

NOT ANOTHER GABAPENTIN MECHANISM!

Gabapentin Activates Presynaptic GABA_B Heteroreceptors in Rat Cortical Slices

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In electrically stimulated rat neocortical brain slices preloaded with [³H]γ-aminobutyric acid (GABA) or [³H]glutamic acid, the pharmacologic actions of 1-(aminomethyl)-cyclohexaneacetic acid (gabapentin, GBP) were compared with those of the GABA_B-receptor agonists baclofen (Bac) and [3-amino-2-(S)-hydroxypropyl]-methylphosphinic acid (CGP 44532). GBP, baclofen, and CGP 44532 all reduced the electrically stimulated release of [³H]glutamic acid [median inhibitory concentration (IC₅₀), 20 μM, 0.8 μM, and 2 μM, respectively]. These effects were sensitive to the GABA_B receptor antago-

nists (+)-(S)-5,5 dimethylmorpholinyl-2-acetic acid (Sch 50911) or *N*-3-[[1-(S)-(3,4-dichlorophenyl)ethyl]amino]-2-(S)-hydroxypropyl-*P*-(cyclo-hexylmethyl)-phosphinic acid (CGP 54626). By contrast, GBP was without effect on the release of [³H]GABA, whereas baclofen (IC₅₀, 8 μM) and CGP 44532 (IC₅₀, 1 μM) inhibited [³H]GABA release. It is concluded that GBP selectively activates presynaptic GABA_B heteroreceptors, but not GABA_B autoreceptors, and may be a useful ligand to discriminate between presynaptic GABA_B-receptor subtypes.

COMMENTARY

Despite a decade of clinical use both as an antiepileptic and antinociceptive agent, the mechanism by which gabapentin (GBP) exerts its pharmacologic effects remains to be determined. It is a structural analogue of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and was originally designed as a GABA_B agonist that could freely cross the blood-brain barrier (1). However, subsequent studies suggested that GBP was without significant activity at GABA receptors. A number of subsequent investigations reported a possible interaction with the synthesis and nonvesicular release of GABA and weak inhibitory effects at multiple cellular targets, including voltage-gated sodium channels, the system L-amino acid transporter, and branched-chain amino acid transferase (2). The first convincing evidence of a clinically significant mechanism came with the identification of a specific binding of GBP in mammalian brain to the auxiliary α₂δ subunit of a voltage-dependent calcium channel. Subsequent functional studies demonstrated calcium channel-blocking properties of the drug at therapeutically relevant concentrations (3,4). Although inhibition of calcium currents may be regarded as the most likely mechanism of GBP action, several recent articles revisited the issue of GBP pharmacology and focused on an unexpected interaction with GABA_B receptors.

GABA_B receptors are distributed throughout the central nervous system, localized both to presynaptic nerve terminals, where they regulate neurotransmitter release, and to postsynaptic cell membranes, where they are responsible for prolonged

hyperpolarizing inhibitory potentials (5). In contrast to the GABA_A receptor, which has an intrinsic chloride-ion sensitive pore, the GABA_B receptor is coupled to a G-protein, with agonist challenge producing indirect hyperpolarization via inhibition of a voltage-gated calcium channel (presynaptic receptors) or activation of an inwardly rectifying potassium channel (postsynaptic receptors). The GABA_B receptor is a heterodimer complex comprising two independent protein subunits, denoted GABA_{B1} and GABA_{B2}, both of which are required for the expression of functional receptors (6). Unlike many other neurotransmitter receptors, the GABA_B receptor does not display significant subunit heterogeneity. GABA_{B1} and GABA_{B2} are the only two gene products identified to date, and although numerous splice variants of the GABA_{B1} subunit exist, only two (GABA_{B1a} and GABA_{B1b}) appear to predominate. The GABA_{B2} subunit is even less well delineated, with no convincing evidence to support the existence of alternative isoforms. Accordingly, only two principal GABA_B receptors, distinguished by the splice variant of the GABA_{B1} subunit, are currently recognized in mammalian brain (6).

