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EFFECT OF DIMETHYLSULFOXIDE ON THE NOCICEPTIVE RESPONSE INDUCED BY TRPV1 ION-CHANNEL STIMULATION IN MICE

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The effect of preventive administration of a single intraperitoneal dose of dimethylsulfoxide (DMSO) at various concentrations (10, 30 and 50%; 10 mL/kg) on the nociceptive response in mice induced by local and systemic activation of TRPV1 ion channels by the TRPV1 agonist capsaicin was studied. DMSO (50%) significantly reduced (by 73.1%) the latency of the response to injection of capsaicin solution into the metatarsus. DMSO in the tail-flick test did not significantly affect the thermal pain threshold, which was elevated after a single subcutaneous injection (above the shoulder blades) of capsaicin at a dose of 1 mg/kg. However, DMSO (30 and 50%) *per se* raised the pain threshold of mice in the tail-flick test. Because externally applied DMSO enhanced the effect of capsaicin in the capsaicin test, the demonstrated analgesic effect of a single intraperitoneal injection of DMSO in the capsaicin test and tail-flick test in mice was due to its central action rather than to its effect on afferent innervation.

Keywords: dimethylsulfoxide (DMSO), TRPV1 ion channels, NMDA receptors, capsaicin, nociceptive response, mice.

Dimethylsulfoxide (DMSO) is a widely used solvent and cryoprotectant that affects the central and peripheral nervous systems, owing to which it is studied as a pharmacological agent for nervous system pathologies. For example, 10% DMSO after a single injection (microinjection of 0.5 μL) into the dorsal periaqueductal grey matter of Wistar rats increased the number of entries (as compared to normal saline) in the elevated plus maze test [1]. Chronic administration of DMSO in small doses (0.01 – 1% DMSO was used instead of drinking water in *ad libitum* mode) led to increased locomotor activity and anxiety- and compulsive-like behavior of C57BL/6 mice [2]. Long-term administration of DMSO (1%) to APP_{SDL} transgenic mice, which form β-amyloid plaque by 18 months of life, was a model of the prolonged prodromal phase of Alzheimer's disease [3], improved spatial memory, normalized olfactory sensitivity, and reduced anxiety-like behavior. Also, DMSO did not affect β-amyloid oligomerization although it increased the dendritic spine density in the hippocampus of these mice (*ex vivo* and *in vivo*). This was associated with its influence on NMDA-subtype glutamate receptors [4]. DMSO at concentrations of 0.5 – 1.5% rapidly and reversibly suppressed the electrophysiological response and calcium influx caused by glutamate, *N*-methyl-*D*-aspartate (NMDA), and α-amino-3-hydroxy-5-methylisoxazole-4 propionate in hippocampal neurons [5].

DMSO applied topically exhibited analgesic activity caused by blockage of neural C-fiber conduction [6]. However, it intensified the nociceptive response caused by activation of primary afferent neuron TRPV1 ion channels in mice [7]. Enhancement of the activity of the TRPV1 ion-channel agonist capsaicin by DMSO, which was used as an absorption activator in a dosage form for external use of *N*-(2-adamantyl)-hexamethyleneimine hydrochloride (hemantane), an NMDA-receptor antagonist, led to a reduced analgesic effect of hemantane in the capsaicin test in mice [8]. Peripheral NMDA-receptors could interact with TRPV1 ion channels in calcium/calmodulin-dependent protein kinase II and protein kinase C cascades. The TRPV1 ion-channel antagonist AMG9810 reduced NMDA-induced mechanical hyperalgesia in rats [9]. The TRPV1 ion-channel agonist capsaicin after a single subcutaneous injection at a dose of 1 mg/kg raised the sensitivity threshold to thermal pain stimulation in mice [10], which was explained by its

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ability to cause long-term desensitization of nociceptors [11]. Preventive administration of either noncompetitive NMDA-receptor antagonists (high-affinity MK-801 and low-affinity hemantane) or the selective TRPV1 antagonist BCTC inhibited the capsaicin-induced increase in the pain threshold [10].

The aim of the present work was to assess the effect of DMSO after intraperitoneal administration on the change of pain sensitivity of mice after topical and systemic activation of TRPV1 ion channels by capsaicin.

EXPERIMENTAL BIOLOGICAL PART

Animals. The work used mature male ICR mice (25 – 28 g) from the Stolbovaya laboratory animal nursery, a branch of the FMBA Scientific Center of Biomedical Technologies (Moscow Region). The work was organized and conducted in compliance with GOST 33216(2014 "Guidelines for accommodation and care of animals. Species-specific provisions for laboratory rodents and rabbits"; GOST 33215(2014 "Guidelines for accommodation and care of animals. Environment, housing and management"; Directive 2010/63/EU of the European Parliament and European Union Council of Sept. 22, 2010, on the protection of animals used for scientific purposes; and rules approved by the Biomedical Ethics Commission, Research Zakusov Institute of Pharmacology.

