Inhibitory Junction Potentials of the Guinea-Pig Duodenum in the Treatment with Catecholamines

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OHKAWA, H. Inhibitory Junction Potentials of the Guinea-Pig Duodenum in the Treatment with Catecholamines. Tohoku J. exp. Med., 1983, 140 (2), 209-219 — The inhibitory junction potentials (IJPs) in response to single and repetitive stimulation were recorded from the smooth muscle cells of the guinea-pig duodenum intracellularly. In adrenaline and noradrenaline (10^-8-10^-5 g/ml), the IJP could be evoked in spite of a hyperpolarization of the cell membrane. The amplitude of the IJP was slightly changed in these agents but not abolished. Similar results were obtained in isoprenaline (10^-5 g/ml) and phenylephrine (10^-5 g/ml). The IJPs evoked by single and repetitive stimulation were not blocked by phentolamine (10^-7 g/ml) and propranolol (10^-5 g/ml). In propranolol (10^-7-10^-5 g/ml), the membrane was depolarized and the amplitude and the rate of hyperpolarization in the IJP were decreased. The membrane potential was decreased and the amplitude of the IJP was slightly increased in the presence of guanethidine (10^-5 g/ml). The amplitude of the IJP was increased with increasing the concentration of tyramine (10^-5-10^-4 g/ml). These results suggest that the transmitter released from the intramural inhibitory nerve in the duodenum is nonadrenergic and this type of inhibition seems to be independent from adrenergic inhibition.

Repetitive stimulation of the sympathetic nerve caused a hyperpolarization of the membrane and decreased spike activity of the intestinal smooth muscle (Gillespie 1962). Burnstock et al. (1966) and Bennett et al. (1966a) found the inhibition of the taenia coli in response to repetitive stimulation of the perivascular nerves, and showed that these nerves had properties of sympathetic postganglionic adrenergic nerves. Hyperpolarization of the membrane and inhibition of the spike activity of the taenia coli were produced by exogenously applied adrenaline (Bülbring and Kuriyama 1963). These results indicate that the intestinal smooth muscle is innervated with adrenergic inhibitory nerves.

The nonadrenergic inhibition due to the inhibitory junction potentials (IJPs) in the gastrointestinal tract was also demonstrated by many authors (Bennett et al. 1966b; Furness 1969; Ito and Kuriyama 1971). The inhibition of membrane activity and the hyperpolarization were produced during the generation of IJPs. The functional relation between these two inhibitory systems is not clear. The present experiments were intended to investigate further the properties of the IJPs of the guinea-pig duodenum under the influence of catecholamines.

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METHODS

The preparation used in the present experiment was obtained by the same procedure as described in the previous paper (Ohkawa 1983). The methods of the recording and stimulating systems and the composition of the modified Krebs' solution were the same as those described in the previous papers (Ohkawa 1981, 1982). The drugs used were; L-adrenaline, guanethidine sulfate, L-isoprenaline hydrochloride, D,L-noradrenaline, phentolamine hydrochloride (Regitine, Ciba), phenylephrine hydrochloride, propranolol hydrochloride (Inderal, Sumitomo) and tyramine hydrochloride.

RESULTS

Effects of adrenaline and noradrenaline on the IJP

When adrenaline or noradrenaline was applied to the tissue, the membrane potential of the cell increased. In a series of experiments, the resting membrane potential was \(-58.4\pm4.9\) mV (M±S.D.; \(n=9\)) in normal solution, \(-59.4\pm4.3\) mV (\(n=7\)) at \(10^{-7}\) g/ml and \(-60.7\pm5.0\) mV (\(n=8\)) at \(10^{-6}\) g/ml of adrenaline. In the other preparation, the resting membrane potential was \(-55.0\pm6.8\) mV (\(n=6\)) in normal solution and \(-63.3\pm2.2\) mV (\(n=6\)) at \(10^{-5}\) g/ml of noradrenaline. Spontaneous spike activity of the cell was inhibited in these solutions.

