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GABA pharmacology—what prospects for the future?

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Abstract

Following the recognition of GABA as an inhibitory neurotransmitter, the discovery of high affinity GABA uptake, and the characterisation of GABA receptors great progress has been made in developing GABA pharmacology. Tiagabide, the first marketed GABA uptake inhibitor may be followed by new and more selective uptake inhibitors. Knowledge of the molecular pharmacology of GABA-A receptors, both synaptic and non-synaptic, may lead to improved anti-anxiety/anticonvulsant agents devoid of the sedative and dependence liabilities of earlier compounds and new hypnotics. Gaboxadol (THIP) is an example of a novel hypnotic that acts on GABA-A receptors by a non-benzodiazepine mechanism. Exploiting neurosteroid interactions with GABAergic mechanisms also holds much future promise.

Keywords: Tiagabide; GABA uptake; GABA-A receptor; Gaboxadol; THIP; Neurosteroids

Readers will have learned from previous contributions of the significant advances made in our understanding of the complexities of GABA pharmacology in recent years, and of the many ways in which these are beginning to be applied to new therapies. The following comments should be regarded as a highly personal account which interspersses some history with comments on future trends.

My own introduction to research on GABA came on joining the Department of Neurobiology at Harvard Medical School as a postdoc in 1965. I was fortunate to work with Ed Kravitz and Masanori Otsuka in putting together one of the final pieces of the jigsaw puzzle to demonstrate that GABA acted as an inhibitory neurotransmitter in the crustacean nervous system. Although GABA was well known to be an important neurochemical in the vertebrate and invertebrate nervous system, the hypothesis that it acted as an inhibitory neurotransmitter was by no means accepted in the 1960s. The consensus of an influential international conference on GABA held in 1960 seemed to be that GABA played a metabolic role rather than a chemical messenger role in the nervous system [1]. But the team at Harvard by a painstaking combination of neurophysiology, microdissection and neurochemical micro-analysis had amassed an impressive body of data showing that GABA was selectively concentrated in inhibitory motor neurons and not in excitatory motor neurons in the lobster nervous system and it exactly mimicked the physiological actions of the inhibitory transmitter[2]. They had even carried this analysis down to a single cell level [3]. We were given the task of showing that GABA was selectively released from inhibitory motor nerves on stimulation. This was made easier by the fact that crustacean muscles receive a dual motor innervation with separate excitatory and inhibitory motor axons, so we were able to look for GABA release from nerve-muscle preparations. The one chosen eventually was the opener muscle of the crusher claw. Although this had a convenient innervation which permitted selective stimulation of inhibitory or excitatory motor inputs, the amounts of GABA released were very small—less than 1 nmol/25 min collection period. The GABA had to be concentrated from the relatively large volumes of sea water (0.5 M NaCl) used to superfuse the preparation and assayed by a sensitive enzyme-coupled assay. Nevertheless, we were eventually successful in showing a highly significant increase in GABA efflux on stimulation of the inhibitory but not the excitatory axon. Furthermore, GABA release was both frequency-dependent and required external calcium [4]. We were excited by these data and believed that it would help finally to gain acceptance of the neurotransmitter status of GABA—although when the results were presented to the Physiological Society on my return to the UK in

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1966, I received a sceptical reaction from the audience, and was only rescued from serious damage by the generous intervention of Bernard Katz! [5].

1. GABA uptake

My interest in GABA was maintained after return to Cambridge, and a collaboration with Mike Neal soon demonstrated the existence of a high affinity uptake of exogenous GABA by slices of rat cerebral cortex [6]. Subsequent work with Snyder [7] showed that radiolabelled GABA was taken up into a distinct subpopulation of synaptosomes that could be separated physically from those accumulating catecholamines by density gradient centrifugation. And with Floyd Bloom electron microscopic autoradiography was used to show that exogenous GABA was taken up by a distinct subpopulation of nerve endings in slices of rat cerebral cortex [8].

It was immediately apparent that GABA uptake represented an interesting drug-discovery target, as the catecholamine and serotonin reuptake systems were already known to be key targets for antidepressants [9]. But this idea was not to see fruition for another 30 years. The medicinal chemistry of inhibitors of GABA uptake was pioneered by Krogsgaard-Larsen et al. in Denmark [10], and Claus Braestrup and coworkers at Novo-Nordisk in Denmark discovered, tiagabide, the first GABA uptake inhibitor to be marketed as a novel anti-epileptic [11]. It is interesting to see that tiagabide, launched initially for use in epilepsy is now being investigated for other possible indications, in the treatment of psychosis [12], generalized anxiety [13], sleep in the elderly [14] and drug addiction [15].

