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The Contractile and Electrical Responses of Guinea-pig's Gastric Smooth Muscle to Serotonin

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= ABSTRACT =

In order to elucidate systematically the effects of serotonin on gastric motility of guinea-pig, the contractile and electrical responses to serotonin were recorded using four kinds of muscle strips prepared from antral circular, antral longitudinal, fundic circular, and fundic longitudinal muscles. Experiments were performed using various methods including isometric contraction recording, transmural electrical field stimulation, junction potential recording, intracellular microelectrode technique, and partition stimulation method.

The results were as follows:

1) The effect of serotonin on spontaneous contractions was inhibitory in the circular muscle strips of the antrum and fundus, while it was excitatory in the longitudinal muscle strips of the antrum and fundus. Serotonin changed mainly phasic contractions of both the circular and longitudinal muscle strips in the antrum, while it changed mainly tonic contractions of both the circular and longitudinal muscle strips in the fundus.

2) On the contractions induced by transmural nerve stimulation, serotonin decreased the amplitude in the circular muscle strips of the antrum, but it increased them in the other three groups of muscle strips(antral longitudinal, fundic circular, and fundic longitudinal).

3) On the contractions induced by direct muscle stimulation, serotonin decreased the amplitude in the circular muscle strips of the antrum and fundus.

4) In the fundic circular muscle strips serotonin potentiated excitatory junction potentials (EJPs), and in the antral circular muscle strips it evoked EJPs after inhibitory junction potentials(IJPs).

5) In the antral circular muscle strips serotonin did not affect the slow wave even at the disappearance of spontaneous contractions. On the contrary it increased the amplitude of the slow wave, when the spike component was potentiated and the second component was inhibited.

6) In the antral circular muscle strips the membrane potential was slightly hyperpolarized, but the membrane resistance was not changed.

From the above results following conclusions could be made.

1) Serotonin inhibits spontaneous contractions of the circular muscle layer and it increases those of the longitudinal one, irrespective of the gastric region.

2) In the guinea-pig stomach there exists a serotoninergic facilitatory neuromodulation system which exerts its effect on cholinergically mediated contraction.

3) The excitation-contraction decoupling was observed in the effect of serotonin.

Key Words: Gastric smooth muscle, Serotonin, Spontaneous Contraction, Slow wave, Membrane potential.

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INTRODUCTION

The action of serotonin in neurons and smooth muscle cells including vascular, respiratory and gastrointestinal ones has been reported to be excitatory or inhibitory according to the investigators, so there is no report showing concordant and constant result. This problem is thought to arise from the difficulties due to following problems. First, the smooth muscle has very diverse characteristics, that is, may be very different according to region or layer in the same tissue. Second, serotonin owes its effect not only to the smooth muscle itself, but also to nerve reflex. Third, serotonin frequently produce tachyphylaxis phenomenon(Douglas, 1985).

Other than the above several factors contribute to making the experiment more difficult. The classification of serotonin receptors is yet incomplete. In 1988 the receptors were classified as 5-HT_1, 5-HT_2 and 5-HT_3 according to the affinities of serotonin to agonists and antagonists(Bradley et al, 1986; Heuven-Nolsen, 1988; Peroutka, 1988) and in 1989 the classification was modified on the basis of receptor cloning in *Xenopus* oocyte(Hartig, 1989). It is reported that ketanserin which is known to be a selective antagonist (Janssen, 1983) and is being used clinically, has an effect on α_1-adrenergic receptor, histamine H_1 receptor, and dopamine receptor as well as 5-HT_2 which can be regarded as main receptor in the smooth muscle(Janssen, 1983), and that ketanserin may not be a main antagonist in the gastrointestinal tract where the receptor system is different from that in the vascular system(Cohen et al, 1985). Methysergide which is also in popular use as a serotonin antagonist has a similar effect as ketanserin. In addition, as ketanserin is a ergot derivative it has vasoconstricting and oxytotic activities and even in high concentration it has a strong nerve stimulating effect as well(Douglas, 1985).

Research articles on the action of serotonin in the stomach have been reported in recent years. In the rabbit stomach acetylcholine induced secretion of serotonin to gastric interstitial fluid by acting on nicotinic receptor(Pairet et al, 1986). In the canine stomach inhibitory effect of β-agonist in contraction was indirect action through β-adrenoceptor of the neuron membrane and the candidate mediator was supposed to be either serotonin or somatostatin(Beck, 1986; 1988). Serotonin was also reported to be a neurotransmitter mediating an action of greater splanchnic nerve(Smironov et al, 1986). Enterochromaffin cells of the gastric mucosa in the mouse were observed to secrete serotonin(Solov'eva et al, 1986). Therefore, it is supposed that the sources of serotonin in the stomach are the intrinsic nervous plexus and gastric mucosa. But other sites like the intestinal mucosa can be serotonin sources via blood stream, considering the fact that subcutaneously administered serotonin affected gastric motility(Shemerovskii & Ovsiannikov, 1988; Groisman et al, 1989).

