Jpn. J. Pharmacol. 83, 143 – 149 (2000)

Inhibitory Mechanism of Papaverine on the Smooth Muscle of Guinea Pig Urinary Bladder

Kazumasa Shimizu, Taeko Ichikawa, Norimoto Urakawa and Shinjiro Nakajyo

Division of Veterinary Pharmacology, Nippon Veterinary and Animal Science University, 7-1 Kyonan-cho 1-Chome, Musashino, Tokyo 180-8602, Japan

Received January 11, 2000 *Accepted March 14, 2000*

ABSTRACT—In guinea pig urinary bladder, the hyperosmotic 65 mM KCl (H-65K$^+$)- or carbachol (CCh)-induced contraction was inhibited by an addition of papaverine in a concentration-dependent manner. The cAMP content of the muscle in the presence of H-65K$^+$ or CCh was increased by papaverine only at the higher concentration of 100 μM, but cGMP content was not affected by papaverine. Forskolin, compared with papaverine, increased cAMP content in a concentration-dependent manner, and nitroprusside did not significantly increase cGMP content. In a fura 2 loaded muscle, papaverine did not affect an increase of $[Ca^{2+}]_i$ level by high K$^+$ or CCh. The increase of oxidized flavoprotein (FPox) fluorescence and muscle contraction in the presence of H-65K$^+$ or CCh was decreased by papaverine (1 – 100 μM), and the increase of pyridine nucleotide (PNred) fluorescence was not affected by papaverine. In summary, it was concluded that papaverine induced relaxation by inhibiting mitochondrial respiration in guinea pig urinary bladder as well as ileum. Moreover, it is proposed that the mechanism of papaverine-induced relaxation in the smooth muscle, which shows predominantly a metabolic dependency on its contraction, is an inhibition of mitochondrial respiration.

Keywords: Metabolic inhibition, Guinea pig urinary bladder, Papaverine, Muscle relaxation

Papaverine, a non-selective smooth muscle relaxant, has been utilized as vasodilator or bronchodilator and as a tool to reveal a mechanism of relaxation in smooth muscle. On the other hand, its mechanism of relaxation in smooth muscle has been explained by the following three modes: 1) an intracellular accumulation of cAMP by inhibiting phosphodiesterase (PDE) (1 – 4), 2) an inhibition of mitochondrial respiration (5 – 8) and 3) effects on Ca^{2+} movement (5, 9 – 11). Although these different mechanisms have been proposed by respective researchers, an effort to compare and evaluate these three concepts in various smooth muscles had been neglected up to the present. Recently, we have suggested that papaverine inhibits smooth muscle contraction mainly by the accumulation of cAMP and/or cGMP due to the inhibition of PDE in the rat aorta, but by acts by inhibiting mitochondrial respiration in guinea pig ileal smooth muscle, and that the relaxing mechanism of papaverine shows an organ difference (12).

On the other hand, we have reported that high K$^+$-induced contractions in various smooth muscles were inhibited by adding metabolic inhibitors, by removing glucose or by isosmotic substitution of K$^+$ for Na$^+$ in medium, and we have suggested that there are three types of inhibition by the high K$^+$/Na$^+$-deficient solution: 1) the inhibition is due to a swelling of the cell, as in rabbit aorta and guinea pig trachea (type 1) (13, 14); 2) the inhibition is due to an inhibition of glucose utilization resulting from Na$^+$ deficiency in medium, as in guinea pig and rat urinary bladders, guinea pig ileum and vas deferens (type 2) (13, 15, 16); 3) the inhibition is due to both the swelling and the inhibition of glucose utilization, as in rabbit trachea, guinea pig gall bladder, seminal vesicle and taenia coli (type 3) (14, 17, 18).

The excitability of a membrane of a smooth muscle belonging to type 1, which does not generate an action potential even with stimulation under a physiological condition, has been indicated to be low, and the dependency on oxygen consumption for a tension development is low. A muscle belonging to type 2 is one that generates an action potential spontaneously. It shows spontaneous contraction and dependency on oxygen consumption for developed tension is high (19). The purpose of the present study is to investigate the mechanism of the inhibitory effect of papaverine in the guinea pig urinary bladder that is a type 2 muscle and shows predominantly a metabolic dependency on smooth muscle contraction.

For this purpose, we examined inhibitory effects of papaverine on high K$^+$- or carbachol (CCh)-induced con-

traction by measuring muscle tension, cAMP or cGMP content, intracellular Ca^{2+} ($[Ca^{2+}]_i$) level, oxidized flavoprotein as an indicator of mitochondrial respiration, and reduced pyridine nucleotides as indicator of glycolytic activity in the guinea pig urinary bladder.

