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Interaction of prostaglandins and clonidine in the rat vas deferens

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Stimulation of presynaptic α_2-adrenoceptors by clonidine may lead to local synthesis of prostaglandins which contribute to the inhibition of noradrenaline release observed with this drug. The present investigation was undertaken to determine the role of prostaglandins in the effect of clonidine and xylazine on the rat vas deferens. Both drugs inhibited the twitch response to field stimulation in this preparation. Inhibition was reversed by yohimbine. This effect of clonidine (but not xylazine) was reduced by preincubating vasa deferentia in Krebs containing indomethacin for 1h. Clonidine (but not xylazine) stimulated the synthesis of prostaglandin-like activity in pieces of intact vas deferens incubated in Krebs containing arachidonic acid. Such stimulation was prevented by inclusion of yohimbine (but not prazosin) in the incubation medium. Clonidine did not stimulate prostaglandin synthesis in a cell-free preparation of sheep seminal vesicle microsomes incubated with arachidonic acid or inhibit PGE_2 catabolism by purified swine lung 15-PGDH. We conclude that clonidine (but not xylazine) stimulates prostaglandin synthesis possibly by activating phospholipase activity and releasing arachidonic acid from membrane phospholipids. This effect on prostaglandin production is secondary to activation of α_2-adrenoceptors.

The release of neurotransmitter from sympathetic nerve terminals may be subject to modulation by noradrenaline acting on presynaptic α_2-adrenoceptors (Langer 1977). Drugs such as clonidine and xylazine selectively stimulate α_2-adrenoceptors thus inhibiting noradrenaline release and reducing the response to nerve stimulation in a variety of sympathetically innervated isolated pharmacological preparations (Starke 1977).

It has been suggested that stimulation of presynaptic α_2-adrenoceptors in guinea-pig vas deferens leads to the synthesis of prostaglandins which contribute to the decrease in neurotransmitter release produced by noradrenaline in this preparation (Starnje 1973). No such interaction with prostaglandins was observed in rabbit heart using the more selective α_2-adrenoceptor agonist, oxymetazoline (Starke & Montel 1973).

Clonidine stimulates in vitro production of prostaglandins in rabbit brain and heart (Taube et al 1976) and increases urinary PGE concentration in dogs (Olsen 1976). The present investigation assesses the part played by prostaglandins in the presynaptic, inhibitory effect of clonidine and xylazine on the isolated rat vas deferens preparation.

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MATERIALS AND METHODS

Preparation of vasa deferentia

Male rats (Wistar, 250–300 g) were killed by a blow to the head and exsanguinated. Vasa deferentia were removed, cleared of connective tissue and the epididymal end mounted in 20 ml organ baths containing Mg^{2+} free Krebs (composition, mM: NaCl 121, KCl 4·7, $CaCl_2$ 2·7, $NaHCO_3$ 25, KH_2PO_4 1·18, glucose 11·1) warmed to 37 °C and aerated with 5% CO_2 in oxygen. Preparations were placed under an initial tension of 0·5 g and contractions recorded isometrically using Grass FTO3 transducers connected to a Devices pen recorder. Vasa deferentia were field stimulated using platinum ring electrodes connected to an SRI stimulator (0·1 Hz, 0·1 ms, 150 V).

As the inhibitory effect of clonidine was slow to reverse, responses to it were obtained cumulatively. Each concentration was kept in contact with the tissue for 5 min. Dose response curves to xylazine were carried out in the normal way with a contact time of 3 min. Vasa deferentia were preincubated in Krebs containing indomethacin (10^{-7} M), or an appropriate volume of 0·5% Na_2CO_3, for 1 h at 37 °C. After this period the response to clonidine and xylazine was re-assessed in the continued presence of indomethacin. Post-synaptic sensitivity of vasa deferentia was determined by addition of

noradrenaline (10^{-6}–10^{-4} M) which was kept in contact with the tissue for 1 min.

Prostaglandin biosynthesis by vasa deferentia

Vasa deferentia were removed and cut into four approximately equal pieces each of 10–20 mg. Tissues were incubated in 1 ml Mg^{2+}-free Krebs solution under a stream of 5% CO_2 in oxygen for 30 min at 37 °C. Incubations contained in addition arachidonic acid (10 μg ml^{-1}) or 10 μl absolute ethanol as control as well as clonidine, xylazine or an appropriate volume of distilled water vehicle. In some experiments, prostaglandin synthesis was prevented by inclusion of indomethacin (10^{-6} M). After 30 min, incubates were cooled on ice, and the vas deferens removed, dried and weighed. The remaining Krebs solution was acidified to pH 3·4 with an equal volume of 1 M formic acid and extracted twice into 2 volumes of ethyl acetate. The combined ethyl acetate phase was evaporated to dryness at 30 °C under a constant stream of air and the extract stored at −20 °C until required. Extracts stored in this way were stable for several weeks. The concentration of prostaglandin-like activity in these extracts was determined by bioassay against authentic PGE_2 on the rat stomach strip as previously described (Hoult & Moore 1977).

