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

Norimichi NAKAHATA, Tomoyuki ONO and Hironori NAKANISHI
Department of Pharmacology, Fukushima Medical College, Fukushima 960, Japan
Accepted December 22, 1986

Abstract—Bradykinin (100 pM to 1 μM) 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 μM), a cyclooxygenase inhibitor. Caffeic acid (10 μM), 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 μM). Prostaglandin (PG) E2 contracted the urinary detrusor in a concentration-dependent manner, and the release was abolished by treatment with indomethacin (5 μM). Arachidonic acid was converted to PGE2 and F2α when it was incubated with the 700×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, 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 x 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%; PGF2α, 3.0%; thromboxane B2, 0.02%; and 6-keto-PGF1α, 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 [14C]-
arachidonic acid (2 \muCi, 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 \mu l of the methanol was
put on silica gel plate (Whatman, LK-50) for
thin layer chromatography (TLC). The com-
position of the solvent for TLC was benzene/
dioxane/acetic acid=60/30/3 in volume. The
authentic standards used were PGE2, PGF2\alpha,
PGD2 and arachidonic acid. After develop-
ment, 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 radio-
activity 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 [14C]arachidonic acid
(4 \muCi) 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 (Dalichi), 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 (Sig-
amain, prostaglandin (PG) E2 (Ono), PGF2\alpha
(Ono), PGD2 (Ono) and arachidonic acid
(Sigma). Other chemicals were obtained from
Wako Pure Chemicals. [3H]PGE2 and [14C]-
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 con-
   traction in rabbit urinary detrusor (Fig. 1).
The threshold concentration of bradykinin
was about 100 pM, and the pD2 value was
8.01±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±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 ap-
peared slowly, and a small relaxation was
sometimes observed just after its application.
Atropine (0.1 \mu M), diphenhydramine (1 \mu M),
FPL-55712 (1 \mu M), methysergide (1 \mu M)
and prazosin (1 \mu M) did not change the
bradykinin-induced contraction (Fig. 2),
indicating that bradykinin elicited con-
traction, not mediating the release of ACh,
histamine, peptide leukotrienes, serotonin or
catecholamine. Tetrodotoxin (0.3 \mu M) did
not influence the contraction induced by
bradykinin (Fig. 2).

![Fig. 1. Dose-response curve for bradykinin (BK)
and acetylcholine (ACh). Ordinate: % of con-
tractile 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 (O) and 6 observations
for acetylcholine (●) with S.E.]
2. Effects of indomethacin and caffeic acid on bradykinin-induced contraction: Indomethacin (5 μM), a cyclo-oxygenase inhibitor, reduced the bradykinin-induced contraction to a great extent (Fig. 3). However, caffeic acid (10 μM), a lipoygenase 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 closely related to the contractile response to bradykinin. Indomethacin (5 μM) 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 μM 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. The 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 pM 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 [14C]-arachidonic acid metabolism in cell-free and intact preparations: [14C]Arachidonic acid was converted to [14C]PGE2 and [14C]PGF2α, by incubation with the 750×g supernatant of the muscle homogenate (Fig. 6). [14C]-PGE2 and [14C]PGF2α converted from [14C]-arachidonic acid were not increased by treatment with bradykinin (1 μM) or ATP (100 μM) (Fig. 6). These data indicated that bradykinin and ATP did not affect cyclooxygenase or conversion of arachidonic acid to PGs. When the intact detrusor was used, bradykinin (1 μM) and ATP (100 μM) increased the conversion of [14C]arachidonic acid to [14C]PGE2 and [14C]PGF2α (Fig. 7). Bradykinin and ATP could stimulate the production of [14C]PGE2 and [14C]PGF2α (only when the phospholipid of the intact muscle was prelabelled with [14C]-arachidonic acid). The augmented synthesis of [14C]PGE2 and [14C]PGF2α was reduced by treatment with indomethacin (5 μM) (Fig. 7).

7. Effects of mepacrine on bradykinin-, ATP- and ACh-induced contractions: Mepacrine (1–100 μM), 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 (8). 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. 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 $[^{14}C]$PGE$_2$ were (dpm/100 mg tissue): control, 948.7; BK (1 nM), 2213.5; ATP (100 $\mu$M), 2905.2; BK+IND (5 $\mu$M), 853.6; ATP+IND, 308.4; and those of $[^{14}C]$PGF$_{2\alpha}$ 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 PGE$_2$ releases (260 ng/g by 100 nM bradykinin) are submaximal concentrations of PGE$_2$ 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 (IP$_3$) and 1,2-diacylglycerol in model system cells (23). IP$_3$ has an ability to increase intracellular Ca$^{2+}$ ions (24). Furthermore, phospholipase A$_2$, which liberates arachidonic acid, is sensitive to Ca$^{2+}$ 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 μM), adenosine 5′-monophosphate (ATP, 100 μM) and acetylcholine (ACh, 30 μM). Ordinate: % of control (the response to each of the drugs before addition of mepacrine was taken as 100%). Abscissa: concentration of mepacrine (μM). Each point represents the mean of 6 observations with S.E. for BK (○), ATP (●) and ACh (□). 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 A2 through some steps after binding to its receptor, and contracts the rabbit urinary detrusor mediated via PGE2 converted from arachidonic acid.

Acknowledgements: The authors are grateful to Ono Pharmaceutical Co. (Osaka) for continuous gifts of prostaglandins and anti-serum, and we thank Mrs. S. Sato for typing this manuscript.

