Pharmacological Research 116 (2017) 32–38

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

GABA Australis, some reflections on the history of GABA receptor research in Australia

Graham A.R. Johnston

Pharmacology, School of Medical Sciences, The University of Sydney, NSW 2006, Australia

ARTICLE INFO

Article history:
Received 6 December 2016
Received in revised form
10 December 2016
Accepted 21 December 2016
Available online 22 December 2016

Keywords:
GABA
Bicuculline
Muscimol
Modulators
Epilepsy
Flavonoids

ABSTRACT

Research on GABA receptors has a long history in Australia dating from 1958 with David Curtis and his colleagues in Canberra. This review traces many of the advances made in Australia guided by highly cited publications and some obscure ones. It covers the discovery of key chemicals with which to investigate GABA receptor function including bicuculline, muscimol, phaclofen, THIP and (+)-CAMP. Also described are findings relevant to the involvement of mutant GABA receptors in inherited epilepsy. The modulation of GABA receptors by a bewildering range of chemicals, especially by flavonoids and terpenoids, is discussed.

We now know that GABA (or to give it its full name γ-amino-*n*-butyric acid or its strict chemical name 4-aminobutanoic acid) is a neurotransmitter of major significance in the brain, being released by up to 40% of neurones to activate chloride channels resulting generally in inhibition of firing [1]. Research carried out in Australia has made highly significant contributions to our understanding of the function of GABA receptors over the last 50 years [2].

GABA was first identified in the brain in 1950 by Eugene Roberts, Sidney Undenfriend, Jorge Awapara and their colleagues in the USA [3]. It had been described earlier in potatoes. This led to Eugene Roberts giving lectures on "why the brain is like a potato"! A flurry of activity investigating the biological properties of this deceptively simple molecule then ensued. Its neuroactive effects were of particular interest. By 1958, Stephen Kuffler, Dominick Purpura, Ed Kravitz and their colleagues in the USA had published seminal papers relating to the likely effects of GABA as an inhibitory neurotransmitter. In that same year, David Curtis and John Phillis at the Australian National University in Canberra published a brief note, less than one page, to Nature entitled "Gamma-Amino-*n*-Butyric Acid and Spinal Synaptic Transmission" in which they described the depressant action of GABA on neuronal firing in the cat spinal cord [4]. They stated "These results make it extremely unlikely that γ-amino-*n*-butyric acid is a transmitter substance in the mammalian spinal cord." Further, they stated "Accordingly, it is probable that γ-amino-*n*-butyric acid has a depressant action on the whole soma-dendritic membrane of centrally located neurones". Thus started the history of GABA research in Australia in an atmosphere of denial regarding its transmitter status and a mystery as to what its functions really were in the CNS.

A relatively independent review of the history of the discovery of GABA (and L-glutamate) as neurotransmitters in the CNS can be found in 'When and why amino acids?' in the *Journal of Physiology* in 2010 by Kresimir Krnjevic [5], who spent a post-doc in Canberra in 1956–1958.

These reflections on the history of GABA receptor research in Australia are a very personal view. The author apologises for the many excellent publications in the field that he has failed to mention. The author has relied on citation analyses by the Institute for Scientific Information's Web of Science and Google Scholar for data on the contributions of Australian scientists to research on GABA receptors and to identify highly cited publications. At the time of writing, there were some 44,234 publications in the Web of Science that had 'GABA' or 'aminobutyric acid' in the title. Of these 964 publications, (2%) had authors listed as from Australia. This is a commendable achievement as it is widely accepted that Australia has approximately 1% of the world's scientists.

The most highly cited GABA research paper authored by an Australian scientist appears to be 'Sequence and functional expression of the $GABA_A$ receptor shows a ligand-gated receptor super-family'

E-mail address: grahamj@mail.usyd.edu.au

http://dx.doi.org/10.1016/j.phrs.2016.12.031
1043-6618/

by Peter Schofield et al. in *Nature* in 1987 [6]. Peter is an Australian scientist who was working at that time at Genentech in California. He subsequently returned to Australia to the Garvan Institute of Medical Research and now heads Neuroscience Research Australia in Sydney. This article received 1475 citations in the Web of Science and 1635 in Google Scholar. (Citation analysis is certainly not an exact science! The Web of Science appears to miss many chapters in books. In addition Google may count all the citations to a particular publication that give the incorrect year, volume or page numbers. It does happen.) This is a seminal paper representing a major breakthrough in our molecular understanding of ionotropic GABA receptors. Many similar studies followed showing the rich diversity of these receptors and enabling studies on recombinant receptors of known subunit composition to be carried out. These insights revolutionised the field.

The most highly cited review covering GABA published from Australia is the extensive review by David Curtis and the author 'Amino acid transmitters in the mammalian central nervous system' in *Ergebnisse der Physiologie, Biologischen Chemie und Experimentellen Pharmakologie* in 1974 [7]. (1131 citations in the Web of Science, 1476 in Google Scholar). This was a particularly timely review covering the actions of GABA in various CNS regions at a time when interest in the field was gaining momentum.

