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The major histocompatibility complex genes impact pain response in DA and DA.1U rats

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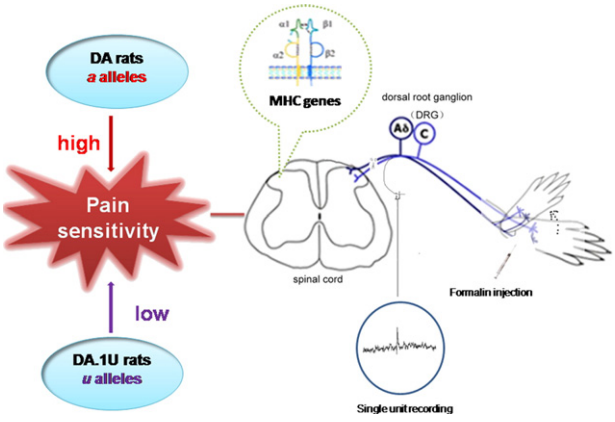
HIGHLIGHTS

- DA and DA.1U rats, whose genetic background is the same except MHC genes
- DA had higher composite pain scores than DA.1U rats in formalin model.
- Formalin induced biphasic increase in discharge rates, especially in DA rats.
- Formalin induced increase of RT1-B in DA rats, but not in DA.1U rats.

GRAPHICAL ABSTRACT



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ABSTRACT

Our recent studies have shown that the difference in basal pain sensitivity to mechanical and thermal stimulation between Dark-Agouti (DA) rats and a novel congenic DA.1U rats is major histocompatibility complex (MHC) genes dependent. In the present study, we further used DA and DA.1U rats to investigate the role of MHC genes in formalin-induced pain model by behavioral, electrophysiological and immunohistochemical methods. Behavioral results showed biphasic nociceptive behaviors increased significantly following the intraplantar injection of formalin in the hindpaw of DA and DA.1U rats. The main nociceptive behaviors were lifting and licking, especially in DA rats ($P < 0.001$ and $P < 0.01$). The composite pain scores (CPS) in DA rats were significantly higher than those in DA.1U rats in both phases of the formalin test ($P < 0.01$). Electrophysiological results also showed the biphasic increase in discharge rates of C and Aδ fibers of L5 dorsal root in the two strains, and the net change of the discharge rate of DA rats was significantly higher than that of DA.1U rats ($P < 0.05$). The mechanical thresholds decreased after formalin injection in both strains ($P < 0.01$), and the net change in the mechanical threshold in DA was greater than that in DA.1U rats ($P < 0.05$). The expression of RT1-B, representation of MHC class II molecule, in laminae I-II of L4/5 spinal cord in DA rats was significantly higher than that in DA.1U rats in the respective experimental group ($P < 0.05$). These results suggested that both DA and DA.1U

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rats exhibited nociceptive responses in formalin-induced pain model and DA rats were more sensitive to noxious chemical stimulus than DA.1U rats, indicating that MHC genes might contribute to the difference in pain sensitivity.

1. Introduction

Pain is very complex and widely investigated for many years. Some researches have shown that pain process can be influenced by a variety of factors, including genetic differences [1–3], but the genetic mechanisms of pain are still poorly understood.

The major histocompatibility complex (MHC), denoted as RT1 in the rat, is located on chromosomes 20 and spans approximately 4 Mb [4]. The major regions of MHC genes contain class I region (the centromeric class I region is RT1-A and the telomeric class I region is RT1-C/E/M), class II region (RT1-B/D), and class III regions [5,6]. It has been shown that MHC genes are involved in the genetic mechanisms for hyperalgesia induced by peripheral or central nervous system injury [7–9]. Our recent study has suggested that the level of MHC II molecular RT1-B might cause the differences in basal pain sensitivity between Dark-Agouti (DA) and cogenic DA.1U rats, whose genetic background is the same as that of DA rats except for MHC genes [10]. The purpose of the present study was to investigate the role of MHC genes in formalin-induced pain model.

The formalin test was used in the present study to observe the pain responses to chemical nociceptive stimulation in the two strains because it is a well-characterized behavioral model of tonic chemogenic pain and widely used in studies on nociceptive processes and analgesic drug effects [11,12]. The behavioral responses including specific and non-specific nociceptive behaviors induced by formalin injection were observed in DA and DA.1U rats. In addition, electrophysiological methods were used to record the afferent discharge rates of C and Aδ fibers from L5 dorsal root. Furthermore, the expression of RT1-B, representation of MHC II molecular, in L4/L5 dorsal horn of spinal cord was also observed to further investigate the role of MHC II molecular in pain sensitivity.

