Inhibitory Effect of Cephalosporins on γ-Aminobutyric Acid Receptor Binding in Rat Synaptic Membranes

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Cephalosporins inhibited γ-aminobutyric acid receptor binding in a concentration-dependent manner in vitro. Scatchard analysis revealed that cefazolin decreased the binding capacity but did not change the affinity of the receptor. It is suggested that this inhibition of γ-aminobutyric acid receptor binding may be involved in the induction of convulsions by cephalosporins.

γ-Aminobutyric acid (GABA) is now a well-demonstrated inhibitory transmitter in the central nervous system of mammals, and the characteristics of its receptor sites have been studied by many workers. It has been proposed that a decline in GABA-mediated inhibitory transmission may increase the excitability of the central nervous system and thus lead to convulsions.

In recent years, many antibiotics have been developed and used for treatment of infectious diseases. Some are known to have neurotoxicities, especially convulsant actions, as their side effects. Penicillins induced convulsions when the agents were administered in massive doses (13) or applied directly to the cerebral cortex of animals (4). The onset of convulsions might be related to their capacity to reduce release of GABA from nerve terminals (5, 12) or inhibit GABA binding to its receptor sites (1, 12).

Cephalosporins (CEPs) have also been reported to induce convulsions clinically and experimentally. For example, the intrathecal administration of cephaloridine has caused convulsions in premature infants (16), and cefazolin has induced convulsions in patients with compromised renal functions (3, 17). In experimental animals, cefazolin induced convulsions which could be reversed by GABA agonists (7, 9, 11, 14). However, there is little evidence to explain clearly the convulsive action of CEPs. In this study, we have examined the effect of CEPs on GABA receptor binding in synaptic membranes in vitro.

Male albino rats were decapitated, and the brains were rapidly removed and homogenized with 0.32 M sucrose solution. The homogenate was centrifuged to obtain crude mitochondrial pellet. Crude synaptic membranes were prepared from disrupted crude mitochondrial pellet by the method of Zukin et al. (18). The membranes were washed five times with 10 mM potassium phosphate buffer (pH 7.4) containing 150 mM NaCl. The resulting pellet was suspended in a suitable volume of 10 mM potassium phosphate buffer (pH 7.4) (ca. 8 mg of protein per ml) and stored at −20°C until use.

For [3H]-GABA binding assay, a 400-μl sample consisting of [3H]-GABA (34.9 Ci/mmol, New England Nuclear Corp.), drugs, and membrane protein (300 to 400 μg) in 10 mM potassium phosphate buffer (pH 7.4) was incubated for 20 min at 4°C (6, 18). The binding reaction was terminated by centrifugation. The pellet was rinsed rapidly and superficially with ice-cold deionized water, and the radioactivity in the pellet was determined with a liquid scintillation counter. Specific [3H]-GABA binding was determined by subtracting nonspecific binding (the amount of [3H]-GABA bound in the presence of 1 mM unlabeled GABA; 20.6 ± 1.3% of total binding) from total binding (the amount of [3H]-GABA bound in the absence of unlabeled GABA). All determinations were carried out in duplicate. Protein determinations were carried out by the method of Lowry et al. (10).

To study the effect of CEPs on specific [3H]-GABA binding, the binding assay was carried out in various concentrations of CEPs. Specific [3H]-GABA binding to crude synaptic membranes was inhibited by CEPs in a concentration-dependent manner (Fig. 1A). Drug concentrations that inhibited 50% of binding were as follows: cefazolin, 1.39 ± 0.08 mM; cephaloridine, 2.99 ± 0.24 mM; cephalaxin, >50 mM (mean plus or minus standard deviation for three experiments).

Scatchard analysis was carried out, using 1.25 to 150 nM [3H]-GABA as ligand, to determine whether inhibition of GABA receptor binding was due to the decrease of binding capacity or to the change of the affinity of the receptor. The analysis revealed that, in the absence of cefazolin, [3H]-GABA was bound to a single class of receptor sites having a dissociation constant (K_d) of 31.7 ± 0.56 nM with a maximum binding capacity of 1.70 ± 0.03 pmol/mg of protein. The addition of cefazolin (1 mM) significantly reduced the maximum binding capacity of the receptor (0.92 ± 0.14 pmol/mg of protein) but did not change the K_d of the receptor (28.1 ± 3.6 nM [mean plus or minus standard deviation for three experiments].

<table>
<thead>
<tr>
<th>Drugs added</th>
<th>[3H]-GABA bound (pmol/mg of protein) in type of medium:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cl⁻ free</td>
</tr>
<tr>
<td>None</td>
<td>0.203 ± 0.003</td>
</tr>
<tr>
<td>Cefazolin (1 mM)</td>
<td>0.122 ± 0.006*</td>
</tr>
<tr>
<td>Pentobarbital (500 μM)</td>
<td>0.229 ± 0.004*</td>
</tr>
<tr>
<td>Cefazolin (1 mM) + pentobarbital (500 μM)</td>
<td>0.127 ± 0.003</td>
</tr>
</tbody>
</table>

* The binding assay was carried out in the medium with or without 100 mM Cl⁻ (KCl) at a single concentration of [3H]-GABA (5 nM). Each value represents the mean ± standard deviation for three separate experiments.

< p < 0.01, in comparison with that in Cl⁻ free medium.

< p < 0.01, in comparison with that in the absence of any drug.

< p < 0.01, in comparison with that in the presence of cefazoline alone.

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Three separate experiments) (Fig. 1B). These results suggested that CEPs inhibited GABA receptor binding by decreasing the binding capacity of the receptor sites.

Since pentobarbital enhances GABA receptor binding (2, 15) and has an anticonvulsive activity against CEP-induced convulsions (8), the effect of the barbiturate on inhibition of GABA receptor binding by cefazolin was studied in vitro. Specific 3H-GABA binding was assayed in the presence of pentobarbital (500 μM) alone or in combination with cefazolin (1 mM), in the medium with or without 100 mM KCl. Pentobarbital markedly increased specific 3H-GABA binding in the medium containing 100 mM Cl− (Table 1), in agreement with the report of Asano and Ogasawara (2). Pentobarbital was able to restore 3H-GABA binding, which had been reduced by cefazolin, to the control level in the Cl−-containing medium. This Cl−-dependent enhancement of GABA receptor binding might be associated with the protective activity of the barbiturate against convulsions induced by CEPs.

The data described above suggest that the convulsive activities of CEPs are related to the reduction of the binding capacity of GABA receptor, with a consequent inhibition of GABA-mediated inhibitory transmission.

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LITERATURE CITED


