

# Comparison of the Analgesic Activity of Antiparkinsonian Aminoadamantane Derivatives Amantadine and Hemantane

Elena Ivanova\*, Inga Kapitsa, Elena Valdman, Tatyana Voronina

Laboratory of Psychopharmacology, Zakusov Institute of Pharmacology, Moscow, Russia  
Email: \*iwanowaea@yandex.ru

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## Abstract

The efficacy of some aminoadamantane derivatives used as neurodegeneration treatments is due to their ability to block NMDA receptors. But this mechanism of pharmacological action can also produce analgesic activity. Analgesic properties of two aminoadamantanes, amantadine (20 mg/kg) and hemantane (20 mg/kg), which were uncompetitive NMDA receptor antagonists, were assessed in rodent models of pain induced by different pain stimuli (tail-flick test, acetic twitches test in mice and formalin test in rats). Additionally, the anti-inflammatory properties of hemantane and amantadine were evaluated in mice with acetic peritonitis and in rats with hind paw edema induced by formalin injection. The results of our study demonstrate that the analgesic activity of the 1-aminoadamantane amantadine differs from the 2-aminoadamantane hemantane. The analgesic activity of amantadine administered intraperitoneally was more pronounced in the case of acute thermal pain in mice compared to hemantane, and only amantadine had a significant analgesic effect on the acute early phase of formalin pain in rats induced by the effect of the algogen on the primary sensory afferents. Hemantane was more effective than amantadine for relieving pain produced by inflammation owing to its pronounced anti-inflammatory activity: only hemantane decreased the amount of acetic twitches in mice that received drugs orally and was effective in the tonic phase of formalin pain in rats.

## Keywords

NMDA Receptor Antagonists, Tail-Flick Test, Acetic Twitches, Formalin Test, Exudative Inflammation

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\*Corresponding author.

## 1. Introduction

Some derivatives of aminoadamantane are known as efficient treatments for neurodegenerative diseases [1]. Amantadine, a derivative of 1-aminoadamantane, is useful as an antiparkinsonian and antidyskinetic drug [2]-[4]. Benefits of therapy with memantine, another 1-aminoadamantane, are confirmed in clinical trials investigating the treatment of Alzheimer's disease [5] [6], vascular dementia [7], and Parkinson's disease [8]. Hemantane, a derivative of 2-aminoadamantane, is a new antiparkinsonian and antidyskinetic agent [9] [10] and currently undergoing clinical trials in the Russian Federation.

The aminoadamantane derivatives act by blocking NMDA receptors [11]-[13]. In addition to the neuroprotective effect, the blocking of NMDA receptors could also produce analgesic activity [14]. Activation of NMDA-type ionotropic glutamate receptors is required for long-term potentiation of nociceptive neurons [15]-[17] and plays a major role in the amplification of a nociceptive input in post-inflammatory conditions [18].

The analgesic effect of the NMDA receptor antagonist amantadine, a 1-aminoadamantane, was demonstrated in patients with diabetic peripheral neuropathy [19]. Another NMDA receptor antagonist, hemantane, a 2-aminoadamantane, demonstrated analgesic properties in the model of visceral pain in mice [20].

The goal of this study is to compare the analgesic effects of the 1-aminoadamantane amantadine and 2-aminoadamantane hemantane in rodent models of pain induced by different pain stimuli.

Amantadine (adamantan-1-amine) is used in two salt forms: amantadine hydrochloride [3] and sulfate [21]. In our work amantadine hydrochloride was used because hemantane was also a hydrochloride, specifically N-2 (adamantyl) hexamethylenimine hydrochloride (**Figure 1**). Comparator drugs were used to objectively assess the analgesic effects of amantadine and hemantane on pain in rodents. Carbamazepine, an anticonvulsant, was used as an effective comparison drug in models of peripheral thermal nociception [22]. Diclofenac sodium, an extensively used and highly effective nonsteroidal anti-inflammatory drug (NSAID) [23]-[25], was used in models of inflammation-induced pain and in additional experiments on exudative inflammation.

