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Review

Cellular mechanisms for antinociception produced by neuropeptides in the rat spinal dorsal horn

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ABSTRACT

Various neuropeptides modulate synaptic transmission in spinal dorsal horn lamina II (substantia gelatinosa; SG) neurons that play a pivotal role in regulating nociceptive transmission from the periphery. The SG neurons receive glutamatergic excitatory, and GABAergic and glycinergic inhibitory transmissions in a mono- and polysynaptic manner through primary-afferent fibers contained in the dorsal root. This review article introduces how several neuropeptides involved in antinociception modulate synaptic transmission in adult rat SG neurons. Nociceptin hyperpolarized the membranes and inhibited spontaneous and monosynaptically-evoked excitatory transmissions by activating opioid receptor-like1 receptors without a change in inhibitory transmission. Similar actions were produced by the activation of μ-opioid receptors by endomorphin-1 and -2. Galanin at low concentrations presynaptically enhanced spontaneous excitatory transmission by activating galanin type-2/3 receptors whereas at high concentrations it produced a membrane hyperpolarization by galanin type-1 receptor activation; inhibitory transmission was not affected by galanin. Galanin type-2/3 but not type-1 receptor activation resulted in monosynaptically-evoked excitatory transmission inhibition. Alternatively, oxytocin produced a membrane depolarization by activating oxytocin receptors, which increased neuronal activity, resulting in the enhancement of spontaneous inhibitory transmission. Excitatory transmission was not affected by oxytocin. In conclusion, neuropeptides inhibit the excitability of SG neurons through various mechanisms, resulting in antinociception.

KEYWORDS: antinociception, endomorphin, excitatory transmission, inhibitory transmission, galanin, nociceptin, oxytocin, patch-clamp, spinal dorsal horn

ABBREVIATIONS

4-AP	:	4-Aminopyridine
DRG	:	Dorsal root ganglion
EC_{50}	:	Effective concentration for half-maximal effect
EM-1	:	Endomorphin-1
EM-2	:	Endomorphin-2
E_K	:	Equilibrium potential for K^+
EPSC	:	Excitatory postsynaptic current
GalR	:	Galanin receptor
V_H	:	Holding potential
IPSC	:	Inhibitory postsynaptic current
ORL1	:	Opioid receptor-like1
sEPSC	:	Spontaneous excitatory postsynaptic current
sIPSC	:	Spontaneous inhibitory postsynaptic current
SG	:	Substantia gelatinosa
TEA	:	Tetraethylammonium
TTX	:	Tetrodotoxin

INTRODUCTION

Information of nociceptive stimuli given to the periphery is transmitted through fine myelinated

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Aδ and unmyelinated C primary-afferent glutamatergic fibers contained in the dorsal root to superficial dorsal horn, especially substantia gelatinosa (SG; lamina II of Rexed) neurons ([1-3]; for review see [4, 5]), and then to the primary-sensory area in the cerebrum (Figure 1A). This transmission to the SG is in origin not only monosynaptic but also polysynaptic through glutamate-, GABA- and/or glycine-containing interneurons ([6]; Figure 1B). The SG neurons play a pivotal role in the modulation of the nociceptive transmission. In support of this idea, a plastic change in glutamatergic primary-afferent inputs to SG neurons occurred in hyperalgesic rats

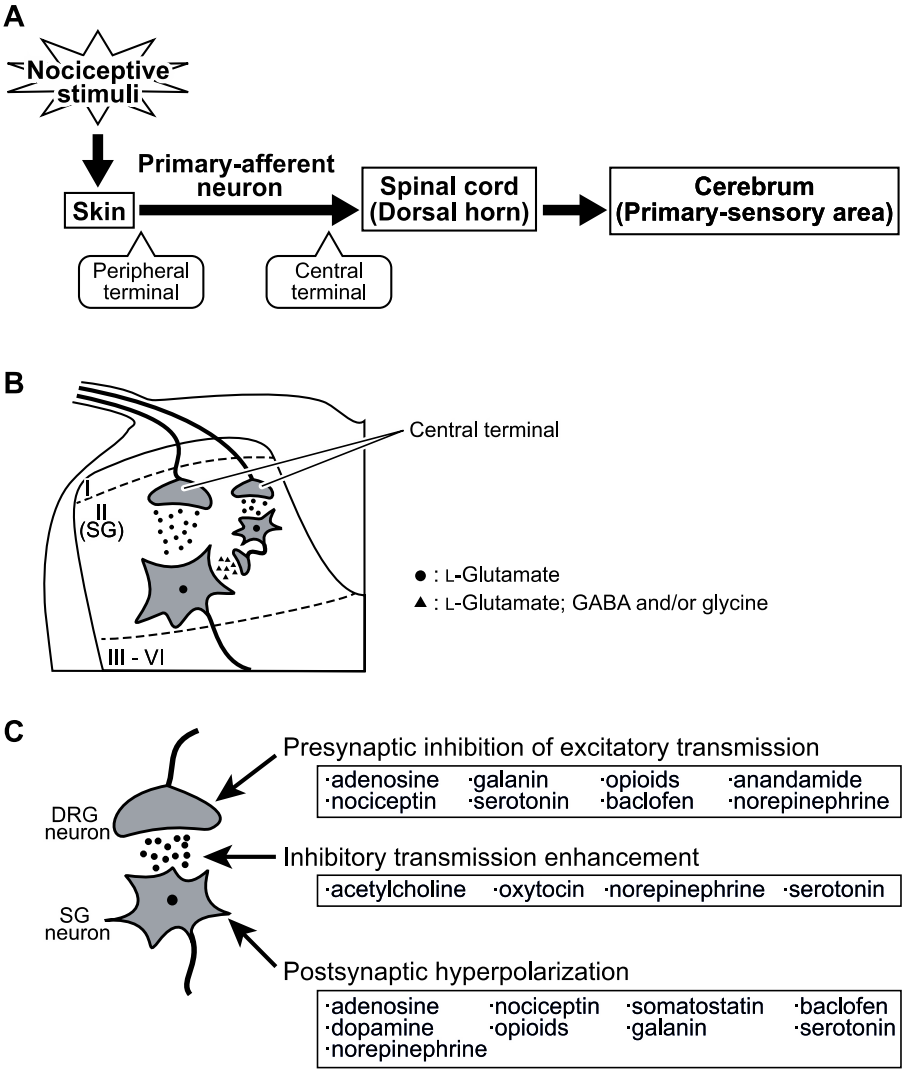

Figure 1. Schematic diagram showing the modulation of nociceptive transmission in the spinal dorsal horn lamina II (substantia gelatinosa, SG). The peripheral terminal of a primary-afferent, i.e., dorsal root ganglion (DRG), neuron receives nociceptive stimuli given to the skin while its central terminal transfers this information to the spinal dorsal horn, particularly SG, and then to the primary-sensory area of the cerebrum (A). The SG neurons receive glutamatergic excitatory, and GABAergic and/or glycinergic inhibitory transmissions in a mono- and polysynaptic manner (B). The modulation in the SG, which is mediated by a variety of endogenous and exogenous analgesics, is due to (1) a presynaptic inhibition of glutamatergic excitatory transmission, (2) a GABA- and/or glycine-mediated inhibitory transmission enhancement and (3) a membrane hyperpolarization in postsynaptic neurons (C). Modified from [43].

that were subject to either an intraplantar injection of complete Freund's adjuvant [7] or ovariectomy [8]. This modulation in SG neurons is performed at pre- and/or postsynaptic sites of excitatory and inhibitory synapses through actions of a variety of chemical substances that are either locally released/ produced in the spinal dorsal horn or released from descending neurons arising from higher centers such as the brain stem and hypothalamus. These sites are also targets of exogenous analgesics used to alleviate pain.

Endogenous and exogenous analgesics, which exhibit antinociception when administrated intrathecally, hyperpolarize membranes of SG neurons and reduce the release of L-glutamate onto SG neurons from nerve terminals, both of which are actions that reduce the membrane excitability of the SG neurons [9]. For example, opioids ([10]; for review see [11]), $GABA_B$-receptor agonist baclofen [12], tramadol [13], norepinephrine [14], serotonin [8, 15], adenosine [16-18], somatostatin [19, 20] and dopamine [21, 22] hyperpolarized the membrane of rat SG neurons (Figure 1C). Inhibition of L-glutamate release onto rat SG neurons was caused by opioids ([23]; for review see [11]), baclofen [24, 25], anandamide [26, 27], norepinephrine [28], serotonin [8, 15] and adenosine ([17, 29, 30]; for review see [16]; Figure 1C). The modulation of inhibitory transmission in SG neurons also plays a role in regulating nociceptive transmission ([31, 32]; for review see [33-35]). In support of this idea, it was shown that spontaneous inhibitory transmission in rat SG neurons was enhanced by ATP [36], acetylcholine [37, 38], norepinephrine [39-41] and serotonin ([15, 42]; Figure 1C), all of which are substances that are involved in antinociception in the spinal dorsal horn.

There are neuropeptides among chemical substances involved in the modulation of nociceptive transmission in the SG. This review article introduces how neuropeptides such as nociceptin, endomorphins, galanin and oxytocin involved in antinociception modulate synaptic transmission in SG neurons. Our previous results included in this article were obtained by applying the blind whole-cell patch-clamp technique to SG neurons of spinal cord slices dissociated from adult (6-7 weeks old) rats that are often used for behavioral studies of analgesics [44-48].

