Pertussis toxin blocks angiotensin II-induced calcium influx but not inositol trisphosphate production in adrenal glomerulosa cell

Itaru Kojima, Hiroshi Shibata and Etsuro Ogata

The Fourth Department of Internal Medicine, University of Tokyo School of Medicine, 3-28-6 Mejirodai, Bunkyo-ku, Tokyo 112, Japan

Received 23 May 1986; revised version received 17 June 1986

Involvement of GTP-binding proteins in angiotensin II-induced mobilization of calcium has been examined in adrenal glomerulosa cells by using pertussis toxin. Pretreatment of glomerulosa cells with pertussis toxin abolishes angiotensin II-induced calcium influx without blocking inositol trisphosphate production. These results suggest a role of the pertussis toxin-sensitive GTP-binding protein in transducing angiotensin II-receptor occupancy into opening of calcium channel.

Angiotensin II *(Adrenal glomerulosa cell)* *Ca^{2+} influx* *Pertussis toxin* *Inositol trisphosphate*

1. INTRODUCTION

Angiotensin II stimulates aldosterone secretion in adrenal glomerulosa cells by a calcium-dependent mechanism [1,2]. This hormone induces two major alterations in cellular calcium handling: a rapid but transient release of calcium from an intracellular non-mitochondrial pool(s) [3] and a sustained elevation of calcium influx rate [4], both of which are important in the action of angiotensin II [5]. Several lines of evidence indicate that calcium release is mediated by inositol 1,4,5-trisphosphate (Ins-P_3): (i) angiotensin II rapidly increases Ins-P_3 by activating phospholipase C specific to phosphatidylinositol 4,5-bisphosphate [6,7]; (ii) Ins-P_3 causes a release of calcium from the non-mitochondrial pool in permeabilized glomerulosa cell [6]; (iii) dantrolene, which inhibits Ins-P_3-induced calcium release in permeabilized cell [6], also attenuates the action of angiotensin II on calcium release in intact glomerulosa cell [3]. Thus, an activation of phospholipase C is the initial step to mobilize calcium from the intracellular pool. In contrast, the mechanism by which angiotensin II stimulates calcium influx remains unknown. It is even unclear whether or not stimulation of calcium influx is a consequence of phosphoinositide breakdown.

Recent studies have demonstrated that GTP-binding proteins are involved in the action of calcium-mobilizing agonists. In cell free system, GTP enhances agonist-induced activation of phospholipase C [8–12]. Further support for the notion that GTP-binding proteins are involved in the action of calcium-mobilizing agonists is obtained by using pertussis toxin, which ADP-ribosylates the inhibitory coupling protein, N_i, and blocks its action [13]. Pertussis toxin is shown to attenuate agonist-induced breakdown of polyphosphoinositide [14–16] and changes in ion fluxes [17,18] in some systems. Thus, pertussis toxin provides a useful tool to assess an involvement of GTP-binding protein, particularly N_i, in the action of calcium-mobilizing agonists.

In the present study, we have examined the effect of pertussis toxin treatment on angiotensin II-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

induced calcium mobilization in adrenal glomerulosa cells. Results indicate that pertussis toxin blocks angiotensin II-mediated increase in calcium influx without inhibiting calcium release from an intracellular pool.

2. MATERIALS AND METHODS

Isolated bovine adrenal glomerulosa cells were prepared by a collagenase digestion method [7]. Cells were incubated in modified Hanks solution containing (in mM) 137 NaCl, 3.5 KCl, 0.44 KH_2PO_4, 4.2 $NaHCO_3$, 0.33 Na_2HPO_4, 0.5 $CaCl_2$ and 20 Hepes/NaOH (pH 7.4), equilibrated with O_2 gas. For pertussis toxin treatment, cells were incubated in modified Hanks solution containing pertussis toxin for 1 h. When cells were incubated in medium containing low extracellular calcium concentration, Ca^{2+}-EGTA buffer was employed [4]. Changes in cytoplasmic free calcium concentration ($[Ca^{2+}]_c$) were measured with a calcium-sensitive photoprotein, aequorin [19]. Aequorin was introduced into glomerulosa cells by the method of Morgan and Morgan [20] as described [21]. Cell suspension containing 10^7 cells in 1 ml was incubated in a cuvette at 37°C under constant stirring and aequorin bioluminescence was measured as described [21]. $[Ca^{2+}]_c$ is not calibrated since no information is available at present as to the spatial distribution of calcium in the cell. Aequorin luminescence is expressed as electric current. Traces presented are representative of at least three experiments with similar results.

