Possible role of prostaglandins in regulating the non-cholinergic, non-adrenergic excitatory neural control of the rabbit urinary bladder

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Possible role of prostaglandins in regulating the non-cholinergic, non-adrenergic excitatory neural control of the rabbit urinary bladder

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SUMMARY

The modulating role of PGs in the atropine-resistant response of the rabbit urinary bladder was investigated. The atropine-resistant responses to single pulses were decreased in magunitude and the rate and extent of the decrease were greater than those in the indomethacin (4×10^{-6}M) or mefenamic acid (4×10^{-6}M) after desensitization of the muscle to ATP (10^{-4}M) developed. Both PGE_1 (10^{-9}—5×10^{-9}M) and E_2 (10^{-9}M) were effective in potentiating the atropine-resistant responses, but not $PGF_{2\alpha}$. ATP produced an initial phasic contraction followed by a late, long-lasting contraction. Indomethacin abolished the long-lasting contraction. PGE_1 and E_2 reversed the indomethacin-induced inhibition of the contractile response to ATP. The initial phasic component of the ATP -induced contraction was found to have some similarities to the atropine-resistant response to nerve stimulation. It is concluded that PGs are more likely to act as modulators of non-cholinergic, non -adrenergic transmission to smooth muscle in the rabbit urinary bladder.

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INTRODUCTION

It is well known that the urinary bladder of mammals receives a dense excitatory innervation via the pelvic nerves[1)2)]. The contractile response of the urinary bladder to electrical stimulation of the pelvic nerves is partially resistant to atropine and other antimuscarinic agents even when they were elevated in concentrations high enough to block the contractile response to exogenously-applied acetylcholine[3)5)] (ACh). In general, the atropine-resistant component in predominant in the contractile responses to stimulation of the pelvic nerves at low frequencies[6)]. Burnstock et al[7)]. observed the atropine-resistant response of the guinea-pig urinary bladder and proposed that the response is mediated by purinergic nerves from which adenosine-5'-triphosphate (ATP) is released as the transmitter. This idea is based on their observation that desensitization of the muscle to ATP resulted in abolition of the atropine-resistant response to the nerve stimulation.

ATP is known to be capable of releasing prostaglandins (PGs) in a number of tissues[8)-12)]. Anderson et al[9)]. have suggested that ATP, whether exogenous or endogenous in origin, causes release of a PG, which in turn exerts its ability to contract the smooth muscle of the urinary bladddder.

However, in almost all studies on the possible involvement of PGs in transmission from the pelvic nerves to smooth muscle cells in the urinary bladder, electrical stimulation of the pelvic nerves has been made at relatively high frequencies.

In this study, we have examined the effects of two inhibitors of PG synthesis and one antagonist of PG receptors on the atropine-resistant responses elicited by stimulating the pelvic nerves in the muscle of the rabbit urinary bladder in an attempt to provide further evidence for their possible role in regulating the neuromuscular transmission.

MATERIALS AND METHODS

Adult rabbits of either sex, weighing 2-3 kg, were stunned by a blow on the neck and bled rapidly from the carotid artery to death. The urinary bladder was exposed by incising along the median line, detached from the adhering connective tissues and fat, and excised by cutting transverse below the trigone. The isolated urinary bladder was incised on both its lateral edges except the fundal portion to give a rectangular sheet of tissue about 15 mm in width and 20 mm in length and pinned serosal side up on a rubber board. Strips of the muscle (about 2 mm wide and 15 mm long) were obtained by cutting along longitudinal muscle fibres of the outer layer. The mucosal layer was removed, but the inner layer retained was attached. The muscle strip was vertically mounted in a 5 ml organ bath in such a way that it was passed through a pair of platinum ring electrodes (5 mm in diameter and about 1 cm apart between the two rings) with one end fixed and the other attached by thread to a transducer. The ring electrodes were used for electrical field stimulation of the intramural nerves. For nerve stimulation, monopolar square wave pulses of 0.5 ms width at supramaximal voltage were delivered from stimulator (Nihon Koden, MSE-3) at an interval of 30 sec. The organ bath was filled with Kreb's solution (mM) ; NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.3, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11.1 ; continously bubbled with a gas mixture of 95% O_2 and 5% CO_2 and kept at 30℃. Atropine (3×10^{-7} M) and guanethidine (10^{-6}M) were added to Kreb's solution to block muscarinic receptors and adrenergic neurones. The initial tone of the muscle strips was adjusted to a force of 0.5g and equilibrated for 30 min before starting the experiment. Changes in tension were recorded isometrically with a force-displacement transducer (Nihon Koden, SB-LT) coupled to a potentiometric pen recorder (Hidachi).

