ELSEVIER

European Journal of Pharmacology 430 (2001) 243–249

ejp

www.elsevier.com/locate/ejphar

Ovariectomy aggravates convulsions and hippocampal γ-aminobutyric acid inhibition induced by cyclosporin A in rats

Koji Tominaga, Atsushi Yamauchi [1], Hideki Shuto, Midori Niizeki, Kazutaka Makino, Ryozo Oishi, Yasufumi Kataoka *

Department of Hospital Pharmacy, Faculty of Medicine, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

Received 12 April 2001; received in revised form 28 August 2001; accepted 30 August 2001

Abstract

The possible cyclosporin A application for rheumatoid arthritis that develops preferentially in middle-aged women raises concerns about adverse effects of cyclosporin A, including neurotoxicity in patients with climacterium. The present study was aimed at elucidating the effect of cyclosporin A on the convulsive activity and γ-aminobutyric acid (GABA) neural activity of the hippocampus in ovariectomized rats, as a menopause/climacterium model. Ovariectomy markedly aggravated the effect of repeated administration of cyclosporin A (40 mg/kg, once a day for 5 or 6 days), convulsions and reduction of the basal GABA levels and aminooxyacetic acid-evoked GABA accumulation. These aggravations were blocked by estradiol replacement. The present findings demonstrated that ovariectomy increased the susceptibility to cyclosporin A-induced convulsions by accelerating an inhibitory action of cyclosporin A on GABA neural activity in the hippocampus, this being blocked by estrogen replacement. Menopause/climacterium is, therefore, included in the risk factors for cyclosporin A-induced neurotoxicity and this risk is lowered by estrogen replacement therapy.

Keywords: Cyclosporin A; Ovariectomy; Convulsion; γ-Aminobutyric acid (GABA); Menopause; Climacterium

1. Introduction

Cyclosporin A, a cyclic 11-amino acid peptide, is widely used as a potent immunosuppressant to prevent allograft rejection in solid organ transplantation and in fatal graft-versus-host disease after bone marrow transplantation and to treat various autoimmune diseases including rheumatoid arthritis, psoriasis and idithic nephrotic syndrome (Kahan, 1989). Recently, the use of cyclosporin A was extended to atopic dermatitis and systemic lupus erythematosus (Naeyaert et al., 1999; Klein et al., 1999). Despite its high efficacy, cyclosporin A induces adverse effects including impaired renal function, cardiovascular disorders, gastrointestinal disorders and neurological complications. These events were found to occur with a relatively high frequency (20–40%) in organ-transplanted patients (Gijtenbeek et al., 1999; Pirsch et al., 1997; U.S. Group, 1994). The adverse neurological effects of cyclosporin A including tremor, seizure and encephalopathy were suggested to be triggered by dysfunction of the P-glycoprotein, a multi-drug efflux pump, and hyperpermeability of the brain capillary endothelial cells (Dohgu et al., 2000; Kochi et al., 1999). We previously reported that cyclosporin A produced convulsions by inhibiting the neural activity of γ-aminobutyric acid (GABA) and binding properties of $GABA_A$ receptors (Shuto et al., 1999). A facilitatory action of cyclosporin A on stimulation-evoked nitric oxide production in glial cells was also attributed to the occurrence of neurotoxicity (Ikesue et al., 2000).

The menopause or climacterium is a critical risk factor for cardiovascular diseases (Greendale et al., 1999), osteoporosis and mental disorders (Pearce and Hawton, 1996). Estrogen treatment of males and females was reported to counter the development of neurological symptoms due to ischemia, stroke epilepsy, Alzheimer's disease and Parkinson's disease (Garcia-Segura et al., 2001; Green and Simpkins, 2000). In rats with ovariectomy, 17β-estradiol de-

* Corresponding author. Present address: Clinical Pharmacy Division, Department of Pharmacy, Faculty of Pharmaceutical Sciences, Fukuoka University, 8-19-1 Nanakuma, Jyounan-ku, Fukuoka 814-0180, Japan. Tel.: +81-92-871-6631x6630; fax: +81-92-863-0389.

E-mail address: ykataoka@cis.fukuoka-u.ac.jp (Y. Kataoka).

[1] Present address: Clinical Pharmacy Division, Department of Pharmacy, Faculty of Pharmaceutical Sciences, Fukuoka University, 8-19-1 Nanakuma, Jyounan-ku, Fukuoka 814-0180, Japan.