MATERIALS AND METHODS

Muscle preparations and tension measurement

Male guinea pigs (Hartley strain, 300 – 400 g; Funabashi Farm, Chiba) were bled after stunning, and then the urinary bladder of each animal was quickly removed. Trigunum vesicae, superficial tissue, fat and mucous layer were removed. The strips of urinary bladder were about 10 – 15 mm in length and 3 – 5 mm in width. One end of each strip was bound to a glass holder and the other end was connected to a strain-gauge transducer (TB-611; Nihon Kohden, Tokyo) with silk threads in an organ bath. The muscle tension was isometrically recorded. The physiological salt solution (PSS) used was a modified Tyrode's solution (136.8 mM NaCl, 5.4 mM KCl, 2.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, 11.9 mM $NaHCO_3$ and 5.5 mM glucose). The solution was aerated with 95% O_2 and 5% CO_2 at 37°C, pH 7.2. Hyperosmotic 65 mM K^+ (H-65K^+) solution was made by increasing the KCl concentration in the PSS. Isosmotic 77 mM K^+ (Iso-77K^+) solution was made by substituting an equimolar amount of K^+ for Na^+ in the PSS.

Assay of cAMP or cGMP content

The cAMP or cGMP content in the urinary bladder was measured by enzyme immunoassay. After incubation of the muscles with papaverine, forskolin or nitroprusside for 10 min in the presence of H-65K^+ or CCh, the muscles were rapidly frozen in liquid nitrogen and stored at −80°C until homogenized in trichloroacetic acid (6%, 0.4 ml). The homogenate was centrifuged at $3000 \times g$ for 15 min and the supernatant was washed with water-saturated diethylether (1.5 ml) three times; the cAMP and cGMP content was assayed by using an enzyme immunoassay system (Amersham Pharmacia Biotech, Tokyo).

Simultaneous measurement of muscle contraction and $[Ca^{2+}]_i$ level

The $[Ca^{2+}]_i$ level was measured simultaneously with muscle contraction as reported previously (20). Muscle strips were incubated with PSS containing 5 μM acetoxymethyl ester of fura 2 (fura 2/AM) for 3 – 4 h at room temperature. Cremophol EL (0.02%), a non-cytotoxic detergent, was also added to increase the solubility of fura 2/AM. One end of the muscle was pinned to the bottom of the organ bath which was filled with PSS (8 ml), and the other end was attached to the transducer with a silk thread. The muscle strip kept horizontally in the organ bath was alternately excited with light at 340 nm or 380 nm by means of a rotating filter wheel, and emission at 500 nm was measured through a band-pass filter with a fluorimeter (CAF-100; Japan Spectroscopic, Tokyo).

Simultaneous measurement of a muscle contraction and oxidized flavoproteins (FPox) or reduced pyridine nucleotides (PNred) fluorescence

The fluorescence of FPox or PNred was measured simultaneously with muscle contraction as reported previously (21). One end of the muscle was pinned to the bottom of the organ bath, which was filled with 8 ml of PSS, and the other end was attached to the transducer with a silk thread. The muscle strip was excited with light at 450 nm and emission at 530 nm was measured by a fluorimeter (CAF-100) to detect FPox, and it was excited with light at 340 nm and emission at 470 nm was measured by the fluorimeter to detect PNred fluorescence.

Chemicals

Chemicals used were papaverine, carbachol, forskolin (Sigma, St. Louis, MO, USA), nitroprusside (Wako Pure Chemical, Osaka), fura 2/AM (Dojindo Laboratories, Kumamoto) and cremophol EL (Nacalai Tesque, Kyoto).

Statistics

Values are expressed as means ± S.E.M., and statistical analyses were performed by Student's *t*-test.

RESULTS

Effects of papaverine on the high-K^+- or CCh-induced contraction in guinea pig urinary bladder

The urinary bladder showed a phasic contraction, followed by a tonic one, in each of the H-65K^+ solutions. When the contractile response to H-65K^+ reached a steady level, papaverine (0.3 – 100 μM) was added cumulatively. Papaverine inhibited the H-65K^+-induced contraction in a concentration-dependent manner. Papaverine almost completely inhibited H-65K^+-induced contractions at 100 μM. The IC_{50} value of papaverine for the H-65K^+-induced contraction is 5.3 μM; and the sustained contractions by 10 μM CCh, a receptor agonist, was inhibited by the application of papaverine in a concentration-dependent manner. Papaverine almost completely inhibited the CCh-induced contractions at 100 μM. The IC_{50} value of papaverine for the CCh-induced contraction is 24.1 μM, which was a somewhat low sensitivity compared with the IC_{50} of the H-65K^+-induced one (Figs. 1 and 2). Forskolin (0.3 – 100 μM), which increases cAMP content by activating adenylate cyclase, inhibited the H-65K^+- and CCh-induced contractions, and the IC_{50} values were 4.3 μM and 7.8 μM, respectively. Nitroprusside (0.1 – 30 μM), which increases

