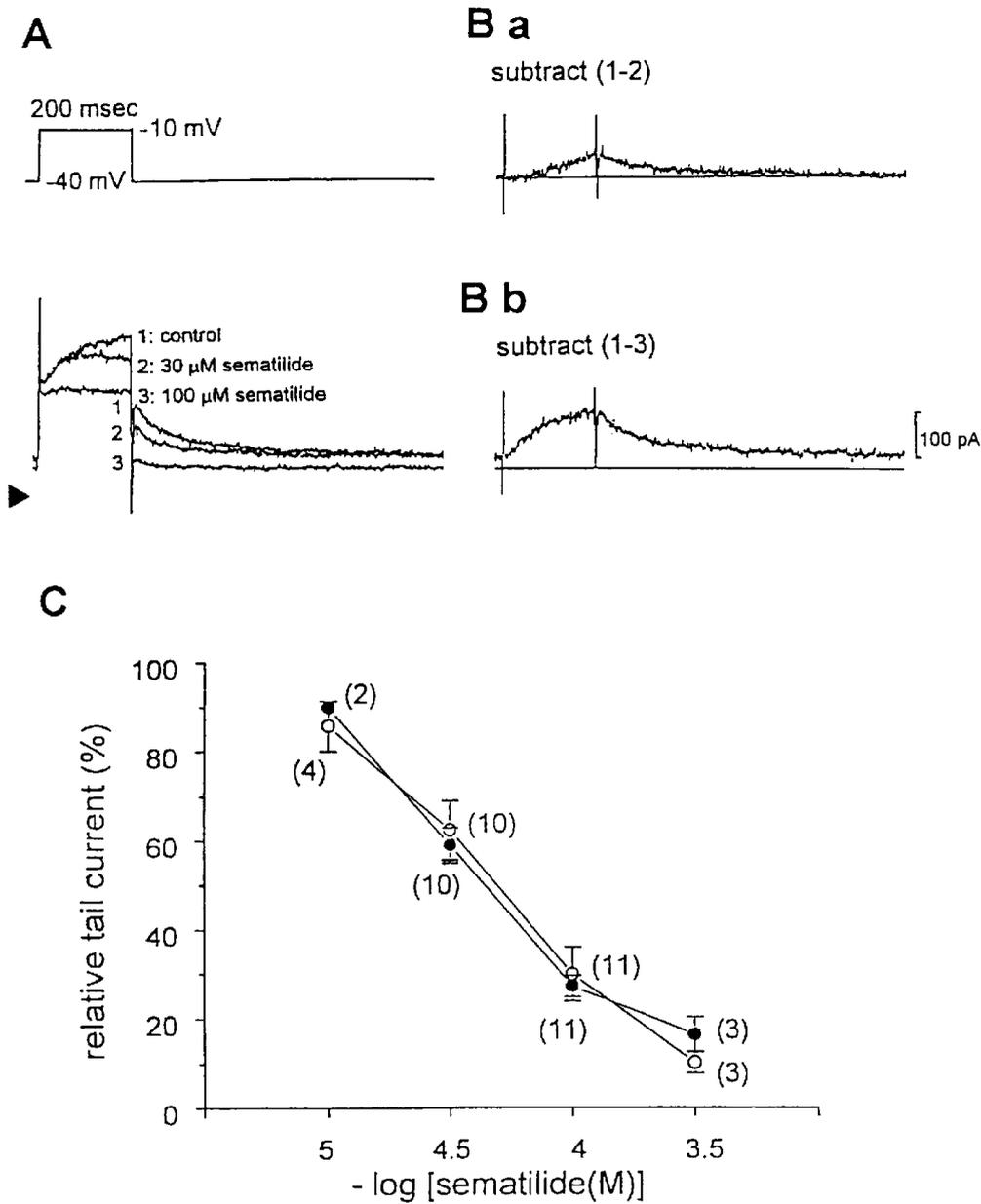


Fig. 1. Effect of sematilide on delayed rectifier K^+ current in guinea pig atrial myocytes. **A:** A myocyte was depolarized every 5 sec for 200 msec from a holding potential of -40 to -10 mV. Application of 30 and 100 μ M sematilide inhibited outward current during depolarization and the tail current (I_{K-tail}) at -40 mV. The zero current level is indicated by a triangle. **B:** 30 (**Ba**) and 100 μ M (**Bb**) sematilide-sensitive current, which were obtained by subtraction of the current in the presence of sematilide from the corresponding control. **C:** Concentration dependent inhibition of I_{K-tail} by sematilide. The I_{K-tail} was recorded upon repolarization to -40 mV after the depolarization for 200 msec from -40 to 0 (open circles) and $+40$ mV (closed circles). The amplitude of I_{K-tail} in the presence of sematilide was normalized by that in the absence and plotted against the concentration of sematilide. The vertical bar on each symbol indicates S.E. The numbers in the parentheses denote number of cells examined.

dependent Ca^{2+} current, 0.3 μ M nifedipine was added to the external solution. Application of 30 μ M sematilide reduced I_K at -10 mV and the tail current at -40 mV (I_{K-tail}). Further addition of sematilide (100 μ M) abolished I_K and I_{K-tail} . Figure 1B (a and b) shows the current sensitive to 30 and 100 μ M 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 to 0 (open circles) and $+40$ mV (closed circles) in the presence

