Mechanisms of Ionic Currents Involved in Suppressive Effect of Isoprenaline on the Action Potential of Guinea-Pig Vas Deferens in Normal Krebs Solution

Tomofumi MIMATA¹ and Hachiro INOMATA²

¹Department of Applied Physiology, Tohoku University School of Medicine, Seiryomachi, Sendai 980, and ²Akita University College of Allied Medical Science, Hondo, Akita 010 Japan

Abstract

The effects of β-adrenoceptor stimulation by isoprenaline (ISO) on action potential and membrane current were studied in the guinea-pig vas deferens in normal Krebs solution by using a double sucrose gap method.

In current-clamp experiments, ISO produced the membrane hyperpolarization by reducing the resistance and modified the pattern of multi-spike activity elicited by long depolarizing currents; an initial spike potential was reduced in the amplitude and the rate of rising phases and the slope of diastolic depolarization was slowed, leading to a prolongation of the spike discharge interval.

In voltage-clamp experiments, ISO greatly enhanced the late outward K⁺ current (IK₂) by increasing the conductance, but hardly affected the peak outward K⁺ current (IK₁). ISO also reduced the inward Ca²⁺ current (ICa) by decreasing the conductance (gₐ) as well as by reducing the driving force for Ca²⁺. While it increased the leakage conductance (gₐ) due to K⁺ associated with hyperpolarizing voltage steps.

In the preparations treated with Ca²⁺ channel blocker such as D-600, the enhanced effect of ISO on the IK was still prominent, suggesting that Ca²⁺ and K⁺ channels appear to be independently regulated during β-adrenoceptor stimulation.

These results suggest that ISO exerts depressant actions on the vas deferens muscle by reduction of the ICa as well as by prominent enhancement of the IK₂ via β-adrenoceptor activation.

Key words: Vas deferens; Smooth muscle; Isoprenaline; K⁺ current; Ca²⁺ current; Voltage-clamp

1. Introduction

In the guinea-pig vas deferens, it is well known that isoprenaline (ISO) has a suppressive effect on contractile responses to chemical and electrical stimulation via β-adrenoceptor activation (see review by Bolton, 1979). However, although many intensive studies on mechanical responses of vas deferens muscles to ISO have been presented, electrophysiological analysis...
of suppressing effects of β-adrenoceptor stimulation have not yet fully been done; no convincing explanation has not been provided of β-adrenoceptor stimulation causing directly a negative inotropic effect. In particular, as to the electrophysiological study of catecholamine action on this muscle (see reviews by Bülbucing and Tomita, 1987 and by McDonald et al., 1994), whereas several voltage-clamped studies on the electrical events via α-adrenoceptor stimulation by noradrenaline (NA) have been reported (Inomata et al., 1989; Mimata and Inomata, 1989; Imaizumi et al., 1991), no voltage-clamped report on those via β-adrenoceptor stimulation by ISO has yet been presented in the guinea-pig vas deferens.

Therefore, the present experiments were performed to investigate the actions of ISO on the action potential and membrane currents of the guinea-pig vas deferens in normal Krebs solution under current- and voltage-clamped conditions using a double sucrose gap method.

Brief accounts of this work have been reported in abstract form (Inomata and Mimata, 1986a and 1986b).

2. Materials and methods

Male guinea-pig weighing about 200~300 g were used. Small bundles of longitudinal smooth muscle tissue were isolated from vas deferens. Typical preparations were 0.4 mm in diameter and 5~8 mm in length.

Current- and voltage-clamped experiments were performed using a double sucrose gap method similar to that described in our earlier publications (Inomata and Kao, 1979; Yamagata et al., 1983). This voltage-clamp method is now considered to have limited value due to difficulty in interpreting results in multicellular preparations (Kao and Inomata, 1986). However, in the present study, this method is useful for comparative purposes in which the properties of multicellular preparations of smooth muscles under experimental conditions are compared with those of the same preparations of muscles under control conditions, as in earlier studies (e.g., Kao and Inomata, 1986; Mimata and Inomata, 1989; Inomata et al., 1991).

Membrane responses to constant-current and constant-voltage steps were displayed on a storage oscilloscope (Tektronix 5111) for photographing and were monitored on a pen-writing recorder (San-Ei Rectigraph 8S) throughout the experiments. The rate of rise and fall of the action potential elicited during current-clamp run was obtained with an electronic differentiator. Some membrane properties obtained during voltage-clamp run were expressed on basis of unit capacitance to facilitate comparison.

