DOI 10.1007/s11094-020-02202-1
Pharmaceutical Chemistry Journal, Vol. 54, No. 4, July, 2020 (Russian Original Vol. 54, No. 4, April, 2020)

EFFECTS OF AMINOADAMANTANE DERIVATIVES ON MORPHINE-INDUCED ANALGESIA IN MICE

L. G. Kolik,[1,*] A. V. Nadorova,[1] I. V. Chernyakova,[1] and E. A. Val'dman[1]

Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 54, No. 4, pp. 15 – 19, April, 2020.

Original article submitted February 8, 2017.

The effects of low-affinity NMDA-receptor antagonists amantadine (1-aminoadamantane hydrochloride) and hemantane [*N*-(2-adamantyl)hexamethyleneimine hydrochloride] on morphine-induced analgesia in C57Bl/6 mice were studied. Amantadine (10 and 20 mg/kg, i.p.) *per se* did not affect the latent period of the response in the hot-plate test while hemantane (10 and 20 mg/kg, i.p.) increased dose-dependently pain thresholds 180 and 240 min after administration. Morphine (20 mg/kg, s.c.) showed a time—effect dependence (30 – 120 min). The aminoadamantanes were administered 90 min after the opioid to assess their effects on morphine-induced antinociception. The responses of the animals were recorded for the next 2.5 h. The aminoadamantanes potentiated and extended the analgesic activity of morphine in the order of efficacy amantadine < hemantane. The results indicated that the aminoadamantanes had different capabilities to cause delayed analgesia and modulated opioid antinociceptive activity at the supraspinal level.

Keywords: hemantane, amantadine, hot plate, morphine-induced analgesia, C57Bl/6.

Antagonists of *N*-methyl-D-aspartate (NMDA) receptors such as ketamine and dextromethorphan are currently used as adjuvant therapy for enhancing the antinociceptive activity of opioid analgesics for pain therapy during post-operative recuperation [1] despite the adverse side effects such as the addiction potential that place several limitations on pharmacotherapy with these drugs. Clinical experience with the use of low-affinity aminoadamantane NMDA-receptor antagonists is equivocal. On one hand, amantadine, a drug that is widely used to treat Parkinson's disease [2], after a single administration in double-blind placebo-controlled trials reduced the demand for morphine in prostatectomy patients [3] and after surgical spinal manipulations [4]. On the other, the effects of amantadine on acute and chronic pain and the amount of used morphine in the post-operative period were not reported [5].

According to experimental studies, blockage of NMDA-receptors by aminoadamantane derivatives can potentiate [6 – 8], diminish [9], or have no effect on [10] analgesia induced by opioids. An analysis of results for the effects of non-competing NMDA-receptor antagonists on acute morphine-induced antinociception led to the conclusion that the results depended on the type of laboratory animal [6, 7], sex [11], sequence and time between administrations [12], selected test protocol, and modality of nociceptor stimulation [6 – 8]. It is noteworthy that most studies on the combined action of NMDA-receptor blockers and opioids on somatic pain used the tail-flick test.

Hemantane is an original domestic aminoadamantane drug with pronounced activity equivalent to amantadine in experimental Parkinsonism models [13 – 15] and in patients in its early stages [16]. Previously, hemantane was shown to have analgesic activity with acute and subchronic administration and electrical pain stimulation and for simulated neuropathic pain in rats [13]. Hemantane exhibited anti-inflammatory activity in *in vivo* tests for a visceral pain model [17]. However, the effect of hemantane on the antinociceptive properties of morphine have not yet been reported. The mechanism of action including blockage of ion channels of NMDA-subtype glutamate receptors [18] suggested that hemantane was effective when combined with opioid analgesics for simulated somatic pain with thermal irritation of nociceptors.

