The hemispheric functional expression of the thyrotropin-releasing-hormone receptor is not determined by the receptors’ physical distribution

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The thyrotropin-releasing-hormone receptor (TRH-R) is a member of a family of the G-protein-coupled receptors that share structural similarities and exert their physiological action via the inositol lipid signal-transduction pathway. The TRH-R when expressed in Xenopus oocytes exhibits marked preference of the response (increased chloride conductance) for the animal hemisphere. Whereas the rat TRH-R functional distribution was strongly asymmetric (animal/vegetal ratio = 9.5), the mouse TRH-R exhibited a significantly lower ratio (3.9). Truncation of the last 59 amino acids of the C-terminal region of the mouse TRH-R did not lead to any changes in the functional hemispheric distribution. Despite the polarization of response, receptor number was similar on both hemispheres. Moreover, the apparent half-life of the functional expression of the TRH-R was approx. 4 h on both hemispheres when the expression was inhibited by a specific antisense oligonucleotide. Inhibition of total protein synthesis with cycloheximide affected hemispheric responses mediated by each of the three TRH-Rs tested in a qualitatively different way. These results suggest that an additional, rapidly degraded, protein modulates the functional hemispheric expression of the TRH-Rs.

INTRODUCTION

Xenopus oocytes are a convenient model system for expression of membrane proteins, including ion channels and cell membrane receptors [1]. In this model system, exogenous receptors that couple to guanine-nucleotide-binding regulatory proteins (G-proteins) can be expressed and their activity quantified by measuring the increase in Ca²⁺-activated chloride conductance. Several laboratories have utilized the oocyte to investigate the structure–function relationship of different receptors [2–4]. We have previously described the expression of muscarinic acetylcholine receptors in Xenopus oocytes [5,6]. In these studies we demonstrated that receptors that display structural similarities exhibit different asymmetry of hemispheric distribution. Moreover, we showed that the ratio of hemispheric activity of the muscarinic receptors reflected the ratio of receptor distribution [5,6]. Inhibition of protein synthesis or glycosylation differentially affected the hemispheric behaviour of these receptors.

We have reported that the rat thyrotropin-releasing-hormone receptor (TRH-R) was expressed in Xenopus oocytes with a marked preference for the animal hemisphere [7]. Here, we describe the functional and physical expression of three TRH-Rs (rat, mouse wild-type and mouse truncated) that have been recently cloned and shown to have a high degree of structural homology [8–10]. Our results suggest that the asymmetry of response is determined by a distal component of the signal-transduction pathway rather than by the receptor distribution. Nevertheless, the effect of this component on the functional expression of TRH-Rs is characteristic of the specific receptor type. Moreover, different effects of inhibition of total protein synthesis compared with specific ablation of TRH-R synthesis suggest that another rapidly degraded protein may be involved in the regulation of TRH-R activity.

MATERIALS AND METHODS

Experimental animals

Adult Xenopus females, purchased from the South African Xenopus facility (Noordhoek, South Africa 7985), were maintained and fed as described previously [11]. The animals were cold-anaesthetized, and ovary fragments were dissected into ND96 medium. Oocytes at stage 5 or 6 were separated and maintained at 20 °C in NDE96 medium. Before microinjection of RNA, oocytes were defolliculated with collagenase [12]. When the same donors were used repeatedly, dissections were spaced 2–3 weeks apart to allow full recovery of the animals.

Microinjection of RNA

TRH-Rs were expressed by injecting either 250–500 ng/cell of total GH₄ rat pituitary-tumour cell RNA (prepared by guanidinium thiocyanate extraction; see [11]) or 0.1–10 ng/cell of RNA transcribed in vitro from cDNA encoding the mouse wild-type TRH-R or the mutant receptor truncated at Cys-335 as described by Straub et al. [10] and Nussenzveig et al. [13]. Oocytes were assayed 24–48 h later.

