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Biochemical Pharmacology 68 (2004) 1549–1555
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

How does a little acronym become a big transmitter?

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Received 4 June 2004; accepted 22 June 2004

Abstract

After an overview of the early, chequered history of the discovery of GABA and its gradual acceptance as inhibitory synaptic transmitter in the brain, the article lists and discusses some of the more unexpected later developments in studies of GABA, especially its role as excitatory transmitter in the immature brain.

Keywords: Gamma-amino-butyric acid; Chloride transport; Developmental changes; History; Synaptic inhibition and excitation; Transmitters

1. Prelude

As place name or personal name, GABA antedates the current acronym; it has been used as a label for lozenges for sore throat and is currently sold as vitamin supplement. For the present purpose, the ω-amino acid known as GABA is of interest as physiological agent, whose main role appears to be that of widespread inhibitory transmitter. But GABA is not exclusively a constituent of nerves: it may have some metabolic function in microorganisms, such as *Escherichia coli* [1]; it is present in insulin-secreting cells of the pancreas, at a remarkably high concentration, but with no known function [2]. Its uneven but wide distribution can be explained by the intimate relation between GABA and glutamate, a major constituent of proteins, from which it is simply obtained by removal of the α-carboxylic group. As an ω-amino acid, unable to form peptide links, GABA could be expected to have a variety of important roles in cellular function. But this does not seem to be the case. Its most prominent function is mediation of synaptic inhibition, and that, curiously, only in crustacean muscle and vertebrate CNS.

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doi:10.1016/j.bcp.2004.06.038

2. Historical

"The present is messy; while history, after historians have finished with it, is always tidy."

"New idea: at first, they said, it can't be true; when it became obvious it was true, they said it wasn't important; and when its importance couldn't be doubted, they said, it wasn't new!"

Inevitably, historical accounts are largely arbitrary and subjective, and reflect the authors' beliefs and prejudices, and, of course, those prevailing at the time of writing. As viewed in retrospect, the story of GABA should have been straightforward, something like the following. Once people accepted the idea that nerve cells do not form a continuous reticulum – largely thanks to Cajal's [3] prodigiously industrious use of Golgi's silver technique (though Golgi himself never accepted it [4]) – the presence of a gap (aptly named 'synapse' by Sherrington [5]) would be a powerful stimulus to research on the question: how are nerve signals carried across the synapse? The simple answer that electrical signals just continue without a break from one neuron to the next would soon prove untenable, especially with regard to inhibition (widely accepted as an essential element in CNS function). Bearing in mind the abundance of GABA in the brain and its

demonstrably powerful and ubiquitous inhibitory action – quickly revealed by the rapid progress in brain studies – everyone would soon realize that GABA *had to be* the principal transmitter at inhibitory synapses. Thus, began the great explosion of studies on every aspect of GABA and GABA-mediated function.

3. Actual history

Things went quite otherwise. Until the middle of the 20th century, most neurophysiologists were much more inclined towards electrical transmission, which seemed both quick and neat – unlike the messy humoral mechanims promoted for peripheral synapses by the school of Dale [6]. Inhibition also was far from being generally accepted as a significant mechanism in the brain.

The story began effectively in 1950 when three groups [7–9] discovered large amounts of GABA in the brain. It was thought likely to have some metabolic function. With no clear supporting evidence, there was little real advance for the next few years, and mainly ups and downs for the rest of the decade. Instead of any kind of linear or monotonic process, the temporal course of ''progress'' was not unlike that of prices on the stock exchange: periods of growth unpredictably alternating with plateaus when nothing much seems to happen, or even recessions when hard-won gains evaporate.

Real growth depended on research that might appear to be of unlikely relevance, the almost simultaneous discovery of a proprioceptive organ in lobsters and crabs [10]. The large and very accessible cells of this stretch receptor, and its double (motor and inhibitory) innervation soon attracted quite wide attention [11], notably of two individuals who were to play major roles in this history: SW. Kuffler and E. Florey. Initially, Kuffler was principally concerned with the motor control of the receptor muscle [12]. Whereas Florey, having studied its physiological and pharmacological properties with Wiersma, in California [13], realized that the steady sensory discharge from the abdominal stretch receptor of crayfish was a good index of changes in receptor excitability and therefore might provide a useful assay of possible excitatory and inhibitory agents. Indeed, extracts of mammalian brain proved to have a potent inhibitory action, which was ascribed to an unknown ''Factor I'' [14]. In a fruitful collaboration with the neurochemist K.A.C. Elliott – at the Montreal Neurological Institute – Florey used the crayfish stretch receptor to assay the inhibitory potency of all known constituents of mammalian brain. Though at first misled by a bad sample of GABA, the authors [15] identified GABA as the agent responsible for Factor I activity and suggested that GABA might be an inhibitory transmitter or modulator of activity. Less direct evidence of some inhibitory action came from the finding that convulsant hydrazides depress brain GABA levels by blocking glutamic decarboxylase activity [16]. In further experiments on crustacean muscle, the action of GABA and synaptic inhibiton were found to be quite similar, resulting in a selective increase in membrane Cl^- conductance [17,18]. The way forward seemed clear. Indeed it was, but only for the crustacean peripheral synapses: systematic investigations by Kuffler and coworkers later demonstrated the specifically high GABA content of inhibitory axons [19] and then its selective release [20].

