Kor J Physiol (1993) 27(2), 139-150

Effect of Dopamine on the Ca^{2+}-dependent K^+ Currents in Isolated Single Gastric Myocytes of the Guinea-pig

Poong Lyul Rhee, Sang Jin Lee, Sung Joon Kim
Insuk So, Sang Ik Hwang and Ki Whan Kim

Department of Physiology and Biophysics, Seoul National University College of Medicine

= ABSTRACT =

We have reported that dopamine potentiates spontaneous contractions dose-dependently in guinea-pig antral circular muscle strips (Hwang et al, 1991). To clarify the underlying excitatory mechanism of dopamine on the gastric smooth muscle, the effects of dopamine on voltage-dependent Ca^{2+} currents and Ca^{2+}-dependent K^+ currents were observed in enzymatically dispersed guinea-pig gastric myocytes using the whole-cell voltage-clamp technique. Experiments were also done using isometric tension recording and conventional intracellular microelectrode techniques.

1) The effect of dopamine on the spontaneous contraction of antral circular muscle strips of the guinea-pig was excitatory in a dose-dependent manner, and was blocked by phentolamine, an α-adrenoceptor blocker.

2) The slow waves were not changed by dopamine.

3) The voltage-operated inward Ca^{2+} current was not influenced by dopamine.

4) The Ca^{2+}-dependent K^+ outward current, which might reflect the changes of intracellular calcium concentration, was enhanced by dopamine. This effect was abolished by phentolamine.

5) The enhancing effect of dopamine on the Ca^{2+}-dependent K^+ current disappeared with heparin which is known to block the action of $InsP_3$.

From these results, it is suggested that dopamine acts via $InsP_3$-mediated Ca^{2+} mobilization from intracellular stores and such action potentiates the spontaneous contraction of guinea-pig gastric smooth muscle.

Key Words: Dopamine, Smooth muscle, Inositol trisphosphate, Calcium-dependent potassium current.

INTRODUCTION

Dopamine is important neurotransmitter in the central nervous system compound and is also present in the peripheral autonomic nervous system, where it acts as a neurotransmitter in the cardiovascular system and gastrointestinal tract (Bech & Hovendal, 1982). Dopamine is the precursor for norepinephrine synthesis in the nervous system and is secreted from the mucosal endocrine cells of the gastrointestinal tract (Hakanson, 1970; Ahone & Penttila, 1974).

The effects of dopamine on gastrointestinal motility are known to vary depending upon animal species, regions and muscle layers (Mukhopadhyay & Weisbrodt, 1977; Cox and Ennis, 1980; Sahyoun et al, 1982; Lefebvre et al, 1984). Moreover, the site and the mechanism of action are unclear. For example, it is still uncertain whether dopamine acts through its own specific receptor (Schuurke et al, 1983) or acts by binding to other catecholamine receptors (Cox & Ennis, 1980; Costall et al, 1982).

From the fact that externally administered dopamine inhibits gastrointestinal motility (Thorner, 1975) and that dopamine is found in

large amounts in human gastric juice, it is suggested that endogenous dopamine might play a physiological role in the regulation of gastrointestinal motility. Clinically, it is thought that dopamine is one of the etiologic factors eliciting gastroesophageal regurgitation and vomiting in newborn infants (Miolan et al, 1983). But there remains controversy concerning the site and mechanism of action of dopamine on gastric motility (Schuurkes & Van Neuten, 1983; Cox & Ennis, 1980).

Rhythmic contractions of the gastrointestinal smooth muscles result from the myogenic electrical activity (phenomenon of periodic depolarization-repolarization of smooth muscle membrane; slow wave). They are modulated by intrinsic and extrinsic nerves as well as circulating hormones and drugs (Demole et al, 1989). The autonomic nervous system is composed not only of sympathetic and parasympathetic nervous systems, but also of non-adrenergic non-cholinergic (NANC) nervous system. It is reported that dopamine is released from the NANC nerve terminal and acts as a neurotransmitter (Jorge & Valenzuela, 1976).

Although the mechanism of smooth muscle contraction is quite different from that of skeletal muscle contraction, the increase of intracellular Ca^{2+} ($[Ca^{2+}]_i$) is still necessary. Two integrated membrane systems are involved in the control of smooth muscle $[Ca^{2+}]_i$: (a) the plasmalemma, which is under the control of the membrane potential (E_m) and agonists such as neurotransmitters hormones and autacoids, and (b) the sarcoplasmic reticulum, which is under the control of second messengers (van Breemen & Saida, 1989).

Agonists are responsible for the generation of intracellular messengers besides Ca^{2+}. Inositol phosphate, especially inositol 1,4,5-trisphosphate, releases Ca^{2+} from the sarcoplasmic reticulum (Berridge & Irvine, 1984). Although the regulation of $[Ca^{2+}]_i$ is a complex process, it is important to clarify the source of increased $[Ca^{2+}]_i$ for the elucidation of the mechanisms.

We have already reported results about the effect of dopamine and the regional differences of the action of dopamine on the contractility of guinea-pig gastric smooth muscle (Hwang et al, 1991). In this study, we tried to elucidate the mechanisms of action of dopamine on the contractility and electrical activity of the guinea-pig antral circular muscle using conventional microelectrode techniques and the whole cell patch-clamp method.

