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The difference in the inhibitory mechanisms of papaverine on vascular and intestinal smooth muscles

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Abstract

Papaverine (0.3–100 μM) more potently inhibited phenylephrine (1 μM)-induced contraction than 65 mM K^+-induced contraction of the aorta, while it equally inhibited contractions induced by 65 mM K^+ and carbachol (1 μM) in ileal smooth muscle. In phenylephrine-treated aorta, papaverine (1–10 μM) increased the cAMP and cGMP content. However, in carbachol-treated ileum, 30 μM papaverine partially increased the cAMP content while it maximally relaxed the preparation. In fura2-loaded aorta, papaverine (0.3–10 μM) inhibited both the contraction and the increase in intracellular Ca^{2+} level ($[Ca^{2+}]_i$) induced by phenylephrine in parallel. However, papaverine inhibited carbachol-induced contraction with only a small decrease in $[Ca^{2+}]_i$. Papaverine (1–30 μM) inhibited the carbachol-induced increase in oxidized flavoproteins, an indicator of increased mitochondrial oxidative phosphorylation, in ileal smooth muscle whereas it did not change the phenylephrine-induced increase in the aorta. These results suggest that papaverine inhibits smooth muscle contraction mainly by the accumulation of cAMP and/or cGMP due to the inhibition of phosphodiesterase in the aorta whereas, in ileal smooth muscle, papaverine inhibits smooth muscle contraction mainly by the inhibition of mitochondrial respiration.

Keywords: Papaverine; Cyclic nucleotide; Oxidative metabolism; Aorta; Ileum

1. Introduction

Papaverine is a non-selective smooth muscle relaxant. Papaverine relaxes $BaCl_2$-induced contraction by inhibiting phosphodiesterase in bovine coronary artery (Kukovetz and Pöch, 1970), and it increases intracellular cAMP in guinea pig taenia coli (Takayanagi et al., 1972). It was generally accepted that the relaxing mechanism of papaverine is correlated with the accumulation of cAMP through inhibition of phosphodiesterase. However, Santi et al. (1963, 1964) pointed out that the relaxing action of papaverine resembled that of metabolic inhibitors, such as anoxia, cyanide or dinitrophenol. Tsuda et al. (1977a,b,c) subsequently showed that papaverine inhibits a high K^+-induced contraction by inhibiting mitochondrial respiration in guinea pig taenia coli. Moreover, papaverine has been shown to increase $^{45}Ca^{2+}$ efflux in taenia coli (Tomiyama et al., 1973) and to inhibit a Ba^{2+} inward current in guinea pig trachea (Iguchi et al., 1992). Therefore, papaverine appears to cause relaxation by multiple mechanisms.

The purpose of the present study is to examine the mechanism of the inhibitory effect of papaverine on contractions of rat aorta and guinea pig ileal smooth muscle. For this purpose, we measured muscle tension, cAMP and cGMP content, $[Ca^{2+}]_i$ level, oxidized flavoproteins as indicator of mitochondrial respiration and reduced pyridine nucleotides as indicator of glycolytic activity.

2. Materials and methods

2.1. Muscle preparations and tension measurement

Male rats (Wistar strain, 250–300 g; Imamichi Institute for Animal Reproduction, Ibaragi) and male guinea pigs (Hartley strain, 300–400 g; Funabashi Farm, Funabashi) were bled after stunning, and then the thoracic aorta and ileum were quickly removed. The aorta was cut into spiral strips and the endothelium was removed by gentle rubbing with absorbent cotton. The strips of aorta were about 2 mm in width and 10 mm in length. The longitudinal smooth muscle was stripped from the circular smooth muscle as

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described by Paton and Aboo Zar (1968). The strips of ileum were about 5–8 mm in width and 15 mm in length. One end of each strip was bound to a glass holder and the other end was connected by a silk thread to a strain-gauge transducer (TB-611T; Nihon Kohden, Tokyo) 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, 1.5 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). A hyperosmotic KCl solution (65 mM) was made by increasing the concentration of KCl in the PSS.

2.2. Assay of cAMP or cGMP content

The cAMP or cGMP content in the aorta or ileal smooth muscle was measured by enzyme immunoassay. After incubation of the muscles with papaverine, forskolin or sodium nitroprusside for 10 min in the presence of phenylephrine (1 μM) or carbachol (1 μM), the muscles were rapidly frozen in liquid nitrogen and stored at −80°C until homogenized in 6% trichloroacetic acid (0.4 ml). The homogenate was centrifuged at $3000 \times g$ for 15 min and the supernatant was washed with 1.5 ml of water-saturated diethylether three times; the cAMP or cGMP content was assayed by using an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI, USA). The cAMP or cGMP content is expressed as picomole per gram wet weight.

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

The $[Ca^{2+}]_i$ level was measured simultaneously with muscle contraction as reported previously (Ozaki et al., 1987). Muscle strips were incubated with PSS containing 5 μM acetoxymethyl ester of fura2 (fura2/AM) for 3 to 4 h at room temperature. Cremophor EL (0.02%), a non-cytoxic detergent, was also added to increase the solubility of fura2/AM. 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 by a silk thread. The muscle strip was kept horizontally in the organ bath. The muscle strip was alternately excited with light at 340 nm and 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, Japan).