Highly cited GABA research papers from Australia include "The excitation and depression of spinal neurones by structurally related amino acids" (738/872 citations) [8], "Bicuculline, an antagonist of GABA and synaptic inhibition receptors in the spinal cord of the cat" (695/709 citations) [9] and "Mutant $GABA_A$ receptor γ-2-subunit in childhood absence epilepsy and febrile seizures" (521/718 citations) [10]. The significance of these publications is discussed below.

1. GABA as a transmitter?

David Curtis was joined in 1958 by Jeff Watkins, an organic chemist from Western Australia who had trained in Cambridge. His arrival led to a very useful collaboration resulting in many pivotal publications on the role of GABA and also L-glutamate in the central nervous system. The problem facing the ANU team was that both GABA and L-glutamate acted on most, if not all, neurones in the CNS. At the time, this evidence seemed like a most un-transmitter-like behaviour. Transmitters like acetylcholine had selective actions on central neurones. Furthermore GABA and L-glutamate were found in abundant quantities in the CNS. To their great credit they soldiered on in their studies. By 1960 Curtis and Watkins published a paper in the *Journal of Neurochemistry* on "The excitation and depression of spinal neurones by structurally related amino acids" [8]. In this paper, they described the key structure-activity properties of GABA and L-glutamate. Amongst their findings was the observation that glycine had a moderately strong depressant action on the firing of spinal neurones.

2. Glycine and strychnine

Glycine proved to be the key to the puzzle. In 1966, Curtis attended a conference of neurobiologists in Stockholm and heard of the studies by Morris Aprison and Robert Werman [11] showing that the concentration of glycine in the ventral grey matter of the cat spinal cord was higher than that of any other amino acid and that this amino acid was associated with some interneurons. Furthermore glycine hyperpolarised spinal motoneurones. As Curtis states in his autobiography [12]: "This very strong indication that glycine could be an inhibitory transmitter in the mammalian spinal cord was indeed a very significant break-through, which subsequently should have been recognised internationally as a major advance in the understanding of mammalian central neurotransmission." Bradley and Eccles had published that spinal inhibition could be blocked with strychnine [13]. David Curtis on his return to Canberra showed that strychnine did indeed block the actions of glycine but had little effect on the activity of GABA in the spinal cord [14]. This and other observations were finally strong evidence that glycine was undeniably an important neurotransmitter in the spinal cord.

The more potent action of GABA compared to that of glycine on spinal neurones that the Canberra group had observed was in cats anaesthetised with barbiturates. We now know that barbiturates enhance the action of GABA but have little effect on glycine on most neurones. Where did this leave GABA? It could not therefore be a transmitter at strychnine-sensitive spinal synapses.

3. Bicuculline as a GABA antagonist

The author joined the Canberra group in 1965. He had been recruited by Jeff Watkins who wished to go England for personal reasons but wanted the synergies between chemistry and physiology in Canberra to continue. Originally from Sydney, the author had a similar background to Watkins as an organic chemist trained in Cambridge. Over time the work was extended to include biochemical pharmacology and neurochemistry. It was a very successful collaboration resulting in some 130 publications, mostly on GABA (38 with Curtis) until he moved to Sydney in 1980. Collaborating with Curtis on the strychnine-glycine investigations, the author began studying other convulsants, reasoning that if GABA was indeed an inhibitory neurotransmitter, agents that blocked GABA-mediated inhibition also might have convulsant actions. As it eventuated, most convulsants examined turned out to be glycine antagonists like strychnine with one exception.

In 1970, bicuculline, one of a number of convulsant isoquinoline alkaloids tested, was shown by the Canberra group to be a GABA antagonist at what became known as $GABA_A$ receptors [9]. These receptors were insensitive to strychnine. Bicuculline was soon shown to inhibit synaptic inhibition in various regions of the CNS. Bicuculline itself was somewhat difficult to use and for many uses it was easier to use a quaternary salt like bicuculline methochloride [15]. Together with many other findings, this provided unequivocal evidence that GABA was a central inhibitory transmitter of major significance. The discovery of bicuculline and related substances as $GABA_A$ receptor antagonists has been covered in an historical review for the *British Journal of Pharmacology* [16].

In his 2006 autobiography [12], writing of the GABA-glutamate neurotransmitter puzzle, David Curtis stated "my negative conclusions related to transmitter functions were unfortunately based on a faulty technique and incorrect assumptions". To be fair, GABA and L-glutamate have turned out to be very different from specialised neurotransmitters like acetylcholine and the biogenic amines. Curtis was right to be cautious in his speculations and his group in Canberra over time provided significant experimental data to support neurotransmitter functions for GABA and L-glutamate.

4. Muscimol as a $GABA_A$ receptor agonist

Muscimol, a psychoactive isoxazole isolated from *Amanita* and related mushrooms, was reported by the Canberra group in 1968 to have a strychnine-insensitive depressant action on neurones [17]. Later it was shown that this depressant action was sensitive to blockade by bicuculline [18]. The GABA-like chemical structure of muscimol that had been recognised by the author [17] was confirmed by X-ray studies by the group in Copenhagen headed by Povl Krogsgaard-Larsen [19]. This led to a very fruitful collabora-