In general the actions of serotonin on the contraction of the gastrointestinal tract are regarded as excitatory in the small intestine and inhibitory in the large intestine and the stomach, although excitatory effect was reported infrequently in latter ones. The similar results were reported in the guinea-pig stomach(Gunning et al, 1986). Considering the fact that the gastric motility and the electrical properties and the distribution of intrinsic nervous system are all different from each other according to gastric regions(Komori & Suzuki, 1986; Meyer, 1987) and the fact that even in the same gastric region histologic structure and function differ from each muscle layer to other(Kuriyama et

al, 1975), the action of serotonin should be investigated using method, which distinguishes the circular muscle layer from the longitudinal one in the antrum and the fundus, and which separates direct muscle effect from nervous one.

Recently, classification of serotonin receptors has been arranged to some degree and research articles on the action of serotonin on the stomach have been reported. Therefore, more systemic investigation is needed now.

In this experiment the gastric smooth muscle of the guinea-pig which is generally known to relax in response to serotonin was divided into four kinds of strips i.e. the antral circular and longitudinal ones, and the fundic circular and longitudinal ones.

MATERIALS AND METHODS

Guinea-pigs of either sex, weighing about 300 g, were stunned and bled. The whole stomach was excised and placed in a bath containing oxygenated 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 mM, pH 7.35) at room temperature. Antral and fundic regions were obtained and cut in the longitudinal direction along the lesser curvature. After contents of the stomach were removed, patches of the muscle coat were obtained by removing mucosal layer off in Tyrode solution. Circular muscle strips, 2 mm wide and 10 mm long, were made dissected along the direction of the circular muscle and longitudinal ones along the direction of the longitudinal muscle in both the antrum and the fundus.

Mechanical contractions were recorded in a vertical chamber which has a capacity of 100 ml. Before main experiment the strip was recovered in oxygenated tris-buffered Tyrode solution(NaCl 147, KCl 4, $MgCl_2 \cdot 6H_2O$ 1.05, $CaCl_2 \cdot 2H_2O$ 2, tris · HCl 5, glucose 5.5 mM, pH 7.35) for 1 hour at 35°C. Isometric contractions were recorded through a Grass FT-03 force transducer connected to a Device physiograph. The optimal length of the strip was determined by length-tension curve of either spontaneous contractions or contractions evoked by electrical field stimulation.

The electrical and mechanical responses were recorded simultaneously using conventional glass capillary microelectrode method in a horizontal chamber which has a capacity of 2 ml. In the chamber the strip was pinned out at one end with tiny pins on a rubber plate and was connected to the Grass FT-03 force transducer at the other free end. The microelectrode which was filled with 3 M KCl and ranged between 40~80 MΩ in tip resistance was impaled from the mucosal side of the strip and the electrical activity was recorded on the Device pen recorder. The strip was perfused constantly at the rate of 2~3 ml/min with tris-buffered normal Tyrode solution that was bubbled with 100% O_2 and kept at 35°C.

Junction potentials were recorded by transmural electrical nerve stimulation method by using a platinum stimulating electrode(diameter 0.5 mm). A stimulation wave produced by a Grass S88 stimulator was a single square pulse with duration of 50~100 μs and intensity of 10~50 V. A recording microelectrode was placed as near the stimulating electrode as possible.

Membrane resistance was estimated from current-voltage graph obtained by partition stimulating method(Abe & Tomita, 1968), where two partitions were made by chlorinated platinum plates so that electrical field stimulation might be carried out in one partition and resulting electrotonic potential might be recorded by a microelectrode placed as near the bound-

ary as possible in the other partition.

The following actions of serotonin were compared and analyzed according to gastric regions and muscle layers.

1) Direct smooth muscle effect was separated from the nervous effect.

2) The effect of serotonin on the slow wave was analyzed to investigate the mechanism acting on the gastric smooth muscle.

3) Membrane resistance was measured to know the change in ionic channels.

In this experiment no statistics was used because the result analysis was qualitative comparison between data groups, and the figures cited in this article were representative ones selected after confirmation of the same results.

Drugs used were as follows; apamin(Sigma), atropine sulfate(Sigma), guanethidine sulfate(Tokyo kasei), ketanserin tartrate (Janssen), serotonin(5-hydroxytryptamine, 5-HT, Sigma), tetrodotoxin(TTX, Sankyo).

RESULTS

Effects of serotonin on the contractile and electrical activities of the antral circular muscle

The dose-response relationship of serotonin and spontaneous contraction of the antral circular muscle strip were presented in Fig. 1. The inhibitory effect of serotonin on the spontaneous contractions has achieved at a concentration of 10^{-8} M. Such effect has become increased dose-dependently until complete suppression of the contractions at a concentration of 10^{-6} M(Fig. 1A). Antagonists such as TTX(3×10^{-7} M), guanethidine(5×10^{-6} M) and atropine(10^{-6} M) had no influence on the effect of serotonin(Fig. 1B). Reduction of the tonic contraction was relatively small in degree compared to that of the phasic contraction.