Normal Krebs solution with the following composition (mM) was used: NaCl, 120.5; KCl, 5.9; CaCl₂, 2.5; MgCl₂, 1.2; NaHCO₃, 15.5; NaH₂PO₄, 1.2; glucose, 11.5. The solution was gassed with 95% O₂ and 5% CO₂, and its pH was adjusted to 7.4. The temperature of the perfused solution was maintained at about 34°C.

The following drugs were used: l-isoprenaline (Nikken Kagaku, Japan) as β-adrenoceptor agonist, and also phentolamine (Ciba—Geigy, Swiss) and propranolol (Sumitomo Kagaku, Japan) as α- and β-adrenoceptor blockers, respectively. To abolish the Ca²⁺ current, D-600 (methoxverapamil; Sigma, USA) was used. The test solution containing drug were prepared by diluting to the final required concentration with the normal Krebs solution. The concentration
of drugs was described in the text. Application of drugs was made for continuous superfusion through the test node from valve-selectable reservoir containing the control or the test solution.

The data were given as mean±S.E. and paired Student's t test was used for statistical analysis.

3. Results

3.1. Effects of ISO on the membrane resting and action potentials

The action potentials evoked by long depolarizing current pulses were characterized by an initial full spike followed by multi-abortive spike discharges in the guinea pig vas deferens in normal Krebs solution (Fig. 1). These electrical membrane patterns were qualitatively similar to those obtained with intracellular microelectrode (Hashimoto and Holman, 1967; Goto et al., 1978).

Effects of ISO were studied in the preparations untreated or pretreated with phentolamine (2.6 μM) in normal Krebs solution. ISO at low concentration (0.01 μM) had no appreciable effect on the resting potential, but caused any change in the spike discharge pattern; reduction in the slope of diastolic depolarization with no change in the spike amplitude, resulting in a prolongation in interval of repetitive spikes (record not shown).

Fig. 1. Membrane potential responses of vas deferens before and during ISO action. Panels A and B illustrate effect of ISO in the preparation before and after pretreating with phentolamine, respectively. Current pulses of alternating polarity were applied to evoke electrotonic potentials; depolarizing pulses for eliciting action potential and hyperpolarizing pulses for estimating membrane conductances of steady state. ISO was applied for period indicated by horizontal bars. The interruption in the records is an interval of 10 min. Upper trace, current intensity; lower trace, membrane potential. Original resting potential indicated by broken line. Panel C shows expanded records of action potential. Records -a, -b, and -c correspond to same letters in panel B, upper trace, first derivatives (dV/dt); middle traces, applied current intensity; bottom traces, membrane responses. Membrane resistance in frames a, b, and c, 619, 571, and 800 kΩ, respectively. Note depolarization of the membrane accompanying with an increase in membrane resistance produced by ISO in unpretreated preparation, while hyperpolarization accompanying a decrease in membrane resistance in phentolamine–pretreated preparation.
However, ISO at high concentrations (1.0 to 10 μM) had variable effects on the resting potential from trial to trial, or among different preparations; five out of twelve preparations tested were hyperpolarized, four preparations depolarized while others were unaffected. Whereas ISO reversibly hyperpolarized the membrane in all the preparations pretreated with phentolamine, although the effect was relatively small (2 to 7 mV). A typical example is shown in Fig. 1. When ISO (10 μM) was applied first in normal solution, the membrane was depolarized accompanying by an increase in membrane resistance, and the action potential was reduced in the amplitude by 13% and the rate of rising phase by 15% (Fig. 1A). After wash out, the perfused medium was switched to one containing phentolamine. After at least 7 min in the latter solution ISO was applied again. The membrane was reversed to hyperpolarization accompanying by a reduction in membrane resistance (Fig. 1B), while the action potential was markedly reduced in the amplitude by 14% and the rate of rising phase by 34%, and the slope of diastolic depolarization reduced by 18% (Fig. 1C). This effect persisted during continuous application of ISO.

