Isoprenaline Enhances Both Voltage-Dependent $\text{Ba}^{2+}$ and $\text{K}^+$ Currents in Guinea-Pig Vas Deferens

HACHIRO INOMATA, TOMOFUMI MIMATA$^1$ and MAKOTO WAKUI$^2$

College of Allied Medical Science, Akita University, Akita 010-8543, $^1$Department of Applied Physiology, Tohoku University School of Medicine, Sendai 980-8575, and $^2$Department of Physiology, Hirosaki University School of Medicine, Hirosaki 036-8562

INOMATA, H., MIMATA, T. and WAKUI, M. Isoprenaline Enhances Both Voltage-Dependent $\text{Ba}^{2+}$ and $\text{K}^+$ Currents in Guinea-Pig Vas Deferens. Tohoku J. Exp. Med., 1998, 184 (4), 317–320 — In the guinea-pig vas deferens, isoprenaline (ISO) can exert significantly enhanced effects on voltage-dependent Ca$^{2+}$ channel current carried by Ba$^{2+}$ ($I_{\text{Ba}}$) via $\beta$-adrenoceptor activation. Voltage-dependent Ba$^{2+}$ ($I_{\text{Ba}}$) and K$^+$ currents ($I_K$) were recorded in the preparation voltage-clamped in a Krebs solution replaced Ca$^{2+}$ by Ba$^{2+}$ (Ba$^{2+}$ solution) of $36^\circ$C. At a holding potential of $-40$ mV, ISO markedly increased $I_{\text{Ba}}$ evoked by depolarizing pulses, whereas it also increased $I_K$ at a holding potential of $-20$ mV so as to inactivate Ca$^{2+}$ channel. This ISO-induced enhancement of $I_{\text{Ba}}$ seems to be contradictory to the suppression of contraction by $\beta$-adrenoceptor activation in normal Krebs solution. —— isoprenaline; barium current; vas deferens © 1998 Tohoku University Medical Press

In the vas deferens, it is well known that isoprenaline (ISO) exerts suppressive effect on barium-induced contraction as well as electrically induced twitch mediated through $\beta$-adrenoceptors (Bülbring and Tomita 1987; Diaz-Toledo and Jurkiewicz 1991). Our previous voltage-clamp study has demonstrated that this underlying mechanism may be interpreted as suggesting that ISO produced a depression of Ca$^{2+}$ channel current responsible for spike generation in normal Krebs solution (Mimata and Inomata 1996) as observed in other smooth muscles (for review see McDonald et al. 1994). In contrast, in a Ba$^{2+}$ containing Krebs (Ba$^{2+}$ solution) in which Ba$^{2+}$ is used as the charge carrier for Ca$^{2+}$ channel current ($I_{\text{Ba}}$), the opposite effects of ISO on $I_{\text{Ba}}$ were seen as described below.

Male guinea-pigs of about 200–300 g were used. Small bundles of the longi-
tudinal muscle layer were isolated from vas deferens. Details of the double sucrose-gap current- and voltage-clamp method used have been described in our earlier publications (Inomata and Kao 1985; Kao and Inomata 1986; Mimata and Inomata 1989). The composition of normal Krebs solution was the following (in mM): 120.5, NaCl; 5.9, KCl; 1.2, MgCl₂; 2.5, CaCl₂; 15.5, NaHCO₃; 1.2, NaH₂PO₄ and 11.5, glucose. A Krebs solution containing Ba²⁺ ions was prepared by replacing 2.5 mM CaCl₂ with 2.5 or 5 mM BaCl₂. All control and test solutions were gassed with 95% O₂ and 5% CO₂ and maintained at 36°C. Drugs used were l-isoprenaline (Nikken Chemical Co., Tokyo), propranolol (Sumitomo Chemical Co., Osaka) and phentolamine (Ciba-Geigy, Basel, Switzerland). A paired Student's t-test was used to compare data, with p < 0.05 considered significant. In all experiments, phentolamine (0.26–2.60 μM) was pretreated to prevent α-adrenoceptor activation by ISO.