Serotonin reduced the amplitude of the

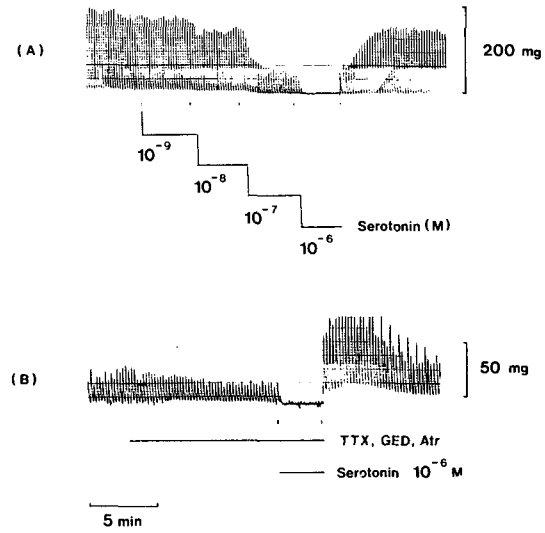

Fig. 1. The effect of serotonin on the spontaneous contractions recorded from the antral circular muscle strip of guina-pig stomach (dose-response relationship).
(A) Serotonin, which was administered cumulatively, reduced the amplitude of spontaneous contractions in a dose-dependent manner. At the concentration of 10^{-6} M serotonin spontaneous contractions were abolished almost completely.
(B) 10 minutes after the pretreatment with 3 neurotransmitter blockers (TTX, GED, Atr) serotonin (10^{-6} M) was administered and the similar result as that before the treatment with above 3 blockers was obtained.
Note: TTX (tetrodotoxin, 3×10^{-7} M)
GED (guanethidine, 5×10^{-6} M)
Atr (atropine, 10^{-6} M)

contraction evoked by the transmural electrical nerve stimulation(Fig. 2A). and atropine also abolished it completely. When the muscle was activated by direct electrical stimulation under the pretreatment with TTX(3×10^{-7} M) the result was similar to that obtained from the transmural nerve stimulation(Fig. 2B).

The junction potential recordings were

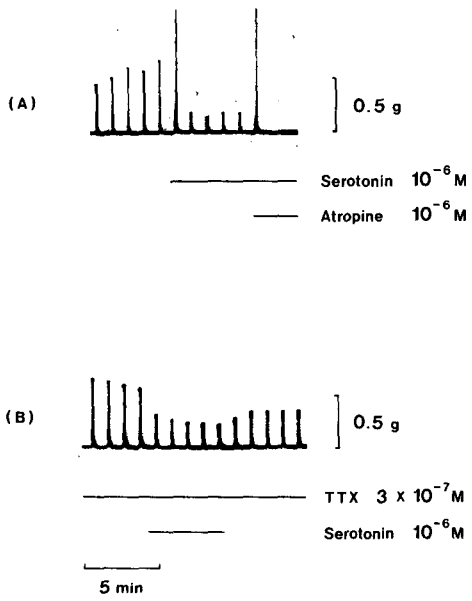

Fig. 2. The effect of serotonin on the contractions evoked by transmural electrical field stimulation of both nerve and muscle in the antral circular muscle strip.
(A) Transmural nerve stimulation was done according to following scheme: pulse duration 1 msec, pulse frequency 3 Hz, intensity 60 V, train duration 5 sec, train interval 1 min.
(B) Direct muscle stimulation was done under the treatment with TTX (3×10^{-7} M) according to following scheme: pulse duration 20 msec, pulse frequency 30 Hz, intensity 70 V, train duration 5 sec, train interval 1 min.

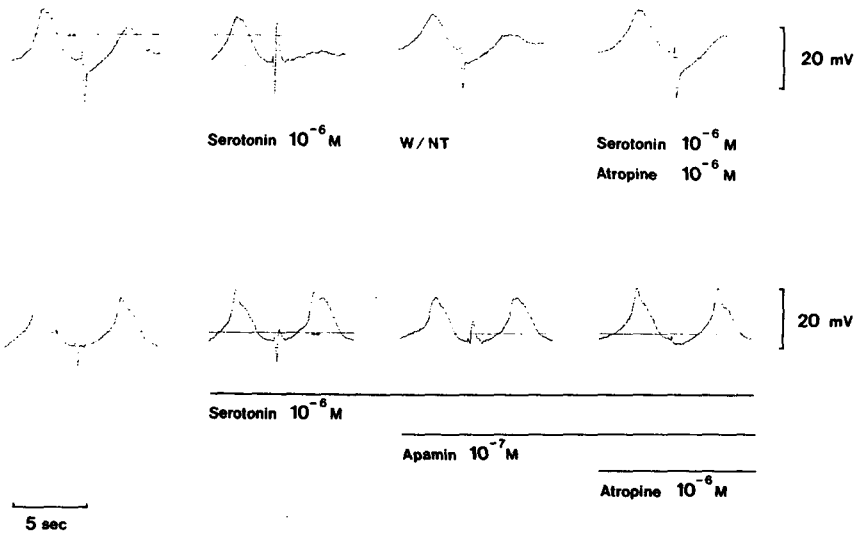

Fig. 3. The effect of serotonin on junction potentials produced by transmural nerve stimulation (50 V in intensity, 50 μs in duration, single square pulse) recorded from the antral circular muscle strip.
Upper panel: serotonin (10^{-6} M) produced both inhibitory junction potential (IJP) and excitatory junction potential (EJP), and atropine (10^{-6} M) abolished only EJP.
Lower panel: apamin (10^{-7} M) abolished IJP and remaining EJP was abolished by atropine (10^{-6} M).
W/NT; washout with normal Tyrode solution