2.4. Simultaneous measurement of muscle contraction and oxidized flavoproteins or reduced pyridine nucleotides

Reduced pyridine nucleotides are fluorescent substances in the cytosol and mitochondria. Oxidized flavoproteins are also fluorescent substances which are localized in mitochondria. In guinea pig taenia coli, hypoxia or an aerobic metabolic inhibitor, cyanide, inhibited the fluorescence of oxidized flavoproteins induced by 40 mM KCl and enhanced the fluorescence of reduced pyridine nucleotides induced by 40 mM KCl (Ozaki et al., 1988). The NADH content of guinea pig taenia coli, measured by an analytical method, was increased under these conditions. Therefore, the intensity of oxidized flavoprotein fluorescence represents mitochondrial activity and the intensity of pyridine nucleotide fluorescence represents glycolytic activity.

In the present experiments, the fluorescence of oxidized flavoproteins or reduced pyridine nucleotides was measured simultaneously with muscle contraction as reported previously (Ozaki et al., 1988). One end of the muscle was pinned to the bottom of the organ bath, which was filled with 5 ml of PSS, and the other end was attached to the transducer by a silk thread. The muscle strip was kept horizontally in the organ bath. The muscle strip was excited with light at 450 nm and emission at 530 nm was measured with a fluorimeter (CAF-100; Japan Spectroscopic) to detect oxidized flavoprotein fluorescence or was excited with light at 340 nm and emission at 470 nm was measured with a fluorimeter to detect reduced pyridine nucleotide fluorescence.

2.5. Chemicals

Chemicals used were papaverine, carbachol, phenylephrine, forskolin (Sigma, St. Louis, MO, USA), sodium nitroprusside (Wako Pure Chemical, Osaka, Japan), fura2/AM (Dojindo Laboratories, Kumamoto, Japan) and cremophor EL (Nacalai Tesque, Kyoto, Japan).

2.6. Statistics

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

3. Results

3.1. Effects of papaverine on contractile responses in aorta and ileal smooth muscles

High K^+ (65 mM) or phenylephrine (1 μM) in rat aorta and high K^+ (65 mM) or phenylephrine (10 μM) in guinea pig aorta induced sustained contractions. The concentrations of high K^+ and phenylephrine selected for these experiments produced maximal responses of similar amplitude in rat or guinea pig aorta. When the contractile response to high K^+ or phenylephrine reached a steady level, papaverine (0.3–100 μM) was added cumulatively. Papaverine inhibited the high K^+- or the phenylephrine-induced contraction in a concentration-dependent manner (Fig. 1A,C). Table 1 summarizes the concentrations of papaverine producing 50% relaxation (IC_{50}) of the high

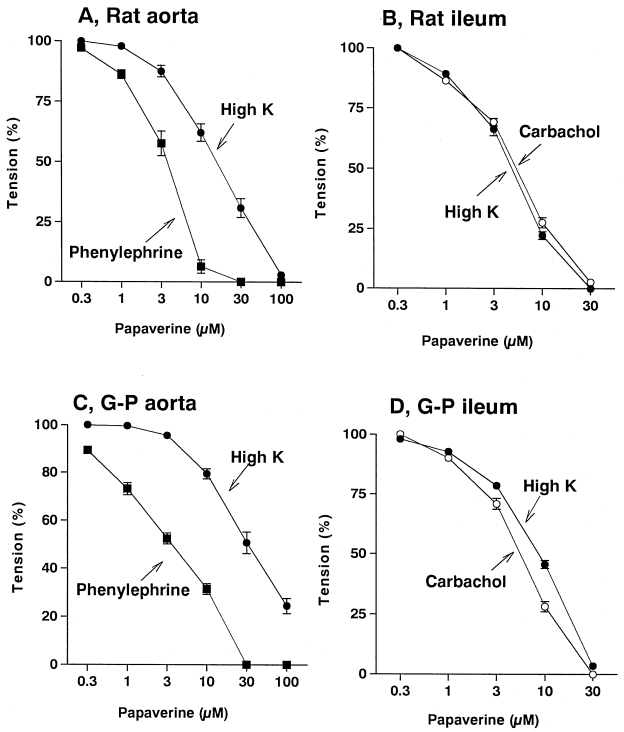

Fig. 1. Effects of papaverine on a contraction induced by high K^+ (65 mM, ●), phenylephrine (1 μM∗, ■) or carbachol (1 μM, ○) in rat aorta (A) and ileum (B), and guinea pig aorta (C) and ileum (D). Preparations were precontracted with high K^+, phenylephrine or carbachol, and then papaverine was added cumulatively. The maximum contraction induced by high K^+, phenylephrine or carbachol in the absence of papaverine was taken as 100%. Vertical bars indicate S.E.M. ∗Around 10 μM phenylephrine was added to guinea pig aorta.

