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The Vasopressin 1b Receptor is Prominent in the Hippocampal Area CA2 Where It Is Unaffected by Restraint Stress or Adrenalectomy

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Abstract

The vasopressin 1b receptor (Avpr1b) is one of two principal receptors mediating the behavioral effects of vasopressin (Avp) in the brain. Avpr1b has recently been shown to strongly influence social forms of aggression in mice and hamsters. This receptor appears to play a role in social recognition and motivation as well as in regulating the hypothalamic-pituitary-adrenal axis. Most of these studies have been performed in knockout mice, a species in which the localization of the Avpr1b has not been described, thus precluding correlations with the behaviors. We performed *in situ* hybridization histochemistry (ISHH) with specific probes and found especially prominent expression within the CA2 pyramidal neurons of the hippocampus, with much lower expression in the hypothalamic paraventricular nucleus and amygdala. Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) confirmed expression in those as well other areas in which the ISHH was not sensitive enough to detect labeled cells (e.g., piriform cortex, septum, caudate-putamen and lower brainstem areas). Mouse Avpr1b transcript levels were not altered in the CA2 field by restraint stress or adrenalectomy. Finally, ISHH and RT-PCR showed expression of the *Avpr1b* gene in the rat and human hippocampi as well. We suggest that the CA2 field may form or retrieve associations (memories) between olfactory cues and social encounters.

Keywords

social memory; aggression; corpora amyloacea; paraventricular nucleus

Abbreviations

Avp, vasopressin; Avpr1a, vasopressin 1a receptor; Avpr1b, vasopressin 1b receptor; ISHH, *in situ* hybridization histochemistry; Oxt, oxytocin receptor; PVN, paraventricular nucleus of the hypothalamus; RT-PCR, reverse-transcriptase-polymerase chain reaction

In species ranging from molluscs to mammals, the neuropeptide vasopressin (Avp) or its homologs participate in the regulation of social behavior (Grober and Sunobe, 1996; Albers and Bamshad, 1998; Dantzer, 1998; Godwin et al., 2000; Bester-Meredith and Marler, 2001; Goodson and Bass, 2001; Pitkow et al., 2001; Semsar et al., 2001). In mammals, aggression, affiliative behavior, and social recognition are regulated by Avp. Avp and related compounds

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have also been shown to play a role in memory (de Wied, 1965; Bohus et al., 1972; de Wied et al., 1991). Pharmacological (Ferris et al., 1985; Ferris et al., 1988; Bluthé and Dantzer, 1992; Engelmann et al., 1992; Alescio-Lautier et al., 1995; Bamshad and Albers, 1996; Dantzer, 1998; Dlužen et al., 1998) and more recent genetic (Bielsky et al., 2004; Egashira et al., 2004) manipulations have implicated the vasopressin 1a receptor (Avpr1a) in the regulation of some of these behaviors.

There is, however, mounting evidence that the vasopressin 1b receptor (Avpr1b) is also an important regulator of social behavior. Analysis of the Avpr1b knockout mouse indicates that this receptor regulates aggression and social memory (Wersinger et al., 2002; Wersinger et al., 2004). Pharmacological antagonism of the Avpr1b produces reduced anxiety and aggression (Griebel et al., 2002; Blanchard et al., 2005; Stemmelin et al., 2005). To date, no radiolabeled ligand has been used to map the distribution of the Avpr1b in any species.

Immunohistochemical studies in rats suggest that the Avpr1b has a limited regional distribution and is expressed at lower levels than the Avpr1a (Hernando et al., 2001). Interestingly, Avpr1b-immunoreactivity in rat is found in regions involved in the regulation of social behavior, including the olfactory tubercle, taenia tecta, cingulate cortex, amygdala and medial preoptic area (Hernando et al., 2001).

ISHH has been used to map the distribution of Avpr1b transcripts in the rat pituitary (Lolait et al., 1995) and brain (Vaccari et al., 1998), but that probe has significant sequence identities with the Avpr1a and oxytocin receptor (Oxtr). Two recent studies using oligonucleotide probes showed significant expression in Avp magnocellular neurons in the rat (Hurbin et al., 1998; Hurbin et al., 2002). However, the wash conditions, given the lengths of the probes, were not appropriately stringent. These shortcomings open the possibility of locations inadvertently assigned as sites of Avpr1b expression. For this reason, we generated more specific Avpr1b probes for the mouse (and rat and human) to examine the distribution of this receptor's transcripts more accurately by both ISHH and RT-PCR.

