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Chemistry and Biology of Orexin Signaling

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Abstract

The orexins are neurohormones that, in concert with their cognate receptors, regulate a number of important physiological processes, including feeding, sleep, reward seeking and energy homeostasis. The orexin receptors have recently emerged as important drug targets. This review provides an overview of recent development in deciphering the biology of orexin signaling as well as efforts to manipulate orexin signaling pharmacologically.

The orexins (also called hypocretins) are neuropeptides that were first discovered in 1998 by two independent research groups^{1, 2}. Orexins A (33 amino acids) and B (28 amino acids) are derived from a single pre-pro-orexin polypeptide³. They mediate their actions via interactions with two closely related GPCRs called Orexin Receptor 1 and 2 (OxR1 and OxR2). OxR1 binds Orexin A with about 100-fold higher affinity than Orexin B whereas OxR2 binds both peptides with approximately the same affinity. The orexins are produced by specialized neurons in the hypothalamus, which project to many different regions of the brain^{4, 5}.

As will be reviewed briefly below, physiological studies over the last ten years have implicated orexin signaling as playing a central role in a variety of important biological processes, including feeding, energy homeostasis, sleep/wake cycles, addiction and reward seeking, amongst others⁶. There is clear evidence for defects in orexin signaling being involved in diet-induced obesity and diabetes^{7, 8}, narcolepsy^{9, 10}, panic anxiety disorder¹¹, drug addiction¹² and Alzheimer's Disease¹³. Thus, there is considerable interest in the drug industry in the development of potent compounds for the manipulation of orexin signaling. Recent progress in understanding and manipulating orexin signaling is reviewed here.

Orexin signaling in coordinating sleep and feeding

The best understood role of orexin signaling is the coordination of feeding and sleep. The level of orexin production in the hypothalamus is inversely correlated with blood glucose levels¹⁴. Orexin signaling, at least in the short term, induces a sensation of hunger and stimulates feeding behavior, probably by activating neurons in the brain that produce neuropeptide Y and functionally related hormones. In addition, orexin signaling is a key component of inducing a state of wakefulness in mammals^{9, 15}. In other words, orexin signaling mandates that when animals are hungry, they are also awake, but when satiated, they can be sleepy. This makes good physiological sense in that animals will want to be foraging for food, not sleeping, when they are hungry.

Genetic ablation of the orexin gene or the receptor in rodents results in a phenotype remarkably similar to human narcolepsy with cataplexy, in which the animal suffers inappropriate intrusion of sleep into its normal wake time, including collapsing at odd

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moments⁹. The same is true of dogs with an inactivating mutation in the OxR2-expressing gene¹⁰. Most, though not all, human narcoleptics, although they do not carry mutations in the genes that encode orexin and its receptors, nonetheless lack detectable orexin. This is thought to be due to an autoimmune attack on the orexin-producing neurons in the brain^{16, 17}. However, this autoimmune model for human narcolepsy remains to be proven and nothing is known about the nature of the putative autoimmune reaction.

The known biology of orexin signaling thus predicts that brain-penetrant OxR2 agonists should stimulate wakefulness and might be useful for the treatment of narcolepsy. Yanagisawa and colleagues have published proof of concept experiments that support this idea¹⁸. They showed that direct intracranial injection of orexin peptide into the brains of orexin-deficient rodents resulted in a heightened state of wakefulness. Conversely, orexin receptor antagonists might be used to treat insomnia¹⁹, though the possible induction of cataplexy, seen in animals and humans with a chronic orexin deficiency, is a concern (vide infra).