Fig. 1. Effects of adrenaline and noradrenaline on the IJP in response to single stimulus. A: IJPs in normal solution (upper), adrenaline \(10^{-8}\) g/ml (middle), and \(10^{-6}\) g/ml (lower). The IJPs were evoked by single stimulus (0.6 and 0.9 msec duration). B: IJPs in normal solution (upper) and noradrenaline \(10^{-5}\) g/ml (lower). The IJPs were evoked by single stimulus (0.5 and 0.7 msec duration). C: Changes in the membrane potential and the parameters on the IJP obtained in adrenaline (\(10^{-8}\) g/ml) and noradrenaline (\(10^{-5}\) g/ml). \(n\) indicates the observed number.
Field stimulation could evoke inhibitory junction potentials (IJPs) in normal solution and adrenaline- and noradrenaline-containing solutions. Fig. 1A shows the IJPs evoked by a short pulse (0.6 and 0.9 msec duration) in normal solution and adrenaline (10^{-8} and 10^{-6} g/ml). Fig. 1B shows the IJPs evoked by a short pulse (0.5 and 0.7 msec duration) at 10^{-5} g/ml of noradrenaline.

The amplitude of the IJP was slightly decreased in adrenaline and noradrenaline (control, 10.9±1.2 mV, n=8; in adrenaline 10^{-6}g/ml, 8.1±0.7 mV, n=9 and control, 24.3±1.0 mV, n=4; in noradrenaline 10^{-5}g/ml, 22.8±0.4 mV, n=4). The rate of hyperpolarization was also decreased (control, 90.6±16.2 mV/sec; in adrenaline, 72.8±6.5 mV/sec and control, 196.5±14.9 mV/sec; in noradrenaline, 129.7±2.5 mV/sec). No change in the latency was observed and the half decay time of the IJP was prolonged (control, 142.6±18.3 msec; in adrenaline, 201±26.3 msec; and control, 226.8±27.9 msec; in noradrenaline, 261±13.2 msec). Fig. 1C shows these changes in the parameters on the IJP in adrenaline (10^{-6}g/ml) and noradrenaline (10^{-5}g/ml).

Fig. 2A shows the effect of field stimulation of low frequency (1 Hz) on the IJPs in adrenaline (10^{-8}g/ml). Each pulse evoked the IJPs but those amplitudes were gradually reduced in normal solution. The amplitude of the IJP in adrenaline was slightly decreased but the time course of the IJP was similar to that in normal solution. Fig. 2B shows the effect of the stimulation of high frequency (25 Hz)
in adrenaline (10^{-8} and 10^{-6} g/ml). In the normal solution, the repetitive stimulation produced a hyperpolarization initially but the membrane potential decreased gradually to the initial level during the stimulation. After the stimulation was stopped, the rebound excitation was observed. The time course and the maximum amplitude of the hyperpolarization due to the repetitive stimulation at 10^{-8} g/ml of adrenaline were similar to the control. The maximum amplitude of the hyperpolarization was decreased in adrenaline 10^{-6} g/ml. The rebound excitation seems to be prevented in adrenaline 10^{-8} g/ml and was abolished completely in adrenaline 10^{-6} g/ml. Similar results on the IJPs evoked by repetitive stimulation were obtained at 10^{-5} g/ml of noradrenaline. The amplitude of successive IJPs in response to repetitive stimulation (0.3 msec duration, 4 Hz and 11 Hz) decreased gradually in normal solution. The rate of decrease in the amplitude was slightly large in noradrenaline.

