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Neuronal Pathways Linking Substance P to Drug Addiction and Stress

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Abstract

Accumulating evidence suggests that the neuropeptide Substance P (SP), and its principal receptor neurokinin 1 (NK1), play a specific role in the behavioral response to opioids and stress that may help to initiate and maintain addictive behavior. In animal models, the NK1 receptor is required for opioids to produce their rewarding and motivational effects. SP neurotransmission is also implicated in the behavioral response to stress and in the process of drug sensitization, potentially contributing to vulnerability to addiction or relapse. However, SP neurotransmission only plays a minor role in opioid-mediated antinociception and the development of opioid tolerance. Moreover, the effects of SP on addiction-related behavior are selective for opioids and evidence supporting a role in the response to cocaine or psychostimulants is less compelling. This review will summarize the effects of SP neurotransmission on opioid-dependent behaviors and correlate them with potential contributing neural pathways. Specifically, SP neurotransmission within components of the basal forebrain particularly the nucleus accumbens and ventral pallidum as well as actions within the ascending serotonin system will be emphasized. In addition, cellular or network level interactions between opioids and SP signaling that may underlie the specificity of their relationship will be reviewed.

Keywords

substance P; neurokinin 1; nucleus accumbens; serotonin; stress

Substance P (SP) is a member of the tachykinin family of neuropeptides. It is one of the most abundant neuropeptides in the brain and one of the earliest to be purified and sequenced (reviewed by (Hokfelt et al., 2001; Nicoll et al., 1980). It was named SP because it was the active component of a purified preparation (Von Euler and Gaddum, 1931), although later the P of SP became tightly linked with the peptides suspected role in the sensation of pain. However, the association of SP with pain appears to be largely a miss-

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statement of what has emerged as a multifaceted role in modulating behavior at many points across the neuroaxis (Hill, 2000).

SP is thought to primarily act at the neurokinin 1 (NK1) receptor. However, there are three peptide and three receptor members of the tachykinin family and potential for cross-talk between them. SP and a second tachykinin, neurokinin A are both encoded by the preprotachykinin A gene. The third tachykinin, neurokinin B is encoded by a separate gene preprotachykinin B. These three peptides act on three receptor subtypes, neurokinin (NK) 1, 2 and 3. The preferential, although perhaps not exclusive receptor for SP is NK1, for neurokinin A, NK2, and for neurokinin B, NK3 (Regoli et al., 1994). As a neuropeptide, SP coexists with other fast neurotransmitters within axons including glutamate, GABA, serotonin and acetylcholine depending on its location in the brain.

The importance of NK1 signaling to addiction-related behaviors was emphasized by studies using mice lacking functional NK1 receptors. That is, opioids no longer appear rewarding in NK1-knockout mice, at least as indicated in two behavioral paradigms, the conditioned place preference test and drug self-administration behavior (De Felipe et al., 1998a; Murtra et al., 2000; Ripley et al., 2002). In the conditioned place preference test, rodents develop a preference for a chamber previously paired with drug administration. The amount of time spent within the drug-paired chamber in preference to an alternative chamber is thought to reflect a prior reward. The propensity to self-administer drugs is also thought to depend on the rewarding effects of the drugs. These tests also involve motivation, such that they are thought to invoke both neural circuits that mediate reward and the motivation to seek that reward.

The loss of rewarding value of opioids in NK1 knockout mice was consistent with prior studies and further supported by subsequent ones. For example, it had been previously shown that NK1 receptor activation by itself is able to produce conditioned place preference (Hasenohrl et al., 1992; Hasenohrl et al., 1998a; Hasenohrl et al., 1998b; Nikolaus et al., 1999). The effects of SP on major neurotransmitter systems involved in addiction-related behaviors including dopamine, serotonin, norepinephrine and acetylcholine had also been known (reviewed by (Goodman, 2008)). In addition more recent studies have shown that lesion of cells bearing NK1 receptors in the amygdala mimics the results in the NK1 knockout mice (Gadd et al., 2003) and administration of NK1 receptor antagonists to normal mice attenuates their response to opioids on the conditioned place preference test (Jasmin et al., 2006). Thus a variety of approaches have converged to support a role for NK1 signaling in opioid addiction-related behaviors

Considering these observations, the most likely interpretation of the effect of the NK1 knockout on opioid-related behaviors is that the administration of opioids elicits an endogenous SP release that activates NK1 receptors. NK1 signaling may then play a permissive role, or possibly enhance the effects of opioids. Supporting this notion there has been a report showing that administration of morphine increases extracellular SP, at least in the periaqueductal gray (Rosen et al., 2004).