Orexin signaling in addictive and other pathological behaviors

The insula is a brain region known to be involved in the development of urges and cravings and generally is involved in reward behavior²⁰. Damage to this region of the brain results in a striking drop in the motivation of smokers to continue their habit²¹. In contrast, abstinence in cigarette smokers is known to activate the insula²². The same region of the brain appears to be involved in reward-seeking behavior involved in addition to morphine, cocaine and alcohol. Orexin-producing neurons densely innervate this region of the brain and orexin signaling plays a key role in these behaviors^{23–25}. For example, administration of the OxR1 selective antagonist SB-334867 (see Figure 5) significantly decreased the drive of rodents to self-inject themselves with nicotine¹². Similar results have been obtained for other addictive compounds. Interestingly, in the nicotine study, pharmacological blockade of OxR1 did not substantially suppress food intake, which, along with wakefulness, is thought to be mediated predominantly by OxR2. Thus, the pre-clinical data in rodents strongly suggests that OxR1 selective antagonists might be interesting anti-addictive drugs.

Orexin signaling has also recently been shown to be involved in panic attacks and anxiety¹¹. Using a rat model in which panic attacks are induced by treatment of the animal chronically with a GABA synthesis inhibitor followed by acute treatment with sodium lactate, it was demonstrated that SB-334867-mediated blockade of OxR1 or siRNA-mediated silencing of orexin production strongly suppresses panic attacks in this model. Moreover, human subjects subject to panic attacks have elevated levels of orexin in their cerebrospinal fluid (CSF)¹¹, consistent with a role of orexin signaling in human anxiety disorders.

Very recently a report appeared linking the sleep/wake cycle with Alzheimer's Disease¹³. Specifically, it was shown that the accumulation of amyloid beta, a hallmark of the disease, is correlated with wakefulness. For example, chronic sleep deprivation increased amyloid beta levels in the CSF of the animal. Interestingly, administration of the orexin receptor antagonist SB-334867 decreased the levels of this neurotoxic intermediate. Thus, it is possible that an appropriate treatment regimen with OxR antagonists could be a viable treatment to slow the development of Alzheimer's Disease, if it were diagnosed early enough.

Orexin signaling in energy homeostasis, diet-induced obesity and diabetes

There is circumstantial evidence that orexin signaling is important in energy homeostasis. For example, narcoleptic humans deficient in orexin exhibit a higher body mass index than narcoleptic individuals with normal levels of orexin²⁶. Genetic ablation of orexin neurons in

mice results in obese animals²⁷ and these mice also develop age-related insulin resistance⁷. These results appear to be counterintuitive in that pharmacological induction of orexin signaling acutely promotes feeding behavior¹. This suggests that the short term and longer-term effects of orexin signaling on energy homeostasis are different.

Recently, this area of orexin biology has been clarified by a landmark study that demonstrated conclusively that orexin signaling strongly opposes diet-induced obesity and the subsequent development of insulin resistance in rodents⁸. It was shown that chronic overexpression of the orexin gene or pharmacological induction of the orexin receptor almost completely blocked the development of obesity and insulin resistance in mice fed a high fat diet. This was shown to be due largely to increased energy expenditure, though there was no change in the respiratory quotient, an indirect indicator of carbohydrate vs. lipid utilization. Chronic stimulation of orexin signaling also reduced food consumption. A variety of both genetic and pharmacological experiments indicated that the majority of this effect was mediated by signaling through OxR2, not OxR1. Finally, a striking finding of this study is that chronic stimulation of orexin signaling had no effect on mice lacking leptin. These animals, when fed a high fat diet, still became obese and insulin resistant even when treated with the orexin receptor agonist. Thus, the protective effect of orexin appears to be rooted in improving leptin sensitivity. This study has obvious therapeutic implications in the treatment of diet-induced obesity and diabetes.

While this study mostly implicated signaling through OxR2 as being important for the obesity-resistant phenotype, some OxR1-mediated effects were noted⁸. The authors found that genetic ablation of OxR1 alone protected against high fat diet-induced hyperglycemia, though not obesity. This indicates that orexin signaling through OxR1 by normal physiological levels of orexin play a permissive role in the development of age- or high fat diet-induced insulin resistance. However, in the non-physiological scenario of sustained orexin overexpression via a transgene, both OxR1- and OxR2-mediated protection against the development of insulin resistance on the high fat diet. This complicated set of results with respect to OxR1 suggests that either the receptor plays different roles under conditions of normal and supraphysiological orexin expression, or that the receptor mediates different physiological effects that can enhance or oppose the development of hyperinsulinemia and that the “winner” of these competing effects is different depending on the level of orexin or the timing and duration of its signaling.

