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# Pregnancy block by MHC class I peptides is mediated *via* the production of inositol 1,4,5-trisphosphate in the mouse vomeronasal organ

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**Summary**

**The vomeronasal organ (VNO) has evolved to link an animal's behavior to its environment in a highly species-specific fashion. In mice, it is thought to be the primary sensory system responsible for the detection of pheromones. Pheromones regulate a variety of responses including mate recognition in the context of selective pregnancy failure. MHC (major histocompatibility complex) class I peptides have been identified as compounds that elicit the pregnancy block effect *via* the VNO. However, the transduction cascade of these molecules is unknown and it is not known if the production of these compounds are androgen dependent. By using male urine and MHC peptides, we show that female mice treated with MHC peptides (in urine or PBS) and urine from castrated males or juvenile mice of different haplotypes respond to the Bruce Effect paradigm in a manner equivalent to female mice exposed to whole urine. In addition to providing new evidence that urine from castrated or juvenile males and MHC peptides can induce pregnancy block, we show correlation of the effect with an increase in inositol 1,4,5-trisphosphate.**

Key words: $Ins(1,4,5)P_3$, MHC peptide, vomeronasal organ (VNO), pregnancy block.

## Introduction

Communication among mammals *via* chemical messengers known as pheromones plays an important role in a variety of social interactions, especially those associated with reproductive behaviors (Wysocki, 1979; Halpern, 1987; Wysocki and Meredith, 1987). In response to pheromones, animals exhibit behavioral repertoires that are often innate and do not require learning or experience. The perception of pheromones is mostly mediated by the vomeronasal organ (VNO). The VNO is a paired, tubular structure divided by the nasal septum, each side having a crescent-shaped lumen lined with receptor neurons on the medial concave side and filled with fluid from the vomeronasal glands (Døving and Trotier, 1998). Lateral to the lumen are large blood vessels and sinuses that are innervated by the autonomic nervous system inducing vasodilations and vasoconstrictions that produce a pump-like action for stimulus access to the lumen (Meredith, 1994). The vomeronasal epithelium is segregated into two distinct zones, which express unique sets of transduction related molecules. The neurons expressing vomeronasal class 1 Receptors (V1Rs) also express the alpha subunit of $G\alpha_{i2}$ and project to the anterior region of the accessory olfactory bulb (AOB), whereas the neurons expressing vomeronasal class 2 Receptors (V2Rs) also express the alpha subunit of $G\alpha_o$ and project to the posterior region of the AOB (Halpern et al., 1995; Wekesa and Anholt, 1999; Rodriguez et al., 1999). V1R receptors respond to volatile compounds such as 2-heptanone and 2, 5-dimethylpyrazine (Leinders-Zufall et al., 2000). V2R receptors have been shown to respond to non-volatile compounds such as major histocompatibility complex (MHC) class I peptides (Leinders-Zufall et al., 2004).

The removal of the VNO (VNX) in males diminishes the robustness of the mating response, but does not eliminate sexual behavior (Powers and Winans, 1975). In females, VNX results in diminished aggression by lactating females, reduction in response to puberty delay pheromones and elimination of pregnancy block (Wysocki and Lepri, 1991; Kelliher et al., 2006). Other innate behaviors, including lordosis in female pigs (*Sus scrofa*) (Dorries et al., 1997) in response to the male hormone, androstenone, or suckling behavior in newborn rabbits (*Oryctolagus cuniculus*) in response to mammary secretions, are unaffected by removal of the VNO (Schaal et al., 2003). These innate behavioral responses are likely to be elicited by pheromones that activate the main olfactory system. Thus, mammals have evolved innate behavioral repertoires that are mediated by pheromones that activate both the main olfactory system and vomeronasal system.

