Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 7330–7334, August 1992
Pharmacology

Stimulation of adenosine A_1 receptors and bradykinin receptors, which act via different G proteins, synergistically raises inositol 1,4,5-trisphosphate and intracellular free calcium in DDT_1 MF-2 smooth muscle cells

(receptor cross talk/phospholipase C/fura-2/pertussis toxin/cAMP)

PÄR GERWINS* AND BERTIL B. FREDHOLM

Department of Pharmacology, Karolinska Institutet, Box 60400, S-104 01 Stockholm, Sweden

Communicated by Viktor Mutt, April 13, 1992 (received for review January 10, 1992)

ABSTRACT **We have examined the cross talk between adenosine and bradykinin receptors in DDT_1 MF-2 smooth muscle cells. Both adenosine and bradykinin mobilized intracellular free calcium via the formation of inositol 1,4,5-trisphosphate in a time- and dose-dependent manner. Adenosine exerted its actions via adenosine A_1 receptors as demonstrated by the observations that N^6-cyclopentyladenosine, a selective A_1 receptor agonist, had an EC_{50} in the low nanomolar range and that a selective adenosine A_1 receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine, counteracted adenosine-mediated responses at concentrations typical for signaling via adenosine A_1 receptors. Adenosine A_1 receptors were coupled to phospholipase C via pertussis toxin-sensitive guanine nucleotide-binding regulatory protein(s) [G protein(s)], whereas bradykinin responses were unaffected by pertussis toxin. When adenosine or N^6-cyclopentyladenosine was combined with bradykinin, the resulting formation of inositol 1,4,5-trisphosphate was more than additive, and the EC_{50} value for adenosine and N^6-cyclopentyladenosine was shifted to the left by bradykinin, the affinity of which was unaltered. Combining N^6-cyclopentyladenosine and bradykinin also synergistically raised intracellular free calcium both at subthreshold levels and at maximal concentrations of the two agonists. The interaction was not dependent upon cAMP. In conclusion, stimulation of adenosine A_1 receptors coupled to pertussis toxin-sensitive G protein(s) and bradykinin receptors coupled to pertussis toxin-insensitive G protein(s) synergistically mobilizes intracellular free calcium and inositol 1,4,5-trisphosphate formation.**

Cells are often simultaneously activated by agonists acting at different receptors, which makes receptor–receptor interactions important. Such interactions can be both synergistic and antagonistic (1). Adenosine is a ubiquitous endogenous modulator, which is known to influence signaling via several hormones and neurotransmitters (2), and acts via A_1 and A_2 receptors (3). It is in the nature of a modulator to interact with the signaling via other cellular regulators. For example, adenosine has been shown to enhance (4–7) or attenuate (8–10) the formation of inositol phosphates induced via several types of receptors. The type of adenosine receptor responsible for these actions, as well as the occasionally reported ability of adenosine *per se* to stimulate inositol phosphate formation (11), is unclear. Recently we reported preliminary evidence that adenosine, acting on adenosine A_1 receptors, stimulated inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] formation in DDT_1 MF-2 cells (12). Adenosine A_1 receptors in these cells also inhibit adenylyl cyclase via pertussis toxin-sensitive guanine nucleotide-binding regulatory protein(s) [G protein(s)], G_{i2} and/or G_{i3} (13, 14). The purpose of the present study was to further characterize the effect of adenosine A_1 receptor agonists and to compare the effect of adenosine to that of bradykinin on Ins(1,4,5)P_3 formation and intracellular calcium levels in DDT_1 MF-2 cells. Bradykinin is known to stimulate Ins(1,4,5)P_3-mediated calcium mobilization in several cells (15, 16) and has been shown to stimulate phosphate incorporation into inositol phospholipids in DDT_1 MF-2 cells (17). We found that both adenosine and bradykinin are able to raise Ins(1,4,5)P_3 and intracellular calcium levels in DDT_1 MF-2 cells, but via different G proteins. Despite this, there was a synergistic interaction between the two substances.

