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Prostaglandin-release impairment in the bladder epithelium of streptozotocin-induced diabetic rats

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Abstract

Isolated epithelial layer preparations were obtained from urinary bladders of 4-week streptozotocin-diabetic rats and used for endogenous prostaglandins E_2 and $F_{2\alpha}$ determination. Tissues were incubated in modified Krebs solution under basal conditions, or in the presence of either indomethacin (5×10^{-7} M), ATP (10^{-5} and 10^{-3} M) or bradykinin (10^{-7} and 10^{-5} M), and samples of incubation medium were collected at 15 and 30 min. In the presence of indomethacin, the release of prostaglandins in the incubation medium was under the detection limit of the enzyme immunoassay (EIA). The epithelium from diabetic rat urinary bladders was thicker and heavier and the absolute amount of endogenous prostaglandins E_2 and $F_{2\alpha}$ was higher than for control animals, but when prostaglandin production was expressed as a fraction of tissue weight, it was reduced in diabetic epithelium. ATP and bradykinin has significantly increased the endogenous release of both prostaglandins from the epithelium when compared with the release under basal conditions. This increase was time-dependent and was higher in diabetic than in control tissues. ATP evoked a phasic and tonic contraction in bladder strips that was abolished by epithelium removal. Concentration–response curves for ATP did not differ among groups. Bradykinin evoked a long-lasting tonic contraction that was reduced significantly by epithelium removal in diabetic rat bladders only. Concentration–response curves for prostaglandin E_2 and $F_{2\alpha}$ in diabetic rat bladder differed significantly from that in controls and epithelium removal did not alter these responses. It is suggested that bradykinin receptors and P2X nucleotide receptors already found in the smooth muscle detrusor might be present in the epithelial layer of the bladder. The prostaglandin-release impairment observed in this study might be responsible, in part, for bladder abnormalities observed in pathological conditions, such as diabetes.

Keywords: Urinary bladder; Epithelium; Prostaglandin; Diabetes

1. Introduction

Epithelium is usually found at the interface between the organism and the environment (epidermis or bronchial), or between an organ and a fluid space. This location implies that regulation of permeability, transport and endocytosis is a major requirement. Nevertheless, the bladder epithelium, similar to bronchial epithelium (Matera et al., 1995), is more than just a passive barrier against urea and ion diffusion. Complex second messenger systems are active in epithelial cells. Both cyclic adenosine monophosphate (Chlapowski, 1975) and arachidonic acid metabolites (Brown et al., 1980) are part of the second messenger system in bladder epithelium. These pathways are important mediators of physiological as well as of pathological functions (Downie and Karmazyn, 1984).

We suggested earlier that, in pathological conditions such as diabetes, bladder epithelium might produce and release prostanoids (Pinna et al., 1992) and non-prostanoic compounds (Pinna et al., 1994) that can modulate bladder responsiveness. The aim of the present study was to assess whether an isolated epithelial layer from urinary bladder of 4-week streptozotocin-induced diabetic rats produced prostaglandin E_2 and $F_{2\alpha}$ in a greater amount than control tissues did. We also investigated whether exogenous bradykinin and ATP can affect prostaglandin production in the isolated epithelium. In fact, bradykinin, a well known mediator of inflammation (Maggi et al., 1989), and ATP, an excitatory cotransmitter in parasympathetic innervation of detrusor smooth muscle (Brown et al., 1979), evoke

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their contractile effects partly by activation of prostanoid synthesis (Nakahata et al., 1987; Kishii et al., 1992).

