EFFECTS OF PROSTAGLANDINS ON EXCITATORY TRANSMISSION IN ISOLATED CANINE TRACHEAL MUSCLE

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Accepted May 24, 1978

Abstract—Contractile response of the isolated canine tracheal muscle to transmural nerve stimulation was considerably depressed by 10⁻⁶ g/ml of prostaglandin A₁ (PGA₁), A₂ (PGA₂), B₂ (PGB₂), E₁ (PGE₁), E₂ (PGE₂) and F₂α (PGF₂α), 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; PGE₁ > PGE₂ > PGA₁ > PGA₂ > PGB₂ > PGF₂α. 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 E₁ (PGE₁) and E₂ (PGE₂) 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. PGE₁ and PGE₂ 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 PGE₁ in rabbits and guinea-pigs (15). PGE₁ and PGE₂ 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 x 0.2 cm) was
suspended in Krebs-Ringer bicarbonate solution (20 ml), bubbled with a mixture of 95% O₂
and 5% CO₂, and kept at 36°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; CaCl₂·2H₂O,
2.5 mM; KH₂PO₄, 1.2 mM; KCl, 4.7 mM; MgSO₄·7H₂O, 1.2 mM; NaHCO₃, 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 be-
tween the values obtained were analyzed using Student's t-test.

Drugs used were acetylcholine chloride (ACh), prostaglandin A₁ (PGA₁), prostaglandin
A₂ (PGA₂), prostaglandin B₂ (PGB₂), prostaglandin E₁ (PGE₁), prostaglandin E₂ (PGE₂),
prostaglandin F₂a (PGF₂a) 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

I. 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 PGE₁
produced a relaxation. The pretreatment (10 min before) of 10⁻⁵ g/ml of PGA₁, PGA₂,
PGB₂ or PGF₂a slightly depressed the contractile response to exogenously administered ACh

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Fig. 1. Effects of PGA₁, PGA₂, PGB₂ and PGF₂a 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). × — ×: Vehicle (equi-volume of the solvent with 10⁻⁵ g/ml of
PGs). ○ — ○: PGA₁ (10⁻⁵ g/ml) treated group. ● — ●: PGA₂ (10⁻⁵ g/
ml) treated group. △ — △: PGB₂ (10⁻⁵ g/ml) treated group. □ — □: PGF₂a
(10⁻⁵ g/ml) treated group. Each point shows the mean±standard error
(Number of animals = 4).
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 PGE₁ or PGE₂ 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 PGE₁ depressed the contractile response to

![Graph of Fig. 2](image)

**Fig. 2.** Effects of PGA₁ and PGA₂ 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). ×—×; Vehicle (equi-volume of the solvent with 10⁻⁵ g/ml of PGs) treated group. ○—○; PGA₁ (10⁻⁵ g/ml) treated group. ●—●; PGA₁ (10⁻⁶ g/ml) treated group. △—△; PGA₂ (10⁻⁵ g/ml) treated group. □—□; PGA₂ (10⁻⁶ g/ml) treated group. Each point shows the mean ± standard error (Number of animals—4). *P<0.05; significantly differed from the value of vehicle treated group.

![Graph of Fig. 3](image)

**Fig. 3.** Effect of PGB₂ 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). ×—×; Vehicle (equi-volume of the solvent with 10⁻³ g/ml of PGB₂) treated group. ○—○; PGB₂ (10⁻⁵ g/ml) treated group. ●—●; PGB₂ (10⁻⁶ g/ml) treated group. Each point shows the mean ± standard error (Number of animals—4). *P<0.05; significantly differed from the value of 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±10.1\% and 47.2±13.8\% (the mean±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). 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; PGE_{1} > PGE_{2} > PGA_{1} > PGA_{2} > PGB_{2} > 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^{-5} 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^{-5} 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±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±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 0.5x10^{-9} g/ml PGE1 produced a relaxation in the muscle contracted by 10^{-8} g/ml of ACh. In contrast to PGE1, PGF2a produced a contraction in human bronchial muscle (20). However, PGF2a 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 PGF₂α-induced contraction in guinea pig tracheal muscle, i.e. the small dose produced a potentiation and the large dose, an inhibition.

Acknowledgement: The authors would like to thank Mr. H. Mori (Ono Pharmaceutical Co. Ltd.) for providing the prostaglandins.

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