However, both depolarizing and hyperpolarizing responses to ISO were abolished by the

| Table 1. Effects of ISO on electrical properties of vas deferens in normal Krebs solution. All items are means±S.E.M. with number preparations in parentheses. P values of Student’s t test based on paired comparison. Data obtained from preparations pretreated with phentolamine. All data except ones obtained from those pretreated with phentolamine. |
|---------------------------------|-----------------|-----------------|
| 1. Resting potential (mV) | -50.5±2.2* (12) | -48.4±1.8* (12) | 0.3 |
| 2. Spike amplitude (mV) | -48.0±1.5 (12) | -53.7±2.0 (12) | 0.001 |
| 3. Spike rise phase (V/s) | 42.6±1.8* (12) | 37.0±1.6* (12) | 0.002 |
| 4. Spike fall phase (V/s) | 41.3±0.9 (12) | 35.8±1.2 (12) | 0.001 |
| 5. Diastolic depolarization (V/s) | 4.1±0.3 (14) | 2.7±0.2 (14) | 0.001 |
| 6. $E_a$ (mV) | +15.0±3.6 (8) | +8.6±3.6 (8) | <0.01 |
| 7. $I_{Ca}$ (μA/μF) | -1.11±0.12 (7) | -0.79±0.05 (7) | 0.02 |
| 8. $g_k$ (μS/μF) | 49.3±12.7 (7) | 46.0±7.0 (7) | <0.05 |
| 9. $E_app$ (mV) | -67.9±3.0 (5) | -67.2±2.7 (5) | 0.3 |
| 10. $g_{k1}$ (μS/μF) | 48.3±10.5 (7) | 48.6±9.8 (7) | 0.05 |
| 11. $g_{k2}$ (μS/μF) | 32.7±7.6 (7) | 37.0±8.1 (7) | 0.005 |
| 12. $g_{k1}$ (μS/μF) | 16.9±1.3 (5) | 17.0±1.8 (5) | 0.3 |
| 13. $g_{k2}$ (μS/μF) | 13.2±0.9 (5) | 15.4±1.1 (5) | 0.01 |
| 14. $g_l$ (μS/μF) | 2.33±0.17 (7) | 3.06±0.12 (7) | 0.01 |

*Estimated from diastolic depolarization followed initial spike potential.

Reversal potential of Ca current ($I_{Ca}$).

Maximum $I_{Ca}$.

Maximum chord conductance of $I_{k1}$, defined as $I_{k1}/(E-E_a)$.

Reversal potential of outward $K$ current ($I_k$).

Maximum chord conductance of peak $I_k$ defined as $I_{k1}/(E-E_a)$.

Maximum chord conductance of late $I_k$ defined as $I_{k2}/(E-E_a)$.

Slope conductance of tail current of $I_{k1}$.

Slope conductance of tail current of $I_{k2}$.

Leakage conductance estimated at 10 mV hyperpolarization.
presence of propranolol (3.4 µM) (record not shown).

In the result, similar findings were obtained in eleven or thirteen other preparations: in particular, in the preparations pretreated with phentolamine, ISO at high concentration hyperpolarized the membrane from a control value of $-48.0 \pm 1.0$ to $-53.7 \pm 2.0$ mV ($n=12$), and reduced the rate of spike rising phase from a control value of $4.1 \pm 0.3$ to $2.7 \pm 0.2$ V/s ($n=14$) and the rate of diastolic depolarization from a control $0.40 \pm 0.04$ to $0.33 \pm 0.04$ V/s ($n=13$), respectively.

The data obtained from above observations are summerized in Table 1 (items 1 to 5).

The membrane potential changes due to ISO observed above can be better appreciated by examining the ionic currents under voltage-clamped conditions.

### 3.2. Effects of ISO on the membrane currents

In the voltage clamp study, following experiments were performed in the solution containing phentolamine (13 µM), $\alpha$-adrenoceptor blocker to avoid ISO-induced $\alpha$-adrenergic effect.

