Effects of Indomethacin and Prostaglandins on the Dog Iris Sphincter and Dilator Muscles

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Effects of indomethacin and prostaglandins (PGs: PG E₁, PG E₂ and PG F₂α) on electrical and mechanical properties of the dog iris sphincter and dilator muscles were studied using isometric tension recording and microelectrode methods. When field stimulations (50 V, 0.4 msec, 10 stimuli at 10 Hz) were applied every 1 min, the muscle tone was gradually elevated in both muscle tissues. Application of 10⁻⁶ M indomethacin gradually reduced the tone of the muscle tissues; however, this reduction was more pronounced in the sphincter muscle. On the contrary, exogenously applied PG F₂α increased the muscle tone in both tissues. Ca-free solution reduced the tone in both muscle tissues. Following pre-treatment with indomethacin, Ca-free solution had no effect on the muscle tone in the sphincter; however, it did reduce the tone in the dilator muscle. Indomethacin (10⁻⁶ M) or PGs (PG E₁, PG E₂ and PG F₂α; up to 10⁻⁷ M) had no effect on the resting membrane potential of both muscles. PG F₂α dose-dependently contracted both of the sphincter and dilator muscles. PG E₁ and PG E₂ had little effect on the mechanical properties of these muscles. Neither atropine nor guanethidine affected the amplitude of contractions evoked by PG F₂α. These results suggest that endogenous PG F₂α may contribute to maintenance of the muscle tone of the sphincter muscle but contributes to a lesser degree in the dilator muscle in the dog iris. PGs apparently act directly on these muscles rather than through the release of adrenergic or cholinergic neurotransmitters. Invest Ophthalmol Vis Sci 29:127-132, 1988

We reported that the dog iris sphincter and dilator muscles receive double reciprocal innervations from adrenergic and cholinergic nervous systems. The cholinergic activation contracts the sphincter and relaxes the dilator, and miosis occurs. On the other hand, adrenergic activation contracts the dilator and relaxes the sphincter, and mydriasis occurs. In the dog iris, activation of adrenergic or cholinergic nervous systems in the presence of atropine or guanethidine could relax the sphincter or dilator muscles, respectively. Furthermore, in both muscle tissues, Ca²⁺-free solution containing 2 mM EGTA gradually reduced the resting tension to a certain level. These findings indicate that the isolated iris sphincter or dilator muscles of the dog maintain the muscle tone to some extent, under in vitro conditions and dependent on extracellular Ca²⁺. Irids of all specimens studied, like all other mammalian tissues with the exception of enucleated red blood cells, synthesize PGs and it was reported that endogenous PGs may contribute to maintenance of the muscle tone of the isolated bovine iris sphincter muscle. In an attempt to clarify the effects of PGs on the muscle tone in the sphincter and dilator muscles, the effects of indomethacin, PG E₁, PG E₂ and PG F₂α on electrical and mechanical properties of these muscles were studied, using isometric tension recording and microelectrode methods. The dog iris provides a good model for studies on the sphincter and dilator muscles, both of which have muscle tone and could be relaxed by activation of adrenergic or cholinergic nervous system, respectively; thus, we performed comparative studies on the action of indomethacin or PGs on the isolated sphincter and dilator muscles. The evidence we obtained suggests that endogenous PG F₂α may play an important role in maintaining the muscle tone of the isolated sphincter, but a less important role in the case of the dilator muscle.

Materials and Methods

Mongrel dogs of either sex weighing 10 to 15 kg were given pentobarbital-Na (30 mg/kg) intravenously and bled from the femoral artery. The eyes were immediately enucleated and placed in oxygenated Krebs solution. At the same time, stomach, ileum, trachea and portal veins were also dissected and used for physiological and pharmacological experiments. Under microscopic observation, ring-
Fig. 1. Effects of electrical field stimulations on the dog iris sphincter (A) and dilator (B) muscles. Field stimulations (50 V, 0.4 msec, 10 stimuli at 10 Hz) were applied every 1 min. The four individual spikes shown are representative of spikes either before or after continuous field stimulation, expanded on the time scale 24 times to show the contractile and relaxant components.

shaped iris sphincter muscle specimens (1 mm wide) and dilator muscle specimens (1 mm wide, 3-4 mm length) were prepared.