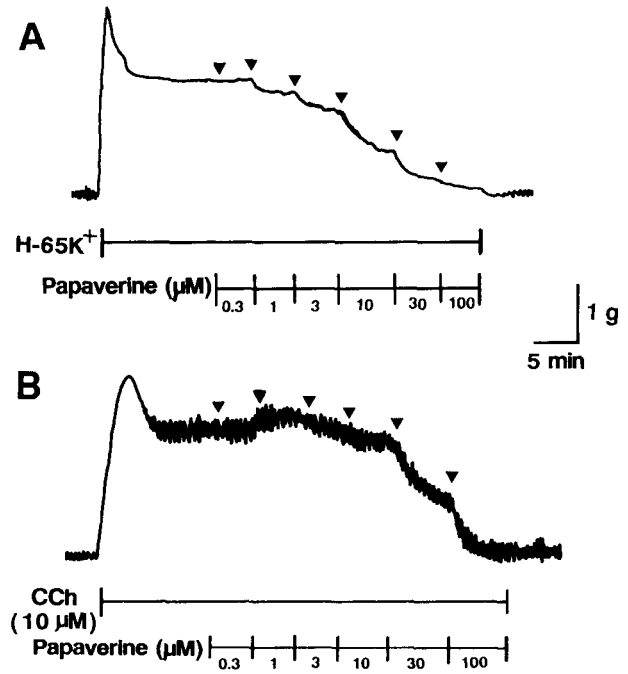

Fig. 1. Effects of papaverine on hyperosmotically added 65 mM KCl (H-65K$^+$) (A)- and carbachol (CCh) (B)-induced increase in muscle tension in guinea pig urinary bladder. After the response to H-65K$^+$ or CCh reached a steady level, papaverine was added cumulatively. Trace of a typical result from 6 experiments.

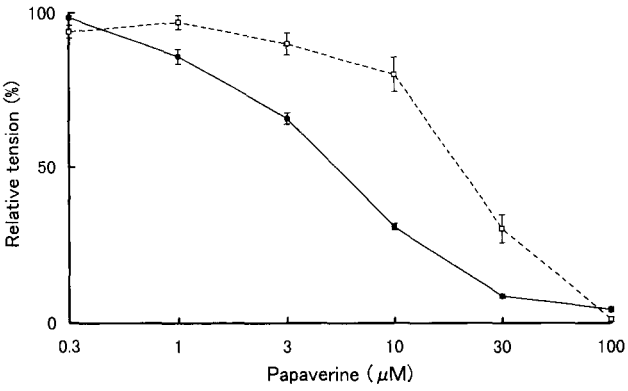

Fig. 2. Effects of papaverine on a contraction induced by hyperosmotically added 65 mM KCl (H-65K$^+$, -●-) or carbachol (10 μM, ---□---) in guinea pig urinary bladder. The maximum contraction induced by H-65K$^+$ or CCh in the absence of papaverine was taken as 100%. Vertical bars indicate S.E.M.

cGMP content by activating guanylate cyclase, did not affect the H-65K$^+$- and CCh-induced contractions (data not shown).

Effects of papaverine on cAMP and cGMP contents

Although papaverine (1 – 30 μM) did not increase the cAMP content above the control in the presence of H-65K$^+$

