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EFFECT OF DIMETHYL SULFOXIDE ON THE NOCICEPTIVE RESPONSE INDUCED BY THE TRPV1-AGONIST CAPSAICIN IN MICE

E. A. Ivanova,[1,*] S. K. Mamonova,[2] and T. A. Voronina[1]

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The effect of cutaneous applications of dimethyl sulfoxide (DMSO) on the nociceptive response induced by the transient receptor potential vanilloid 1 (TRPV1) agonist capsaicin in mice was studied at various concentrations (5, 10, 20, 50, and 100%) and application times (1, 10, 30, and 60 min before injection of the algogen). DMSO applied 1 min before administration of capsaicin solution did not significantly change the pain sensitivity at all studied concentrations. DMSO 10 min after application at concentrations of 50 and 100% significantly increased the response time of mice to an injection of capsaicin solution. Application of DMSO at concentrations from 10 to 100% 30 min before administration of the algogen significantly enhanced the reaction of mice in a dose-dependent manner. The effect of the algogen was significantly enhanced only at 100% DMSO when it was applied 60 min before injection of capsaicin solution.

Keywords: dimethyl sulfoxide (DMSO), transient receptor potential cation channel subfamily V member 1 (TRPV1), capsaicin, pain reaction, mice.

Dimethyl sulfoxide (DMSO) is widely used as a solvent and cryoprotectant. It is one of the first and most well-studied compounds used to enhance absorption during development of drugs for external use [1, 2]. Diclofenac sodium solution for topical application (Pennsaid®, Mallinckrodt Inc., Hazelwood, MO) and idoxuridine solution for topical application (Herpid®, Astellas Pharma, Staines, UK) are FDA-approved medicines in which it is used as an absorption enhancer [2]. Also, DMSO exhibits anti-inflammatory and analgesic activity and is used as a medicine for musculoskeletal diseases and in dermatology [3] and urology [4]. The mechanism of pharmacological action of DMSO remains unclear, despite greater than 40 years of use as a medicine (the first commercial DMSO medicine was approved in 1978 [2]). DMSO possesses antioxidant properties, acting as a scavenger of reactive oxygen species [5, 6], and attenuates activation of NLRP3 inflammasomes [7]. It dose-dependently reduces the production of interleukin (IL)-8 in lipopolysaccharide-stimulated human whole blood [8] and the expression of IL-1α, pro-IL-1β, and IL-6 in lipopolysaccharide-stimulated brain macrophages [7] and increases secretion of IL-1β and IL-6 in lipopolysaccharide-stimulated human whole blood [8] and mononuclear peripheral blood cells [9]. Blockage of peripheral nerve C fiber conduction is a possible mechanism of DMSO analgesia [10].

DMSO is included as an absorption enhancer in the gel dosage form for cutaneous application of the analgesic and anti-inflammatory drug *N*-(2-adamantyl)hexamethyleneimine hydrochloride (hemantane) that was developed at Zakusov Institute of Pharmacology [11]. The analgesic and anti-inflammatory activity of hemantane was enhanced by addition of DMSO to its gel in experiments on rats with topical application of the dosage form 60 min before subplantar injection of formalin solution into the paw of the animals, which caused a two-phase pain reaction and tissue edema [12]. The hemantane gel with DMSO as an absorption activator reduced the duration of a capsaicin-induced pain reaction in mice with application 60 min but not 10 min before injection of the algogen. However, an analgesic effect of the drug with application both 10 and 60 min before induction of the pain response by capsaicin appeared if DMSO was excluded from the hemantane gel formulation [13]. Capsaicin

[1] Zakusov Institute of Pharmacology, 8 Baltiiskaya St., Moscow, 125315 Russia.
[2] Sechenov University, 8/2 Trubetskaya St., Moscow, 119991 Russia.
[*] e-mail: iwanowaea@yandex.ru

