The Effect of Dexamethasone on Spinal Glutamine Synthetase and Glutamate Dehydrogenase Expression in Morphine-Tolerant Rats

Gong-Jhe Wu, MD*†
Zhi-Hong Wen, PhD‡
Wu-Fu Chen, MD§
Yi-Chen Chang, MS‡∥
Chen-Hwan Cherng, MD, DMSc∥
Chih-Shung Wong, MD, PhD∥

BACKGROUND: Excitatory amino acids play an important role in morphine tolerance. Recently, we demonstrated that a single morphine challenge induces an increase in spinal cerebrospinal fluid excitatory amino acid concentrations in morphine-tolerant rats, and that dexamethasone inhibits the development of morphine tolerance. We further examined the effect of intrathecal dexamethasone infusion on the development of morphine tolerance and on expression of the intracellular glutamate metabolizing enzymes, glutamate dehydrogenase and glutamine synthetase, in the spinal cord.

METHODS: Male Wistar rats, implanted with an intrathecal catheter, were divided into four groups that were infused for 5 days with intrathecal morphine (15 μg/h), saline (1 μL/h), dexamethasone (2 μg/h), or dexamethasone (2 μg/h) plus morphine (15 μg/h). On Day 5, the spinal cords were removed and prepared for Western blot analysis of glutamate dehydrogenase and glutamate synthetase.

RESULTS: Glutamate dehydrogenase and glutamate synthetase concentrations were downregulated in the morphine-tolerant rat spinal cords. Concurrent infusion of dexamethasone attenuated morphine tolerance and the associated glutamate dehydrogenase and glutamate synthetase downregulation.

CONCLUSION: Intrathecal dexamethasone attenuates long-term morphine infusion-induced glutamate dehydrogenase and glutamate synthetase downregulation and antinociceptive tolerance.

(Long-term opioid administration results in tolerance, which is manifested by a reduction in the analgesic effect of opioid. The mechanism of morphine tolerance has been extensively investigated, but remains unclear. In addition to the G-protein uncoupling and receptor downregulation, the excitatory amino acid (EAA), glutamate, also plays a critical role in opioid tolerance (1,2). In our previous studies (2,3), we found that the N-methyl-D-aspartate (NMDA) receptor antagonist, MK-801, attenuates morphine tolerance and prevents the morphine challenge-induced EAA increase in the cerebrospinal fluid (CSF). Glutamate-induced neuroplasticity is suggested to be one of the molecular mechanisms of morphine tolerance.

Glutamate is a primary excitatory neurotransmitter in the nociceptive signal transduction pathway in the spinal cord. Tight control of the extracellular glutamate concentration in the synapse is crucial, not only for nociception transmission, but also for preventing glutamate-induced neurotoxicity. Glutamate is taken up by glial cells, thus preventing over-excitation of neurons and excitotoxicity. It is then metabolized by glutamine synthetase and glutamate dehydrogenase into neutral metabolites glutamine and 2-oxoglutarate, respectively. Glutamate dehydrogenase is an enzyme that links the glutamine cycle with the astrocytic tricarboxylic acid (TCA) cycle (4). Glutamine has no direct access to the neuronal TCA cycle. It must be transported out of the astrocyte via glutamate transporter into neurons. Once inside the neuron, glutamine will be converted to glutamate by glutaminase in the glutamine cycle (5). Glutamate homeostasis is maintained by the balance between glutamate production from glutamine and glutamate metabolism via the TCA cycle.)
Adrenocorticotropic hormone has been demonstrated to prevent development of morphine tolerance (9). Takahashi et al. (10) found that stress inhibits the development of morphine tolerance in naïve mice, but not in adrenlectomized mice. Zhu et al. (11) also demonstrated that dexamethasone enhances glutamate uptake in rat cerebral cortex synaptosomes and neuroblastoma SH-N-SH cells. Moreover, in our previous study (12), we found that dexamethasone inhibits morphine tolerance, glutamate transporter downregulation, and the associated EAA increase in the spinal CSF dialysate seen after morphine challenge in morphine-tolerant rats. Studies have shown that glucocorticoid treatment upregulates glial glutamate dehydrogenase and glutamate synthetase expression, and suggests that these enzymes are involved in the normalization of extracellular glutamate levels (13,14). Rauen and Wießner (15) suggest that cortisol participates in the common transcriptional regulation on glutamate synthetase and glutamate dehydrogenase. Their results suggest that astrocytic glutamate metabolism, in addition to expression, may play a role in clearing the accumulated EAs from the synaptic cleft into glial cells, and thus attenuates morphine tolerance. Glucocorticoids might influence the expression of glutamate transporters, glutamate synthetase, and glutamine dehydrogenase, which are responsible for glutamate–glutamine cycling.