MATERIALS AND METHODS

Materials. Cell culture media, fetal calf serum, and cell culture flasks were from NordCell (Bromma, Sweden). D-*myo*-[2-^{3}H]Inositol 1,4,5-trisphosphate {[^{3}H]Ins(1,4,5)P_3; 51.4 Ci/mmol; 1 Ci = 37 GBq} was from Amersham, and [2,8-^{3}H]adenosine 3′,5′-cyclic monophosphate ([^{3}H]cAMP; 44.5 Ci/mmol) was from New England Nuclear. N^6-Cyclopentyladenosine (CPA), bovine serum albumin, cAMP, EDTA, EGTA, 8-cyclopentyl-1,3-dipropylxanthine, 1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2′-amino-5′-methylphenoxy)ethane-*N,N,N′,N′*-tetraacetic acid pentaacetoxy methyl ester (fura-2 AM), Hanks' balanced salt solution, Hepes, and forskolin were all from Sigma. Adenosine was purchased from Aldrich-Europe (Belgium), and Ins(1,4,5)P_3 was from Boehringer Mannheim. Pertussis toxin was from List Biological Laboratories (Campbell, CA). Rolipram [4-(3-cyclopentyloxymetoxyphenyl)-2-pyrrolidone] was a gift from Schering.

Cell Culture. DDT_1 MF-2 smooth muscle cells, originally isolated from a steroid-induced leiomyosarcoma of Syrian hamster vas deferens (18), were obtained from the American Type Culture Collection. Cells were grown in suspension, maintained in Dulbecco's modified Eagle's medium with 4.5 g of glucose per liter and also containing 5% fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and 2 mM L-glutamine at 37°C in 5% CO_2/95% air. Cells were subcultured three times weekly and used at a density of ≈10^5 cells/ml. Cell viability was >90% as assessed by the exclusion of trypan blue.

Determination of Ins(1,4,5)P_3. Cells were washed once in assay medium (Dulbecco's modified Eagle's medium without

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Ins(1,4,5)P_3, inositol 1,4,5-trisphosphate; G protein, guanine nucleotide-binding regulatory protein; CPA, N^6-cyclopentyladenosine; fura-2 AM, 1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2′-amino-5′-methylphenoxy)ethane-*N,N,N′,N′*-tetraacetic acid pentaacetoxy methyl ester.

*To whom reprint requests should be addressed.

$NaHCO_3$ and buffered with 20 mM Hepes at pH 7.4) and resuspended in assay medium to a concentration of 3×10^6 cells per ml. Aliquots (0.2 ml = 6×10^5 cells) were transferred to test tubes and preincubated for 20 min at 37°C in a water bath before addition of indicated drugs (0.1 ml). Lithium was omitted to avoid any interaction with the formation of inositol phosphates. Reactions were terminated by the addition of perchloric acid to a final concentration of 0.4 M and placed on ice for 1 hr. Samples were neutralized with 4 M KOH/1 M Tris/60 mM EDTA and frozen until analyzed.

The quantitation of Ins(1,4,5)P_3 was performed with a competitive binding assay as described (19) with the exception that bound [^{3}H]Ins(1,4,5)P_3 was separated from free by filtration over filters for receptor binding (Skatron, Tranby, Norway) by using a Skatron 1719 cell harvester. Filters were transferred to scintillation vials and counted in a liquid scintillation spectrometer.

cAMP Assay. After being washed once with assay medium (Dulbecco's modified Eagle's medium without $NaHCO_3$ buffered with 20 mM Hepes at pH 7.4), cells were resuspended in the same medium to a density of 1.4×10^5 cells per ml. Aliquots (0.35 ml = 0.5×10^5 cells) were transferred to test tubes, and the indicated drugs were added, together with the phosphodiesterase inhibitor rolipram (30 μM), to a final volume of 0.5 ml. After incubation at 37°C, reactions were terminated by the addition of perchloric acid to a final concentration of 0.4 M. Samples were neutralized with KOH, and the cAMP content in the supernatants was determined with a protein binding assay (20), where bound [^{3}H]cAMP was separated from free by rapid filtration over glass fiber filters by using a Skatron 1719 cell harvester and filters were counted in a liquid scintillation spectrometer.

Measurement of Intracellular Concentrations of Free Calcium. Cells were washed and resuspended in Hanks' balanced salt solution (1.2 mM $CaCl_2$, 0.1% bovine serum albumin, and 20 mM Hepes at pH 7.4) to a concentration of 10^6 cells per ml and loaded with 5 μM fura-2 AM for 40 min at 37°C. After the loading period, cells were washed twice in Hanks' balanced salt solution and resuspended to a concentration of 10^6 cells per ml. Prior to the measurements, cells were washed once more and then placed in a cuvette (10^6 cells in 2 ml of Hanks' balanced salt solution), and the intracellular calcium concentration was determined at 30°C in a dual-wavelength Sigma ZFP22 fluorometer by using the ratio of the fluorescence intensity obtained with an excitation wavelength of 334 nm to that obtained with an excitation wavelength of 366 nm with an emission cutoff at 500 nm. Free calcium concentration was calculated as described (21).

