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Anti-inflammatory and Analgesic Activity of Azaadamantanes – Analogues of Amantadine

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Abstract

The anti-inflammatory and analgesic activity of three azaadamantane analogues of amantadine **1** was investigated. The tested azaadamantanes containing nitrogen atoms in the polycyclic framework were: 5,7-dimethyl-1,3-diazaadamantane-6-one **2**, 6-amino-5,7-dimethyl-1,3-diazaadamantane **3** and 7-amino-1,3,5-triazaadamantane **4**. Experiments were carried out on male mice of CD1 and CBA lines. Compounds were administered intraperitoneally at doses of 20 and 50 mg/kg. Anti-inflammatory activity was studied on the standard models of inflammatory edema induced by histamine or concanavaline inflammation by injecting the phlogogens in the aponeurosis of the hind paw of mice. The analgesic activity was assessed using the hot plate and acetic writhing tests. It is shown that the introduction of heteroatoms into the adamantane core does not reduce the anti-inflammatory activity of diazaadamantanes (**2**, **3**) and enhances that of triazaadamantane (**4**) compared to amantadine. Modification of the adamantane backbone with nitrogen atoms leads to a change in the analgesic properties of the molecule, which is manifested as hyperalgesia in the acetic writhing test. This phenomenon is presumably related to the effect of azaadamantanes on nocigenic transient receptor potential channels (TRPV1).

Keywords: amantadine, azaadamantanes, anti-inflammatory, analgesic activity

INTRODUCTION

The derivatives of adamantane remain in the focus of attention as a source of biologically active compounds with diverse kinds of pharmacological activity. These compounds attract much attention during the recent decades, first of all as the modulators of glutamate AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and NMDA (N-methyl-*D*-aspartate) receptors for the purpose of pharmacological correction of neurodegenerative processes and other pathologies with similar symptoms, including post-covid syndrome. The application of blockers of NMDA-receptors based on the amino-derivatives of adamantane, namely amantadine, memantine, gimantan and their synthetic analogues, is evidence in favour of the reasonability of further search for agents within this class of compounds. In spite of the fact that amantadine remains at attractive platform for obtaining new modulators of neurotransmitter systems, promising neurotropic agents may also be azaadamantanes, nitrogen-containing analogues of adamantane with one or two carbon atoms in the framework substituted by nitrogen. They are less lipophilic than adamantane analogues, which affects their bioavailability and interaction with biological targets. For azaadamantane derivatives, the existence of diverse kinds of biological activity including analgesic [1, 2], antiviral [3, 4], anti-

bacterial [5, 6], antitumour [7] and others [8], in combination with moderate toxicity and simplicity of synthesis, makes them promising objects for synthesis and investigation as new biologically active compounds. At present, there are no published data on the studies of neuroprotective properties of adamantane derivatives containing nitrogen atom in the polycyclic framework of adamantane.

The goal of the present work was to study anti-inflammatory and analgesic activity of azaadamantane series. It is known that these kinds of pharmacological activity may contribute substantially to the mechanisms of neuroprotective action of the drugs used in treating Parkinson, Alzheimer diseases, senile dementia, as well as remitting multiple sclerosis [9–11].

EXPERIMENTAL

Materials

The compounds under investigation were commercially available aminoadamantane hydrochloride (amantadine, **1**, Roth, ≥99%) and its polycyclic nitrogenous analogues containing nitrogen atoms in the nodal sites of the adamantane framework: 5,7-dimethyl-1,3-diazaadamantane-6-one **2**, 6-amino-5,7-dimethyl-1,3-diazaadamantane **3** and 7-amino-1,3,5-triazaadamantane **4**, synthesised according to the procedures described in [2, 12] and sown in Fig. 1.

Experiments were carried out with CD-1 and CBA line male mice with 25–30 g body weight, obtained from the vivarium of the FRC ICG SB RAS (Novosibirsk). The animals were kept under standard conditions with controlled temperature and humidity on a 12 h light-dark cycle, with free access to water and standard granulated food. All manipulations with the animals were carried out in agreement with the legislation of the Russian Federation according to GOST 33044-2014 "Principles of Good Laboratory Practice" and provisions of the Directive 2010/63/EU of the Parliament of the European Union and the Council of the European Union of 22.09.2010 concerning the protection of animals used for research purposes. Experiment design and methods applied in the work were approved by the bioethical commission of the Novosibirsk Institute of Organic Chemistry SB RAS (Novosibirsk), Protocol No. P-14-2023-04-01.