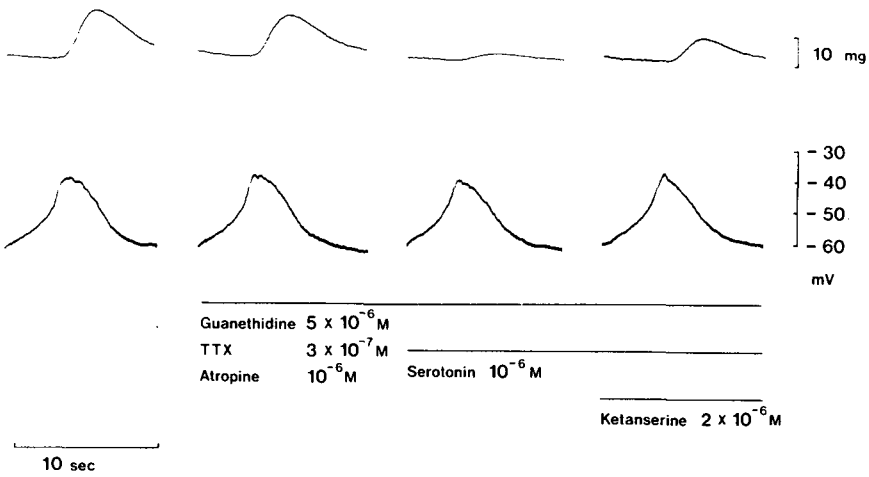

Fig. 4. The effect of serotonin on the contractile and electrical activities in the antral circular muscle strip (group I).
In the presence of guanethidine (5×10^{-6}M), TTX (3×10^{-7}M), and atropine (10^{-6}M) the contractile and electrical activities were almost the same as in the absence of above 3 blockers. Serotonin (10^{-6}M) reduced the amplitude of spontaneous contraction and ketanserin (2×10^{-6}M) partially restored it (upper trace), while there was no change in slow waves (lower trace).

presented in Fig. 3. Though the inhibitoty junction potential(IJP) was always recorded in the antral circular muscle, the excitatory junction potential(EJP) after IJP was developed by the administration of serotonin. In general, serotonin either did not change IJP or did it by less than 1 mV. The EJP caused by serotonin was potentiated by apamin which had an effect of blocking IJP. The potentiation was antagonized by atropine(10^{-6}M).

The isometric tension and the membrane potential of the antral circular muscle strip was simultaneously recorded using the intracellular microelectrode technique. Serotonin reduced muscle tone with hyperpolarization of the membrane potential from -65 mV to -70 mV, and abolished spontaneous contractions in spite of the increased amplitude of the slow wave. The degree of hyperpolarization was not large.

Under the treatment with TTX(3×10^{-7} M), guanethidine(5×10^{-6}M) and atropine (10^{-6}M) the effect of serotonin on the slow wave was studied. The slow wave could be divided into two categories depending upon the response to serotonin: group I, in which the slow wave did not change remarkably(Fig. 4) and group II in which the amplitude of the slow wave was increased(Fig. 5). In group II the slow wave was increased in the spike component by serotonin and this effect was partially blocked by ketanserin.

Electrotonic potentials were recorded through the microelectrode inserted intracellularly 0.15 mm apart from the stimulating electrode by partition stimulation method and the current-voltage relationship was ploted in graph(Fig. 6). The membrane resistance did not show remarkable difference between control and serotonin treatment.

Effects of serotonin on the contractile and electrical activities of fundic circular muscle

The effects of serotonin on the sponta-

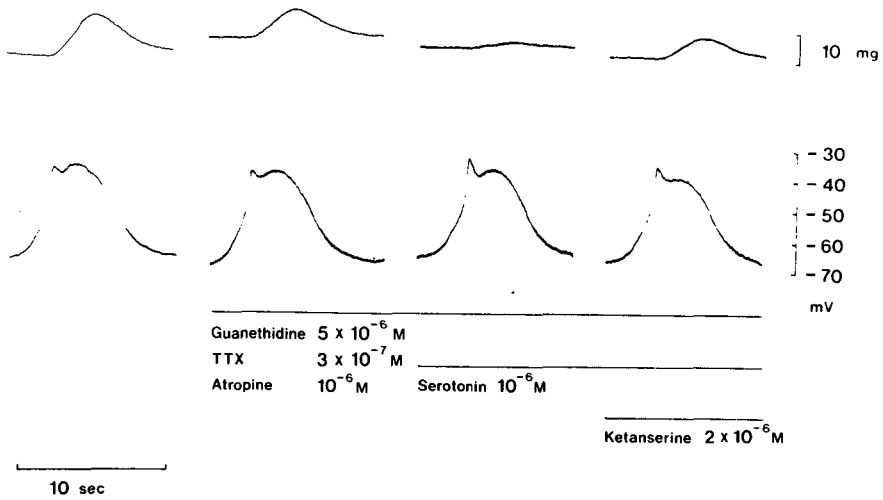

Fig. 5. The effect of serotonin on the contractile and electrical activities in the antral circular muscle strip (group II).
In the presence of guanethidine (5×10^{-6} M), TTX (3×10^{-7} M), and atropine (10^{-6} M) the contractile and electrical activities were almost the same as in the absence of above 3 blockers. Serotonin (10^{-6} M) increased the amplitude of slow waves and ketanserin (2×10^{-6} M) decreased it. (refer to main text.)

