Vol. 195, No. 1, 1993 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 31, 1993 Pages 415-421

UTP ACTIVATES PHOSPHOLIPASE C-Ca^{2+} SYSTEM THROUGH A RECEPTOR DIFFERENT FROM THE 53-KDa ATP RECEPTOR IN PC12 CELLS

Mohammed Abdul Majid, Fumikazu Okajima, and Yoichi Kondo

Department of Physical Biochemistry, Institute of Endocrinology
Gunma University, Maebashi 371, Japan

Received July 12, 1993

Extracellular ATP (a purine nucleotide) and UTP (a pyrimidine nucleotide) both activated phospholipase C with a similar potency and efficacy; however, in contrast to ATP which induced a remarkable norepinephrine release, UTP-induced norepinephrine release was small in PC12 cells, a rat pheochromocytoma cell line. ATP, its derivatives (2-methylthioadenosine 5'-triphosphate (MeSATP) and 2'- and 3'-*O*-(4-benzoylbenzoyl)adenosine 5'-triphosphate (BzATP)) and UTP increased intracellular Ca^{2+} in the presence of 2 mM extracellular Ca^{2+} with the potency order of ATP > MeSATP > BzATP = UTP. Under the low extracellular Ca^{2+} conditions, the Ca^{2+} response to purine nucleotides was markedly reduced, but the UTP response was not. The [^{32}P]BzATP labeling of a 53-kDa putative ATP receptor coupled to a channel system (Majid, M.A., Okajima, F., and Kondo, Y. (1992) Biochim. Biophys. Acta 1136, 283-289) was markedly inhibited by ATP, but not by UTP. These results suggest that UTP activates the phospholipase C-Ca^{2+} system through a receptor different from the 53-kDa ATP receptor.

ATP is co-stored with neurotransmitters in synaptic vesicles of neuronal cells and with hormones in granules of endocrine cells, and released into extracellular space upon the stimulation of these cells (1). Extracellular ATP thus released interacts with its specific receptor (P_2-purinoceptor) on the surface of many cell types and induces a variety of biological responses (1). For example, ATP stimulates glycogen metabolism in hepatocytes (2), iodide metabolism in thyroid cells (3), secretion of norepinephrine and dopamine in PC12 cells (4-7), exocytosis of β-glucuronidase and O_2^- generation in neutrophils as well as HL60 cells (8,9), and arachidonate cascade in both thyroid cells (10) and renal mesangial cells (11). Althugh changes in cyclic AMP and cyclic GMP metabolism are, in some cases, associated with the ATP actions (2,10,12), intracellular Ca^{2+}

Abbreviations: BzATP, 2'- and 3'-*O*-(4-benzoylbenzoyl)adenosine 5'-triphosphate; MeSATP, 2-methylthioadenosine 5'-triphosphate; $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration.

0006-291X/93 $4.00

($[Ca^{2+}]_i$) increase seems to be a common feature in all the cellular responses to ATP. On the other hand, the $[Ca^{2+}]_i$ rise, is caused in some cases, by the activation of phospholipase C, but in other cases, by the stimulated ion fluxes or channel systems.

UTP, a pyrimidine nucleotide, is also stored in granules of some cells such as platelets and released into extracellular space upon cell stimulation (1,13,14). The extracellular UTP exerts similar actions to those of ATP on target cells and appears to share the same receptor as that for ATP in rat renal mesangial cells (11) and human airway epithelial cells (15). These results suggest the presence of 'nucleotide' receptor which has a high affinity for both purine and pyrimidine nucleotides (13,14). In liver (16), HL60 cells (9) and neutrophils (8), however, UTP actions are somehow different from those of ATP. For example, in neutrophils, the UTP actions are more sensitive to pertussis toxin and cAMP producing agents than those of ATP (8). In additon, cellular responses to UTP are increased during HL60 cell differentiation but those to ATP are not (9). These complex observations may be explained by assuming the presence of the nucleotide receptor for both purine and pyrimidine nucleotides in addition to the P_2-purinoceptor specific for purine nucleotides (13,14). In renal mesangial cells (11) and airway epithelial cells (15), only the nucleotide receptor might exist, while in liver cells, HL60 cells and neutrophils, both nucleotide and P_2 receptors might be present.

