EFFECT OF CEPHALOSPORINS ON γ-AMINOBUTYRIC ACID RECEPTOR BINDING WITH OR WITHOUT NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

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Cephalosporins have been well known to have potent convulsant activity. We studied the mechanism of the convulsions induced by cephalosporins. Cefazolin, cephaloridine, cefpirome and cefmetazole inhibited the receptor binding of γ-aminobutyric acid (GABA), an inhibitory transmitter in the mammalian central nervous system. As fluoroquinolones also inhibited GABA receptor binding and this inhibition was enhanced in the presence of non-steroidal anti-inflammatory drugs (NSAIDs), we studied the effect of cephalosporins on GABA receptor binding in the presence of NSAIDs. The inhibitory activity of cephalosporins was not enhanced in the presence of NSAIDs. These results suggested that cephalosporins might induce convulsions through the inhibition of GABA receptor binding, and that concurrent administration of cephalosporins and NSAIDs might not enhance the convulsant activity of cephalosporins.

Recently many antibiotics have been developed and used for treatment of infectious diseases. Cephalosporins are well known to have potent convulsant activity. For example, intrathecal injection of cephaloridine have been reported to induce convulsions[1], and massive administration of cefazolin in the patients with compromised renal functions induced convulsions[2,3]. KAMEI *et al.* have reported that cephalosporins could induce convulsive patterns in electroencephalogram of rats[4]. However, there have been few reports to show the neurochemical mechanism of convulsions induced by cephalosporins.

γ-Aminobutyric acid (GABA) is now well demonstrated to be an inhibitory transmitter in mammalian central nervous system (CNS), and the reduction of GABA-mediated inhibitory transmission should increase the excitability in CNS and lead to convulsions[5]. We have reported that cefazolin, cephaloridine inhibited GABA receptor binding in rat synaptic membranes and suggested that this inhibition of GABA receptor binding might be related to the onset of the convulsions induced by cephalosporins[6]. Furthermore, we reported that new quinolones inhibited GABA receptor binding and the inhibitory activity was enhanced in the presence of non-steroidal anti-inflammatory drugs (NSAIDs)[7].

Cefpirome is a newly developed cephalosporin[8]. In order to know whether this agent has convulsant activity, we studied the effect of cefpirome, as well as other cephalosporins, on GABA receptor binding. In addition, we studied the effect of these agents on GABA receptor binding in the presence of NSAIDs.

Materials and Methods

Materials

[^{3}H]Muscimol (495.8 GBq/mmol) was purchased from New England Nuclear Co. Cefpirome was kindly donated by Nippon Roussel Co., Ltd., and cefmetazole from Sankyo Co., Ltd. Cefazolin and cephaloridine were purchased from Sigma Chemical Co. Acetylsalicylic acid, flurbiprofen, indomethacin

and 4-biphenylacetic acid were purchased from Sigma Chemical Co., and conventional male albino mice were supplied by Nisseizai Co.

Other reagents were of analytical grade.

Preparation of Mouse Synaptic Membranes

Male albino mice (ddY strain, 20~25 g) were decapitated, and their brains were removed rapidly. The brains were homogenized with 9 volumes of ice-cold 0.32 M sucrose solution using Teflon-glass homogenizer. The homogenate was centrifuged at $1,000 \times g$ for 10 minutes, and the supernatant was centrifuged $15,000 \times g$ for 15 minutes to obtain a crude mitochondrial pellet. The crude mitochondrial pellet was dispersed and disrupted with 20 volumes of 10 mM potassium phosphate buffer (KPB, pH 7.4), and centrifuged at $9,000 \times g$ for 15 minutes to get crude synaptic membrane fraction[9]. The crude synaptic membrane suspension was frozen and thawed. Then, it was washed with 20 volumes of ice-cold 10 mM KPB (pH 7.4) containing 150 mM NaCl for 5 times to remove endogenous GABA. Mouse synaptic membranes obtained were suspended in 10 mM KPB (pH 7.4, about 1 g brain/1 ml) and stored at $-20°C$ until use.

GABA Receptor Assay

Prior to the GABA receptor assay, mouse synaptic membranes were thawed and washed with 10 mM KPB (pH 7.4) for three times. GABA receptor binding was determined as specific [^{3}H]muscimol binding in mouse synaptic membranes. Four hundred μl aliquot, consisting of membrane, [^{3}H]muscimol (final concentration 3 nM) and cephalosporins with or without NSAIDs, was incubated at 4°C for 15 minutes. The reaction was terminated by filtration on glass-filter (Whattman GF/B) under vacuum. After washing the filter with 3 ml of ice-cold deionized water for two times, the amount of radioisotope on glass-filter was determined with a liquid scintillation counter. Specific [^{3}H]muscimol binding was obtained by subtracting non-specific binding (the amount of [^{3}H]muscimol binding in the presence of 0.1 mM unlabeled GABA) from total binding (the amount of [^{3}H]muscimol binding in the absence of unlabeled GABA). Each assay was carried out in duplicate.

