Role of PGE₂ in Neurotransmission from Pre- to Post-Ganglionic Hypogastric Nerves of Guinea Pigs

Akikazu Fujita, Tadayoshi Takeuchi, Fumiaki Hata* and Osamu Yagasaki

*Department of Veterinary Pharmacology, College of Agriculture,
University of Osaka Prefecture, Sakai 591, Japan*

Received August 19, 1991 Accepted October 14, 1991

ABSTRACT—The hypogastric nerve to guinea pig vas deferens was stimulated pre- or post-ganglionically by adjusting the position of the suction electrode. Both stimulations induced a biphasic contraction consisting of a rapid transient phase and a delayed tonic phase. Indomethacin partially inhibited the contraction induced by pre-ganglionic stimulation, but did not inhibit that induced by post-ganglionic stimulation. Prostaglandin (PG) E₂ counteracted the inhibitory effect of indomethacin. Mepacrine also inhibited the contraction induced by pre-ganglionic stimulation. Arachidonic acid and PGE₂ both reversed the inhibition. The PGE₂-receptor antagonist SC-19220 inhibited the contraction induced by pre-ganglionic, but not post-ganglionic nerve stimulation. These results suggested that endogenous PGE₂ is important in neurotransmission in the pelvic ganglion of guinea pigs.

Prostaglandins (PGs) modulate adrenergic transmission by inhibiting the release of nor-epinephrine (NE) (for reviews, see refs. 1 and 2). PGs are also known to stimulate (3, 4) or inhibit (5–9) cholinergic transmission in various organs. Previously we reported the characteristic effects of PGE₂ on neurotransmission in a hypogastric nerve-vas deferens preparation of the guinea pig: PGE₂ (57 nM) selectively delayed the transmission mediated by ATP and enhanced smooth muscle contraction elicited by ATP or NE (10). The pelvic ganglion is close to the vas deferens, and careful positioning of a suction electrode enabled us to stimulate the hypogastric nerve pre- or post-ganglionically. In the present work, we studied the physiological role of PGs in neurotransmission of the hypogastric nerve-vas deferens preparation by examining the effects

of indomethacin, an inhibitor of PG synthesis. The results indicated the importance of endogenous PGs in the neurotransmission from pre- to post-ganglionic hypogastric nerves.

MATERIALS AND METHODS

Preparations

Male guinea pigs (450–700 g) were used. They were stunned by a blow on the head and bled via the carotid artery. Their abdomen was opened, and the vas deferens from the prostatic end to the epididymal end with the hypogastric nerve, which runs in the mesentery (11, 12), was removed and placed in Krebs-Ringer solution of the following composition: 119 mM NaCl, 4.5 mM KCl, 2.5 mM CaCl₂, 25 mM NaHCO₃, and 11 mM glucose, (13). A 3–4-cm length of the hypogastric nerve was freed from adherent tissue and aspirated into a suction electrode to a position

*To whom correspondence should be addressed.

about 3 cm or 3 mm from the vas deferens. This arrangement made pre- or post-ganglionic nerve stimulation possible. The vas deferens connected to the nerve was suspended in an organ bath (15 ml) of a Magnus apparatus, aerated with 95% O₂–5% CO₂ and equilibrated with Krebs-Ringer for 30 min at 37°C.

Recording of the contractile response

Contractile responses were recorded isometrically with a force-displacement transducer (TM-651T, Nihon Kohden, Tokyo, Japan) in a recticorder. A load of 0.75 g was applied as a resting tension. Contractile responses were recorded successively with 10-min intervals between tests. The hypogastric nerve was stimulated by trains of 140 pulses of 0.1 msec at a frequency of 20 Hz and supramaximal voltage (50 V).

Other methods

Indomethacin, PGE₂ and arachidonic acid were dissolved in ethanol, and 15 µl of each solution was added to the organ bath, resulting in 1,000-fold dilutions. The resulting concentration of 0.1% ethanol during the tests did not affect the contractile response. Contraction is expressed as g per g wet wt. of vas deferens. Results were analyzed statistically by the paired *t*-test, and a value of *P* < 0.05 was regarded as significant.

Drugs

PGE₂ was a gift from Ono Pharmaceutical Co. (Osaka, Japan). Indomethacin, arachidonic acid, and mepacrine dihydrochloride were from Sigma Chemical Co. (St. Louis, U.S.A.).

