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The β-lactam antibiotics, penicillin-G and cefoselis have different mechanisms and sites of action at $GABA_A$ receptors

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1 The action of the β-lactam antibiotics, penicillin-G (PCG) and cefoselis (CFSL) on $GABA_A$ receptors ($GABA_A$-R) was investigated using the two-electrode voltage clamp technique and *Xenopus* oocyte expressed murine $GABA_A$-R.

2 Murine $GABA_A$-Rs were expressed in *Xenopus* oocytes by injecting cRNA that encoded for each subunit (α1, β2, and γ2) and the effects of PCG and CFSL on the α1β2γ2s subunit receptors were examined using two-electrode voltage clamp. Using the α1β2γ2s $GABA_A$-R, PCG and CFSL inhibited GABA-induced currents in a concentration-dependent manner, with IC_{50}s of 557.1 ± 125.4 and 185.0 ± 26.6 μM, respectively. The inhibitory action of PCG on GABA-induced currents was non-competitive whereas that of CFSL was competitive.

3 Mutation of tyrosine to phenylalanine at position 256 in the β2 subunit ($\beta2_{Y256F}$), which is reported to abolish the inhibitory effect of picrotoxin, drastically reduced the potency of PCG ($IC_{50} = 28.4 \pm 1.42$ mM) for the α1$\beta2_{Y256F}$γ2s receptor without changing the IC_{50} of CFSL (189 ± 26.6 μM).

4 These electrophysiological data indicate that PCG and CFSL inhibit $GABA_A$-R in a different manner, with PCG acting non-competitively and CFSL competitively. The mutational study indicates that PCG might act on an identical or nearby site to that of picrotoxin in the channel pore of the $GABA_A$-R.

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Abbreviations: CFSL, cefoselis sulphate; DMSO, dimethylsulphoxide; $GABA_A$-R, gamma-amino butyric acid type A receptor; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; PCG, penicillin-G; Tricaine, 3-aminobenzoic acid ethyl ester

Introduction

The $GABA_A$-R plays a major role in inhibitory synaptic transmission within the central nervous system, and is a member of the ligand gated ion channel superfamily that includes glycine, acetylcholine and $5HT_3$ receptors. The $GABA_A$-R is presumed to be a hetero-pentameric receptor, with molecular cloning techniques identifying 19 genes that encode for the subunits; these have been divided into seven classes (Barnard *et al*., 1998). The different combinations of $GABA_A$-R subunits show varying levels of sensitivity to different drugs that include convulsants, sedatives, and general anaesthetics (Franks & Lieb, 1994; Sieghart, 1995; Barnard *et al*., 1998).

Antibiotics can have serious adverse effects on the central nervous system (CNS), producing confusion, twitching and convulsions (Wallace, 1997). Indeed, penicillins have been widely reported to induce convulsions in clinical situations, with this effect described as an example of antibiotic-induced neurotoxicity (Curtis *et al*., 1972; Weinstein *et al*., 1964). The effects of penicillins on the CNS have been thoroughly investigated both *in vitro* and *in vivo*, and appear to be mediated by suppression of inhibitory postsynaptic responses, predominantly at $GABA_A$-R (Wallace, 1997). The fact that positive modulators of $GABA_A$-R such as benzodiazepines and barbiturates can prevent or treat antibiotic-induced convulsions is consistent with this hypothesis (Barrons *et al*., 1992; Zeng *et al*., 1992; Wallace, 1997). In addition, antibiotics, including β-lactams such as penicillin and cephalosporines have been shown to inhibit GABA-induced currents in $GABA_A$-R neurons (Twyman *et al*., 1992; Tsuda *et al*., 1994; Fujimoto *et al*., 1995), however, the molecular mechanism and site of action of these antibiotics at the $GABA_A$-R remains obscure.

In examining $GABA_A$-R function, care should be taken that a single mutation of tyrosine to phenylalanine at position 256 ($\beta2_{Y256F}$) on the β2 subunit, when combined with wild type α and γ subunits, produces picrotoxin insensitivity (Gurley *et al*., 1995). This mutation is at a position predicted to be near the centre of the M2 region, which may be deep within the channel pore (Gurley *et al*., 1995). As studies indicate that penicillin may inhibit $GABA_A$-R in a non-competitive manner like the ion channel blocker picrotoxin (Fujimoto *et al*., 1995; Twyman *et al*., 1992),

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examining the effects of penicillin on GABA-induced currents in this $\beta 2$ mutant could reveal important information on the molecular site of action of penicillin. In the present study, the effects of PCG and a newly developed cephalosporin, CFSL (Mine *et al*., 1993a, b), were investigated electrophysiologically using *Xenopus* oocytes expressed $GABA_A$-R. The effects of PCG and CFSL on GABA-induced currents were studied using the $\alpha 1\beta 2\gamma 2$s and $\alpha 1\beta 2_{Y256F}\gamma 2$s subunit combinations to try and delineate the mechanism and site of action of these antibiotics.