In current-clamped conditions, by replacing 2.5 mM Ca²⁺ with 2.5 mM Ba²⁺, almost preparations known to be usually quiescent in normal Krebs solution, produced spontaneous spike discharge and then became completely depolarized and quiescent. However, when the normal resting potential was re-established by hyperpolarizing the preparation, a Ba²⁺ action potential with prolonged repolarization developed with depolarizing current pulses (records not shown) (Inomata and Mimata 1983). Therefore, the effects of ISO were examined on membrane currents in the preparations voltage-clamped at two different holding potentials in Ba²⁺ solution; i.e., 440 msec depolarizing pulses were applied in the preparation first at the level of Ba²⁺-induced depolarization (Fig. 1A) and then held by the feedback system to the original resting potential in normal Krebs solution (Fig. 1B) before (control) and after (test) exposure to 5 μM ISO. In the preparation held at −20 mV which was level of the Ba²⁺-induced depolarization, there was no tendency for initial inward current to occur in response to step depolarizations because of depolarized Ca²⁺ channel inactivation. After 3 minutes exposure to ISO, all outward currents due to K⁺ (I_K) elicited by different step depolarizations were significantly enhanced whereas initial inward current possibly due to Ba²⁺ in response to smaller step depolarization appreciably appeared. On the other hand, in the same preparation held at −40 mV which was the original resting potential, depolarizing pulses elicited time-dependent inward currents (I_{Ba}), which were separated into two phases: an early fast inactivating phase and a long lasting one. After ISO exposure, the former phase of I_{Ba} appreciably enhanced, but the latter remained unaffected. As shown in Fig. 1C and D, these features are clearer in the current-voltage relations for I_{Ba} and for I_K, respectively. The peak I_{Ba} enhanced at any membrane voltage, but its zero-current reversal potential (E_a) remained about the same during ISO exposure (Fig. 1C). Whereas the late inward current at end of 440 msec step depolarization because of slowed inactivation of early current remains unchanged for voltage between −30 and −16 mV, the late outward current (largely I_K) at membrane potentials above −16 mV
enhanced (Fig. 1D). Likewise, the late outward current within the range of pulses tested in the same preparation held at −20 mV significantly enhanced. These similar effects were obtained in other preparations: before and during exposure to 1−5 μM ISO, the values of the peak $I_{Ba}$ estimated from the current-voltage relations obtained from seven preparations averaged $-0.9 \pm 0.1$ (mean ± s.e.) and $-1.1 \pm 0.2 \mu A/\mu F$ ($p < 0.01$), respectively, while the values of the $E_a$ obtained from eight preparations averaged $+11.0 \pm 2.7$ and $+10.4 \pm 2.0$ mV ($p = 0.1$), respectively.

Since the enhancements by ISO on $I_{Ba}$ and $I_K$ were hardly detected in the preparations pretreated with 1 μM propranolol in other experiments (data not shown), these changes can be considered to be mediated through β-adrenoceptor activation similarly to those in cardiac muscles (McDonald et al. 1994). Whereas noradrenaline (NA) is well known to exert suppressive effects on the $Ca^{2+}$ current via α-adrenoceptor activation in the same tissue (Inomata et al. 1989; Mimata and Inomata 1989), the enhancement by NA of the $I_{Ba}$ was found to be mediated
possibly via $\beta$-adrenoceptor activation in some of all myocytes tested in other experiments with whole-cell patched-clamp method (Kamimura et al. 1996).

However, in the vas deferens muscle, the enhanced effects of ISO on the $I_{Ba}$ seem to be contradictory to the suppression of contraction possibly due to a reduction of Ca$^{2+}$ channel current via $\beta$-adrenoceptor activation usually observed in normal Krebs solution (Bülbring and Tomita 1987; Mimata and Inomata 1996). Recently, similar enhancement in the $I_{Ba}$ by ISO has been reported in vascular muscles, which relaxed via $\beta$-adrenoceptor activation (Fukumitsu et al. 1990; McDonald et al. 1994). As suggested in these muscles, in the vas deferens muscle also, intracellular mechanisms in addition to an alteration of membrane function caused by ISO should be noticed.

Thus, in the vas deferens muscle, further studies are required to clarify the mechanism involved in the unexpected $\beta$-actions.

References