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Distribution of vasopressin and oxytocin binding sites in the brain and upper spinal cord of the common marmoset

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ABSTRACT

The aim of this study was to label selectively and to map central vasopressin (AVP) and oxytocin (OT) binding sites in the common marmoset. [^{125}I]VPA, a compound selective in rodents and human for the AVP V_{1a} receptor, yielded the same labeling pattern as [^{3}H]AVP, thus suggesting that most AVP receptors present in the marmoset brain are of the V_{1a} subtype. Numerous areas exhibited AVP binding sites, among which the olfactory bulb, the accumbens nucleus, the bed nucleus of the stria terminalis, the hypothalamic suprachiasmatic, arcuate and ventromedial nuclei, the medial amygdaloid nucleus, the nucleus of the solitary tract and the cerebral cortex. Binding sites for [^{125}I]OTA, a selective OT receptor antagonist in rat and human, were markedly less abundant than [^{125}I]VPA ones, and, to a few exceptions, expressed in different areas. Neither AVP, nor OT binding sites were detected in the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei identified by neurophysin immunoreactivity. Marked species-related differences were observed in the distribution of both AVP and OT binding sites. Altogether, our data provide a morphological basis to investigate the function of central AVP and OT in the marmoset.

The distribution of central receptors for vasopressin (AVP) and oxytocin (OT) displays remarkable species-related differences [6,11–13,17–19,23,25,32,38–41,43,45,48]. This is consistent with results of recent studies which demonstrate that both peptides are involved in the regulation of complex social and affective behaviors showing a high degree of variability from one species to the other [4,8,10,20,24,29,35,44–47]. Interestingly, some evidence suggests that AVP and OT may play a critical role in the establishment of social and affective bonds in human as well [5,21,22,31,42]. The aim of the present study was to provide comprehensive and comparative maps of central AVP and OT binding sites in the common marmoset (*Callithrix jacchus*). The marmoset, commonly used as a model of human physiology and disease, has a complex behavioral repertoire [1,28] and may therefore represent a useful model to investigate the respective behavioral roles of AVP and OT. AVP and OT binding sites – presumably receptors [36] – were detected using in vitro receptor binding autoradiography.

Six unfixed brains were provided to us already frozen by the Anthropology Institute of Zurich, Switzerland and by the INSERM Unit 816, Lilles, France. Three were from adult males, 3, 5 and 7 years old; 1 from an 11-year-old adult female; 2 from young adults [28], a 12-month-old male and a 14-month-old female. Housing conditions and procedures for euthanasia were in accordance with NIH guidelines for animal research. Series of alternate coronal sections, 14 μm thick, were cut, mounted on chrome-alum-gelatine-coated slides and stored at −80 °C until use.

The binding procedure was performed as previously described [37]. AVP binding sites were labeled with the radioiodinated V_{1a} receptor antagonist HO-Phaa^{1}-*D*-Tyr(Me)^{2}-Phe^{3}-Gln^{4}-Asn^{5}-Arg^{6}-Pro^{7}-Arg^{8}-NH_2 (^{125}I-VPA) [3] at 0.02 nM and tritiated AVP (^{3}H-AVP, PerkinElmer Life Sciences, Boston, MA, USA) at 2 nM; OT ones with the radioiodinated OT antagonist d$(CH_2)_5$[Tyr(Me)^{2},Thr^{4},Tyr^{9}-NH_2]OVT (^{125}I-OTA) [14] at 0.02 nM. The selectivity profile of ^{125}I-VPA and ^{125}I-OTA in marmoset brain tissue was investigated by using [Thr^{4},Gly^{7}]OT, a selective OT receptor agonist [2,27], and [Phe^{2},Orn^{8}]VT, a V_1 selective agonist [2,27], as competitors. According to results of these competition studies, ^{125}I-VPA and ^{125}I-OTA were subsequently used with respectively 75 nM [Thr^{4},Gly^{7}]OT and 20 nM [Phe^{2},Orn^{8}]VT. ^{3}H-AVP which has a similar high affinity for V_{1a}, V_{1b} and OT receptors was used with 20 nM [Thr^{4},Gly^{7}]OT to prevent its binding to OT receptors. Non-specific binding was determined in the presence of 2 μM AVP or OT. Sections were apposed to βmax hyperfilms (Amersham, Buckinghamshire, UK) for 1–4 days or tritium-sensitive hyperfilms (Amersham) for 3 months, then stained with Cresyl Violet. Labeled structures were identified according to Stephan et al. [34] and Paxinos and Watson [30].

