MORPHOLOGY AND PATHOMORPHOLOGY

Effect of the Blockade of Capsaicin-Sensitive Nerves on the Development of the Exudative Reaction in Immune Response and Aseptic Inflammation

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The neurotoxin capsaicin, which depletes some sensory neurons of neuropeptides, is used to explore the role of these neurons in the development of the exudative response induced by zymosan and diphtheria-tetanus-pertussis modified vaccine (DTP-M) in mice. Four hours after the induction of inflammation in capsaicin-treated mice the reaction to DTP is reduced by 52% and delayed-type hypersensitivity to DTP by 26%. No effect of capsaicin is noted on a zymosan-induced exudative reaction.

Key Words: *delayed-type hypersensitivity; substance P; zymosan; capsaicin; diphtheria-tetanus-pertussis vaccine*

Recently, a large body of evidence has been accumulated on a local "effector" function of peripheral nerve endings of sensory neurons. They contain and release upon stimulation some neuropeptides, in particular substance P, which participate in the regulation of vasodilation, vascular permeability, and neurogenic inflammation and in the modulation of metabolic and immune processes [8]. Data have been gathered on the activating effect of substance P on immunocompetent cells, chemotaxis, synthesis of immunoglobulins, and release of histamine and other substances from mast cells [3,6]. It is known that capsaicin, a selective blocker of small afferent peptidergic neurons with C-type naked fibers, as well as surgical denervation prevents the development of neurogenic inflammation caused by various agents [7,9]. Reports on the role of capsaicin-sensitive neurons in immune reactions indicate both the inhibition of the antigen-induced increase in vascular permeability [5] and increased edema and cellular infiltration [4] in capsaicin-treated animals.

In the present study we investigated the effect of capsaicin blockade of peptidergic neurons on the development of the exudative reaction in zymosan-induced aseptic inflammation, the immediate-type immune response, and delayed-type hypersensitivity (DTH) induced by DTP-M vaccine.

MATERIALS AND METHODS

Male BALB/c mice weighing 25-30 g received an injection of inductors of inflammation in a volume of 50 μl to the pad of the right hind paw. Groups 1 and 2 were injected with zymosan (0.5 mg in physiological saline), and groups 3 and 4 with DTP-M vaccine (Biomed) diluted 3-fold with physiological saline. The mice of groups 5 and 6 were immunized with DTP-M vaccine (60 μl diluted 5-fold with physiological saline, intraperitoneally) and 8 days later they were injected with

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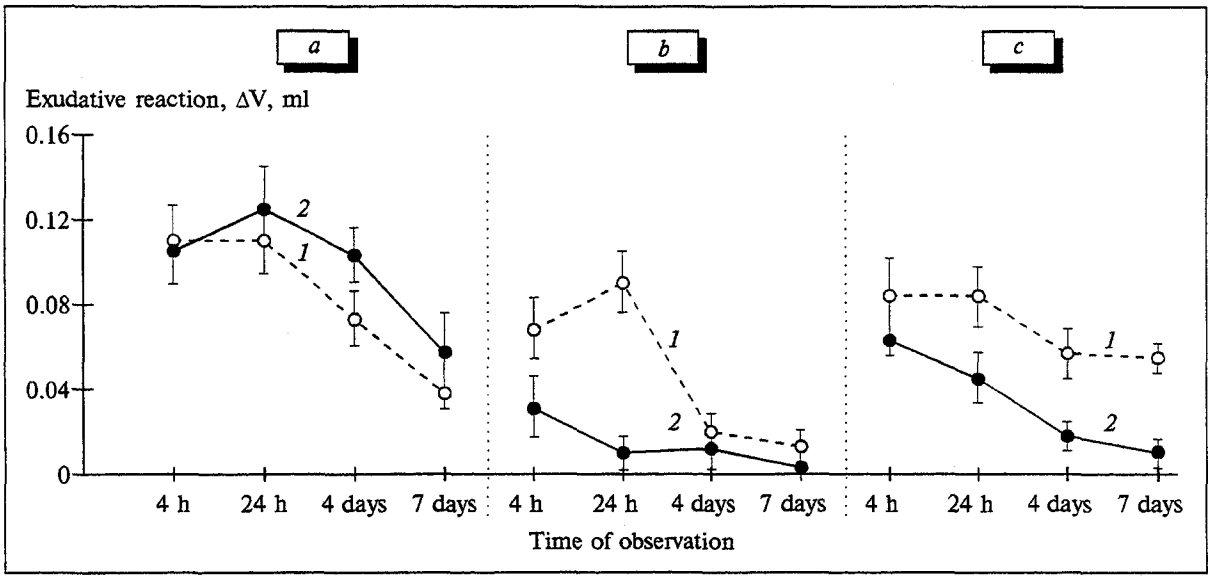