RESULTS

Contractions of guinea pig vas deferens evoked by pre- and post-ganglionic stimulations of the hypogastric nerve

A suction electrode was positioned about 3 cm and 3 mm from the vas deferens for pre- and post-ganglionic stimulations of the hypogastric nerve, respectively. These stimulations were confirmed by complete inhibition of the contraction by hexamethonium in the former position and its ineffectiveness in the latter (Fig. 1). Pre-ganglionic nerve stimulation (PRNS) and post-ganglionic nerve stimulation (PONS) both evoked a biphasic contraction, consisting of a fast transient and a delayed tonic contraction (Fig. 1). Height and shape of the contraction in each preparation were nearly constant during the following four to five stimulations. Tetrodotoxin (1 µM) completely inhibited the contractions (data not shown).

Effects of indomethacin, mepacrine and SC-19220 on the contractions evoked by pre- and post-ganglionic nerve stimulations

Fig. 1. Contraction of guinea pig vas deferens evoked by pre- or post-ganglionic hypogastric nerve stimulation. The hypogastric nerve was electrically stimulated pre- (Pre Stimu.) or post-ganglionically (Post Stimu.). Triangles indicate the beginning of the 7-sec stimulation. Treatment with hexamethonium (C₆, 500 µM) was for 8 min. For further details, see Methods.

Fig. 2. Effects of indomethacin on the contractions evoked by pre- or post-ganglionic nerve stimulations. Treatment with indomethacin (IND, 5.6 μ M) were for 8, 18 and 28 min in the 1st, 2nd and 3rd trials, respectively. For further details, see Methods and the legend of Fig. 1.

At 5.6 μ M, indomethacin, an inhibitor of cyclooxygenase, inhibited the contractions evoked by PRNS time-dependently. Although the inhibition was not large, it was clear and significant in all preparations tested (Figs. 2 and 3). This concentration of indomethacin seemed to be optimal under the present experimental conditions: 2.8 μ M indomethacin had a small effect (for example, contraction after 8 min-treatment was 97.8% of the control); and at higher concentration than 11.2 μ M, ethanol affected the contractile response (see Methods). The inhibitions by indomethacin were equipotent in the fast transient phase and delayed tonic phase (Figs. 2 and 3). On the other hand, indomethacin did not affect the contractions evoked by PONS (Fig. 2). PGE₂ alone at concentrations of up to 11.4 nM did not affect the contraction of the vas deferens, but it reversed the inhibitory effect of indomethacin at 5.7 nM (Fig. 3). Arachidonic acid at 1 μ M did not affect the contractions in the absence (data not shown) or presence (Fig. 3) of indomethacin. Treatment of the vas deferens with 5 μ M mepacrine, an inhibitor of phospholipase A₂ (14), also inhibited the contraction evoked by PRNS. The inhibitions by mepacrine were also equipotent in the two contractile responses. Mepacrine at this concentration had a stronger effect than

Fig. 3. Inhibitory effect of indomethacin and its reversion by PGE₂. The hypogastric nerve was stimulated pre-ganglionically (○) in the absence (C) and presence of 5.6 μ M indomethacin. Rapid phasic contraction was expressed as a percent of the control. Treatment with PGE₂ (5.7 nM, □) or arachidonic acid (1 μ M, ●) was for 8 min before the third trial. Points and bars are means and standard deviations for 3–6 experiments. Dashed line without symbols and deviations indicates inhibition in delayed tonic contraction. *Significantly different from the value before indomethacin treatment (control) by the paired *t*-test ($P < 0.05$). **Significantly different from the value before indomethacin treatment ($P < 0.02$). ***Significantly different from the value before PGE₂ treatment (2nd trial in the presence of indomethacin, $P < 0.05$). For further details, see Methods and the legend of Fig. 2.

indomethacin: in the first trial, mepacrine treatment induced about 15% inhibition, which was similar to that in the third trial after indomethacin treatment. Both $1\text{ }\mu\text{M}$ arachidonic acid and 5.7 nM PGE_2 reversed the contraction to almost the control level (Table 1).