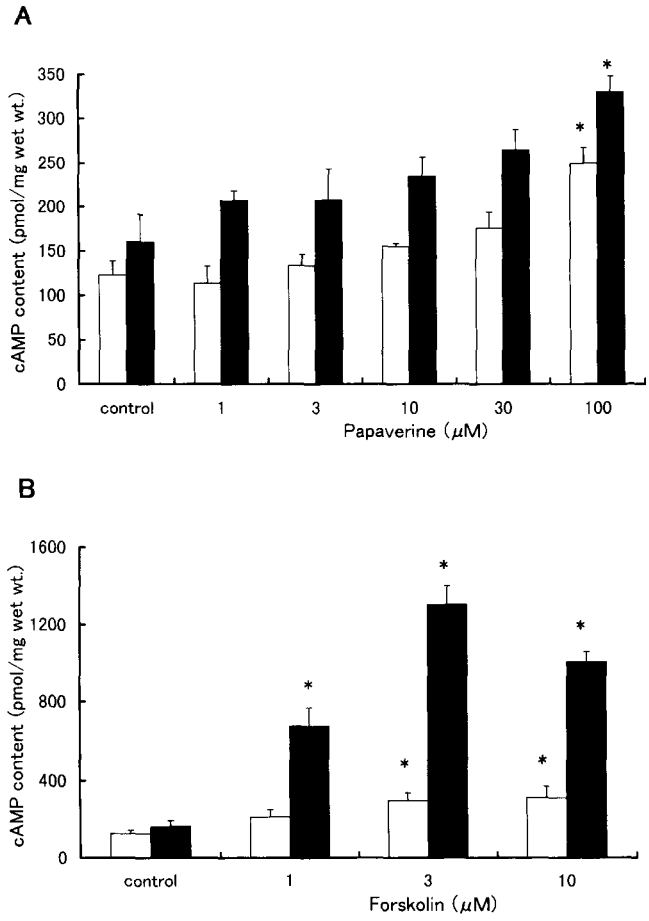

Fig. 3. Effects of papaverine (A) and forskolin (B) on cAMP content of the urinary bladder in the presence of H-65K$^+$ (□) or 10 μM CCh (■). *: Significant difference from each respective control with $P<0.05$. Each point represents the mean of 4 experiments. Vertical bars indicate S.E.M.

or CCh (10 μM), papaverine only at the higher concentration of 100 μM significantly increased cAMP content in the both cases (Fig. 3A). Forskolin (3 and 10 μM) significantly increased cAMP content in the presence of H-65K$^+$, and forskolin at 1, 3 or 10 μM also significantly increased cAMP content in the presence of CCh (Fig. 3B). On the other hand, papaverine (1 – 100 μM) did not affect cGMP content in the presence of H-65K$^+$ or CCh, respectively, and nitroprusside (1, 3 and 10 μM) did not increase cGMP content significantly (Fig. 4: A and B). Concerning the relationship between the inhibition of the H-65K$^+$- or CCh-induced contraction and the increase in cAMP content in the presence of papaverine, papaverine at a concentration inducing a medium-sized relaxation did not increase cAMP content, though 100 μM papaverine that induced a maximum relaxation significantly increased cAMP content. However, papaverine at a concentration of 100 μM did not significantly increase the cGMP content.

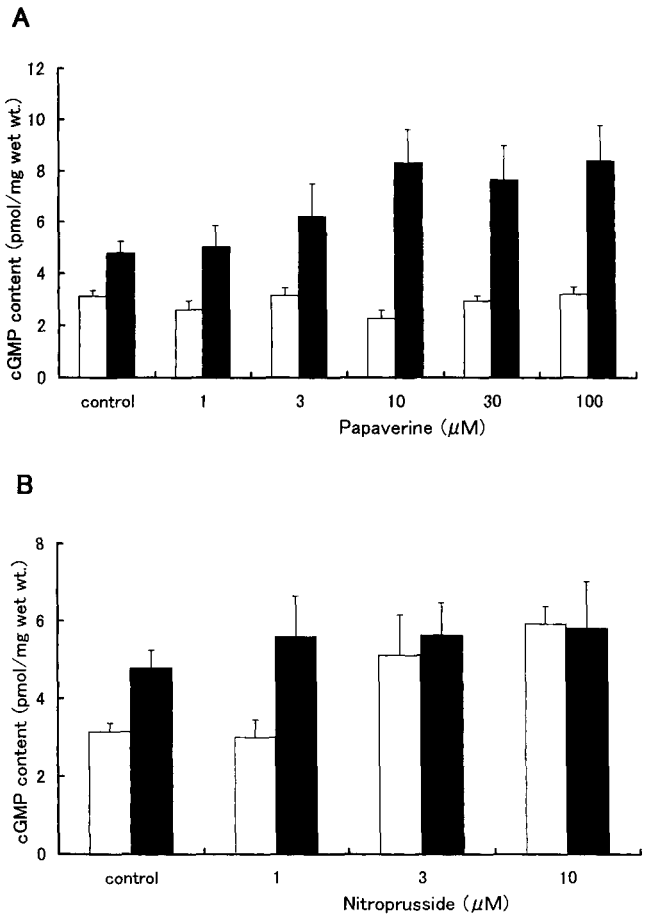

Fig. 4. Effects of papaverine (A) and nitroprusside (B) on cGMP content in the presence of H-65K$^+$ (□) or CCh (■). Each point represents the mean of 4–8 experiments. Vertical bars indicate S.E.M.