0014-2999/01/$ - see front matter
PII: S0014-2999(01)01377-2

layed the onset of kainic acid-induced clonic seizures and remedied the neuronal kainic acid-induced degeneration in the hippocampus (Veliskova et al., 2000). These findings suggest that the extremely low level of estrogen at the menopause may aggravate the risk of cyclosporin A-induced neurotoxicity. Furthermore, the possible cyclosporin A application for the rheumatoid arthritis that develops with high incidence in middle-aged women suggested concerns about cyclosporin A-induced adverse effects including neurotoxicity for patients during menopause or climacterium.

In the present study, we investigated the effect of cyclosporin A on convulsive activity and GABA neural activity in the hippocampus, known to be responsible for the induction of seizures (Green, 1986) in ovariectomised rats, as a menopause/climacterium model.

2. Materials and methods

2.1. Animals

Female Wistar rats weighting 160–200 g were purchased from Kyudo (Saga, Japan). The animals were maintained on a 12-h light/dark schedule (lights on 7:00 a.m.) at a temperature of 23 ± 2 °C with free access to food and water. This study was reviewed by the ethics committee regarding animal experiments at the Faculty of Medicine, Kyushu University and was performed according to the Guidelines for Animal Experiments in the Faculty of Medicine, Kyushu University, and the law (No. 105) and notification (No. 6) of the Japanese Government.

2.2. Drugs

The pharmaceutical formulations of the immunosuppressant agent and estradiol used were as follows: cyclosporin A (Sandimmun® injection, 250 mg/5 ml/ampule, Novartis Pharma, Tokyo, Japan) and estradiol valerate (Pelanin Depot®, 10 mg/ml/ampule, Mochida Pharmaceutical, Tokyo, Japan). *O*-Phthalaldehyde and 2-mercaptoethanol were purchased from Wako (Osaka, Japan). Aminooxyacetic acid and 3-mercaptopropionic acid were from Sigma (St. Louis, MO, USA). The vehicle solution for cyclosporin A consisted of 13% polyoxyethylene castor oil (Cremophor EL®, Sigma), 7% ethanol and 80% saline (the same mixture as the vehicle of the Sandimmun® injection). The original cyclosporin A injection was diluted with saline immediately before use.

2.3. Ovariectomy and estradiol treatment

Rats were bilaterally ovariectomized rats or sham-operated rats (sham rats) under sodium pentobarbital anesthesia (50 mg/kg, i.p.). At 7 days post-operation, ovariectomized rats were divided into two groups and the treatment of each group with vehicle (sesame oil) or estradiol valerate 1.0 mg/kg was initiated (estradiol/ovariectomized rats). Vehicle and estradiol were injected intramuscularly in a volume of 0.1 ml/100 g body weight once per week for 3 weeks starting 7 days post-operation. Sham rats were treated with vehicle from 7 days after operation. At 28 days post-operation, the animals were used for the behavioral and biochemical experiments.

2.4. Convulsions tests

Cyclosporin A (20 and 40 mg/kg) or vehicle was intraperitoneally (i.p.) administered once a day for 6 days to rats in a volume of 0.5 ml/100 g body weight. Sixty minutes after the 6th injection of cyclosporin A or vehicle, each rat was placed individually in a transparent plastic cage ($28 \times 44 \times 18$ cm) and the root of the tail was pinched with the forceps for 10 s. Behaviour was observed for 60 min. The intensity of convulsions was scored using the five-point semiquantitative rating scale described by Loskota et al. (1974) with a minor modification (Shuto et al., 1999) as follows: 0, no convulsion; 1, mild convulsions (motor arrest with more pronounced convulsions); 2, moderate convulsions (posture restraint, rigidity of extremities); 3, severe convulsions (clonic–tonic seizure with loss of righting reflex); 4, lethal seizure.

