The ghrelin receptor is known from in vitro studies to signal in the absence of the hormone ghrelin at almost 50% of its maximal capacity. But, as for many other 7-transmembrane receptors, the in vivo importance of this ligand-independent signaling has remained unclear. In this issue of the JCI, Pantel et al. find that a natural mutation in the ghrelin receptor, Ala204Glu, which is associated with a selective loss of constitutive activity without affecting ghrelin affinity, potency, or efficacy, segregates in 2 families with the development of short stature (see the related article beginning on page 760). By combination of the observations from this study with those related to the phenotype of subjects carrying another natural ghrelin receptor mutation, Phe279Leu, having identical molecular-pharmacological properties, it is proposed that selective lack of ghrelin receptor constitutive signaling leads to a syndrome characterized not only by short stature, but also by obesity that apparently develops during puberty.

Seven-transmembrane segment (7TM) or G protein–coupled receptors can signal without any agonist present (1). This phenomenon is a simple reflection of the molecular activation mechanism where the receptor is in an equilibrium between inactive and active conformations and can relatively easily access the active conformation without the presence of an agonist (2). The degree of ligand-independent or constitutive signaling varies among receptors but in most cases only represents a small fraction of the maximal signaling capacity and is consequently generally ignored. However, among receptors involved in the control of appetite and energy expenditure, such constitutive activity appears to have important functional consequences. The cannabinoid type 1 (CB1) receptor, which is the target for the novel antiobesity drug rimonabant — acting as a combined antagonist and inverse agonist — and the ghrelin receptor both signal with around 50% activity in the absence of ligand (3–5). Nevertheless, the physiological importance of such constitutive signaling has been very hard to establish in vivo, mainly because of the lack of appropriate pharmacological tools. Inverse agonists, i.e., ligands that inhibit the constitutive receptor signaling, in most cases also act as antagonists, i.e., they also block the action of the endogenous agonist. Thus, it is very hard in the in vivo setting to differentiate between an effect on constitutive receptor signaling and a blocking effect on receptor access to an endogenous ligand. In this issue of

Nonstandard abbreviations used: CB1, cannabinoid type 1; GH, growth hormone; 7TM, 7-transmembrane segment.

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comments
Expenditure (7–9). Besides being a trans
innovated in growth hormone (GH) release
receptor in the development of normal
and control of food intake and energy

Ghrelin is a hormone and neuropeptide
signaling of the ghrelin receptor
Agonist-dependent and constitutive

— that are significantly more potent as inverse agonists than as antagonists (4).

However, compounds have been described — for example, in the ghrelin receptor system
7TM receptors in general, antagonists generally also act as inverse agonists, a phenomenon
both the agonist-induced signaling and the constitutive signaling. It should be noted that for
the effect of a combined antagonist and inverse agonist (dotted curve in blue), which blocks
of an inverse agonist, which inhibits constitutive signaling; the effect of a pure antagonist,
important in the fasting state and pre-meal situation (10). (Figure 1)

Variations in plasma ghrelin concentration (conc.) depicted as

-3–36, etc. (10).

The other novel, uncharacterized ghrelin receptor variant reported in the German
study — a Phe279Leu mutation — was identified in a child with short stature

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Figure 1
Constitutive and hormone-mediated signaling of the ghrelin receptor in relation to the dynamic
pattern of ghrelin secretion. (A) Variations in plasma ghrelin concentration (conc.) depicted as
a dynamic range in relation to a meal. Note the pre-meal surge in ghrelin secretion followed
by inhibition related to the presence of food in the upper gastrointestinal tract. Illustration
based on work of Cummings and coworkers (12, 13). (B) Illustration of the almost 50% con-
stitutive, ligand-independent signaling of the ghrelin receptor as measured, for example, in
inositol phosphate accumulation assays and the agonist response mediated by the ghrelin hor-
mones (4). To the upper left is indicated that the high constitutive ghrelin receptor signaling is
expected to be dominant in the inter-meal period, while the ghrelin-mediated signaling is most
important in the fasting state and pre-meal situation (10). (C) Diagram of the theoretical effect
of an inverse agonist, which inhibits constitutive signaling; the effect of a pure antagonist,
which blocks the agonist-mediated signaling without affecting the constitutive signaling; and
the effect of a combined antagonist and inverse agonist (dotted curve in blue), which blocks
both the agonist-induced signaling and the constitutive signaling. It should be noted that for
7TM receptors in general, antagonists generally also act as inverse agonists, a phenomenon
whose salience depends on the receptor displaying a reasonable degree of constitutive signal-
ing. However, compounds have been described — for example, in the ghrelin receptor system
— that are significantly more potent as inverse agonists than as antagonists (4).