The following drugs were used : acetylcholine chloride (Wako), adenosine-5'-triphosphate (Sigma), atropine sulphate (Wako), guanethidine sulphate (Ciba), indomethacin (Sigma), mefenamic acid (Sankyo), prostaglandin E_1, E_2 and $F_{2\alpha}$ (Sigma), SC19220 (AM, French, G.D. Searle). Prostaglandins, indomethacin and SC19220 were initially dissolved in 70% ethanol to keep as stock solution, and the stock solutions were diluted with Kreb's solution to an appropriate concentration just before use. ATP was dissolved in 0.9% NaCl solution containing 0.1M phosphate buffer (pH=7.2). A certain amount of the concentrated drug solution was added to the bathing solution to give a desired concentration. Student's t test was used for all comparisons between mean values, and differences were regarded as statistically significant if $P<0.05$. Values in the text refer to the mean ± S.E.

RESULTS

Atropine-resistant contraction

Electrical field stimulation with single pulses produced rapid twitch-like contractions of the muscle strip, as shown in Fig. 1. The contractile response reached a maximal tension within 10 sec after the start of stimulation, which was abolished with tetrodotoxin (3×10^{-7}M) and nerve-mediated. Atropine (3×10^{-7}M) did not inhibit the contractile response even when the drug was used in concentrations of 10 times or more the concentration required to antagonize completely the action of ACh (5×10^{-5}M).

Desensitization of the muscle to ATP

To test involvement of ATP in the atropine-resistant response, the muscle was exposed repeatedly to ATP, while twitch-like responses were elicited by stimulating the nerves with single pulses at an interval of 30 sec in the presence of atropine (3×10^{-7}M). ATP (10^{-4}M) applied first by adding to the bathing medium, produced a contraction which reached a peak in 20-30 seconds and faded slowly

to the base line tension. The muscle was not apparently desensitized to ATP : The magnitude of the first ATP-induced contraction was not significantly different from that of the fourth one. However, this treatment with ATP increased spontaneous mechanical activity of the muscle and reduced the maximal tension of the twitch-like responses to 50-80% of that of the control responses recorded immediately before the first application of ATP (Fig. 1A). There is evidence that ATP induced PG synthesis and PGs potentiate ATP-induced contractions in the smooth muscle of the guinea-pig urinary bladder. The failure to demonstrate desensitization of the action of ATP may be assumed to be due to potentiation of the action of ATP by an PG which was released by the applied ATP. After the muscle strip was pre-treated with indomethacin (4×10^{-6}M), an inhibitor of PG synthesis for 30 min., ATP induced a contraction whose magnitude and duration were decreased greatly. An application of ATP was repeatedly, the response became smaller and finally the muscle rendered insensitive to ATP even if the concentration was increased up to 5×10^{-4}M (desensitization). Similar results were obtained in other eight experiments. In the muscle desensitized to ATP, the twitch-like response to stimulation of the intramural nerves was also decreased in magnitude, and the rate and extent of the decrease were greater than in the absence of indomethacin (Fig. 1B). This effect of indomethacin suggests involvement of endogenous PG(s) in determining the ATP-induced and the nerve-mediated contractions.