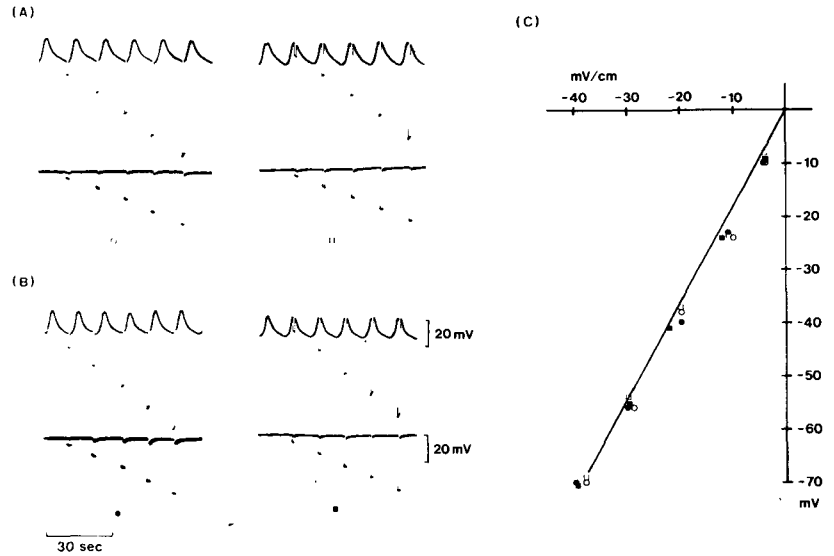

Fig. 6. Electrotonic potentials produced by 5 different intensities of inward current pulse (1 sec in duration) recorded from a circular muscle strip of the guinea-pig gastric antrum.
(A) Control recording.
(B) Recordings taken after application of serotonin (10^{-6} M) for 10 min.
(C) Current-voltage relationship. The amplitude of the electrotonic potential was plotted against the intensity of the current shown by mV/cm.

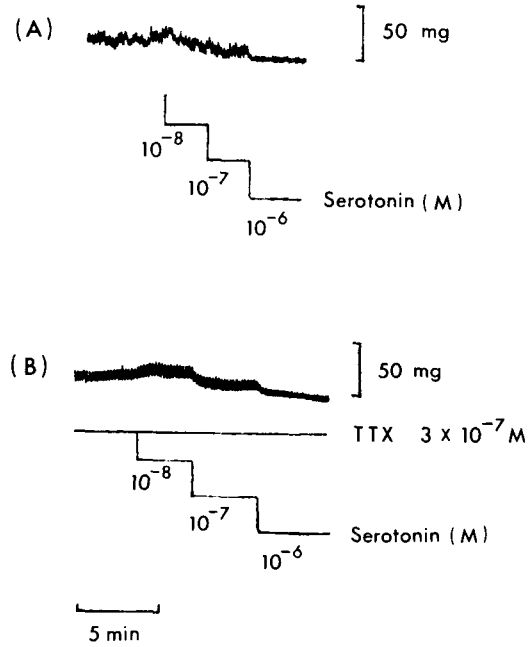

Fig. 7. The effect of serotonin on the spontaneous contractions recorded from the fundic circular muscle strip(dose-response relationship).
(A) Serotonin, which was administered cumulatively from 10^{-8}M to 10^{-6}M, reduced the amplitude of spontaneous contractions and also basal tone in a dose-dependent manner.
(B) 10 min after the pretreatment with TTX(3×10^{-7}M) serotonin was administered cumulatively from 10^{-8}M to 10^{-6}M, obtaining the same result as that before the pretreatment with TTX.

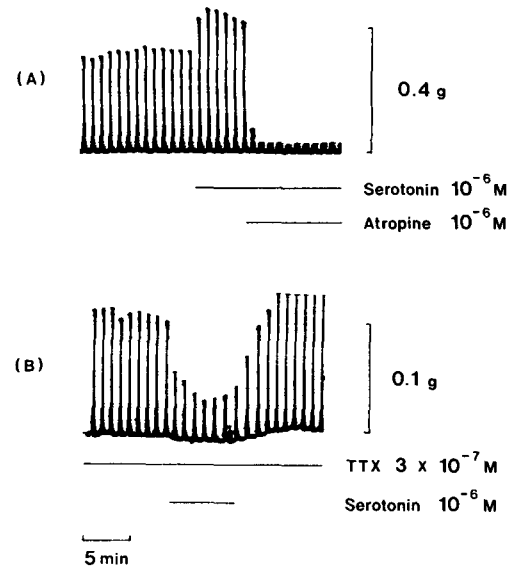

Fig. 8. The effect of serotonin on the contractions evoked by transmural electrical field stimulation of both nerve and muscle in fundic circular muscle strip.
(A) Transmural nerve stimulation(pulse duration 1 msec, pulse frequency 10 Hz, intensity 60 V, train duration 5 sec, train interval 1 min).
(B) Transmural direct muscle stimulation (pulse duration 20 msec, pulse frequency 30 Hz, intensity 70 V, train duration 5 sec, train interval 1 min) was done under the treatment with TTX(3×10^{-7}M).

neous contractions were observed in the fundic circular muscle strip(Fig. 7). Serotonin, administered cumulatively from the concentration of 10^{-8}M to 10^{-6}M, reduced both the amplitude of phasic contraction and the basal tone in a dose-dependent manner. The reduction of basal tone was more distinct than that of phasic contraction. These changes were more distinct under the treatment with TTX(3×10^{-7}M).

