Effects of Electrolytes and Drugs on the Inhibitory Junction Potentials Recorded from the Antrum of Guinea-pig Stomach

Yong Sook Goo, Suk Hyo Suh*, Suk-Ho Lee**
Sang-Ik Hwang** and Ki Whan Kim**

Department of Physiology, College of Medicine, Chungbuk National University, Dongkuk University, Seoul National University***

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ABSTRACT

The effects of electrolytes, adenosine, ATP, 5-hydroxytryptamine (5-HT, serotonin) and ketanserin on the inhibitory junction potentials (IJPs) were investigated to clarify the interactions of these drugs with the neurotransmitters released from non-adrenergic, non-cholinergic nerves in the antrum of guinea-pig stomach. Electrical responses of antral circular muscle cells were recorded intracellularly using glass capillary microelectrode filled with 3 M KCl. All experiments were performed in Tris-buffered Tyrode soluition which was aerated with 100% O_2 and kept at 35℃.

The results obtained were as follows:

1) Inhibitory junction potential (IJP) was recorded in antral strip, while excitatory junction potential (EJP) was recorded in fundic strip.

2) IJP recorded in antral strip was not influenced by atropine (10^{-6} M) and guanethidine (5×10^{-6} M).

3) The amplitude of IJP increased in high Ca^{2+} solution, while that of IJP decreased in high Mg^{2+} solution or by Ca^{2+} antagonist (verapamil). Apamin, Ca^{2+}-activated K^+ channel blocker blocked IJP completely.

4) ATP and adenosine decreased the amplitude of IJP.

5) 5-HT decreased the amplitude of IJP with no change of the amplitude of slow waves, while ketanserin (5-HT type 2 blocker) decreased the amplitude of slow waves markedly with no change in that of IJP.

From the above results, the following conclusions could be made.

1) IJP recorded in antral strip is resulted from neurotransmitters released from non-adrenergic, non-cholinergic nerves.

2) An increase in the concentration of external Ca^{2+} enhances the release of neurotransmitters from non-adrenergic, non-cholinergic nerves which activate the Ca^{2+}-dependent K^+ channel.

Key Words: Inhibitory junction potential (IJP), Excitatory junction potential (EJP), Non-adrenergic non-cholinergic nerves, Ca^{2+} dependent K^+ channel

INTRODUCTION

The presence of non-adrenergic non-cholinergic nerves has been established throughout the gastro-intestinal tract of many vertebrate species (Burnstock et al, 1966; Campbell & Burnstock, 1968) as well as in other organs including lung, urinary bladder, tra-

chea, esophagus, anococcygeus, seminal vesicles and parts of the cardiovascular system (Burnstock, 1969; 1979b; Campbell, 1970; Furness & Costa, 1973). Non-adrenergic non-cholinergic nerves are concerned with the propulsion of material through the alimentary canal, participating in reflex opening of lower esophageal and internal anal sphincters, receptive relaxation of the stomach and descending inhibition during intestinal peristalsis (Burnstock & Costa, 1973).

Nerves utilizing ATP as the principal transmitter were termed purinergic ones (Burnstock, 1971) and it has been suggested that polypeptides such as enkephalin, vasoactive intestinal polypeptide (VIP) and other substances including serotonin, GABA, dopamine, substance P, bombesin, somatostatin, neurotensin, and cholecystokinin (CCK) are autonomic neurotransmitters (Burnstock, 1981).

By recording junction potentials evoked by the stimulation of the intramural nerve in guinea-pig stomach, it was established that there were three types of junction potentials, namely cholinergic excitatory, adrenergic inhibitory, and non-adrenergic non-cholinergic inhibitory junction potentials (Kuriyama, 1970; Beani et al, 1971; Shuba & Vladimirova, 1980). There existed regional differences in junction potentials recorded in the circular muscles of the guinea-pig stomach, that is cholinergic excitatory junction potential (EJP) in the fundic region and non-adrenergic non-cholinergic inhibitory junction potential (IJP) in the antral region.

The EJP was blocked and instead IJP was evoked on nerve stimulation in the atropine-treated fundic region. The thresholdal concentration of Ach required to depolarize the membrane was about 1000 times higher in the antral region than in the fundic one. So it was concluded that the regional differences in junction potentials may be due to different sensitivities of Ach receptor of the guinea-pig stomach, in other words its higher sensitivity evokes the EJP in the fundic region and the lower sensitivity evokes the IJP in antral region, in response to nerve stimulation (Komori & Suzuki, 1986).

There are also differences in membrane potentials and slow waves between smooth muscles in fundic region and those in antral region. In fundic region, resting membrane potential (RMP) is relatively low (−50 mV) and there are no slow waves. On the contrary in the antral region, RMP is high (about −65 mV) and there are regular slow waves and action potentials sometimes appear at the peak of slow waves (Komori & Suzuki, 1986; Rhie & Kim, 1987).

Thus in this paper, we tried to confirm the regional differences between fundic and antral regions. Then we observed the effects of electrolytes such as Ca^{2+} and Mg^{2+}, adenosine, ATP, 5-hydroxytryptamine (5-HT, serotonin) and ketanserin on the IJP to clarify the interactions of these drugs with the neurotransmitters released from non-adrenergic non-cholinergic nerves in the antrum of guinea-pig stomach.