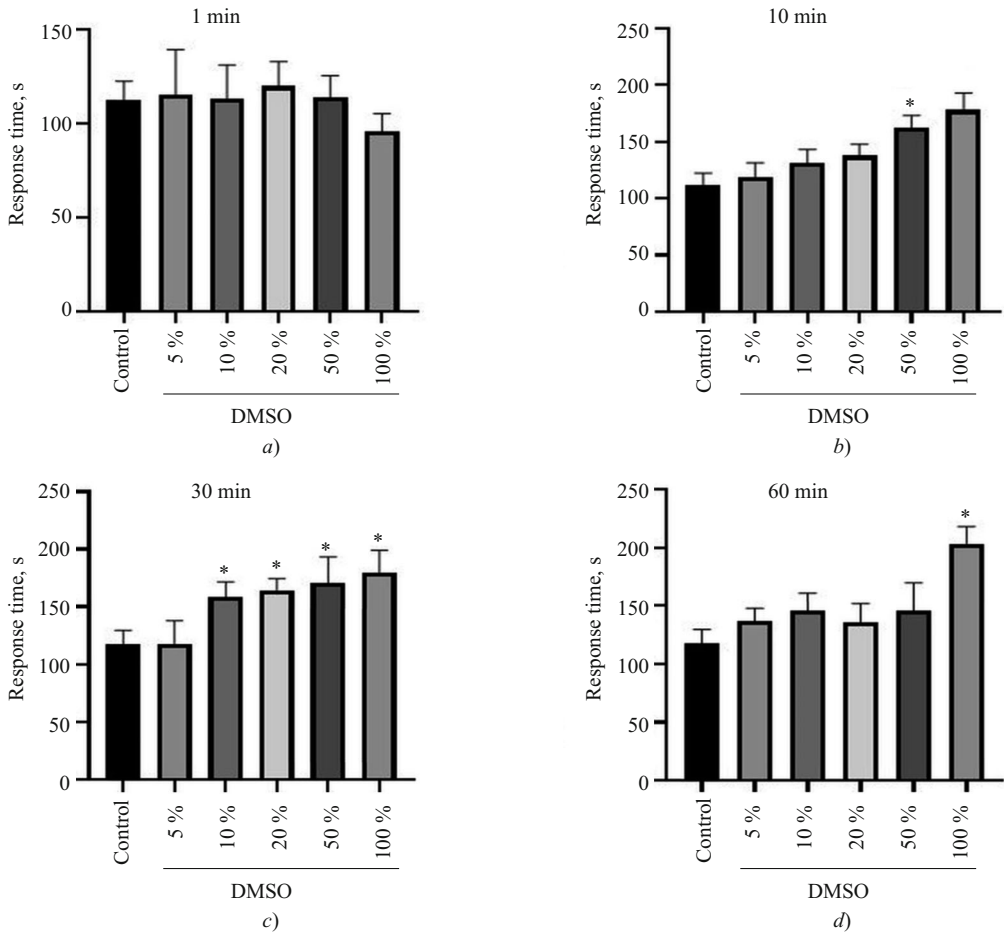

Fig. 1. Effect of cutaneous applications of dimethyl sulfoxide at concentrations 5, 10, 20, 50, and 100% on response time of mice to intraplantar injection of capsaicin solution with applications 1 (*a*), 10 (*b*), 30 (*c*), and 60 min (*d*) before injection of capsaicin solution. Data are given as means ± errors of the means; * $p \le 0.05$ vs. the control group, Student *t*-criterion.

is an agonist of transient receptor potential cation channel subfamily V member 1 (TRPV1), which is expressed by two subpopulations of primary afferent neurons with small-diameter unmyelinated projections that express anti-inflammatory peptides or have an affinity for IB4 lectin and are activated by vanilloid compounds, protons, or temperatures >43°C [14, 15]. They can be affected by the analgesic effect of DMSO after topical application. Therefore, the experimental data obtained by us [13] provided a basis for further studies of the action of DMSO on TRPV1 ion channels to expand our understanding of its mechanism of action.

The present work was aimed at evaluating the effect of DMSO on the pain response caused by the TRPV1-agonist capsaicin in mice as a function of concentration and time of cutaneous applications.

EXPERIMENTAL BIOLOGICAL PART

Animals. The work used mature male ICR mice (26 – 28 g) obtained from the nursery of laboratory animals, Stolbovaya Branch, Scientific Center of Biomedical Technologies FMBA (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, Zakusov Institute of Pharmacology.

Studied drugs, doses, and administration pathways. The work used Dimexide, concentrate for preparation of solution for external use, 99% (Tatkhimfarmpreparaty Inc., Russia). The solvent was normal saline (Sodium chloride, solution for infusion, 0.9%; NPK ESKOM, Russia). DMSO at concentrations of 5, 10, 20, 50, and 100% in a volume of 20 μL/mouse was applied externally as an application in a nonwoven patch (1 × 1 cm) to the metatarsal area for 1 min at time intervals corresponding to 1, 10, 30, and 60 min before injection of capsaicin solution.

Capsaicin test. Capsaicin (1.6 μg, Sigma Aldrich, USA) [16] diluted in a mixture (20 μL) of normal saline and EtOH (9:1, v/v) was injected into mice in the hind metatarsal region using a Hamilton syringe. The animals were placed into individual transparent Plexiglas cages on a stand under which a mirror was situated to observe the hind extremities through the cage floor. The time (s) of mouse behavior indicating pain, i.e., licking the paw, shaking, rearing, was recorded starting immediately after injecting the capsaicin solution using RealTimer software (timer procedure, Otkrytaya Nauka SPC, Russia) for 5 min.