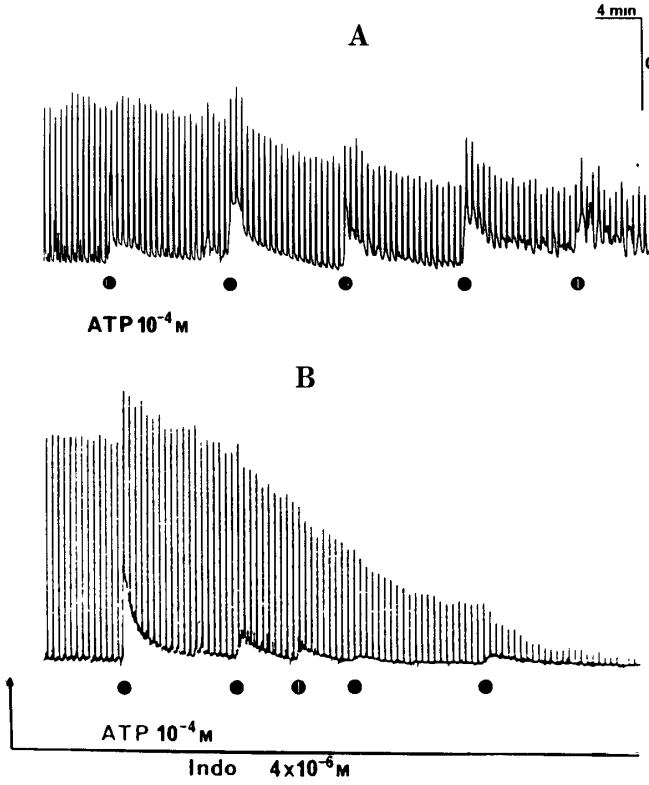

Fig. 1 The effects of ATP (10^{-4}M) applied repeatedly on contractile responses to electrical stimulation of the intramural nerves with single pulses every 30 sec. A and B, records obtained before and after treatment for 30 min with indomethacin (Indo, 4×10^{-6}M). Notice development of desensitization of the muscle to ATP and a marked decline in magnitude of atropine-resistant twitch-like responses after treatment with indomethacin.

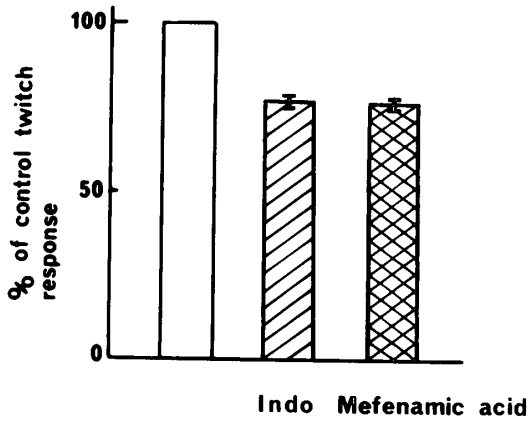

Fig. 2 The effects of indomethacin and mefenamic acid on atropine-resistant twitch-like responses to stimulation of the intramural nerves with single pulses every 30 sec. Ordinate, relative magnitude of the contractile response expressed as a percentage of the maximal tension of nerve-mediated contraction before application of the inhibitors of PG synthesis. Open column, control. Stippled column, after treatment with indomethacin (Indo, 4×10^{-6} M) for 30 min. Meshed column, after treatment with mefenamic acid (4×10^{-6}M) for 30 min. Each value represents the mean from four to nine experiments, and vertical lines indicate the S.E. mean.