#### 3.2.1. Early Ca$^{2+}$ and outward K$^+$ currents

Figure 2A shows the typical ionic currents recorded from one individual preparation before and during ISO action. Test pulses were applied to $-35$ to $+10$ mV, in 5 mV increments, from
a holding potential of $-40 \text{ mV}$. Application of a command depolarizing pulses above $-35 \text{ mV}$ evoked both inward Ca\textsuperscript{2+} ($I_{\text{Ca}}$) and outward K\textsuperscript{+} currents ($I_K$). The outward current consisted of a transient large outward K\textsuperscript{+} current (peak K\textsuperscript{+} current, $I_{K1}$) and subsequently generated tonic outward current (late K\textsuperscript{+} current, $I_{K2}$), as described previously (Mimata and Inomata, 1989). After the stable recording of a series of membrane currents at various voltage levels under control conditions, this series of currents was periodically recorded under test conditions. When ISO was applied, the holding current level was slightly shifted to the outward direction and the leakage conductance ($g_1$) increased, as suggested by the superimposed inward currents due to hyperpolarizing steps in Fig. 2A. With application of ISO, the inward Ca\textsuperscript{2+} current was appreciably suppressed, while the late outward K\textsuperscript{+} current enhanced at all potentials tested. But, the peak outward K\textsuperscript{+} current remained unaffected. These features are clearer in the current–voltage relationships (I–V curve) as illustrated in Fig. 2B. Indeed, during action of ISO, the peak inward current is reduced at all potentials, and this inhibition was accompanied by a slight negative shift in the zero-current reversal potential ($E_a$) (Table 1, item 6). It also is clear that with the range of the pulses tested the peak outward K\textsuperscript{+} current is unaffected by application of ISO, but that the late outward K\textsuperscript{+} current is appreciably activated at membrane potentials more positive to $-20 \text{ mV}$.

Leakage current. To further evaluate a reduction in membrane resistance by ISO observed above (Fig. 1B, C), changes of the leakage current ($I_1$) associated with a hyperpolarizing step of 20 to 80 mV were measured before and during ISO action (Fig. 3A). A Typical record from one of the muscles is shown in Fig. 3A. Inward currents due to various hyperpolarizing pulses were found to increase after exposure to ISO. This finding is clear in the associated current–voltage relationship for hyperpolarizing pulses as shown in Fig. 3B. Although the I–V relationship was linear for hyperpolarizing pulses both normally and in the presence of ISO,

![Fig. 3. Families of membrane currents associated with hyperpolarizing step before (Aa) and during (Ab) ISO action and current–voltage relation (B). A. Zero current indicated by broken line. 440 msec clamp pulses from HP of $-40 \text{ mV}$ in 5 mV steps were applied at a rate of 10 sec in control and test solution; both solutions contained 2.6 $\mu$M phentolamine. Note that presence of ISO slightly shifted holding current in outward direction in frame b, (compared to holding current level in frame a). B. Current (ordinate)–voltage (abscissa) relations in control (open symbols) and in test solution (filled symbols). Circles and triangles are obtained from two different experiments. Note increased $g_1$ in test condition (see also Table 1).]

Fig. 4. Families of outward K⁺ currents associated with depolarization step in the solution containing 2 µM D-600 before (A) and during (B) ISO action and current–voltage relation (C). 440 ms depolarizing clamp pulses from HP of −35 mV were applied in 10 mV steps up to +10 mV. Iₒₑ was imperfectly blocked by D-600, as be visible as initial downward deflexion of outward current traces. C, current–voltage relations for late Iₒ (D-600 resistant Iₒ) in control (open triangles) and in test solution (filled triangles) of panel A and B. Outward currents at end of various voltage steps at 440 msec were plotted after subtracting with leakage current. Arrow indicates HP. Note that outward current was increased throughout the entire duration and at all voltages.

the slope of the line was increased by ISO. In each experiment done in two different preparations, the leakage conductance (g₁) estimated from the slope of the I/V changed from 4.3 to 4.9 µS and from 5.2 to 5.8 µS, respectively (see also to Table 1, item 14).

In addition, as it is evidenced in current-clamp experiments, these observed actions of ISO also were prevented in the preparations treated with propranolol (3.4 µM) (data not shown here).

