Increased affiliative response to vasopressin in mice expressing the V$_{1a}$ receptor from a monogamous vole

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Arginine vasopressin influences male reproductive and social behaviours in several vertebrate taxa through its actions at the V$_{1a}$ receptor in the brain. The neuroanatomical distribution of vasopressin V$_{1a}$ receptors varies greatly between species with different forms of social organization. Here we show that centrally administered arginine vasopressin increases affiliative behaviour in the highly social, monogamous prairie vole, but not in the relatively asocial, promiscuous montane vole. Molecular analyses indicate that gene duplication and/or changes in promoter structure of the prairie vole receptor gene may contribute to the species differences in vasopressin-receptor expression. We further show that mice that are transgenic for the prairie vole receptor gene have a neuroanatomical pattern of receptor binding that is similar to that of the prairie vole, and exhibit increased affiliative behaviour after injection with arginine vasopressin. These data indicate that the pattern of V$_{1a}$-receptor gene expression in the brain may be functionally associated with species-typical social behaviours in male vertebrates.

Arginine vasopressin (AVP) and its non-mammalian homologue, arginine vasotocin (AVT), are involved in many species-typical, male social behaviours, including communication, aggression, sexual behaviour and, in monogamous species, pairbonding and paternal care. Pharmacological studies using selective antagonists indicate that the behavioural effects of AVP are mediated by the V$_{1a}$ receptor. The V$_{1a}$ receptor is a member of a family of evolutionarily related receptors for the neurohypophysial peptides, arginine vasopressin and oxytocin, which includes the V$_{1a}$, V$_{1b}$ and V$_{2}$ receptors, all of which are G-protein-coupled proteins comprising seven transmembrane domains. The V$_{1a}$ receptor is exceptional in that its pattern of expression in the brain is phylogenetically plastic, with the neuroanatomical distribution of this receptor being unique in virtually every species examined.

Voles provide a useful model to test the relationship between receptor expression patterns and social behaviour. Prairie voles (Microtus ochrogaster) are highly affiliative, biparental and monogamous; whereas montane voles (Microtus montanus) are relatively asocial, non-paternal, and promiscuous. In the male prairie vole, cohabitation and/or mating with a female stimulates both the central release of AVP and the development of a pairbond and paternal care. Central blockade of the vasopressin receptors during mating with a selective V$_{1a}$ antagonist prevents the normal development of pairbonds between mates and paternal care in male prairie voles. Prairie and montane voles have strikingly different distributions of V$_{1a}$-receptor binding in the brain. We tested the hypothesis that these differences in V$_{1a}$-receptor binding patterns would confer species differences in behavioural response to AVP. Using an ovariec-tomized stimulus female placed in one side of a two-chambered arena, 2 ng of AVP delivered by intracerebroventricular (i.c.v.) injection to male prairie voles resulted in a significant increase in affiliative behaviour (offactory investigation and grooming) toward the stimulus female (P < 0.01). In contrast, the identical treatment had no effect on affiliative behaviour, compared with control injections, in male montane voles.

To investigate the mechanisms of the species differences in regional gene expression, we compared the structures of the prairie and montane vole V$_{1a}$-receptor genes, including the 5′ flanking regions (Fig. 2a). The V$_{1a}$-receptor gene encodes a protein of 420 amino-acid residues that is 99% homologous between the vole species. The binding kinetics of the receptor protein and its second messenger coupling are identical in both species. Both 5′ and 3′ rapid amplification of complementary DNA ends (RACE) were used to identify the transcriptional boundaries of the gene (data not shown). The prairie vole V$_{1a}$ messenger RNA is composed of a 232-base-pair (bp) 5′ untranslated region (UTR), a 1,260-bp coding region followed by a 36-bp 3′ UTR. No CAAT or TATA enhancer elements were found in the promoter region of the prairie vole V$_{1a}$ gene. In contrast, the montane vole V$_{1a}$ gene contains a 301 bp CAAT box region and a 60 bp TATA box region. These findings indicate that the pattern of V$_{1a}$-receptor gene expression in the brain may be functionally associated with species-typical social behaviours in male vertebrates.
elements were found near the transcriptional initiation site. Northern analysis using poly (A) + RNA from brain demonstrate that the transcript length is similar in prairie and montane voles (data not shown). Both species have a 2.5-kilobase (kb) intron located between the sixth and seventh transmembrane domains. In contrast to the similarities in the coding sequences, the 5′ flanking region of the V1a gene displays marked differences between the species. In the gene for the prairie vole V1a-receptor, this region contains a 428-bp sequence that is rich in repetitive di- and tetra-nucleotide sequences, or microsatellite DNA, between −1,151 and −723 relative to the transcription start site. This sequence is not found in the montane vole V1a gene and sequences on either side of the expansion are contiguous in the montane vole gene.

