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$G\alpha_{11}$ and $G\alpha_q$ guaninine nucleotide regulatory proteins differentially modulate the response to thyrotropin-releasing hormone in *Xenopus* oocytes

Dafna Lipinsky[a], Marvin C. Gershengorn[b] and Yoram Oron[a]

[a]*Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv 69978, Israel* and [b]*Division of Molecular Medicine, Department of Medicine, Cornell University Medical College and The New York Hospital, New York, NY 10021, USA*

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Xenopus oocytes that express mouse thyrotropin-releasing hormone receptors (TRH-Rs) after injection if RNA transcribed from TRH-R cDNA respond to THR by a depolarizing current. This response is transduced by activation of phosphoinositide-specific phospholipase C and utilizes an as yet unidentified endogenous guanine nucleotide-binding regulatory (G) protein(s). The α subunit of G_{11} and G_q have recently been shown to couple receptors to activation of phospholipase C. To determine whether there are functional differences between these proteins, we have co-expressed the TRH-R with either α_{11} or α_q. α_{11} potentiated the response to TRH (by 61±16%), while α_q inhibited the response (by 37±9%). The changes in amplitudes were accompanied by inverse changes in response latencies. These data show that α_{11} and α_q differentially modulate signal transduction in *Xenopus* oocytes.

G-protein; TRH receptor; *Xenopus* oocyte

1. INTRODUCTION

Receptors that belong to the seven transmembrane-spanning segment family couple to heterotrimeric guanine nucleotide-binding regulatory (G) proteins [1]. It has been suggested that G-proteins of the G_o/G_i class, which are sensitive to pertussis toxin (PTX), are involved in responses that are transduced by activation of a phospholipase C that hydrolyzes phosphatidylinositol 4,5- bisphosphate [2–7]. In many cell types, however, the resulting responses are not affected by PTX [8–11]. Hence, G-proteins that lack the PTX-modified ADP-ribosylation site seem to be logical candidates for this regulatory function for some receptors.

Recently, several laboratories have demonstrated that two members of a new sub-family of G-protein α subunits, α_{11} and α_q [12] can activate phospholipase C [13–17]. These experiments were performed either by in vitro reconstitution or by transfection into mammalian cells. In these studies, no differences in function were found for α_{11} and α_q. *Xenopus* oocytes are an excellent model system for mechanistic studies of signal transduction pathways and have been used to study G-protein coupling [3,18]. Here we use *Xenopus* oocytes to study the coupling of α_{11} and α_q to the TRH-R. Our results show that α_{11} and α_q differentially modulate the TRH-R response in *Xenopus* oocytes.

Correspondence address: Y. Oron, Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv 69978, Israel.

2. MATERIALS AND METHODS

The electrophysiological methods and the response to TRH in oocytes injected with RNA coding for the TRH-R have been described in detail previously [19–21].

In the present series of experiments, we have co-injected in vitro transcribed RNAs encoding the cloned TRH-R [21] and either the α_{11} or the α_q G-protein subunits [12]. The responses to THR were assayed 12–96 h after the injection. Amplitudes and latencies of the responses were determined. All results are expressed as mean ± S.E.M. % of control responses assayed in oocytes of the same donor and injected with identical amount of TRH-R RNA alone. The number of oocytes assayed was denoted by *n* and the number of different experiments by *N*. Different experiments were mostly performed on oocytes from different donors.

The cloned α_{11} and α_q were a generous gift of Dr. Melvin Simon. TRH and collagenase (type IA) were purchased from Sigma. All other chemicals were of analytical grade.

3. RESULTS

3.1. *The experimental paradigm*

TRH-Rs are expressed in *Xenopus* oocytes following injection of cloned TRH-R RNA [20]. The activation of TRH-R is manifested by a depolarizing current resulting from calcium-induced opening of chloride channels

subsequent to phospholipase C-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate [19]. This current can be characterized by its maximal amplitude and the time between the addition of TRH and the onset of the response (latency). Responses can be observed within 12 h of the injection of TRH-R RNA and optimal responses can be observed within 24–48 h at 1–10 ng TRH-R RNA injected/oocyte. These observations indicate that exogenous TRH-R couples effectively to an unknown native G-protein(s) in oocytes.