Selective loss of ghrelin receptor
constitutive activity associated
with short stature

In a systematic search among subjects
with short stature, Pantel and coworkers
found an identical missense mutation
— Ala204Glu — in the ghrelin receptor in
2 unrelated Moroccan subjects, one with
idiopathic short stature and another with
isolated GH deficiency (6). Surprisingly,
ghrelin bound with normal (high) affinity
and stimulated signal transduction with normal potency and normal apparent
efficacy at the Ala204Glu receptor variant. Importantly, however, the character-
istic high constitutive signaling activity of the receptor was eliminated by
the mutation, and the receptor numbers at the cell surface were somewhat lower
compared with the wild-type (6). In the 2 families, the mutation lacking constit-
tive activity segregated with short stature
in a dominant manner with a penetrance
of approximately 70% (6). Interestingly,
one of the probands, who developed
short stature during the first years of her
life and subsequently developed obesity
around puberty, was homozygotic for a consanguineous
relationship between her heterozygotic
parents, both of whom were not only very
short, but also obese. Nevertheless, Pantel
and coworkers were — based on the lim-
ited patient material — not able to associ-
ate the obese phenotype directly to the
receptor mutation (6).

The loss of ghrelin receptor
constitutive activity may be part
of a broader syndrome

The Ala204Glu allele of the ghrelin recep-
tor had already been described previously
in a German subject by Hinney, Hebe-
brand, and coworkers, but without any
functional characterization (15). In a
study on the occurrence of ghrelin recept-
or variants in populations of obese and
underweight subjects, as well as subjects
with short stature, they identified 2 novel
missense variants of the ghrelin receptor
of which the Ala204Glu variant was found
to be heterozygous in a very obese child.
This observation adds further support to
the notion that obesity could be part of
the phenotype of carriers of the Ala204Glu
variant of the ghrelin receptor.

The other novel, uncharacterized ghrelin
receptor variant reported in the German
study — a Phe279Leu mutation — was
identified in a child with short stature

the JCI, Pantel and coworkers provide very
strong evidence for a physiological role
of the constitutive activity of the ghrelin
receptor in the development of normal
height in humans (6).

Agonist-dependent and constitutive
signaling of the ghrelin receptor

Ghrelin is a hormone and neuropeptide
involved in growth hormone (GH) release
and control of food intake and energy
expenditure (7–9). Besides being a trans-
mitter in discrete neuronal networks in,
for example, the hypothalamus, ghrelin
functions as a hormonal “hunger signal”
from enteroeendocrine cells in the stom-
ach to various target cells located in affer-
ent vagal neurons, the brain stem, and
the arcuate nucleus of the hypothalamus
(10, 11). The dynamic pattern of ghre-
lin concentrations in plasma is opposite
that of gastrointestinal tract hormones
in general: a surge before the first meal
of the day is followed by a prolonged
nadir caused by the inhibitory effect of
food being present in the upper gastroin-
testinal tract (Figure 1) (12, 13). Thus, it
has been suggested that, between meals,
constitutive GH secretagogue receptor
activity could play an important role in
modulating the orexigenic signals in the
regulatory pathways that are integrat-
ing anorexigenic signals such as leptin,
insulin, and peptide YY13–36, etc. (10).
The amount of constitutive signaling is
directly proportional to the expression
level of the receptor, and the expression
of the ghrelin receptor is — like that of
the CB1 receptor — highly regulated, for
example by fasting (10, 14).
of severity comparable to that seen in the French-Moroccan study (15). However, during a systematic structure-function analysis of residues located in the main ligand-binding pocket of the ghrelin receptor, Phe279 (PheVI:16) was recently identified as being especially important for the constitutive signaling activity of the receptor (16). That is, Phe279 is part of an unusual cluster of aromatic residues located on the inner faces of transmembrane segments VI and VII, which are essential in holding the ghrelin receptor in the active conformation. Removal of the aromaticity at position 279 (VI:16) selectively eliminates the constitutive activity of the receptor (16). Thus, the German child with short stature carries a different ghrelin receptor mutant — Phe279Leu — which, however, has a molecular-pharmacological profile similar to that of the Ala204Glu mutant found in the French-Moroccan subjects with a similar degree of short stature (Figure 2).