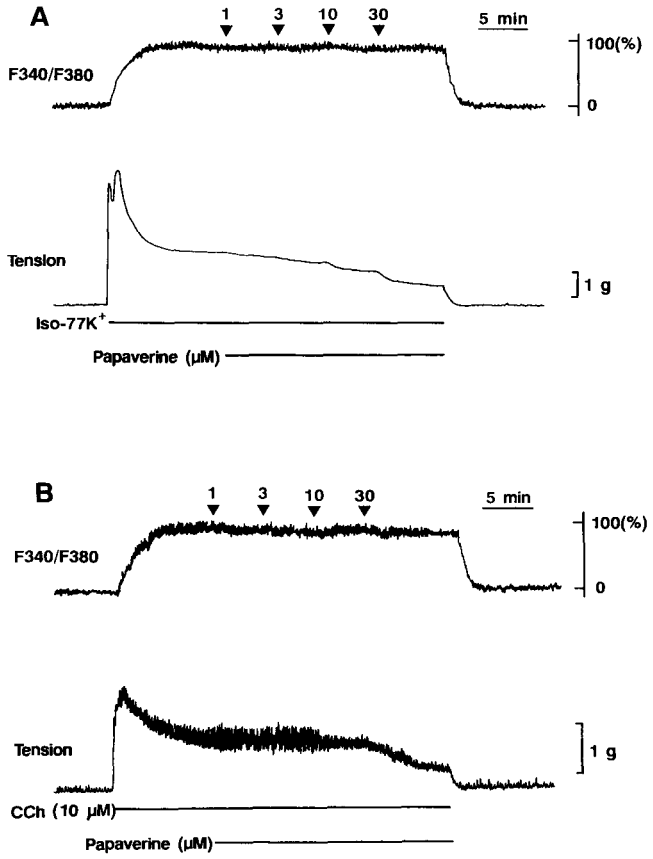

Fig. 5. Effects of papaverine on isosmotically added 77 mM KCl (Iso-77K$^+$) (A)- and 10 μM CCh (B)-induced increase in $[Ca^{2+}]_i$ (F340/F380, upper trace) and muscle tension (lower trace) in guinea pig urinary bladder. The increase in $[Ca^{2+}]_i$ induced by Iso-77K$^+$ or CCh before addition of papaverine was taken as 100%. After the response to Iso-77K$^+$ or CCh reached a steady level, papaverine was added. Trace of a typical result from 4 experiments.

Effects of papaverine on the elevated $[Ca^{2+}]_i$ level and muscle contraction

The effect of papaverine on a $[Ca^{2+}]_i$ level was measured simultaneously with muscle contraction, using a fluorescent Ca^{2+} indicator, fura 2. Iso-77K$^+$ solution and CCh (10 μM) induced an increase in $[Ca^{2+}]_i$ level or muscle contraction, respectively. When the $[Ca^{2+}]_i$ level and muscle contraction induced by Iso-77K$^+$ or CCh reached a steady level, addition of papaverine (1–30 μM) decreased the muscle contraction in a concentration-dependent manner. However, papaverine did not change the $[Ca^{2+}]_i$ level in both cases, even at a concentration of 30 μM (Fig. 5).

Effect of papaverine on FPox fluorescence or PNred one

The effect of papaverine was investigated on FPox or PNred fluorescence measured simultaneously with a muscle contraction. When papaverine was cumulatively applied, it inhibited the H-65K$^+$-induced contraction and the increase in FPox fluorescence in a concentration-dependent manner (Fig. 6A). CCh (10 μM) induced a transient contraction followed by a sustained one. FPox fluorescence increased before initiation of the contraction. Papaverine (1–100 μM) inhibited both the CCh-induced contraction and the stimulated FPox fluorescence in a concentration-dependent manner (Fig. 6B). On the other hand, on the increase in PNred fluorescence and muscle contraction by H-65K$^+$ or CCh, papaverine inhibited the contraction but did not affect the PNred fluorescence (data not shown).

DISCUSSION

Papaverine, a typical non-selective smooth muscle relaxant, can relax high-K$^+$ or agonist-induced contractions in smooth muscles of various organs of many animal species. In the present experiment, papaverine inhibited the H-65K$^+$- or CCh-induced contraction in a concentration-