## 2. Materials and Methods

### 2.1. Animals

Male white outbred rats weighing 240 to 295 g at the age of 2.5 months and male white outbred mice weighing 27 to 30 g at the age of 2 months were obtained from the Stolbovaya branch of the Scientific Centre of Biomedical Technology of Federal Biomedical Agency (Moscow Region, Russia). Mice were used in the tail-flick test and the acetic twitches test; the formalin test was done in rats. Animals were housed under standard conditions: constant temperature ( $22^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ), humidity (relative, 30%) and a 12 h light/dark cycle and were allowed to adapt to the laboratory conditions for 7 days before the onset of the experiments. The use of the animals and protocol procedures were approved and supervised by the Ethics Committee for the Use of Laboratory Animals at the Zakusov Institute of Pharmacology, Moscow, Russia.

### 2.2. Drugs under Investigation

Amantadine and hemantane were synthesized in the department of experimental technology of the Zakusov Institute of Pharmacology under the direction of the leading researcher Dr. N.I. Avdyunina. The compounds were administered at the dose of 20 mg/kg. Determination of the dose was based on previous experimental results. Hemantane at the dose of 20 mg/kg demonstrated analgesic and anti-inflammatory properties in mice [20]. Amantadine at the dose of 20 mg/kg exhibited antiparkinsonian action in some animal models of Parkinson's



**Figure 1.** Structural formulas of the 1-aminoadamantane amantadine and 2-aminoadamantane hemantane.

disease [26] [27]. The dose of the comparator drug carbamazepine produced by ALSI Pharma (Russia) was determined in our preliminary results at 20 mg/kg, identical with the dose of aminoadamantanes under investigation. The comparator drug diclofenac sodium produced by Hemofarm (Serbia) was administered at the dose of 10 mg/kg. Determination of the dose of diclofenac sodium is based on literature [28] [29] and our previous results.

### 2.3. The Tail-Flick Test

The tail-flick test was used as a model of acute thermal pain in mice [30]. The tail-flick response was tested using a TSE Systems tail-flick apparatus (Germany). A thermal stimulus was applied at a 25% intensity, which corresponds to a temperature of 50°C to 55°C. This temperature produces tail-flick latencies of 4 to 5 sec while preventing tissue damage. In the absence of a withdrawal reflex, the stimulus was cut off at 10 sec to avoid possible tissue damage [31]. Experimental animals were divided into four groups of 10 mice each: control group, hemantane group, amantadine group and group of the comparison drug carbamazepine. Tail-flick latencies were evaluated 30, 60 and 90 min after an injection of hemantane (20 mg/kg), amantadine (20 mg/kg), carbamazepine (20 mg/kg) or saline (control group) intraperitoneally (i.p.).

### 2.4. The Acetic Twitches Test

The model of acetic twitches in mice was chosen as a model of persistent pain produced by acute inflammation. Twitches were induced by an i.p. injection of 1% acetic acid, 1 ml per 100 g of animal weight, and were counted for 15 min after the injection [32]. Mice were divided into 4 groups of 12 mice each. 40 minutes before the acetic acid injection, animals of the control group were given saline i.p.; mice of the comparison drug group received the NSAID diclofenac sodium i.p. 10 mg/kg, mice of the third group received hemantane i.p. 20 mg/kg, and mice of the fourth group received amantadine i.p. 20 mg/kg.

### 2.5. Evaluation of Exudative Inflammation in Mice after Acetic Acid Injection

Exudate from the abdominal cavity of mice was collected at 3 hours after the i.p. acetic acid injection, and its amount was measured in order to compare the anti-inflammatory effect of hemantane with amantadine and diclofenac sodium [32].

### 2.6. Evaluation of the Analgesic Activity of Oral Hemantane and Amantadine in Mice

Two additional experiments were performed to evaluate the analgesic activity of oral hemantane and amantadine. The tail-flick test and acetic twitches test in mice were described above. Control group animals were given oral saline; mice of the second group received oral hemantane 20 mg/kg, and mice of the third group received oral amantadine 20 mg/kg 40 minutes before the tests. There were 8 to 10 animals in each experimental group.