To measure the isometric tension development, the muscle preparations were mounted in the organ bath (1.5 ml volume) through which the test solution, at a temperature of 35°C, flowed continuously (0.3 ml/sec). The preparation was positioned vertically and the ends were tied with silk thread. One end of the strip was tied to a mechano-transducer (Nihon-Koden Ltd., RCA 5734, Tokyo, Japan) and the other end to a hook at the bottom of the bath, with a resting tension of 20-70 mg. To investigate the neural effects on the motility of the dog iris sphincter and dilator muscle, transmural electrical field stimulations were applied through a pair of electrodes placed in the apparatus. These electrodes consisted of silver plates, separated by 5 mm and placed so that the current pulse would pass transversely across the tissue. Single and repetitive stimulations at 10 Hz were applied, using a current pulse of 0.1-0.8 msec in duration, 30-100 V in strength.

To record the membrane potential, the muscle preparations (0.4-0.8 mm wide and 4-6 mm length) were mounted in a 2 ml volume organ bath with a volume of 2 ml through which the solution flowed continuously at a rate of 3 ml/min, at 35°C. A conventional glass microelectrode with a resistance of 40-70 mΩ and filled with 3 M KCI was used.

Modified Krebs solution of the following ionic concentration was used (mM): Na⁺ 137.4, K⁺ 5.9, Mg²⁺ 1.2, Ca²⁺ 2.5, Cl⁻ 134.0, HCO₃⁻ 15.5, H₂PO₄⁻ 1.2 and glucose 11.5. The solution was aerated with 97% O₂ and 3% CO₂ and the pH was adjusted to 7.2-7.3. Excess [K]o solution was prepared by replacing equimolar NaCl with KCl, isotonically. The Ca-free 3 mM EGTA-containing solution was prepared by replacing CaCl₂ with equimolar MgCl₂ and adding 3 mM EGTA.

The following drugs were used: tetrodotoxin (TTX, Sankyo, Tokyo), atropine sulphate (Daiichi, Tokyo), guanethidine (Tokyo Kasei, Tokyo), prostaglandin E₁, E₂ and F₂α (Ono, Tokyo), indomethacin (Sigma, St. Louis, MO) and ethyleneglycolbis-(β-aminoethyl-ether)-N,N'-tetraacetic acid (EGTA, Dozin, Kumamoto, Japan).

All experiments were performed according to the ARVO Resolution on the Use of Animals in Research.

Results

Specimens of the dog iris sphincter and dilator muscles, mounted in an organ bath, gradually relaxed to a steady level after 30 to 60 min superfusion with Krebs solution and the muscle tone remained at the same level for several hours. Spontaneous mechanical responses did not occur during these procedures. One hour after the muscle tone had reached a steady level, electrical field stimulations (50 V, 0.4 msec, ten stimuli at 10 Hz) were applied every 1 min. Electrical field stimulation evoked biphasic mechanical responses in both the iris sphincter and dilator muscles, i.e., an initial twitch contraction followed by a long lasting relaxation. In the iris sphincter muscle, the amplitude of contraction was larger than that of relaxation; however, in the dilator, the amplitude of relaxation was much larger than that observed in the sphincter. As shown in Figure 1, during continuous electrical field stimulations, the muscle tone was gradually elevated and the relaxation evoked by field stimulation was enhanced in the sphincter muscle (Fig. 1A). On the other hand, in the dilator muscle, increase in the muscle tone was slight in comparison to that of the sphincter, but a greater enhancement of the relaxation was evident (Fig. 1B). These changes were not seen when field stimulation was done in the presence of 10⁻⁶ M indomethacin.