METHODS

Animal Preparation and Antinociceptive Test

Male Wistar rats (350–375 g) were used. Using the method described by Yaksh and Rudy (16), an intrathecal catheter was inserted via the atlantooccipital membrane into the intrathecal space and down to the level of the lumbar enlargement of the spinal cord. Rats were excluded from the study if they showed evidence of gross neurological injury or of fresh blood in the intrathecal catheter. The use of animals in this study conformed to the Guiding Principles in the Care and Use of Animals of the National Defense Medical Center, and was approved by the Care and Use Committee of our institute.

The hot water tail immersion test (52°C ± 0.5°C) was used to measure the antinociceptive effect. At this temperature, the mean tail flick latency was 2.2 ± 0.2 s in naïve rats. An automatic cut-off was set at 10 s to prevent tissue injury. Rats were placed in plastic restrainers for the antinociception test. Continuous intrathecal morphine infusion (15 µg/h, MT) for 5 days was used for tolerance induction, while concurrent infusion of dexamethasone (2 µg/h) with morphine (15 µg/h, DEX + MT) was used to examine the effect of dexamethasone on morphine tolerance development. Rats received either intrathecal saline or intrathecal dexamethasone (2 µg/h) as controls. Tail flick latencies were examined before drug infusion and daily during drug infusion for 5 days. All drugs were infused at a rate of 1 µL/h via a miniosmotic pump (model 2001; Alzet, Palo Alto, CA) implanted in the interscapular region. Tolerance was defined as a decrease in, or loss of, the antinociceptive effect of morphine infusion. The latencies were converted into the maximum percent effect (MPE) using the equation:

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\text{MPE} (%) = \frac{\text{Test response time} - \text{Basal response time}}{\text{Cut-off time} - \text{Basal response time}} \times 100\%
\]

Data and Statistical Analysis

All data are presented as the mean ± sem. For immunoreactivity data, the intensity of each test band was assigned the MPE values of 0% or 100%, respectively.

Western Blot of Glutamate Dehydrogenase and Glutamate Synthetase

On Day 5 of infusion, the rats were anesthetized with isoflurane and rapidly decapitated. The dorsal portion of the lumbar enlargement of the spinal cord was removed and stored at −80°C until use. For Western blot analysis, the samples were homogenized in ice-cold lysis buffer (50 mmol/L Tris, pH 7.5, 150 mM NaCl, 2% Triton X-100, 100 µg/mL of phenylmethylsulfonyl fluoride, 1 µg/mL of aprotinin), then centrifuged at 150,000 g for 30 min at 4°C, and the supernatants were used for Western blot analysis. Protein concentrations were determined using a DC protein assay kit (Bio-Rad, Hercules, CA) (17). Western blotting was performed as in our previous study (12) with primary antibody against glutamate dehydrogenase (1:1000 dilution; Biogenesis, cat no.: 4670-5488) and glutamate synthetase (1:1000 dilution; Biogenesis, cat no.: 4673-5007) and horseradish peroxidase-conjugated secondary antibody (dilution 1:2000; Transduction Laboratories, Lexington, KY). The glutamate synthetase antibody was recognized at a band at approximately 45 kDa in spinal cord homogenates and the glutamate dehydrogenase antibody at a band at approximately 55 kDa. The membranes were re-probed with a monoclonal mouse anti-β-actin antibody (1:2500; Sigma, St. Louis, MO, product no. A5441) as the loading control.

RESULTS

As in our previous report (12), intrathecal morphine infusion produced a maximal antinociceptive effect on Day 1. There was complete loss of the analgesic effect by Day 3 (data not shown). This reduction in the analgesic effect was significantly attenuated by the concurrent intrathecal dexamethasone infusion (Fig. 1).
Intrathecal dexamethasone infusion alone did not produce any antinociceptive effect.

On Day 5 of morphine infusion, significant down-regulation of glutamate synthetase and glutamate dehydrogenase protein expression was seen in the dorsal portion of the lumbar enlargement of the rats' spinal cords. This effect was blocked by concurrent intrathecal infusion of dexamethasone (Fig. 2). Intrathecal dexamethasone infusion alone also had no effect on the glutamate synthetase and glutamate dehydrogenase expression.

