Bradykinin-induced transient accumulation of inositol trisphosphate in neuron-like cell line NG108-15 cells

Koh Yano, Haruhiro Higashida*, Hiroaki Hattori and Yoshinori Nozawa

Department of Biochemistry, Gifu University School of Medicine, Tsukasamachi-40, Gifu 500, and **Department of Pharmacology, Cancer Research Institute, Kanazawa University, 13-1 Takaramachi, Kanazawa 920, Japan*

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Studies were undertaken to further elucidate the mechanism(s) by which bradykinin-dependent phosphoinositide metabolism takes place in neuroblastoma × glioma hybrid NG108-15 cells [(1984) J. Biol. Chem. 259, 10201–10207] using [^{3}H]inositol-labelled cells. Bradykinin produced net increases in the level of [^{3}H]inositol phosphates, especially of [^{3}H]inositol trisphosphate which is formed transiently and most rapidly. The results indicate that bradykinin activates a phosphodiesterase to break down phosphatidylinositol 4,5-bisphosphate, generating two recently recognized intracellular messengers, 1,2-diacylglycerol and inositol trisphosphate.

Neuroblastoma × glioma *Bradykinin* *Inositol trisphosphate* *Phosphodiesterase*

1. INTRODUCTION

In many cell systems, Ca^{2+}-mobilising hormones and neurotransmitters can cause a rapid breakdown of PIP_2 through activation of a phosphodiesterase [1]. It has become increasingly apparent that the products of the phosphodiesteratic hydrolysis of PIP_2 may serve as intermediates in the transmembrane signalling [1]: 1,2-diacylglycerol activates protein kinase C [2] and (1,4,5)IP_3 acts as a mobiliser of intracellular Ca^{2+} [3–9]. These two putative second messengers are thought to synergistically activate the cells to carry out their physiological functions [10,11].

A previous report from our laboratory [12] showed that bradykinin induces a rapid loss of radioactivity from PIP_2, independently of a rise in intracellular Ca^{2+} concentration, in $^{32}P_i$-labelled neuroblastoma × glioma hybrid NG108-15 cells. However, because of the multiple pathways in which phosphoinositides are metabolized [13], studies using ^{32}P labelling were unable to lead us to the conclusion as to the enzyme(s) which causes the loss of PIP_2.

The aim here, therefore, is to establish the metabolic pathway by which PIP_2 is hydrolyzed in the bradykinin-stimulated NG108-15 cells. To achieve this, we have studied the effects of bradykinin on the accumulation of [^{3}H]inositol phosphates in the hybrid cells prelabelled with [^{3}H]inositol. The results show that bradykinin rapidly enhances the level IP_3 which is subsequently degraded to IP_2 and further to IP. Here, we can conclude that the bradykinin-induced PIP_2 breakdown in NG108-15 cells is due to activation of a phosphodiesterase.

Abbreviations: IP_3, inositol trisphosphate; (1,4,5)IP_3, inositol 1,4,5-trisphosphate; IP_2, inositol bisphosphate; IP, inositol monophosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate

2. MATERIALS AND METHODS

2.1. *Cell culture*

NG108-15 cells were cultured essentially as described previously [14,15].

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2.2. *Studies with [^{3}H]inositol-labelled cells*

NG108-15 cells which had been grown for 6 days in monolayer cultures were released from culture dishes with a rubber policeman. The harvested cells were washed once with Tris-buffered saline (TBS) consisting of 20 mM Tris–HCl (pH 7.4), 150 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$ and 25 mM glucose. The washed cells (3×10^7 cells) were then incubated in 20 ml of the same buffer supplemented with 120 μCi of [^{3}H]inositol (Amersham) at 37°C. After 2.5 h, the medium was replaced with 20 ml of fresh TBS with no inositol and further incubated for 1 h. The labelled cells were washed two times with TBS and finally suspended in TBS containing 10 mM LiCl at a final concentration of 4×10^6 cells/ml. The inclusion of LiCl was to inhibit IP phosphatase [16,17]. Aliquots (0.25 ml) of the cell suspension were transferred to siliconized tubes and after 5 min preincubation at 37°C, bradykinin (Protein Research Foundation, Osaka) solution (0.05 ml) was added to give a final concentration of 1×10^{-5} M. For control incubations, the same volume of vehicle (TBS) alone was always added. At designated times, the reaction was terminated by the addition of 0.1 ml of 10% (w/v) perchloric acid [18]. The acid-soluble extract was neutralized with 0.15 ml of 1.53 M KOH–75 mM Hepes, and perchloric acid was precipitated on ice and removed by a brief centrifugation. The aqueous extract was then diluted to 5 ml with distilled water and was applied to a column (0.6×4.0 cm) of AG-1×2, 200–400 mesh (Bio-Rad), which had been converted to formate form. After free inositol and glycerophosphoinositol were eluted with 16 ml of 5 mM sodium tetraborate/60 mM ammonium formate, total inositol phosphates were eluted with 12 ml of 1.0 M ammonium formate/0.1 M formic acid, or individual inositol phosphates were eluted sequentially using 16 ml of 0.2 M ammonium formate/0.1 M formic acid for IP, 20 ml of 0.4 M ammonium formate/0.1 M formic acid for IP_2, and 12 ml of 1.0 M ammonium formate/0.1 M formic acid for IP_3 as described by Berridge et al. [19]. Radioactivity in each fraction was counted with a Beckman liquid scintillation counter, LS 7500 model, in the gel phase using 60% (v/v) Aquasol (New England Nuclear) [20]. Usually, 4-ml samples of the eluates were collected and mixed with Aquasol. In the case of 1.0 M ammonium formate/0.1 M formic acid eluates, however, 2-ml samples were diluted to 4 ml with distilled water and then Aquasol was added so that the gel phase can be formed.

