VIEWPOINT

Is the anticonvulsant mechanism of valproate linked to its interaction with the cerebral γ-hydroxybutyrate system?

Philippe Vayer, Christopher D. Cash and Michel Maitre

There is now evidence that γ-hydroxybutyrate (GHB) may be a neuromodulator in the CNS. Administration of this compound to various mammals at sub-anaesthetic doses induces brain electrical activity resembling that of human absence epilepsy. This effect is antagonized by the anticonvulsant drugs valproate and ethosuximide, and by the opiate antagonist naloxone. In vitro *valproate and ethosuximide reduce the depolarization-induced release of GHB from rat hippocampal slices, and* in vivo *valproate antagonizes the increase in hippocampal cGMP levels induced by prior GHB administration.* Michel Maitre and colleagues *therefore propose that the anticonvulsant action of valproate may be linked to its interaction with the endogenous GHB system.*

γ-Hydroxybutyrate (GHB) is a normal brain metabolite derived primarily from GABA. Recent biochemical and pharmacological data (see Ref. 1) lend support to the hypothesis that this substance may play a neuromodulator role in brain.

GHB is heterogenously distributed in brain tissue, where it is synthesized by a specific enzyme located exclusively in neurones. The highest concentration of this substance is found in the synaptosomal fraction[2]. GHB is released by depolarization of brain tissue slices by a Ca^{2+}-dependent mechanism and is transported by a high-affinity, energy- and Na^{+}-dependent system. High-affinity specific GHB binding sites are primarily located on the rostral area of the brain, the richest region being the hippocampus[3]. *In vivo*, GHB administration induces accumulation of cGMP in rat brain hippocampus[4]. Moreover, its half-life in rat brain (about 28 min) is at least as short as those of established neurotransmitters.

Administration of GHB to animals and humans induces various neuropharmacological and neurophysiological effects, the most salient of which are: (1) modulation of dopaminergic activity[5] (especially in the striatum) and an increase in 5-HT turnover[6], and (2) a pronounced induction of sedation leading at higher GHB doses to loss of consciousness and anaesthesia[7]. This latter property has been exploited clinically.

However, our principal interest here lies in the electroencephalographic perturbations induced by GHB administration. These effects have been most studied in the cat[8] and in the rat[9]. In these animals, GHB induces authentic experimental epilepsies which are characterized by mono- and polyphasic discharges. These discharges are analogous to those observed in human petit mal epilepsy[10]. The abnormal electroencephalograms induced by GHB are antagonized by various anticonvulsant drugs including valproate and also by ethosuximide and trimethadione[11]. These epileptic phenomena may be considered as the result of a hyperactivity of an endogenous brain system and, taking into account its pharmacological and biochemical properties, we would suggest that GHB plays a role in the resultant hyperactivity. Thus, we propose that the mechanism of action of some antiepileptic drugs is due to their interactions with the GHB system in the brain. We shall concentrate our attention on the specific effects of valproate on cerebral GHB functions and propose that these biochemical actions indicate a heuristic molecular model for the mode of action of certain anticonvulsant drugs.

Anticonvulsant mechanism of valproate: hypothesis

In rodents, valproate induces an increase in the cerebral GABA level[12], coinciding with a protective effect against various forms of experimental epilepsy. This GABA increase has been ascribed to the inhibitory effect of valproate on the GABA shunt enzymes, GABA transaminase[12] and succinic semialdehyde dehydrogenase[13] (see Fig. 1). The inhibitory effect is more pronounced on the latter enzyme and is competitive with respect to the substrate, succinic semialdehyde[13] – a metabolite which has only been detected in minute quantities in brain tissue. Valproate has no effect on GABA release from cortical synaptosomes preloaded with labelled GABA[14].

Concomitant with the GABA level increase induced by valproate is a marked reduction in cerebral aspartate level[15]. However, no interaction of valproate with possible excitatory synapses which function with aspartate has been demonstrated. The increase in the cerebellar cGMP level frequently observed in numerous forms of experimental epilepsy is greatly diminished by prior administration of valproate, whereas cAMP levels are unchanged[16]. This increase in cGMP level is considered as playing a role in the induction and continuation of epileptogenic activity[17].