Method

Xenopus *oocyte electrophysiology*

Preparation of cRNA for $GABA_A$ receptor subunits Mouse cDNAs encoding for $\alpha 1$, $\beta 2$, and $\gamma 2$s $GABA_A$-R subunits were kindly provided by Dr J Yang (University of Rochester, Rochester, U.S.A.) and Dr D Burt (University of Maryland, Baltimore, U.S.A.). All subunits were subcloned into the transcription vector, modified pBluescript (pBluescriptMXT), with the multiple cloning sites flanked by the β-globulin of *Xenopus laevis* in order to facilitate stable mRNA expression in oocytes. Plasmid cDNAs were purified using Qiagen's plasmid preparation kit (Qiagen, Chatworth, CA, U.S.A.), resuspended in sterile water and the cloned DNAs for the different subunits verified by restriction digest. Each cDNA template was linearized by restriction digest (Bgl I for $\beta 2$ and $\beta 2_{Y256F}$; Pvu II for $\alpha 1$ and $\gamma 2$s; Wako, Osaka, Japan). Capped mRNA was synthesized *in vitro* using Ambion's T3 RNA message machine kit (Ambion, Austin, TX, U.S.A.) by following the manufacturer's recommended protocol. Stock mRNA's were stored in RNAse-free water at -80°C until use.

Site-directed mutagenesis of the $\beta 2$ $GABA_A$-R subunit

Site-directed mutagenesis of the $\beta 2$ subunit (tyrosine to phenylalanine at position 256 of the amino acid sequence) was performed using Stratagene's QuikChange™ Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, U.S.A.) as per the manufacturer's protocol. The primers, 5′-CGGGTTGCATTAGGAATT<u>TTC</u>ACTGTCCTAACAAT-GACC-3′ and 5′-GGTCATTGTTAGGACAGT<u>GAA</u>AATT-CCTAATGCAACCCG-3′, were designed to incorporate the base sequence for phenylalanine instead of tyrosine at position 256 of the $\beta 2$ subunit (the mis-match base pairs are underlined). The modified DNA sequence was verified using an automated sequencer (Applied Biosystems).

Xenopus *oocyte expression* In accordance with the study protocol approved by the Animal Research Committee of Osaka University Medical School, female frogs (*Xenopus laevis*) were anaesthetized with 1% tricaine (3-aminobenzoic acid ethyl ester), and surgery performed on ice under sterile conditions. Oocytes were harvested through a 5 mm laparotomy incision and frogs returned to the main tank after 2 days in isolation. Oocytes were manually defolliculated with forceps and treated with collagenase type 1A (1.5 mg ml^{-1}) (Sigma, St Louis, MO, U.S.A.) for 30 min at room temperature in Ca^{2+}-free ND96 (in mM: NaCl 96, KCl 2, HEPES 5, $MgCl_2$1). Healthy oocytes at stage 4 and 5 were selected and thoroughly rinsed with ND96. The desired combination of $GABA_A$-R subunit cRNAs (0.1 mg ml^{-1}) were mixed in equal ratios and 50–100 nl injected into oocytes using a Nanoject injector (Drummond Scientific, Broomall, PA, U.S.A.). Prior to electrophysiological experiments, oocytes were incubated for 24–72 h at 20°C in ND96 containing $CaCl_2$(1.8 mM) and sodium pyruvate (2.5 mM).