2.5. Estimation of GABA neural activity in the hippocampus

Effects of single and subchronic treatment with cyclosporin A on GABA neural activity in the rat hippocampus was estimated by measuring the basal GABA levels and GABA accumulation at 60 min after the injection of aminooxyacetic acid, a GABA-transaminase inhibitor as previously described (Grattan and Selmanoff, 1994). Rats were subjected to single and subchronic (once a day for 5 days) treatment with cyclosporin A (40 mg/kg, i.p.) or vehicle. Sixty minutes after the single or 5th injection, each of four separate groups (single vehicle, single cyclosporin A, subchronic vehicle and subchronic cyclosporin A injection) was divided into two sub-groups; one was decapitated to obtain the basal GABA levels 60 min after vehicle or cyclosporin A injection and the other was injected with aminooxyacetic acid (100 mg/kg, i.p.) 60 min after vehicle or cyclosporin A injection followed by decapitation after 60 min to measure GABA accumulation. To prevent non-specific postmortem synthesis of GABA, the rats were injected with 3-mercaptopropionic acid (1.2 mmol/kg i.p.) 2.5 min before decapitation to block glutamic acid decarboxylase, the rate-limiting enzyme of GABA synthesis. The brain was removed immediately after decapitation and the hippocampus was dis-

sected on an ice-cold glass plate. The tissues were rapidly frozen in liquid nitrogen and stored at −80 °C. The frozen tissue was homogenized in 1.8 ml of 0.1 M perchloric acid, with a part stored at −80 °C until assay of tissue protein content using a Bio-Rad DC protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA). The homogenized mixture was centrifuged at 20,000 × *g* at 4 °C for 15 min. The supernatant was filtered through a membrane filter (0.45 μm) and stored at −20 °C. GABA concentrations were determined in a high-performance liquid chromatography (HPLC) system with fluorometric detection after pre-column derivatization with *O*-phthalaldehyde and 2-mercaptoethanol. Fifty microliters of filtrate was reacted with 10 μl of 0.1 M carbonate buffer containing 4 mM *O*-phthalaldehyde and 5.7 mM 2-mercaptoethanol for 5 min. The reaction mixture was injected into a HPLC system consisting of two multi-pumps (CCPM-II, Tosoh, Tokyo, Japan), a controller (PX-8020, Tosoh), a degasser (SD-8023, Tosoh), a reversed phase column (Eicompak MA-5ODS, 4.6 × 150 mm, Eicom, Kyoto, Japan), and fluorometric detector (RF-550, Shimadzu, Kyoto, Japan). The excitation wavelength was 340 nm and the emission wavelength was 445 nm. The mobile phase was 0.02 M acetate buffer containing 35% acetonitrile (pH = 4.1), and flow rate was 1.0 ml/min.

2.6. Statistical analysis

Values are shown as the means ± S.E.M. The behavioral data were analyzed by the Kruskal–Wallis rank test, and individual comparisons were performed using the Mann–Whitney *U*-test, if a significant difference was shown in the Kruskal–Wallis rank test. GABA neural activity was analyzed by one-factor analysis of variance (ANOVA) followed by post-hoc Scheffe' F test. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Effect of ovariectomy on cyclosporin A-induced convulsions

Fig. 1A shows the time course of subchronic treatment with cyclosporin A (40 mg/kg, once a day for 6 days)-induced convulsions in sham-, ovariectomized- and estradiol/ovariectomized rats. Convulsions appeared gradually and the intensity of convulsions increased with time from the 4th to 6th injection. In ovariectomized rats, cyclosporin A significantly increased the convulsion score at the 5th injection, reaching a maximum at the 6th injection. The effects of ovariectomy and estradiol replacement on convulsions induced by subchronic treatment (1 × 6 days) with cyclosporin A are shown in Fig. 1B. Cyclosporin A (20 and 40 mg/kg) dose-dependently produced convulsions (score 0.2 and 1.5, respectively) in sham rats. Ovariectomy markedly increased the intensity of cyclosporin A (20 and 40 mg/kg)-induced convulsions to 3.5- and 2.5-fold convulsion scores in sham rats, respectively. A significant difference between sham- (1.5 ± 0.4) and ovariectomized- (3.8 ± 0.2) rats was observed for cyclosporin A (40 mg/kg)-induced convulsions. When ovariectomized rats were injected intramuscularly with estradiol valerate 1.0 mg/kg once per week for 3 weeks before the start of subchronic treatment with cyclosporin A, no facilitatory effect of ovariectomy on cyclosporin A-induced convulsions was observed and the score was decreased significantly to the level of that for sham rats (1.4 ± 0.4 in estradiol/ovariectomized rats).

