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Expression and electrophysiological identification of the receptor for bombesin and gastrin-releasing peptide in *Xenopus laevis* oocytes injected with polyA$^+$ RNA from rat brain

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The receptor for bombesin and the related peptide, gastrin-releasing peptide (GRP) has been induced in frog oocytes by injection of polyA$^+$ RNA from rat brain. The primed oocytes responded to peptides of the bombesin family (GRP, neuromedin C of bombesin) by showing dose-dependent oscillations in membrane currents as recorded by the voltage-clamp method. The induced membrane changes were suppressed when oocytes were pretreated with a bombesin-receptor antagonist.

Bombesin receptor; Gastrin-releasing peptide receptor; Thyrotropin-releasing hormone receptor; Serotonin receptor; mRNA; Microinjection; Voltage-clamped oocyte; (Rat brain)

1. INTRODUCTION

The amphibian tetradecapeptide bombesin and its mammalian homologue, the twenty-seven residue gastrin-releasing peptide (GRP) [1,2], exert a diverse range of biological effects. These include roles in thermoregulation, blood-sugar stasis and sympathetic outflow, each mediated via the central nervous system [3], while in the gastrointestinal tract GRP potentiates the release of gastrointestinal hormones [4,5]. Further, in pulmonary neuroendocrine cells and in small cell carcinomas in vivo, GRP has been reported to function as an autocrine growth factor [6–8]. Putative receptors for bombesin and GRP have been demonstrated in various tissues and organs including brain [9–11]. In Swiss 3T3 cells the receptor for peptides of the bombesin family appears to be linked to the inositol triphosphate second messenger system which in turn mediates the intracellular mobilization of Ca^{2+} [12].

Recently we have demonstrated the expression of receptors for certain neuropeptides in voltage-clamped *Xenopus* oocytes primed with mRNA from receptor-containing tissues [13,14]. Positive responses were observed for those receptors known to respond to ligand activation in vivo by triggering inositol phospholipid breakdown leading to Ca^{2+} release from intracellular stores. Apparently certain receptors when induced in frog oocytes are capable of interacting with endogenous G-protein(s), which probably function(s) as coupling (a) element(s) in the intracellular signaling pathway between the receptor and endogenous Ca^{2+}-activated ion channels. Here we report that the brain receptor for peptides of the bombesin family represents a further example of a receptor that can be expressed and electrophysiologically identified in frog oocytes, primed in this case with brain mRNA.

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2. MATERIALS AND METHODS

Bombesin, GRP, GRP_{14-27}, neuromedin C (GRP_{18-27}), [Lys^3]-bombesin, *N*-α-acetyl-GRP_{20-27} and the bombesin receptor antagonist [D-Phe^{12},Leu^{14}]bombesin were purchased from Bachem, Basel, and Peninsula, St. Helens, England.

2.1. *PolyA⁺ RNA preparation and oocyte injections*

PolyA⁺ RNA was prepared from rat brain and 50 nl RNA solution (0.5 μg/ml in 10 mM Tris-HCl, pH 7.5, 100 mM NaCl) injected into stage V oocytes of *Xenopus laevis* as reported previously [13]. Oocytes were kept in Barth's medium at 20°C for 1–4 days. For further purification, polyA⁺ RNA was size-fractionated on a NaDodSO₄ sucrose-density gradient as described earlier [14]. The collected fractions were precipitated twice with ethanol, taken up in 10 μl of H_2O and 50 nl aliquots used for injection into oocytes [14].

2.2. *Voltage clamping of oocytes*

Whole-cell current measurements were performed at room temperature with a conventional two-microelectrode voltage clamp in Ringer's solution [15]. The membrane potential was adjusted to −60 mV and voltage clamped. The membrane current signal was filtered with a corner frequency of 0.3 kHz. Ligands were perfused, at the concentrations indicated, in Ringer solution or applied directly to the oocyte bath with a syringe.

3. RESULTS

In order to establish if the receptor for the bombesin-related family of peptides can be expressed and functionally detected by the voltage-clamp method, polyA⁺ RNA was prepared from rat brain and injected into *Xenopus laevis* oocytes. When exposed to bombesin in the incubation bath, the injected oocytes responded by showing oscillations in membrane currents, indicating that expression of the bombesin receptor can be functionally detected in frog oocytes (fig.1). The bombesin-evoked response was not observed with oocytes sham-injected with buffer.

