Contribution of Prostaglandin E₂ to Bradykinin-Induced Contraction in Rabbit Urinary Detrusor

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Abstract—Bradykinin (100 pM to 1 μ M) contracted the rabbit urinary detrusor in vitro. The sensitivity to bradykinin was about 1000 times higher than that to acetylcholine (ACh) on a molar basis. The contractile response to bradykinin was unaffected by atropine, diphenhydramine, FPL-55712, methysergide, prazosin or tetrodotoxin, indicating that the contraction was not mediated via the release of ACh, histamine, peptide leukotrienes, serotonin or catecholamine. The bradykinin-induced contraction was, however, inhibited by indomethacin (5 μ M), a cyclooxygenase inhibitor. Caffeic acid (10 μ M), a lipoxygenase inhibitor, did not affect the contraction. Bradykinin (1 nM to 100 nM) stimulated the release of prostaglandin E₂ from the detrusor in a concentration-dependent manner, and the release was abolished by treatment with indomethacin (5 μ M). Prostaglandin (PG) E₂ contracted the urinary detrusor with an EC₅₀ of about 0.1 μ M. Adenosine 5'-triphosphate (ATP), a stimulator of PG synthesis, also contracted the muscle with an EC₅₀ of about 100 μ M. [¹⁴C]Arachidonic acid was converted to PGE₂ and F_{2 α} when it was incubated with the 700 $\times$ g supernatant of the muscle homogenate. However, neither bradykinin nor ATP stimulated the PG synthesis in the supernatant. These results showed that bradykinin and ATP did not affect the cyclooxygenase and/or PG degradation system. On the other hand, when the intact detrusor muscle was incubated with [¹⁴C]arachidonic acid, bradykinin and ATP stimulated the PG synthesis, and the stimulated synthesis was inhibited by indomethacin. Mepacrine, a phospholipase A₂ inhibitor, more potently inhibited the bradykinin- and ATP-induced contractions than the ACh-induced one. Therefore, bradykinin as well as ATP would stimulate phospholipase, probably the A₂-type, after binding of its receptor, and contract rabbit urinary detrusor mediated via PGE₂ converted from arachidonic acid.

Bradykinin elicits contraction in non-vascular smooth muscles, including uterine (1), trachea (2) and urinary bladder (3). In the intestine, the biphasic response to bradykinin was observed (4). Bradykinin, however, elicits relaxation in vascular smooth muscle, and two mechanisms of the bradykinin-induced relaxation have been proposed (5): one is mediated by PGs, and the other is by endothelial cell derived relaxing factors (EDRF). The former is observed in rabbits and cats, and the latter is in dogs and humans (5). While there have been a number of investigations about the mechanisms of

bradykinin-induced vasodilation (6–8), there is yet insufficient data for understanding the mechanism of bradykinin-induced effects on smooth muscle preparations of urinary bladder.

In rabbit urinary detrusor, Downie and Rouffignac (9) showed that bradykinin elicited a contraction partially mediated through the release of PGs, based on the observations that naproxen, a cyclooxygenase inhibitor, inhibited the contractile response to low concentrations of bradykinin. In the bladder, bradykinin could elicit contraction at low concentrations (9, 10),

indicating its importance in the physiological and/or pathological state. In the present study, therefore, we investigated the bradykinin-induced contraction in rabbit urinary detrusor in vitro and show that bradykinin elicits contraction accompanied with PG generation through an activation of phospholipase A₂.

Materials and Methods

Mechanical activity: Rabbits of either sex, weighing about 2.5 kg, were anesthetized with intramuscular injection of sodium pentobarbital (30 mg/kg) and were bled from the carotid arteries. Urinary bladder was resected, and the muscle was removed from the mucosa. The muscular segment (0.2×1.5 cm) in longitudinal section was suspended in an organ bath containing 20 ml of Krebs-Ringer solution. The organ bath was bubbled with a mixture of 95% O₂ and 5% CO₂ and kept at 37°C. The composition of the solution was (mM) NaCl, 119; KCl, 4.7; CaCl₂, 2.5; KH₂PO₄, 1.2; MgSO₄, 1.2; NaHCO₃, 25; and glucose, 11. The tension development was isometrically recorded on a pen recorder through an FD pick-up (Nihon Kohden, SB-1T) and a carrier amplifier (Nihon Kohden, RP-3). Tension of the muscle was adjusted to 2.0 g at the beginning of the experiment, and the drugs were tested at least 1 hr after the suspension of the muscle. The tension of the muscle decreased to 0.2 to 1.0 g during the experiment.

