models with a tetrapeptide library made up of a total of 6 250 000 peptides (200 mixtures made up of 125 000 tetrapeptides each) (Dooley *et al*, 1998; Houghten *et al*, 2006, 2008). Mixtures ranging from 2500 to 125 000 tetrapeptides have yielded clear *in vivo* activity that is not necessarily related to classic *in vitro* target-based screening. For mixture-based small molecule libraries the process can be improved by careful selection of those libraries guided by theoretical calculation of their drug-like properties. Over the past 10 years a process termed cassette testing (Liu *et al*, 2008, and references cited therein) has been used to study *in vivo* ADME with small mixture sets (typically 5–10 related compounds) to facilitate the early elimination of compounds with poor drug-like profiling in PK profiling.

The concept of using large, highly diverse mixture-based libraries for the identification of inherently more advanced 'hits' by the direct *in vivo* testing is both exciting and promising. It remains to be seen if these recent early preliminary successes will fulfill their current potential promise.

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Targeting the serotonin 2C receptor for the treatment of obesity and type 2 diabetes

The neurotransmitter serotonin (5-hydroxytryptamine; 5-HT) has a well-established role in energy homeostasis. The clinical potential of beneficially manipulating the 5-HT system is best illustrated by the efficacy of compounds such as fenfluramine and sibutramine, both of which increase 5-HT bioavailability, in the pharmacological treatment of obesity. The success of these compounds in reducing food intake and body weight has stimulated interest in dissecting the mechanisms whereby 5-HT influences energy balance. Early pharmacological studies implicated the 5-HT_{2C} receptor (5-HT_{2C}R; previously classified as the 5-HT_{1C} receptor) in these effects, a suggestion supported by the later observation that genetic inactivation of the 5-HT_{2C}R, but not other 5-HT receptors, produces hyperphagia and obesity in the mouse (Tecott *et al*, 1995). 5-HT_{2C}R knockout mice also display blunted responses to fenfluramine, indicating that action at these receptors is required for the anorectic effect of this compound (Vickers *et al*, 1999). Collectively, these findings motivated attempts to develop selective 5-HT_{2C}R agonists for the treatment of obesity.

Unfortunately, the high degree of sequence homology between the 5-HT_{2C}R and 5-HT_{2B}R has proved a major challenge in efforts to generate a truly specific high affinity 5-HT_{2C}R agonist that does not stimulate 5-HT_{2B}Rs. Activity at the 5-HT_{2B}R is a particular concern because action at these receptors is thought to contribute to the valvular heart disease reported in some patients following fenfluramine-phentermine use (Fitzgerald *et al*, 2000). Nevertheless, recent studies using combined pharmacological and genetic approaches in murine models have recently rekindled pharmaceutical interest in drug discovery programs focusing on generating more selective high affinity 5-HT_{2C}R agonists; for example, it has been shown that fenfluramine influences appetite through the melanocortin system (Heisler *et al*, 2002). This brain pathway, specifically acting through the melanocortin 4 receptor, is critical for the normal regulation of energy balance, and integrates inputs from many other neuropeptides and neurotransmitters.

More recently, a distinct role for the 5-HT_{2C}Rs in glucose homeostasis has also been reported in rodents. Specifically, both a classic 5-HT_{2C}R agonist (with binding affinity for other 5-HT receptors) and a more selective and high affinity 5-HT_{2C}R agonist were demonstrated to reduce elevated insulin levels and improve glucose tolerance and insulin sensitivity in both genetically obese mice and in mice with diet-induced obesity, both with impaired glucose tolerance and insulin resistance (Zhou *et al*, 2007). Importantly, these effects were achieved at concentrations of the compounds which were too low to influence food intake, energy expenditure, locomotor activity, or body weight. These findings indicate that the 5-HT_{2C}R may be a mechanistically novel target for the treatment of type 2 diabetes. This has been corroborated by genetic inactivation of the 5-HT_{2C}R in mice, which, either alone or in combination with leptin deficiency, impairs glucose homeostasis (Wade

et al, 2008). These findings identify a specific 5-HT receptor of relevance to a prevalent metabolic disease.

Together, these data indicate that the 5-HT_{2C}R is an attractive and tractable potential drug target for the treatment of obesity and/or type 2 diabetes. Recent pharmaceutical efforts have led to the development of at least one compound that is currently in clinical trials for obesity treatment. Results from these trials are awaited with considerable interest.

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Turning up the pace of ion channel screening in drug discovery

Ion channels represent an important family of integral membrane proteins involved in many diverse physiological processes and are also implicated in a number of pathological conditions in particular of the nervous, cardiovascular, and inflammatory systems. These proteins have proven to be attractive targets for drug discovery with approximately 13% of marketed drugs having their mechanism of action attributed to activity at ligand- or voltage-gated ion channels (Overington *et al*, 2006). Although this success is noteworthy, there is a general consensus in the field of ion channel-targeted drug discovery that progress has been significantly hampered because of the low-throughput nature of the gold standard assay for electrophysiological assessment of ion channel activity, ie, manual patch clamp electrophysiology in mammalian cell lines expressing channels of interest. Recent innovations in the development of enabling technologies supporting higher throughput and fully automated patch clamp electrophysiology (Dunlop *et al*, 2008; Lu and An, 2008) have provided for a reenergizing of ion channel drug discovery with unprecedented capabilities for compound screening.

Two different approaches to achieving automation of manual patch clamp electrophysiology have recently emerged taking advantage of the so-called planar array of multi-well configurations in either a plate- or chip-based format allowing for multiple parallel recordings replacing the single channel recording typical of manual patch clamp. The IonWorks platform (Schroeder *et al*, 2003) was the first major innovation to be introduced and although this technology did not recapitulate the tight gigaohm seal quality typical of manual recordings, a number of assays have been successfully transferred onto this platform. Most notably, it has been possible to screen small compound libraries using the IonWorks (John *et al*, 2007), representing perhaps the best example of how such technologies have revolutionized ion channel screening as such a feat would be unimaginable with manual recording approaches. The second series of technologies to be introduced in the form of the PatchXpress, QPatch, and Patchliner (Dunlop *et al*, 2008) have successfully recapitulated the gigaohm quality seals typical of manual recordings. Until recently, these systems have relied on the parallel recording of up to 16 cells, in of itself a significant increase in screening capability. A recent innovation toward unprecedented screening capacity has been introduced in the form of a 48-channel QPatch system, a major advance over the manual recording approach where one can only imagine having 48 different individuals operating manual recording set-ups.

Despite the obvious advantages associated with fully automated ion channel screening there are challenges associated with the implementation of these technologies. Not to be underestimated is the often significant time to generate a cell line compatible with each platform, and not necessarily the cell line you have been using for many years in others applications. This process together with assay optimization and validation can be lengthy and resource intensive. However, these challenges are clearly outweighed by the now unprecedented screening capability to support ion channel-targeted drug discovery, holding much promise for expediting the discovery of new ion channel-targeted drugs.

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