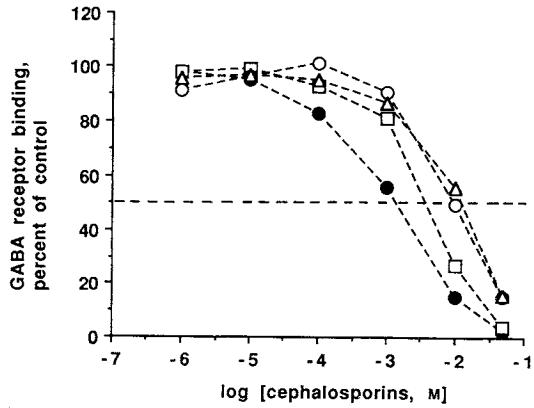
Results

GABA receptor assay was carried out in the presence of various concentrations of cephalosporins. Cephalosporins inhibited specific [^{3}H]muscimol binding in a concentration-dependent manner (Fig. 1). The concentrations that inhibited 50% of the binding (IC_{50}s) were: 1.6×10^{-3} M for cefazolin, 4.3×10^{-3} M for cephaloridine, 1.1×10^{-2} M for cefpirome and 1.5×10^{-2} M for cefmetazole.

To know the effect of NSAIDs on the inhibitory activity of cephalosporins on GABA receptor binding, the assays were carried out in the presence of both cephalosporins and NSAIDs. The final concentration of cephalosporins was 2×10^{-3} M, and that of NSAIDs was 10^{-4} M. Cefazolin (2×10^{-3} M) inhibited GABA receptor binding to 56% of control, but the addition of 4-biphenylacetic acid, indomethacin, flurbiprofen or acetylsalicylic acid did not affect the inhibitory activity of cefazolin

Fig. 1. Effect of cephalosporins on the receptor binding of γ-aminobutyric acid.

● Cefazolin, □ cephaloridine, ○ cefpirome, △ cefmetazole.



A 400-μl aliquot consisting of membrane protein, [^{3}H]muscimol (3 nM, final concentration) and various concentrations of drugs was incubated at 4°C for 15 minutes. The incubation was terminated by filtration on Whattman GF/B glass filter, and the amount of radioisotope on the filter was determined with a liquid scintillation counter. The experiment was carried out in duplicate and each point shows the mean of three separate experiments.

Table 1. Effect of non-steroidal anti-inflammatory drugs on the inhibitory activity of cephalosporins on GABA receptor binding.

	No NSAIDs	Acetylsalicylic acid	Flurbiprofen	Indomethacin	4-Biphenylacetic acid
No cephalosporins	100±6.6	103.5±2.0	97.5±4.9	104.1±10.5	101.6±10.2
Cefazolin	55.6±4.1	63.6±1.9	63.0±1.9	60.4±5.8	56.9±3.8
Cephaloridine	75.9±6.8	77.8±6.7	63.0±1.9	75.5±5.8	72.5±6.6
Cefpirome	86.7±1.1	87.0±6.7	81.7±4.7	89.3±3.1	77.3±6.3
Cefmetazole	87.1±10.2	91.9±2.1	95.3±4.6	87.2±3.4	85.8±7.5

Each value represents percent of the specific [^{3}H]muscimol binding without any drugs (mean±standard deviation for three separate experiments).

Each assay was carried out in duplicate. The concentrations of cephalosporins and non-steroidal anti-inflammatory drugs were 2×10^{-3} M and 1×10^{-4} M, respectively.

NSAIDs: non-steroidal anti-inflammatory drugs.

(Table 1). Cephaloridine, cefpirome and cefmetazole slightly inhibited GABA receptor binding, and the inhibitory activity of these cephalosporins was not affected by the addition of NSAIDs. And NSAIDs themselves (10^{-4} M) did not inhibit specific [^{3}H]muscimol binding (Table 1).

Discussion

Penicillins and cephalosporins have been reported to induce convulsions in human and experimental animals[1~4,10]. However, there have been few reports to show the neurochemical mechanism of the convulsions induced by penicillins and cephalosporins. Penicillins have been reported to inhibit GABA receptor binding[11,12]. CURTIS *et al.* reported the convulsant activity of penicillin and they suggested the conformational similarity between penicillin and bicuculline, a GABA receptor antagonist[13]. We have reported that cefazolin and cephaloridine inhibited GABA receptor binding and suggested that the inhibition of GABA receptor binding might be responsible to the onset of the convulsions induced by cephalosporins[6]. In this study, we tried to reveal the convulsant activity of cephalosporins including cefpirome, one of the new cephalosporins. As shown in Fig. 1, cephalosporins inhibited GABA receptor binding in a concentration-dependent manner. This result suggests that these agents might induce convulsions through the inhibition of GABA receptor binding, when they are accumulated in the CNS.

The Ministry of Public Health and Welfare in Japan have reported that concurrent administration of enoxacin, a fluoroquinolone and fenbufen (NSAID) induced convulsions in the patients without any convulsive disorders. We have reported that fluoroquinolones inhibited GABA receptor binding, and that this inhibitory activity of fluoroquinolones on GABA receptor binding was enhanced in the presence of NSAIDs[7], especially with the addition of 4-biphenyl acetate, an active metabolite of fenbufen. We also suggested that concurrent administration of fluoroquinolones and NSAIDs might induce convulsions in lower concentrations of fluoroquinolones. As the concurrent administration of cephalosporins and NSAIDs is relatively common, it is important to know whether NSAIDs enhance the inhibitory activity of cephalosporins on GABA receptor binding. To solve this problem, we determined the inhibitory activity of cephalosporins (2×10^{-3} M) in the presence of various NSAIDs (10^{-4} M). The inhibitory activity of the cephalosporins was not enhanced, even in the presence of NSAIDs (Table 1).

From these results, we suggested that cephalosporins might induce convulsions when they accumulate in the CNS. However, concurrent administration of cephalosporins and NSAIDs might not enhance the convulsant activity of cephalosporins.

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