muscle contractions in both guinea pig ileum and mouse vas deferens several times greater than Leu-enkephalinamide. This solid-phase route to these compounds is leading to the further exploration of combinatorial libraries based on glycopeptides.

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High-throughput screening

Assay miniaturization for 384-well applications

In the first part of this two-part report on 384-well microtitre-plate-based HTS, liquid-handling systems were outlined [Rogers, M.V. *Drug Discovery Today* (1997) 2, 395–396]. In this second part, two examples of specific HTS assays screened in the 384-well format at Glaxo Wellcome, Stevenage are described.

Based on our experience at Stevenage, we have found that assay miniaturization for HTS can significantly increase screen capacities and throughput and at the same time be very cost-effective. At Stevenage, we now routinely develop cell-based reporter assays and certain biochemical assay types in the 384-well format. Compound supply and screening data handling systems are being upgraded and improved to cope with the movement from the 96- to 384-well plate format. In particular, compound supply will be totally automated and samples held in a high-density store containing 384-well blocks. This will allow direct interfacing with the screening robots.

384-Well biochemical assays on the R2 robotic system

Glaxo Wellcome, like other major pharmaceutical companies, is committed to integrated automation of drug screening. We have several large integrated robots at the research centre in Stevenage. One of these robots, 'R2', built by Thurnall (Manchester, UK), was described in a recent article in *Drug Discovery Today* [Harding, D. *et al. Drug Discovery Today* (1997) 385–390]. One of the two cells on R2 was designed exclusively for running non-radioactive assays in either 96- or 384-well formats.

'Cold R2' consists of a CRS robotic arm on a linear track. Around the track are positioned a Tecan Genesis pipetting station, ambient and 37°C incubators, a plate mixer, colourimeter, fluorimeter, bulk reagent dispenser, delidders and tip carousels. When the 'cold R2' robot became operational earlier in the year, one of the first assays to run on it was designed to detect activators of protein C. The high cost of using protein C at the concentration required for a kinetically valid assay precluded development of a cost-effective screen in the 96-well format. Reducing the volumes of all assay components fourfold for the 384-well format enabled a much more cost-effective screening strategy to be devised. Data relating to throughput of the 'robotized' protein C activator screen was presented at the MIPTEC conference on automation of HTS held in Washington, 23–27 June 1997. This screen was run in transparent, square-well, 384-well Nunc plates. Each sample plate for this screen was prepared from four 96-well plates using a Matrix Technologies PlateMate™, which dispensed 2 μl of compounds diluted in DMSO into dry wells with a high degree of precision.

The primary screen consisted of >100,000 assay points comprising discrete compounds and library samples. "The run took four days to complete on R2, which is almost five times faster than the current Robolab 9600 (Robocon, Austria) system ('R1') maximum for this assay", says David Mobbs of the assay design team. R1 is a robotic system capable of performing the same assay, but only in the 96-well plate format.

In order to achieve maximum sample throughput during an automated HTS campaign, all assay reagents should remain stable in the refrigerated reagent holding vessels on the robots for approximately 24 hours. This is a common problem and an efficient assay validation and robotization process is required in order to ensure optimal use of the robots. Movement to the miniaturized formats reduces reagent consumption and makes it possible to devise better ways of storing the smaller reagent stocks on the robot.

Semi-automated cell-based assay

We are currently building an integrated robot 'R4' specifically designed to perform HTS on a variety of different cell-based assay types in the 96- or 384-well format. At present 384-well cell-based screening is semi-automated and has been successfully used for a variety of assays including a cytokine receptor activation assay. In this case, a cell line was constructed with an episomal reporter construct containing a promoter sequence specific for transcription initiation following selective activation of the cytokine receptor. The reporter gene secreted alkaline phosphatase (SPAP) was positioned downstream of the promoter. Activation of the cytokine receptor leads to the secretion of SPAP into the cell culture medium. The assay was transferred from the project biologists to the assay design team in a 96-well format and was simplified and redeveloped into a 384-well format.

It is not possible to screen 384-well plates manually using hand-held pipetting devices and, therefore, automated liquid dispensing is required. A 'stand alone' Matrix Technologies PlateMate was used for translation of compounds dissolved in DMSO from 96-well stock plates into dry, square-well Nunc 384-well plates for the assay. Assay reagents were added to these plates using a 384-well Labsystems Multidrop™. Both instruments achieved a high degree of accuracy typically returning C.V.'s of <5%. The basic protocol involved addition of compounds using the PlateMate, addition of cells using the Multidrop and then an overnight incubation step at 37°C. Buffer containing a chromogenic substrate for SPAP was then added using the Multidrop and assay plates incubated for 5 hours; wells in which SPAP

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had been secreted could be identified by colour change, which is measured spectrophotometrically at 405 nm.

