

Original Article

Effect of Dextromethorphan on Antinociception and Tolerance Induced by Swim-Stress in the Formalin Test

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Background: In the present study, the effect of dextromethorphan on antinociception and tolerance induced by water swim-stress in the formalin test was investigated.

Methods: Swim- stress at 8°C induces antinociception in both phases of the formalin test. Intraperitoneal administration of dextromethorphan (60 mg/kg) also induces antinociception in the second phase of the formalin test. The lower doses of dextromethorphan (1.25, 2.5, and 5 mg/kg) which did not induce antinociception alone, but did so in combination with swim- stress (40 second), showed antinociceptive effect in both phases of the test. Exposure to water swimming stress with a period of 20 sec, once daily for three days, altered swim- stress- induced antinociception in the formalin test, when tested on the fourth day.

Results: In these animals, exposure to either water swimming stress alone or water swimming stress in combination with dextromethorphan showed potentiation of antinociception induced by swim- stress up to 20 second and decreased the response induced by 40 and 60 second swim- stress, indicating a tolerance induction. Dextromethorphan (20 mg/kg) did not alter the changes induced by three days exposure to swim- stress.

Conclusion: The results may indicate a possible involvement of n-methyl-d-aspartate receptor mechanism in the antinociception but not tolerance induced by swim- stress at 8°C.

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Keywords: Dextromethorphan • formalin test • mice • swim-stress-induced antinociception • tolerance

Introduction

Different types of swim- stress-induced antinociception (SIA) have been recognized. The antinociceptive properties of the water swimming stress (WSS) depends on the severity of swim parameters. Antinociception induced by WSS at 4°C may be an opioid-mediated type which is reversed by naloxone and other opioid receptor antagonists.

Thus, endogenous opioids and δ -opioid receptors in the spinal cord have been proposed to be involved in the opioid form of SIA.^{1,2} Repeated exposure to cold WSS at 4°C, produces an opioid-mediated antinociception^{1,2}; the induced tolerance may be due to a reduction of opioid receptors in the brainstem, midbrain, and spinal cord regions.³

Exposure of animals to swimming stress in the 20°C water, produces antinociception measured by the formalin test, which has been suggested to be mediated through nonopioid mechanism by some investigators.⁴ Nonopioid form of SIA is insensitive to the opioid receptor antagonists. Dopamine D₂ receptors and n-methyl-d-aspartate (NMDA) receptors have also been suggested to be involved in the nonopioid form.^{4,5} An opioid-nonopioid form of SIA has also been suggested.⁶⁻⁹ Mixed and often conflicting results have been reported concerning the involvement of NMDA receptor in the opioid-mediated antinociception. It

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is not clear whether the use of an NMDA receptor antagonist would enhance or decrease the antinociceptive effect of opioids.¹⁰ However, there is a report that the NMDA receptor antagonist, dextromethorphan alleviates chronic pain.¹¹ On the other hand, the drug may reduce the development of morphine tolerance and dependence in rats.¹² Previously, we have shown a cross-tolerance between antinociception induced by swim-stress at 20°C and morphine.

In the present study, the effect of an NMDA receptor antagonist, dextromethorphan, on antinociception and tolerance induced by the swim-stress at low temperature (8°C) in the formalin test was investigated. In the present study, swim-stress at 8°C was used since 30% of the animals had died at 4°C.

Materials and Methods

Animals

Male NMRI mice weighing 25 – 30 g, from Pasteur Institute of Iran (Tehran, Iran) were used for these experiments. The animals were housed ten per cage (43×30×15 cm) in a room maintained at 23±1°C with an alternating 12 hours light/dark cycle. Food and water were freely available except during the experiments. Each mouse was used only once and was euthanized immediately after the experiment.

Water swimming stress (WSS)

The mice were forced to swim in 8°C water in a container 15 cm in diameter and 20 cm tall with water filled to a depth of 11 cm (at 10 am), for a period of 20, 40, and 60 sec, in order to induce antinociception or a period of 20 sec, once daily for three days in order to induce tolerance to antinociception induced by swim-stress. After the swimming, the mice were gently dried by patting the body with a towel for two minute. The swim-stress at 8°C was chosen since about 30% of the animals died at 4°C. Formalin was injected 15 min after swim-stress and pain scores were recorded immediately after formalin administration. The control groups had similar treatment without water swimming.