In PC12 cells, both ATP and UTP activate phospholipase C in a similar fashion, suggesting that these actions are mediated by a single species of receptor (17). We have recently found that only in the presence of extracellular Ca^{2+}, BzATP could induce norepinephrine release from PC12 cells (7). By photolabeling of the cell membranes with this photosensitive ATP analogue, we have identified a 53-kDa membrane protein as a putative ATP receptor coupled to a channel system (7). Here, we further characterized the UTP actions in PC12 cells and found that UTP activated phospholipase C as effectively as ATP did, but UTP did not affect the 53-kDa protein labeling by [^{32}P]BzATP, which ATP markedly inhibited. The results suggest that UTP stimulates the phospholipase C-Ca^{2+} system through a nucleotide receptor which is activated by UTP as well as by ATP, but different from the 53-kDa ATP receptor for purine nucleotides.

MATERIALS AND METHODS

Materials: UTP was purchased from Sigma (St. Louis, MO). The sources of other materials were as described in (7).

Cell culture: PC12 cells (provided by the Japanese Cancer Research Resorces Bank, ID No. IFO 50278) were cultured as described previously (7).

Measurement of $[Ca^{2+}]_i$: The cells were harvested from the dishes with phosphate buffered saline containing 4 mM EDTA. $[Ca^{2+}]_i$ was measured as described previously (3). In brief, the cells were incubated with 1μM Fura 2/AM for 20 min at 37° C, and then washed with Hepes-buffered medium [10 mM Hepes (pH 7.4), 134 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.0 mM $CaCl_2$, 2.5 mM $NaHCO_3$, 5 mM glucose and 0.1% bovine serum albumin (Fraction V)]. The fluorescence change (excitation, 340 nm; emission, 510 nm) induced by agonists was monitored in the same buffer at 37° C. In the experiments where the effect of extracellular Ca^{2+} depletion was examined, 2.4 mM EGTA was applied 1 min before the application of the agonists.

Measurement of [^{3}H]inositol phosphate production and [^{3}H]norepinephrine release: Procedures were essentially the same as those described in the previous paper (7).

Photoaffinity labeling: Photoaffinity labeling was performed as described previously (7). In brief, PC12 cell membranes were photolyzed with [^{32}P]BzATP on ice in the presence of ATP or UTP for 15 min, under a low intensity long wave (366 nm) ultraviolet lamp. The radiolabeled membranes were sedimented, dissolved in the Laemmli sample buffer (18) and subjected to SDS-polyacrylamide gel electrophoresis. The gel plates were then exposed to X-ray films for 24 h. The exposed films were scanned by a laser densitometer (LKB 2202) to measure the radiolabeling of the 53-kDa protein band.

RESULTS AND DISCUSSION

In accordance with the previous report (17), UTP activated phospholipase C as effectively as ATP did (Fig. 1 A). The enzyme activation by ATP was accompanied by a remarkable norepinephrine release, however, the release caused by UTP was small, but significant (Fig. 1 B). As shown previously (7), ATP

