Short communication

Changes in the expression of glial glutamate transporters in the rat brain accompanied with morphine dependence and naloxone-precipitated withdrawal

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Abstract

The expression of mRNAs for the glial glutamate transporters, GLT-1 and GLAST, in the rat brain accompanied with morphine dependence and naloxone-precipitated withdrawal was investigated by Northern blot analysis. The expression of GLT-1 mRNA was significantly decreased in the striatum and thalamus of morphine-dependent rats, and significantly increased in the striatum 2 h after the naloxone-precipitated withdrawal, compared with that of naive rats. On the other hand, there were no significant changes in GLAST mRNA level in any brain region. These results suggest the involvement of GLT-1 in the development of morphine dependence and the expression of morphine withdrawal.

Theme: Neural basis of behaviour

Topic: Drugs of abuse: opioids and others

Keywords: Morphine; Dependence; Withdrawal; Glutamate transporter; GLT-1; GLAST

Morphine is widely used for therapeutic purposes because of its potent analgesic effect. However, it is known that chronic use of morphine leads to tolerance, physical and psychological dependence, and addiction [19]. Although the mechanisms have been vigorously investigated, they still remain unclear. Recent evidence supports that excitatory amino acid, particularly glutamatergic, systems participate in them [32]. It has been shown that several non-competitive and competitive N-methyl-D-aspartate (NMDA) receptor antagonists such as MK-801 [11,29], ketamine [10] and LY274614 [17], AMPA receptor antagonists [18], and metabotropic glutamate receptor antagonists [4] attenuated the development of tolerance to the analgesic effect of morphine and physical dependence on morphine. The expression of the NR1 subunit mRNA was increased in the locus coeruleus and the hypothalamic paraventricular nucleus of morphine-dependent rats [31]. Furthermore, neurochemical studies using the in vivo microdialysis method have directly shown an elevation of extracellular glutamate level within the locus coeruleus during naloxone-precipitated morphine withdrawal [1].

Extracellular glutamate levels are primarily regulated by sodium-dependent glutamate transport into glia and neurons in the mammalian central nervous system. To date, five subtypes of glutamate transporters (GLT-1, GLAST, EAAC1, EAAT4 and EAAT5) have been identified by molecular cloning from mammalian tissues [5,23]. GLT-1 and GLAST are mainly expressed in astrocytes, whereas EAAC1 and EAAT4 are mainly in neurons [21,23]. EAAT5 exists predominantly in retina [23]. It has been shown that the glial glutamate transporters, GLT-1 and GLAST, play important roles in maintaining low extracellular glutamate, rather than neuronal glutamate transporters [5,20].

In the present study, to examine the involvement of glial glutamate transporters in the development of morphine dependence and the expression of morphine withdrawal, we investigated changes in the expression of mRNAs for the glial glutamate transporters, GLT-1 and GLAST, in the...
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rat brain accompanied with morphine dependence and naloxone-precipitated withdrawal.

Male Sprague–Dawley rats weighing 200–250 g were used. They were kept at a constant ambient temperature of 24±1°C under a 12-h light/dark cycle with free access to food and water. The procedures for induction of morphine dependence and precipitation of naloxone-precipitated withdrawal were previously described [13]. Briefly, morphine pellets each containing 75 mg of morphine base (Takeda Chemical Industries, Osaka, Japan) were prepared according to the method of Gibson and Tingstad [6]. Under light ether anesthesia, rats had a placebo or morphine pellet implanted subcutaneously in the back of the neck (day 1). Twenty-four hours later (day 2), the rats received a second placebo or morphine pellet. Seventy-two hours after the second pellet was implanted (day 5), the placebo-treated and morphine-dependent rats were killed by decapitation.

For precipitation of morphine withdrawal, the rats were intraperitoneally administered with naloxone (Sigma, St Louis, USA) at a dose of 3 mg/kg, and were decapitated 2 h after the administration of naloxone. The brains were rapidly dissected into eight regions; that is, the cerebral cortex, hippocampus, striatum, thalamus, hypothalamus, midbrain, cerebellum and pons–medulla. Then, they were frozen in liquid nitrogen and stored at −80°C until use.

The rat GLT-1 and GLAST cDNAs were cloned from the cortex of Sprague–Dawley rats by a reverse transcription–polymerase chain reaction-based method. The DNA fragments were subcloned into pBluescript II SK (−) (Stratagene, La Jolla, USA) and confirmed to be a partial cDNAs for GLT-1 and GLAST by sequence analysis. The plasmids linearized at HincII site were used as templates to generate antisense RNA probes for GLT-1 and GLAST. 32P-labeled antisense RNA probes for GLT-1 and GLAST were synthesized in the presence of [α-32P]uridine triphosphate (15 TBq/mmol, Amersham, Buckinghamshire, UK) using T3 RNA polymerase (Promega, Madison, USA).

