Biochemical Pharmacology, Vol. 57, pp. 649–652, 1999.

ISSN 0006-2952/99/$–see front matter
PII S0006-2952(98)00326-8

SHORT COMMUNICATION

Mediation by G Protein βγ Subunits of the Opioid Stimulation of Adenylyl Cyclase Activity in Rat Olfactory Bulb

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ABSTRACT. In the rat olfactory bulb, activation of opioid receptors enhances basal adenylyl cyclase (EC 4.6.1.1) activity and potentiates enzyme stimulation by G_s-coupled neurotransmitter receptors in a pertussis toxin-sensitive manner. In the present study, we investigated the involvement of G protein βγ subunits by examining the effects of βγ scavengers and exogenously added βγ subunits of transducin ($\beta\gamma_t$). The QEHA fragment of type II adenylyl cyclase (50 μM), a peptide that binds to and inactivates βγ, inhibited the maximal stimulation of adenylyl cyclase activity elicited by Leu-enkephalin (Leu-enk) by about 50%. Similarly, the GDP-bound form of the α subunit of transducin (5 nM–1.5 μM), another βγ scavenger, reduced both the opioid stimulation of basal adenylyl cyclase activity and the potentiation of vasoactive intestinal peptide-stimulated enzyme activity. Under the same experimental conditions, these agents failed to affect the stimulation of the enzyme activity elicited by activation of β-adrenergic receptors with 1-isoproterenol. Moreover, the addition of $\beta\gamma_t$ (400 nM) stimulated basal adenylyl cyclase by 80%, and this effect was not additive with that produced by Leu-enk. The data indicate that opioids enhance adenylyl cyclase activity in rat olfactory bulb by promoting the release of βγ subunits from pertussis toxin-sensitive G proteins G_i/G_o. BIOCHEM PHARMACOL **57**;6:649–652, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. opioid receptors; adenylyl cyclase; βγ subunits of transducin; βγ scavengers; vasoactive intestinal peptide; rat olfactory bulb

Although activation of opioid receptors has generally been found to inhibit cyclic AMP formation [1], increasing evidence indicates that opioids can also cause stimulation of adenylyl cyclase (EC 4.6.1.1) activity in different cell systems. Thus, in mouse spinal cord-ganglion explants and F-11 neuroblastoma-sensory neuron hybrid cells, opioid receptor agonists, besides inhibiting forskolin-stimulated adenylyl cyclase activity, have been reported to significantly enhance basal cyclic AMP accumulation [2, 3]. Similarly, in guinea pig longitudinal muscle/myenteric plexus preparation, the μ-selective agonist sufentanil stimulated and inhibited cyclic AMP formation at nanomolar and micromolar concentrations, respectively [4, 5]. In these studies, tissue treatment with pertussis toxin, which uncouples opioid receptors from G proteins of the G_i/G_o family [6], prevented the inhibition of cyclic AMP but enhanced the stimulatory effects. Conversely, exposure to cholera toxin, which causes a persistent activation of the adenylyl cyclase stimulatory G protein G_s [6], has been reported to prevent the opioid stimulation of cyclic AMP. These findings have been taken as an indication that in these tissues opioid receptors stimulated adenylyl cyclase through a direct coupling to G_s [3, 5].

We previously reported that in membranes of rat olfactory bulb activation of δ and μ, but not κ, opioid receptors stimulated basal adenylyl cyclase activity, potentiated G_s-coupled neurotransmitter receptors, and inhibited forskolin- and Ca^{2+}/calmodulin-stimulated enzyme activities [7, 8]. Unusually, the opioid stimulatory effect was prevented by pertussis toxin treatment, indicating that G_i/G_o, rather than G_s, mediated the positive coupling to adenylyl cyclase [8]. To explain this unique response, we hypothesized [8] that opioid receptors increase cyclic AMP through the release of βγ subunits from G_i/G_o and the consequent activation of type II adenylyl cyclase, a Ca^{2+}-independent βγ-stimulated enzyme isoform that is highly expressed in rat olfactory bulb [9].

In the present study, we investigated this possibility by examining the effects of βγ scavengers and detergent-free βγ subunits of retinal G protein transducin on the opioid stimulation of adenylyl cyclase in membranes of rat olfactory bulb.

MATERIALS AND METHODS

Materials

[α-^{32}P]ATP (30–40 Ci/mmol) and [2,8-^{3}H]cyclic AMP (25 Ci/mmol) were obtained from Dupont-NEN.

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Received 29 April 1998; accepted 7 August 1998.

α_{tGDP}* and $\beta\gamma_t$, each purified from bovine retina, were kindly provided by Dr. Heidi E. Hamm, University of Illinois at Chicago. The purity of each subunit preparation was checked by SDS-polyacrylamide gel electrophoresis and Coomassie blue staining. The peptide corresponding to amino acids 956-982 of type II adenylyl cyclase (QEHA peptide) [10] was custom-synthesized by Bio-Synthesis. The purity of the peptide was 94% by HPLC. Leu-enk and VIP were from Peninsula Laboratories. The other chemicals were from Sigma Chemical Co.

