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Change of intracellular calcium of neural cells induced by extracellular ATP

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Exposure of various neural cells to ATP increased intracellular Ca^{2+} and the production of inositol trisphosphate. The Ca^{2+} responses were also observed in the absence of extracellular Ca^{2+}, suggesting that a part of Ca^{2+} mobilization took place from cytosolic storage. Since adenosine had no effect on intracellular Ca^{2+} increment, ATP appears to act through a P_2-purinergic receptor. Islet-activating protein or pertussis toxin pretreatment hardly influenced the increase in intracellular Ca^{2+} and inositol trisphosphate production induced by ATP, suggesting that IAP-sensitive GTP-binding proteins do not play a practical role in this reaction.

ATP; Neural cell line; Intracellular calcium; Inositol trisphosphate

1. INTRODUCTION

Extracellular ATP exhibits many biological effects through P_2-purinergic receptors [1,2], which is distinguished from a P_1 receptor for adenosine, in a variety of cell types [3–6] including hepatocytes [7], and FRTL-5 thyroid cells [8–10]. For example, extracellular ATP activates glycogen phosphorylase in hepatocytes [7] and activation of iodine metabolism in FRTL-5 thyroid cells [8–10]. These responses to ATP were associated with the activation of a phospholipase C-Ca^{2+} system [3–9]. In neurons ATP is localized in synaptic vesicles and released from nerve endings during nerve stimulation [1,2]. It is defined that ATP works as a co-neurotransmitter by modulating the release or action of other classical transmitters such as norepinephrine [1,2], however, the mechanism through which ATP works is not well characterized. In this study we examine the effect of ATP on several neural cell lines, and found that ATP but not adenosine was a potent inducer of Ca^{2+} mobilization in these cells.

2. MATERIALS AND METHODS

2.1. *Materials*

NG 108-15, mouse neuroblastoma-rat glioma hybrid cells were a gift of Dr. H. Higashida of Kanazawa University (Ishikawa, Japan) [11]. GOTO, human neuroblastoma cells, were a gift of Dr. M. Sekiguchi of Institute of Medical Science, University of Tokyo (Tokyo, Japan) [12]. Rat glioma C_6 and rat pheochromocytoma PC12 were provided by the Japanese Cancer Research Resources Bank (Tokyo, Japan). Human neuroblastoma cell lines, NB-1, TNB-1, SCMC-N3 were gifts of Dr. N. Kanda of Tokyo Women's Medical College (Tokyo, Japan) [13].

ATP and adenosine were purchased from Sigma. Islet-activating protein (IAP), pertussis toxin, was kindly given by Dr. Michio Ui of University of Tokyo (Tokyo, Japan). The sources of other reagents used were described previously [7–10].

2.2. *Cell culture*

NG 108-15 cells were cultured in Dulbecco's modified Eagle medium (DMEM) with 5% fetal bovine serum (FBS), 100 μM hypoxanthine, 1 μM aminopterin and 1.6 μM thymidine. GOTO, NB-1, TNB-1 and SCMC-N3 cells were cultured in DMEM with 10% FBS. PC12 cells were cultured in RPMI 1640 with 10% horse serum and 5% FBS. C_6 cells were cultured in Ham's F-10 medium with 15% horse serum and 2.5% FBS. All cells were incubated under 5% CO_2 at 37°C.

2.3. *Measurement of intracellular calcium*

Intracellular Ca^{2+} ($[Ca^{2+}]_i$) was measured using a fura-2 method according to [14] as modified previously [8]. Pretreatment of the cells with IAP (10 ng/ml) was carried out for 24 h in their complete media at 37°C. In some experiments extracellular Ca^{2+} was omitted and $[Ca^{2+}]_i$ measurement was performed in the presence of EGTA (0.1 mM).

2.4. *Measurement of inositol phosphate production*

The cells were cultured for 2 days in the presence of 2.5 μCi/ml of [^{3}H]inositol in 6-well plates (Corning) in the case of PC12, C_6 and GOTO cells and in 10 cm dishes (Corning) in the case of NG108-15 cells. Where indicated, IAP (10 ng/ml) was added to the culture medium 1 day before the experiment. All the cells except NG108-15 were used for experiments without harvesting. NG108-15 cells were harvested by being treated with 4 mM EGTA before experiments. These cells were washed with the HEPES buffer [8] and incubated in the same buffer containing 10 mM LiCl for 10 min at 37°C as described previously [9]. NG 108-15 and GOTO cells were exposed to 100 μM ATP for 15 s. C_6 and PC12 cells were exposed to the same stimulant for 30 s and 60 s, respectively. Termination of the reaction and the separation of various inositol phosphates were performed as described previously [7,15].