The recent article by Parker and colleagues reported an interaction between GBP and GABA_B receptors on glutamatergic nerve terminals (heteroreceptors) in rat neocortical brain slices, resulting in a reduction in the evoked release of glutamate. This effect, which did not extend to GABA_B receptors on GABAergic nerve terminals (autoreceptors), was observed at clinically relevant concentrations and was reversed by the coapplication

of recognized GABA_B antagonists. These findings would suggest that GBP exerts its effects, at least in part, by activation of a subset of GABA_B receptors, leading to the selective reduction of excitatory neurotransmitter release and relative sparing of inhibitory neurotransmission. This study also implies that different isoforms of the GABA_B receptor are expressed on excitatory and inhibitory nerve terminals and that GBP may be a useful tool in the pharmacologic differentiation of GABA_B receptors. These conclusions are consistent with previous reports that proposed a preferential effect of GBP on GABA_B receptors containing the GABA_{B1a} subunit (7,8).

At face value, this experimental evidence is intriguing. It supports a selective effect of GBP on excitatory neurotransmitter release, which might account for many of its anticonvulsant and antinociceptive actions, and affords a novel insight into the molecular pharmacology and cellular distribution of GABA_B receptors. However, an equivalent volume of data, which reports no interaction between GBP and GABA_B receptors, also exists (9,10), the most compelling of which is the long-acknowledged failure of the drug to undergo high-affinity binding to the GABA recognition site on the GABA_B receptor. It is possible that GBP interacts with an, as yet, unidentified site on the GABA_B receptor complex, the existence of which would explain why the drug exerts differential pharmacologic effects on distinct GABA_B receptor subunits with identical agonist binding domains (9,10). It is equally plausible, however, that the inconsistent findings are the result of an experimental artifact or the manifestation of a currently inadequate understanding of GABA_B-receptor heterogeneity.

Amid fanciful notions of alternative binding sites, it would appear that a somewhat more obvious explanation might have been overlooked. GBP binds with high affinity to the $\alpha_2\delta$ subunit of the voltage-gated calcium channel, but these channels are functionally associated with presynaptic GABA_B receptors and are intimately involved in neurotransmitter release. As a result, selective inhibition of specific GABA_B-linked presynaptic calcium channels containing the $\alpha_2\delta$ subunit could, in theory, explain the findings of Parker and colleagues and equally support all evidence to the contrary. The only argument against this simplistic proposition would be the ability of GABA_B antagonists to reverse the effects of GBP, a phenomenon that could be accounted for by indirect physiologic antagonism, particularly in brain-slice preparations in which endogenous GABA may act as a residual agonist. Low-level GABA contamination of GBP samples has been suggested as being responsible for a similar phenomenon in recombinant receptor studies (10).

A selective reduction in the release of excitatory neurotransmitters, with relative sparing of GABAergic neurotransmission, was previously reported with lamotrigine (11). It is possible that

other antiepileptic agents, such as topiramate and pregabalin, which also block high-voltage activated calcium currents, will have a similar profile. It should come as no real surprise that recognized calcium channel blockers can inhibit neurotransmitter release. Perhaps only the selectivity of this effect, most likely the result of calcium channel-subunit heterogeneity, is worthy of comment. Although little doubt exists that Parker and colleagues have considered their findings in a careful and judicious manner, it seems they have subscribed to a common failing in discussions of antiepileptic drug pharmacology and overlooked the relation between calcium channel blockade and neurotransmitter release. Thus it would appear that, despite a recent resurgence in interest, the mechanisms of action of GBP are no clearer. On current evidence, the antiepileptic and antinociceptive activities of the drug are most likely to be conferred by multiple cellular effects, maybe not through activation of GABA_B receptors, but almost certainly involving blockade of voltage-gated calcium channels.

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