The pregnancy block or Bruce Effect is one of the best-known examples of olfactory imprinting in adult vertebrates (Bruce, 1959). Pregnancy block occurs when a recently inseminated female is exposed to a strange male or his urine and terminates the current pregnancy. The pregnancy block effect depends on the formation and maintenance of a pheromonal recognition memory by the vomeronasal system

(Brennan et al., 1990; Hudson, 1993; Kaba et al., 1994). As a result of this memory, males made familiar by mating are recognized by the females, thus mitigating pregnancy block. Murine MHC class I peptide ligands are the first identified vomeronasal stimuli that can mediate the pregnancy block effect (Leinders-Zufall et al., 2004; Boehm and Zufall, 2006). These ligands are nonvolatile molecules, typically nine amino acids residues in length, presented by MHC molecules at the cell surface. These peptides–MHC complexes are released into the extracellular space and appear in urine and other bodily secretions (Singh et al., 1987). In 2004, Leinders-Zufall et al. (Leinders-Zufall et al., 2004) demonstrated that female mice experience pregnancy block when exposed to MHC class I peptides representing a different strain of mouse than the male which had mated with the female. Using electrophysiological studies, they reported whole-cell current recording from vomeronasal sensory neurons (VSNs) producing membrane depolarization and subsequent action potentials when MHC class I peptides were applied.

In order to investigate the transduction pathways of MHC class I peptides, Kelliher et al. (Kelliher et al., 2006) used mice with a homozygous deficiency in the transient receptor potential channel 2 (TRPC2) cation channel gene. TRPC2 has been previously shown to play a crucial role in the signal transduction mechanism of at least some VSNs (Stowers et al., 2002; Leypold et al., 2002; Lucas et al., 2003). They found that the loss of the TRPC2 channel did not influence the formation of social memories in the context of the Bruce effect, thus indicating that TRPC2 is not part of the transduction cascade of social cues by peptide-sensitive VSNs located in the basal zone of the VNO. Their results suggest an alternative, TRPC2-independent signal transduction mechanism in the detection of molecular cues required for the Bruce Effect (Kelliher et al., 2006).

Here we show that we can induce the Bruce Effect in C57BL/6 female mice by using urine from BALB/c males. This effect can also be induced by using only the H-$2^d$ haplotype peptide of BALB/c males without urine. In addition to providing new evidence that urine (whole, or from castrated or juvenile males) and MHC peptides can induce pregnancy block, we show correlation of the effect with an increase in inositol 1,4,5-trisphosphate [Ins(1,4,5)$P_3$] production mediated by these compounds.

## Materials and methods

### *Animals*

C57BL/6 and BALB/c mice (*Mus musculus* L.) were obtained from Charles River Laboratories (Kingston, NY, USA) and maintained in a breeding colony in the Department of Biological Sciences at Alabama State University. Animals were housed in Institutional Animal Care and Use Committee (IACUC) inspected and approved facilities and cared for according to the NIH Guide for Care and Use of Laboratory Animals. Food and water were provided *ad libitum.*

### *Chemical stimuli*

Ligands were chosen to correspond to prototypical representatives for the two disparate H-2 haplotypes, namely AAPDNRETF (for the H-$2^b$ haplotype of C57BL/6 mice) and SYFPEITHI (for the unrelated H-$2^d$ haplotype of BALB/c mice). Control peptides in which the characteristic anchor residues of the two MHC class I ligands were replaced by alanines (i.e. AAPD**A**RET**A** and S**A**FPEITH**A**, respectively) were used as negative controls (Leinders-Zufall et al., 2004).

The synthetic peptides were purchased from Sigma-Genosys (The Woodlands, TX, USA). Peptide concentration was 250 μmol $l^{-1}$ in phosphate-buffered saline (PBS). Whole urine was collected from adult, castrated and juvenile males (21–22 days of age), pooled by strain, then stored frozen at –80°C until needed. For pregnancy block assays, peptide mixtures in PBS (H-$2^b$ haplotype AAPDNRETF, AAPDARETA; H-$2^d$ haplotype SYFPEITHI, SAFPEITHA) were mixed 1:1 with urine prior to use. PBS consists of 8 g NaCl, 0.2 g KCl, 1.44 g $Na_2HPO_4$ and 0.24 g $KH_2PO_4$ in 1 l $dH_2O$. Chemicals were purchased from Fisher Scientific (Suwanee, GA, USA) and Sigma-Aldrich (Milwaukee, WI, USA). The peptides were also used without mixing with urine.