Methods of investigation

The experimental models of acute inflammation and pain, recommended for preclinical trial, were used in the work [13]. The compounds under study **1–4** were introduced intraperitoneally at the doses of 20 and 50 mg/kg as a suspension in water for injections with 0.5% Tween-80. Non-steroidal anti-inflammatory agent indomethacin (Fluka Bio Chemika) was administered to the animals of the comparison group at the effective dose of 20 mg/kg [14], while the animals of the control group received an equivalent amount of water with Tween-80.

In the model of histamine-induced acute exudative inflammation, the CD-1 line mice were randomly divided into 10 groups, with 8 animals per each group. The aqueous solution of histamine in the concentration of 0.01% (Sigma Aldrich) was injected into the aponeurosis of the hind paws of all animals in the volume of 0.05 mL 1 h later after the injection of the agents under investigation. Collateral paw remained intact. Five hours later, the mice were sacrificed through cervical dislocation, both hind paws were dissected, and the mass of each paw was determined. The anti-inflammatory effect was estimated as the value of edema index (EI), which was determined as a ratio of the mass difference between inflamed and intact paws to the mass of the intact paw, expressed in per cent.

In the model of pseudo-allergic inflammation induced by concanavalin A, 80 CBA male mice with 25–30 g body weight were used. The animals were divided into 10 groups. The agents under investigation were introduced twice at 24 h interval, as indicated above. One hour after the last injection, a solution of concanavalin A (Sigma Aldrich) with the concentration of 0.5 mg/mL was injected subplantarly in the volume of 0.01 mL

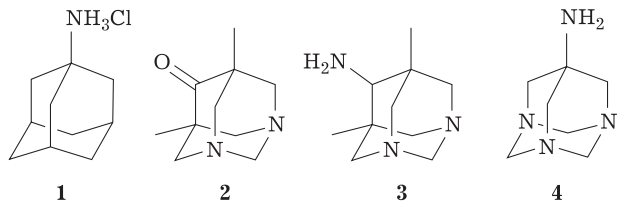

Fig. 1. Chemical structure of the compounds under investigation: **1** – aminoadamantane hydrochloride (amantadine); **2** – 5,7-dimethyl-1,3-diazaadamantane-6-one; **3** – 6-amino-5,7-dimethyl-1,3-diazaadamantane; **4** – 7-amino-1,3,5-triazaadamantane.

per 10 g body weight into the hind paws of the animals of all groups. Collateral paws remained intact. One hour later, the mice were sacrificed through cervical dislocation, both hind paws were dissected, each paw was weighted to determine the paw EI as described in the previous model.

The hot plate test was used in the model of thermal pain. The CD-1 line mice were randomised into 10 groups, with 6 animals per group. Thermal irritation was imposed 1 h after the injection of the compounds under investigation, by placing an animal onto a plate heated to 54 °C, and the time until the first paw withdrawal (licking) or jumping was measured.

In the model of visceral pain, the acetic writhing test was applied. The CD-1 mice were divided into 10 groups, with 6 animals in each group. Visceral pain was caused by the intraperitoneal introduction of the 0.75% solution of acetic acid in the amount of 0.1 mL per 10 g of body weight in an hour after the injection of compounds under investigation. The intensity of acute pain reaction was determined by the number of writhes within the first 5 min.

The statistical treatment of results was carried out by nonparametric analysis using the Statistica 8.0 software package (StatSoft Inc., USA). The data are presented as the median and quartile range. The pairwise comparison between groups was performed with the help of Mann-Whitney U-test. The differences were considered to be statistically significant at $p \le 0.05$.

RESULTS AND DISCUSSION

Anti-inflammatory activity of the nitrogenous analogues of amantadine

In the model of exudative inflammation, histamine was used as phlogogen. Under natural conditions, histamine is released by the sensory nerve endings and effector cells in the focus of inflammation. Histamine causes vasodilatation of microcirculatory vessels, enhancement of vessel well permeability, and sensitisation of the endings of pain nerves. As a result, hyperemia, pain and the exudative edema of tissues arise [15]. Efficient pharmaceuticals, in this case, are non-steroidal anti-inflammatory drugs. In our experiment, preventive injection of azaadamantanes **2–4** and amantadine **1** caused a reliable decrease in the paw edema value in mice, and the effect did not exhibit a significant dependence on dose (Table 1).

The highest activity in the series of adamantane derivatives was caused by compound **4**, which caused a decrease in EI by 38%, while other compounds caused a decrease by not more than 28%. The efficiency of all the agents under investigation were reliably behind indomethacin (a decrease in EI by 58%), so their anti-inflammatory activity within the dose range of 20–50 mg/kg may be considered as moderate (see Table 1).

