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Species Differences in Central Oxytocin Receptor Gene Expression: Comparative Analysis of Promoter Sequences

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Abstract

The distribution of oxytocin binding sites in the brain is highly variable among mammals. Using two species of microtine rodents (voles) with strikingly different patterns of oxytocin binding sites in the brain, we demonstrate that these differences are due to differences in region specific gene expression and not post-translational processing. The distribution of oxytocin receptor mRNA closely resembles the distribution of oxytocin receptor binding sites in both species. Analysis of the 5′ flanking region of the oxytocin receptor gene from both species reveals few differences in potential regulatory elements which could explain the differences in gene expression. These data suggest that species differences in oxytocin receptor binding are due to species differences in: i) distant DNA sequences further upstream or downstream which may influence expression; ii) the distribution of regulatory proteins such as transcription factors in the brain or iii) epigenetic factors, such as prenatal and perinatal environment which may affect gene expression in the adult.

The oxytocin receptor (OTR), which has been recently cloned and sequenced (1, 2), appears to be a member of the large family of G-protein coupled receptors with seven hydrophobic domains expressed in the mammalian brain. In contrast to most members of this family, the OTR may be distinguished by great species variability in its neuroanatomic expression, as measured by radioligand binding. Indeed, each of the 9 mammalian species described thus far shows a unique pattern of OTR regional localization and regulation in the brain (3). As the brain OTR has been implicated in the control of reproductive, maternal, and affiliative behavior (4), these species differences in neural distribution and regulation might contribute to the evolution of species-specific behavior patterns (5). Although previous studies have demonstrated species differences in binding of a selective iodinated ligand (^{125}I-d$(CH_2)_5$[Tyr$(Me)_2$,Tyr-$NH_2{}^9$]OVT, designated ^{125}I-OTA), it remains unclear if these contrasting binding maps reflect i) different receptors encoded by distinct genes but sharing a high affinity for ^{125}I-OTA, ii) different cell specific expression of the same gene due to species differences in transcriptional regulation, or iii) different region specific post-translational processing or neuronal transport.

To address these issues, we studied the OTR gene in two closely related, but behaviorally distinct species of voles: the monogamous prairie vole (*Microtus ochrogaster*) and the promiscuous montane vole (*Microtus montanus*). We have previously reported that these species exhibit striking differences in oxytocin receptor distribution in brain as determined by receptor autoradiography (6). In the first part of the present study, we use RT-PCR to amplify a fragment of the vole OTR to create a probe to determine the neuroanatomical distribution of OTR mRNA in prairie and montane voles. Then, to investigate one possible molecular mechanism which could give rise to mRNA differences, we analyze the 5′ flanking region of the OTR gene of both species to detect species differences in potential regulatory elements in the promoters.

Results and Discussion

The ^{125}I-OTA binding autoradiograms replicated the results previously reported in these species (6). *In situ* hybridization using the uterine derived antisense OTR probe (Fig. 1) resulted in specific labeling of localized neuronal groups while the sense probe resulted in a uniform background. The neuroanatomical distribution of the OTR mRNA expression in both species matched the distribution of ^{125}I-OTA binding sites (Fig. 2). Quantitative analysis of both techniques reveal a significant correlation in both species between ^{125}I-OTA binding sites and OTR mRNA content for each brain region (Table 1): i.e. animals with relatively high levels of ^{125}I-OTA binding in a given area tended to have relatively high levels of mRNA in that region as well. The similarity in the distribution of binding sites and OTR mRNA suggests that in voles, OTR protein remains near the cell body after synthesis rather than being transported to distant regions of the brain via neuronal processes as has been suggested in the rat (7). In the cortex and hippocampus, however, ^{125}I-OTA binding tended to be more diffuse than the mRNA localization suggesting some