EXPERIMENTAL PROCEDURES

Animals

Adult C57BL/6J and 129/P3J strains of mice were obtained from the Jackson Laboratory (Bar Harbor, ME). Avpr1b $+/+$ and $-/-$ mice of mixed background (C57BL/6J and 129/SvJ) were created as described previously (Wersinger et al., 2002) and bred in our own colony. Six adrenalectomized and six sham-operated C57BL/6 mice were obtained from Taconic (Germantown, NY). Their brains were removed 7 days after surgery. Adult Sprague-Dawley rats were also obtained from Taconic.

All animals were given food and water *ad libitum*. The adrenalectomized mice were also supplied with 0.9% saline. All animal procedures were approved by the NIMH Animal Care and Use Committee and were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996).

For ISHH (see below), three C57BL/6J, two 129/P3J and 3 mixed C57BL/6J-129/SvJ mice were sectioned through the entire extent of the neuraxis from the medulla to olfactory bulbs. Another C57BL/6J and a mixed mouse were examined throughout the extent of the hippocampus. These series sampled sections every 100 μm . Over 20 C57BL/6 mice were examined at the level of the dorsal hippocampus, from approximately 1 to 2.5mm behind the bregma. Two Sprague-Dawley rats were serially sectioned from the medulla to the olfactory bulbs as well.

Human tissues

Hippocampal tissue blocks and samples were obtained from the Human Brain Tissue Bank (Budapest, Hungary). One was from a 36 year-old female and the other from a 50 year-old male. An isolated punch sample for RT-PCR was obtained from a 35 year-old male. None of these subjects had a known neurological disease and the tissues were obtained within 2 hours of death. After being removed from the skull, brains were rapidly frozen, dissected and the samples were stored at -70°C until use. The protocol of tissue sampling and retrospective assessments was approved by the Institutional Review Board of the Semmelweis University, Budapest. A pituitary from a 63 year-old male with a 16 hour post-mortem interval was obtained from the NIMH Human Brain Collection. The patient was an alcoholic with no known neuropathology.

In situ hybridization histochemistry

ISHH was performed as described (Bradley et al., 1992; Young and Mezey, 2006). Briefly, rodent brains and pituitaries were removed and frozen on powdered dry ice. They were then stored at -80°C until $12\mu\text{m}$ sections were cut at -15°C and thaw-mounted onto SuperFrost Plus slides (VWR, Batavia, IL). Sections from human samples were cut at a thickness of $16\mu\text{m}$. They were then stored at -80°C until warmed to room temperature, fixed in 4% formaldehyde in phosphate-buffered saline, treated with acetic anhydride and defatted through a series of alcohols. Radiolabeled riboprobe was applied to the sections for 20–24 hours at 55°C before treating with RNase and stringent washes at 65°C .

The sections were apposed to phosphorimaging plates for up to 2 weeks before scanning in a Cyclone system (Perkin-Elmer, Boston, MA). Sections for quantitation were analyzed using the Cyclone software. Image intensities were well within the linear range of the phosphorimaging system. Results were analyzed using unpaired t-tests (InStat, GraphPad Software, San Diego, CA). Most slides were subsequently coated with Ilford K.5D (Polysciences, Warrington, PA) or Kodak NTB (Rochester, NY) nuclear emulsions and exposed for up to 4 months before developing and staining with 0.2% toluidine blue.

Riboprobes

Two separate riboprobes were designed for use in mice to avoid any cross reactivity with related receptor sequences, especially the Avpr1a and Oxtr. A 5' probe that targeted bases 628–825 of the mouse Avpr1a mRNA (GenBank accession #NM_011924) was used initially and then a longer 3' probe was made and used for the majority of ISHH that targeted bases 1635–2057. Analogous 3' probes targeted bases 1549–1982 of the rat receptor (GenBank accession #U27322) and 1318–1824 of the human receptor (GenBank accession #D31833).

A rat corticotropin-releasing factor (Crh) probe that targeted bases 223–720 of the mouse mRNA (97% sequence identity, GenBank Accession #NM_205769) was used in adjacent sections from the mice that underwent restraint stress or adrenalectomy to gauge the effectiveness of the treatments. All probes, including sense versions, were labeled using ^{35}S -UTP and the appropriate RNA polymerases.