[1] V. V. Zakusov State Institute of Pharmacology, Russian Academy of Medical Sciences, 8 Baltiiskaya St., Moscow, 125315 Russia.
[*] e-mail: lgkolik@mail.ru

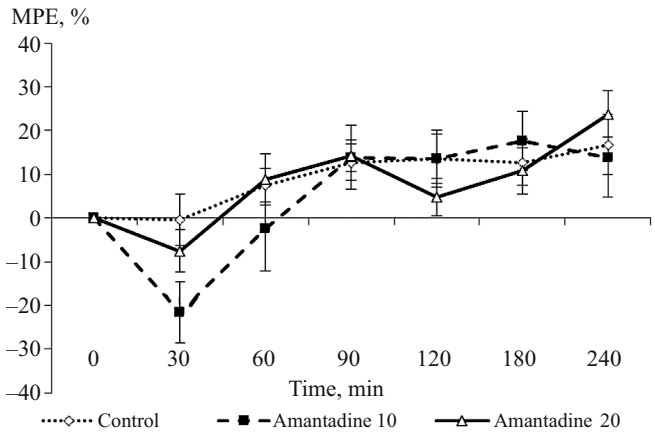

Fig. 1. Effects of amantadine at doses of 10 and 20 mg/kg on latent period of response to thermal irritation of nociceptors in C57Bl/6 mice ($M \pm SEM$). No statistically significant differences vs. control, ANOVA; 10 animals per group.

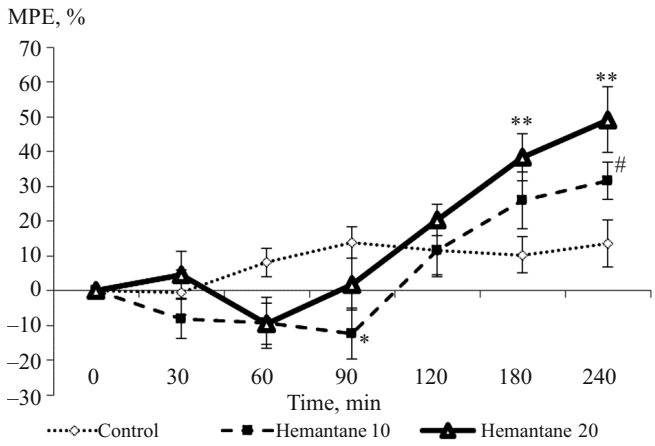

Fig. 2. Effects of hemantane at doses of 10 and 20 mg/kg on latent period of response to thermal irritation of nociceptors in C57Bl/6 mice. $M \pm SEM$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{\#}p < 0.1$, statistically significant vs. control, ANOVA, Duncan test; 9 animals per group.

The goal of the present work was to study the antinociceptive activity of hemantane and amantadine and their effects on morphine-induced analgesia in the hot-plate test in mice.

EXPERIMENTAL PART

The experiments used outbred male C57Bl/6 mice (18 – 22 g; Scientific Center for Biomedical Technologies, Federal Medical and Biological Agency, Stolbovaya Branch). The animals (15) were kept in standard T/3 cages under vivarium conditions at V. V. Zakusov State Institute of Pharmacology (SIP) (21 – 23°C, relative humidity 40 – 60%) with natural lighting and free access to water and feed pellets for 10 d before the start of testing. The work was organized and performed according to Ministry of Health of Russia Order No. 199 dated Apr. 10, 2016, "On approval of Good Laboratory Practice rules." Animals were kept in compliance with SP 2.2.1.3218-14 "Sanitary and epidemiological requirements to arrangement, equipment and maintenance of biological clinics (vivariums)" dated Aug. 29, 2014, No. 51. Experiments were approved by the Biomedical Ethics Committee, V. V. Zakusov SIP.

Hemantane [*N*-(2-adamantyl)hexamethyleneimine hydrochloride, drug substance, synthesized at V. V. Zakusov SIP] at i.p. doses of 10 and 20 mg/kg; amantadine (1-aminoadamantane hydrochloride, drug substance, Sigma-Aldrich) at i.p. doses of 10 and 20 mg/kg as a reference drug; and morphine hydrochloride (Minmedbioprom Combine Khimkentbiofarm, drug substance) at an s.c. dose of 20 mg/kg were dissolved in H_2O for injection and administered once calculated for 0.1 mL/10 g of animal mass. Control animals received H_2O for injection. The morphine doses and the experimental protocol were selected based on literature data [12].