K^+- or phenylephrine-induced contraction. These results showed that papaverine more potently inhibited the phenylephrine-induced contraction than the high K^+-induced contraction.

High K^+ (65 mM) or carbachol (1 μM) induced a transient contraction followed by a sustained contraction in rat or guinea pig ileum. The concentrations of high K^+ and carbachol selected for these experiments produced maximal responses of similar amplitude in rat or guinea pig ileum. When the contraction induced by high K^+ or carbachol reached a steady level, papaverine (0.3–30 μM) was added cumulatively. Papaverine inhibited the high K^+- or carbachol-induced contraction in a concentration-dependent manner (Fig. 1B,D). The IC_{50} values of papaverine for the high K^+- or the carbachol-induced contraction are presented in Table 1. Papaverine inhibited the high K^+- and the carbachol-induced contractions to a similar extent in the ileum.

Table 1
IC_{50} of papaverine for a contraction induced by high K^+, phenylephrine or carbachol

		Stimulant	IC_{50} (μM)	*n*
Rat	aorta	High K^+ (65 mM)	13.4 (6.6–27.3)	12
		Phenylephrine (1 μM)	2.8 (1.9–4.1)	16
	ileum	High K^+ (65 mM)	4.7 (2.1–7.4)	10
		Carbachol (1 μM)	4.1 (2.3–6.9)	12
Guinea pig	aorta	High K^+ (65 mM)	38.5 (29.4–42.3)	10
		Phenylephrine (10 μM)	4.9 (3.5–8.2)	10
	ileum	High K^+ (65 mM)	7.0 (5.2–10.4)	16
		Carbachol (1 μM)	5.1 (3.0–8.8)	18

Numbers in parentheses indicate 95% confidence limits.

3.2. Effects of forskolin and sodium nitroprusside on the high K^+- or receptor agonist-induced contraction in rat aorta and guinea pig ileum

In rat aorta, forskolin (0.001–10 μM) or sodium nitroprusside (0.001–10 μM) inhibited the high K^+ (65 mM)- or the phenylephrine (1 μM)-induced contraction in a concentration-dependent manner (Fig. 2A,B). The IC_{50} for forskolin was 8.5 μM for the high K^+ (65 mM)-induced contraction and 40 nM for the phenylephrine-induced contraction. The IC_{50} for sodium nitroprusside was higher than 10 μM for the high K^+-induced contraction and 8.9 nM for the phenylephrine-induced contraction. These results suggest that forskolin and sodium nitroprusside more strongly inhibited the phenylephrine-induced contraction than the high K^+-induced contraction in rat aorta.

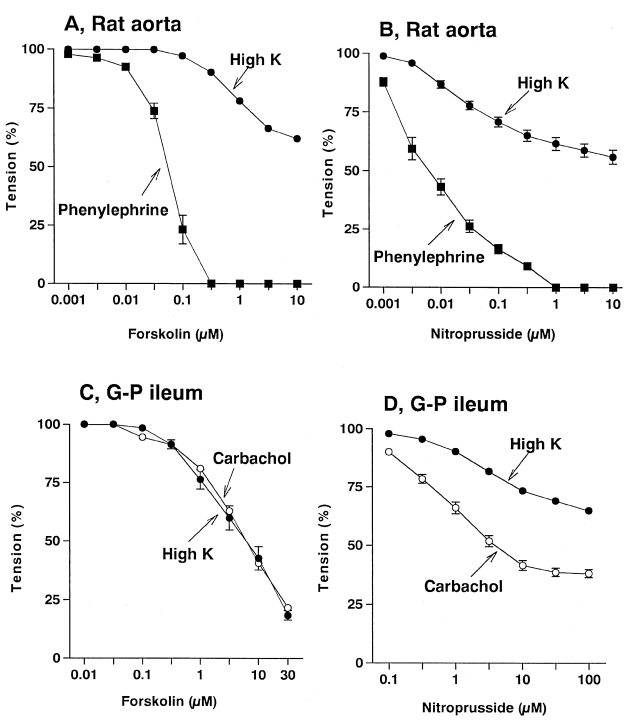

Fig. 2. Effects of forskolin or sodium nitroprusside on the contraction induced by high K^+ solution (65 mM, ●), phenylephrine (1 μM, ■) or carbachol (1 μM, ○) in rat aorta (A,B) and guinea pig ileum (C, D). Preparations were precontracted with high K^+ or phenylephrine and the agents were then added cumulatively. The maximum contractions induced by high K^+, phenylephrine or carbachol in the absence of the agents were taken as 100%. Vertical bars indicate S.E.M.

In guinea pig ileum, forskolin (0.01–30 μM) or sodium nitroprusside (0.1–100 μM) also inhibited the high K^+ (65 mM)- or carbachol (1 μM)-induced contraction in a concentration-dependent manner (Fig. 2C,D). The IC_{50} for forskolin was 5.1 μM for the high K^+-induced contraction and 5.7 μM for the carbachol-induced contraction. The IC_{50} for sodium nitroprusside was higher than 100 μM for the high K^+-induced contraction or 8.3 μM for the carbachol-induced contraction. Although sodium nitroprusside more potently inhibited the carbachol-induced contraction than the high K^+-induced contraction, forskolin inhibited these contractions to a similar extent in the guinea pig ileum.