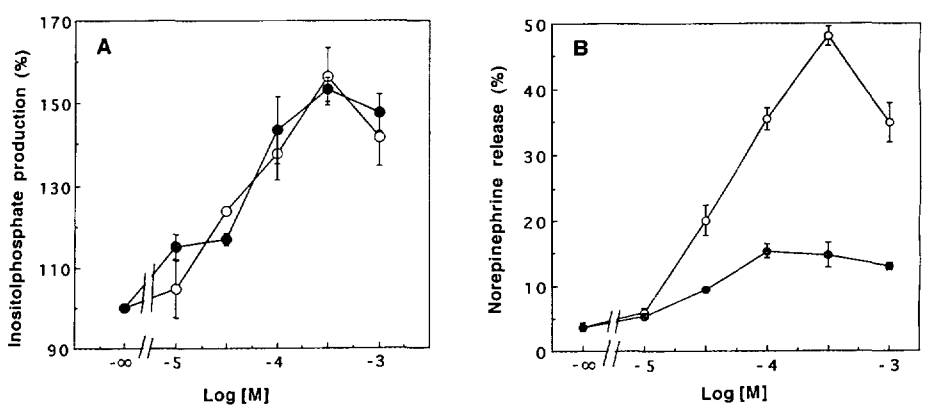

Fig. 1. Dose response curve of ATP and UTP on [^{3}H]inositol phosphates (IP_2 + IP_3) production (A) and [^{3}H]norepinephrine release (B). The cells were incubated for 1 min in (A) and 2 min in (B) with the indicated dose of ATP (○) or UTP (●) as described in (7). Data are expressed as percentages of the IP_2 + IP_3 radioactivity produced in the control cells in (A) and are expressed as percent release of total radioactivity incorporated in the cells in (B). Values are means ± S.E. of three separate experiments performed in triplicate.

derivatives, MeSATP and BzATP, also stimulated norepinephrine release without a significant phospholipase C activation. These results suggest that the phospholipase C activation is not enough to induce a full response of norepinephrine release and a mechanism other than the phospholipase C activation is involved in the norepinephrine release by ATP. The additional mechanism may be the activation of Ca^{2+} influx through a channel (4-6). Thus, the norepinephrine releasing response to ATP and its derivatives, especially MeSATP and BzATP almost totally depends on extracellular Ca^{2+} (7).

To clarify the source of Ca^{2+} in these nucleotides action, we compared the agonist-induced change of $[Ca^{2+}]_i$ in the presence of 2 mM extracellular Ca^{2+} with that in a Ca^{2+}-depleted medium. In the presence of 2 mM Ca^{2+}, ATP and its derivatives, MeSATP and BzATP induced a marked $[Ca^{2+}]_i$ rise with the potency order of ATP>MeSATP>BzATP (Fig. 2 A and C). Under low extracellular Ca^{2+} conditions where KCl (a depolarizing agent)-induced effects were completely lost (data not shown), the Ca^{2+} responses to ATP and its derivatives were markedly reduced. The peak values for 100 μM ATP, MeSATP and BzATP under low extracellular Ca^{2+} conditions were 13, 6 and 1% respectively of that in the presence of 2 mM extracellular Ca^{2+} (Fig. 2 B and D). Thus, as expected from the norepinephrine release experiments, the $[Ca^{2+}]_i$ increase by purine nucleotides is markedly dependent on extracellular Ca^{2+}.

UTP also induced a $[Ca^{2+}]_i$ rise in PC12 cells. In the presence of 2 mM extracellular Ca^{2+}, the efficacy of the intracelllular Ca^{2+} response was much less than that of ATP or MeSATP, but similar to that of BzATP (Fig. 2 A and C). In contrast to purine nucleotides, the UTP action was not influenced by the low extracellular Ca^{2+} conditions. The UTP-induced peak $[Ca^{2+}]_i$ rise was the same as that in the presence of 2 mM Ca^{2+} (Fig. 2 A and B), and both potency and efficacy of UTP were similar to those of ATP in the low Ca^{2+} medium (Fig. 2 D). This result suggests that UTP mobilizes Ca^{2+} solely from the intracellular pool at least at the early stage of cell stimulation.

The results suggest that there are at least two mechanisms for $[Ca^{2+}]_i$ rise in PC12 cells, i.e. the phospholipase C-mediated Ca^{2+} mobilization from an internal pool and the Ca^{2+} influx probably through an ion channel. UTP appears to activate only the former pathway, MeSATP and BzATP the latter one, and ATP the both. This idea was confirmed by inhibition experiments of BzATP labeling of a 53-kDa protein. In the previous report (7), we identified a 53-kDa membrane protein which is photolabeled by BzATP as a putative ATP receptor coupled to a