### 2.7. The Formalin Test

The formalin test in rats simulates pain produced by surgery dissections and includes two phases of pain behavior: the early acute phase and the late tonic phase [33] [34]. Pain behavior was measured every 5 minutes on a scale from 0 to 4 with the following interpretation 0: no reaction; 1: the paw is on the ground but the rat does not stand on it; 2: the paw is raised; 3: the rat is licking, flinching or shaking its paw [30]. Data were recorded in the early acute phase 0 to 5 minutes after the injection of 100 µl of a 2% formalin solution and in the late tonic phase 20 to 60 minutes after the injection [33]. 1 hour before the formalin injection, animals of control group were given saline i.p.; mice of the comparison drug group received diclofenac sodium (Hemofarm, Serbia) i.p. 10 mg/kg; mice of the third group received hemantane i.p. 20 mg/kg; and mice of the fourth group received amantadine i.p. 20 mg/kg. There were 8 to 10 animals in each experimental group.

### 2.8. Evaluation of Paw Edema in Rats Induced by Formalin Injection

Additionally, the effect of hemantane, amantadine and diclofenac sodium on test paw edema was recorded. The baseline diameters of the hind paws were measured at the metatarsal level before the formalin injection using a caliper. The diameters of the hind paws that developed edema were determined 4 hours after the injection by

measuring the dorsal-plantar foot thickness at the metatarsal [31] [33]. An increase in the paw's diameter 4 hours after the formalin injection was a sign of inflammation.

## 2.9. Data Analysis

Statistica 8 software was used to perform statistical analyses. Normality was checked by the Shapiro-Wilk test with further estimation of dispersion congruence using the Levene test. The Newman-Keuls test was used for parametric analysis; the Kruskal-Wallis test with post-hoc Dunn's multiple comparison test and the Mann-Whitney test were used for non-parametric analysis. Statistical significance was set at  $p < 0.05$ . Data in the tables and figures are expressed as median, 25% ÷ 75%.

## 3. Results

### 3.1. Evaluation of the Analgesic Activity of Intraperitoneal Hemantane and Amantadine in the Tail-Flick Test

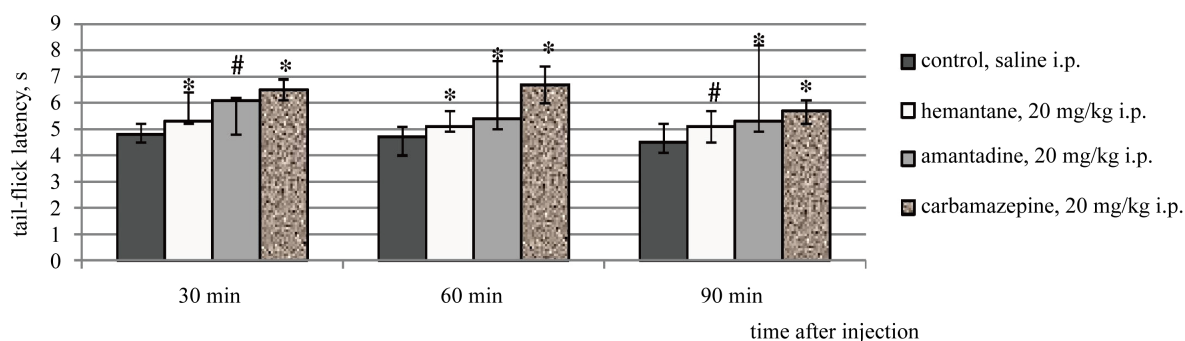
In the model of acute thermal pain, median tail-flick latency in the control group (receiving saline i.p.) was 4.5 - 4.8 seconds during the 90 minutes of experiment. At 30 and 60 minutes after the administration of hemantane (20 mg/kg i.p.), the median tail-flick latency significantly increased by 10.4% and 8.5% compared to the control group ( $p < 0.05$ ). At 90 minutes there was a 13.3% tendency ( $p < 0.1$ ) toward an increase of the pain threshold. The efficacy of amantadine (20 mg/kg i.p.) was more pronounced than hemantane (20 mg/kg i.p.). The tail-flick latency increase relative to the control group was insignificant at 27.1% ( $p < 0.1$ ) at 30 minutes after the injection and significant at 14.9% and 17.8% at 60 and 90 minutes of observation respectively ( $p < 0.05$ ). The comparator drug carbamazepine (20 mg/kg i.p.) exhibited the most pronounced analgesic effect in the tail-flick test. It significantly increased pain threshold in mice by 35.4% at 30 minutes, 42.6% at 60 minutes and 26.7% at 90 minutes after the injection compared to the control group ( $p < 0.05$ ) (Figure 2).