To determine whether there are differences in coupling of α_{11} and α_q, we co-expressed these two mammalian G-protein α subunits with the TRH-R. We have monitored the efficiency of coupling by comparing the amplitude and latency of the response to those observed in oocytes injected with TRH-R RNA alone.

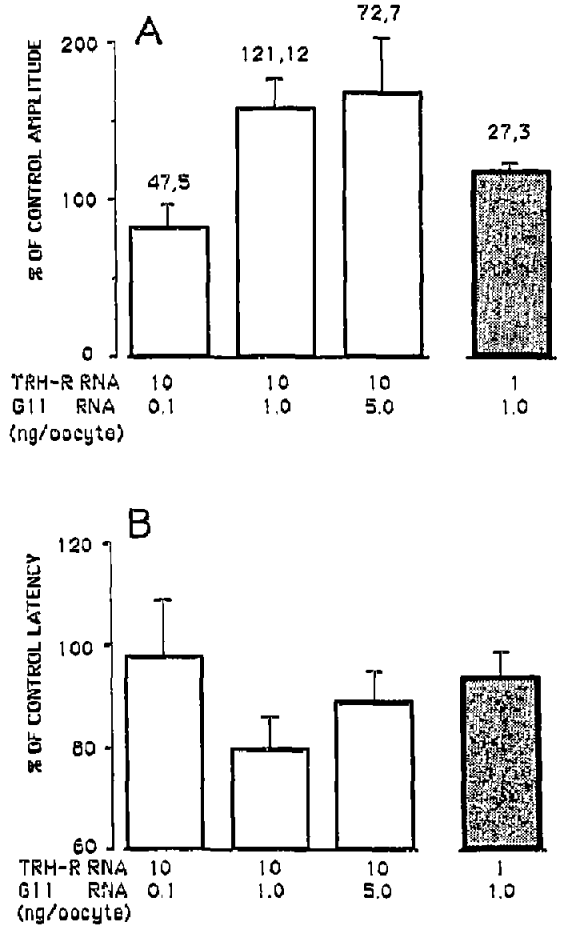

Fig. 1. The effect of α_{11} on amplitude and latency of the TRH response. The bars represent the mean amplitude±S.E. (panel A) or latency (panel B) in oocytes injected with 10 ng/cell of TRH-R RNA and the indicated amount of α_{11} RNA. The cross-hatched bar represents the results obtained in oocytes injected with 1 ng TRH-R RNA. Responses to 1 μM TRH were measured. All results were normalized as % of matched controls. Control response amplitudes and latencies ranged from 296±96 to 6594±1172 nA and 1.3±0.2 to 16.7±2.0 s, respectively. The numbers above the bars denote the number of treated oocytes assayed and the number of experiments performed (n,N).

3.2. *The effect of α_{11} co-expression*

Co-expression of α_{11} and TRH-R resulted in a significant potentiation of the response without affecting its pattern. In a paired comparison in 12 experiments of oocytes from 9 different donors injected with 10 ng TRH-R RNA and 1 ng α_{11} RNA, the amplitude of the response increased to 159±18% (P<0.01). This increase was dependent on the amount of α_{11} RNA co-injected. Thus, in oocytes co-injected with 0.1 ng α_{11} RNA, the amplitude did not increase (83±14% of controls, n=5), while in oocytes co-injected with 5 ng of RNA, the amplitude was 164±33% of matched controls (n=7, P<0.1). These results are shown in Fig. 1A.

The increase in amplitude was accompanied by a decrease in the latency of the response. In oocytes co-injected with 1 ng α_{11} the latency decreased to 80±6% of control values (n=12, P<0.005). In oocytes co-injected with 0.1 ng RNA there was no change in latency (98±11% of control, N=5), while those co-injected with 5 ng did not exhibit a further decrease in latency (91±6% of control, n=6). These results are shown in Fig. 1B.

The effect of co-expression of α_{11} was much less when a lower amount of TRH-R was expressed. In oocytes injected with 1 ng each of TRH-R and α_{11} RNAs, the amplitude of the response increased to 118±5% and the latency was 94±5% of control values (n=3, see Fig. 1A,B).

