INHIBITORY EFFECTS OF PROSTAGLANDIN E₁ AND E₂ ON CHOLINERGIC TRANSMISSION IN ISOLATED CANINE TRACHEAL MUSCLE

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Abstract—The contractile response of the isolated canine tracheal muscle to the transmural nerve stimulation was depressed by atropine and augmented by physostigmine, indicating that the response was predominantly mediated via the parasympathetic nerve. The contractile response to the transmural nerve stimulation was inhibited by prostaglandin E₁ (PGE₁) and E₂ (PGE₂) (10⁻⁷ to 10⁻⁶ g/ml) and the inhibitory action of PGE₁ was more potent than that of PGE₂. On the other hand, the contractile response of the tracheal muscle to exogenously administered ACh was unaffected by 10⁻⁶ g/ml of PGE₁ and PGE₂. These findings lend support to the hypothesis that the PGE series, in a manner similar to adrenergic transmission, are involved in a negative feed-back control mechanism for the transmitter release in cholinergic transmission.

It has been shown that exogenously administered prostaglandin E series inhibit the response to sympathetic nerve stimulation in various tissues (1-4). Prostaglandin E₁ (PGE₁) and E₂ (PGE₂) are released by the sympathetic nerve stimulation (5, 6). These findings led to an assumption that the prostaglandin E series released from the organ regulate the amount of chemical transmitter to be released by the nerve impulses (7). Recently, a similar negative feed-back regulation was proposed in parasympathetic nerve innervating rabbit heart (8). On the other hand, the inhalation of PGE₁ and PGE₂ relieved the increase of pulmonary resistance induced by cholinomimetics (9). PGE₁ depressed the contractile response of isolated guinea-pig tracheal muscle to acetylcholine (ACh) (10). PGE₂ has a bronchodilating action in guinea pig and monkeys, but not in dogs (11). The present study, therefore, dealt with the effect of PGE₁ and PGE₂ on the nerve-mediated and ACh-induced contractions of the canine tracheal muscle, in vitro, in order to verify applicability of the aforementioned hypothesis.

MATERIALS AND METHODS

Mongrel dogs (either sex), weighing 7 to 15 kg, were anaesthetized with an intravenous injection of pentobarbital sodium (35 mg/kg) and exsanguinated from bilateral common carotid arteries. The cervical trachea was then excised. A strip of tracheal circular muscle (approx. 1.0 cm x 0.2 cm) was hung between two platinum rings to be stimulated transmurally. To obtain the dose-response curve of ACh using a cumulative method (12), 2 or 3 strips of the tracheal circular muscle approx. 2.5 cm x 0.2 cm were connected with
cotton thread. The preparation was immersed in Krebs-Ringer bicarbonate solution (20 ml), bubbled with 100% oxygen and kept at 36°C. The composition of the solution was as follows: NaCl, 119 mM; CaCl₂, 2.5 mM; KH₂PO₄, 1.2 mM; KCl, 4.7 mM; MgSO₄·7H₂O, 1.2 mM; NaHCO₃, 25 mM and glucose, 11 mM. The pH of the solution was between 7.6 and 8.0. Square wave pulses (stimulator; Nihon Kohden MSE-3R) were applied to stimulate the preparation transmurally. The stimulation parameters used were 30 Hz, 1 msec, 25 volts/cm and for 30 sec, except in the studies concerning the relationship between the contractile response and stimulation frequency, pulse-width or intensity. The isotonic contraction of the muscle was recorded on a kymograph via a lever (0.8 g loaded). The final concentration of the drugs is expressed in g/ml. The differences between the values obtained were analysed by Student t-test.

Drugs used were acetylcholine chloride (ACh), atropine sulfate, noradrenaline (Sankyo, Co.), physostigmine hydrochloride, prostaglandin E₁ (PGE₁) and prostaglandin E₂ (PGE₂). Tolazoline hydrochloride (Imidalin®, Yamanouchi Co.), ACh, atropine and physostigmine were dissolved in distilled water. PGE₁ and PGE₂ were dissolved as 2%, solution in 20% ethyl alcohol (vehicle), and diluted with distilled water before administration.

RESULTS

I. Contractile response to transmural electrical stimulation

1) Effects of stimulation parameters

Stimulation frequency: The canine tracheal muscle in vitro exhibited no spontaneous activity and the transmural stimulation produced a contraction. The amplitude of the contraction varied according to stimulation frequencies. Therefore, the effect of stimulation frequency on the contractile response of the tracheal muscle to the transmural stimulation was studied, fixing pulse-width at 1 msec, stimulation intensity at 25 volts/cm and stimulation time at 30 sec or at constant pulses numbering 600. The optimal stimulation frequency for the contractile response to transmural stimulation varied between 30 and 60 Hz (Fig. 1). Contraction could not be maintained with continuous stimulation, especially at a higher frequency.