Calcium influx rate was obtained by measuring an initial uptake of ^{45}Ca into glomerulosa cells [4]. Protein content was measured according to Bradford [22] using bovine γ-globulin as standard.

Production of Ins-P_3 was measured using [^{3}H]inositol-labeled cells as described [21]. After a 2 h labeling period, cells were washed and further incubated for 1 h in modified Hanks solution containing 10 mM inositol and 10 mM lithium chloride in the presence and absence of pertussis toxin. Cells were then stimulated by angiotensin II for 20 and 60 s and the reaction was terminated by adding perchlorate (final concentration of 10%). Cells were homogenized by repetitive aspirations through a 26 G needle. The homogenate was centrifuged at 800 × g for 5 min, supernatant neutralized by adding 1 M KOH and applied onto an anion exchange column. [^{3}H]Inositol trisphosphate was eluted as described by Berridge et al. [23].

Pertussis toxin was a generous gift of Professor Michio Ui, Hokkaido University, Sapporo, Japan. Aequorin was purchased from Dr J.R. Blinks of Mayo Foundation (Rochester, MN). Angiotensin II was obtained from Sigma (St. Louis, MO). [^{3}H]Inositol and $^{45}CaCl_2$ were obtained from New England Nuclear (Boston, MA).

3. RESULTS AND DISCUSSION

When aequorin-loaded adrenal glomerulosa cells are stimulated by 1 nM angiotensin II, $[Ca^{2+}]_c$ increases immediately after the addition of angiotensin II. As demonstrated in fig.1A, angiotensin II-mediated increase in $[Ca^{2+}]_c$ is only transient and the decrease in $[Ca^{2+}]_c$ is characterized by two phases, a rapid fall followed by a second slowly declining phase. $[Ca^{2+}]_c$ returns to the resting value within 2 min. The second slow phase greatly depends on calcium influx since this phase disappears when cells are stimulated in medium containing 1 μM extracellular calcium (fig.1B), under which angiotensin II-induced calcium influx is abolished [4].

Pertussis toxin stimulates ADP-ribosylation of α-subunit of N_i and, thereby, prevents agonist-induced dissociation of the protein into active subunits [13]. Pertussis toxin is also known to ADP-ribosylate other GTP-binding proteins [24,25]. When pertussis toxin-pretreated cells are stimulated by angiotensin II, time course of changes in $[Ca^{2+}]_c$ is similar to that in pertussis toxin-untreated cells stimulated in low calcium containing medium (fig.1C). Thus, pertussis toxin appears to modify angiotensin II-mediated calcium influx. To examine this possibility, aequorin-loaded cells are stimulated in calcium free medium and extracellular calcium is subsequently restored by adding calcium. In pertussis toxin-untreated cells, restoration of extracellular calcium in the presence of angiotensin II results in a large increase in $[Ca^{2+}]_c$ (fig.2A). This increase is due to angiotensin II-induced calcium influx since restoration of calcium in the absence of angiotensin II results in a gradual increase in $[Ca^{2+}]_c$ (fig.2B).