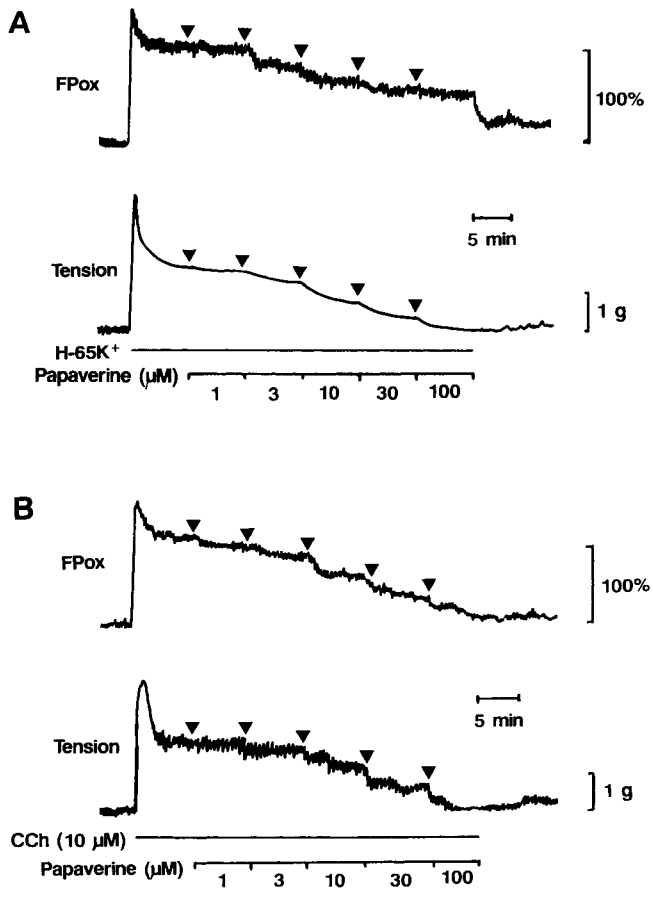

Fig. 6. Effects of papaverine on H-65K$^+$(A)- and CCh (B)-induced increase in oxidized flavoprotein (FPox) fluorescence (upper trace) and muscle tension (lower trace) in guinea pig urinary bladder. The FPox fluorescence induced by H-65K$^+$ or CCh before addition of papaverine was taken as 100%. After the response to H-65K$^+$ or CCh reached a steady level, papaverine was added. Trace of a typical result from 4 experiments.

dependent manner in guinea pig urinary bladder. The IC_{50} for papaverine was 5.3 μM for the H-65K$^+$-induced contraction and 24.1 μM for the CCh-induced contraction. The difference of IC_{50} of papaverine between H-65K$^+$-induced contraction and CCh-induced one may be due to a difference of metabolic dependency on them.

There are three mechanisms of relaxation by papaverine: 1) an intracellular cAMP accumulation, 2) inhibition of cellular respiration and 3) effects on Ca^{2+} movement. Since Kukovetz and Pöch (4) have reported that papaverine induced relaxation of bovine coronary artery by inhibiting PDE which hydrolyses cAMP or cGMP, it has been shown that there is a correlation between the relaxation and the increase in cAMP or cGMP content elicited by papaverine in vascular (12, 22), tracheal (23) and ureteral smooth muscles (24). In the measurement of cAMP or cGMP content in the present paper, papaverine at a concentration lower than 30 μM did not increase significantly the cAMP content above the control in the presence of H-65K$^+$ or CCh. However, papaverine increased cAMP content only at the higher concentration of 100 μM which induced a maximum relaxation. A similar result was reported in guinea pig taenia coli; that is, papaverine at the concentration of 30 μM, which increases the cAMP content, induced a maximum relaxation (25, 26). Moreover, papaverine did not significantly increase the cGMP content even at a concentration of 100 μM. Forskolin, an adenylate cyclase activator, increased the cAMP content of the muscle strips in the presence of H-65K$^+$ or CCh in a concentration-dependent manner. However, nitroprusside, a soluble guanylate cyclase activator, did not increase the cGMP content. We have observed that papaverine inhibited muscle contraction mainly by the accumulation of cAMP and/or cGMP due to the inhibition of PDE in rat aorta, but not in guinea pig ileum (12). From these results, it is suggested that the relaxation induced by papaverine is not predominately related to the accumulation of cAMP and/or cGMP due to the inhibition of PDE in the urinary bladder and the mechanism of papaverine-induced relaxation in the urinary bladder is similar to that in guinea pig ileum.

Papaverine has been shown to increase ^{45}Ca efflux in taenia coli (11) and to inhibit a Ba^{2+} inward current in guinea pig trachea (9). It seems that the decrease in $[Ca^{2+}]_i$ by papaverine is due to two modes of action: 1) papaverine directly blocks Ca^{2+} influx by the inhibition of voltage-dependent Ca^{2+} channels (5) and 2) papaverine increases the level of cAMP and/or cGMP as a result of inhibition of PDE, and then indirectly decreases $[Ca^{2+}]_i$. There are several reports about the decreases in $[Ca^{2+}]_i$ produced by cAMP and cGMP (27–29). In the present paper, high K$^+$ or CCh induced a contraction with an increase in $[Ca^{2+}]_i$ in fura 2-loaded muscle. When the increased $[Ca^{2+}]_i$ level and muscle contraction induced by high K$^+$ or CCh reached a steady level, an addition of papaverine decreased the muscle contraction in a concentration-dependent manner, but did not change the $[Ca^{2+}]_i$ level. Moreover, in the β-escin-permeabilized muscle, papaverine had no effect on Ca^{2+}-induced contraction (data not shown). These results suggest that the relaxation induced by papaverine is not related either directly or indirectly, to Ca^{2+} movements in the urinary bladder.