Membrane Preparation

A mitochondrial fraction (P2) was prepared from olfactory bulbs and striata of male Sprague–Dawley rats. This fraction was lysed in a hypotonic buffer containing 10 mM HEPES/NaOH and 1 mM EGTA (pH 7.4). The tissue was then centrifuged at 27,000*g*, and the final pellet was resuspended in the same buffer supplemented with 100 kallikrein inhibitor units/mL of aprotinin and used immediately.

Adenylyl Cyclase Assay

The enzyme activity was assayed in a reaction mixture (final volume 50 μL) containing 50 mM HEPES/NaOH buffer (pH 7.4), 0.2 mM EGTA, 2.0 mM $MgCl_2$, 0.2 mM [α-^{32}P]ATP (70–80 cpm/pmol), 0.5 mM [^{3}H]cyclic AMP, 1 mM 3-isobutyl-1-methylxanthine, 100 μM GTP, 5 mM phosphocreatine, 50 units/mL creatine phosphokinase, 5 μg of bacitracin, and 5 kallikrein inhibitor units of aprotinin. The reaction was started by adding the tissue preparation (15–20 μg of protein) and was carried out at 30° for 10 min. When the effects of α_{tGDP} and $\beta\gamma_t$ were examined, the tissue (10 μL) was preincubated with an equal volume of a solution containing either the transducin subunits or the appropriate vehicle for 60 min at ice-bath temperature. Thereafter, the receptor agonists were added immediately, followed by the addition of the reaction mixture. The concentrations of the transducin subunits reported in the figures refer to the final concentrations in the adenylyl cyclase assay. [^{32}P]cyclic AMP was isolated as previously reported [7].

RESULTS AND DISCUSSION

Effects of the QEHA Peptide

The QEHA peptide has previously been shown to block the stimulatory effects of $\beta\gamma$ subunits on different effector systems, including type II adenylyl cyclase, phospholipase C-β3, potassium channels, and β-adrenergic receptor kinase [10]. As shown in Fig. 1, in rat olfactory bulb membranes the peptide (50 μM) significantly inhibited the stimulation of basal adenylyl cyclase activity caused by the opioid receptor agonist Leu-enk. The net stimulation elicited by 1 μM Leu-enk was reduced by 48 ± 4% in the presence of QEHA peptide (opioid-stimulated enzyme activities, expressed as pmol cyclic AMP/min/mg protein ± SEM, were: control 44.1 ± 1.5, QEHA, 23.0 ± 1.0, $P < 0.01$). Moreover, the peptide increased the EC_{50} of Leu-enk from 65 ± 6 to 183 ± 11 nM ($P < 0.01$). At the concentration used, the peptide failed to reduce either basal adenylyl cyclase activity or the enzyme stimulation elicited by 1-isoproterenol (Fig. 1).

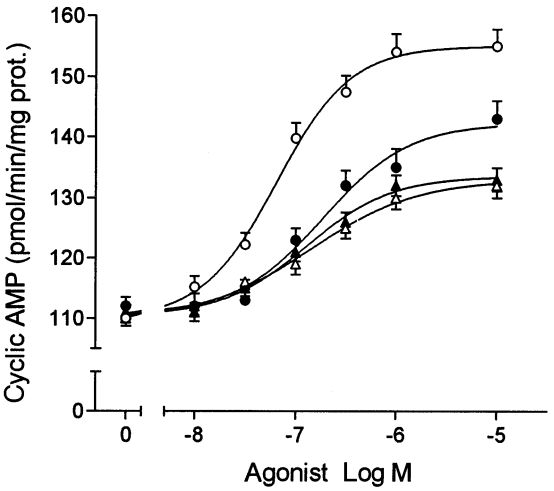

FIG. 1. Inhibition of opioid stimulation of adenylyl cyclase by the QEHA peptide. The enzyme activity was assayed at the indicated concentrations of Leu-enk (○, ●) and 1-isoproterenol (△, ▲) in the absence (open symbols) and in the presence (closed symbols) of 50 μM QEHA peptide. Values are the means ± SEM of three experiments. $P < 0.05$ for the difference between the Leu-enk concentration–response curves by analysis of variance.