Regarding the question of whether obesity is part of the phenotype associated with a ghrelin receptor lacking constitutive activity, it is particularly interesting that the mother of the German proband, who also was a carrier of the Phe279Leu mutant receptor, was not only very short (157 cm) but also very obese (BMI = 34.6) (ref. 15, and personal communication with A. Hinney and J. Hebebrand, University of Duisburg-Essen, Essen, Germany). As discussed above, obesity was frequently observed among members of the Moroccan families, especially in adult carriers (6). Interestingly, the Ala204Glu homozygotic proband was somewhat overweight as a child but became severely obese only around puberty (see Figure 3 in ref. 6). Thus, based on the 2 different but functionally similar mutations, and the 4 families in which these are found (6, 15), we suggest that selective loss of ghrelin receptor constitutive activity causes a syndrome of short stature and obesity, of which the obesity appears to develop around puberty.

How can loss of ghrelin receptor function lead to obesity?
It can rather easily be rationalized that loss-of-function mutations in the ghrelin

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**Figure 2**
Natural mutations selectively eliminating the constitutive signaling of the ghrelin receptor that are associated with short stature and possible obesity. (A) Serpentine model of the human ghrelin receptor in which residues that are identical (white on black) or structurally conserved (white on gray) between the ghrelin receptor and its closest homologue, the motilin receptor, are indicated. Note that a Cys residue in the middle of extracellular loop 2 forms a disulfide bridge with a Cys residue at the extracellular end of transmembrane segment III (TM-III), which is a highly conserved structural feature of 7TM receptors. The location of the Ala204Glu mutation in the part of extracellular loop 2 connecting the extracellular end of TM-III with TM-V, which Pantel et al. describe in this issue of the JCI (6), is highlighted with a red circle (6). As indicated in the red box at the top left, this mutation selectively eliminates constitutive signaling by the ghrelin receptor and is associated with short stature (6). This mutation has previously been identified in a very obese child by Wang et al. (15). Also highlighted with a red circle is the location of the Phe279Leu mutation in TM-VI. The constitutive activity of the ghrelin receptor is highly dependent on the presence of an aromatic residue at position VI:16 (16), and Wang et al. found the Phe279Leu mutation in a child with short stature and in his mother, who was also of short stature (157 cm) and who was very obese (BMI = 34.6) (15). (B) Schematic diagram of the effects of these natural mutations in eliminating the constitutive signaling of the ghrelin receptor (red arrow to the left) without affecting the potency or efficacy of the natural ligand, ghrelin (dotted vertical line corresponding to the EC$_{50}$).
receptor would lead to the development of short stature through reduced activity at the hypothalamic-pituitary-GH axis — although, in the present case, the precise mechanism of this is not entirely clear (6). In contrast, with respect to the control of appetite and energy expenditure, it would be expected that loss-of-function mutations in the ghrelin receptor would lead to a lean and not an obese phenotype, because ghrelin is a potent hunger signal. In accordance with this inference, recent studies in mice have shown that knockout of either the ghrelin hormone or the ghrelin receptor protects against obesity induced by a high-fat diet (11, 17, 18).