Some reports investigated the possibility that the relaxing mechanism of papaverine is involved in the inhibition of mitochondrial respiration (6–8, 30–32). Tsuda et al. (6–8) showed that papaverine inhibited high K$^+$-induced contraction and O_2 consumption in guinea pig taenia coli and elucidated that papaverine inhibited mitochondrial respiration by blocking the transduction of an electron between NADH and coenzyme Q and by inhibiting NADH, NADHP-diaphorase. Further, Ishida and Takagi (30)

demonstrated that papaverine decreased the content of ATP and phosphocreatine in guinea pig taenia coli in a concentration-dependent manner. On the other hand, Ozaki et al. (21) reported that high K^+-induced contraction was accompanied by an increase in FPox fluorescence or PNred fluorescence in guinea pig taenia coli. They suggested that the change in FPox fluorescence represented mitochondrial respiration activity and that PNred fluorescence represented glycolysis activity. In the present experiment, papaverine inhibited both the H-65K^+- or CCh-induced contraction and the increase in FPox fluorescence in a concentration-dependent manner in the urinary bladder. There was a positive correlation between the muscle contraction and the FPox fluorescence in the presence of papaverine. Furthermore, there was a no correlation between muscle contraction and PNred fluorescence in the presence of papaverine in it. Papaverine showed the same results in guinea pig ileal longitudinal muscle (12). These results suggest that papaverine inhibits smooth muscle contraction mainly by the inhibition of mitochondrial respiration in guinea pig urinary bladder as well as guinea pig ileum.

From these data, it is proposed that the relaxing mechanism of papaverine on a smooth muscle such as guinea pig urinary bladder or ileum, which shows high spontaneous activity and high respiratory dependency on a tension development, is mainly due to an inhibition of mitochondrial respiration, and the mechanism in a muscle as rat aorta, which shows no spontaneous activity and low respiratory dependency of tension development, mainly involves the inhibition of cAMP-PDE.

REFERENCES

1 Andersson RS, Nilsson K, Wikberg J, Jogansson S, Mohme-Lundholm E and Lundholm L: Cyclic nucleotides and the contraction of smooth muscle. Adv Cyclic Nucleotide Res **5**, 491 - 518 (1975)

2 Beavo JA and Reifsnyder DH: Primary sequence of cyclic nucleotide phosphodiesterase isoenzyme and the design of selective inhibitors. Trends Pharmacol Sci **11**, 150 - 155 (1990)

3 Diamond J: Role of cyclic nucleotides in control of smooth muscle contraction. Adv Cyclic Nucleotide Res **9**, 327 - 340 (1978)

4 Kukovetz WR and Pöch G: Inhibition of cyclic-3',5'-nucleotide-phosphodiesterase as a possible mode of action papaverine and similarly acting drugs. Naunyn Schmiedebergs Arch Pharmacol **267**, 189 - 194 (1970)

5 Takagi K, Takayanagi I and Tomiyama A: Action of dibutyryl cyclic adenosine monophosphate, papaverine and isoprenaline on the intestinal smooth muscle. Jpn J Pharmacol **21**, 477 - 483 (1971)

6 Tsuda S, Urakawa N and Saito Y: The inhibitory effect of papaverine on respiration-dependent contracture of guinea pig taenia coli in high-K medium: I. The relationship between contracture and respiration. Jpn J Pharmacol **27**, 833 - 843 (1977)

7 Tsuda S, Urakawa N and Fukami J: The inhibitory effect of papaverine on respiration-dependent contracture of guinea pig taenia coli in high-K medium: II. Inhibition of mitochondrial respiration. Jpn J Pharmacol **27**, 845 - 853 (1977)

8 Tsuda S, Urakawa N and Fukami J: The inhibitory effect of papaverine on respiration-dependent contracture of guinea pig taenia coli in high-K medium: III. The differential effect of papaverine and rotenone on DT diaphorase. Jpn J Pharmacol **27**, 855 - 863 (1977)

9 Iguchi M, Nakajima T, Hisada T, Sugimoto T and Kurachi Y: On the mechanism of papaverine inhibition of the voltage-dependent Ca^{++} current in isolated smooth muscle cells from the guinea pig trachea. J Pharmacol Exp Ther **263**, 194 - 200 (1992)

10 Sunagane N, Uruno T and Kubota K: Mechanism of relaxant action of papaverine. 2. Differences between modes of relaxant effects of papaverine and amytal in guinea-pig taenia coli. J Pharmacol Dyn **6**, 209 - 216 (1983)

11 Tomiyama A, Takayanagi I and Takagi K: Relaxation of intestinal smooth muscle and calcium movements. J Pharm Pharmacol **25**, 65 - 68 (1973)

12 Kaneda T, Shimizu K, Nakajyo S and Urakawa N: The difference in the inhibitory mechanism of papaverine on vascular and intestinal smooth muscles. Eur J Pharmacol **355**, 149 - 157 (1998)

13 Shimizu K, Yaguchi S, Kometani A, Nakajyo S, Ozaki H and Urakawa N: A comparative study on the contraction induced by high K^+/Na^+ deficient solution in the trachea, gall bladder and urinary bladder in guinea pig. Jpn J Pharmacol **34**, 231 - 239 (1984)