Fig.1 shows that oocytes primed with rat brain polyA⁺ RNA also express the receptors for serotonin and thyrotropin-releasing hormone (TRH) [13] as indicated by responses to the respective ligands when added to the incubation bath. Additionally, small but significant signals were obtained in response to substance P and histamine, suggesting the presence of induced receptors for these ligands in the injected oocytes (not shown).

In general the observed oscillating current response for serotonin was at least a magnitude of order larger than those evoked either by bombesin

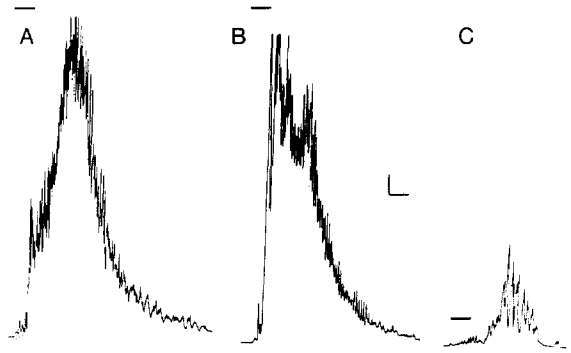

Fig.1. Whole cell current traces of *X. laevis* oocytes. After microinjection of 50 nl of rat brain polyA⁺ RNA (0.5 μg/μl) into oocytes the latter were voltage clamped at −60 mV, perfused with frog Ringer's solution and exposed to 1 μM bombesin (A), 0.1 μM serotonin (B) or 10 μM TRH (C). The bars denote the duration of ligand application. Time and current scales are given by the rectangle: horizontal, 1 min; vertical, 10 nA.

or TRH which in turn were significantly greater than those for substance P and histamine. This may reflect a significantly higher abundance of the mRNA encoding the serotonin receptor compared to that for the bombesin, TRH, substance P or histamine receptors. Furthermore, oocytes when exposed to serotonin exhibited a bi-phasic response with an initial rapid and transient depolarizing current, followed by a prolonged fluctuating current of smaller amplitude. This initial rapid depolarizing current is significantly reduced or even absent in the case of the bombesin- and TRH-evoked response.

Fig.2 shows membrane current responses of frog oocytes evoked by peptides of the bombesin family (table 1). Oocytes previously injected with rat brain polyA⁺ RNA responded to bombesin (A), [Lys^3]bombesin (B), GRP (C), GRP_{14-27} (D), GRP_{18-27} (neuromedin C; E) and *N*-[α-]acetyl GRP_{20-27} (F), respectively. In most experiments maximal response was observed with bombesin; N-terminally truncated GRP_{20-27} was as active as full length GRP suggesting that the C-terminus of the mammalian hormone GRP is essential for its interaction with the brain receptor.

When brain polyA⁺ RNA was size-fractionated on SDS-sucrose density gradients as reported recently [13,14] and aliquots of individual fractions were injected into oocytes, the major activity peak travelled at or slightly ahead of the position