3.3. Effects of papaverine, forskolin and sodium nitroprusside on cAMP and cGMP contents

In rat aorta, papaverine (1–10 μM) or forskolin (0.03–0.3 μM) increased the cAMP content above the control in the presence of phenylephrine (1 μM) in a concentration-dependent manner (Table 2). Papaverine (1–10 μM) or sodium nitroprusside (0.01–1 μM) also increased the cGMP content of rat aorta in the presence of phenylephrine (1 μM) in a concentration-dependent manner (Table 2). There was a positive correlation between the inhibition of the phenylephrine-induced contraction and the increase in cAMP content elicited by papaverine or forskolin in rat aorta (Fig. 3A). Although the inhibition of the phenylephrine-induced contraction was correlated with the increase in cGMP content elicited by sodium nitroprusside

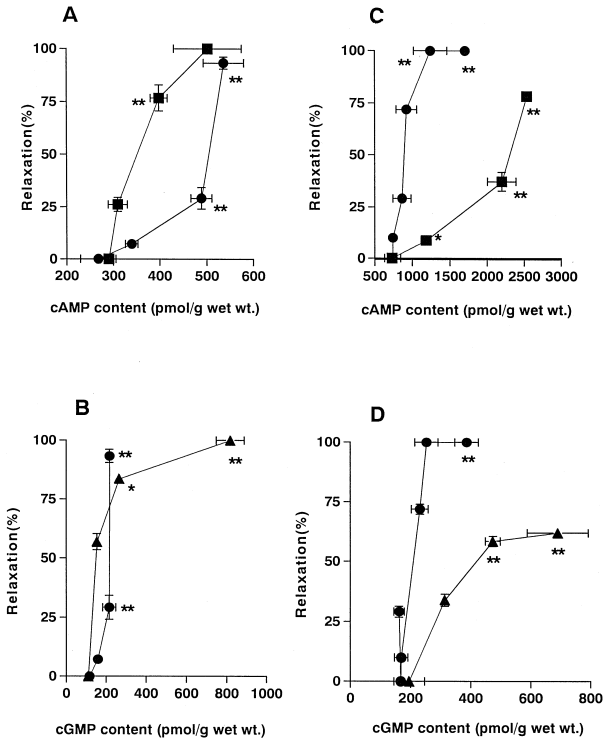

Fig. 3. Relationship between the relaxation (ordinate) and the cAMP or cGMP content (abscissa) in rat aorta (A,B) or guinea pig ileum (C, D) in the presence of the stimulants, ●; papaverine, ■; forskolin, ▲; nitroprusside. Vertical bars indicate S.E.M. of relaxation and horizontal bars indicate S.E.M. of cyclic nucleotide content. ∗, ∗∗: Significant difference from each respective control with $P < 0.05$, $P < 0.01$, respectively in cyclic nucleotide content.

Table 2
Effect of papaverine, forskolin or sodium nitroprusside on the cyclic nucleotide content of rat aorta

Agents	cAMP (pmol/g wet wt.)	cGMP (pmol/g wet wt.)
Papaverine		
Control	267.6±38.2	116.7±10.6
1 μM	339.6±13.7	158.3±10[a]
3 μM	488.7±22.2[b]	215.3±33.1[a]
10 μM	536.1±43.1[b]	216.5±18.9[b]
Forskolin		
Control	290.2±9.4	no data
0.03 μM	309.6±20.8	no data
0.1 μM	398.2±17.9[b]	no data
0.3 μM	502.1±72.5[b]	no data
Sodium nitroprusside		
Control	no data	110.9±22.7
0.01 μM	no data	153.6±21
0.1 μM	no data	265.2±21.6[b]
1 μM	no data	502.1±72.5[b]

[a,b]Significant difference from each respective control with $P < 0.05$, $P < 0.01$.
The muscle strips were contracted with 1 μM phenylephrine and were treated with papaverine, forskolin or sodium nitroprusside for 10 min. Values are the means±S.E.M. Each values represents the mean of four experiments.

in rat aorta, the correlation for papaverine was considerably lower than that for sodium nitroprusside (Fig. 3B).

As shown in Table 3, papaverine (1, 3 and 10 μM) did not increase significantly the cAMP content above control in the presence of carbachol in guinea pig ileum. However, the increase of cAMP elicited by papaverine at a concentration of 30 μM, which induced a maximum relaxation, was 1.7 times the control and the increase of cAMP elicited by 100 μM papaverine was 2.3 times higher. Forskolin increased the cAMP content of the muscle strips in the presence of carbachol in a concentration-dependent manner (Table 3).

In guinea pig ileum, papaverine at a concentration lower than 30 μM did not increase significantly the cGMP content above the control in the presence of carbachol (Table 3). However, the increase of cGMP elicited by 100μM papaverine was 2.3 times the control. There was a positive correlation between the inhibition of the carbachol-developed tension and the increase in cAMP or cGMP content produced by forskolin or sodium nitroprusside in guinea pig ileum (Fig. 3C,D). However, papaverine at a concentration which induced a medium-sized relaxation did not significantly increase the cAMP or cGMP content (Fig. 3C,D).