The amplitude of contractions driven by the transmural electrical nerve stimulation was increased by serotonin(Fig. 8A), whereas that driven by direct muscle stimulation was decreased by it(Fig. 8B). The former effect was blocked by atropine, showing that serotonin owed its effect to cholinergic nervous system.

Serotonin increased EJP in the recording of junction potentials(Fig. 9). The resting potential was -60 mV and the top of EJP was raised from -40 mV to -30 mV with the prolongation of EJP in duration. This observation was well coincident with

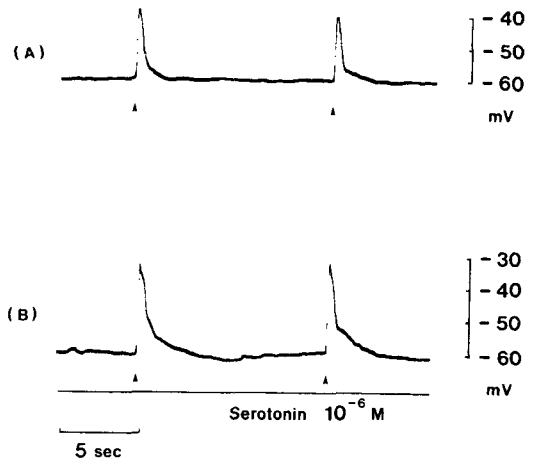

Fig. 9. The effect of serotonin on junction potentials produced by transmural nerve stimulation(50 V in intensity, 50 μs in duration, single square pulse) recorded from the fundic circular muscle strip.
(A) Control recording. Stimulations were done at arrows and produced EJPs.
(B) 10 min after the administration of serotonin(10^{-6} M) the recorded EJPs were more potentiated in amplitude. EJPs were increased in amplitude and persisted longer.

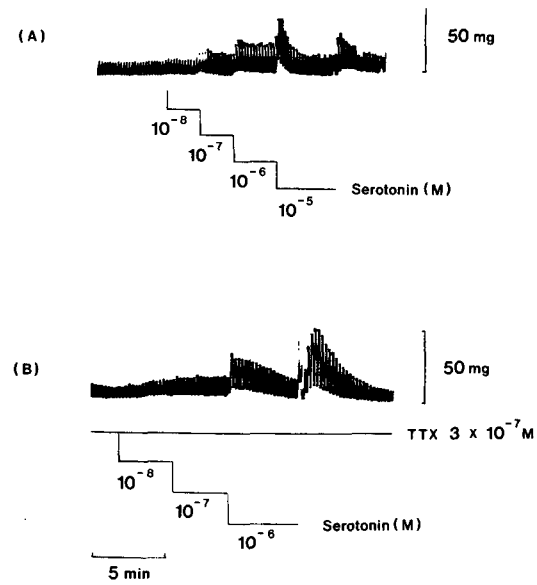

Fig. 10. The effect of serotonin on the spontaneous contractions recorded from the antral longitudinal muscle strip(dose-response relationship).
(A) Serotonin, which was administered cumulatively from 10^{-8} M to 10^{-5} M, increased the amplitude of spontaneous contractions in a dose-dependent manner. Maximum contractions were obtained at the concentration of 10^{-6} M.
(B) 10 min after the pretreatment with TTX(3×10^{-7} M) serotonin was administered cumulatively from 10^{-8} M to 10^{-6} M, showing the same result as that before the pretreatment with TTX.

the result of electrical field stimulation, which showed the existance of a serotoninergic facilitatory neuromodulation system mediated by cholinergic system.

Effects of serotonin on the contractility of antral longitudinal muscle

The effects of serotonin on the spontaneous contractions were studied in the antral longitudinal muscle strip(Fig. 10). The effect of serotonin was excitatory, contrary to that in the circular muscle strip. The amplitude of phasic contraction was biggest instantly at 10^{-5}M serotonin and at 10^{-6}M serotonin that was sustained enduringly. The amplitude of tonic contraction was biggest at 10^{-5}M serotonin instantly and was not maintained. Under the pretreatment with TTX(3×10^{-7} M) the effects were identical.

Serotonin increased the contraction driven by transmural nerve stimulation, which was blocked by TTX(3×10^{-7}M) or by atropine(10^{-6}M)(Fig. 11).

Effects of serotonin on the contractility of fundic longitudinal muscle

The effects of serotonin on the spontaneous contraction were studied in the

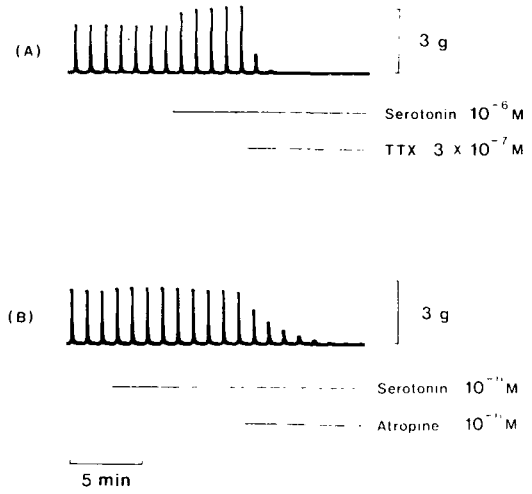

Fig. 11. The effect of serotonin on the contractions evoked by transmural nerve stimulation in antral longitudinal muscle strip. Under the condition of transmural nerve stimulation(pulse duration 1 msec, pulse frequency 10 Hz, intensity 60 V, train duration 5 sec, train interval 1 min) serotonin(10^{-6} M) was administered and the result was the increase in amplitude of contractions which was blocked completely by either TTX 3×10^{-7} M (A) or atropine 10^{-6} M (B).