2. Materials and methods

2.1. Tissues preparation

Forty male Sprague–Dawley rats weighing 200–225 g (initial age ca. 2 months) were used for the experiments. Procedures involving animals and their care were conducted in conformity with the Institutional Guidelines that are in compliance with National (D.L. n. 116 G.U. suppl. 40, 18 Feb. 1992) and International Laws and Policies (EEC Council Directive 86/609, OJL 358, 1, 12 Dec. 1987; NIH Guide for the Care and Use of Laboratory Animals, NHI Publication No. 85-23, 1985). Diabetes was induced in half of the animals by a single injection of streptozotocin (65 mg/kg i.v.), as described previously (Pinna et al., 1992). The remaining rats were injected with the vehicle. At the end of 4 weeks, blood samples were collected from the tail for serum glucose determination using a glucose test (Glu-cinet, Sclavo diagnostics, Milan, Italy). Only rats with plasma glucose higher than 300 mg/dl were used for the experiments. The animals were killed by asphyxiation with CO_2, the urinary bladder was quickly removed, placed in cold modified Krebs solution and opened by a ventral incision from the urethra to the dome. The bladder and the urethra were then separated by a transverse cut at the level of the bladder neck and each bladder was cut into four longitudinal strips. The epithelial layer was removed under a dissecting microscope using forceps and scissors, in 80% of the strips. Bladder epithelium was also removed from half of the remaining intact strips and preparations, with or without epithelium, were used for contractile studies in organ baths.

2.2. Light microscopy

For histological studies, tissues from both control and diabetic rats were stored for 48 h in 7% sucrose in a phosphate saline buffer (PBS) containing 0.01% sodium azide at 4°C. The tissues were then frozen in Tissue–Tek (OCT embedding compound, BDH Laboratory Supplies) and stored in liquid nitrogen. Frozen 10-μm sections of the bladder body were then cut in a cryostat, thawed onto gelatine-coated slides and air-dried for 30 min. Sections were stained with hematoxylin (Hemalum Mayer's, BDH Laboratory Supplies) and eosin (Eosin yellowish, BDH Laboratory Supplies), and then examined by light microscopy (Axioplan, Zeiss).

2.3. Prostaglandin determination

The epithelial layers from control and diabetic rat bladder strips were first washed three times with fresh Krebs solution to remove prostanoids released by tissues during the mechanical trauma. Each tissue was then incubated in a glass vial with 2-ml modified Krebs solution gassed with 95% O_2 and 5% CO_2 at 37 ± 0.5°C and gently shaken. Samples of the medium were collected for prostaglandins E_2 and $F_{2\alpha}$ determination at the beginning of the experiment and after 15 and 30 min of incubation using a specific enzyme immunoassay (EIA), according to the manufacturer's instructions (Cayman, Ann Arbor, MI, USA). Prostaglandins were not detected in the incubation medium at the start of the experiments. In some experiments, tissues were incubated in the presence of either indomethacin (5×10^{-7} M), ATP (10^{-5} and 10^{-3} M) or bradykinin (10^{-7} and 10^{-5} M) to study the effect of these drugs on prostaglandin release. In the presence of indomethacin, the release of prostaglandins was under the detection limit of the EIA assay. Since it was known from our previous study that smooth muscle synthesizes and releases prostaglandins, some detrusor smooth muscle strips without epithelium were incubated under the same conditions and samples were collected at the same incubation times for prostaglandin determination, as positive controls.

2.4. Organ bath experiments

Each strip was attached to a holder by tying one end with a silk ligature and the other end to an isometric force transducer dynamometer coupled to a two-channel recorder (Gemini 7070, Basile, Italy). The strips were equilibrated for 1 h in a 5-ml organ bath containing modified Krebs solution gassed with 95% O_2 and 5% CO_2 at 37 ± 0.5°C. The strips were initially loaded to a tension of 1.5 g (14.7 mN). This resting tension for optimal force development was determined in preliminary experiments in which detrusor strips were stretched stepwise and stimulated with acetylcholine (3×10^{-5} M) at each length. After the equilibration period, each preparation was exposed to acetylcholine (EC_{50}: 3×10^{-5} M) until two reproducible contractions were obtained. Non-cumulative concentration–response curves to ATP, bradykinin, prostaglandin E_2 and $F_{2\alpha}$ were run. Each preparation was challenged with only one drug. The tissues were then washed several times with fresh Krebs solution and left in the bath until the resting tone had recovered and tonic contractions had ceased. After this equilibration period (not less than 1 h), the tissues were incubated for 1 h with indomethacin (5×10^{-7} M) and the concentration–response curves for ATP and bradykinin were repeated to confirm the involvement of prostaglandins in their responses.