The coupling of the orexin effect to leptin signaling is interesting with respect to another study published recently in which it was found that supraphysiological levels of leptin allow diabetic rodents completely lacking insulin to thrive²⁸. Previously, leptin was known to lower blood glucose level by potentiating residual levels of endogenous insulin in streptozotocin (STZ)-induced diabetic rats with partial insulin deficiency²⁹. However, the idea that leptin alone could rescue insulin-deficient animals from diabetic symptoms had never been tested and this finding came as a major surprise. The similar phenotypes induced by chronic treatment of animals with supraphysiological levels of orexin and leptin, coupled with the finding that orexin appears to function by improving leptin sensitivity, leads one to wonder if these two studies were different sides of the same coin and that stimulation of the orexin/leptin signaling pathways may indeed be a highly promising treatment for type I or type II diabetes.

Does orexin signaling have effects in the periphery?

Most of the effects of orexin signaling described above are thought to occur in the hypothalamus. The roles of orexin signaling, if any, outside the nervous system are controversial. The expression of the orexins and their receptors have been detected in

various peripheral tissues, including the intestine, pancreas, kidney, adrenals, adipose tissue and reproductive tract^{30–33}, but functional proof of a role for orexin signaling in the periphery is rare.

A recent study showed that the incidence of OxR1-expressing cells in the pancreatic islets increased with streptozotocin (STZ)-induced hyperglycemia in rats and co-localized with glucagon³⁴. Moreover, cleaved caspase-3 co-localized immunochemically with OxR1 in the islets. Animals lacking orexin, on the other hand, exhibited reduced hyperglycemia and better glucose tolerance than wild-type animals. These results suggest that orexin signaling through OxR1 in the pancreas may contribute to beta cell apoptosis and the development of diabetes in response to STZ treatment. While the connection is tenuous, it is possible that this effect could explain the observations of Funato, et al., mentioned above, that genetic ablation of OxR1 was protective against high fat diet-induced hyperglycemia and hyperinsulinemia⁸.

The orexin signaling cascade

While orexin signaling has been studied intensively at the physiological level, there has been much less effort devoted to characterizing the intracellular events triggered by binding of the orexin hormones to their receptors. It is known that binding of the hormone to its receptor triggers an influx of calcium, which is coupled to activation of Erk³⁵. The receptors also couple to a phospholipase C (PLC)-mediated pathway that releases intracellular calcium stores.

The most thorough analysis of the gene transcription programs triggered by orexin signaling was reported in 2007³⁶. This study employed global gene expression profiling to identify genes strongly up- or down-regulated in HEK293 cells stably expressing OxR1. 260 genes were found to be up-regulated and 64 down-regulated by two-fold or more when sampled both two and four hours after orexin stimulation. The gene annotations indicated that about half of the highly regulated genes were involved in cell growth (30%) or metabolism (27%). A pathway analysis using the commercial Ingenuity program implicated several pathways as being regulated by orexin signaling (Figure 1). The canonical TGF- β /Smad/BMP, FGF, NF- κ B, and hypoxic signaling pathways were the most prominent. This study focused in detail on the hypoxic pathway, which was an odd “hit” in this study since the cells were cultured under normoxic conditions. Nonetheless, the Hypoxia-Inducible Factor 1- α (HIF-1 α) transcription factor, which had previously been known to rise in response to hypoxia, and many of its target genes were highly induced by orexin treatment. In hypoxic cancer cells, where HIF-1 α action has been best studied, it partners with HIF-1 β to form a heterodimeric transcription factor (HIF-1) that drives a reprogramming of cellular metabolism³⁷. In particular, it massively up-regulates glucose import and glycolysis, having binding sites upstream of almost all of the genes involved in these events. In hypoxic cells, HIF-1 acts to shunt the product of glycolysis, pyruvate, to the production of lactate rather than conversion to acetyl-CoA and entry into the TCA cycle and oxidative phosphorylation. This is done, at least in part, by the HIF-1-dependent up-regulation of the expression of lactate dehydrogenase A (LDH-A), the enzyme that mediates the conversion of pyruvate to lactate, as well as pyruvate dehydrogenase kinase (PDHK), which inactivates PDH, the enzyme that mediates the transformation of pyruvate to Acetyl Co-A (see Figure 2). Surprisingly however, the orexin-treated cells appeared to push most of their metabolic flux through the TCA cycle and oxidative phosphorylation³⁶, resulting in a strongly enhanced production of ATP and, presumably other biosynthetic intermediates attendant with activation of these pathways. It is not known how orexin signaling and hypoxia can both stimulate HIF-1 α activity, yet only a subset of genes commonly activated by this transcription factor in hypoxia are activated in orexin-treated cells.

