J. Pharm. Pharmacol. 1997, 49: 520–524
Received November 18, 1996
Accepted January 30, 1997



# Effects of ATP on Phosphoinositide Hydrolysis and Prostaglandin $E_2$ Generation in Rabbit Astrocytes

HIROMI ISHIMOTO, NORIMICHI NAKAHATA*, ISAO MATSUOKA AND HIRONORI NAKANISHI

*Department of Pharmacology, Fukushima Medical College, Hikarigaoka 1, Fukushima 960-12 and *Department of Pharmaceutical Molecular Biology, Faculty of Pharmaceutical Sciences, Tohoku University, Aoba, Sendai 980-77, Japan*

## Abstract

Extracellular ATP secreted from stimulated nerves plays a role in neurotransmission. This study examined the effects of extracellular ATP on phospholipase $A_2$ and C signalling pathways in rabbit astrocytes.

ATP caused prostaglandin $E_2$ ($PGE_2$) generation and phosphoinositide hydrolysis in a time- and concentration-dependent manner. A $P_{2y}$ purinoceptor-selective agonist, 2-methylthio-ATP also caused phosphoinositide hydrolysis, but not $PGE_2$ generation. A $P_{2x}$ purinoceptor-selective agonist, $\alpha,\beta$-methylene-ATP did not cause either phosphoinositide hydrolysis or $PGE_2$ generation. Although pertussis toxin had no effect on 2-methylthio-ATP-induced phosphoinositide hydrolysis, it markedly decreased ATP-induced $PGE_2$ generation, with significant inhibition of phosphoinositide hydrolysis. Dexamethasone and indomethacin which potently inhibited ATP-induced $PGE_2$ generation, caused partial inhibition of phosphoinositide hydrolysis, suggesting that pertussis toxin-sensitive component of ATP-induced phospholipase C activation is mediated by cyclo-oxygenase metabolites of arachidonic acid.

These results suggest that a stimulation of $P_{2y}$ receptor results in phospholipase C activation in a pertussis toxin-insensitive manner, and that a $P_2$ receptor other than the $P_{2y}$ or $P_{2x}$ subtypes is involved in ATP-induced phospholipase $A_2$ activation via a pertussis toxin-sensitive G protein.

ATP stored in synaptic vesicles is released into the synaptic cleft on nerve stimulation (Burnstock & Sneddon 1985). In addition to its well-known intracellular function as energy source for many important reactions, extracellular ATP stimulates specific membrane receptors, termed $P_2$ purinoceptors, and plays a role as a messenger in several tissues to evoke physiological responses, such as smooth muscle contraction and secretion (El-Moatassim et al 1992). $P_2$ purinoceptors are classified into several subclasses according to their agonist selectivity and, more recently, according to molecular cloning (Dubyak & El-Moatassim 1993). Recent studies have defined signal transduction systems coupled to $P_2$ purinoceptors. For example, stimulation of $P_{2y}$ and $P_{2u}$ purinoceptors results in phosphoinositide hydrolysis and $Ca^{2+}$ mobilization via heterotrimetric G proteins (Cooper et al 1989). $P_{2x}$ purinoceptors, currently classified into seven different subclasses, are ligand-gated ion channels (Valera et al 1994; Evans et al 1995; Kennedy & Leff 1995). $P_{2z}$ purinoceptor, which is activated by 3′-(*O*)-(4-benzoyl benzoyl)ATP and by $ATP^{4-}$, also has intrinsic cation-channel activity (Kaiho et al 1996). Recently, we have shown that a $P_2$ purinoceptor activates adenylyl cyclase in neuroblastoma × glioma hybrid NG108-15 cells (Matsuoka et al 1995).

Astrocytes have neurotrophic action mediated by neurotrophic factors, cell surface molecules and the extracellular matrix (Yoshida et al 1992). Astrocytes express $P_2$ purinoceptors that initiate phosphoinositide hydrolysis, $Ca^{2+}$ mobilization and prostaglandin generation (Petroni et al 1991). Bruner & Murphy (1993) have shown that $P_{2y}$ purinoceptors couple directly to phospholipase $A_2$ activation in rat astrocytes. The correlation between phosphoinositide hydrolysis and prostaglandin generation induced by ATP in astrocytes remains unknown, however.

