The mechanism of action of probiotics and prebiotics is likely to involve modulation of key body functions relevant to the prevention or management of disease-prone situations. The first target of these effects is the intestinal microflora. However, according to more recent research, they may also modulate immune functions, in addition to essential regulatory processes, particularly those mediated by gastrointestinal peptides [5].

Probiotics and prebiotics sit at the interface between nutrition and pharmacology. They are currently predominantly used in foods, but tomorrow they shall find their place in medicine, even if they will probably never be therapeutic drugs.

Drug Discovery Today aims to bring to the attention of the medical community new developments that help patient care. In that context, a review on probiotics and prebiotics is important to inform, to stimulate interest, to create opportunities for further research and to hopefully help patients.

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Ligand-selective signaling and high-content screening for GPCR drugs

Intracellular signals that are observed following stimulation of G protein-coupled receptors (GPCRs) by synthetic ligands are sometimes unpredictable. Hence, as elegantly delineated in the recent review by Graham Milligan [1] and the subsequent update article by Terry Kenakin [2], GPCRs-directed drug screening should not rely on a single assay, because lack of efficacy for a certain biochemical event does not necessarily indicate lack of receptor activation. Rather, drug-screening protocols should employ a multitude of biochemical events, including measurements of different second messengers, downstream events, receptor internalization and related gene transcription. Employing such high-content screening (HCS) protocols should enable better identification of promising GPCR compounds in drug development projects.

We would like to illustrate this point by presenting our decade-old experience with the identification of AF102B (Cevimeline) as a selective M1 agonist with a unique signaling profile [3,4], long before HCS platforms became available. When assayed in Chinese hamster ovary (CHO) fibroblasts stably transfected with the M1 muscarinic receptor gene, AF102B behaved as a classical M1 antagonist when measuring adenylate cyclase, fully blocking its activation by the non-selective cholinergic agonist carbachol [4].

However, it was soon realized that AF102B was far from being an antagonist. When measuring activation of phosphatidyl-inositol specific phospholipase C or phopsholipase A2 in the same cell line, AF102B behaved as a partial agonist [4]. The most amazing observation, however, was from confocal microscopy imaging of intracellular free calcium ions. Here, AF102B behaved as a super-agonist, yielding a stronger rise in calcium ions than carbachol [4]. Notably, these assays were conducted using similar assay conditions in the same cells. Thus, our studies illustrated that a single ligand, by activating a particular GPCR, was capable of exhibiting a signaling profile of an antagonist, a partial-agonist, or even a super-agonist, depending on the signal being measured. Subsequent studies, which explored late events related to M1 receptor activation and using the neuronal cell line PC12M1 – such as secretion of the amyloid precursor protein [5], NGF-induced neurite outgrowth [6] or inhibition of *tau* phosphorylation [7] – indicated that, indeed, AF102B was capable of slightly surpassing the efficacy of the classical agonist carbachol in mediating certain late cellular events.

We coined the term 'ligand-selective signaling' to describe such novel observations, and suggested that they represented a universal aspect of GPCR activation [4]. Namely, we proposed that the discrete activation profiles of second-messenger signaling pathways reflected, at least in part, the capacity of rigid ligands, such as AF102B, to enable only a limited subset of ligand–receptor conformations, compared with the larger scope allowed by full agonists, which are typically more flexible molecules [4]. Our suggestion that such 'ligand-selective signaling' via M1 muscarinic receptors reflects different ligand–receptor conformations remains unproven, and should await X-ray analysis of purified M1 receptors crystallized in the presence of different ligands. However, clues for discrete ligand-dependent signaling profiles were subsequently demonstrated for other GPCRs, such as β-2 adrenergic [8] and α-2 adrenergic receptors [9].

Moreover, the realization of ligand-dictated gene transcription profiles for nuclear steroid hormone receptors led to the development of selective estrogen

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receptor modulators (SERMs) as promising hormone replacement therapy drugs [10]. Thus, phenomena of unique signaling profiles dictated by particular ligands at a given receptor are not limited to the GPCR family. Such phenomena seem to be imperative for drug development and we believe that depiction of ligand-selective signaling profiles should become a key part of preclinical drug development. Seemingly, compounds exhibiting selective signaling profiles would be more likely to exhibit reduced desensitization and fewer side-effects, making them superior candidates for further drug development.

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The language of screening evolves

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The value of HTS to the drug discovery arena was debated widely at the *Society for Biomolecular Screening (SBS) 9th Annual Conference and Exhibition*, in Portland, OR, USA, (21–25 September 2003). The solution could lie, in part, in the diversity of SBS sessions beyond traditional HTS single-point assay technology.

As Jeff Pasley from Wyeth (http://www.wyeth.com) discussed in a session titled *Point/Counterpoint: Is HTS Worth the Cost?*, HTS is moving beyond activity assays into the ADME arena for determination of compound characteristics *in vitro*. However, as counter proponent Mel Reichman (drughunter@aol.com) added, HTS is more a commodity in the rapidly evolving oligopoly pharmaceutical discovery market. With the low barrier to entry, many academic institutions are now implementing HTS to protect their target intellectual property (see http://iccb.med.harvard.edu/screening/index.htm).

Furthering HTS

Within small molecule discovery at many pharmaceutical companies, HTS is now being supplemented and iteratively used with a variety of new methodologies, including computational modelling of compound activity, data quality, systems biology and mechanisms of action at the cellular and organism level. HTS is evolving beyond a search for a needle in a haystack to become a generic, efficient method for testing a hypothesis. Screening assay data is being quantified, both in range and quality, so all results, both negative and positive, can be used to better understand the target, compound selectivity and the systems biology underlying the *in vivo* therapeutic interaction.

The SBS sessions investigated two major avenues:

- A better understanding of systems biology in order to more accurately select and screen the drugable therapeutically relevant targets
- A more efficient selection of compounds that will be potent against the target as well as readily bioavailable as an oral therapeutic.

Meeting highlights

- Systems biology for increased understanding of physiology and target identification

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