news and views

GABA and benzodiazepine receptors

from Leslie L. Iversen

MINOR tranquillisers of the benzodiazepine series (diazepam = Valium for example) are the most widely prescribed of all psychotropic drugs. The discovery last year (Squires & Braestrup *Nature* **255**, 732; 1977; Möhler & Okada *Science* **198**, 849; 1977; see *News and Views* **266**, 678; 1977) of specific high affinity binding sites for ^{3}H-diazepam in mammalian brain, possibly representing a novel type of 'benzodiazepine receptor', was thus an exciting breakthrough.

Previous work on this group of drugs had suggested that they might mimic or enhance the synaptic actions of the inhibitory amino acid transmitter γ-aminobutyric acid (GABA) in the brain. At first, biochemical studies failed to reveal any direct connection between benzodiazepines and GABA at the receptor level. Thus benzodiazepines did not influence the binding of ^{3}H-GABA to its receptor sites in brain membrane preparations, and conversely GABA and related drugs did not influence ^{3}H-diazepam binding. A series of recent studies, however, now suggests that some interaction may exist between the two types of receptor. Thus, Tallman *et al* (*Nature* **274**, 383; 1978) report that GABA and the GABA-like drug muscimol cause an increase in the apparent affinity of binding sites for ^{3}H-diazepam in rat cortical membranes *in vitro*. This effect is blocked by the GABA antagonist bicuculline, which itself lowers the affinity of the drug receptor sites for ^{3}H-diazepam.

Other studies suggest that, as with enkephalins and opiate receptors, brain normally contains an endogenous ligand for the benzodiazepine sites. Thus, Karobath *et al.* (*Eur. J. Pharmac.* **49**, 323; 1978) and Marangos *et al.* (*Life Sci.* **22**, 1893; 1978) describe the existence in acid acetone extracts of bovine brain of substance(s) (molecular weight less than 1000) which inhibit the binding of ^{3}H-diazepam. The specificity of these compounds, however, is not yet clear. The substance described by Karobath *et al.* for example, was present not only in brain but could be detected in even larger amounts in peripheral tissues such as heart and skeletal muscle.

A very interesting proposal has been made by Toffano *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **75**, 4024; 1978), (see also Guidotti *et al. Alfred Benzon Foundation Symposium GABA Neurotransmitters*, 22–25 May, 1978, Munksgaard, Copenhagen, in press; Guidotti *et al.* this issue of *Nature*, page 535), who describe the purification from rat brain of a protein which seems to act both as a ligand for benzodiazepine receptors and as a modulator of GABA receptors. They studied the binding of ^{3}H-GABA to synaptic membranes from rat brain *in vitro*, and confirmed what others have found (Enna & Snyder *Molec. Pharmac.* **13**, 442; 1977; Greenlee *et al. Life Sci.* **22**, 1653; 1978), namely that high affinity binding of ^{3}H-GABA is not detectable in freshly prepared membranes but becomes evident only after the membranes have been subjected to exhaustive washing. In fresh membrane preparations only a limited amount of specific ^{3}H-GABA binding, with relatively low affinity (K_d ~ 200 nM) can be detected, whereas after repeated washing, freezing and detergent treatment specific binding increases and a high affinity component (K_d ~ 20 nM) is revealed. The supernatant fractions from fresh membrane preparations contained a substance which inhibited high affinity GABA binding when added to washed membranes. Chromatographic purification of this material yielded a 500-fold enrichment of a heat stable protein, with apparent molecular weight 15,000. The possible relation of this endogenous inhibitor of GABA receptors to benzodiazepine receptors stems from the observation that the purified substance is able to inhibit the binding of ^{3}H-diazepam when brain membranes are preincubated in its presence. Furthermore, incubation of fresh membrane preparations with quite low concentrations of various pharmacologically active benzodiazepines seems to displace the endogenous inhibitor and converts the GABA binding properties of such membranes to the high affinity type normally seen after removal of the inhibitor protein by washing. Thus, the benzodiazepines might act normally to enhance the actions of GABA at its receptors by displacing an endogenous modulator protein which forms part of a macromolecular complex constituting the GABA receptor-ionophore.

This attractive hypothesis will certainly generate a great deal of interest and further work. It is perhaps surprising that small amounts of the endogenous GABA receptor inhibitor could be detected in non-cerebral tissues such as liver. The relevance of this mechanism to the pharmacological actions of benzodiazepines also rests critically on the correlation between the known pharmacological actions of various benzodiazepines *in vivo* and their ability to displace the endogenous modulator protein from brain membranes; so far only a limited number of such drugs have been tested.

Our understanding of the cellular localisation and molecular nature of the benzodiazepine receptors remains limited. Labelling of benzodiazepine receptors *in vivo* has been demonstrated using ^{3}H-flunitrazepam (Chang & Snyder *Eur. J. Pharmacol.* **48**, 213; 1978) and ^{3}H-diazepam (Wililamson *et al.* page 533, this issue of *Nature*). The findings of Chang *et al.* (*Proc. natn. Acad. Sci. U.S.A.* in the press) suggest that these receptors are located in large part on glial cells in rat brain. They report that a variety of chemical or surgical lesions which destroy the neuronal elements in corpus striatum or cerebellum of rat brain fail to alter the density of benzodiazepine binding sites in these brain areas. Furthermore, benzodiazepine sites were enriched in subcellular fractions containing glial cells, and like Guidotti *et al.*, Chang *et al.* were able to detect high affinity benzodiazepine binding sites in membranes prepared from rat C6 glioma cells. The postulated glial localisation of benzodiazepine sites, however, is not easy to reconcile with the notion that these sites interact directly or indirectly with GABA receptors, which are thought to be located largely on neurones. The fact that ^{3}H-diazepam and ^{3}H-GABA binding sites do not show parallel distributions among brain regions in any case suggests that they are not necessarily linked—and indeed may not even occur on the same cellular elements. □

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