Finally, valproate has no effect

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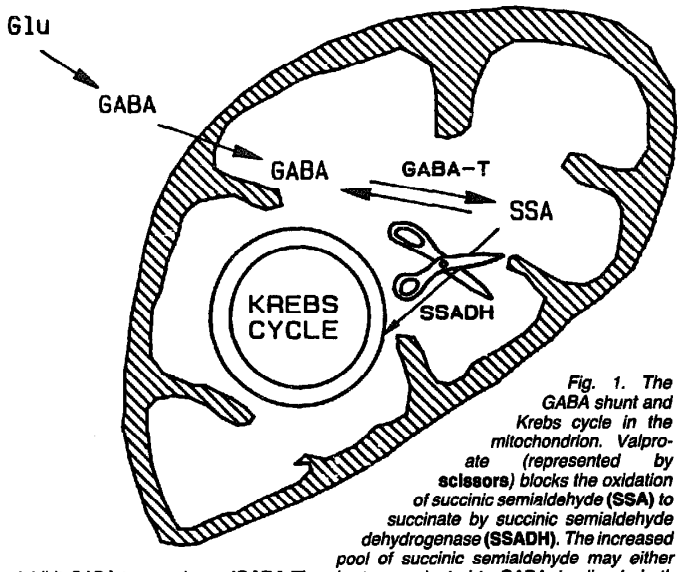

Fig. 1. The GABA shunt and Krebs cycle in the mitochondrion. Valproate (represented by **scissors***) blocks the oxidation of succinic semialdehyde* **(SSA)** *to succinate by succinic semialdehyde dehydrogenase* **(SSADH)**. *The increased pool of succinic semialdehyde may either inhibit GABA-transaminase* **(GABA-T)** *or be transaminated to GABA, leading in both cases to raised GABA levels.*

on the $GABA_A$ receptor nor on benzodiazepine receptors, although it displaces the convulsant dihydropicrotoxin from its binding site[18].

In general, it is thought that valproate potentiates GABAergic neurotransmission. However, it decreases its turnover rate and it does not modify its release. A possible direct GABA-like agonist action of valproate on a postsynaptic site has yet to be demonstrated[18], although a direct action of sodium valproate on action potentials of cultured neurons has been reported[19].

Interactions of valproate with cerebral GHB system

Several workers have reported that valproate administration to rodents brings about an increase in the brain GHB level[20]. This increase is time- and dose-dependent, and appears to be due to two related factors: direct inhibition of catabolic enzymes and a reduction in synaptic release.

As the principal precursor of GHB is GABA, the mechanism of this GHB increase could be due to inhibition of the mitochondrial enzyme succinic semialdehyde dehydrogenase (EC1.2.1.24), which causes the pool of succinic semialdehyde (the direct precursor of GHB) to be elevated. The enzyme which reduces succinic semialdehyde to GHB (specific succinic semialdehyde reductase) is unaffected by valproate[21]. In fact the increase in GABA levels induced by valproate has been ascribed to succinic semialdehyde dehydrogenase inhibition[13]. However, succinic semialdehyde dehydrogenase is a mitochondrial enzyme whereas specific succinic semialdehyde reductase is cytoplasmic. Thus the hypothesis that valproate increases GHB levels via an increased synthesis due to higher precursor availability would depend on an as yet unknown mechanism whereby excess succinic semialdehyde is transported out of the mitochondria into the cytoplasm (Fig. 1).

A more plausible mechanism for valproate-induced increases in GHB levels is the powerful inhibition of nonspecific succinic semialdehyde reductase by valproate[21]. [This enzyme is identical to the previously described ALR_1, also referred to as glucuronate reductase or SSR_1 (Ref. 22).] It is reported to be responsible for the catabolism of GHB to succinic semialdehyde both *in vitro*[22] and *in vivo*[23]. Under physiological conditions *in vitro*, GHB is degraded to GABA via nonspecific succinic semialdehyde reductase and GABA transaminase[22]. The operation of this catabolic pathway explains why inhibitors of nonspecific semialdehyde reductase such as valproate or ethosuximide, or GABA transaminase inhibitors such as γ-vinyl GABA, increase GHB levels[2] (Fig 2).

Valproate and ethosuximide also considerably reduce the Ca^{2+}-dependent depolarization-induced release of GHB from hippocampal and striatal slices from rat brain[24]. The IC_{50}s for these two drugs in this test are compatible with their brain levels after administration of anticonvulsant doses.

The latter phenomenon explains why the epileptogenic effects of GHB administration are attenuated by valproate or ethosuximide[11], and also explains the paradox that drugs such as valproate increase GHB brain levels[25] whilst antagonizing its effects.