Actions of nociceptin on synaptic transmission in SG neurons

Nociceptin (a 17 amino acid peptide), also called as orphanin FQ, is an endogenous ligand [49, 50] for the G-protein coupled opioid receptor-like1 (ORL1) receptor [51, 52]. Nociceptin has negligible affinities to G-protein coupled μ, δ and κ opioid receptors while it has a high affinity to the ORL1 receptor [50]. The ORL1 receptor is structurally similar to the opioid receptors and is negatively coupled to adenylyl cyclase [52]. Additionally, activation of the ORL1 receptor results in opening inwardly-rectifying K^+ channels in different types of neurons including rat dorsal raphe [53], locus coeruleus [54, 55], periaqueductal grey [56], suprachiasmatic nucleus [57], hippocampal [58] and guinea-pig hypothalamic arcuate nucleus neurons [59]. Moreover, nociceptin inhibits voltage-gated Ca^{2+}-channel currents in rat hippocampal [60] and dorsal root ganglion (DRG) neurons ([61]; for review see [62]).

There is much evidence supporting the idea that nociceptin plays an important role in modulating nociceptive transmission in the spinal cord level (for review see [63]). According to behavioral studies in adult rats, intrathecal administration of nociceptin produces an antinociceptive effect in the tail flick test [64, 65] and attenuates hyperalgesia in a model of nerve injury [66] as well as of inflammation [67, 68]. Alternatively, nociceptin inhibited A-fiber and C-fiber-evoked responses [69] and also C-fiber-evoked wind-up phenomena (increase in pain over time in response to repeated nociceptive stimuli) in the rat spinal dorsal horn [70]. In support of a role of nociceptin in the spinal cord, nociceptin precursor mRNA [71], nociceptin peptide and mRNA [72] and the ORL1 receptor [73] are densely distributed in the superficial dorsal horn of the rodent spinal cord.

In adult rat SG neurons, nociceptin produced an outward current at a holding potential (V_H) of -70 mV (Figure 2A) in a concentration-dependent manner (effective concentration for half-maximal effect, EC_{50}: 0.23 μM). This nociceptin-induced current was due to the opening of inwardly-rectifying K^+ channels. This was because this current reversed at a potential which is close to

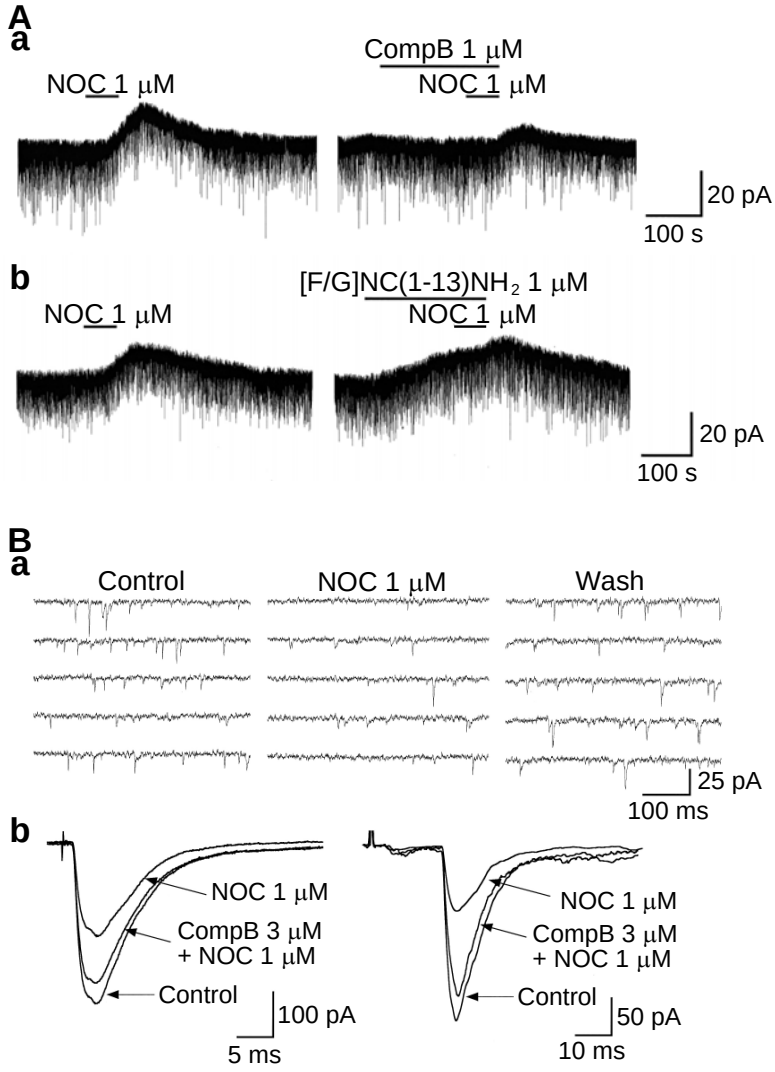

Figure 2. Actions of nociceptin (NOC; 1 μM) on glutamatergic excitatory transmission in adult rat SG neurons. (A) NOC produced outward currents in a manner sensitive to CompB (1 μM; Aa) and [F/G]NC(1-13)NH_2 (1 μM; Ab). (B) NOC inhibited excitatory transmission in a presynaptic manner. (Ba) Recordings of spontaneous excitatory postsynaptic currents (EPSCs) in the absence and presence of NOC. (Bb) Monosynaptic Aδ-fiber (left) and C-fiber evoked EPSCs (right) under the action of NOC in Krebs solution without and with CompB (3 μM). Holding potential (V_H) = -70 mV. (A) and (B): modified from [46] and [47], respectively.

the equilibrium potential for K^+ (E_K), as calculated by the Nernst equation. Moreover, this current was inhibited in amplitude by Ba^{2+} (0.1 mM) but not by 4-aminopyridine (4-AP; 1 mM) and tetraethylammonium (TEA; 5 mM), all of which are K^+-channel blockers. The nociceptin current was not affected by a voltage-gated Na^+-channel blocker tetrodotoxin (TTX), indicating that this current was not due to the production of action potentials and thus due to an increase in neuronal activity. A non-specific opioid-receptor antagonist naloxone (1 μM) had no effect on the nociceptin current. The peak amplitude of the nociceptin current was reduced by a nociceptin precursor product nocistatin (1 μM) and also by a nonpeptidyl ORL1-receptor antagonist CompB (1-[(3*R*, 4*R*)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2H-benzimidazol-2-one; 1 μM;

Figure 2Aa) without a change in holding currents. On the other hand, an ORL1-receptor antagonist [F/G]NC(1-13)NH_2 ([$Phe^1\psi(CH_2\text{-}NH)Gly^2$]-nociceptin-(1-13)-$NH_2$, 1 μM, which is a derivative of nociceptin) by itself induced an outward current, during which the nociceptin current was suppressed in amplitude (Figure 2Ab). These results indicate that nociceptin activates a K^+ channel, thereby exhibiting an inwardly-rectification through the activation of ORL1 receptor in adult rat SG neurons [46].

With respect to synaptic transmission in adult rat SG neurons, nociceptin (1 μM) decreased the frequency of glutamatergic spontaneous excitatory postsynaptic current (EPSC) without a change in its amplitude (Figure 2Ba). Nociceptin (1 μM) also reduced the amplitude of EPSCs that were monosynaptically evoked by stimulating primary-afferent Aδ or C fibers in SG neurons of a spinal cord slice with an attached dorsal root, where a hyperpolarizing effect of nociceptin was blocked postsynaptically. The inhibition of C-fiber EPSCs was larger in extent than that of Aδ-fiber EPSCs (Figure 2Bb). This could be due to the fact that an inhibitory action of nociceptin on voltage-gated Ca^{2+} channels, which is possibly involved in the presynaptic inhibition as suggested in hippocampal neurons [60], differs in extent between small and large DRG neurons [61]. Each of the nociceptin actions was concentration-dependent in a range of 0.1 to 1 μM, and was largely suppressed by CompB (3 μM; Figure 2Bb). Both electrically-evoked and spontaneous inhibitory postsynaptic currents (IPSCs), mediated by either $GABA_A$ or glycine receptors, were unaffected by nociceptin (1 μM). These results indicate that nociceptin inhibits excitatory but not inhibitory transmission in adult rat SG neurons through the activation of the ORL1 receptor; this action is presynaptic in origin. Considering that the SG is the main part of termination of Aδ- and C-fibers transmitting nociceptive information, these finding would account for at least a part of the inhibitory action of nociceptin on nociceptive transmission with its hyperpolarizing effect. Nociceptin was suggested to inhibit more potently slow-conducting than fast-conducting nociceptive transmission [47].

Consistent with our observation in adult rats, Lai *et al.* [74] have demonstrated in SG neurons of young (25-30 days old) rats that nociceptin produces a presynaptic inhibition of excitatory postsynaptic potentials and a postsynaptic hyper-polarization under the current-clamp condition. Although the presynaptic action of nociceptin was analyzed in detail by Liebel *et al.* [75] in young (7-14 days old) rats, they did not note such a postsynaptic action of nociceptin as that reported by Lai *et al.* [74]. Zeilhofer *et al.* [76] have reported a similar presynaptic inhibition of glutamatergic transmission and the lack of the action of nociceptin on inhibitory transmission in young rat spinal dorsal horn neurons. The latter and our observations were contrary to that of Vaughan *et al*. [56] that nociceptin suppresses inhibitory transmission in rat periaqueductal grey neurons.