In this study we have investigated the signal transduction of stimulation of a $P_2$ purinoceptor to clarify the correlation between phosphoinositide hydrolysis and prostaglandin $E_2$ ($PGE_2$) generation in rabbit astrocytes. A selective $P_{2y}$ purinoceptor agonist, 2-methylthio-ATP, and a selective $P_{2x}$ purinoceptor agonist, $\alpha,\beta$-methylene-ATP, were used to analyse subclass-specific signal transduction. The results suggest that $P_{2y}$ purinoceptor is coupled to phosphoinositide hydrolysis via a pertussis toxin-insensitive G protein, and that a $P_2$ purinoceptor other than the $P_{2y}$ or $P_{2x}$ subtypes is involved in ATP-induced phospholipase $A_2$ activation via a pertussis toxin-sensitive G protein.

Correspondence: H. Ishimoto, Department of Pharmacology, Fukushima Medical College, Hikarigaoka 1, Fukushima 960-12, Japan.

## Materials and Methods

### *Materials*

Dulbecco's modified Eagle's medium and Eagle's minimum essential medium were obtained from Nissui Pharmaceutical (Tokyo, Japan), foetal calf serum from Whittaker Bioproducts (Walkersville, MD), mouse anti-glial fibrillary acidic protein antibody from Medac Gesellschaft für Klinische Spezialpräparate GmbH, and fluorescein isothiocyanate-labelled goat anti-mouse IgG antibody from Amersham Japan (Tokyo, Japan). ATP, indomethacin, trichloroacetic acid and diethyl ether were from Wako Pure Chemical Industries (Osaka, Japan), 2-methylthio-ATP from Research Biochemicals (Natick, MA) and dexamethasone from Bany Pharm (Tokyo, Japan), pertussis toxin from Funakoshi (Tokyo, Japan). $\alpha,\beta$-Methylene-ATP was from Sigma (St Louis, MO), anti-$PGE_2$ serum from Ono Pharmaceutical Company (Osaka, Japan);

[$^3$H]myo-inositol (16·5 Ci mmol$^{-1}$) from American Radiolabeled Chemicals (St Louis, MO) and [$^3$H]$PGE_2$ (200 Ci mmol$^{-1}$) from Du Pont NEN Products (Boston, MA). Other chemicals used were of reagent grade or the highest quality available.

*Cell culture*

Astrocytes were prepared from rabbit brain as previously described (Nakahata et al 1992). Briefly, the cerebral cortex was cut into small pieces in Eagle's minimum essential medium buffered with HEPES (20 mM; pH 7·4), and was treated with 0·02% trypsin (w/v) at 37°C for 15 min. The trypsinized cells were collected by centrifugation at 180 *g* for 1 min and washed several times with the HEPES-buffered Eagle's minimum essential medium. The cells (concentration of $10^7$ cells mL$^{-1}$) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum, penicillin (50 units mL$^{-1}$) and streptomycin (50 μg mL$^{-1}$) in a humidified atmosphere of 5% $CO_2$–95% air at 37°C. The medium was changed every 3–4 days. After 2–3 weeks, over 90% of the cells grown were astrocytes, as determined by binding of anti-glial fibrillary acidic protein antibody.

