J. Pharm. Pharmacol. 1998, 50 (Supplement): 77

Radioligand–receptor binding assays in the search for bioactive principles from plants

J. DAVID PHILLIPSON

Department of Pharmacognosy, The School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX

Rapid sensitive *in vitro* bioassays using specific enzymes and radioligand-receptor binding form the basis of industrial screening for potential novel drug entities. In collaboration with two multi-national pharmaceutical companies, we have applied receptor ligand binding assays using either animal or human cloned receptors to investigate plants for a range of CNS activities.

Selected Chinese plants and sources of CNS active compounds

Ten species from six Chinese genera were selected because of their use in the treatment of CNS disorders or because of close relationship to such plants. Extracts prepared in 70% ethanol were screened for their ability to inhibit binding of radioligands to 18 different receptors obtained from animal tissues. Bioassay-guided fractionation of *Schefflera bodinieri* resulted in the isolation of ten bioactive novel triterpenoids and *Clerodendrum mandarinorum* yielded fourteen known compounds identified as triterpenoids, steroids, flavonoids, pyrones and saccharides. *Uncaria rhynchophylla* yielded known heteroyohimbines and polyphenols as active principles. Compounds were identified on the basis of spectral data, mainly ^{1}H and ^{13}C nmr and ms. All of the isolated compounds had IC_{50} values in the μM range in contrast to the nM values of the radioligands.

Should plants be selected for biological testing or collected at random?

Many plants have traditional reputations for the treatment of pain and more than 20 endogenous neuropeptides are implicated in the mediation of pain. The nine amino acid neuropeptide bradykinin II is implicated in the pathophysiological processes accompanying tissue damage i.e. inflammation and hyperalgesia. BK II human cloned receptors expressed in CHO cells were used to compare the activities of 300 plant species selected for their traditional use in relief of "pain" and 300 plants with no such reputations. Methanol extracts of the selected plants gave a 22% "hit rate" in comparison with 7.3% for the non-selected plants. These values were reduced to 6% and 0.6%, respectively, when polyphenolic compounds were removed from the extracts.

Should polyphenols (tannins) be removed from plant extracts prior to testing?

In some industrial screens polyphenols are removed from plant extracts before screening takes place. Twenty pure polyphenols were examined for their activity in 16 radioligand-receptor binding assays. At 10^{-5}M concentrations all of the polyphenols failed to inhibit binding of radioligands to 10 of the 16 receptors under the assay conditions and 16 of the polyphenols were active against single receptors. Further research is needed to investigate the specificity of polyphenol-receptor interactions. Active compounds may well be missed when screening is carried out on extracts from which polyphenols are removed.

Conclusion

Radioligand-receptor assays are useful biological tests for guiding fractionation of plant extracts in the search for bioactive compounds. In theory, the technology is available to screen all 250,000 species of higher plant on Earth for a range of biological activities. In practice, many plants have been screened but because of weak activity, or lack of specificity, of isolated compounds, the results are buried in company files. The objective for industry is to identify novel drug entities, or leads to them, by selecting compounds with activity against one subset of receptor and not against related receptors. Some 50,000 such tests can be done on a daily basis. Academic research cannot match such numbers and hence needs to be targeted, perhaps by following ethnomedical leads using a narrow range of biological activities. Radioligand-receptor binding assays are powerful analytical tools which can be used to identify bioactive compounds from plant extracts. In addition to searching for novel drug entities, there is a need to provide scientific evidence for the use of traditional plant medicines.