Electrophysiology and drug application

Twenty-four to 72 h after mRNA injection, oocytes were placed in a small well and continuously perfused with frog Ringer's solution (in mM: NaCl 115, KCl 2.5, $CaCl_2$ 1.8, HEPES 10 at pH 7.4) at a rate of 10 ml min^{-1}, using a perfusion system constructed of polyethylene tubing. Oocytes were impaled with two glass electrodes (2–5 MΩ) filled with 3 M KCl, and voltage clamped at -80 mV using a two-electrode voltage amplifier (Nihon Khoden, Tokyo, Japan). All electrophysiological experiments were performed at room temperature. Drug solutions were applied by switching three-way stopcocks from frog Ringer's solution to an otherwise identical solution containing the test drug at the desired concentration. Drugs were applied for at least 20 s to obtain peak currents, with GABA applications separated by varying intervals (1–10 min) depending upon the drug concentration used, avoiding receptor desensitization. To test for cumulative desensitization, a low concentration of GABA (5 μM) was applied following the response to higher concentrations. Currents were digitally recorded with AxoScope software (Axon Instruments, Burlingame, CA, U.S.A.), running on an IBM personal computer. To construct concentration-response curves for GABA-induced currents and inhibition curves for antibiotics, observed peak amplitudes were normalized and plotted, and the data fitted to the following equation using Origin software (Microcal Software, Northampton, MA):

$$I = I_{max}/(1 + (ED_{50}{}^{n}/[GABA])^{n}) \quad (1)$$

where I is the peak current at a given concentration of GABA, I_{max} is the maximum current and ED_{50} and n denote the concentration of GABA eliciting a half-maximal response and the Hill coefficient, respectively. For inhibition studies with PCG and CFSL the data were fitted to the following equation:

$$I = [1 - [AB]^{n}/(IC_{50}{}^{n} + [AB]^{n})] \quad (2)$$

where I is the reduced current normalized with control data at a given concentration of antibiotic (AB) and IC_{50} denotes the concentration of antibiotics that produce half maximal currents.

Data analysis

All data are expressed as mean ± s.e.mean and statistical analysis was performed using a 2-tailed *t*-test or one-way analysis of variance (ANOVA), with $P<0.05$ indicating significance.

Materials

PCG was purchased from Sigma (St. Louis, MO, U.S.A.), and CFSL was synthesized by the Fujisawa Pharmaceutical Co., Ltd. (Osaka, Japan). GABA, picrotoxin, bicuculline

methiodide, baclofen and diazepam were from Wako (Osaka, Japan). In the electrophysiological experiments all drugs except diazepam were directly dissolved in frog Ringer's solution. Diazepam was dissolved in dimethylsulfphoxamide (DMSO) and then diluted with frog Ringer's solution to the desired concentration. The final concentration of DMSO never exceeded 0.05%, which itself had no effect. When PCG and CFSL were dissolved in frog Ringer's solution, the pH was re-adjusted to pH 7.4 with either 1 N HCl or NaOH, respectively.

Results

The effects of PCG and CFSL on $\alpha1\beta2\gamma2s$ $GABA_A$ receptors.

The application of GABA (1–300 μM) to recombinant $\alpha1\beta2\gamma2s$ $GABA_A$-R expressed in oocytes, evoked inward currents in a concentration-dependent manner. Following a maximal response to GABA (300 μM), constant responses to 5 μM GABA were obtained between recordings to exclude receptor desensitization. In addition, ED_{20} GABA-induced currents were blocked by 10^{-5} M bicuculline methiodide and 10^{-5} M picrotoxin (data not shown; Gurley *et al*., 1995; Sieghart, 1995; Whiting *et al*., 1995). Expression of the $\gamma2s$ subunit was confirmed by the potentiation of GABA-induced currents by 10^{-6} M diazepam using the $\alpha1\beta2\gamma2s$ receptor (data not shown; Verdoorn *et al*., 1990).

PCG and CFSL both inhibited GABA-induced currents in a concentration-dependent manner (Figure 1A,B). The application of either PCG (up to 1 M) or CFSL (up to 10 mM) to oocytes expressing the $\alpha1\beta2\gamma2s$ subunit $GABA_A$-R (i.e., in the absence of GABA) did not generate any measurable currents (data not shown). Concentration-response curves for GABA in the absence and presence of PCG (500 μM) are shown in Figure 1C. The ED_{50} values for GABA from the concentration-response curves using the $\alpha1\beta2\gamma2s$ subunit receptor were 17.4 ± 1.8 μM and 16.0 ± 1.1 μM in the absence and presence of PCG, respectively. PCG suppressed the maximum response induced by GABA without changing the ED_{50} values, with the Line-