2.5. Solutions and drugs

The modified Krebs solution was composed as follows (mM): NaCl, 133; KCl, 4.7; $CaCl_2$, 2.5; NaH_2PO_4, 1.4; $NaHCO_3$, 16.4; $MgSO_4$, 0.6; glucose, 7.7.

Acetylcholine hydrochloride, ATP, bradykinin, indomethacin, prostaglandin E_2 and $F_{2\alpha}$ were all purchased from Sigma–Aldrich (Milan, Italy). Streptozotocin was a gift from Upjohn (Caponago, Italy). Stock solutions of the drugs were prepared in distilled water, except streptozotocin, which was prepared in citrate buffer.

2.6. Data analysis

All the data in Table 1 are the means ± SEM of six individual observations, and represent the average amount of prostaglandin E_2 or $F_{2\alpha}$ released by an epithelial preparation excised from a detrusor strip. Epithelial preparations from the same bladder were used in different experiments. For instance, the four epithelial preparations obtained from each bladder were used as follows: first and second tissue for prostaglandin E_2 determination in the presence of either ATP or bradykinin, third and fourth tissue for prostaglandin $F_{2\alpha}$ determination in the presence of either ATP or bradykinin. Data were expressed as pg mg^{-1} wet tissue for 15 min or 30 min of incubation. Concentration–response curves were prepared with the help of the computer programme PRISM: for each curve the programme calculates the lower and upper plateau, the slope, the EC_{50} and the pD_2 value ± SEM. Contractile responses to ATP, bradykinin and prostaglandins were expressed as tension (mN mg^{-1} wet tissue), and each point on the curves represents the mean of six observations. To evaluate the long-lasting tonic contraction evoked by bradykinin in the detrusor strips, the first peak and the amplitude of contractions 1 and 3 min after bradykinin administration were measured. Then, the mean of the three values was used as the correct bradykinin-induced contraction. A one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test was used to compare pD_2 values and maximal responses to drugs. When two concentration–response curves were compared, a two-way ANOVA was used according to Ludbrook (1994). The data in Table 1 were analysed with a multi-way ANOVA (general linear model), by means of the computer programme, MINITAB. $P < 0.05$ was considered significant.

3. Results

Bladders from 4-week diabetic rats were significantly larger than those from age-matched controls: 225 ± 16 vs. 127 ± 6 mg wet weight ($P < 0.005$, $n = 20$). Detrusor strips from diabetic rats were thicker and heavier (12-mm length, 4-mm width and 40.6 ± 1.7 mg wet weight) than control detrusor strips (10-mm length, 3-mm width and 16.6 ± 0.9 mg wet weight). Epithelium excised from strips of diabetic tissues was also thicker and heavier than that from the controls, weighing 10.7 ± 1.05 and 4.04 ± 0.31 mg wet weight, respectively ($P < 0.005$, $n = 30$), (Fig. 1).

3.1. ATP-induced responses

Fig. 2 shows typical tracings of the response induced by a submaximal concentration of ATP (EC_{80}: 10^{-3} M) in control (A) and diabetic (B) detrusor smooth muscle strips with (left) and without epithelial layer (right). ATP elicited

Table 1
Endogenous release (pg mg^{-1} wet tissue) of prostaglandin E_2 (A) and $F_{2\alpha}$ (B) from epithelial preparations of control and 4-week diabetic rat bladders. Prostaglandin release was evoked by ATP, bradykinin (BK) or under basal conditions. Each value represents the mean ± SEM of six individual observations. Samples of incubation medium were collected for analysis after 15 and 30 min of incubation