METHODS

Guinea-pigs of either sex, weighing about 300 g, were stunned and bled. And then the stomach was excised and cut in the longitudinal direction along the lesser curvature. The mucosal layer was removed from the muscle layers in phosphate-buffered Tyrode solution (saturated with 100% O_2 at room temperature). The strip of circular muscle preparation, 2 mm in width and 10 mm in length, was isolated together with the longitudinal layer along the direction of circular muscle. After one hour recovery, the preparation was mounted on the horizontal experimental chamber using tiny pins. The experimental chamber had a volume of about 2 ml, and the tissues were superfused with 35℃ Tris-buffered Tyrode solution at a flow rate of 4～5 ml/min. After another one hour recovery, electrical responses of smooth muscle cells were recorded with glass capillary microelectrodes filled with 3 M KCl. The tip resistance of electrodes

ranged between 40 and 80 MΩ.

The electrode was advanced from the mucosal side of the preparation. The electrical responses were displayed on a pen recorder (Gould RS 3200). Field stimulation was applied transmurally to stimulate intramural nerves by using a pair of platinum wires (0.5 mm in diameter) placed on both sides of the preparation. Electrical current pulses of 0.05~0.1 ms in duration and 10~50 V in intensity were applied from an electric stimulator (Grass S 88). The glass microelectrode put into the tissue about 0.3 mm apart from platinum electrode (Fig. 1).

All experiments were done at 35℃.

The ionic composition of the solutions used were as follows (mM): Phosphate-buffered Tyrode solution (NaCl 147, KCl 4, $MgCl_2 \cdot 6H_2O$ 1.05, $CaCl_2 \cdot 2H_2O$ 2, $NaH_2PO_4 \cdot 2H_2O$ 0.42, $Na_2HPO_4 \cdot 12H_2O$ 1.81, Glucose 5.5, pH 7.35), Tris-buffered Tyrode solution (NaCl 158, KCl 4, $CaCl_2 \cdot 2H_2O$ 2, $MgCl_2 \cdot 6H_2O$ 1.05, Tris·HCl 10, Glucose 5.5, pH 7.35).

Drugs used were as follows: adenosine (Sigma), apamin (Sigma), atropine sulfate (Sigma), guanethidine sulfate (Tokyo kasei), ketanserin tartrate (Janssen), 5-hydroxytryptamine (5-HT, serotonin, Sigma), tetrodotoxin (TTX, Sankyo), verapamil (isoptin, Knoll AG).

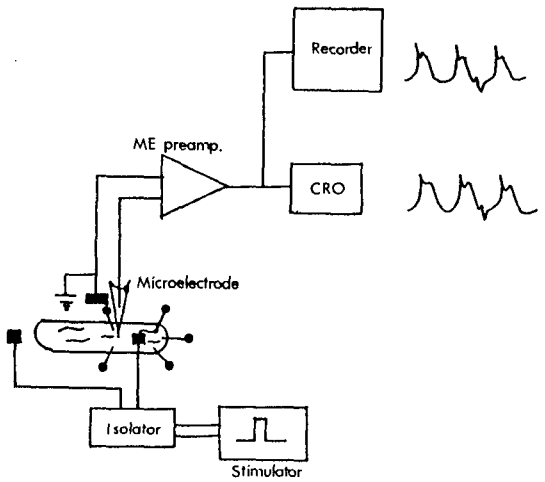

Fig. 1. Schematic representation of electrical activity recording system. Junction potentials were generated by field stimulation of transmural nerves. The microelectrode puncture technique for intracellular recording of the electrical activities was employed in this experiment.

RESULTS

1. Regional differences of slow waves and junction potentials

Fig. 2 shows slow waves and junction potentials in different regions of the guinea-pig stomach. In the antral region, the resting membrane potentials (RMPs) were spontaneously fluctuated periodically, that is the slow waves were recorded and the amplitudes were about 20 mV. On the contrary, in the fundic region the slow waves were not recorded or the amplitudes were very small, if any (Fig. 2A).

Transmural stimulation of nerves evoked an inhibitory junction optential (IJP) in the antral region and an excitatory junction potential (EJP) in the fundic region (Fig. 2B).

2. Effects of neurotransmission blockers on junction potentials

Fig. 3 shows the effects of atropine, guanethidine, and TTX on the IJP recorded in the antral region.

Atropine blocked the effects of parasympathetic nerve stimulation and guanethidine blocked those of sympathetic nerve stimulation. 15 minutes after the application of atropine (10^{-6} M) and guanethidine (5×10^{-6} M), the amplitudes of IJP were never affected (Fig. 3B). The IJPs were completely blocked by additional treatment of nonspecific nerve blocker, TTX (3×10^{-7} M) to the solution containing atropine and guanethidine (Fig. 3C).

In the following experiments, we executed the pretreatment of atropine (10^{-6} M) and guanethidine (5×10^{-6} M).