Frequently, knockout of molecular elements in complex, redundant physiological systems will lead to compensatory changes in related pathways as a compensation for the lost function. Normally, such compensation will result in a diminished or total lack of the expected phenotype, but not an “overshoot,” i.e., the development of the opposite phenotype. In the case of subjects expressing a ghrelin receptor that lacks constitutive signaling, there is, however, a special possibility regarding such compensation: the expression of the ghrelin receptor itself could become upregulated if it is normally controlled by an intracellular feedback mechanism requiring constitutive signaling. Because the mutant ghrelin receptor retains normal affinity and response to ghrelin, such an upregulation of receptor molecules could result in enhanced net ghrelin response, and, for example, a corresponding increase in hunger. These are obviously speculations that need to be tested experimentally. Nevertheless, it is interesting that loss of ghrelin receptor constitutive activity leads to the expected phenotype with respect to growth — i.e., short stature — but apparently leads to the opposite of the expected phenotype with respect to appetite and energy expenditure — i.e., obesity.

We have previously suggested that an inverse agonist, which eliminates the constitutive activity of the ghrelin receptor, would be particularly interesting as an antiobesity agent (10). However, apparent predisposition to obesity among subjects expressing a ghrelin receptor lacking constitutive activity could indicate that an inverse agonist approach in itself may not be optimal. But it is difficult to draw this conclusion, because the obesity could be a consequence of the persistent lack of constitutive activity during a particular period in the development of the carrier. Moreover, it should be noted that, in general, most inverse agonists also act as antagonists, i.e., they block the hormone-mediated response. Thus, a combined antagonist/inverse agonist would perhaps be the preferred antiobesity agent in the ghrelin system.

Cross-fertilization between genetic studies and systematic structure-function analysis

The significance of Phe279 in ghrelin receptor function was initially identified through a systematic biochemical analysis (Figure 2), which could be performed relatively easily based on knowledge about the general structure and function of 7TM receptors (16). Thus, in this case, the basic, molecular-pharmacological analysis could help to elucidate the clinical phenotype of the patient. Conversely, the Ala204Glu mutation, which was found through a clinical genetic approach by screening of patients with a particular phenotype, has provided significant structure-function information about residues in extracellular loop 2 of the receptor (Figure 2). Importantly, this particular epitope would have been very difficult to identify through classical structure-functional analysis, because it is located outside the region that has been most intensively investigated in 7TM receptors. It is likely that a broad analysis of subjects with short stature — and especially those who develop obesity around puberty — will identify more mutations in the ghrelin receptor that, like Ala204Glu and Phe279Leu, selectively eliminate its constitutive signaling. In fact, a number of such mutations with the expected molecular-pharmacological phenotype have been identified through site-directed mutagenesis of the ghrelin receptor. It will be interesting to see whether some of these substitutions are in fact found as natural mutations among short, obese subjects.

Constitutive 7TM receptor signaling is physiologically important

Recently, Vaisse and coworkers showed that substitutions at the hottest spot for natural missense mutations in the melanocortin-4 receptor — Arg18 — as well as a couple of other sites in the N-terminal, extracellular segment of the receptor, surprisingly have no effect on α-melanocyte-stimulating hormone (α-MSH) binding and no effect on α-MSH potency and efficacy but, instead, selectively eliminate the constitutive activity of the receptor (19). The obese phenotype of carriers of such mutations indicates that the constitutive activity of the melanocortin-4 receptor is important in vivo, conceivably in providing a tonic hypothalamic satiety signal. Similarly, the study by Pantel et al. in this issue of the JCI (6) demonstrates that the constitutive activity of the ghrelin receptor also is important in vivo, in this case in providing a tonic signal required for the development of normal height, conceivably through an effect on the GH axis (6).

These are both landmark studies, as they for the first time clearly demonstrate that constitutive signaling of 7TM receptors is not just an in vitro artifact or epiphenomenon but rather serves important functions of these receptors in vivo. Consequently, inverse agonists should be taken seriously, not only as pharmacological tools, but as important drug candidates.

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