Effects of α_{tGDP}

Incubation of olfactory bulb membranes with increasing concentrations of α_{tGDP}, another effective scavenger of $\beta\gamma$ subunits [11, 12], caused a progressive decrease in the opioid stimulation of adenylyl cyclase (Fig. 2A). The maximal inhibition was reached with 1.5 μM α_{tGDP} and corresponded to an 82 ± 6% reduction ($P < 0.01$) of the stimulatory effect elicited by 1 μM Leu-enk. At the higher concentrations, α_{tGDP} also slightly inhibited basal adenylyl cyclase activity by 14%. At the same concentrations inhibiting the opioid stimulation of adenylyl cyclase, α_{tGDP} failed to affect the stimulation of the enzyme by 1-isoproterenol (10 μM) (result not shown). As previously reported [13], Leu-enk (1 μM) significantly enhanced the adenylyl cyclase stimulation elicited by VIP. This facilitatory effect was completely blocked by membrane treatment with 0.5 μM α_{tGDP} (Fig. 2B) which, conversely, failed to significantly affect the stimulation by VIP.

* *Abbreviations:* α_{tGDP}, GDP-bound form of the α subunit of transducin; $\beta\gamma_t$, $\beta\gamma$ subunits of transducin; Leu-enk, Leu-enkephalin; VIP, vasoactive intestinal peptide; and QEHA, peptide corresponding to amino acids 956-982 of type II adenylyl cyclase.

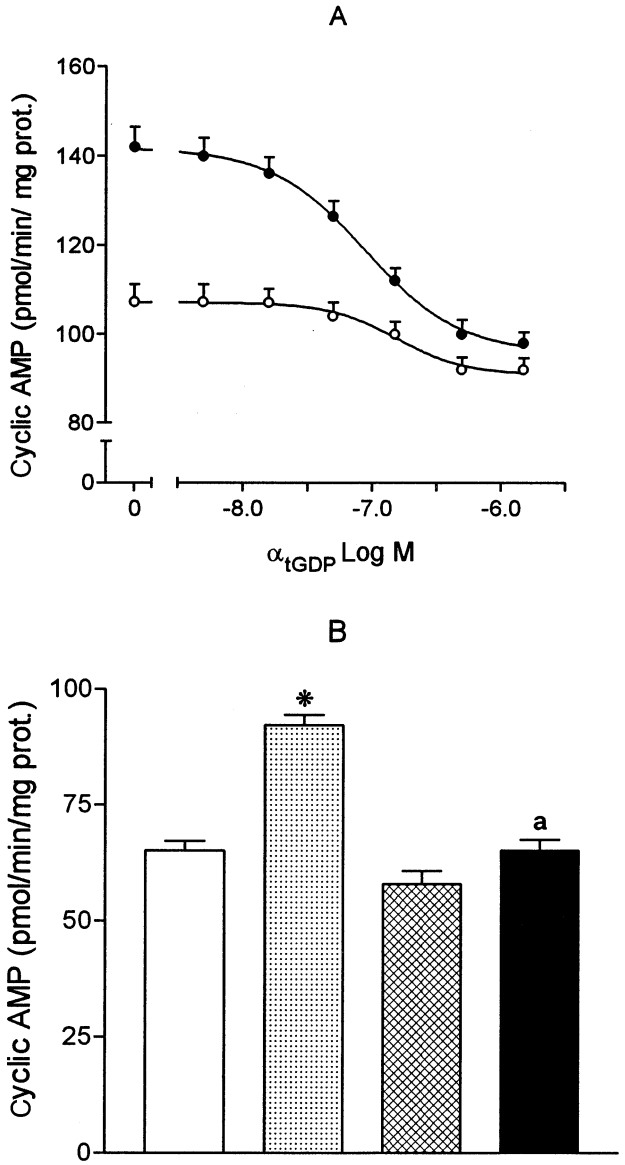

FIG. 2. (A) Inhibition of opioid stimulation of basal adenylyl cyclase activity by α_{tGDP}. Membranes were preincubated with the indicated final concentrations of α_{tGDP} for 60 min at ice-bath temperature. Thereafter, adenylyl cyclase activity was assayed in the absence (○) and in the presence (●) of 1 μM Leu-enk. Values are the means ± SEM of three experiments. (B) Blockade of opioid potentiation of VIP-stimulated adenylyl cyclase activity by α_{tGDP}. The net enzyme activity stimulated by 1 μM VIP was determined in membranes preincubated for 60 min with either vehicle (empty and dotted columns) or 0.5 μM α_{tGDP} (dashed and filled columns) in the absence (empty and dashed columns) and in the presence (dotted and filled columns) of 1 μM Leu-enk. Values are the means ± SEM of three experiments. *P < 0.01 versus VIP alone; [a] not significantly different from VIP alone in either vehicle- or α_{tGDP}-treated membranes (two-tail Student's *t*-test).

