New Topics in Vasopressin Receptors and Approach to Novel Drugs: Vasopressin and Pain Perception

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Abstract. Arginine vasopressin (AVP) activates three vasopressin receptors and it also has an agonistic activity on the oxytocin receptor. For an accurate description of the target receptor subtype(s) responsible for complex AVP and oxytocin actions, a careful evaluation of ligand specificity and receptor activities are required, particularly when these receptors are co-expressed in the central nervous system. Previous studies suggest that AVP plays a regulatory role in nociception through the direct activation of central vasopressin receptors and also through the receptors that reside in the peripheral tissues. Genetically altered rodent models, including the AVP-deficient mutant Brattleboro rat and gene knockout mice lacking an endogenous opioid peptide, advanced the understanding of the interactions between the pain perception process and AVP system. This report reviews previous findings in this important field and reconciles them with the findings of recent gene knockout/knockdown studies.

Keywords: arginine vasopressin, oxytocin, pain, nociception, V1a receptor, V1b receptor

Introduction

Neurohypophyseal hormones, such as arginine vasopressin (AVP) and oxytocin (OT), have a wide range of effects on the central nervous system (CNS), including nociception, learning and memory process, social recognition, central regulation of the cardiovascular system, and stress response (1 - 13). These CNS functions of AVP are not directly related to their peripheral roles as circulating hormones (14). Indeed, neurons containing AVP peptides extend to extrahypothalamic structures, which are important for pain perception (15 - 19). OT, on the other hand, is present in several thalamic nuclei, the mesencephalic central gray nucleus, the substantia nigra, the locus coeruleus, the raphe nucleus, the nucleus of the solitary tract, and the spinal cord (20, 21).

Because AVP is a principal agonist to all vasopressin receptors and also has agonistic activity on OT receptors, it has been difficult to define the target receptor responsible for the complex AVP actions in the CNS. There are three types of G-protein-coupled vasopressin receptors, termed V1a, V1b, and V2 receptors (22); and a single OT-receptor gene has been identified (20). V1a, V1b, and OT receptors activate Gq heterotrimeric GTP binding protein and V2 stimulates Gs protein (22). The main receptors for AVP in the brain could be presumably of the V1 type (23); no V2-specific ligand binding or V2 mRNA were detected in previous reports (24, 25), while other studies suggest that there is a V2-like receptor in the CNS (3, 26). A relatively good correlation was reported between the autoradiographic distribution of mRNA transcripts for the V1a and autoradiographic distribution reported for V1-specific binding sites (25, 27). The V1b receptor transcript, on the other hand, is localized in several brain regions, including the olfactory bulb, CA2 pyramidal neurons in the hippocampus, supraoptic, suprachiasmatic and dorsomedial hypothalamic nuclei, piriform and entorhinal cortices, substantia nigra, and dorsal motor nucleus of the vagus (28 - 30). The functional interactions between AVP/OT peptides and their receptor system have been shown to provide diverse opportunities to modulate the efficiency of sensory transmission (31). This review will...
discuss the roles of endogenous and external AVP in nociception. Owing to limitations of space, this article is not a comprehensive overview of the subject. For discussions concerning the variety of neurophysiological roles of central AVP and OT, other previous reviews should also be consulted (3, 14, 20, 21, 32 – 35).

**Effect of AVP in pain perception**

The pain perception process can be altered by a variety of peptide and non-peptide neurotransmitters (33). Using rodent withdrawal reflex tests, such as the tail flick test, or more complex behavioral tests, such as the hot plate test, in which supraspinal motivational processing is involved, AVP and OT were shown to modify the nociception threshold induced by these noxious heat stimuli (33). In early reports, the antinociceptive activity was observed after either the intraventricular or subcutaneous administration of lysine vasopressin (LVP) in the rat or after intraperitoneal injection of AVP in mice (1, 36, 37). Des-glycinamide-LVP, a vasopressin analog with no apparent pressor or antidiuretic action, or des-α-vasopressin, a vasopressin analog with minimal pressor activity but greatly enhanced antidiuretic activity, was also relatively ineffective (36, 37). Later, intracerebroventricular injections of AVP or OT were shown to lead to antinociceptive effects in rats and in human cancer patients (38 – 40).

