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Prostaglandins involved in contractions by angiotensin II and bradykinin of isolated dog sphincter pupillae

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1 The dog isolated sphincter pupillae contracted in response to acetylcholine, angiotensin II (AII), bradykinin, prostaglandins $F_{2\alpha}$, D_2, E_2 and I_2, and thionate thromboxane A_2 ($sTXA_2$) in a concentration-dependent manner.

2 AII-induced contractions were suppressed by treatment with saralasin, indomethacin, aspirin and diphloretin phosphate (DPP), a prostaglandin receptor antagonist. Contractions induced by bradykinin were also attenuated by indomethacin, aspirin and DPP. The amount of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in the bathing media was increased approximately 41% following stimulation of the preparations by bradykinin.

3 The potency of contractile responses was in the order of $PGF_{2\alpha} > PGD_2 = sTXA_2 > PGE_2 >$ arachidonic acid $> PGI_2$. Contractions induced by $PGF_{2\alpha}$ were not significantly affected by treatment with indomethacin and ONO3708, an antagonist of the vasoconstrictor effect of prostaglandins, but appreciably attenuated by DPP. Arachidonic acid-induced contractions were inhibited by indomethacin.

4 Contractions of dog iris sphincter muscle in response to AII and bradykinin may be mediated via substances synthesized by cyclo-oxygenase from arachidonic acid. The distribution and nature of the prostaglandin receptors appear to differ markedly in iris sphincter and vascular smooth muscles.

Introduction

Iris sphincter and dilator muscles control the size of pupils via autonomic nerves and possibly by endogenous substances, including amines, peptides, fatty acids, etc. The sphincter muscles are contracted physiologically by activation of cholinergic nerves; electrical stimulation of the nerves and acetylcholine markedly contract the isolated sphincter muscle preparation (Yamauchi *et al.*, 1973; Ueda *et al.*, 1981; Narita & Watanabe, 1981). However, prostaglandins and thromboxane B_2 (TXB_2) have been chemically detected in the bovine iris, choroid and ciliary body (Fujiwara, 1983), and active metabolites of arachidonic acid produced by the cyclo-oxygenase pathway are considered to modulate the function of uveal tissues and to be involved in functional disorders, such as inflammation and trauma (Beitch & Eakins, 1969; Cole & Unger, 1973; Eakins, 1977; Unger *et al.*, 1977; Bhattacherjee, 1980).

Biologically active polypeptides, such as angiotensin II (AII) and bradykinin, release prostaglandins from blood vessels (Blumberg *et al.*, 1977; Toda, 1981; Toda *et al.*, 1987), cultured vein smooth muscle cells (Alexander & Gimbrone, 1976) and other tissues (Ercan & Türker, 1977; Ercan *et al.*, 1978; Türker, 1982), which possibly mediate physiological and pathophysiological actions of the peptides. These polypeptides present in plasma and produced locally would also be expected to act on receptors on sphincter muscle cells. Hence, the present study was undertaken to determine the actions of AII, bradykinin, prostaglandins and a TXA_2 analogue, in comparison with acetylcholine, on the dog isolated sphincter muscle preparation in order to clarify the mechanism of action of the polypeptides in relation to the release of prostaglandins.

Methods

Preparation

Mongrel dogs of either sex, weighing 7–14 kg, were anaesthetized with intravenous injections of sodium

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pentobarbitone (30 mg kg^{-1}) and killed by bleeding from the common carotid arteries. Eyes were rapidly removed. One strip of the iris sphincter was isolated from each eye. The strip was fixed vertically between hooks in the muscle bath of 20 ml capacity containing a modified Ringer-Locke solution, which was maintained at 37 ± 0.3°C and aerated with a mixture of 95% O_2 and 5% CO_2. The hook anchoring the upper end of the strip was connected to the lever of a force-displacement transducer (Nihonkohden Kogyo Co., Tokyo, Japan). The resting tension was adjusted to 50 mg. Constituents of the Ringer-Locke solution were (mM); NaCl 120, KCl 5.4, $NaHCO_3$ 25.0, $CaCl_2$ 2.2, $MgCl_2$ 1.0 and dextrose 5.6. The pH of the solution was 7.3–7.4. Before the start of the experiments the strips were allowed to equilibrate for 60 to 90 min in control media, during which time the solutions were replaced every 10 to 15 min.