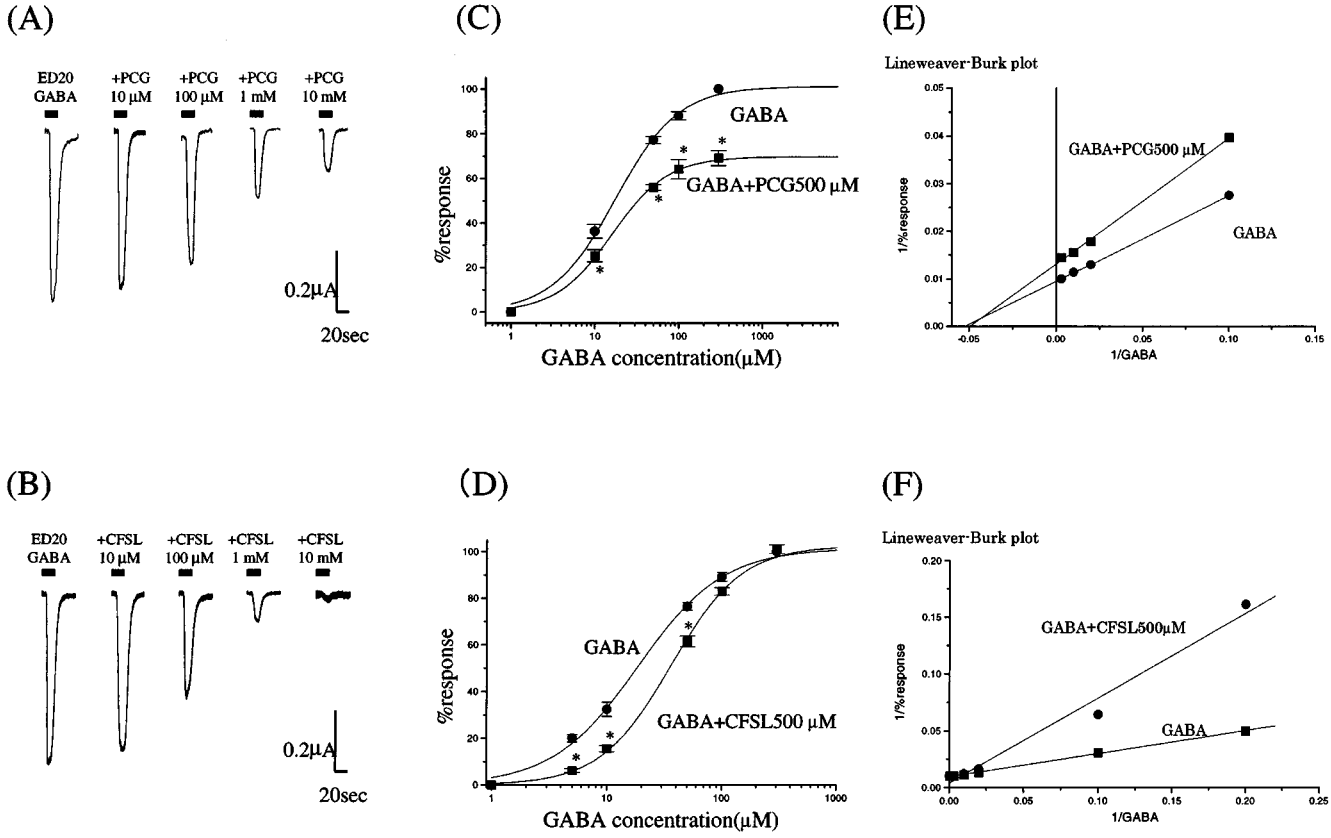

Figure 1 The effect of PCG and CFSL on GABA-induced currents on $\alpha1\beta2\gamma2s$ $GABA_A$ receptors expressed in *Xenopus* oocytes. (A,B) Co-application of GABA (EC_{20} : 5 μM) and PCG or CFSL resulted in a concentration dependent reduction in current amplitude. The bars over the current traces indicate the duration of drug application. (C–F) The effects of PCG (500 μM) and CFSL (500 μM) on the concentration-response curves of GABA. The ED_{50} values of GABA calculated from the dose-response curves using the $\alpha1\beta2\gamma2s$ subunit receptor were 17.4 ± 1.8 and 16.0 ± 1.1 μM, in the absence and presence of PCG respectively. PCG suppressed the maximum response induced by GABA without changing the ED_{50} values (C). In contrast, CFSL shifted the dose response curve to the right without affecting the maximum response. The ED_{50} value shifted from 18.5 ± 1.5 μM to 36.1 ± 2.4 μM in the presence of CFSL (D). Lineweaver-Burk plots of concentration-response curves show that inhibition by PCG was non-competitive (E) and that by CFSL was competitive (F). All the GABA responses were normalized to the peak current amplitude induced by 300 μM GABA alone. Each data point shows the average from five to seven oocytes, and is expressed as mean$\pm$s.e.mean, with asterisks indicating significant differences ($P<0.05$).

weaver-Burk plots (Figure 1E) showing that inhibition by PCG was non-competitive. In contrast, CFSL shifted the dose response curve to the right without affecting the maximum response (Figure 1D). The ED_{50} value for GABA changed from 18.5 ± 1.5 μM to 36.1 ± 2.4 μM in the presence of CFSL (500 μM), with the Lineweaver-Burk plot (Figure 1F) showing that inhibition by CFSL was competitive.