The hot-plate test was used to evaluate the nociceptive reaction. An analgesimeter (Ugo Basile, Italy) was used to record the latent time of the response (licking hind paws or jumping). Animals were selected 1 – 2 h before the start of the test based on the baseline response under the conditions of the particular experimental model. Mice remaining on the heated plate (up to 56 ± 5°C) longer than 15 sec were excluded from the test. A latent period of 20 sec (maximum exposure time) was evaluated as 100% analgesia. The response times of the mice were recorded 30, 60, 90, 120, 180, and 240 min after a single administration of the tested drugs. The results were expressed in percent of the maximum possible effect (MPE). The MPE equaled (the latent period of the response after administration of the drug minus the baseline latent period of the response) divided by (the maximum exposure time minus the baseline latent period of the response) times 100%.

EXPERIMENTAL PROTOCOLS:

1. Effects of aminoadamantane derivatives *per se* with thermal irritation of nociceptors were studied after a single administration 30 min before the test.

2. Effects of aminoadamantane derivatives with morphine-induced analgesia were studied 90 min after a single administration of morphine and 30 min after a single administration before testing on the hot plate, i.e., without an independent baseline analgesic effect of the aminoadamantane derivatives.

Results were processed using the Mann—Whitney test, multiple comparisons of means test for all groups, and single-factor dispersion analysis (ANOVA) followed by a Duncan test. Normal distributions of data were checked us-

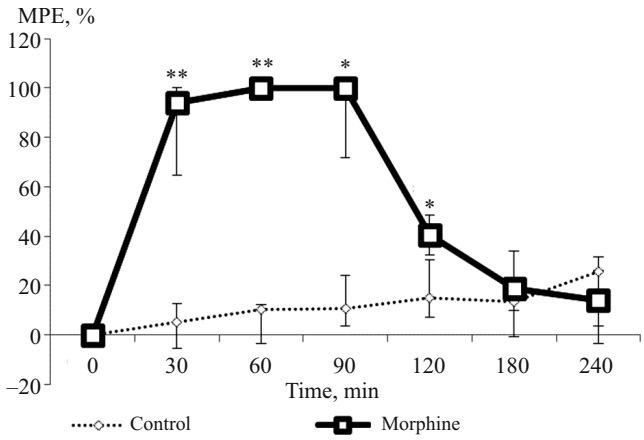

Fig. 3. Effects of morphine on latent period of response to thermal irritation of nociceptors in C57Bl/6 mice. Me (25q; 75q). * $p < 0.01$, ** $p < 0.001$, statistically significant vs. control, Mann—Whitney test; 10 animals per group.

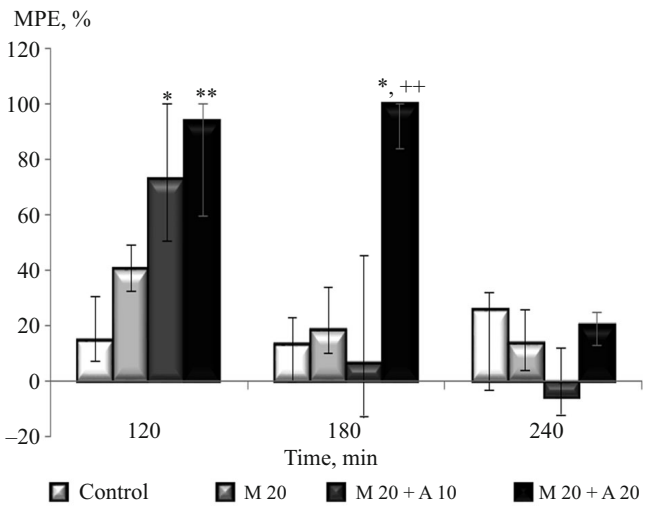

Fig. 4. Effects of amantadine at doses of 10 and 20 mg/kg on morphine-induced analgesia in C57Bl/6 mice in hot-plate test. Me (25q; 75q). * $p < 0.01$, ** $p < 0.001$ vs. control, ++ $p < 0.01$ vs. M 20 group, according to multiple comparisons test of means for all groups; 9 – 10 animals per group.

ing the Shapiro—Wilk criterion. The critical significance level was $\alpha = 0.05$.