Recording

Isometric contractions were recorded on an ink-writing oscillograph (Nihonkohden Kogyo Co.). The contractile response to 30 mM K^+ was first obtained, and the preparations were washed three times with control media and equilibrated for 40–50 min. Concentration-response curves for acetylcholine, bradykinin, prostaglandins, a TXA_2 analogue or arachidonic acid were obtained by adding the agent directly to the bathing media in cumulative concentrations. To determine the concentration-response relationship for AII, the response to 10^{-7} M AII was obtained twice, and then the response to AII in a single concentration (5×10^{-9} to 5×10^{-7} M) was obtained. Preparations were treated for 30 min with diphloretin phosphate (DPP) or ONO3708 and for 20 min with other pharmacological inhibitors, before the response to the agonist was obtained.

Measurement of prostaglandin $F_{2\alpha}$

Two sphincter muscle strips obtained from eyes of the same dogs were used for the paired analysis of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) release in the bathing media. After 20 min preincubation at 37°C, one of the strips was treated with 10^{-7} M bradykinin, and the other strip was treated with vehicle. The preparation was incubated for 15 min before adding indomethacin (10^{-6} M) to stop the further production of $PGF_{2\alpha}$. The bathing medium was then quickly collected by aspiration and stored frozen at below −60°C until the assay. Samples were concentrated ten times by SEP-PAK C18 column (Waters, MA, U.S.A.) before the assay. $PGF_{2\alpha}$ was measured by radioimmunoassay using a commercial kit ([^{3}H]-$PGF_{2\alpha}$ assay system, Amersham, England).

Statistics and drugs

Results shown in the text and figures are expressed as mean ± s.e.mean. Statistical analyses were made by use of Student's paired and unpaired t test and Tukey's method after one-way analysis of variance. Drugs used were $PGF_{2\alpha}$, PGE_2, PGD_2, PGI_2, $sTXA_2$ (9,11-epithio-11,12-methano TXA_2) and ONO3708 ((9,11),(11,12)-dideoxa-9α,11α-di-methyl-methano-11,12-methano-13,14-dihydro-13-azo-14-oxo-15-cyclo-pentanyl-16, 17,18,19,20-pentanor-15-epi-TXA_2) (Ono Pharmaceutical Co., Osaka, Japan), diphloretin phosphate (Leo Co., Helsingborg, Sweden), arachidonic acid and indomethacin (Sigma, St. Louis, MO, U.S.A.), angiotensin II, [Sar^1, Ala^8]angiotensin II(saralasin) and bradykinin (Peptide Institute Inc., Minoh, Japan), acetylsalicyclic acid (Nakarai Chemicals, Kyoto, Japan) and sodium pentobarbitone (Abbott Lab., North Chicago, IL, U.S.A.).

Results

Response to angiotensin II

The addition of AII (5×10^{-9} to 10^{-7} M) produced a concentration-dependent contraction in the dog isolated sphincter muscle (Figure 1). The contraction developed rapidly and did not persist for long. The rate of contraction was related directly to the magnitude of contraction. The magnitude of the contraction was quantitatively compared to that induced by acetylcholine (see Figure 2). No tachyphylaxis developed even after repeated applications of AII.

The contractile response to AII (10^{-7} M) was abolished almost completely by treatment with 10^{-7} M saralasin, but the inhibition was reversed by removal of the antagonist (Figure 1). Treatment with 10^{-7} M indomethacin reduced the response. The inhibition was also reversed by repeated rinsing of the preparation with fresh media. The quantitative data with saralasin, indomethacin (10^{-6} M), aspirin and diphloretin phosphate (DPP), a prostaglandin antagonist (Eakins, 1971; Toda, 1984), are summarized in Figure 3. Treatment with 10^{-7} M saralasin and 10^{-5} M DPP did not significantly alter the contraction induced by 30 mM K^+ ($n = 3$ and 4, respectively). Indomethacin (10^{-6} M) failed to inhibit the response to $PGF_{2\alpha}$, as shown later.