When pertussis toxin-pretreated cells are

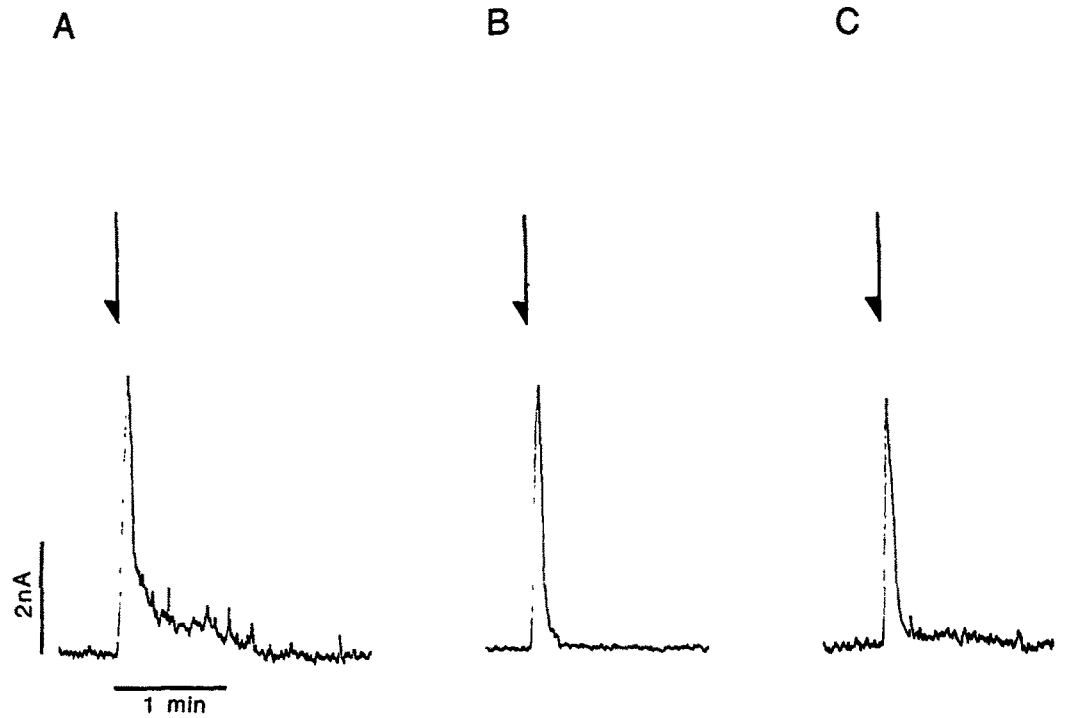

Fig.1. Effect of pertussis toxin pretreatment on angiotensin II-induced changes in cytoplasmic free calcium concentration. Aequorin-loaded cells were incubated for 60 min with (C) or without (A,B) 100 ng/ml pertussis toxin. Cells were then stimulated by 1 nM angiotensin II as indicated by an arrow. Concentration of extracellular calcium was 0.5 mM (A,C) or 1 μM (B). Aequorin bioluminescence is expressed as electric current.

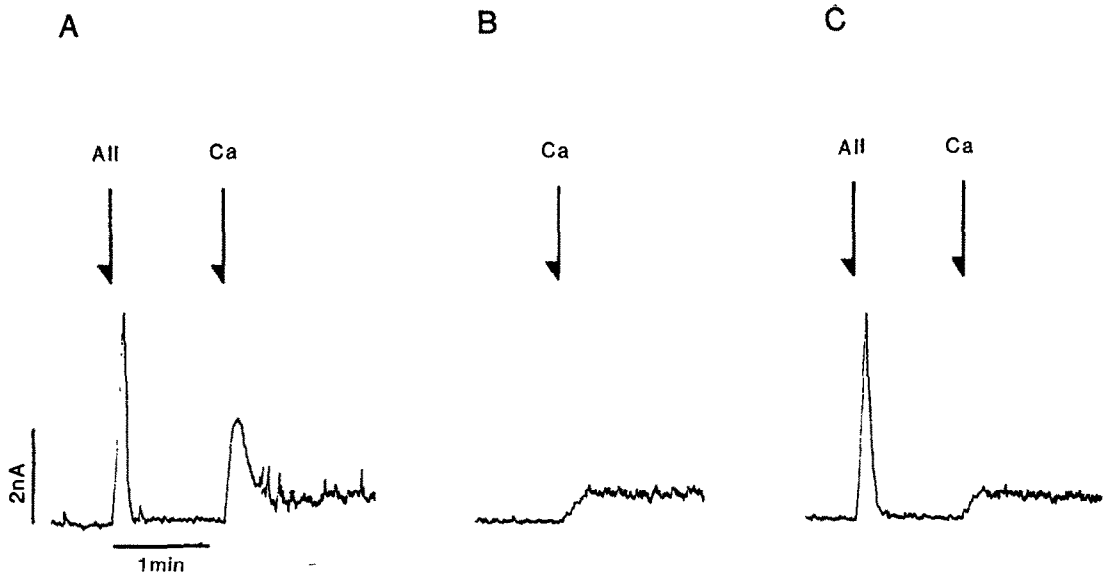

Fig.2. Effect of pertussis toxin pretreatment on changes in cytoplasmic free calcium concentration induced by restoration of extracellular calcium. Aequorin-loaded cells were incubated for 60 min with (C) or without (A,B) 100 ng/ml pertussis toxin. Cells were then incubated in calcium free modified Hanks solution and 1 nM angiotensin II was added in (A,C) as indicated by an arrow. Extracellular calcium concentration was then restored to 0.5 mM by adding calcium chloride.