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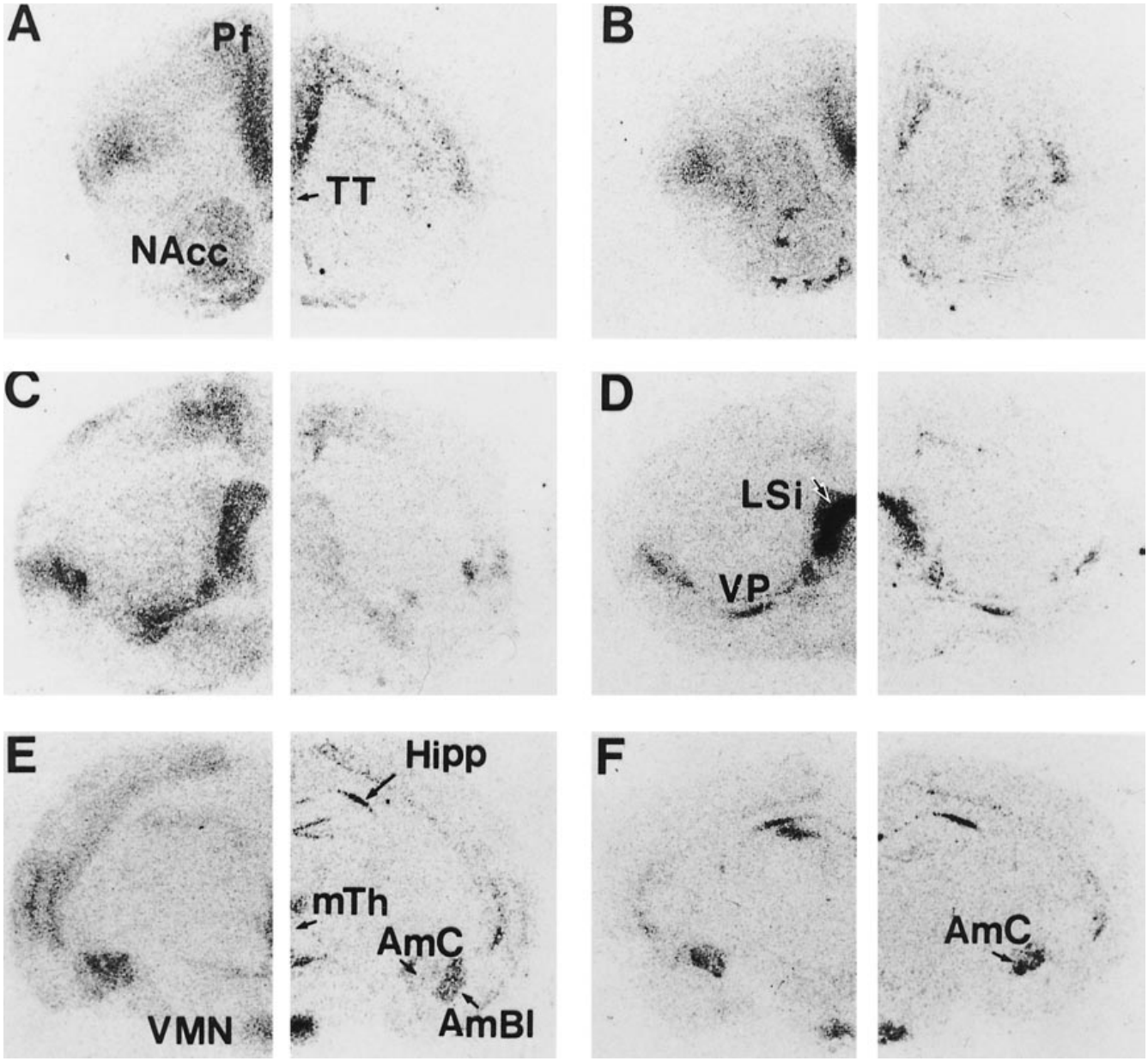

FIG. 2. Comparison of ^{125}I-OTA binding and oxytocin receptor mRNA distribution in prairie vole (A, C, E) and montane vole (B, D, F). Each panel consists of a composite of OTR protein as determined by ^{125}I-OTA binding sites (left hemisphere) and OTR mRNA expression (right hemisphere) in adjacent sections. Notice the similarity in the distribution of binding sites and mRNA. AmBl = basolateral nucleus of the amygdala, AmC = central nucleus of the amygdala, Hipp = hippocampus, LSi = lateral septum (intermediate zone), mTh = midline thalamus, NAcc = nucleus accumbans, Pf = prefrontal cortex, TT = tenea tecta, VMN = ventromedial nucleus of the hypothalamus, VP = ventral pallidum.

been demonstrated to result in species differences in gene expression (13–15), the striking conservation in the immediate 5′ flanking region of the vole OTR gene suggests that proximal promoter variability may not be responsible for the species differences in gene expression in the vole brain. It should be noted that distant regulatory elements located both upstream and downstream of the proximal promoter often play an important role in directing tissue-specific gene expression. For example, sequences up to 13 kb upstream of the transcription start site and 1.5 kb of the 3′ untranslated region are required for correct expression of the vasopressin gene the brain (16). Alternative mechanisms to explain the species specific pattern of gene expression in voles include: i) DNA sequences further upstream or downstream (e.g. intronic or 3′ untranslated regions) differ between the species (17); ii) genetic differences which result in differences in the distribution of regulatory proteins such as transcription factors in the brain; or iii) epigenetic factors, such as prenatal and perinatal environment (e.g. steroid hormones) could affect gene expression in the adult (18, 19).