Reverse Transcription-Polymerase Chain Reaction

Total RNA was isolated from tissues using the RNeasy Kit (Qiagen, Valencia, CA) and stored frozen in water at -20°C until used. 100 ng of RNA were used in each assay. The mouse Avpr1b primers were GCTGGCCCAAGTCCTCATCTTCTG and GCGGTGACTCAGGGAACGT producing a 322 bp product from mRNA (and 2095 bp from genomic DNA). To control for RNA loading, the mouse samples were checked using primers against b-actin (CCAGGTCATCACTATTGGCAACG and CTCAGGAGGAGCAATGATCTTGA) that

produced a 266 bp product from mRNA (and 343 bp from genomic DNA). This was done by distributing the samples among a set of wells in 10 μ l (containing 200 μ g) and then removing 5 μ l for another set of wells in the same plate. An RT-PCR reaction mix was made, divided in two and, after the appropriate primer pairs were added, aliquoted to the RNA samples. The reactions were subsequently run on the same gel from parallel wells. The rat primers were GCGGTGCTCACAGCTTGCTACGG and CTGTTGAAGCCCATGTAGATCCAG and produced 384 bp and 9083 bp products from mRNA and genomic DNA, respectively. The human primers were GGCCCTCACCTTCCACCTTAGCTG and CTGTTGAAGCCCATGTAGATCCAG and produced 283 bp and 5721 bp products from mRNA and genomic DNA, respectively. The reactions were run using the Invitrogen One-Step RT-PCR system (Carlsbad, CA) at 50°C $\times$ 30 min, 94°C $\times$ 2 min, (94°C $\times$ 45 s, 55°C $\times$ 45 s, 72°C $\times$ 30 s) for 40 cycles, and 72°C $\times$ 5 min. The samples were then run on 1.5% agarose gels or 2% NuSieve (FMC BioProducts, Rockland, ME) gels.

Restraint stress

Six C57BL/6J mice were restrained in DecapiCones (Braintree Scientific, Braintree, MA) for one hour. They were then returned to their cages for another three hours at which time their brains were rapidly removed. Five control C57BL/6J mice were left in their cages until their brains were removed.

RESULTS

The mouse, rat and human Avpr1b riboprobes were tested first on the respective pituitaries and showed good cellular labeling (Fig. 1). The brain distributions of Avpr1b transcripts were the same in C57BL/6J and 129/P3J strains or in the mixed backgrounds that the Avpr1b $-/-$ mice were maintained on, with either the 5' or 3' probe. In the brain, labeling of the dorsal hippocampal CA2 pyramidal cells was the highest (Fig. 2) at 20–40 grains per 100 μ m² after a 4 month exposure. In fact, at the level of resolution of the phosphorimager, the only labeling within the brain that was seen is in this portion of hippocampus. In the most rostral coronal sections of the hippocampus, pyramidal cell neurons of the medial portion of the CA2 that is separated by the CA1 field were also labeled. The lateral portion of CA2 pyramidal cell layer has a sharp medial border of expression with the CA1 field. However, some labeled pyramidal cells “bleed” into the CA3 field at that interface. At approximately 2.5mm behind the level of the bregma and further caudal, the labeling disappeared (about half-way in the rostral-caudal extent of the hippocampus and roughly one third of the CA2 pyramidal cells) leaving the ventral hippocampus unlabeled.

Only 1–3 neurons were ever labeled in the dorsomedial subdivision of the paraventricular nucleus (PVN), and only 2 neurons total were found in the anterior amygdaloid area in our series (Fig. 3). No labeling was detected with the sense probe (Fig. 2). After 4 month exposures to nuclear emulsion, we generally found between 6–12 grains per 100 μ m² for PVN neurons. Background (including over CA1 and CA3 neurons) was less than 1 per 100 μ m². Some sections from Avpr1b $-/-$ mice were probed initially using the shorter 5' probe and no labeling was observed in those sections (data not shown).

RT-PCR was also used to confirm and extend the ISHH results (Fig. 4). In agreement with the ISHH, Avpr1b expression was present in mouse tissue from the hippocampal formation (dorsal and ventral combined) and hypothalamus. However, RT-PCR also revealed expression in the piriform cortex, caudate-putamen, septum, midbrain, pons, cerebellum and medulla. Faint bands were observed with total RNA from the parietal cortex and amygdala. No expression was detected in the vomeronasal organ (3 different animals), olfactory bulb (2 different animals), thalamus, cervical spinal cord, trigeminal ganglion, adrenal, kidney, or liver.