Studied materials, doses, and administration pathways. The work used Dimexide, concentrate for preparing solution for external use, 99% (manufacturer AO Tatkhimfarmpreparaty, Russia). The solvent was normal saline (Sodium chloride, solution for infusions, 0.9%; manufacturer: OAO NPK ESKOM, Russia). DMSO at concentrations of 10, 30, and 50% was injected intraperitoneally (10 mL/kg) 60 min before administration of capsaicin solution.

The TRPV1 ion-channel agonist capsaicin (Sigma Aldrich, USA) was diluted in normal saline and EtOH (9:1, v/v) and administered in the capsaicin test locally into the metatarsus in the amount of 1.6 μg/mouse [12] in a volume of 20 μL; in the tail-flick test, systemically subcutaneously above the shoulder blades at a dose of 1 mg/kg (in a volume of 10 mL/kg), at which it raised the pain sensitivity threshold of the mice [10].

Capsaicin test. Mice were injected with capsaicin solution in the metatarsus of the left hind paw using a Hamilton syringe [12] and then placed into individual transparent Plexiglas cages on a stand under which a mirror was arranged to observe the hind extremities through the cage floor. The time (s) indicative of a pain response of the mice, i.e., licking paws, shaking, rearing, was recorded for 5 min using RealTimer software (procedural timer, NPK Otkrytaya Nauka, Russia) starting immediately after injecting the capsaicin solution.

Thermal tail-flick test. The tail-flick test was based on a spinal flexor reflex in response to progressively increasing thermal effects of radiation on the skin [13]. The pain stimulus was applied locally to the tail using thermal radiation from a TSE-system analgesia meter (Germany). The stimulus intensity corresponded to a gradual temperature increase from 51 to 61°C in 15 sec. A latency period (LP) of 15 sec was considered the maximum allowed time for applying the stimulus. The maximum possible effect (MPE) was calculated using the formula:

$$\mathrm{MPE}(\%) = \frac{(\mathrm{LP_t} - \mathrm{LP_c})}{(\mathrm{MAX_{time}} - \mathrm{Lp_c})} \times 100,$$

where LP_t is the latency period of the response of the mice 30 min after administration of capsaicin solution or 60 min after administration of DMSO; LP_c, latency period of the control group of mice that received solvent; MAX_{time}, the maximum allowed time for applying the stimulus (15 sec).

The effect of DMSO on the change of pain response threshold of mice in the tail-flick test was studied in two stages. In the first stage, the effect of DMSO on the rise in the pain response threshold after capsaicin administration was assessed 30 min after administering the TRPV1 ion-channel agonist. In the second stage, the effect of DMSO on the sensitivity of mice to the thermal stimulus applied to the tail was assessed 60 min after intraperitoneal injection. The control groups used mice injected with an equivalent volume (10 mL/kg) of solvent, i.e., the solvent for capsaicin in the

TABLE 1. Effect of DMSO After a Single Intraperitoneal Injection on Response Time of ICR Mice to Intraplantar Injection of Capsaicin Solution (Capsaicin Test), Median (Q1; Q3)

Group	Number of mice in group	Response time of mice, s	Reduction of response time of mice vs. control, %
Normal saline + capsaicin	8	91.0 (86.0; 119.5)	-
10% DMSO + capsaicin	10	105.0 (91.0; 119.0)	–15.4
30% DMSO + capsaicin	10	57.0 (36.0; 94.0)$^{\#}$	37.4
50% DMSO + capsaicin	14	24.5 (7.0; 45.0)*$^{\#}$	73.1

* $p < 0.05$ vs. "normal saline + capsaicin" group; Mann(Whitney criterion; $^{\#}$ $p < 0.05$ vs. "10% DMSO + capsaicin" group, Mann(Whitney criterion.

first experiment was a mixture of normal saline and EtOH (9:1, v/v) or DMSO diluted with normal saline; in the second experiment, normal saline.

Experimental results were statistically processed using the Statistica 10.0 program. Data were checked for normal distributions using the Shapiro(Wilk criterion followed by an estimate of the intergroup equivalence of the dispersions using the Levene criterion. Because data in the groups did not have normal distributions, the Kruskal(Wallis criterion was used to find statistically significant differences between groups that were then compared pairwise using the Mann(Whitney criterion. Differences between groups were considered statistically significant for $p \le 0.05$. Tables 1 – 3 present the results as medians (first and third quartiles, Q1 and Q3).