Fig. 1. Exudative reaction in intact (*1*) and capsaicin-treated (*2*) mice after injection of zymosan (*a*) and DTP-M to nonsensitized (*b*) and sensitized (*c*) animals.

vaccine in the paw similarly to groups 3 and 4. The mice of groups 2, 4, and 6 were twice injected with capsaicin under ether anesthesia 10-14 days before the induction of inflammation (first day - 25 mg/kg, second day - 35 mg/kg in a solution containing 10% ethanol, 10% Tween-80, and 80% physiological saline). The volume of the left and right (control and experimental) hind paws was determined after 4 and 24 hours and 4 and 7 days by measuring the volume of displaced liquid with a capillary graduated to 0.001 ml and attached to a measuring vessel. The difference between the control and experimental paws for each animal was taken as the volume of edema and expressed in ml. Statistical processing of the results was performed using the Student *t* test.

RESULTS

In intact mice an exudative reaction to injection of inductors of inflammation was found to develop intensively during the first few hours and to remain maximal during 24 hours. Twenty-four hours after the injection of zymosan or DTP-M to sensitized or nonsensitized animals the volumes of experimental paws were 176, 167, and 156%, respectively, in comparison with the control paws. The volume of edema in intact and capsaicin-treated animals is presented in Fig. 1.

Zymosan in the applied doses induced a more pronounced reaction than DTP-M. The inflammatory reaction to DTP-M in intact sensitized mice during the first 24 hours was similar to that in nonsensitized animals, but during the subsequent days it was reliably more pronounced.

Capsaicin produced swelling of control paws (not treated with inductors of inflammation). In intact animals of groups 1, 3, and 5 the mean volumes of the control paws were 0.140±0.007, 0.125±0.005, and 0.144±0.006 ml, respectively, while in capsaicin-treated animals they were 0.180±0.007, 0.181±0.009, and 0.152±0.008 ml (data not shown). Bleeding cracks in the skin were visually detected in interphalangeal folds. These changes may indicate capsaicin-induced disturbances in the trophism of the skin, which is in conformity with the data on the trophic role of capsaicin-sensitive nerves in the skin [10]. The dynamics of inflammatory edema in capsaicin-treated mice depended on the type of inductor of inflammation that was used. Zymosan-induced edema in capsaicin-treated animals practically did not differ from the reaction of intact mice during the first 24 hours and slightly surpassed that during the following observation times (Fig. 1). The exudative reaction to DTP-M injection in capsaicin-treated mice was weaker in comparison with that in intact animals. This decrease was most pronounced in nonsensitized mice: after 4 hours the degree of edema was only half that in intact animals and subsequently did not differ from the control level. The exudative reaction in DTH in capsaicin-treated animals after 4 hours was 26% lower than in intact animals and then progressively decreased, until after one week the paw volume normalized.

Thus our findings suggest that treatment of peptidergic afferent neurons with capsaicin weakens the exudative reaction in the immediate immune response and DTH and has practically no effect on the exudative reaction in zymosan-induced aseptic

inflammation. The different effects of capsaicin on inflammatory processes is apparently due to mechanisms of the inductors of inflammation used. Zymosan, acting via lectin, mannose-fucose, and C_3-receptors, stimulates resident macrophages, which triggers granulomatous inflammation with macrophage-leukocyte infiltration and edema due to an increase in endothelial permeability by cell-derived transmitters [1,2]. The DTP-M vaccine, containing dead pertussis microbes and diphtheria and tetanus anatoxins, induces a primary immune inflammatory reaction of the exudative-destructive type with a prevailing exudative component in nonsensitized mice and DTH in sensitized animals.

The results attesting to decreased edema in the immune response of capsaicin-treated mice are in conformity with previous data on inhibition of the antigen-dependent increase in vascular permeability in guinea pigs with capsaicin-induced substance P deficiency [5]. It is known that enhanced functional activity of peptidergic nerve endings, for instance, caused by electrical stimulation, is accompanied by increased vascular permeability and an increased content of mast cells in the tissue [3].

The findings suggest a modulating effect of afferent peptidergic neurons on the development of the exudative stage of inflammation in the immediate-type immune response and DTH.

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