

EDITORIAL

The benzodiazepines: recent trends¹

The benzodiazepines have now been available to the clinician for over 20 years, since the introduction of chlordiazepoxide (Librium) in 1960. The broad and unique spectrum of activity of these compounds as anxiolytics, hypnotics/sedatives, anticonvulsants and muscle relaxants, together with their low toxicity and an essential absence from peripheral side-effects, has resulted in compounds of this class becoming the most frequently prescribed of all psychotropic drugs. Although it is difficult to obtain an accurate estimate of their usage in England, in Canada 1 in 10 of the population receives a prescription for these drugs each year, while over 30% of hospitalized patients is given one or other of these agents (Ban *et al.* 1981).

USE OR OVER-USE

The indications are that the benzodiazepines are suffering a significant though small decline in their popularity, but their continued widespread use has resulted in some disquiet concerning their over-use. A number of cases of dependence on these compounds has been reported, though with respect to their widespread usage the percentage of such difficulties is extremely small and rarely becomes apparent without long-term administration of the compounds. However, with regard to their anxiolytic activity recent evidence from Davis *et al.* (1981) in Oxford would contra-indicate such prescription habits. It is well known that animals subjected to certain stressful situations are able to adapt to their new environment over a period of time. The Oxford group has found that the benzodiazepines are able to inhibit this adaptation response, and the results of experiments so far indicate that such inhibition can occur in situations normally experienced by man (J. A. Gray, personal communication). This would imply that, while short-term usage of the benzodiazepines to overcome some acute anxiety problem is acceptable, continued ingestion of the compounds would reduce the ability to cope with stressful situations.

THE PROLIFERATION OF COMPOUNDS

There is little evidence that the pharmacological profile of any single compound among the many now available to the clinician is significantly different from any other: i.e. all produce sedation/anxiolysis in approximately the same proportions. It is, however, possible to use an appropriate drug to accentuate a particular effect by relying on the differing pharmacokinetic profiles of the compounds; for example, the choice of one of short biological half-life for night time sedation and a compound with a longer biological half-life if the drug effects are to be protracted, as in the treatment of anxiety. The fact that all of the compounds currently available display the varying aspects of their pharmacological profile to a similar extent has provided a considerable impetus for research into the mechanisms of action of these drugs, in the hope that compounds might be found with a more restricted spectrum of activity.

THE BENZODIAZEPINE RECEPTOR: EARLY DAYS

The observations made independently in 1977 by Squires & Braestrup in Copenhagen and Möhler & Okada in Basle, that the mammalian brain possesses specific receptors for the benzodiazepines,

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produced a remarkable explosion of research interest in these compounds. These initial experiments showed, with the use of radioactively labelled diazepam, that binding sites existed on cell membranes in the mammalian CNS which recognized the benzodiazepines specifically and with high affinity. Many further experiments have since been carried out which suggest that these 'binding-sites' are part of the receptor complex through which the benzodiazepines exert their effects.

Initially, it was thought that the benzodiazepine receptors consisted of a single homogeneous population, and the inference was therefore that all of the pharmacological actions of these drugs were mediated via this single receptor. However, work by Squires *et al.* (1979) indicated that the triazolopyridazine CL 218872 (see Fig. 1) was able to displace diazepam from its binding sites in