Some hypothalamic sections used to generate autoradiograms were fixed for 20 min in 4% paraformaldehyde in 0.1 M PBS (pH 7.4),

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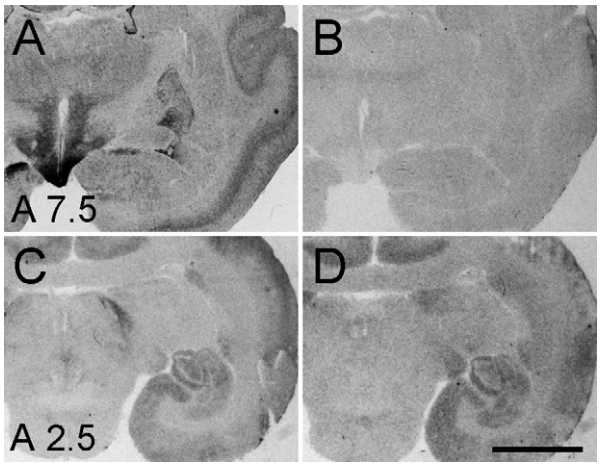

Fig. 1. Specific and non-specific binding of [^{125}I]VPA and [^{125}I]OTA. (A and B) Adjacent sections incubated with 0.02 nM [^{125}I]VPA alone (A) or with 2 μM AVP (B); (C and D), adjacent sections incubated with 0.02 nM [^{125}I]OTA alone (C) or with 2 μM OT (D). A 7.5 and A 2.5: antero-posterior levels [34]. Bar: 5 mm.

rinsed in PBS and incubated 24 h with an anti-neurophysin serum (AB948, Chemicon, Temecula, CA, USA) diluted at 1:5000 in PBS containing 0.3% Triton X-100. Immunostaining was performed by using the ABC-Elite kit (Vector, Burlingame, CA, USA).

Non-specific binding was low with ^{125}I-VPA (Fig. 1 A and B) and ^{3}H-AVP (not illustrated), higher with ^{125}I-OTA, particularly in gray matter (Fig. 1C and D). Preliminary experiments revealed that ^{125}I-OTA binding sites were labeled as well with ^{125}I-VPA (Fig. 2A and C). However, ^{125}I-VPA could be displaced, in regions labeled by both radioligands, by low concentrations of [Thr^4,Gly^7]OT (Fig. 2D and E), which indicates that ^{125}I-VPA binds to OT binding sites. Consequently, to enhance their receptor subtype selectivity [9,27], ^{125}I-VPA and ^{125}I-OTA were thereafter used with [Thr^4,Gly^7]OT and [Phe^2,Orn^8]VT respectively. Fig. 3 describes the distribution of ^{125}I-VPA and ^{125}I-OTA binding observed in these conditions. ^{3}H-AVP (in the presence of [Thr^4,Gly^7]OT) yielded the same labeling pattern as ^{125}I-VPA (not illustrated). Results obtained were similar in the 6 brains investigated.

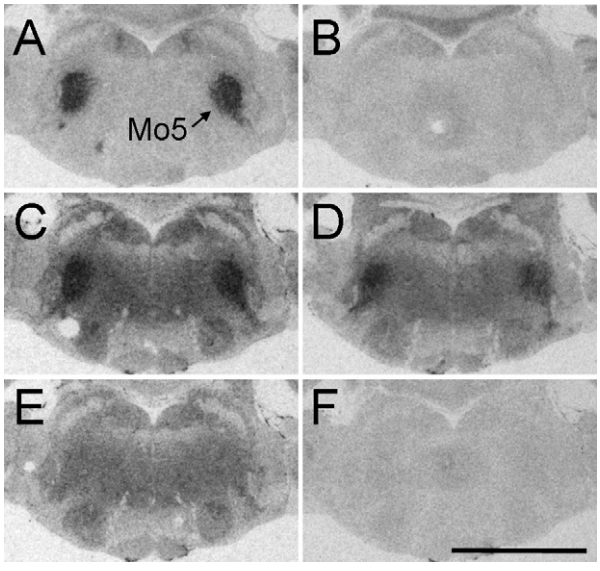

Fig. 2. Binding of [^{125}I]VPA to OT binding sites in six adjacent sections of the motor trigeminal nucleus (Mo5). (A) 0.02 nM [^{125}I]OTA alone; (B) 0.02 nM [^{125}I]OTA and 2 μM OT; (C) 0.02 nM [^{125}I]VPA alone; (D) 0.02 nM [^{125}I]VPA and 20 nM [Thr^4,Gly^7]OT; (E) 0.02 nM [^{125}I]VPA and 75 nM [Thr^4,Gly^7]OT; (F) 0.02 nM [^{125}I]VPA and 2 μM AVP. Bar: 5 mm.

