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Research Report

Influence of the dopamine D2 receptor knockout on pain-related behavior in the mouse

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Abstract

We studied the role of the dopamine D2 receptor in physiological regulation of pain-related behavior. The experiments were performed in dopamine D2 receptor knockout mice and in their wild-type controls. Baseline sensitivity to thermal nociception was determined by measuring the response latency in the hot plate at three different stimulus temperatures and by determining the radiant-heat-induced paw withdrawal. Mechanical sensitivity was assessed by determining paw withdrawal responses to stimulation with a calibrated series of monofilaments. Intracolonic capsaicin was used to produce sustained pain-related behavior and referred hypersensitivity to mechanical stimulation. The hot plate response latencies were not significantly different between the dopamine D2 receptor knockout and wild-type animals, although the stimulus temperature-dependent decrease in the response latency was steeper in the wild-type group. The radiant-heat-induced paw withdrawal latency was slightly longer in the knockout animals. The number of capsaicin-induced behavioral responses or the latency to the occurrence of the first capsaicin-induced response was not different between the experimental groups. Dopamine D2 receptor knockout animals were more sensitive to mechanical stimulation of the hindpaws than wild-type animals both in the baseline condition and following development of capsaicin-induced referred hypersensitivity in the hindpaws. The results indicate that dopamine D2 receptors influence baseline nociception in the mouse, although this effect is weak and submodality selective. Additionally, dopamine D2 receptors may contribute to attenuation of referred hypersensitivity caused by sustained nociception.

Theme: Sensory systems
Topic: Pain modulation: pharmacology

Keywords: Capsaicin; Dopamine D2 receptor; Pain modulation; Referred hyperalgesia

1. Introduction

There is considerable amount of evidence indicating that dopamine influences pain sensitivity. Parkinson's disease, which is associated with hypofunction of the nigrostriatal dopaminergic system, commonly causes pain [7,10,22] and is accompanied by increased sensitivity to painful stimuli [4]. In line with this, hypoactivity of the nigrostriatal dopaminergic system is associated with chronic orofacial pain syndrome [17], and dopaminergic compounds, such as levodopa or bupropion, attenuate chronic pain [5,19,33]. Dopaminergic influence on pain is predominantly due to action on dopamine D2 receptors as indicated by the following findings. In healthy subjects, dopamine D2 receptor binding in the striatum is associated with indivi-

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dual's pain sensitivity and pain modulatory capacity [11,26,31]. In patients with chronic orofacial pain, striatal dopamine D2 receptor binding potential is lower than in their age-matched controls [12,13]. In animal studies, selective dopamine D2 receptor agonists have produced antinociceptive effects following administration at a supraspinal [25,34; however, 2] and a spinal site [2,6,9] or systemically [2,8,27,28,38]. Paradoxically, antinociceptive effects have been reported also following administration of dopamine D2 receptor antagonists, possibly due to interaction with opioidergic systems [32,37,38].

Pharmacological studies in experimental animals indicate that synthetic dopaminergic compounds modulate nociception due to action on dopamine D2 receptors. However, the previous studies do not reveal the possible physiological role of dopamine D2 receptors in regulation of nociception. In the present study, we attempted to find out whether dopamine D2 receptors have a significant role in physiological regulation of nociception by comparing pain-related behavior of dopamine D2 receptor knockout mice [1] and their wild-type controls in baseline conditions and during a capsaicin-induced sustained nociception.

2. Materials and methods

2.1. Experimental animals

Animals were bred under standard animal housing conditions in a 12 h light/dark cycle. Food and water were available ad libitum. All experiments were carried out according to the guidelines of the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

The experiments were performed using male dopamine D2 receptor knockout and wild-type control mice of 15–21 weeks of age. The generation of dopamine D2 receptor knockout mice has been reported previously [1]. The D2R−/− mice used in this study were obtained by backcrossing mice in a 50% C57/Bl6/50% 129SV background for six consecutive generations with pure C57/Bl6 mice resulting into 98.5% C57/Bl6–1.5% 129SV animals. Age-matched wild-type littermates in the same genetic background were used as control animals. Genotypes were performed by Southern analyses of DNA extracted by tail biopsies, as previously described [1]. Absence of D2 receptor expression was assessed at the mRNA and protein levels as described [1].

For all behavioral experiments, the animals were allowed to habituate for 5 days to the testing environment prior to the first testing day and for 15 min before the actual testing during each testing day. During the 5-day habituation period, the animals were exposed daily for 15 min to different testing devices (hot plate, plantar test, elevated mesh screen for von Frey hair testing). During the habituation period, noxious stimuli were not delivered to the animals. Mice were studied blinded to the genotype. All the animals were given identity codes by a laboratory technician who did not participate in the execution of the study. Identity codes revealing the different genotypes were given to the investigators after all the behavioral assessments were performed. The genotype of the mouse was not revealed by the appearance of the mouse.

2.2. Assessment of thermal nociception

Hot plate test was performed using a standard hot plate (Ugo Basile, Varese, Italy). Each animal was tested using three different temperatures of the hot plate: 52, 55, and 58 °C. Each stimulus temperature was tested once on separate days. Latency to licking of hindpaws or jumping was measured. The order of testing different stimulus temperatures was counterbalanced between the mice. The cut-off latencies in the hot plate test were 40, 30, and 20 s (from the lowest to the highest stimulus temperature, respectively).

To assess limb withdrawal latency to radiant heat, mice were placed on a glass plate, and radiant heat was applied from below to the plantar surface of each hindpaw (Plantar Test Apparatus, Ugo Basile, Varese, Italy; [15]). Paw withdrawal latency was measured electronically. The cut-off latency was 12 s. Three measurements were performed in each hindpaw spaced at least 1 min apart to determine mean withdrawal latency for each animal.

2.3. Assessment of pain-related behavior and hypersensitivity induced by capsaicin

Intracolonic capsaicin-induced spontaneous pain-related behavior and referred hypersensitivity were assessed using the method developed by Laird and her co-workers [23]. The spontaneous behavior was observed directly during 20 min following intracolonic capsaicin (0.1%/50 μl). Postures defined as pain-related behavior were licking of the abdomen, stretching of the abdomen, squashing of the lower abdomen against the floor, and abdominal retractions. The latency of the first pain-related behavior was recorded, as were the number and type of behaviors displayed. To assess the development of referred hypersensitivity, the frequency of withdrawal responses to the application of von Frey hairs to the abdomen and the hindpaws was examined prior to and 20 min after the administration of capsaicin. Six hairs with forces of 0.06, 0.16, 0.69, 1.2, 2.0, and 3.6 g were applied 5 times each in ascending order of force, and the frequency of responses at each force was calculated. The data from the left and right hindpaw were pooled. Due to a long-lasting spontaneous pain-related behavior induced by intracolonic capsaicin [23], hypersensitivity to mechanical stimulation cannot be