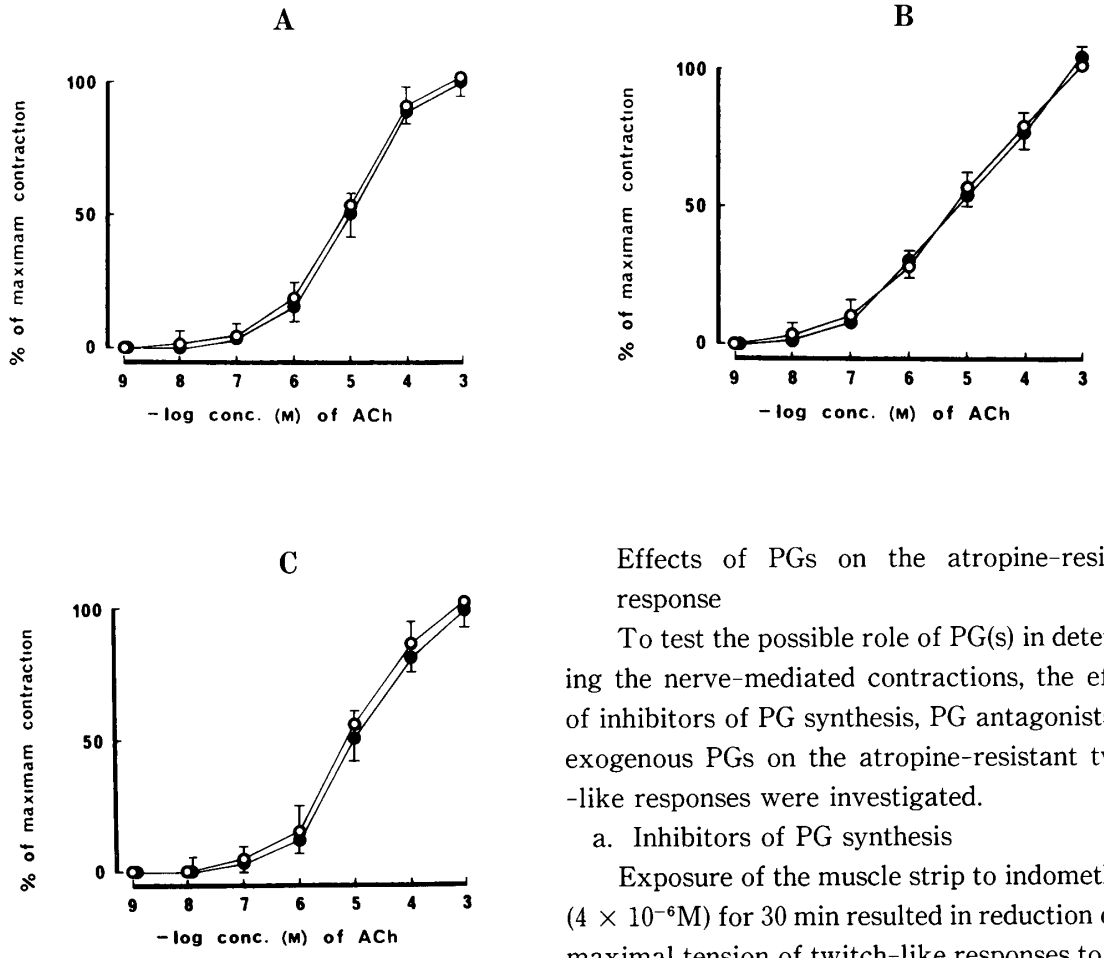

Fig. 3. The concentration-responses for ACh before (○) and after (●) treatment with indomethacin, mefenamic acid and SC19220. A, indomethacin (4×10^{-6}M). B, mefenamic acid (4×10^{-6}M). C, SC19220 (5×10^{-5}M). Ordinate, relative magnitude of expressed as a percentage of the maximal tension of the contraction produced by ACh (10^{-3}M). Each value represents the mean from four experiments, and vertical lines indicate the S.E. mean.

Effects of PGs on the atropine-resistant response

To test the possible role of PG(s) in determining the nerve-mediated contractions, the effects of inhibitors of PG synthesis, PG antagonists and exogenous PGs on the atropine-resistant twitch-like responses were investigated.

a. Inhibitors of PG synthesis

Exposure of the muscle strip to indomethacin (4×10^{-6}M) for 30 min resulted in reduction of the maximal tension of twitch-like responses to stimulation of the intramural nerves so 77.4±2.6% (n=9) of the control, the maximal inhibition being obtained within 20 min (Fig.2). Indomethacin also inhibited the spontaneous mechanical activity. With another inhibitor of PG synthesis, mefenamic acid (4×10^{-6}M), the maximal tension of twitch-like responses was reduced to 76.1±1.2% (n=4). On the other hand, the concentration-response curve for ACh remained unaltered in the presence of either of these inhibitors (Fig. 3).

b. PG antagonist

After treatment of the muscle strip with SC19220 (5×10^{-5}M), the magnitude of twitch-like responses was reduced to 69.3 ± 6.3% (n=4) of the control, and the effect reached a maximum within less than 10 min (Fig. 4). Figure 3C shows no appreciable effect SC19220 on the concentration-response curve for ACh. The inhibitory effects on the atropine-resistant responses may result from elimination of the excitatory action of endogenous PG(s).

