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Prostaglandin E_1 action on canine isolated tracheal muscle

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Prostaglandin E_1 (PGE_1) inhibits contractions of dog isolated tracheal muscle stimulated by different agents, but the degree of inhibition varies with the agent used. Low concentrations of PGE_1 completely block the stimulant effect of 5-hydroxytryptamine, but even large concentrations of PGE_1 do not completely antagonize the contractions caused by acetylcholine. The inhibitory effect of PGE_1 is blocked by methysergide and not by propranolol, morphine or dihydroergotamine. PGE_1 does not relax depolarized smooth muscle, although bradykinin and isoprenaline do. It is concluded that in tracheal smooth muscle, PGE_1 interacts with cell membranes close to the 5-hydroxytryptamine D receptors. This causes activation of the smooth sarcoplasmic reticulum, leading to accumulation of calcium ions and relaxation.

Prostaglandin E_1 usually contracts isolated smooth muscle, but it relaxes tracheal muscle (see review by Bergström, Carlson & Weeks, 1968). We have found that when dog isolated tracheal muscle is contracted by acetylcholine or by 5-hydroxytryptamine (5-HT), the inhibition caused by prostaglandin E_1 (PGE_1) is quantitatively different. The present study deals with this difference and with the mechanism of action of PGE_1.

EXPERIMENTAL

Methods

Tracheae were obtained from normal mongrel dogs anaesthetized with sodium pentobarbitone (30 mg/kg i.v.). A tracheal ring was mounted in Tyrode solution ($NaCl$ 8·0; $NaHCO_3$ 1·0; KCl 0·2; $CaCl_2$ 0·2; $MgCl_2$ 0·1; Na_2HPO_4 0·05; and glucose 1·0 g/litre) in a 10 ml organ bath at 37° according to Akçasu (1959) and bubbled with O_2. Isomeric responses were measured with a Grass force-displacement transducer and recorded on a Beckman Dynograph Type RB. The muscle was allowed to equilibrate in Tyrode solution for 3–4 h while subjected to a passive stretch of 1 g.

In a few experiments the NaCl in Tyrode solution was replaced with isotonic KCl and in other experiments calcium content of the salt solution was varied. The following drugs were used: acetylcholine chloride, 5-hydroxytryptamine creatinine sulphate, methysergide, dihydroergotamine methyl sulphate, propranolol hydrochloride, ouabain and morphine sulphate. Prostaglandin E_1 was a gift from Upjohn. All drug concentrations were expressed as the free base.

RESULTS

Acetylcholine (10 ng/ml) and 5-HT (40 ng/ml) contracted the dog trachea to the same extent. The inhibitory action of PGE_1 was more marked on the 5-HT-contracted smooth muscle, 10 ng/ml of PGE_1 abolishing the response to 40 ng/ml of 5-HT, while

30 ng/ml of PGE_1 reduced response to 10 ng/ml of acetylcholine by about one-half (Table 1). In the presence of 10 ng/ml of acetylcholine, 512 ng/ml of PGE_1 was required to relax the muscle to 70% of its resting state, while in the presence of 40 ng/ml of 5-HT, only 8 ng/ml of PGE_1 was needed (Fig. 1).

Table 1. *Inhibition of dog isolated tracheal muscle by prostaglandin* E_1

Concentration of PGE_1 (ng/mg)	% Inhibition on muscle contracted by acetylcholine (10 ng/ml) (mean ± s.e.)	% Inhibition on muscle contracted by 5-HT (40 ng/ml) (mean ± s.e.)
0·5	5·0 ± 0·2 (n = 5)	20·0 ± 0·8 (n = 10)
1·0	8·0 ± 0·4 (n = 6)	45·0 ± 0·7 (n = 10)
2·0	15·1 ± 0·8 (n = 5)	80·4 ± 0·6 (n = 10)
4·0	25·3 ± 0·5 (n = 7)	95·5 ± 0·8 (n = 10)
8·0	33·0 ± 0·8 (n = 6)	100 (n = 10)
16·0	41·0 ± 0·7 (n = 6)	
32·0	53·0 ± 0·9 (n = 8)	
64·0	58·0 ± 0·6 (n = 10)	
128·0	65·7 ± 0·7 (n = 10)	
512·0	70·0 ± 0·6 (n = 10)	