Northern blot analysis was carried out as previously described [13]. Briefly, total RNA was extracted from tissues of each brain region using ISOGEN (Nippon gene, Tokyo, Japan). Total RNA samples 10 μg each were fractionated by electrophoresis in 1.2% agarose gels containing 6% formaldehyde, transferred onto nylon membranes Biodyne (Pall, Glen Cove, USA) and baked at 80°C for 2 h. The membranes were prehybridized for 2–3 h at 67°C and then hybridized to 32P-labeled antisense RNA probes for GLT-1 or GLAST for 16–18 h at 67°C. The membranes were washed twice in 2×saline sodium citrate (SSC)/0.1% sodium dodecyl sulfate (SDS) for 5 min at 67°C, then washed twice in 0.1×SSC/0.1% SDS for 5 min at 67°C and then washed twice in 0.1×SSC/0.1% SDS for 30 min at 67°C. The membranes were exposed to X-ray film at −80°C with an intensifying screen. Quantitative analysis of film autoradiograms was conducted with a Bioimaging analyzer BAS2000 (Fuji Photo Film, Japan). The obtained value of each brain region in placebo-treated rats served as the control (100%) and the results are presented as the mean of the percentage of the control±S.E.M. for each region. Statistical analysis was performed using the Mann–Whitney U-test. P<0.05 was considered significant.

First, the expressions of GLT-1 and GLAST mRNAs in the brain of normal rats was examined (Fig. 1). The expression of GLT-1 mRNA was generally high throughout all brain regions, except for the cerebellum. On the other hand, GLAST mRNA was expressed the most intensely in the cerebellum, intermediately in the cerebral cortex, hippocampus, striatum, thalamus and hypothalamus, weakly in the midbrain and pons–medulla. These distribution patterns are in accordance with reported patterns of the glutamate transporters [21].

In morphine-dependent rats, the expression of GLT-1 mRNA was significantly decreased in the striatum and thalamus, compared with that of placebo-treated rats (P<0.05). Also, GLT-1 mRNA levels in the hippocampus, hypothalamus and cerebellum tended to decrease, although these changes were not statistically significant. In naloxone-precipitated withdrawal rats, the expression of GLT-1 mRNA in the striatum was significantly higher than that of placebo-treated and morphine-dependent rats (P<0.05), while GLT-1 mRNA level in the thalamus was signifi-

Fig. 1. Distribution of glial glutamate transporter mRNAs in the rat brain: (A) GLT-1; (B) GLAST.Brains from two rats were removed and dissected into eight regions (cerebral cortex, hippocampus, striatum, thalamus, hypothalamus, midbrain, cerebellum and pons–medulla). Northern blot analysis was carried out with 10 μg of total RNA for each lane. The size of GLT-1 and GLAST mRNA was 11 kbp and 4.5 kbp, respectively.
cantly lower than that of placebo-treated rats (P<0.01). No significant changes in GLT-1 mRNA level were detected in the cerebral cortex, hippocampus, hypothalamus, midbrain, cerebellum andpons-medulla of naloxone-precipitated withdrawal rats (Table 1). On the other hand, there were no significant alterations in the expression of GLAST mRNA in any brain region of both morphine-dependent and naloxone-precipitated withdrawal rats (Table 1).

It is well known that extracellular glutamate released from presynaptic terminals is removed from synaptic clefts by glutamate transporters in neurons and glial cells, thereby terminating the glutamatergic signal transmission and protecting neurons from an excitotoxic action of glutamate [8,9]. The glial glutamate transporters, GLT-1 and GLAST, are thought to play an essential role in modulating the extracellular glutamate level in the brain. For instance, the knock-down of GLT-1 or GLAST by antisense oligonucleotides elevated extracellular glutamate concentrations [20]. The present study showed that the expression of GLT-1 mRNA was significantly decreased in the striatum and thalamus of morphine-dependent rats.

In the nucleus accumbens, which is located near the striatum and included in the sections we examined, a large population of opioidergic neurons and high densities of opioid receptors are present [12], and acute morphine administration decreases the extracellular glutamate level [3,24]. The striatum is reported to partly participate in the expression of naloxone-precipitated withdrawal syndrome [2,28], and the nucleus accumbens is well known to be a substrate for opioid reward [15]. On the other hand, it has been reported that injection of naloxone into the medial thalamus precipitated withdrawal syndrome in morphine-dependent rats [28,30], and that morphine withdrawal produced marked increase in the c-fos mRNA level in the thalamus [13]. Thus, the thalamus is also considered to be involved in opioid addiction.