Antinociceptive testing

Animals were allowed to acclimatize for 30 min before formalin injection. Forty microliter of formalin (1%) was injected subcutaneously into the dorsal surface of the right hind paw of the mouse using a microsyringe (Hamilton Co, Reno, NV)

with a 27-G needle and pain behavior was recorded immediately after formalin injection. The behavior was that originally described by Dubuisson and Dennis¹³ and reiterated by Abbott et al.¹⁴ as 0=normal weight bearing on the injected paw; 1=limping during locomotion or resting the paw lightly on the floor, or elevation of the injected paw so that at most, the nails touch the floor; 2=licking; and 3=biting, or shaking the injected paw.

The animals were observed each for 15 sec and pain scores were recorded one to five min (the first phase; 0 – 60 scores) and 15 – 60 min (the second phase; 0 – 540 scores) after the injection. Each mouse was observed by a separate observer who was unaware of treatments and doses given.

Drug

Dextromethorphan hydrobromide (Sigma-Aldrich, Germany) 5 mL/kg was injected intraperitoneally (IP), 20 min before formalin injection. The control groups received saline.

Drug Treatment

The animals were treated as follows:

Swim- SIA in mice

Four groups of mice had WSS at 8°C, for three durations of time (20, 40, and 60 sec) or no stress, and pain scores were recorded 15 min after swim-stress for a period of one hour.

Antinociceptive effect of dextromethorphan

Four groups of mice received IP injection of either saline (5 mL/kg) or different doses of dextromethorphan (20, 40, and 60 mg/kg) 20 min before formalin injection. Pain scores were recorded immediately after formalin injection for a period of one hour

Antinociceptive effect of dextromethorphan in mice exposed to different periods of water swimming stress

Mice were injected with saline (5 mL/kg) or different low doses of dextromethorphan (2.5 and 5 mg/kg) 20 min before formalin injection and divided into four groups. Each group received either no swim-stress or stress for different durations of time (20, 40, and 60 sec) 15 min prior to formalin injection and pain scores were recorded immediately after formalin injection, for a period of one hour.

Antinociceptive effect of different doses of dextromethorphan in mice exposed to a duration of water swimming stress

Mice were injected with saline (5 mL/kg) or different low doses of dextromethorphan (1.25, 2.5, and 5 mg/kg) 20 min before formalin injection and divided into two groups. Each group received either no swim-stress or stress with duration of 40 sec, 15 min before formalin injection; pain scores were recorded immediately after formalin administration for one hour.

Antinociceptive effect of swim-stress in the animals which received repeated exposure to swim-stress in the presence or absence of dextromethorphan

In these experiments, three groups of mice had either no stress (control) or swim-stress for a duration of 20 sec, once daily for three days in the presence or absence of dextromethorphan (5 mg/kg). Each group was divided into four sub-groups, receiving either no stress or stress with different durations of time (20, 40, and 60 sec) 15 min before formalin injection; pain scores were recorded immediately after formalin injection for one hour.

Statistical analysis

Data were analyzed only for the early (five min) and late (15 – 60 min) phases of the formalin test. One-way ANOVA was used for the comparison of the antinociceptive effects of swim-stress with different durations of time and also the effects of different doses of dextromethorphan with the controls. Two-way ANOVA was used for evaluation of interactions between the effects of swim-stress and administration of dextromethorphan. Two-tailed Newman-Keuls test was used as the *post hoc* test. $P < 0.05$ was considered statistically significant.

Results

Swim-SIA in mice

Figure 1 shows the antinociceptive effect of the swim-stress. Three groups of animals received WSS at 8°C, with three durations of time (20, 40, and 60 sec) 15 min before formalin injection and compared with no stress group (control). Pain scores were recorded immediately after formalin injection for one hour. One-way ANOVA indicated a significant difference between antinociception induced in the control and the three