RESULTS AND DISCUSSION

The average baseline level of the nociceptive response for C67Bl/6 mice in the hot-plate test was 6.8 ± 0.3 sec. Animals with a latent period of the response >10 sec were excluded from the experiment.

Evaluation of antinociceptive activities of amantadine and hemantane. Amantadine at the studied doses with thermal irritation of C57Bl/6 mouse nociceptors at the supraspinal level in the hot-plate test did not affect the latent period from 30 to 240 min of observation after a single systemic administration according to the lack of statistically significant differences from the control group (Fig. 1).

Hemantane, in contrast to amantadine, showed dose-dependent analgesic activity starting from 180 to 240 min of observation. The drug was effective at a dose of 20 mg/kg and increased statistically significantly ($p < 0.01$) the threshold pain response in mice in the hot-plate test (Fig. 2).

Hemantane at a dose of 10 mg/kg demonstrated a tendency to increase the response latent period from 180 to 240 min ($p < 0.1$), despite the slight reduction of the pain threshold at 90 min.

Figure 3 shows the dynamics of the analgesic activity of the opioid analgesic morphine hydrochloride under the given test conditions. Pronounced analgesia that lasted for 120 min developed under the influence of morphine already 30 min after a single administration. The threshold pain response 180 min after administration did not differ for the test and control groups.

Effects of amantadine and hemantane on morphine-induced analgesia. Statistically significant dose-dependent increases of pain thresholds as compared to the control group were observed at 120 and 180 min during the evaluation of the effects of amantadine on the antinociceptive response on the background of morphine action. The independent analgesic effect of the drug disappeared 180 min after morphine administration. However, distinct extended and potentiated antinociceptive activity of morphine was observed ($p < 0.01$) after amantadine administration at the maximum dose of 20 mg/kg. Amantadine at a dose of 10 mg/kg did not affect morphine-induced analgesia. The latent period of the response in test animals at 240 min did not differ from that of the controls (Fig. 4).

Hemantane administered on the background of morphine caused a dose-dependent increase of the antinociceptive activity of the opioid analgesic at 120 and 180 min. Hemantane at a dose of 10 mg/kg increased statistically significantly the latent period of the response in mice at 120 and 180 min ($p < 0.001$ and $p < 0.05$, respectively) as compared to the control. Hemantane potentiated and extended morphine-induced analgesia at 120 and 180 min ($p < 0.05$ and $p < 0.01$, respectively) if the hemantane dose was increased to 20 mg/kg. The threshold pain sensitivity did not change after 240 min in any of the test groups (Fig. 5).

Parkinson's disease is considered a multi-system disease for which motor disruptions are observed together with symptoms unrelated to motor disorders, as a rule, characteristic of the prodromal stage of the disease [19]. Pain is considered one of the most common nonmotor symptoms encountered by various estimates in 53 – 60% of patients diagnosed with Parkinson's disease [19, 20]. As a rule, dopaminergic therapy is optimized and anti-inflammatory agents and opioid analgesics are used for pharmacological correction of skeletal muscular, neuropathic, and central pain [20].