METHODS

Preparation of tissue and intracellular recording of electrical activity

Albino guinea-pigs of either sex, weighing 200~250 g, were stunned and bled. The stomach was isolated and cut in the longitudinal direction along the lesser curvature. The contents of the stomach were removed, and the mucosal layer was separated from the muscle layers in 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. $Na_2HPO \cdot 12H_2O$ 1.81, glucose 5.5 mM, pH 7.35) at room temperature. Strips of muscle (2 mm wide, 10 mm long) were cut parallel to the circular fibers, and set in a 100 ml vertical chamber. One end was fixed and the other was connected to a force transducer (Isometric Transducer, Harvard Bioscience, USA) to measure isometric contraction (Fig. 1-A). Another strip was mounted in a 2 ml horizontal chamber. The strips were pinned out at one end with tiny pins on a rubber plate, and the other end was connected to the force transducer (Fig. 1-B). The strip was constantly perfused at a rate of 2~3 ml/min with Tris-buffered normal Tyrode solution (NaCl 147, KCl 4, $CaCl_2 \cdot 2H_2O$ 2, $MgCl_2 \cdot 6H_2O$ 1.05, tris·HCl 5, glucose 5.5 mM, pH 7.35) bubbled with 100% O_2 and maintained at 35℃. Electrical responses of smooth muscle cells were recorded by means of glass microelectrodes filled with 3 M KCl. Microelectrodes with a tip resistance of 40~80 MΩ were used. Mechanical and electrical responses of smooth muscle cells were simultaneously recorded by a pen recorder (MX-6, Device Ltd, Britain).

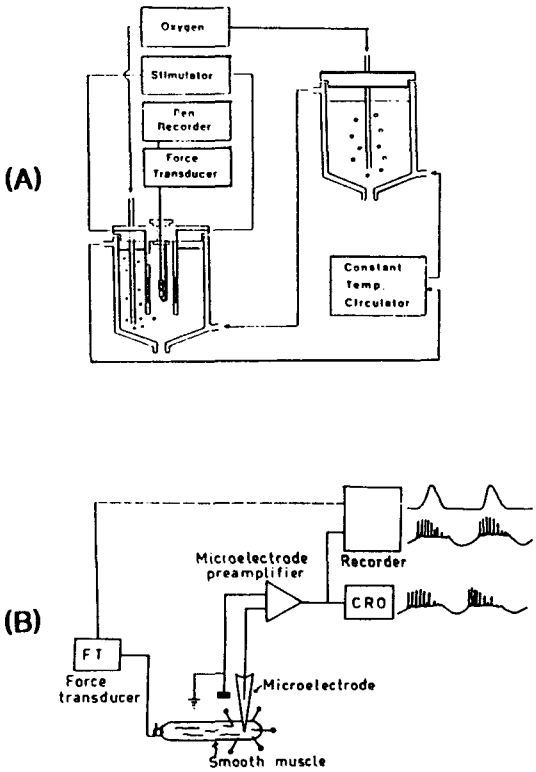

Fig. 1. (A) A schematic representation of the 100 ml vertical chamber and the isometric recording system. The chamber was maintained at a constant temperature and saturated with 100% O_2.
(B) A schematic representation of simultaneous recording system for isometric contractions and electrical activity. The intracellular microelectrode was used to record the changes of membrane potential, while simultaneously recording isometric contractions with the tension transducer.
**CRO: cathode ray oscilloscope*

Patch clamp experiments

Isolation of cells: The circular muscle layer was dissected from the longitudinal layer and small segments of the tissues were made. The muscles were incubated in Ca^{2+}-free PSS (physiological salt solution) for 30 min at room temperature. Then, small segments were incubated for 20~30 min in Ca^{2+}-free PSS containing 0.1% collagenase, 0.1% trypsin inhibitor, and 0.2% bovine serum albumin at 35℃. After digestion, single cells were dispersed by gentle agitation with a glass pipette in the Krafts-Brühe (KB) solution. Isolated gastric myocytes were kept in KB medium at 4℃. All experiments were carried out within 12 hours of harvesting cells and performed at room temperature (Fig. 6).

Membrane currents measurement: Isolated cells were transferred to a small chamber on the stage of an inverted microscope (IMT-2, Olympus, Japan). The chamber was perfused with PSS (2~3 ml/min). Glass pipettes with a resistance of 2~5 MΩ were used to make a giga seal of 5~10 GΩ. Standard patch clamp techniques were used (Hamill et al, 1981). An axopatch-clamp amplifier (Axon instruments, USA) was used to record membrane currents. The data were displayed on a digital oscilloscope (PM 3350, Philips, Netherland), a pen recorder (Recorder 220, Gould, USA) and stored on a videotape recorder (BR-6400, Victor, USA) with a pulse code modulator (RP-880, NF, USA).

Solutions: Ca^{2+}-free PSS contained (mM) NaCl 134.8, KCl 6.2, $CaCl_2$ 0, glucose 12.2, HEPES 0.4 and pH was adjusted to 7.3 by Tris. PSS contained 2.3 mM $CaCl_2$ in the Ca^{2+}-free PSS. KB solution contained (in mM) L-glutamate KCl 50, taurine 20, KH_2PO_4 20, $MgCl_2$ 3, glucose 10, HEPES 10, EGTA 0.5 and pH was adjusted to 7.3 by KOH. Pipette solution consisted of (in mM) K^+-aspartate 110, Mg-ATP 5, di-Tris-creatine phosphate 5, KCl 20, $MgCl_2$ 1, ethyleneglycol-bis (β-aminoethyl ether)-N, N, N′, N′-tetraacetic acid (EGTA) 0.1, HEPES 5, pH 7.4. For studies in which K^+ currents were blocked, pipette solution contained (in mM) Cs-aspartate 110, Mg-ATP 5, di-Tris-creatine phosphate 2.5, di-Na-creatine phosphate 2.5, $MgCl_2$ 1, HEPES 0.1, tetraethylammonium (TEA)-Cl 20, EGTA 5, pH 7.4.

