Effects of Aniracetam on Bladder Overactivity in Rats with Cerebral Infarction

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ABSTRACT

Aniracetam has been used to improve the mental condition of patients with cerebrovascular disease. Previous studies have demonstrated that aniracetam activates the residual functions of cholinergic neurons in damaged brain areas. In this study, the effects of aniracetam on bladder overactivity after left middle cerebral artery occlusion were assessed through oral or i.c.v. administration in sham-operated and cerebral infarcted rats. Oral administration of aniracetam (100 and 300 mg/kg) resulted in a significant and dose-dependent increase in bladder capacity in cerebral infarcted rats but had no effect on bladder capacity in sham-operated rats. Intracerebroventricular administration of aniracetam (0.25 and 2.5 μg/rat) resulted in a significant and dose-dependent increase in bladder capacity in cerebral infarcted rats but not in sham-operated rats. Aniracetam had no significant effect on bladder contraction pressure or micturition threshold pressure in either sham-operated or cerebral infarcted rats. Furthermore, i.c.v. administration of atropine (1 μg/rat), a muscarinic acetylcholine receptor antagonist, completely inhibited the enhancing effects of aniracetam on bladder capacity in cerebral infarcted rats. The effects of aniracetam on bladder overactivity are thought to be mediated in part by activation of cholinergic inhibitory mechanisms in the brain. These results indicate that aniracetam may improve the neurogenic voiding dysfunction observed in patients with cerebrovascular disease.

Urinary frequency and incontinence are often observed in patients with brain ischemia, because damage to the neural circuitry in the forebrain can produce overactivity of the urinary bladder and urinary incontinence (Tsuchida et al., 1983; Kitada et al., 1991; Griffiths et al., 1994). It has been reported in cats that decerebration above the inferior colliculus induces an overactive bladder (Tang and Ruch, 1956). This overactivity has been attributed to the interruption of inhibitory pathways from the forebrain to the micturition center in the brainstem [i.e., the pontine micturition center (PMC)]. An experimental model to study the effect of forebrain lesions on voiding function has recently been developed in rat by occluding the middle cerebral artery (MCA) under pentobarbital or halothane anesthesia (Ishiura, 1996; Yokoyama et al., 1997, 1998, 1999). One-half hour after MCA occlusion, bladder capacity in cerebral infarcted rats was markedly reduced, indicating an overactive bladder (Yokoyama et al., 1997).

In animals with an intact neuraxis, a supraspinal reflex pathway passing through the PMC in the brainstem initiates micturition, and transmission in the PMC is modulated by cortical-diencephalic mechanisms (Noto et al., 1989). Glutamate is the principal excitatory transmitter in the ascending and descending limbs of the micturition reflex pathway (Yoshiyama et al., 1993). Other neurotransmitters that regulate transmission in the micturition reflex pathway include γ-aminobutyric acid (GABA), enkephalins, dopamine, and acetylcholine (ACh). ACh has been found to have both excitatory and inhibitory effects on the micturition pathway (De Groot, 1998). Anticholinergic (antimuscarinic) drugs are generally used for the treatment of urinary frequency and incontinence, but their influence on this pathway in the brain has not been clarified. Recently, Yamamoto et al. (1995) reported a decrease in bladder capacity in a rat model of cholinergic denervation in the cerebral cortex. These results suggest that the cholinergic pathway in the cerebral cortex plays an important role in the regulation of the micturition reflex. We therefore speculated that the effect of a cholinergic activating agent on the cerebral cortex would be capable of increasing bladder capacity in cerebral infarcted rats.