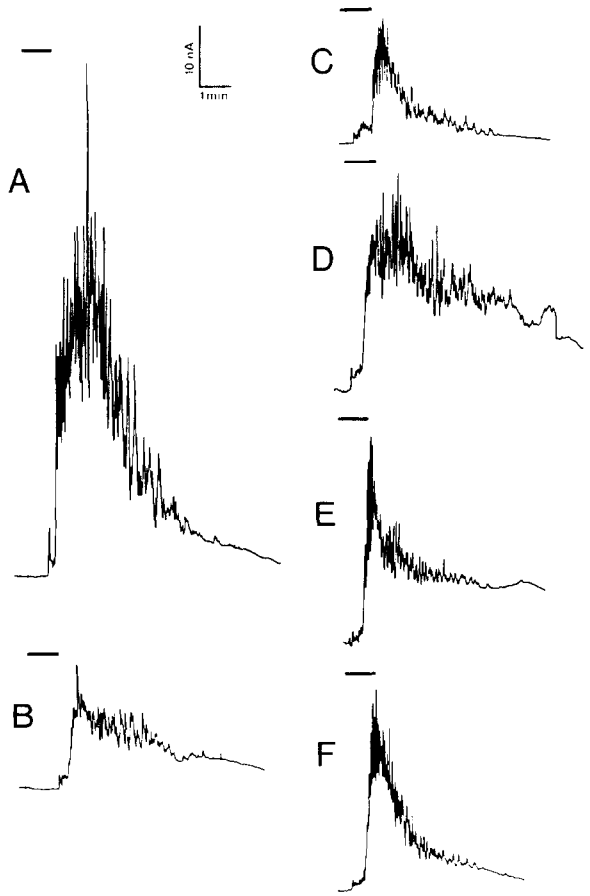

Fig.2. Membrane current responses of a brain polyA$^+$ RNA primed *X. laevis* oocyte evoked by peptides of the bombesin family. A voltage-clamped oocyte previously injected with rat brain polyA$^+$ RNA was subsequently treated with 0.1 μM bombesin (A), 0.1 μM [Lys^3]bombesin (B), 1 μM GRP (C); 1 μM GRP_{14-27} (D), 1 μM GRP_{18-27} (neuromedin C) (E), and 1 μM *N*-α-acetyl-GRP_{20-27} (F) as indicated by the bars. The oocyte was perfused 15 min with frog Ringer's solution prior to peptide applications.

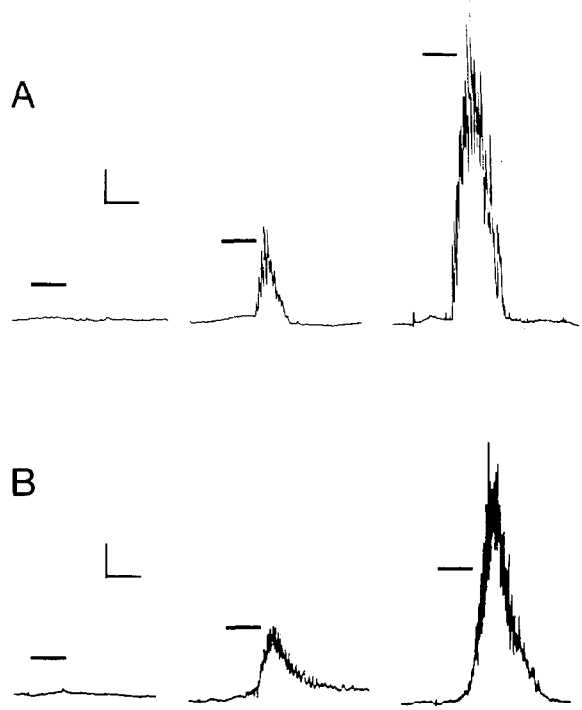

Fig.3. Effect of a bombesin-receptor antagonist, [D-Phe^{13},Leu^{14}]bombesin- and *N*-α-acetyl-GRP_{20-27}-evoked whole cell current responses. Membrane current responses of voltage-clamped oocytes to challenge with 0.1 μM bombesin (A) or 1 μM *N*-α-acetyl-GRP_{20-27} (B) in the presence and absence of a bombesin receptor antagonist. In A the antagonist was used at 100-fold (left) and 25-fold (middle) molar excess; in B a 5-fold (left) and 10-fold (middle) molar excess was used. Responses in the absence of antagonist are shown on the right. Following each treatment oocytes were perfused with frog Ringer's solution for 15 min. The time and current scale is given by rectangles: horizontal, 1 min; vertical, 10 nA.

of the 18 S rRNA suggesting that the mRNA encoding the bombesin receptor is greater than 2 kb in length.

To determine if the bombesin receptor functionally expressed in frog oocytes shows similar pharmacological properties to the brain receptor, a bombesin-receptor antagonist [D-Phe^{12},Leu^{14}]-bombesin was applied together with either the ligand bombesin or *N*-α-acetyl GRP_{20-27}. Fig.3 shows that the antagonist suppressed the bombesin and *N*-α-acetyl GRP_{20-27}-specific response in a dose-dependent manner.

4. DISCUSSION

The data presented here show that polyA$^+$ RNA isolated from rat brain encodes a receptor that recognizes peptides of the bombesin family and which can be functionally expressed and electrophysiologically recorded in oocytes from *X. laevis*. The expressed receptor shows similar pharmacological properties to that from the brain, insofar as the electrophysiological response can be triggered by bombesin and GRP agonists while a bombesin-receptor antagonist blocks this effect.

Bombesin and GRP are known to induce inositol triphosphate-mediated Ca^{2+} release from in-

tracellular stores [11,12,16] probably by activating a phospholipase C which hydrolyzes phosphatidylinositol-4,5-bisphosphate to yield 1,2-diacylglycerol, and inositol-1,4,5-trisphosphate second messengers. This signal transduction mechanism can be mimicked in frog oocytes leading ultimately to Ca^{2+}-mediated ion-channel opening probably of the chloride type [17]. The fact that a number of other receptors can be expressed similarly in the frog oocyte [13–15,18–24] suggests that certain transmitter and peptide hormone signal transduction pathways proceed via a generalized and conserved mechanism.

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