RESULTS

Mechanical contractions

Dopamine increased the basal tone and the

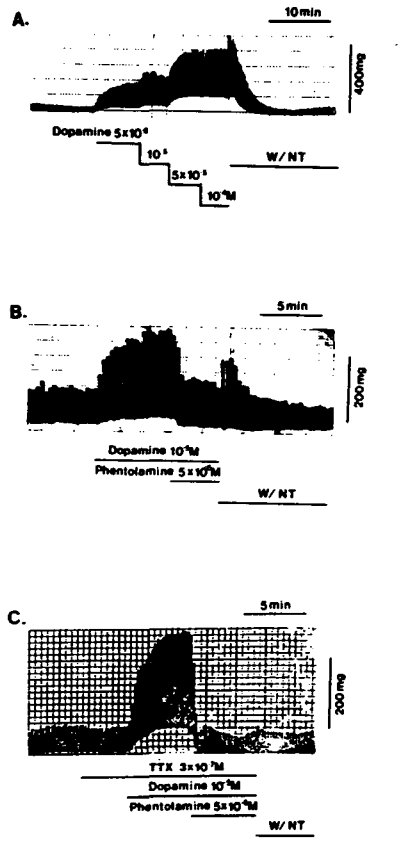

Fig. 2. The effect of dopamine on the spontaneous contractions recorded from an antral circular muscle strip of guinea-pig stomach. Dopamine enhanced the spontaneous contractions in a dose-dependent manner (A), and the excitatory effect of the dopamine was completely suppressed by the α-blocker, phentolamine (B). This dopamine-induced excitatory effect was not blocked by TTX (C).

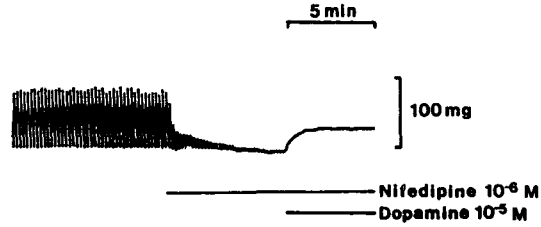

Fig. 3. The effect of dopamine on the spontaneous contractions in the presence of the Ca^{2+} channel blocker, nifedipine. However, dopamine (10 μM) increased the tonic contractions even in the presence of nifedipine.

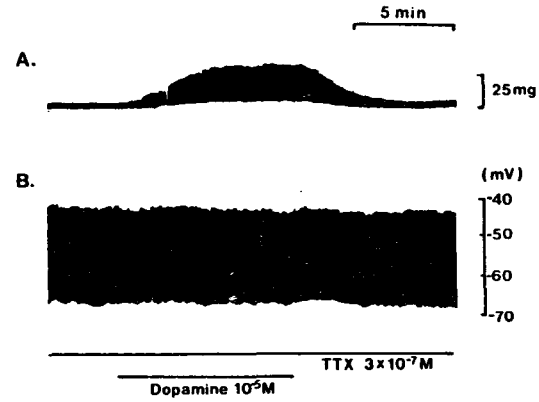

Fig. 4. The effect of dopamine on isometric tension and membrane potential in an antral circular muscle strip of guinea-pig stomach. The spontaneous contractions were enhanced by the application of 10 μM dopamine (A), whereas the simultaneously recorded membrane potential was not altered (B).

spontaneous contraction of the antral circular muscle in dose-dependent manner, and exerted a maximal effect at a concentration of 50 μM (Fig. 2-A). Such effect was almost completely blocked by 5 μM phentolamine (α-adrenergic blocker, Fig. 2-B), but not by TTX (tetrodotoxin, neuronal action blocker, Fig. 2-C). Dopamine increased the basal tension even in the presence of nifedipine which abolished the spontaneous contractions (Fig. 3).

Spontaneous electrical activity

Circular smooth muscles of the antral region show spontanous electrical activity, which consists of a slow depolarization, upstroke and plateau potential and repolarization, sometimes with superimposed spike potentials (Tomita, 1981). Dopamine had no effect on the amplitude or frequency of the electrical activity of this tissue (Fig. 4). But with a fast recording speed, a little more depolarization of the plateau potential was observed (Fig. 5).

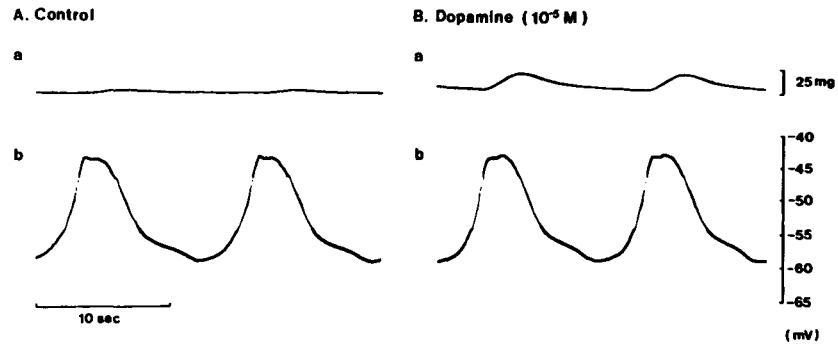

Fig. 5. The effect of dopamine on the contractile and electrical activity with a fast recording speed in antral circular muscle strip of guinea-pig stomach. In a detail view, the plateau portion of the slow wave was slightly depolarized by dopamine (B-b) compared with control (A-b).