Electrophysiology

Electrophysiological methods were described in detail previously [11,12,14]. Briefly, oocytes were assayed for chloride current under two-electrode voltage clamp by using a DAGAN 8500 or Axoclamp intracellular clamp. Oocytes were routinely voltage-clamped at −100 mV to minimize the K+ current. TRH (1 μM, the concentration that elicits maximal responses [11]) was added rapidly and directly to a special perfusion bath in 1 ml of ND96.
Assay of hemispheric responsiveness to TRH
A special Lucite (Perspex) perfusion bath was constructed with depressions (0.8–1.2 mm diameter; 3 mm depth) drilled in its bottom. An oocyte was placed in an orifice that was slightly smaller than its diameter, in a way that allowed the exposure of only one hemisphere of the cell to the agonist [5].

Radioligand binding
The protocol was similar to that described previously by us [5,6,12,15]. Briefly, oocytes were incubated for at least 3 h in ND96 solution (20 μl/oocyte) with 25 nM [methyl-3H]His-TRH at 4 °C. This concentration was chosen on the basis of preliminary experiments, which demonstrated that the specific binding was the same with 15, 25 and 40 nM ligand (results not shown). After the incubation, oocytes were washed in 3 × 4 ml of ice-cold ND96 solution and counted for radioactivity. Non-specific binding was determined in the presence of 25 μM unlabelled TRH (45 ± 9% of total binding).

Assay of hemispheric receptor distribution
Each oocyte was inserted into an orifice (1 mm in diameter) made in an agar disc (5 mm in diameter, 3 mm thickness; 2.5% agar dissolved in hot ND96) with the equator perpendicular to the surface of the disc. The oocyte in the disc was quickly frozen on a block of solid CO₂. The two hemispheres were cut apart, under a binocular microscope, with a precooled razor blade and the halves were counted for radioactivity. Oocytes incubated in the presence of unlabelled TRH to determine non-specific binding were processed in the same way. The mean non-specific binding of each hemisphere was subtracted from the corresponding mean total binding.

Intracellular injection of antisense oligonucleotide
The oligonucleotide was synthesized with a DNA synthesizer, on the basis of the published sequence of the mouse TRH-R [10]. The antisense oligonucleotide was complementary to the N-terminal portion of the receptor (bp 268–303). The oligonucleotide was dissolved in distilled water, and 10 ng was injected into a single oocyte (50 ml) by using a VWR digital microdispenser with a capillary pulled to a 15–20 μm tip. Control oocytes were sham-injected with the same volume of distilled water.

Incorporation of amino acids
Oocytes were preincubated with cycloheximide (CHX) for the desired time period, then injected with 0.05–0.25 μCi/cell of a 3H-labelled mixture of amino acids (leucine, lysine, phenylalanine, proline, tyrosine; sp. radioactivity 75–190 Ci/mmol). After 2 h, individual oocytes were homogenized in 10% trichloroacetic acid and filtered. The filters were washed with cold 10% trichloroacetic acid and counted for radioactivity in 4.5 ml of Hydroluma (LUMAC) scintillator. The non-specific adsorption of label on the filters (Whatman GF/A) was 2% of incorporation.

Effects of CHX on hemispheric expression
At 24 h after the injection of TRH-Rs mRNAs, oocytes were incubated for 2–4.5 h in the presence of CHX and assayed for responses or binding and compared with untreated controls. Binding studies were performed in the presence of CHX. The final concentration of CHX was 10 μg/ml, which has been previously shown to inhibit fully total protein synthesis in oocytes [16,17].

Analysis of results
All experiments were repeated several times in oocytes from different donors. The number of oocytes assayed for each condition was denoted by n and the numbers of donors by N. All results were represented as mean ± S.E.M. Statistical analyses were performed by Student’s t test.