Pulse-width: Effect of pulse-width on the contractile response of the tracheal muscle to transmural stimulation was studied, fixing stimulation frequency at 30 Hz, stimulation intensity at 25 volts/cm and stimulation time at 30 sec. The lowest pulse-width which produced a contractile response was 0.1 msec. The increase of pulse-width up to 3 msec...
augmented the amplitude of the contraction in response to transmural stimulation (Fig. 2A).

For following pharmacological studies, the pulse-width was kept at 1 msec to stimulate the intramural nerve selectively (13).

**Stimulation intensity:** Effect of stimulation intensity on the contractile response of the tracheal muscle to transmural stimulation was studied, fixing the stimulation frequency at 30 Hz, the pulse-width at 1 msec and the stimulation time at 30 sec. The lowest stimulation intensity to produce a contraction was 10 volts/cm. As the stimulation increased, the amplitude of the contraction increased and reached a maximum at 25 volts/cm (Fig. 2B). For following pharmacological studies using the transmural nerve stimulation, the stimulation parameters were fixed as follows; 30 Hz in stimulation frequency, 1 msec in pulse-width, 25 volts/cm in stimulation intensity and for 30 sec.

2) **Effects of atropine, physostigmine and tolazoline**

Atropine, in doses of more than $10^{-6}$ g/ml, reduced the contractile response of the tracheal muscle to transmural stimulation. The contractile response was almost completely abolished by $10^{-6}$ g/ml of atropine 15 min after the administration (Fig. 3). The depressive effect of atropine progressed according to the time-course after the administration of the drug. The depressive effect disappeared with repeated washings. Physostigmine, in a dose of $10^{-6}$ g/ml, remarkably potentiated the contractile response of the tracheal muscle to transmural stimulations. The potentiating effect of physostigmine also progressed according to the time-course after the administration of the drug. Tolazoline, in a dose of $10^{-4}$ g/ml slightly potentiated the contractile response to transmural stimulation, 27%, 28%, and 34%, at 3, 10 and 15 min after the administration of the drug, respectively (N=3). Noradrenaline, in a dose of $10^{-5}$ g/ml, had no effect on the muscular tone in the three preparations.

3) **Effects of PGE₁ and PGE₂**

PGE₁ and PGE₂, in doses of $10^{-7}$ and $10^{-6}$ g/ml, had no direct effect on the muscular
FIG. 4. Effect of prostaglandin E₁ on the contractile response of the isolated canine tracheal muscle to transmural stimulation. ×—×; Vehicle (equi-volume with 10⁻⁶ g/ml of prostaglandin E₁). The mean of eight experiments. ●—●; Prostaglandin E₁ 10⁻⁷ g/ml. The mean of five experiments. ○—○; Prostaglandin E₁ 10⁻⁶ g/ml. The mean of five experiments. Vertical bars represent the standard errors. * P<0.05 and ** P<0.02; significantly differed from the value of the vehicle-treated group.

FIG. 5. Effect of prostaglandin E₂ on the contractile response of the isolated canine tracheal muscle to transmural stimulation. ×—×; Vehicle (equi-volume with 10⁻⁸ g/ml of prostaglandin E₂). The mean of eight experiments. ●—●; Prostaglandin E₂ 10⁻⁷ g/ml. The mean of four experiments. ○—○; Prostaglandin E₂ 10⁻⁶ g/ml. The mean of five experiments. Vertical bars represent the standard errors. * P<0.05; significantly differed from the value of the vehicle-treated group.

tone. However, the contractile response of the tracheal muscle to transmural stimulation was depressed by the treatment of PGE₁ or PGE₂ in doses of 10⁻⁷ to 10⁻⁶ g/ml. The depressive effect of PGE₁ was more potent than that of PGE₂. The depressive effects gradually increased according to the time-course after the drug-administration and disappeared with washout of the preparation. The administration of the vehicle which was equi-volume with 10⁻⁶ g/ml of PGE₁ or PGE₂, had almost no effect on the contractile response to transmural stimulation (Figs. 4 and 5).

II. Contractile response to ACh

Effect of ACh: ACh, in doses greater than 10⁻⁵ g/ml, produced a sustained contraction of the tracheal muscle. The amplitude of the contraction to ACh was dose-dependent, so that a cumulative method (12) was applied. Sometimes, a phasic contraction of the tracheal muscle was observed by the administration of higher doses (more than 10⁻³ g/ml) of ACh. The pD₂ (−log ED₅₀) of ACh for the contractile response of canine tracheal muscle was 4.66±0.19 g/ml (mean and standard error of five experiments).