### 3.2. Evaluation of the Analgesic Activity of Intraperitoneal Hemantane and Amantadine in the Acetic Twitches Test

In the model of acetic twitches, the median number of twitches in the control group (saline i.p.) was 54. Hemantane (20 mg/kg) significantly reduced pain reaction by 32.4% compared to the control group ( $p < 0.05$ ). The effect of amantadine (20 mg/kg) was similar to hemantane: in the amantadine group the number of twitches dropped by 33.3% ( $p < 0.05$ ). The analgesic activity of both adamantane derivatives did not differ significantly from the effect of the NSAID diclofenac sodium (10 mg/kg i.p.), which reduced the median number of twitches by 48.1% compared to the control group ( $p < 0.05$ ) (Table 1).

### 3.3. Evaluation of the Analgesic Activity of Oral Hemantane and Amantadine in Mice

Two additional experiments were performed to evaluate the analgesic activity of the adamantane derivatives administered orally in the tail-flick test and acetic twitches test in mice. Hemantane (20 mg/kg) was found to



**Figure 2.** Tail-flick latency at 30, 60 and 90 minutes after injections of hemantane, amantadine, carbamazepine or saline (control group). Data were analyzed by the Mann-Whitney test. Data are expressed as median (25% ÷ 75%). \* $p < 0.05$  compared to the control group; # $p < 0.1$  compared to the control group.

significantly reduce pain reaction in both tests ( $p < 0.05$ ). In the tail-flick test the pain threshold in mice receiving hemantane increased by 11.4% (Table 2). In the model of persistent pain produced by acute inflammation, hemantane induced a 28.6% drop in the number of twitches, and its effect did not significantly differ from the effect of diclofenac sodium (10 mg/kg) (Table 1). Amantadine (20 mg/kg) by administered orally 40 minutes before the experiments failed to exhibit analgesic properties in any of the tests (Table 1 and Table 2).

### 3.4. Evaluation of the Analgesic Activity of Intraperitoneal Hemantane and Amantadine in the Formalin Test

In the formalin test, the maximum pain score in rats of all experimental groups observed at the moment of algogen injection (minute 0); pain reaction decreased at minute 5 of the acute phase. Significant reduction of pain behavior at minute 5 of observation compared to the rats receiving saline was observed only in rats that received amantadine (20 mg/kg i.p.) ( $p < 0.05$ ). Amantadine was the only compound to significantly reduce the average score in the acute early phase of formalin pain by 33.3% relative to the control group ( $p < 0.05$ ) (Table 3).

**Table 1.** The number of twitches in mice with acetic peritonitis.

	The number of twitches with different routes of administration	
	intraperitoneally	orally
Control, saline	54.0 (50.0 ÷ 64.0)	70.0 (52.0 ÷ 84.0)
Hemantane, 20 mg/kg	36.5 (24.0 ÷ 50.0)*	50.0 (41.0 ÷ 53.0)#
Amantadine, 20 mg/kg	36.0 (21.0 ÷ 47.0)*	74.0 (69.0 ÷ 80.0)
Diclofenac sodium, 10 mg/kg	28.0 (17.0 ÷ 33.0)*	42.0 (28.0 ÷ 58.0)#

Data were analyzed by the Newman-Keuls test (for parametric analysis) and by the Mann-Whitney test (for non-parametric analysis). Data are expressed as median (25% ÷ 75%). \* $p < 0.05$  compared to the control group, Mann-Whitney test; # $p < 0.05$  compared to the control group, Newman-Keuls test.

**Table 2.** Tail-flick latency after the oral administration of hemantane, amantadine or saline.

	Tail-flick latency (s)
Control, saline orally	4.4 (3.5 ÷ 4.7)
Hemantane, 20 mg/kg orally	4.9 (4.5 ÷ 5.7)*
Amantadine, 20 mg/kg orally	4.5 (4.3 ÷ 4.9)

Data were analyzed by the Mann-Whitney test. Data are expressed as median (25% ÷ 75%). \* $p < 0.05$  compared to the control group.