Solutions and chemicals
The composition of ND96 was (mM): NaCl 96, KCl 2, MgCl₂ 1, CaCl₂ 1.8, Na-Hepes 5, pH 7.5. NDE96 included additionally sodium pyruvate (2.5 mM) and cefotaxim (50 μg/ml). TRH, collagenase (type IA), puromycin and CHX were purchased from Sigma. [methyl-3H]His-TRH was from New England Nuclear. All other chemicals were of analytical grade.

Table 1 Comparison of hemispheric response amplitudes and TRH-R binding
Hemispheric responses in oocytes injected with either GH₃-cell RNA (300 ng/oocyte) or mouse TRH-R RNA transcribed in vitro (0.1 or 10 ng/oocyte) were determined. The last line describes the results of binding experiments in oocytes injected with 10 ng of mouse TRH-R RNA. The high S.E.M. represents the high variability of binding sites among the different donors.

<table>
<thead>
<tr>
<th>Receptor type</th>
<th>Animal</th>
<th>Vegetal</th>
<th>A/V ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat TRH-R (300 ng/oocyte)</td>
<td>1758 ± 274</td>
<td>217 ± 31</td>
<td>9.5 ± 1.6</td>
</tr>
<tr>
<td>Mouse TRH-R (0.1 ng/oocyte)</td>
<td>828 ± 285</td>
<td>210 ± 58</td>
<td>4.0 ± 0.8</td>
</tr>
<tr>
<td>Mouse TRH-R (10 ng/oocyte)</td>
<td>1625 ± 217</td>
<td>464 ± 87</td>
<td>3.9 ± 0.5</td>
</tr>
<tr>
<td>TRH-R binding (fmol/hemisphere)</td>
<td>1.2 ± 0.6</td>
<td>1.1 ± 0.5</td>
<td>0.94 ± 0.08</td>
</tr>
</tbody>
</table>

Figure 1 Response-amplitude–receptor-number relationship
Response amplitude of oocytes injected with either 300 ng of total GH₃-cell RNA (□) or 10 ng of mouse TRH-R RNA transcribed in vitro (■) were plotted against receptor number determined in the same batch of oocytes. Each point represents the mean amplitude and receptor number determined in a different donor on at least 5 oocytes.
RESULTS

Hemispheric asymmetry of responses

We investigated the functional distribution of TRH-Rs in oocytes expressing either rat TRH-R (in oocytes injected with total GH3-cell RNA) or mouse TRH-R (in cells injected with RNA transcribed in vitro from TMT-tumour cell TRH-R cDNA [10]). We have previously reported that the response to TRH was much greater on the animal hemisphere [7]. Here, we confirmed the marked polarity of responsiveness to TRH in oocytes injected with GH3-pituitary-cell RNA. The mean responses obtained at the animal and vegetal hemispheres were 1758 ± 274 nA and 217 ± 31 nA respectively. The mean animal/vegetal (A/V) ratio was 9.5 ± 1.5 (Nanimal = Nvegetal = 68, N = 21). The mouse TRH-R also exhibited marked asymmetry of responses, with preference for the animal hemisphere. The mean amplitudes were 1625 ± 217 nA and 464 ± 87 nA on the animal and vegetal hemispheres respectively (Nanimal = Nvegetal = 68, N = 8). Despite the high amount of RNA injected (10 ng/oocyte) compared with GH3-cell RNA, the responses remained unchanged on the animal hemisphere and increased 2-fold on the vegetal hemisphere. The mean A/V ratio was 3.9 ± 0.5 (range 2.6–5.8). This was markedly lower than the ratio observed for the rat TRH-R (P < 0.025). To test whether the increased response on the vegetal hemisphere reflected insertion of a larger number of receptors, due to the large amount of mRNA injected (thus leading to the low ratio), we injected oocytes with 0.1 ng/cell of cloned mouse mRNA. The responses obtained at the animal and vegetal hemispheres were 828 ± 285 nA and 210 ± 58 nA respectively (Nanimal = Nvegetal = 37, N = 5). The ratio, however, remained unchanged (4.0 ± 0.8, range 2.0–6.2). These results are shown in Table 1. We could not exclude the possibility that the markedly different hemispheric response ratios observed in oocytes expressing the two rodent TRH-Rs may have been due to co-expression of additional GH3-cell proteins. This possibility is further explored in the Discussion section.

