Characteristics of $GABA_B$ receptor binding sites on rat whole brain synaptic membranes

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Commentary by

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Reflecting on the past is often affected by an incomplete recall of the events – one always remembers the good parts but amnesia can sometimes influence recall of the less exciting and more tedious aspects. The work which culminated in the report on $GABA_B$ receptor binding, which appeared in the January 1983 issue of the Journal, had its exciting moments but there were times when we wondered if the binding site would ever be detected. In fact, we were not the first to *attempt* the detection of $GABA_B$ binding sites using a radiolabelled ligand but the failure of the previous group did tend to reduce our confidence of ever being able to detect the site on CNS neurones. Nevertheless, we were certain that the $GABA_B$ receptor was present in the brain (Bowery *et al* 1980) and, therefore, felt that we should be able to label it under appropriate conditions.

Prior to 1980 only one receptor for the inhibitory amino acid, GABA, existed (see Curtis & Johnston, 1974; Krnjevic, 1974). This was coupled to Cl^- channels in neuronal membranes and could be modulated by benzodiazepines and barbiturates. This receptor was present not only within the brain but also on neurones of the peripheral autonomic system (Bowery & Brown 1974; Adams & Brown 1975). As a consequence, I wondered if its presence on sympathetic ganglion neurones might extend to the nerve terminals where their activation could provide a model for chloride-dependent presynaptic inhibition in the spinal cord. Since it was impossible to measure Cl^- conductance in sympathetic nerve terminals we measured the neurally-evoked release of ^{3}H-noradrenaline from rat isolated atria (Bowery & Hudson 1979). The outcome of these experiments was that GABA did reduce the release of transmitter as predicted, but the process was not Cl^- dependent and, most importantly, the action of GABA was not blocked by bicuculline, the established GABA receptor antagonist. It seemed to us that a distinct receptor was involved and so we proceeded to characterise it in peripheral tissues (Bowery *et al* 1981). The possibility of a separate receptor for GABA was disputed by other groups and although we felt that we were able to demonstrate the receptor in the CNS, as well as in the periphery, by measuring radiolabelled neurotransmitter release, it was difficult to convince everyone that a novel receptor was responsible. The advent of a binding assay made all the difference so much so that one adversary even wrote to admit defeat when this binding paper appeared in the Journal.

We had wanted to develop a binding assay for the $GABA_B$ site for more than a year but we could not see how we could use ^{3}H-GABA as this had already been employed to label the bicuculline-sensitive $GABA_A$ receptor (Zukin *et al.*, 1974). It seemed essential that we obtained the radiolabelled form of baclofen, which we had previously shown to be selective for $GABA_B$ sites (Bowery *et al.*, 1981) to avoid any contamination by $GABA_A$ site binding. CIBA-GEIGY were the only source of this radioligand but we were unable to obtain a sample of the compound even though I kept up a constant 'request line' to them. Unbeknown to me they were trying to do the experiments we wanted to do. Eventually, after nearly one year, I received a letter with a sample of tritiated baclofen from Basle. The letter contained details about all the conditions that had been tried in an attempt to obtain the binding of ^{3}H-baclofen to neuronal membranes. All had failed and they decided that the time had come to stop. This was very useful information for us because it meant that we did not have to try the conditions ourselves but could branch off in another direction. All of the conditions tested thus far had been analogous to those

used previously for other ligand binding assays and were not really physiological. We decided that because our functional assays were all performed in a physiological solution we would perform the binding assay in the same solution, namely Krebs-Henseleit solution. Fortunately, we knew that baclofen is not an inhibitory substrate for the GABA transport process and we confirmed that ^{3}H-baclofen is not accumulated by neurones. We could therefore leave sodium ions in the buffer solution.

Even in our first experiment we obtained a specific component of binding which, although small (~17% of total) meant it was worth pursuing. The rest of the story is contained in the article from 1983 but it rapidly became apparent that binding was dependent on divalent cations. This explained why $GABA_B$ site binding had never been detected in previous studies with ^{3}H-GABA, as a tris citrate solution was invariably used (see Enna & Snyder 1977). This would chelate the essential divalent cations. On realising this we added Ca^{++} to a tris hydrochloride buffer solution and used ^{3}H-GABA as the ligand. The amount bound was almost double that obtained in the absence of Ca^{++} but the characteristic of this extra binding component was completely different from the Ca^{++} -independent portion. These two components exhibited pharmacological profiles which were consistent with the $GABA_A$ and $GABA_B$ characteristics previously shown in the functional assays. Thus, although we used ^{3}H-baclofen in the beginning to detect the binding site, the information obtained subsequently enabled us to use ^{3}H-GABA to label both receptor types.

The results described in the paper really established the existence of $GABA_B$ receptors in higher centres and provided the reassurance needed to perform further studies in the brain. It was soon after this that the synaptic role of the $GABA_B$ receptor was described (Dutar & Nicoll 1988) and the actions of agonists and antagonists for the receptor established.

It is rare that one has the opportunity of discovering a new functional receptor but it is important that the scientific community accepts the observation. This is now the case such that our $GABA_A$/$GABA_B$ nomenclature is used routinely world-wide but at the beginning it was not easy to get complete support for the idea - but this is surely the basis of scientific rigour!

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