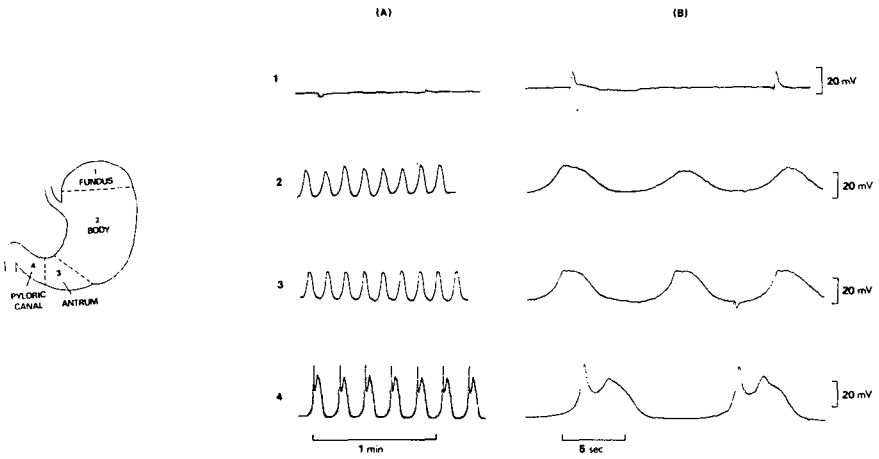

Fig. 2. Electrical activities (slow waves, junction potentials) recorded from smooth muscle cells in the regions shown in the schematic representation of guinea-pig stomach.
(A) Slow waves recorded with slow tracing speed.
(B) Junction potentials recorded with fast tracing speed.
Note that the smooth muscle cells in the fundic region show electrically quiescent responses and excitatory junction potentials (EJP), whereas those in the antral region show regular slow waves and inhibitory junction potentials (IJP).

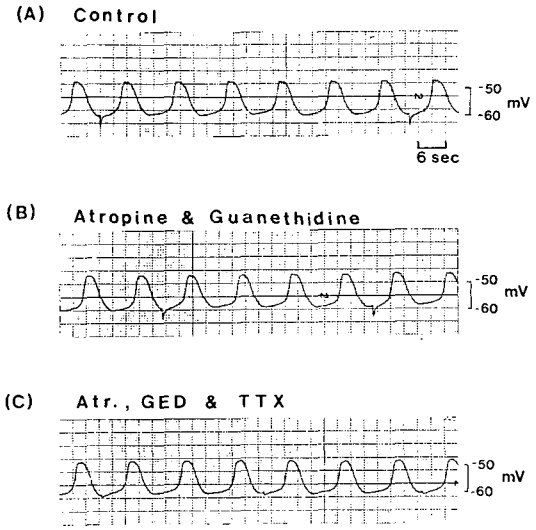

Fig. 3. Effects of atropine, guanethidine and TTX on inhibitory junction potentials (IJPs) induced by transmural nerve stimulation (50 V in intensity, 50 μs in duration, single pulse) recorded from the antrum of guinea-pig stomach.
It shows the result without blockers (A). In the presence of atropine (10^{-6} M) and guanethidine (5×10^{-6} M), there was no change in IJPs (B). Additional application of TTX (3×10^{-7} M) inhibited IJPs (C).

3. Effects of electrolytes on junction potentials

1) Ca^{2+} effect: Fig. 4 shows the effect of Ca^{2+} on the amplitudes of IJPs and slow waves. In the high Ca^{2+} (7 mM) solution, the amplitudes of slow waves increased to 13 mV (control 9 mV) and those of IJPs increased to 20 mV (control 10.5 mV) (Fig. 4B1). In the other antral strip, the amplitudes of slow waves were not affected with the application of high Ca^{2+} solution but those of IJPs increased to 4 mV (control 2.5 mV) and abortive spikes appeared (Fig. 4B2).

After the treatment with high Ca^{2+} solution, we applied Ca^{2+} free Tyrode solution to confirm the effect of Ca^{2+}. In 7 mM Ca^{2+} solution, the amplitudes of slow waves increased to 24 mV (control 15 mV) and those of IJPs increased to 5 mV (control 3 mV). Then with the application of Ca^{2+} free Tyrode solution, the amplitudes of slow waves decreased to 7 mV and those of IJPs also decreased to 2 mV (Fig. 5).

2) Mg^{2+} effect: Mg^{2+} is considered as a natural physiologic Ca^{2+} antagonist and it influences the

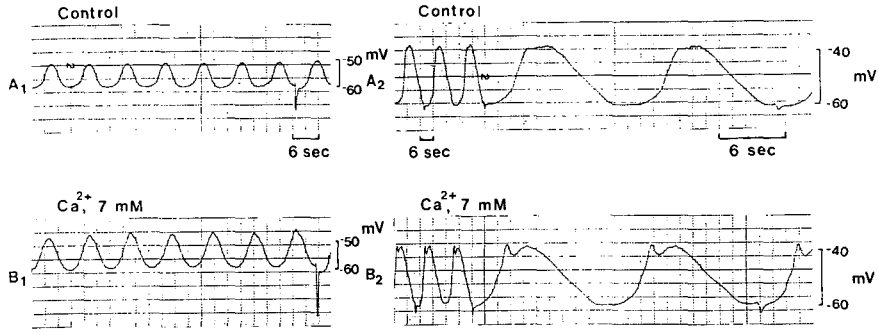

Fig. 4. Effects of high Ca^{2+} (7 mM) on the amplitudes of the slow waves and IJPs induced by transmural nerve stimulation (50 V in intensity, 50 us in duration, single pusle) in the antrum of guinea-pig stomach. The amplitudes of slow waves and IJPs were greatly increased in high Ca^{2+} solution. (A_1) (B_1), (A_2) (B_2) were different antral strips.