**Table 3.** Pain behavior in the acute early phase of the formalin test (score).

	Time after the formalin injection, min		Average score
	0 min	5 min	
Control, saline	3.0 (3.0 ÷ 3.0)	1.5 (1.0 ÷ 2.0)	4.5 (4.0 ÷ 5.0)
Hemantane, 20 mg/kg	3.0 (3.0 ÷ 3.0)	1.0 (0.0 ÷ 2.0)	4.0 (3.0 ÷ 5.0)
Amantadine, 20 mg/kg	3.0 (2.5 ÷ 3.0)	0.0 (0.0 ÷ 1.0)*	3.0 (3.0 ÷ 3.5)*
Diclofenac sodium, 10 mg/kg	3.0 (3.0 ÷ 3.0)	1.0 (0.0 ÷ 1.0)	4.0 (3.0 ÷ 4.0)

Data were analyzed by the Kruskal-Wallis test with post-hoc Dunn's multiple comparison test. Data are expressed as median (25% ÷ 75%). \* $p < 0.05$  compared to the control group.

Pain reaction in the control animals reached a peak at minute 20 of the tonic phase, thereafter decreasing gradually up to minute 30 of observation. Specifically, the score was 2.5 at minute 25 and 2 at minute 30 after the formalin injection. The median pain score in the control group was stable from minute 30 to minute 60 of observation. The average score in the tonic phase of formalin pain (from minute 20 to minute 60 after the formalin injection) in the control group was 18.5 (Table 4).

Hemantane (20 mg/kg i.p.) delayed the development of tonic pain by decreasing pain reaction compared to the control group significantly at minute 20 ( $p < 0.02$ ) and insignificantly at minute 25 ( $p < 0.3$ ). In the diclofenac sodium (10 mg/kg i.p.) group the intensity of pain behavior was also reduced significantly at minutes 20 and 25 of observation ( $p < 0.05$ ). There were no significant differences between the control group and the groups of rats receiving hemantane or diclofenac sodium at minutes 30 to 60 after the formalin injection (Table 4). The average score for the tonic phase of formalin pain for the hemantane and diclofenac sodium groups decreased significantly ( $p < 0.05$ ). Hemantane reduced the intensity of pain behavior by 27%; diclofenac sodium, by 30% compared to the control group (Table 4). The 2-aminoadamantane amantadine 20 mg/kg failed to produce a significant effect on the tonic phase of formalin pain (Table 4).

### 3.5. Evaluation of the Anti-inflammatory Activity of Hemantane and Amantadine in Rats with Paw Edema Induced by Formalin Injection

After the formalin injection the edema of the damaged hind paw was progressing, and at 4 hours the median paw diameter in the control group increased by 1.2 mm. Hemantane (20 mg/kg) significantly decreased the exudative reaction by 42% compared to the control group ( $p < 0.05$ ). Its effect was non-inferior to the effect of the NSAID diclofenac sodium (10 mg/kg), which reduced the edema of the affected hind paw by 17% relative to the control group ( $p < 0.05$ ). Amantadine (20 mg/kg) did not exhibit significant anti-inflammatory activity (Figure 3).

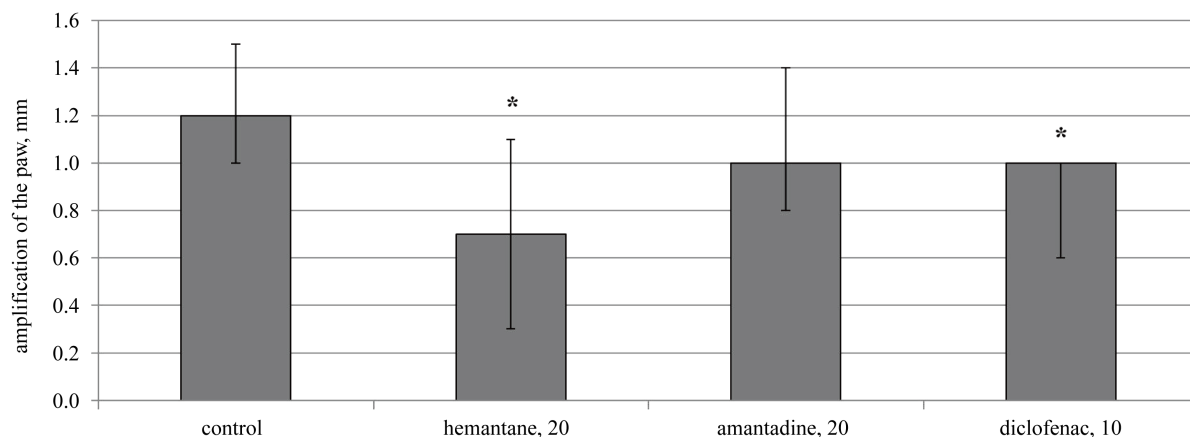
### 3.6. Evaluation of the Anti-inflammatory Activity of Hemantane and Amantadine in Mice with Peritonitis Induced by Acetic Acid Injection