DISCUSSION

The present study showed that tolerance to intrathecal morphine was associated with downregulation of spinal glutamate dehydrogenase and glutamate synthetase expression, and that concurrent intrathecal infusion of dexamethasone not only attenuated the antinociceptive tolerance, but also inhibited the downregulation of expression of these enzymes. In our prior studies, we found that dexamethasone or the NMDA receptor antagonist MK-801 attenuated morphine challenge in morphine-tolerant rats (3). In our previous study, we further demonstrated that concurrent intrathecal infusion of dexamethasone with morphine prevents the downregulation of glial glutamate transporters, GLAST and GLT-1, but not the neuronal glutamate transporter EAAC1, seen in morphine-tolerant rat spinal cords (12). Moreover, in the same study, we found an upregulation of GLT-1 transporter expression by the concurrent intrathecal dexamethasone infusion. An increase of intracellular glutamate was found to inhibit glutamate transporter expression, particularly the neuronal EAA transporters, but also by the glutamate synthetase and glutamate dehydrogenase activity. In our present study, we found that long-term intrathecal morphine infusion induced downregulation of glutamate dehydrogenase and glutamate synthetase protein expression. Taken together, these results suggest that extracellular EAA are not only counterbalanced by glutamate transporters, but also by the glutamate synthetase and glutamate dehydrogenase activity. Glutamate transporters and the glutamate-metabolizing enzymes, glutamate synthetase and glutamate dehydrogenase, are widely expressed in glial cells, particularly in astrocytes that surround glutamatergic synapses, where they maintain the extracellular EAA concentration in a normal range. The glutamate synthetase gene contains a glucocorticoid response element and glucocorticoids promote glutamate synthetase expression at the transcriptional level (21). It is surprising that dexamethasone alone did not increase glutamate synthetase expression in controls in our present study, in contrast to many previous reports that glucocorticoids regulate glutamate synthetase expression (22,23). Moreover, Hardin-Pouzet et al. (13) also found that glucocorticoids upregulate glutamate dehydrogenase gene expression by activating the glucocorticoid response element. These results support the notion that glucocorticoids might directly regulate glutamate synthetase and glutamate dehydrogenase expression. Immunohistochemical studies have shown that glutamate dehydrogenase and glutamate synthetase are found mainly in astrocytes and are weakly expressed in neurons (24,25). Our present and previous (12) results suggest that the glucocorticoid, dexamethasone, acts by two mechanisms, upregulating GLT-1 expression and...
Figure 2. Glutamate dehydrogenase and glutamine synthetase expression in the dorsal portion of the lumbosacral segment of the rat spinal cords. Rats were infused with saline (1 μL/h, intrathecal, C), morphine (15 μg/h, intrathecal, MT), morphine (15 μg/h, intrathecal) plus dexamethasone (2 μg/h, intrathecal, MT + dexamethasone), or dexamethasone alone (2 μg/h, intrathecal, dexamethasone) for 5 days. The corresponding glutamate synthetase (a) or glutamate dehydrogenase (b) protein bands on the Western blots are shown above each column. The optical density of each protein band was quantified by densitometry and the relative optical density was calculated from the average value of samples from five rats, and takes the density of the control band as 100%. Glutamate dehydrogenase and glutamate synthetase levels were significantly downregulated 5 days after morphine infusion (*P < 0.05 compared with control) and this effect was prevented by dexamethasone co-infusion.
preventing the glutamate synthetase/glutamate dehydrogenase downregulation, thus maintaining the synaptic EAA concentration in the physiological range and, subsequently, attenuating morphine tolerance and preserving its antinociceptive effect.

In conclusion, long-term intrathecal morphine administration results in antinociceptive tolerance. Tolerance is associated with downregulation of spinal glutamate transporters and the glutamate-metabolizing enzymes, glutamate synthetase and glutamate dehydrogenase. This downregulation is responsible for the increase in synaptic EAA levels, particularly glutamate, that follows a morphine challenge in tolerant rats. These phenomena are prevented by concurrent intrathecal infusion of dexamethasone. These results, and our previous report (12), suggest that concurrent intrathecal dexamethasone infusion attenuates morphine tolerance by preventing the downregulation of the glial glutamate transporters, GLAST and GLT-1, and the glutamate metabolizing enzymes, glutamate synthetase and glutamate dehydrogenase. Dexamethasone thus maintains synaptic EAA levels within normal limits by enhancing glutamate uptake and metabolism in glial cells.

REFERENCES