stimulated by angiotensin II in the absence of extracellular calcium and then extracellular calcium is restored, $[Ca^{2+}]_c$ increases only gradually (fig.2C). These results suggest that pertussis toxin blocks angiotensin II-mediated calcium influx without affecting calcium release from an intracellular pool. To directly demonstrate the effect of pertussis toxin on calcium influx, calcium influx rate was determined by measuring initial uptake of ^{45}Ca into the cell. As shown in fig.3, pertussis toxin pretreatment inhibited angiotensin II-induced calcium influx in a dose-dependent manner. Treatment with 100 ng/ml pertussis toxin almost completely abolishes angiotensin II-mediated calcium influx. It is noteworthy that basal calcium influx is not affected by toxin pretreatment. In contrast to its action on calcium influx, pertussis toxin has little effect on polyphosphoinositide breakdown. As shown in table 1, angiotensin II elicits 3-fold increase in $[^3H]Ins\text{-}P_3$ in pertussis toxin-pretreated cells, which is comparable to that observed in toxin-untreated cells.

The involvement of GTP-binding proteins in the action of angiotensin II on adrenal glomerulosa cells has been suggested by the following observations: (i) binding of angiotensin II to its receptor is influenced by GTP [26]; (ii) angiotensin II decreases cAMP response to ACTH [27]. The results in the present study extend this notion by showing that angiotensin II-mediated calcium influx is sensitive to pertussis toxin. Unlike some other systems [14–16], pertussis toxin does not affect agonist-induced activation of phospholipase C. Our present results agree with a previous report by Murayama et al. [18]. They have shown in 3T3 fibroblasts that pertussis toxin inhibits angiotensin II-induced calcium uptake without inhibiting the activation of phospholipase C. Although pertussis toxin does not affect the activation of phospholipase C, we have recently found that angiotensin II-induced activation of phospholipase C observed in plasma membrane fraction is enhanced by GTP-γS (Kojima, I., unpublished). Thus, as demonstrated in hepatocytes [10,11,28], multiple GTP-binding proteins may be involved in signal transduction of angiotensin II in adrenal glomerulosa cells.

At present, the mechanism by which angiotensin II stimulates calcium influx is unknown. We have previously shown that angiotensin II and

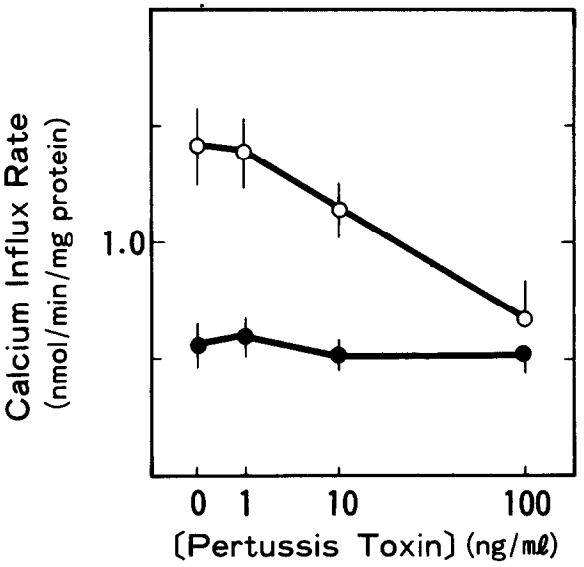

Fig.3. Dose-response curve for pertussis toxin-induced inhibition of calcium influx. Adrenal glomerulosa cells were incubated for 60 min in the presence of various doses of pertussis toxin. Calcium influx rate was measured as described in section 2 in the presence (○) and absence (●) of 1 nM angiotensin II. Values are the mean ± SE for 4 determinations.

Table 1

Effect of pertussis toxin on angiotensin II stimulation of inositol trisphosphate production in adrenal glomerulosa cells

Pertussis toxin	Inositol trisphosphate (% increase induced by angiotensin II)	
	20 s	60 s
Untreated	290 ± 60	304 ± 29
Treated	302 ± 53	320 ± 40

$[^3H]$Inositol-labeled cells were incubated for 60 min with or without 100 ng/ml pertussis toxin. Cells (10^7 cells in 1 ml) were then stimulated for indicated time by 1 nM angiotensin II in the absence of lithium chloride. Radioactivity in inositol trisphosphate fraction is expressed as percent of that in unstimulated cells. Radioactivities in unstimulated cells were 196 ± 42 dpm in pertussis toxin-untreated cells and 178 ± 33 dpm in toxin-treated cells. Values are the mean ± SE for 7 determinations using 3 cell preparations

potassium act on the common calcium channel, presumably voltage-dependent calcium channel [4]. In GH_3 cells, TRH induces an opening of voltage-dependent calcium channels by modulating potassium channels [29]. Given the fact that a pertussis toxin-sensitive protein is involved in the regulation of potassium channel by carbachol [17], it seems possible that GTP-binding protein acts as a transducer of angiotensin II action on calcium influx by modulating potassium channel.