3.2. Effect of ovariectomy on cyclosporin A-decreased GABA neural activity in the hippocampus

The basal GABA levels just before aminooxyacetic acid injection in the hippocampus were 5.2 ± 0.9, 3.6 ± 0.5 and

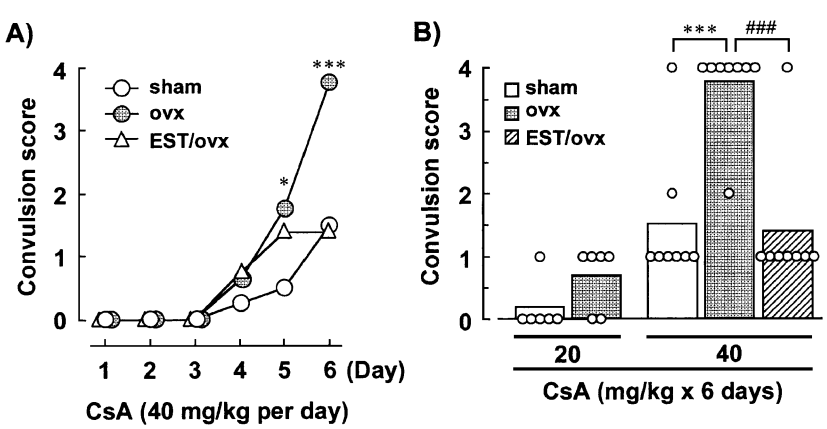

Fig. 1. Time course for the intensity of convulsions induced by subchronic treatment (once a day for 6 days) with cyclosporin A (CsA, 40 mg/kg) in sham-operated (sham), ovariectomized (ovx) and estradiol-treated ovariectomized rats (EST/ovx) (A) and effects of ovariectomy and estradiol replacement on convulsions 60 min after the 6th injection of cyclosporin A in rats. Open circles in panel B represent convulsion score for each rat. Values are the means ± S.E.M. for six to eight rats. $^{*}P < 0.05$ and $^{***}P < 0.001$; significant difference between ovariectomized rats and sham rats.

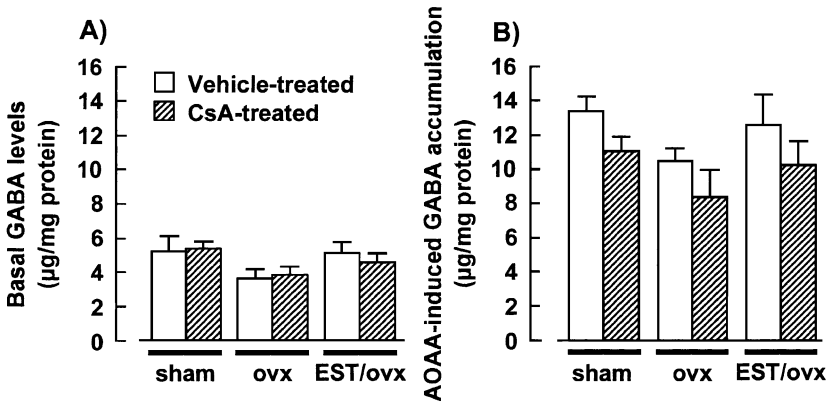

Fig. 2. Effect of single treatment with cyclosporin A (40 mg/kg) on the basal GABA levels (A) and the aminooxyacetic acid (AOAA, 100 mg/kg)-induced GABA accumulation during a 60-min period (B) in the hippocampus of sham-operated (sham), ovariectomized (ovx) and estradiol-treated ovx rats (EST/ovx). The basal levels and aminooxyacetic acid-induced accumulation of GABA were measured 60 min after, and during a period of 60 to 120 min, after cyclosporin A injection, respectively. Values are the means ± S.E.M. for five to six animals.

5.1 ± 0.6 μg/mg protein in vehicle-treated sham-, ovariectomized- and estradiol/ovariectomized rats, respectively (Fig. 2A). Ovariectomy moderately, although non-significantly, decreased GABA levels to 70% of those of sham rats, and this event was blocked by estradiol replacement. Single treatment with cyclosporin A (40 mg/kg) failed to influence the hippocampal GABA levels in any of the three groups, when compared with the effect of each corresponding vehicle. The amounts of GABA accumulation including basal at 60 min after aminooxyacetic acid injection in vehicle-treated sham-, ovariectomized- and estradiol/ovariectomized rats were 13.4 ± 0.8, 10.5 ± 0.7 and 12.6 ± 1.8 μg/mg protein, respectively (Fig. 2B). Cyclosporin A decreased GABA levels by 17–20% of that with the vehicle at 60 min after aminooxyacetic acid injection in all three groups. There was no significant difference among these groups for the decrease of GABA accumulation induced by a single injection of cyclosporin A.