One hour of restraint stress yielded no change in the levels of Avpr1b transcripts in the CA2 field when examined three hours later (3744 ± 495 units $\pm$ S.E.M. for the control vs. 3712 ± 712 for the restrained). This contrasts with the 56% elevation of Crh transcripts in the PVN ($11,959$ units $\pm$ 849 S.E.M. for the control vs. $18,614 \pm 1865$ for the restrained; $p=0.011$, $df=11$).

Similarly, one week after adrenalectomy, there was no change in the levels of Avpr1b transcripts in the CA2 field (5669 ± 520 units $\pm$ S.E.M. for the control vs. 5906 ± 762 for the adrenalectomized). Again, this contrasts with the 151% elevation of Crh transcripts in the PVN (7946 units $\pm$ 529 S.E.M. for the control vs. $19,929 \pm 4612$ for the operated; $p=0.024$, $df=12$).

Human and rat hippocampi were also studied to look for evolutionary conservation at this site of expression. As noted above, the rat and human pituitaries, as controls, were positive by ISHH (Fig. 1) and RT-PCR (data not shown) as expected. In the rat, labeled pyramidal cells were prominent in the CA2 field just as in the mouse but reduced labeling seemed to persist further into the CA3 field than in the mouse (Fig. 5). Labeled neurons were also present in the human hippocampal CA2 and CA3 fields, but more sparsely (Fig. 6A). Further, they were generally located adjacent to the pyramidal cells in the stratum radiatum and less often in the pyramidal cell layer. Interestingly, the majority of labeled human neurons contained corpora amylacea (Fig. 6B) as identified by their intense periodic acid Schiff (PAS) staining (data not shown). RT-PCR confirmed their presence in the rat and human hippocampi (data not shown).

DISCUSSION

The present study revealed expression in the mouse by ISHH in a much more restricted distribution than we had anticipated, being especially prominent in the CA2 field of the hippocampus. This result contrasts with the previous results in the rat using a riboprobe (Vaccari et al., 1998). The discrepancies are not likely due to species differences as the CA2 pyramidal layer was the only region we observed labeled in the rat (Fig. 5). In fact, using a mouse probe analogous to the original rat probe gave a more extensive signal in the mouse brain and only the CA2 field labeling was greatly reduced in sections from Avpr1b $-/-$ mice (data not shown). It is likely that this additional labeling results from cross-hybridization with the Avpr1a and Oxtr mRNAs in the case of the original riboprobe as it has significant stretches of identity with those receptors (vs. Avpr1a: 123 total bases with 87% identity; vs. Oxtr: 108 total bases with 85% identity). Even with our longer, more heavily labeled riboprobes, we failed to see expression of the Avpr1b in the magnocellular neurons of the supraoptic nucleus as reported with the oligonucleotide probes (Hurbin et al., 1998; Hurbin et al., 2002). Examination of the sequences used by NCBI Blast (<http://www.ncbi.nlm.nih.gov/BLAST>) and IDT SciTools OligoAnalyzer 3.0 (<http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/Default.aspx>) revealed numerous sequences potentially recognized by the oligonucleotide probes at the wash stringency employed (165mN Na^+ at 45°C) that might have contributed to the ISHH signal. We did find a few labeled neurons in the PVN. Their locations suggest that they may be parvocellular neurons and studies are in progress to examine this issue and to see whether their expression increases after stress or adrenalectomy.

It is worth commenting that the RT-PCR reveals more areas of expression than the ISHH, the most parsimonious explanation being that very low levels of expression are not detected by the ISHH. That there are areas in which we could not detect cells by ISHH but have been reported to contain protein in rat (Hernando et al., 2001; e.g., parietal cortex, cerebellum) and mRNA by RT-PCR (this study) suggests that the Avpr1b is very stable requiring little ongoing synthesis to maintain adequate receptor levels. The adrenal gland, where we do not find Avpr1b mRNA, has been reported to have (Grazzini et al., 1998) and not have (Lolait et al., 1995) the Avpr1b transcripts by RT-PCR in the rat. This may reflect strain and species differences.

The relatively high expression of Avpr1b in the CA2 field compared to the rest of the hippocampus (and brain) is, to our knowledge, unique. Numerous substances are seen to be more concentrated or absent in the CA2 field or have abrupt boundaries between the edge of CA2 field and either CA1 or CA3 fields (for example, refs. (Lein et al., 2004) and (Lein et al., 2005)). Unlike the CA3 field, the CA2 field does not receive a rich mossy fiber input and CA2 pyramidal neurons lack the thorny excrescences that are characteristics of CA3 pyramidal neurons (Lorente de Nó, 1934; Tamamaki et al., 1988).