*Assay of [$^3$H]inositol phosphates*

Phosphoinositide hydrolysis was monitored by measuring [$^3$H]inositol phosphates, as previously described (Nakahata & Nakanishi 1988). Rabbit astrocytes were sub-cultured into 12-well plates at a density of $10^5$ cells/well and used 3–4 days after subculture. The cells were labelled with 1 μCi mL$^{-1}$ [$^3$H]myo-inositol in Dulbecco's modified Eagle's medium containing 10% (v/v) foetal calf serum for 18 h. The labelled cells were washed twice with Eagle's minimum essential medium buffered with HEPES (20 mM; pH 7·4) and pre-incubated in the medium at 37°C for 10 min. The reaction was started by adding drugs to the medium in the presence of LiCl (10 mM), and was terminated by addition of ice-cold 5% (w/v) trichloroacetic acid after aspiration of the medium. The trichloroacetic acid extracts were washed three times with diethyl ether to remove trichloroacetic acid, and the samples were then applied to a column (bed volume, 0·6 mL) of anion exchange resin (AG 1-X8, 100–200 mesh, formate form; Bio-Rad). Total [$^3$H]inositol phosphates were eluted by ammonium formate (1 M) in formic acid (0·1 M) and were determined by liquid scintillation counting.

*Assay of $PGE_2$*

$PGE_2$ was determined by radioimmunoassay as described previously (Nakahata & Nakanishi 1988) with minor modification. Rabbit astrocytes were sub-cultured into 12-well plates at a density of $10^5$ cells well$^{-1}$ and were used 3–4 days after subculture. The cells were washed twice with Eagle's minimum essential medium buffered with HEPES (20 mM; pH 7·4) and pre-incubated in the medium at 37°C for 10 min. The reaction was started by adding drugs in the medium and was terminated by transferring the incubation medium into an ice-cold tube. $PGE_2$ accumulated in the medium was extracted twice with ethyl acetate (1 mL) after acidification to pH 4·0 with 1 M HCl. After evaporation of the ethyl acetate with a stream of nitrogen, the sample was dissolved in 10 mM Tris-HCl (pH 7·6). The assay mixture (0·3 mL) of sample, anti-$PGE_2$ antiserum (600-fold dilution) and [$^3$H]$PGE_2$ (22 000 d min$^{-1}$) in 100 mM Tris-HCl (pH 7·6) containing 0·5% (w/v) bovine serum albumin was incubated at 4°C for 18 h. [$^3$H]$PGE_2$ bound to antibody was assayed by liquid scintillation counting after sedimentation of free [$^3$H]$PGE_2$ with dextran-coated charcoal.

*Data analysis*

Results were expressed as means ± s.e.m. Statistically significant differences were determined by Student's *t*-test.

## Results

*Effects of ATP and its analogues on phosphoinositide hydrolysis and $PGE_2$ generation*

ATP (100 μM) induced formation of [$^3$H]inositol phosphates and generation of $PGE_2$ in astrocytes (Fig. 1). Time-course analysis revealed that ATP at 100 μM resulted in the slow accumulation of [$^3$H]inositol phosphates from 653·3 ± 0·4 to 1168·8 ± 0·2 d min$^{-1}$/well. In contrast with its effect on [$^3$H]inositol phosphate-formation, ATP (100 μM) caused rapid generation of $PGE_2$ from 0·25 ± 0·04 to 0·59 ± 0·03 ng/well within 2 min; the amount generated then increased slowly until 10 min. These results indicate that ATP evokes both phospholipase C and phospholipase $A_2$ activation.

We next compared the effects of ATP on phosphoinositide hydrolysis and $PGE_2$ generation with those of a $P_{2y}$ purinoceptor agonist, 2-methylthio-ATP and a $P_{2x}$ purinoceptor agonist, α,β-methylene-ATP. ATP caused formation of [$^3$H]inositol phosphates and $PGE_2$ generation in a concentration-dependent manner with an EC50 value of approximately 10 mM. 2-Methylthio-ATP induced formation of [$^3$H]inositol phosphates with an EC50 value of approximately 0·3 μM, but it failed to produce $PGE_2$ (Fig. 2). α,β-methylene-ATP had no effect on the formation of [$^3$H]inositol phosphates or $PGE_2$ generation (Fig. 2). These results indicate that 2-methylthio-ATP-sensitive $P_{2y}$ purinoceptor couples to phospholipase C, but not to phospholipase $A_2$ activation. An α,β-methylene-ATP-sensitive $P_{2x}$ purinoceptor might not be present in rabbit