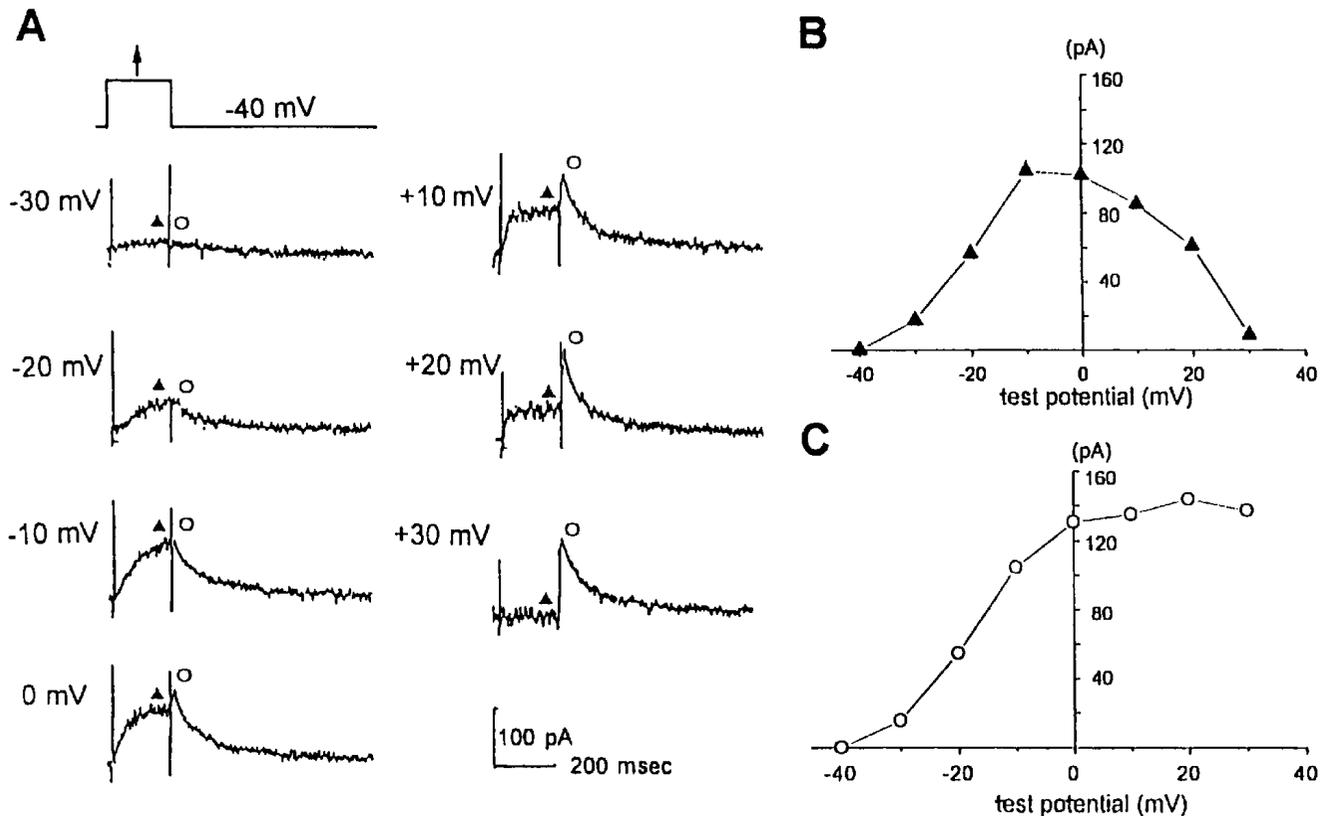


Fig. 2. Sematilide-sensitive current and its current-voltage relationship. **A:** A myocyte was depolarized for 200 msec from -40 mV to various test potentials between -30 and $+30$ mV with 10 mV step. A sematilide-sensitive current (I_{sema}) was obtained by subtraction of current recorded in the presence of $100 \mu\text{M}$ sematilide from the corresponding control. Closed triangles or open circles indicate the peak of I_{sema} during depolarization or its tail current, respectively. **B** and **C:** Current-voltage relationships of I_{sema} (**B**) and its tail current (**C**), respectively.

of sematilide was normalized by that in the absence and plotted against the concentration of sematilide. According to the relationships, the inhibition of I_K by sematilide was not affected by depolarizing potentials of 0 and $+40$ mV, and the concentration of sematilide required for a 50% decrease in I_{K-tail} (IC_{50}) was approximately $50 \mu\text{M}$ at both potentials.

Figure 2 shows I_{sema} elicited upon depolarization to various potentials between -30 and $+30$ mV. I_K and I_{K-tail} were recorded under the same experimental conditions as those in Fig. 1 and I_{sema} was obtained by subtraction. The current-voltage (I - V) relationship of peak I_{sema} 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 I_{K-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 I_{K-tail} , the half activation voltage for I_{sema} was approximately -15 mV ($n=10$).

To estimate the contribution of I_{sema} 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 $100 \mu\text{M}$ sematilide reduced a part of the outward current. The I_{sema} was obtained by subtraction of the current in the presence of $100 \mu\text{M}$ 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 $100 \mu\text{M}$ sematilide are superimposed. I_{sema} exhibited a marked inward rectification and reached a maximum around -30 mV.

In the present study, sematilide caused an inhibition of I_K in guinea pig atrial myocytes. Sematilide-sensitive current (I_{sema}) is considered to be a I_{K_r} , because of the following results: i) I_{sema} shown in Fig. 3 had a marked inward rectification that is characteristic of I_{K_r} ; ii) the half activation voltage of I_{sema} (Fig. 2B, ~ -15 mV) was almost

