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RELEASE OF PROSTAGLANDIN E FROM THE ISOLATED URINARY BLADDER OF THE GUINEA-PIG

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1 Release of prostaglandin E (PGE) from guinea-pig urinary bladder *in vitro* has been demonstrated both in the resting state and during electrical stimulation.

2 The electrically evoked release of PGE was significantly higher than the resting release and was frequency-dependent.

3 The released substance was characterized as PGE pharmacologically by (a) blockade of its response by SC-19220 on guinea-pig ileum, (b) reduction of the amount of the released substance by indomethacin and (c) the inhibitory effect of the released substance on adrenergic neurotransmission in guinea-pig vas deferens.

4 The prostaglandin seemed to originate from the muscle since tetrodotoxin treatment did not abolish the release during direct muscle stimulation; however, concomitant release from neuronal tissue could not be excluded in the present experiments.

5 Indomethacin failed to inhibit the mechanical responses of the bladder to transmural stimulation.

6 The present experiments suggest that PGE is not involved in mediating the non-cholinergic non-adrenergic neurotransmission in the guinea-pig urinary bladder.

Introduction

Both acetylcholine (ACh) and adenosine 5'-triphosphate (ATP) have been shown to be liberated from the isolated urinary bladder of the guinea-pig during field stimulation (Chesher, 1967; Burnstock, Cocks, Crowe & Kasakov, 1978). Evidence exists for the involvement of prostaglandins in the atropine-resistant transmission of rabbit and monkey urinary bladders (Hill, 1976; Anthony & Paton, 1977) and for the maintenance of tone by prostaglandins in human bladder (Hill, 1976). Recently Burnstock *et al.* (1978) found evidence for involvement of prostaglandin E (PGE) in modulating the responses of the guinea-pig urinary bladder to ATP and to non-adrenergic, non-cholinergic nerve stimulation. We describe here the release of an E type prostaglandin from the guinea-pig urinary bladder as characterized by the pharmacological properties of the released substance.

Methods

Guinea-pigs of either sex (wt. 300 to 500 g) were stunned by a blow on the head and exsanguinated. The whole urinary bladder was dissected out and its interior was washed out with Krebs solution through the urethra. Tissues were equilibrated for 1 h in the organ bath with repeated draining of the bathing fluid. Electrical field stimulation of the bladder was performed with two platinum electrodes, one of which was inserted into the bladder through the urethra while the other formed a ring around the exterior of the mid-region of the bladder. Nerve-mediated responses of the bladder were obtained by electrical stimulation with supramaximal voltage of 50 to 70 μs duration at various frequencies. Direct muscle stimulation was carried out by the method of Burnstock *et al.* (1978), the pulse duration being increased to 5 to 7 ms at a time when the nerve-induced responses were totally blocked by incubation with 0.31 μM tetrodotoxin (TTX).

The bladder was stimulated initially at a frequency of 20 Hz for 5 s every 3 min for 1 h before collection of any sample. After repeated draining of the bathing fluid, electrical stimulation was performed at a chosen frequency for the first 15 s of every min for 20 min in order to collect the sample. The 'resting sample' was obtained by collecting the bathing fluid of the urinary bladder after 20 min of contact with the tissue in the absence of electrical stimulation.

The sample was usually assayed for prostaglandin-like activity in terms of PGE_1 equivalents on the isolated stomach strip of the rat (Vane, 1964). In addition, a set of resting and stimulated (20 Hz) samples were assayed on the guinea-pig ileum against stan-

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dard PGE_1 in order to obtain a quantitative correlation in two different bioassay preparations. The Krebs solution for the stomach strip and the Tyrode solution for the guinea-pig ileum contained indomethacin (2.8 μM), atropine (0.14 μM), mepyramine (0.25 μM), cyproheptadine (0.57 μM), dibenzyline (0.3 μM) and propranolol (3.4 μM).

Transmurally stimulated preparations of the isolated vas deferens of the guinea-pig were set up according to the method described earlier (Alkondon, Vedasiromoni, Mukherjee & Ganguly, 1978). Transmural electrical stimulation was with supramaximal pulses of 0.5 ms duration at a frequency of 5 Hz, repeated every 3 min.