We now realize that there are at least three different GABA transporters, located in both neurons and glial cells [16]. The one targeted by tiagabide (GAT1) is neuronal while the others are on glia and other non-neural cells [16]. There must be a rich scope for the future development of selective inhibitors of these other sites of GABA uptake—yielding new pharmacological tools of as yet unknown profiles or utility.

2. GABA-A receptors

2.1. Benzodiazepine-sensitive GABA-A receptors

The next phase of my life with GABA started on joining Merck Research Laboratories where I helped to establish their Neuroscience Research Centre at Terlings Park in England in 1983. One of our first projects was in collaboration with Danish scientists at what was then Ferrosan with the aim of developing drugs that acted as partial agonists at benzodiazepine receptors. The benzodiazepine tranquilisers had represented a major advance in psychopharmacology in the 1960s and 1970s. For a period of almost 10 years diazepam (Valium®) was the biggest selling prescription drug in the world and there were many other look-alikes. But by the 1990s the shortcomings of these drugs were beginning to be appreciated. The benzodiazepines were sedative and more importantly there was a significant dependence liability. Although as with so many psychopharmaceuticals the benzodiazepines were discovered by serendipity, their mode of action through the GABA receptor in brain was already well-established. In Switzerland, Haefely and coworkers at Roche with Möhler had pioneered the concept that improvements might be made by developing compounds that acted as partial agonists at the benzodiazepine receptor [17]. Their idea was that full agonists were required to see the full spectrum of benzodiazepine pharmacology, including sedation, muscle relaxation and dependence liability. Compounds that acted as partial agonists, however, might retain the beneficial effects of an anti-anxiety and anticonvulsant profile while no longer being sedative, ataxic or having dependence liability. It was an attractively simple hypothesis, and compounds with partial agonist properties were reported to have improved profiles in whole animal behavioural models [17,18].

In our own collaboration with Ferrosan we discovered novel partial agonist benzodiazepine compounds. For example, the imidazobenzodiazepine derivative FG8205 had a high affinity for the benzodiazepine receptor, acted as a partial agonist, and had an attractive behavioural profile—with potent anticonvulsant and anxiolytic effects but much less ataxia and less propensity to cause dependence in rodent or primate models [19]. Unfortunately the compound failed in early preclinical toxicology and none of the other attempts by us or by others to achieve this goal succeeded in producing a useful drug.

But of course this all occurred before we understood the molecular complexities of the GABA-A receptor and its multiple subtypes [20]. The idea of developing a ‘‘partial agonist for the benzodiazepine receptor’’ no longer had much meaning when one realized that this receptor came in multiple different forms. Instead the focus switched to discovering compounds that acted as selective agonists or modulators at one or other of the different GABA-A receptor subtypes [21]. The Merck team, along with Möhler and coworkers in Switzerland and many others set about the painstaking research needed to build the basis of this new pharmacology [22,23]. Great progress has been made in defining the GABA-A receptor subtypes that are important for the sedative as opposed to anti-anxiety actions of benzodiazepines through the development of genetically engineered animal models [23,24] and subtype selective compounds, e.g. the α_2,α_3,α_5-subtype-selective L-838,417 that has no efficacy at α_1-subunit forms of the receptor [25]. This helped to define the importance of GABA-A receptors containing the α_1-subunit in mediating sedative effects, and those containing α_2/α_3-subunits for anti-anxi-

ety/anticonvulsant effects. But the prize of a new medicine based on these concepts has so far still proved elusive—although there must be every hope that this will eventually happen.

Meanwhile the demand for prescription sleeping pills has continued to grow at a rate of almost 25% a year in the USA [26]. The USA market alone was worth about $1.5 billion in 2002 [26]. This field has been dominated in recent years by compounds which are non-benzodiazepine chemically but which act as more or less selective ligands for benzodiazepine binding sites in GABA-A receptors containing the α_1-subunit. These new drug classes include the cyclopyrrolones (zopiclone), imidazopyridines (zolpidem) and pirazolopyrimidines (zaleplon). Waiting in the wings are indiplon and eszopiclone (the active *S*-isomer of zopiclone). While these short-acting hypnotics represent effective sleeping aids they continue to suffer from the memory impairment, abuse potential and hangover effects that have traditionally affected benzodiazepines [27,28].