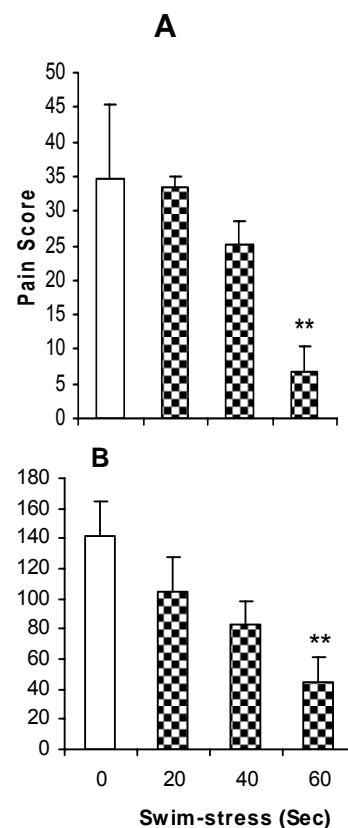


Figure 1. Antinociception induced by swim-stress in mice. Mice were forced to swim in 8°C water, for different durations of time (20, 40, and 60 sec), 15 min before formalin injection. Pain scores were recorded immediately after formalin injection in the first phase (A) or the second phase (B) of the formalin test. Bars and error bars represent respectively, the mean and SEM of scores of pain response for 10 mice. ** $P < 0.01$ different from the control group.

other groups of animals, in the first phase ($P < 0.01$), and in the second phase ($P < 0.05$) of the formalin test. *Post hoc* analysis showed that swim-stress with duration of 60 sec induced antinociception in both phases of the formalin test.

Antinociceptive effect of dextromethorphan in mice

Figure 2 indicates the antinociceptive response of dextromethorphan. Four groups of mice received IP injections of either saline (5 mL/kg) or different doses of dextromethorphan (20, 40, and 60 mg/kg) 20 min before formalin injection; pain scores were recorded immediately after formalin injection for one hour. One-way ANOVA indicated a significant difference between responses induced in the three groups of animals

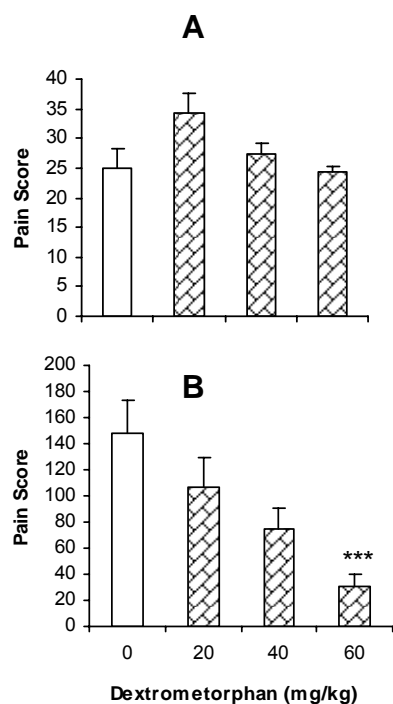


Figure 2. Antinociception induced by dexamethorphan in mice. Animals were treated intraperitoneally with either saline (5 mL/kg) or different doses of dexamethorphan (20, 40, and 60 mg/kg), 20 min before formalin injection. Pain scores were recorded immediately after formalin injection in the first phase (A) or the second phase (B) of the formalin test. Bars and error bars represent respectively, the mean and SEM of scores of pain response for 10 mice. *** $P < 0.001$ different from the control group.

which received dexamethorphan (20, 40, and 60 mg/kg) and that of the control group in the first phase ($P < 0.05$), and in the second phase ($P < 0.01$) of the formalin test. *Post hoc* analysis showed that the higher dose of dexamethorphan (60 mg/kg) induced antinociception in the second phase of the test.

Antinociceptive effect of swim-stress in the presence or absence of different doses of dexamethorphan

Figure 3 shows the effect of dexamethorphan on swim- SIA in mice. Mice were injected with saline (5 mL/kg) or lower doses of dexamethorphan (2.5 and 5 mg/kg) and divided into four groups. Each of the groups received either no swim- stress or stress with different durations of time (20, 40, and 60 sec) 15 min before formalin injection; pain scores were recorded immediately after formalin injection for one hour. Two-way