The apparent lack of linear relationship between the amount of injected message (a 100-fold increase) and the amplitude of the response (2-fold increase; see Table 1) confirmed our previous study in which we performed RNA dose–response experiments [18]. This phenomenon implied that the extent of the response may be limited by a distal component(s) of the signal-transduction pathway, rather than the number of expressed receptors.

Hemispheric distribution of the TRH-Rs

Receptor number was determined by using [methyl-3H]His-TRH. Despite the relatively large response amplitudes, the number of receptors expressed in oocytes injected with GH3-cell RNA was quite low (0.68 ± 0.18 fmol/cell; n = 100, N = 10). The mean non-specific binding was 45 ± 9%. This level of expression was too low to allow determination of hemispheric distribution. Despite the large amount of RNA injected, the number of receptors in oocytes expressing mouse TRH-R was only slightly larger than in oocytes expressing rat TRH-Rs. The mean value was 0.9 ± 0.2 fmol/oocyte (n = 183, N = 26); the non-specific binding was 29 ± 3%. This level of expression was sufficient for determination of physical hemispheric distribution. The A/V ratio obtained in 7 different experiments was 0.94 ± 0.08 (n = 56, N = 7; see Table 1). The ratio was markedly different even from the lowest ratio of activity.

Moreover, no correlation was found between response amplitudes and receptor number in oocytes from different donors (Figure 1). Only when a large number of oocytes of the same donor was assayed for response amplitudes and radioligand binding was a weak correlation between the two parameters observed (Figure 2). These results, taken together, strongly suggest that neither response amplitudes nor their hemispheric ratios are determined by the number of expressed receptors. Hence, another component, distal to the receptor, appears to be limiting in the signal-transduction cascade.

Effect of protein-synthesis inhibition on the functional hemispheric distribution of TRH-Rs

In order to determine the half-life of the TRH-Rs, we incubated oocytes with 10 μg/ml CHX. This concentration decreased the incorporation of 3H-labelled amino acids by 97% (26824 ± 3630 d.p.m. and 767 ± 45 d.p.m. for control and CHX-treated oocytes respectively). Oocytes injected with GH3-cell RNA (300 ng/cell) or cloned mouse TRH-R RNA (10 ng/cell) were assayed 24–48 h later. After incubation for 2–4.5 h with CHX, response amplitudes were measured. In oocytes expressing the rat TRH-R, the response on the animal hemisphere decreased, with a half-life of approx. 2 h (Figure 3). The response on the vegetal hemisphere, on the other hand, remained unchanged during the
incubation time (up to 4.5 h). Oocytes expressing mouse TRH-R were incubated in the same manner for 2-6 h with CHX. A major decrease in the response amplitudes at the animal hemisphere was observed (to 37.5% and 15% respectively, \( n = 14 \) for each condition, \( N = 2 \)). The effect of CHX on protein synthesis was confirmed by experiments in which we used puromycin. These results suggested that the half-life of the TRH-R on the animal hemisphere was less than 2 h. Surprisingly, the response measured on the vegetal hemisphere increased after incubation for 3 h with CHX to 210±10% of untreated controls (\( n = 43 \) for each condition, \( N = 6 \); see Figure 4). Hence, the conflicting effects of protein-synthesis inhibition on hemispheric receptor activity were difficult to interpret in terms of receptor degradation alone.