	15-min incubation		30-min incubation	
	Control	Diabetic	Control	Diabetic
(A) Prostaglandin E_2 release				
Basal	1626 ± 96	506 ± 32[a]	1744 ± 140	511 ± 36[a]
+ ATP 10 M	1937 ± 50	782 ± 93[a]	2142 ± 54	1025 ± 100[a]
+ ATP 1 mM	2532 ± 104[b,c]	1002 ± 133[a,b]	2908 ± 145[b,c]	1240 ± 94[a,b]
+ BK 0.1 M	1860 ± 150	1108 ± 109[a,b]	2449 ± 55[b,d]	1216 ± 102[a,b]
+ BK 10 M	3228 ± 140[b,e]	1229 ± 48[a,b]	4112 ± 176[b,e]	1504 ± 34[a,b]
(B) Prostaglandin $F_{2\alpha}$ release				
Basal	254 ± 12	150 ± 14	291 ± 26	236 ± 38[d]
+ ATP 10 M	343 ± 48	183 ± 15	598 ± 48[b,d]	458 ± 30[d]
+ ATP 1 mM	690 ± 68[b,c]	301 ± 8[a,b]	1277 ± 151[b,c,d]	534 ± 17[a,b,d]
+ BK 0.1 M	653 ± 59[b]	426 ± 56[b]	1032 ± 23[b,d]	724 ± 109[b,d]
+ BK 10 M	720 ± 103[b]	575 ± 63[b]	1232 ± 156[b,d]	1043 ± 72[b,d]

[a] $P < 0.05$ (vs. control, same treatment, same incubation time).
[b] $P < 0.05$ (vs. basal conditions, same incubation time).
[c] $P < 0.05$ (vs. ATP 10 μM, same incubation time).
[d] $P < 0.05$ (vs. 15 min, same treatment).
[e] $P < 0.05$ (vs. BK 0.1 μM, same incubation time).

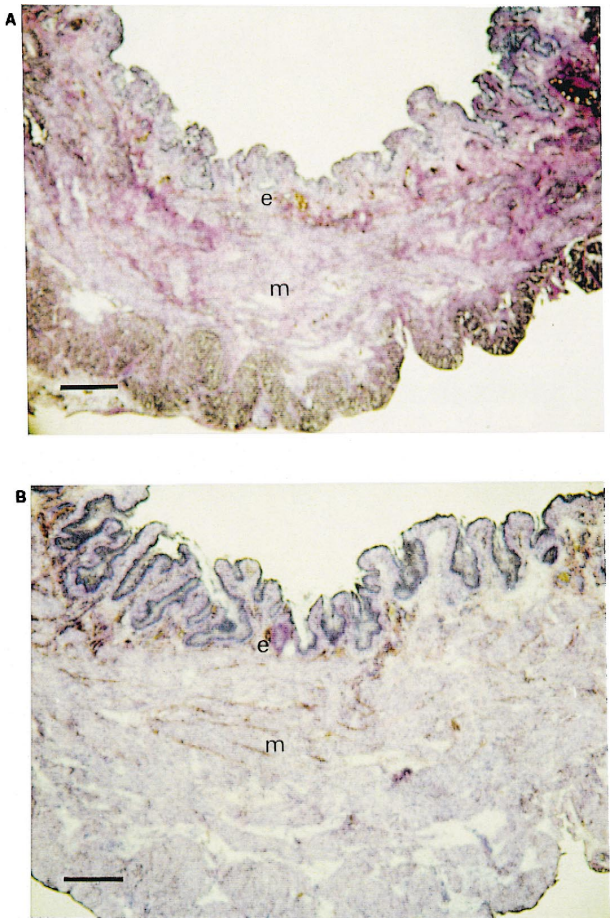

Fig. 1. Light-microscope micrograph of section of urinary bladder strips from control (A) and 4-week diabetic rats (B). Diabetic strips were thicker than those of controls. Epithelium (e) and smooth muscle layer (m). Scale bar = 300 μm.