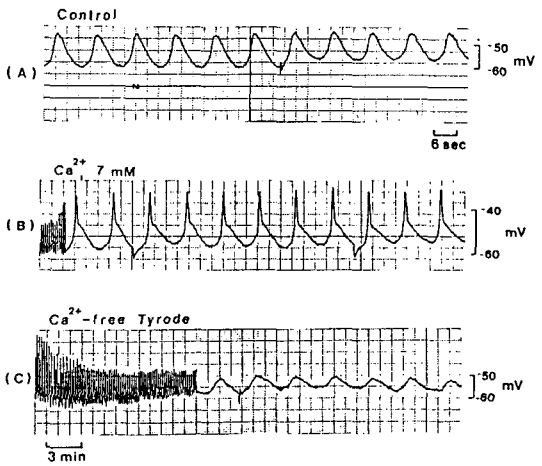

Fig. 5. Effects of Ca^{2+} on the amplitudes of slow waves and IJPs induced by transmural nerve stimulation (50 V in intensity, 50 μs in duration, single pulse) in the antrum of guinea-pig stomach.
In high Ca^{2+} solution (7 mM), the amplitudes of slow waves and IJPs increased greatly and abortive spitke potentials appeared (B).
In Ca^{2+}-free Tyrode solution, the amplitudes of both slow waves and IJPs decrease (C).

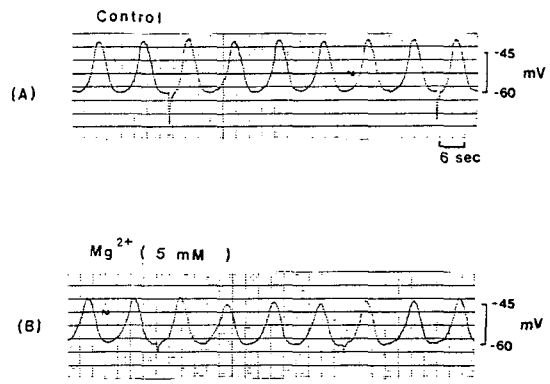

Fig. 6. Effects of high Mg^{2+} (5 mM) on the amplitudes of slow waves and IJPs induced by transmural nerve stimulation (40 V in intensity, 40 μs in duration, single pulse) in the antrum of guinea-pig stomach. The amplitudes of slow waves decresed slightly while those of IJPs decreased prominently (B).

distribution as well as the influx/efflux of Ca^{2+} (Altura et al, 1986). Ca^{2+} and Mg^{2+} have effects on the stability of smooth muscle cell membrane (Helga et al, 1973; Palaty, 1974; Karzaki et al, 1985; Altura, 1986; Filliponi, 1987). Mg^{2+} has more powerful binding capacity with cell membrane than Ca^{2+} does (Triggle, 1971) and potentiates the stability of cell membrane (Smith et al, 1972). If extracellular Mg^{2+} concentration is increased, Ca^{2+} permeability is diminished (Helga, 1973).

Fig. 6 shows the effects of Mg^{2+} on the amplitudes of slow waves and IJPs. 10 minutes after the application of high Mg^{2} solution (5 mM) the amplitudes of slow waves slightly decreased (89% of control state), whereas those of IJPs greatly decreased (29% of control state).

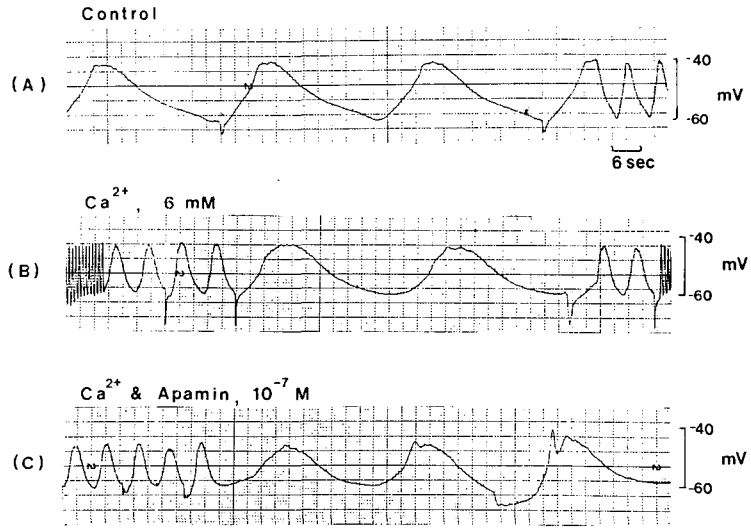

Fig. 7. Effects of apamin on the amplitudes of IJPs induced by transmural nerve stimulation (40 V in intensity, 40 μs in duration, single pulse) in the antrum of guinea-pig stomach.
Apamin blocked IJPs completely (C) which were enhanced prominently by high Ca^{2+} solution (B).

4. Effects of apamin on junction potentials

Apamin, the extract of bee venom, is known as a Ca^{2+} dependent K^+ channel blocker (Banks et al, 1979).

In Fig. 7 the IJP completely disappeared with additional application of apamin (10^{-7} M) to high Ca^{2+} solution (6 mM). It suggests that the neurotransmitters released from non-adrenergic non-cholinergic nerves may activate Ca^{2+} dependent K^+ channel and then the hyperpolarization of the membrane may take place to generate IJPs.