Data Analysis. Dose–response curves were generated by using the GRAPHPAD (ISI Software) program. Statistical comparisons between different drug treatments were made by using Student's t test or analysis of variance using the STATGRAPHICS (Statistical Graphics Corporation) program with a confidence level of 95%. Data are expressed as the mean ± SEM.

RESULTS

Formation of Ins(1,4,5)P_3. Bradykinin (EC_{50} = 150 ± 58 nM; n = 4; Fig. 1*A*), adenosine (EC_{50} = 246 ± 8 nM; n = 4; Fig. 1*B*), and the A_1 receptor selective agonist CPA (EC_{50} = 9.4 ± 0.4 nM; n = 3; Fig. 1*B*) all caused concentration-dependent increases in the formation of Ins(1,4,5)P_3. The increase in Ins(1,4,5)P_3 formation was rapid and transient (Fig. 2). The response to bradykinin was unaffected by pertussis toxin (Fig. 1*A*), whereas adenosine A_1 responses were completely abolished after treatment of cells with pertussis toxin at 200 ng/ml for 4 hr (Fig. 1*B*). This treatment completely inactivates the two substrates G_{i2} and G_{i3} in DDT_1 MF-2 cells (13, 14). The effect of bradykinin was potentiated

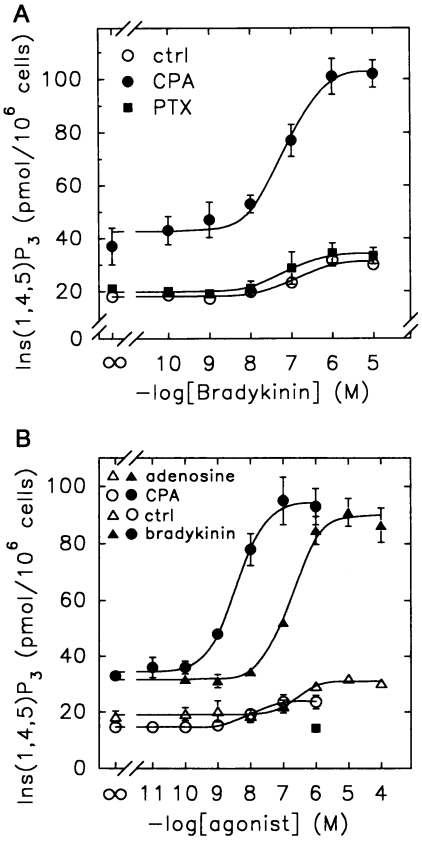

FIG. 1. (*A*) Concentration–response curves for bradykinin-induced Ins(1,4,5)P_3 formation alone and in combination with the adenosine A_1 agonist CPA and the influence of pertussis toxin (PTX). DDT_1 MF-2 cells were activated for 30 sec with bradykinin alone (○) (EC_{50} = 150 ± 58 nM; n = 4) or in combination with 100 nM CPA (●) (EC_{50} = 86 ± 27 nM; n = 5). When cells were pretreated with pertussis toxin (200 ng/ml, 4 hr) before the addition of bradykinin (■) (EC_{50} = 186 ± 78; n = 3), the EC_{50} value was not different from that of the control. Data are presented as the mean ± SEM; each experiment was performed in triplicate. (*B*) Concentration–response curves for adenosine A_1 receptor agonist-induced Ins(1,4,5)P_3 formation alone or in combination with bradykinin. DDT_1 MF-2 cells were activated for 30 sec with increasing concentrations of the selective adenosine A_1 receptor agonist CPA in the absence (○) (EC_{50} = 9.4 ± 0.4 nM; n = 3) or presence (●) (EC_{50} = 3.9 ± 1.0 nM; n = 3) of 1 μM bradykinin or with increasing concentrations of adenosine in the absence (△) (EC_{50} = 246 ± 8 nM; n = 4) or presence (▲) (EC_{50} = 153 ± 21 nM; n = 5) of 1 μM bradykinin. Pertussis toxin (200 ng/ml, 4 hr) completely abolished the CPA-induced Ins(1,4,5)P_3 accumulation (■). Data are presented as the mean ± SEM; each experiment was performed in triplicate. ctrl, Control.

by the addition of adenosine or CPA (Figs. 1 and 2), with no change in the apparent potency of bradykinin. Conversely, when adenosine or CPA was combined with 1 μM bradykinin, there was a reduction in the dose of adenosine or CPA required for half-maximal effect (for adenosine a reduction from 246 ± 8 nM, n = 4 to 153 ± 21 nM, n = 5 and for CPA a reduction from 9.4 ± 0.4 nM, n = 3 to 3.9 ± 1.0 nM, n = 3; $P < 0.05$, Student's t test for unpaired data). All synergistic effects were lost after treatment of cells with pertussis toxin (200 ng/ml, 4 hr; data not shown).

