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SHORT REPORT

Disruption of the Fifth Melanocortin Receptor Alters the Urinary Excretion of Aggression-modifying Pheromones in Male House Mice

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Abstract

The preputial glands of house mice express the gene for the fifth melanocortin receptor (MC5-R) and are a primary source of urinary pheromones involved in inter-male aggression. A 'resident-intruder' behavioral model was used to compare the responses of resident males to urine from mice with an engineered disruption of the fifth melanocortin receptor (MC5-RKO) with residents' responses to urine from wild-type mice (WT). Each type of urine was presented in combination with a castrated intruder male to provide the appropriate biological context. Resident males responded with a longer latency to bite when the urine was from gonadally intact WT males compared with urine from MC5-RKO mice. These results are consistent with the hypothesis that activation of the fifth melanocortin receptor in the preputial glands of male house mice causes excretion of urinary pheromones that delay aggressive responses by other males.

Introduction

Inter-male aggression is a key component in the reproductive ecology of male house mice, where it influences the partitioning of food, mates and shelter (Anderson and Hill, 1965; Bronson, 1979). An individual mouse's propensity to engage in aggressive behavior is influenced by genetic background, previous fighting experience, body mass and the perception of aggression-related odor cues (Sandnabba, 1985; 1986), including urinary pheromones from the preputial glands.

The paired preputial glands of house mice are modified sebaceous glands located near the base of the penis. The size of these glands is testosterone dependent, whereas acute secretory activity appears to be regulated by hormones from the anterior pituitary gland, specifically by α-melanocyte-stimulating hormone (α-MSH) (Thody and Shuster, 1973). It has been demonstrated that chemical cues secreted into the urine from the preputial glands modify aggressive interactions (McKinney and Christian, 1970). Socially dominant male mice have larger preputial glands than do subordinate males (Bronson and Marsden, 1973). Moreover, preputial-glandectomized males fail to establish social dominance as readily as sham-operated males (Ingersoll *et al.*, 1986; Hayashi, 1987). These and other results have been interpreted to suggest that the preputial glands are the source of a chemical signal that promotes aggression. However, the current study demonstrates that robust preputial signals might cause a recipient of these signals to delay the onset of aggressive behavior, thus reducing the recipient's risk of physical injury resulting from fighting with a dominant male.

The fifth melanocortin receptor (MC5-R) is a 7-transmembrane G-coupled protein receptor that is found in a variety of exocrine glands, including the preputial glands (Chen *et al.*, 1997; Tatro and Reichlin, 1987). The endogenous ligands for the MC5-R are the melanocortins, including α-MSH (Grantz *et al.*, 1994; Griffon *et al.*, 1994; Labbe *et al.*, 1994; Fathi *et al.*, 1995; Hruby *et al.*, 1995). The availability of mice with a disruption of the fifth melanocortin receptor (MC5-RKO) provided a unique opportunity to examine the pheromonal contribution of the preputial glands to urine. Because the preputial glands are the only component of the urogenital system known to express the MC5-R gene, it was hypothesized that its disruption would alter the aggression-modifying qualities of urine from MC5-RKO male mice.

Materials and methods

Strains of *Mus musculus domesticus* (Taconic) were used in

this experiment: ICR male 'retired breeders' were used as resident males (n = 16), and castrated males of the C57/B6 strains were used as intruders (n = 3). Gonadally intact WT (n = 6) and MC5-RKO (n = 6) male mice of the C57/B6 strain provided stimulus urine. The production of the knockout mice is described by Chen *et al.* (Chen *et al.*, 1997). All mice were individually housed at the University of North Carolina at Greensboro Animal Facility in polypropylene cages of size 25 × 20 × 15.5 cm. Animals were maintained in a 14:10 light:dark reverse light cycle, with food (Harlan TEKLAD Rodent Diet) and tap water provided *ad libitum*. All testing was conducted during the dark part of the light:dark cycle in recognition of the nocturnal behavior patterns of house mice. Proposals for all aspects of this work were institutionally reviewed and approved.

A modified resident–intruder model was used to compare the aggressive responses of 'resident' males to urine previously collected from WT males and MC5-RKO males. The urine had been collected, pooled by genotype and frozen at –20°C until tested. Resident males were prescreened for aggressive behavior 1 week prior to testing by dangling a mouse in front of them for up to 1 min on four consecutive days; residents were classified as aggressive if they attacked the dangling mouse on one or more of these four days. Sixteen of 20 males were deemed 'aggressive' based on these criteria.

To provide the biological context for testing pheromone activity, castrated 'intruder' males were placed into a urine-scented protective cage, hereafter called the 'corral', which was a double-walled cylindrical chamber made of hardware cloth. Thirty microliters of urine from either WT or MC5-RKO males was pipetted onto clean paper and wire 'twist ties' attached to the ends of the corral. Qualitative observations made prior to this experiment verified that resident males did not attack the corral in the absence of the intruder male or urine.

The behavioral tests were 15 min in duration and several behavioral measurements were recorded. Measurements of aggressive behavior included latencies (time elapsed to first occurrence) and frequencies (total number) of 'bites' (resident's mouth placed on the wires of the corral) and 'attacks' (resident's front paws placed on the corral simultaneously with a vigorous bite). To compare baseline activity in the presence of different odor stimuli, locomotor activity (number of times the resident's nose crossed a midline) was measured. Finally, in an attempt to ascertain the interaction of the residents with the urine stimuli, the following measurements were recorded: climbing onto the top of the corral, and latencies and durations of proximity (resident's nose within 1 cm) to the corral and to the stimuli. A latency score of 900 s (the duration of the test) was assigned for any behaviors that did not occur.