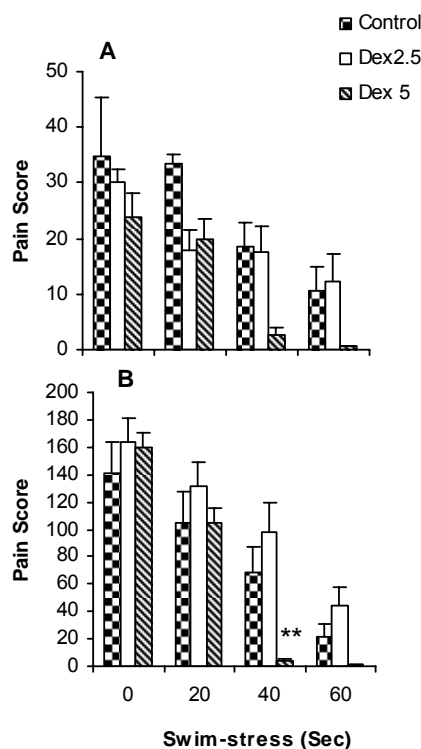


Figure 3. Effect of dexamethorphan on swim-stress with different periods of time in mice. Animals were exposed to swim-stress for different durations of time (0, 20, 40, and 60 sec), 15 min before formalin injection and saline, or dexamethorphan (2.5 and 5 mg/kg) was administered 20 min before formalin injection. Pain scores were recorded immediately after formalin injection in the first phase (A) or the second phase (B) of the formalin test. Bars and error bars represent respectively, the mean and SEM of score of pain response for 10 mice. ** $P < 0.01$ different from the control group.

ANOVA indicated no significant difference between responses induced in the control group of animals with those which received dexamethorphan (2.5 mg/kg) in the first ($P > 0.05$) and the second phase ($P > 0.05$) or with dexamethorphan (5 mg/kg) in the first phase ($P > 0.05$); there was, however, significant difference in the second phase ($P < 0.05$) of the formalin test. *Post hoc* analysis showed that the higher dose of dexamethorphan (5 mg/kg) potentiated SIA induced with 40 sec in the second phase of the test.

Effect of different doses of dexamethorphan on SIA

Figure 4 presents the antinociceptive effect of dexamethorphan on SIA by a 40-sec swim. Two-way ANOVA showed a significant difference

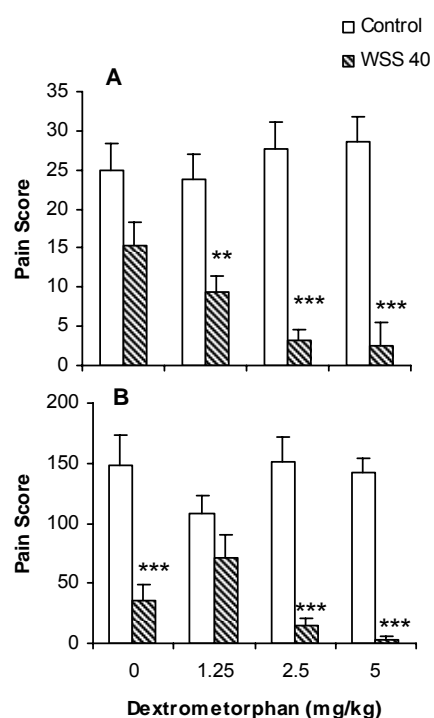


Figure 4. Effect of swim-stress in the presence or absence of low doses of dextromethorphan on the formalin test in mice. Animals were exposed to swim-stress for 40 sec, after either saline, or dextromethorphan (1.25, 2.5, and 5 mg/kg). Animals were exposed to swim-stress 15 min prior to formalin injection and were administered dextromethorphan 20 min before formalin injection. Pain scores were recorded immediately after formalin injection in the first phase (A) or the second phase (B) of the formalin test. Bars and error bars represent respectively, the mean and SEM of score of pain response for 10 mice. ** $P < 0.01$, *** $P < 0.001$ different from the control group.

between SIA (40 sec) in the presence or absence of different low doses of dextromethorphan (1.25, 2.5, and 5 mg/kg) in the first ($P < 0.05$) and the second phases ($P < 0.01$) of the formalin test. *Post hoc* analysis showed that different doses of dextromethorphan increased the SIA in both phases of the formalin test.