### *Bruce Effect experiments*

#### *Pregnancy failure test*

Animals included adult, virgin female mice of the C57BL/6 strain, and sexually experienced males of either C57BL/6 or BALB/c strains. Mice were kept in Nalgene cages 26 cm×21 cm×14 cm, at 20°C room temperature and a reversed 12 h:12 h light:dark cycle (lights on at 11:00 h and off at 23:00 h). Each C57BL/6 female was paired with a single C57BL/6 or BALB/c male and checked four times during the ensuing 12 h period for vaginal plugs indicating that mating had occurred. Mated females remained with the males until 08:00 h the following day at which time the females were moved to clean cages and assigned to groups based on stimulus to be administered. Exposure to urine, supplemented urine, or peptide alone was achieved by depositing 30 μl of liquid on the oronasal groove while holding the female by the nape of the neck. Stimulant was delivered four times per day for 2 days at regular intervals. Eight days after mating, the females were sacrificed, the uteri surgically removed and examined for implantation sites or the presence of embryos. After completing the dissections, results for each group were recorded and the percentage of pregnancy failures was calculated. A significant effect was based on a >60% pregnancy failure rate ($N$=5). Statistical analysis was done by analysis of variance (ANOVA).

#### *Membrane preparations*

VNOs from female mice (C57BL/6), between 40 and 60 days of age, were dissected from their crevices in the nasal cavity, removed from the cartilaginous capsule, and frozen on dry ice. The tissues were then minced with a razor blade and subjected to sonication for 2–5 min in ice-cold PBS. The

resulting suspension was layered on a 45% (w/w) sucrose cushion and centrifuged at 4°C for 30 min at 3000 ***g*** in a Beckman SW55Ti rotor. The membrane fraction on top of the sucrose was collected and centrifuged as before for 15 min to pellet the membranes. The membranes were re-suspended in 100 μl of ice-cold PBS. Protein concentration was determined according to the method of Lowry et al. (Lowry et al., 1951), using bovine serum albumin as standard. The procedure used for the preparation of microvillar membranes is modeled after well-established methods (Anholt, 1995; Wekesa and Anholt, 1999; Wekesa et al., 2003). These preparations have been previously characterized (Anholt et al., 1986; Anholt, 1995; Wekesa and Anholt, 1997) and are sufficiently enriched in chemosensory membranes.

*Second messenger assays*

For Ins(1,4,5)$P_3$ assays, reactions were incubated for 1 min at 37°C in 25 mmol $l^{-1}$ Tris–acetate buffer pH 7.2, 5 mmol $l^{-1}$ magnesium acetate, 1 mmol $l^{-1}$ dithiothreitol (DTT), 0.5 mmol $l^{-1}$ ATP, 0.1 mmol $l^{-1}$ $CaCl_2$, 0.1 mg ml $l^{-1}$ bovine serum albumin, 10 μmol $l^{-1}$ GTP, and 20 μg VNO membrane proteins. Reactions were terminated by the addition of 1 mol $l^{-1}$ trichloroacetic acid. Ins(1,4,5)$P_3$ was measured with a kit from Perkin Elmer, Inc. (Boston, MA, USA) according to the manufacturer's instructions and is based on displacement of [$^3$H]Ins(1,4,5)$P_3$ from a specific Ins(1,4,5)$P_3$ binding protein. Differences between experimental and control animals were analyzed by analysis of variance (ANOVA).

## Results

### *Pregnancy failure using whole urine and urine mixed with MHC class I peptides*

These experiments closely followed previously established protocols by Leinders-Zufall et al. (Leinders-Zufall et al., 2004) and Kelliher et al. (Kelliher et al., 2006). We first examined pregnancy failure that occurs when recently mated females are exposed to the urine of a strange male. Female C57BL/6 (H-2$^b$ haplotype) mice were mated with C57BL/6 or BALB/c males and then exposed to urine taken from BALB/c males (unfamiliar male urine; H-2$^d$ haplotype). Application of the unfamiliar urine resulted in a 100% pregnancy failure rate (Fig. 1, experiment 1), whereas application of familiar urine did not result in pregnancy failure (Fig. 1, experiment 4). The effect of unfamiliar urine could be mimicked by adding MHC class I peptides of disparate haplotype to familiar urine samples (Fig. 1, experiments 3 and 5). By contrast, addition of cognate peptides (H-2$^b$) did not induce pregnancy failure (Fig. 1, experiment 2). Using a blend of C57BL/6 and BALB/c male urines resulted in a pregnancy failure rate of 60% (Fig. 1, experiment 6).