An adenosine A_1 receptor selective antagonist, 1,3-dipropyl-8-cyclopentylxanthine, antagonized the ability of adenosine (100 nM) to potentiate Ins(1,4,5)P_3 formation induced by bradykinin (1 μM) in a concentration-dependent manner. The calculated K_i value for the antagonist was 4.3 nM, which is typical for actions on adenosine A_1 receptors (13).

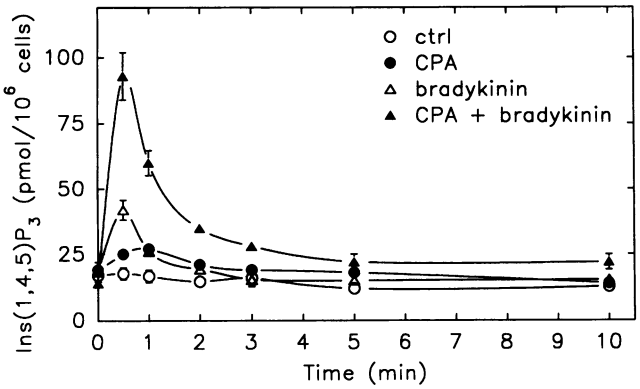

FIG. 2. Time course for bradykinin- and CPA-induced Ins(1,4,5)P_3 formation in DDT_1 MF-2 cells. Cells were activated with vehicle (○), 100 nM CPA (●), 1 μM bradykinin (△), or the combination of 100 nM CPA and 1 μM bradykinin (▲) for the indicated times, and the amount of Ins(1,4,5)P_3 formed was analyzed as described in *Materials and Methods*. Results are expressed as the mean ± SEM from three independent experiments each performed in triplicate. Analysis of variance (95% confidence level) showed that CPA and bradykinin significantly increased the formation of Ins(1,4,5)P_3 compared to the control and that the combination of CPA and bradykinin further increased the Ins(1,4,5)P_3 formation compared to when they were added separately. ctrl, Control.

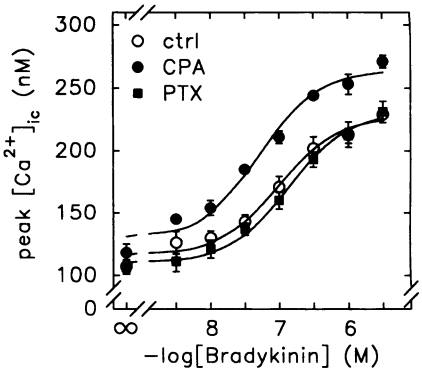

FIG. 3. Bradykinin-induced increases in intracellular free calcium. DDT_1 MF-2 cells were loaded with the fluorescent dye fura-2 AM, and intracellular changes in free calcium were measured as described in *Materials and Methods*. Cells were activated with increasing concentrations of bradykinin in the absence (○) (EC_{50} = 241 ± 140 nM; n = 4) or presence (●) (EC_{50} = 57 ± 12 nM; n = 5) of 1.8 nM CPA. Cells were also pretreated with pertussis toxin (200 ng/ml, 4 hr) and then incubated with increasing concentrations of bradykinin prior to calcium measurements (■) (EC_{50} = 161 ± 38 nM; n = 4). Data are presented as the maximal increase in intracellular calcium concentration ($[Ca^{2+}]_{ic}$), measured at the top of the initial peak. PTX, pertussis toxin; ctrl, control.

The synergy could not be explained by an interference of adenosine A_1 receptors with adenylyl cyclase since the synergy was still observed after raising intracellular levels of cAMP with 10 μM forskolin for 20 min prior to the addition of bradykinin and CPA (Table 1). Furthermore, neither bradykinin, adenosine, nor CPA caused any significant change in basal levels of cAMP (Table 1).

Mobilization of Intracellular Free Calcium. Bradykinin caused a concentration-dependent increase in intracellular free calcium (EC_{50} = 241 ± 140 nM; n = 4; Fig. 3), a response that was unaffected by treatment of cells with pertussis toxin (200 ng/ml) for 4 hr prior to the experiments (EC_{50} = 161 ± 38 nM; n = 3; Fig. 3). CPA caused a concentration-dependent, pertussis toxin-sensitive increase in intracellular calcium (EC_{50} = 3.7 ± 0.9 nM; n = 4; data not shown). The CPA-induced calcium increase was completely blocked by the A_1 receptor selective antagonist 1,3-dipropyl-8-cyclopentylxanthine, whereas the bradykinin response was unaffected (data not shown).