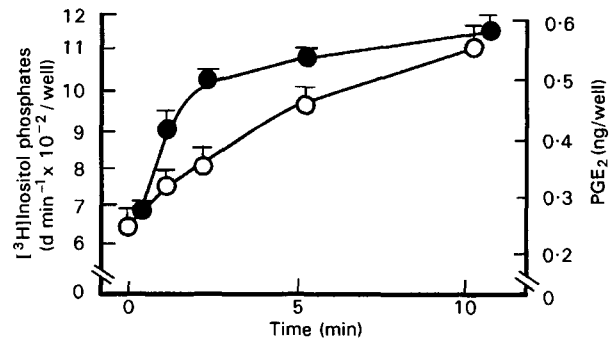


FIG. 1. Time-course of ATP (100 μM)-induced phosphoinositide hydrolysis and $PGE_2$ generation in rabbit cultured astrocytes. Astrocytes were incubated at 37°C in Eagle's minimum essential medium buffered with HEPES (20 mM; pH 7·4) in the presence of 100 μM ATP for the time indicated. Cells were labelled overnight with 1 μCi mL$^{-1}$ [$^3$H]inositol and stimulated with ATP. The accumulated [$^3$H]inositol phosphates were separated with AG 1-X8 (○). $PGE_2$ produced by ATP was incubated overnight at 4°C with anti-$PGE_2$ antiserum and [$^3$H]$PGE_2$. [$^3$H]$PGE_2$ bound to antiserum was assessed by the dextran-coated charcoal method (●). Results represent the means ± s.e.m. of three experiments.

astrocytes, or might not communicate with either phospholipase C or phospholipase $A_2$.

*Effects of dexamethasone and indomethacin on phosphoinositide hydrolysis and $PGE_2$ generation*

Table 1 shows the effects of dexamethasone and indomethacin on ATP-induced formation of [$^3$H]inositol phosphates or generation of $PGE_2$. The treatment of cells with dexamethasone (100 ng $mL^{-1}$) resulted in partial inhibition of ATP-induced formation of [$^3$H]inositol phosphates and an appreciable decrease in $PGE_2$ generation. Indomethacin partially inhibited ATP-induced formation of [$^3$H]inositol phosphates and severely attenuated $PGE_2$ generation. The result suggests that cyclooxygenase metabolites produced by ATP might activate phospholipase C.

*Effect of pertussis toxin on phosphoinositide hydrolysis and $PGE_2$ generation*

To examine the role of G protein in $P_2$ purinoceptor-mediated phosphoinositide hydrolysis and phospholipase $A_2$ activation, we measured the formation of [$^3$H]inositol phosphates and generation of $PGE_2$ in response to ATP, 2-methylthio-ATP and $\alpha,\beta$-methylene-ATP after pertussis toxin treatment. Whereas ATP-induced formation of [$^3$H]inositol phosphates was partially inhibited by the pertussis toxin treatment, 2-methylthio-ATP-induced phosphoinositide hydrolysis was unaffected (Table 2). The treatment of the cells with pertussis toxin potently inhibited the $PGE_2$ generation induced by ATP. $\alpha,\beta$-Methylene-ATP had no effect on formation of [$^3$H]inositol phosphates, and pertussis toxin did not modify the formation (data not shown). These results suggest that $P_{2y}$ purinoceptor is coupled to phospholipase C in a pertussis toxin-insensitive manner, and a $P_2$ purinoceptor other than $P_{2y}$ or $P_{2x}$ is associated with phospholipase $A_2$ via pertussis toxin-sensitive G proteins such as Gi and Go.

Table 1. Effects of dexamethasone and indomethacin on ATP-induced phosphoinositide hydrolysis and $PGE_2$ generation.

| | [$^3$H] Inositol phosphates (d $min^{-1}$ × $10^{-2}$/well) | $PGE_2$ (ng/well) |
|---|---|---|
| None | 7·2 ± 0·2 | 0·6 ± 0·2 |
| Dexamethasone | 5·4 ± 0·7 | 0·4 ± 0·4 |
| Indomethacin | 7·4 ± 0·5 | 0·5 ± 0·7 |
| ATP | 24·7 ± 1·3 | 2·3 ± 0·6 |
| ATP + dexamethasone | 17·1 ± 2·1* | 0·9 ± 0·2* |
| ATP + indomethacin | 17·3 ± 1·4* | 0·7 ± 0·1* |

Cells were pre-incubated with dexamethasone (100 ng $mL^{-1}$) for 24 h or indomethacin (5 $\mu$M) for 10 min. The cells were stimulated with 100 $\mu$M ATP for 10 min. *$P < 0.01$, significantly different from the response to ATP alone.