Effects of $\beta\gamma_t$

To further examine the role of βγ subunits, we investigated whether exogenously added βγ could reproduce the opioid stimulation of adenylyl cyclase. Incubation of olfactory bulb membranes with the hydrophilic detergent-free $\beta\gamma_t$ (400 nM) resulted in a marked stimulation (78 ± 4% increase, $P < 0.01$) of basal enzyme activity (Fig. 3). Under the same experimental condition, $\beta\gamma_t$ failed to significantly affect basal adenylyl cyclase activity of rat striatum (result not shown). When adenylyl cyclase activity of olfactory bulb membranes was activated by preincubation with $\beta\gamma_t$, no further stimulation could be elicited by the addition of a maximally effective concentration (10 μM) of Leu-enk (Fig. 3).

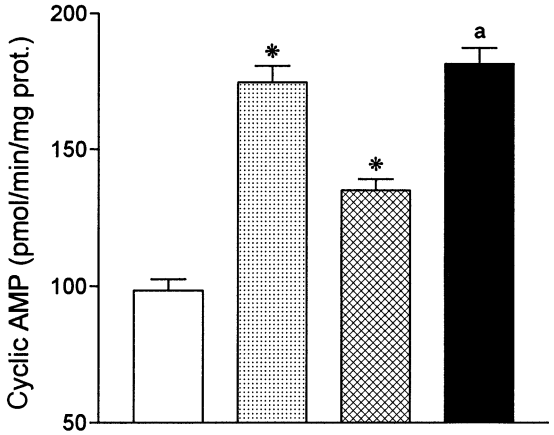

FIG. 3. Stimulation of adenylyl cyclase activity by $\beta\gamma_t$ and lack of additivity with opioid stimulation. Membranes were preincubated with either vehicle (empty and dashed columns) or $\beta\gamma_t$ (final concentration: 400 nM) (dotted and filled columns) for 60 min at ice-bath temperature. Thereafter, adenylyl cyclase activity was assayed in the absence (empty and dotted columns) and in the presence (dashed and filled columns) of 10 μM Leu-enk. Values are the means ± SEM of three experiments. *P < 0.01 versus vehicle alone; [a] not significantly different from $\beta\gamma_t$ alone (two-tail Student's *t*-test).

The data obtained in the present study strongly indicate that βγ subunits mediate the opioid stimulation of adenylyl cyclase activity in rat olfactory bulb. The opioid stimulatory effect is reduced by the addition of βγ scavengers, such as the QEHA peptide and α_{tGDP}, and is not additive with that produced by exogenously added $\beta\gamma_t$. In addition, membrane treatment with α_{tGDP} prevents the opioid potentiation of VIP-stimulated adenylyl cyclase activity. This indicates that the potentiation results from the synergistic interaction of βγ subunits released by opioid receptors and $G_{s\alpha}$ activated by VIP receptors on a common pool of adenylyl cyclase.

The specificity of the actions of QEHA peptide and α_{tGDP} is supported by the finding that in the same membrane preparation these agents do not affect the enzyme stimulation elicited by G_s-coupled receptors, such as β-adrenergic and VIP receptors. Both the potency and the maximal stimulatory effect of Leu-enk are reduced by QEHA peptide. This type of inhibition is consistent with an action of QEHA on the signal transduction mechanism of the opioid receptor. In fact, the βγ scavenger is expected to effectively block the response elicited by threshold agonist concentrations (thereby shifting the response curve to the right) because a limited amount of βγ is released

under this condition. However, at higher receptor occupancy, only a fraction of the βγ released may be complexed by QEHA, resulting in a reduction of the maximal response. A similar pattern of inhibition of receptor-mediated effects has been observed for the brain α_{GDP} in *Xenopus* oocytes [14].

The stimulatory effect of $\beta\gamma_t$ on olfactory bulb adenylyl cyclase is consistent with the presence in this brain area of enzyme isoforms, such as types II and IV, which can be activated by G protein βγ subunits [9, 15]. On the other hand, the finding that $\beta\gamma_t$ does not stimulate enzyme activity in membranes of rat striatum correlates with the low expression of the type II isoform in this brain region and with the presence of the βγ-insensitive type V adenylyl cyclase [16, 17]. As $\beta\gamma_t$ is structurally different from the βγ subunits expressed in the brain, it is important to note that it cannot be considered as the physiological partner of brain type II and IV adenylyl cyclases. However, there is abundant evidence that $\beta\gamma_t$, although less potent, is functionally similar to brain βγ subunits [12, 18, 19].

The possibility that opioid receptors stimulate adenylyl cyclase through βγ subunits is also supported by the observations that in cells transiently transfected with the cloned μ receptor and type II adenylyl cyclase, opioids increased cyclic AMP accumulation when either G_i or G_o, but not G_s, were coexpressed [20]. The present study is the first to provide evidence that this opioid receptor signaling mechanism can occur in native brain membranes where receptors, G proteins, and cyclase isoforms are naturally expressed.

We are grateful to Dr. H. E. Hamm, Department of Physiology and Biophysics, University of Illinois at Chicago for the generous gift of transducin subunits.

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