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Could Low-Molecular-Weight TSH Receptor Antagonists Become a Therapy for Graves' Orbitopathy?

van Zeijl CJ, van Koppen CJ, Surovtseva OV, de Gooyer ME, Plate R, Conti P, Karstens WJ, Timmers M, Saeed P, Wiersinga WM, Miltenburg AM, Fliers E, Boelen A. Complete inhibition of rhTSH-, Graves' disease IgG-, and M22-induced cAMP production in differentiated orbital fibroblasts by a low-molecular-weight TSHR antagonist. J Clin Endocrinl Metab 2012, March 14, 2012 [Epub ahead of print]. doi: 10.1210/jc.2011-2931.

SUMMARY

Background

Although some G-protein–coupled receptors are activated when their ligands bind at sites in crevices within their seven membrane-spanning domains, the TSH receptor is activated when TSH or TSH receptor (TSHR)–stimulating immunoglobulins bind to its extracellular domain. Small-molecule agonists/antagonists for several glycoprotein hormone receptors, including the TSHR, have now been reported that appear to act within the receptors' membrane-spanning domains and do not compete for TSH binding (1, 2). These compounds are proving useful for understanding how hormones act, but they also present the exciting prospect of a new approach for treating some endocrine diseases.

The small polycyclic compound Org-274179-0 was recently shown to act in FRTL-5 thyrocytes as a potent antagonist of the cyclic AMP responses to TSH and also to M22, a TSHR-binding human monoclonal antibody (1). Furthermore, in CHO cells expressing the human TSHR, Org-274179-0 blocked the cyclic AMP and also the phospholipase C responses to TSH and M22. It also blocked these responses in CHO cells expressing constitutively active human TSHR mutants (2). M22 did not stimulate CHO cells expressing LH or FSH receptors. In the current article, Org-274179-0 was tested for its effects on the release of cyclic AMP from adipocytes that were prepared from fibroblasts obtained from retroorbital fat removed from patients undergoing decompressive surgery for Graves' orbitopathy.

Methods

Cultures of Graves' orbital fibroblasts obtained from eight patients with active disease who had recently received large doses of corticosteroids and from six patients with inactive disease that had required no treatment for the previous 6 months were treated with factors that promote the fibroblasts' differentiation into adipocytes that express the TSHR. After three to seven passages, the adipocytes were grown to near-confluence, then preincubated with the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine for 30 minutes, then with Org-274179-0 (presumably dissolved in 6% DMSO) for 5 minutes, and finally they were exposed for 6 hours to: (1) recombinant human TSH; (2) IgG from a patient with high TSHR-antibody–binding activity or IgG from a control patient; or (3) M22 or to a control monoclonal IgG. Data on the mean (±SEM) levels of cyclic AMP in the culture medium were provided, but the number of wells of adipocytes from each patient assayed with each agent was not clear. Cyclic AMP levels were measured in the culture medium (which may not parallel cyclic AMP levels in intracellular compartments).

Results

Adipocytes from five patients were incubated with human TSH (hTSH; 10 mU/ml) plus increasing concentrations of Org-274179-0: the cyclic AMP response was reduced by 50% at about 2.5 nM, and complete inhibition was obtained at about 1 μM. Org-274179-0 had no effect on cyclic AMP released from cells incubated with vehicle alone. Adipocytes from three patients were incubated with IgG (1 mg/ml) from a

continued on next page

patient with high TSHR-antibody–binding activity. Org-274179-0 reduced the cyclic AMP response by 50% at about 1 nM, but even at 1 μM, cyclic AMP levels remained somewhat elevated, probably reflecting the fact that the normal control IgG also increased cyclic AMP levels, both in the absence of inhibitor and also at the highest two concentrations of inhibitor. Adipocytes from a single patient were incubated with monoclonal M22 (500 ng/ml). Org-274179-0 reduced the cyclic AMP response by 50% at about 0.5 nM, but even at 1 μM, the cyclic AMP level remained somewhat elevated, probably reflecting the fact that the normal control human monoclonal antibody also increased cyclic AMP levels, both in the absence of inhibitor and at the highest concentrations of inhibitor. The inhibitor did not block the ability of forskolin to directly activate adenylate cyclase. It is not clear why samples from more patients were tested with hTSH than with M22 and IgG, nor is it clear which cultures came from patients with active versus inactive Graves' orbitopathy.

Conclusions

Nanomolar concentrations of Org-274179-0 inhibit the ability of TSHR to generate cyclic AMP in response to TSH, M22, or thyroid-stimulating IgG when added to adipocytes differentiated from Graves' retroorbital fibroblasts.

ANALYSIS AND COMMENTARY

Administration of small-molecule antagonists could become a clinically important form of therapy for Graves' disease if they are specific for the human TSHR in vivo and if they do not have major side effects at doses that are clinically effective. Blocking the actions of thyroid-stimulating immunoglobulins on orbital fibroblasts could also have a role in treating Graves' orbitopathy. However, orbitopathy does not develop in patients whose serum TSH levels are chronically elevated unless they also have Graves' disease, so it is clear that factors in addition to the prolonged stimulation of TSHR by immunoglobulins are at work. In this regard, the higher levels of hyaluronic acid (HA) that are known to be present in Graves' orbital fat and connective tissue are of interest. The authors previously reported that Graves' IgGs stimulate HA release from a much higher percentage of adipocytes from patients with Graves' disease than the percentage of these adipocytes that respond to hTSH (3). A recent report indicates that when Graves' adipocytes are exposed to M22 for 48 hours—in the absence of a phosphodiesterase inhibitor—the release of HA is mediated by phosphoinositide-3-kinase rather than by cyclic AMP–dependent protein kinase activity (4). It will therefore be important to learn how Org-274179-0 affects the HA and phospholipase C responses in adipocytes incubated under the conditions described in the current article. In passing, we note that incubation with Org-274179-0 (1 μM for four hours) slightly reduced the release of labeled TSH previously bound to membranes from TSHR-expressing CHO cells (2), which might indicate that high levels of the agent can affect the association of TSHR with membrane proteins, or the formation of TSHR multimers. Data in the current article indicated that a high concentration of Org-274179-0 increased cyclic AMP production in response to the single control IgG and monoclonal antibody used, yet it did not alter the response to M22 (or TSH) in the absence of the control IgG and monoclonal antibody. Perhaps this simply reflects a fluke in both of the controls, but it would be interesting to know whether similar effects are produced by other control IgGs, such as were used in the authors' previous study (3). Furthermore, high levels of Org-274179-0 actually increased expression of a cyclic AMP reporter in CHO cells that stably express the LH receptor as well (2). It will be fascinating to learn the results of studies of these agents, in both in vivo and additional in vitro models.

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