Motivation and Reward Pathways

Relevant NK1 signaling may occur within several brain areas associated with reward. These include a cadre of structures in the ventral forebrain sometimes referred to as the extended amygdala composed of the bed nucleus of the stria terminalis (BNST), amygdala, nucleus accumbens (posterior shell) and major efferent targets of the extended amygdala particularly the ventral pallidum, lateral hypothalamus and cortex (De Vries and Shippenberg, 2002; Heimer and Alheid, 1991; Koob, 1999; McBride et al., 1999; Panagis et al., 1995; Shippenberg and Elmer, 1998). Key control of these areas is exerted by the mesolimbic dopamine system, the primary component of incentive or motivational processes (Koob, 2009; Spanagel and Weiss, 1999). This system is composed of neurons that contain dopamine located in the ventral tegmental area (VTA) that send their axons to the nucleus accumbens. Disruption of the mesolimbic pathway significantly impairs motivation to seek any positive reward be it food, sex or drugs (Berridge, 2004; Fibiger et al., 1986; Hnasko et al., 2005). These groups of structures together are tightly interconnected functionally, as well as anatomically.

An important SP-NK1 interaction relevant for reward and motivational state may occur in the shell region of the nucleus accumbens (Fig. 1). Morphine activates neurons in the nucleus accumbens and promotes the appearance of certain gene products, such as Fos B in this area. Morphine's activation of Fos B in the nucleus accumbens is substantially decreased in NK1 knockout mice (Murtra et al., 2000). Within the nucleus accumbens SP is present in the local axon collaterals of medium spiny projection neurons (Lee et al., 1997; Napier et al., 1995). SP-containing axons form synapses with aspiny neurons in this area that contain the NK1 receptor, many of which are cholinergic (Martone et al., 1992; Pickel et al., 1976). Activating NK1 receptors drives cholinergic neurons (Bell et al., 1998), which subsequently leads to increased activation of spiny projection neurons within the nucleus (Galarraga et al., 1999). Large cholinergic cells in the nucleus accumbens are active in response to a cue signaling a reward and thus have been suspected to play a role in associative learning (Elliott et al., 1986; Graybiel et al., 1994).

The ventral pallidum is also implicated as an important site for SP signaling. The ventral pallidum has received increasing attention as the "final common pathway" for reward and motivational processes (Smith et al., 2009) and SP precursors are expressed in many neurons in the nucleus accumbens that project to the ventral pallidum (Lu et al., 1998; Napier et al., 1995). Indeed, the SP-containing axons massively innervate the ventral pallidum (Fig. 2) (Groenewegen and Russchen, 1984; Marksteiner et al., 1992) and at least in part terminate on ventral pallidum cholinergic neurons potentially bearing NK1 receptors (Zaborszky and Cullinan, 1992). SP injected directly into this area induces place preference behavior (Hasenohrl et al., 1992; Hasenohrl et al., 1998a; Hasenohrl et al., 1998b; Nikolaus et al., 1999).

In the nucleus accumbens, ventral pallidum and associated areas, NK1 receptors are prominently located on cholinergic neurons (Kaneko et al., 1993; Parent et al., 1995; Pickel et al., 1976). These cholinergic neurons also contain the third vesicular glutamate transporter, VGLUT3 (Commons, 2009; Freneau et al., 2002; Gras et al., 2002; Schafer et

al., 2002), and may participate in a selective projection pathway to the basolateral amygdala (Nickerson Poulin et al., 2006). The importance of the basolateral amygdala is in its involvement in learning that has an emotional component. For example learning paradigms including inhibitory avoidance, Pavlovian fear conditioning, food and amphetamine place preference, and stimulus-drug reinforcement associations all utilize circuits within the basolateral amygdala (McGaugh, 2004; Power et al., 2003). Moreover, acetylcholine specifically in the basolateral amygdala contributes to the consolidation of emotionally-motivated learning (McGaugh, 2004).

Thus the basal forebrain provides at least two likely areas where SP actions on NK1 receptors may play a role in addiction-related behavior, the nucleus accumbens and ventral pallidum. Cholinergic neurons within these areas commonly have the NK1 and also VGLUT3 and may engage emotional memory circuits in the basolateral amygdala. However, these are by no means exclusive sites where SP signaling may influence reward and motivation. Rather there are several additional sites of action suggesting this could be a distributed function of SP signaling. For example, there may be actions within the amygdala itself, and ablation of NK1-bearing cells in the amygdala mimics deficits seen in NK1 receptor knockout mice (Gadd et al., 2003). Moreover, SP has effects directly within the VTA on dopamine neuronal activity. Injection of SP into the VTA increases cell-firing rates of neurons (Korotkova et al., 2006) and dopamine release in forebrain areas including the prefrontal cortex and nucleus accumbens (Cador et al., 1989; Elliott and Iversen, 1986).