Inter-genus species differences in OTR expression. Differences in OTR expression between voles and the rat are worth noting. The neuroanatomical distribution of OTR in rat brain as

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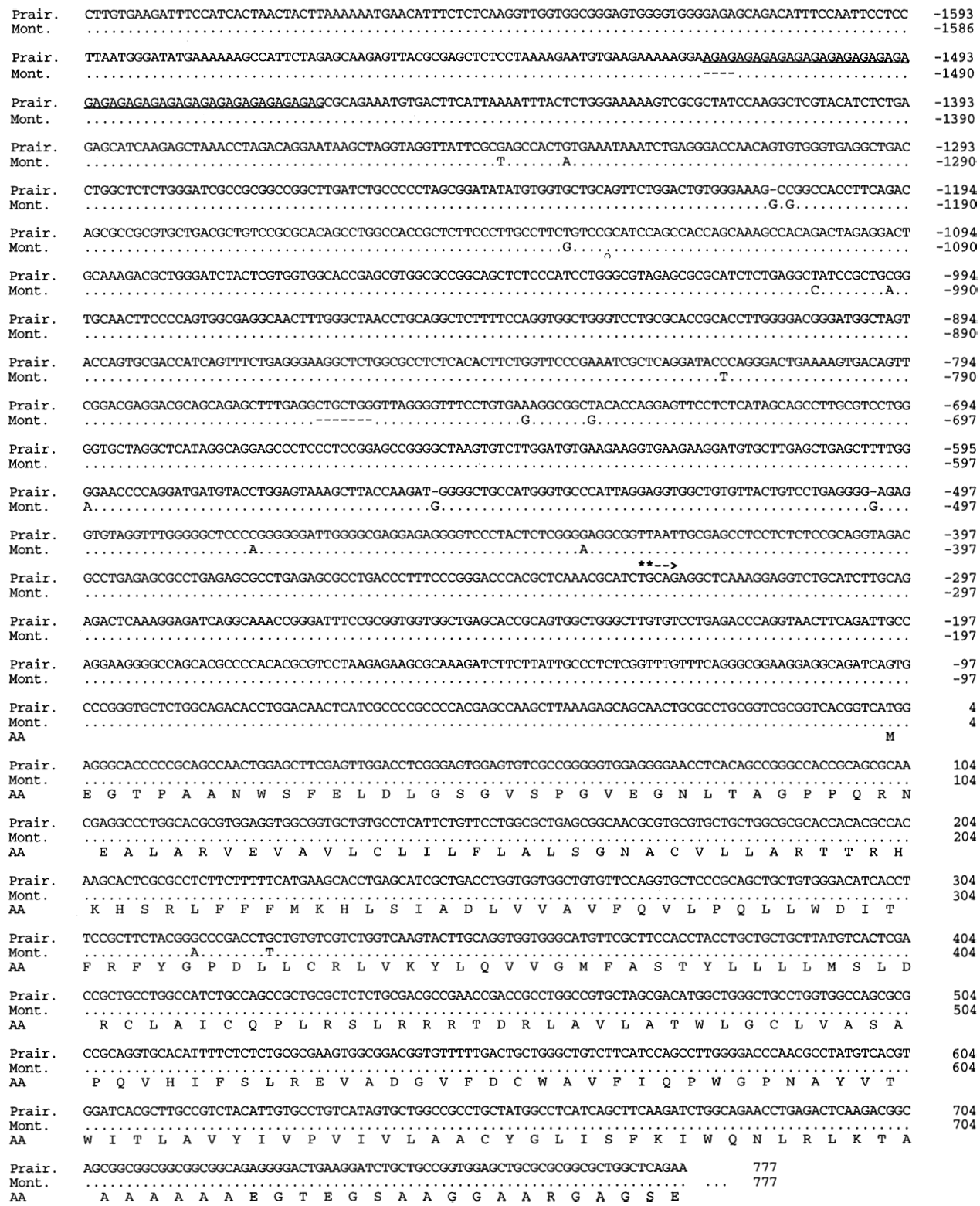

FIG. 4. DNA and deduced amino acid sequence of the prairie vole and montane vole OTR genomic clones. Dots indicate sequences that are identical to the prairie vole sequence while dashes indicate breaks in the alignment due to insertions or deletions. Asterisks are positioned above the sequence corresponding to the transcription initiation site of the rat (2). The microsatellite DNA sequences are underlined.

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