Subchronic treatment (1 × 5 days) with vehicle induced the same changes in the basal levels and aminooxyacetic acid-induced accumulation of GABA (Fig. 3) as those obtained with single vehicle injection in each of the three groups (Fig. 2). The repeated administration of cyclosporin A (40 mg/kg/day × 5 days) in ovariectomized rats significantly decreased the basal GABA levels to 61.7 + 2.7% of those with the vehicle, but this effect was not observed in sham- and estradiol/ovariectomized rats (Fig. 3A). Cyclosporin A-treated sham-, ovariectomized- and estradiol/ovariectomized rats had 8.6 ± 1.2, 4.0 ± 0.4 and 8.1 ± 0.4 μg/mg protein for GABA accumulation, including the basal level at 60 min after aminooxyacetic acid injec-

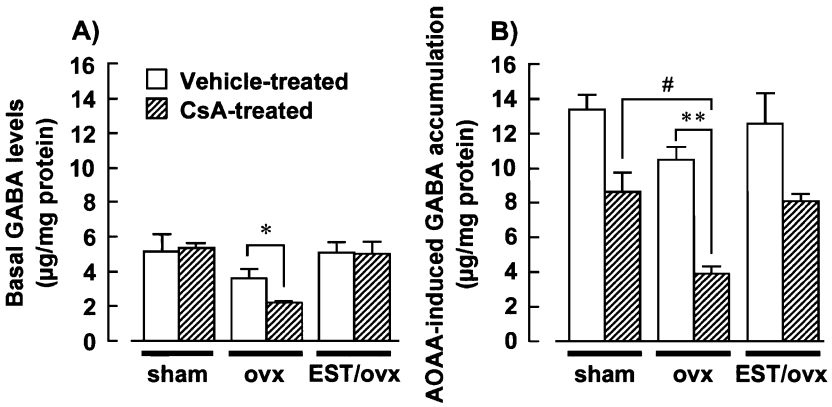

Fig. 3. Effect of subchronic treatment with cyclosporin A (40 mg/kg once a day for 5 days) on the basal GABA levels (A) and the aminooxyacetic acid (AOAA, 100 mg/kg)-induced GABA accumulation during a 60-min period (B) in the hippocampus of sham-operated (sham), ovariectomized (ovx) and estradiol-treated ovx rats (EST/ovx). The basal levels and aminooxyacetic acid-induced accumulation of GABA were measured 60 min after and during a period of 60 to 120 min after, the 5th injection of cyclosporin A, respectively. Values are the means ± S.E.M. for five to eight animals. $^{*}P < 0.05$ and $^{**}P < 0.01$; significant difference from each corresponding vehicle-treated group. $\#P < 0.05$; significant difference from cyclosporin A-treated sham rats.

tion, respectively (Fig. 3B). Subchronic treatment with cyclosporin A significantly inhibited aminooxyacetic acid-induced increase in GABA levels by 65% in ovariectomized rats. This inhibition was attenuated by estrogen replacement.

4. Discussion

In the present study, ovariectomy markedly increased the convulsions induced by subchronic treatment with cyclosporin A, when compared to those in sham rats. Estradiol replacement blocked cyclosporin A-increased convulsions in ovariectomized rats. The mechanisms mediating cyclosporin A-induced neurotoxicity are still not understood. Permeation of cyclosporin A across the blood–brain barrier is restricted by a drug efflux pump, P-glycoprotein, on the luminal membrane of the cerebral capillary endothelial cells. However, prolonged of mouse brain endothelial cells to cyclosporin A inhibited the function and expression of P-glycoprotein (Kochi et al., 1999). Astrocytes are known to positively maintain the barrier function of cerebral endothelial cells (Cancilla et al., 1993). It was shown that 17β-estradiol protects astrocytes against glutamate-induced cell death (Shy et al., 2000). Therefore, ovariectomy may lower the function of the blood–brain barrier, to cause a rise in brain cyclosporin A concentrations and/or an extravasation of fluid and proteins into the brain, leading to aggravation of neurotoxicity, including convulsions and encephalopathy.