Reversal potential and the outward K⁺ tail current. To study more of the nature of the actions of ISO on the outward K⁺ current, two kinds of experiments were carried out. First, the effect on the outward K⁺ current (late K⁺ current, Iₒₑ) due to depolarization steps not associated with the inward Ca²⁺ current was examined in the normal Krebs solution containing 2 µM D-600. A series of clamp pulses were applied before and during exposure to ISO. As shown in Fig. 4, under the conditions suppressing the inward Ca²⁺ current, ISO markedly enhanced the outward K⁺ currents to all depolarizing steps with no change in residuary Ca²⁺ currents. This finding suggests that an increase in the outward K⁺ current (chiefly Iₒₑ) by ISO may be independent of Ca²⁺ current. Secondly, to compare the nature of the actions of ISO on peak and late components of the outward K⁺ current, using a double command pulses method (Inomata and Kao, 1979), tail current analysis for both components before and during exposure to ISO were examined. After a 40 mV depolarizing pulse lasting 80 ms and 400 ms, both durations at which peak and late outward K⁺ currents were respectively activated, different test potentials were imposed (Fig. 5A). The superimposed current tails of both outward currents shown in Fig. 5A illustrate that it relaxes in a time– and voltage–dependent fashion.
Fig. 5. K⁺ tail currents at different potentials of repolarization before (Aa and c) and during (Ab and d) ISO action. In A, the preparation was maintained at −40 mV, a depolarizing pulse (V₁) to 0 mV was applied for 400 msec (a, b) and 70 msec (c, d), followed by a second pulse (V₂) that varied in amplitude. In B, reversal potential (Eₑ) and current (ordinate)–voltage (abscissa) relation of tail currents after repolarization, corrected for leakage and residual capacitance before (open symbols) and during (filled symbols) ISO action (see Fig. 5 in Inomata and Kao, 1979). With V₁ of 400 msec to activate late IK (IK₂), triangles; with V₁ of 70 msec to activate peak IK (IK₁). Presence of ISO enhances the slope conductance (Gₑ₂) for IK₂, but does not alter one (Gₑ₁) for IK₁. Note that Eₑ is unaffected by ISO application.

In Fig. 5B is shown the ‘instantaneous’ current–voltage relations of both currents obtained from records in Fig. 5A. The zero–current reversal potential (E₀) of both currents, which were mostly Eₑ, is practically identical (Table 1, item 9). With application of ISO, the slope conductance (Gₑ₁) of the tail current for peak component was unaffected, although the inward

Fig. 6. Relation between membrane potential and conductance of Ca and K current channels before (open symbol) and during (filled symbol) ISO action. A, average gₐ (circles) of seven preparations before and during ISO action. Presence of ISO decreases conductance at each step as well as gₑ. B, average gₑ₁ (inverted triangles) and average gₑ₂ (triangles) of same preparations as those in A. Note small depression of gₑ₁ in voltage negative to −15 mV without any change in gₑ₂, but an appreciable increase in conductance at each step as well as gₑ. Mean±S.E.M. of seven preparations are shown. *p<0.05; **p<0.03 (compared to corresponding controls).
Ca\(^{2+}\) current was appreciably inhibited, while the slope conductance (\(G_{K2}\)) for late component was markedly enhanced (Table 1, items 12 and 13). \(E_n\) did not shift measurably before and during its application (Table 1, item 9).

3.2.2. Conductances of the Ca\(^{2+}\) and K\(^+\) current channels

The effects of ISO on the characteristics of membrane conductances are more clearly observed when the membrane conductances are plotted on a logarithmic scale as a function of the membrane potential. Figure 6 illustrates the voltage-conductance relations obtained from seven preparations both normally and in the presence of ISO. For membrane potentials between \(-35\) and \(-15\) V, the presence of ISO causes a slight decrease in the relative conductance (\(g_a\)) for the Ca current channels. For membrane potentials more positive than \(-15\) mV, ISO causes a significant decrease of the Ca\(^{2+}\) conductance with the maximum \(g_a\) (\(g_a\)). The calculated values of the maximum \(g_a\) were thereby reduced by about 10\% (Table 1, item 8). On the other hand, in the presence of ISO, the relative conductances (\(g_{k1}\)) for the peak K\(^+\) current channels at any level of membrane potential remain unchanged, indicating that the maximum \(g_{k1}\) (\(g_{k1}\)) is almost the same (Table 1, item 10). There was, however, an appreciable increase in the relative conductance (\(g_{k2}\)) at any membrane voltage, causing significant increase of the maximum \(g_{k2}\) (\(g_{k2}\)) by 13\% (Table 1, item 11), as also can be supported by the slope conductance (\(G_{K2}\)). In agreement with the enhanced \(I_{k2}\) at any fixed membrane voltage, the relative conductance of the \(I_{k2}\) is higher under test condition than under control condition.