5. Effects of Ca^{2+} antagonist on junction potentials

Fig. 8 shows the effects of Ca^{2+} antagonist, verapamil on the amplitudes of slow waves and IJPs, which both were increased in 7 mM Ca^{2+} solution. By the application of the Tyrode solution containing 7 mM Ca^{2+} for 5 minutes, the amplitudes of slow waves increased to 13 mV (control 10 mV) and those of IJPs incresed to 19.5 mV (control 16 mV) (Fig. 8B). With the additional application of verapamil (10^{-5} M) to 7 mM Ca^{2+}-containing solution for 25 minutes, the membrane potential was depolarized (not shown in figure) and the abortive spikes appeared and the amplitudes of IJPs were increased to 22 mV (Fig. 8C1). 40 minutes after the application of verapamil the abortive spike potential disappeared and the amplitudes of slow waves decreaed to 7 mV (70% of control level), whereas those of IJPs increased to 21 mV (Fig. 8C2). With the application of verapamil for 45 minutes the amplitudes of slow waves decreased to 4 mV (40% of control level) but those of IJPs were still higher than control level (16 mV). These results suggest that increased IJP amplitudes due to membrane depolarization masked the effects of verapamil on the IJPs.

6. Effects of adenosine and ATP on junction potentials

Fig. 9 and 10 show the effects of adenosine on the junction potentials. With the application of adenosine (5×10^{-6} M) for 15 minutes, the changes in

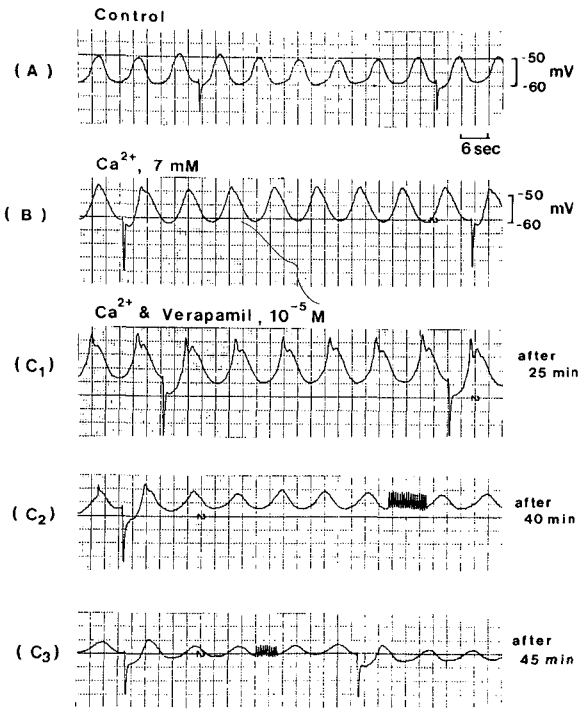

Fig. 8. Effects of Ca^{2+} and verapamil on the amplitudes of slow waves and IJPs induced by transmural nerve stimulation (40 V in intensity, 40 μs in duration, single pulse) in the antrum of guinea pig stomach.
In high Ca^{2+} solution (7 mV), the amplitudes of slow waves and IJPs greatly increased (B). 25 min after additional application of verapamil (10^{-5} M) to high Ca^{2+} solution, the amplitudes of slow waves and IJPs increased more and abortive spike potential appeared due to Ca^{2+} effect (C_1). 40 min after verapamil application, the amplitudes of slow waves decreased to 70% of control level but those of IJPs were larger than control level (C_2). 45 min after verapamil application, the amplitudes of slow waves decreased to 40% of control level but those of IJPs were still larger than control level (C_3).

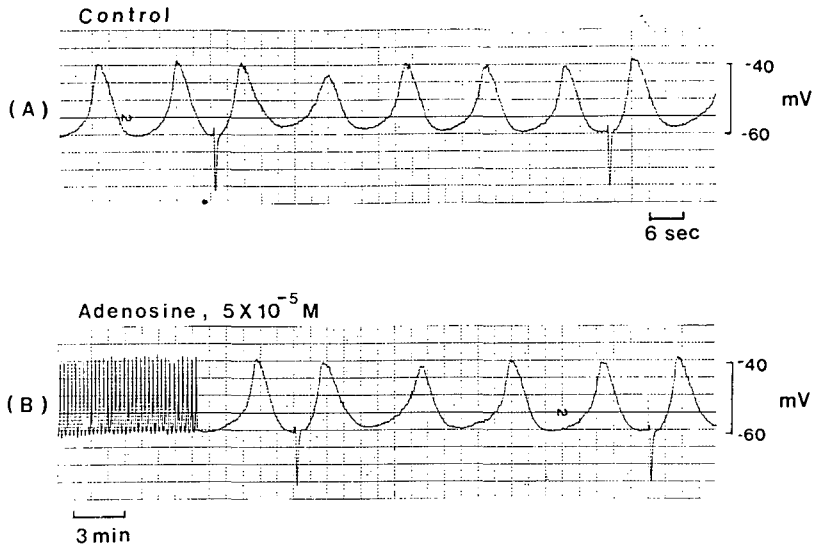

Fig. 9. Effects of adenosine (5×10^{-5} M) on the amplitudes of slow waves and IJPs induced by transmural nerve stimulation (50 V in intensity, 50 μs induration, single pulse) in the antrum of guinea-pig stomach.
The amplitudes of slow waves did not change but those of IJPs decreased to 89% of control level (B).

the amplitudes of slow waves were not observed but those of IJPs slightly decreased to 16.5 mV (control 18.5 mV) (Fig. 9). To confirm the inhibitory effects of adenosine on IJPs, adenosine was added to 7 mM Ca^{2+} solution. In the solution containing 7 mM Ca^{2+} for 10 minutes, the amplitudes of IJPs increased to 13.5 mV (control 10 mV). And then with adenosine added to 7 mM Ca^{2+}-containing solution for 10 minutes, the amplitudes of IJPs decreased to 12 mV (89% of the value in the solution containing 7 mM Ca^{2+}) (Fig. 10). The inhibitory effects of adenosine was not conspicuous. It was supposed that 10 minute duration of application was too short for adenosine to reverse the Ca^{2+} effect.