We have not observed a clear relationship between the time after injection of the RNAs and the magnitude

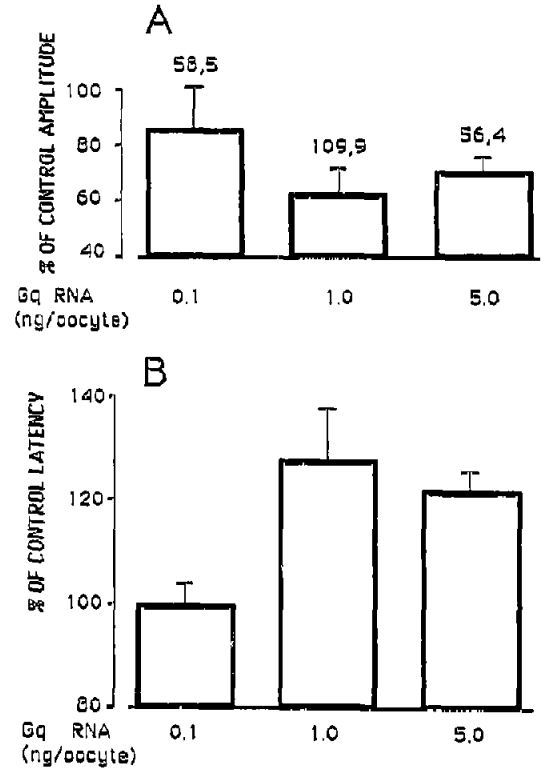

Fig. 2. The effect of α_q on amplitude and latency of the response. For experimental conditions see legend to Fig. 1.

of the effects on either amplitude or latency (not shown). Hence, it may be assumed that TRH-R and α_{11} are expressed at approximately the same rate within 12–96 h after the injection.

3.3. *The effect of α_q co-expression*

To further assess the specificity of exogenous G-proteins to TRH-R, we have conducted similar experiments with the closely homologous α subunit of G_q. In contrast to α_{11}, co-expression of α_q inhibited the response to TRH. In oocytes injected with 10 ng of TRH-R RNA and 1 ng of α_q RNA, the response to TRH was 63±9% of control (n=9, P<0.025). This property of α_q showed a degree of dose dependence. Thus, oocytes co-injected with 0.1 ng α_q RNA exhibited 86±15% of control amplitude (n=5) and those co-injected with 5 ng, 71±5% (n=4, P<0.025). The decrease in amplitude was accompanied by a parallel increase in the latencies of the responses. The latency was 100±4, 128±10 (P<0.05) and 122±8% (P<0.025) of control in oocytes injected with 0.1, 1.0 and 5.0 ng of α_q RNA, respectively. These results are shown in Fig. 2A,B.

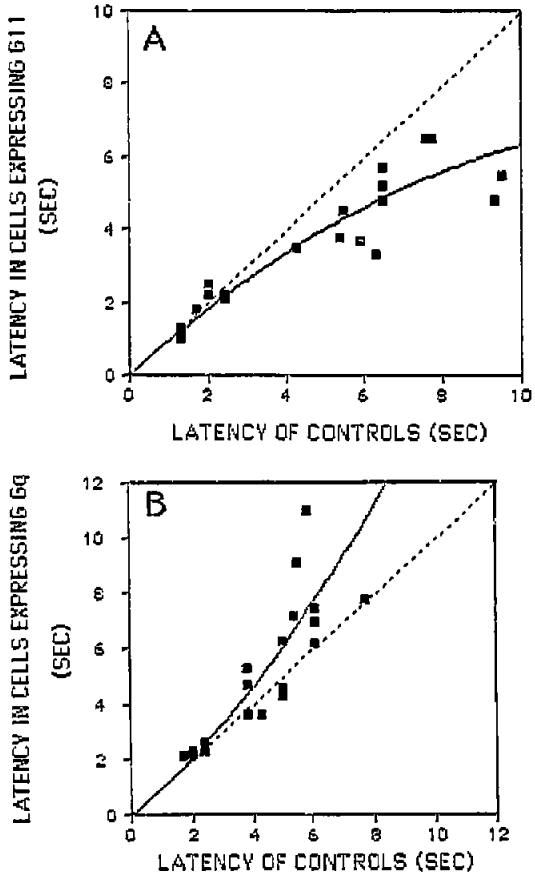

Fig. 3. The effect of the latencies on control responses on the magnitude of the latencies in oocytes expressing α_{11} or α_q. Mean latencies of responses in oocytes co-expressing the TRH-R and either α_{11} (panel A) or α_q (panel B), from individual experiments described in Figs. 1 and 2, were plotted against the matching control latencies. The broken line describes the theoretical curve that would have been obtained if α_{11} or α_q had no effect on latency.