2.3. *Expression of data*

Data in table 1 and fig.1 are expressed as the mean ± SE of 4 samples from two experiments, each performed in duplicate.

3. RESULTS AND DISCUSSION

The effect of bradykinin on the level of [^{3}H]inositol phosphates is presented in table 1. Bradykinin caused an increase in [^{3}H]inositol phosphates to 3.1-fold of control at 5 min. This finding indicates that bradykinin is capable of activating phosphodiesterase(s) which degrades phosphoinositides.

In order to determine the primary substrate of the bradykinin-stimulated phosphoinositide breakdown, the time course of the effect of bradykinin on the release of individual inositol phosphates was examined. As shown in fig.1, the addition of bradykinin to [^{3}H]inositol-labelled cells induced a rapid occurrence of IP_3, which reached a peak (3.4-fold increase of control) at 10 s. There was a subsequent accumulation of IP_2 and it was maximally enhanced by 3.5-fold at 30 s. The levels of these two inositol phosphates then gradually declined and returned to near-control levels by 5 min. The increase of IP level, in contrast, was small at the onset but became greater with an incubation time up to 5 min. This time sequential generation of inositol phosphates in the order of IP_3, IP_2 and IP, taken together with the previous observation [12] that bradykinin causes a preferential loss of ^{32}P-PIP_2 from prelabelled cells, in-

Table 1

Bradykinin-stimulated increase in the content of total inositol phosphates

	Control	Bradykinin
[^{3}H]Inositol phosphates (dpm/10^6 cells)	1249 ± 265	3918 ± 318

[^{3}H]Inositol-labelled NG108-15 cells were incubated at 37°C for 5 min with or without 1×10^{-5} M bradykinin. Total inositol phosphates were separated as described in section 2

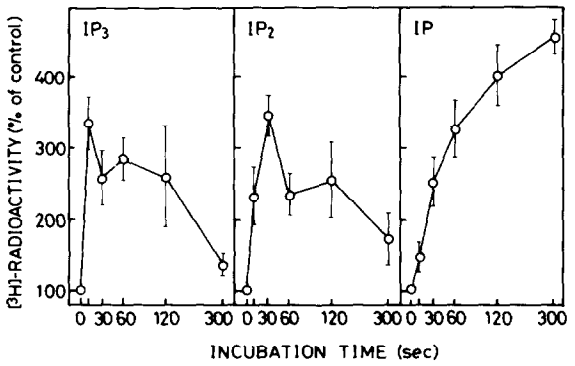

Fig.1. Time course of the effect of bradykinin on the production of individual inositol phosphates in [^{3}H]inositol-labelled NG108-15 cells. [^{3}H]Inositol-labelled NG108-15 cells were incubated at 37°C for 5 min with or without 1×10^{-5} M bradykinin. The individual inositol phosphates were separated as described in section 2. Data are expressed as percentages of their respective controls. Control values at zero time for [^{3}H]IP_3, [^{3}H]IP_2 and [^{3}H]IP (dpm/10^6 cells) were 156 ± 47, 369 ± 74 and 660 ± 48, respectively.

dicates that the primary substrate of the bradykinin-activated phosphodiesterase is PIP_2. The increase in the content of IP_2 and IP, therefore, is most likely to be the result of the sequential degradation of IP_3 to IP_2 and IP_2 to IP by their respective phosphatases [19,21,22]. However, the possibility of phosphatidylinositol 4-phosphate breakdown to yield IP_2 cannot be excluded.

The results obtained here demonstrate that bradykinin-receptor interaction in NG108-15 cells can produce two putative second messengers, IP_3 and 1,2-diacylglycerol by a stimulated phosphodiesteratic hydrolysis of PIP_2. Diacylglycerol is thought to play a pivotal role in cell activation by phosphorylating target protein(s) through protein kinase C [11], and IP_3 by releasing Ca^{2+} from internal store(s) [1]. We have shown previously that bradykinin provokes a depolarization preceded by a transient hyperpolarization in NG108-15 cells, ultimately leading to the release of acetylcholine [12]. In this context, it is of great interest to note the observations which indicate that (1,4,5)IP_3 induces a marked depolarization in the ventral photoreceptors of *Limulus,* usually provoked by light [23,24]. The precise role of these two putative second messengers in the physiological functions of NG108-15 cells remain to be clarified. Studies using Quin-2 on Ca^{2+} mobilisation in the bradykinin-stimulated NG108-15 cells are currently under investigation in our laboratory.

Bradykinin has emerged as a potential neurotransmitter in the central nervous system [25], and a recent work by Perry and Snyder [26] substantiated the presence of bradykinin in mammalian brain, especially in a higher quantity in hypothalamus. Whether the action of bradykinin observed in the neuron-like cell line NG108-15 reflects the events occurring in the central nervous system awaits further exploration.

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