EFFECTS OF PROSTAGLANDINS ON EXCITATORY TRANSMISSION IN ISOLATED CANINE TRACHEAL MUSCLE

Hironori NAKANISHI, Hirohide YOSHIDA*, Norimichi NAKAHATA
and Takehiko SUZUKI

*Department of Pharmacology, Fukushima Medical College and *Pharmacy,
Fukushima Medical College Hospital, Fukushima 960, Japan*

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Abstract—Contractile response of the isolated canine tracheal muscle to transmural nerve stimulation was considerably depressed by 10^{-6} g/ml of prostaglandin A_1 (PGA_1), A_2 (PGA_2), B_2 (PGB_2), E_1 (PGE_1), E_2 (PGE_2) and $F_{2\alpha}$ ($PGF_{2\alpha}$), while the response to exogenously administered ACh was only slightly affected by the pretreatment with prostaglandins (PGs). The order of the potency of the depressive effect was as follows; $PGE_1 > PGE_2 > PGA_1 > PGA_2 \div PGB_2 \div PGF_{2\alpha}$. Therefore, if a negative feedback control mechanism through prostaglandins exists in the excitatory transmission in canine tracheal muscle, the PGE series may predominantly operate the mechanism. Indomethacin in a dose of 10^{-6} g/ml produced a potentiation of the contractile responses to transmural nerve stimulation and to exogenously administered ACh.

It has been observed that prostaglandin E_1 (PGE_1) and E_2 (PGE_2) suppressed cardiac acceleration and release of noradrenaline (NA) in response to sympathetic nerve stimulation in isolated rabbit hearts (1-5). Similar findings have also been observed in the spleen (3, 6), vas deferens (3, 7, 8) and blood vessels (9) in various species. PGE_1 and PGE_2 are also released by the nerve stimulation (10, 11). Therefore, it has been postulated that the released PGE series act on the prejunctional site and control the subsequent release of NA (5). Recently, the existence of a similar negative feedback mechanism was suggested in parasympathetic nerves innervating rabbit and mouse hearts (12-14).

On the other hand, broncho-constriction *in situ* in response to parasympathetic nerves was prevented by PGE_1 in rabbits and guinea-pigs (15). PGE_1 and PGE_2 prevented the contractile response of the isolated canine tracheal muscle to transmural nerve stimulation (16).

The present study was undertaken to investigate the effects of other prostaglandins (PGs) on the contractile responses to transmural nerve stimulation and to exogenously administered ACh in isolated canine tracheal muscle.

MATERIALS AND METHODS

Mongrel dogs of either sex, weighing 8 to 15 kg were anesthetized with pentobarbital sodium (35 mg/kg, i.v.) and exsanguinated from the bilateral common carotid arteries. The cervical trachea was excised. Recording of the muscle contraction was made using

the same method previously described (16). Briefly, in order to obtain the cumulative dose-response curve of ACh, a strip of tracheal circular muscle (approx. 1.0×0.2 cm) was suspended in Krebs-Ringer bicarbonate solution (20 ml), bubbled with a mixture of 95% O_2 and 5% CO_2 , and kept at $36^\circ C$. To stimulate the nerve transmurally, the muscular strip was hung between two platinum rings and immersed in Krebs-Ringer bicarbonate solution. The composition of the solution was as follows; NaCl, 119 mM; $CaCl_2 \cdot 2H_2O$, 2.5 mM; KH_2PO_4 , 1.2 mM; KCl, 4.7 mM; $MgSO_4 \cdot 7H_2O$, 1.2 mM; $NaHCO_3$, 25 mM and glucose, 11 mM. Contraction of the muscle was recorded on a smoked drum, using an isotonic lever. The stimulation parameters used were 30 Hz, 1 msec, 25 volts/cm and for 30 sec. The final concentration of the drugs was expressed in g/ml. The differences between the values obtained were analyzed using Student's *t*-test.

Drugs used were acetylcholine chloride (ACh), prostaglandin A_1 (PGA_1), prostaglandin A_2 (PGA_2), prostaglandin B_2 (PGB_2), prostaglandin E_1 (PGE_1), prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and indomethacin (Sumitomo Chemical Co.). Prostaglandins (PGs, Ono Pharmaceutical Co.) were stocked as 2% solution in 20% ethyl alcohol (vehicle), and diluted with distilled water just before administration. Indomethacin was dissolved in a weak basic solution (pH 8.5) adjusted by NaOH, and used as a stock solution.