As shown in Figure 2, application of 10⁻⁶ M indomethacin gradually reduced the muscle tone of the sphincter and dilator. In the sphincter muscle, the relaxation evoked by field stimulation was blocked by 10⁻⁶ M indomethacin (Fig. 2A). On the other hand, in the dilator muscle, the reduction in the muscle tone was slight in comparison to that of the sphincter, but a greater enhancement of the relaxation was evident (Fig. 1B). These changes were not seen when field stimulation was done in the presence of 10⁻⁶ M indomethacin.
sphincter and dilator muscles. The effects of Ca-free solution and of indomethacin on the muscle tone of the sphincter and dilator muscles were compared. Ca-free 3 mM EGTA-containing solution reduced the muscle tone and abolished the mechanical responses evoked by field stimulation in both these muscles and the effects were reversible (Fig. 3A, B). Indomethacin (10^{-6} M) reduced the muscle tone of both muscles, as shown in Figure 2. However, after treatment with indomethacin for 50 min, Ca-free solution did not further reduce the muscle tone of the sphincter (Fig. 3A), but did reduce that of the dilator muscle (Fig. 3B). When the amplitude of the tone of these muscles evoked in Krebs solution relative to that obtained in Ca-free solution was taken as 1.0, the effect on the muscle tone of sphincter and dilator induced by indomethacin was a reduction to near zero (n = 4) and 0.68 ± 0.15 (±SD, n = 5), respectively. These observations indicate that endogenous products sensitive to indomethacin contribute to maintenance of the muscle tone of the sphincter muscle but contribute less in case of the dilator muscle.

To investigate the contribution of PGs on the muscle tone, effects of various PGs on the mechanical and electrical properties of the sphincter and dilator muscle were studied in the presence of indomethacin (10^{-6} M). The resting membrane potentials of the iris sphincter and dilator muscles were −59.3 ± 1.7 (±SD, n = 38) and −49.7 ± 2.1 (n = 63) mV, respectively, and indomethacin (10^{-6} M) or PGs (PG E₁, PG E₂ and PG F₂α; up to 10^{-7} M) had no effect on the resting membrane potential of these muscles (Table 1).

Figure 4A shows the effect of PGs on the mechanical properties of the dog iris sphincter muscles. PG F₂α dose-dependently provoked contraction, and the minimum concentration of PG F₂α required to generate the contraction was 10^{-10} M. However, the minimum concentration of PG E₂ required to generate the contraction was 10^{-8} M. PG E₁ neither modified the muscle tone nor generated the contraction in concentrations up to 10^{-7} M. Figure 4B shows an example of the effects of PGs on the sphincter muscle; here electrical field stimulation was applied every minute. In the presence of PG E₂ or PG F₂α, field stimulation evoked biphasic mechanical responses. In the presence of 10^{-8} M PG F₂α, the amplitude of contraction evoked by field stimulation was markedly reduced due to the contraction evoked by PG F₂α.

As shown in Figure 5, PG E₁ did not modify the resting tone of the dilator muscle in a concentration up to 10^{-7} M. PG E₂ evoked contraction only when the concentrations were increased up to 10^{-7} M. PG F₂α evoked the contraction in a dose-dependent manner and the minimum concentration of PG F₂α required to generate the contraction was 10^{-10} M, as in the case of the sphincter muscle. Figure 5B shows an example of the effect of PGs on the dilator muscle, under conditions where the electrical field stimula-

Table 1. Effects of indomethacin and PGs on the resting membrane potential of the dog iris sphincter and dilator muscles