4. DISCUSSION

α_{11} and α_q are two members of a sub-family of G-protein α subunits that are not sensitive to PTX and activate phosphoinositide-specific phospholipase C. In transfection studies in mammalian cells [15] and reconstitution experiments in vitro [17], these proteins appear to be equally effective in interaction with receptors. It has been shown, however, that heterotrimeric G-proteins can couple under certain condition to effector molecules — enzymes, channels, etc. — with which they do not interact in vivo under usual conditions. For example, different muscarinic receptors can exhibit anomalous coupling to G-proteins upon transfection and overexpression in mammalian cells [22]. We attempted to determine whether there were any differences in the interaction of α_{11} and α_q with receptors or effectors, or both, in intact cells in which the level of the expression could be readily controlled. We used *Xenopus* oocytes for these studies, because we could monitor the kinetics of the response to TRH with excellent resolution. We previously reported that the increase in amplitude was inversely related to the latency of the response [23].

We found there is a difference between the effect of α_{11} and α_q on the TRH response in *Xenopus* oocytes. α_{11} enhanced the response to TRH, exhibited as an increase in response amplitude and shortening of latency, whereas α_q diminished the response. Thus, it appears that there are differences in the properties of these two G-protein α subunits.

The mechanism of enhancement by α_{11} and inhibition by α_q of the TRH response are not known. Any hypothesis must take into account the productive interaction between the TRH-R and endogenous G-proteins in oocytes. Endogenous G-proteins of the G_i/G_o family have been cloned from oocytes (24,25). It is possible that because both α subunits [12] and the TRH-R [21] used in this experiment are murine in origin there is a greater affinity between the receptor and these exogenous α subunits than between the mammalian TRH-R and endogenous amphibian G-proteins. This could explain the enhancement of the TRH response by α_{11}, assuming that receptor-activated α_{11} effectively stimulates the phospholipase C.

To explain the mechanism of inhibition of the TRH response by α_q we suggest that activated α_q does not activate the phospholipase C as efficiently as endogenous G-proteins. That is, α_q competes for binding for the TRH-R with endogenous G-proteins but is less effective in eliciting a response. The idea that there is competition between exogenous and endogenous G-protein α subunits is supported by our data. In Fig. 3, we compare the TRH response latencies in oocytes injected with TRH-R RNA alone to those in oocytes injected with TRH-R and α subunit RNAs. We chose to use latencies rather than response amplitudes for these

comparisons because latencies exhibit less variability and, in our experience, correlate better than amplitudes with the number of receptors expressed [26], Lipinsky and Oron, unpublished). It is apparent that the relative change in latency due to expression of exogenous α subunits is related to the duration of latency in matched control oocytes injected with TRH-R alone. That is, the effects of α_{11} and α_q are most prominent in oocytes in which the TRH response is smaller, i.e. in responses that exhibit longer latencies. A possible interpretation of these data is that in oocytes that possess a large quantity of endogenous G-proteins, that is those that exhibit the most robust responses with the shortest latencies, the exogenous α subunits fail to compete effectively for binding to the TRH-R and, therefore, do not influence the response.

In conclusion, we showed that α_{11} and α_q differentially affect the electrophysiological response to TRH in *Xenopus* oocytes expressing either of these subunits. We have not yet delineated the mechanism(s) of these effects. Nevertheless, our data show that these closely related members of the α_q sub-family can have different effects under certain circumstances and suggest that these proteins may not subserve the same function(s) in mammalian cells. To the best of our knowledge, this is the first description of functional differences between α_{11} and α_q.

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