Extraction and assay of PGE₂: The muscle bundle was incubated in Krebs-Ringer solution, in the same way as the case when mechanical activity was recorded, except with a bath volume of 10 ml. The muscle was preincubated for 10 min, followed by 3 min incubation with drugs. Then, Krebs-Ringer solution was rapidly collected. When indomethacin was used, the muscle was incubated with indomethacin (5 μM) for 10 min, and the incubated solution was discarded. Then, the muscle was incubated in a new Krebs-Ringer solution containing indomethacin (5 μM) for 10 min, followed by 3 min incubation with drugs. The collected solution was kept at -20°C until extraction. PGs were extracted twice with 10 ml of

ethyl acetate after the solution was acidified to pH 4.0 with 1 N HCl. Ethyl acetate was evaporated under reduced pressure at 40°C, using a rotary evaporator. The dried sample was dissolved in 1 ml of 10 mM Tris-buffer (pH 7.6), and 0.1 ml of the sample solution was immediately prepared for radioimmunoassay (RIA). Methods of RIA for PGE₂ are similar to our previous report on determining the E type of PGs (11). In brief, the assay mixture consisted of 0.1 ml of sample or standard, 0.1 ml of anti-serum (450 times dilution) and 0.1 ml of 0.1 μCi/ml of [³H]-PGE₂. Anti-serum to PGE₂ was kindly given to us by Ono Pharmaceutical Co., Ltd. (Japan), and its cross-reactivity for other PGs is as follows: PGE₁, 51%; PGF_{2α}, 3.0%; thromboxane B₂, 0.02%; and 6-keto-PGF_{1α}, 0.2%. Specific activity of [³H]PGE₂ used was 160 Ci/mmol. Anti-serum and labelled PGE₂ were dissolved in 0.1 M Tris-buffer (pH 7.6) containing 0.5% bovine serum albumin (BSA). Sample and standard were dissolved in 10 mM Tris-buffer (pH 7.6). The mixture was incubated at 4°C overnight. After addition of 0.5 ml of dextran-coated charcoal (1 g of Norit Extra, 150 mg of dextran and 1 g of BSA in 300 ml of water) to the mixture, the preparation was centrifuged for 2 min. [³H]PGE₂ bound to anti-serum in the supernatant (0.5 ml) was counted with a liquid scintillation counter (Aloka, LSC-900) using toluene-based scintillator, the composition being as follows: 4.0 g 2,5-diphenyloxazole (PPO) and 0.1 g 1,4-(2-(5-phenyl-oxazolyl))-benzene (POPOP) in 1000 ml of toluene and 500 ml of triton X-100. The recovery of PGE₂ (5 ng) added during the whole procedure was 110.5 ± 4.65% (n=6). Although anti-serum to PGE₂ crossreacts to PGE₁ by 51%, PGE determined here was expressed as PGE₂, because the endogenous substrate for phospholipase A₂ is mainly phospholipids containing arachidonic acid.

Arachidonic acid metabolism: Analysis of the metabolites of [¹⁴C]arachidonic acid was similar to the previous report (12). For the assay of cyclo-oxygenase, the 700×g supernatant of the homogenate in 50 mM Tris-buffer (pH 7.9) containing 10 mM MgCl₂ was used. One ml of the supernatant

(6.6 mg protein) was incubated with [14 C]-arachidonic acid (2 μ Ci, specific activity of 58 mCi/mmol) for 40 min in the presence or absence of drugs. The reaction was terminated by acidification to pH 4.0 by 1 N HCl, and arachidonic metabolites were extracted twice with ethyl acetate. After ethyl acetate was volatilized in a stream of nitrogen gas, the metabolites were dissolved in 0.1 ml of methanol, and 70 μ l of the methanol was put on silica gel plate (Whatman, LK-50) for thin layer chromatography (TLC). The composition of the solvent for TLC was benzene/dioxane/acetic acid=60/30/3 in volume. The authentic standards used were PGE₂, PGF_{2 α} , PGD₂ and arachidonic acid. After development, the plate was dried and iodine was sprayed on the plate. The authentic standards were stained a dark color. The silica gel was cut in 5 mm wide pieces, and the radioactivity of each silica gel fragment was counted in a liquid scintillation counter.