"The primary screen consisted of >100,000 discrete compounds. This was achieved in five experiments, taking just 10 days to complete. Assay cost of the whole primary screen was less than £1000", says Phil Robinson, a member of the design team.

The conversion of this 96-well assay to a 384-well format resulted in significant savings in time from reduced handling of plates and reduced demand on tissue culture. This in turn reduced the cost of the assay sevenfold during assay development and optimization, rather than the anticipated fourfold reduction.

In our experience the combined use of 384-well plates and automation represents a major advance in reducing costs and increasing sample throughput and precision.

New high-density plates

Greiner Labortechnik (Frickenhausen, Germany) described their 1536-well plates at the MIPTEC conference. The 1536-well plate was developed in close collaboration with Bayer (Leverkusen, Germany) to fulfil future demands in HTS and is likely to generate much interest from the HTS fraternity. These plates have the length, width and height of a standard 96-well plate. Each well has an internal well area of 2.3 mm^2 with a total well volume of 14 µl. Several plate types are available including transparent, black or white (also available with clear bottom) and tissue-culture treated and sterile plates. Greiner also produce a range of 384-well plates.

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Emerging molecular targets

Immortalized human CNS cell line

A source of neural cells is frequently a limiting factor for research or drug discovery efforts for CNS conditions. Dinah Say, Jasodhara Ray and Fred Gage from Signal Pharmaceuticals (La Jolla, CA, USA) have now demonstrated that a possible solution exists for this dilemma. They report the establishment of an immortalized CNS progenitor cell line from brain tissue of 13-week-old fetuses [*Nat. Biotechnol.* (1997) 15, 574–580].

Two cell lines were obtained using a tetracycline (Tc)-responsive v-*myc* oncogene to immortalize the cells. One of the cell lines possessed a default differentiation into neurons. The other cell line could be differentiated into either neurons or astrocytes. The investigators believe that the development of these cell lines clearly 'demonstrate that bipotent precursor cells exist in the human brain' and that it is possible to establish CNS cells in culture such that 'the fate of these cells can be directed by their environment'. Such cell lines should be an important source of human CNS drug targets as well as a model system for studies on the differentiation of CNS precursor cells.

Y_5-like receptors and epilepsy

Neuropeptide Y binds to a family of G-protein-coupled receptors widely distributed throughout the central nervous system. So far, six different subtypes of the receptor have been identified, all of which appear to act through the inhibition of adenylate cyclase. The receptors for neuropeptide Y are associated with numerous physiological functions, including stimulation of food intake, memory, cardiovascular function and anxiolysis [see *Annu. Rev. Pharmacol. Toxicol.* (1993) 32, 309–352 for a comprehensive overview].

Several lines of evidence support the notion that neuropeptide Y may also function as an endogenous anticonvulsive agent:

- mice deficient in neuropeptide Y sometimes develop spontaneous seizures;
- such mice are more sensitive to the induction of seizures by GABAergic antagonists than those with normal levels of neuropeptide Y; and
- when seizures occur an increase in the level of neuropeptide Y has been observed, possibly as a compensatory mechanism to correct the seizure state.

Now David P.D. Woldbye and associates at the University of Copenhagen (Denmark) and at H.S. Lundbeck A/S (Copenhagen-Valby, Denmark) have provided direct evidence that administration of neuropeptide Y can block seizures in rats that have been induced by kainic acid [*Nat. Med.* (1997) 3, 761–764].

Kainic acid-induced seizures in rats is a well characterized seizure model. Such seizures are caused by the direct perturbation of kainic acid receptors as well as the release of glutamate triggered by kainic acid. Woldbye and coworkers found that a dose of 1.5 nmol or greater of neuropeptide Y – administered through a 22 gauge cannula directly into the right lateral ventricle – completely inhibited kainic acid-induced motor seizures. By comparing the effectiveness of various neuropeptide Y structural analogs, they concluded that the antiepileptic effects were achieved through the perturbation of the Y_5 receptor subtype, the same subtype that has been identified as regulating feeding behavior. They believe that their findings are important; the Y_5 receptor may be an effective target for the discovery of new antiepileptic drugs. However, they caution that weight gain may be an unwanted side effect, because Y_5 antagonists would be expected to increase feeding behavior. They further caution that their data suggest that targeting the Y_5 receptor for development of drugs to decrease appetite may result in a drug with proconvulsant properties. Certainly an area of future research will be an attempt to differentiate the anticonvulsant activity of Y_5 receptors from their effects on feeding behavior, either through the development of chemical compounds with differential activity or the further dissection of subtypes of the Y_5 receptor.

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