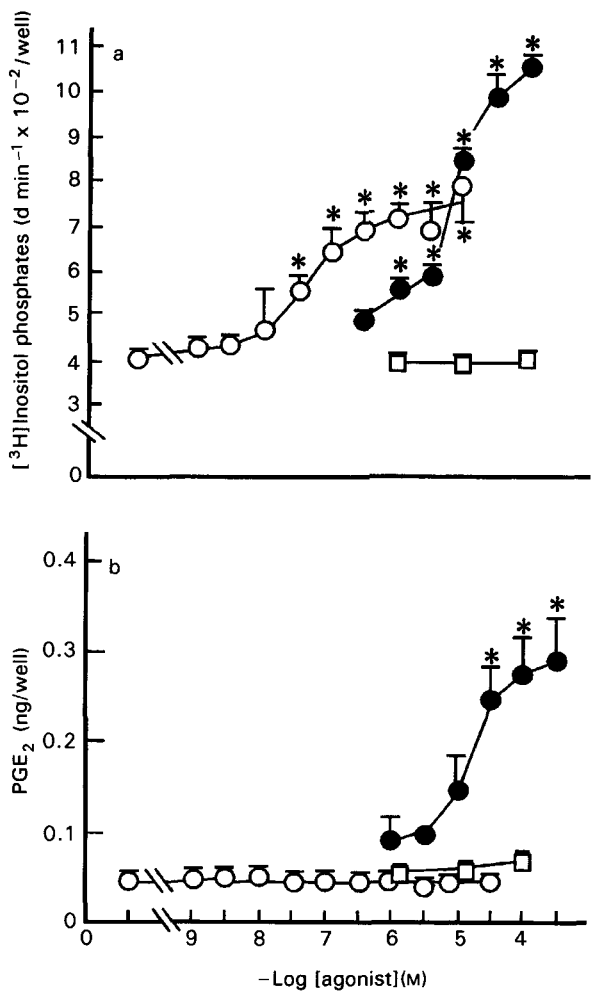


FIG. 2. Effects of ATP and ATP analogues on phosphoinositide hydrolysis and PGE2 generation. Cells were incubated with various concentrations of agents for 10 min. Panels (a) and (b) show the formation of [$^3$H]inositol phosphates and generation of $PGE_2$ induced by ATP (●), 2-methylthio-ATP (○) and $\alpha,\beta$-methylene-ATP (□), respectively. Results represent the means ± s.e.m. of three experiments. *$P < 0.05$, significant compared with the value without drug.

Table 2. Effects of pertussis toxin on formation of [$^3$H]inositol phosphates and generation of $PGE_2$ induced by ATP and its analogues.

| Agonist | [$^3$H]inositol phosphates (d $min^{-1}$ × $10^{-2}$/well) | | $PGE_2$ (ng /well) | |
|---|---|---|---|---|
| | Control | Pretreated | Control | Pretreated |
| None | 7·1 ± 0·2 | 5·7 ± 1·5 | 0·8 ± 1·0 | 0·5 ± 0·2 |
| ATP | 27·1 ± 3·6 | 15·5 ± 3·1 | 1·9 ± 0·2 | 0·9 ± 0·3* |
| 2-Methylthio-ATP | 11·6 ± 0·6 | 10·2 ± 0·5 | 1·1 ± 0·3 | 1·0 ± 0·4 |

Control cells and cells pre-treated with pertussis toxin (0·1 $\mu$g $mL^{-1}$) for 24 h were stimulated with 100 $\mu$M ATP or 1 $\mu$M 2-methylthio-ATP, respectively, for 10 min. Results represent the mean ± s.e.m. from three experiments. *$P < 0.01$, significantly different from the control response.