STRESS

Reward and motivation to seek that reward, also called incentive salience, are two fundamental components of drug addiction. However, these functions are critically modified by other factors including stress. There are at least three ways of describing the importance of stress to addiction-related behaviors. First stress has important effects on mood, and dysregulation of mood can contribute to the initial motivation for drug-seeking behavior. Second, stress can produce a sensitization to the effects of drugs of abuse, enhancing their rewarding value. Third, stress is known to be an important contributor to relapse to drug-seeking behavior. While the involvement of SP in mediating the effects of stress on addiction-related behaviors has not been systematically studied, there is substantial evidence that endogenous SP signaling is activated as a consequence of stress.

Initially it was observed that when SP was injected into the lateral ventricle of a rodent, it produces a constellation of behaviors reminiscent of a response to pain or stress (Unger et al., 1988). This includes grooming behaviors such as face washing and hind limb grooming. These behaviors are accompanied by increases in sympathoadrenal activity, including increases in mean arterial pressure and heart rate and regional changes in blood flow with vasoconstriction occurring in the viscera and dilation occurring in the hind-limbs, as if in preparation for a 'fight or flight' response (Culman and Unger, 1995a). While pharmacological administration of SP produces these effects, inhibition of NK1 receptors attenuates the cardiovascular response to noxious stimulus indicating that endogenous SP signaling is activated as a consequence of a pain-associated stress (Culman et al., 1997).

The hallmark of stress is activation of the hypothalamic-pituitary-adrenal (HPA) axis, and the paradigms most often used to evoke a stress response include a painful stimulus, as in the case of many of the previously mentioned studies. However, endogenous SP signaling may not be restricted to pain-associated stress. Indeed, emotional stressors increase SP efflux in forebrain areas including the amygdala and septum (Ebner et al., 2004). Restraint stress leads to a decrease in NK1 receptors in the amygdala (Takayama et al., 1986) probably due to receptor internalization (Ebner et al., 2004; Smith et al., 1999). Ebner and colleagues (Ebner et al., 2008b) suggests that SP may be released within the lateral septum in response to swim stress, and blocking NK1 receptors in the septum increases active coping strategies during the swim. These findings suggest that endogenous SP may not be restricted to the behavioral response to pain, but may play a more wide-spread function in helping to coordinate the behavioral response to stress. Supporting this conclusion aversive or stressful situations change SP content or receptor binding in several areas of the brain (Culman and Unger, 1995b).

Stress and drugs of abuse likely act within many overlapping neural circuits that influence reward and motivation (Solomon and Corbit, 1974). That is, both stress and drugs of abuse provide strong motivation for behavior, although of opposing valence. With stress, the motivation is centered on avoiding the aversive qualities of stressful circumstances, while the relief from stress may have a positive hedonic value. With drugs of abuse, motivation focuses on an initial reward, although cessation of drug use can be aversive (Haddjeri and Blier, 2008). Thus effects of SP within similar neural circuits within the basal forebrain and mesolimbic dopamine system may link relevant effects of stress and addiction-related behaviors (Carlezon and Thomas, 2009).

Two additional candidate regions where SP signaling as a consequence of stress may be relevant to addiction-related behaviors are the serotonergic dorsal raphe nucleus (DR) and the noradrenergic locus coeruleus (LC). Altered forebrain serotonergic and noradrenergic neurotransmission is associated with the behavioral response to different types of stress (Kirby et al., 1997; Valentino et al., 1993; Will et al., 2004). In addition, NK1 receptor signaling has been associated with modifying mood through actions on these midbrain serotonergic and noradrenergic nuclei (Haddjeri and Blier, 2008; Santarelli et al., 2001). These observations raise the possibility that SP signaling in these regions could contribute to the negative affective consequences of stress that promotes drug-seeking behavior.

SP is enriched in the DR where it acts on a population of glutamatergic cells in the DR that have the NK1 receptor. When SP activates these cells there is an increase in excitatory glutamatergic postsynaptic potentials in serotonin neurons (Liu et al., 2002). Like basal forebrain cholinergic cells, NK1 cells in the DR are distinguished by their content of the third vesicular glutamate transporter, VGLUT3 (Commons, 2009). Through these NK1 bearing cells, SP activates some serotonin neurons. Activated serotonin neurons then in turn release 5-HT onto their neighbors leading to a subsequent 5-HT_{1A}-mediated inhibition of many cells in the DR (Valentino et al., 2003). Correlating with these effects, SP in the DR decreases extracellular serotonin in the frontal cortex, as detected using microdialysis techniques in conscious mice, and these effects are blocked by both glutamate receptor and 5-HT_{1A} receptor antagonists in the DR (Guiard et al., 2007).