Effect of clonidine on PG synthesis and inactivation in vitro

The effect of clonidine on prostaglandin formation by sheep seminal vesicle (SSV) microsomes was also determined. Incubations (total volume 0·2 ml) contained SSV microsomes (5 mg ml^{-1}), resuspended in Tris-HCl buffer (pH 7·4), arachidonic acid (10 μg ml^{-1}), reduced glutathione (3 mM) as cofactor and an appropriate concentration of clonidine or vehicle. After 1 h incubation at 37 °C the reaction was terminated by adding 0·2 ml ethanol, acidified, extracted and bioassayed as described above.

Prostaglandin catabolism was determined in incubations containing 0·2 ml Tris-HCl buffer (pH 7·4), PGE_2 (10 μg ml^{-1}), NAD^+ (5 mM) and 1 μl purified swine lung 15-PGDH (equivalent to 16·3 μg protein). Clonidine or vehicle was added and incubation allowed to proceed for 45 min at 37 °C before extraction and bioassay as described above.

Statistical analysis of data

Results are mean ±s.e.m. Number of observations are shown in parentheses. Significant differences between groups was determined by Student's *t*-test. A probability (P) value of 0·05 or less was taken to indicate a significant difference.

Drugs and chemicals

Prostaglandin E_2 and arachidonic acid (Sigma) were stored in ethanol (1 mg ml^{-1}; −20 °C) and diluted in Krebs when required. Clonidine (Boehringer Ingelheim) and xylazine (Bayer) were dissolved in Krebs or distilled water. Yohimbine (Sigma) was dissolved after warming in distilled water. Prazosin (Pfizer) was dissolved in ethanol: water (1:1 v/v). (−)-Noradrenaline bitartrate and indomethacin were from Sigma. Purified swine lung 15-PGDH was obtained from BDH and sheep seminal vesicle microsomes from Miles Laboratories.

RESULTS

Field stimulation of the rat epididymal vas deferens produced a rapid twitch response which was maintained for several hours. The tension developed per twitch was 265 ± 13 mg, n = 20. Contractions due to electrical stimulation were prevented by guanethidine (10^{-6} M) and tetrodotoxin (5×10^{-7} M) at concentrations which failed to affect the response to exogenous noradrenaline. Thus, contractions to field stimulation were nerve-mediated and not due to direct stimulation of the muscle.

Pre-synaptic and post-synaptic activity on the rat vas deferens

Clonidine and xylazine produced dose-dependent inhibition of the twitch response to field stimulation (Fig. 1). The concentration of each drug required to produce 50% inhibition of the twitch response (ID50) as determined from the dose response curve was $2·6 \pm 0·1 \times 10^{-9}$ M, n = 7, and $8·0 \pm 0·4 \times$

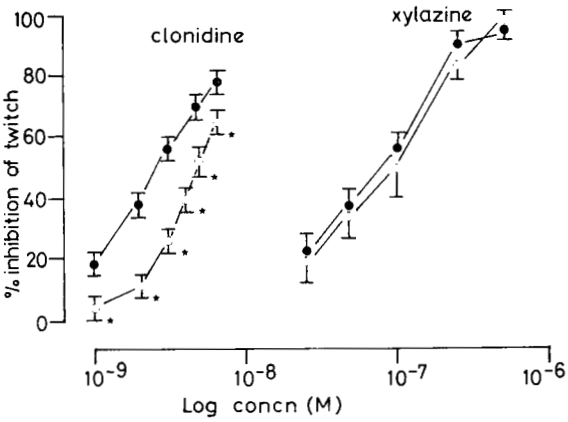

FIG. 1. Inhibition of field stimulated rat vas deferens preparation by clonidine and xylazine in the presence (open symbols) and absence (closed symbols) of indomethacin (10^{-6} M). Preparations were preincubated with indomethacin or 0·5% Na_2CO_3 for 1 h as described in Methods. Results show mean ± s.e.m., n = 5–7. *P <0·001.

10^{-8} M, n = 6 for clonidine and xylazine respectively. Inhibition was rapidly reversed following the addition of yohimbine (10^{-7} M). At concentrations sufficient to inhibit electrically-induced contractions, neither clonidine nor xylazine contracted the vas deferens or potentiated the response to exogenous noradrenaline (10^{-6}–10^{-4} M). This confirms the selectivity of clonidine and xylazine for the α_2-adrenoceptor in this dose range. However, at higher concentrations both clonidine and xylazine contracted the rat vas deferens by an action on post-synaptic α_1-adrenoceptors. The threshold concentration for spasmogenic activity was 5×10^{-6} and 10^{-5} M respectively. Contractions were abolished by prazosin (10^{-8} M).