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<tr>
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<th>Sphincter muscle*</th>
<th>Dilator muscle†</th>
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<tr>
<td>Control</td>
<td>−59.3 ± 1.7 mV †</td>
<td>−49.7 ± 2.1 mV</td>
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<tr>
<td>Indomethacin (10^{-6} M)</td>
<td>−58.9 ± 1.9 mV</td>
<td>−49.9 ± 2.2 mV</td>
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<tr>
<td>PG E₁ (10^{-8} M)</td>
<td>−59.4 ± 1.9 mV</td>
<td>−50.2 ± 2.2 mV</td>
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<tr>
<td>PG E₂ (10^{-7} M)</td>
<td>−59.2 ± 2.1 mV</td>
<td>−50.0 ± 2.1 mV</td>
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<tr>
<td>PG F₂α (10^{-8} M)</td>
<td>−59.2 ± 1.9 mV</td>
<td>−49.4 ± 1.9 mV</td>
</tr>
<tr>
<td>PG F₂α (10^{-7} M)</td>
<td>−58.7 ± 1.4 mV</td>
<td>−49.2 ± 2.4 mV</td>
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* n = 20-43.
† n = 16-63.
‡ Mean ± SD.
tions were applied every 1 min. In the presence of PG E₂ (10⁻⁷ M) or PG F₂α (10⁻⁸ M), contraction occurred and, as a consequence, the contraction and relaxation evoked by field stimulation were reduced and enhanced, respectively.

Figure 6 shows the effects of PG F₂α on the mechanical properties of the sphincter and dilator muscles in the presence of indomethacin (10⁻⁶ M). In the sphincter muscle, electrical field stimulation evoked only the twitch contraction following pretreatment with indomethacin (10⁻⁶ M). However, application of 10⁻⁹ M PG F₂α evoked the contraction, and the electrical field stimulation evoked biphasic responses, i.e., a twitch contraction followed by a long-lasting relaxation (Fig. 6A). In the dilator muscle, electrical field stimulation evoked biphasic responses in the presence of indomethacin (10⁻⁶ M). Application of 10⁻⁸ M PG F₂α contracted the muscle and reduced or enhanced the amplitude of contraction or relaxation evoked by field stimulation, respectively (Fig. 6B).

In the presence of indomethacin (10⁻⁶ M), atropine (10⁻⁶ M) blocked the twitch contraction of the sphincter muscle evoked by field stimulation. Application of 10⁻⁹ M PG F₂α contracted the muscle and field stimulation evoked relaxation. On the other hand, guanethidine (5 x 10⁻⁶ M) did not affect the twitch contraction evoked by field stimulation in the presence of indomethacin (10⁻⁶ M). Application of 10⁻⁹ M PG F₂α also generated the contraction but field stimulation evoked only contraction, while the relaxation was abolished by 5 x 10⁻⁶ M guanethidine. However, neither atropine nor guanethidine modified the amplitude of contraction evoked by PG F₂α (Fig. 7A). Figure 7B shows the effects of atropine or guanethidine on the mechanical response of the iris dilator muscle. Atropine and guanethidine selectively blocked the relaxation or contraction evoked by field stimulation, respectively. Atropine and guanethidine had no effect on the contraction evoked by 10⁻⁸ M PG F₂α. These findings suggest that PG F₂α has a direct action on the iris sphincter and dilator muscles, but that these responses are not due to activations of cholinergic or adrenergic nervous systems.

**Discussion**

In the present experiments, indomethacin, a cyclooxygenase inhibitor, greatly reduced the muscle tone of the sphincter and abolished the relaxation evoked by field stimulation. On the other hand, in case of the dilator muscle, indomethacin slightly re-
duced the muscle tone and reduced the relaxation evoked by field stimulation. These results indicate that the muscle tone of the sphincter and dilator could be regulated by endogenous PGs, at least in in vitro experimental conditions. When field stimulations were applied every 1 min, the muscle tone increased in both sphincter and dilator muscles and indomethacin abolished this increase. Thus, the synthesis of PGs may be accelerated during field stimulation. It was reported that topical application of indomethacin prevents surgically-induced miosis and it was considered that endogenous PGs may be increased during eye surgery, thus causing miosis. However, in general, topical application of indomethacin does not affect the pupillary diameter in vivo, under normal conditions, thereby indicating that the synthesis of PGs in the vicinity of the iris is inadequate to change the pupillary diameter. On the other hand, PG synthesis may be greatly enhanced in this in vitro experimental condition because the tissue is inevitably traumatized. PG contraction is likely a pathological mechanism in trauma inflammation, and not a normal physiological mechanism related to the regulation of pupillary function. It was reported that the PGs or PG-like activity in the aqueous humor is increased in patients with acute anterior uveitis, Behçet’s disease or glaucomato-cyclitic crises, and by surgical trauma, which may cause atropine-resistant miosis.