**Effects of phenylephrine on the IJP**

Phenylephrine (10^{-5} g/ml) slightly increased the membrane potential of the cell. The membrane potential was $-58.2 \pm 6.0 \text{ mV (n=13)}$ in normal solution

![Fig. 3. Effects of phenylephrine on the IJP in response to single and repetitive stimulation.](image)

- **A**: Left, three examples of control IJPs evoked by 0.5 msec stimulation in normal solution. Right, three examples of the IJPs in phenylephrine 10^{-5} g/ml.
- **B**: Changes in the membrane potential and the amplitude of the IJP in phenylephrine 10^{-5} g/ml.
- **C**: Upper, control IJPs evoked by 0.5 msec duration at 3 Hz in normal solution. Lower, phenylephrine 10^{-5} g/ml.
- **D**: Upper, control IJPs evoked by 0.5 msec stimulation at 5 Hz in normal solution. Lower, phenylephrine 10^{-5} g/ml. The value at the left side on each panel indicates the membrane potential in mV.
and $-59.2\pm4.9$ mV ($n=14$) at $10^{-5}$ g/ml of phenylephrine. Fig. 3A shows examples of the IJPs evoked by brief pulses (0.5 msec duration) in normal solution and phenylephrine. The amplitude of the IJP was $17.1\pm5.3$ mV ($n=10$) in normal solution and $21.7\pm2.9$ mV ($n=11$) in phenylephrine (Fig. 3B). Figs. 3C and D show the effect of repetitive stimulation (0.5 msec duration, 3 Hz and 5 Hz) on the generation of the IJPs. The amplitude of the IJP evoked by repetitive stimulation in phenylephrine was similar to that obtained in normal solution. The rebound excitation was not inhibited in phenylephrine.

**Effects of isoprenaline on the IJP**

The membrane potential was increased by the treatment with isoprenaline. The membrane potential was $-58.1\pm4.8$ mV ($n=14$) in normal solution and $-61.9\pm4.6$ mV ($n=16$) at $10^{-5}$ g/ml of isoprenaline. The IJPs evoked by single stimulus (0.5 msec duration) in normal solution and isoprenaline are shown in Fig. 4A. The amplitude of the IJP in normal solution was $11.6\pm3.0$ mV ($n=13$) and $10.4\pm1.8$ mV ($n=17$) in isoprenaline. The rate of hyperpolarization was increased slightly. However, the half decay time was decreased and the latency was not changed in isoprenaline ($10^{-5}$ g/ml). Fig. 4B shows the effect of repetitive stimulation (0.5 msec duration, 3 Hz and 5 Hz) in isoprenaline. The amplitude of successive IJPs was slightly reduced in isoprenaline. The rebound excitation in these cells was observed in normal solution but it was prevented in isoprenaline. Several spikes due to the rebound excitation were observed even at $10^{-5}$ g/ml of isoprenaline.

**Fig. 4.** Effects of isoprenaline on the IJP in response to single and repetitive stimulation. 
A: Left, three examples of the IJPs evoked by 0.5 msec stimulation in normal solution. Right, three examples of the IJPs in isoprenaline $10^{-5}$ g/ml. 
B: Upper, Control IJPs evoked by 0.5 msec stimulation at 3 Hz in normal solution. Middle, two examples of the IJPs in isoprenaline $10^{-5}$ g/ml (initial and later stages). Lower, IJPs evoked by 0.5 msec stimulation at 5 Hz in isoprenaline $10^{-5}$g/ml.
Effects of adrenergic blockers on the IJP

The IJP was not blocked by phentolamine and propranolol. Fig. 5A shows the effect of phentolamine (10⁻⁷ g/ml) for long perfusion up to 120 min. The stimulation (0.3 msec duration) was applied repeatedly at 1 Hz. The membrane potential was not changed by phentolamine and the amplitude of the IJPs was not changed either.