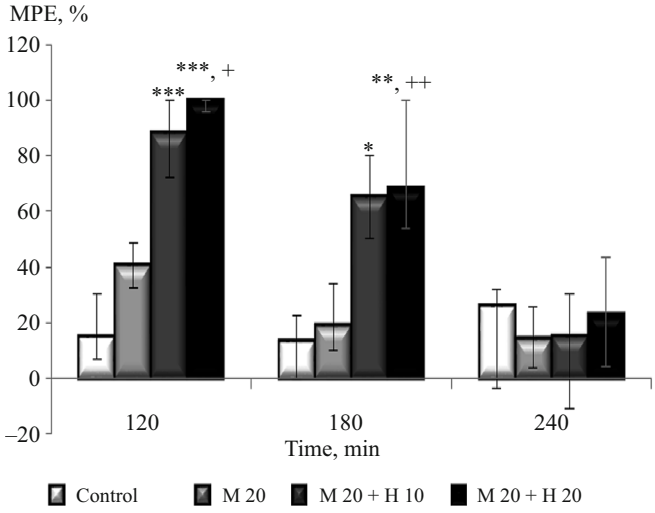

Fig. 5. Effects of hemantane at doses of 10 and 20 mg/kg on morphine-induced analgesia in C57Bl/6 mice in hot-plate test. Me (25q; 75q). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{\times}p < 0.001$ vs control, $^{+}p < 0.05$, $^{++}p < 0.01$ vs. M 20 group, according to multiple comparisons test of means for all groups; 9 – 10 animals per group.

The antinociceptive properties of hemantane with simulated supraspinal somatic pain were studied in the present work. Hemantane, in contrast with the reference drug amantadine, which has been used for a long time in clinical practice [2], demonstrated moderate delayed analgesic activity at doses that did not disrupt motor coordination [13]. Such an effect has not been reported in the literature. Despite positive results with aminoadamantane derivatives during recuperation of chronic neuropathic pain [13, 21], non-competing NMDA-receptor antagonists, including memantine (3,5-dimethyladamantane-1-amine), did not exhibit independent activity in the acute nociceptive response model [12, 22]. Apparently, this was explained by the methodological specifics of the testing and a delayed antinociceptive response. Keeping in mind the complex mechanism of action of hemantane, including non-competing blockage of NMDA-receptors, the dopaminergic component [23], and its spectrum of activity including neuroprotective [24] and anti-inflammatory properties [17] and the ability to eliminate cognitive disorders in the initial stage of Parkinson's disease without movement disorders [14], the results for the antinociceptive activity expand concepts about the pharmacological profile of hemantane and indicate that use of it in the early stage of Parkinson's disease is advantageous.

The opioid analgesic and two aminoadamantane derivatives, i.e., amantadine and hemantane, were shown to interact pharmacodynamically with thermal irritation of nociceptors in mice. This manifested as statistically significant potentiation and extension (by 60 min) of the antinociceptive activity of morphine in the hot-plate test. The effects found for the aminoadamantanes showed a clear dose-dependence that was most evident at a dose of 20 mg/kg. A comparison of the abilities of amantadine and hemantane to potentiate the analgesic effect of morphine showed that hemantane had an advantage because it at a dose of 20 mg/kg possessed anti-Parkinson's activity. The effect of morphine increased statistically significantly at 120 and 180 min. The drug at a dose of 10 mg/kg was only slightly less efficacious (Fig. 5). Amantadine exhibited an analogous effect only at a dose of 20 mg/kg at 180 min of observation (Fig. 4). The results agreed with previous data [12] where the non-competing aminoadamantane NMDA-receptor antagonist memantine (10 mg/kg, i.p.) with an analogous administration protocol enhanced ($p < 0.05$) the analgesic action of morphine in the tail-flick test. According to the researchers, morphine potentiation was noted only if the opioid was used at the maximum dose of 20 mg/kg and was not observed in the dose range 1 – 10 mg/kg. It also depended on the time interval between morphine and memantine injections [12].

Clinical enhancement of the analgesic effect of morphine in the presence of NMDA-receptor antagonists could be explained by analyzing morphine metabolism in the presence of amantadine and dextromethorphan in patients that did not show effects of the drugs on the morphine degradation rate and the concentrations of its metabolites [25]. This was confirmed by test results in rats [7]. The results as a whole for hemantane pharmacodynamics suggested that its interaction with co-administered morphine was apparently determined by the interaction of hemantane with NMDA-receptors.

