J. Pharm. Pharmacol. 1983, 35: 184–186
Communicated August 27, 1982

0022–3573/83/030184–03 $02.50/0

Effect of 6 keto-prostaglandin E_1 on sympathetic neurotransmission in the vas deferens

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Prostaglandins of the E series inhibit noradrenaline release from sympathetic nerve terminals and potentiate the postsynaptic response to noradrenaline and other agonists in a variety of pharmacological preparations (Hedqvist 1977). Recently, a novel metabolite of prostacyclin has been detected in the effluent of perfused rabbit liver (Wong et al 1979). This metabolite, 6-keto-prostaglandin E_1 (6K-PGE_1) is a potent vasodilator (Quilley et al 1979) and inhibits platelet aggregation (Wong et al 1979). We have now studied the effect of 6K-PGE_1 and 6-keto-prostaglandin $F_{1\alpha}$ (6K-$PGF_{1\alpha}$) on sympathetic transmission in the guinea-pig and rabbit vas deferens.

Method

Guinea-pigs (Dunkin Hartley, 400–500 g) and rabbits (New Zealand White, 1·5–2·5 kg) were killed by a blow to the head and exsanguinated. Vasa deferentia (epididymal end) were removed, cleared of connective tissue amd mounted in 20 ml organ baths containing warmed (37 °C), aerated (95% O_2/5% CO_2) Mg^+-free Krebs solution (composition, mM: NaCl 121, KCl 4·7, $CaCl_2$ 2·7, $NaHCO_3$ 25, KH_2PO_4 1·18, glucose 11·1). 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 square wave stimulator (0·1 Hz, 1 ms, 150V). To determine post-synaptic sensitivity, noradrenaline was added to the bath and kept in contact with the tissue for 0·5 min (guinea-pig) or 3 min (rabbit). A higher dose was added 3 min (guinea-pig) or 15 min (rabbit) after wash out. The sensitivity of the tissue to noradrenaline was determined 15 min after addition of prostaglandins to the Krebs.

Results

Noradrenaline elicited single, monophasic contractions of the guinea-pig vas deferens but produced rhythmic, unsustained contractions of the rabbit vas deferens. In the latter preparation the effect of noradrenaline was quantitated by summating the tensions developed by each contraction over the 3 min contact time as described by Poyser & Swan (1981).

Electrical stimulation of both guinea-pig and rabbit vas deferens produced rapid twitch responses which were maintained for several hours. The average tension developed per twitch was 285 ± 12 mg (n = 15) and 300 ± 13 mg (n = 12) for guinea-pig and rabbit respectively. Contractions due to field stimulation were prevented by tetrodotoxin (0·5 μM) or guanethidine (1 μM) in both preparations at concentrations that did not affect the response to exogenous noradrenaline. Thus, contractions were nerve-mediated and not due to direct stimulation of the muscle.

6K-PGE_1 produced dose-dependent inhibition of the twitch response to field stimulation of the rabbit vas deferens. The concentration required for 50% inhibition of contractions (ID50) was 60·4 ± 5·3 ng ml^{-1} (n = 6). PGE_1 and PGE_2 also inhibited electrically-induced contractions in this tissue and were slightly more potent than 6K-PGE_1 with ID50 values of 44·6 ± 5·4 ng ml^{-1} (n= 5) and 46·4 ± 3·9 ng ml^{-1} (n = 5) respectively (Fig 1A). However, the dose response curves were not parallel and the rank order of potency at, for example 80% inhibition of contractions (ID80) was PGE_1 = 6K-PGE_1 > PGE_2. The maximum inhibitory effect of these prostaglandins was produced after 3–4 min and was maintained until wash out (1–2 min later). In contrast, prostacyclin the precursor of 6K-PGE_1 produced only a small (<10%) inhibition of the twitch response at high concentrations

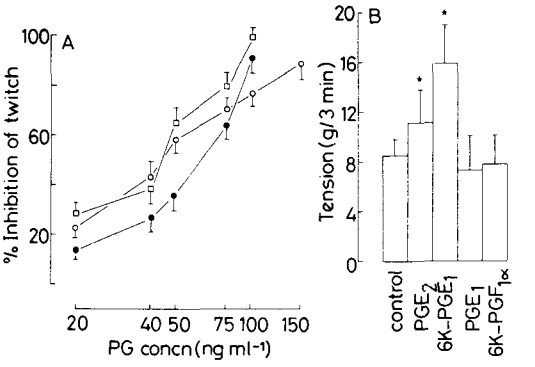

FIG. 1. A. Inhibition of field stimulated rabbit vas deferens by 6K-PGE_1 (—●—). PGE_1 (—□—) and PGE_2 (—○—). Results are mean ± s.e., n = 5–8. B. Potentiation of the spasmogenic effect of noradrenaline (8 μg ml^{-1}) on the rabbit vas deferens. Contractions were estimated as g tension developed over 3 min contact time. Concentration of all prostaglandins was 75 ng ml^{-1}. Results are mean ± s.e., n = 8–20. $P < 0.05$ by Student's *t*-test.