The intrahippocampal connections of CA2 pyramidal cells are somewhat characteristic as they give rise to a projection to the CA1 field that may serve to augment that from CA3 pyramidal neurons (Ishizuka et al., 1990; Sekino et al., 1997). Collaterals of CA2 axons distribute to the polymorphic layer of the dentate gyrus. Commissural fibers give off branches to the ipsilateral septum (triangular and lateral nuclei) before terminating primarily in the stratum oriens of the contralateral hippocampus (Tamamaki et al., 1988).

The CA2 field receives a direct innervation by the perforant path fibers from neurons of the entorhinal cortex (Bartesaghi and Gessi, 2004). In addition, it receives prominent neuronal inputs from the posterior hypothalamus, particularly substance P innervation from the supramammillary nucleus (Borhegyi and Leranth, 1997). Although vasopressin is consistently found in the dorsal hippocampus and its levels are influenced by physiology (Landgraf et al., 1991), no known direct innervation by vasopressinergic neurons has been described; therefore, Avp might reach the hippocampus by extracellular fluid flow (Herkenham, 1987). Alternatively, the Avpr1b may be transported to the axon terminals in the lateral septum where vasopressin innervation (De Vries and Buijs, 1983; Caffé et al., 1987) could affect neurotransmission and behavior.

There is evidence for distinguishing features of the CA2 field relative to the rest of the hippocampus. For example, the perforant pathway provides direct innervation to the CA2 field (Bartesaghi and Gessi, 2004), bypassing the granule cell layer. Also, a relative resistance to CA2 pyramidal cell loss that has been noted in human head trauma (Maxwell et al., 2003) and intractable epilepsy (Fried et al., 1992; Mathern et al., 1995; El Bahh et al., 1999) and, for example, in some (Kirino and Sano, 1984; Sadowski et al., 1999), but not all (Schmidt-Kastner and Freund, 1991), models of ischemia. Similarly, there is protection provided by the more persistent down-regulation of the N-methyl-D-aspartate (NMDA) receptor following transient ischemia (Yamashita et al., 2003) in the CA2 field.

A potential role for the CA2 field in social behavior is suggested by our studies of the Avpr1b knockout mouse. This knockout mouse has deficits in social forms of aggression, recognition and motivation, but not in spatial memory (Morris water maze), prey recognition, or thirst-motivated drinking (Wersinger et al., 2002; Wersinger et al., 2003; Wersinger et al., 2004). Perhaps this particular hippocampal field helps to either form or retrieve memories of social encounters, much as other hippocampal pyramidal cells establish memories of place (O'Keefe and Dostrovsky, 1971). As discussed above, the CA2 field receives a direct input from the entorhinal cortex, a region that processes individual social odors (Petrulis et al., 2005). Place cell firing is modulated by context conferred by simple sensory stimuli, including olfactory (Anderson and Jeffery, 2003), as well as by more complex situations, such as task demand (Smith and Mizumori, 2006) and motivation (Kennedy and Shapiro, 2004). Our results with the Avpr1b knockout mouse could be explained if each encounter failed to associate context from the social experience (e.g., social odor inputs from the entorhinal cortex to the CA2 field were not assimilated). This would be consistent with our previous suggestion that the Avpr1b serves to couple the incoming olfactory stimuli with the appropriate social response (Wersinger et al., 2004). Thus, normally, olfactory stimuli may become an integral part of an event representation and may stimulate retrieval of a previous social memory (e.g., that an intruder

male is a threat) in the hippocampus. Of course, non-olfactory sensory input may play lesser or greater roles in this situation depending on the species. Maaswinkel and colleagues, who have shown decreased social investigation and recognition in fimbria-transected rats (Maaswinkel et al., 1996), have posited a very similar role for the hippocampus (Maaswinkel et al., 1997).