Response to bradykinin

Bradykinin (10^{-10} to 10^{-7} M) contracted sphincter muscle preparations in a concentration-dependent manner (Figure 4). The contraction was maintained

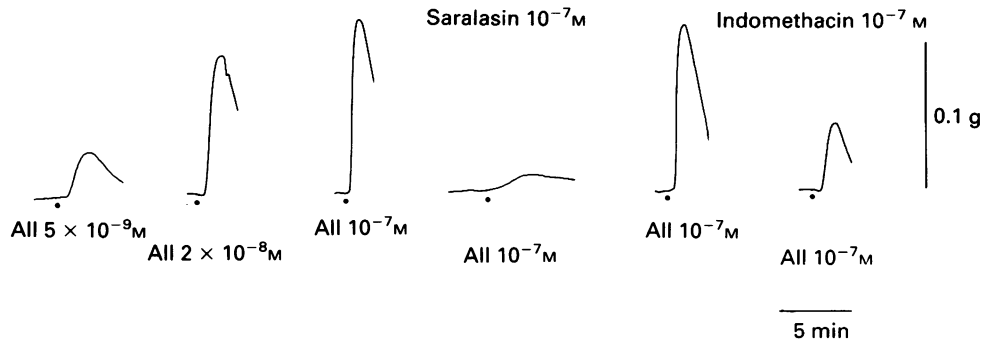

Figure 1 Concentration-dependent contractions induced by angiotensin II (AII, 1st to 3rd tracings) and modifications of the response to 10^{-7} M angiotensin II by 10^{-7} M saralasin and 10^{-7} M indomethacin (3rd to 6th tracings) in a dog iris sphincter strip.

with the peptide in concentrations up to 10^{-9} M, but did not persist long with the higher concentrations. Maximal contractions were obtained at 10^{-7} M; the mean value was 123 ± 21.1% (n = 7) of the contraction induced by 10^{-4} M acetylcholine. The median effective concentration (EC_{50}) of bradykinin was 3.3 ± 1.3 × 10^{-9} M (n = 5). The concentration-response curve was reproducible after a second series of trials; thus, the second curve was taken as a control. The contractile response to the peptide was markedly attenuated by treatment with 10^{-6} M indomethacin (Figure 4). Significant attenuation of the response was also obtained in preparations treated with 5 × 10^{-5} M aspirin (n = 12); the contractions induced by 10^{-9}, 10^{-8} and 10^{-7} M bradykinin were inhibited by 69.1 ± 7.4, 47.4 ± 10.2 ($P < 0.001$) and 38.9 ± 10.9% ($P < 0.01$), respectively. The concentration-response curve for bradykinin was shifted to the right by treatment with DPP (Figure 5). The K_B value of DPP was 7.1 ± 0.4 × 10^{-6} M (n = 5). Treatment with 3 × 10^{-7} M ONO3708, a

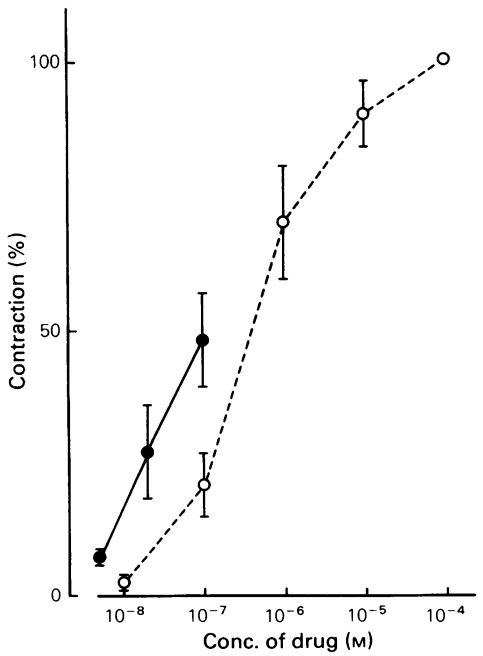

Figure 2 Concentration-response curves for angiotensin II (●, n = 8) and acetylcholine (○, n = 8) in the dog iris sphincter muscle. Contractions induced by 10^{-4} M acetylcholine were taken as 100%; the mean absolute (±s.e.mean) value was 183.6 ± 13.2 mg (n = 8). Vertical lines represent s.e.mean.