Table 1. cAMP levels and the influence of cAMP on Ins(1,4,5)P_3 formation in DDT_1 MF-2 cells

Addition	cAMP, pmol per 10^6 cells	Ins(1,4,5)P_3, pmol per 10^6 cells
Vehicle	30 ± 3	18 ± 2
Bradykinin	31 ± 2	32 ± 2
CPA	23 ± 2	31 ± 3
Bradykinin + CPA	24 ± 5	95 ± 4
Forskolin	138 ± 21	18 ± 1
Forskolin + bradykinin	142 ± 10	33 ± 2
Forskolin + CPA	36 ± 6	32 ± 8
Forskolin + bradykinin + CPA	31 ± 4	68 ± 7

DDT_1 MF-2 cells were activated with the indicated drugs, and the amounts of cAMP (pmol per 10^6 cells) and Ins(1,4,5)P_3 (pmol per 10^6 cells) formed were measured as described in *Materials and Methods*. The concentrations of drugs used were 1 μM bradykinin, 100 nM CPA, and 10 μM forskolin. In cAMP experiments, cells were activated for 10 min in the presence of the phosphodiesterase inhibitor rolipram (30 μM) except for experiments with forskolin where cells were activated for 20 min with forskolin and the indicated agonist. The amounts of Ins(1,4,5)P_3 were measured after a 30-sec stimulation, and in the forskolin experiments cells were preincubated with forskolin and rolipram (30 μM) for 20 min before a 30-sec stimulation with agonists. Data presented are the mean ± SEM from three or four experiments, each performed in triplicate. Forskolin reduced the Ins(1,4,5)P_3 response to the combination of CPA and bradykinin (Student's t test, $P < 0.05$), but the effect of the two agonists was still synergistic.

Bradykinin and CPA caused an initial peak in intracellular calcium concentration that was followed by a sustained plateau phase; the latter was abolished if extracellular calcium was removed by the addition of 3 mM EGTA (Fig. 4 *A* and *B*). At concentrations of CPA and bradykinin that by themselves were insufficient to raise intracellular calcium, the two agonists were able to cause a small increase in calcium when they were combined (Fig. 4*C*). The combination of half-maximal doses of bradykinin and CPA had synergistic effects and showed an initial peak of intracellular calcium, something that was not seen when they were added separately (Fig. 4*D*). The response to half-maximal concentrations of CPA was not affected by EGTA (data not shown), indicating that even in the absence of a clear initial peak the response is due to mobilization of intracellular calcium. Even at maximal concentrations of bradykinin and CPA, a synergistic action was seen (Fig. 4*E*).

DISCUSSION

In the present study we have confirmed our preliminary finding (12) that activation of adenosine A_1 receptors causes an increase in intracellular levels of Ins(1,4,5)P_3 and intracellular free calcium in a time- and dose-dependent manner. The potency of the selective A_1 receptor agonist CPA on Ins(1,4,5)P_3 accumulation (9.4 nM) and calcium mobilization (3.7 nM) is typical for adenosine A_1 receptors and is in agreement with the affinity of CPA as judged by receptor binding studies to DDT_1 MF-2 smooth muscle cells (13). The effect of the natural ligand adenosine, which activates both adenosine A_1 and A_2 receptors, was inhibited by a selective adenosine A_1 receptor antagonist, 1,3-dipropyl-8-cyclopentylxanthine, with the expected potency. To our knowledge, this is the first clear-cut demonstration that the A_1 receptors are coupled to phospholipase C. The effect was completely blocked by pertussis toxin at a concentration that completely inhibits the two pertussis toxin substrates G_{i2} and G_{i3} in these cells (13, 14). It has been previously shown that activation of