Fig. 11 shows the effects of ATP on IJPs. With the application of ATP (10^{-5} M) for 23 minutes, the amplitudes of slow waves did not change but those of IJPs decreased to 3.8 mV (76% of control level).

7. Effects of 5-HT and ketanserin on junction potentials

5-HT is known as one of the most possible neurotransmitter together with ATP, released from non-adrenergic non-cholinergic nerves.

With the application of 5-HT (10^{-6} M) for 15 minutes, the amplitudes of slow waves were same to

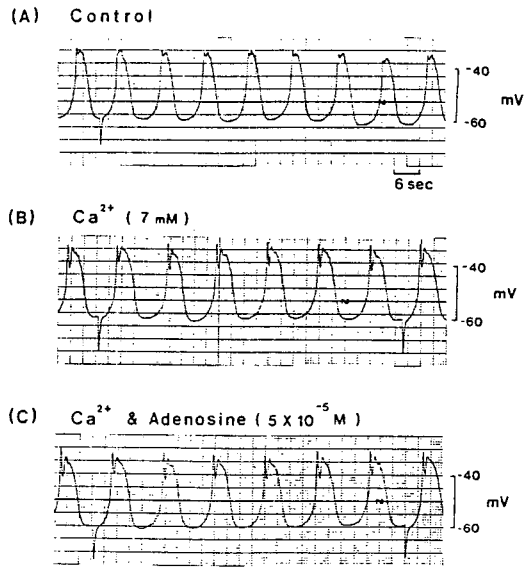

Fig. 10. Effects of Ca^{2+} and adenosine on the amplitudes of slow waves and IJPs induced by transmural nerve stimulation (40 V in intensity, 40 μs in duration, single pulse) in the antrum of guinea-pig stomach.
In high Ca^{2+} solution, the amplitudes of slow waves and IJPs increased and abortive spike potentials appeared (B).
10 minutes after additional application of adenosine (5×10^{-5} M), the amplitudes of slow waves did not change but those of IJPs decreased slightly (C).

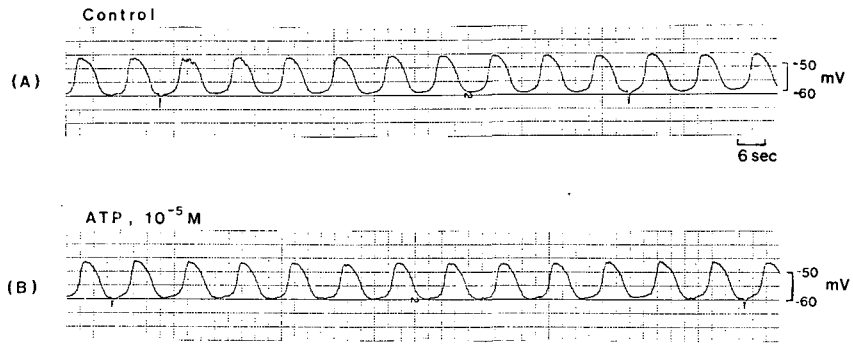

Fig. 11. Effects of ATP (10^{-5} M) on the amplitudes of slow waves and IJPs induced by transmural nerve stimulation (50 V in intensity, 50 μs in duration, single pulse) in the antrum of guinea-pig stomach.
The amplitudes of slow waves did not change but those of IJPs decreased slightly (89% of control level) (B).

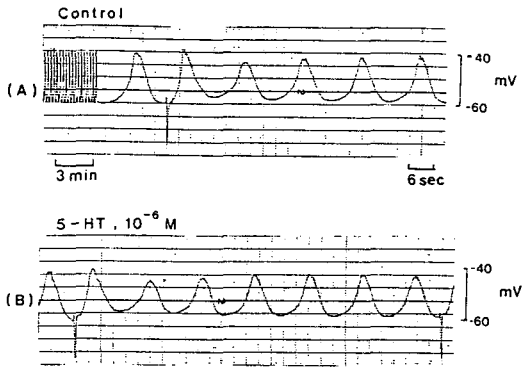

Fig. 12. Effects of 5-HT (10^{-6} M) on the amplitudes of slow waves and IJPs induced by transmural nerve stimulation (50 V in intensity, 50 μs in duration, single pulse) in the antrum of guinea-pig stomach.
The amplitudes of slow waves did not change but those of IJPs decreased slightly (89% of control level) (B).

