Bradykinin stimulates GTP hydrolysis in NG108-15 membranes by a high-affinity, pertussis toxin-insensitive GTPase

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In membranes of neuroblastoma × glioma hybrid (NG108-15) cells, bradykinin ($EC_{50} \cong 5$ nM) stimulates GTP hydrolysis by a high-affinity GTPase ($K_m \cong 0.2$ μM). The octapeptide, des-Arg^9-bradykinin, was inactive. Stimulation of GTP hydrolysis by bradykinin and an opioid agonist was partially additive. Treatment of NG108-15 cells with pertussis toxin, which inactivates N_i, eliminated GTPase stimulation by the opioid agonist but not by bradykinin. The data suggest that bradykinin activates in NG108-15 membranes a guanine nucleotide-binding protein which is not sensitive to pertussis toxin and which may be involved in bradykinin-induced stimulation of phosphoinositide metabolism in these cells.

Bradykinin *(NG108-15 cell)* *Guanine nucleotide-binding protein* *GTPase* *Pertussis toxin* *Phosphoinositide metabolism*

1. INTRODUCTION

The signal transduction of various hormones and neurotransmitters, which regulate cellular functions by inducing the formation of intracellular second messengers, has been shown to involve the coupling actions of guanine nucleotide-binding proteins [1]. At least two of these proteins, namely N_s and N_i, the respective stimulatory and inhibitory coupling proteins of the hormone-sensitive adenylate cyclase system, have been purified and partially characterized with regard to their functions [2]. These proteins not only bind guanine nucleotides but also hydrolyze GTP. When affected by hormone-activated receptors, GTP hydrolysis is increased, apparently due to an increase in the turnover of these proteins from the inactive, GDP-bound to the active, GTP-bound states. The hormone-stimulated GTP hydrolysis is inhibited following ADP-ribosylation of N_s and N_i by cholera and pertussis toxin, respectively [2]. A variety of data suggest that the hormone-induced stimulation of phosphoinositide metabolism also involves a guanine nucleotide-binding coupling protein [3–7], which, however, has not yet been identified. In some membrane systems, a hormone-induced stimulation of GTP hydrolysis has been demonstrated, which appears to be related to the stimulation of phosphoinositide metabolism in these cells [8–10].

Bradykinin has been shown to cause a rapid degradation of phosphatidylinositol 4,5-bisphosphate in neuroblastoma × glioma hybrid (NG108-15) cells [11], which was accompanied by a rapid and transient accumulation of inositol trisphosphate [12]. To investigate whether bradykinin receptors couple to a guanine nucleotide-binding protein, we studied the regulation of GTP hydrolysis by bradykinin in NG108-15 membranes. Three types of hormone receptors (α_2-adrenoceptors, muscarinic cholinergic and opioid receptors) have been shown to inhibit adenylate cyclase in these cells [13], an action mediated by the inhibitory coupling protein N_i [14]. Opioid receptors

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have been reported to stimulate GTP hydrolysis by a high-affinity GTPase in NG108-15 membranes [15]. This stimulation was abolished by treatment of the cells with pertussis toxin [16]. We report here that bradykinin increases GTP hydrolysis by a high-affinity GTPase in NG108-15 membranes and that, in contrast to opioid stimulation, bradykinin-induced stimulation is essentially not impaired by pertussis toxin.

2. MATERIALS AND METHODS

Bradykinin, des-Arg^9-bradykinin and cholera toxin were from Sigma. ATP, GTP and adenylyl imidodiphosphate were obtained from Boehringer. D-Ala^2,D-Leu^5-enkephalin (DADL) was from Peninsula. Pertussis toxin was purified as in [17]. [γ-^{32}P]GTP was prepared as in [18]. Culturing of NG108-15 cells, kindly donated by Dr B. Hamprecht, Tübingen, was carried out essentially as in [13]. Pertussis toxin treatment of intact NG108-15 cells was for 16 h at 37°C at a concentration of 50 ng toxin/ml. NG108-15 membranes were prepared by nitrogen cavitation, followed by low-speed centrifugation (1 min at 1000 × g) of the homogenate to remove nuclei. The supernatant was centrifuged for 10 min at 30000 × g. The membrane-containing pellet was washed twice in 10 mM triethanolamine-HCl, pH 7.4, containing 5 mM EDTA, finally resuspended in 10 mM triethanolamine-HCl, pH 7.4, and frozen in small aliquots at −70°C.