REFERENCES

1. M. Suzuki, *Curr. Opin. Anaesthesiol.*, **22**(5), 618 – 622 (2009).
2. G. Hubsher, M. Haider, and M. S. Okun, *Neurology*, **78**(14), 1096 – 1099 (2012).
3. D. G. Snijdelaar, G. Koren, and G. Katz, *Anesthesiology*, **100**(1), 134 – 141 (2004).
4. B. Bujak-Gizycka, K. Kacka, M. Suski, et al., *Pain Med.*, **13**(3), 459 – 465 (2012).
5. J. Yazdani, *Pain Med.*, **6**(3), e35900 (2016).
6. E. Kozela, W. Danysz, and P. Popik, *Eur. J. Pharmacol.*, **423**(1), 17 – 26 (2001).
7. D. G. Snijdelaar, C. M. van Rijn, P. Vinken, and T. F. Meert, *Pain*, **119**(1 – 3), 159 – 167 (2005).
8. D. Malec, M. Mandryk, and S. Fidecka, *Pharmacol. Rep.*, **60**(2), 149 – 155 (2008).
9. Y. Chen, M. Evola, and A. M. Young, *Psychopharmacology*, **225**(1), 187 – 199 (2013).
10. E. Kozela, A. Pilc, and P. Popik, *Psychopharmacology*, **165**(3), 245 – 251 (2003).
11. J. E. Grisel, S. Allen, K. V. Nemmani, et al., *Pharmacol. Biochem. Behav.*, **81**, 131 – 138 (2005).
12. I. V. Belozertseva, O. A. Dravolina, O. N. Neznanova, et al., *Eur. J. Pharmacol.*, **396**, 77 – 83 (2000).
13. E. A. Val'dman, Author's Abstract of a Doctoral Dissertation in Medical Sciences, Moscow (2001).
14. A. V. Nepoklonov, I. G. Kapitsa, and E. A. Ivanova, *Eksp. Klin. Farmakol.*, **75**(11), 3 – 6 (2012).
15. E. A. Ivanova, I. G. Kapitsa, E. A. Val'dman, and T. A. Voronina, *Byull. Eksp. Biol. Med.*, **159**(3), 362 – 365 (2015).

16. E. V. Katunina, A. V. Petrukhova, G. N. Avakyan, et al., *Zh. Nevrol. Psikhiatr. im. S. S. Korsakova*, **108**(6), 24 – 27 (2008).
17. E. A. Ivanova, I. G. Kapitsa, A. V. Nepoklonov, et al., *Khim.-farm. Zh.*, **47**(10), 12 – 15 (2013).
18. M. V. Elshanskaya, A. I. Sobolevskii, E. A. Val'dman, and B. I. Khodorov, *Eksp. Klin. Farmakol.*, **64**(1), 18 – 21 (2001).
19. S. S. O'Sullivan, D. R. Williams, D. A. Gallagher, et al., *Mov. Disord.*, **23**, 101 – 106 (2008).
20. A. Q. Rana, A. Kabir, M. Jesudasan, et al., *Clin. Neurol. Neurosurg.*, **115**(11), 2313 – 2317 (2013).
21. I. O. Medvedev, A. A. Malyshkin, I. V. Belozertseva, et al., *Neuropharmacology*, **47**(2), 175 – 183 (2004).
22. K. E. Redwine and K. A. Trujillo, *Pharmacol. Biochem. Behav.*, **76**(2), 361 – 372 (2003).
23. I. A. Zimin, I. O. Logvinov, T. A. Antipova, and G. I. Kovalev, *Eksp. Klin. Farmakol.*, **74**(1), 11 – 14 (2011).
24. I. O. Logvinov, T. A. Antipova, A. V. Nepoklonov, and E. A. Val'dman, *Eksp. Klin. Farmakol.*, **79**(1), 12 – 14 (2016).
25. M. Suski, B. Bujak-Gizycka, J. Madej, et al., *Folia Med. Cracov.*, **49**(3 – 4), 111 – 121 (2008).