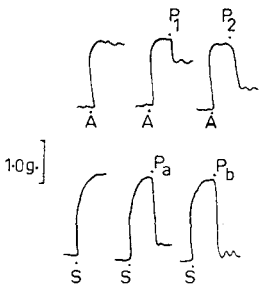

FIG. 1. Responses of isolated tracheal muscle. Initial tension 1·0 g. *Upper Panel:* A 10 ng/ml acetylcholine. P_1 8 ng/ml PGE_1. P_2 512 ng/ml PGE_1. *Lower Panel:* S 40 ng/ml 5-HT. P_a 2 ng/ml PGE_1. P_b 8 ng/ml PGE_1. Bar at left indicates muscle tension of 1·0 g.

Effect of drugs on the inhibitory action of PGE_1 in acetylcholine-constricted muscle

PGE_1 (128 ng/ml) reduced the submaximal contractions caused by acetylcholine (10 ng/ml) by 65·7 ± 0·7% (n= 10) of the control value. After exposure of the muscle to 1 ηg/ml of methysergide for 10 min, the inhibition caused by 128 ng/ml of PGE_1 was only 32·8 ± 2·0 (n = 6). At 10 μg/ml, methysergide completely blocked the effect of 5-HT and slightly potentiated the effect of acetylcholine on the tracheal muscle. PGE_1, in the presence of 10 μg/ml of methysergide, inhibited the acetylcholine-induced contraction by only 13·4 ± 1·07% (n = 10). When the muscle was contracted with 20 mM KCl instead of acetylcholine, PGE_1, 128 ng/ml, caused a relaxation to 23·7 ± 0·8% (n = 6). Contraction to 5-HT was not tested, because of antagonism by methysergide. Propranolol (5 μg/ml), morphine (10 μg/ml) and dihydroergotamine, given 10 min before and ouabain (5·5 μg/ml) given 2 h before PGE_1 had no effect on relaxation.

Effect of ions

Decreasing calcium ion concentration in Tyrode solution to 0·45 mM or addition of 2×10^{-3} M ethylenediamine tetra-acetate (EDTA) in a calcium-free Tyrode solution, had no effect on the relaxant response to PGE_1 in an acetylcholine-contracted muscle. The

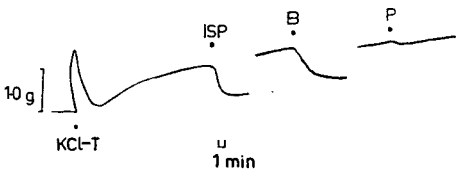

FIG. 2. Responses of dog isolated tracheal muscle depolarized by KCl. Initial tension 1·0 g. KCl-T replacing NaCl in normal Tyrode solution by isotonic KCl. ISP represents 10 ng/ml isoprenaline. B represents 50 ng/ml bradykinin. P represents 512 ng/ml PGE_1. Bar at left indicates muscle tension of 1·0 g.

degree of contraction was not different from that obtained in normal Tyrode. When the muscle was depolarized by replacing the salt solution with isotonic KCl Tyrode, the tracheal ring first contracted rapidly then partially relaxed. Addition of 512 ng/ml of PGE_1 had no further relaxant effect, while 10 ng/ml of isoprenaline and 50 ng/ml of bradykinin caused further relaxation (Fig. 2).

DISCUSSION

Prostaglandin E_1 relaxes dog tracheal muscle as it does tracheal muscle from other species (Horton & Main, 1965). However, the relaxation differs quantitatively when the muscle tonus is increased by equipotent amounts of acetylcholine or 5-HT. When these agonists were given to produce equal degrees of contraction, 8 ng/ml of PGE_1 produced 100% relaxation after 5-HT; while 512 ng/ml produced only a 70% relaxation after acetylcholine. These results indicate that the effect of PGE_1 is not a simple type of drug-response reaction.

In a study on 5-HT receptors in uterine and ileal smooth muscle preparations, Gaddum & Picarelli (1957) subdivided these into two classes; M receptors, possibly on nerve endings and blocked by morphine, and D receptors, possibly on smooth muscle membranes and blocked by dihydroergotamine and lysergic acid diethylamide. Gyermek (1962) reported that tracheal smooth muscle response to 5-HT was not blocked by morphine. Thus it has been concluded that only D receptors are present (Constantine & Knott, 1964).