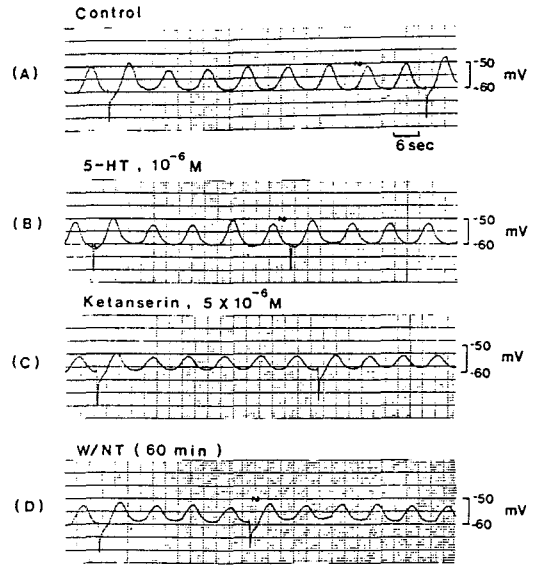

Fig. 13. Effects of 5-HT (10^{-6} M) and ketanserin (5×10^{-6} M) on the ampltudes of slow waves and IJPs induced by transmural nerve stimulation (50 V in intensity, 50 μs in duration, single pulse) in the antrum of guinea-pig stomach.
After treatment of 5-HT (10^{-6} M) the amplitudes of slow waves did not change but those of IJPs decreased (B). On the contrary, after treatment of ketanserin (5×10^{-6} M), the amplitudes of IJPs did not change but those of slow waves decreased (C).
After wash out with normal tyrode solution, the amplitudes of slow waves did not recover to control level until 60 minutes (D).

the control level but those of IJPs decreased to 16.5 mV from 18.5 mV (89% of control level) (Fig. 12).

In the other strip, the amplitudes of slow waves were 9 mV and those of IJPs were 13.5 mV (Fig. 13). After application of 5-HT for 15 minutes, the amplitudes of slow waves never changed but those of IJPs decreased to 10.5 mV (78% of control level).

Ketanserin has been known as a specific antagonist of 5-HT type 2 receptor. With the application of ketanserin opposite results occurred. With the treatment of ketanserin (5×10^{-6} M) for 5 minutes, the amplitudes of slow waves decreased to 6 mV (67% of control level) but those of IJPs did not change. When ketanserin was washed out for 60 minutes with normal Tyrode solution the amplitudes of slow waves were not recovered to control level.

DISCUSSION

With regard to the regional differences of electrical activities recorded in guinea-pig stomach, the membrane potential oscillates periodically, that is, slow waves are recorded in the antral region. On the contrary there are no slow waves in the fundic region (Fig. 2). The characteristics of slow waves recorded in circular muscle of antrum are as follows: The amplitudes and the frequency were 10~30 mV and 6/min at 35℃, respectively. The slow waves had not any change with the application of atropine (10^{-6} M), guanethidine (5×10^{-6} M), and TTX (3×10^{-7} M). So it is confirmed that the slow waves may be originated from the gastric smooth muscle cells themselves regardless of nerves.

It has been reported that the slow waves have

three components (Ohba et al, 1975; 1977). Namely the slow waves have the first, the second, and the spike components recorded in the circular muscle cells of guinea-pig stomach: The first one makes up the bottom part of slow wave and determines the frequency of slow waves. It is originated by electrogenic Na-Ca enchange mechanism and is generated by potential independent process. The secone one is potential dependent process, which is mainly due to an increase in Ca^{2+} conductance initiated by the depolarization through the first component. The spike one at the top of the second component is generated by Ca^{2+} channel activation, so the Ca^{2+} antagonists block the spike component prominently (Tomita, 1981). In our experiment the abortive spikes disappeared with the treatment of verapamil (10^{-5} M) in the first place (Fig. 8). In the experiment treating with the solution containing high Ca^{2+}, the frequency of slow waves did not change whereas the amplitudes increased and abortive spike potentials appeared (Fig. 4, 5, 8, 10). It is supposed that these results are due to the increase of Ca^{2+} influx through the Ca^{2+} channel.

The IJPs were recorded in the antral region, whereas the EJPs were recorded in the fundic region (Fig. 2). Even after the treatment of both atropine (10^{-6} M) and guanethidine (5×10^{-6} M), the IJPs were recorded in the antral region. So it may be supposed that the EJPs have cholinergic nature recorded in the fundic region whereas the IJPs have non-adrenergic non-cholinergic nature recorded in the antrum. And it confirms the results of the other authors (Komori & Suzuki, 1986).

The amplitudes of junction potentials are determined by the amount of neurotransmitters released during nerve stimulation. As extracellular Ca^{2+} concentration increases, the amplitudes of IJPs increase. So it is supposed that Ca^{2+} ions facilitate the release of neurotransmitters from nerve terminal. From the result that high Mg^{2+} and Ca^{2+} free Tyrode solution diminished the amplitudes of IJPs, we confirmed the Ca^{2+} effect on the release of neurotransmitters.

ATP is known as one of the neurotransmitters released from non-adrenergic non-cholinergic nerve (Burnstock, 1971). The other putative neurotransmitters are 5-HT, GABA, dopamine and several polypeptides. There exist certain fundamental differences between putative neurotransmitters and classical ones: Classical neurotransmitters can be synthesized locally at the site of usage whereas the putative ones are supposed to have its origin in the cell body and be released from a nerve ending. Furthermore, whereas an efficient reuptake mechanism operates at the cell membrane of the presynaptic nerve ending for the classical transmitters, there is no such evidence for a similar reuptake mechanism for putative ones.