Aniracetam is widely used for the treatment of a variety of neuropsychiatric symptoms in patients suffering from cerebrovascular disease. Aniracetam has been used to improve the mental condition of patients with cerebrovascular disease. Previous studies have demonstrated that aniracetam activates the residual functions of cholinergic neurons in damaged brain areas. In this study, the effects of aniracetam on bladder overactivity after left middle cerebral artery occlusion were assessed through oral or i.c.v. administration in sham-operated and cerebral infarcted rats. Oral administration of aniracetam (100 and 300 mg/kg) resulted in a significant and dose-dependent increase in bladder capacity in cerebral infarcted rats but had no effect on bladder capacity in sham-operated rats. Intracerebroventricular administration of aniracetam (0.25 and 2.5 μg/rat) resulted in a significant and dose-dependent increase in bladder capacity in cerebral infarcted rats but not in sham-operated rats. Aniracetam had no significant effect on bladder contraction pressure or micturition threshold pressure in either sham-operated or cerebral infarcted rats. Furthermore, i.c.v. administration of atropine (1 μg/rat), a muscarinic acetylcholine receptor antagonist, completely inhibited the enhancing effects of aniracetam on bladder capacity in cerebral infarcted rats. The effects of aniracetam on bladder overactivity are thought to be mediated in part by activation of cholinergic inhibitory mechanisms in the brain. These results indicate that aniracetam may improve the neurogenic voiding dysfunction observed in patients with cerebrovascular disease.
brovascular or Alzheimer's disease, and its therapeutic effects on behavioral abnormalities and emotional disturbances have been demonstrated in two double-blind studies with patients with cerebral infarction (Otomo et al., 1987, 1991). This drug is classified as a cholinergic and glutamatergic modulator because it enhances ACh release from the hippocampus and glutamatergic transmission via α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate (KA) receptors (Gouliaev and Senning, 1994). The identified effects of aniracetam are limited to the central nervous system, and its influence on peripheral cholinergic neurotransmission has not been investigated (Himori et al., 1986). Aniracetam can ameliorate overactivity of the bladder as reported in some clinical cases (Ueda, 1998); however, there are no critical studies focusing on the pharmacological and action mechanisms of this drug in an experimental model of overactive bladder. We therefore initiated this study to evaluate the effect of oral and i.c.v. administration of aniracetam on bladder overactivity induced by MCA occlusion and to determine its site of action.

Materials and Methods

Animals. The experiments were performed on 132 female Sprague-Dawley rats weighing between 220 and 270 g (Japan SLC Inc., Hamamatsu, Japan). They were housed at a constant temperature (23 ± 2°C) and relative humidity (50–60%) under a regular 12-h light/dark schedule (lights on 7:00 AM–7:00 PM). Tap water and standard rat chow were freely available. All experiments were performed in strict accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC).

Cystometrography (CMG) in Conscious Rats. The method of Yaksh and coworkers (1986) for cystometry in conscious rats was used. Animals were anesthetized with halothane, and the bladder was exposed via a midline incision in the abdomen. One end of a polyethylene tube (size 4; i.d. 0.8 mm, o.d. 1.3 mm; Kunii Co. Ltd., Tokyo, Japan) was softened by heating to create a collar and passed through a small incision at the apex of the bladder dome. A suture was tied around the collar of the catheter and the other end of the catheter was passed through the s.c. tissue and extracted through the skin at the back of the neck. After suturing of the abdominal skin, the rats were placed in a restraining cage (Ballman Cage, KN-326 Type 3; Natsume Seisakusho Co. Ltd., Tokyo, Japan) and allowed to recover from halothane anesthesia. The CMG catheter was connected to a pump (TE-311; Terumo Co. Ltd., Tokyo, Japan) for the continuous infusion of saline and to a pressure transducer (TP-200T; Nihon-Kohden Co. Ltd., Tokyo, Japan) by means of a polyethylene T-tube. Cystometric recording was achieved by infusing physiological saline at room temperature into the bladder at a rate of 0.04 ml/min and by collecting and measuring the saline voided from the urethral meatus to determine the voided volume. Evacuating the bladder through the CMG catheter enabled us to measure residual volume after the micturition reflex. Three cystometric parameters (bladder capacity, bladder contraction pressure, and micturition threshold pressure) were determined from each cystometry. Bladder capacity was defined as the sum of the voided and residual volumes. The protocol for the experiments is shown in Fig. 1.