* Correspondence.

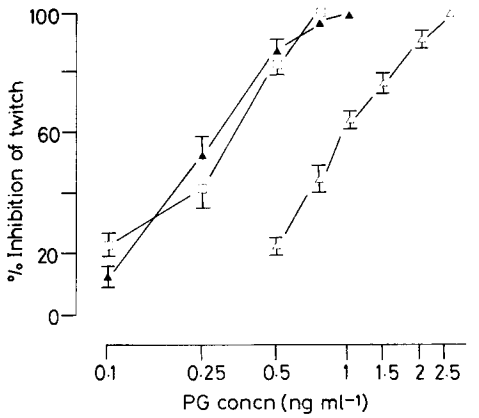

FIG. 2. Inhibition of field stimulated guinea-pig vas deferens by 6 K-PGE_1 (— △ —) PGE_1 (— □ —) and PGE_2 (— ▲ —). Results are mean ± s.e., n = 6.

(400 ng ml^{-1}) and this effect spontaneously reversed after 30–60 s 6K-$PGF_{1\alpha}$ (400 ng ml^{-1}) was inactive.

Since noradrenaline produced pendular contractions of the rabbit vas deferens it was not possible to obtain a maximum effect. However, the relationship between spasmogenic activity and log concentration of noradrenaline was linear over the dose range (4–16 μg ml^{-1}). The postsynaptic response to noradrenaline (8 μg ml^{-1}) was determined in the presence and absence of prostaglandins. 6K-PGE_1 and PGE_2 added to the Krebs solution at a concentration (75 ng ml^{-1}) which produced about 70% inhibition of the electrically-induced twitch response also potentiated the effect of added noradrenaline. PGE_1 and 6K-$PGF_{1\alpha}$ (75 ng ml^{-1}) were inactive and the effect of PGI_2 on the postsynaptic response to noradrenaline was not determined (Fig. 1B).

Qualitatively similar results were obtained using the guinea-pig isolated vas deferens although much lower concentrations of prostaglandins were required to inhibit contractions to field stimulation. ID50 concentrations for 6K-PGE_1, PGE_1 and PGE_2 were 0·78 ± 0·05 ng ml^{-1} (n = 6), 0·30 ± 0·05 ng ml^{-1} (n = 6) and 0·28 ± 0·03 ng ml^{-1} (n = 6) respectively (Fig. 2). PGI_2 (5–75 ng ml^{-1}) also produced dose related inhibition of the field-stimulated guinea-pig vas deferens although as with the rabbit preparation this effect was short lived (maximum after 30–60 s) and the twitch response spontaneously reversed before wash out. The threshold dose for inhibition varied considerably (5–50 ng ml^{-1}) but a dose of 75 ng ml^{-1} produced 50–80% inhibition. Thus it was not possible to obtain an accurate ID50 value for PGI_2 on this preparation. 6K-$PGF_{1\alpha}$ (up to 100 ng ml^{-1}) was inactive.

The postsynaptic spasmogenic effect of noradrenaline on the guinea-pig vas deferens was potentiated by PGE_1 (0·5 ng ml^{-1}) but unaffected by 6K-PGE_1 (1·5 ng ml^{-1}) and 6K-$PGF_{1\alpha}$ (100 ng ml^{-1}) (Fig. 3).

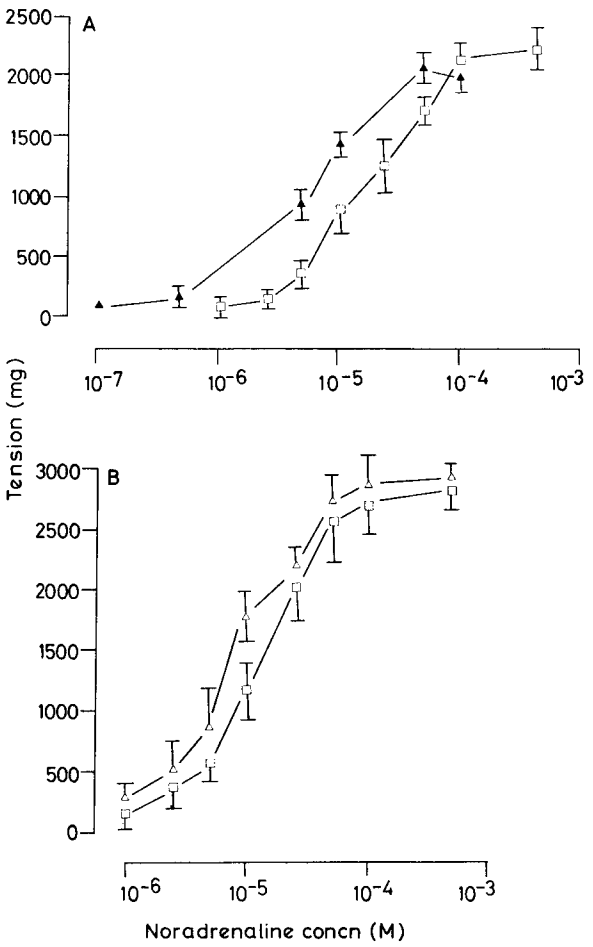

FIG. 3. Effect of noradrenaline on the guinea-pig vas deferens. Contractions were estimated as mg tension developed for 0·5 min contact time (A) control (— □ —), PGE_1 (0·5 ng ml^{-1}) (— ▲ —); (B) control (— □ —), 6 K-PGE_1 (1·5 ng ml^{-1}) (— △ —). Results are mean ± s.e., n = 7.