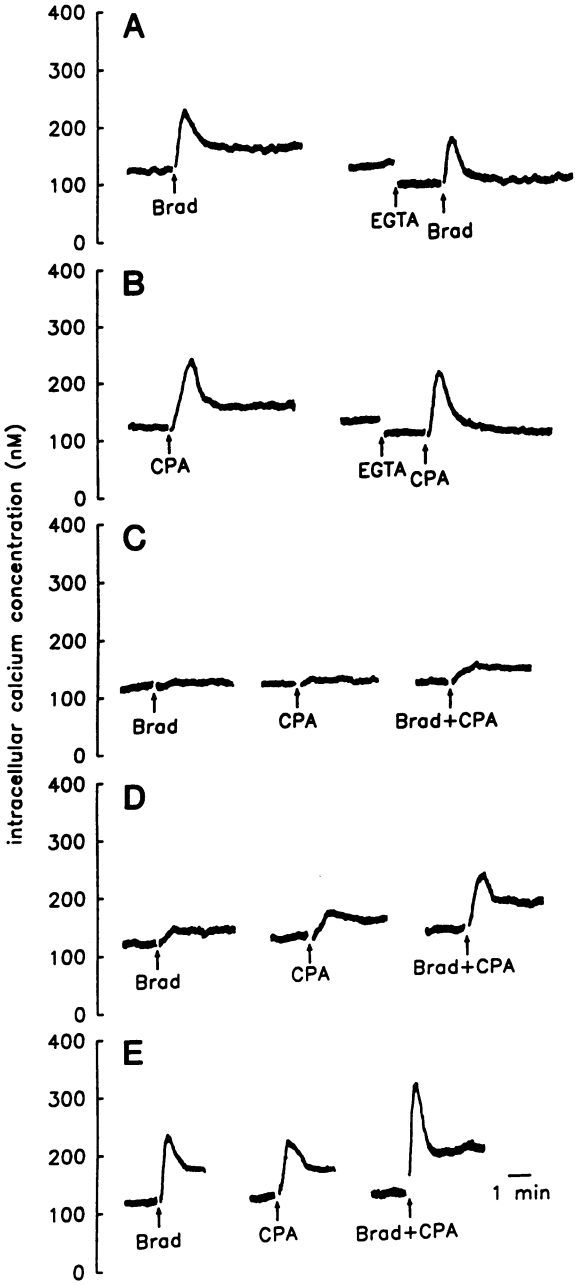

FIG. 4. Representative tracings of bradykinin and adenosine A_1 receptor-stimulated intracellular calcium increases in fura-2-loaded DDT_1 MF-2 cells. (*A*) Bradykinin (1 μM) induced calcium mobilization in the absence (left trace) and presence (right trace) of 3 mM EGTA. (*B*) CPA (100 nM) stimulated calcium increases in the absence (left trace) or presence (right trace) of 3 mM EGTA. (*C*) The effects of 1 nM bradykinin (left trace) or 1 nM CPA (middle trace) or the combination of the two (right trace) are shown. (*D*) Bradykinin (18 nM; left trace) and CPA (2 nM; middle trace) were added alone or in combination (right trace). (*E*) The maximal effects of concentrations of bradykinin (3.2 μM; left trace), or CPA (100 nM; middle trace), or the combination of the two (right trace) are shown. Brad, bradykinin.

A_1 receptors can interact with both of these G proteins (22), inhibit adenylyl cyclase (3), stimulate potassium conductance (23), and, via as of yet poorly defined pertussis toxin-sensitive G proteins, inhibit calcium conductances (24).

In addition, we have shown that in the same cells bradykinin stimulated Ins(1,4,5)P_3 formation and elevated intracellular calcium. The previously demonstrated bradykinin-stimulated phosphate incorporation into inositol phospholipids in these cells (17) is therefore probably secondary to phospholipid hydrolysis. The receptor involved has not been examined, but by analogy with other cells it is reasonable to assume that a B_2 type receptor is involved, even though the EC_{50} values [150 nM for Ins(1,4,5)P_3 formation and 241 nM for calcium mobilization] we found are rather higher than those typically reported for these receptors (25–27).

The effect of bradykinin in DDT_1 MF-2 cells was completely unaffected by pertussis toxin treatment. There is good evidence that a group of G proteins (G_q, G_{11}) that lack pertussis toxin-sensitive ADP-ribosylation sites are able to stimulate phospholipase C (28). In previous studies the pertussis toxin sensitivity of bradykinin-mediated phospholipase C activation has been variable, showing both complete insensitivity (16, 27, 29–31) and partial block by pertussis toxin (15, 27, 32). It is interesting to speculate that the pertussis toxin-sensitive synergistic interaction between adenosine and bradykinin may help explain some of this variability especially since adenosine is often present in media and body fluids (33).

When adenosine A_1 and bradykinin receptors were activated simultaneously, the Ins(1,4,5)P_3 formation was potentiated. Thus, the maximal effect induced by activating both receptors was larger than the sum of the two effects separately. Furthermore, the dose–response curves for adenosine- and CPA-induced Ins(1,4,5)P_3 formation were shifted to the left if 1 μM bradykinin was present.