By contrast, a consensus emerged from several independent groups that GABA could not be the physiological inhibitor in mammalian brain. On closer inspection, Florey decided that GABA could not account for Factor I activity [21,22]. Applications of GABA to the cortex did not yield a clear answer: the effects were interpreted as inactivation of excitatory synapses [23] or selectively restricted to very superficial cortical layers [24]; very extensive studies by Hayashi [25] (with 125 collaborators) confirmed GABA's anticonvulsant action, but this was considered an indirect effect, the result of its transformation to γ-amino-β-hydroxybutyric acid. Though the first tests of GABA by microiontophoresis, on spinal neurons, demonstrated strong inhibition of all neuronal firing; several features, such as the lack of hyperpolarization and insensitivity to strychnine were deemed incompatible with a physiological role as transmitter at inhibitory synapses [26,27]. By the early 1960s, albeit still viewed as a potential intracellular modulator of excitability [28], GABA was largely discounted as synaptic transmitter, at least in the vertebrate CNS.

A more positive trend developed on the basis of iontophoretic studies on cortical and cerebellar neurons. In view of GABA's uniquely fast, quickly reversible and ubiquitous action, mediated by minute iontophoretic applications, Krnjević and Phillis [29] concluded that GABA was the most likely inhibitory transmitter. This idea was supported by the demonstration that the GABA content of individual synaptosomes was sufficient to elicit inhibition of cell firing [30], as well as increasing evidence that GABA is released in by neocortical [31] and cerebellar activation [32]. A major advance came with intracellular recording which permitted a more direct comparison between IPSPs and the action of GABA, in cortical [33,34]; and Deiters neurons [35]. After further pharmacological tests, which revealed selective block by picrotoxin [35,36] and then bicuculline [37], GABAergic transmssion could be demonstrated at a wide variety of sites throughout the CNS, and thus rapidly became generally accepted as the principal transmitter of inhibition, especially in the brain.

To reach this point, research had followed a far from simple logical path. GABA was not known to be a transmitter, in vertebrates or invertebrates. Inevitably, people speculated about various humoral agents identified in studies on vertebrate peripheral junctions. When it was discovered in the brain, as a close relative of glutamate, GABA seemed likely to play some role in intermediary

metabolism. Its very abundance was held as an argument in favour of a metabolic function rather than signal transduction. By a stroke of enlightened luck – there was no particular reason to suspect that the same transmitters might operate at synapses on crustacean muscle and in the mammalian brain – Florey tested brain extracts on the crayfish receptor and discovered Factor I. Only a few years earlier, this might have passed unnoticed. General opinion among neurophysologist was well expressed by a widely authoritative text [38], according to which direct inhibition played only a minor role in brain function and ''the humoral concepts have outlived their usefulness''. By the early 1950s, however, J.C. Eccles, the most outspoken opponent of chemical transmission in the brain, realized that synaptic potentials recorded intracellularly from spinal neurons could not be easily explained by electrical transmission [39]. The resulting shift in the climate of opinion among neurophysiologists – a Kuhnian ''paradigm shift'' – made chemical transmission respectable and triggered the search for putative transmitters.

4. Further progress

There was no going back from that time on. Further progress was along increasingly diverse lines as new fndings and technical possibilities opened new areas of research. Single channel recording [40] threw new light on the kinetics of Cl^- channel opening and closing, and provided more precise information on their anionic selectivity. Others were quite unexpected. Very early, it became clear that the action of GABA could be greatly potentiated by some anxiolytic drugs (benzodiazepines) and general anaesthetics [41,42], as well as some endogenous steroid hormones [43]. Bicuculline-resistant effects of GABA led to the discovery of a distinct $GABA_B$ receptor [44]: unlike the usually dominant $GABA_A$ action, this activated K^+ rather than Cl^- conductance and its slow action was mediated by a G-protein linked receptor. Once cloned and analyzed, the $GABA_A$ receptor was found to be largely homologous to the receptor for the other inhibitory transmitter, glycine. This seemed logical in view of the great similarity between their membrane effects, both causing inhibition by activating Cl^- conductance [40,45,46]. More surprizing was the substantial homology with the nicotine receptor, a strictly cationic channel. But speculation that all ligand-gated ionotropic receptors all belong to one superfamily [47] was cut short when ionotropic glutamate receptors proved to be structurally quite distinct [48].

