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ANTICONVULSANT DRUG ACTIONS ON GABA RESPONSES AND SUSTAINED REPETITIVE FIRING OF NEURONS IN CELL CULTURE

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We have studied barbiturate, benzodiazepine (BDZ), β-carboline, triazolopyridazine, piperazine carboxylate, hydantoin, iminostilbene, valproate, and succinmide actions on GABA responses and sustained, high frequency repititive firing of mouse neurons in cell culture. Barbiturates, clinically used BDZ, zopiclone and Cl 218,872 enhanced GABA responses. Ro 15-1788 and propyl-β-carboline were partial agonists at BDZ receptors while DMCM was an inverse agonist. Phenytoin, carbamazepine, phenobarbital, diazepam and valproic acid limited sustained repetitive firing. We suggest that enhancement of GABAergic inhibition and limitation of sustained high frequency repetitive firing may be anticonvulsant mechanisms of action.

Enhancement of GABAergic synaptic transmission has been proposed as a common mechanism of action of anticonvulsant drugs. We have assessed the pharmacological actions of the commonly used anticonvulsant drugs on postsynaptic GABA responses of mouse neurons in cell culture to identify which drugs act at relevant therapeutic CSF and free serum concentrations. In addition, we have identified electrophysiological actions of anticonvulsant drugs which do not involve GABAergic synaptic transmission in an attempt to explain the selectivity of these drugs in human and experimental seizures.

We have identified two actions of anticonvulsant drugs which appear to occur at therapeutic free serum and CSF concentrations. Of all the major drugs used, only barbiturates and benzodiazepines enhanced postsynaptic GABA responses at appropriate free serum and CSF concentrations. Valproic acid may enhance presynaptic release of GABA. However, phenytoin, carbamazepine, primidone, phenobarbital, valproic acid, diazepam and clonazepam all limited sustained high frequency repetitive firing of action potentials at concentrations relevant for their anticonvulsant action. Ethosuximide did not modify either repetitive firing or postsynaptic GABA responses. We will review the actions of barbiturates and benzodiazepines on postsynaptic GABA responses and repetitive firing and attempt to correlate these actions with behavioral effects.

Barbiturate Actions

Phenobarbital and the anesthetic barbiturate pentobarbital both enhanced GABA responses of spinal cord neurons. Pentobarbital had a threshold of action at 4 μM and produced a 50% enhancement at 30 μM. Phenobarbital threshold for enhancement was 20 μM with a 50% enhancement at 150 μM. Phenobarbital limited sustained high frequency repetitive firing of spinal cord neurons above 200 μM. At somewhat higher concentrations, pentobarbital (25 - 600 μM) and phenobarbital (100 - 5000 μM) reduced the duration of calcium-dependent action potentials by blocking membrane voltage-dependent calcium conductance. At higher concentrations both pentobarbital (100 - 5000 μM) and phenobarbital (500 - 4000 μM) directly increased membrane chloride conductance. This action of both barbiturates was blocked by the GABA receptor antagonists penicillin and picrotoxin.

Benzodiazepine Receptor Ligand Actions

We have studied the action of a variety of benzodiazepine receptor ligands on postsynaptic GABA responses recorded from spinal cord neurons. The benzodiazepines, diazepam and clonazepam, were full agonists and enhanced GABA responses with a threshold at 1 nM and peak response was achieved at 10 to 100 nM. At higher concentrations, the enhancement was reduced. Diazepam and clonazepam are highly protein-bound in serum and likely have free serum concentrations of 5 - 10 nM when total serum concentrations are in the therapeutic range. Nitrazepam was somewhat less potent with threshold increases at 10 nM. The benzodiazepine, Ro 15-1788, and the β-carboline, β-CCPr, both blocked the diazepam enhancement of GABA responses as well as enhancing GABA responses at high concentrations. Therefore these two compounds were partial agonists. The β-carbolines, β-CCMe and β-CCEt, both antagonized diazepam enhancement of GABA responses without having direct actions on GABA responses at concentrations up to 10 μM. The β-carboline, DMCM, antagonized GABA responses consistent with an inverse agonist action. In addition, the triazolopyridazine, CL 218872

and the piperazine carboxylate, zopiclone, both weakly enhanced GABA responses.

In addition to having actions on GABA responses, diazepam reduced high frequency sustained repetitive firing of mouse spinal cord neurons at concentrations above 35 nM. At 175 nM virtually all neurons had limited repetitive firing. At concentrations above 1 μM, diazepam reduced the spontaneous activity of spinal cord neurons. At 10 μM, diazepam reduced the duration of calcium-dependent action potentials of mouse neurons.

In contrast, the convulsant benzodiazepine, Ro 5-4864, blocked GABA responses at concentrations above 10 nM. The antagonism of GABA responses was not reversed by Ro 15-1788. In addition to antagonizing GABA responses, Ro 5-4864 reduced sustained high frequency repetitive firing at concentrations slightly above those effective in antagonizing GABA responses and decreased the duration of calcium-dependent action potentials at concentrations somewhat higher than those blocking GABA responses.

Other Anticonvulsant Drug Actions

Phenytoin, carbamazepine and valproic acid all limited sustained, high frequency repetitive firing at concentrations below 10 μM but did not enhance GABA responses below 20 μM. The reduction of repetitive firing was due to use-dependent and voltage-dependent sodium channel block. Ethosuximide did not alter repetitive firing or GABA responses at concentrations up to 1 mM.

Correlation of Anticonvulsant Drug Actions with Behavioral Effects

Anticonvulsant drugs have selective actions in treatment of the human epilepsies and in treatment of experimentally-induced seizures in animals. Phenytoin, carbamazepine and primidone are effective against generalized tonic-clonic (GTC) seizures and partial seizures in man and against maximal electroshock (MES) seizures in animals but have little action on generalized absence (GA) seizures in man and pentylenetetrazol (PTZ) induced seizures in experimental animals. Phenobarbital, valproic acid and clonazepam have mixed actions on human and experimentally-induced seizures. Phenobarbital has actions against GTC and partial seizures but little action against GA seizures. Valproic acid has effectiveness against both type of seizures in humans while clonazepam is primarily effective in GA seizures. All three anticonvulsant drugs have effectiveness against MES and PTZ seizures in animals. Finally, ethosuximide is selective for GA seizures in man and for PTZ seizures in experimental animals.

We suggest that both barbiturates and benzodiazepines have anticonvulsant action against PTZ and GA seizures by enhancing GABA responses. We further suggest that phenytoin, carbamazepine, barbiturates, valproic acid and benzodiazepines are effective against MES and GTC seizures through a non-GABA mechanism, the limitation of sustained high frequency repetitive firing. The mechanism of action of ethosuximide remains unclear. We propose that at least part of the sedative-anesthetic actions are mediated by reduction in presynaptic calcium entry, and therefore, in release of presynaptic neurotransmitter.