In contrast to the paucity of studies of CA2 field, the role of the Avpr1b in regulating adrenocorticotropin hormone from the anterior pituitary in response to stress, especially chronic, has been extensively studied (for reviews, see (Engelmann et al., 2004; Volpi et al., 2004)) ever since the receptor was first described (Antoni et al., 1984). The expression of the receptor in anterior pituitary rises in response to an acute restraint stress (Rabadan-Diehl et al., 1995) but recovers from an initial decrease six days after adrenalectomy (Rabadan-Diehl et al., 1997). We sought to determine whether or not the expression of Avpr1b in the CA2 field of the mouse was similarly affected, but there were no changes after acute restraint stress or one week after adrenalectomy in the CA2. While not ruling out a role for the CA2 Avpr1b in stress, these findings and preliminary unpublished data showing an increase in expression in paraventricular neurons of the hypothalamus after stress are consistent with its more direct role within the hypothalamo-pituitary axis.

Finally, the presence of Avpr1b mRNA in the CA2 field of three species, including human, leads us to believe that whatever role that it serves there is likely to have been conserved throughout evolution. Continued studies of the receptor in the CA2 field using conditional knockout and selective lesions hold the promise that insights gained from rodents might be applicable to humans. Two key questions should be answerable using these more selective approaches: whether the defects in social behavior we have observed in the Avpr1b knockout mice are related to the CA2 field and whether the phenotype is the result of the absence of the receptor during development. Recent use of an Avpr1b antagonist suggests that at least the lack of aggression may not be of developmental origin (Blanchard et al., 2005).

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Figure 1.

Avpr1b transcripts are present in cells of the mouse (A), rat (B) and human (C) anterior pituitaries as revealed using the 3' probes. The mouse and rat photomicrographs are brightfield and the human photomicrograph darkfield illuminations. The 3' probe was used for this ISHH and exposures were for 1 month.

Figure 2.

In mice, brightfield (A,C,D) and darkfield (B,E) photomicrographs showing *Avpr1b* transcripts in the CA2 pyramidal cells of the far rostral (~1.1 mm behind the bregma; A,B) and slightly more caudal dorsal (~2.0 mm behind the bregma; C–F) hippocampus in coronal sections. Panels C and F show higher magnification of CA2 pyramidal cells from adjacent sections probed with antisense and sense probes, respectively. The 3' probe was used for this ISHH and exposures were for 4 months. The small arrows show the CA2–CA3 pyramidal cell borders and the large arrow the CA1–CA2 pyramidal cell border. DG is the dentate gyrus. At the rostral level (A,B), the hippocampal topography places the CA3 area between portions of the CA2

area, in agreement with observations by Lein et al. (Lein et al., 2005) who nicely demonstrate this unfamiliar arrangement.

Figure 3. Cells containing *Avpr1b* transcripts are found occasionally in the mouse paraventricular nucleus of the hypothalamus (A) and rarely in the anterior amygdaloid area (B). The 3' probe was used for this ISHH and exposures were for 4 months.

Figure 4.

Reverse transcriptase-polymerase chain reaction examination of total RNA isolated from various mouse brain regions and peripheral tissues (wildtype mice unless noted otherwise). The RNA for each sample was divided equally between 2 reactions, one for Avpr1b and one for β -actin to which reaction mix and the appropriate primers pairs were added. The RT-PCR reactions were run in the same 48-well plate and subsequently paired on the same two-comb gels. Specific products are 322 and 266 bp in length for the Avpr1b (top row of each pair) and β -actin (bottom row of each pair) bands, respectively. Row A: 1, cervical spinal cord; 2, medulla; 3, trigeminal ganglion; 4, cerebellum; 5, pons; 6, midbrain; 7, caudate-putamen; 8, septum; 9, hypothalamus; 10, piriform cortex; 11, amygdala. Row B: 12, thalamus; 13, dorsal hippocampus; 14, parietal cortex; 15, olfactory bulb; 16, adrenal; 17, kidney; 18, pituitary; 19, knockout pituitary; 20, vomeronasal organ; 21, liver; 22, no RNA.

Figure 5. Avpr1b transcripts in rat hippocampal CA2 field shown in brightfield (A) and darkfield (B) photomicrographs. The small arrow shows the lateral CA2–CA3 pyramidal cell border and the large arrow the medial CA1–CA2 pyramidal cell border. The exposure was for 4 months.

Figure 6.

Several cells in the human hippocampus contain vasopressin 1b receptor transcripts (arrows in panel A). A single labeled CA2 neuron in the human hippocampus at higher magnification shows the typical association with a corpus amylacea (B). This carbohydrate rich accumulation is in the homogenous structure to the left of the cell nucleus (the arrow indicates the lower boundary between the nucleus and the corpus amylacea). The exposure was for 4 months.