The effects of PCG and CFSL on mutant $GABA_A$ receptors ($\alpha1\beta2_{Y256F}\gamma2s$).

Application of GABA to oocytes expressing the $\alpha1\beta2_{Y256F}\gamma2s$ subunit receptor, resulted in currents with similar pharmacological properties to the native $\alpha1\beta2\gamma2s$ subunit receptor. The ED_{50} for GABA was 17.4 ± 1.8 μM for the $\alpha1\beta2\gamma2s$ subunit receptor and 15.2 ± 1.8 μM for the $\alpha1\beta2_{Y256F}\gamma2s$ subunit receptor. GABA-induced currents in both receptors were blocked by bicuculline, but the $\alpha1\beta2_{Y256F}\gamma2s$ subunit receptor was insensitive to picrotoxin as reported previously (Figure 2A,B; Gurley *et al*., 1995). The effects of PCG on the $\alpha1\beta2\gamma2s$ and $\alpha1\beta2_{Y256F}\gamma2s$ receptors were different (Figure 2D), with the $\beta2_{Y256F}$ subunit having a drastically reduced sensitivity to PCG. The IC_{50} of PCG for inhibition of GABA-induced currents was 557.1 ± 125.4 μM for the $\alpha1\beta2\gamma2s$ subunit receptor, and 28.4 ± 1.42 mM for the $\alpha1\beta2_{Y256F}\gamma2s$ subunit receptor. However, mutation of the $\beta2_{Y256F}$ subunit did not change the affinity of CFSL for $\alpha1\beta2\gamma2s$ receptors. Figure 2C shows concentration-dependent response curves for CFSL inhibition of GABA-induced currents at these receptors. The IC_{50} of CFSL for inhibition of GABA-induced currents was 185.0 ± 26.6 μM for the $\alpha1\beta2\gamma2s$ subunit receptor, and 189.5 ± 25.2 μM for the $\alpha1\beta2_{Y256F}\gamma2s$ subunit receptor. This indicates that the single mutation of the $\beta2$ subunit ($\beta2_{Y256F}$) responsible for picrotoxin insensitivity can play a critical role in the modulation of PCG, but not CFSL on $GABA_A$ receptor function.