Actions of endomorphins on synaptic transmission in SG neurons

Endomorphin-1 (EM-1: Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (EM-2: Tyr-Pro-Phe-Phe-NH_2), which were isolated from mammalian brain in 1997 ([77]), exhibit high affinity and selectivity for the μ-opioid receptor as compared to the δ- and κ-opioid receptors (for review see [78]). There is much evidence demonstrating that EM-1 and EM-2 play an important role in inhibiting nociceptive transmission at the spinal cord level, which are as follows. (1) Intrathecal administration of EM-1 and EM-2 produced antinociception in the tail-flick, paw-withdrawal, tail-pressure and flexor-reflex tests in adult rodents [77, 79-86]. (2) EM-1 and EM-2-like immunoreactive fibers have been shown to exist in the superficial laminae of the rat spinal cord [87-90] and in rat primary-afferent fibers [91, 92]. (3) EM-2-like substances were released from the rat spinal dorsal horn in response to electrical stimulation applied to the dorsal root entry zone [93]. (4) The superficial dorsal horn contains high density of μ-opioid receptors [94-96]. (5) Axon terminals containing EM-2-like immunoreactivity make synapses with neurons immunostained for μ-opioid receptors in the rat spinal dorsal horn [97].

EM-1 and EM-2 are different by only one amino acid residue (see above) and thus exhibit similar antinociceptive potency at the spinal cord level in mice [83, 86] and rats [80, 81]. On the other hand, antinociceptive effects produced by them are

distinct in the development of acute tolerance [86], in the extent [79, 82] and in the duration [80] from each other. Both EM-1 and EM-2 reduce primary-afferent C-fiber-mediated responses while EM-1 but not EM-2 inhibits Aβ-fiber-mediated ones in rat dorsal horn neurons [98]. Such a distinction may not be unexpected, because there is a difference between EM-1 and EM-2 in the affinity to μ-opioid receptors, as determined by binding experiments [77]. The activation of K^+-channels through cloned μ-opioid receptors differed in extent between EM-1 and EM-2 actions [99]. Human μ-opioid receptors fused to $G_{i1}\alpha$ or $G_{i2}\alpha$ in transfected HEK 293 cells exhibited binding affinities which were different by 3-8-fold between EM-1 and EM-2 [100]. Behavioral studies have suggested that EM-1 and EM-2 may activate μ-opioid receptor subtypes different from each other, such as μ_1 and μ_2 (for review see [101]), which are pharmacologically distinct in the spinal dorsal horn [83, 84], although there is no evidence for the presence of the μ-opioid receptor subtypes. A similar idea has been also applied to a difference between EM-1 and EM-2 in motivational effects and conditioned place preference responses that are produced by their intracerebroventricular administrations [102, 103]. There is a difference between EM-1 and EM-2 immunoreactivities in the distribution in the spinal dorsal horn such that EM-2 exists at higher density than EM-1, suggesting a different role of EM-1 and EM-2 in spinal antinociception [88]. It was likely that the difference in antinociceptive effects between EM-1 and EM-2 is due to a distinction in their modulatory effects on synaptic transmission in SG neurons.

In about half of the adult rat SG neurons examined, EM-1 and EM-2 (1 μM each) produced an outward current having a similar amplitude at -70 mV (Figure 3Aa, b) with almost the same EC_{50} value (0.19-0.21 μM). Both of them reversed at a potential close to E_K; the current-voltage relation exhibited a slight inward rectification. The EM-1 and EM-2 currents were reduced in amplitude by Ba^{2+} (0.1 mM) and 4-AP (1 mM), and also by a μ-opioid receptor antagonist CTAP (D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2; 1 μM) with similar extents (Figure 3Aa, b). The EM-2 but not EM-1 current was increased in amplitude by a dipeptidyl peptidase IV inhibitor diprotin A (IIe-Pro-IIe; 30 μM; [44]). One μM each of EM-1 and EM-2 reduced the frequency of spontaneous EPSC (sEPSC) with a similar time course and extent without altering its amplitude; these actions were not in the presence of CTAP (1 μM; Figure 3Ba, b). Yajiri and Huang [104] have reported an inhibition of excitatory transmission in SG neurons by EM-1 or EM-2, although their actions were not compared with each other. These results indicate that EM-1 and EM-2 hyperpolarize membranes by opening inwardly-rectifying K^+ channels and inhibit the release of L-glutamate from nerve terminals in the SG, both of which are mediated by μ-opioid receptors, in a manner quantitatively similar to each other. The difference in antinociceptive effects between EM-1 and EM-2 could not be attributed to a distinction in their effects on excitatory transmission in SG neurons, and may be explained by a difference in their enzymatic degradation [11, 44]. Opioids other than EMs are reported to hyperpolarize membranes and inhibit excitatory but not inhibitory transmission in adult rat SG neurons ([10, 23]; see also [13]).

In young (2-4 weeks old) rats, it has been reported that both EM-1 and EM-2 hyperpolarize membranes [90] and inhibit excitatory transmission in SG neurons [90, 105].

Actions of galanin on synaptic transmission in SG neurons

Galanin (a 29/30 amino acid peptide) was first extracted from porcine upper intestines in 1983 [106] and was then reported to extensively exist in the peripheral and central nervous systems [107]. Galanin serves as a neurotransmitter or neuromodulator in various physiological functions including feeding and pain [107-109]. There are three types of G protein-coupled metabotropic receptor (GalR1, 2, 3) for galanin [107]. There is much evidence for the idea that galanin plays a role in regulating nociceptive transmission to the spinal dorsal horn from the periphery, which are as follows. (1) Galanin immunoreactivity, GalR1, 2, 3 mRNAs and proteins exist in the rat DRG and the spinal dorsal horn [110-116]. (2) Intrathecally-administrated galanin modulated nociceptive responses in rats [117-120]. (3) The expression of galanin was upregulated in DRG neurons after nerve

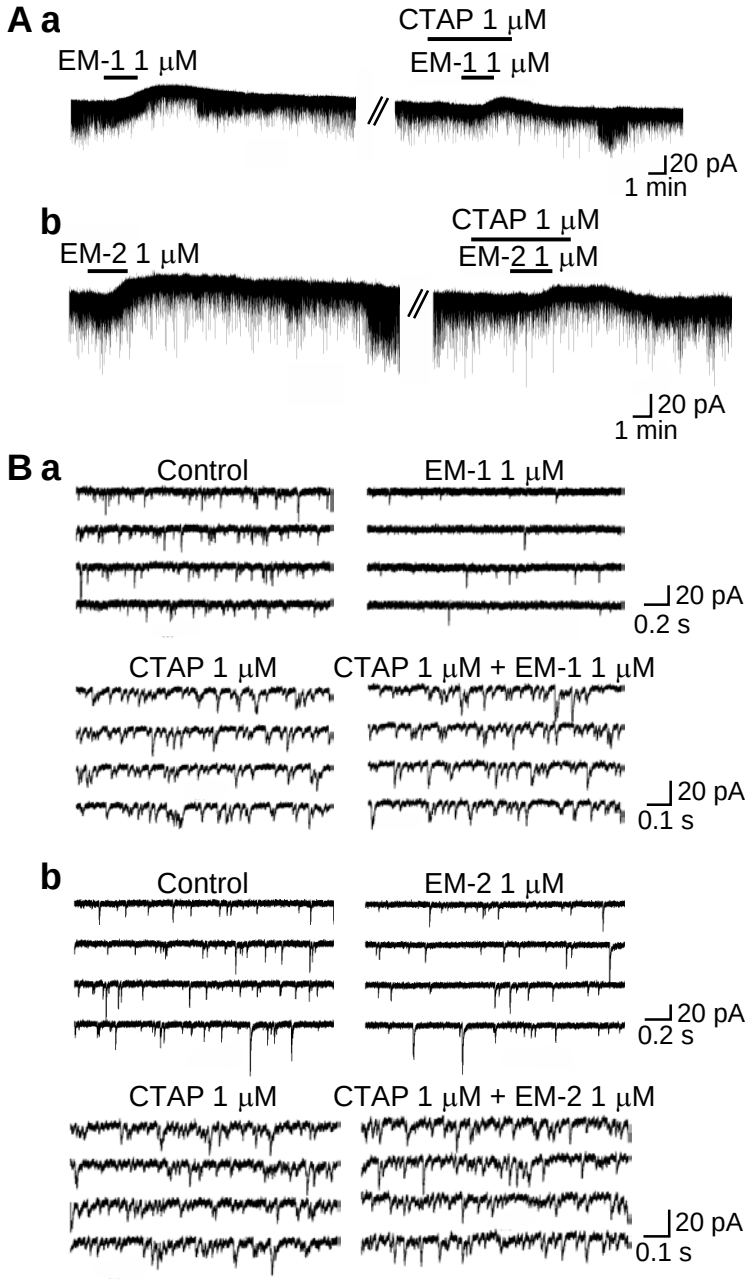

Figure 3. Actions of EM-1 and EM-2 (1 μM each) on spontaneous excitatory transmission in adult rat SG neurons. (Aa, b) EM-1 (Aa) and EM-2 produced outward currents (Ab) in a manner sensitive to CTAP (1 μM). (Ba, b) Four consecutive traces of sEPSCs in the absence (left) of EM-1 (Ba) or EM-2 (Bb) and under its action (right) in Krebs solution without (upper) and with CTAP (1 μM; lower). V_H = -70 mV. Modified from [44].

injury and in dorsal horn neurons after inflammation [107, 109]. For instance, peripheral inflammation induced by the injection of carrageenan into the hindpaw of rats increased the number of galanin mRNA-positive neurons in the superficial dorsal horn [121]. (4) Transgenic mice overexpressing galanin in a population of DRG neurons exhibited nociceptive responses different from those of wild-type controls [122]. (5) According to behavioral studies, the intrathecal administration of galanin

produced such biphasic effects as nociception at low doses and antinociception at high doses [119, 120], for which the cellular mechanisms are mentioned below.