14 Suzuki T, Karaki H and Urakawa N: Inhibition of contraction by swelling of vascular smooth muscle in high KCl, low Na solution. Arch Int Pharmacodyn Ther **250**, 195 - 203 (1981)

15 Kamizaki J, Shimizu K, Nakajyo S and Urakawa N: A comparative study on the contraction induced by high K^+/Na^+ deficient solution in rat uterus or urinary bladder. Jpn J Pharmacol **47**, 179 - 188 (1988)

16 Yaguchi S, Shimizu K, Kometani A and Nakajyo S: Study on the contraction and oxygen consumption induced by high K, Na-deficient solution in the urinary bladder and gall bladder of guinea pig. Jpn J Pharmacol **36**, 407 - 412 (1984)

17 Suzuki T, Karaki H and Urakawa N: Mechanism of inhibition of contraction by high K, Na deficient solution in smooth muscle of guinea pig taenia coli. Arch Int Pharmacodyn Ther **248**, 43 - 49 (1980)

18 Ueda F, Kishimoto T, Karaki H and Urakawa N: High K-induced contraction in rabbit and monkey tracheal smooth muscle. Jpn J Smooth Muscle Res **19**, 541 - 549 (1983)

19 Golenhofen K: Theory of P and T system for calcium activation in smooth muscle. *In* Physiology of Smooth Muscle, Edited by Bülbring E and Shuba MF, pp 197 - 207, Raven Press, New York (1976)

20 Ozaki H, Sato K, Sato T and Karaki H: Simultaneous recording of calcium signals and mechanical activity using fluorescent dye fura 2 in isolated strips of vascular smooth muscle. Jpn J Pharmacol **45**, 429 - 433 (1987)

21 Ozaki H, Sato T, Karaki H and Ishida Y: Regulation of metabolism and contraction by cytoplasmic calcium in the intestinal smooth muscle. J Biol Chem **263**, 14074 - 14079 (1988)

22 Silver PJ, Lepore RE, O'Conner B, Lemp BM, Hamel LT and Bentley RG: Inhibition of the Km cyclic AMP phosphodieterase and action of the cyclic AMP system in vascular smooth muscle by milrinone. J Pharmacol Exp Ther **247**, 34 - 42 (1988)

23 Harris AL, Connell MJ, Ferguson EW, Wallace AM, Gordon RJ, Pagani ED and Silver PJ: Role of low Km cyclic AMP phosphodiesterase inhibitor in tracheal relaxation and bronchodilation in the guinea pig. J Pharmachol Exp Ther **251**, 199 - 206 (1989)

24 Stief CG, Taher A, Truss M, Becker AJ, Schulz-Knappe P, Meyer M, Uckert S, Forsmann W-G and Jonas U: Phosphodiesterase isoenzyme in human ureteral smooth muscle: Identification, characterization and functional effects of various phosphodiesterase inhibitors in vitro. Urol Int **55**, 183 - 189 (1995)

25 Inatomi N, Takayanagi I, Uchida M and Takagi K: Intracellular cyclic AMP level and intestinal smooth muscle relaxation. Eur J Pharmacol **26**, 73 - 76 (1974)

26 Takayanagi I, Uchida M, Inatomi N, Tomiyama A and Takagi K: Intracellular cyclic adenosine 3′,5′-monophosphate and relaxing effects of isoprenaline and papaverine on smooth muscle of intestine. Jpn J Pharmacol **22**, 869 - 871 (1972)

27 Rapoport RM: Cyclic guanosine monophosphate inhibition of contraction may be mediated through inhibition of phosphatidylinositol hydrolysis in rat aorta. Circ Res **58**, 407 - 410 (1986)

28 Smith TK, Ward SM, Zhang L, Buxton ILO, Gerthoffer WJ, Sanders KM and Keef KD: β-Adrenergic inhibition of electrical and mechanical activity in canine colon: role of cAMP. Am J Physiol **264**, G708 - G717 (1993)

29 Twort CHC and van Breeman C: Cyclic guanosine monophosphate-enhanced sequestration of Ca^{2+} by sarcoplasmic reticulum in vascular smooth muscle. Circ Res **62**, 961 - 964 (1988)

30 Ishida Y and Takagi K: Interference of papaverine with energy producing of the guinea-pig taenia caecum. Jpn J Pharmacol **36**, Suppl 178P (1984)

31 Santi R, Contessa AR and Ferrari M: Spasmolytic effect of papaverine and inhibition of the oxidative phosphorylation. Biochem Biophys Res Commun **11**, 156 - 159 (1963)

32 Santi R, Ferrari M and Contessa AR: On the mechanism of spasmolytic effect of papaverine and certain derivatives. Biochem Biophys Res Commun **13**, 153 - 158 (1964)