In view of its crucial importance for the control of cellular firing, GABAergic inhibition is surprizingly labile: repetitive activation in the hippocampus that induces seizure activity is associated with a rapid fading of IPSPs [49]. Originally thought to reflect some form of desensitization, more recent studies have brought to light a curious mechanism linking post-synaptic depolarization with a retrograde selective depression of GABA release [50], which, by all accounts is mediated by some endogenous cannabinoid(s) [51–53].

A particularly interesting finding is that GABA and glycine can be co-released at a given synapse, not only from the same terminal but even from individual vesicles [54,55]. Even less expected is the co-release of GABA with glutamate from hippocampal mossy fibres [56]. Perhaps less surprizing are the extrasynaptic effects of GABA, mediated by higher affinity receptors [57]. Such receptors appear to mediate especially general anaesthetic potentiation of GABAergic activity; as well as effects of GABA either leaking from cells by reversed transport or from neighbouring synapses (''spilling-over''). A comparable leakage of glycine and its agonist taurine may also exert a significant tonic modulation of neuronal firing [58]. This may be especially important in the perinatal phase of early development when taurine levels and leakage are exceptionally high [59].

No systematic list can be attempted here of the manyfold advances over the last five decades: many of these topics are covered by other participants at this symposium. But one discovery, that during early ontogeny GABA acts as an excitatory transmitter, deserves a closer look.

5. GABA as depolarizing agent

Since some of the earliest studies of GABA, various authors have reported depolarizing excitant effects of GABA. Some proconvulsant actions observed by Hayashi [25] led him to conclude that GABA could not be the physiological inhibitor. After it was shown that primary afferent terminal depolarization – which accompanies presynaptic inhibition in the spinal cord – is probably mediated by GABA [60,61], De Groat et al. [62] found that dorsal root ganglion cells are also depolarized by GABA. How could these findings be explained if $GABA_A$ receptors mediate only Cl^- flux? From the beginnings of intracellular recording, it was noted that the direction of both IPSPs [39] and GABA effects [26,33] soon change from hyperpolarizing to depolarizing when recorded with microelectrodes containing a high Cl^- concentration. Leakage of Cl^- from the electrodes evidently reversed the normal inwardly directed electrochemical gradient for Cl^- to an outward gradient. The equilibrium potential for Cl^- (E_{Cl}) is usually only a little more negative than the resting membrane potential of central neurons. To move E_{Cl} above the resting potential does not require a huge Cl^- influx. However, such a reversal does not necessarily result in functional reversal from inhibition to excitation. The very large increase in membrane conductance caused by GABA [34,63] tends to clamp the membrane near E_{Cl}: as long as E_{Cl} is more negative than the threshold for spikes, firing is inhibited. Even if the threshold is exceeded, strong depolarization may suppress firing by inactivation of Na

current. That this more than idle speculation is shown by GABA's role as mediator of the conspicuous presynaptic inhibition at primary afferent synapses in the spinal cord [60].

6. What determines E_{GABA}?

Frequently observed depolarizing effects of GABA – for example more often at dendritic than somatic levels – had led to the suggestion that GABA may partly activate Na^+ conductance at certain sites. As first pointed out by Kaila [64], even when the Cl^- gradient is inward, depolarizing IPSPs can be generated by a relatively large efflux of bicarbonate ions. Though significant permeation of inhibitory Cl^- channels by HCO_3^- was known [40,46], its possible importance had not been realized. So perturbations of acid–base balance – such as might be caused by changes in metabolism or oxygen supply – can lead to corresponding changes in IPSPs.