In adult rat SG neurons, galanin concentration-dependently increased the frequency of sEPSC (EC_{50} = 2.0 nM) without a change in amplitude, indicating a presynaptic effect. This effect reduced in extent in Ca^{2+}-free or a voltage-gated Ca^{2+}-channel blocker La^{3+} (30 μM)-containing Krebs solution, and was produced by a GalR2/R3 agonist galanin 2-11 but not a GalR1 agonist M617 [galanin(1-13)-Gln^{14}-bradykinin (3-9)amide; 0.03 μM each; Figure 4Aa, b]. Galanin also concentration-dependently produced an outward current at -70 mV (EC_{50} = 44 nM), although this appeared to be contaminated by a small inward current. This outward current was mimicked by M617 but not galanin 2-11 (1 μM each; Figure 4B, C). Moreover, galanin (0.1 μM) reduced monosynaptically-evoked Aδ-fiber and C-fiber EPSC amplitudes; the former reduction was larger than the latter one. A similar action was produced by galanin 2-11 but not M617 (0.1 μM each; Figure 4D, E). Spontaneous and focally-evoked inhibitory transmissions were unaffected by galanin (0.1 μM). These results indicate that galanin at lower concentrations enhances the spontaneous release of L-glutamate from nerve terminals by Ca^{2+} entry from external solution following GalR2/R3 activation while at higher concentrations it also produces a membrane hyperpolarization by activating GalR1. Moreover, galanin reduces L-glutamate release onto SG neurons from primary-afferent fibers by activating GalR2/R3 [48]. These effects could contribute to at least a part of the behavioral effect of galanin.

In SG neurons of young adult rats, Alier *et al.* [123] have reported that galanin inhibits excitatory transmission evoked by stimulating the dorsal root entry zone.

Actions of oxytocin on synaptic transmission in SG neurons

A posterior pituitary hormone oxytocin (a nine amino acid peptide) has various actions including social interaction and antinociception, other than milk ejection during lactation and uterine contraction during parturition [124-127]. There is much evidence showing that oxytocin plays a role in regulating nociceptive transmission to the spinal dorsal horn from the periphery, which are as follows. (1) There are oxytocin-immunoreactive fibers to the spinal superficial dorsal horn from the hypothalamic paraventricular nucleus [128, 129]. (2) Oxytocinergic axons make synaptic contacts with spinal superficial dorsal horn neurons [130]. (3) Oxytocin-binding sites densely exist in the spinal dorsal horn [131-135]. (4) The electrical stimulation of the anterior part of the hypothalamic paraventricular nucleus increased oxytocin concentration in cerebrospinal fluid and produced antinociception [136]. (5) Intraperitoneal or intrathecal administration of oxytocin reportedly produced antinociception in rats [137, 138]. (6) Somatic noxious stimulation activated hypothalamic paraventricular oxytocinergic neurons projecting to the spinal dorsal horn [139].

In adult rat SG neurons, oxytocin (0.5 μM) unaffected sEPSC frequency and amplitude, and monosynaptically-evoked primary-afferent Aδ-fiber and C-fiber EPSC amplitude. On the other hand, oxytocin produced an inward current at -70 mV (Figure 5A) in a concentration-dependent manner (EC_{50} = 0.022 μM). Oxytocin also concentration-dependently increased GABAergic and glycinergic spontaneous IPSC (sIPSC) frequency with a small increase in its amplitude (Figure 5C). Their EC_{50} values were 0.024 and 0.038 μM, respectively. These activities were repeated with a slow recovery from desensitization and mimicked by a G-protein coupled oxytocin-receptor agonist TGOT ([Thr^4,Gly^7]-oxytocin; 0.5 μM; Figure 5B, D). The oxytocin current was inhibited by an oxytocin-receptor antagonist dVOT ([$d(CH_2)_5^1$,$Tyr(Me)^2$,Thr^4,Orn^8, des-Gly-NH_2^9]vasotocin; 1 μM; Figure 5A), intracellular GDP-β-S, U-73122 (an inhibitor of phospholipase C coupled to G_q protein; 10 μM), 2-aminoethoxydiphenyl borate (an IP_3-induced Ca^{2+}-release inhibitor; 200 μM) but not dantrolene (a Ca^{2+}-induced Ca^{2+}-release inhibitor; 10 μM), chelerythrine (a protein kinase C inhibitor; 10 μM), dibutyryl cyclic-AMP (1 mM), CNQX (a non-NMDA receptor antagonist; 10 μM), Ca^{2+}-free and TTX (0.5 μM). On the other hand, the spontaneous inhibitory transmission enhancements were depressed by dVOT (1 μM; Figure 5C) and

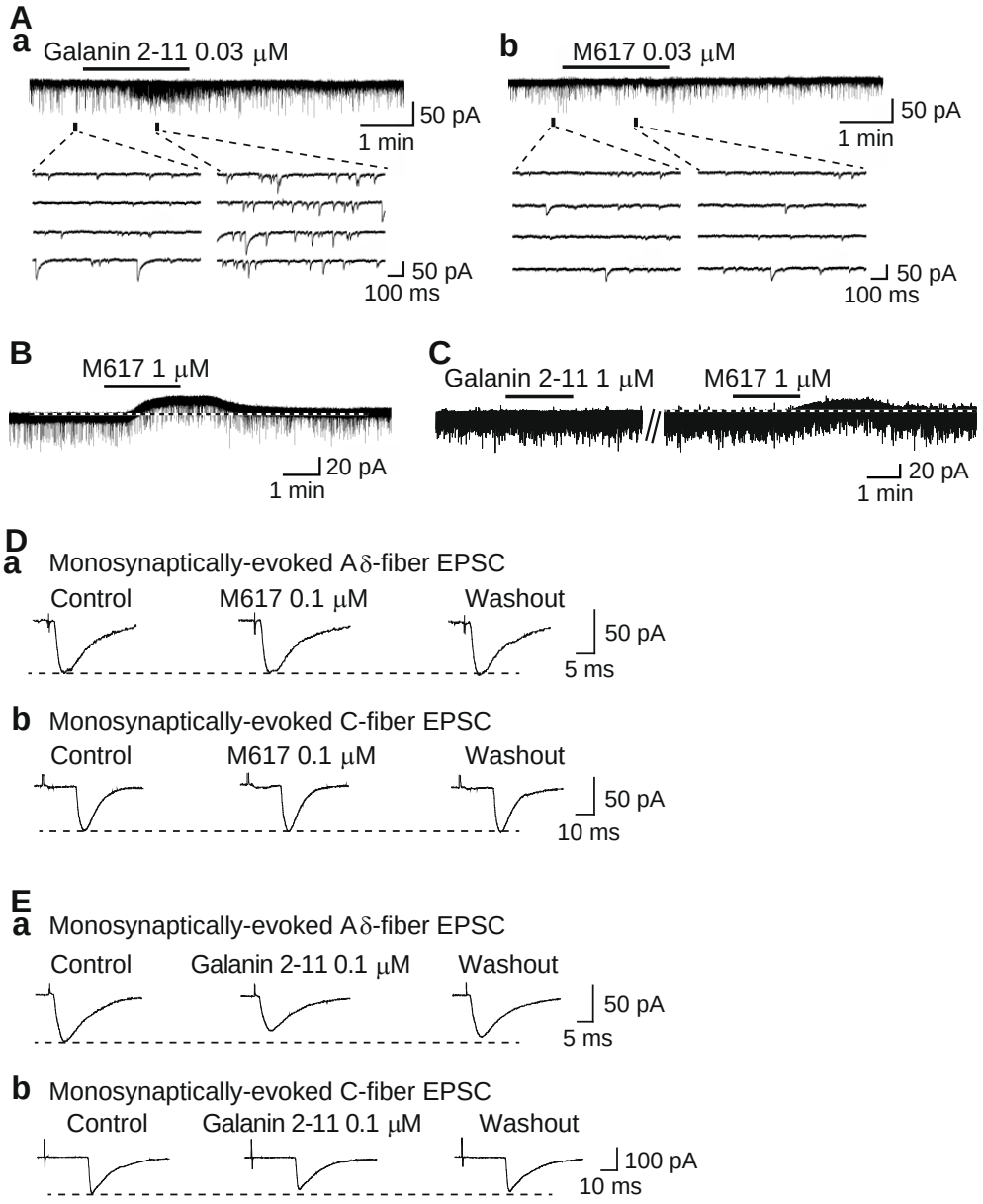

Figure 4. Actions of galanin receptor (GalR) agonists on spontaneous excitatory transmission in adult rat SG neurons. (Aa, b) Recordings of sEPSCs in the absence and presence of a GalR2/R3 agonist galanin 2-11 (Aa) or a GalR1 agonist M617 (Ab; 0.03 μM each). Four consecutive traces of sEPSCs for a period indicated by a short bar located below the chart recording are shown in an expanded scale in time. (B, C) M617 (B and C) but not galanin 2-11 (1 μM each; C) produced an outward current. (D, E) Galanin 2-11 but not M617 (0.1 μM each) inhibited monosynaptically-evoked Aδ-fiber and C-fiber EPSCs. V_H = -70 mV. Modified from [48].