When ATP released from purinergic nerve endings during nerve stimulation binds to postsynaptic P_2 receptor, the Ca^{2+} permeability of muscle membrane changes and successively the Ca^{2+} dependent K^+ channel opens so that membrane hyperpolarizes transiently. As a result the IJPs are recorded (Banks et al, 1979).

The reversal potential for the IJPs is about −89 mV and it increases at depolarization whereas it decreases at hyperpolarization (Komori & Suzuki, 1986). With the application of apamin, Ca^{2+} dependent K^+ channel blocker, IJPs were inhibited completely in our experiment.

There have been known to be two kinds of purinergic receptor: One is presynaptic P_1 purinoceptor, which adenosine binds to and methylxanthines competitively blocks the binding of adenosine to. The other is postsynaptic P_2 purinoceptor, which ATP binds to, and its competitive antagonists are quinidine, 2, 2'-pyridilisatogen, 2-substituted imidazoline compounds (antazoline, phentolamine), apamin.

When adenosine binds to P_1 purinoceptor, it inhibits the release of ATP from purinergic nerve, namely that presynaptic inhibition appears (Burn-

stock, 1981). With the application of adenosine (5×10^{-5} M) the amplitudes of IJPs decreased in our experiment (Fig. 9, 10). So we could conclude that adenosine did have the effect through the presynaptic inhibition.

With the application of ATP (10^{-5} M) the amplitudes of IJPs decreased (Fig. 11). It is supposed that ATP hyperpolarized membrane potential so that the amplitudes of IJPs decreased secondarily.

The other possible neurotransmitter is 5-HT, released from non-adrenergic non-cholinergic nerves. The fast EJP is due to 5-HT recorded in the small intestine of guinea-pig with single nerve stimulation (Wood & Mayer, 1979). When 5-HT is applied iontophoretically to enteric ganglion cell, the responses are three types: First, it is a major response. The membrane potential is depolarized consistently, which is not desensitized with the repetitive application of 5-HT. Second, the membrane potential is depolarized transiently, which is desensitized with repetitive application of 5-HT. Third, the membrane potential is hyperpolarized.

With the treatment of 5-HT, the amplitudes of IJPs decreased in our experiment (Fig. 12, 13). Following explanation could be possible: Binding of 5-HT to the receptor on circular muscle results in membrane hyperpolarization and then lowers the amplitudes of IJPs secondarily. But the explanation has not been confirmed, because the value of the membrane potential and the amplitudes of IJPs had no change even with the application of ketanserin, specific antagonist of 5-HT type 2 receptor. Until now, have never been established the kinds and the distribution of serotoninergic receptor in gastrointestinal tract. Further study should be preceded on it so that specific antagonists would be found for each receptor. And we could expect the confirmation of the possibility of 5-HT as a neurotransmitter by means of pretreatment of specific antagonists iontophoretically and observation of change of amplitudes of IJPs.

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국문초록 =

기니피그 유문동에서 기록되는 억제성 접합부 전압에 미치는 전해질과 약물의 효과

충북대학교, 동국대학교* 및 서울대학교** 의과대학 생리학교실

구용숙 · 서석효* · 이석호 · 황상익** · 김기환****

기니피그 유문동 부위를 절제한 뒤 점막층을 박리하고 윤상근 주행방향으로 길이 10 mm, 너비 2 mm 되는 조직 절편을 만들어 수평형 실험용기에 넣어 핀으로 고정하였다. 유리미세전극을 세포내에 삽입하여 서파를 기록하면서 조직양편에 설치한 백금자극전극(직경 0.5 mm)에 강도 10 ~50 V, 기간 50~100 μs 되는 자극파를 주어 신경-근 부위의 접합부 전압을 기록하여 다음과 같은 결과를 얻었다.

1) 위저부에서는 흥분성 접합부 전압이, 유문동에서는 억제성 접합부 전압이 기록되었고 유문동의 억제성 접합부 전압은 atropine(10^{-6} M)과 guanethidine(5×10^{-6} M)을 동시 처치했을 때 영향을 받지 않았다.

2) 세포외 Ca^{2+} 농도를 높였을 때(7 mM)는 억제성 접합부 전압의 크기가 증가하고 세포외 Mg^{2+} 농도를 높였을 때(5 mM)와 verapamil(10^{-5} M)을 주었을 때는 억제성 접합부 전압의 크기가 감소하였다.

3) 아데노신을 투여하였을 때와 ATP를 투여했을 때는 모두 억제성 접합부 전압의 크기가 감소하였다.

4) 5-HT(10^{-6} M)을 투여했을 때는 서파크기에는 변화없이 억제성 접합부 전압의 크기만 감소하였고 5-HT type 2 길항제인 ketanserin(5×10^{-6} M)을 투여했을 때는 서파크기는 현저히 감소한 반면 억제성 접합부 전압크기는 변화가 없었다.

이상의 결과로부터 유문동에서 기록되는 억제성 접합부 전압은 비아드레날린, 비콜린 동작성 신경에 의해 유발되며 Ca^{2+}은 비아드레날린 비콜린 동작성 신경에서 신경흥분전달물질의 유리를 촉진시키고 분비된 신경흥분전달물질로 인해 Ca^{2+} 의존성 K^+ 통로가 활성화되어 억제성 접합부 전압의 크기를 증가시킨다고 사료된다.