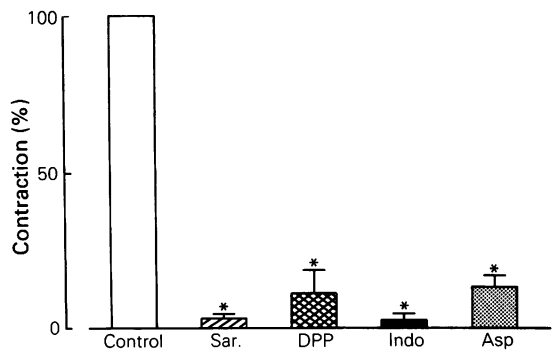

Figure 3 Modification by 10^{-7} M saralasin (Sar) (hatched column, n = 7), 10^{-5} M diphloretin phosphate (DPP) (cross-hatched column, n = 6), 10^{-6} M indomethacin (Indo) (solid column, n = 10) and 5 × 10^{-5} M aspirin (Asp) (stippled column, n = 7) of the angiotensin II (10^{-7} M)-induced contraction in iris sphincter strips. Contractions induced by angiotensin II in control preparations (open column, n = 10) were taken as 100%; the mean absolute (±s.e.mean) value was 49.2 ± 9.4 mg (n = 10). Significantly different from controls; *$P < 0.001$. Vertical lines indicate s.e.mean.

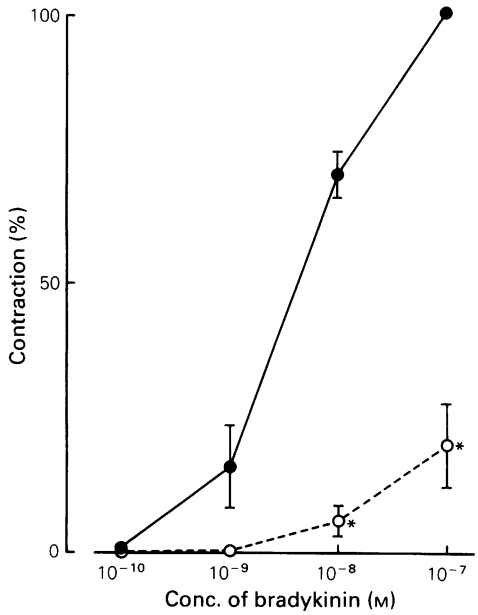

Figure 4 Modification by 10^{-6} M indomethacin (○, $n = 7$) of the contractile response to bradykinin in iris sphincter strips. Contractions induced by 10^{-7} M bradykinin in control media (●, $n = 7$) were taken as 100%; the mean absolute (±s.e.mean) value was 117.0 ± 14.4 mg ($n = 7$). Significantly different from controls; $*P < 0.001$. Vertical lines indicate s.e.mean.

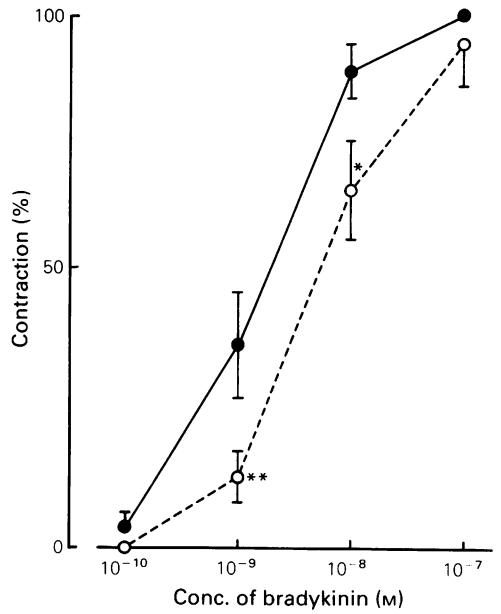

Figure 5 Modification by 10^{-5} M diphloretin phosphate (○, $n = 6$) of the contractile response to bradykinin in iris sphincter strips. Contractions induced by 10^{-7} M bradykinin in control media (●, $n = 6$) were taken as 100%; the mean absolute (±s.e.mean) value was 179.8 ± 17.0 mg ($n = 6$). Significantly different from controls; $*P < 0.025$, $**P < 0.05$. Vertical lines indicate s.e.mean.

TXA_2 analogue which suppresses the contractile response of dog isolated arteries to prostaglandins and thionate TXA_2 ($sTXA_2$; Toda *et al.*, 1986), did not attenuate the contraction induced by bradykinin ($n = 4$).

Responses to prostaglandins, a TXA_2 analogue and arachidonic acid

Concentration-response curves for the contractile effects of prostaglandins (2×10^{-10} to 2×10^{-6} M), $sTXA_2$ (2×10^{-10} to 10^{-7} M), a stable analogue of TXA_2, and arachidonic acid (10^{-8} to 10^{-5} M) obtained under resting conditions are summarized in Figure 6. The contractile potency was in the order of $PGF_{2\alpha} > PGD_2 = sTXA_2 > PGE_2 >$ arachidonic acid $> PGI_2$.