GTPase activity of the NG108-15 membranes (2–6 μg protein/tube) was determined with a reaction mixture containing [γ-^{32}P]GTP (0.1 μCi/tube) at the indicated concentrations, 2 mM $MgCl_2$, 0.1 mM EGTA, 0.1 mM ATP, 1 mM adenylyl imidodiphosphate, 5 mM creatine phosphate (Tris salt), 0.4 mg/ml creatine kinase and 2 mg/ml bovine serum albumin in 50 mM triethanolamine-HCl, pH 7.4, in a total volume of 100 μl. After 10 min preincubation of the membranes with the reaction mixture at 25°C, measurement of GTPase activity was initiated by the addition of GTP without and with the hormones studied and continued for 10 min at 25°C. Stopping of the reaction and isolation of $^{32}P_i$ were carried out as in [17]. High-K_m GTPase activity was determined with 50 μM GTP and subtracted from the total GTPase activity measured at low GTP concentrations as described [15–17]. The assays were performed in triplicate with intra-assay variations of less than 3% of the means and were repeated at least twice with results comparable to those shown here.

3. RESULTS

The hydrolysis of [γ-^{32}P]GTP by NG108-15 membranes in the absence and presence of 1 μM bradykinin is shown in fig.1. At all GTP concentrations below 10 μM, bradykinin increased hydrolysis, but not at higher concentrations. Both curves plateaued at about 10 μM GTP. Thus, as shown before [15], the NG108-15 membranes contain a high-affinity, hormone-sensitive GTPase(s) and a low-affinity, hormone-insensitive GTPase(s). Fig.2 shows the high-affinity GTPase activity as a function of substrate concentrations, measured in the absence and presence of 1 μM bradykinin. The enzyme exhibited an apparent K_m value for GTP of about 0.2 μM, which was not significantly changed by bradykinin. Instead, the nonapeptide increased the V_{max} value of the enzyme. The stimulatory effect of bradykinin on GTP hydrolysis by the high-affinity GTPase(s) in

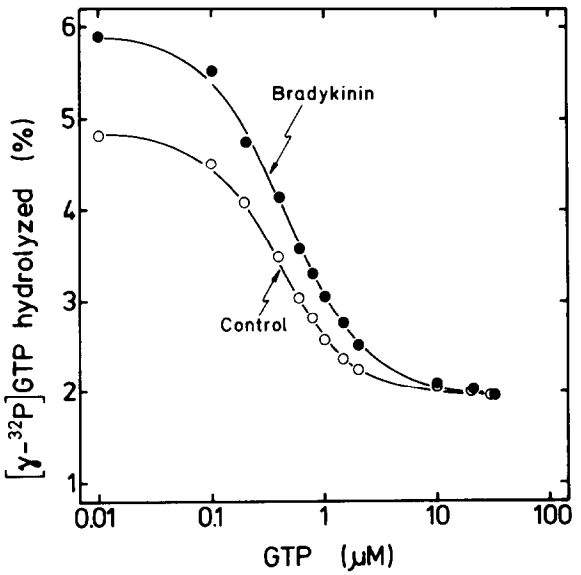

Fig.1. Influence of bradykinin on hydrolysis of [γ-^{32}P]GTP in NG108-15 membranes. Hydrolysis of [γ-^{32}P]GTP was determined in NG108-15 membranes without (○) and with 1 μM bradykinin (●) at the indicated concentrations of unlabelled GTP.

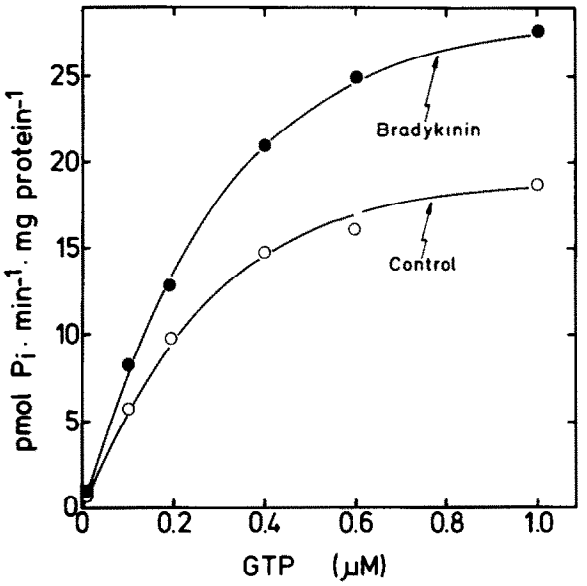

Fig.2. Influence of bradykinin on GTP hydrolysis by high-affinity GTPase. GTP hydrolysis by high-affinity GTPase was determined in NG108-15 membranes without (○) and with 1 μM bradykinin (●) at the indicated concentrations of GTP. Values were calculated from data in fig.1 as described in section 2.