a phasic contraction followed by a secondary tonic component that was present in intact tissues of control and diabetic rats, and was almost totally abolished in strips devoid of epithelium. The secondary tonic component was also inhibited by incubation of tissues with indomethacin (5×10^{-7} M). Fig. 2C shows concentration–response curves for ATP in all groups. Maximal responses to ATP (mN/mg wet tissue) were: 0.37 ± 0.05, 0.47 ± 0.08, 0.27 ± 0.05, 0.33 ± 0.09 (ANOVA, $P = 0.25$, $n = 6$) and pD_2 values were: 2.83 ± 0.4, 2.93 ± 0.15, 3.10 ± 0.08 and 2.90 ± 0.26 (ANOVA, $P = 0.9$, $n = 6$) in control, with and without epithelium, diabetic, with and without epithelium, respectively. The curves for ATP did not differ significantly among groups (ANOVA, $P = 0.2$, $n = 6$).

3.2. Bradykinin-induced responses

Fig. 3 shows the long-lasting tonic contraction evoked by bradykinin (10^{-5} M) in control (A) and diabetic (B) detrusor smooth muscle strips with (left) and without epithelial layer (right). Epithelium removal did not alter significantly the bradykinin-induced tonic response in control tissues, whereas it greatly reduced the bradykinin response in diabetic tissues. The amplitude of the bradykinin tonic contraction was also greatly reduced by incubation with indomethacin (5×10^{-7} M). Fig. 3C shows concentration–response curves to bradykinin for all groups. Maximal responses to bradykinin (mN/mg wet tissue) were: 0.32 ± 0.05, 0.28 ± 0.08, 0.26 ± 0.04, 0.16 ± 0.03 (ANOVA, $P = 0.2$, $n = 6$) and pD_2 values were: 6.72 ± 0.1, 7.47 ± 0.14, 7.75 ± 0.36 and 6.30 ± 0.3 in control, with and without epithelium, diabetic, with and without epithelium, respectively. Significant differences between pD_2 values were found in controls without epithelium versus diabetics without epithelium (ANOVA, $P < 0.05$, $n = 6$) and in diabetics with epithelium vs. diabetics without epithelium (ANOVA, $P < 0.01$, $n = 6$). The curve for bradykinin in diabetic strips with epithelium differed sig-

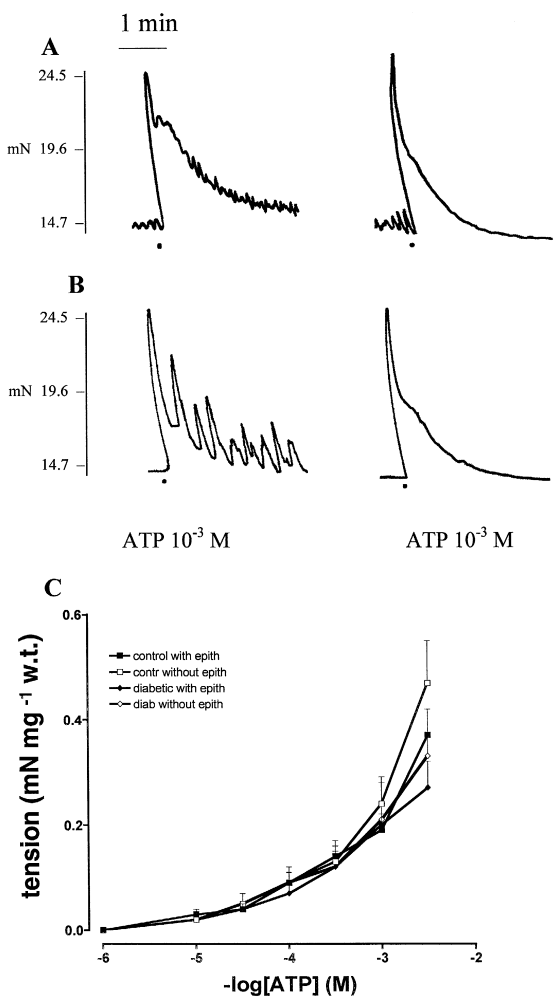

Fig. 2. Original tracing of responses to ATP (10^{-3} M) in detrusor smooth muscle strips of control (A) and diabetic rats (B). Responses in intact preparations are represented in the left panel, responses in epithelial-free preparations are in the right panel. Concentration–response curves for ATP in all groups (C) were not significantly different (ANOVA, $P > 0.05$, $n = 6$).