also by TTX (0.5 μM). Current-voltage relation for the oxytocin current reversed at negative potentials more than E_K or around 0 mV. The oxytocin current was depressed in high-K^+ (10 mM), low-Na^+ (decreased by 117 mM) or Ba^{2+} (1 mM)-containing Krebs solution. Although a vasopressin V_{1A}-receptor antagonist ($d(CH_2)_5^1$,$Tyr(Me)^2$, Arg^8) vasopressin (1 μM) inhibited the oxytocin (0.5 μM) current, there was no correlation in amplitude between a vasopressin-receptor agonist [Arg^8] vasopressin (0.5 μM) and oxytocin (0.5 μM) responses. These results indicate that oxytocin

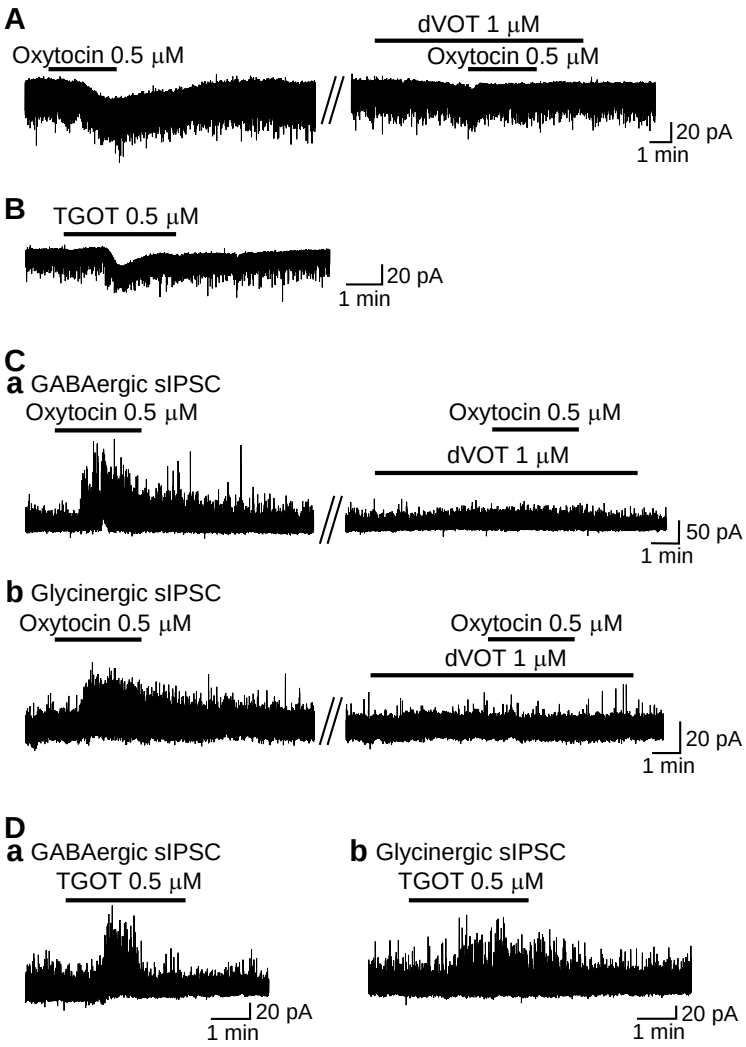

Figure 5. Oxytocin (0.5 μM) produced inward currents and enhanced GABAergic and glycinergic spontaneous inhibitory transmissions by activating oxytocin receptors in adult rat SG neurons. (A) Changes in holding currents produced by oxytocin (0.5 μM) in the absence (left) and presence of an oxytocin-receptor antagonist dVOT (right; 1 μM). (B) An oxytocin-receptor agonist TGOT (0.5 μM) as well as oxytocin produced an inward current. (C) Recordings of GABAergic (a) and glycinergic spontaneous inhibitory postsynaptic currents (sIPSCs; b) in the absence and presence of oxytocin (0.5 μM) in Krebs solution without (left) and with dVOT (1 μM; right). (D) Recordings of GABAergic (a) and glycinergic sIPSCs (b) in the absence and presence of TGOT (0.5 μM). V_H = -70 mV (A, B) or 0 mV (C, D). The GABAergic and glycinergic sIPSCs were recorded in the presence of a glycine-receptor antagonist strychnine (0.5 μM) and a $GABA_A$-receptor antagonist bicuculline (10 μM), respectively. Modified from [45].

produces a membrane depolarization mediated by oxytocin but not vasopressin-V_{1A} receptors, which increases neuronal activity, resulting in the enhancement of inhibitory transmission, a possible mechanism for antinociception. This depolarization is due to a change in membrane permeabilities to K^+ and/or Na^+, which is possibly mediated by phospholipase C and IP_3-induced Ca^{2+} release [45]. This idea is illustrated in figure 6.

In spinal superficial dorsal horn neurons of young (2-4 weeks old) rats, Breton *et al.* [141] have reported that TGOT increases the spontaneous release of L-glutamate on GABAergic interneurons, resulting in GABA release enhancement, a cellular

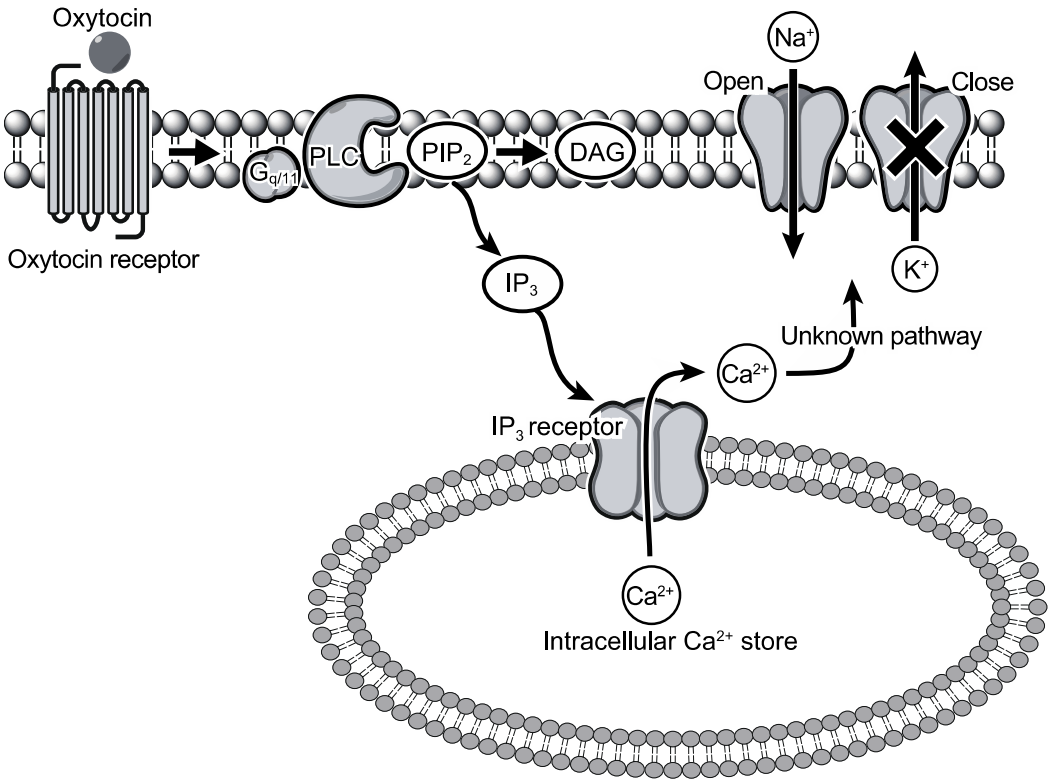

Figure 6. Schematic illustration demonstrating how oxytocin produces an inward current in adult rat SG neurons. DAG: diacylglycerol; PIP_2: phosphatidylinositol 4,5-bisphosphate; PLC: phospholipase C. Modified from [140].

mechanism for antinociception produced by oxytocin. Although an increase in L-glutamate release produced by oxytocin has been reported in neonatal rat spinal superficial dorsal horn neurons in culture [131], Robinson *et al*. [142] have demonstrated that oxytocin inhibits primary-afferent evoked excitatory transmission in adult mouse spinal superficial dorsal horn neurons by activating oxytocin receptors. On the other hand, Schorscher-Petcu *et al*. [134] have reported that antinociception produced by systemically administrated oxytocin is mediated by vasopressin V_{1A} but not oxytocin receptors in the mouse spinal dorsal horn. There may be a difference between rat and mouse and also a developmental change in cellular mechanisms for antinociception produced by oxytocin. These issues remain to be further examined.