Using polymerase chain reaction (PCR) primers positioned on either side of the expansion, we found that the V1a-receptor gene of the monogamous pine vole (Microtus pinetorum), which has a prairie vole-like pattern of V1a-receptor expression, has a similar 5′ flanking sequence to that of the prairie vole gene (Fig. 2b). In contrast, the non-monogamous meadow vole (Microtus pennsylvanicus), which has a montane-vole-like pattern of receptors in the brain, did not have this sequence. In addition to the change in the 5′ flanking region, two distinct V1a loci were isolated from prairie vole genomic libraries, indicating that the V1a-receptor gene from the prairie vole has been duplicated (Fig. 2a). Southern blot analysis of genomic DNA from both prairie and montane voles confirms the duplication (not shown). The second V1a-receptor locus has a 589-bp 5′ truncated fragment of an L1 long interspersed nuclear element (LINE) just upstream of the expansion. The LINE, a mobile genetic element or retrotransposon, inserted in the centre of a 10-bp palindrome (TTCTT/AAGAA). A deletion of a single base in the 5′-flanking sequence to that of the prairie vole gene (Fig. 2b). In contrast, the level of expression of the prairie vole V1a transgene in the mice was similar to that of montane voles (data not shown). The intensity of binding in the transgenic mouse was not elevated in brain regions that normally express V1a receptor in the mouse, such as the diagonal band, lateral septum or the lateral hypothalamus (Fig. 3f). The level of expression of the prairie vole V1a transgene in the mice was similar to that found in prairie voles. Thus, any changes in response to AVP should be attributable to the expression of the receptor in new areas of the mouse brain and not simply to increased numbers of receptors in regions that are normally responsive to AVP in the mouse.

In a behavioural model similar to that used in the vole experiments, 2 ng of AVP increased the expression of affiliative behaviour, as measured by olfactory investigation and grooming, in transgenic but not non-transgenic mice (P < 0.01) (Fig. 4a), a response similar to that found in prairie mice.

Figure 2 Structure of the V1a-receptor gene in voles. V1a-receptor genes from montane vole (M. mon) and prairie vole (M. och) were isolated from genomic DNA libraries. Transcription begins at +1 and polyadenylation (pA) occurs at +1,623 of the prairie vole gene. The box area within these sites represents the coding region and the vertical bars in the coding region represent the location of the transmembrane domains. Two distinct loci were isolated from prairie vole DNA. The sequences of the genomic clones have been deposited in GenBank (accession number AF069304).

Figure 3 The pattern of V1a-receptor binding of mice that are transgenic for the V1a-receptor gene from the prairie vole is similar to that of the prairie vole. Receptor autoradiography illustrates the distribution of V1a-receptor binding in wild-type (a) and transgenic (b) mice. Although V1a-receptor binding is altered, oxytocin-receptor binding is identical in wild-type (c) and transgenic (d) mice. Binding in the cingulate cortex (Ci), lateral dorsal (LD) and ventroposterior (VP) thalamic nuclei, claustrum (Cla) and several other regions of transgenic mice is similar to that found in the brain of the prairie vole (e). Quantification of V1a-receptor binding in wild-type mice (open bars), transgenic mice (striped bars) and prairie vole (filled bars) (n = 6–7 per group) demonstrates that receptor density in transgenic mice is not elevated in areas that normally express receptors in mice (for example, lateral hypothalamus (LH), diagonal band (DB) and lateral septum (LS)); nd indicates no detectable specific binding in wild-type mice.