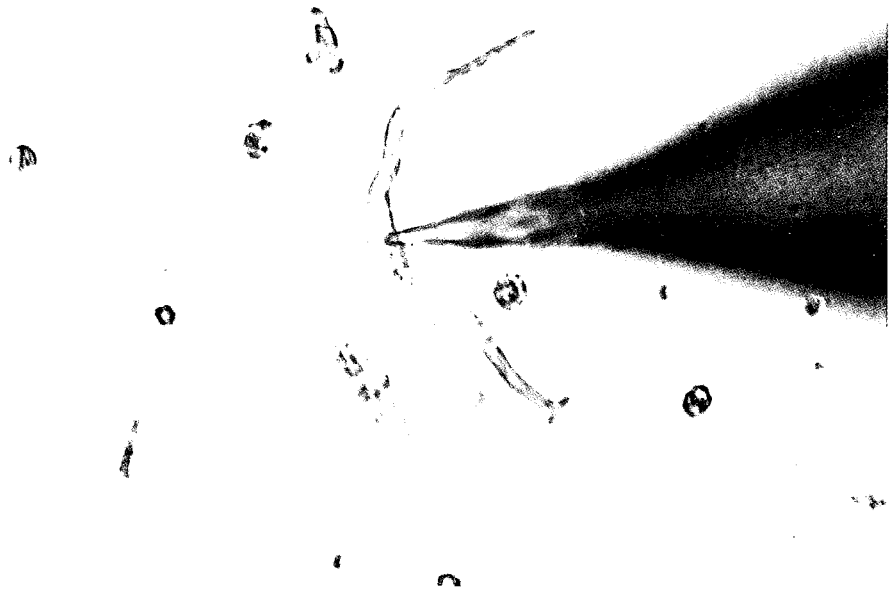

Fig. 6. The microscopic features of enzymatically dispersed single cells obtained from the guinea-pig stomach. The single myocyte showed a typicl spindle shape, about 200 μm in length and 10 μm in width. A glass microelectrode approaches to the cells for whole-cell voltage clamp experiement.

Inward Ca^{2+} currents

Isolated single cells were 5~10 μm in diameter and 100~200 μm in lenght. Figure 6 shows the light microscopic appearance of single smooth muscle cells of the antrum. Electrophysiological properties of the isolated myocytes were studied using the patch clamp technique (Hamill et al, 1981). For recording only inward currents, we used cesium-aspartate solution with 20 mM TEA-Cl in the pipette (Mitchell et al, 1987). At this condition, mainly inward currents were observed by clamping membrane potentials from the holding potential (-60 mV) to various levels (-40~60 mV). Because TTX (tetrodotoxin) had no effect

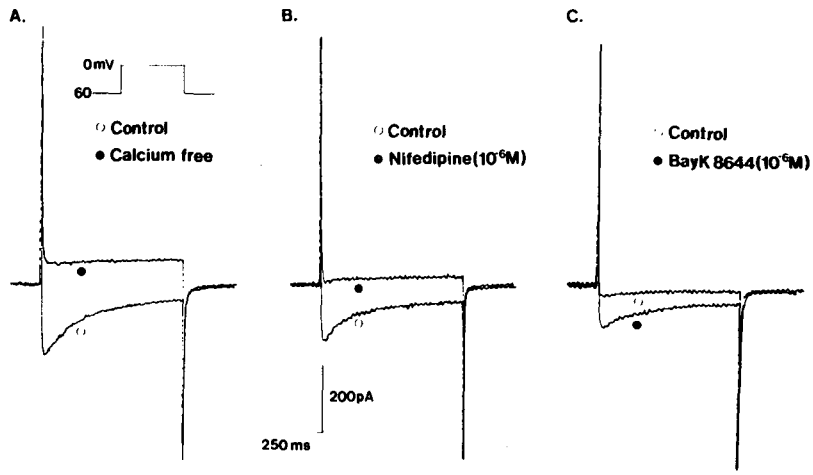

Fig. 7. The effects of Ca^{2+}-free bathing solution and a Ca^{2+} channel blocker, nifedipine on the inward Ca^{2+} currents. Inward currents were completely abolished either by the elimination of Ca^{2+} from the bathing solution or by 1 μM nifedipine. This inward current was increased by 1 μM Bayk 8644 (C).

on the shape of these inward currents, we did not used TTX to exclude Na^+-currents. The inward currents proved to be Ca^{2+} currents (I_{Ca}) because the currents were completely blocked by removal of Ca^{2+} in the bath solution or by the application of nifedipine or verapamil (Fig. 7).

A family of I_{Ca} activated by different depolarizing pulses from a holding potential of −60 mV is shown in Fig. 8. I_{Ca} became apparent near −30 mV, reached its maximum at 10 mV, and became outward at membrane voltages between 40 and 50 mV. In the same cell, dopamine had no effect on these inward currents (Fig. 8-B). Typical current-voltage relationships for I_{Ca} of this experimant are shown in Fig. 9.

Outward K^+ currents

To record Ca^{2+}-dependent K^+-outward current, the intrapipette solution changed to high K^+-low EGTA (0.1 mM)-solution. Depolarization of cells to 0 mM from the holding potential of −60 mV for 3 seconds elicited a large initial transient outward (TO) and following oscillatory outward (OO) currents which were superimposed on sustained outward current (Fig. 10-A). These spontaneous transient outward

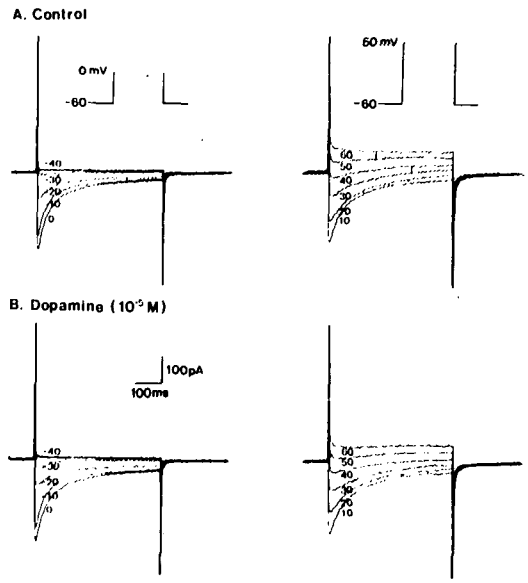

Fig. 8. The effect of dopamine on voltage-dependent inward Ca^{2+} currents in a guinea-pig gastric myocyte. The Ca^{2+} currents were activated by various levels of depolarizing pulses from the holding potential of −60 mV, using a Cs-TEA solution containing 5 mM EGTA in the pipette and PSS (Physiological salt solution) in the bath. These Ca^{2+} currents were not influenced by 10 μM dopamine (B) compared with control (A).