Additionally, the anti-inflammatory properties of hemantane were confirmed by measuring the level of peritoneal exudation 3 hours later after the injection of 1% acetic acid in mice in the acetic twitches test. Hemantane (20 mg/kg) demonstrated a significant anti-inflammatory effect when administered i.p. and orally ( $p < 0.05$ ). Intraperitoneal injection of hemantane reduced exudation by a median of 32.8% compared to the control group. Administration of hemantane 20 mg/kg orally reduced the intensity of acetic peritonitis by 30.5% relative to control group animals. The effects of hemantane (20 mg/kg i.p.) and orally did not significantly differ from the effects of the comparator drug diclofenac sodium (10 mg/kg). Intraperitoneal injection of the NSAID reduced inflammation by 60.2% ( $p < 0.005$ ), and its oral administration reduced the pathological reaction by 57.4% ( $p < 0.005$ ) compared to the control group. The adamantane derivative amantadine (20 mg/kg) had a significant anti-inflammatory effect only when administered i.p. ( $p < 0.05$ ). The medicine exhibited the weakest anti-exudative properties among the compounds under investigation and reduced inflammation by 18.6% compared to the control group (Table 5).

**Table 4.** The pain behavior in the late tonic phase of formalin test (score).

	Time after the formalin injection, min									Average score	
	20 min	25 min	30 min	35 min	40 min	45 min	50 min	55 min	60 min		
Control, saline	3.0 (2.0 ÷ 3.0)	2.5 (2.0 ÷ 3.0)	2.0 (2.0 ÷ 2.0)	2.0 (2.0 ÷ 2.0)	2.0 (2.0 ÷ 2.0)	2.0 (2.0 ÷ 2.0)	2.0 (2.0 ÷ 2.0)	2.0 (2.0 ÷ 2.0)	2.0 (1.0 ÷ 2.0)	2.0 (0.5 ÷ 2.0)	18.5 (17.5 ÷ 19.5)
Hemantane, 20 mg/kg	1.0 (0.0 ÷ 2.0)*	2.0 (2.0 ÷ 2.0)	2.0 (2.0 ÷ 2.0)	2.0 (2.0 ÷ 2.0)	2.0 (2.0 ÷ 2.0)	1.5 (1.0 ÷ 2.0)	2.0 (1.0 ÷ 2.0)	1.0 (1.0 ÷ 2.0)	0.5 (1.0 ÷ 2.0)	0.5 (0.0 ÷ 2.0)	13.5 (12.0 ÷ 16.0)*
Amantadine, 20 mg/kg	1.5 (1.0 ÷ 2.0)	2.0 (1.5 ÷ 2.0)	2.0 (2.0 ÷ 2.5)	2.0 (1.0 ÷ 2.0)	2.0 (1.5 ÷ 2.0)	1.0 (1.0 ÷ 2.0)	1.5 (1.0 ÷ 2.0)	1.5 (1.0 ÷ 2.0)	1.5 (1.0 ÷ 2.0)	2.0 (1.0 ÷ 2.0)	15.5 (12.5 ÷ 17.5)
Diclofenac sodium, 10 mg/kg	1.0 (1.0 ÷ 1.0)*	2.0 (1.0 ÷ 2.0)*	2.0 (2.0 ÷ 2.0)	2.0 (2.0 ÷ 2.0)	1.5 (1.0 ÷ 2.0)	1.0 (1.0 ÷ 2.0)	1.0 (1.0 ÷ 2.0)	1.0 (1.0 ÷ 2.0)	1.0 (0.0 ÷ 2.0)	1.0 (0.0 ÷ 2.0)	13.0 (8.0 ÷ 17.0)*

Data were analyzed by the Kruskal-Wallis test with post-hoc Dunn's multiple comparison test. Data are expressed as median (25% ÷ 75%). \* $p < 0.05$  compared to the control group.