In the model of pseudo-allergic inflammation imitating immediate-type hypersensitivity reaction, polyvalent lectin concanavalin A causes the direct degranulation of mast cells, which leads to the rush of inflammation mediators, leukotrienes

TABLE 1

Edema index (EI) of the paws of mice in the model of exudative inflammation

Group	Dose, mg/kg	EI	EI with respect to the reference, %
Reference	–	35.9±6.1	100
1	20	$26.5\pm9.2^{***\#\#}$	74
	50	$33.4\pm10.4^{\#\#\#}$	93
2	20	$25.7\pm3.7^{*\#}$	72
	50	$28.8\pm4.5^{**\#\#\#}$	80
3	20	$32.3\pm6.2^{\#\#\#}$	90
	50	$25.7\pm2.4^{***\#\#\#}$	72
4	20	$23.2\pm5.0^{***\#\#\#}$	65
	50	$22.4\pm13.0^{***\#}$	62
Indomethacin	20	$15.1\pm4.4^{***}$	42

Note. The data are presented in the form: median ± quartile range.
$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ – differences from the reference are significant.
$^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ – differences from indomethacin are significant.

TABLE 2

Edema index (EI) of the paws of mice in the model of pseudo-allergic inflammation

Group	Dose, mg/kg	EI	EI with respect to the reference, %
Reference	–	25.8±7.9	100
1	20	20.1±6.4	78
	50	29.1±5.4$^{\#}$	113
2	20	23.3±6.2	90
	50	20.9±4.4	81
3	20	23.0±9.3$^{\#}$	89
	50	23.1±7.1	89
4	20	21.6±10.8	84
	50	24.5±9.0	95
Indomethacin	20	17.6±3.7**	68

Note. The data are presented in the form: median ± quartile range.
**$p < 0.01$ – differences from the reference are significant.
$^{\#}p < 0.05$ – differences from indomethacin are significant.

and other vasoactive agents inducing local inflammation. In this case, the advantageous means are those blocking the membrane receptors of mast cells and preventing their degranulation. Under the conditions of this model, amantadine **1** and its nitrogen-containing analogues **2–4** did not exhibit significant anti-inflammatory activity, unlike indomethacin, which caused not very large but reliable decrease in EI by 32% (Table 2).

Analgesic activity of the nitrogenous analogues of amantadine

The hot plate test serves as a model of nociception with the involvement of spinal and supraspinous structures controlling the behavioural reactions of the animals (paw licking, jumping, vocalisation) [13]. The parameter characterising the analgesic effect in this case is an increase in pain threshold for temperature stimulus.

No reliable differences in the threshold of temperature sensitivity were detected between the reference and experimental groups of animals in the test (Table 3). The obtained data provide evidence that azaadamantanes and amantadine introduced within the dose range of 20–50 mg/kg cannot modulate pain perception caused by thermal action, both in sensitive afferent pathways and in the descending antinociceptive system. According to literature data, analgesic effect arises within this model under the action of the agonists of central μ- and δ-opioid receptors, as well as the blockers of nonselective cation channels of the transient receptor potential (TRP) in the sensory nerve endings [16]. The absence of the analgesic effect of indomethacin (see Table 3) points to the fact that the pain reaction in this test is not related to inflammation.

In the acetic writhing test, the somatogenic pain syndrome is induced, which is connected with the contraction of peritoneal muscles under irritation with a diluted acetic acid solution. The model reproduces acute visceral and deep somatic pain, which corresponds to the clinical features of peritonitis. The number of spasmodic body motions was calculated for 5 min after the introduction of the algogenic agent.

Test results show that the injection of the nitrogenous analogues of amantadine **2–4** in mice

TABLE 3

Pain sensitivity threshold in the hot plate test

Group	Dose, mg/kg	Pain sensitivity threshold, s*
Reference	–	7.0±1.0
1	20	6.0±1.0
	50	7.0±1.0
2	20	7.0±3.0
	50	6.5±2.0
3	20	6.5±2.0
	50	6.0±3.0
4	20	7.0±1.0
	50	8.0±1.0
Indomethacin	20	8.5±2.0

Note. The data are presented in the form: median ± quartile range.
*$p > 0.05$ – differences from the reference are insignificant.