RESULTS AND DISCUSSION

Capsaicin stimulated TRPV1 ion channels, causing nociceptive pain after injection into the metatarsus of mice that was manifested as characteristic behavior aimed at decreasing the pain response and eliminating the algogen. They licked, shook, and raised their paws. Previously, DMSO was shown by us to dose-dependently enhance the effect of capsaicin after topical application [7]. However, DMSO at concentrations of 10 – 50% after a single intraperitoneal injection did not intensify and even, conversely, dose-dependently reduced the nociceptive response in mice caused by activation of TRPV1 ion channels. For example, while DMSO (10%) did not affect the response time of animals to the capsaicin injection, a tendency ($p = 0.08$ as compared to the control group, Mann(Whitney criterion) toward its reduction was observed in mice injected with DMSO (30%). The response times of the mice decreased significantly by 73.1% for DMSO (50%) (Table 1).

The sensitivity threshold of mice to a thermal pain stimulus rose sharply in the tail-flick test producing a spinal flexor reflex after a single subcutaneous injection above the shoulder blades of capsaicin at a dose of 1 mg/kg. The LP of the tail flick after capsaicin injection increased by 2.4 times as compared to the group of mice that received solvent [normal saline + normal saline and EtOH (9:1, v/v)]. The sensitivity in the tail-flick test was not significantly affected as compared to the group of animals that received the TRPV1 ion-channel agonist and normal saline after preventive intraperitoneal injection to mice of DMSO at concentrations of 10 – 50% 30 min before capsaicin injection. For example, the tail-flick LP of mice injected with DMSO and capsaicin rose by 2.3 – 3.0 times as compared to the group of mice that received solvent. Moreover, the maximum rise of the pain sensitivity threshold to the thermal pain stimulus was recorded in the group of animals that were injected with 50% DMSO before capsaicin injection (Table 2).

DMSO *per se* at concentrations of 30 and 50% could raise the sensitivity threshold to the thermal pain stimulus after a single intraperitoneal injection to mice 60 min before testing. DMSO at a concentration of 30% significantly increased the tail-flick LP of the mice by 28.3%; at a concentration of 50%, by 30.4% as compared to the control group of animals that were injected with normal saline. DMSO (10%) did not have a significant effect on the sensitivity threshold of the mice to the thermal pain stimulus in the tail-flick test (Table 3).

Thus, DMSO exhibited an analgesic effect in mice in the capsaicin test after intraperitoneal injection but not after topical application (for which the reverse effect was recorded). The pain sensitivity threshold in the tail-flick test producing a spinal flexor reflex rose with increasing DMSO concentration after intraperitoneal injection. Therefore, the mechanism of analgesic action of DMSO involved its central action and not effects on afferent innervation. These results agreed with those showing that DMSO after peroral administration or injection into the cerebral ventricle of mice raised the pain sensitivity threshold to thermal and chemical stimuli [14]. However, the pain intensified in mice after local subcutaneous injection into the dorsal surface of the hind paw 10 min but not 30 min before formalin injection. Formalin affected TRPA1 ion channels [15] that were co-expressed with TRPV1 ion channels that affected them [16, 17]. It was noteworthy that addition of DMSO as an absorption activator to a hemantane gel led to disappearance of the analgesic effect of the gel after application 10 min before but not 60 min before injection of capsaicin solution in the capsaicin test in mice [8]. How-

TABLE 2. Effect of DMSO After a Single Intraperitoneal Injection on Rise of Threshold Nociceptive Sensitivity After Capsaicin Injection in Thermal Tail-Flick Test in ICR Mice, Median (Q1; Q3)

Group	Number of mice in group	Latency period (LP) of tail flick, s	MPE, %
Normal saline + normal saline/EtOH	8	4.8 (4.6; 5.1)	–0.5 (–2.0; 2.9)
Normal saline + capsaicin 1 mg/kg	8	11.7 (8.1; 14.2)*	67.2 (31.96; 91.7)*
10% DMSO + capsaicin 1 mg/kg	10	12.6 (9.4; 15.0)*	76.0 (45.1; 100.0)*
30% DMSO + capsaicin 1 mg/kg	9	11.0 (7.5; 15.0)*	60.8 (26.5; 100.0)*
50% DMSO + capsaicin 1 mg/kg	10	14.5 (10.8; 15.0)*	94.6 (58.8; 100.0)*

*$p < 0.001$ vs. "normal saline + normal saline/EtOH group, Mann(Whitney criterion.