In the present study, ovariectomy moderately decreased the basal GABA levels and aminooxyacetic acid-induced GABA accumulation in the rat hippocampus, a decrease that was restored to the level in sham rats by estradiol replacement. A single cyclosporin A injection decreased GABA accumulation in sham-, ovariectomized- and estradiol/ovariectomized rats. Interestingly, subchronic treatment with cyclosporin A significantly lowered the basal GABA levels in ovariectomized rats but not in sham or in estradiol/ovariectomized rats. The inhibitory action of cyclosporin A on aminooxyacetic acid-induced GABA accumulation was accelerated by subchronic administration in all three groups, this being most apparent in ovariectomized rats. We previously reported that cyclosporin A inhibited GABA neural activity in the mouse brain and binding properties of the $GABA_A$ receptor in the cultured rat cerebellar granule cells (Shuto et al., 1999). These findings suggest that ovariectomy facilitates the cyclosporin A-induced inhibition of the hippocampal GABA neural activity, thereby elevating the susceptibility to cyclosporin A-induced convulsions. This notion is further supported by the present findings that a time-dependent increase in subchronic cyclosporin A injection-induced convulsions was well correlated with the development of cyclosporin A-induced suppression in GABA neural activity in ovariectomized rats. In the hippocampal CA1 subfields of ovariectomized rats, estradiol increased the levels of mRNA for glutamic acid decarboxylase, the rate-limiting enzyme of GABA synthesis (Weiland, 1992); these mRNA levels are known to be dependent on the degree of GABA neuronal activation (Erlander and Tobin, 1991). The findings of the present study suggest that ovariectomy led to a decreased GABA neural activity in the hippocampus. Schumacher et al. (1989) demonstrated that estradiol increased [^{3}H]muscimol binding to $GABA_A$ receptors in specific regions of the hippocampal CA1 subfields which paralleled the localization of the estrogen receptors in ovariectomized plus adrenalectomized rats, suggesting that ovariectomy induces down-regulation of the $GABA_A$ receptors. The present findings taken together with these previous observations suggest that cyclosporin A can decrease the low activity in the hippocampal GABA neurotransmission in ovariectomized rats to a level under the threshold for the induction of convulsions more easily than the moderate activity maintained in sham and estradiol/ovariectomized rats. There is a report that estrogen produces negative modulation of the NMDA receptor of rat hippocampal neurons and protects neurons against NMDA excitotoxicity (Weaver et al., 1997). Therefore, the possibility that this antagonistic action on NMDA receptors contributes to raising the threshold level for the induction of convulsions in estradiol/ovariectomized rats cannot be excluded. Not only estrogen but also progesterone is depleted by ovariectomy, this phenomenon being accompanied by a rise in follicle-stimulating hormone and luteinizing hormone (Rannevik et al., 1995). Progesterone synthesized by endocrine glands in the periphery and glial cells in the brain has the potential to influence the activity of $GABA_A$ receptors (Lambert et al., 1995). Clinical study suggests that progesterone therapy is effective in the management of women with epilepsy (Herzog, 1995). Although the present findings demonstrated that estrogen replacement significantly protected against exacerbation of cyclosporin A-induced convulsions in ovariectomized rats, ovariectomy-induced progesterone depletion may be considered in the mediation of the effect of ovariectomy observed here. Tacrolimus and cyclosporin A inhibit neurotoxicity of NMDA in primary cultures of rat cortical neurons (Dawson et al., 1993) and protect the cortex from focal cereberal ischemia in rats (Butcher et al., 1997). The clinical relevance of these findings is still being evaluated. Contrary to these findings, cyclosporin A induced neuronal apoptosis and selective oligodendrocyte death in mouse cortical cultures (McDonald et al., 1996), supporting the clinical observation of cyclosporin neurotoxicity, using computed tomography and magnetic resonance imaging (Gijtenbeek et al., 1999). Cyclosporin A aggravates convulsions induced by pentylentetrazol and electroshock in rats (Asanuma et al., 1995; Racusen et al., 1990). In fact, cyclosporin A induces neurological side-effects including convulsions and encephalopathy in up to 40% of patients

(Gijtenbeek et al., 1999). Therefore, the neuroprotective action of cyclosporin A remains controversial.