References
1 Walaszek, E.J.: The effect of bradykinin and kallidin on smooth muscle. In Handbook of Experimental Pharmacology, Edited by Erdös, E.G., Vol. XXV, p. 421–429, Springer, New York (1970)
2 Collier, H.O.J.: Kinins and ventilation of the lung. In Handbook of Experimental Pharmacology, Edited by Erdös, E.G., Vol. XXV, p. 409–420, Springer, New York (1970)
3 Matsumura, S., Taira, N. and Hashimoto, K.: The pharmacological behavior of the urinary bladder and its vasculature of the dog. Tohoku J. Exp. Med. 96, 274–285 (1968)
4 Bonta, I.L. and Hall, D.W.R.: Potentiation of the biphasic bradykinin response of the guinea-pig ileum. Br. J. Pharmacol 49, 1616–1622 (1973)
5 Cherry, P.D., Furchgott, R.F., Zawadzki, J.V. and Jothianandan, D.: Role of endothelial cells in a relaxation of isolated arteries by bradykinin. Proc. Natl. Acad. Sci. U.S.A. 79, 2106–2110 (1982)
6 Furchgott, R.F.: The role of endothelium in the response of vascular smooth muscle to drugs. Annu. Rev. Pharmacol. Toxicol. 24, 175–197 (1984)
7 Chand, N. and Attura, B.M.: Acetylcholine and bradykinin relax intrapulmonary arteries by acting on endothelial cells: Role in lung vascular diseases. Science 213, 1376–1379 (1981)
8 Gordon, J.L. and Martin, W.: Endothelium-dependent relaxation of the pig aorta: relationship to stimulation of 45Rb efflux from isolated endothelial cells. Br. J. Pharmacol. 79, 531–541 (1983)
9 Downie, J.W. and Rouffignac, S.: Response of rabbit detrusor muscle to bradykinin. Life Sci. 28, 603–608 (1981)
10 Longhurst, P.A., Belis, J.A., O’Donnell, J.P., Galie, J.G. and Westfall, D.P.: A study of the atropine-resistant component of the neurogenic response of the rabbit urinary bladder. Eur. J. Pharmacol. 99, 295–302 (1984)
11 Nakahata, N., Nakanishi, H. and Suzuki, T.: A possible negative feedback control of excitatory transmission via prostaglandins in canine small intestine. Br. J. Pharmacol. 68, 393–398 (1980)
12 Nakahata, N., Ono, T. and Nakanishi, T.: Possible involvement of a product of the 5-lipoxygenase pathway in mediation of indomethacin-induced inhibition of cholinergic transmission in guinea-pig ileum. Eur. J. Pharmacol. 104, 133–138 (1984)
13 Rossum, L.M.V.: Cumulative dose-response curve II. Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. Arch. Int. Pharmacodyn. Ther. 143, 299–330 (1963)
14 Needleman, P., Minkes, M.S. and Douglas, J.R.: Stimulation of prostaglandin biosynthesis by adenine nucleotides. Profile of prostaglandin release by perfused organs. Circ. Res. 34, 455–460 (1974)
15 Ferreira, S.H., Moncada, S. and Vane, J.R.: Prostaglandins and the mechanism of analgesia
produced by aspirin-like drugs. Br. J. Pharmacol. 49, 86–97 (1973)

16 Burnstock, G., Dumsday, B. and Smythe, A.: Atropine resistant excitation of the urinary bladder: the possibility of transmission via neurons releasing a purine nucleotide. Br. J. Pharmacol. 44, 451–461 (1972)

17 Burnstock, G., Cocks, T., Crowe, R. and Kasakov, L.: Purinergic innervation of the guinea-pig urinary bladder. Br. J. Pharmacol. 63, 125–138 (1978)

18 Westfall, D.P., Fedan, J.S., Colby, J., Hogaboom, G.K. and O'Donnel, J.P.: Evidence for a contribution by purines to the neurogenic response of guinea-pig urinary bladder. Eur. J. Pharmacol. 87, 415–422 (1983)

19 Dean, D.M. and Downie, J.W.: Interaction of prostaglandins and adenosine 5'-triphosphate in the noncholinergic neurotransmission in rabbit detrusor. Prostaglandins 16, 254–261 (1978)

20 Nakanishi, H. and Nakahata, N.: Role of adenosine triphosphate in excitatory transmission in rabbit detrusor. Japan. Smooth Muscle Res. 18, 307–308 (1982) (in Japanese)

21 Nakanishi, H., Watanabe, Z., Kanno, R., Ono, T. and Nakahata, N.: Effects of various prostaglandins and leukotriene C4 on isolated detrusor muscle in rabbits. Fukushima J. Med. Sci. 30, 111–121 (1984)

22 Nakanishi, H., Shineha, H., Ohtani, H., Ono, T. and Nakahata, N.: The utility of adenylyl imidodiphosphate for desensitization of the presynaptic P1 and postsynaptic P2-purinergic receptors in rabbit detrusor. Fukushima J. Med. Sci. 30, 11–18 (1984)

23 Vicentini, L. and Villereal, M.L.: Serum, bradykinin and vasopressin stimulate release of inositol phosphate from human fibroblasts. Biochim. Biophys. Res. Commun. 123, 663–670 (1984)

24 Streb, H., Irvine, R.F., Berridge, M.J. and Schulz, I.: Release of Ca2+ from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol 1,4,5-triphosphate. Nature 306, 67–69 (1983)

25 Derksen, A. and Cohen, P.: Patterns of fatty acid release from endogenous substrates by human platelet homogenates and membrane. J. Biol. Chem. 250, 9342–9347 (1975)