TABLE 3. Effect of DMSO After a Single Intraperitoneal Injection on Threshold of Nociceptive Sensitivity in Thermal Tail-Flick Test in ICR Mice, Median (Q1; Q3)

Group	Number of mice in group	LP of tail flick, s	MPE, %
Normal saline	9	4.6 (4.2; 4.9)	0.0 (– 3.9; 2.9)
10% DMSO	7	4.8 (4.6; 5.5)	1.9 (0.0; 8.7)
30% DMSO	9	5.9 (4.8; 6.3)*	12.5 (1.9; 16.4)*
50% DMSO	9	6.0 (5.6; 8.3)**,#	13.5 (9.6; 35.6)**,#

*$p < 0.05$ vs. "normal saline" group, Mann(Whitney criterion; **$p < 0.0005$ vs. "normal saline" group, Mann(Whitney group; #$p < 0.01$ vs. "10% DMSO" group, Mann(Whitney criterion.

ever, topical application of hemantane solution 1 min before injection of capsaicin solution decreased the response time of the animals to the injection [18].

The effects of NMDA-receptor antagonists were not repeated in mice that were injected systemically with capsaicin after preventive intraperitoneal injection of DMSO. DMSO, like NMDA-receptor antagonists [10], did not reduce analgesia caused by desensitization of nociceptors by capsaicin in the tail-flick test. In contrast to DMSO, NMDA-receptor antagonists attenuated afferent innervation. The high-affinity NMDA-receptor antagonist MK-801 and the low-affinity NMDA-receptor antagonist hemantane after topical application reduced the duration of the response of mice to an injection of the TRPV1 ion-channel agonist capsaicin into the metatarsus [18]. Thus, DMSO after a single intraperitoneal injection and topical application did not affect the change of pain sensitivity of mice, like the effects of NMDA-receptor antagonists, although it suppressed the electrophysiological response and calcium influx in neurons of primary hippocampus cell culture [5]. DMSO reduced calcium levels increased by various experimental conditions not only in neurons but also in other cells, which suggested it had nonspecific action [19] that was most probably also due to the effect of DMSO on hippocampal neurons that was previously reported [5]. The reduction in the intracellular Ca^{2+} level could also explain the increase in the response time of mice to capsaicin injection after DMSO application because the influx of Ca^{2+} into the cell was considered necessary for desensitization of the nonselective TRPV1 ion channel [20, 21]. The closed state of the TRPV1 ion channel could stabilize phosphatidylinositol lipids [22]. They were dissolved in DMSO, which freely penetrated through the membrane and could affect the TRPV1 ion channel because of this.

The increase in the pain sensitivity threshold in the tail-flick test in mice that was observed in the present study after a single intraperitoneal injection of DMSO (30% and greater) was most probably due to its toxic effect on the CNS. The facts that DMSO after a single peroral administration (10 mg/kg) and injection into the cerebral ventricle (5 μL/mouse) reduced the locomotor activity of mice [14], after a single intraperitoneal injection to C57BL/6 mouse pups at P7 caused dose-dependent (0 – 10 mL/kg) apoptosis of neurons, and after administration at a dose of 10 mL/kg led to lethal outcomes [23] argued in favor of this. The pharmacological and toxic effects of DMSO, which can manifest even after a single injection of it, must be considered for planning animal experiments in which it is used as a solvent over a broad concentration range up to 30% [24].

Thus, DMSO (10 – 50%) after a single intraperitoneal injection was found to increase dose-dependently the pain sensitivity threshold of mice in the capsaicin test and thermal tail-flick test although it did not significantly affect the increased pain sensitivity threshold after subcutaneous administration of capsaicin in the tail-flick test. The analgesic effect of DMSO that was recorded in the capsaicin test and thermal tail-flick test was due to its central action and not an effect on afferent innervation because DMSO after topical application enhanced the effect of capsaicin in the capsaicin test in mice. Also, DMSO after a preventive single intraperitoneal injection did not cause an effect typical of NMDA-receptor antagonists in mice that received systemic capsaicin although DMSO was reported to suppress electrophysiological responses and intracellular influx of Ca^{2+} in hippocampal neurons [5].

Conflict of interest

We declare no conflict of interest.

Contributions of authors

EAI formulated the problem, performed the experimental work, analyzed and interpreted the results, and wrote the text; SKM performed the experimental work and analyzed and interpreted the results; TAV formulated the problem and edited and finalized the manuscript.

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Approval of the ethics committee for animal experiments

The experimental protocol was approved by the Commission on Biomedical Ethics of Research Zakusov Institute of Pharmacology.

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