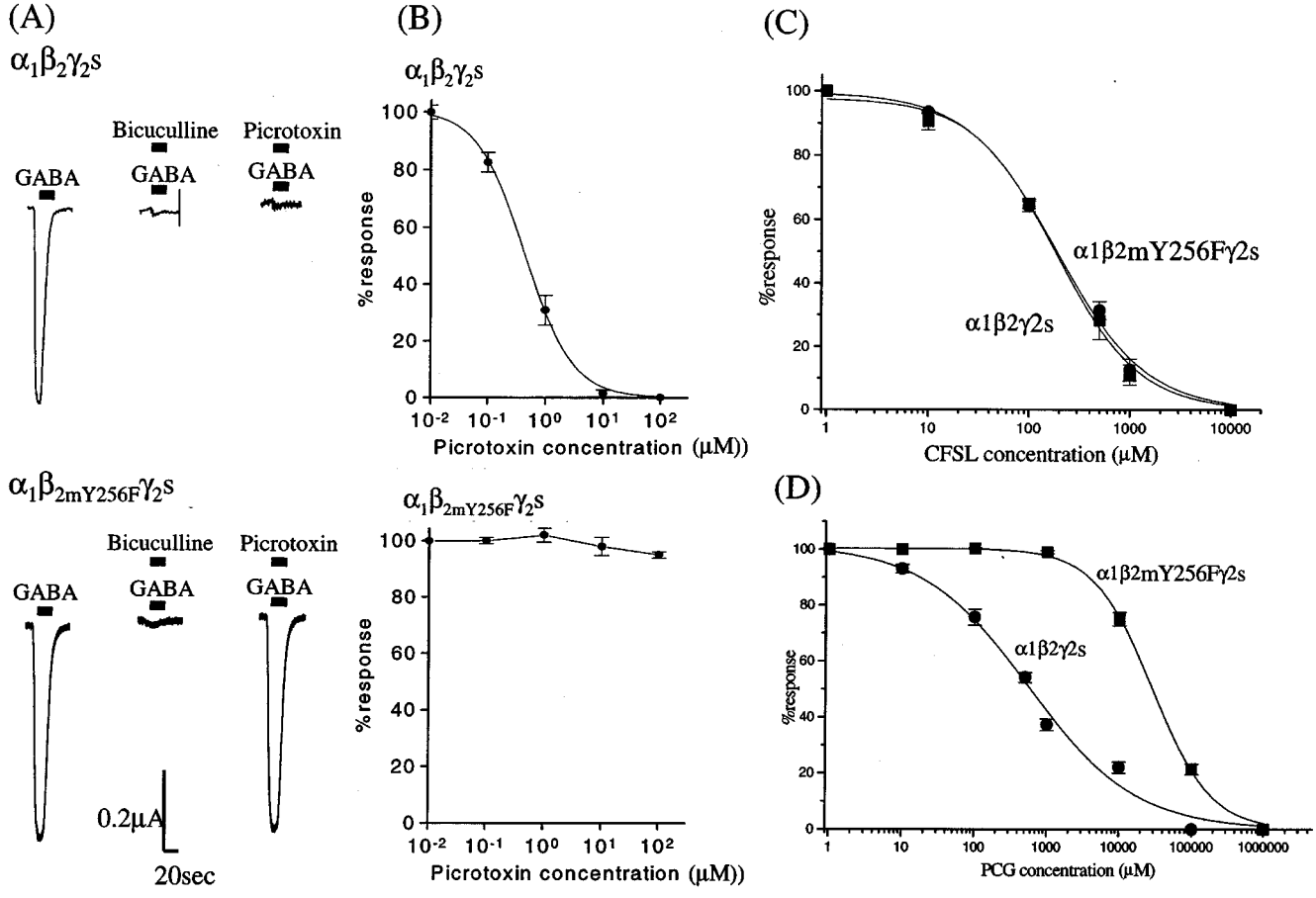

Figure 2 The effects of the mutation ($\beta2_{Y256F}$) on the $\alpha1\beta2\gamma2s$ subunit $GABA_{A-R}$. (A) Bicuculline methiodide (10 μM) completely blocked GABA (ED_{20})-induced currents in both $\alpha1\beta2\gamma2s$ and $\alpha1\beta2_{Y256F}\gamma2s$ $GABA_{A-R}$. Picrotoxin (10 μM) completely inhibited GABA-induced currents in the $\alpha1\beta2\gamma2s$ $GABA_{A-R}$, but not in the $\alpha1\beta2_{Y256F}\gamma2s$ mutant $GABA_{A-R}$. (B) Concentration-response curves for inhibition of GABA (ED_{20})-induced currents in $\alpha1\beta2\gamma2s$ and $\alpha1\beta2_{Y256F}\gamma2s$ $GABA_{A-R}$ by picrotoxin. Each data point represents the average from four to seven oocytes. The IC_{50}s for picrotoxin at the $\alpha1\beta2\gamma2s$ receptor were 0.44 ± 0.03 and 1.05 ± 0.06 μM, respectively. Using the $\alpha1\beta2_{Y256F}\gamma2s$ subunit $GABA_{A-R}$ mutant, picrotoxin (0.01–100 μM) did not inhibit GABA-induced currents. (C,D) Inhibition of GABA (ED_{20})-induced currents in $\alpha1\beta2\gamma2s$ and $\alpha1\beta2_{Y256F}\gamma2s$ $GABA_{A-R}$ by PCG (C) and CFSL (D). Each data point represents the average from five to seven oocytes. IC_{50}s values for CFSL at the $\alpha1\beta2\gamma2s$ and $\alpha1\beta2_{Y256F}\gamma2s$ subunit receptors were 185.0 ± 26.6 μM and 189.5 ± 25.2 μM, respectively. This mutation of the $\beta2$ subunit produced no significant alteration in the affinity CFSL. The IC_{50} of PCG for inhibition of GABA-induced currents in the $\alpha1\beta2\gamma2s$ subunit $GABA_{A-R}$ was 557.1 ± 125.4 μM. In contrast, PCG had a markedly reduced affinity (IC_{50}; 28.4 ± 4.42 mM) for the $\alpha1\beta2_{Y256F}\gamma2s$ subunit receptor. The asterisks indicate significant difference ($P<0.05$).