Figs. 5B-E show the effect of propranolol on the IJPs in response to single or repetitive stimulation. The membrane potential was decreased by a perfusion of propranolol and the depolarization increased with increasing the concentration of propranolol; -61.9±7.3 mV (n=42) in normal solution, -55.9±7.7 mV (n=24) at 10⁻⁷ g/ml, -52.3±7.3 mV (n=19) at 10⁻⁶ g/ml and -48.5±5.2 mV (n=3) at 10⁻⁵ g/ml of propranolol. The amplitude of the IJP evoked by single stimulus (0.5 msec duration) was reduced in propranolol (Fig. 5B); 11.9±1.0 mV (n=18) in normal solution and 6.8±1.3 mV (n=24) at 10⁻⁷ g/ml, 6.7±1.0 mV (n=8) in normal solution and 6.8±1.3 mV (n=24) at 10⁻⁷ g/ml, 6.7±1.0 mV (n=8) in normal

Fig. 5. Effects of phentolamine and propranolol on the IJPs in response to single and repetitive stimulation.

A: *Left,* control IJPs evoked by 0.3 msec stimulation at 1 Hz in normal solution. *Right,* IJPs in phentolamine 10⁻⁷ g/ml. Times indicate the perfusion period.

B: *Upper,* two examples of the IJPs evoked by 0.5 msec stimulation in normal solution. *Lower,* two examples of the IJPs in propranolol 10⁻⁵ g/ml.

C: IJPs evoked by 0.5 msec stimulation at 1 Hz in normal solution (upper) and in propranolol 10⁻⁵ g/ml (lower).

D: IJPs evoked by 0.5 msec stimulation at 4 Hz in normal solution (upper) and in propranolol 10⁻⁵ g/ml (lower).

E: IJPs evoked by 0.5 msec stimulation at 6 Hz in normal solution (upper) and at 12 Hz in propranolol 10⁻⁵ g/ml (lower).
solution and \(3.6 \pm 0.5\) mV \((n=13)\) at \(10^{-6}\) g/ml and \(31.1 \pm 1.0\) mV \((n=4)\) in normal solution and \(23.1 \pm 0.8\) mV \((n=5)\) at \(10^{-5}\) g/ml of propranolol. The decrease in amplitude was large at \(10^{-5}\) g/ml of propranolol. The latent time was nearly the same (56.0 \pm 7.4\) msec, \(n=4\) in normal solution and 57.8 \pm 7.5\) msec, \(n=5\) at \(10^{-5}\) g/ml of propranolol). The rate of hyperpolarization of the IJP was decreased \((256.7 \pm 18.4\) mV/sec, \(n=4\) in normal solution and 187.2 \pm 1.0\) mV/sec, \(n=5\) at \(10^{-5}\) g/ml of propranolol). The half decay time of the IJP was nearly the same.

The amplitude of the IJPs in response to repetitive stimulations \((0.5\) msec duration) at \(1\) Hz (Fig. 5C), at \(4\) Hz (Fig. 5D) and at \(12\) Hz (Fig. 5E) was slightly reduced in propranolol \((10^{-5}\) g/ml). The amplitude of successive IJPs decreased gradually in normal solution and this decrease in amplitude of the IJPs was more potentiated in propranolol.

The IJPs could be evoked in guanethidine. Fig. 6 shows the IJPs obtained by single and repetitive stimulation in guanethidine. The membrane potential was not altered by guanethidine; \(-61.2 \pm 4.6\) mV \((n=17)\) in normal solution and \(-60.1 \pm 4.9\) mV \((n=19)\) at \(10^{-5}\) g/ml of guanethidine. The IJPs in response to single stimulus \((0.5\) msec duration) observed in normal solution and in guanethidine \((10^{-5}\) g/ml) were shows in Fig. 6A. The amplitudes of these IJPs were nearly the

![Image](image-url)

**Fig. 6.** Effects of guanethidine on the IJP in response to single and repetitive stimulation.

A: Three examples of the IJPs evoked by 0.5 msec stimulation in normal solution (left) and guanethidine \(10^{-5}\) g/ml (right).

B: Changes in the membrane potential and the amplitude of the IJPs in guanethidine \(10^{-5}\) g/ml.