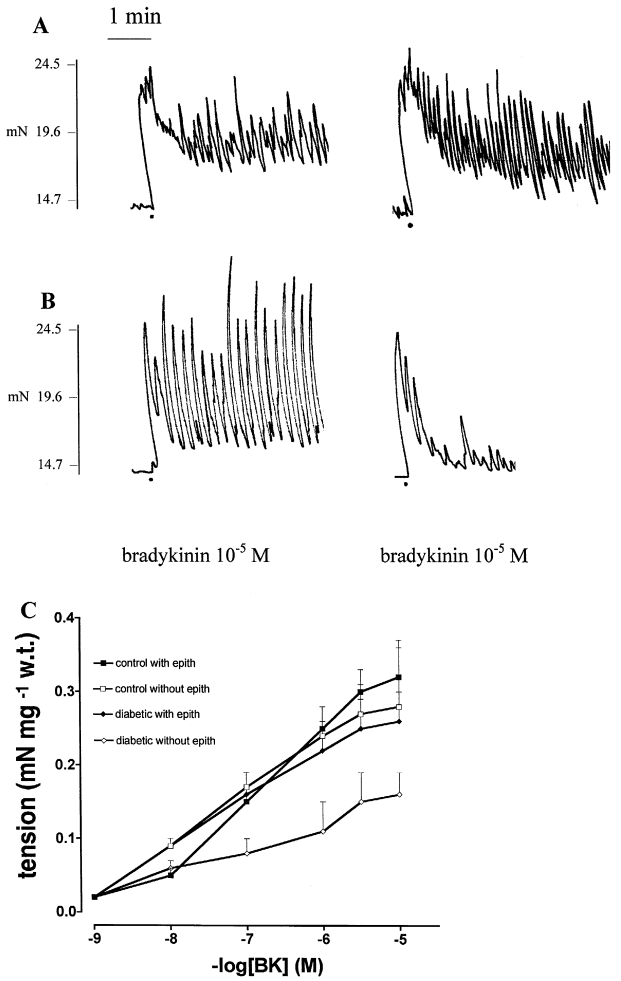

Fig. 3. Original tracing of responses to bradykinin (10^{-5} M) in detrusor smooth muscle strips of control (A) and diabetic rats (B). Responses in intact preparations are represented in the left panel, responses in epithelial-free preparations are in the right panel. Concentration–response curve to bradykinin (C) for epithelium-free bladders of diabetic rats differed significantly from curve obtained for intact bladder of diabetic rats (ANOVA, $P < 0.05$, $n = 6$).

nificantly from that of diabetic strips without epithelium (ANOVA, $P < 0.01$, $n = 6$).

3.3. Prostaglandin-induced responses

Fig. 4 shows a concentration–response curve for prostaglandin E_2 in all groups. Maximal responses to prostaglandin E_2 (mN/mg wet tissue) were: 1.08 ± 0.09, 1.30 ± 0.1, 0.96 ± 0.04, 1.05 ± 0.07 (ANOVA, $P = 0.2$, $n = 6$) and pD_2 values were: 8.77 ± 0.18, 8.54 ± 0.8, 6.51 ± 0.34 and 6.97 ± 0.14 in control strips, with and without epithelium, diabetic strips, with and without epithelium, respectively. Significant differences between pD_2 values were found in controls, with or without epithelium vs. diabetic strips with epithelium (ANOVA, $P < 0.05$, $n = 6$). The curves for prostaglandin E_2 in control tissues differed significantly from that of diabetic strips (ANOVA, $P < 0.01$, $n = 6$). Epithelium removal did not affect significantly the concentration–response curves. Prostaglandin F_2-induced contractions and curves in all groups were similar in amplitude and trend to those observed with prostaglandin E_2.