Among neuropeptides involved in the descending pain control in the spinal dorsal horn that originates from the hypothalamus [143], there are not only oxytocin but also orexin-A (hypocretin-1) and orexin-B (hypocretin-2; 33 and 28 amino acid peptides, respectively; [144, 145]). In support of this idea, both orexin-A and orexin-B are present in the rat spinal cord, albeit the latter's content is higher than the former's one [146-148]. The intrathecal administration of orexin-A produced antinociception in the hot plate test in rats, both inflammatory [149, 150] and neuropathic pain rat models [151-153]. Orexin-B also exhibited a similar action, albeit this peptide was less effective than orexin-A [151, 154]. The orexin-A activity appears to be mediated by G-protein coupled orexin-1 and orexin-2 receptors while the orexin-B activity is mediated by orexin-2 receptor [151], because orexin-2 receptor binds to both orexin-A and orexin-B with a similar affinity whereas orexin-1 receptor is more responsive (by about 10 fold) to orexin-A than orexin-B [145]. The orexin-1 and orexin-2 receptors are located in the rat spinal cord [155-157]. A non-specific

muscarinic acetylcholine-receptor agonist carbachol-induced stimulation of the hypothalamus produced antinociception in a neuropathic pain model in a manner sensitive to an orexin-1 receptor antagonist SB-334867 [*N*-(2-methyl-6-benzoxazolyl)-*N*'-1,5-naphthyridin-4-yl urea] [158]. In order to reveal cellular mechanisms responsible for the antinociception produced by orexin-A and orexin-B, their effects on synaptic transmissions in SG neurons have been examined in young rats. Orexin-A (0.1 μM) reduced Aδ-fiber and C-fiber-evoked monosynaptic EPSC amplitudes in a manner sensitive to an orexin-1 receptor antagonist SB-674042 ([5-(2-fluorophenyl)-2-methyl-4-thiazolyl] [2(S)-2-[(5-phenyl-1,3,4-oxadiazol-2-yl)methyl-1-pyrrolidinyl] methanone; 1 μM]) but not to an orexin-2 receptor antagonist EMPA (*N*-ethyl-2-[(6-methoxy-3-pyridinyl)[(2-methylphenyl)sulfonyl] amino]-*N*-(3-pyridinylmethyl)-acetamide; 1 μM). Orexin-A (0.1 μM) also increased sEPSC frequency and produced an inward current at -70 mV; the latter action was inhibited by both SB-674042 and EMPA [159]. Orexin-B (1 μM) produced an inward current at -60 mV, increased sEPSC frequency without a change in the peak amplitude of EPSC evoked by stimulating the dorsal root and increased sIPSC frequency [160]. Orexin-A and orexin-B appeared to affect synaptic transmission in SG neurons in a manner different from each other and also from oxytocin. This issue remains to be further examined.

CONCLUSION

Various neuropeptides modulate synaptic transmission in SG neurons that play a pivotal role in regulating nociceptive transmission from the periphery. The SG neurons receive glutamatergic excitatory, and GABAergic and glycinergic inhibitory transmissions in a mono- and polysynaptic manner through the dorsal root. Nociceptin, EM-1 and EM-2 hyperpolarized membranes and inhibited excitatory transmissions by activating their receptors without a change in inhibitory transmission. On the other hand, galanin affected excitatory transmission in a biphasic concentration-dependent manner with no effect on inhibitory transmission. Galanin at low concentrations presynaptically enhanced spontaneous excitatory transmission by activating GalR2/R3 while at high concentrations it produced a membrane hyperpolarization by GalR1 activation. GalR2/R3 but not GalR1 activation inhibited monosynaptically-evoked excitatory transmission. Oxytocin produced a membrane depolarization by activating oxytocin receptors, resulting in spontaneous inhibitory transmission enhancement; this was not accompanied by a change in excitatory transmission. In conclusion, neuropeptides inhibit the excitability of SG neurons through various mechanisms, leading to antinociception.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

REFERENCES

1. Schneider, S. P. and Perl, E. R. 1988, J. Neurosci., 8, 2062.
2. Sugiura, Y., Lee, C. L. and Perl, E. R. 1986, Science, 234, 358.
3. Yoshimura, M. and Jessel, T. M. 1990, J. Physiol., 430, 315.
4. Todd, A. J. 2010, Nat. Rev. Neurosci., 11, 823.
5. Willis, W. D. Jr. and Coggeshall, R. E. 1991, Sensory Mechanisms of the Spinal Cord, 2nd Edition, Plenum, New York.
6. Yoshimura, M. and Nishi, S. 1993, Neuroscience, 53, 519.
7. Nakatsuka, T., Park, J.-S., Kumamoto, E., Tamaki, T. and Yoshimura, M. 1999, Pain, 82, 39.
8. Ito, A., Kumamoto, E., Takeda, M., Takeda, M., Shibata, K., Sagai, H. and Yoshimura, M. 2000, J. Neurosci., 20, 6302.
9. Fürst, S. 1999, Brain Res. Bull., 48, 129.
10. Yoshimura, M. and North, R. A. 1983, Nature, 305, 529.
11. Fujita, T., Nakatsuka, T. and Kumamoto, E. 2007, Cellular and Molecular Mechanisms for the Modulation of Nociceptive Transmission in the Peripheral and Central Nervous Systems, E. Kumamoto (Ed.), Research Signpost, Kerala, 87.

12. Yang, K., Kumamoto, E., Furue, H., Li, Y.-Q. and Yoshimura, M. 2000, Neuropharmacology, 39, 2185.
13. Koga, A., Fujita, T., Totoki, T. and Kumamoto, E. 2005, Br. J. Pharmacol., 145, 602.
14. Sonohata, M., Furue, H., Katafuchi, T., Yasaka, T., Doi, A., Kumamoto, E. and Yoshimura, M. 2004, J. Physiol., 555, 515.
15. Abe, K., Kato, G., Katafuchi, T., Tamae, A., Furue, H. and Yoshimura, M. 2009, Neuroscience, 159, 316.
16. Kumamoto, E. and Fujita, T. 2005, Recent Res. Develop. Physiol., 3, S. G. Pandalai (Ed.), Research Signpost, Kerala, 39.
17. Li, J. and Perl, E. R. 1994, J. Neurophysiol., 72, 1611.
18. Liu, T., Fujita, T., Kawasaki, Y. and Kumamoto, E. 2004, Brain Res. Bull., 64, 75.
19. Jiang, N., Furue, H., Katafuchi, T. and Yoshimura, M. 2003, Neurosci. Res., 47, 97.
20. Nakatsuka, T., Fujita, T., Inoue, K. and Kumamoto, E. 2008, J. Physiol., 586, 2511.
21. Tamae, A., Nakatsuka, T., Koga, K., Kato, G., Furue, H., Katafuchi, T. and Yoshimura, M. 2005, J. Physiol., 568, 243.
22. Taniguchi, W., Nakatsuka, T., Miyazaki, N., Yamada, H., Takeda, D., Fujita, T., Kumamoto, E. and Yoshida, M. 2011, Pain, 152, 95.
23. Kohno, T., Kumamoto, E., Higashi, H., Shimoji, K. and Yoshimura, M. 1999, J. Physiol., 518, 803.
24. Ataka, T., Kumamoto, E., Shimoji, K. and Yoshimura, M. 2000, Pain, 86, 273.
25. Iyadomi, M., Iyadomi, I., Kumamoto, E., Tomokuni, K. and Yoshimura, M. 2000, Pain, 85, 385.
26. Luo, C., Kumamoto, E., Furue, H., Chen, J. and Yoshimura, M. 2002, Neurosci. Lett., 321, 17.
27. Morisset, V. and Urban, L. 2001, J. Neurophysiol., 86, 40.
28. Kawasaki, Y., Kumamoto, E., Furue, H. and Yoshimura, M. 2003, Anesthesiology, 98, 682.
29. Lao, L.-J., Kumamoto, E., Luo, C., Furue, H. and Yoshimura, M. 2001, Pain, 94, 315.
30. Lao, L.-J., Kawasaki, Y., Yang, K., Fujita, T. and Kumamoto, E. 2004, Neuroscience, 125, 221.
31. Coull, J. A. M., Boudreau, D., Bachand, K., Prescott, S. A., Nault, F., Sik, A., de Koninck, P. and de Koninck, Y. 2003, Nature, 424, 938.
32. Moore, K. A., Kohno, T., Karchewski, L. A., Scholz, J., Baba, H. and Woolf, C. J. 2002, J. Neurosci., 22, 6724.
33. Kohno, T. 2007, Cellular and Molecular Mechanisms for the Modulation of Nociceptive Transmission in the Peripheral and Central Nervous Systems, E. Kumamoto (Ed.), Research Signpost, Kelara, 131.
34. Sandkühler, J. 2009, Physiol. Rev., 89, 707.
35. Zeilhofer, H. U., Wildner, H. and Yévenes, G. E. 2012, Physiol. Rev., 92, 193.
36. Rhee, J.-S., Wang, Z.-M., Nabekura, J., Inoue, K. and Akaike, N. 2000, J. Physiol., 524, 471.
37. Baba, H., Kohno, T., Okamoto, M., Goldstein, P. A., Shimoji, K. and Yoshimura, M. 1998, J. Physiol., 508, 83.
38. Takeda, D., Nakatsuka, T., Papke, R. and Gu, J. G. 2003, Pain, 101, 13.
39. Baba, H., Goldstein, P. A., Okamoto, M., Kohno, T., Ataka, T., Yoshimura, M. and Shimoji, K. 2000, Anesthesiology, 92, 485.
40. Baba, H., Shimoji, K. and Yoshimura, M. 2000, Anesthesiology, 92, 473.
41. Liu, T., Fujita, T. and Kumamoto, E. 2011, J. Neurophysiol., 106, 233.
42. Fukushima, T., Ohtsubo, T., Tsuda, M., Yanagawa, Y. and Hori, Y. 2009, J. Neurophysiol., 102, 1459.
43. Fujita, T. and Kumamoto, E. 2011, Curr. Top. Pharmacol., 15, 27.
44. Fujita, T. and Kumamoto, E. 2006, Neuroscience, 139, 1095.
45. Jiang, C.-Y., Fujita, T. and Kumamoto, E. 2014, J. Neurophysiol., 111, 991.
46. Luo, C., Kumamoto, E., Furue, H. and Yoshimura, M. 2001, Neuroscience, 108, 323.
47. Luo, C., Kumamoto, E., Furue, H., Chen, J. and Yoshimura, M. 2002, Neuroscience, 109, 349.
48. Yue, H.-Y., Fujita, T. and Kumamoto, E. 2011, J. Neurophysiol., 105, 2337.