Induction of Cerebral Infarction in Rats. Immediately after completion of the first cystometric recording, the rats were anesthetized with halothane, and the left carotid bifurcation was exposed through a midline incision in the neck. After ligation of the left common carotid artery, the left internal carotid artery (ICA) was isolated and carefully separated from the adjacent vagus nerve. The pterygopalatine branch of the left ICA was then ligated close to its origin. A 4-0 monofilament nylon thread with the tip rounded by exposure to a flame was inserted into the left ICA and advanced a distance of 17 mm from the carotid bifurcation as far as the origin of the left MCA, where it occluded the blood flow and thus induced an infarction on the left side of the brain (Longa et al., 1989). In sham-operated animals, the left carotid bifurcation was exposed through a midline incision in the neck, but no additional procedures were performed. These surgeries were performed within one-half hour from induction of halothane anesthesia. After suturing of the incision

\[\text{Fig. 1. }\] Protocol for the experiments. Hatched columns show the recovery period from halothane anesthesia. After surgical implantation of the CMG catheter (group 1), rats were recovered from anesthesia within one-half hour. After surgical implantation of the CMG catheter and i.c.v. cannula (groups 2 and 3), rats were recovered from anesthesia within 1 h. After MCA occlusion or sham operation, rats were recovered from anesthesia within one-half hour. The first and second CMG recordings were performed after rats regained consciousness.
in the neck, the rats were placed in a restraining cage again and allowed to recover from halothane anesthesia. The second cystometric recording was performed after identification of consciousness recovery.

**Intracerebroventricular Administration of Drug.** The implantation of an injection tube into the intracerebroventricle was performed immediately after implantation of the CMG catheter in continuation of halothane anesthesia. The rats were positioned in a stereotactic frame (ST-7; Narishige Co. Ltd., Tokyo, Japan), the incisor bar was set at −3.3 mm (Paxinos and Watson, 1986), a scalp incision was made over the sagittal suture, and a hole (diameter approximately 1.0 mm) was drilled in the right parietal bone to expose the dural surface 1.0 mm lateral and 0 mm anterior from the bregma (Paxinos and Watson, 1986). A sterile stainless steel cannula (o.d. 0.6 mm, i.d. 0.3 mm, length 10.5 mm) was lowered 5.3 mm ventrally from the skull surface with the end of a micromanipulator. By using a small screw placed in the skull as an anchor, the cannula was fixed to the skull with dental acrylic. The surgeries, including implantation of the CMG catheter and injection tube into the intracerebroventricle, were performed within 1 h from induction of halothane anesthesia.

**Evaluation of the Effects of Drug.** The drug was administered orally (5 ml/kg) or i.c.v. (5 µl/rat) as a single dose to the conscious rats. Two hours after the left MCA occlusion or sham operation, the effects of a single dose of aniracetam (30–300 mg/kg p.o. or 0.025–2.5 µg/rat i.c.v.), atropine (a muscarinic acetylcholine receptor (mAChR) antagonist, 1 µg/rat i.c.v.), or vehicle [0.5% solution of methyleneblue or artificial cerebrospinal fluid (aCSF); 138.6 nM NaCl, 3.35 nM KCl, 1.26 nM CaCl2, 1.16 nM MgCl2, 11.9 nM NaHCO3, pH 7.0–7.2] on bladder activity were examined in the awake rats after the control cystometric recording. To examine the interactions between aniracetam and atropine, atropine (1 µg/rat) was i.c.v. administered 10 min after the oral administration of aniracetam (100 mg/kg) in the cerebral infarcted rats. After the effects of the drugs had been evaluated, the rats were euthanized by decapitation, and their brains were removed. The forebrain was cut into five coronal slices, each 2-mm thick, by using a Brain Matrix RBM-4000C (Activational Systems Inc., Warren, MI), and immersed in a 2% solution of 2,3,5-triphenyltetrazolium chloride in saline at 37°C. After 15 to 30 min, the slices were photographed and the area of ischemic lesion was quantified by computer-assisted image analysis using NIH Image Version 1.61. The corrected infarction volume was then calculated with the method of Golanov and Reis (1995).