Specific inhibition of receptor synthesis with antisense oligonucleotides

To clarify whether the half-life of activity measured after total inhibition of protein synthesis was due to degradation of the receptor or of an additional protein, we injected oocytes expressing mouse TRH-R with antisense oligonucleotide that was complementary to residues 10-45 of the N-terminal portion of the receptor. At 24 h after injection of 0.1 ng of TRH-R mRNA/oocyte, oocytes were injected with 10 ng of the oligonucleotide/cell. Response amplitudes were measured 2.5-3 h later. The oocytes exhibited 63±6% and 60±12% of control response on the animal and vegetal hemispheres, respectively (\( n_{\text{animal}} = n_{\text{vegetal}} = 30, N = 4 \); Figure 4). In contrast with the results obtained with CHX, this experiment demonstrated that the half-life of TRH-R was similar on both hemispheres and was approx. 4 h or less. This provided further support for the hypothesis that other protein(s), different from the receptor, may determine the ratio of functional expression.

Effect of the CHX on the functional expression of the truncated TRH-R mutant

Although the overall amino acid residue identity of the two rodent TRH-Rs is very high, they exhibited different characteristics (A/V ratio of response, effect of CHX treatment). The functional expression of a receptor may reflect additional modulatory factors, such as phosphorylations or its expression density. We therefore studied a mouse TRH-R mutant that was truncated at the C-terminal portion of the receptor molecule (stop codon in position 335). In our previous paper [13], we demonstrated that the deletion of this region, possibly due to the loss of four consensus phosphorylation sequences, increased the proportion of TRH-Rs in the plasma membrane in transfected mammalian cells. This was confirmed in oocytes, too (N. Matus-Leibovitch, D. Nussenzveig, M. C. Gershengorn and Y. Oron, unpublished work).

Oocytes were injected with 5 ng/cell of either wild-type or mutant TRH-R mRNA transcribed in vitro and hemispheric responses were determined. The mean amplitude measured on the animal hemisphere of oocytes expressing the mutant TRH-R was 456±80 nA, and on the vegetal hemisphere 130±20 nA (\( n = 69 \) for each hemisphere, \( N = 12 \)). The decreased responses were not accompanied by any change in the A/V ratio (3.7±0.3, versus 3.9±0.5 for the wild-type). The decreased response was probably due to desensitization (N. Matus-Leibovitch, D. Nussenzveig, M. C. Gershengorn and Y. Oron, unpublished work).

In order to assess the role of the C-terminal domain in the phenomena that follow the inhibition of protein synthesis, oocytes expressing the mutant receptor were incubated in CHX for 2-4 h. Electrophysiological measurements after this period of incubation revealed potentiation of the response at both hemispheres, to 221±20% and 268±86% of wild-type response (2 h, for animal and vegetal hemispheres respectively) and 355±98% and 227±97% (4 h, for animal and vegetal hemisphere respectively). Hence, inhibition of total protein synthesis resulted in a major increase (361±102%) in the whole-cell response to TRH. This was markedly different from the results of the CHX effect on responses in oocytes expressing the wild-type TRH-R (48.3±13% of untreated controls at 4 h of incubation with CHX). To investigate this increase, radioligand-binding studies were performed in the presence or absence of CHX. As expected, oocytes exhibited decreased receptor number after incubation with CHX for 3 h (1.1±0.5 fmol/cell, versus 3.0±0.8 fmol/cell for control). These results suggest that CHX may inhibit the synthesis of a rapidly degraded protein, which regulates the response of the receptor. Considering the low amplitudes of responses, we could not obtain a favourable oligonucleotide/TRH-R RNA ratio that will allow assessment of the true half-life of the mutant receptor.

DISCUSSION

In eukaryotic cells, there are several cellular systems that display polar distribution of membrane proteins [19-21]. The most widely investigated are the Madin–Darby canine kidney cells and hepatocytes [22,23]. Asymmetric distribution can be a result of different cellular processes, mainly vectorial transport [24], transcytosis and insertion [25], or maintenance [26]. Most of the reports deal with the distribution of viral proteins. Much less is known about the sorting mechanisms of membrane receptors for hormones and neurotransmitters. We have recently suggested that Xenopus oocytes may serve as a convenient model system to study the asymmetric functional expression of the G-protein-coupled receptors [6]. Oocytes allow hemispheric determination of both the first component of the signal-transduction pathway, i.e. the distribution of membrane receptors, and the final physiological output, i.e. the chloride response. Since the polarity of the response does not necessarily have to reflect asymmetric receptor distribution, this type of assay can also reveal the
distribution of more distal components of the signal-transduction pathway.