4. Discussion

It has already been documented that ISO causes a negative inotropic action via \(\beta\)-adrenoceptor activation in the vas deferens muscle (Hedqvist and Von Euler, 1976; Diaz-Toledo and Jurkiewicz, 1991; see review by Bülbring and Tomita, 1987).

In the electrophysiological studies, it has been shown with the sucrose gap method that in the guinea-pig vas deferens the negative inotropic effect induced by ISO appears with a reduction in spike amplitude and/or with more or less change in membrane potential. There are qualitative differences between the results obtained in different laboratories (Magaribuchi et al., 1971; Sjöstrand, 1973). These discrepancies can be due to some problem of the sucrose gap technique used and can be explained by a clear difference in drug action depending on its concentration.

From our present current-clamped experiments, it was usually found that lower concentration of ISO did not cause detectable change in the membrane potential, but caused the action potential due to long depolarizing current pulse characterized by a prolongation in interspike-interval, but by no change in spike-height (records not shown). But, higher concentration of ISO produced not only the depolarization accompanying by an increase in membrane resistance, but also the action potential characterized by a shortening in interspike-interval and by a decrease in spike height and upstroke (Table 1, items 1 and 2). These effects were seemingly similar to those produced by application of noradrenaline (NA) (Wakui and Inomata, 1985; Inomata and Mimata, 1989; Mimata and Inomata, 1989). However, since this depolarizing
responses were reversed to hyperpolarizing ones by exposure to $\alpha$-blocker such as phentolamine, as is evident in Fig. 1A, this depolarizing effect seems likely due to $\alpha$-adrenoceptor activation in the muscle by either ISO itself as proposed in the guinea-pig (Hedqvist and Von Euler, 1976) and the rat vas deferens (Vohra, 1979) or NA released from nerve terminal via its $\beta$-adrenoceptor activation (Wooton, Thoa, Kopin and Axelrod, 1973; Adler-Graschinky and Langer, 1975; Stjärne and Brundin, 1976). Both responses were blocked by exposure to $\beta$-blocker such as propranolol, indicating that those are caused by $\beta$-adrenoceptor activation.

From above observations, we confirmed that $\beta$-adrenoceptor activation of the membrane by ISO produce an appreciable suppression of the slow diastolic depolarization (i.e. the pacemaker potential) and upstroke of the evoked spike potential, and in part, some hyperpolarization of the membrane as clearly shown in Fig. 1B-C. In particular, since our preliminary experiment showed that the threshold concentration of ISO for suppressing the diastolic depolarization (i.e. the negative chronotropic effect) was about 100 times lower than that for suppressing spike potential (records not shown), the decreased slope of the diastolic depolarization may be a primary characteristic of the mechanisms underlying the $\beta$-adrenoceptor activation, as be pointed out in the guinea-pig taenia coli (Bülbring and Den Hertog, 1984).

Throughout the present voltage-clamped results, ionic mechanisms underlying membrane response to $\beta$-adrenoceptor activation by ISO can be inferred, corresponding to the change in the action potential produced by ISO.

Electrophysiological results on Ca$^{2+}$ current due to $\beta$-adrenoceptor activation are variant in some smooth muscles (review by McDonald et al., 1994); in the porcine coronary artery in which $\beta$-adrenoreceptor stimulation has mechano-relaxing effect, ISO enhanced effect (Fukumitsu et al., 1990), while it caused either an enhanced or a suppressive effect in the rabbit portal vein (Xiong et al., 1994).

In the present experiments on the was deferens, ISO at lower concentration (0.01 $\mu$M) enough to affect the outward K$^+$ current (I_K) produced no effect on voltage-dependent inward current (I_{Ca}), but ISO at concentration higher than 4.0 $\mu$M produced suppressive effect. This can be considered to be due to a decrease of inward Ca$^{2+}$ current, but not due to an increase in overlapping K$^+$ current, because ISO had a negligible effect on the peak K$^+$ current (I_{K2}) as clearly shown in Fig. 2. The observed reduction in the maximum inward current by ISO can be due to a reduction in the $g_a$ (Table 1, items 6, 7 and 8). Moreover, this change also may be attributed to a reduction of driving force for Ca$^{2+}$, possibly due to a rise in cytoplasmic Ca$^{2+}$, since the E_a was significantly more negative than that in the control solution (Table 1, item 6), in agreement with a marked reduction in the spike amplitude in test solution (Table 1, item 2). Thus, it can be likely that in the was deferens depression of inward Ca$^{2+}$ current via $\beta$-adrenoceptor activation may be essentially similar to those via $\alpha$-adrenoceptor activation (Inomata et al., 1989; Imaizumi et al., 1991).