## Discussion

This study demonstrates that the stimulation of $P_2$ purinoceptors results in phosphoinositide hydrolysis and $PGE_2$ generation in rabbit astrocytes. Although both ATP and 2-methylthio-ATP cause formation of [$^3$H]inositol phosphates, there are two signalling pathways which are discriminated by pertussis toxin. Because phosphoinositide hydrolysis induced by 2-methylthio-ATP, a $P_{2y}$ purinoceptor agonist, is resistant to pertussis toxin treatment, $P_{2y}$ purinoceptor might couple with a pertussis toxin-insensitive G protein to elicit phosphoinositide hydrolysis. Because maximum phosphoinositide hydrolysis by ATP is greater than that by 2-methylthio-ATP, and pertussis toxin reduces ATP-induced phosphoinositide hydrolysis to the level of 2-methylthio-ATP-induced hydrolysis, it is likely that ATP-induced hydrolysis is divided into two mechanisms. It is assumed that one is a $P_{2y}$ purinoceptor-mediated, pertussis toxin-insensitive activation, the other a $P_2$ purinoceptor-mediated, pertussis toxin-sensitive activation. It has recently been shown that there are two signalling pathways in heterotrimeric G protein-mediated activation of phospholipase C. The $\alpha$-subunit of $G_{q/11}$, which is not a pertussis toxin substrate, activates phospholipase C-$\beta_1$ (Lee et al 1992). On the other hand, the $\beta\gamma$-subunit of Gi activates phospholipase C-$\beta_2$ in a pertussis toxin-sensitive manner (Camps et al 1992). The current results indicate that ATP might stimulate the above two signalling pathways mediated via different receptor subtypes.

ATP-induced $PGE_2$ generation was potently inhibited by treatment of cells with dexamethasone or indomethacin, or both. One of the mechanisms of action of dexamethasone is thought to be the production of inhibitory proteins of phospholipase $A_2$, such as macrocortin from macrophages, lipomodulin from neutrophils and renocortin from medullary interstitial cells (Hirata et al 1981; Blackwell et al 1982; Russo-Marie & Duval 1982; Gupta et al 1984), although recent lines of evidence suggest that dexamethasone inhibits the expression of cyclooxygenase 2 (Tordjiman et al 1995). Because dexamethasone inhibits $PGE_2$ generation more potently than arachidonic acid release in rabbit astrocytes (Nakahata et al 1996), both mechanisms might be involved in the inhibition by dexamethasone of ATP-induced $PGE_2$ generation. The treatment of astrocytes with indomethacin or dexamethasone, or both, partially reduced ATP-activated phospholipase C, indicating that cyclooxygenase products released by stimulation of $P_2$ purinoceptors might participate in phospholipase C as a positive feedback mediator. One cyclooxygenase product eliciting phosphoinositide hydrolysis is likely to be $TXA_2$, because: $PGE_2$ does not stimulate phosphoinositide hydrolysis in these cells (Ishimoto et al, unpublished observation); $STA_2$, a stable $TXA_2$ analogue, stimulates phosphoinositide hydrolysis in rabbit astrocytes (Nakahata et al 1992); and ATP causes the release of $TXB_2$, a stable metabolite of $TXA_2$ (Ishimoto et al 1996).

It has recently been shown that mitogen-activated protein kinase is involved in the process of phospholipase $A_2$ activation (Lin et al 1993), and mitogen-activated protein kinase is activated through $G_i$ and $p21^{ras}$ pathways (Alblas et al 1993). The $P_2$ purinoceptor which mediates phospholipase $A_2$ activation is, therefore, likely to couple with $G_i$, stimulation of which results in the activation of the mitogen-activated protein kinase-phospholipase $A_2$ pathway in addition to phosphoinositide hydrolysis in rabbit astrocytes. Several reports reveal that $P_2$ purinoceptors without definition of subtypes are coupled to prostanoid synthesis via a pertussis toxin-sensitive stimulation of phospholipase $A_2$ in various tissues or cells (Takikawa et al 1990; Gebicke-Haerter 1991); this is in agreement with our results.