Discussion

β-lactam antibiotics such as penicillins and cephalosporins are frequently used in the clinical treatment of various infectious diseases. Treatment with β-lactams can produce adverse effects such as convulsions, that are thought to be due to suppression of inhibitory postsynaptic responses, mainly mediated by GABA (Curtis *et al*., 1972; Wallace, 1997). However, the mechanism and site of action of antibiotics at $GABA_A$-R remain obscure. Among the β-lactams, the penicillins have been relatively well investigated, with electrophysiological and biochemical studies on native neurons showing that penicillins appear to inhibit $GABA_A$-R in a non-competitive and voltage-dependent manner (Pickles & Simmonds, 1980; Tsuda *et al*., 1994, Fujimoto *et al*., 1995). Studies using single channel recordings from neurons, indicate that open channel block of $GABA_A$-R is the mechanism by which penicillin inhibits GABA-induced currents (Twyman *et al*., 1992). Other β-lactam antibiotics, such as the cephalosporins, which also have strong epileptogenic activities (Wallace, 1997), have been less well characterized. Previous binding studies indicated that some cephalosporins may act as competitive inhibitors of $GABA_A$-R function (Hori *et al*., 1985), however there was no data on the effects of the newly developed cephalosporins, such as CFSL. These observations suggest that not all antibiotics inhibit $GABA_A$-R by the same mechanism of action.

In the present study, the electrophysiological data from recombinant $GABA_A$-R suggest that PCG and CFSL act differently at $GABA_A$-R. We demonstrated using recombinant $\alpha1\beta2\gamma2$s subunit $GABA_A$-R that PCG and CFSL both inhibit GABA-induced currents in a concentration dependent manner with similar potency, but that their mode of actions were non-competitive and competitive, respectively. Furthermore, the results from the experiments using the mutated β subunits not only support the evidence for the different mechanism of actions of PCG and CFSL, but also give new information on the molecular site of action of PCG at the $GABA_A$-R. Mutation of tyrosine to phenylalanine at position 256 on the $\beta2$ $GABA_A$-R subunit was reported to abolish the inhibitory effect of the classical non-competitive inhibitor, picrotoxin, without general impairment of channel function (Gurley *et al*., 1995). This mutation of the β subunit also drastically reduced the ability of PCG to inhibit GABA-induced currents when co-expressed with the α and γ subunits but its effects were not completely abolished like that of picrotoxin (Gurley *et al*., 1995). This indicates that the binding site of PCG site on the $GABA_A$-R might be in close proximity to that of picrotoxin. Further mutagenesis studies around position 256 of the β subunit are required to clarify whether it is possible to distinguish between sites for PCG and picrotoxin. Tyrosine 256 is predicted to be near the centre of the M2 region, which may lie deep within the channel pore (Gurley *et al*., 1995). Our data strongly suggests that PCG is similar to picrotoxin and can inhibit GABA-induced currents by acting at a site deep within the channel pore. As the Y256F mutation did not decrease the potency of the natural agonist ligand GABA, these data imply that the hydroxy group of the Tyr256 residue does not directly interact with GABA, or play any major role in maintaining the active conformation of the $GABA_A$-R. In sharp contrast, this single mutation drastically reduced the potency of the antagonist PCG, suggesting that it could interact directly with the hydroxy group of Tyr256. However, this study does not rule out the possibility that the Y256F mutation might result in a critical conformational change in an allosteric binding site for PCG, which may be located on the surface of the receptor, without affecting the conformation of the agonist binding site (Twyman *et al*., 1992)

In summary, this is the first report on the $GABA_A$-R that uses recombinant receptors and site directed mutagenesis to demonstrate that the antibiotics PCG and CFSL inhibit $GABA_A$-R function *via* different mechanisms, with the former acting non-competitively and the latter competitively. Site directed mutagenesis of the β subunit also revealed that the site of action of PCG on the $GABA_A$-R might be closely related to the picrotoxin binding site in the M2 region of the $\beta2$ subunit.

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References

BARNARD, E.A., SKOLNICK, P., OLSEN, R.W., MOHLER, H., SIEGHART, W., BIGGIO, G., BRAESTRUP, C., BATESON, A.N. & LANGER, S.Z. (1998). International Union of Pharmacology. XV. Subtypes of gamma-aminobutyric acid A receptors: classification on the basis of subunit structure & receptor function. *Pharmacol. Rev.,* **50,** 291 – 313.