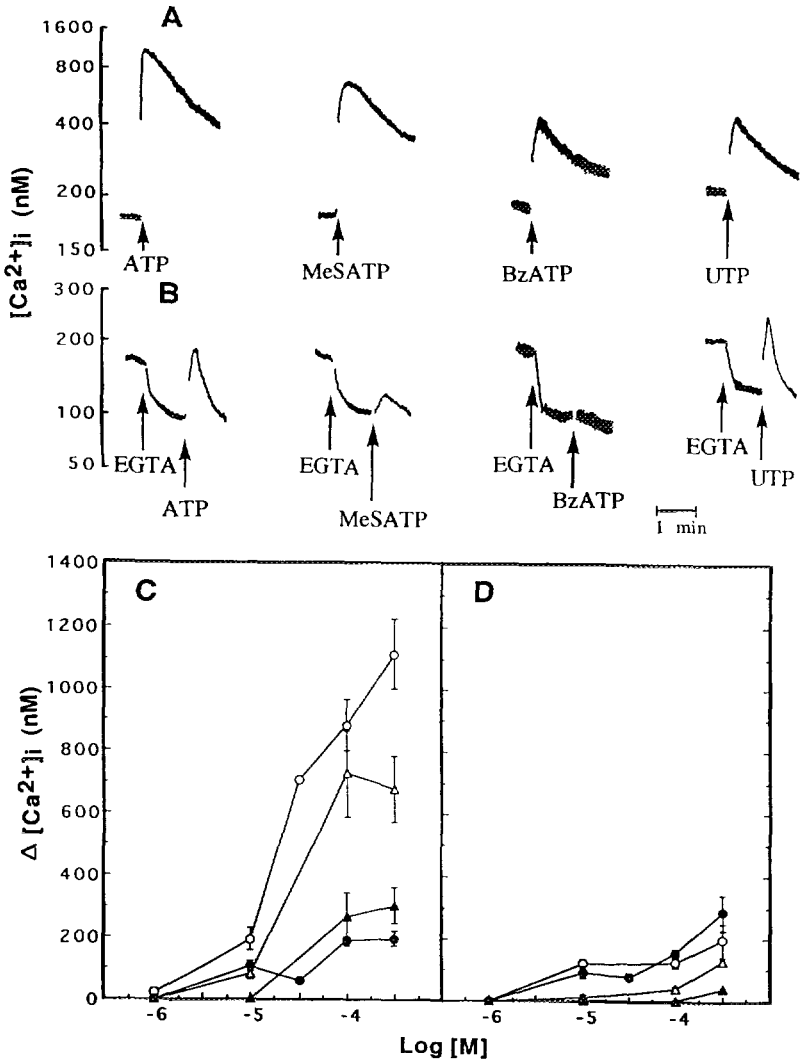

Fig. 2. Effect of ATP, MeSATP, BzATP and UTP on $[Ca^{2+}]_i$ in the presence of 2 mM Ca^{2+} (A,C) or under low extracellular Ca^{2+} conditions (B,D). Time courses of the change in $[Ca^{2+}]_i$ of typical experiments are shown in A and B. At the arrows 100 μM of ATP, MeSATP, BzATP, UTP or 2.4 mM EGTA was applied. In C and D, the cells were maintained in 2 mM Ca^{2+} contained medium (C) or exposed to 2.4 mM EGTA for 1 min (D), then stimulated with the indicated doses of ATP (○), MeSATP (△), BzATP (▲) and UTP (●). Peak rises in $[Ca^{2+}]_i$ are plotted. Results are means ± S.E. of four values or means (without error bar) in duplicate experiments.

channel system. This was based on the parallelism between potency order of the nucleotides in the induction of extracellular Ca^{2+}-dependent norepinephrine release and their potency order in the inhibition of the [^{32}P]-BzATP labeling of a 53-kDa protein. As shown in Fig. 3, UTP, however, failed to inhibit the labeling even at 10 μM, whereas ATP inhibited the BzATP labeling of a 53-kDa protein in

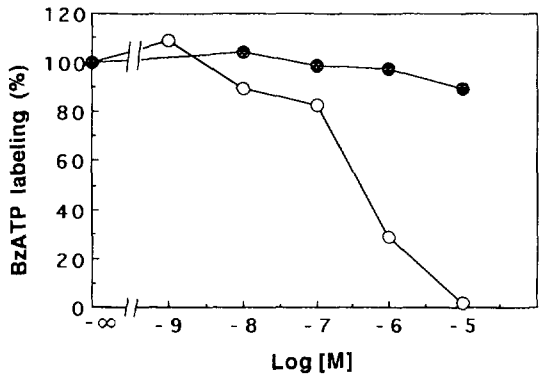

Fig.3. Dose-resonse curves of ATP and UTP for the inhibition of [^{32}P]BzATP labeling of a 53-kDa protein. PC12 cell membranes were photolysed with 8 nM [^{32}P]BzATP in the presence of the indicated dose of ATP (○) or UTP (●) for 15 min on ice as described in Materials and Methods. Data are means of values obtained from two separate experiments.

a dose-dependent fashion. This result definitely indicates that UTP has no ability to interact with the 53-kDa ATP receptor protein.