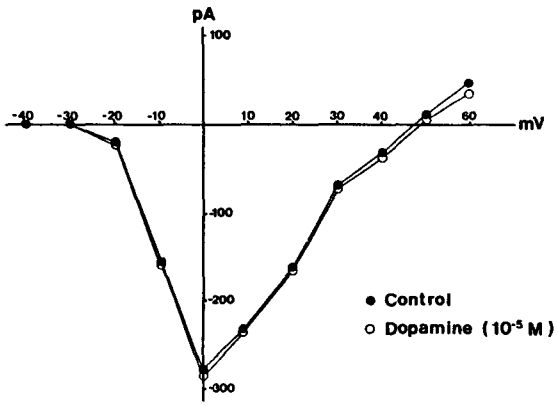

Fig. 9. The current-voltage relationship of the voltage-dependent Ca^{2+} inward current before and after the application of dopamine. The Ca^{2+} currents (●) were not influenced by 10 μM of dopamine (○) in the whole range of test potentials.

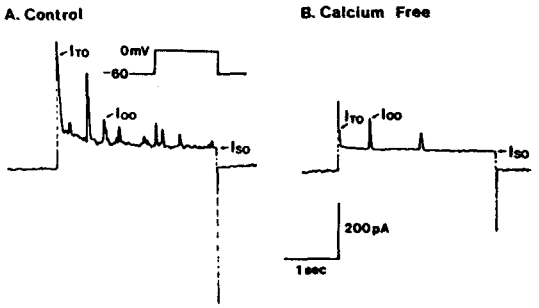

Fig. 10. Ca^{2+}-dependent K^+-outward currents and their extracellular Ca^{2+} dependency. These currents were elicited by a depolarizing pulse to 0 mV from a holding potential of −60 mV, using a high K solution containing 0.1 mM EGTA in the pipette and PSS in the bath. With the elimination of Ca^{2+} in the bathing solution, the amplitude of the current was diminished and also the frequency of the oscillatory outward component (I_{OO}) was decreased (B).

currents (STOCs, Benham & Bolton, 1986) have been described in many other smooth muscle cells studied and at present are thought to be due to the activation of Ca^{2+}-dependent K^+ channels by cyclic Ca^{2+}-release from the sarcoplamic reticulum (Benham & Bolton, 1986; Okabe et al, 1987; Bolton & Lim, 1989).

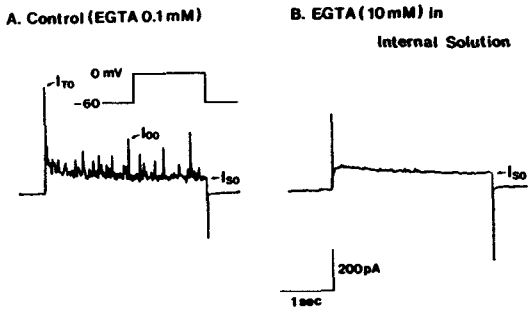

Fig. 11. Ca^{2+}-dependent K^+-outward currents and their intracellular Ca^{2+} dependency. These currents were elicited by a depolarizing pulse to 0 mV from the holding potential of −60 mV, using a K-Asp solution containing 0.1 mM EGTA in the pipette and PSS in the bath. The outward currents were completely abolished by increasing the level of EGTA in the pipette up to 10 mM from 0.1 mM using the internal perfusion technique (B).

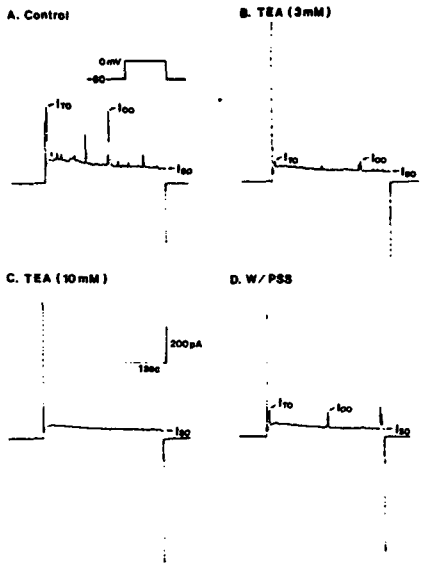

Fig. 12. The effect of a potassium channel blocker, TEA-Cl on the Ca^{2+}-dependent K^+-outward currents. These currents were elicited by a depolarizing pulse to 0 mV from the holding potential of −60 mV, using a high K solution containing 0.1 mM EGTA in the pipette and PSS in the bath. By increasing the TEA-Cl level in the bath solution, the outward currents decreased in a dose-dependent manner (A, B, C). However, these effects were reversible by washing out with PSS (D).