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Table I

Change in $[Ca^{2+}]_i$ (nM) by 100 μM of extracellular ATP

Cell lines	Regular		Calcium-Free		IAP	
	Base	Δ	Base	Δ	Base	Δ
NG108-15	179 ± 51	161 ± 45[a]	91 ± 31	59 ± 25[a]	215 ± 81	127 ± 80[b]
PC12	198 ± 59	736 ± 305[a]	119 ± 20	36 ± 11[b]	196 ± 28	755 ± 294[a]
C_6	176 ± 22	109 ± 27[a]	102 ± 15	22 ± 6[a]	227 ± 28	104 ± 31[a]
GOTO	87 ± 16	39 ± 11[a]	34 ± 10	11 ± 3[b]	75 ± 15	45 ± 2[b]

Basal and increased values (increment from base: Δ) by ATP in regular and Ca^{2+}-free medium are shown. IAP (10 ng/ml) was added to culture medium before cell harvest and $[Ca^{2+}]_i$ was measured in the regular medium. Data are means ± SE of at least 6 determinations. Statistical evaluation of increased values was performed with paired-sample *t*-test. Statistically significant values from base are indicated: [a]$P<0.01$, [b]$P<0.05$.

3. RESULTS AND DISCUSSION

Addition of extracellular ATP (100 μM) caused a transient increase of $[Ca^{2+}]_i$ in the neural cell lines tested (Fig. 1). In PC12 and C_6 cells $[Ca^{2+}]_i$ level declined after their peak and plateaued at the higher state than the base. The basal values and increased values by ATP (increment from base) are shown in Table I. In other neuroblastoma cell lines, NB-1, TNB-1 and SCMC-N3 cells, a small but significant increase in $[Ca^{2+}]_i$ was also induced by extracellular ATP (100 μM) (data not shown). Adenosine (100 μM) had no effect on the $[Ca^{2+}]_i$ of all cell lines tested (data not shown). It suggests that ATP exerts Ca^{2+} increase through P_2-receptor but not P_1-receptor.

There are at least three Ca^{2+}-mobilizing mechanisms caused by extracellular ATP, e.g. (1) phospholipase C-mediated Ca^{2+} mobilization from intracellular stores, (2) increase of membrane permeability and (3) opening of Ca^{2+} channel. In NG 108-15, PC12, C_6 and GOTO cells, the smaller but statistically significant increase of $[Ca^{2+}]_i$ by ATP was also observed in the absence of extracellular Ca^{2+} (Fig. 1, Table I). ATP-induced increase in $[Ca^{2+}]_i$ was also observed in TNB-1 and SCMC-N3 cells even in the absence of Ca^{2+} in the extracellular medium (data not shown). These results indicate that a part of Ca^{2+} mobilization took place from intracellular stores via inositol phosphates produced by phospholipase C activation. This was confirmed by the fact that ATP enhanced the production of inositol trisphosphate in these cells (Fig. 2). In NG 108-15, PC12 and C_6 cells extracellular ATP increased the amount of inositol trisphosphate by about 20%, 60% and 80%, respectively, although in GOTO cells significant increase was not observed. However, increased values of $[Ca^{2+}]_i$ induced by ATP in the absence of extracellular Ca^{2+} were much smaller than those in the presence of extracellular Ca^{2+} in all cell lines tested, suggesting that a substantial portion of Ca^{2+} increment might depend on the influx of Ca^{2+} across the plasma membrane as well. Indeed ATP is known to activate a Ca^{2+} channel in PC12 cells [16].

We next examined the effect of IAP. In many cells such as FRTL-5 [8-10], neutrophil [3], HL-60 [4], endothelial cells [5] and smooth muscle cells [6], the $[Ca^{2+}]_i$ increase by extracellular ATP is IAP-sensitive, although the rate of inhibition by the toxin varied from cell line to cell line. It indicates the involvement of an IAP substrate GTP-binding protein(s) such as G_i or G_o in the signal transduction mechanism of P_2-receptor. However, the inhibition of the Ca^{2+} response to ATP in IAP-treated cells was not statistically significant in our experiments on neural cells (Table I). In addition, the same treatment of these cell lines with IAP did not affect their ATP-induced inositol trisphosphate production (Fig. 2). These results indicate no participation of IAP substrate GTP-binding proteins.