It was suggested that the distribution of Ca\textsuperscript{2+}-activated chloride channels favours the animal hemisphere [27]. Our own data, however, suggest similar channel densities on either hemisphere, but different kinetic characteristics of the response [28]. Although the hemispheric distribution of chloride channels appears to be controversial, their polarity cannot account for the different hemispheric response ratios observed for a variety of expressed receptors.

We chose to characterize the hemispheric polarization of the response to TRH mediated by three different TRH-Rs. We have previously shown that the functional expression of rat TRH-R in oocytes exhibited marked preference for the animal hemisphere [7]. Here we confirmed these results for the recently cloned mouse TRH-R. The much lower activity ratio (A/V) of the mouse TRH-R (3.9 versus 9.5) may reflect differences between the two receptor structures. Recently, the rat TRH-R has been cloned and sequenced [8,9]. Although there is 96% homology to the mouse TRH-R [10], it contains additional unique sequence in the putative cytoplasmic region of the C-terminus of the receptor [8,9]. Another possibility is that the higher hemispheric response ratio of the rat TRH-R reflects the contribution of additional pituitary proteins, co-expressed in oocytes injected with total GH\textsubscript{3}-cell RNA. Since cloned rat TRH-R was not available, we could not exclude this possibility. Alternatively, an interaction between the expressed TRH-Rs and an endogenous regulatory protein could generate the observed polarity of responses.

In contrast with the report by Peter et al. [29], who showed that the asymmetry reflected the kinetics of ion-channels expression, we did not find differences in the A/V ratio when activities were measured at different times after injection of RNA. Moreover, the A/V ratio remained unchanged regardless of the amount of RNA injected. We can conclude that TRH-Rs reach equilibrium quite early after the injection of RNA.

Our previous studies on muscarinic acetylcholine receptors [5,6] indicated that the ratio of activity reflected receptor distribution. Similar results were obtained for the Torpedo nicotinic receptor [30]. Hence, it was surprising to find that the physical distribution of the mouse TRH-R (A/V ratio 0.94) markedly differed from the functional distribution (A/V ratio 3.9). A possible explanation for this phenomenon may be that the ratio of activity reflects asymmetric distribution of a distal, limiting, component of the signal-transduction pathway, such as G-protein(s), inositol-trisphosphate-sensitive Ca\textsuperscript{2+} store [28], regulatory protein kinase(s) etc. In this context, the lack of a linear correlation between the amount of RNA injected and the response amplitude [15] further supports the existence of a post-receptor limiting step.

We have previously shown that the turnover of muscarinic receptors in oocytes was quite rapid [6]. Using the same approach, we specifically inhibited receptor synthesis with an antisense oligonucleotide, or total protein synthesis with CHX. In contrast with the muscarinic receptors, which exhibited a parallel rapid decrease in receptor number and activity at both hemispheres when the two methods were employed, the functional expression of the TRH-Rs was differentially affected. Although a specific inhibition of receptor synthesis led to an equivalent loss of response amplitudes at both hemispheres, yielding a n of approx. 4 h, inhibition of total protein synthesis with CHX produced different results at the two hemispheres. Oocytes expressing rat TRH-R exhibited a rapid decrease in activity at the animal hemisphere (t\textsubscript{1/2} = 2 h), with no decrease on the vegetal hemisphere. In oocytes expressing mouse TRH-Rs, the decrease in the activity at the animal hemisphere was accompanied by a more than 2-fold increase in the response at the vegetal hemisphere. It is possible that protein-synthesis inhibition leads to a rapid degradation of an additional protein in the signal-transduction cascade, which normally limits the activity of the TRH-R.