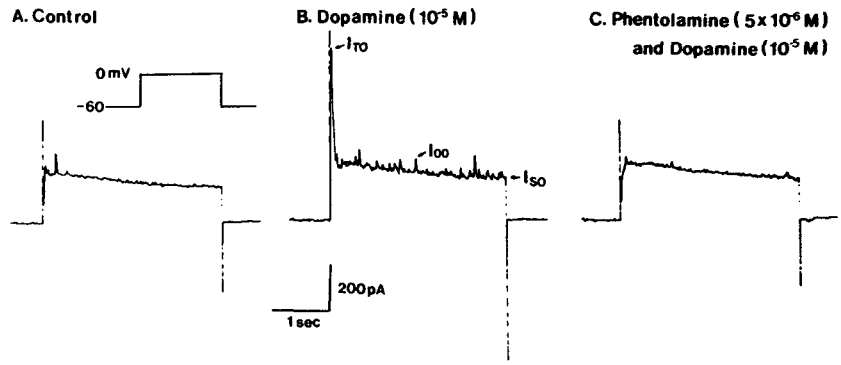

Fig. 13. The effect of dopamine on the Ca^{2+}-dependent K^+-outward currents. These currents were elicited by a depolarizing pulse to 0 mV from the holding potential of −60 mV, using a high K solution containing 0.1 mM EGTA in the pipette and PSS in the bath. The outward currents were enhanced by 10 μM dopamine (B). The enhancing effect was abolished by adding 5 mM phentolamine (C).

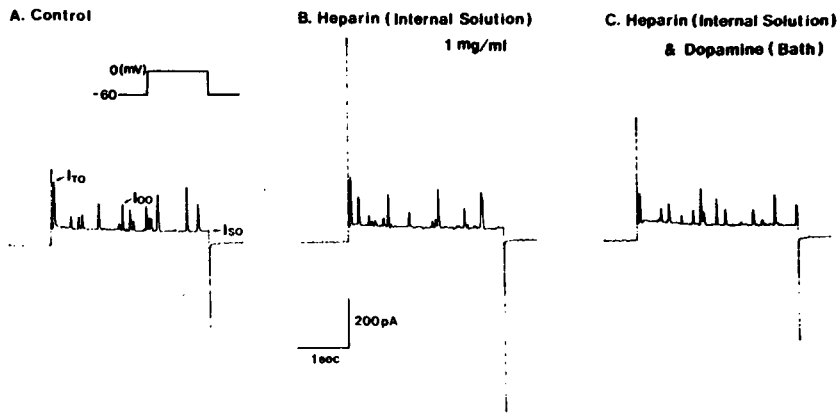

Fig. 14. The effect of heparin in the pipette on the dopamine-induced enhancing of Ca^{2+}-dependent K^+-current. There was no difference between control (A) and heparin in the pipette (B) using an internal perfusion technique. But the enhancing effect of dopamine was abolished by the heparin (C).

To characterize these STOCs further, we omitted Ca^{2+} from the extracellular fluid. In this nominally Ca^{2+}-free state, both the size and frequency of the STOCs were decreased (Fig. 10-B). Also when we increased the concentration of EGTA in the pipette solution (designed to strongly buffer intracellular Ca^{2+}), the STOCs (TO and OO) were abolished almost completely (Fig. 11). Both the sustained outward current and STOCs were blocked in a dose dependent manner by bathapplied TEA. This effect was reversible, and STOCs were more sensitively blocked by TEA (Fig. 12). From the above results we conclude that the STOCs are K^+-currents activated by the increase of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$).

Effects of dopamine on the Ca^{2+}-dependent K^+ current

Figure 13 shows the effects of dopamine on

the Ca^{2+}-dependent K^+ currents elicited by a commanding pulse from −60 mV to various levels (−40 mV ~ +10 mV) with duration of 400 ms. By the application of dopamine (10^{-5} M) in the bath solution, transient and spontaneous oscillatory outward currents were increased. Such increases were blocked by treatment with phentolamine, which was consistent with the results from mechanical tension recording (Fig. 2-B).

Recently, it was reported that heparin is a specific blocker for the $InsP_3$ receptors of sarcoplamsic reticulum (Kobayashi et al, 1989). We thought that heparin could therefore be used to block the $InsP_3$-mediated Ca^{2+}-release which might be triggered by the activiation of α-receptors by dopamine. Firstly, we observed the control Ca^{2+}-dependent K^+ currents, and then, the pipette solution was changed to heparin-containing solution using the internal perfusion technique. No change was found in the shape of outward current, and in this condition, dopamine could not evoke the increase of STOCs (Fig. 14).

DISCUSSION

In this experiment, we found that dopamine enhanced the contractility of guinea-pig stomach. Dopamine receptors are classified into CNS-type and peripheral type, and each of them are subclassified according to their mode of action. There are many conflicting classifications of CNS-type receptors (Cools & Van Rossu, 1980; Kebabian, 1984; Seeman, 1980). Dopamine receptors of the peripheral type have been observed in many organs (Willems, 1973; Enero & Langer, 1975; Hope et al, 1978) since the first description in 1972 (Goldberg, 1972). Although they were classified into DA1 or DA2 according to their mode of action, such classification was done on the basis of the results from blood vessels (Goldberg et al, 1979). In addition, there have been controversies about the effect of dopamine on the contractility of G-I smooth muscles (Sahyon et al, 1982; Costall et al, 1983), and even about the presence of real dopamine receptors in G-I smooth muscles (Van Nueten et al, 1978; Schuurk et al, 1983; Cox et al, 1980).

In our experiment, dopamine-induced enhancement of mechanical contraction was almost completely reversed by the application of phentolamine, an α-adrenoceptor blocker, and these results were not influenced by the pretreatment with TTX (Fig. 2). Therefore, these results suggest either that α-adrenergic receptors in smooth muscle mediate the actions of dopamine or that phentolamine blocked a kind of dopamine receptor. We have not study further the types of dopamine receptors in guinea-pig stomach. Dopamine and norepinephrine are both catecholamines, and their receptors are both G protein-coupled receptors. G protein coupled receptors constitute a superfamily of proteins that includes the visual pigment rhodopsin, muscarinic ACh receptors, α- and β-adrenergic receptors, and receptors for serotonin, dopamine, and a variety of neuropeptides. All the receptor proteins of this superfamily show homologous 7 transmembrane domains and amino acid sequences (Barritt, 1992). Therefore there can be nonspecific binding and nonspecific actions within this receptor-agonist superfamily, and the blockade of the action of dopamine by phentolamine in this experiment might be one such example of nonspecificity. The α-adrenergic action of norepinephrine in this tissue also showed potentiation of mechanical contraction while its effect on electrical activity was rather different (Jun et al, 1992).