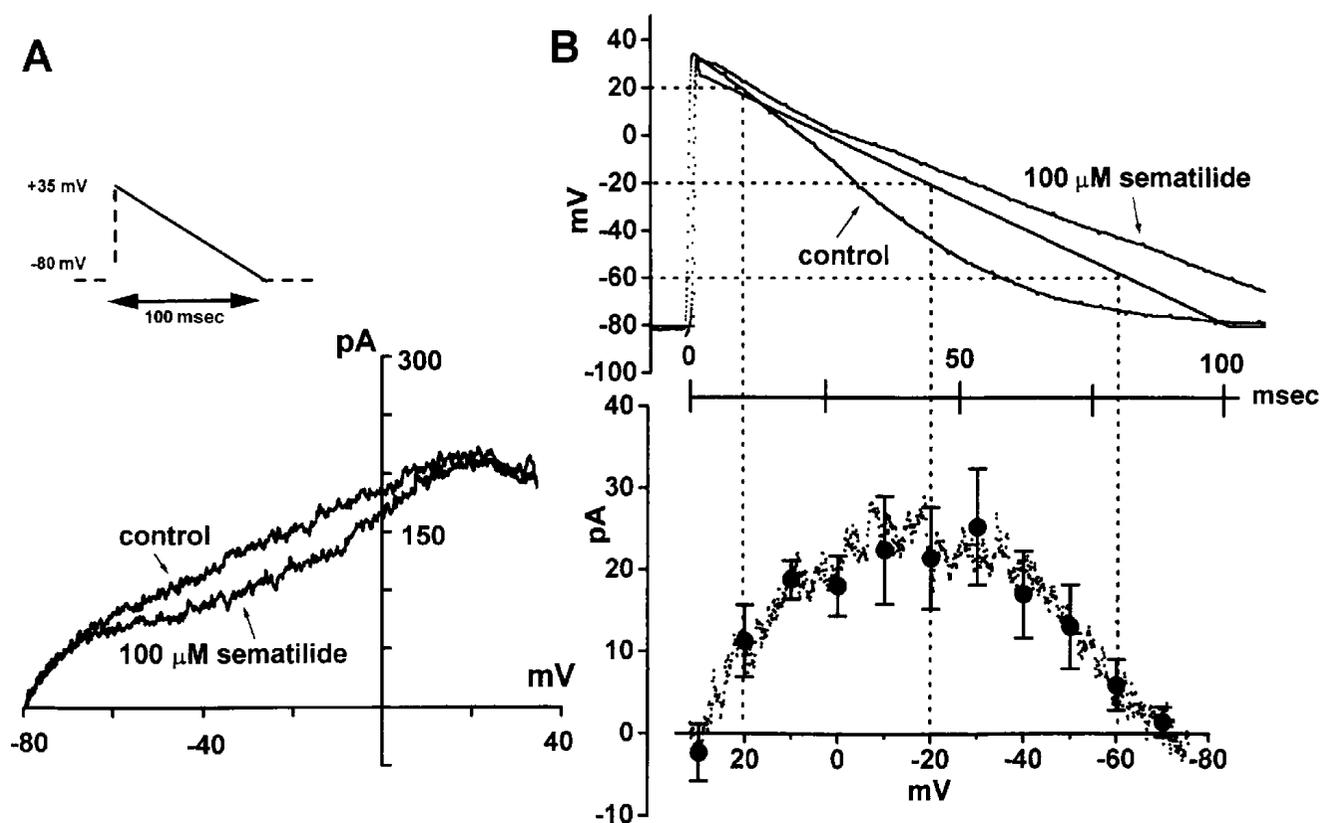


Fig. 3. Current-voltage relationship of sematilide-sensitive current obtained using a triangular voltage command. **A:** The current recorded during repolarization in the absence and presence of $100 \mu\text{M}$ sematilide was expressed as a function of repolarizing potential. A myocyte was depolarized from -80 mV to $+35 \text{ mV}$ and then repolarized to -80 mV at a rate of 1.1 V/sec . The inset indicates the triangular voltage command. **B:** Summarized current-voltage relationship of sematilide-sensitive current (I_{sema}) elicited by a triangular voltage command which mimics action potential (AP). In the upper panel, APs recorded in a guinea pig atrial preparation using a microelectrode in the absence and presence of $100 \mu\text{M}$ sematilide and the triangular voltage command are superimposed. In the lower panel, dotted points and closed circles denote the average amplitude of I_{sema} from five separate cells at each potential. The bar on each closed circle indicates S.E. Note that I_{sema} shows a strong inward rectification.