c . Exogenous PG

Figure 5 shows potentiation of the twitch-like responses with PGs. Concentrations in 5×10^{-10} to 5×10M of PGE_2 were effective in potentiating the twitch-like restponses. The effect increased in a concentration-dependent manner and reached a peak within 10 min after application. In a

concentration of 5×10^{-10}M, PGE_2 increased the maximal tension of the twitch-like responses to 110.0 ±4.2% (n=4) of the control. When the concentration of PGE_2 was increased to 10^{-9}M or 5×10^{-9}M, the maximal tension was increased to 130.2±4.2% (n=4) or 140.0 ± 6.7% (n=5), respectively. With concentrations higher than 5×10^{-9} M, PGE_2 increased spontaneous mechanical activity of the muscle which prevented stable measurements of the responses to stimulation of the intramural nerves. PGE_1 (2×10^{-9}M) increased the maximal tension of the twitch-like responses to 127.4 ± 4.1% (n=5) of the control and also slightly increased spontaneous mechanical activity in some preparations. $PGE_{2\alpha}$, when used in concentrations of up to 5×10^{-8}M, had no effect on the twitch-like responses.

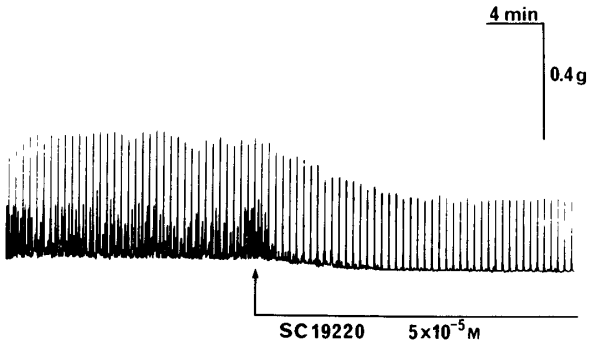

Fig. 4. The effect of SC19220 (5×10^{-5}M) on atropine-resistant twitch-like response to stimulation of the intramural nerves with single pulses every 30 sec.

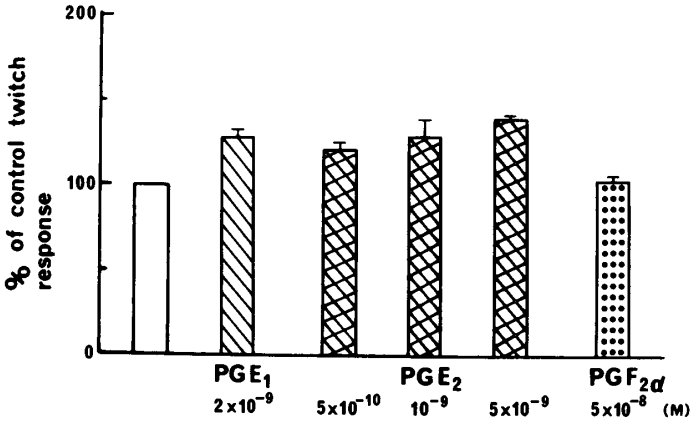

Fig. 5. Potentiation by PGs of atropine-resistant twitch-like response to stimulation of the intramural nerves with single pulses every 30 sec. Open column, control. Stippled column, after application of PGE_1 (2×10^{-9}M). Meshed column, after aplication of PGE_2 (5×10^{-10}, 10^{-9} and 5×10^{-9}M, respectively). Dotted column, after application of $PGF_{2\alpha}$ (5×10^{-8}M). Ordinate, relative magnitude expressed as a percentage of the maximal tension of nerve-mediated contraction before application of the PGs. Each value represents the mean from four experiments, and vertical lines indicate the S.E. means.

Effect of exogenous PG on indomethacin-induced inhibition.

When inhibition of the twitch-like responses produced by indomethacin (4×10^{-6}M) reached a steady-state level, PGs were applied to test their effect. PGE_2 (5×10^{-10} to 5×10^{-9}M) reversed the indomethacin-induced inhibition (Fig. 6). In fact, the maximal tension of the twitch-like response was increased to 128.8 ± 1.3% (n=4), 180.0 ± 8.7% (n=4) and 250.3 ± 17.3% (n=4) with PGE_2 in concentrations of 5×10^{-10}, 10^{-9} and 5×10^{-9}M, respectively, as a percentage of the maximal tension of the steady-state twitch-like response in the presence of indomethacin. PGE_1 (2×10^{-9}M) and $F_{2\alpha}$ (10^{-8}M) had similar effects, and the maximal tension of the twitch-like responses was increased to 182.0 ± 2.4% (n=5) and 133.0 ± 6.9% (n=4), respectively.

Effects of indomethacin and PGE_2 on contractile responses to ATP.