Experiments with the isolated taenia coli of the guinea-pig were carried out in the conventional way. The Krebs solution for this preparation contained atropine sulphate (0.14 μM) and guanethidine sulphate (3.4 μM).

Drugs

The following drugs were used: acetylcholine chloride (E. Merck), adenosine 5'-triphosphate (Sigma), atropine sulphate (E. Merck), cyproheptadine hydrochloride (Merck, Sharp & Dhome), dibenzyline hydrochloride (Smith, Kline & French), guanethidine sulphate (Ciba), indomethacin (Merck, Sharp & Dhome), mepyramine maleate (May & Baker), propranolol hydrochloride (ICI), prostaglandin E_1 (Upjohn), SC-19220 (Searle) and tetrodotoxin (Sigma). Concentrations of drugs are expressed in terms of molarity of the salts.

Results

Biological activity of the bladder perfusate

The perfusate (or sample) collected from the isolated bladder both under resting conditions and during field stimulation caused contraction of the rat isolated stomach strip in the presence of the antagonists, indomethacin, atropine, mepyramine, cyproheptadine, dibenzyline and propranolol. The response of the sample was characterized by a delayed onset (15 to 20 s), failure to reach the maximum amplitude in 90 s and persistence of increased tone after washout. The sample produced contraction of the guinea-pig ileum in the presence of these antagonists and also produced contraction of the guinea-pig isolated taenia coli in the presence of atropine and guanethidine. The time course and the nature of the response of the sample typically resembled the responses to exogenous PGE_1 in all of the test organs.

In view of the fact that PGE is known to cause inhibition of adrenergic neurotransmission at very

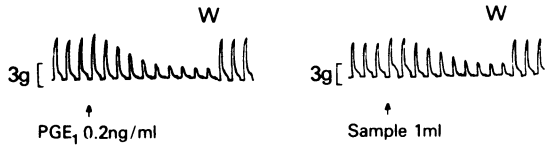

Figure 1 Typical blocking effects of prostaglandin E_1 (PGE_1) and the sample on the responses of the isolated vas deferens of the guinea-pig to transmural stimulation at 3 min intervals.

low concentrations (for review, see Starke, Taube & Borowski, 1977), the effect of the perfusate from the urinary bladder was examined on the responses of the transmurally stimulated isolated vas deferens of the guinea-pig in the presence of atropine. The sample invariably produced inhibition of the adrenergically mediated responses of the vas deferens and the nature of this effect was qualitatively similar to that of exogenous PGE_1 as shown in Figure 1. However, the inhibitory influence of the sample on neurotransmission of guinea-pig vas deferens cannot be solely attributed to PGE action, since $PGF_{2\alpha}$ also produces a similar effect although of lesser intensity (Baum & Shropshire, 1971).

Pharmacological identification of prostaglandin

That the responses induced by the sample were caused by PGE could be shown pharmacologically by the use of indomethacin and SC-19220. When the Krebs solution bathing the urinary bladder contained indomethacin (2.8 μM), a prostaglandin synthesis inhibitor (Vane, 1971), the resting as well as the stimulated release (20 Hz) of the PGE-like substance was significantly inhibited as shown in Figure 2. An equivalent amount of the vehicle (ethanol) used for dissolving indomethacin had no effect on the release of the active substance from the bladder.

The specific PGE-antagonistic effect of SC-19220 in the guinea-pig ileum (Sanner, 1971) was used for further identification of the released substance as PGE. SC-19220 (6 to 30 μM) blocked the responses to exogenous PGE_1 and to the released substance as a function of its concentration, at a time when the responses to exogenous ACh remained unaltered. The effect of SC-19220 (30 μM) on submaximal responses to the sample, to ACh and to PGE_1 is illustrated in Figure 3.