Effects of PGE₁ and PGE₂ on ACh-induced response: The dose response curves for ACh obtained by the cumulative method were not affected by pretreatment (10 min before) of 10⁻⁶ g/ml of PGE₁, PGE₂ or vehicle (equi-volume with 10⁻⁶ g/ml of PGE₁ or PGE₂) (Figs. 6 and 7). The pD₂ values of ACh in the presence of 10⁻⁶ g/ml of PGE₁, PGE₂ and the vehicle being almost the same, such were compared with that of the control (Table 1). Namely, PGE₁ and PGE₂ had almost no effect on the contractile response of the tracheal muscle to ACh.
FIG. 6. The dose-response curves of ACh in the isolated canine tracheal muscle (cumulative method). •; control, ×; in the presence of vehicle (equi-volume with 10^{-6} g/ml of prostaglandin E,). O; in the presence of 10^{-4} g/ml of prostaglandin E,.

Each value shows the mean of five experiments. Vertical bars represent the standard errors. The difference between each of the values obtained with the same dose of ACh was statistically insignificant.

FIG. 7. The dose-response curves of ACh in the isolated canine tracheal muscle (cumulative method). •; control, ×; in the presence of vehicle (equi-volume with 10^{-6} g/ml of prostaglandin E,). O; in the presence of 10^{-4} g/ml of prostaglandin E,.

Each value shows the mean of five experiments. Vertical bars represent the standard errors. The difference between each of the values obtained with the same dose of ACh was statistically insignificant.

TABLE 1. The pD2 values of ACh for the contractile response of the isolated canine tracheal muscle

|                          | pD2 of ACh |
|--------------------------|------------|
| control                  | 4.66±0.19  |
| in the presence of vehicle| 4.63±0.20  |
| in the presence of PGE1 10^{-4} g/ml | 4.55±0.15 |
| in the presence of PGE2 10^{-4} g/ml | 4.61±0.12 |

The mean±the standard error of five experiments. The difference between each of the pD2 values was statistically insignificant. Vehicle was equi-volume with 10^{-6} g/ml of prostaglandin E, or E,.

N=5

DISCUSSION

The contractile response of the canine tracheal muscle to transmural stimulation was predominantly mediated via the cholinergic nerve, since the response was blocked by atropine and potentiated by physostigmine. The finding also indicates that the cholinergic nerve innervating the canine tracheal muscle is not resistant to atropine. A higher dose (10^{-6} g/ml) of atropine almost completely abolished the contractile response to the transmural stimulation. However, the finding may not entirely exclude the existence of some other excitatory nerve, because a high dose of atropine has a potent non-specific spasmolytic activity (14). The potentiating effect of tolazoline on the contractile response to transmural stimulations may be due to anticholinesterase activity (15). The optimal stimulation frequency for the contractile response was between 30 and 60 Hz, which was somewhat
higher than that for other cholinergic nerves (16). Loofbourrow et al. (17) have reported that the optimal stimulation frequency for the constrictor response of canine tracheal muscle in situ to the cervical vagal stimulation was 40 Hz. It is of interest that the in situ tracheal muscle of the dog reveals a spontaneous slow rhythmic contraction (17) which was not noted in the present in vitro preparation.

The most significant finding obtained from the present experiments is that the contractile response of the tracheal muscle to the transmural stimulation was considerably depressed by PGE₁ and PGE₂, while the contractile response to ACh was unaffected by these drugs. Wennmalm and Hedqvist (18) reported that the response of rabbit heart in vitro to the parasympathetic nerve stimulation was inhibited by PGE₁, while the response to ACh was unaffected. Therefore, PGE₁ may act on a prejunctional site of the nerve or parasympathetic ganglion. Main (11) has also reported that the bronchoconstrictions of rabbit, guinea pig and cat in response to the vagal stimulation were inhibited by PGE₁. On the other hand, Turker and Khairallah (19) found that the contractile response of the canine isolated tracheal muscle to ACh was reduced only slightly by PGE₁. Recently, Junstad and Wennmalm (8) reported that electrical stimulation of the parasympathetic nerve innervating the rabbit heart releases PGE₁ and PGE₂ which suggests the existence of the negative feed-back mechanism concerning the liberation of the cholinergic transmitter as well as was proposed for sympathetic transmission (7). The present study may support this hypothesis, and further study is under way to determine the release of PGE₁ or PGE₂ from the parasympathetic nerve innervating the canine tracheal muscle.

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