In the preparations partially contracted with acetylcholine or $PGF_{2\alpha}$, PGI_2, a potent vasodilator (Toda, 1980), in concentrations up to 10^{-6} M did not produce a relaxation. The maximal contractile response to $PGF_{2\alpha}$ was approximately 1.4 times as large as that induced by 10^{-4} M acetylcholine (Figure 6), and 352 ± 69.5% ($n = 7$) of that induced by 30 mM K^+.

The contractile response to $PGF_{2\alpha}$ tended to be

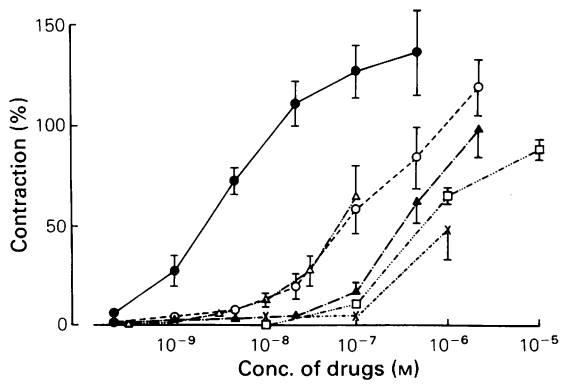

Figure 6 Concentration-response curves for prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; ●, $n = 7$), PGD_2 (○, $n = 7$), PGE_2 (▲, $n = 7$), thionate thromboxane A_2 (△, $n = 6$), PGI_2 (×, $n = 4$) and arachidonic acid (□, $n = 8$) in iris sphincter strips. Contractions induced by 10^{-4} M acetylcholine were taken as 100%; the mean absolute (±s.e.mean) value was 184.8 ± 16.2 mg ($n = 15$). Vertical lines indicate s.e.mean.

potentiated by treatment with 10^{-6}M indomethacin (Figure 7a); however, the difference was not statistically significant. The $PGF_{2\alpha}$-induced contraction was significantly inhibited by 10^{-5}M DPP (Figure 7b). The K_B value of DPP was $1.7 \pm 0.8 \times 10^{-5}$M ($n = 5$). Similar attenuation was also obtain with the contractions induced by PGE_2 ($n = 2$), PGD_2 ($n = 2$) and $sTXA_2$ ($n = 1$). The inhibitory effect was reversed by washing the preparations with fresh media. Treatment with 3×10^{-7}M ONO3708, failed to alter significantly the contraction induced by $PGF_{2\alpha}$ ($n = 5$).