Table 3
Effect of papaverine, forskolin or sodium nitroprusside on the cyclic nucleotide content of the guinea pig ileum

Agents	cAMP (pmol/g wet wt.)	cGMP (pmol/g wet wt.)
Papaverine		
Control	736.3 ± 106.4	167.3 ± 13.1
1 μM	742.2 ± 35.7	169.3 ± 22.4
3 μM	864.1 ± 119.4	162.4 ± 17.5
10 μM	922.2 ± 43.1	230.7 ± 27.6
30 μM	1239.3 ± 222.7[a]	252.8 ± 38.3
100 μM	1705.4 ± 58.9[b]	386.2 ± 39[b]
Forskolin		
Control	728.8 ± 46.8	no data
0.3 μM	1183.9 ± 68.2[b]	no data
3 μM	2200.8 ± 191.7[b]	no data
30 μM	2534.9 ± 46.4[b]	no data
Sodium nitroprusside		
Control	no data	193.5 ± 50.2
1 μM	no data	313.6 ± 11.3
10 μM	no data	473.9 ± 24.9[b]
100 μM	no data	690.4 ± 101.5[b]

[a,b]Significant difference from each respective control with $P < 0.05$, $P < 0.01$.
The muscle strips were contracted with 1 μM carbachol and were treated with papaverine, forskolin or sodium nitroprusside for 10 min. Values are the means ± S.E.M. Each values represents the mean of four experiments.

3.4. Effects of papaverine, forskolin or sodium nitroprusside on elevated $[Ca^{2+}]_i$ produced by a receptor agonist

The effects of papaverine, forskolin and sodium nitroprusside on $[Ca^{2+}]_i$ were measured simultaneously with muscle tension, using a fluorescent Ca^{2+} indicator, fura2. In rat aorta, phenylephrine (1 μM) induced a contraction with an increase in $[Ca^{2+}]_i$ as indicated by F340/F380. Papaverine (0.3–10 μM) inhibited the phenylephrine-induced contraction by decreasing $[Ca^{2+}]_i$ in a concentration-dependent manner (Fig. 4A). Forskolin (0.3 μM) and sodium nitroprusside (1 μM) also inhibited the phenylephrine-induced increase in $[Ca^{2+}]_i$ and muscle contraction almost to the resting level.

In guinea pig ileum, carbachol (1 μM) increased $[Ca^{2+}]_i$ and muscle tension. Papaverine (30 μM) inhibited the carbachol-induced increase in $[Ca^{2+}]_i$ by only 8.1% but it completely inhibited the carbachol-induced contraction (Fig. 4B). Forskolin (30 μM) and sodium nitroprusside (100 μM) inhibited the carbachol-induced contraction by 66.4 ± 2.5% and 55.0 ± 3.1%, respectively, and decreased $[Ca^{2+}]_i$ by 21.8 ± 4.3% and 8.5 ± 1.7%, respectively.

3.5. Effects of papaverine, forskolin or sodium nitroprusside on change of oxidized flavoprotein fluorescence or reduced pyridine nucleotide fluorescence

The effects of papaverine, forskolin and sodium nitroprusside were investigated on oxidized flavoprotein fluorescence or reduced pyridine nucleotide fluorescence mea-

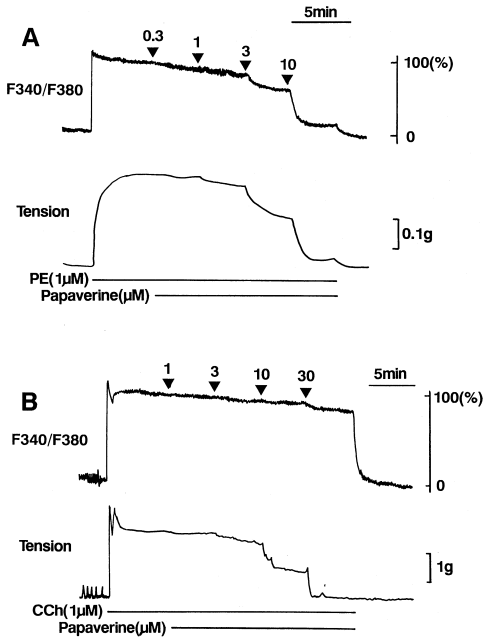

Fig. 4. Effects of papaverine on the phenylephrine- or carbachol-induced increase in $[Ca^{2+}]_i$ (F340/F380, upper trace) and muscle tension (lower trace) in rat aorta(A) or guinea pig ileum (B). The increase in $[Ca^{2+}]_i$ induced by phenylephrine or carbachol before addition of papaverine was taken as 100%. After the responses to phenylephrine or carbachol reached steady levels, papaverine was added. Trace of typical result in four experiments.