Effect of SIA in the presence or absence of repeated exposure to swim-stress or swim-stress plus dextromethorphan

Figure 5 indicates the effect of repeated swim-stress (WSS) in the presence or absence of dextromethorphan on SIA. In these experiments, three main groups of mice had either no stress or WSS for 20 sec, once daily for three days in the presence or absence of dextromethorphan (5 mg/kg). SIA was tested on the fourth day of

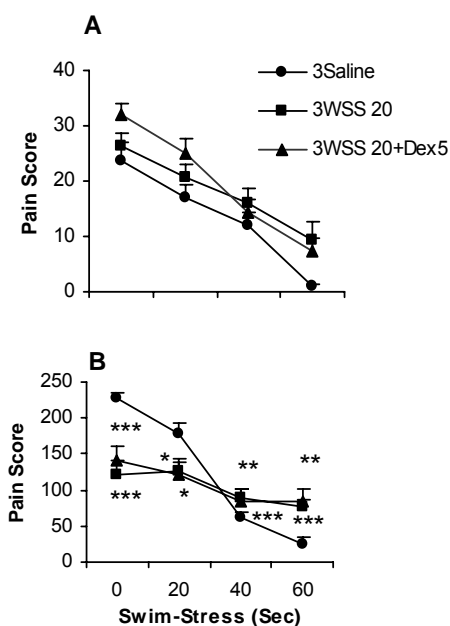


Figure 5. Antinociceptive effect of swim-stress in the animals which were exposed to repeated swim-stress (WSS) in the presence or absence of dextromethorphan. Mice were exposed to 20 min WSS after saline (■) or dextromethorphan (Dex, ▲; 5 mg/kg) administration, once daily for three days. The control animals received no WSS (●). On the test day, animals received swim-SIA for different durations of time (0, 20, 40, and 60 sec) 15 min before formalin injection. Pain scores were recorded immediately after formalin injection in the first phase (A) or the second phase (B) of the formalin test. Bars and error bars represent respectively, the mean and SEM of score of pain response for 10 mice. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ different from the control group.

exposure to swim-stress with different durations (20, 40, and 60 sec). Two-way ANOVA showed that SIA was altered in the presence of WSS (chronic WSS for 20 sec) in the second phase ($P < 0.0001$), but not in the first phase ($P > 0.05$) of the formalin test. Analysis also showed a significant difference between the SIA in the presence of chronic WSS plus dextromethorphan (5 mg/kg) in the second phase ($P < 0.0001$), but not in the first phase ($P > 0.05$) of the test. *Post hoc* analysis showed that chronic WSS caused antinociception on the fourth day and potentiated SIA with lower duration (20 sec), while reduced SIA induced by higher durations of time (40 and 60 sec).

Discussion

The formalin test is used widely as an animal model for evaluating the antinociceptive effects of mild analgesic drugs.¹⁵ Formalin injections into a paw in mice produces a biphasic nociceptive response consisting of a transient early phase—acute pain—followed by a tonic late phase—chronic pain.^{5,16} In the present study, the effect of dextromethorphan on antinociception and on the response of repeated exposure to WSS at 8°C, in the formalin test was investigated.

Our data showed that WSS at 8°C for 60 sec induced antinociception (SIA) in both phases of the formalin test. This is in agreement with previous results that swim-stress produces antinociception.³ Exposure to WSS at different degrees of temperature may cause antinociception with different mechanisms. Therefore, it has been shown that cold WSS at 4°C, induces antinociception through endogenous opioid peptides and the stimulation of $\delta 2$ -opioid receptors in the spinal cord.^{1,2} However, exposure of animals to swimming stress in 20°C water, produces antinociception, which has been suggested to be mediated through nonopioid mechanism, and through dopamine D₂ receptors and NMDA receptors.^{4,5} It may be interesting to examine whether the opioid or nonopioid mechanism is involved in the SIA induced at 8°C.

In the present experiments, in agreement with others^{11,17} administration of dextromethorphan, an NMDA receptor antagonist, also induced antinociception in the second phase of the formalin test. Our data showed that lower doses of dextromethorphan which did not induce any responses by themselves, potentiated SIA. Since SIA induced at the low temperature may be mediated through the opioid receptor system,^{1,2} and it has also been shown that the drug potentiates morphine's antinociception in the hot plate test,¹⁸ the possibility may exist that both opioid and nonopioid mechanisms may be involved in the SIA induced at 8°C.