The increase in cGMP levels in certain brain regions (such as the hippocampus) commonly seen after administration of convulsant drugs is concomitant with the

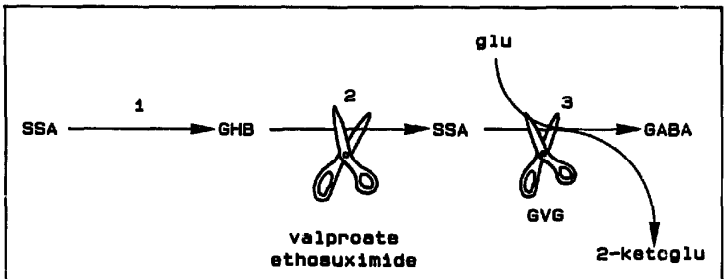

Fig. 2. Inhibition of GHB catabolism by **valproate, ethosuximide** *and γ-vinyl GABA* **(GVG)**. **(1)**, *Specific succinic semialdehyde reductase;* **(2)**, *nonspecific succinic semialdehyde reductase;* **(3)**, *GABA-transaminase.* **Valproate** *and* **ethosuximide** *directly inhibit nonspecific succinic semialdehyde reductase, whereas* **GVG** *inhibits GABA-transaminase leading to an increase in succinic semialdehyde* **(SSA)**, *which is a product inhibitor of the dehydrogenase.*

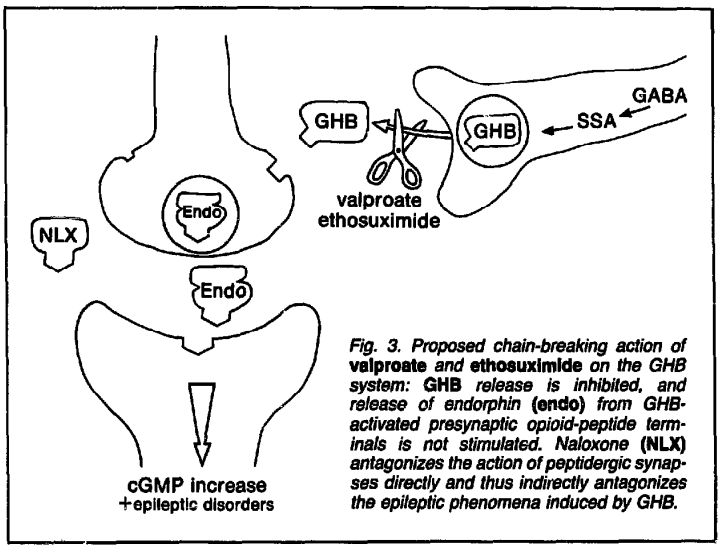

Fig. 3. Proposed chain-breaking action of **valproate** *and* **ethosuximide** *on the GHB system:* **GHB** *release is inhibited, and release of endorphin* **(endo)** *from GHB-activated presynaptic opioid-peptide terminals is not stimulated. Naloxone* **(NLX)** *antagonizes the action of peptidergic synapses directly and thus indirectly antagonizes the epileptic phenomena induced by GHB.*

commencement and generalization of massive depolarization phenomena[17]. The increase we have observed in hippocampal cGMP after GHB administration[4] could be considered as either a side-effect or a precursor to the evolution of the epilepsy induced by GHB, the latter directly or indirectly provoking a perturbation in the membrane polarization of the hippocampus, a region rich in high-affinity GHB binding sites[3]. In various experimental epilepsies, therapeutic doses of valproate antagonize increases in cGMP levels; valproate also antagonizes, both *in vivo* and *in vitro*, the increase in cGMP levels seen after GHB administration[4]. Ethosuximide also antagonizes this increase[4] and thus it can be inferred that their anticonvulsant effects are mediated by the inhibition of Ca^{2+}-dependent release of GHB.

In addition, the opiate receptor antagonist naloxone inhibits the GHB-induced cGMP increase[4] and attenuates the abnormal EEG activity[27]. Taking into account the fact that GHB administration causes an increase in dynorphin level in the hippocampus[27], a region rich in μ-receptors, and that administration to the rat of certain opiates induces epileptic seizures which are antagonized by both valproate and ethosuximide[28], it could be suggested that these anticonvulsants act via a GHB-ergic mechanism which is linked to the endogenous opioid system (Fig. 3).

GHB administration to animals most often brings about epileptic spiking activity. These phenomena may represent functional overload of synapses which release GHB. There is much support for the existence of such synapses in the CNS. Valproate and ethosuximide modify the characteristics and functions of this group of synapses by inhibiting the degradation of GHB and by blocking its Ca^{2+}-dependent depolarization-induced release. Increases in cGMP brought about by GHB are blocked by valproate, ethosuximide and also by naloxone. These three drugs antagonize the epileptogenic activity of GHB. The effect of naloxone may indicate that endorphins participate in the aetiology of this epileptic phenomenon. This biochemical mechanism could constitute a model for anticonvulsant agents. It would thus be of interest to synthesize new molecules which either reduce synaptic release of GHB or are antagonists at its receptor sites.

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