By comparison SP actions in the locus coeruleus (LC), the major source of forebrain norepinephrine, are rather straightforward. Noradrenergic neurons in the LC express high levels of NK1 receptor (Chen et al., 2000) and are directly activated by SP (Velimirovic et al., 1995). However, there is the potential that SP signaling could also act through indirect means to influence norepinephrine. For example, SP contributes to activation of the paraventricular nucleus (Ebner et al., 2008a; Spitznagel et al., 2001) the principal coordinator in both the neural and endocrine response to stress via release of corticotropin releasing factor (CRF). This raises the possibility that SP contributes to activating endogenous CRF signaling and consequent behavioral responses to stress.

Stress- or Drug-Induced Behavioral Sensitization

NK1 knockout mice do not exhibit sensitization produced by previous drug exposure. Unfortunately however, the effect of a loss of NK1 receptors on stress-induced sensitization or relapse has not been fully characterized. However, SP actions within the ascending serotonergic DR could also contribute to behavioral sensitization, at least as a consequence of prior drug exposure. Serotonin has the capacity to modify activation of the mesolimbic dopamine system (Yan and Yan, 2001) and has been associated with sensitization to cocaine (Cunningham et al., 1992), amphetamine (Auclair et al., 2004), and nicotine (Olausson et al., 2001). With respect to opioids, serotonin is associated with sensitization produced by previous drug exposure (Sills and Fletcher, 1997) as well as with stress-induced sensitization to opioids (Bland et al., 2003; Will et al., 2004). Both the 5-HT_{2A} and 5-HT_{1A} receptors have been implicated in behavioral sensitization to drugs of abuse (Auclair et al., 2004; Lanteri et al., 2008; Salomon et al., 2006).

SPECIFICITY OF THE ROLE OF SP

There are many behavioral effects of opioids, which are independent from one another in their underlying mechanism. In addition to rewarding and motivational effects, opioids have analgesic effects that are accompanied by other endocrine and somatic adjustments including respiratory depression. Chronic opioid administration leads to the development of dependence and tolerance. Dependence is defined by the appearance of withdrawal symptoms after drug cessation and is distinct from addiction-related behavior both in humans and in animal models. For example, dependence can be seen in very ill human patients that nevertheless do not exhibit signs of addiction such as craving and drug seeking. An important clinical problem is opioid tolerance, or the decreasing efficacy of opioid to produce analgesia with chronic exposure, and this can also be dissociated from addiction-related behaviors and the development of dependence.

The effects of SP or NK1 receptor manipulations on opioid-dependent behaviors reveal a role in a specific subset of behaviors. Mice lacking NK1 receptors do not self-administer or exhibit conditioned place preference or behavioral sensitization to opioids, yet they exhibit a fairly normal antinociceptive response to opioids (De Felipe et al., 1998b; Murtra et al., 2000). In addition, NK1 receptors are not necessary for the development of opioid tolerance and many, although not all, symptoms of dependence. This suggests that NK1 receptors may

only be critical for the effect of opioids within a subset of neural systems, and these appear to selectively involve motivation, reward, and sensitization.

This functional specificity in opioid-dependent behaviors suggests interactions only in specific pathways. There are many potential mechanisms that could underlie such a relationship. To illustrate one possibility, there could be interactions based upon the colocalization of mu opioid receptors (MOR) and NK1 receptors within the same subsets of cells. This could set the stage for cell-autonomous interactions between MOR and NK1 receptors, where the presence or activation of NK1 receptors influences MOR function by affecting trafficking (Yu et al., 2009) or by heterodimerization (Pfeiffer et al., 2003). Consistent with potential interactions between these two proteins, MORs and NK1 receptors are expressed together within certain subsets of neurons that could be positioned to have an important effect on reward. These include neurons in the LC and the nucleus accumbens shell region (Fig. 1) (Gadd et al., 2003; Jabourian et al., 2005; Nakaya et al., 1994; Pickel et al., 2000; Svingos et al., 2001). Indeed, recent studies have suggested that NK1 receptor activation inhibits internalization and functional desensitization of MOR by various ligands (Yu et al., 2009). The underlying mechanism is proposed to be sequestration of arrestins on endosomes containing the NK1 receptor (Yu et al., 2009). Likewise, previous studies had shown an interaction between signaling mechanisms of these two receptors when located on the same cells (Velimirovic et al., 1995).