When animals exposed to chronic swimming stress for 20 sec, once daily for three days, SIA induced by a lower duration of time was potentiated while that induced by a higher duration of time was reduced. However, the alteration induced due to repeated exposure to SIA, was not changed by low doses of dextromethorphan. The data may also show that dextromethorphan as an NMDA receptor antagonist, is not involved in the tolerance by repeated SIA.

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References

- Mizoguchi H, Narita M, Kampine JP, Tseng LF. [met5]enkephalin and delta2-opioid receptors in the spinal cord are involved in the cold water swimming-induced antinociception in the mouse. *Life Sci.* 1997; **61**: PL81 – PL86.
- Mizoguchi H, Narita M, Nagase H, Tseng LF. Antisense oligodeoxynucleotide to a delta-opioid receptor blocks the antinociception induced by cold water swimming. *Regul Pept.* 1995; **59**: 255 – 259.
- Kim KW, Choi SS, Woo RS, Suh HW. Development of antinociceptive tolerance and changes of opioid receptor ligand binding in central nervous system of the mouse forced to single and repeated swimming in the cold water. *Brain Res Bull.* 2003; **61**: 93 – 97.
- Vendruscolo LF, Takahashi RN. Synergistic interaction between mazindol, an anorectic drug, and swim-stress on analgesic responses in the formalin test in mice. *Neurosci Lett.* 2004; **355**: 13 – 16.
- Vaccarino AL, Marek P, Sternberg W, Liebeskind JC. NMDA receptor antagonist mk-801 blocks nonopioid stress-induced analgesia in the formalin test. *Pain.* 1992; **50**: 119 – 123.
- O'Connor P, Chipkin RE. Comparisons between warm and cold water swim-stress in mice. *Life Sci.* 1984; **35**: 631 – 639.
- Terman GW, Morgan MJ, Liebeskind JC. Opioid and nonopioid stress analgesia from cold water swim: importance of stress severity. *Brain Res.* 1986; **372**: 167 – 171.
- Kest B, Marek P, Liebeskind JC. The specific n-methyl-d-aspartate (NMDA) receptor antagonist mk-801 blocks u-50,488, but not morphine antinociception. *Brain Res.* 1992; **589**: 139 – 142.
- Lapo IB, Konarzewski M, Sadowski B. Effect of cold acclimation and repeated swimming on opioid and nonopioid swim stress-induced analgesia in selectively bred mice. *Physiol Behav.* 2003; **8**:345 – 350.
- Mao J. NMDA and opioid receptors: their interactions in antinociception, tolerance, and neuroplasticity. *Brain Res Brain Res Rev.* 1999; **30**: 289 – 304.
- Fisher K, Coderre TJ, Hagen NA. Targeting the n-methyl-d-aspartate receptor for chronic pain management. Preclinical animal studies, recent clinical experience, and future research directions. *J Pain Symptom Manage.* 2000; **20**: 358 – 373.
- Mao J, Price DD, Caruso FS, Mayer DJ. Oral administration of dextromethorphan prevents the development of morphine tolerance and dependence in rats. *Pain.* 1996; **67**: 361 – 368.
- Dubuisson D, Dennis SG. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brainstem stimulation in rats and cats. *Pain.* 1977; **4**: 161 – 174.
- Abbott FV, Franklin KB, Westbrook RF. The formalin test: scoring properties of the first and second phases of

- the pain response in rats. *Pain*. 1995; **60**: 91 – 102.
- 15** Hunskaar S, Fasmer OB, Hole K. Formalin test in mice, a useful technique for evaluating mild analgesics. *J Neurosci Methods*. 1985; **14**: 69 – 76.
- 16** Rosland JH, Tjolsen A, Maehle B, Hole K. The formalin test in mice: effect of formalin concentration. *Pain*. 1990; **42**: 235 – 242.
- 17** Berrino L, Oliva P, Massimo F, Aurilio C, Maione S, Grella A, et al. Antinociceptive effect in mice of intraperitoneal n-methyl-d-aspartate receptor antagonists in the formalin test. *Eur J Pain*. 2003; **7**: 131 – 137.
- 18** Grass S, Hoffmann O, Xu XJ, Wiesenfeld-Hallin Z. N-methyl-d-aspartate receptor antagonists potentiate morphine's antinociceptive effect in the rat. *Acta Physiol Scand*. 1996; **158**: 269 – 273.

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