C: IJPs evoked by 0.5 msec stimulation at 3 Hz in normal solution (left upper), guanethidine \(10^{-5}\) g/ml (left lower) and at 5 Hz in guanethidine \(10^{-5}\) g/ml (right).
same; 14.6±2.7 mV (n=22) in normal solution and 16.4±4.1 mV (n=18) at 10⁻⁵ g/ml of guanethidine (Fig. 6B).

Fig. 6C shows the IJPs evoked by repetitive stimulation (0.3 msec duration and 3 Hz or 5 Hz) at 10⁻⁵ g/ml guanethidine. The response in guanethidine was similar to that obtained in normal solution. The rebound excitation was observed in guanethidine.

Effects of tyramine on the IJP

The effect of tyramine on the generation of the IJPs was examined. The membrane potential was slightly decreased by the treatment with tyramine, i.e., -57.3±8.2 mV (n=8) in normal solution, -55.1±4.3 mV (n=8) at 10⁻⁶ g/ml and -51.9±3.8 mV (n=11) at 10⁻⁵ g/ml of tyramine. When a pulse with very short duration (0.05 msec) was applied in normal solution, a small hyperpolarization was produced but the amplitude and the time course of the IJPs were not clear. However, after the addition of tyramine 10⁻⁶ g/ml, the same pulses generated more clearly the responses. The amplitude of these responses increased with increasing the concentration of tyramine, as shown in Fig. 7A. Similar results on the changes in the amplitude of the IJPs evoked by 0.3 msec stimulation at 1 Hz (Fig. 7B) and 5 Hz (Fig. 7C) were obtained at 10⁻⁶ g/ml and 10⁻⁵ g/ml of tyramine.

Fig. 7. Effects of tyramine on the IJPs in response to repetitive stimulation.
A: IJPs evoked by 0.05 msec stimulation at 1 Hz in normal solution (upper), tyramine 10⁻⁶ g/ml (middle) and 10⁻⁵ g/ml (lower).
B: IJPs evoked by 0.3 msec stimulation at 1 Hz in normal solution (upper), tyramine 10⁻⁶ g/ml (middle) and 10⁻⁵ g/ml (lower).
C: IJPs evoked by 0.3 msec stimulation at 5 Hz in normal solution (upper) and tyramine 10⁻⁶ g/ml (lower).
amplitudes of the IJPs in response to 0.1 msec stimulation at 1 Hz were 4.2±0.3 mV \((n=5)\) in normal solution, 7.2±1.0 mV \((n=5)\) at 10^{-6} g/ml and 8.7±0.9 mV \((n=7)\) at 10^{-5} g/ml of tyramine. The amplitudes of the IJPs in response to 0.3 msec stimulation at 1 Hz were 8.9±0.5 mV \((n=5)\) in normal solution, 16.1±1.3 mV \((n=3)\) at 10^{-6} g/ml and 19.6±1.5 mV \((n=2)\) at 10^{-5} g/ml of tyramine. The rebound excitation was observed in tyramine.

**DISCUSSION**

In taenia coli, the IJP was not influenced by the treatment with phentolamine and propranolol perfused for a long duration (Ito and Kuriyama 1971). In the present experiment the amplitude of the IJP was decreased slightly after long perfusion but not abolished by phentolamine and propranolol. The IJP recorded from the taenia coli was not blocked by guanethidine (Bennett et al. 1966b). Similar result on the guanethidine effect was obtained in spite of a small depolarization of the cell membrane. The possibility that these exogenously applied blockers do not reach the regions of neuromuscular junction may be excluded because of long perfusion of these agents at high concentrations. The obtained results suggest that a transmitter released from terminals of the intramural inhibitory nerve is nonadrenergic.