So far, it has been reported that various factors in vivo and in vitro could be regulated the expression of glial glutamate transporters [5]. For example, transient ischemia decreased the expression of GLT-1 mRNA and protein in the rat hippocampus [27]. In addition, it was reported that the disruption of cortical glutamatergic pathways led to down-regulation of GLT-1 and GLAST, but not EAAC1, proteins in the rat striatum and hippocampus [7]. These findings suggest that glutamate released from glutamatergic nerve endings may be capable of affecting the expression of glial glutamate transporters in surrounding astrocytes. Recently, Thorlin et al. reported that sustained treatment of cultured astrocyte with DPDPE, a selective δ-opioid receptor agonist, decreased the expression of GLT-1 mRNA in them [26]. Therefore, a possible mechanism for decreases in GLT-1 mRNA in morphine-dependent rats is presumed to be due to the direct effect of morphine on astrocytes. Taken together, the inhibition of glutamate release by morphine is suggested to result in the decreases of GLT-1 mRNA in the striatum and thalamus, which cause to attenuate glutamate uptake. Thus, we consider that the attenuation of glutamate uptake might be partly involved in the elevation of glutamate level just after precipitation of morphine withdrawal. In fact, we found that extracellular glutamate level was elevated both in the striatum and nucleus accumbens during naloxone-precipitated morphine withdrawal (our unpublished data).

On the other hand, the expression of GLT-1 mRNA was significantly increased in the striatum of naloxone-precipitated withdrawal rats 2 h after the injection. It was reported that sustained treatment of cultured astrocytes with glutamate resulted in up-regulation of GLT-1 mRNA [26]. Thus, excessive glutamate in the synaptic clefts during morphine withdrawal might, at least partly, induce the expression of GLT-1 mRNA in the striatum. Furthermore, in cultured astrocytes treatment with dibutyryl cyclic AMP increased the expression of GLT-1 and GLAST [22,25]. It is known that cyclic AMP overshoot phenomenon, that is, compensatory elevation of cyclic AMP level is observed during opioid withdrawal [14–16]. These findings also

### Table 1

<table>
<thead>
<tr>
<th>Region</th>
<th>GLT-1 Dependence</th>
<th>Withdrawal (%)</th>
<th>GLAST Dependence</th>
<th>Withdrawal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>85.0±5.2</td>
<td>95.7±19.7</td>
<td>89.4±5.0</td>
<td>96.2±4.9</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>79.4±10.4</td>
<td>84.1±4.3</td>
<td>107.0±3.9</td>
<td>103.5±2.5</td>
</tr>
<tr>
<td>Striatum</td>
<td>66.4±14.8*</td>
<td>155.4±26.7*</td>
<td>96.5±4.7</td>
<td>95.5±4.8</td>
</tr>
<tr>
<td>Thalamus</td>
<td>50.0±6.4*</td>
<td>53.9±4.2**</td>
<td>98.2±4.0</td>
<td>101.7±10.6</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>77.3±15.4</td>
<td>109.9±4.3</td>
<td>103.0±20.1</td>
<td>132.4±36.0</td>
</tr>
<tr>
<td>Midbrain</td>
<td>86.8±11.2</td>
<td>116.2±21.3</td>
<td>92.0±8.7</td>
<td>88.0±12.1</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>79.0±10.6</td>
<td>74.9±16.9</td>
<td>116.6±11.8</td>
<td>103.9±15.7</td>
</tr>
<tr>
<td>Pons–medulla</td>
<td>96.9±8.3</td>
<td>104.9±24.1</td>
<td>95.8±4.7</td>
<td>100.7±5.7</td>
</tr>
</tbody>
</table>

Animals in placebo-treated and morphine-dependent group were implanted with either two placebo or morphine pellets for 5 days, respectively, as described in the text. For the morphine-withdrawal group, the morphine-dependent rats were inaperitoneally administered with naloxone (3 mg/kg). In each brain region, the expression of GLT-1 or GLAST mRNA in placebo-treated rats was assigned a value of 100%. Data are presented as means of the percentage of the placebo-treated group±S.E.M. of 4–6 separate experiments. *P<0.05, **P<0.01 compared with the placebo-treated group; *P<0.05 compared with the morphine-dependent group (Mann–Whitney U-test).
suggest the involvement of cyclic AMP overshoot in the induction of GLT-1 mRNA. In addition, we found that isoproterenol, a β-adrenergic agonist, and dopamine, which can activate adenyl cyclase system, increased the expression of GLT-1 mRNA in the rat cortical cultured astrocytes (our unpublished data), suggesting the involvement of adrenergic and dopaminergic systems in the changes of GLT-1 mRNA level.

In conclusion, we observed alterations in the expression of GLT-1, but not GLAST, mRNA in some brain regions of morphine-dependent and naloxone-precipitated withdrawal rats. These results suggest that the changes in the expression of GLT-1, which alter the glutamate uptake and affect the glutamatergic transmission efficiency, are involved in the development of morphine dependence and the expression of morphine withdrawal.

Acknowledgements

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References