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 $+40 \text{ mV}$), 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 $10 \mu\text{M}$ 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 IC_{50} of sematilide to inhibit $I_{\text{K-tail}}$ was approximately $50 \mu\text{M}$ and voltage-independent between 0 and $+40 \text{ mV}$. 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 $10 \mu\text{M}$ sematilide. Sematilide, therefore, may affect the APD and refractory period in a multi-cellular preparation at slightly lower concentrations than that for $I_{\text{K-tail}}$ inhibition in single myocytes. Although the reason for this is not completely clear, the relation be-

tween 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 alomakalant (13).

The I-V relationship of I_{sema} shown in Fig. 3 indicates that the activation of I_{Kr} 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_{K1}) 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_{Kr} 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.

REFERENCES

- Ishii Y, Muraki K, Kurihara A, Imaizumi Y and Watanabe M: Effects of sematilide, a novel class III antiarrhythmic agent, on action potential in guinea pig atrium. *Jpn J Pharmacol* **68**, 175–182 (1995)
- Argentieri TM, Carroll MS and Sullivan ME: Cellular electrophysiological effects of the Class III antiarrhythmic agents sematilide and clofilium on rabbit atrial tissues. *J Cardiovasc Pharmacol* **18**, 167–174 (1991)
- Suzuki R, Kodama I, Iwata H, Kamiya K and Toyama H: Frequency-dependent effects of a new class-III antiarrhythmic agent, sematilide, on the action potential duration of rabbit ventricular muscles. *Environ Med* **38**, 57–60 (1994)
- Sawanobori T, Adania H, Namiki T and Hiraoka M: Rate-dependent effects of sematilide on action potential duration in isolated guinea-pig ventricular myocytes. *J Pharmacol Exp Ther* **271**, 302–310 (1994)
- Argentieri TM: Sematilide. *Cardiovasc Drug Rev* **10**, 182–198 (1992)
- Giles WR and Imaizumi Y: Comparison of potassium currents in rabbit atrial and ventricular cells. *J Physiol (Lond)* **405**, 123–145 (1988)
- Muraki K, Imaizumi Y, Watanabe M, Habuchi Y and Giles WR: Delayed rectifier K^+ current in rabbit atrial myocytes. *Am J Physiol* **269**, H524–H532 (1995)
- Hamill OP, Marty A, Neher E, Sackman B and Sigworth J: Improved patch-clamp techniques for high resolution current recording from cell free membrane patches. *Pflugers Arch* **391**, 85–100 (1981)
- Sanguinetti MC and Jurkiewicz NK: Delayed rectifier outward K^+ current is composed of two currents in guinea pig atrial cells. *Am J Physiol* **260**, H393–H399 (1991)
- Jurkiewicz NK and Sanguinetti MC: Rate-dependent prolongation of cardiac action potentials by a methanesulfonanilide class III antiarrhythmic agent; Specific block of rapidly activating delayed rectifier K^+ current by dofetilide. *Circ Res* **72**, 75–83 (1993)
- Nakaya H, Tohse N, Takeda Y and Kanno M: Effects of MS-551, a new Class-III antiarrhythmic drug, on action potential and membrane currents in rabbit ventricular myocytes. *Br J Pharmacol* **109**, 157–163 (1993)
- Spector PS, Curran ME, Keating MT and Sanguinetti MC: Class III antiarrhythmic drugs block HERG, a human cardiac delayed rectifier K^+ channel. *Circ Res* **78**, 499–503 (1996)
- Carmeliet E: Use-dependent block and use-dependent unblock of the delayed rectifier K current by almokalant in rabbit ventricular myocytes. *Circ Res* **73**, 857–868 (1993)
- Hume JR and Uehara A: Ionic basis of the different action potential configurations of single guinea pig atrial and ventricular myocytes. *J Physiol (Lond)* **368**, 525–544 (1985)