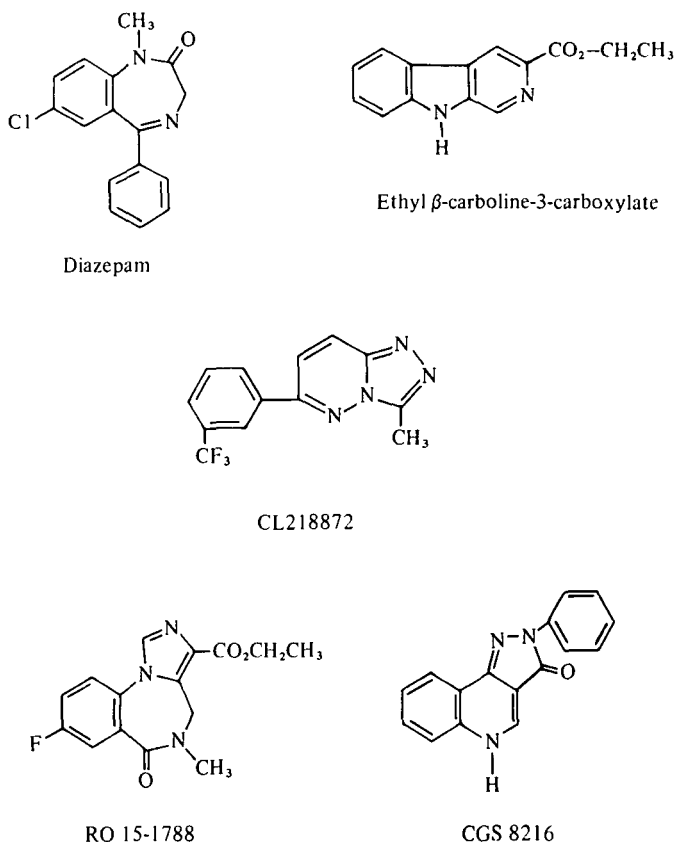


FIG. 1.

a manner which indicated that two binding sites existed: sites which could be differentiated by this compound but not by diazepam. The further claim (not since confirmed) that this compound produced anxiolytic and anti-convulsant effects but little sedation aroused intense interest, the hypothesis being that one of the receptor subtypes was responsible for the sedative effects of the benzodiazepines, while the other mediated the anxiolytic and anti-convulsant actions. Further support was added to the multiple site hypothesis by studies on the thermal inactivation of benzodiazepine receptors, the stability of which could not be adequately explained by the assumption that only a single homogeneous population of receptors existed (Squires *et al.* 1979).

THE BENZODIAZEPINE RECEPTOR: ONE OR TWO?

In searching for the endogenous ligand for the benzodiazepine receptor Braestrup *et al.* (1980) obtained a compound ethyl β -carboline-3-carboxylate (β -CCE) from human urine (see Fig. 1). Although it is now clear that this compound was an experimental artefact, β -CCE nevertheless has an extremely high affinity for the benzodiazepine receptor and has proved to be an extremely valuable experimental tool.

This compound was found to be potent in displacing radiolabelled diazepam from CNS membranes, but its potency varied depending on which brain region had been used to prepare the membranes. It was suggested that the explanation of this phenomenon was that two subtypes of benzodiazepine existed, a Bz₁ type with a high affinity for β -CCE (1 nM) and a Bz₂ type with a lower affinity (about 12 nM), though both subtypes appeared to show the same affinity for the benzodiazepines (Nielsen & Braestrup, 1980). The numbers of these two subtypes varied independently in different brain regions, thus explaining the variation in potency for the displacement of the diazepam. Thus further support was obtained for the multiple receptor hypothesis. Subsequent behavioural work, however, revealed that β -CCE was a proconvulsant: i.e. the compound appeared to have actions diametrically opposed to those of the benzodiazepines (Oakley & Jones, 1980; Tenen & Hirsch, 1980; Cowan *et al.* 1981). Further derivatives were made and subsequent work with these compounds has shown the methyl ester to be a convulsant, while the *n*-propyl ester exhibits no overt actions alone but, like the benzodiazepines, is able to block the convulsions produced by the methyl ester (Jones & Oakley, 1981).

While the β -carboline-3-carboxylates have markedly different actions with regard to anti-convulsant activity, their complete behavioural profile has not yet been fully investigated, due to the extremely short biological half-life of these compounds. It is clear, however, that β -CCE is able to suppress the increase in drinking produced by benzodiazepines under conditions thought to be predictive of anxiolytic activity (Brown & Johnson, 1982) and to reverse the sedative effects of flurazepam (Cowan *et al.* 1981). However, all three of these esters are able to differentiate between the two proposed subtypes of benzodiazepine receptor in binding experiments with the same degree of preference, making the significance of these two receptor classes unclear.