Parallel studies of hypothalamic slices obtained from wild-type or OXR1 knock-out mice generally concurred with these conclusions and validated that the effects seen were the result of orexin signaling³⁶. In some ways, this result is appealing in that it explains a conundrum in our understanding of orexin biology at the physiological level. If we equate a state of wakefulness with high neuronal metabolic activity, which seems reasonable, it is odd that this would occur when the brain is bathed in low levels of glucose. Therefore, it makes some sense that wakefulness requires an override of this situation via a mechanism that increases the efficiency of glucose uptake and processing. An orexin-mediated up-regulation of cellular metabolism would also appear to be consistent with the observation of increased energy expenditure and resistance to high fat diet-induced obesity⁸. However, it is important to remember that this study focused on OXR1-expressing cells and neuronal slices obtained from wild-type or OXR1 knock-out mice. Wakefulness and resistance to diet-induced obesity, as mentioned above, is now thought to be more of an effect of OXR2-mediated signaling. It may be that the pathways observed are more relevant to reward behavior and other processes that appear to be dominated by OXR1-mediated signaling. Much more work will be required in various cell types and tissues to sort out these issues. But the idea that orexin signaling is a major regulator of energy metabolism is an interesting model that will guide future studies.

Is orexin signaling important in cancer?

The finding that orexin signaling is capable of turbo charging glucose consumption suggests a possible link to cell proliferation in dividing cells and thus to cancer. However, the published results discussed above indicate that orexin can stimulate oxidative energy metabolism³⁶, while many cancer cells employ anaerobic glycolysis for the bulk of their metabolism³⁸. One possibility is that cancer cells that do employ oxidative metabolism could employ orexin signaling in an autocrine or paracrine fashion to stimulate their metabolism and proliferation and, if so, orexin receptor antagonists would be interesting therapeutic options. On the other hand, if orexin signaling forces metabolic flux through TCA and oxidative phosphorylation pathways at the expense of anaerobic glycolysis, then activation of this pathway might be toxic to tumor cells “addicted” to a glycolytic lifestyle.

There are some indications of a role for orexin signaling in cancer cells and, as one might predict from the above analysis, the effect of orexin signaling appears to be different in different kinds of cancer cells. For example, orexins suppress cell growth by inducing apoptosis in human colon cancer, neuroblastoma cells, and rat pancreatic tumor cells³⁹. On the other hand, expression of OXR1 and OXR2 are higher in adenomas than the normal adrenal cortex. Orexin A and B can stimulate cell proliferation in these cells and the effects were more pronounced in cultured adenomatous than normal adrenocortical cells⁴⁰. While these reports are interesting, our understanding of the importance of orexin signaling in cancer, if any, is in its infancy and far more work will be required to determine if the orexin receptors or downstream effectors of the signaling pathway represent viable targets for cancer chemotherapy.