3.4. Prostaglandin E_2 and $F_{2\alpha}$ release

Table 1 shows the amount of prostaglandin E_2 (panel A) and $F_{2\alpha}$ (panel B) released by the epithelium of control and diabetic urinary bladder in the incubation medium, and expressed as pg/mg wet tissue. The release of prostaglandin was measured under basal conditions, or in the presence of either indomethacin (5×10^{-7} M), ATP (10^{-5} M and 10^{-3} M) or bradykinin (10^{-7} M and 10^{-5} M), after 15 and 30 min of incubation. In the presence of indomethacin, the level of both prostaglandins was below the detection limit of EIA. The absolute amount of the endogenous prostaglandins E_2 and $F_{2\alpha}$ was higher than that of control animals, but when prostaglandin production was expressed as fraction of tissue weight, it was reduced in diabetic epithelium.

ATP and bradykinin significantly increased the release of endogenous prostaglandins E_2 and $F_{2\alpha}$ both in control and in diabetic rat bladder epithelium compared to the basal release. The increase in prostaglandin E_2 release induced by ATP was higher in diabetic tissues than in controls, being 54% vs. 19% (at 10 μM ATP) and 98% vs. 56% (at 1 mM ATP) in diabetic vs. control tissues, respectively. The increase in prostaglandin release induced by bradykinin, was greater than that evoked by ATP, being 119% vs. 14% (at 0.1 μM of bradykinin), and 142% vs. 98% (at 10 μM of bradykinin) in diabetic vs. control tissues, respectively. Prostaglandin release was also time-dependent. Smooth muscle detrusor strips from diabetic

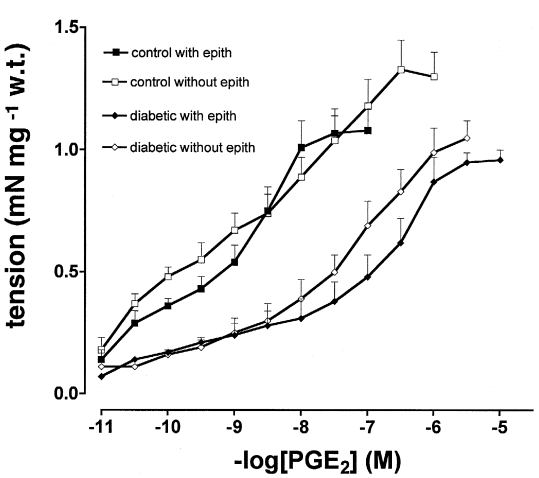

Fig. 4. Concentration–response curve for prostaglandin E_2 in all groups. Epithelium-free bladders of diabetic rats differed significantly from curve obtained with intact bladder of diabetic rats (ANOVA, $P < 0.05$, $n = 6$).

and control rats were also used in some experiments for prostaglandin determination. Strips of epithelium-free bladder from diabetic rats released similar amount of prostaglandin E_2 when compared with controls (4958 ± 770 pg and 5057 ± 600 pg), whereas the release of prostaglandin F_2 appeared to be increased in diabetic strips, being 5313 ± 660 pg and 2865 ± 520 pg in diabetic and control animals.

4. Discussion

Bladder epithelium, similar to other epithelia, is more than just a passive barrier, and it produces second messengers such as prostaglandins $F_{2\alpha}$ and E_2 and probably some other non-prostanoic compounds (Pinna et al., 1992, 1994) which are important mediators of bladder function under both physiological and pathological conditions. Under normal conditions prostanoids elicit contractions directly in the smooth muscle and act on sensory fibres as local modulators of urinary bladder function (Maggi, 1992), but an increased synthesis of prostanoids during epithelial irritation may produce hyperactivity or spasm of the smooth muscle detrusor (Hoyle, 1994).