RESULTS

1. Effects of PGs on the contractile response to exogenously administered ACh

PGs rarely produced any effect on the tone of the isolated tracheal muscle. However, when the muscular tone was elevated by 10^{-6} g/ml of ACh, more than 10^{-6} g/ml of PGE_1 produced a relaxation. The pretreatment (10 min before) of 10^{-5} g/ml of PGA_1 , PGA_2 , PGB_2 or $PGF_{2\alpha}$ slightly depressed the contractile response to exogenously administered ACh

FIG. 1. Effects of PGA_1 , PGA_2 , PGB_2 and $PGF_{2\alpha}$ on the contractile response of isolated tracheal muscle of dogs to exogenously administered ACh. Ordinate; % of maximum response obtained in untreated group. Abscissa; doses of ACh (log g/ml). $\times$ — $\times$; Vehicle (equi-volume of the solvent with 10^{-5} g/ml of PGs). $\circ$ — $\circ$; PGA_1 (10^{-5} g/ml) treated group. $\bullet$ — $\bullet$; PGA_2 (10^{-5} g/ml) treated group. $\triangle$ — $\triangle$; PGB_2 (10^{-5} g/ml) treated group. $\square$ — $\square$; $PGF_{2\alpha}$ (10^{-5} g/ml) treated group. Each point shows the mean $\pm$ standard error (Number of animals = 4).

(cumulative method). However, the inhibition was statistically not significant from the inhibition induced by the vehicle (equi-volume of the solvent with 10^{-5} g/ml of PGs) (Fig. 1). The pretreatment with 10^{-6} g/ml of PGE_1 or PGE_2 had almost no effect on the contractile response to ACh, thus confirming the previous finding (16).

II. Effects of PGs on the contractile response to transmural nerve stimulation

The administration of 10^{-6} to 10^{-5} g/ml of PGA_1 depressed the contractile response to

FIG. 2. Effects of PGA_1 and PGA_2 on the contractile response of isolated tracheal muscle to field stimulation in dogs. Ordinate; % of control (the height of contraction induced by field stimulation before drug administration was 100). Abscissa; time course after drug administration (min). $\times$ — $\times$; Vehicle (equi-volume of the solvent with 10^{-5} g/ml of PGs) treated group. $\bigcirc$ — $\bigcirc$; PGA_1 (10^{-5} g/ml) treated group. $\bullet$ — $\bullet$; PGA_1 (10^{-6} g/ml) treated group. $\triangle$ — $\triangle$; PGA_2 (10^{-5} g/ml) treated group. $\square$ — $\square$; PGA_2 (10^{-6} g/ml) treated group. Each point shows the mean $\pm$ standard error (Number of animals=4). * $P < 0.05$; significantly differed from the value of vehicle treated group.

FIG. 3. Effect of PGB_2 on the contractile response of isolated tracheal muscle of dogs to field stimulation. Ordinate; % of the control (the height of contraction induced by field stimulation before drug administration was 100). Abscissa; time course after drug administration (min). $\times$ — $\times$; Vehicle (equi-volume of the solvent with 10^{-5} g/ml of PGB_2) treated group. $\bigcirc$ — $\bigcirc$; PGB_2 (10^{-5} g/ml) treated group. $\bullet$ — $\bullet$; PGB_2 (10^{-6} g/ml) treated group. Each point shows the mean $\pm$ standard error (Number of animals=4). * $P < 0.05$; significantly differed from the value of vehicle treated group.

FIG. 4. Effects of PGE_1 , PGE_2 and $\text{PGF}_{2\alpha}$ on the contractile response of isolated tracheal muscle to field stimulation in dogs. Ordinate; % of the control (height of contraction induced by field stimulation before drug administration was 100). Abscissa; time course after drug administration (min). $\times \times \times$; Vehicle (equi-volume of the solvent with 10^{-6} g/ml of PGs) treated group. $\bigcirc$ $\bigcirc$; $\text{PGF}_{2\alpha}$ (10^{-7} g/ml) treated group. $\bullet \text{---} \bullet$; $\text{PGF}_{2\alpha}$ (10^{-6} g/ml) treated group. $\triangle \cdots \triangle$; PGE_1 (10^{-6} g/ml) treated group. $\square \cdots \square$; PGE_2 (10^{-6} g/ml) treated group. Each point shows the mean $\pm$ standard error (Number of animals=4). * $P < 0.05$, ** $P < 0.02$; significantly differed from the value of the vehicle treated group.

transmural nerve stimulation. The depressive effect progressed according to the time course after administration of the drug. The contractile response was reduced to $71.5 \pm 10.1\%$ and $47.2 \pm 13.8\%$ (the mean $\pm$ S.E., $N=4$), respectively, 15 min after the administration of 10^{-6} and 10^{-5} g/ml of PGA_1 (Fig. 2). These values were statistically significant from the value obtained by the vehicle ($P < 0.05$).