The PGE_2 receptor antagonist SC-19220 (5 ng/ml) inhibited the contraction evoked by PRNS, but not that by PONS (Fig. 4): the contractions evoked by PRNS before and after

treatment with SC-19220 for 8 min were 122.5 ± 6.9 and $106.5 \pm 7.4\text{ g/g}$ vas deferens, respectively (means $\pm$ S.D., $n = 6$, $P < 0.01$).

DISCUSSION

The effects of PGE_2 on the responses of guinea pig vas deferens have mainly been studied in preparations without the hypogastric nerve (15–19) by electrical transmural stimulation of nerve terminals. The results of these studies have suggested several characteristic effects of PGE_2 on transmitter release from hypogastric nerve terminals and contraction of the vas deferens. We prepared guinea pig vas deferens with the hypogastric nerve to stimulate the nerve pre- or post-ganglionically. In this study, we found that indomethacin partially inhibited the contraction evoked by PRNS, but not that by PONS. It seems likely that it inhibits neurotransmission from pre- to post-ganglionic hypogastric nerves. In this study, we used indomethacin at $5.6\text{ }\mu\text{M}$ ($2\text{ }\mu\text{g/ml}$), which inhibits most of the PG synthesis in guinea pig ileum (20, 21) and is sufficiently free of unwanted side effects (22). This inhibitory effect of indomethacin is probably due to a decrease in the level of PGs. This idea is supported by the finding that PGE_2 reversed the inhibitory effect of indomethacin. We used a low concentration of PGE_2 , 5.7

Table 1. Inhibition by mepacrine of the contraction evoked by pre-ganglionic stimulation and its reversal by arachidonic acid or PGE_2

	Contraction (g/g tissue)
Control	124.4 ± 6.3 (9)
Mepacrine	106.4 ± 6.9 (9)]***
Mepacrine + A.A.	119.9 ± 7.9 (6)]**
Mepacrine + PGE_2	119.4 ± 6.9 (3)]*

Treatment with mepacrine ($5\text{ }\mu\text{M}$) with or without arachidonic acid (A.A., $1\text{ }\mu\text{M}$) or PGE_2 (5.7 nM) was for 8 min. Values indicate the contraction of the fast transient phase and they are means $\pm$ S.D. for the numbers of experiments shown in parentheses. Indicated values were significantly different from the control value, (** $P < 0.001$) and from the value with mepacrine alone (** $P < 0.01$ and * $P < 0.05$). For further details, see Methods and the legend of Fig. 3.

Fig. 4. Typical contractile responses in the presence of SC-19220 evoked by pre- or postganglionic nerve stimulation. Treatment with SC-19220 (5 ng/ml) were for 8, 18 and 28 min in the 1st, 2nd and 3rd trials respectively. For further details, see Methods and the legend of Fig. 2.

nM (2 ng/ml), which did not have any significant effects on the response evoked by PONS. These results indicate that the tissue contains a sufficient amount of PGs to maintain the excitability of the post-ganglionic hypogastric nerve; and therefore, exogenous PGs act only after treatment of the tissue with indomethacin. The inhibitory effect of SC-19220 on the contraction evoked by PRNS, but not that by PONS, also supports the role of PGs in neurotransmission from the pre- to the post-ganglionic hypogastric nerve. This inhibitory effect of SC-19220 tended to be more rapid and potent than that of indomethacin. A probable explanation of this finding is that addition of indomethacin to the bathing fluid did not decrease the PGs level rapidly, whereas SC-19220 rapidly and completely blocked the PG receptors. As arachidonic acid and PGE₂ had similar effects in reversing the inhibitory effect of mepacrine, PGE₂ rather than arachidonic acid seems to be important. Thus, these results indicate an important role of PGE₂ in neurotransmission from pre- to post-ganglionic hypogastric nerves. The mechanism of action of PGs is, however, still unknown. Previously, we reported that indomethacin inhibits acetylcholine release from the myenteric plexus of guinea pig ileum (23) and suggested that endogenous prostaglandins maintain the excitability of the somal membranes of myenteric cholinergic neurons (23). We have also reported that indomethacin did not affect the release of acetylcholine from nerve terminals induced by electrical stimulation (24). PGs may modulate transmitter release from pre-ganglionic nerve terminals, but it seems more likely from our results that they maintain the excitability of the somal membranes of post-ganglionic hypogastric neurons.

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