Cyclosporin A, when which binds to cyclophilin, inhibits the activity of calcineurin, a calcium- and calmodulin-dependent protein phosphatase (Yakel, 1997). Immunosuppressant inhibition of calcineurin either facilitates or suppresses neurotransmitter release and synthesis, the effect depending on which calcineurin substrate phosphoproteins, including synaptic vesicle-associated proteins and nitric oxide synthase, are affected (Snyder et al., 1998). Various mechanisms including inhibition of GABA synthesis, release and uptake are involved in mediating the reduction of basal levels and accumulation of GABA induced in the hippocampus by subchronic treatment with cyclosporin A. Further studies will be required to clarify its precise mechanisms.

In conclusion, the present findings demonstrated that ovariectomy elevated the susceptibility to cyclosporin A-induced convulsions by accelerating the inhibitory action of cyclosporin A on GABA neural activity in the hippocampus and that this was blocked by estrogen replacement. The possibility that menopause/climacterium is included in the risk factors for cyclosporin A-induced neurotoxicity and that this risk is lowered by estrogen replacement therapy should be considered.

Acknowledgements

This study was supported, in part, by Grants-in-Aid for Scientific Research ((C)(2)12672218) from the Ministry of Education, Science, Sports and Culture, Japan.

References

Asanuma, M., Ogawa, N., Nshibayashi, S., Kondo, Y., Mori, A., 1995. Effects of repeated injection of cyclosporin A on pentylenetetrazol-induced convulsion and cyclophilin mRNA levels in rat brain. Neurochem. Res. 20, 101–105.

Butcher, S.P., Henshall, D.C., Teramura, Y., Iwasaki, K., Sharkey, J., 1997. Neuroprotective actions of FK506 in experimental stroke: in vivo evidence against an antiexcitotoxic mechanism. J. Neurosci. 17, 6939–6946.

Cancilla, P.A., Bready, J., Berliner, J., 1993. Brain endothelial–astracyte interaction. In: Pardridge, W.M. (Ed.), The Blood–Brain Barrier. Cellular and Molecular Biology. Raven Press, New York, pp. 25–46.

Dohgu, S., Kataoka, Y., Ikesue, H., Naito, M., Tsuruo, T., Oishi, R., Sawada, Y., 2000. Involvement of glial cells in cyclosporine-increased permeability of brain endothelial cells. Cell. Mol. Neurobiol. 20, 781–786.

Dawson, T.M., Steiner, J.P., Dawson, V.L., Dinerman, J.L., Uhl, G.R., Snyder, S.H., 1993. Immunosuppressant FK506 enhances phosphorylation of nitric oxide synthase and protects against glutamate neurotoxicity. Proc. Natl. Acad. Sci. U. S. A. 90, 9808–9812.

Erlander, M.G., Tobin, A.J., 1991. The structural and functional heterogeneity of glutamic acid decarboxylase: a review. Neurochem. Res. 16, 215–226.

Garcia-Segura, L.M., Azcoitia, I., DonCarlos, L.L., 2001. Neuroprotection by estradiol. Prog. Neurobiol. 63, 29–60.

Gijtenbeek, J.M.M., Van den Bert, M.J., Vecht, Ch.J., 1999. Cyclosporine neurotoxicity: a review. J. Neurol. 246, 339–346.

Grattan, D.R., Selmanoff, M., 1994. Castration-induced decrease in the activity of medial preoptic and tuberoinfundibular GABAergic neurons is prevented by testosterone. Neuroendocrinology 60, 141–149.

Green, A.R., 1986. Changes in g-amminobutyric acid biochemistry and seisure threshold. Ann. N. Y. Acad. Sci. 462, 105–119.

Green, P.S., Simpkins, J.W., 2000. Neuroprotective effects of estrogens: potential mechanisms of action. Int. J. Dev. Neurosci. 18, 347–358.

Greendale, G.A., Lee, N.P., Arriola, E.R., 1999. The menopause. Lancet 353, 571–580.

Herzog, A.G., 1995. Progesterone therapy in women with complex partial and secondary generalized seizures. Neurology 45, 1660–1662.

Ikesue, H., Kataoka, Y., Kawashi, R., Dohgu, S., Shuto, H., Oishi, R., 2000. Cyclosporine enhances α_1-adrenoreceptor-mediated nitric oxide production in C6 glioma cells. Eur. J. Pharmacol. 407, 221–226.

Kahan, B.D., 1989. Cyclosporine. N. Engl. J. Med. 321, 1725–1738.