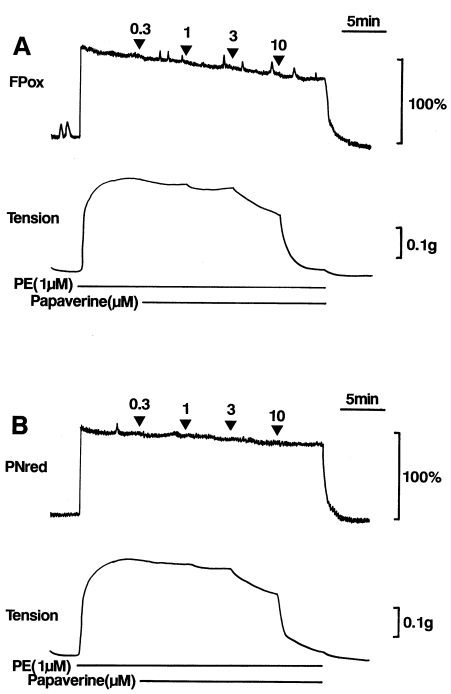

Fig. 5. Effects of papaverine on phenylephrine-induced increase in oxidized flavoprotein (FPox) fluorescence (A) and reduced pyridine nucleotide (PNred) fluorescence (B) (upper trace) in rat aorta. The FPox fluorescence or PNred fluorescence induced by phenylephrine before addition of papaverine was taken as 100%. After the responses to phenylephrine reached steady levels, papaverine was added. Trace of typical result in four experiments.

sured simultaneously with muscle contraction. After the addition of phenylephrine (1 μM) to rat aorta, oxidized flavoprotein fluorescence increased before initiation of the contraction. In contrast, pyridine nucleotide fluorescence increased after the initiation of the contraction induced by phenylephrine (Fig. 5A,B). Although papaverine (0.3–10 μM) inhibited the phenylephrine-induced muscle contraction in a concentration-dependent manner, it did not show any effect on the increase in oxidized flavoprotein fluorescence and reduced pyridine nucleotide fluorescence in rat aorta (Figs. 5 and 6A,B). Forskolin (0.3 μM) and sodium nitroprusside (1 μM) also had no effect on oxidized flavoprotein fluorescence and reduced pyridine nucleotide fluorescence (data not shown).

Carbachol (1 μM) induced a transient contraction followed by a sustained contraction in guinea pig ileum. Oxidized flavoprotein fluorescence increased before initiation of the contraction. Papaverine (30 μM) inhibited both the carbachol-induced contraction and the stimulated oxidized flavoprotein fluorescence to the resting level (Fig. 7A). When papaverine (1–30 μM) was cumulatively applied, it inhibited the carbachol-induced contraction and the increase in oxidized flavoprotein fluorescence in a concentration-dependent manner (Fig. 6C). The reduced pyridine nucleotide fluorescence increased after initiation

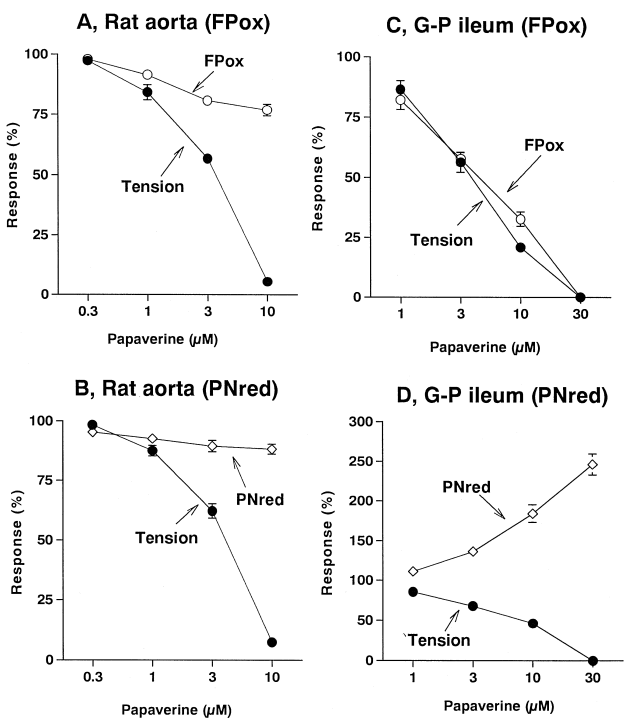

Fig. 6. Effects of papaverine on muscle tension (●) and increases in oxidized flavoprotein (FPox) fluorescence (○) and reduced pyridine nucleotide (PNred) fluorescence (◇) induced by phenylephrine(1 μM) in rat aorta (A, B) or by carbachol (1 μM) in guinea pig ileum (C, D). The developed tension and FPox fluorescence or PNred fluorescence in the presence of the receptor agonists were taken as 100%, and the resting tension and FPox fluorescence or PNred fluorescence before addition of the agonist to the bath were taken as 0%.