Considered another way however, the colocalization of mu opioid and NK1 receptors complicates the implication that they work together in promoting reward behaviors. This is because typically mu opioids open a potassium conductance inhibiting cell activity whereas NK1 activation closes the same channel, promoting excitation. However the summation of these signals within single cells may non-linear (Velimirovic et al., 1995), which could have complex effects on network function. In addition, at least in the case of the nucleus accumbens, it may be inaccurate to characterize the electrophysiological effects of mu opioids this simply. In fact, a previous study reported several actions of mu opioids, including a postsynaptic potentiation of currents through NMDA receptors (Martin et al., 1997). NMDA-receptor activation requires postsynaptic depolarization, which would be enhanced by convergent excitatory effects of NK1 receptor activation.

All drugs of abuse are thought to act on similar pathways in the brain, in particular the mesolimbic dopamine system as well as other monoamine systems that regulate mood and arousal. Likewise non-opioid drugs of abuse including cocaine have been implicated in activating the endogenous opioid system to participate in their rewarding effects (Bain and Kornetsky, 1987; Bilsky et al., 1992; Corrigall and Coen, 1991; Schroeder et al., 2007). The effects of NK1 receptors are also specific for opioids and are not apparent with cocaine. Mice lacking NK1 receptors show normal place preference and self-administration behavior in response to cocaine (Murtra et al., 2000; Ripley et al., 2002). This specificity for opioid but not cocaine place preference was replicated by lesions of NK1 bearing cells in the amygdala (Gadd et al., 2003).

A simplistic resolution to this observation may be that while cocaine may also invoke endogenous opioid signaling, it could have actions that over-ride the required permission or

help of SP. While activation of NK1 receptors can include reinstatement of cocaine seeking behavior, NK1 antagonists do not block cocaine-induced reinstatement, consistent with the idea that their actions are parallel but independent (Placenza et al., 2004). SP and cocaine use similar mechanisms to depress excitatory synaptic in the nucleus accumbens and their effects occlude each other (Kombian et al., 2009). Cocaine by itself activates Fos expression in cholinergic neurons in the accumbens (Berlanga et al., 2003). These observations support the notion that the distinct molecular mechanisms that cocaine uses to activate reward and motivational circuits may obviate a required participation of endogenous SP signaling.

Conclusions

SP signaling plays a specific role in the behavioral response to opioids, suggesting interactions in pathways contributing to addiction-dependent behaviors. Neurons, including many cholinergic neurons, in the nucleus accumbens and the ventral pallidum represent likely candidates to participate in this process. In addition, actions in modulatory serotonergic and noradrenergic nuclei in the pons and medulla are implicated in mediating SP effects. These are also likely sites for stress related effects of SP. Although SP is more than likely involved in the behavioral response to pain and stress, as well as in the motivational and rewarding properties of opioids, the interplay between these functions remains poorly understood. However, a fuller understanding of the role of SP in these functions will likely improve strategies to treat addiction.

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Figure 1.

Neurons immunolabeled for NK1 receptor (green) also contain the mu opioid receptor (MOR, red) in the nucleus accumbens shell region. A. Merged view of immunolabeling for NK1 (green), MOR (red) and tyrosine hydroxylase (blue). Both NK1 and MOR immunolabeling is detected within large aspiny neurons and their dendritic processes (arrows). Additional processes appear immunolabeled for NK1 receptor only (arrowhead), while MOR is also more diffusely present through the neuropil. NK1 immunolabeling detected with a guinea pig antisera against NK1 (US Biologicals, 1:1000) combined with a rabbit antisera to MOR (Immunostar, 1:1000) and a mouse antisera to tyrosine hydroxylase (Immunostar, 1:1000) detected with fluorescence secondary antisera on sections from a paraformaldehyde fixed rat brain. Bar = 100 μ m.

Figure 2.

Substance P immunolabeled processes (red) densely innervate the ventral pallidum (VP). Substance P overlies dense immunolabeling for leu-enkephalin (blue) and reticular NK1 immunolabeled processes (green, panel A''). Substance P was detected using a guinea pig anti-substance P (Peninsula Laboratories, 1:1000) rabbit anti-NK1 (Novus Biologicals, 1:1000) and mouse anti-leu enkephalina (Fitzgerald Industries, 1:100).

Aca= anterior commissure, HDB= horizontal limb of the diagonal band, Cpu= caudate putament. Bar = 500 um.