We applied nifedipine to block the voltage-activated Ca channel, which result in the inhibition of the characteristic spontaneous phasic contractions of this smooth muscle. With nifedipine, dopamine could still increase the tonic contraction (Fig. 3). Also dopamine had little effect on the shape of slow waves (Fig. 4, Fig. 5), and did not change the size of voltage operated calcium current or their current-voltage relationships (Fig. 8). Such results suggest that the increase of $[Ca^{2+}]_i$, which is necessary for the dopamine-induced contraction, was not

attained through the enhancement of the activity of the L-type voltage operated calcium channel (VOCC). Considering alternative ways of increasing $[Ca^{2+}]_i$, one possibility is to increase the plasmalemma Ca^{2+} permeability, and the other is to increase the release of Ca^{2+} from intracellular Ca^{2+} store site such as the sarcoplasmic reticulum. Depending on the cell type and the agonist, several hypotheses about the receptor-operated Ca^{2+}-influx have been advanced but the mechanisms and regulation of Ca^{2+} influx are still controversial and the nature of such a pathway is still unknown (capacitive Ca^{2+} entry: Jacob, 1990, receptor-operated channel: Benham & Tsien, 1987; Inoue, 1991; Pacaud & Bolton, 1991, second messenger-mediated Ca-influx: Von Tscharner et al, 1986; Kuno & Gardner, 1987; Morris et al, 1987). But above hypotheses are controversial and beyond the aim of this experiment, so we did not do experiments to check those possibilities.

It is now widely accepted that inositol 1, 4, 5-trisphosphate ($InsP_3$) is the intracellular messenger which links cell surface receptor stimulation to the release of intracellular Ca^{2+} pools (Berridge & Irvine, 1984; Berridge, 1987), and it is known that the α-adrenergic action on the smooth muscle occurs through $InsP_3$-mediated Ca^{2+} release (Kitazawa et al, 1989). On the other hand, the spontaneous transient outward currents (STOCs) we have observed in our experiments (Fig. 10, Fig. 11, Fig. 12) are thought to be due to the activation of Ca^{2+}-dependent K^+ channels by Ca^{2+} released from the sarcoplasmic reticulum (Benham & Bolton, 1986; Ohya et al, 1987; Bolton & Lim, 1989). And it is known that heparin can be used as a blocker for $InsP_3$-receptors in the sarcoplasmic reticulum (Kobyashi et al, 1989). From the above facts, we postulated that if dopamine can increase the size and frequency of STOCs, and if such an increase is blocked by the intracellular application of heparin, we would be able to conclude that the effect of dopamine on the contractility of gastric smooth muscle is due, at least partly, to Ins_3P-mediated release of intracellular calcium. The experimental results support this hypothesis (Fig. 13, Fig. 14).

From these results, it is concluded that in the guinea-pig stomach, dopamine produces excitatory effects on the contractility of mucosa-free smooth muscle cells. These responses are thought to be due to increase of intracellular Ca^{2+} mediated by $InsP_3$-production which is activated by α-adrenoceptor stimulation by dopamine.

REFERENCES

Ahone A & Penttila A (1974) Development of enterochromaffin and dopamine cells in the gastrointestinal tract of the calf. *Experientia* **30,** 1460-1462

Barritt GJ (1992) The plasma-membrane receptors and GTP-binding proteins. In: Barrit GJ (ed) *Communication within Animal Cells,* Oxford University Press, New York, p53-90

Bech K & Hovendal CP (1982) Effect of dopamine on bethanichol-stimulated gastric fistula. *Scand J Gastroenterol* **17,** 945-951

Benham CD & Bolton TB (1986) Spontaneous transient outward currents in single visceral and vascular smooth muscle cells of the rabbit. *J Physiol (London)* **381,** 385-406

Benham CD & RW (1987) A novel receptor operated Ca^{2+} permeable channel activated by ATP in smooth muscle. *Nature* **328,** 275-278

Berridge MJ (1987) Inositol trisphosphate and diacylglycerol: two interacting second messengers. *Ann Rev Biochem* **56,** 159-193

Berridge MJ & Irvine RF (1984) Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature* **312** (22), 315-321

Bolton TB & Lim SP (1989) Properties of calcium stores and transient outward currents in single smooth muscle cells of the rabbit intestine. *J Physiol (London)* **409,** 375-401

Cools AR & Van Rossum JM (1980) Multiple receptors for brain dopamine in behavior regulation: concept of dopamine-E and dopamine-I receptors. *Life Sci* **27,** 1237-1253

Costall B, Gunning SJ, Naylor RJ & Simpson KH (1983) A central site of action for benzamide

facillitation of gastric emptying. *Eur J Pharmacol* **91,** 197-205

Cox B & Ennis C (1980) Mechanism of action of dopamine on the guinea-pig. *Br J Pharmacol* **70,** 140-146

Demol P, Ruoff HJ & Weithrauch TR (1989) Rational pharmacotherapy of gastrointestinal motility disorders. *Eur J Pediatr* **148,** 489-495

Enero MA & Langer SZ (1975) Inhibition by dopamine of ^{3}H-noradrenaline release elicited by nerve stimulation in the isolated cat's nictating membrane. *Naunyn-Schmiedeberg Arch Pharmakol* **289,** 179-203