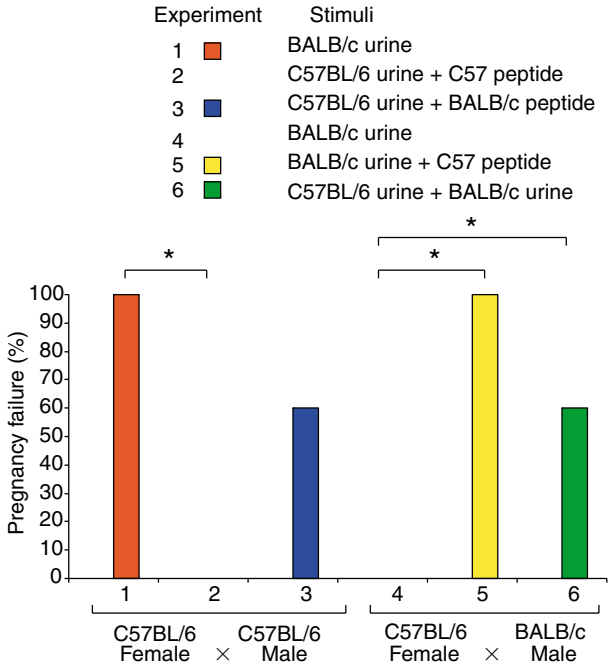


Fig. 1. Pregnancy failure after exposure to whole urine and urine mixed with MHC class I peptides. MHC class I peptides function as individual identification signals in the circumstance of pregnancy block in mice. A significant pregnancy failure rate was observed when female C57BL/6 mice were mated with C57BL/6 male mice and then exposed to BALB/c male urine or the H-2$^d$ haplotype peptide in C57BL/6 urine (*$P$<0.05). Pregnancy block in females exposed to familiar male urine or the H-2$^b$ haplotype peptide was not significant. A significant effect was not observed when C57BL/6 female mice were mated with BALB/c male mice and then exposed to familiar urine. Female C57BL/6 mice mated with BALB/c male mice and exposed to BALB/c male urine supplemented with H-2$^b$ haplotype peptide or C57BL/6 male urine showed a significant pregnancy failure rate (*$P$<0.05).

### *Pregnancy failure by MHC peptides only*

Next, we examined the occurrence of pregnancy failure when MHC peptides alone were administered. C57BL/6 females were mated with either C57BL/6 or BALB/c males and exposed to the cognate peptides dissolved in PBS. Application of disparate peptides resulted in a 100% pregnancy failure rate (Fig. 2, experiments 1 and 3). The inactive forms of the peptides were ineffective at inducing pregnancy failure (Fig. 2, experiments 2 and 4). Leinders-Zufall et al. (Leinders-Zufall et al., 2004) previously reported that these peptides without urinary background were not inducive to the pregnancy block. In our experiments, we were able to induce pregnancy block using only the active form of the peptide. This positive result is possibly due to the increased number of times the peptide was administered (four times per day for 2 days *versus* twice per day for 2 days) reflecting an increased exposure concentration in the sensory system.

In a series of experiments designed to disrupt the ability of MHC ligands to induce the Bruce Effect we first tried boiling the urine and second, digesting the constituents contained in the urine with trypsin. Boiling the urine for 5 min had no effect in disrupting the MHC ligands ability to induce pregnancy