In Ca-free solution containing EGTA, the muscle tone of sphincter and dilator muscles was greatly reduced. However, following application of indomethacin, Ca-free solution did not modify the muscle tone of the sphincter but did reduce it in the dilator. Therefore, mechanisms which maintain the muscle tone differ between these muscles, and PGs probably have a more important role in maintaining the muscle tone in the sphincter.

Exogenously applied indomethacin or PGs had no effect on the resting membrane potentials of sphincter and dilator muscles, yet reduced the muscle tone or generated contraction in both muscle tissues. Much the same response was noted in other visceral smooth muscles, eg exogenously applied carbachol or noradrenaline evoked contraction or relaxation, respectively, without affecting the resting membrane potential in the dog iris sphincter muscle, with the reversed sequence of mechanical responses being observed in the dilator muscle. These phenomena, termed pharmaco-mechanical coupling, also occur in smooth muscles of the rabbit pulmonary artery, and in the porcine and dog coronary arteries.

Exogenously applied PG E1 and PG E2 had little effect on the muscle tone, but PG F2α produced contraction of sphincter and dilator muscles. Furthermore, in the presence of indomethacin, exogenously applied PG F2α reversed the effect of indomethacin, ie, it produced contraction and enhanced the relaxation evoked by field stimulation. Thus, PG F2α may have an important role in maintaining the muscle tone of these muscles, at least in in vitro experimental conditions.

In the cat eye, topical application of PGs evoked miosis and PG F2α was the most potent among the various PGs. In the dog iris sphincter muscle, the contraction induced by 10^{-7} M PG F2α was larger than the contraction evoked by field stimulation; however, in the dilator, the contraction evoked by PG F2α was relatively small. Thus, the sphincter muscle seems to be more sensitive to PGs. On the other hand, it was also reported that topical application of PGs did not modify the diameter of the monkey and human pupils. In vitro studies revealed that PG E2 was more potent than PG F2α in generating contraction in the bovine iris sphincter muscle. Exogenously applied PGs were found to evoke relaxation in the rabbit iris sphincter. We found that PG F2α was the most potent in contracting the dog iris sphincter muscle and PG E1 or PG E2 had little effect. Thus, there are marked species differences concerning the effect of PGs on the iris sphincter and dilator muscles.

The iris sphincter muscle of the dog is innervated by cholinergic excitatory and adrenergic inhibitory nerve fibers, and the dilator muscle receives adrenergic excitatory and cholinergic inhibitory innervations. PGs are known to modulate neuro-effector transmission in smooth muscle tissues from various species. In the bovine iris sphincter muscle, PG E2 enhances the transmitter release from cholinergic nerve terminals. In the rabbit iris sphincter muscle, PGs enhance the transmitter release from non-cho-
linergic non-adrenergic nerve terminals. In the present investigations, PGs or indomethacin modified the mechanical responses evoked by field stimulation. However, these agents also altered the resting muscle tone of both muscles, and this action was not affected by either atropine or guanethidine. It appears, therefore, that PGs act directly on these muscles rather than through the release of adrenergic or cholinergic neurotransmitters.

Key words: iris sphincter, iris dilator, muscle tone, indomethacin, prostaglandins

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References