BARRONS, R.W., MURRAY, K.M. & RICHEY, R.M. (1992). Populations at risk for penicillin-induced seizures. *Ann. Pharmacother.,* **26,** 26 – 29.

CURTIS, D.R., GAME, C.J.A., JOHNSTON, G.A.R., MCCULLOUCH, R.M. & MACLACHLAN, R.M. (1972). Convulsive action of penicillin. *Brain Res.,* **43,** 242 – 245.

FRANKS, N.P. & LIEB, W.R. (1994). Molecular and cellular mechanisms of general anesthesia. *Nature,* **367,** 607 – 614.

FUJIMOTO, M., MUNAKATA, M. & AKAIKE, N. (1995). Dual mechanisms of $GABA_A$ response inhibition by β-lactam antibiotics in the pyramidal neurons of the rat cerebral cortex. *Br. J. Pharmacol.,* **116,** 3014 – 3020.

GURLEY, D., AMIN, J., ROSS, P.C., WEISS, D.S. & WHITE, G. (1995). Point mutation in the M2 region of the α, β, or γ subunit of the $GABA_A$ channel that abolish block by picrotoxin. *Receptors Channels,* **3,** 13 – 20.

HORI, S., KURIOKA, S., MATSUDA, M. & SHIMADA, J. (1985). Inhibitory effect of cephalosporins on gamma-aminobutyric acid receptor binding in rat synaptic membranes. *Antimicrob. Agents Chemother.,* **27,** 650 – 651.

MINE, Y., WATANABE, Y., SAKAMOTO, H., HATANO, K., KUNO, K., HIGASHI, Y., KAMIMURA, T., MATSUMOTO, Y., TAWARA, S., MATSUMOTO, F. & KUWAHARA, S. (1993a). In vitro antibacterial activity of FK037, a novel pareteral broad-spectrum cephalosporin. *J. Antibiotics.,* **46,** 71 – 87.

MINE, Y., WATANABE, Y., SAKAMOTO, H., HATANO, K., WAKAI, Y., KAMIMURA, T., TAWARA, S., MATSUMOTO, S., MATSUMOTO, F. & KUWAHARA, S. (1993b). In vivo antibacterial activity of FK037, a novel parenteral broad-spectrum cephalosporin. *J. Antibiotics,* **46,** 88 – 98.

PICKLES, H.G. & SIMMONDS, M.A. (1980). Antagonism by penicillin of GABA depolarizations at presynaptic sites in rat olfactory cortex and cuneate nucleus *in vitro*. *Neuropharmacology,* **19,** 35–38.

SIEGHART, W. (1995). Structure and pharmacology of γ-aminobutyric acid A receptor subtypes. *Pharmacol. Rev.,* **47,** 181–234.

TSUDA, A., ITO, M., KISHI, K., SHIRAISHI, H., TSUDA, H. & MORI, C. (1994). Effect of penicillin on GABA-gated chloride ion influx. *Neurochem. Res.,* **19,** 1–4.

TWYMAN, R.E., GREEN, R.M. & MACDONALD, R.L. (1992). Kinetics of open channel block by penicillin of single $GABA_A$ receptor channels from mouse spinal cord neurons in culture. *J. Physiol.,* **445,** 97–127.

VERDOORN, T.A., DRAGUHN, A., YMER, S., SEEBURG, P.H. & SAKMANN, B. (1990). Functional properties of recombinant rat $GABA_A$ receptors dependent upon subunit composition. *Neuron,* **4,** 919–928.

WALLACE, K.L. (1997). Antibiotic-induced convulsions. *Crit. Care Clin.,* **13,** 741–762.

WEINSTEIN, L., LERNER, P.I. & CHEW, W.H. (1964). Clinical and bacteriologic studies of the effect of ''Massive'' doses of penicillin G on infections caused by Gram-negative bacilli. *N. Engl. J. Med.,* **271,** 525–533.

WHITING, P.J., MCKERNAN, R.M. & WAFFORD, K.A. (1995). Structure and pharmacology of vertebrate $GABA_A$ receptor subtypes. *Int. Rev. Neurobiol.,* **38,** 95–138.

ZENG, Y.C., PEZZOLA, A., SCOTTI DE CAROLIS, A. & SAGRATELLA, S. (1992). Inhibitory influence of morphinans on ictal and interictal EEG changes induced by cortical application of penicillin in rabbits: a comparative study with NMDA antagonists and pentobarbitone. *Pharmacol. Biochem. Behav.,* **43,** 651–656.

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