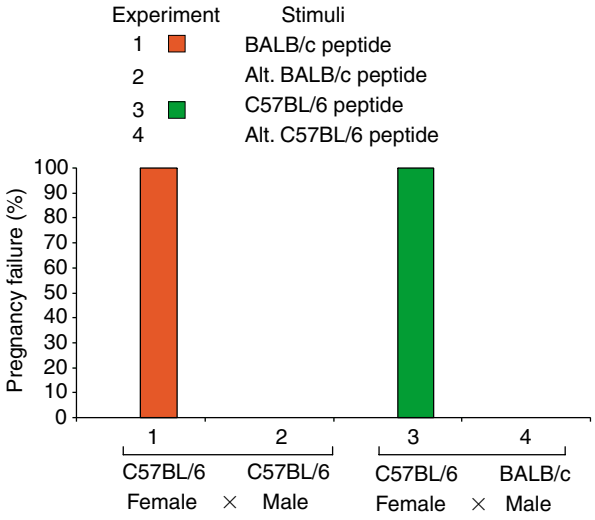


Fig. 2. Pregnancy failure caused by MHC peptides only. Prototypical representative ligands for H-$2^b$ and H-$2^d$ haplotypes induce pregnancy failure when administered without urinary constituents. C57BL/6 female mice mated with C57BL/6 male mice and exposed to the active form of the MHC peptide specific for the H-$2^d$ haplotype resulted in a significant pregnancy failure rate. The inactive H-$2^d$ peptide failed to block pregnancy. C57BL/6 female mice mated with BALB/c male mice and exposed to the H-$2^b$ haplotype peptide also showed a significant pregnancy failure rate. The inactive form of the H-$2^b$ peptide failed to block pregnancy. Alt C57BL/6 and Alt. BALB/c refers to the control peptides, which had their characteristic anchoring residues changed to alanines, i.e. AAPDARETA and SAFPEITHA, respectively.

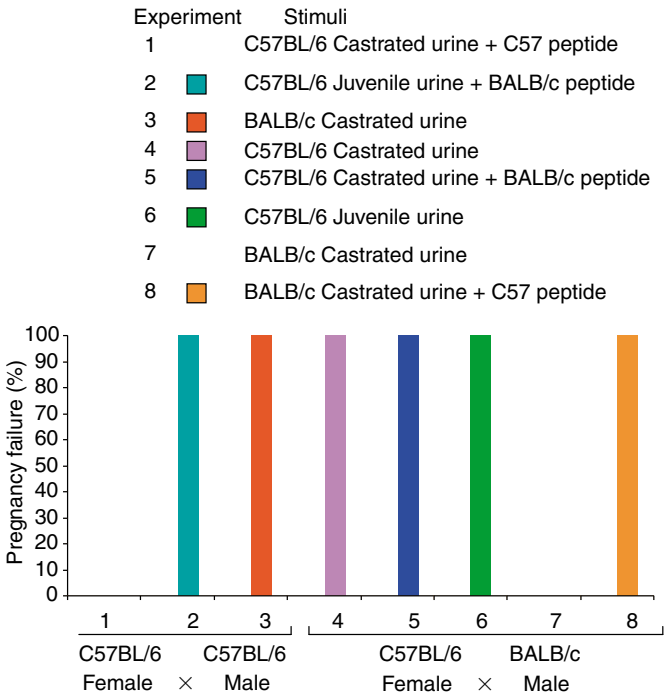


Fig. 3. Pregnancy failure after exposure to urine of juvenile or castrated male mice. MHC class I peptides and urine, in the absence of an androgen influence, are able to induce pregnancy failure. C57BL/6 females mated with C57BL/6 male mice and exposed to C57BL/6 castrated male urine supplemented with the H-$2^b$ peptide failed to block pregnancy. However, a significant effect was observed when C57BL/6 females mated with C57BL/6 males were exposed to C57BL/6 juvenile urine supplemented with the H-$2^d$ peptide. A significant effect was observed when C57BL/6 females mated with C57BL/6 males were exposed to urine from BALB/c castrated males. Exposure of C57BL/6 female mice mated with BALB/c males to urine of castrated or juvenile C57BL/6 males resulted in a significant pregnancy failure rate, whereas, exposure to urine of BALB/c castrated males failed to block pregnancy. A significant pregnancy failure rate was observed when C57BL/6 female mice were mated with BALB/c males and exposed to urine of C57BL/6 castrated males supplemented with the H-$2^d$ peptide. A significant result was again observed when urine from BALB/c castrated males supplemented with the H-$2^b$ peptide was applied to the C57BL/6 females mated with the BALB/c males.

block due to their already small, linear structure. However, digesting the proteins/peptides did eliminate the ligands ability to induce the Bruce Effect by cutting the peptides into even smaller fragments making them nonfunctional (data not shown).