The present results support the existence of a nucleotide receptor for either pyrimidine nucleotides such as UTP or purine nucleotides such as ATP in addition to a purine-nucleotide-specific 53-kDa receptor in PC12 cells. This nucleotide receptor couples to the phospholipase C-Ca^{2+} system, but not to the channel systems. On the other hand, ATP activates both systems prefering the channel systems. The activation of the channel systems by ATP is probably mediated by the 53-kDa receptor protein. The ATP-induced activation of phospholipase C might be mediated by the putative nucleotide receptor as suggested by others (17). In relation to this, it is noted that a 53-kDa protein photolabeled by BzATP exists in several cell types including turkey erythrocytes where this protein appears to couple to phospholipase C through a GTP-binding protein (19), although there is no evidence that the 53-kDa protein in turkey erythrocytes is identical with that present in PC12 cells. Quite recently, cDNA encoding the nucleotide receptor which has high affinity for both UTP and ATP in NG 108-15 cells, has been isolated and the molecular mass of the protein is predicted to be 42-kDa (20). The result further suggests the nucleotide receptor for UTP and ATP in PC12 being a different species of molecule from the 53-kDa ATP receptor.

ACKNOWLEDGMENTS

This work was supported, in part, by a Grant-in-Aid for Scientific Research of the Ministry of Education, Science and Culture of Japan. M.A.Majid is a Japanese Government Scholarship Student.

REFERENCES

1. Gordon, J.L. (1986) Biochem. J. 233, 309-319.
2. Okajima, F., Tokumitsu, Y., Kondo, Y., and Ui, M. (1987) J. Biol. Chem. 262, 13483-13490.
3. Okajima, F., Sho, K., and Kondo, Y. (1988) Endocrinology 123, 1035-1043.
4. Inoue, K., Nakazawa, K., Fujimori, K., and Takanaka, A. (1989) Neurosci. Lett. 106, 294-299.
5. Fasolato, C., Pizzo, P., and Pozzan, T. (1990) J. Biol. Chem. 265, 20351-20355.
6. Sela, D., Ram, E., and Altas, D. (1991) J. Biol. Chem. 266, 17990-17994.
7. Majid, M.A., Okajima, F., and Kondo, Y. (1992) Biochim. Biophys. Acta 1136, 283-289.
8. Seifert, R., Burde, R., and Schultz, G. (1989) Biochem. J. 259, 813-819.
9. Stutchfield, J., and Cockcroft, S. (1990) FEBS Lett. 262, 256-258.
10. Okajima, F. Sato, K., Nazarea, M., Sho, K., and Kondo, Y. (1989) J. Biol. Chem. 264, 13029-13037.
11. Pfeilschifter, J. (1990) Biochem. J. 272, 469-472.
12. Okajima, F., and Kondo, Y. (1990) J. Biol. Chem. 265, 21741-21748.
13. Seifert, R., and Schultz, G. (1989) Trends Pharmacol. Sci. 10, 365-369.
14. O'Connor, S.E., Dainty, I.A., and Leff, P. (1991) Trends Pharmacol. Sci. 12, 137-141.
15. Brown, H.A., Lazarocoski, E.R., Boucher, R.C., and Harden, T.K. (1991) Mol. Pharmacol. 40, 648-655.
16. Haussinger, D., Stehle, T., and Gerok, W. (1987) Eur. J. Biochem. 167, 65-71.
17. Murrin, R.J.A., and Boarder, M.R. (1992) Mol. Pharmacol. 41, 561-568.
18. Laemmli, U.K. (1970) Nature 227, 680-685.
19. Boyer, J.L., Cooper, C.L., and Harden, T.K. (1990) J. Biol. Chem. 265, 13515-13520.
20. Lustig, K.D., Shiau, A.K., Brake, A.J., and Julius, D. (1993) Proc. Natl. Acad. Sci. USA 90, 5113-5117.