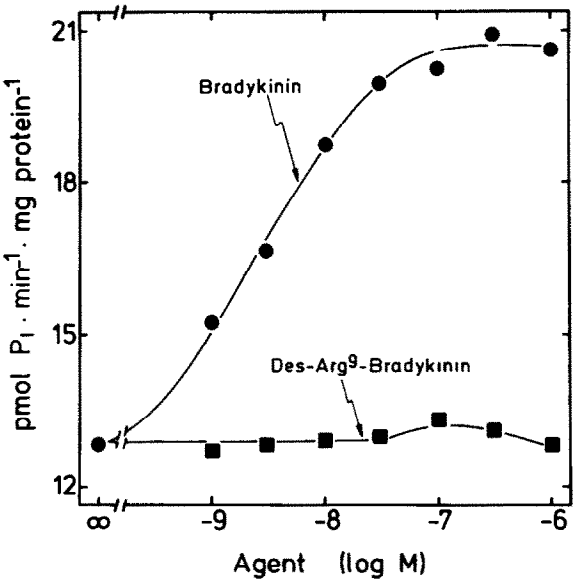

Fig.3. Concentration-response curve for bradykinin-induced stimulation of GTP hydrolysis. GTP hydrolysis by high-affinity GTPase was determined in NG108-15 membranes at the indicated concentrations of bradykinin (●) and des-Arg^9-bradykinin (■) with 0.3 μM GTP as enzyme substrate.

NG108-15 membranes was half-maximal at 3–5 nM (fig.3). Maximal stimulation was seen with 100 nM bradykinin. In contrast, the octapeptide, des-Arg^9-bradykinin, was ineffective at concentrations up to 1 μM. In studies on opioid stimulation of NG108-15 GTPase, it has been reported that Na^+ is essential for maximal hormonal activation [19]. Therefore, we studied the influence of NaCl on bradykinin-induced stimulation of GTP hydrolysis by the high-affinity GTPase. As described in [19], NaCl (40 mM) reduced unstimulated GTPase activity, but the maximal extent of stimulation by bradykinin was not significantly increased by NaCl (fig.4). On the contrary, in the presence of NaCl (40 mM), the potency of bradykinin was reduced by about 3-fold. Similar data with regard to reduction in hormone potency by NaCl have been reported before for stimulation of GTP hydrolysis in membranes of cyc^- S49 lymphoma cells by somatostatin, which couples through N_i to adenylate cyclase [20]. Furthermore, in the NG108-15 membrane preparation used, the opioid stimulation of high-affinity GTPase was not dependent on the presence of NaCl.

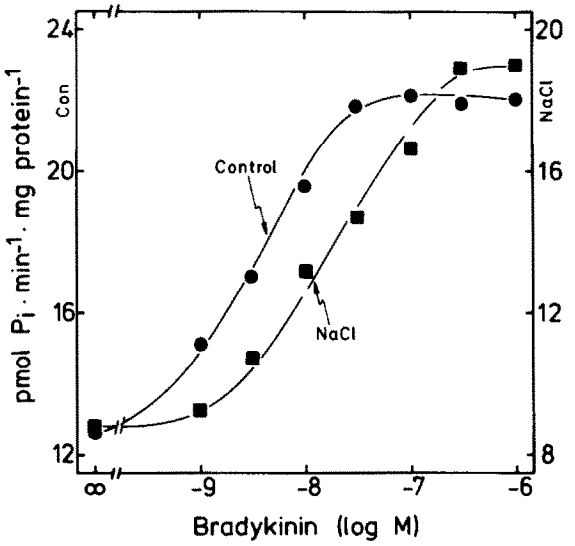

Fig.4. Influence of NaCl on bradykinin-induced stimulation of GTP hydrolysis. GTP hydrolysis by high-affinity GTPase was determined in NG108-15 membranes at the indicated concentrations of bradykinin in the absence (●) and presence of 40 mM NaCl (■) with 0.5 μM GTP as enzyme substrate. GTPase activity measured without and with NaCl is given on the left and right ordinate, respectively.