**Figure 3.** Paw edema in the formalin test. Data were analyzed by the Mann-Whitney test. Data are expressed as median (25% ÷ 75%). \* $p < 0.05$  compared to the control group.

**Table 5.** Mass of peritoneal exudate in mice with acetic peritonitis (mg).

	Mass of exudate with different routes of administration	
	intraperitoneally	orally
Control, saline	796.0 (705.0 ÷ 968.0)	912.0 (746.0 ÷ 989.0)
Hemantane, 20 mg/kg	535.0 (424.0 ÷ 800.0)*	634.0 (434.0 ÷ 772.0)#
Amantadine, 20 mg/kg	648.0 (558.0 ÷ 714.0)*	995.5 (787.0 ÷ 1234.0)
Diclofenac sodium, 10 mg/kg	317.0 (267.0 ÷ 419.0)**	388.0 (272.0 ÷ 527.0)##

Data were analysed by the Mann-Whitney test (for non-parametric analysis) and by the Newman-Keuls test (for parametric analysis). Data are expressed as median (25% ÷ 75%). \* $p < 0.05$  compared to the control group, Mann-Whitney test; \*\* $p < 0.005$  compared to the control group, Mann-Whitney test; # $p < 0.05$  compared to the control group, Newman-Keuls test; ## $p < 0.005$  compared to the control group, Newman-Keuls test.

## 4. Discussion

Aminoadamantane derivatives are known to have antinociceptive properties. Memantine, a 1-aminoadamantane derivative and an uncompetitive NMDA receptor antagonist, had an antinociceptive effect in the vincristine-induced peripheral neuropathy model in rats at a dose of 10 mg/kg [35]. Some derivatives of 1-aminoadamantane are investigated as potential analgesic compounds because of their agonist activity at CB<sub>2</sub> or/and CB<sub>1</sub> cannabinoid receptors [36] [37]. Replacement of the phenyl ring in molecule of paracetamol (acetaminophen) with an adamantyl moiety produced a new 1-aminoadamantane derivative with analgesic properties that can only be explained by selective TRPA<sub>1</sub> channel antagonist activity rather than by COX inhibition or by direct interaction with cannabinoid receptors [38]. Molecules containing 2-aminoadamantane-1-carboxylic acid and 2-aminoadamantane fragments have antinociceptive properties owing to the inhibition of P<sub>2</sub>X<sub>7</sub>-triggered glutamate release [39]. Furthermore, one of the molecules under investigation was tested in the carrageenan paw edema model in rats, exhibiting a capacity to reduce the volume of exudative edema [39].

In our investigation the analgesic properties of two aminoadamantane-class uncompetitive NMDA receptor antagonists were assessed. The analgesic effect of amantadine, a 1-aminoadamantane derivative, differed from the effect of hemantane, a 2-aminoadamantane derivative (Table 6). To wit, both of them exhibited analgesic activity in the tail-flick test in mice when administered i.p.; the effect of amantadine was more pronounced but did not surpass the analgesic activity of carbamazepine (Figure 2). The stronger analgesic action of amantadine is probably best explained by its greater capacity for the inhibition of serotonin reuptake compared to hemantane [40].

Hemantane and amantadine i.p. both exhibited analgesic activity in the test on persistent pain produced by acute inflammation. However, when given orally in the acute thermal pain test and in the acetic acid-induced persistent pain test, hemantane administered 40 minutes before the tests was the only drug to significantly reduce

**Table 6.** Influence of hemantane and amantadine on different types of experimental pain\*.

Models of pain	Aminoadamantane derivatives and their mode of administration			
	Hemantane, 20 mg/kg		Amantadine, 20 mg/kg	
	Intraperitoneally	Orally	Intraperitoneally	Orally
Acute thermal pain	+	+	+	0
Persistent pain produced by acute inflammation	+	+	+	0
Formalin pain	Hemantane, 20 mg/kg intraperitoneally		Amantadine, 20 mg/kg intraperitoneally	
The acute early phase of formalin pain	0		+	
The late tonic phase of formalin pain	+		0	

\*“+” means significant ( $p < 0.05$ ) analgesic effect, “0” means absence of significant analgesic effect.

pain. Amantadine should probably be administered earlier before the test to take effect. For example, amantadine was administered subcutaneously 100 min prior to L-DOPA to demonstrate its anti-dyskinetic activity in the model of L-DOPA-induced dyskinesia in animals [41]-[43]. Remarkably, simultaneous administration of amantadine 20 mg/kg with levodopa 10 mg/kg and benserazide 15 mg/kg i.p. accelerated the onset of L-DOPA-induced dyskinesia in rats and increased its severity rather than reducing it [10].