**Drugs.** Aniracetam, 1-p-anisoyl-2-pyrrolidinone, was obtained from Toyama Chemical Co., Ltd. (Tokyo, Japan). Atropine sulfate was obtained from Sigma Chemical Co. (St. Louis, MO). These drugs were of the highest purity commercially available. For the oral administration, aniracetam was suspended in 0.5% methylcellulose solution. For the i.c.v. administration, the two drugs were dissolved in distilled water and diluted with aCSF to adjust the concentration.

**Data Analysis.** Data are expressed as mean values ± S.E. Statistical comparisons were performed by one-way or two-way ANOVA, with subsequent individual comparisons by Fischer’s protected least significant difference test. In some cases, two-tailed paired Student’s t test was used. A level of P < .05 was considered statistically significant.

**Results**

**Effect of Left MCA Occlusion on Cystometrogram.** We examined the effect of the MCA occlusion on bladder capacity in the awake rats. The bladder capacity before and 2 h after the sham operation was 0.44 ± 0.05 and 0.45 ± 0.07 ml, respectively, and the corresponding values for the occlusion were 0.50 ± 0.05 and 0.24 ± 0.03 ml. Cerebral infarcted rats thus showed a significant reduction in bladder capacity (P < .01 by two-tailed paired Student’s t test). Residual volumes in cerebral infarcted and sham-operated rats were very small and insignificant, and the micturition volume was nearly equivalent to bladder capacity.

Bladder contraction pressure was 34.2 ± 5.3 cm of water before and 29.1 ± 3.5 cm of water 2 h after the sham operation compared with 36.0 ± 6.8 and 26.6 ± 4.0 cm for the occlusion (not significant). Bladder contraction pressure was therefore not affected by the MCA occlusion.

Micturition threshold pressure was 4.56 ± 0.60 cm of water before and 4.11 ± 0.48 cm of water 2 h after the sham operation compared with 5.33 ± 0.83 and 5.10 ± 0.48 cm for the occlusion. The cerebral infarcted rats thus also showed a significant decrease in micturition threshold pressure (P < .05 by two-tailed paired Student’s t test). On the other hand, the micturition threshold pressure of the sham-operated rats was not affected by the operation.

**Effects of Oral Administration of Aniracetam on Sham-Operated Rats and Cerebral Infarcted Rats.** Aniracetam increased bladder capacity in a dose-dependent manner in cerebral infarcted rats (F3,24 = 7.92, P < .01 by two-way ANOVA, Fig. 2B). Statistical analysis was based on the percentage increase in bladder capacity at a dose of 300 mg/kg, which induced the maximum response. In the cerebral infarcted rats, the oral administration of aniracetam significantly increased bladder capacity in comparison with administration of the vehicle, with an increase in bladder capacity of 34.3 ± 15.3% for 100 mg/kg and of 40.9 ± 11.6% for 300 mg/kg 30 min after administration of aniracetam doses (F3,24 = 4.81, P < .01 by two-way ANOVA, P < .01, and P < .01 by Fischer’s protected least significant difference test). In the sham-operated rats, the oral administration of aniracetam did not significantly increase bladder capacity in comparison with administration of the vehicle (F3,20 = 1.01, P = 0.394 by two-way ANOVA, Fig. 2A). Any dose of aniracetam produced small and insignificant increases in the residual volume in both cerebral infarcted and sham-operated rats.