The opioid receptor agonist, DADL (10 μM), increased GTP hydrolysis by a high-affinity GTPase in NG108-15 membranes to an extent similar to that of bradykinin (fig.5). Interestingly, when DADL at the maximally effective concentration (10 μM) [15] was combined with bradykinin, also at a maximally effective concentration (1 μM), their effects were partially additive. These data suggested that bradykinin activates, at least partially, another high-affinity GTPase than DADL. Since the opioid receptor interacts with N_i in NG108-15 membranes and, thereby, increases GTP hydrolysis, we studied whether pertussis toxin, which inactivates N_i [14] and blocks opioid stimulation of GTPase [16], would also affect bradykinin stimulation of GTP hydrolysis. As shown in fig.5, pretreatment of intact NG108-15 cells with pertussis toxin eliminated DADL-induced stimulation of GTP hydrolysis, regardless of being measured without or with NaCl present (not shown). However, the bradykinin stimulation of GTP hydrolysis was essentially not affected by the pertussis toxin treatment. Even at a submaximal concentration (10 nM), bradykinin still increased GTP hydrolysis. In the toxin-treated membranes, the partial additivity of the effects of bradykinin and DADL was also lost. Neither DADL nor bradykinin-induced stimulation of GTP hydrolysis was affected by treatment of NG108-15 membranes with cholera toxin (not shown), which causes inactivation of N_s GTPase activity [2].

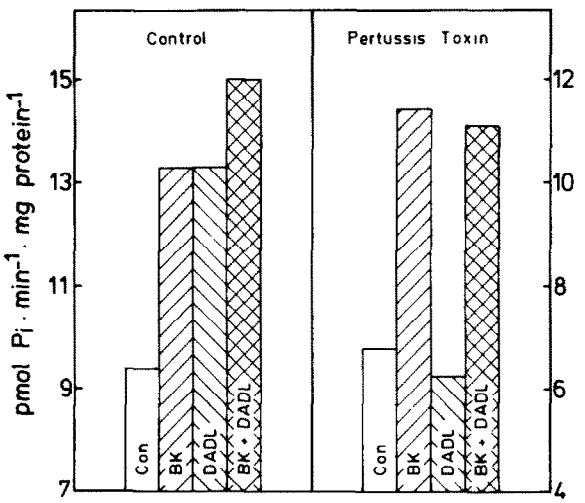

Fig.5. Influence of pertussis toxin on hormone-stimulated GTP hydrolysis. GTP hydrolysis by high-affinity GTPase was determined in membranes of control (left panel and ordinate) and pertussis toxin-treated (right panel and ordinate) NG108-15 cells in the absence (Con) and presence of 1 μM bradykinin (BK), 10 μM DADL or bradykinin plus DADL (BK + DADL) as indicated. GTP was 0.25 μM.

4. DISCUSSION

The present data indicate that NG108-15 membranes contain a hormone-sensitive high-affinity GTPase which is not sensitive to pertussis toxin. Since the hormone-stimulated GTP hydrolysis of N_i, the inhibitory guanine nucleotide-binding coupling protein of the adenylate cyclase system, is impaired by treatment with pertussis toxin, as reported in [16] and shown here, the data suggest that the bradykinin-stimulated hydrolysis of GTP in NG108-15 membranes is essentially not related to N_i. However, we have recently observed, that bradykinin can also inhibit adenylate cyclase in NG108-15 membranes in a GTP-dependent manner and evidence has been accumulated that this inhibition involves N_i (P.Z. and K.H.J., in preparation). These data and those presented herein, therefore, suggest that the bradykinin receptors in NG108-15 membranes can couple to two GTP-binding proteins, namely to N_i and another GTP-hydrolyzing protein. Our failure to detect a reduction in bradykinin stimulation of GTP hydrolysis by pertussis toxin treatment does not exclude the possibility that bradykinin receptors also interact with N_i in these membranes. The contribution of N_i to GTP hydrolysis stimulated by bradykinin may have been too small to be detected. As shown by others [16], stimulation of N_i GTPase activity by epinephrine in NG108-15 membranes is much smaller than opioid stimulation, although epinephrine via N_i inhibits adenylate cyclase in these membranes [14]. That bradykinin receptors also interact with N_i in stimulating GTP hydrolysis is additionally suggested by the fact that the stimulations of GTP hydrolysis by bradykinin and DADL were not completely but only partially additive.

The question arises as to what the nature and function of the other GTP-hydrolyzing protein which is affected by bradykinin receptors. Since bradykinin has been shown to activate the

phosphoinositide metabolism in these cells [11,12] and since data from other cell types suggest that a guanine nucleotide-binding protein is involved in this signal transduction mechanism [3–7], it is feasible that the GTP-hydrolyzing protein affected by bradykinin is involved in bradykinin stimulation of phosphoinositide metabolism. Therefore, studies in NG108-15 membranes are in progress to determine whether the bradykinin-stimulated hydrolysis of membrane polyphosphoinositides is regulated by guanine nucleotides and whether such a regulation is affected by pertussis toxin.

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