It is, however, possible to distinguish the propyl ester (anti-convulsant) from the methyl ester (convulsant) by the fact that the affinity of the latter compound is significantly decreased by the presence of GABA and sodium chloride, while under identical conditions the affinity of the propyl ester is increased, in a manner similar to that displayed by the benzodiazepines (Doble *et al.* 1982*b*). It is therefore possible to identify 'benzodiazepine-like' activity in the test tube by the fact that the affinity of such compounds for the receptor is increased in the presence of GABA and sodium chloride, while the affinity of compounds with the opposite behavioural actions is decreased. The effects of GABA suggest some interaction of benzodiazepines with this important inhibitory neurotransmitter in the brain. It is now widely believed that the activation of benzodiazepine receptors serves in some way to enhance GABA actions in brain.

While there is now considerable support for the hypothesis of multiple subtypes of the benzodiazepine receptor, other biochemical evidence suggests that the observations may be explained by different states, or conformations of the same receptor (Doble *et al.* 1982*a*). The significance of the existence of multiple subtypes of the receptor, in any case, remains unclear, although it continues to provide a considerable impetus to the research effort.

BENZODIAZEPINE ANTAGONISTS

Changes in the basic structure of the benzodiazepine molecule by the group at Hoffman la Roche have led to the development of antagonists to the actions of these compounds (Hunkeler *et al.* 1981). Ro 15-1788 (see Fig. 1) is one such example, having a high affinity for the benzodiazepine receptor. It is assumed that its remarkable ability to reverse specifically the actions of the

benzodiazepines is due to its interaction at the receptor itself. However, the compound is also able to reverse effectively the actions of β -CCE, and this antagonism of two diametrically opposed actions has led to considerable speculation as to the nature of the interaction of these compounds with the benzodiazepine receptor (Nutt *et al.* 1982). While Ro 15-1788 is structurally related to the benzodiazepines, the compound CGS 8216 (see Fig. 1), from Ciba-Geigy, shows little such resemblance, though it exhibits a very high affinity for the receptor. Little further information is available about this compound so far, except that it is reported to antagonize the actions of the benzodiazepines by a direct interaction at the benzodiazepine receptor itself (Czernik *et al.* 1981).

ENDOGENOUS LIGANDS FOR THE BENZODIAZEPINE RECEPTOR

As the discovery of opiate receptor sites in the brain led, in turn, to the discovery of endogenous opioids (the enkephalins), so it was hoped that it might be possible to identify endogenous benzodiazepine-like compounds in the brain. This area of research, however, has proved to be a graveyard of dashed hopes (Martin, 1980), and arguments have been put forward concerning the necessity to envisage such a compound (see Möhler, 1981). The most recent reports concern a polypeptide found in the small intestine and bile duct which is able to displace competitively the benzodiazepines from their binding sites (Woolf & Nixon, 1981). This compound, named nepenthin, has now been purified to apparent homogeneity and appears to have a molecular weight of ~ 16000 , though the stability of its displacing activity after treatment with proteases suggests that a lower molecular weight fragment retains activity. Little is yet known about the compound, though anti-bodies prepared to the purified material have been used in double antibody labelling experiments on rat brain slices, the result of which suggest that an immunologically similar material is present in some cells of deep cortical regions of the forebrain.

SUMMARY AND CONCLUSIONS

The discovery of non-benzodiazepinoid compounds which are able to displace effectively the benzodiazepines from their receptors, together with the apparent existence of multiple types of this receptor, have resulted in new hopes that the different behavioural actions of these drugs can be effectively separated. Indeed, chemical modifications of the molecule have resulted in the preparation of effective antagonists and to compounds with a greater anxiolytic/sedative ratio than those currently available to the clinician. However, although we have a great deal of information concerning the neurochemical actions of these compounds, a detailed understanding of the structure-activity relationships in this series can only be attempted with similarly detailed clinical/behavioural evidence concerning the profile of action of the individual compounds. Such a task is, of course, a daunting one but, unless we are to continue to rely on serendipity to provide us with compounds which exhibit more restricted profiles of action, this information must be obtained. Therefore it is perhaps in this field of endeavour where greater efforts and support must be sought in the future.

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