We do not know how this synergy is achieved. Even though cAMP may inhibit phospholipase C in some cells (34), the synergy between adenosine A_1 and bradykinin receptors was still present after intracellular levels of cAMP were raised with forskolin. This excludes the possibility that adenosine A_1 receptors enhance bradykinin-induced Ins(1,4,5)P_3 formation via the known inhibition of adenylyl cyclase in DDT_1 MF-2 cells (13, 14). Experiments with EGTA, indomethacin, and dexamethasone (P.G., unpublished data) also tend to eliminate a role for extracellular calcium or of arachidonic acid metabolites. Acute treatment of cells with phorbol ester did not mimic the effect of adenosine receptor activation, and long-term treatment, to down-regulate protein kinase C, did not inhibit the synergistic interaction (P.G., unpublished data), indicating that protein kinase C is not directly involved either. It is an intriguing possibility that there is some direct interaction at the G-protein level. When activated, the G protein dissociates into one α subunit that is assumed to be the primary activator of the effector systems (e.g., adenylyl cyclase, phospholipase C, and ion channels) and one $\beta\gamma$ subunit. The latter may also have some signaling functions (35). In particular, it has been shown for some forms of adenylyl cyclase (36) that $\beta\gamma$ subunits generated via one pathway amplify the stimulatory effect of an α_s subunit. The same may be true for phospholipase C, but experiments to study this possibility have not been carried out.

The synergy on Ins(1,4,5)P_3 formation had functional consequences on the mobilization of intracellular free calcium. The characteristics of the bradykinin- and CPA-induced calcium increase with an initial peak and a sustained plateau phase are in agreement with an Ins(1,4,5)P_3-mediated initial peak and a plateau phase caused by influx of calcium. This is further supported by the finding that the peak in intracellular calcium coincides with the peak in Ins(1,4,5)P_3 formation and that the plateau phase is eliminated if extracellular calcium is removed by the addition of the calcium chelator EGTA. Concentrations of bradykinin and CPA that were unable to raise intracellular calcium *per se* were able to cause a small increase when combined. The combination of half-maximal doses had additive effects when combined, and a typical initial peak could be observed, something that was not seen when they were added separately. Even at maximal

concentrations of each agonist, an additive effect was seen. It is difficult to know if the present findings have a direct physiological significance. However, it has been shown that adenosine is able to increase the contractile responses to α_1-adrenergic agonists in rodent vas deferens preparations (37, 38) and increase the Ins(1,4,5)P_3 response in intact vas deferens (7). In summary, the present study shows that two receptors that activate phospholipase C, one via pertussis toxin-sensitive the other via pertussis toxin-insensitive G protein(s), act synergistically on the formation of Ins(1,4,5)P_3 and mobilization of intracellular free calcium. This type of interaction may provide another mechanism for the physiologically important synergistic interactions between hormones, neurotransmitters, and local modulators.

We thank S. Orrenius and G. Kass (Department of Toxicology, Karolinska Institutet) for their help with calcium measurements. These studies were supported by the Swedish Medical Research Council (project no. 2553, K92-04P-09717-02), by the Swedish Association for Medical Research, and by Karolinska Institutet.