In conclusion, there are at least two kinds of purinoceptor, with distinct signalling pathways, in rabbit astrocytes. One is the $P_{2y}$ purinoceptor that activates phospholipase C in a pertussis toxin-insensitive manner. Another is the $P_2$ purinoceptor other than $P_{2y}$ or $P_{2x}$ purinoceptors which causes activation of phospholipase C and phospholipase $A_2$ via a pertussis toxin-sensitive G protein.

*Acknowledgements*

We are very grateful to Dr Junko Kimura for helpful discussion. We are also grateful to Ono Pharmaceutical Co. Ltd (Osaka, Japan) for donation of $PGE_2$ and its antibody. The technical help of Ms Sanae Sato is greatly appreciated. This work was partly supported by a Grant-in-Aid for Scientific Research (08672496 to N. N. and 20223034 to H. I.) from the Ministry of Education, Science and Culture of Japan.

## References

Alblas, J., Corven, E. J. V., Hordijk, P. L., Milligan, G., Moolenaar, W. H. (1993) Gi-mediated activation of the $p21^{ras}$-mitogen-activated protein kinase pathway by $a_2$-adrenergic receptors expressed in fiblobalsts. J. Biol. Chem. 268: 22235–22238

Blackwell, G. J., Carnuccio, R., DiRosa, M., Flower, R. J., Langham, C. S. J., Parente, L., Persico, P., Russell-Smith, N. C., Stone, D. (1982) Glucocorticoids induce the formation and release of anti-inflammatory and anti-phospholipase proteins into the peritoneal cavity of the rat. Br. J. Pharmacol. 76: 185–194

Bruner, G., Murphy, S. (1993) Purinergic $P_{2y}$ receptors on astrocytes are directly coupled to phospholipase $A_2$. Glia 7: 219–224

Burnstock, G., Sneddon, P. (1985) Evidence for ATP and noradrenaline as cotransmitters in sympathetic nerves. Clin. Sci. 68 (Suppl 10): 89s–92s

Camps, M., Carozzi, A., Schnabel, P., Scheer, A., Parker, P. J., Giershik, P. (1992) Isozyme-selective stimulation of phospholipase C-$b_2$ by G protein bg-subunits. Nature 360: 684–686

Cooper, C. L., Morris, A. J., Harden, T. K. (1989) Guanine nucleotide-sensitive interaction of a radiolabeled agonist with a phospholipase C-linked $P_{2y}$-purinergic receptor. J. Biol. Chem. 264: 6202–6206

Dubyak, G. R., El-Moatassim, C. (1993) Signal transduction via $P_2$-purinergic receptors for extracellular ATP and other nucleotides. Am. J. Physiol. 265: C577–606

El-Moatassim, C., Dornand, J., Mani, J. (1992) Extracellular ATP and cell signalling. Biochim. Biophys. Acta 1134: 31–45

Evans, R. J., Lewis, C., Buell, G., Valera, S., North R. A., Surprenant, A. (1995) Pharmacological characterization of heterologously expressed ATP-gated cation channels ($P_{2x}$ purinoceptors). Mol. Pharmacol. 48: 178–183

Gebicke-Haerter, P. J., Schobert, A., Hertting, G. (1991) Pertussis and cholera toxins inhibit prostaglandin synthesis in rat astrocyte cultures at distinct metabolic steps. Biochem. Pharmacol. 42: 1267–1271

Gupta, C., Katsumata, M., Goldman, A. S., Herold, R., Piddington, R. (1984) Glucocorticoid-induced phospholipase $A_2$-inhibitory proteins mediate glucocorticoid teratogenicity in vitro. Proc. Natl Acad. Sci. USA 81: 1140–1143

Hirata, F., Schiffmann, E., Venkatasubramanian, K., Salomon, D., Axelrod, J. (1981) Phospholipid methylation and phospholipase $A_2$ activation in cytotoxicity by human natural killar cells. Proc. Natl Acad. Sci. USA 78: 3839–2536