The administration of 10^{-5} to 10^{-6} g/ml of PGA_2 or PGB_2 also depressed the contractile response, but the depressive effects of these drugs were weaker than that of PGA_1 (Figs. 2 and 3). As previously reported (16), PGE_1 and PGE_2 in the dose of 10^{-6} g/ml strongly depressed the contractile response. The depressive effect of PGE_1 was more potent than that of PGE_2 (Fig. 4). $\text{PGF}_{2\alpha}$ had a slight depressive effect on the contractile response (Fig. 4). The order of the potencies of the depressive effect was as follows; $\text{PGE}_1 > \text{PGE}_2 > \text{PGA}_1 > \text{PGA}_2 \div \text{PGB}_2 \div \text{PGF}_{2\alpha}$. The depressive effects of PGs on the contractile response to the transmural nerve stimulation disappeared after washout of PG.

III. Effect of indomethacin on the contractile response to exogenously administered ACh and transmural nerve stimulation

Indomethacin in a dose of 10^{-6} g/ml slightly reduced the muscular tone of some of the strips. Pretreatment (30 min before) with indomethacin in a dose of 10^{-6} g/ml, potentiated the contractile response to exogenously administered ACh (Fig. 5) and to the transmural nerve stimulation (Fig. 6). PGE_1 in a dose of 10^{-6} g/ml remarkably depressed the augmented contractile response to the transmural nerve stimulation in the presence of 10^{-6} g/ml of indomethacin. However, the increase of the dose of indomethacin to 10^{-5} g/ml attenuated its enhancing effect on the contractile response to exogenously administered ACh (not shown in figure) and to transmural nerve stimulation (Fig. 6).

FIG. 5. Effect of pretreatment (30 min before) with indomethacin on the contractile response of isolated tracheal muscle to exogenously administered ACh in dogs. Ordinate; % of the control (the height of contraction induced by 10^{-5} g/ml of ACh was 100). Abscissa; doses of ACh (log g/ml). ○—○; Control (untreated) group. ●—●; Indomethacin (10^{-6} g/ml) treated group. Each point shows the mean $\pm$ standard error (Number of animals=4). * $P<0.05$; the difference between the values obtained in the same dose of ACh was statistically significant.

FIG. 6. Effect of pretreatment with indomethacin on the contractile response of isolated tracheal muscle to field stimulation. Ordinate; % of the control (the height of contraction induced by field stimulation before drug administration was 100). Abscissa; time course after drug administration (min). ○—○; Indomethacin (10^{-6} g/ml) treated group. ●—●; Indomethacin (10^{-5} g/ml) treated group. Each point shows the mean $\pm$ standard error (Number of animals=4). * $P<0.05$; significantly differed from the control.

DISCUSSION

Existence of a feedback control mechanism in sympathetic nerve transmission through endogenous PGE series has been suggested (10). A similar mechanism has also been suggested in parasympathetic nerves (12). Our previous study showed that contractile response of the isolated canine tracheal muscle to the transmural nerve stimulation was predominantly mediated through cholinergic nerves (16). PGE₁ and PGE₂ prevented contractile response to the transmural nerve stimulation, while the response to exogenously administered ACh was almost unaffected or was only slightly depressed. Therefore, the aforementioned feedback control mechanism may work in parasympathetic nerves innervating canine tracheal muscle. Comparison with other PGs, PGE series, particularly PGE₁ had the most potent inhibitory action on sympathetic nerves (2) and parasympathetic nerves (12, 14, 17) in the heart. The present study also demonstrated that the PGE series was the most potent inhibitory substance among PGs. Therefore, it seems that, if the feedback mechanism does exist in the autonomic nerves, PGE series is a specific substance involved in the mechanism. The resting tone of the tracheal muscle used in the present study was low, and PGE₁ produced a relaxation only when the muscular tone was elevated by ACh. The finding is consistent with the results that PGs caused relaxation, when the muscle had an isometric tone (18). Türker and Khairallah (19) also observed that in a dose of over 0.5×10^{-9} g/ml PGE₁ produced a relaxation in the muscle contracted by 10^{-8} g/ml of ACh. In contrast to PGE₁, PGF_{2 α} produced a contraction in human bronchial muscle (20). However, PGF_{2 α} did not contract canine tracheal muscle.

Indomethacin inhibits the biosynthesis of PGs (21). In the presence of indomethacin, the contractile response to the transmural nerve stimulation was considerably potentiated, and such may be due to a partial removal of the negative feedback control through the PGE series (22). However, at least in part, indomethacin may act on postsynaptic sites since exogenously administered ACh was also potentiated by the drug. The potentiating effect of indomethacin on both nerve-mediated and ACh-induced contraction weakened as the administered dose was increased. Farmer et al. (23) also reported that the biphasic action of indomethacin on $\text{PGF}_{2\alpha}$ -induced contraction in guinea pig tracheal muscle, i.e. the small dose produced a potentiation and the large dose, an inhibition.

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