Pharmacological Control of Orexin Signaling

The biology reviewed above suggests that agonists, antagonists and potentiators of orexin signaling could be of significant interest clinically. For example, the treatment of narcolepsy and cataplexy brought about by a lack of orexin production should be treatable using an orexin receptor agonist. On the other hand, addictive behavior should be treatable using an orexin receptor antagonist. Diet induced-obesity and diabetes might be combated with either an orexin receptor agonist or perhaps a positive allosteric potentiator. Several major

pharmaceutical companies have undertaken the development of molecules that target the orexin receptors ⁴¹.

Orexin Receptor Antagonists

Since orexin signaling in the brain promotes wakefulness, it stands to reason that pharmacological blockade of this pathway should result in sleepiness and thus orexin receptor antagonists might be useful drugs for treating insomnia. However, a major concern would be the induction of cataplexy. Indeed, the central question in the development of therapeutically useful orexin receptor antagonists is whether transient pharmacological inhibition of the orexin receptor will phenocopy the narcoleptic and cataplectic phenotype of chronic orexin deficiency.

The pre-clinical and clinical data available so far argue that the answer is “no”. Most of the data come from studies of Almorexant (Figure 3; also known as ACT-078573), which is being developed by Actelion Pharmaceuticals for the treatment of insomnia. Almorexant is an orally available tetrahydroisoquinoline that potently antagonizes both OxR1 and OxR2. Actelion published a preliminary study in early 2007 that reported the successful treatment of rat, dog and human subjects with Almorexant or a classical GABA receptor agonist, zolpidem ¹⁹. Almorexant was shown to be safe and well tolerated in this study. It induced subjective and objective physiological signs of sleep. In the rat experiments, it was demonstrated that Almorexant induced both non-REM and REM sleep. This is significant in that zolpidem does not induce REM sleep. Importantly, no signs of cataplexy were observed in any of the experimental animals or human patients.

At the end of 2009, Actelion announced the completion of an extensive Phase III study of a two-week treatment of adult and elderly subjects with primary chronic insomnia. The company claims that the primary endpoint of the trial, superior effectiveness of Almorexant over the placebo, was met, as were several secondary endpoints (see <http://www1.actelion.com/en/our-company/news-and-events/index.page?newsId=1365361>). However, a cryptic line in the news release stated that “...certain safety observations were made that will require further evaluation and assessment in longer-term Phase III studies.” It will be interesting to know what this means when the data become available.

Almorexant was the result of an extensive development program that began with the tetrahydroisoquinoline **1** (Figure 3) ⁴². This compound arose as a primary hit from a high throughput screen using a FLIPR-based calcium assay in Chinese Hamster Ovary (CHO) cells expressing high levels of human OxR1 or OxR2. Note that compound **1** is fairly selective for OxR1. Indeed, this medicinal program produced many different potent compounds, some of which were dual receptor antagonists, while some were reasonably selective inhibitors of either OxR1 or OxR2 (see Figure 3). Quite large swings in the degree of selectivity were observed as a result of modest structural alterations. For example, the replacement of one of the methoxy groups in “upper left” aromatic ring of **1** with an isopropyl ether resulted in a completely OxR1 specific antagonist. Another interesting contrast is compound **3**, which is rather selective for OxR2, but shares many structural features with the dual antagonist Almorexant. Unfortunately, there are no structural data on the orexin receptor and thus the molecular basis of this selectivity is unknown.

The tetrahydroisoquinolines are far from the only orexin receptor antagonists reported. For example, Actelion has reported structurally distinct compounds such as sulfonamides **4** and **5**, which are potent dual and OxR2 selective antagonists, respectively (Figure 4) ⁴³. Through inspection of the structures, which include a highly modified glycine core, one is tempted to

conclude that the tetrahydroisoquinolines and sulfonamides may occupy overlapping sites on the receptor, but to the best of our knowledge, this has not been tested.