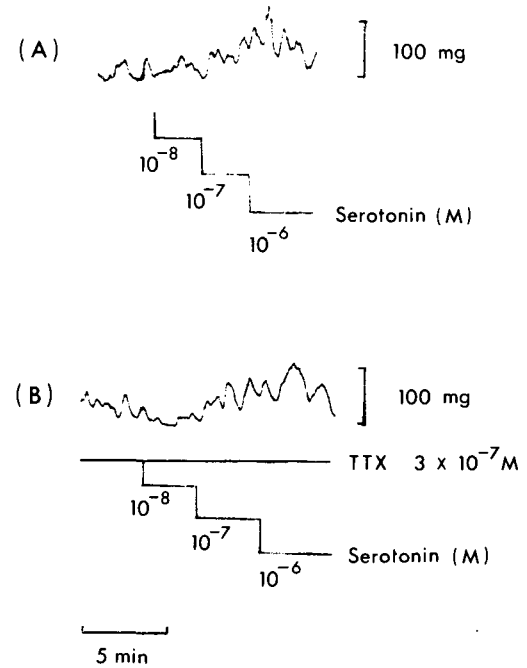

Fig. 12. The effect of serotonin on the spontaneous contractions recorded from the fundic longitudinal muscle strip(dose-response relationship).
Serotonin was administered cumulatively from the concentration of 10^{-8} M to 10^{-6} M without TTX(3×10^{-7} M) (A) and with TTX (B). Spontaneous contractions were absent and basal tone was increased in both cases as serotonin was administered.

fundic longitudinal muscle strip(Fig. 12). Without spontaneous contractions only tonic contraction increased as the concentration of serotonin was raised from 10^{-8} M to 10^{-6} M. Such effect was not altered by the treatment with TTX(3×10^{-7} M).

Serotonin potentiated the contraction driven by transmural nerve stimulation, which was blocked by TTX(3×10^{-7} M) or by atropine(10^{-6} M)(Fig. 13). This results were the same as that observed in the antral longitudinal muscle.

DISCUSSION

Serotonin is a autacoid discovered at first as a vasoconstricting substance as the name mean it. But it also has a vasodilating effect in the striated muscle and the skin. This bidirectional effect of serotonin is thought to be due to multi-factors. First, serotonin receptors whose classification is yet incomplete are known to have at least four types(Heuven-Nolsen, 1988). Second, the effect of serotonin in the gastrointestinal tract is such a combined one on the smooth muscle itself and the intrinsic nervous system, thus it is very difficult to separate the direct ef-

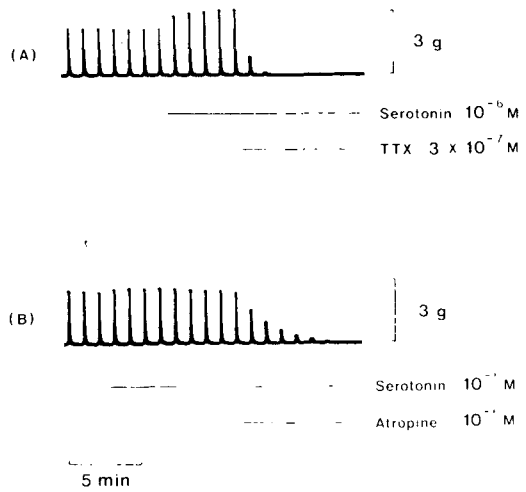

Fig. 13. *The effect of serotonin on the contractions evoked by transmural nerve stimulation in fundic longitudinal muscle strip.*
Under the condition of transmural nerve stimulation(pulse duration 1 msec, pulse frequency 10 Hz, intensity 60 V, train duration 5 sec, train interval 1 min) serotonin(10^{-6} M) was administered and the result was the increase in amplitude of contractions, which was blocked completely by either TTX(3×10^{-7} M) (A) or atropine(10^{-6} M) (B).

fect on the smooth muscle from the nervous one.

The effects of serotonin on the stomach reported recently are as follows: Serotonin induced contraction in the antrum of the mouse stomach(Lidberg, 1985; Lidberg et al, 1985) and in the fundus of the mouse stomach(Cohen & Wittenauer, 1985; Cohen et al, 1985; Cohen & Fludzinski, 1987), while it induced relaxation in the other investigation using the mouse fundus(Radomirov & Venkova, 1986). Serotonin induced contraction in the antrum of the canine stomach(Beck, 1986), while it induced relaxation(Beck, 1988) or relaxation after initial contraction(Beck & Andersen, 1985). In addition, serotonin induced relaxation in the antrum of the rabbit stomach(Cohen & Fludzinski, 1987).