It has been suggested that the atropine-resistant response is mediated by ATP released from purinergic nerves, and that ATP serves not only as the transmitter but also as a releaser of PGs. If so, the contractile effect of ATP on the muscle strip should be affected by indomethacin and PGE_2 in the same way as the nerve-mediated contraction was modified. ATP (10^{-4}M) produced a biphasic contraction, consisting of an initial phasic contraction followed by a late, long, -lasting contraction

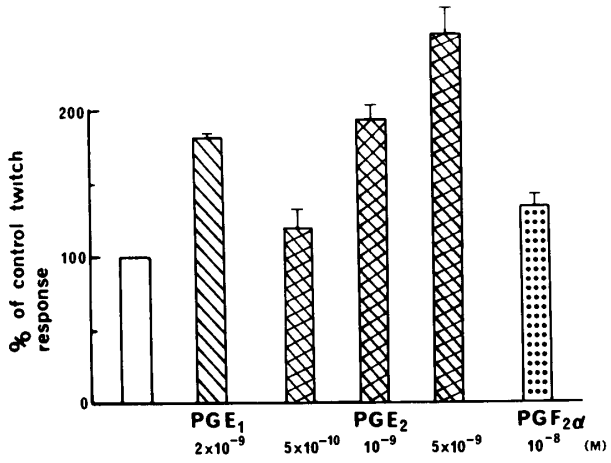

Fig. 6. Reversal by PGs of indomethacin-induced inhibition of atropine-resistant twitch-like response to stimulation of the intramural nerves with sigle pulses every 30 sec. Open column, control. Stippled column, after application of PGE_1 (2×10^{-9}M). Meshed column, after application of PGE_2 (5×10^{-10}, 10^{-9} and 5×10^{-9}M, respectively). Dotted column, after application of $PGP_{2\alpha}$ (10^{-8}M). Ordinate, relative magnitude expressed as a percentage of the maximal tension of the steady-state, nerve-mediated contraction in the presence of indomethacin (Indo. 4×10^{-6}M). Each value represents the mean from four experiments, and vertical lines indicate the S.E. means.

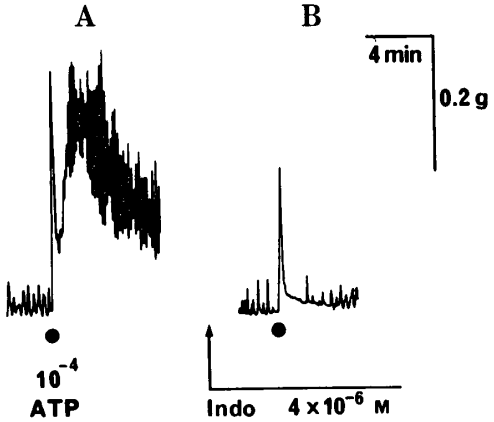

Fig. 7. Effect of indomethacin on contractile response to ATP (10^{-4}M). A and B correspond, respectively, to before and after treatment with indomethacin (Indo. 4×10^{-6}M) for 30 min. Note that the long-lasting contraction to ATP is virtically abolished, but the phasic contraction still occurs.

(Fig. 7). The phasic contraction begin within 1 sec after application of ATP and reached a peak tension within the next 5 sec. The long-lasting contraction reached a peak tension within 10 sec after the first peak tension and the second peak tension was maintained up to 2 min. Indomethacin (4×10^{-6}M) abolished the long-lasting contraction (Fig. 8) with reduction of the first peak tension to 83.6 ± 5.8% (n=6) of the control. PGE_2 in a concentration of 10^{-9}M increased the peak tension of the phasic contraction to 122.2 ± 5.9% (n=5) of the control without changing the long-lasting contraction. When applied in the presence of indomethacin, PGE_2 reversed the indomethacin-induced inhibition of the contractile response to ATP. The peak tension of the phasic contraction was increased to 167.2 ± 10.8% (n=5) of that of the maximal contraction producing ATP in the presence of indomethacin alone. Thus, the initial, phasic component of the ATP-induced contraction was found to have some similarities to the atropine-resistant response to stimulation of the intramural nerves. To determine whether these effects of PGE_2 and indomethacin were specific on the response to ATP or