Figure 4a. Response similar...
to that observed in prairie voles (Fig. 1). This dose of AVP, identical to that used in the vole studies, did not change locomotor or autogrooming behaviour. When not in contact with the female, males of both genotypes actively explored the arena. To determine whether the increase in olfactory investigation was specific for a social stimulus, we tested another set of male mice with the same model but substituted cotton balls that had been scented with lemon extract. No genotype (F = 0.129, P > 0.05) or treatment (F = 2.763, P > 0.05) effects were found in olfactory exploration of this new stimulus. In a further test, we used cotton balls scented with bedding from ovariectomized females and still observed no effect of genotype (F = 0.443, P > 0.05) or treatment (F = 0.038, P > 0.05) (Fig. 4b). Thus, the increase in olfactory investigation and grooming after AVP injection in the transgenic mice seemed to indicate an increase in affiliative behaviour rather than a non-specific increase in investigation of new olfactory stimuli. At higher doses, transgenic mice, but not non-transgenic littermates, exhibited a dose-dependent increase in autogrooming behaviour after AVP injection (significant at 100 ng, data not shown).

Our results show that centrally administered AVP increases affiliative behaviour in the monogamous male prairie vole, but not in the montane vole. This acute effect of AVP may be essential for the long-lasting effects of AVP in monogamous mammals, such as the formation of pairbonds1. The species difference in response to AVP is likely to be due to differences in the distribution of V1a-receptor expression in the brain as (1) vole species with similar social organization have similar receptor patterns2, and (2) transgenic induction of the prairie vole pattern of V1a receptors in mice results in a pro-social response to AVP. Although many genes are likely to be involved in the evolution of complex social behaviours such as monogamy, our data indicate that changes in the expression of a single gene can have an impact on the expression of components of these behaviours, such as affiliation. These observations, together with the species diversity in the neuroanatomical distribution of V1a receptors in the brain, indicate that changes in the pattern of V1a-receptor gene expression in the brain may be a common mechanism to alter the behavioural response to AVP, enabling adaptation to changing socioecological situations.

Methods

Animals and behaviour tests. Prairie and montane voles used in this study were laboratory-reared animals derived from field-caught specimens. Transgenic mice were generated by pronuclear injection in FVB/N embryos.

The testing arena comprised two standard mouse cages connected by a tube to allow free movement between the cages. Ovariectomized, conspecific females were used in all tests to eliminate sexual attractiveness as a possible confounding variable. In both vole and mouse experiments, male subjects were lightly anaesthetized with Metofane and injected i.c.v. with 4 μl of either artificial cerebrospinal fluid (CSF) or 2 ng arginine vasopressin dissolved in the same volume of CSF. After a 5-min recovery period, the subjects were placed in the empty cage of the testing arena. Behaviour was recorded for 5 min beginning at the moment when the subject first entered the cage containing the stimulus female. This time line was chosen based on preliminary observations of the duration of other AVP effects, such as autogrooming and locomotion. Affiliative behaviour was defined as olfactory investigation or grooming of the stimulus animal. In the experiments with voles, it was necessary to anaesthetize the female during the test as females typically show some aggregative behaviour during the first few minutes of contact with a male. In the experiments with mice, the females were not anaesthetized but were tethered to one side of the cage by a restrictor. In some experiments, female mice were replaced with cotton balls scented with either 10 μl of lemon extract or with soiled bedding from ovariectomized female cages. All experiments included 5–8 animals per treatment. Duration of affiliative behaviour was analysed using a two-way analysis of variance (ANOVA) with species or genotype and treatment as factors, followed by Fisher’s least significant difference (LSD) post-hoc test.

Molecular analysis. Genomic clones were isolated from EMBL3 libraries derived for montane and prairie vole genomic DNA (Clontech), and sequenced on both strands. Amplification of the 5′ flanking region of the V1a receptor was done using PCR from approximately 100 ng of genomic DNA isolated from the liver of prairie, montane, and meadow pine voles. Ten per cent of the reaction was electrophoresed on a 1.2% agarose gel, transferred onto nylon membrane, and probed with a 32P-labelled fragment of the prairie vole V1a-receptor gene containing the amplified region.

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