In addition to the capacity of administered AVP to show antinociception, studies on nociceptive condition of Brattleboro rats, which are deficient in mature vasopressin, are informative concerning the role of AVP in pain sensitivity. When examined using the flinch-jump threshold test, Brattleboro rats were in a hyperalgesic state and stress analgesia was impaired in comparison to controls (41). These analgesic deficits observed in Brattleboro rats was vasopressin-dependent; LVP introduced into the lateral cerebral ventricle, or subcutaneously at a high dose, induced an antinociceptive effect (42). One of other methods to inhibit AVP action in vivo is the administration of antiserum against AVP directly into the cerebral ventricle or brain nucleus. Administration of antiserum to the cerebral ventricle resulted in a small reduction of tail-flick latency from 3.9 s in the vehicle administration to 3.3 s at a high radiant heat level (50°C), but it prolonged the latency at moderate (46°C) heat (43).

In the CNS, AVP is released synaptically in the lateral septum, hippocampus, amygdala, habenula, and several other brain structures (15). Therefore, the brain region(s) where AVP acts as antinociceptive neurotransmitter is of large concern. The amygdala, an important region regulating emotional responses, such as anxiety or fear, has a critical role in conditional pain perception. When AVP was injected into the central nucleus of the amygdala and the nociceptive jaw opening reflex monitored in freely moving rats, AVP showed an analgesic effect in diagnostic electromyograms, which was inhibited by a V1, but not V2, receptor antagonist (44). Other studies examined the effect of AVP in nociception by injecting it into the periaqueductal gray and observed an increase in pain threshold (26). AVP increased the endorphin and enkephalin concentration in liquid samples perfused through the periaqueductal gray (45). Not only intracranial injection, but also intrathecal administration of AVP showed an antinociceptive effect (46, 47). However, another study reported that AVP failed to influence nociceptive thresholds or to modify the antinociceptive action of morphine (48). Intrathecal AVP also produced scratching bouts and suppression of hindbody motor function (47).

**Opioid-dependency of AVP-induced antinociception**

So far, evidence has been accumulating that AVP administered into the intracranial and, in some reports, intrathecal spaces causes an increase in the pain threshold. The next question to be answered is whether the pain-inhibiting effect of AVP could be mediated by intrinsic opioid peptide–receptor systems. To clarify this, a selective μ-opioid–receptor antagonist, naloxone, or several opioid agonists, such as morphine and other peptide agonists, were used together with AVP. The series of results so far have been equivocal. Naloxone inhibited AVP-induced analgesia in several studies (44, 49, 50), but in other studies, the antinociceptive actions of AVP were not mediated by opioids (1, 37, 38, 46).

Apart from the intracranial regions, anterior and posterior pituitary functions are closely related to AVP and OT. Since AVP is secreted under stress conditions and several reports describe evidence of AVP-secreting stimulation also causing secretion of opioid peptides. In the posterior pituitary where AVP and OT neurons terminate, the hypophysial nerve terminals contain enkephalin peptides together with AVP or OT in rat pituitary (51). Dynorphin immunoreactivity was also localized in AVP and OT neurons (52). These reports suggested that AVP and opioid peptides could be secreted from the posterior pituitary and in the CNS. On the other hand, AVP acts as stimulator of adrenocorticotropic secretion in the anterior pituitary. The adrenocorticotropic peptide is processed from the pro-opiomelanocortin (POMC) gene. Although the POMC gene can also produce β-endorphin in the CNS of the human and rat pituitary, a lack of an appreciable amount of active β-endorphin has been reported in basal condi-
tions and under corticotropin-releasing stimuli, such as AVP administration or insulin-induced hypoglycemia (53). Therefore, in mice the pituitary gland is one of the major sources of peripherally circulating β-endorphin, but its role in analgesia remains uncertain (54). Previous studies have shown that hypophysectomy may in fact enhance most types of opioid analgesia (55). Furthermore, the lack of stress-induced analgesia documented in β-endorphin–knockout mice may be due to the loss of central β-endorphin rather than loss of pituitary-derived β-endorphin (54).