After 1h preincubation in Krebs solution containing indomethacin (10^{-6} M), the pre-synaptic inhibitory effect of xylazine was unchanged ($8{\cdot}5 \pm 0{\cdot}5 \times 10^{-8}$ M, n = 6) whilst that of clonidine was reduced ($4{\cdot}6 \pm 0{\cdot}3 \times 10^{-9}$ M, n = 7, $P < 0{\cdot}001$). Preincubation of tissues in Krebs solution containing an appropriate volume of 0·5% Na_2CO_3 (the vehicle for indomethacin) for 1 h did not change the inhibitory potency of either drug. Indomethacin pretreatment did not affect the post-synaptic sensitivity of the rat vas deferens to exogenous noradrenaline.

Effect on PG formation by vas deferens in vitro

Prostaglandin formation in pieces (10–20 mg) of rat vas deferens was determined as described in Materials and Methods. In control experiments, vasa deferentia incubated in 0·2 ml Krebs solution for 30 min at 37 °C generated small, but detectable amounts of prostaglandin-like material ($0{\cdot}58 \pm 0{\cdot}16$ ng E_2 equiv/30 min mg^{-1}, n = 6). Arachidonic acid (10 μg ml^{-1}) added to the incubation medium significantly increased the yield of bioassayable activity ($1{\cdot}0 \pm 0{\cdot}09$ ng E_2 equiv/30 min mg^{-1}, n = 29, $P < 0{\cdot}05$). In the presence of both arachidonic acid and indomethacin (10^{-6} M) prostaglandin synthesis was greatly decreased ($0{\cdot}29 \pm 0{\cdot}06$ ng E_2 equiv/30 min mg^{-1}, n = 6, $P < 0{\cdot}01$). No biological activity was detected in extracts from samples that had been incubated with arachidonic acid but without tissue for 30 min (limit of detection of bioassay, $1{\cdot}1 \pm 0{\cdot}15$ ng E_2 equiv ml^{-1}). In all subsequent experiments, arachidonic acid was routinely added to the incubation mixture.

Clonidine (6×10^{-8} and 6×10^{-7} M) produced a dose-related stimulation of prostaglandin formation (Table 1). Xylazine ($2{\cdot}3 \times 10^{-6}$ M) failed to increase vas deferens prostaglandin synthesis. In order to study the α-adrenoceptor selectivity of this effect, pieces of rat vas deferens were incubated with clonidine (6×10^{-8} M) and either yohimbine (5×10^{-7} M) or prazosin (10^{-7} M). The selective α_2-adrenoceptor antagonist, yohimbine, prevented the clonidine-induced stimulation of prostaglandin synthesis whilst the selective α_1-adrenoceptor antagonist, prazosin, was without effect. Furthermore, yohimbine (5×10^{-7} M) did not affect prostaglandin formation from arachidonic acid in the absence of clonidine (Table 1).

Table 1. Stimulation of PG synthesis in rat vas deferens. Estimation of prostaglandin synthesis by pieces of rat vas deferens incubated in 0·2 ml Krebs containing arachidonic acid (10 μg ml^{-1}). Synthesis of prostaglandin-like activity determined after acidification and extraction against authentic PGE_2 on the rat stomach strip preparation. Results show mean ± s.e.m., No. of observations in parentheses.

	Biosynthesis of PGE_2 activity (ng PGE_2 mg^{-1}/30 min)
Control	1·0 ± 0·09 (29)
Control + yohimbine (5×10^{-7} M)	0·94 ± 0·32 (7)
Clonidine (6×10^{-8} M)	2·16 ± 0·29 (20)*
Clonidine (6×10^{-7} M)	4·53 ± 0·55 (15)*
Xylazine ($2{\cdot}3 \times 10^{-6}$ M)	1·16 ± 0·05 (12)
Clonidine (6×10^{-8} M) + yohimbine (5×10^{-7} M)	0·58 ± 0·09 (12)
Clonidine (6×10^{-8} M) + prazosin (10^{-7} M)	2·35 ± 0·26 (12)*

* $P < 0{\cdot}001$.

Effect of clonidine on in vitro prostaglandin synthesis by sheep seminal vesicles (SSV) and inactivation by 15-PGDH

Prostaglandin biosynthesis from arachidonic acid by resuspended sheep seminal vesicle microsomes was determined as described in Materials and Methods. Control incubations lacking drug additions synthesized $46{\cdot}3 \pm 4{\cdot}3$ ng PGE_2 equiv ml^{-1} SSV h^{-1}, n = 13. No statistically significant inhibition or potentiation of cyclooxygenase activity in this cell free system was observed in the presence of clonidine at concentrations that stimulated prostaglandin formation by intact vas deferens. The yield of bioassayable PGE_2-like activity was $39{\cdot}8 \pm 2{\cdot}3$ ng PGE_2 equiv mg^{-1} SSV h^{-1}, n = 16 and $48{\cdot}2 \pm 6{\cdot}8$ ng PGE_2 equiv mg^{-1} SSV h^{-1}, n = 8 in the presence of 6×10^{-8} and 6×10^{-7} M clonidine respectively.