Resting and evoked release of prostaglandin E

The mean resting release of PGE from guinea-pig urinary bladder in terms of PGE_1 as assayed separately on the rat stomach strip and guinea-pig ileum in two sets of experiments was 11.1 ± 2.14 ng/h (13 experiments) and 11.3 ± 3.05 ng/h (4 experiments) re-

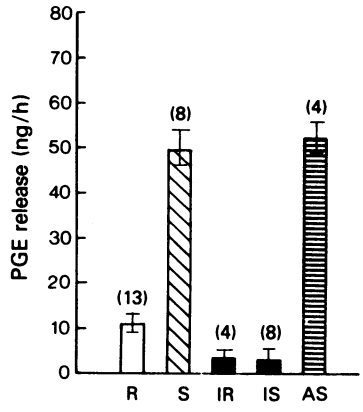

Figure 2 Histogram illustrating the release of prostaglandin E (PGE) from guinea-pig urinary bladder (expressed as PGE_1-equivalents) under resting conditions (R); during electrical stimulation at 20 Hz (S); during resting conditions and electrical stimulation in presence of and after preincubation with indomethacin (IR and IS respectively) and during stimulation (20 Hz) in presence of ethanol, the vehicle for indomethacin (AS). Vertical lines show s.e. mean. Figures in parentheses indicate number of experiments.

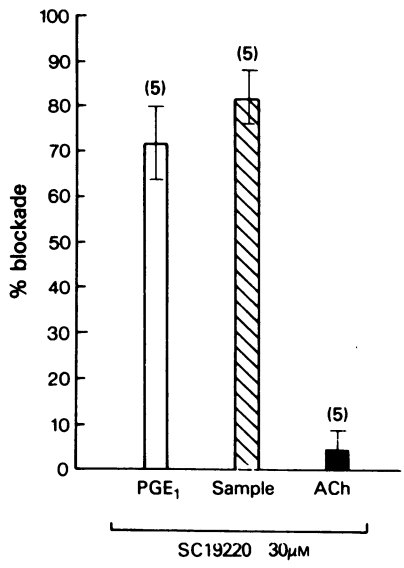

Figure 3 Histogram illustrating the percentage-blockade of the responses of the guinea-pig ileum to prostaglandin E_1 (PGE_1), the sample and acetylcholine (ACh) after preincubation (5 min) with SC-19220 (30 μM). Vertical lines show s.e. mean.

spectively. On stimulation at 20 Hz the release of PGE when assayed on rat stomach strip and guinea-pig ileum increased to 50.3 ± 4.05 ng/h (15 experiments) and 51.7 ± 6.51 ng/h (6 experiments) respectively.

The release of PGE from the bladder increased progressively with the increase of stimulation frequency up to 50 Hz which was the maximum frequency used in the present study (Table 1).

Source of the released prostaglandin E

Experiments with TTX (0.31 μM) were carried out to determine whether or not the source of released PGE was neuronal. The spontaneous or the resting release of PGE was unaffected in the presence of TTX (Table 2). Incubation with TTX (0.31 μM) did not affect the spontaneous mechanical rhythmic contractions of the bladder. However, the evoked release of PGE was blocked by TTX (0.31 μM) at a time when the responses of the bladder to field stimulation were completely abolished in the presence of TTX (Table 2). When, under such conditions, the mechanical responses of the bladder were elicited by stimulating muscle directly by increasing the pulse duration of shocks from 50 μs to 5 ms, the release of PGE was restored (Table 2).

Effects of indomethacin on mechanical responses

While 2.8 μM of indomethacin abolished the spontaneous and evoked release of PGE from the urinary bladder (Figure 2), it failed to inhibit the mechanical responses of the bladder to field stimulation in the presence of atropine (0.14 μM) and guanethidine (3.4 μM) even in a concentration of 56 μM. The contractile responses of the bladder to exogenous ACh also remained unaffected in the presence of indomethacin (up to 56 μM).

Effects of ATP on rat stomach strip

The influence of exogenous ATP was examined on the rat isolated stomach strip in order to exclude the possibility of its interference in the bioassay of the released PGE in our experiments, since ATP is released from the guinea-pig urinary bladder (Burnstock *et al.*, 1978). ATP up to 1.8 μM failed to produce any contraction of the muscle and the only noticeable effect of ATP was some relaxation.