In the sham-operated rats, the dose of 300 mg/kg aniracetam significantly reduced bladder contraction pressure when compared with the effect of the vehicle (F3,20 = 6.49, P < .01 by two-way ANOVA, P < .01 by Fischer’s protected least significant difference test), but statistical analysis based on percentage change in bladder contraction pressure at a dose of 300 mg/kg did not show any significant change in comparison with the bladder contraction pressure before the administration (Fig. 2C). In the cerebral infarcted rats, the dose of 300 mg/kg aniracetam showed similar results without any significant change in comparison with the bladder contraction pressure before the oral administration (Fig. 2D). Thus, aniracetam was found to have no influence on the bladder contraction pressure in sham-operated and cerebral infarcted rats. In neither the sham-operated nor the cerebral infarcted rats did the effects of aniracetam on micturition threshold pressures differ from those of the vehicle (Fig. 2, E and F).

**Effects of i.c.v. Administration of Aniracetam on Sham-Operated Rats and Cerebral Infarcted Rats.** Aniracetam increased bladder capacity in a dose-dependent manner in the cerebral infarcted rats (Table 1). Statistical analysis was based on the percentage increase in bladder capacity at a dose of 2.5 µg/rat, which induced the maximum response. In the cerebral infarcted rats, the i.c.v. administration...
of aniracetam did not significantly increase bladder capacity in comparison with administration of the vehicle ($F_{3,20} = 0.77, P = .53$ by one-way ANOVA, Table 1). The bladder capacity with i.c.v. administration of aniracetam did not show any response at 120 min after administration ($F_{3,20} = 0.67, P = .57$ by two-way ANOVA, Fig. 3A). Effects of aniracetam on either bladder contraction pressure or micturition threshold pressure in the sham-operated and cerebral infarcted rats did not differ from those of the vehicle (data not shown).

**Antagonistic Effects of Atropine on Aniracetam-Induced Increase in Bladder Capacity.** To study the interactions between aniracetam and atropine, we administered a fixed dose of atropine to construct a cumulative dose-response curve for aniracetam. This systemic administration paradigm was necessary because atropine ($1 \mu g/rat$ i.c.v.) at the dose used here is known to have a protective effect on the cholinergic neuronal activity in the brain. This dose was selected because it did not cause any significant changes in bladder capacity in the cerebral infarcted rats (Fig. 4). Single doses of aniracetam ($100 \, \text{mg/kg}$) produced an increase in bladder capacity that reached its maximum value within 30 min after oral administration and did not return to control levels within 2 h (Fig. 2B).

For experiments designed to identify the pharmacological interaction between aniracetam and atropine, atropine was given 10 min after oral administration of aniracetam. Intracerebroventricular administration of aCSF after an oral administration of aniracetam resulted in a statistically significant increase in bladder capacity ($F_{3,24} = 22.93, P < .01$ by two-way ANOVA, Fig. 4). In contrast, i.c.v. administration of atropine completely nullified the aniracetam-induced increase in bladder capacity. There was a significant difference in the percentage increase in bladder capacity between the aniracetam + aCSF and the vehicle + aCSF groups ($P < .01$ by Fischer’s protected least significant difference test), and between the aniracetam + aCSF and the aniracetam + atropine groups ($P < .01$ by Fischer’s protected least significant difference test).

**Effects of Aniracetam on Corrected Infarction Volume in Cerebral Infarcted Rats.** As described by Longa et al. (1989), MCA occlusion results in ischemic damage in the frontal cortex, parietal cortex, striatum, and subcortical basal ganglia. These areas showed the morphological characteristics of infarction during 7 h after MCA occlusion. Figure 5 shows the effect of aniracetam on the corrected infarction volume measured in terms of the area of ischemic damage in the cerebral cortex and striatum at different stereotaxic levels. No significant differences in cerebral infarction volume were found between the vehicle- and aniracetam-treated groups, nor were any such differences found in the study of the interactions between aniracetam and atropine.