Prostaglandin catabolism by purified swine lung 15-PGDH was also determined. Metabolism of PGE_2 (10 μg ml^{-1}) in the absence of added clonidine was $0{\cdot}09 \pm 0{\cdot}002$ μg μg^{-1} protein min^{-1}, n = 8. Clonidine (6×10^{-7} M) did not affect the rate of

breakdown of PGE_2 (0·09 ± 0·002 μg μg^{-1} protein min^{-1}, n = 8).

DISCUSSION

Clonidine is believed to exert an antihypertensive effect by interacting with central α-adrenoceptors (Schmitt 1977). However, the reduction in blood pressure induced by centrally administered clonidine in rats may be reduced by pre-treating the animals with prostaglandin synthesis inhibitors such as paracetamol or indomethacin (Siren & Karppanen 1980). Since clonidine elevates rabbit brain prostaglandin concentration (Taube et al 1976) and intracerebroventricular injection of prostaglandins may either increase or decrease rat blood pressure (Kondo et al 1979) it is conceivable that at least part of the antihypertensive action of clonidine in these species may be secondary to stimulation of prostaglandin synthesis in the brain.

Similarly, clonidine produces water diuresis in animals (Humphreys & Reid 1974) which may also be secondary to elevated intra-renal synthesis of vasodilator, natriuretic prostaglandins. In conscious dogs, clonidine-induced diuresis was associated with increased urinary PGE excretion. Both effects were prevented by intravenous infusion of indomethacin (Olsen 1976). However, species differences may be important since clonidine-induced diuresis in the rat was not antagonized by indomethacin (Kauker & Barr 1981).

In this paper, we present evidence that clonidine, but not xylazine, stimulates prostaglandin biosynthesis by the intact rat vas deferens. Prostaglandins of the E series and prostacyclin (PGI_2) inhibit contractions of the rat or guinea-pig vas deferens to field stimulation (Hedqvist 1977; Wennmalm 1980). Pretreatment with indomethacin at a concentration known to produce selective inhibition of prostaglandin biosynthesis (Flower 1974) reduced the ability of clonidine to inhibit the field stimulated rat vas deferens. Thus, part of the inhibitory effect of clonidine in this preparation may be attributed to induction of prostaglandin formation at or near to the sympathetic nerve terminals of the intramural plexus. However, high concentrations of clonidine inhibit the field-stimulated, indomethacin-treated rat vas deferens which suggests either incomplete inhibition of prostaglandin synthesis in the preparation or that clonidine reduces noradrenaline release by an α_2-receptor–mediated, prostaglandin-independent mechanism. Since the formation of prostaglandins from exogenous arachidonic acid by intact rat vas deferens was inhibited more than 70% by indomethacin at the concentration used in the bath, we favour the existence of a prostaglandin-independent component of clonidine action. Moreover, xylazine which does not stimulate prostaglandin synthesis by rat vas deferens in vitro also reduces contractions to field stimulation in this preparation. We did not use a higher concentration of indomethacin for fear of inhibiting other enzymes such as 3′,5′-cAMP phosphodiesterase and 15-hydroxyprostaglandin dehydrogenase which is responsible for inactivation of prostaglandins (Flower 1974; Moore & Hoult 1982).

The mechanism whereby clonidine stimulates rat vas deferens prostaglandin biosynthesis is not known. Synthesis of prostaglandins from arachidonic acid by resuspended sheep seminal vesicle microsomes was not stimulated by clonidine. Furthermore, it is unlikely that the observed effect of this drug on prostaglandin synthesis was secondary to inhibition of prostaglandin catabolism since clonidine did not influence the activity of purified 15-PGDH in vitro. Yohimbine but not prazosin prevented the stimulation of prostaglandin synthesis by clonidine in the rat vas deferens. Thus, it seems that activation of the presynaptic α_2-adrenoceptor is a necessary step before stimulation of prostaglandin synthesis. Previous authors have demonstrated that PGE_2 biosynthesis follows activation of β-adrenoceptors in the guinea-pig trachea possibly the result of phospholipase A_2 activation and liberation of arachidonic acid (Omini et al 1981). A similar mechanism may account for the results obtained in this study. However, xylazine neither stimulates prostaglandin formation in vitro nor shows reduced potency in the indomethacin rat vas deferens which suggests that this drug may combine with a separate subpopulation of α_2 adrenoceptors.

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