49. Meunier, J.-C., Mollereau, C., Toll, L., Suaudeau, C., Moisand, C., Alvinerie, P., Butour, J.-L., Guillemot, J.-C., Ferrara, P., Monsarrat, B., Mazarguil, H., Vassart, G., Parmentier, M. and Costentin, J. 1995, Nature, 377, 532.
50. Reinscheid, R. K., Nothacker, H.-P., Bourson, A., Ardati, A., Henningsen, R. A., Bunzow, J. R., Grandy, D. K., Langen, H., Monsma, F. J. Jr. and Civelli, O. 1995, Science, 270, 792.
51. Lachowicz, J. E., Shen, Y., Monsma, F. J. Jr. and Sibley, D. R. 1995, J. Neurochem., 64, 34.
52. Mollereau, C., Parmentier, M., Mailleux, P., Butour, J.-L., Moisand, C., Chalon, P., Caput, D., Vassart, G. and Meunier, J.-C. 1994, FEBS Lett., 341, 33.
53. Vaughan, C. W. and Christie, M. J. 1996, Br. J. Pharmacol., 117, 1609.
54. Connor, M., Vaughan, C. W., Chieng, B. and Christie, M. J. 1996, Br. J. Pharmacol., 119, 1614.
55. Connor, M., Vaughan, C. W., Jennings, E. A., Allen, R. G. and Christie, M. J. 1999, Br. J. Pharmacol., 128, 1779.
56. Vaughan, C. W., Ingram, S. L. and Christie, M. J. 1997, J. Neurosci., 17, 996.
57. Allen, C. N., Jiang, Z.-G., Teshima, K., Darland, T., Ikeda, M., Nelson, C. S., Quigley, D. I., Yoshioka, T., Allen, R. G., Rea, M. A. and Grandy, D. K. 1999, J. Neurosci., 19, 2152.
58. Amano, T., Matsubayashi, H., Tamura, Y. and Takahashi, T. 2000, Brain Res., 853, 269.
59. Wagner, E. J., Rϕnnekleiv, O. K., Grandy, D. K. and Kelly, M. J. 1998, Neuroendocrinology, 67, 73.
60. Knoflach, F., Reinscheid, R. K., Civelli, O. and Kemp, J. A. 1996, J. Neurosci., 16, 6657.
61. Abdulla, F. A. and Smith, P. A. 1997, J. Neurosci., 17, 8721.
62. Darland, T., Heinricher, M. M. and Grandy, D. K. 1998, Trends Neurosci., 21, 215.
63. Yamamoto, T., Nozaki-Taguchi, N., Sakashita, Y. and Kimura, S. 1999, Prog. Neurobiol., 57, 527.
64. Hao, J.-X., Wiesenfeld-Hallin, Z. and Xu, X.-J. 1997, Neurosci. Lett., 223, 49.
65. Xu, X.-J., Hao, J.-X. and Wiesenfeld-Hallin, Z. 1996, NeuroReport, 7, 2092.
66. Yamamoto, T., Nozaki-Taguchi, N. and Kimura, S. 1997, Neurosci. Lett., 224, 107.
67. Erb, K., Liebel, J. T., Tegeder, I., Zeilhofer, H. U., Brune, K. and Geisslinger, G. 1997, NeuroReport, 8, 1967.
68. Yamamoto, T., Nozaki-Taguchi, N. and Kimura, S. 1997, Brain Res., 754, 329.
69. Faber, E. S. L., Chambers, J. P., Evans, R. H. and Henderson, G. 1996, Br. J. Pharmocol., 119, 189.
70. Stanfa, L. C., Chapman, V., Kerr, N. and Dickenson, A. H. 1996, Br. J. Pharmacol., 118, 1875.
71. Houtani, T., Nishi, M., Takeshima, H., Nukada, T. and Sugimoto, T. 1996, Biochem. Biophys. Res. Commun., 219, 714.
72. Neal, C. R. Jr., Mansour, A., Reinscheid, R., Nothacker, H.-P., Civelli, O. and Watson, S. J. Jr. 1999, J. Comp. Neurol., 406, 503.
73. Anton, B., Fein, J., To, T., Li, X., Silberstein, L. and Evans, C. J. 1996, J. Comp. Neurol., 368, 229.
74. Lai, C. C., Wu, S. Y., Dun, S. L. and Dun, N. J. 1997, Neuroscience, 81, 887.
75. Liebel, J. T., Swandulla, D. and Zeilhofer, H. U. 1997, Br. J. Pharmacol., 121, 425.
76. Zeilhofer, H. U., Muth-Selbach, U., Gühring, H., Erb, K. and Ahmadi, S. 2000, J. Neurosci., 20, 4922.
77. Zadina, J. E., Hackler, L., Ge, L.-J. and Kastin, A. J. 1997, Nature, 386, 499.
78. Horvath, G. 2000, Pharmacol. Ther., 88, 437.
79. Goldberg, I. E., Rossi, G. C., Letchworth, S. R., Mathis, J. P., Ryan-Moro, J., Leventhal, L., Su, W., Emmel, D., Bolan, E. A. and Pasternak, G. W. 1998, J. Pharmacol. Exp. Ther., 286, 1007.
80. Grass, S., Xu, I. S., Wiesenfeld-Hallin, Z. and Xu, X.-J. 2002, Neurosci. Lett., 324, 197.
81. Horvath, G., Szikszay, M., Tömböly, C. and Benedek, G. 1999, Life Sci., 65, 2635.
82. Ohsawa, M., Mizoguchi, H., Narita, M., Nagase, H., Kampine, J. P. and Tseng, L. F. 2001, J. Pharmacol. Exp. Ther., 298, 592.

83. Sakurada, S., Zadina, J. E., Kastin, A. J., Katsuyama, S., Fujimura, T., Murayama, K., Yuki, M., Ueda, H. and Sakurada, T. 1999, Eur. J. Pharmacol., 372, 25.
84. Sakurada, S., Hayashi, T., Yuhki, M., Fujimura, T., Murayama, K., Yonezawa, A., Sakurada, C., Takeshita, M., Zadina, J. E., Kastin, A. J. and Sakurada, T. 2000, Brain Res., 881, 1.
85. Sakurada, S., Hayashi, T., Yuhki, M., Orito, T., Zadina, J. E., Kastin, A. J., Fujimura, T., Murayama, K., Sakurada, C., Sakurada, T., Narita, M., Suzuki, T., Tan-no, K. and Tseng, L. F. 2001, Eur. J. Pharmacol., 427, 203.
86. Stone, L. S., Fairbanks, C. A., Laughlin, T. M., Nguyen, H. O., Bushy, T. M., Wessendorf, M. W. and Wilcox, G. L. 1997, NeuroReport, 8, 3131.
87. Martin-Schild, S., Zadina, J. E., Gerall, A. A., Vigh, S. and Kastin, A. J. 1997, Peptides, 18, 1641.
88. Martin-Schild, S., Gerall, A. A., Kastin, A. J. and Zadina, J. E. 1999, J. Comp. Neurol., 405, 450.
89. Wang, Q.-P., Zadina, J. E., Guan, J.-L., Kastin, A. J., Funahashi, H. and Shioda, S. 2002, Neuroscience, 113, 593.
90. Wu, S.-Y., Dun, S. L., Wright, M. T., Chang, J.-K. and Dun, N. J. 1999, Neuroscience, 89, 317.
91. Martin-Schild, S., Gerall, A. A., Kastin, A. J. and Zadina, J. E. 1998, Peptides, 19, 1783.
92. Pierce, T. L., Grahek, M. D. and Wessendorf, M. W. 1998, NeuroReport, 9, 385.
93. Williams, C. A., Wu, S.-Y., Dun, S. L., Kwok, E. H. and Dun, N. J. 1999, Neurosci. Lett., 273, 25.
94. Besse, D., Lombard, M. C., Zajac, J. M., Roques, B. P. and Besson, J. M. 1990, Brain Res., 521, 15.
95. Rahman, W., Dashwood, M. R., Fitzgerald, M., Aynsley-Green, A. and Dickenson, A. H. 1998, Develop. Brain Res., 108, 239.
96. Stevens, C. W., Lacey, C. B., Miller, K. E., Elde, R. P. and Seybold, V. S. 1991, Brain Res., 550, 77.
97. Wang, Q.-P., Zadina, J. E., Guan, J.-L. and Shioda, S. 2003, Neurosci. Lett., 341, 107.
98. Chapman, V., Diaz, A. and Dickenson, A. H. 1997, Br. J. Pharmacol., 122, 1537.
99. Gong, J., Strong, J. A., Zhang, S., Yue, X., DeHaven, R. N., Daubert, J. D., Cassel, J. A., Yu, G., Mansson, E. and Yu, L. 1998, FEBS Lett., 439, 152.
100. Massotte, D., Brillet, K., Kieffer, B. L. and Milligan, G. 2002, J. Neurochem., 81, 1372.
101. Pasternak, G. W. and Wood, P. J. 1986, Life Sci., 38, 1889.
102. Narita, M., Ozaki, S., Ioka, M., Mizoguchi, H., Nagase, H., Tseng, L. F. and Suzuki, T. 2001, Neuroscience, 105, 213.
103. Wu, H.-E., MacDougall, R. S., Clithero, A. D., Leitermann, R. J., Terashvili, M. and Tseng, L. F. 2004, Neurosci. Lett., 365, 157.
104. Yajiri, Y. and Huang, L.-Y. M. 2000, J. Biomed. Sci., 7, 226.
105. Wu, S.-Y., Ohtubo, Y., Brailoiu, G. C. and Dun, N. J. 2003, Br. J. Pharmacol., 140, 1088.
106. Tatemoto, K., Rökaeus, Å., Jörnvall, H., McDonald, T. J. and Mutt, V. 1983, FEBS Lett., 164, 124.
107. Lang, R., Gundlach, A. L. and Kofler, B. 2007, Pharmacol. Ther., 115, 177.
108. Hökfelt, T. 2005, Neuropeptides, 39, 125.
109. Liu, H.-X. and Hökfelt, T. 2002, Trends Pharmacol. Sci., 23, 468.
110. Brumovsky, P., Mennicken, F., O'Donnell, D. and Hökfelt, T. 2006, Brain Res., 1085, 111.
111. Ch'ng, J. L. C., Christofides, N. D., Anand, P., Gibson, S. J., Allen, Y. S., Su, H. C., Tatemoto, K., Morrison, J. F. B., Polak, J. M. and Bloom, S. R. 1985, Neuroscience, 16, 343.
112. Kerekes, N., Mennicken, F., O'Donnell, D., Hökfelt, T. and Hill, R. H. 2003, Eur. J. Neurosci., 18, 2957.
113. Landry, M., Bouali-Benazzouz, R., André, C., Shi, T. J. S., Léger, C., Nagy, F. and Hökfelt, T. 2006, J. Comp. Neurol., 499, 391.
114. O'Donnell, D., Ahmad, S., Wahlestedt, C. and Walker, P. 1999, J. Comp. Neurol., 409, 469.