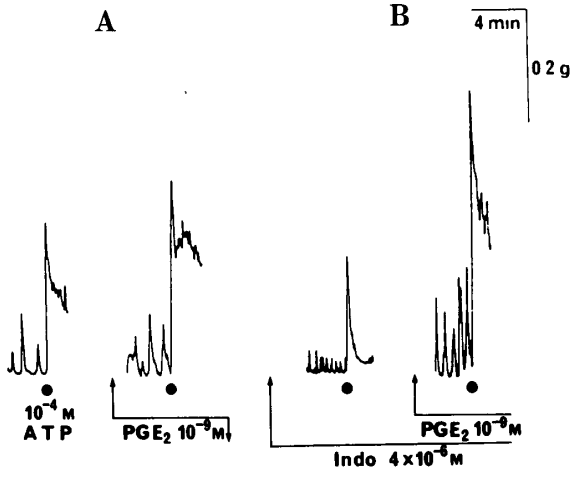

Fig. 8. Reversal by exogenous PGE_2 (10^{-9}M) of indomethacin-induced inhibition of contractile response to ATP (10^{-4}M). A and B correspond, respectively, to before and after treatment with indomethacin (Indo. 4×10^{-6}M) for 30 min.

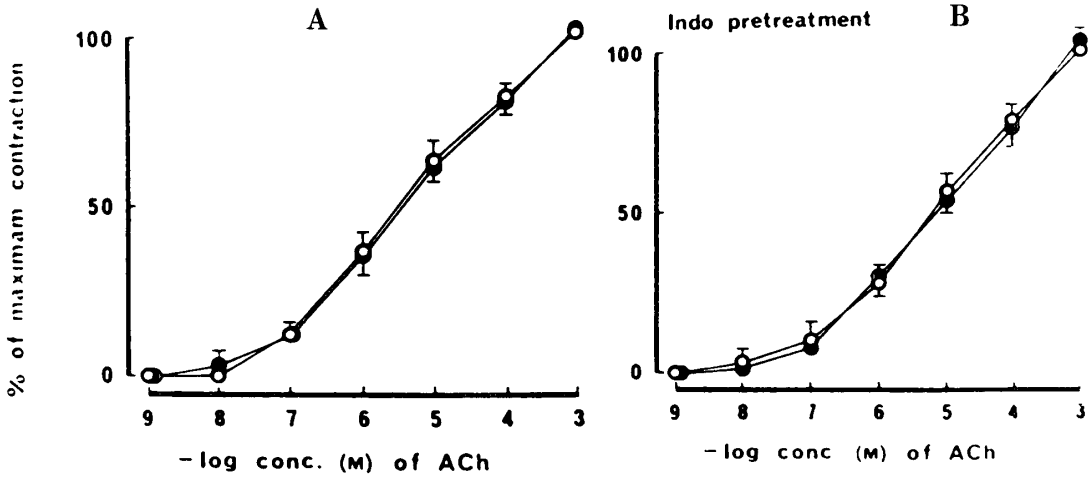

Fig. 9. Effect of PGE_2 and PGE_2 plus indomethacin on concentration-response curve for ACh. A correspond to before (○) and after (◐) application of PGE_2 (10^{-9}M). B correspond to before (○) and after (●) application of PGE_2 (10^{-9}M) plus indomethacin (Indo. 4×10^{-6}M). Ordinate, relative magnitude expressed as a percentage of the maximal tension of the contraction produced by ACh (10^{-3}M) in normal solution. Each value represents the mean from four experiments, and vertical lines indicate the S. E. means.

not, effects of PGE_2 and PGE_2 plus indomethacin on the concentration-response curve for ACh were observed. After treatment with either PGE_2 alone or PGE_2 plus indomethacin, the concentration -response relation of ACh was unaffected (Fig. 9).

DISCUSSION

It seems likely that endogenous PGs act as a modulator of atropine-resistant transmission from the intramural excitatory nerves to the smooth muscle in the rabbit urinary bladder. This view stems from the following findings : 1) the exogenous PGs, PGE_1, PGE_2 and $PGF_{2\alpha}$ showed their ability to potentiate the atropine-resistant responses to nerve stimulation ; 2) the inhibitors of PG synthesis and the antagonist of PG receptor inhibited the atropine-resistant responses to nerve stimulation.