Statistical processing of experimental results used the Statistica 10.0 program. The normalcy of distributions was checked by the Shapiro–Wilk criterion followed by an assessment of the intergroup equivalence of the dispersions using the Levene criterion. The significance of differences in groups of animals that were treated with DMSO patches as compared to the control group was assessed using the Student *t*-criterion because the distribution of data in groups was normal and the intergroup dispersions were equivalent. The results in Fig. 1 are given as the mean ± the error of the mean. Differences between groups were considered statistically significant for $p \le 0.05$.

RESULTS AND DISCUSSION

The average duration of the mouse response to injection of capsaicin solution in control groups, i.e., animals that did not have a DMSO patch, was 112.1 – 117.9 sec (Fig. 1). Use of DMSO patches at concentrations of 5 – 100% for 1 min before injection of capsaicin solution did not significantly change the duration of the response of the animals to its injection (Fig. 1*a*). However, application of DMSO at concentrations of 50 and 100% 10 min before injection of the algogen significantly increased the duration of the response by 44.7 and 59.0%, respectively. Application of DMSO at concentrations of 10 and 20% 10 min before injection of capsaicin solution produced a slight increase of the response to injection of the algogen (by 16.9 and 23%, respectively). However, it was statistically insignificant ($p > 0.05$) as compared to the control group (Fig. 1*b*). Use of DMSO patches at concentrations from 10 to 100% for 30 min before injection of capsaicin solution led to a significant dose-dependent increase of the pain response in the animals. For example, application of DMSO at a concentration of 10% increased the response time of the animals to injection of capsaicin solution by 34.8%; 20%, by 39.3%; 50%, by 45.1%; and 100%, by 52.8%. The duration of the response of mice treated with a DMSO patch at a concentration of 5% 30 min before injection of capsaicin solution was practically the same as the duration of the reaction in control animals (Fig. 1*c*). Use of DMSO patches 60 min before injection of capsaicin solution increased statistically significantly the response time as compared to the control group only for application of DMSO at a concentration of 100% (Fig. 1*d*).

Asynchronous excitation of axons for 1 – 5 min is known to occur immediately after applying DMSO at a concentration of 5%. This leads to a brief decrease in the response amplitude of C-fibers. C-fibers are blocked by applying DMSO at a concentration of 10% and greater for several minutes [10]. Our experiments results indicated that the capsaicin-induced pain response recorded in the mice 10 min after DMSO application was affected at concentrations of 50 and 100% but not at concentrations of 20% and less. Use of a DMSO patch even at the maximum concentration 1 min before injection of capsaicin solution did not lead to a significant change in the mouse sensitivity. A significant effect of DMSO application at concentrations from 10 to 100% (but not at 5%) on the pain response induced by the TRPV1 agonist capsaicin was recorded in the mice 30 min after their application although the response of the mice after injection of the TRPV1 agonist capsaicin manifested already in the first seconds after its injection. Therefore, the mediated action of DMSO on TRPV1 ion channels was responsible for the effect of DMSO on the pain response caused by activation of TRPV-1 ion channels that was recorded in this and previous research on the use of DMSO as an activator of hemantane absorption from the gel [13].

TRPV1 is a nonselective ion channel with high permeability for Ca^{2+} [17]. Influx of Ca^{2+} into the cell is considered necessary for desensitization of the ion channel over time [18, 19], particularly because its opening is inhibited [20]. DMSO can lower an intracellular Ca^{2+} level that is elevated by various experimental factors [21]. Therefore, it can be proposed that a reduction of the intracellular Ca^{2+} concentration caused the increased duration of the mouse response to injection of the TRPV1 agonist capsaicin with application of DMSO. Also, the polar aprotic solvent DMSO could affect phosphatidylinositol lipids that presumably could stabilize the open state of the ion channel and were occupying the capsaicin binding site in the closed state of the ion channel [22].

Thus, the experimental studies found that DMSO with external use could dose-dependently enhance the pain response in mice caused by activation of TRPV1 ion channels. This effect manifested a certain time after cutaneous application of DMSO (after 10 min and more in the present research, depending on the DMSO concentration). Use of DMSO at concentrations up to 50% gave short-lived responses. The ability of DMSO to enhance activation of TRPV1 ion channels was important to consider in choosing it as the solvent for performing studies assessing the effects of compounds on physiological processes involving these ion channels and of compounds acting or potentially acting on TRPV1 ion channels to avoid incorrect results.

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