Although in the was deferens, hyperpolarization due to ISO is smaller than that observed in spontaneously active muscles (rat myometrium: Kroeger and Marshall, 1973; guinea-pig taenia coli: Kao et al., 1975; guinea-pig taenia coli: Kao et al., 1975), this hyperpolarizing change may reinforce the depressant effects of ISO on the vas deferens. From the above voltage clamped observation that the leakage conductance ($g_l$) was increased while the reversal potential for the late (chiefly K$^+$) current (E_o)
remained unchanged (Table 1, item 9) this hyperpolarization is probably associated with some increase in the resting $K^+$ conductance as accepted in some other smooth muscles (Somlyo et al., 1970; Kroeger and Marshall, 1973; review by Bulbring and Tomita, 1987).

The ionic current mechanisms responsible for the generation of diastolic depolarization have not yet clarified in smooth muscles, whereas those have been intensively analyzed in cardiac muscles (for review see Noble, 1984). The voltage clamp study on these muscles has suggested that there are at least three currents flowing during diastolic depolarization: $I_{si}$ (analogous to $I_{ca}$) hypothesis, $g_K$ decay hypothesis, and $I_h$ (hyperpolarization-activated current) hypothesis. Similarly, in the vas deferens muscle, ionic currents such as $I_{ca}$, $I_K$ and $I_l$ can be suggested to play an important role during the diastolic depolarization. But, from the present voltage-clamped results described above, it appears difficult to explain the attribution of $I_{ca}$ and $I_l$ to the diastolic depolarization.

In our previous study (Inomata and Mimata, 1983; Mimata and Inomata, 1989), we suggested that in the vas deferens, a peak component ($I_{k1}$) of voltage-dependent outward $K^+$ current may be associated with repolarization of spike potential and a late component ($I_{k2}$) possibly associated with diastolic depolarization. Enhanced effect by ISO on two kinds of outward $K^+$ currents has been postulated in some other myocytes (myometrium: Anwer et al., 1992, Kao et al., 1989; taenia coli: Fan et al., 1993). Present analysis of the voltage-dependent outward current showed that ISO at all concentrations used produced a marked enhancement of the $g_{K2}$, but that ISO even at higher concentrations had no effect on the $g_{K1}$. Although no change in the $I_{k1}$ by ISO can be seemingly observed, a possibly enhanced effect on the $I_{k1}$ cannot be excluded. It seems likely that this unaltered $I_{k1}$ may be due to balance of decreased effect mediated with reduced $I_{ca}$ and enhanced effect by ISO. Although we have no information on the kinetic aspects of the outward current, in agreement with the increased $I_K$ by ISO, the slope conductance of the tail current is higher (e.g. Table 1, item 13). The increased $I_K$ due to an increase of the $g_K$ and $G_K$ (e.g. Table 1, items 11 and 13) measured during the time course of the diastolic depolarization may be adequate to explain basis features of suppression in the diastolic depolarization by ISO; that is, the increased $I_l$ by ISO probably hyperpolarizes the maximum diastole potential and increased $I_K$ (chiefly $I_{k2}$) suppresses the slope of diastolic depolarization. Anyhow, it seems that a combination of the activation in $g_K$ and the enhancement in $I_l$ induced by ISO could suppress the diastolic depolarization in the guinea-pig vas deferens.

In conclusion, at least, three ionic mechanisms underlying the suppression of multi-spike discharges via $\beta$-adrenoceptor activation in normal Krebs solution may be involved: (1) marked enhancement of the outward $K^+$ current responsible for the slowed diastolic depolarization; (2) increase of the leakage $K^+$ conductance (due to hyperpolarizing steps) responsible for the membrane hyperpolarization; (3) depression of the inward $Ca^{2+}$ current responsible for the reduced spike amplitude.

These findings will serve a suggestive information for the subsequent study in aid with whole cell clamp method.
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