A variety of tissues are capable of releasing PGs in response to various stimuli[13)~15)]. Hills[16)] has demonstrated that in the isolated rabbit urinary bladder, an increase in activity of cholinergic neurons by physostigmine results in PGs release into the bathing medium. The enhancing effect of PGs on neuro-effector transmission, as in the rabbit urinary bladder, has been observed on the transmission between cholinergic nerves and smooth muscle cells in the guinea-pig ileum[13)]. It is conceivable that release of PGs leads to operation of a common mechanism by which neuro-effector transmission is altered to be more efficient.

The question as to how the atropine-resistant transmission in the rabbit urinary bladder is accelerated by PGs remains to be answered. A possible explanation is that endogenous PGs may act at the postjunctional site to render the smooth muscle more sensitive to the transmitter, as previously described for the possible role of PGs in the excitatory neurotransmission of other mammalian urinary bladder[7)12)]. Another explanation is that endogenous PGs may cause an increase in release of the transmitter from the non-cholinergic excitatory nerve endings.

The ATP hypothesis has been proposed[2)8)] that the atropine-resistant responses to nerve stimulation in the urinary bladder of the rabbit and guinea-pig are mediated by ATP released as the

transmitter from the purinergic nerves. However, earlier workers[7)9)] reported that the atropine -resistant contractile response to nerve stimulation were little affected even after desensitization of the smooth muscle to ATP, although both responses obtained after treatment of indomethacin had similar characteristics. This disimilarity between the responses to nerve stimulation and ATP has been frequently taken by some workers[11)12)] as evidence against the ATP hypothesis. In the present experiments, neither of the contractile responses to nerve stimulation or ATP was reduced after application of ATP had been repeated ; they were altered similarly by indomethacin and PGE_2, and the contractile response to nerve stimulation was abolished after desensitization to ATP of the muscle pre-treated with indomethacin. These similarities corroborate the ATP hypothesis.

Burnstock et al[7)]. argued a mutual relationship between the purinergic nerves and endogenous PGs in the guinea-pig urinary bladder : stimulation of the purinergic nerves releases ATP, ATP exerts its action to trigger synthesis and release of PGs as well as its principal action as an excitatory neurotransmitter to the smooth muscle, and in turn PGs cause a contraction of the smooth muscle which may superimpose or may be preceded by the ATP-induced principal contraction. Anderson[8)] measured PGs during the ATP-induced contraction of the rabbit urinary bladder and provided evidence for release of PGs. Our finding that the long-lasting component, but not the phasic component, of the ATP-induced contraction was abolished after treatment with indomethacin suggests that endogenous PGs are responsible for generation of the former component . Thus, it is highly probable that PGs are involved in the atropine-resistant neuromuscular transmission in the rabbit urinary bladder, just as in the guinea-pig urinary bladder.

In conclusion, the results presented here provide further evidence for a possible role of PGs in regulating the neuromuscular transmission in which the transmitter-receptor interaction is atropine -resistant in the rabbit urinary bladder.

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ウサギ膀胱の非コリン非アドレナリン作動性興奮神経支配調節におけるプロスタグランディンの役割

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ウサギ膀胱のアトロピン耐性反応に対するプロスタグランディン（ＰＧ）の調節的役割が調べられた。単一パルス刺激によるアトロピン耐性反応は，ＡＴＰに対する筋の感受性を低下させると，インドメサジン（4×10^{-6}M）あるいはメフェナム酸（4×10^{-6}M）の非存在下よりも存在下の方が反応高は大きく減少し，その減少速度及び程度も顕著であった。PGE_1（$10^{-9}\sim5\times10^{-9}$M）と$E_2$（$10^{-9}$M）はアトロピン耐性反応を効果的に増強したが，$PGF_{2\alpha}$にはこの様な効果は認められなかった。ＡＴＰは初期の速い時間経過の収縮と引き続いて緩徐な時間経過の収縮を惹き起こした。インドメサジンは緩徐な収縮を消失させた。PGE_1とE_2はインドメサジンによるＡＴＰの収縮抑制を元に回復させた。ＡＴＰによる速い収縮要素は神経刺激によるアトロピン耐性反応と類似していることが明らかとなった。ウサギ膀胱において，ＰＧが非コリン非アドレナリン作動性神経と平滑筋との情報伝達の調節因子として作用していると結論される。