Discussion

Other authors have previously shown that prostaglandins of the E series inhibit sympathetic neurotransmission in the vas deferens (Baum & Shropshire 1971; Hedqvist 1977; Hedqvist & von Euler 1972). PGE_1 and PGE_2 similarly inhibit noradrenaline release in other sympathetically innervated organs such as spleen, heart, blood vessels and portal vein (Hedqvist 1973, 1977; Westfall 1977; Starke 1977).

In contrast, PGI_2 did not inhibit noradrenaline release in response to nerve stimulation in the cat mesenteric and rabbit renal vascular beds (Lippton et al 1979; Hedqvist 1979). However, Poyser & Swan (1981) have shown inhibition of the twitch response to electrical stimulation of the rabbit vas deferens by prostacyclin. We cannot confirm this finding since in our

experiments the response to PGI_2 rapidly reversed before wash out presumably due to chemical hydrolysis to yield inactive 6K-$PGF_{1\alpha}$.

6K-PGE_1 is a potent vasodepressor (Quilley et al 1979), inhibitor of platelet aggregation (Wong et al 1979), bronchodilator (Spannhake et al 1981) and renin secretogogue (McGiff et al 1982). To date, its effect on sympathetic neurotransmission has not been studied. We report here that 6K-PGE_1, like PGE_1 and PGE_2 but unlike prostacyclin is a potent inhibitor of contractions of the field stimulated guinea-pig and rabbit vas deferens. Since 6K-PGE_1 either potentiates (rabbit) or does not affect (guinea-pig) the post-synaptic response to noradrenaline one may tentatively conclude that this prostaglandin inhibits electrically-induced contractions of the vas deferens by a pre-synaptic mechanism. These results suggest that if 6K-PGE_1 synthesis occurs in the vicinity of the sympathetic nerve ending then it may have a physiological role to play in regulating noradrenaline release.

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J. Pharm. Pharmacol. 1983, 35: 186–188
Communicated September 9, 1982

0022–3573/83/030186–03 $02.50/0

Identification of a seven day biological cycle in the rat

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The swelling that is an integral component of the inflammatory response is commonly investigated in the rat by the measurement of hindpaw volume (Garcia-Leme & Roch e Silva 1972; Van Arman 1976). The swelling induced may be acute and localized as after the injection of carageenan into a hindpaw (Winter et al 1968; Vinegar et al 1976; Labrecque et al 1979), or it may be delayed and disseminated as occurs in adjuvant disease when the injection of mycobacteria in oil into one hindpaw causes inflammatory lesions of the injected and three uninjected paws after an interval of about 10 days (Pearson & Chang 1979; Chang et al 1980; Muir & Dumonde 1982). In the pharmaceutical industry, measurement of the hindpaw volume in rats with adjuvant disease is widely used in the assessment of anti-inflammatory drugs (Winter et al 1968; Garcia-Leme & Rocha e Silva 1972; Vinegar et al 1976). As part of an investigation into the nature of the swelling associated with paw joint inflammation in adjuvant disease, we measured hindpaw volumes of control rats injected with oil alone, without the mycobacteria. Here we report that these hindpaw volumes varied throughout the experiment with a biological cycle of 7 day period. The cycle was exhibited by both male and female rats; the amplitude was greater in the latter. Thus we provide evidence of a hitherto unreported cycle. A possible mechanism for these findings is variation in water content of the paw. It is suggested that the findings may have implications for the conduct of studies in experimental pharmacology and therapeutics.

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Method

Highly inbred SK Wistar rats were bred and housed in one room that was light and heat controlled. There were 16 h of light daily with total darkness for the remaining 8 h. The temperature varied between 22·5 to 24 °C in the experimental period. All measurements and injections were begun at 9.30 a.m. which was 2½ h after the onset of the light phase. Rats were individually identified. Paw volume measurements were performed with a mercury plethysmometer designed to specification by Photon Ltd. (Somerset) and connected to a pen recorder. Hindpaws were marked on the lateral prominence of the astragalus, dipped in mercury up to the mark and held in position until a horizontal tracing was obtained. A representative recording of one cage of rats is shown in Fig. 1. A tracing of a Perspex rod calibrated in ml was made for each cage of rats; the accuracy of