As mentioned in the Introduction, ATP is supposed to be transmitted from nerve endings together with

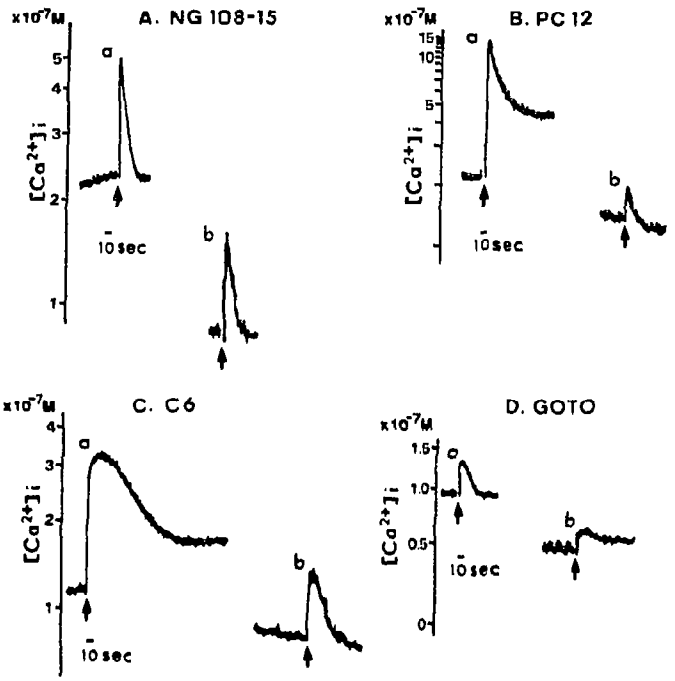

Fig. 1. Effects of extracellular ATP on $[Ca^{2+}]_i$ of 4 cultured neural cell lines in the presence (a) or absence (b) of extracellular Ca^{2+}. At the time indicated by arrows, ATP (100 μM) was added to the incubation medium.

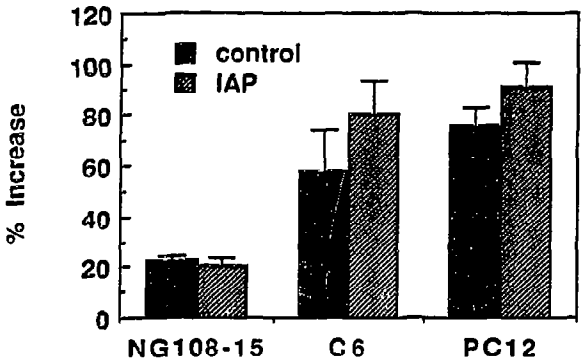

Fig. 2. Effect of extracellular ATP on inositol trisphosphate production of the cultured cell lines treated with or without IAP (10 ng/ml). The value obtained in the absence of ATP was assigned to 100%. ATP-induced increase (%) over this control value is shown. Results are means ± SE of three separate experiments. IAP treatment significantly affects neither the incorporation of [^{3}H]inositol into lipid fraction nor [^{3}H]inositol trisphosphate content in the absence of ATP.

other classical neurotransmitters such as norepinephrine and acetylcholine [1,2]. ATP thus released can be rapidly hydrolyzed to adenosine and this nucleoside interacts with the ATP-donor cells to inhibit the neurotransmitter release [18]. Adenosine interacts also with many types of postsynaptic cells to induce a variety of biological reactions [18]. For example, in cultured thyroid cells adenosine synergistically enhances GTP- and norepinephrine-induced phospholipase C activation and Ca^{2+} mobilization [9,10]. Thus, a role of adenosine has been addressed on both presynaptic and postsynaptic actions, while that of ATP only on postsynaptic actions. ATP has been shown to exert, without hydrolysis to adenosine, a variety of physiological responses in many types of peripheral cells. Here, we showed that ATP but not adenosine is a potent Ca^{2+} mobilizer in neural cell lines as well. These results are consistent with recent works with astrocytes [19], adrenal chromaffin cells [20] and primary culture of rat hippocampus [21], in which ATP markedly increased $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ increase in presynaptic neurons is thought to be responsible for neurotransmitter release [22], therefore we assume that ATP released from presynaptic neurons, via P_2-receptors, might facilitate neurotransmitter release in an autocrine fashion. Indeed, our preliminary experiments showed that ATP markedly induced norepinephrine release from PC12 cells. Thus, the neural cell lines used in the present study will be useful as a model system to further investigate the action mechanism of ATP in a nervous system.

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