Another experiment was performed using intact muscles (about 250 mg) in order to examine phospholipase activity. The muscle was mounted on a stainless steel rod, and it was preincubated with [14 C]arachidonic acid (4 μ Ci) for 40 min in 1 ml of Krebs-Ringer solution bubbled with a mixture of 95% O₂ and 5% CO₂. Krebs-Ringer solution removed from the muscle was acidified to pH 4.0 by 1 N HCl at 5 min after drug application. Extraction or analysis of the arachidonic acid metabolites was performed in the same manner as described above.

Drugs and statistical analysis: Drugs used were bradykinin (Sigma), acetylcholine chloride (Daiichi), atropine sulfate (Wako), diphenhydramine hydrochloride (Sigma), FPL-55712 (Fujisawa), methysergide (Sandoz), prazosin (Eisai), tetrodotoxin (Sigma), indomethacin (Sumitomo), caffeic acid (Sigma), adenosine 5'-triphosphate disodium (ATP, Yamasa), mepacrine (Sigma), prostaglandin (PG) E₂ (Ono), PGF_{2 α} (Ono), PGD₂ (Ono) and arachidonic acid (Sigma). Other chemicals were obtained from Wako Pure Chemicals. [3 H]PGE₂ and [14 C]-arachidonic acid were obtained from Amersham.

The statistical significance of difference between values obtained was determined

using the paired Student's *t*-test. The pD₂ value, negative logarithm of EC₅₀, was calculated according to Rossum (13).

Results

1. Effects of bradykinin and ACh on rabbit urinary detrusor: Bradykinin elicited contraction in rabbit urinary detrusor (Fig. 1). The threshold concentration of bradykinin was about 100 pM, and the pD₂ value was 8.01 $\pm$ 0.17 (n=8). The pD₂ value of ACh, which has been assumed to be one of the transmitters in parasympathetic pelvic nerves, was 4.68 $\pm$ 0.12 (n=6). The sensitivity to bradykinin was about a thousand times higher than that to ACh on a molar basis. The contraction induced by bradykinin appeared slowly, and a small relaxation was sometimes observed just after its application. Atropine (0.1 μ M), diphenhydramine (1 μ M), FPL-55712 (1 μ M), methysergide (1 μ M) and prazosin (1 μ M) did not change the bradykinin-induced contraction (Fig. 2), indicating that bradykinin elicited contraction, not mediating the release of ACh, histamine, peptide leukotrienes, serotonin or catecholamine. Tetrodotoxin (0.3 μ M) did not influence the contraction induced by bradykinin (Fig. 2).

Fig. 1. Dose-response curve for bradykinin (BK) and acetylcholine (ACh). Ordinate: % of contractile response (the response to 3 mM ACh was taken as 100%). Abscissa: concentration of agonist ($-\log(M)$). Each point represents the mean of 8 observations for bradykinin (○) and 6 observations for acetylcholine (●) with S.E.

Fig. 2. Effects of various antagonists on bradykinin (BK)-induced contraction. Bradykinin of 50 nM was applied. The concentration of each antagonist is shown in the figure.

2. Effects of indomethacin and caffeic acid on bradykinin-induced contraction: Indomethacin (5 μ M), a cyclo-oxygenase inhibitor, reduced the bradykinin-induced contraction to a great extent (Fig. 3). However, caffeic acid (10 μ M), a lipoxygenase inhibitor, did not affect the contraction. These findings show that bradykinin elicits a generation of the PGs, and the PGs produce a contraction.

3. Release of PGE₂ by bradykinin: As indomethacin reduced the bradykinin-induced contraction, it was examined whether bradykinin stimulated the PG synthesis or not by direct determination of PGE₂. Bradykinin was able to stimulate the release of PGE₂ in a dose-dependent manner (Fig. 4). The contractile response of the preparation used for PG release by bradykinin is also shown in Fig. 4. The PGE₂ release was

Fig. 3. Effects of indomethacin (I) and caffeic acid (C) on bradykinin (BK)-induced contraction. Upper traces are typical ones. Summarized data are shown in the lower figure. Ordinate: % of contractile response (the response to 50 nM bradykinin was taken as 100%). Indomethacin (5 μ M) treatment was for 20 min, and caffeic acid (10 μ M) treatment was for 10 min. The data are shown as the mean of 6 observations with S.E. *: Significant difference from control ($P < 0.05$).

closely related to the contractile response to bradykinin. Indomethacin (5 μ M) inhibited the bradykinin-induced release of PGE₂, as well inhibiting the contraction. When 100 nM of bradykinin was applied, bradykinin-induced release of PGE₂ (increase from control) was about 260 ng/g, which was about 0.74 μ M if the specific gravity of the tissue was calculated as 1.