ACKNOWLEDGEMENTS

Authors are indebted to Professor Michio Ui for his generous gift of pertussis toxin. This work was supported in part by grants from Ministry of Education, Science and Culture of Japan, The Workshop for Cardiovascular System and Calcium Signal, and The Japanese Intractable Disease Research Foundation.

REFERENCES

[1] Fakunding, J., Chow, R. and Catt, K. (1979) Endocrinology 105, 327–333.
[2] Foster, R., Lovo, M.V., Rasmussen, H. and Marusic, E.T. (1981) Endocrinology 109, 2196–2200.
[3] Kojima, I., Kojima, K. and Rasmussen, H. (1985) Am. J. Physiol. 247, E36–E43.
[4] Kojima, I., Kojima, K. and Rasmussen, H. (1985) J. Biol. Chem. 260, 9171–9177.
[5] Kojima, I., Kojima, K. and Rasmussen, H. (1985) J. Biol. Chem. 260, 9177–9183.
[6] Enyedi, P., Buki, B., Mucsi, I. and Spät, A. (1985) Mol. Cell. Endocrinol. 41, 105–112.
[7] Kojima, I., Kojima, K., Kreutter, D. and Rasmussen, H. (1984) J. Biol. Chem. 259, 14448–14457.
[8] Litosch, I., Wallis, C. and Fain, J. (1985) J. Biol. Chem. 260, 5464–5471.
[9] Martin, T.F.J., Lucas, D.O., Bajjalieh, S.M. and Kowalchyk, J.A. (1986) J. Biol. Chem. 261, 2918–2927.
[10] Wallace, M.A. and Fain, J.N. (1985) J. Biol. Chem. 260, 9527–9530.
[11] Uhing, R.J., Prpic, V., Jiang, H. and Exton, J.H. (1986) J. Biol. Chem. 261, 2140–2146.
[12] Smith, C.D., Lane, B.C., Kusaka, I., Verghese, M.W. and Snyderman, R. (1985) J. Biol. Chem. 260, 5875–5878.
[13] Ui, M. (1984) Trends Pharmacol. Sci. 5, 277–279.
[14] Nakamura, T. and Ui, M. (1983) Biochem. Pharmacol. 32, 3435–3441.
[15] Nakamura, T. and Ui, M. (1985) J. Biol. Chem. 260, 3584–3593.
[16] Okajima, F. and Ui, M. (1984) J. Biol. Chem. 259, 13863–13871.
[17] Pfaffinger, P.J., Martin, J.M., Huter, D.D., Nathanson, N.M. and Hill, B. (1985) Nature 317, 536–538.
[18] Murayama, T. and Ui, M. (1985) J. Biol. Chem. 7226–7233.
[19] Blinks, J.R. (1978) Ann. NY Acad. Sci. 307, 71–85.
[20] Morgan, J.P. and Morgan, K.G. (1982) Pflügers Arch. 395, 75–77.
[21] Kojima, I., Shibata, S. and Ogata, E. (1986) Biochem. J., in press.
[22] Bradford, M. (1976) Anal. Biochem. 72, 248–254.
[23] Berridge, M.J., Dawns, R.M.C., Downs, C.P., Heslop, J.P. and Irvine, R.F. (1983) Biochem. J. 220, 345–360.
[24] Sternweis, P.C. and Robishow, J.D. (1984) J. Biol. Chem. 259, 13806–13813.
[25] Manning, D.R., Fraser, B.A., Kahn, R.A. and Gilman, A.G. (1984) J. Biol. Chem. 259, 749–756.
[26] Lean, A., Ong, H., Gutkowska, J., Schiller, P.W. and McNicoll, N. (1984) Mol. Pharmacol. 26, 498–508.
[27] Marie, J. and Jard, S. (1983) FEBS Lett. 159, 97–101.
[28] Pobner, B.F., Hewlett, E.L. and Garrison, J.C. (1985) J. Biol. Chem. 260, 16200–16209.
[29] Barros, F., Katz, G.M., Kaczorowski, G.J., Vandlen, R.L. and Reuben, J.P. (1985) Proc. Natl. Acad. Sci. USA 82, 1108–1112.