115. Skofitsch, G. and Jacobowitz, D. M. 1985, Brain Res. Bull., 15, 191.
116. Waters, S. M. and Krause, J. E. 2000, Neuroscience, 95, 265.
117. Cridland, R. A. and Henry, J. L. 1988, Neuropeptides, 11, 23.
118. Kuraishi, Y., Kawamura, M., Yamaguchi, T., Houtani, T., Kawabata, S., Futaki, S., Fujii, N. and Satoh, M. 1991, Pain, 44, 321.
119. Liu, H.-X., Brumovsky, P., Schmidt, R., Brown, W., Payza, K., Hodzic, L., Pou, C., Godbout, C. and Hökfelt, T. 2001, Proc. Natl. Acad. Sci. USA, 98, 9960.
120. Wiesenfeld-Hallin, Z., Villar, M. J. and Hökfelt, T. 1989, Brain Res., 486, 205.
121. Ji, R.-R., Zhang, X., Zhang, Q., Dagerlind, Å., Nilsson, S., Wiesenfeld-Hallin, Z. and Hökfelt, T. 1995, Neuroscience, 68, 563.
122. Holmes, F. E., Bacon, A., Pope, R. J. P., Vanderplank, P. A., Kerr, N. C. H., Sukumaran, M., Pachnis, V. and Wynick, D. 2003, Proc. Natl. Acad. Sci. USA, 100, 6180.
123. Alier, K. A., Chen, Y., Sollenberg, U. E., Langel, Ü. and Smith, P. A. 2008, Pain, 137, 138.
124. Gimpl, G. and Fahrenholz, F. 2001, Physiol. Rev., 81, 629.
125. Lee, H.-J., Macbeth, A. H., Pagani, J. H. and Young, W. S. 3rd. 2009, Prog. Neurobiol., 88, 127.
126. Raggenbass, M. 2001, Prog. Neurobiol., 64, 307.
127. Stoop, R. 2012, Neuron, 76, 142.
128. Cechetto, D. F. and Saper, C. B. 1988, J. Comp. Neurol., 272, 579.
129. Sawchenko, P. E. and Swanson, L. W. 1982, J. Comp. Neurol., 205, 260.
130. Rousselot, P., Papadopoulos, G., Merighi, A., Poulain, D. A. and Theodosis, D. T. 1990, Brain Res., 529, 178.
131. Jo, Y.-H., Stoeckel, M.-E., Freund-Mercier, M.-J. and Schlichter, R. 1998, J. Neurosci., 18, 2377.
132. Liu, X., Tribollet, E., Ogier, R., Barberis, C. and Raggenbass, M. 2003, Eur. J. Neurosci., 17, 1833.
133. Reiter, M. K., Kremarik, P., Freund-Mercier, M. J., Stoeckel, M. E., Desaulles, E. and Feltz, P. 1994, Eur. J. Neurosci., 6, 98.
134. Schorscher-Petcu, A., Sotocinal, S., Ciura, S., Dupré, A., Ritchie, J., Sorge, R. E., Crawley, J. N., Hu, S.-B., Nishimori, K., Young, L. J., Tribollet, E., Quirion, R. and Mogil, J. S. 2010, J. Neurosci., 30, 8274.
135. Uhl-Bronner, S., Waltisperger, E., Martínez-Lorenzana, G., Condés-Lara, M. and Freund-Mercier, M. J. 2005, Neuroscience, 135, 147.
136. Martínez-Lorenzana, G., Espinosa-López, L., Carranza, M., Aramburo, C., Paz-Tres, C., Rojas-Piloni, G. and Condés-Lara, M. 2008, Pain, 140, 265.
137. Lundeberg, T., Uvnäs-Moberg, K., Ågren, G. and Bruzelius, G. 1994, Neurosci. Lett., 170, 153.
138. Yang, J., Yang, Y., Chen, J.-M., Liu, W.-Y., Wang, C.-H. and Lin, B.-C. 2007, Peptides, 28, 1113.
139. Condés-Lara, M., Rojas-Piloni, G., Martínez-Lorenzana, G. and Rodríguez-Jiménez, J. 2009, Eur. J. Neurosci., 30, 1056.
140. Jiang, C.-Y., Fujita, T., Xu, Z.-H., Ohtsubo, S., Matsushita, A. and Kumamoto, E. 2014, Pain Res., 29, 215.
141. Breton, J.-D., Veinante, P., Uhl-Bronner, S., Vergnano, A. M., Freund-Mercier, M. J., Schlichter, R. and Poisbeau, P. 2008, Mol. Pain, 4, 19.
142. Robinson, D. A., Wei, F., Wang, G. D., Li, P., Kim, S. J., Vogt, S. K., Muglia, L. J. and Zhuo, M. 2002, J. Physiol., 540, 593.
143. van den Pol, A. N. 1999, J. Neurosci., 19, 3171.
144. de Lecea, L., Kilduff, T. S., Peyron, C., Gao, X.-B., Foye, P. E., Danielson, P. E., Fukuhara, C., Battenberg, E. L. F., Gautvik, V. T., Bartlett, F. S. II, Frankel, W. N., van den Pol, A. N., Bloom, F. E., Gautvik, K. M. and Sutcliffe, J. G. 1998, Proc. Natl. Acad. Sci. USA, 95, 322.
145. Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R. M., Tanaka, H., Williams, S. C., Richardson, J. A., Kozlowski, G. P., Wilson, S., Arch, J. R. S., Buckingham, R. E., Haynes, A. C., Carr, S. A., Annan, R. S., McNulty, D. E., Liu, W.-S., Terrett, J. A., Elshourbagy, N. A., Bergsma, D. J. and Yanagisawa, M. 1998, Cell, 92, 573.

146. Cutler, D. J., Morris, R., Sheridhar, V., Wattam, T. A. K., Holmes, S., Patel, S., Arch, J. R. S., Wilson, S., Buckingham, R. E., Evans, M. L., Leslie, R. A. and Williams, G. 1999, Peptides, 20, 1455.
147. Date, Y., Mondal, M. S., Matsukura, S. and Nakazato, M. 2000, Neurosci. Lett., 288, 87.
148. Guan, J.-L., Wang, Q.-P. and Shioda, S. 2003, Brain Res., 987, 86.
149. Yamamoto, T., Nozaki-Taguchi, N. and Chiba, T. 2002, Br. J. Pharmacol., 137, 170.
150. Yamamoto, T., Saito, O., Shono, K. and Hirasawa, S. 2003, Eur. J. Pharmacol., 481, 175.
151. Cheng, J.-K., Chou, R. C.-C., Hwang, L.-L. and Chiou, L.-C. 2003, J. Pharmacol. Exp. Ther., 307, 1065.
152. Kajiyama, S., Kawamoto, M., Shiraishi, S., Gaus, S., Matsunaga, A., Suyama, H. and Yuge, O. 2005, Brain Res., 1044, 76.
153. Yamamoto, T., Saito, O., Shono, K., Aoe, T. and Chiba, T. 2003, Neurosci. Lett., 347, 183.
154. Mobarakeh, J. I., Takahashi, K., Sakurada, S., Nishino, S., Watanabe, H., Kato, M. and Yanai, K. 2005, Peptides, 26, 767.
155. Cluderay, J. E., Harrison, D. C. and Hervieu, G. J. 2002, Regul. Pept., 104, 131.
156. Guan, J.-L., Wang, Q.-P., Hori, T., Takenoya, F., Kageyama, H. and Shioda, S. 2004, Peptides, 25, 1307.
157. Hervieu, G. J., Cluderay, J. E., Harrison, D. C., Roberts, J. C. and Leslie, R. A. 2001, Neuroscience, 103, 777.
158. Jeong, Y. and Holden, J. E. 2009, Neuroscience, 159, 1414.
159. Jeon, Y., Park, K. B., Pervin, R., Kim. T. W. and Youn, D.-h. 2015, Neurosci. Lett., 604, 128.
160. Grudt, T. J., van den Pol, A. N. and Perl, E. R. 2002, J. Physiol., 538, 517.