4. Effects of PGE₂ and ATP on rabbit urinary detrusor: PGE₂ contracted the rabbit urinary detrusor in a dose-dependent manner (Fig. 5). The pD₂ value of PGE₂ was 7.03 ± 0.12 ($n=8$). The sensitivity of the detrusor to PGE₂ was about ten times lower than that to bradykinin on a molar basis. However, bradykinin could release PGE₂, concen-

Fig. 4. Effect of bradykinin (BK) on prostaglandin E₂ (PGE₂) release. PGE₂ release into Krebs-Ringer solution is shown in the open columns. Exact values of PGE₂ were as follows (ng/g of wet weight tissue): control, 50.8 ± 9.68; 1 nM BK, 110.2 ± 12.66; 10 nM BK, 240.3 ± 38.82; 100 nM BK, 313.1 ± 28.05; 100 nM BK + 5 µM indomethacin, 12.5 ± 2.27. Bradykinin-induced contraction is shown in the hatched columns, when PGE₂ was assayed. The data are shown as the mean of 6 observations with S.E. See Methods for details. *: Significant difference from control ($P < 0.05$).

Fig. 5. Dose-response curves for prostaglandin E₂ (PGE₂) and adenosine 5'-monophosphate (ATP). Ordinate: % of contractile response (the response to 3 µM PGE₂ or to 3 mM ATP was taken as 100%). Abscissa: concentration of agonist (-log(M)). Each point represents the mean of 8 observations for PGE₂ (○) and 7 observations for ATP (●) with S.E.

trations of which were enough to produce contraction (Figs. 4 and 5). On the other hand, ATP, a known stimulator of PG synthesis (14), also contracted rabbit urinary

detrusor in a dose-dependent manner. The pD₂ value was 4.17 ± 0.13 (n=7). The sensitivity to ATP was about ten thousand times lower than that to bradykinin.

5. Effects of bradykinin and ATP on [14 C]-arachidonic acid metabolism in cell-free and intact preparations: [14 C]Arachidonic acid was converted to [14 C]PGE₂ and [14 C]PGF_{2 α} by incubation with the 750 $\times$ g supernatant of the muscle homogenate (Fig. 6). [14 C]-PGE₂ and [14 C]PGF_{2 α} converted from [14 C]-arachidonic acid were not increased by treatment with bradykinin (1 μ M) or ATP (100 μ M) (Fig. 6). These data indicated that bradykinin and ATP did not affect cyclo-

oxygenase or conversion of arachidonic acid to PGs. When the intact detrusor was used, bradykinin (1 μ M) and ATP (100 μ M) increased the conversion of [14 C]arachidonic acid to [14 C]PGE₂ and [14 C]PGF_{2 α} (Fig. 7). Bradykinin and ATP could stimulate the production of [14 C]PGE₂ and [14 C]PGF_{2 α} (only when the phospholipid of the intact muscle was prelabelled with [14 C]-arachidonic acid). The augmented synthesis of [14 C]PGE₂ and [14 C]PGF_{2 α} was reduced by treatment with indomethacin (5 μ M) (Fig. 7).

7. Effects of mepacrine on bradykinin-, ATP- and ACh-induced contractions: Mepacrine (1–100 μ M), a phospholipase A₂ inhibitor, reduced the contractile responses to bradykinin and ATP in a dose-dependent manner (Fig. 8). Mepacrine also inhibited ACh-induced contraction to a small extent. The inhibitory effect of mepacrine on bradykinin- or ATP-induced contraction is significantly different from its effect on the ACh-induced one.

Discussion

The present study showed that bradykinin elicits contraction mediated via PG synthesis in rabbit urinary detrusor. The increased release of PGE₂ by bradykinin is closely correlated to the contraction. The concentration of bradykinin to produce contraction was extremely low (the EC₅₀ of about 10 nM), as compared with ACh, ATP and PGE₂. Similar observations that bradykinin elicited contraction at low concentrations in rabbit urinary bladder were reported by Downie and Rouffignac (9). It has been well known that bradykinin elicits pain, and the pain is partially weakened by anti-inflammatory drugs (15). If the pain would be augmented by bradykinin-induced contraction in the case of an inflammation of the bladder, an anti-inflammatory drug, such as indomethacin, could attenuate the pain in a peripheral site of action to avoid the contraction.