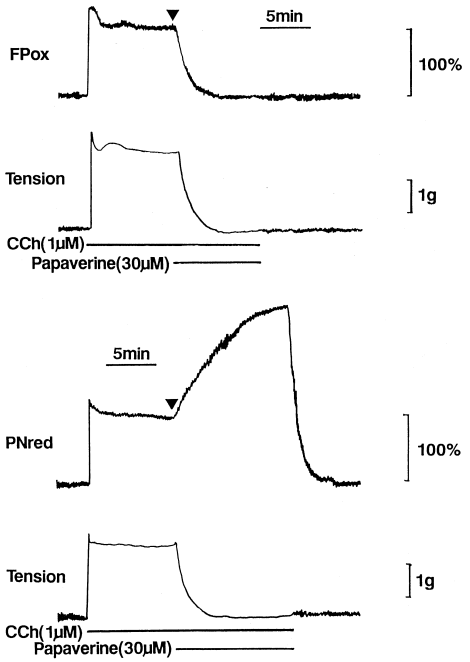

Fig. 7. Effects of papaverine on carbachol-induced increases in oxidized flavoprotein (FPox) fluorescence(A) and reduced pyridine nucleotide (PNred) fluorescence (B) (upper trace) in guinea pig ileum. FPox fluorescence or PNred fluorescence induced by carbachol before addition of papaverine was taken as 100%. After the responses to carbachol reached steady levels, papaverine was added. Trace of typical result in 4–5 experiments.

of the contraction induced by carbachol. Papaverine (30 μM) inhibited the carbachol-induced contraction almost to the resting level, but it enhanced the increase in reduced pyridine nucleotide fluorescence by 246.4 ± 13.4% (Fig. 7B). Cumulative application of papaverine (1–30 μM) inhibited the carbachol-induced contraction and enhanced the increase in reduced pyridine nucleotide fluorescence in a concentration-dependent manner (Fig. 6D). Forskolin (30 μM) and sodium nitroprusside (100 μM) had no effect on oxidized flavoprotein fluorescence and reduced pyridine nucleotide fluorescence (data not shown).

4. Discussion

4.1. Effect of papaverine on phosphodiesterase

In the present experiment, papaverine, forskolin and sodium nitroprusside more strongly inhibited the α_1-adrenoceptor agonist-induced contraction than the high K^+-induced contraction in rat and guinea pig aorta, which is consistent with earlier reports of the effect of forskolin (Abe and Karaki, 1989) or sodium nitroprusside (Karaki et al., 1988) on the two contractions. However, papaverine inhibited the high K^+-induced contraction and the carbachol-induced contraction in rat or guinea pig ileum to a similar extent. These data suggest that papaverine pre-

dominantly inhibits muscle contraction through cAMP- or cGMP-mediated mechanisms in the aorta, but not in the ileum.

Kukovetz and Pöch (1970) suggested that papaverine induced relaxation of bovine coronary artery by inhibiting phosphodiesterase, which hydrolyses cAMP or cGMP. It has since been shown that there is a correlation between the relaxation and the increase in cAMP or cGMP content elicited by papaverine in vascular (Silver et al., 1988), tracheal (Harris et al., 1989) and ureteral smooth muscle (Stief et al., 1995). In the present experiment, papaverine and forskolin increased the cAMP content in a concentration- dependent manner in rat aorta and there was a correlation between the relaxation and the increase in cAMP content produced by these agents. However the increase in cGMP content induced by papaverine in the rat aorta was much smaller than that induced by sodium nitroprusside. Papaverine at a concentration (30 μM) which increases the cAMP content by inhibiting phosphodiesterase activity induced a maximum relaxation in guinea pig taenia coli (Takayanagi et al., 1973; Inamoto et al., 1974) and in the ileum of rats and guinea pigs (present study). From these reports, it is difficult to find a relationship between relaxation and inhibition of phosphodiesterase by papaverine in intestinal smooth muscle. In contrast, there was a correlation between both parameters for forskolin in guinea pig ileum. Papaverine did not significantly increase the cGMP content in the ileum, even at a concentration of 30 μM. There is a possibility that cyclic nucleotides which are increased by papaverine are localized in ileal smooth muscle cells. However, milrinone(type III phosphodiesterase inhibitor) and Ro20-1724(type IV phosphodiesterase inhibitor), which increased the cAMP and cGMP content to the same extent as 30 μM papaverine, reduced the carbachol-induced contraction to less than 20% (Kaneda et al., 1997). Therefore, it is unlikely that the cyclic nucleotides are compartmentalized.

These results suggest that papaverine inhibits muscle contraction mainly by the accumulation of cAMP and/or cGMP due to the inhibition of phosphodiesterase in the aorta; however the relaxation seems not to be related to the inhibition of phosphodiesterase in ileal smooth muscle.