Differences in the properties of noradrenaline receptors between the junctional and extrajunctional regions in the smooth muscles have been reported in the artery (Holman and Surprenant 1979; Hirst and Neild 1980) and vas deferens (Hotta 1969). In the vas deferens, it was assumed that alpha- or beta-blockers had no effect on the junctional receptors (Hotta 1969). It is not clear whether the location of receptors on the cell membrane of duodenal smooth muscle for the generation of the IJP is junctional or extrajunctional. If the region of receptors is junctional and resistant to adrenergic blocking agents, the possibility that a transmitter for the IJP is adrenergic still remains. However, this is unlikely because the IJP could be evoked by a brief pulse and the IJP’s amplitude was still large in a high concentration of adrenaline and noradrenaline, nevertheless the cell membrane was hyperpolarized by these agents.

Holman and Surprenant (1979) proposed the different types of noradrenaline receptors on the arterial smooth muscle which seem to respond in a different manner to noradrenaline released from the nerves and to that applied exogenously. In the smooth muscle of the taenia coli, the hyperpolarization in response to perivascular inhibitory nerve stimulation was blocked by guanethidine (Bennett et al. 1966a). Therefore the intestinal smooth muscle seems to respond to noradrenaline applied endogenously and exogenously in a similar manner to the mesenteric vein (Suzuki 1981).

When a brief pulse is given to the tissue, the terminals of adrenergic and non-adrenergic nerves are activated at the same time but it seems that the characteristics of the IJP were not influenced by adrenergic transmission. There is no class of the IJP which disappears or is strongly inhibited by the perfusion of adrenaline,
noradrenaline and isoprenaline. This observation confirmed the previous results (Read and Burnstock 1969; Furness 1969). A sparse sympathetic inhibitory innervation to the smooth muscle of the duodenum was suggested in the previous work (Ohkawa 1983) similarly to that in the taenia coli (Bennett and Rogers 1967).

At a concentration of $10^{-5} \text{g/ml}$ noradrenaline, the membrane potential of the cell was $-63 \text{mV}$. In this solution, the amplitude of the IJP evoked by single stimulus reached up to $23 \text{mV}$. The peak potential of the IJP was $-86 \text{mV}$ and this value was nearly the same as the potassium equilibrium potential in the smooth muscle (Casteels 1970). It has been reported that the hyperpolarization induced by exogenously applied adrenaline and noradrenaline and by an IJP was due to an increase in potassium conductance (Bulbring et al. 1966; Jenkinson and Morton 1967; Tomita 1972). However, the desensitization of the cell membrane to nonadrenergic transmitter was remarkable in the duodenal smooth muscle (Ohkawa 1983).

The concentration of noradrenaline released from adrenergic nerve terminals was estimated to be as high as $10^{-9} \sim 10^{-4} \text{M}$ in the vascular tissues (Ljung 1969; Bell and Vogt 1971; Suzuki 1981). These concentrations were higher than those of noradrenaline used in the present experiment. However, it is unlikely that such high concentrations of noradrenaline produce a hyperpolarization over 20–30 mV. The IJP generation in most of the duodenal smooth muscles does not seem to be influenced by endogenous adrenergic transmitter.

Propranolol decreased the amplitude of the IJP. This suggests that propranolol inhibits the interaction between the nonadrenergic transmitter and its receptors and prevents an increase of potassium conductance due to nonadrenergic transmitter. The amplitude of the IJP, in general, decreased when the membrane was hyperpolarized by agents except propranolol, and vice versa. Tyramine mimics the physiological effects of adrenergic nerve stimulation by releasing endogenous noradrenaline from its stores at sympathetic nerve terminals (Ambache et al. 1972). If tyramine acts on the terminals of nonadrenergic inhibitory nerve in a similar way to that on the adrenergic nerve and increases the release amount of a nonadrenergic transmitter, the amplitude of the IJP may increase depending on the concentration of tyramine.

References
5) Bennett, M.R., Burnstock, G. & Holman, M.E. (1966b) Transmission from intramural
inhibitory nerves to the smooth muscle of the guinea-pig taenia coli. J. Physiol., 182, 541–558.