The transmitter of atropine-resistant excitatory nerves in the urinary bladder still remains unknown (10). ATP is one of the candidates for the transmitter of the nerves (16–20). ATP might stimulate phospholipase A₂ and prostaglandin generation in rabbit

Fig. 6. Effects of bradykinin (BK) and adenosine 5'-triphosphate (ATP) on conversion of [14 C]-arachidonic acid by incubation with the 700 $\times$ g supernatant of muscle homogenate. Data are expressed as % of total radioactivities extracted by ethyl acetate. The radioactivities of [14 C]PGE₂ were (dpm/mg protein): control, 245.5; BK (1 μ M), 246.2; ATP (100 μ M), 235.1; and those of [14 C]-PGF_{2 α} were: control, 217.1; BK, 241.1; ATP, 213.5. It is noteworthy that neither BK (1 μ M) nor ATP (100 μ M) stimulates PG synthesis in a cell-free preparation. See Methods for details.

Fig. 7. Stimulation of conversion of [^{14}C]arachidonic acid by bradykinin (BK) or adenosine 5'-monophosphate (ATP) in incubation with intact muscle and its inhibition by indomethacin (IND). Data are expressed as % of total radioactivities extracted by ethyl acetate. The radioactivities of [^{14}C]PGE₂ were (dpm/100 mg tissue): control, 948.7; BK (1 μM), 2213.5; ATP (100 μM), 2905.2; BK+IND (5 μM), 853.6; ATP+IND, 308.4; and those of [^{14}C]PGF_{2 α} were: control, 406.5; BK, 1159.5; ATP, 1335.7; BK+IND, 406.8; ATP+IND, 167.7. Note that both BK and ATP stimulate PG synthesis in intact muscles. See Methods for details.

urinary detrusor, because the contractile response and prostaglandin generation were reduced by treatment with indomethacin (21). Although bradykinin elicits contraction of rabbit detrusor at low concentrations, bradykinin is unlikely to be the transmitter of the nerve. The contractile pattern of bradykinin is slower than that induced by nerve stimulation. Bradykinin takes some time to produce a contraction, probably because PG generation occurs some steps after the binding of bradykinin to its receptors. Stimulation of the nerve produced a rapid phasic contraction followed by slow tonic contraction (22). The slow tonic contraction was inhibited by treatment with an anti-inflammatory drug (22). ATP showed the similar contractile pattern to that of nerve stimulation (22), but bradykinin did not. Therefore, bradykinin would not be the transmitter in the atropine resistant excitatory nerves of rabbit urinary bladder.

As shown in the results, bradykinin is a

potent stimulator of PG generation in rabbit urinary detrusor. The increased PGE₂ releases (260 ng/g by 100 nM bradykinin) are submaximal concentrations of PGE₂ to elicit contraction. However, bradykinin failed to stimulate PG generation in a cell-free preparation (Fig. 6). Furthermore, it took some time for bradykinin to produce contraction. These observations mean that bradykinin stimulates the PG synthesis through some steps after binding to its receptor, as discussed above. Recently, it has been reported that bradykinin stimulates phosphoinositides breakdown and accumulates inositol 1,4,5-triphosphate (IP₃) and 1,2-diacylglycerol in model system cells (23). IP₃ has an ability to increase intracellular Ca²⁺ ions (24). Furthermore, phospholipase A₂, which liberates arachidonic acid, is sensitive to Ca²⁺ ions (25). There is so far little evidence about phosphoinositides breakdown by bradykinin in rabbit urinary bladder. Therefore, more experiments

Fig. 8. Inhibitory effect of mepacrine on contractile response to bradykinin (BK, 50 μ M), adenosine 5'-monophosphate (ATP, 100 μ M) and acetylcholine (ACh, 30 μ M). Ordinate: % of control (the response to each of the drugs before addition of mepacrine was taken as 100%). Abscissa: concentration of mepacrine (μ M). Each point represents the mean of 6 observations with S.E. for BK ($\circ$), ATP ($\bullet$) and ACh ($\square$). Mepacrine significantly inhibited ACh-, BK- and ATP-induced contractions, but there is a difference in inhibitory potency of mepacrine (*: significant difference between ACh and BK or between ACh and ATP).

are necessary to clarify the mechanism of the bradykinin-induced effect on rabbit urinary detrusor.

In conclusion, bradykinin stimulates phospholipase A_2 through some steps after binding to its receptor, and contracts the rabbit urinary detrusor mediated via PGE_2 converted from arachidonic acid.

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