4.2. Effect of papaverine on Ca^{2+} movement

In the present experiment, papaverine inhibited the phenylephrine-induced contraction, accompanied by a large decrease in $[Ca^{2+}]_i$ in rat aorta. 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 causing the direct inhibition of voltage-dependent Ca^{2+} channels (Iguchi et al., 1992). (2) cAMP and/or cGMP, the levels of which increase as a result of inhibition of phosphodiesterase by papaverine, indirectly decrease $[Ca^{2+}]_i$. In rabbit and rat aorta, verapamil, an inhibitor of voltage-dependent Ca^{2+} channels, inhibited more selectively a high K^+-induced contraction than a α_1-adrenoceptor agonist-induced contraction (Karaki and Weiss, 1984), whereas forskolin or sodium nitroprusside inhibited more potently a α_1-adrenoceptor agonist-induced contraction than a high K^+-induced contraction (Abe and Karaki, 1989; Karaki and Weiss, 1984). In the present experiment, papaverine, forskolin or sodium nitroprusside inhibited more potently the phenylephrine-induced contraction than the high K^+-induced contraction in rat aorta. Moreover, forskolin (Abe and Karaki, 1989) and sodium nitroprusside (Karaki et al., 1988) inhibited more strongly the α_1-adrenoceptor agonist-induced increase in $[Ca^{2+}]_i$ than the high K^+-induced contraction in rat aorta. From these results, it is suggested that the decrease in the phenylephrine-induced increase in $[Ca^{2+}]_i$ by papaverine in the aorta is related to cAMP and/or cGMP- mediated mechanisms, but to the inhibition of voltage-dependent Ca^{2+} channels. Several mechanisms have been proposed for the decrease in $[Ca^{2+}]_i$ produced by cAMP and cGMP, namely, membrane hyperpolarization due to the opening of K^+ channels (Haeusler and Thorens, 1975; Smith et al., 1993), an increase in Ca^{2+} uptake into intracellular Ca^{2+} stores (Twort and Van Breemen, 1988; Abe and Karaki, 1989), an increase in Ca^{2+} efflux (Zsoter et al., 1977; Mueller and Van Breemen, 1979), or inhibition of phosphatidyl-inositol turnover mediated by the receptor (Rapoport, 1986; Ahn et al., 1992). Our results suggest that the decrease in $[Ca^{2+}]_i$ induced by papaverine is related to cAMP and/or cGMP-mediated mechanisms in rat aorta. However, in guinea pig ileum, papaverine inhibited the carbachol- induced contraction accompanied by an extremely small decrease in $[Ca^{2+}]_i$. These results suggest that the relaxation produced by papaverine is not predominately related to Ca^{2+} movements in guinea pig ileum.

4.3. Effect of papaverine on energy metabolism

Santi et al. (1963) reported that papaverine inhibited mitochondrial respiration in rat liver. Tsuda et al. (1977a,b,c) showed that papaverine or rotenone inhibited 40 mM KCl-induced contraction and mitochondorial O_2 consumption. They also found that this inhibition was decreased by succinate. Therefore, they elucidated that papaverine inhibited mitochondrial respiration in guinea pig taenia coli and showed that papaverine induced inhibition of high K^+-induced contraction by blocking the transduction of an electron between NADH and coenzyme Q, and by inhibiting NADH, NADPH-diaphorase. Ishida and Takagi (1984) showed that papaverine decreased the content of ATP and phosphocreatinase in guinea pig taenia coli in a concentration-dependent manner, though there was a report that papaverine did not decrease the content of ATP and phosphocreatinase in guinea pig taenia coli (Takayanagi et al., 1980).

Ozaki et al. (1988) reported that high K^+-induced contraction accompanied by increase in oxidized flavoprotein

fluorescence or reduced pyridine nucleotide fluorescence in guinea pig taenia coli. They suggested that the change in oxidized flavoprotein fluorescence represented mitochondrial respiration and that reduced pyridine nucleotide fluorescence represented glycolysis. In the present experiment, papaverine inhibited both the carbachol-induced contraction and the increase in oxidized flavoprotein fluorescence in a concentration-dependent manner, and there was a positive correlation between the muscle tension and the oxidized flavoprotein fluorescence in the presence of papaverine in guinea pig ileum. Further, there was a negative correlation between muscle tension and reduced pyridine nucleotide fluorescence in guinea pig ileum treated with papaverine.

In rat aorta, papaverine at a concentration which inhibited the phenylephrine-induced contraction did not have a pronounced effect on the increase in oxidized flavoprotein fluorescence or reduced pyridine nucleotide fluorescence. These results suggest that the relaxation induced by papaverine is related to the inhibition of mitochondrial respiration in guinea pig ileum not but in rat aorta. The fact that the correlation coefficient between the magnitude of muscle contraction and the increase in oxygen consumption is large in intestinal smooth muscle and small in large-diameter arteries (Paul, 1987) probably explains the difference in the effects of papaverine on respiration in the aorta and ileum.

As neither forskolin nor sodium nitroprusside had a pronounced effect on oxidized flavoprotein fluorescence or reduced pyridine nucleotide fluorescence in rat aorta and guinea pig ileum, it would seem that an increase in the cAMP or cGMP content is not related to inhibition of mitochondrial respiration.

In summary, it is suggested that papaverine inhibits muscle contraction mainly by the accumulation of cAMP and/or cGMP due to the inhibition of phosphodiesterase in the aorta, but mainly by inhibiting mitochondorial respiration in ileal smooth muscle, and that the relaxing mechanisms of papaverine show tissue differences.

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