**OT and analgesia**

Physiological stimulations that induce a large increase in OT concentrations in the blood, such as parturition and vaginal dilation, are known to raise the pain threshold (21). The analgesic effect of this type of OT action is not a morphine-sensitive process and peripheral injections of OT have no analgesic effect (21). OT, however, attenuates the development of tolerance to the analgesic action of morphine (56). Lesions of the hypothalamic paraventricular nucleus deplete endogenous immuneactive AVP and OT from the rat spinal cord, but fail to modify the nociceptive thresholds (48). Therefore, complex interactions are suggested between the central OT systems and opioid analgesia.

Using a human neuroblastoma cell line the relationship of opioid- and OT-dependent cellular signaling was recently examined in our laboratory to determine the molecular mechanism for the enhanced nociceptive effect produced by OT. The neuroblastoma cells express both the μ-opioid receptor (MOR1) and δ-opioid receptor (DOR), in addition to the OT receptor (Fig. 1). OT receptor–Gq coupling and Ca⁡²⁺ responses are evoked by application of a specific OT-receptor agonist, which

**Fig. 1.** The co-expression of MOR1 and DOR, but not κ (KOR), opioid receptors and OT oxytocin receptor transcripts in a human neuroblastoma cell line. Real time PCR analysis detected MOR1 and DOR (A) and OT receptor (B) transcripts as the predominant opioid receptors and vasopressin/OT receptor, respectively, in human neuroblastoma cells. C: The specific OT-receptor agonist TG stimulate d [Ca²⁺], mobilized and desensitized the corresponding receptor, resulting in no Ca²⁺ response upon subsequent AVP (1 μM) application. The TG-induced [Ca²⁺] mobilization was inhibited by pretreatment of the cells with 2 μM Mpomeovt, a selective OT-receptor antagonist (D), but Mpomeovt failed to inhibit the angiotensin II–stimulated Ca²⁺ response. Mpomeovt: 1-deamino-2-O-methyl-tyrosyl-8-ornithine-1-(β-mercapto-(β,β-cyclopentamethylene)propionic acid)oxytocin; TG, [Thr⁴,Gly⁷] oxytocin.

**Fig. 2.** The inhibition of cyclicAMP production by OT, AVP, and co-application of AVP and morphine. A: The inhibitory effects of OT and AVP against forskolin-induced cyclic AMP production. The cells were stimulated at the indicated concentrations of OT or AVP for 5 min at ambient temperature and reactions were terminated by heating cells at 100°C for 5 min. Cyclic AMP concentrations of the cellular extracts were examined by an enzyme-linked immunosorbent assay. B: The co-applications of morphine and AVP enhanced inhibition of adenylate cyclase activities. In the presence of 1 μM AVP, the inhibition of forskolin-stimulated adenylate cyclase by morphine was significantly enhanced. *P<0.05.
are inhibited by an antagonist, M promise (Fig. 1: C and D). Interestingly, OT inhibits forskolin-induced cyclic AMP production in a concentration-dependent manner (Fig. 2A). The co-application of 1 μM AVP and morphine enhances adenylyl cyclase inhibition, when the effect is compared with morphine alone (Fig. 2B). These previously unpublished results from our investigations suggested that when expressed in the same cell and stimulated simultaneously, MOR1, DOR, and OT receptors cooperatively enhance the Gi-signaling pathway. These results might be one of the possible mechanisms for the analgesic effect of central OT-receptor activation.

**Concluding remarks**

A majority of the studies performed in vivo on the roles of central AVP and OT receptors suggested that these intrinsic peptides show analgesic effects through an undetermined mechanism. The complexities in delineating the AVP- and OT-receptor functions could partly originate from mutual receptor–ligand interactions. In addition, direct interactions between receptor molecules, resulting in homomer and heteromer receptor complexes, as well as indirect intracellular signaling cross talks, have also been suggested (57, 58). To obtain a more detailed picture, both genetically modified animal models and specific pharmacological tools continue to be useful by perturbing one signal domain so that the other remaining one could be more clearly demonstrated. In this regard, mice lacking the V1a- or V1b-receptor gene are important animal models for unequivocally identifying receptor subtype(s) responsible for AVP actions in the CNS [see the following review article in this issue by K Honda and Y Takano (ref. 59)].

**References**


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