### *Pregnancy failure caused by urine from juvenile and castrated males*

In order to determine the androgen dependency of MHC peptide production, we did a series of experiments to determine the ability of urine from juvenile and castrated males (hereafter referred to as juvenile and castrated urine, respectively) to induce pregnancy block. C57BL/6 females mated with BALB/c males and exposed to unfamiliar juvenile urine, resulted in a 100% pregnancy failure rate (Fig. 3, experiment 6). Next, C57BL/6 females mated with C57BL/6 males and exposed to familiar juvenile urine supplemented with the disparate peptide resulted in 100% pregnancy failure (Fig. 3, experiment 2). To investigate the ability of castrated male urine to induce pregnancy block, we mated C57BL/6 females with either C57BL/6 or BALB/c males, and then exposed the pregnant females to familiar or unfamiliar castrated urine (Fig. 3, experiments 3, 4 and 7). In order to determine the effects of the MHC peptides in urine from castrated males, we administered familiar castrated urine supplemented with the cognate peptide to C57BL/6 females paired with C57BL/6 males (Fig. 3, experiment 1). The exposure to familiar urine resulted in no pregnancy failure (Fig. 3, experiment 7), whereas the unfamiliar urine resulted in 100% pregnancy failure rates (Fig. 3, experiments 3 and 5). Urine from castrated males supplemented with the cognate peptide resulted in no pregnancy failure (Fig. 3, experiment 1). Finally, C57BL/6 females mated with BALB/c males and then exposed to familiar or unfamiliar urine supplemented with either cognate or disparate peptide, respectively, resulted in 100% pregnancy failure (Fig. 3, experiments 5 and 8). These results suggest that the production of MHC class I peptides is not androgen dependent.

*Administration of MHC peptides and castrated urine results in the production of Ins(1,4,5)$P_3$*

In an effort to determine whether MHC class I peptides induce the production of Ins(1,4,5)$P_3$ in the VNO, we performed radioreceptor assays. We observed an increase in Ins(1,4,5)$P_3$ when stimulating the C57BL/6 female VNO preparation with the BALB/c haplotype peptide ($P$<0.05) (Fig. 4), whereas, the inactive peptides, as well as the cognate peptides, failed to stimulate any significant increase in Ins(1,4,5)$P_3$. The amount of Ins(1,4,5)$P_3$ induced by the peptides was significantly lower than the amount induced by urine. This is due to urine containing a mixture of different pheromones that stimulate either all or a higher subset of pheromone receptors. Although not statistically significant, the BALB/c peptide induced more Ins(1,4,5)$P_3$ than the C57 peptide, possibly because of activation of strain-related receptor networks.

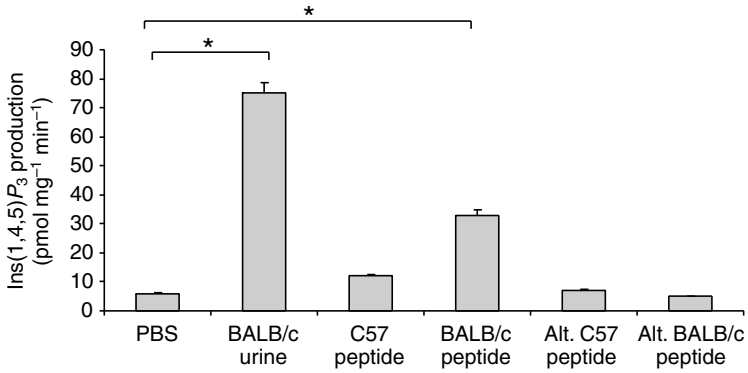


Fig. 4. Production of Ins(1,4,5)$P_3$ by MHC class I peptides. MHC class I peptides have the ability to stimulate the production of Ins(1,4,5)$P_3$ in vomeronasal organ (VNO) microvillar preparations from female C57BL/6 mice. Reactions were performed without stimulus (PBS only), with whole urine, or active and inactive forms of the H-$2^b$ and H-$2^d$ haplotype peptides. Synthetic BALB/c peptide (H-$2^d$) stimulates the production of Ins(1,4,5)$P_3$ in the VNO (*$P$<0.05). The synthetic C57 peptide (H-$2^b$) does not significantly increase Ins(1,4,5)$P_3$ production, and the alternative forms of both peptides result in Ins(1,4,5)$P_3$ levels approximately equal to basal levels, indicating their inability to stimulate Ins(1,4,5)$P_3$ production in the VNO. Values are means ± s.e.m. of at least five independent experiments, each consisting of duplicate measurements.