Dense ^{125}I-VPA binding was found in the olfactory bulb (Fig. 3A, A 17 and A 15), Islands of Calleja (Fig. 3A, A 11), bed nucleus of the stria terminalis (Fig. 3A, A 10 and A 8.5), hypothalamic suprachiasmatic nucleus (Fig. 3A, A 10), medial amygdaloid nucleus (Fig. 3A, A 10–A 8), hypothalamic ventromedial nucleus (Fig. 3A, A 8 and A7), hypothalamic arcuate nucleus (Fig. 3A, A 8 and A7), nucleus of the solitary tract (Fig. 3A, P3) and choroid plexus (Fig. 3A, A 8–A 2.5). Labeling was slightly less intense in the nucleus accumbens (Fig. 3A, A 12.5), diagonal band of Broca (Fig. 3A, A 11 and A10), a restricted area of the lateral septum (Fig. 3A, A 11), anterodorsal preoptic nucleus (Fig. 3A, A 10), basal nucleus of Meynert (Fig. 3A, A 10–A 8) and globus pallidus (Fig. 3A, A 10–A 8). Layer 6 of cerebral cortex was moderately labeled (Fig. 3A, A 12.5–AP 0). Diffuse ^{125}I-VPA binding slightly above non-specific levels was found in central gray of the brainstem (Fig. 3A, A 2.5–P 4) and spinal cord (Fig. 3A, C1).

^{125}I-OTA labeling was most intense in the accumbens nucleus (Fig. 3B, A 12.5), limitans thalamic nucleus (Fig. 3B, A 2.5), motor trigeminal nucleus (Fig. 3B, AP 0), sustantia gelatinosa of the spinal trigeminal nucleus (Fig. 3B, P 4) and of the spinal cord (Fig. 3B, C1). Moderate labeling was found in a restricted area of the caudate putamen (Fig. 3B, A 11), the caudal part of the diagonal band of Brocca (Fig. 3B, A 10 and A 8.5), superficial layer of the superior colliculus (Fig. 3B, A 0.5), inferior olive nucleus (Fig. 3B, A 0.5) and dorsal motor nucleus of vagus (Fig. 3B, P 3).

^{125}I-VPA and ^{125}I-OTA binding levels were not above non-specific ones in the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei (Fig. 4).

First described as ligands selective respectively for V_{1a} and OT receptors in the rat [2,3,14], ^{125}I-VPA and ^{125}I-OTA have since been used extensively in other species. However, we found that ^{125}I-VPA has not the same receptor selectivity in the marmoset since it labeled OT binding sites nearly as efficiently as ^{125}I-OTA (Fig. 2). This observation emphasizes the need to be cautious when extrapolating pharmacological data obtained in one species to other ones [9,27]. Using ^{125}I-VPA and ^{125}I-OTA in the presence of analogues selective for OT or AVP receptors has allowed us to discriminate OT and AVP binding sites in marmoset brain sections and to establish their comparative maps. ^{3}H-AVP and ^{125}I-VPA yielded the same labeling pattern which suggests that central AVP receptors are predominantly of the V_{1a} subtype in the marmoset.

AVP binding sites were found in numerous structures, most of those previously reported by Wang et al. [43], and in addition in the anterior olfactory nucleus, islands of Calleja, nucleus of the solitary tract, cerebral cortex and choroid plexus. OT binding sites were strikingly less abundant than AVP ones and distributed in different areas, to the exception of accumbens nucleus and nucleus of diagonal band of Broca. Of particular interest is the similar absence in marmoset and human [25] of ^{125}I-OTA binding in regions rich in OT receptors in most rodents, for instance amygdala, where OT receptor activation facilitates social recognition in rat [7] and mouse [15]. Interestingly, in the human also OT exerts prosocial effects by modulating amygdala activity [5,21,31]. In the latter studies however, the receptor involved was not characterized pharmacologically, and OT has possibly activated V_{1a} receptors [25].

Our results do not support the presence of AVP or OT receptors in the magnocellular nuclei SON and PVN. Although data obtained in the rat suggest that locally released AVP and OT regulate magnocellular neuronal activity [26], AVP and/or OT receptor expression by these neurons is still a subject of controversy [33,45]. Interestingly, OT binding sites could be detected in the PVN and SON of lactating female rats following intra-cerebro-ventricular injection of a selective OT receptor antagonist which probably induced the up-regulation of OT receptors [16]. The expression of AVP and/or OT receptors in magnocellular nuclei is possibly under constant down-regulation by locally released AVP and OT, therefore making their detection hazardous.