The results obtained in the present study confirm the view that the epithelial layer produces and releases prostanoids, as already shown (Brown et al., 1980; Downie and Karmazyn, 1984). Our results also gave evidence that 4-week streptozotocin-induced diabetes significantly altered the production of prostaglandins E_2 and $F_{2\alpha}$ from the bladder epithelium. The absolute amount of endogenous prostaglandins E_2 and $F_{2\alpha}$ was higher compared to that from control animals, but when prostaglandin production was expressed as fraction of tissue weight, it was reduced in diabetic epithelium. Our previous study (Pinna et al., 1992) and others (Tammela et al., 1994) have shown an increase in spontaneous activity of bladder strips from streptozotocin-diabetic rats that was suppressed or reduced by indomethacin. This increased activity observed in ''in vitro'' experiments is probably attributable to a direct action of endogenous prostaglandins on the smooth muscle rather than on sensory endings located on the tissue. Evidence indicates that prostaglandins are generated in the bladder as a result of neurotransmitter activity (Hoyle et al., 1994). Two forms of cyclo-oxygenase activity are present in mammalian cells (Harris et al., 1994; Kargman et al., 1996). Cyclooxygenase-1 is expressed in many normal tissues, whereas cyclooxygenase-2, not detected in healthy tissues, is induced in response to pro-inflammatory cytokines, lipopolysaccharide and growth factors. The increase in prostaglandin production observed in the bladder epithelium during diabetes might be due to the inflammatory condition associated with diabetes, and might be considered as a compensatory mechanism against diabetic neuropathy affecting sensory and motor nerves.

Our results also showed that bradykinin and ATP were able to stimulate the release of prostaglandins in the epithelium. ATP elicited a biphasic response in intact detrusor strips, the phasic component of the ATP-induced response was not affected significantly by epithelium removal. On the contrary, the tonic contraction evoked by ATP was almost totally abolished by epithelium removal both in control and in diabetic tissues. Epithelium removal did not significantly affect the tonic contraction evoked by bradykinin in control tissues, whereas it significantly reduced the response in diabetic tissues. A possible explanation for the difference observed between ATP-and bradykinin-evoked prostaglndin release might be related to the fact that ATP is a physiological cotransmitter and bradykinin is a mediator of inflammation which may activate not only the constitutive form of cyclooxygenase, but also the inducible form, leading to the observed increase in prostaglandin production. These data confirm a smooth muscle localization of both bradykinin receptors and P2X nucleotide receptors and also suggest their localization in the epithelial layer. This finding is in agreement with the results of Zenser et al. (1988), who found a bradykinin-induced release of prostaglandin E_2 in primary cultures of human epithelial cells.

There are still a few papers dealing with the involvement of epithelium in the modulation of bladder responsiveness, but some new studies are consistent with the hypothesis put forth in Section 1. Ferguson et al. (1997) have found that epithelial cells are able to synthesize and release ATP during the filling phase of the bladder. More recently, another group has found that bladder epithelium can release nitric oxide (Birder et al., 1998). Such a release induced by noradrenaline and capsaicin may provide a mechanism for modulation of bladder and urethral function. These studies might lead to new approaches for the development of more active drugs against bladder dysfunctions.

In conclusion, the present results confirm the view that bladder epithelium is a source for prostaglandin synthesis and show that diabetes impairs endogenous prostaglandin E_2 and $F_{2\alpha}$ release from epithelial preparations. The dysfunctions of urinary bladder in streptozotocin-diabetic rats, such as urinary retention, are believed to be related to alterations in both afferent and efferent neurons of the autonomic system (Öztürk et al., 1996). Since prostaglandins act directly on the smooth muscle, but also as modulators of the sensory signal from the bladder to the central nervous system, it is possible that the impairment in prostaglandin release seen in the bladder epithelium might be in part responsible for bladder abnormalities occurring during diabetes. Our results also showed that ATP and bradykinin are able to increase the release of endogenous prostaglandins from the bladder epithelium, suggesting the presence of both bradykinin-receptors and P2X nucleotide receptors not only in the detrusor smooth muscle but also in the epithelial layer.

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