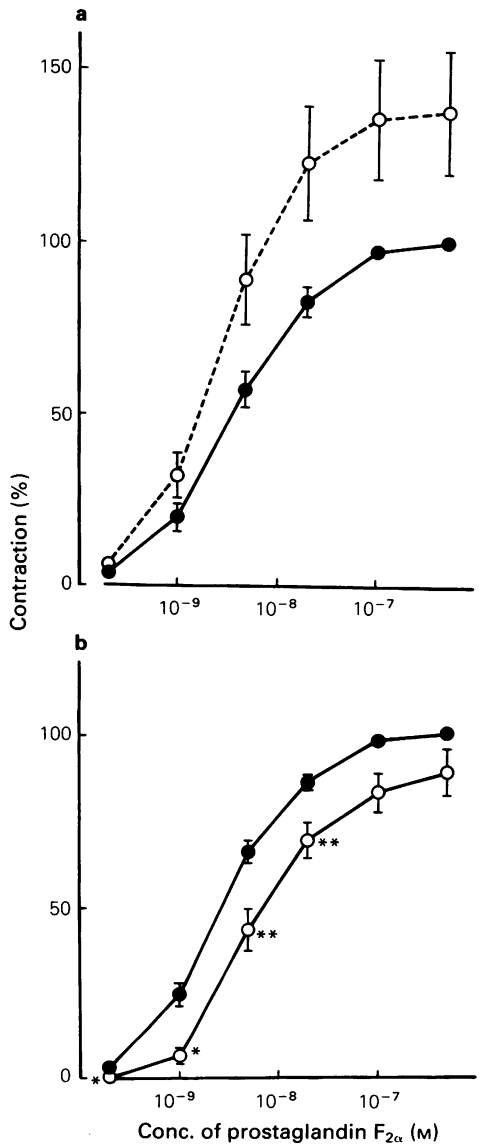

Figure 7 Modification by 10^{-6}M indomethacin (a, ○, $n = 7$) and 10^{-5}M diphloretin phosphate (b, ○, $n = 6$) of the contractile response to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in iris sphincter strips. Contractions induced by 5×10^{-7}M $PGF_{2\alpha}$ in control media (●) were taken as 100%; mean absolute (±s.e.mean) values in (a) and (b) were 143.8 ± 17.2 mg ($n = 7$) and 205.6 ± 24.4 mg ($n = 6$), respectively. Significantly different from controls; $*P < 0.005$, $**P < 0.025$. Vertical lines indicate s.e.mean.

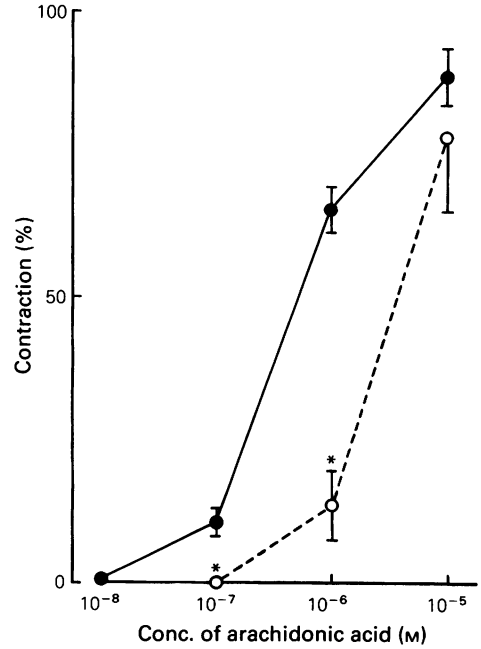

Figure 8 Concentration-response curves for arachidonic acid in the presence (○, $n = 8$) and absence (●, $n = 8$) of 10^{-6}M indomethacin in iris sphincter strips. Responses were compared in pairs of strips obtained from the same dogs. Contractions induced by 10^{-4}M acetylcholine were taken as 100%; the mean (±s.e.mean) value was 216.0 ± 23.0 mg ($n = 8$). Significantly different from controls; $*P < 0.001$.

Tachyphylaxis developed to the contractile response to arachidonic acid. Therefore, the responses of pairs of muscle preparations obtained from the same dogs were compared in the presence and absence of indomethacin. The concentration-response curve for arachidonic acid was shifted to the right by treatment with 10^{-6}M indomethacin (Figure 8).

Release of $PGF_{2\alpha}$ by bradykinin

Paired comparisons were made of the amount of $PGF_{2\alpha}$ in the bathing media in which sphincter muscle preparations from the same dogs were

treated with either bradykinin or its vehicle. The amount of $PGF_{2\alpha}$ released from control preparations in the media averaged $95.7 \pm 7.6\,pg\,mg^{-1}$ wet tissue weight ($n = 8$). Treatment with bradykinin significantly increased the release by $40.5 \pm 8.6\%$ ($n = 8$, $P < 0.001$).

Discussion

AII contracted the dog isolated sphincter muscle in a concentration-dependent manner. The contraction appears to be mediated by activation of the AII receptor as saralasin, a selective antagonist of the AII receptor, suppressed the peptide-induced contraction but not the contraction evoked by K^+. Indomethacin and aspirin, cyclo-oxygenase inhibitors, markedly inhibited the AII-induced contraction but did not significantly alter the contraction induced by $PGF_{2\alpha}$. The contractions produced by AII and $PGF_{2\alpha}$, but not those induced by K^+, were attenuated by DPP in a concentration (10^{-5} M) sufficient to inhibit significantly the contractile response of dog coronary arteries to $PGF_{2\alpha}$ and carbocyclic TXA_2 (Toda, 1982; 1984). Therefore, AII probably contracts dog sphincter muscle by activation of AII receptors, which then results in the release of prostaglandins and TXA_2 intracellularly synthesized from arachidonic acid. AII has been demonstrated to release prostaglandins from isolated and perfused vasculature (McGiff *et al.*, 1970; Aiken & Vane, 1973; Needleman *et al.*, 1975; Toda & Miyazaki, 1981) and from cultured vein smooth muscle cells (Alexander & Gimbrone, 1976).