Urine as a whole is the most potent source of pheromones, but not all of the pheromonal constituents are androgen related; by using castrated urine in our assay we provide evidence that some components are able to stimulate the production of Ins(1,4,5)$P_3$ independent of an androgen presence (Fig. 5). The urine used in these experiments was collected from BALB/c males that had been castrated 4 months prior to the collection and use period. Ins(1,4,5)$P_3$ induction by urine from castrated males was significantly lower than that produced by urine from uncastrated males. This reduced production is most likely due to the absence of pheromones that are androgen related, which are found in urine from normal mice. Supplementation of castrated urine with the BALB/c peptide slightly increased the production of Ins(1,4,5)$P_3$, although this was not statistically significant.

## Discussion

Leinders-Zufall et al. (Leinders-Zufall et al., 2004) reported that MHC peptide ligands are not only detected by neuronal populations in the VNO, but also by sensory neurons of the main olfactory epithelium (MOE). However, pregnancy block is absent in mice in which the VNO has been surgically removed, demonstrating that recognition of MHC peptides *via* the MOE does not replace VNO function in the context of the Bruce Effect. In support of these results, previous findings showing that transgenic mice exhibiting a selective ablation of the MOE respond normally to urine stimuli in the context of the pregnancy block test (Ma et al., 2002).

Clear phenotypic discrepancies have been observed between genetically modified mice and those with surgical VNO lesions (McCarthy and Auger, 2002; Brennan and Keverne, 2004; Pankevich et al., 2004). For example, impaired sexual behavior toward females has been reported in male mice after VNO lesions (Clancy et al., 1984), whereas no such deficits were reported in *Trpc2*$^{-/-}$ mice (Leypold et al., 2002; Stowers et al., 2004; Pankevich et al., 2004). Similarly, in sexually naïve male mice, VNO removal prevents ultrasonic vocalizations in response to female chemosignals (Wysocki and Lepri, 1991), whereas robust vocalizations are produced by *Trpc2*$^{-/-}$ males (Stowers et al., 2002). It was initially thought that the transduction of all pheromones was mediated via the TRPC2 channels (Lucas et al., 2003). Therefore it was assumed that the VNO would be impaired if this channel were knocked out.

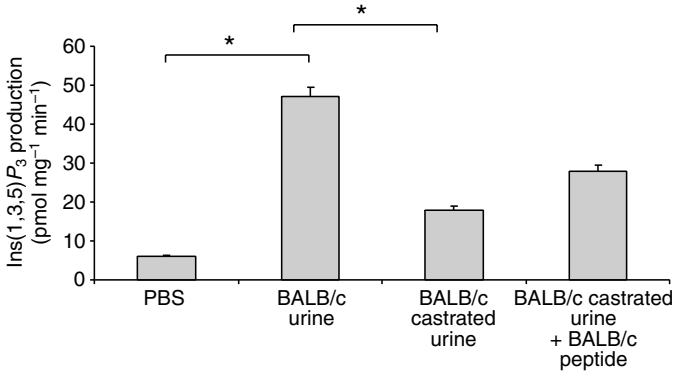


Fig. 5. Production of Ins(1,4,5)$P_3$ after exposure to urine from castrated males. C57BL/6 female vomeronasal organ (VNO) microvillar membranes responded to whole urine and urine from BALB/c castrated males with an increase in the production of Ins(1,4,5)$P_3$ (*$P$<0.05). The addition of H-$2^d$ peptide further increased the production of Ins(1,4,5)$P_3$, though not significantly. Reactions were performed without stimulus, in the presence of whole urine, urine from castrated males, and urine from castrated males supplemented with MHC peptide. Values are means ± s.e.m. of at least five independent experiments, each consisting of duplicate measurements.

Considerable debate remains over the role of the VNO and TRPC2 channel mediating behaviors such as the pregnancy block effect.