Klein, M., Radhakrishnan, J., Appel, G., 1999. Cyclosporine treatment of glomerular diseases. Annu. Rev. Med. 50, 1–15.

Kochi, S., Takanaga, H., Matsuo, H., Naito, M., Tsuruo, T., Sawada, Y., 1999. Effect of cyclosporine A or tacrolimus on the function of blood–brain barrier cells. Eur. J. Pharmacol. 372, 287–295.

Lambert, J.J., Belelli, D., Hill-Venning, C., Peters, J.A., 1995. Neurosteroids and $GABA_A$ receptor function. Trends Pharmacol. Sci. 16, 295–303.

Loskota, W.J., Lomax, P., Rich, S.T., 1974. The gerbil as a model for the study of the epilepsies: seizure patterns and ontogenesis. Epilepsia 15, 109–119.

McDonald, J.W., Goldberg, M.P., Gwag, B.J., Chi, S., Choi, D.W., 1996. Cyclosporine induces neuronal apoptosis and selective oligodendrocyte death in cortical cultures. Ann. Neurol. 40, 750–758.

Naeyaert, J.M., Lachapelle, J.M., Degreef, H., de la Brassinne, M., Heenen, M., Lambert, J., 1999. Cyclosporine in atopic dermatitis: review of the literature and outline of Belgian consensus. Dermatology 198, 145–152.

Pearce, M.J., Hawton, K., 1996. Psychological and sexual aspects of the menopause and HRT Baillieres. Clin. Obstet. Gynecol. 10, 385–399.

Pirsch, J.D., Miller, J., Deierhoi, M.H., Vincenti, F., Filo, R.S., 1997. A comparision of tacrolimus (FK 506) and cyclosporine for immunosuppression after cadaveric renal transplantation. Transplantation 63, 977–983.

Racusen, L.C., McCrindle, B.W., Christenson, M., Fivush, B., Fisher, R.S., 1990. Cyclosporine lowers seizure threshold in an experimental model of electroshock-induced seizures in munich-wistar rats. Life Sci. 46, 1021–1026.

Rannevik, G., Jeppsson, S., Johnell, O., Bjerre, B., Laurell-Borulf, Y., Svanberg, L., 1995. A longitudinal study of the perimenopausal transition: altered profiles of steroid and pituitary hormones, SHBG and bone mineral density. Maturitas 21, 103–113.

Schumacher, M., Coirini, H., McEwen, B.S., 1989. Regulation of high affinity $GABA_A$ receptors in the dorsal hippocampus by estradiol and progesterone. Brain Res. 487, 178–183.

Shuto, H., Kataoka, Y., Fujisaki, K., Nakao, T., Sueyasu, M., Miura, I., Watanabe, Y., Fujiwara, M., Oishi, R., 1999. Inhibition of GABA system involved in cyclosporine-induced convulsions. Life Sci. 65, 879–887.

Shy, H., Malaiyandi, L., Timiras, P.S., 2000. Protective action of 17β-estradiol and tamoxifen on glutamate toxicity in glial cells. Int. J. Dev. Neurosci. 18, 289–297.

Snyder, S.H., Sabatini, D.M., Lai, M.M., Steinet, J.P., Hamilton, G.S., Suzdak, P.D., 1998. Neuronal actions of immunophilin ligands. Trends Pharmacol. Sci. 19, 21–26.

The U.S. Multicenter FK506 Liver Study Group, 1994. A comparision of tacrolimus (FK 506) and cyclosporine for immunosuprresion in liver transplantation. N. Engl. J. Med. 331, 1110–1115.

Veliskova, J., Velisek, L., Galanopooulou, A.S., Sperber, E.F., 2000. Neuroprotective effects of estrogens on hippocampal cells in adult female rats after status epilepticus. Epilepsia 41, S30–S35.

Weaver Jr., C.E., Park-Chung, M., Gibbs, T.T., Farb, D.H., 1997. 17β-Estradiol protects against NMDA-induced excitotoxicity by direct inhibition of NMDA receptors. Brain Res. 761, 338–341.

Weiland, N.G., 1992. Glutamic acid decarboxylase messenger ribonucleic acid is regulated by estradiol and progesterone in the hippocampus. Endocrinology 131, 2697–2702.

Yakel, J.L., 1997. Calcineurin regulation of synaptic function: from ion shannels to transmitter release and gene transcription. Trends Pharmacol. Sci. 18, 124–134.