Bradykinin-induced contractions were approximately 1.5 times as large as the maximum contraction elicited by acetylcholine. Treatment with indomethacin, aspirin and DPP significantly reduced the peptide-induced contraction. Thus, the contraction elicited by bradykinin appears to be mediated by products of the cyclo-oxygenase pathway, as is the response to AII. In fact, biochemical assay data indicate that the release of $PGF_{2\alpha}$ from sphincter muscle is appreciably increased in response to bradykinin. Bradykinin has been demonstrated to release $PGF_{2\alpha}$, PGE_2 and PGI_2 from blood vessels (Aiken, 1974; Needleman *et al.*, 1975; Messina *et al.*, 1975; Türker, 1982; Förstermann *et al.*, 1986; Toda *et al.*, 1987). The antagonism by indomethacin of the response to bradykinin and AII was appreciably greater than that of the response to arachidonic acid (cf. Figures 3, 4 and 8). Such a difference may be explained by the following: (a) the susceptibility to cyclo-oxygenase inhibition differs in the conversion of endogenous and exogenous arachidonic acid to prostaglandins; or (b) the response to arachidonic acid is mediated by cyclo-oxygenase products and is also associated with possible alterations in the perturbation of cell membranes and thus ionic conductance (Chan & Fishman, 1982).

$PGF_{2\alpha}$, PGE_2, PGD_2 and the TXA_2 analogue, $sTXA_2$, contracted the dog sphincter muscle dose-dependently. PGI_2 did not produce a relaxation in muscle preparations even in those previously contracted with acetylcholine or $PGF_{2\alpha}$. The potency in producing contractions was in the order of $PGF_{2\alpha} > PGD_2 = sTXA_2 > PGE_2 > PGI_2$. However, in dog coronary arteries the potency is in the order of TXA_2 analogues, such as $sTXA_2$ (Toda *et al.*, 1986) and carbocyclic TXA_2 (Toda, 1982), $\gg PGE_2 = PGA_2 > PGF_{2\alpha} > PGD_2$, and PGI_2 relaxes these arteries (Toda, 1984). $PGF_{2\alpha}$-induced contractions in sphincter muscle were approximately 130–140% of the contraction due to 30 mM K^+, whereas those in dog cerebral, coronary, mesenteric and femoral arteries are 60–80% of the K^+-induced contraction (Toda & Miyazaki, 1978). Despite such a marked difference in the potency and the magnitude of contraction, contractile responses to prostaglandins of both the sphincter and the arterial smooth muscle were significantly attenuated by DPP, a prostaglandin antagonist. ONO3708 has a TXA_2-like structure (Kawahara *et al.*, 1983) and is a potent inhibitor of the vasoconstrictor effect of prostaglandins in arterial muscle (Toda *et al.*, 1986). The ED_{50} value of $sTXA_2$ was 0.93×10^{-9} M in dog coronary arteries (Toda *et al.*, 1986) and approximately 10^{-7} M in dog sphincter pupillae (if it is assumed that the high concentrations of $sTXA_2$ produce contractions to the level attained by $PGF_{2\alpha}$, Figure 6). Therefore, the affinity of the TXA_2 analogue, ONO3708, in sphincter muscle was assumed to be about 1/100 of that in coronary arteries. ONO3708 3×10^{-9} M moderately attenuated the coronary arterial contraction induced by $sTXA_2$ and $PGF_{2\alpha}$ (Toda *et al.*, 1986), whereas the antagonist in a concentration 100 times higher (3×10^{-7} M) did not reduce the $PGF_{2\alpha}$-induced contraction of sphincter muscle. These findings may indicate a marked difference in the distribution and nature of prostaglandin receptors in dog sphincter and arterial smooth muscle.

The presence of prostaglandins in bovine uveal tissues has been detected chemically (Fujiwara, 1983), and their quantity increases in inflammatory reactions (Bhattacherjee, 1980). The present study revealed a possible production of prostaglandins from arachidonic acid in sphincter muscle, since the fatty acid-induced contraction was evidently reduced by treatment with indomethacin. Arachidonic acid would be released by endogenous peptides intracellularly, such as AII and bradykinin. Bradykinin and $PGF_{2\alpha}$ can contract dog sphincter muscle to an appreciably greater extent than acetylcholine. These peptides and prostaglandins may have a physiological and pathophysiological role in the pupil and surrounding tissues.

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