In the present study, we provide further evidence that the MHC class I peptides have a role in mediating pregnancy block *via* the VNO. Whole urine, juvenile urine and castrated urine (Figs 1 and 3) are all capable of inducing pregnancy block suggesting that the production of this chemsensory cue is not androgen dependent (Fig. 5). This is unlike pheromonal effects such as puberty acceleration and aggression, which are androgen dependent (Drickamer and Murphy, 1978). The likelihood of female attraction to juvenile or castrated adult male mice is minimal based on findings reported by Lin et al. (Lin et al., 2005). This study shows that adult male urine contains a volatile compound (methyl-thio-methanethiol, MTMT) that is highly attractive to female mice. This compound activates the main olfactory system and is not found in female urine, suggesting that the production may depend on testosterone. Therefore, females would not be attracted to castrated males and therefore not be exposed to MHC peptides that induce the pregnancy block effect. This concept illustrates how pheromonal-induced behaviors work in concert with each other in order to produce the desired effect.

The transduction mechanism of MHC peptides is still unclear. Our results suggest that MHC class I peptides stimulate the female VNO *via* the production of Ins(1,4,5)$P_3$. This result complements other studies that show Ins(1,4,5)$P_3$ levels increase in the VNO membrane preparations during stimulation with urinary pheromones (Sasaki et al., 1999; Kroner et al., 1996; Wekesa and Anholt, 1997; Wekesa et al., 2003; Inamura et al., 1997a; Inamura et al., 1997b). The role of Ins(1,4,5)$P_3$ in the production of calcium and generation of an action potential is still uncertain along with the role of calcium once it has entered the cell. Previous studies (Liman, 2003) show that VNO microvilli have calcium-activated cation channels which may be opened by calcium ions that either enter the cell through the TRPC2 channel or from the endoplasmic reticulum (ER). Opening of this channel would enhance the receptor potential and therefore amplify the signal. The TRPC2 channel has been shown to induce the flow of calcium ions in response to pheromones such as 2-heptanone and 2,5-dimethylpyrazine. The genetic ablation of TRPC2 either eliminates (Stowers et al., 2002) or strongly reduces (Leypold et al., 2002) the sensory response of the VNO to urine or small, volatile pheromones. In 2003, it was proposed (Lucas et al., 2003) that the primary electrical response to pheromones depends on diacylglycerol (DAG) and not on Ins(1,4,5)$P_3$ or arachidonic acid. It is possible that DAG may activate certain pathways which are currently unknown. However, a recent study (Kelliher et al., 2006) shows that the pregnancy block effect can still occur in the absence of the TRPC2 channel. This suggests that not all pheromonal responses in the VNO are mediated by the TRPC2 channel. The MHC class I-induced increase in Ins(1,4,5)$P_3$ implies a role for calcium in vomeronasal transduction. It is possible that the Ins(1,4,5)$P_3$ produced might function in the classical manner by which it binds to the Ins(1,4,5)$P_3$ receptor on the ER initiating the release of calcium from within the ER. Alternatively, as described in a new study in B cells (Dellis et al., 2006), Ins(1,4,5)$P_3$ may play two roles, as it was shown to be present on both the endoplasmic reticulum and the plasma membrane. This would suggest that calcium influx into the cell may be regulated by Ins(1,4,5)$P_3$ receptors in the plasma membrane with the help of other channels (Dellis et al., 2006).

The present study introduces novel results elicited by MHC peptides and urine collected from juvenile and castrated male mice, indicating an active role in pregnancy failure (the Bruce Effect). These stimuli are capable of inducing pregnancy failure in females mated to different haplotype males, while the female haplotype is irrelevant in this behavioral response. The ability of urine from castrated and juvenile males to induce the pregnancy block provides evidence that the production of MHC class I peptides is not androgen dependent. Although, we are unable to define the transduction mechanism utilized in this response, we do show that the production of Ins(1,4,5)$P_3$ in the VNO coincides with the pregnancy failure response.

## List of abbreviations

| | |
|---|---|
| AOB | accessory olfactory bulb |
| DAG | diacylglycerol |
| DTT | dithiothreitol |
| ER | endoplasmic reticulum |
| Ins(1,4,5)$P_3$ | inositol 1,4,5-trisphosphate |
| MHC | major histocompatibility complex |
| TRPC2 | transient receptor potential channel 2 |
| VNO | vomeronasal organ |
| VNX | vomeronasal organ removal |
| VSN | vomeronasal sensory neuron |

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