The direction of net Cl^- transport is the most important factor determining the level of E_{Cl} and therefore the polarity of IPSPs. How Cl^- is transported has become fully clear only during the last decade. Consistent differences between resting potential and E_{Cl} could be satisfactorily explained only by some form of active Cl^- transport. As early as 1966, recordings in snail neurons with Cl^--sensitive electrodes revealed different internal [Cl^-] in cells that gave depolarizing and hyperpolarizing responses to acetylcholine [65]. Inwardly or outwardly directed Cl^- transport thus explained how the same transmitter, acting on identical ionic channels, could evoke synaptic potentials of opposite polarity in different neurons. Thus, in dorsal root ganglia of vetebrates, co-transport of Cl^- with Na^+ and K^+ leads to a relatively positive E_{Cl} [66], which accounts for the depolarizing action of GABA on primary afferents [60–62,67]. The same transporter is responsible for the depolarizing action of GABA observed during early development [68–71]; but with maturation, it becomes restricted to dendritic regions [72]. During maturation, expression of the K^+–Cl^--cotransporter KCC2 – the outward Cl^- carrier long postulated as necessary for a low neuronal E_{Cl} [64–66,73] and first identified and cloned by Payne et al. [74] – ensures a negative shift of E_{Cl} in the neuronal cell body [75–77]. The uneven distribution of these two transporters explains why GABA tends to cause hyperpolarization of somata and depolarization of dendrites [68,78,79].

7. GABA is an excitatory transmitter in immature animals

Immature neurons consistently give depolarizing responses to GABA. In hippocampal pyramidal cells, such responses are still predominant during the first post-natal week of rabbits [68,69] and rats [70,71], but in guinea-pigs only well before birth [75]. As the cells mature, the effect of GABA and the somatic IPSPs gradually change to hyperpolarization. The Cl^--mediated synaptic response generated by glycine alo evolve during ontogeny from depolarizing to hyperpolarizing PSPs [70].

GABAergic excitation in the immature CNS is important for two reasons. First, because neuronal activity promotes and shapes the growth, differentiation, maturation and preservation of neurons and their synaptic connections [80–84]. The crucial point is that active neurons and synapses generate signals that trigger long-term changes in morphology and function. As in the growth cones of new axons [80,82], the main signal is probably Ca^{2+} influx, and the subsequent generation of oscillations of cytoplasmic [Ca^{2+}] and activation of Ca^{2+}-sensitive elements, including calmodulin and CaMKII, resulting in changes in the cytoskeleton – events that are now seen as underlying various forms of synaptic plasticity in the mature CNS. The second important point is that the main excitatory pathways, mediated by AMPA receptor-type synapses are largely inoperative in immature CNS. In contrast to the mature brain, where synaptic plasticity is typically a feature of AMPAR-mediated glutamatergic transmission, the paucity of functional synapses of this type in the very young places the burden on the GABA-mediated depolarizing responses. These are reinforced by NMDARs, which are present quite early on, and therefore significantly enhance the Ca^{2+} influx that can be generated by GABAergic (and glycinergic) action. A recent report [85] provides a striking illustration of such GABA-triggered plasticity at poorly developed mossy fibre synapses on immature CA3 pyramidal neurons. A characteristic feature of these cells is the network-generated ''giant depolarizing potential'', which reflects activation of GABA and NMDA receptors and is therefore associated with substantial Ca^{2+} influx. When mossy fibre stimulation was synchronyzed with such spontaneous giant potentials, there was a selective and lasting potentiation of the active synapses.

8. Postlude

The original prediction that GABA is the main inhibitory transmitter in the brain of mammals is still valid. As such, GABA plays a major role as regulator of neural function, both as global moderator, preventing excessive activity, and in the minutiae of selective activation of certain cells and pathways. It is therefore a crucial element in all aspects of CNS function, from sensation to locomotion, and not least the multifacetted intermediate processes that are reflected in cognition, emotions and sleep. For the treatment of various disorders, an ever larger GABA-based pharmocopoea has developed, involving vast financial resources. Undoubtedly, many who suffer from insomnia

or anxiety have benefitted. So have some epileptics; many more, no doubt, will benefit as better drugs become available. If GABAergic systems also play a significant role in schizophrenia – as long believed by Eugene Roberts [86], one of GABA's co-discoverers – an even wider range of mental disorders may be amenable to GABA-inspired therapy.

The overall theme, inhibition, has survived over the last six decades or so; but the alarmingly quasi-exponential growth of research related to GABA has resulted in such a profusion of detailed information that it can hardly be encompassed by one individual. Even more sobering is the discomfiture of virtually all the ''simple beliefs'' held in earlier stages of this story: that GABA acts exclusively as an inhibitory agent; that GABAergic terminals release only GABA; that it activates only Cl^--permeable channels; and so on. The human mind thirsts for simple explanations that reduce diversity to general laws [87]; but what life scientists have to face is ''the higgledy-piggledy outcome of natural selection and the competition between many interacting factors'' [88]. The bewildering complexity of biological data, of which GABA-mediated events are only one of many examples, presents the greatest challenge. A new page in the story of GABA will begin when even greater emphasis is given to the search for a broader understanding of inhibitory function.

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