GSK reported one of the first orexin receptor antagonist, SB-334867^{44–46} (Figure 5), a compound that is relatively selective for OxR1 and remains the most commonly used “tool compound” in research laboratories studying orexin biology. They also reported a relatively distant relative of SB-334867, **6**, which includes a proline unit, with much better potency against both receptors, but retaining reasonable OxR1 selectivity⁴⁷. It has been speculated that **6** might be a close relative of a compound of undisclosed structure that GSK took into clinical trials for the treatment of insomnia called SB-674042⁴¹. GSK announced the progression of this compound to Phase II clinical trials in 2007, but no further clinical data have been forthcoming to the best of our knowledge.

A Merck team has developed proline-containing orexin receptor antagonists⁴⁸. Compound **7** (Figure 5) arose from a high-throughput screen and evinced excellent in vitro potency against OxR2 as well as modest activity against OxR1, but in vivo exhibited poor blood brain barrier penetration. This was found to be the result of it being a substrate for the P glycoprotein. They found that this problem could be ameliorated by methylation of the benzimidazole nitrogen^{41, 48}. Compound **8**, which also contains a phenyl ring rather than a pyrrole moiety in the “upper right” quadrant of the molecule, is a potent dual receptor antagonist with excellent blood brain barrier penetration. **8** has been shown to active in rats.

Other pharmaceutical companies have reported a few compounds with in vitro activity against orexin receptors, but these will not be reviewed here.

It is tempting to speculate that the same compounds being developed as sleep aids might also be useful for the treatment of chronic addictions, though an obvious side effect of this treatment regime would be that it will make the subjects sleepy if they are dual receptor antagonists. However, the discovery of the role of orexin signaling in addictive behavior is relatively recent and, to the best of our knowledge, no clinical trials have been initiated using the compounds discussed above for this indication.

Orexin Receptor Agonists and Potentiators

As discussed above, Yanagisawa and colleagues have provided proof of principle in animal models that narcolepsy should be treatable with an orexin receptor agonist¹⁸. They employed a modified orexin peptide in these studies. Curiously however, no non-peptide agonists have yet been reported in the literature to the best of our knowledge. It is difficult to imagine that screens for such compounds have not been carried out, given the extensive amount of work done on receptor antagonists. Thus, it seems likely that potent agonists are difficult to find, though it is not clear why this is so.

Very recently, the first positive allosteric potentiator of the orexin receptor, **9** (Figure 6), was identified. This compound was discovered fortuitously during in the course of a modest medicinal chemistry campaign aimed at increasing the potency of a peptoid-based orexin receptor antagonist⁴⁹. Peptoid **10** was identified in a screen in which cells that do or do not express OxR1, but are otherwise identical, were labeled with different colored dyes (red and green, respectively) and hybridized to a microarray displaying several thousand peptoids immobilized on a glass slide. Compounds that bound the OxR1-expressing cells selectively were identified by a high red:green ratio of the fluorescent dyes captured on that spot (see ref.⁵⁰ for the development of this methodology). A sarcosine scanning experiment⁵¹ revealed that only two of the nine side chains present in the molecule was essential for binding to the receptor-expressing cells (highlighted in red in Fig. 6). Subsequent synthesis and analysis of compound **11** confirmed that it was the “minimal pharmacophore” for

receptor binding. **11** is a very weak antagonist of both orexin receptors with an IC_{50} of only approximately 300 μ M in vitro.

The structure of **11** seemed to suggest a weak similarity to Almorexant (Figure 6) in that the compound contained a piperonyl ring attached to a glycine unit, whereas almost the same units, but with a dimethoxybenzyl ring in the place of piperonyl, are present in Almorexant. Using this model as a guide, several derivatives of **11** were synthesized in which hydrocarbon units were added to the amine nitrogen via reductive amination. A benzyl derivative, **12**, in which an additional methylene had also been inserted between the aromatic ring and the nitrogen proved to be a much improved antagonist, albeit still with only modest potency (Fig. 6), suggesting that the model may be correct. Competition studies to determine if the peptoid derivative **12** and Almorexant compete with one another for binding to the receptor have not been reported.