**Discussion**

Focal neurogenic deficits often occur after an episode of cerebral artery occlusion or hemorrhage, and voiding dysfunction is also common in patients with cerebrovascular disease. The most common chronic expression of voiding dysfunction is a sense of urgency with or without urinary incontinence caused by an overactive bladder. Clinical investigations have also suggested that the frontal lobes and basal
ganglia are involved in the inhibitory regulation of bladder activity (Kitada et al., 1991; Griffiths et al., 1994). Impairment of this regulatory system after cerebral artery occlusion or hemorrhage is likely to be responsible for the development of an overactive bladder (Tsuchida et al., 1983). Disruption of the forebrain seems to be involved in the overactive bladder associated with cerebrovascular disease. Patients with an overactive bladder have been treated with anticholinergic agents, which suppress the detrusor muscle at the peripheral neuromuscular junction. Although anticholinergic agents are useful for the suppression of overactivity of the bladder, the effect is not always satisfactory and some patients suffer from side effects such as dry mouth, flushing, and a small urinary stream.

To study the mechanisms underlying neurogenic bladder overactivity, we have developed an animal model of voiding dysfunction induced by experimental cerebral infarction (Ishiiura, 1996; Yokoyama et al., 1997). This model has proved to be useful for the quantitative evaluation of drug actions on micturition reflex. Aniracetam, a nootropic drug, can ameliorate urinary frequency and nocturia as reported in some clinical cases (Ueda, 1998); however, there are no critical studies focusing on the pharmacological and action mechanism of this drug in an experimental model of voiding dysfunction. Therefore, our experimental model was used to investigate the effects of aniracetam on the overactive bladder.

Our study showed that oral and i.c.v. administration of aniracetam significantly increased the bladder capacity in the cerebral infarcted rats. In the sham-operated rats, however, no significant change was seen in the bladder capacity between the vehicle- and aniracetam-treated groups. The site of action of aniracetam is believed to be the forebrain, which influences the micturition reflex, because aniracetam appears to have no peripheral side effects in general pharmacological terms (Himori et al., 1986) and was found to specifically bind to the cortical membrane (Fallarino et al., 1995).

### TABLE 1

Acute effects of i.c.v. administration of aniracetam on sham-operated and cerebral infarcted rats

Values represent the mean ± S.E. of six to seven determinations.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Bladder Capacity</th>
<th>% of Control</th>
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<tr>
<td></td>
<td>µg/rat ml</td>
<td></td>
</tr>
<tr>
<td>Sham-operated rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.512 ± 0.087</td>
<td>43.7 ± 0.078</td>
</tr>
<tr>
<td>Aniracetam</td>
<td>0.025 0.471 ± 0.070</td>
<td>33.3 ± 0.043</td>
</tr>
<tr>
<td></td>
<td>0.25 0.488 ± 0.050</td>
<td>33.5 ± 0.046</td>
</tr>
<tr>
<td></td>
<td>2.5 0.509 ± 0.060</td>
<td>32.3 ± 0.049</td>
</tr>
<tr>
<td>Cerebral infarcted rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.257 ± 0.016</td>
<td>31.3 ± 0.031</td>
</tr>
<tr>
<td>Aniracetam</td>
<td>0.025 0.242 ± 0.026</td>
<td>23.0 ± 0.044</td>
</tr>
<tr>
<td></td>
<td>0.25 0.194 ± 0.014</td>
<td>37.4 ± 0.030</td>
</tr>
<tr>
<td></td>
<td>2.5 0.224 ± 0.035</td>
<td>43.7 ± 0.068</td>
</tr>
</tbody>
</table>

* P < .05 compared with bladder capacity in control (two-tailed paired Student’s t test).
* P < .01 compared with bladder capacity in control (two-tailed paired Student’s t test).
* P < .05 compared with percentage of control in vehicle-treated group (Fischer’s protected least significant difference test) after one-way ANOVA.
* P < .01 compared with percentage of control in vehicle-treated group (Fischer’s protected least significant difference test) after one-way ANOVA.