2. Materials and methods

2.1. Animals

Experiments were performed on inbred DA (originating from Zentralinstitut Fur Versuchstierzucht, Hannover, Germany) and DA.1U rats (originating from Lond University, Sweden) of either gender. All rats were 12–16 weeks old, and weighed 150–220 g. All experimental procedures were approved by the Institutional Animal Care Committee of Xi'an Jiaotong University, and were in accordance with ethical guidelines of the International Association for the Study of Pain [13]. The animals were kept in three per polystyrene cage in specific pathogen free animal facilities on 12 h light–dark cycles with food and water available ad libitum.

2.2. Behavioral tests

Behavioral observations were carried out by the same investigators from 9 to 11 am on experimental days in the same room. The animals were habituated for 30 min before formalin test in a clear 40 × 30 × 30 cm plastic box with a mirror behind the box at a 45° angle to allow an unobstructed view of the opposite paw. The animals were divided into normal saline (NS) group (n = 8 for each rat strain) and formalin (FM) groups (n = 15 for each rat strain). In FM group, formalin (2.5%, 50 μl) was injected into right hindpaw intraplantarly using a 30 g needle. In NS group, NS (50 μl) was injected intraplantarly in the same way.

The nociceptive behavioral responses were quantified by counting the accumulative time (in seconds) spent in specific and non-specific nociceptive behaviors in the injected hindpaw during each 5 min period for 1 h after formalin injection. The specific nociceptive behaviors included favoring, lifting and licking, while non-specific nociceptive behaviors included resting or sleeping, still but alert, walking and grooming. Favoring meant injected paw had little weight and rested on the floor without pressure on the footpad; during locomotion there was a definite limp. Lifting indicated that the injected paw was elevated without touching the floor. Licking indicated that the injected paw was licked or bitten. The composite pain score (CPS) was calculated according to the following numerical scale: 0, no pain (normal weight bearing on the injected paw); 1, favoring; 2, lifting; and 3, licking [14–16]. The nonspecific behaviors for each 5 min block in each of the four behavioral categories were counted simultaneously: 0, rest or sleeping; 1, still but alert; 2, walking; and 3, grooming any part of the body except the injected paw [17]. Then, the CPSs for specific and non-specific nociceptive behaviors, ranging from 0 to 3 were calculated by multiplying the time spent in each category by the category weight, summing these products and dividing by the total time in seconds for each 5 min block of time.

$$\mathrm{CPS} = \sum \text{score for each behavior} \times \text{duration of each behavior in every 5 min}/300$$

2.3. Electrophysiological experiment

Rats were anesthetized initially with urethane (1.0 g/kg i.p.) and supplemental doses (0.05 g/kg/h) were given as needed to maintain areflexia. Hair on the back was shaved off and a skin incision was made longitudinally along the median line. A T13–L3 laminectomy was performed in order to expose L5 dorsal root and cut it proximally. A pool was formed by raising the skin flaps and filling the space with warm paraffin oil (37 °C). Rectal temperature was maintained at approximately 37 ± 0.5 °C using a servo-controlled heating blanket.

The distal end of the L5 dorsal root was placed on platinum bipolar electrodes for recording. On a small platform, the nerve was mechanically desheathed and teased apart under a dissecting microscope. Small filaments were repeatedly split with sharpened watchmaker forceps until single unit activity was obtained. Neural activity was recorded, then amplified (Biophysical Amplifier AVB-11A, Nihon Kohden, Japan), filtered (30–3000 Hz), and displayed on an oscilloscope (VC-11, Nihon Kohden, Japan) for monitoring the action potential's waveform and amplitude. The signals were also fed into a computer based data acquisition system (Spike2, Cambridge Electronic Design Limited, Cambridge, UK) that allowed continuous monitoring of discharges as well as off-line data analysis.

The mechanical threshold (MT) of each unit was measured with a set of calibrated von Frey's filaments (Stoelting Company, Wood Dale, IL, USA), with bending forces from 3.92 mN to 254.8 mN, applied to the unit's receptive field (RF) and expressed as the minimum force (mN) needed to evoke a response in ≥50% of the trials [18–22]. The location of most sensitive point of RF to mechanical stimulation was marked and targeted for drug injection. The 28 g needle with PE10 tubing was inserted into the most sensitive point and connected to microinfusion pump (WZ-50, Zhejiang Medical university, China). After the spontaneous discharge became stable, the 5 min discharge