Ishimoto, H., Matsuoka, I., Nakanishi, H., Nakahata, N. (1996) A comparative study of arachidonic acid metabolism in rabbit cultured astrocytes and human astrocytoma cells (1321N1). Gen. Pharmacol. 27: 313–317

Kaiho, H., Kimura, J., Matsuoka, I., Kumasaka, T., Nakanishi, H. (1996) ATP-activated nonselective cation current in NG108-15 cells. J. Neurochem. 67: 398–406

Kennedy, C., Leff, P. (1995) How should $P_{2x}$ purinoceptors be classified pharmacologically? Trends Pharmacol. Sci. 16: 168–174

Lee, C. H., Park, D., Wu, D., Rhee, S. G., Simon, M. I. (1992) Members of the Gqa subunit gene family activate phospholipase C-b isozymes. J. Biol. Chem. 267: 16044–16047

Lin, L.-L., Wartman, M., Lin, A. Y., Knopf, J. L., Seth, A., Davis, R. J. (1993) $cPLA_2$ is phosphorylated and activated by MAP kinase. Cell 72: 269–278

Matsuoka, I., Zhou, Q., Ishimoto, H., Nakanishi, H. (1995) Extracellular ATP stimulates adenylyl cyclase and phospholipase C through distinct purinoceptors in NG108-15 cells. Mol. Pharmacol. 47: 855–862

Nakahata, N., Nakanishi, H. (1988) Bradykinin-induced contraction is inhibited by tiaramide, an anti-inflammatory drug, with an inhibition of increase in intracellular free calcium. J. Pharmacol. Exp. Ther. 246: 635–640

Nakahata, N., Ishimoto, H., Kurita, M., Ohmori, K., Takahashi, A., Nakanishi, H. (1992) The presence of thromboxane $A_2$ receptors in cultured astrocytes from rabbit brain. Brain Res. 583: 100–104

Nakahata, N., Imata, K., Okawa, T., Watanabe, Y., Ishimoto, H., Ono, T., Ohizumi, Y., Nakanishi, H. (1996) Mastoparan elicits prostaglandin $E_2$ generation and inhibitions inositol phosphate accumulation via different mechanisms in rabbit astrocytes. Biochim. Biophys. Acta 1310: 60–66

Petroni, A., Blasevich, M., Visioli, F., Zancocchia, B., Caruso, D., Galli, C. (1991) Arachidonic acid cyclooxygenase and lipoxygenase pathways are differently activated by platelet activating factor and the calcium-ionophore A23187 in a primary culture of astroglial cells. Brain Res. 63: 221–227

Russo-Marie, F., Duval, D. (1982) Dexamethasone-induced inhibition of prostaglandin production does not result from a direct action on phospholipase activities but is mediated through a steroid-inducible factor. Biochim. Biophys. Acta 712: 177–185

Takikawa, R., Kurachi, Y., Mashima, S., Sugimoto, T. (1990) Adenosine-5′-triphosphate-induced sinus tachycardia mediated by prostaglandin synthesis via phospholipase C in the rabbit heart. Pflügers Arch. 417: 13–20

Tordjiman, C., Coge, F., Andre, N., Riwue, H., Spedding, M., Bonnet, J. (1995) Characterization of cyclooxygenase 1 and 2 expression in mouse resident peritoneal macrophages in vitro; interactions of non steroidal anti-inflammatory drugs with COX2. Biochim. Biophys. Acta 1256: 249–256

Valera, S., Hussy, N., Evans, R. J., Adami, N., North, R. A., Surprenant, A., Buell, G. (1994) A new class of ligand-gated ion channel defined by $P_{2x}$ receptor for extracellular ATP. Nature 371: 516–519

Yoshida, K., Kakihana, M., Chen, L. S., Ong, M., Baird, A., Gage, F. H. (1992) Cytokine regulation of nerve growth factor-mediated cholinergic neutrophic activity synthesized by astrocytes and fibroblasts. J. Neurochem. 59: 919–931