Fig. 3. Effects of i.c.v. administration of aniracetam on bladder capacity in sham-operated (A, n = 6 per group) and cerebral infarcted (B, n = 7 per group) rats. Aniracetam was i.c.v. administered at single doses of 0.025 (●), 0.25 (▲), and 2.5 µg/rat (●) for each group at 0 min. The vehicle-treated group (○) received i.c.v. administration of aCSF alone. Values represent the mean ± S.E. of six to seven determinations. *P < .05; **P < .01; significantly different from the vehicle-treated group.
Fig. 4. Antagonistic effects of atropine (ATR) on aniracetam-induced increase in bladder capacity in cerebral infarcted rats (n = 7 per group). ATR (1 μg/rat) or aCSF was i.c.v. administered 10 min after oral administration of aniracetam (100 mg/kg) or vehicle. This experiment involved the following four groups: vehicle + aCSF (○), vehicle + ATR (●), aniracetam + aCSF (▲), and aniracetam + ATR (▲). Values represent the mean ± S.E. of seven determinations. **P < .01; significantly different from vehicle + aCSF group. *P < .01; significantly different from aniracetam + ATR group.

Previous reports showed that aniracetam improved dysfunctions of the cholinergic systems in the brain (Spignoli and Pepeu, 1987; Toide, 1989). However, aniracetam did not interact, either as a receptor agonist or antagonist, with cholinergic receptors (Martin and Hafely, 1993). Aniracetam has a modulating effect on the presynaptic terminal of the cholinergic neuron, resulting in an increase in ACh release (Giovannini et al., 1993). Based on the effect of aniracetam on cholinergic activation, it is used as a cognitive enhancer in animals (Cumin et al., 1982) and for human neuropsychiatric disorders (Frostl and Maitre, 1989; Senin et al., 1993). Aniracetam is also classified a glutamatergic modulator because it enhances glutamatergic transmission via the AMPA/KA receptors (Gouliav and Senning, 1994). Positive modulation of the AMPA/KA receptors by aniracetam provides a molecular substrate, which explains the second clinical effect of the drug, namely, as a memory and cognition enhancer.

What accounts for the inhibitory effects of aniracetam on the overactive bladder after cerebral infarction? In previous reports we suggested that there were two glutamatergic pathways that regulate bladder activity (Yokoyama et al., 1997). The first is an inhibitory pathway that originates from the forebrain, and the second is an excitatory pathway that is located at a more caudal site, possibly in the brainstem or in the spinal cord. Bladder overactivity after cerebral infarction is reportedly related to the activation of glutamatergic excitatory input to the micturition reflex pathway mediated by N-methyl-d-aspartate receptors (Yokoyama et al., 1997). Furthermore, glutamatergic mechanisms controlling the micturition reflex have been reported to be modulated by dopaminergic systems (Yoshiyama et al., 1994; Yokoyama et al., 1999). Therefore, other neurotransmitter systems besides the dopaminergic ones may interact with these glutamatergic systems. Recent studies have shown that muscarinic activation inhibited the release of glutamate in the corticostriatal pathway (Niittykoski et al., 1999). Cholinergic neurons may thus influence the glutamatergic pathways that regulate the micturition reflex in the brain.

We reported that a selective AMPA/KA receptor antagonist, 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzof[quinoxaline-7-sulfonamide, increased bladder capacity in cerebral infarcted rats (Mizuno et al., 1999). These effects were the opposite of the glutamatergic modulating effects of aniracetam. In this connection the AMPA/KA receptor modulating effects of aniracetam were lower than that of its cholinergic activating effects. The cholinergic activating effects of aniracetam may play a more dominant role than the AMPA/KA receptor-modulating effects in ameliorating bladder overactivity in cerebral infarcted rats.