Since the relaxing effect of PGE_1 is blocked by methysergide and not by morphine, this must indicate that the lipid is acting at the membrane surface, at or close to the specific 5-HT D receptor site, preventing the binding of 5-HT. This receptor site differs from α- and β-adrenergic receptors since dihydroergotamine and propranolol have no effect on responses to PGE_1. We have demonstrated an interaction between PGE_1. and 5-HT receptors in rat isolated duodenum (Khairallah, Page & Türker, 1967). PGE_1 relaxes rat duodenum owing to release of catecholamines, since the response was converted to a contractile one in the presence of α- and β-adrenergic blocking agents or after reserpine pretreatment. Only bromolysergic acid (BOL), another D receptor blocking agent, abolished the contractile response. In no other tissue has PGE_1 been reported to interact with 5-HT receptors. Using isolated mesenteric arterial strips, Strong & Bohr (1967), found that lysergic acid diethylamide had no effect on the response to PGE_1. Thus, interaction of the lipid with 5-HT D receptors may be limited to the muscle of rat duodenum and dog trachea.

The relaxant effect of PGE_1 is significantly decreased and then abolished when KCl replaces NaCl in the bathing medium. KCl-depolarized tracheal smooth muscle does not relax to PGE_1 but does relax to bradykinin and isoprenaline. The latter has

been shown by Schild (1964, 1967) to relax KCl-depolarized uterine muscle, an action which is antagonized by a β-adrenergic blocker, dichloroisoprenaline (DCI). The author concluded that isoprenaline acts independently of membrane potential. PGE_1, on the other hand, is not active in depolarized tracheal smooth muscle, and probably once it is bound to its receptor sites, it leads to depolarization of the normal membrane potential, but would have no effect in a previously depolarized membrane. PGE_1 also acts independently of external calcium ions. Even addition of a chelating agent, EDTA, to the physiological salt solution does not block the relaxing effect seen after PGE_1, although the relaxant effect of isoprenaline in depolarized uterine muscle is abolished in the absence of calcium (Schild, 1967). He has proposed a hypothesis explaining this. Isoprenaline activates a factor which leads to accumulation of calcium ions, thus lowering the concentration of free sarcoplasmic Ca^{++} below a threshold necessary for contraction. This leads to relaxation. The action of isoprenaline is independent of membrane polarization, but requires external calcium ions. A similar hypothesis can be developed to explain the relaxing action of PGE_1. The lipid binds to the cell membrane at or very close to 5-HT D receptors. This prevents the full binding of 5-HT, and thus activates a factor leading to accumulation of calcium in the smooth muscle sarcoplasmic reticulum (Carsten, 1968), lowering free calcium ion concentration and thus producing relaxation. The link between the receptor and the calcium pump in the sarcoplasmic reticulum is related to membrane depolarization and is independent of external Ca^{++} concentration, or the sodium pump, thus differing from isoprenaline.

REFERENCES

AKÇASU, A. (1959). *Archs int. Pharmacodyn. Thér.*, **122**, 201–207.

BERGSTRÖM, S., CARLSON, L. A. & WEEKS, J. R. (1968). *Pharmac. Rev.*, **20**, 1–48.

CARSTEN, M. E. (1968). *Proc. int. Union physiol. Sci.*, **7**, 76 (Abstract No. 227).

CONSTANTINE, S. W. & KNOTT, C. (1964). *J. Pharmac. exp. Ther.*, **146**, 400–403.

GADDUM, J. H. & PICARELLI, Z. P. (1957). *Br. J. Pharmac. Chemother.*, **12**, 323–328.

GYERMEK, L. (1962). *Proc. int. Union physiol. Sci.*, **1**, 28.

HORTON, E. W. & MAIN, I. H. M. (1965). *Br. J. Pharmac. Chemother.*, **24**, 470–476.

KHAIRALLAH, P. A., PAGE, I. H. & TÜRKER, R. K. (1967). *Archs int. Pharmacodyn. Thér.*, **169**, 328–341.

SCHILD, H. O. (1964). *Pharmacology of Smooth Muscle*, 2nd Inter. Pharmacological Meeting. Editor: Bülbring, E. **6**, 95–104.

SCHILD, H. O. (1967). *Br. J. Pharmac. Chemother.*, **31**, 578–592.

STRONG, G. G. & BOHR, D. F. (1967). *Am. J. Physiol.*, **213**, 725–733.