Discussion

It is known that ACh, ATP and perhaps noradrenaline are released neuronally from the urinary bladder

(for references, see Edge, 1955; Chesher, 1967; Burnstock *et al.*, 1978).

The present results demonstrate, for the first time, release of prostaglandin-like substance, characterized pharmacologically as PGE, from the isolated urinary bladder of the guinea-pig. The substance released from the bladder produced contractions of PGE-sensitive smooth muscles i.e. rat stomach strip, guinea-pig taenia coli and ileum in the presence of antagonists which more or less eliminated the interference of other substances, except ATP. A good quantitative correlation could be obtained for the PGE released from the guinea-pig urinary bladder on two different bioassay preparations, the rat stomach strip and guinea-pig ileum. The contractile property of the substance released from the bladder is not attributable to ATP since exogenous ATP did not produce contraction of the stomach strip. A relaxant effect of ATP on the guinea-pig stomach strip has been observed by others (Okwuasaba, Hamilton & Cook, 1977). Moreover, the sample did not produce any relaxation of guinea-pig taenia coli as ATP does. The inhibition of adrenergic neurotransmission by the released substance, a property shared by PGE, was further evidence for identifying the released substance as PGE. Confirmation of release of PGE was obtained by incubation with indomethacin which markedly diminished the PGE-like activity of the sample. In addition, the responses to standard PGE_1 and to the sample on the guinea-pig ileum were selectively inhibited by SC-19220, a specific antagonist of PGE_1 in the guinea-pig ileum (Sanner, 1971). The use of indomethacin in the bioassay medium (in addition to other antagonists) excluded the possibility of an indirect action of the sample through liberation of endogenous PGE from the rat stomach strip (Bennett, Friedmann & Vane, 1967; Coceani, Pace-Asciak, Volta & Wolfe, 1967).

Table 1 Release of prostaglandin E (PGE) from the guinea-pig urinary bladder under resting conditions and during electrical stimulation at various frequencies as assayed on the rat isolated stomach strip

Conditions	*Prostaglandin output (ng/h)*	*No. of expts*
Resting	11.1 ± 2.14	13
Stimulation 5 Hz	20.5 ± 12.35	4
Stimulation 10 Hz	30.0 ± 10.04	6
Stimulation 20 Hz	50.3 ± 4.05	15
Stimulation 50 Hz	81.2 ± 11.55	6

Prostaglandin output is given as PGE_1 equivalents; mean ± s.e. mean.

While it can be concluded that PGE is released from non-neuronal sources in the urinary bladder, the possibility of its release from nerve endings could not be eliminated in the present experiments since the increased release of PGE by field stimulation was abolished in the presence of TTX (Table 2). However, it is possible that PGE-release from the bladder is a consequence of stimulus-contraction coupling at the postsynaptic level.

That the non-cholinergic, non-adrenergic transmission in the guinea-pig urinary bladder (Chesher & Thorp, 1965; Ambache & Zar, 1970; Burnstock, 1972) is not mediated through the release of PGE was evident as indomethacin failed to inhibit transmission. This is in contrast to the results of Anthony & Paton (1977) who observed blockade of transmurally stimulated responses of the atropinized urinary bladder by indomethacin in the monkey and the rabbit. In the present experiments, no involvement of endogenous PGE was observed in maintaining the tone and the spontaneous rhythmic activity of the bladder since preincubation with indomethacin (up to 56 μM) did not affect them. In the light of our findings a modulatory role of PGE on the release of ACh and ATP in the guinea-pig urinary bladder may be postulated.

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Table 2 Effect of tetrodotoxin (TTX, 0.31 μM) on the release of prostaglandin E (PGE) from the guinea-pig urinary bladder under various conditions

Experimental conditions	*Prostaglandin output (ng/h)* *Normal*	*After TTX*	*No. of expts*
Resting	13 ± 3.2	18 ± 2.9	3
Nerve stimulation (50 μs; 20 Hz)	52 ± 3.8	16 ± 2.5	3
Muscle stimulation (5 ms; 20 Hz)	94 ± 5.6	99 ± 4.3	3

Prostaglandin output is given as PGE_1 equivalents; mean ± s.e. mean.

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