In random order, over 2 days, each of the 16 resident male subjects was tested with each type of stimulus urine. The type of urine tested was unknown to the observer. The raw behavioral responses of the residents were analyzed by one-way analysis of variance for genotypic differences, using JMP statistical software (SAS Institute, 1995).

Results

Urine from MC5-RKO mice was associated with a significantly decreased latency to bite [$F(1,32) = 6.7940$; $P = 0.02$; mean latencies to bite ± SEM were 319.7 ± 89.6 versus 142.5 ± 36.8 s for WT and MC5-RKO, respectively; see Figure 1]; and a significant decrease in the number of times the residents climbed on top of the corral [$F(1,32) = 6.7960$; $P = 0.02$; mean frequencies ± SEM were 23.5 ± 1.5 versus 18.6 ± 1.8 observations for WT and MC5-RKO, respectively]. There were no statistically significant differences in locomotor activity, or in the latency and duration of time spent in proximity to the stimuli, indicating that all subjects had equivalent access to the stimuli. In addition, there were no significant differences in the total number of bites or attacks, indicating that the primary effects of disrupting MC5-R were to alter the excretion of signals that modify the onset, rather than full expression, of aggressive behaviors.

Discussion

This experiment demonstrated that the urine from MC5-RKO mice was associated with accelerated onset of aggressive behavior compared with urine from WT mice.

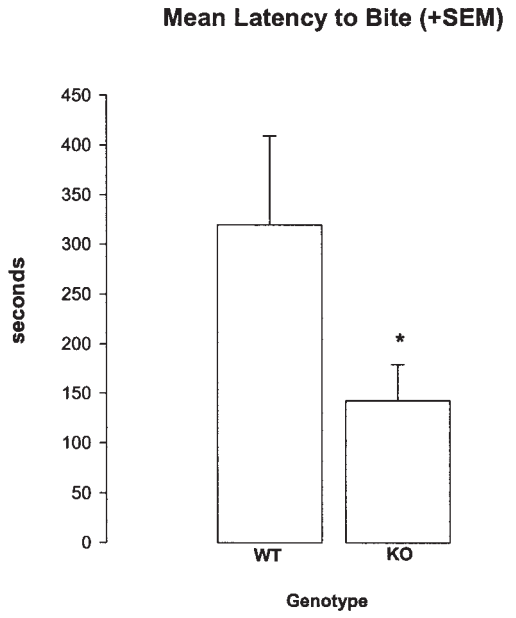

Figure 1 The latency of wild-type resident male house mice (n = 16) to bite at the castrated male intruder's protective cage (corral) was significantly less when the corral was scented with urine from males with a genetic disruption of the fifth melanocortin receptor (KO), compared with urine from wild-type males (WT), suggesting that melanocortins in the preputial glands can alter the excretion of aggression-modifying pheromones (*P = 0.02; mean ± SEM values are 319.7 ± 89.6 s with the WT stimulus and 142.5 ± 36.8 s with the KO stimulus).

This result is consistent with earlier experiments in which urine from 'stressed' and 'unstressed' MC5-RKO mice was associated with a decreased latency to bite and attack compared with urine from WT mice (Caldwell *et al.*, 2001). Over the full duration of the tests, there were no differences in the frequency of bites and attacks in response to the urine from the two genotypes, suggesting that the delayed onset of aggressive behavior in the presence of WT urine does not result in an overall reduction in the display of aggressive behavior. However, the rapid onset of aggressive responses could influence the outcome of interactions between males.

The preputial glands are the only urogenital glands known to express MC5-R (Tatro and Reichlin, 1987; Labbe *et al.*, 1994; Chen *et al.*, 1997), so they are likely to be the source of apparent 'aversion pheromones' or aggression-inhibiting pheromones excreted by dominant male mice. The idea that these signals inhibit or reduce inter-male aggression is supported by previous experiments demonstrating that male mice avoid cage areas that have been 'scent-marked' with urine from a dominant male (Jones and Nowell, 1973). Specifically, the farnesenes are likely candidates for the such chemosignals because α- and β- isomers of the farnesenes, while absent in bladder urine, are present in the preputial glands and in the excreted urine of dominant mice (Novotny *et al.*, 1990). As a behavioral demonstration of the effects of farnesenes, it was shown that socially subordinate male mice were attracted to test areas 'marked' with urine from female mice; however, when exposed to female urine to which farnesene has been added, mice avoided the test area (Novotny *et al.*, 1990). Additional support that WT mice can excrete chemosignals absent in MC5-RKO urine comes from a recent report that MC5-RKO mice have a reduction in volatile compounds, including farnesenes, in their preputial gland extracts (Morgan *et al.*, 1999). Direct tests of farnesene isomers using the resident–intruder model could reveal their possible role in delaying the onset of aggression.

In summary, these data support the hypothesis that melanocortins control the excretion of preputial-gland products that may function to delay the onset of aggression. In house mouse populations, the deployment of such aversion pheromones might allow the most aggressive males to maintain dominance in a social hierarchy, with fewer adversaries initiating high-risk aggressive encounters culminating in physical attacks.

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