In this study, the effect of aniracetam (100 mg/kg p.o.) on bladder overactivity was completely suppressed by the i.c.v. administration of atropine (1 μg/rat) at a dose that had no effect on bladder activity in cerebral infarcted rats. This indicates that aniracetam activates cortical ACh release, and that cholinergic systems in the frontal cortex recover the regulatory influence on the micturition reflex. Yamamoto et al. (1995) reported a decrease in bladder capacity in a rat model of cholinergic denervation in the central nervous system. This model was prepared by inducing severe neuronal damage to the nucleus basalis magnocellularis in the basal forebrain, which resulted in a reduction in choline-acetyltransferase activity in the prefrontal and sensorimotor cortices. Cholinergic systems in the cortex are thought to be one of the inhibitory pathways that regulate bladder activity, so that impairment of this regulatory system after cerebral infarction is very likely responsible for the development of the overactive bladder. The quantitative analysis of mAChR and subtype mRNA in the acute phase of a MCA occlusion model demonstrated a tendency toward a reduction in mRNA levels in the acute ischemic region without a reduction in mAChR (Kuji et al., 1997). These results indicate that the cholinergic neurons in the ischemic regions are viable but hypometabolic. Therefore, the ameliorating effects of aniracetam could be based on the activation of the cholinergic inhibitory pathways in the frontal cortex.

Aniracetam has been reported to facilitate central cholinergic neurotransmission via an increase in ACh release (Giovannini et al., 1993). In other investigations, acetylcholinesterase inhibitors increased regional cerebral blood flow (rCBF) in the bilateral cortices of rats subjected to unilateral MCA occlusion (Scremin and Scremin, 1986). These findings raise the possibility that aniracetam induces cerebral vasodilation by means of activation of cholinergic neurons. The effect of vasodilator agents such as nifedipine to suppress bladder overactivity after MCA occlusion implies the importance of rCBF restoration in the ischemic penumbra (Nakamura et al., 1999). However, the influence of aniracetam on rCBF has not been studied in an ischemic animal model. It is uncertain whether aniracetam ameliorates the rCBF in the ischemic penumbra or not.

Other investigators indicated the neuroprotective property of aniracetam by attenuating hydroxyl free-radical formation in the ischemic brain (Himori et al., 1995). This study was
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Fig. 5. Effects of aniracetam on corrected infarction volume in cerebral infarcted rats. Corrected infarction volume was calculated by measuring the area of ischemic lesions in the coronal sections of the brain. Aniracetam was administered orally (A) or i.c.v. (B). The result of the experiment to study the interactions between aniracetam and atropine (ATR) in cerebral infarcted rats is shown in C. ATR (1 µg/rat) or aCSF was i.c.v. administered 10 min after oral administration of aniracetam (100 mg/kg) or vehicle. This experiment involved four groups: vehicle + aCSF, vehicle + ATR, aniracetam + aCSF, and aniracetam + ATR. Values represent the mean ± S.E. of seven determinations.

performed in the acute phase of focal ischemia, and the neuroprotective effect of aniracetam could not be verified. No significant differences in the volume of the cerebral infarct were found between the vehicle- and aniracetam-treated groups.

It has been reported that aniracetam has modulating effects on neurotransmitter systems and neuroprotective effects in the brain, but these effects have not been clarified to be dependent on direct or indirect action (Gouliava and Senning, 1994; Himori et al., 1995). Nakamura et al. (1998) suggested that aniracetam indirectly induced the activation of dopamine release in the striatum by interacting with glutamatergic systems. D1 dopaminergic mechanism has inhibitory influence on the micturition reflex (Yokoyama et al., 1999). The possibility exists that aniracetam produces an increase in bladder capacity in cerebral infarcted rats via activation of D1 dopaminergic system.

In conclusion, oral or i.c.v. administration of aniracetam, a cholinergic modulator, significantly increased bladder capacity in cerebral infarcted rats but not in sham-operated rats. This enhancing effect was completely inhibited by mAChR antagonist. It seems reasonable to conclude that the cholinergic pathway in the cerebral cortex has a role in this inhibitory influence on the micturition reflex. Aniracetam has no side effects, such as dry mouth, flushing, and small urinary stream, often seen in the clinical use of anticholinergic agents for the overactive bladder. Therefore, aniracetam may be expected to be beneficial for the treatment of the overactive bladder caused by cerebrovascular diseases.

References


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