Serotonin is generally known to have a relaxing effect on the stomach of the guinea-pig. In the circular muscle the effect of serotonin is inhibitory on the spontaneous contractions of the antrum and fundus, as seen in Figs. 1 and 7. This effect is regarded as direct effect on the smooth muscle because it is blocked by lysergic acid derivative(LSD) and neither by TTX nor by morphine(Yamaguchi, 1972). However, it is uncertain whether TTX could completely abolish neural effect, because the inhibitory effect of serotonin might be achieved through the activation of the inhibitory ganglion cells (Douglas, 1985). In contrast the effect of serotonin was excitatory in the longitudinal muscle(Figs. 10 & 12). This effect was also blocked by LSD and neither by TTX nor by morphine, implying that it was direct effect on the smooth muscle(Yamaguchi, 1972).

Selective stimulation of nerve or muscle is possible by controlling pulse duration, frequency and intensity of transmural electrical field stimulation(Goo et al, 1990). Serotonin potentiated the contractions driven by nerve stimulation in all preparations except for the antral circular muscle where contractions were, on the contrary, reduced by serotonin(Figs. 2, 8, 11 & 13). This can be interpreted in several ways. First, serotonin made a strong inhibition of the antral circular muscle, directly. Second, serotonin acted mainly on inhibitory ganglion cells. Third, cholinergic system might be primitive in the antral circular muscle, resulting in predominant inhibitory action in it. In fact there are several evidences that cholinergic action is very weak in the antral circular muscle, where electrical field stimulation

has produced relaxation in many cases, non-adrenergic non-cholinergic(NANC) inhibitory junction potential(IJP) was recorded(Komori & Suzuki, 1986), the excitatory junction potential(EJP) was not always provoked by serotonin, and the sensitivity of acetylcholine receptor was by far lower than in the fundus(Komori & Suzuki, 1986). Meanwhile, the potentiating effect of serotonin was blocked by either TTX or atropine in the antral circular muscle, suggesting that serotonin exerted its effect via cholinergic system. This fact is well coincident with the report that monoamines including serotonin act as neuromodulators or trophic factors rather than neurotransmitters because monoamines are synthesized in the cell body and transported to axon terminal but hardly reuptaken after release(Burnstock, 1981).

The contractions driven by direct muscle stimulation were inhibited by serotonin in both antral and fundic circular muscles(Figs. 2 & 8). In general the effect of serotonin on the GI smooth muscle was known to be antagonized by LSD and that on the ganglion cell presenting within the GI smooth muscle layer was known to be antagonized by morphine(Goth, 1981). Therefore, although the treatment with TTX alone could not rule out completely the inhibitory effect of endogenous serotonin(Gunning et al, 1986), it was quite possible that serotonin made direct relaxation of the smooth muscle, considering the fact that methysergide, one of LSD family, could make a partial blockage of the effect of serotonin(Douglas, 1985).

Serotonin potentiated EJP and also the contractions driven by nerve stimulation in the fundic circular muscle(Figs. 8 & 9) even if it reduced the contractions driven by direct muscle stimulation(Fig. 8). These results propose that a cholinergically mediated contraction is due to serotonin, which was reported in the longitudinal muscle of mouse stomach(Sanger, 1985).

The slow wave was recorded to investigate the action of serotonin on the membrane potential of the antral circular muscle. Serotonin hyperpolarized the membrane potential and increased the amplitude of the slow wave. In accordance with the observations that the reversal potential of IJP was -89 mV and its amplitude increased by depolarization and decreased by hyperpolarization(Komori & Suzuki, 1986), serotonin reduced IJP with membrane hyperpolarization(Fig. 3). The hyperpolarization could be interpreted to be responsible for the decreased tonic contraction. But the excitation-contraction decoupling phenomenon i.e. the contraction has disappeared in spite of the increased amplitude of the slow wave, requires more investigations.

The response of the slow wave in the antral circular muscle to serotonin could be divided into two categories; group I (Fig. 4) which showed no change noticeable and was most frequent findings, and group II(Fig. 5) in which the amplitude of the slow wave has increased. The slow wave has been reported to be consisted of the first, second and spike components (Ohba et al, 1975; 1977). On the basis of this viewpoint, serotonin increased the spike component and decreased the second component(Fig. 5). But this alteration was exaggerated in 4 mM Ca-Tyrode solution, and the small change in components and membrane potential in normal condition is thought to be a secondary change. Namely, rather than acting directly on the receptor operated channel(ROC), serotonin might control the intracellular Ca concentration by acting through an intracellular second messenger and followed secondary change of ionic channels brought about the alteration of the slow wave. If so, the hyperpolarization might result from the activation of K-channel or Cl-channel(Lübbert et al, 1987).

Recent studies on the receptors of serotonin reported that 5-HT_1 made an effect through adenylate cyclase and 5-HT_2 through phosphoinositide and 5-HT_3 through ROC(Berridge, 1985; Hartig, 1989). 5-HT_1 and 5-HT_2 were reported to be important receptors in smooth muscle, and the salivary gland to make a secretion via intracellular second messenger in the blowfly (Berridge, 1985). Considering our results together with above observations, the role of intracellular second messenger is thought to be important in the action of serotonin.

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