TABLE 4

Visceral pain parameters in the acetic writhing test

Group	Dose, mg/kg	Number of writhes per 5 min
Reference	–	7.0±4.0
1	20	$10.0\pm8.0^{\#}$
	50	$10.5\pm8.0^{\#\#}$
2	20	$13.5\pm7.0^{*\#\#}$
	50	$13.0\pm7.0^{\#}$
3	20	$11.5\pm6.0^{*\#\#}$
	50	$11.0\pm9.0^{\#}$
4	20	$13.0\pm4.0^{**\#\#}$
	50	$11.0\pm4.0^{*\#\#}$
Indomethacin	20	$1.5\pm3.0^{*}$

Note. The data are presented in the form: median ± quartile range.

$^{*}p < 0.05$, $^{**}p < 0.01$ – differences from the reference re significant.

$^{\#}p < 0.05$, $^{\#\#}p < 0.01$ – differences from indomethacin are significant.

caused enhancement of the acute visceral pain reaction, which was dose-dependent (Table 4). In the dose of 20 mg/kg, all azaadamantanes exhibited statistically significant (with respect to the reference) increase in the number of writhes (by a factor of 1.5–1.9), while in the dose of 50 mg/kg the effect was either statistically insignificant (for agents **2** and **3**) or decreased with the conservation of reliable difference from the control (for compound **4**) (see Table 4). An increase in the pain reaction was unreliable in both doses for amantadine **1**. The reference preparation, indomethacin, caused a substantial decrease in the pain reaction under the same conditions: by a factor of 4.5 with respect to the control (see Table 4).

The chemical irritation of peritoneum causes local inflammatory response, which is accompanied by the activation of second-type cyclooxygenase and arachidonic acid cascade. In the focus of inflammation, the level of endogenous algogens (prostaglandins, histamine, serotonin, bradykinin) and vaniloids increases, and acidosis develops. The activation of acid-sensitive nocigenic channels of the transient receptor potential (TRPV1) and their ligands occurs under these conditions. One of them is leukotriene B4 (LTB4), causing chemotaxis, aggregation and degranulation of neutrophils, as well as an increase in vascular permeability [17]. The activation of TRPV1-channels causes an increase in the activity of nociceptors of the peripheral sensitive conductors, which enhances the conduction of excitement in afferent pathways. It has been reported that the activation of TRPV1 may enhance glutamatergic signalling, which was observed in some brain regions, including striatum, paraventricular nucleus, descending antinociceptive brainstem pathways, dorsolateral region of the periaqueductal grey matter and spinal marrow [18]. Relying on the above-described considerations, one may suppose that hyperalgesia caused in the visceral pain test by the introduction of the nitrogenous analogues of amantadine is due to their possible crosstalk with TRPV1-channels, as well as to insufficiently high anti-inflammatory activity of azaadamantanes. In this respect, attention should be paid to the pronounced analgesic effect of indomethacin (see Table 4), achieved due to the high anti-inflammatory activity of the preparation.

It may be concluded on the basis of the results obtained in the work that moderate anti-inflammatory activity of anti-exudative type has been revealed for the nitrogenous analogues of amantadine within the dose range of 20–50 mg/kg. Amantadine also exhibits this kind of activity, which is confirmed by the literature data on its ability to decrease the production of pro-inflammatory cytokines in the models of autoimmune encephalomyelitis [19, 20]. The introduction of nitrogen heteroatoms into the adamantane core does not reduce the anti-inflammatory activity of compounds **2** and **3**, and enhances that of triazaadamantane **4** in comparison with amantadine. Anti-inflammatory properties revealed in the studied azaadamantanes may be significant under the conditions of the pathologies of the central nervous system caused by a primary or secondary inflammatory process.

Modification of the adamantane framework by nitrogen atoms causes changes in the analgesic properties of the molecule, which was exhibited as hyperalgesia in the acetic writhing test. This phenomenon, possibly related to the effect of aza analogues of amantadine on the nocigenic channels of the transient receptor potential (TRPV1), makes these compounds interesting as possible modulators of neurotransmission in the correction of pathological processes in the tissues of the nervous system. This issue requires further investigation of specific pharmacological models.

CONCLUSION

1. Di- and triaza-derivatives of adamantane within the dose range of 20–50 mg/kg exhibit moderate anti-inflammatory activity in the model of histamine inflammation. Diazaadamantanes are not

less active than amantadine, while triazaadamantane even exceeds it in the anti-exudative action.

2. Di- and triaza-derivatives of adamantane, similarly to amantadine, do not have anti-inflammatory effect in the model of allergenic inflammation caused by concanavalin A and do not affect the threshold of thermal pain in the hot plate test.

3. The nitrogenous derivatives of adamantane, unlike amantadine, enhance pain perception in the acetic writhing model of visceral pain, presumably due to the interaction with TRPV1-channels. The effect decreases with an increase in the dose from 20 to 50 mg/kg.

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