Goldberg LI (1972) Cardiovascular & renal actions of dopamine: potential clinical applications. *Pharmacol Rev* **24,** 1-29

Hakanson R (1970) New aspects of the formation and function of histamine, 5-hydroxytryptamine and dopamine in gastric mucosa. *Acta Physiol Scand (Suppl)* **79,** 340-348

Hamill OP, Marty A, Neher E, Sakman B, & Sigworth FJ (1981) Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pflü gers Arch* **391,** 85-100

Hope W, McCulloch MW, Rand MJ & Story DF (1978) Modulation of noradrenergic transmisson in the rabbit ear artery by dopamine. *Br J Pharmacol* **64,** 527-537

Hwang SI, Oh Y & Kim KW (1991) Effects of dopamine on the contractiliy of the circular muscles in the guinea-pig gastric antrum and fundus. *Seoul J Med* **32** (4): 199-207

Inoue, R (1991) Ion channels involved in reponses to muscarinic receptor activation in smooth muscle. In: Sperelakis N. & Kuriyama H. (Ed) *Ionic Channels of Vascular Smooth Muscle.* Elsevier, p81-91

Irvine RF & Moor RM (1986) Microinjection of inositol 1, 3, 4, 5-tetrakisphosphate activates sea urchin eggs by a mechanism dependent on external calcium. *Biochemical J* **240,** 917-920

Jacob R (1990) Agonist-stimulated divalent cation entry into single cultured human umbilical vein endothelial cells. *J Physiol (London)* **421,** 55-77

Jorge E & Valenzuela MD (1976) Dopamine as a possoble neurotransmitter in gastric relaxation. *Gastroenterology* **71,** 1019-1022

Jun JY, Lee SJ, Hwang SI, Kim KW (1992) Effects of noradrenaline on the spontaneous contractions and Ca^{2+} current in the isolated antral circular muscle of guinea-pig. *J Physiol (London)* **446,** 247P

Kebabian JW (1984) Pharmacological and biochemical characterization of two categories of dopamine receptors. In: Poste G & Crooke T (ed) *Dopamine Receptor Agonists,* Plenum Press, New York, p3-22

Kitazawa T, Kobayashi S, Horiuti K, Somlyo AV & Somlyo AP (1989) Receptor coupled, permeabilized smooth muscle: Role of the phosphatidylinositol cascade, G proteins and modulation of the contractile response to Ca^{2+}. *J Biol Chem* **264,** 5339-5342

Kuno M & Gardner P (1987) Ion channels activated by inositol 1, 4, 5-trisphophate in plasma membrane of human T-lymphocytes. *Nature* **326,** 923-926

Lefever RA, Williems JL & Bogaert MG (1984) Inhibitory effect of dopamine on canine gastric fundus. *Naunyn-Schmiedeberger's. Arch Pharmacol* **326,** 22-28

Mitchell MR, Powell T, Terrar DA & Twist VW (1987) Calcium-activated inward current and contraction in rat and guinea pig ventricular myocytes. *J Physiol (London)* **391,** 545-560

Mukhopadhyay AK & Weisbrodt N (1977) Effect of dopamine on esophaseal motor function. *Am J Physiol* **232,** E19-E24

Miolan JP, Lajard Am, Rega P & Roman C (1983) Vagal control of gastrointestinal tract during vomiting. In: Roman C (ed) *Gastrointestinal Motility,* MTP Press Limited, Lancaster, England, p167-176

Okabe K, Kitamura K & Kuriyama H (1987) Cellular calcium regulates outward currents in rabbit intestinal smooth muscle cell. *Am J Physiol* **251,** C335-346

Pacaud P & Bolton TB (1991) Relation between muscarinic receptor cationic current and internal calcium in guinea-pig jejunal smooth muscle cells. *J Physiol (London)* **441,** 447-490

Sahyoun HA, Costall B & Naylor RJ (1982) Benzamide actio at α_2-adreniceptors modifies catacholamine-induced contraction and relaxation of circula muscle from guinea-pig stomach. *Naunyn-Schmiedeberger's Arch Pharmacol* 319, 8-11

Schuurkes JAJ & Van Neuten JM (1983) Myogenic control of antroduodenal coordination: cholin-

ergic and dopaminergic modulation. In: Labo G and Bortolotti M (Ed) *Gastrointestinal Motility* Cortina International, Verona, p167-170

Seeman P (1980) Brain dopamine receptors. *Pharmacol Rev* **32,** 229-313

Thorner MO (1975) Dopamine is an important neurotransmitter in the autonomic nervous system. *Lancet* **22,** 662-665

Tomita T (1981) Electrical activity (spike and slow waves) in gastrointestinal smooth muscles. In: Bülbring E, Brading A, Jones AW & Tomita T (ed) *Smooth Muscle: an Assessment of Current Knowledge* E Arnold, London, p127-156

Van Breemen C & Saida K (1989) Cellular mechanisms regulating $[Ca^{2+}]_i$ smooth muscle. *Annu Rev Physiol* **51,** 315-329

Van Neuten JM, Ennis C, Helsen L, Lanuron PM & Janssen PAJ (1978) Inhibition of dopamine receptors in the stomach: an explanation of the gastrokinetic properties of domperidon. *Life Sci* **23,** 453-457

Von Tscharner V, Prod'hom B, Baggiolini M & Reuter H (1986) Ion channels in human neutrophils activated by a rise in cytosolic calcium concentration. *Nature* **324,** 369-372

Willems JL (1973) Dopamine-induced inhibition of synaptic transmission in lumber paravertebral ganglia of the dog. *Naunyn-Schmiedeberg Arch Pharmakol* **279,** 115-126