2.2. Non-synaptic GABA-A receptors and benzodiazepine-insensitive GABA-A receptors

An unexpected discovery was that several categories of GABA-A receptor appear to be associated mainly with non-synaptic locations in the mammalian CNS. For example, GABA-A receptors containing the α_5-subunit are expressed principally in the hippocampus where neurophysiological evidence suggests that they mediate tonic inhibition [29]. These receptors have a high affinity for GABA and desensitize only slowly—properties that make them well suited to respond to the low ambient concentrations of GABA in extracellular space [29]. These receptors may play a role in the functions of the hippocampus in spatial learning and memory. Mice genetically engineered to lack the α_5-subunit of the GABA-A receptor exhibited enhanced learning and memory in a water maze model [30]. Furthermore an α_5-selective GABA-A receptor inverse agonist enhanced spatial learning and memory while lacking the anxiogenic or convulsant effects associated with non-selective inverse agonists [31]. Compounds of this type could be novel cognition enhancers in the treatment of Alzheimer's disease and related dementias [31].

GABA-A receptors containing α_4- and α_6-subunits also appear to be related largely to non-synaptic sites. The α_6-subunit in combination with β- and δ-subunits is a benzodiazepine-insensitive form of the receptor which is tonically activated by low concentrations of GABA and modifies the excitability of cerebellar granule cells [32].

Receptors containing the $\alpha_4\beta\delta$-subunbit combination are localized to thalamus and hippocampus and are also benzodiazepine-insensitive. These receptors exhibited unusual pharmacological properties, as defined in a stable cell line expressing this form of the GABA-A receptor [33]. In particular they showed high sensitivity to GABA and the GABA analogue THIP (4,5,6,7-tetrahydroisothiazolo-[5,4-*c*]pyridine-3-ol). THIP, which acted as a partial agonist on various other GABA-A receptor subtypes, had greater efficacy than GABA itself on the $\alpha_4\beta\delta$-subtype. Other studies have also suggested that THIP acts on GABA-A receptors that are largely insensitive to benzodiazepines [34]. THIP (''Gaboxadol'') is currently the subject of revived interest as a sleeping aid. It appears to improve the quality of sleep according to EEG measures and in elderly subjects suffering from a reduction in non-REM sleep it normalized sleep patterns [28,35]. There may be significant advantages in hypnotic drugs that act on sites other than the traditional benzodiazepine/barbiturate mechanisms.

The $\alpha_4\beta\delta$ GABA-A receptor subtype was also found to be particularly sensitive to positive modulation by neurosteroids of the pregnane class [33]. The neurosteroids exert a number of subtle controls over GABA receptor sensitivity and much remains to be learned of this bridge between the endocrine system and CNS function [34]. Such understanding could offer rich opportunities for novel GABA pharmacology in the future.

3. Conclusions

GABA pharmacology has already yielded many important drugs that are widely used in the treatment of anxiety and panic disorders, epilepsy, muscle spasticity, sleep disorders and as anaesthetics. There is every hope that the new understanding of the molecular pharmacology of GABAergic transmission will lead to a new generation of more selective drugs with improved safety profiles—and that entirely new indications will be discovered. Research on GABA-B receptors, not reviewed here, is also an exciting field for innovative pharmacology (see contributions from N. Bowery, R. Nicoll, W. Froestl, and S. Enna at this meeting).

In looking at the past 60 years from the broader perspective of how advances in basic biomedical research become translated into practical therapeutic benefits several useful lessons can be learned.

Firstly: Scientific/medical advances often do not proceed in a straight line. In reality advances are made in fits and starts, and there are many blind alleys that lead nowhere. Rather than a linear advance, the progress of science more closely resembles a children's game of ''snakes and ladders''. The same holds true in the later stages of translation of new ideas into practical therapies, where again many pitfalls wait. Drug development is not easy—and there are more opportunities for failures than there are for successes. *Secondly:* There seems to be a ''thirty year rule'' which states that on average it takes around 30 years for a new scientific discovery to find its way to a new generally available therapy. In the context of GABA pharmacology

about 30 years elapsed between the discovery of a high affinity uptake mechanism for GABA and the launch of tiagabide, the first GABA uptake inhibitor. The fruits of the radical advances in understanding the molecular pharmacology of GABA receptors some 20 years ago are only now finding their way to the clinic. Who knows what progress we will see in 10 years time at the 70 years anniversary of GABA!

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