The C-terminus of the mouse TRH-R contains four potential regulatory phosphorylation sites [10]. Moreover, the major difference between the mouse and the rat TRH-Rs is in the C-terminal domain [8,9]. This led us to hypothesize that this domain may be involved in receptor activity regulation. We used a truncated TRH-R in order to assess the role of the C-terminal region in the determination of hemispheric distribution. The A/V ratio of the truncated receptor was similar to that described for the wild-type; hence the sorting or insertion of the mouse TRH-R is not affected by changes in the cytoplasmic domain. Upon total protein-synthesis inhibition, there was an increase in the response at the vegetal hemisphere (in agreement with the results observed for the wild-type receptor), but also an increase in the response on the animal hemisphere, despite a decrease in receptor number.

In order to integrate the results presented here into a generalized scheme, we propose that the limiting factor in the signal-transduction pathway is the amount of TRH-R. Our data on receptor levels are the apparent values derived from binding experiments at saturating ligand concentrations, which may be influenced by the amounts of guanine-nucleotide-ligated G-protein(s). However, increasing the amount of injected RNA causes an increase in receptor binding and in response amplitude [18,31]. Similarly, co-expression of G\textsubscript{a\textsubscript{16}} causes an increase in response amplitude only when large amounts of TRH-R are expressed [32]. These data confirm that in oocytes, as in many other cell types, the amount of G-protein(s) is not limiting. This assumption is compatible with the data obtained previously for the native muscarinic receptors [6] and is supported by the symmetrical decrease in function following specific inhibition of the synthesis of the receptor alone. The asymmetry in hemispheric responses has to be attributed to asymmetric distribution of another component of the signal-transduction cascade, e.g. a kinase that phosphorylates the receptor and decreases its activity. This putative kinase would have to be more active on the vegetal hemisphere, and thus create the functional expression ratio of 9.5 in oocytes injected with GH\textsubscript{3}-cell RNA. The cloned mouse wild-type and mutated TRH-Rs may be less good substrates for this putative kinase. This results in a modified A/V activity ratio of 3.7–3.9. Indeed, the ratio changes mainly due to the increase in the activity on the vegetal hemisphere.

Inhibition of total protein synthesis would have two opposing effects on receptor-mediated activity. On the one hand, a decrease in receptor number would inhibit the response, whereas a decrease in the putative kinase would potentiate it, on the other. For the rat TRH-Rs, this balance of opposing effects produces a rapid decrease on the animal hemisphere and virtually no change on the vegetal side. For the mouse TRH-Rs, the decrease in receptor number on the vegetal hemisphere is masked by a more robust decrease in the negative modulation. For the mutant receptor, expressed to a density that is 6-fold higher than the wild-type, even a major decrease in receptor number might leave enough residual receptors to activate fully the subsequent transduction components. This corresponds well to the apparent non-linearity between receptor number and response amplitude. Hence, only the decrease in negative modulation by the kinase prevails, leading to an increase of responses on both hemispheres.

In this work we present evidence pointing to distal components of the signal transduction as determinants of the hemispheric distribution of response. This finding is different from our results for the native muscarinic receptors [5,6], where receptor dis-
tribution correlated with response polarity. Eukaryotic cells may utilize different mechanisms to produce asymmetric profiles of physiological responses. In the oocyte, the different mechanisms, including the contribution of cytoskeletal elements [33], appear to be receptor-specific. The emergence of appropriate experimental tools, such as specific antibodies for various components of the signal-transduction cascade, will greatly help in the future investigation of functional polarity resulting from the activation of a complex pathway. Although we cannot directly extrapolate the validity of our findings to mammalian cells, the evidence that has accumulated so far indicates that mammalian proteins expressed in the *Xenopus* system appear to reflect faithfully their properties in the cells and tissues of origin.

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