During further optimization efforts, compound **9** was synthesized, which differed from **12** only in that the piperonyl ring was opened up to two methoxy units and the single methylene linker between the glycine nitrogen and the aromatic ring that had been present in compound **10** was restored. Remarkably, **9** did not antagonize OxR1 in a cell culture assay but appeared to slightly increase the expression of an orexin-dependent reporter gene. The assays were therefore repeated at lower orexin concentrations (EC_{20} of the hormone) and the results demonstrated clearly that **9** is a positive potentiator of orexin-mediated activation of the receptor. At this hormone concentration, **9** exhibited an EC_{50} of about 120 nM. When the assay was conducted at saturating levels of the potentiator **9** and the level of orexin was titrated, it was found that the EC_{50} of orexin was decreased approximately four-fold and that the maximal level of reporter gene expression was about three-fold greater than that brought about by saturating levels of orexin. Similar results were obtained in experiments using cells expressing OxR2, showing that **9** is a dual receptor potentiator.

The discovery of the first orexin receptor potentiator is potentially exciting with regard to possible applications as an anti-obesity/diabetes treatment⁸, though to date no *in vivo* experiments have been reported using this compound. As mentioned above, Yanagisawa has shown that a peptide-based orexin receptor agonist blocks diet-induced obesity and the development of type II diabetes in rodents⁸. Since these animals and, presumably, over nourished humans still express normal levels of the orexin hormone, a positive potentiator may be useful in stimulating the favorable natural action of the hormone in these individuals. Of course, a potentiator is unlikely to be of interest in the treatment of narcolepsy, since affected individuals produce little or no orexin.

Summary

It is now clear that the neuropeptide orexin and its cognate receptors play a central role in regulating feeding, sleeping, energy expenditure, reward seeking and a variety of other behaviors. Little is known about the intracellular events set off by orexin signaling. There is clearly great promise medically in the pharmacological manipulation of orexin signaling and a number of pharmaceutical companies and academic laboratories have active programs in this area. Though no such compounds are yet in the clinic, the potent dual orexin receptor antagonist Almorexant has completed what was apparently a successful phase III clinical trial for the treatment of insomnia. As yet, no orexin receptor agonists have been reported. It seems likely that there will be a great deal of activity in this area over the next several years.

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Figure 1. Summary of the findings of a gene expression analysis of the effect of orexin on cells expressing the orexin receptor 1. Top: Pie chart summarizing annotated functions of genes affected significantly by orexin signaling. Bottom: Proposed signaling pathways affected by orexin signaling, as derived from an Ingenuity-based analysis of the expression data. (Adapted from a figure provided by D. Sikder).

Fig. 2. Schematic summary of the major effect of orexin signaling on glucose metabolism. OxSig = orexin signaling. The orange oval represents a glucose transporter. Arrows indicate a stimulation of the indicated process and the perpendicular lines represent an inhibition.

Figure 3.

Summary of the development of Almorexant, a clinical candidate for the treatment of insomnia, and related compounds with different selectivity for the two orexin receptors.

Figure 4.
Structures of other orexin receptor antagonists developed by Actelion Pharmaceuticals.

Figure 5.
Structures of orexin receptor antagonists developed by Glaxo Smith Kline (GSK) and Merck.

Figure 6.

Development of an orexin receptor positive potentiator. The structure of the peptoid that arose as a primary hit from a binding screen is shown at the top. The part of the molecule found to be important for receptor binding is highlighted in red. This putative minimal pharmacophore (**11**), was synthesized and shown to have slightly better activity than the peptoid. The part of **11** with a putative relationship to Almorexant (see box) is shown in blue. The structure of an improved peptoid antagonist (**12**) and a positive allosteric potentiator (**9**) are also shown. The moieties on these molecules thought to be related to the trimethylfluoro-substituted aromatic ring of Almorexant are depicted in gold.