1. Fuxe, K. & Agnati, L. F. (1987) *Receptor-Receptor Interactions*, Wenner-Gren International Symposium Series (Macmillan, London).
2. Fredholm, B. B., Ahlberg, S., Altiok, N., Gerwins, P., Parkinson, F., van der Ploeg, I., Kvanta, A., Nordstedt, C. & Dunwiddie, T. (1991) in *Role of Adenosine and Adenine Nucleotides in the Biological System*, eds. Imai, S. & Nakazawa, M. (Elsevier, Amsterdam), pp. 173–182.
3. Linden, J. (1991) *FASEB J.* **5**, 2668–2676.
4. Hollingsworth, E. B., de la Cruz, R. A. & Daly, J. W. (1986) *Eur. J. Pharmacol.* **122**, 45–50.
5. Nazarea, M., Okajima, F. & Kondo, Y. (1991) *Eur. J. Pharmacol.-Mol. Pharmacol.* **Q206**, 47–52.
6. Häggblad, J. & Fredholm, B. B. (1987) *Neurosci. Lett.* **82**, 211–216.
7. El-Etr, M., Cordier, J., Glowinski, J. & Premont, J. (1989) *J. Neurosci.* **9**, 1473–1480.
8. Alexander, S. P. H., Kendall, D. A. & Hill, S. J. (1989) *Br. J. Pharmacol.* **98**, 1241–1248.
9. Kendall, D. A. & Hill, S. J. (1988) *J. Neurochem.* **50**, 497–502.
10. Delahunty, T. M., Cronin, M. J. & Linden, J. (1988) *Biochem. J.* **255**, 69–77.
11. Burnatowska-Hledin, A. & Spielman, W. S. (1991) *Am. J. Physiol.* **260**, C143–C150.
12. Gerwins, P. & Fredholm, B. B. (1991) *Br. J. Pharmacol. Proc. Suppl.* **104**, 302P (abstract).
13. Gerwins, P., Nordstedt, C. & Fredholm, B. B. (1990) *Mol. Pharmacol.* **38**, 660–666.
14. Gerwins, P. & Fredholm, B. B. (1991) *Mol. Pharmacol.* **40**, 149–155.
15. Portilla, D., Morrissaey, J. & Morrison, A. R. (1988) *J. Clin. Invest.* **81**, 1896–1902.
16. Perney, T. M. & Miller, J. M. (1989) *J. Biol. Chem.* **264**, 7317–7327.
17. Leeb-Lundberg, L. M. F., Cotecchia, S., DeBlasi, A., Caron, M. G. & Lefkowitz, R. J. (1987) *J. Biol. Chem.* **262**, 3098–3105.
18. Norris, J. S., Gorski, J. & Kohler, P. O. (1974) *Nature (London)* **248**, 422–424.
19. Palmer, S. & Wakelam, M. J. O. (1990) in *Methods in Inositide Research*, ed. Irvine, R. F. (Raven, New York), pp. 127–134.
20. Nordstedt, C. & Fredholm, B. B. (1990) *Anal. Biochem.* **189**, 231–234.
21. Grynkiewicz, G., Poenie, M. & Tsien, R. Y. (1985) *J. Biol. Chem.* **260**, 3440–3450.
22. Freissmuth, M., Selzer, E. & Schütz, W. (1991) *Biochem. J.* **275**, 651–656.
23. Kurachi, Y., Nakajima, T. & Sugimoto, T. (1986) *Pflügers Arch.* **407**, 264–274.
24. Scott, R. H. & Dolphin, A. C. (1989) in *Adenosine Receptors in the Nervous System*, ed. Ribeiro, J. A. (Taylor & Francis, London), pp. 151–158.
25. Hepler, J. R., Nakahata, N., Lovenberg, T. W., DiGuiseppi, J., Herman, B., Earp, H. S. & Harden, T. K. (1987) *J. Biol. Chem.* **262**, 2951–2956.
26. Bascands, J. L., Emond, C., Pecher, C., Regoli, D. & Girolami, J. P. (1991) *Br. J. Pharmacol.* **102**, 962–966.
27. Fu, T., Okano, Y. & Nozawa, Y. (1988) *Biochem. Biophys. Res. Commun.* **157**, 1429–1435.
28. Taylor, S. J., Chae, H. Z., Rhee, S. G. & Exton, J. H. (1991) *Nature (London)* **350**, 516–518.
29. Voyno-Yasenetskaya, T. A., Tkachuk, V. A., Cheknyova, E. G., Panchenko, M. P., Grigorian, G. Y., Vavrek, R. J., Stewart, J. M. & Ryan, U. S. (1989) *FASEB J.* **3**, 44–51.
30. Monck, J. R., Williamson, R. E., Rogulja, I., Fluharty, S. J. & Williamson, J. R. (1990) *J. Neurochem.* **54**, 278–287.
31. Mihara, S.-I., Shigeri, Y. & Fujimoto, M. (1989) *FEBS Lett.* **259**, 79–82.
32. Bueb, J.-L., Mousli, C., Bronner, C., Rouot, B. & Landry, Y. (1990) *Mol. Pharmacol.* **38**, 816–822.
33. Gustafsson, L., Fredholm, B. B. & Hedqvist, P. (1981) *Acta Physiol. Scand.* **111**, 269–280.
34. Takai, Y., Minakuchi, R., Kikkawa, U., Sano, K., Kaibuchi, K., Yu, B., Matsubara, T. & Nishizuka, Y. (1982) *Prog. Brain Res.* **56**, 287–301.
35. Jelsema, C. L. & Axelrod, J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3623–3627.
36. Tang, W.-J. & Gilman, A. G. (1991) *Science* **254**, 1500–1503.
37. Hedqvist, P. & Fredholm, B. B. (1976) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **293**, 217–223.
38. Holck, M. I. & Marks, B. H. (1978) *J. Pharmacol. Exp. Ther.* **205**, 104–117.