Amantadine produced a pronounced analgesic effect on the acute early phase of formalin pain induced by the action of algogen on the primary sensory afferents [44], but had no impact on the second phase of pain. In contrast, hemantane had no effect on the early phase of formalin pain while reducing the intensity of the late tonic phase of formalin pain mediated by peripheral inflammation [33] [34] (Table 6). The effect of hemantane on the tonic phase of formalin pain was quite similar to diclofenac sodium. The comparator medicine showed an analgesic effect typical for NSAID in this model of pain by only reducing the intensity of the tonic phase of pain. The effect of hemantane 20 mg/kg on the tonic phase of formalin pain was due to its significant anti-inflammatory activity, which was demonstrated in the tests of acetic peritonitis in mice and formalin paw edema in rats (Table 5, Figure 3). Earlier it was shown that hemantane i.p. at the dosage range 10 - 40 mg/kg reduced exudative inflammation in mice with acetic peritonitis but its effect was less pronounced than the effect of diclofenac sodium i.p. 10 mg/kg [45]. Hemantane i.p. at three doses of 10, 20 and 40 mg/kg also significantly decreased the pseudoallergic reaction in the model of concanavalin A-induced edema in mice [46]. The mechanism of anti-inflammatory action of hemantane is associated with its capacity to reduce the elevated level of proinflammatory cytokines in blood plasma in mice and rats with experimental pathology [46], and with its antiradical effect [26]. Additionally, it was observed that hemantane reduced elevated proline-specific endopeptidase (EC 3.4.21.26.) activity in the plasma of rats with rotenone-induced Parkinson's disease to the level representative for intact rats [47]. The activity of the enzyme is known to correlate with the level of proinflammatory interleukins in rat models and in cirrhotic patients [48].

Amantadine demonstrated anti-inflammatory activity in the experiments described above only when administered i.p. in the test of acetic peritonitis in mice, and its effect was less pronounced than the anti-inflammatory effect of hemantane (Table 5). According to the literature, amantadine can alleviate local inflammation in the brain tissue of animals with experimental Parkinson's disease. Specifically, amantadine 20 mg/kg exhibited antiradical activity by reducing the intensity of lipid peroxidation and increasing glutathione content in the substantia nigra of rats with 6-hydroxydopamine-induced Parkinson's disease [49]. This drug reduced lipopolysaccharide- and 1-methyl-4-phenylpyridinium ion (MPP+)-induced toxicity of dopamine neurons through 1) the inhibition of the release of microglial pro-inflammatory factors, 2) an increase in expression of neurotrophic factors such as GDNF from astroglia [50]. Consequently, the anti-inflammatory properties of amantadine and hemantane contribute to their neuroprotective activity. Moreover, as both medicines inhibit NMDA receptors, they could be hypothesized to modulate COX-2 activity, and therefore also inflammation, via NMDA receptors [51].



## 5. Conclusion

In summary, our results demonstrate that the analgesic activity of amantadine (20 mg/kg), a derivative of 1-aminoadamantane, differs from the effect of hemantane (20 mg/kg), a 2-aminoadamantane derivative. Amantadine was more effective in the model of acute thermal pain in mice. In addition, only amantadine exhibited a significant analgesic effect on the acute early phase of formalin pain in rats induced by the influence of the algogen on the primary sensory afferents. Abatement of pain produced by acute inflammation (the late tonic phase of formalin pain in rats and acetic twitches in mice) was stronger with 2-aminoadamantane hemantane treatment owing to its pronounced anti-inflammatory activity. Differences in the analgesic activity of amantadine and hemantane revealed in this experimental study necessitate further investigations which aim at identifying the underlying pharmacodynamic mechanisms.

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