improve rhythms in aged animals. In humans, this question has been addressed by Lavie and colleagues who, using elderly poor sleepers, have shown a correlation between reductions in sleep quality and melatonin levels[21]. Furthermore, they have shown recently that seven days' treatment with melatonin (2 mg daily) can improve sleep patterns in elderly patients with insomnia[22]. Whilst melatonin itself does not appear to be overtly hypnotic, recent studies have indicated that it may have sleep promoting properties[23,24], which may be a useful adjunct to its effects on the circadian timing system.

Thus, not only have melatonin receptors come of age as a therapeutic target for age-related sleep disorders, but the clock is ticking for the traditional treatments for insomnia in this population.

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Receptor state and ligand efficacy

Two-state model of receptor activity does not explain ligand efficacy

Some antagonists at G protein-coupled receptors exhibit properties seemingly opposite to those of the corresponding agonists, at least with respect to GTPase activity, effector regulation and receptor–G protein allostery. This phenomenon, referred to as inverse agonism (or negative antagonism), provides a novel means to study G protein-coupled signal transduction. In a recent issue of *TiPS*, Kenakin discusses the implications of inverse agonism on the concept of efficacy[1]. I would like to comment on two points raised in his letter. Several papers are referred to (e.g. Ref. 2) in which 'previously classified antagonists have been shown to possess the ability to actively destabilize spontaneous complexes of receptors and G proteins'[1]. This is one possible interpretation of the observations cited, which were made using whole cells or crude membrane preparations. However, other explanations cannot be ruled out, as the molecular events underlying inverse agonism remain to be investigated in detail. That the inhibition of spontaneous receptor activity requires the destabilization of receptor–G protein complexes has not been shown thus far, and some findings exist which are not obviously consistent with such a mechanism.

Apparent increases in either antagonist affinity, or binding capacity or both, in the presence of guanyl nucleotides typically are assumed to reflect increases in the availability of 'free' receptors (e.g. Refs 1, 3, 4). Antagonists with negative intrinsic efficacy and guanyl nucleotides thus are both thought to promote receptor–G protein dissociation. It should be noted, however, that nucleotide-induced changes in binding do not necessarily indicate that such dissociation has taken place. For example, guanylate imidodiphosphate has been shown to reduce agonist binding to solubilized cardiac muscarinic acetylcholine receptors under conditions where the nucleotide fails to decrease receptor–G protein co-immunoprecipitation[5]. Furthermore, the 'atypical' antagonists carvedilol and bucindolol exhibit reduced affinity for the β_2-adrenoceptor in the presence of guanyl nucleotides[6] (i.e. they display agonist-like binding), but they also inhibit spontaneous β_2-adrenoceptor activity in membranes[7]. Also, nucleotide-induced decreases in agonist binding and increases in the binding of ligands with negative intrinsic activity at the 5-HT_{2C} receptor do not occur at equivalent concentrations of nucleotide[3,8]. For example, the concentration of guanylate imidodiphosphate required to produce a half-maximal effect on inverse agonist binding is 400 times greater than that required to produce

a half-maximal effect on agonist binding[3]. Moreover, the rank order of potency of guanyl nucleotides differs with respect to these two phenomena at the 5-HT_{2C} receptor[3,8]. In contrast, guanyl nucleotides appear to be without effect on the binding of either the agonist [^{3}H]bradykinin or the inverse agonist [^{3}H]NPC17731 to the bradykinin B_2 receptor in bovine myometrial membranes[4]; also, while a ligand with intrinsic activity might be expected to yield different binding patterns in competition experiments with these two radioligands (see Ref. 3), no such differences were found with either inverse agonists or unlabelled bradykinin using primary cultures of inverse agonist-sensitive rat myometrial cells[4]. The foregoing observations indicate that the relationship between inverse agonism, nucleotide-induced changes in binding, and the stability of receptor–G protein complexes requires further study.

The second point I would like to address is the idea of a dividing line, or 'knife edge', separating ligands with positive intrinsic activity from those with negative intrinsic activity[1]. In our study[2], we found that several ligands considered to be weak β-adrenoceptor agonists, namely labetalol, pindolol, and alprenolol, can inhibit spontaneous β_2-adrenoceptor activity in membranes from insect and mammalian cells. The same ligands, however, failed to decrease intracellular cAMP levels in whole Sf9 cells: pindolol and alprenolol had negligible effects, while labetalol actually increased intracellular cAMP. In contrast, ligands that exhibited greater negative intrinsic activity in membranes (i.e. propranolol and timolol), were found to decrease intracellular cAMP. Interestingly, efficacy in membranes was found to correlate with that in whole cells, even though some drugs differed in the two preparations with respect to their relationship to the proposed dividing line. In addition, a negative correlation was observed between inverse efficacy in membranes and reported levels of intrinsic sympathomimetic activity, the latter being a reflection of β-adrenoceptor partial agonism *in vivo* (the correlation is negative because intrinsic sympathomimetic activity represents the 'opposite' of inverse efficacy). While the discovery that some ligands possess dual activity at first may seem to obscure the difference between agonism and inverse agonism, these correlations suggest that a continuum exists between the two phenomena and that the null point may be variable rather than static[2].

Thus, it appears that ligands which bind to the β_2-adrenoceptor may be distinguished as having positive, negative, or neutral effects on receptor activity in a given experiment, but that these distinctions may be condition-dependent. It remains to be determined whether or not this is a general property of G protein-coupled receptors. The finding that a given ligand can stimulate a receptor in one type of experiment and inhibit its spontaneous activity in another is difficult to reconcile with a 'two-state' model of receptor activity wherein differential binding affinities for active and inactive forms of the receptor dictate whether positive or negative regulation will occur with a particular drug[2]. It follows that such models may be overly simplistic and that one or more additional 'states' may be required to account for all of the observed ligand-dependent changes in receptor activity.

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Chemical name

NPC17731: DArg[Hyp^3-DHyp^7(transpropyl)-Oic^8] bradykinin

Kenakin replies

Efficacy as the molecular property of a ligand

The nature of efficacy is central in pharmacology. The major point of my letter to *TiPS* was to set the sights for the expectation of efficacy in molecules beyond the production of observable response. Specifically, to view efficacy as the molecular property of a ligand (when it is bound to the receptor), that changes the behaviour of the receptor towards other proteins. This viewpoint comes from a bias as an industrial pharmacologist where ligand-specific (but system-independent) information is required to guide medicinal chemists.

The 'knife edge' refers to molecular properties of ligands and not necessarily to observed effects. In his letter, Chidiac raises an important point in that the observed properties of agonism, antagonism and inverse agonism may be reflections of the molecular properties of negative and positive efficacy which may be affected by the type and setpoint of the system. For example, the β-adrenoceptor ligand prenalterol can function as a full agonist, partial agonist, or neutral antagonist in tissues of varying receptor coupling efficiency[1]. However, in molecular terms, the knife edge should exist.