Nociceptin Augments $K^+$ Currents in Hippocampal CA1 Neurons by Both ORL-1 and Opiate Receptor Mechanisms

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Madamba, Samuel G., Paul Schweitzer, and George Robert Siggins. Nociceptin augments $K^+$ currents in hippocampal CA1 neurons by both ORL-1 and opiate receptor mechanisms. J. Neurophysiol. 82: 1776–1785, 1999. We previously reported (see also the accompanying paper) that dynorphin A significantly enhanced the voltage-dependent $K^+$ M-current ($I_M$) in CA3 and CA1 hippocampal pyramidal neurons (HPNs). Because the opioid-receptor-like-1 (ORL-1) receptor shares a high sequence homology with opioid receptors and is expressed in rat hippocampus, we examined the effects of orphanin FQ or nociceptin, the endogenous ligand for the ORL-1 receptor, using the rat hippocampal slice preparation and intracellular voltage-clamp recording. Current-voltage ($I-V$) relationships from CA1 HPNs revealed that nociceptin superfusion induced an outward current reversing near the equilibrium potential for $K^+$ ions. Bu$^2^+$ (2 mM) blocked this effect. The nociceptin-induced current was largest at depolarized membrane potentials, where $I_M$ is largely activated. Nociceptin concentrations of 0.5–1 $\mu$M (but not 0.1 $\mu$M) significantly increased $I_M$ relaxation amplitudes with recovery on washout. Interestingly, both the general opiate antagonist naloxone and the $\delta$ receptor antagonist nor-binaltorphimine (nBNI) inhibited the nociceptin-induced $I_M$ increases and outward currents in the depolarized range but not the inward current induced at hyperpolarized potentials. The putative ORL-1 receptor antagonist, [Phe$^1$Ψ(CH$_2$-NH)Gly$^2$]NC(1–13)NH$_2$ (hereafter ORLAn), blocked most of the nociceptin current near rest but not the $I_M$ increase. However, ORLAn alone had direct effects similar to those of nociceptin, indicating that ORLAn might be a partial agonist. Our results suggest that nociceptin postsynaptically modulates the excitability of HPNs through ORL-1 and $\kappa$-like opiate receptors linked to different $K^+$ channels.

INTRODUCTION

The primary structure of the recently identified heptadcapeptide called nociceptin (Meunier et al. 1995) or orphanin FQ (Reinscheid et al. 1996) (hereafter nociceptin) shares a high sequence homology with opioid peptides, and especially dynorphin A (Meunier et al. 1995). Indeed, Zhang and Yu (1995) have identified dynorphin A as a potential ligand for the opioid-receptor-like-1 (ORL-1) receptor expressed in Xenopus oocytes. This receptor also shares a high sequence homology with opiate receptors (Bunzow et al. 1994) and is localized in rat hippocampus (Anton et al. 1996; Bunzow et al. 1994). Nociceptin has been shown to hyperpolarize neurons by activating an inwardly rectifying $K^+$ conductance in neurons of locus coeruleus (Connor et al. 1996a), dorsal raphe (Vaughan and Christie 1996), hypothalamus (Lee et al. 1997), and CA3 hippocampus (Ikeda et al. 1997). Nociceptin also inhibits Ca$^{2+}$ currents in cultured hippocampal neurons (Knobil et al. 1996), reduces field excitatory postsynaptic potentials (EPSPs) and prevents the induction of long-term potentiation (LTP) in CA1 hippocampus (Yu et al. 1997; for review see Meunier 1997).

Recent electrophysiological studies from our laboratory (Madamba et al. 1997; Moore et al. 1994; see also the accompanying paper, Madamba et al. 1999) showed that dynorphin A enhanced the noninactivating voltage-dependent $K^+$ M-current ($I_M$) in both CA3 and CA1 hippocampal pyramidal neurons (HPNs). This effect of dynorphin was blocked by naloxone and nor-binaltorphimine (nBNI). In most CA3 and some CA1 HPNs, the $\kappa$ agonist U50,488h also increased $I_M$. However, U69,593, a selective $\kappa$ receptor agonist, did not increase $I_M$ relaxation amplitudes in CA1 HPNs, suggesting that a novel $\kappa$ receptor subtype or ORL-1 might be involved. Although $\delta$ receptor agonists decreased $I_M$ in CA3, opiate agonists selective for $\delta$ and $\mu$ receptors had no effect on $I_M$ or other postsynaptic membrane properties in CA1.

Therefore because of the similarities between dynorphin and nociceptin, and the possibility that ORL-1 receptors might mediate the dynorphin $I_M$ effect, we examined the effects of nociceptin on CA1 neurons of the hippocampal slice preparation. Here we report that nociceptin, like dynorphin A, enhances $I_M$ by a $\kappa$-like opiate receptor but also activates another $K^+$ conductance via a nonopiate, putative ORL-1 receptor.

METHODS

Slice preparation

We used standard intracellular voltage-clamp recording techniques in the rat hippocampal slice, prepared as described previously (Madamba et al. 1996; Schweitzer et al. 1993) and in the accompanying paper (Madamba et al. 1999). In brief, male Sprague-Dawley rats (100–170 g) were anesthetized with halothane (3%), decapitated, and their brains rapidly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) gassed with 95% O$_2$-5% CO$_2$. We used sharp glass micropipettes filled with 3 M KCl (tip resistance, 73 ± 3 MΩ; mean ± SE) to penetrate CA1 neurons. Methods of superfusion, voltage-clamp recording, cell identification, drug administration, and data analysis were as described previously (Madamba et al. 1996; Schweitzer et al. 1993) and in the accompanying paper (Madamba et al. 1999).

Electrophysiological methods

Current and voltage records were acquired, stored, and analyzed as described in the accompanying paper (Madamba et al. 1999). Tetrodotoxin (TTX; 1 $\mu$M) was added to the superfuse to block Na$^+$-dependent action potentials and inhibit synaptic transmission.
Electrophysiological protocols

CURRENT-VOLTAGE (I-V) ANALYSIS. We evaluated I-V relationships from a holding potential of -61 ± 1 mV with hyperpolarizing and depolarizing steps (1.5 s duration, 7 s apart). The I-V curves were constructed from the current values measured at the end of the voltage steps (steady-state) just before command offset, and the values obtained in control condition were subtracted from those in the presence of the tested substance to obtain the net current elicited.

M-CURRENT ANALYSIS. In CA1 HPNs, \( I_M \) is commonly seen with holding potentials around -45 mV and hyperpolarizing steps of 5-25 mV and 1-s duration (Halliwell and Adams 1982; Moore et al. 1994); it then appears as a slow inward “relaxation” following the instantaneous (ohmic) inward current. We measured \( I_{relax} \) amplitude with software (Clampfit, Axon Instruments) that fitted second-order exponential curves to the \( I_{relax} \) relaxation and used the difference between the current values just after command onset and just before command offset to quantify the amplitude of \( I_{relax} \).

Q-CURRENT ANALYSIS. We elicited the inwardly-rectifying \( I_Q \) (or \( I_b \)) from a holding potential around -60 mV using a series of hyperpolarizing voltage steps in increments of 15 mV and 1.5 s duration. The \( I_Q \) relaxation amplitudes were analyzed identically to those of \( I_{relax} \).

Drug administration

Drugs and peptides were added from a stock solution to the ACSF in known concentrations immediately before administration to the slice chamber. In this study the cells were depolarized to a mean of -47 ± 0.5 mV for \( I_{relax} \) analysis but were held near resting potential between these periods (control, drug, and wash out) to avoid the instabilities that may develop with prolonged depolarization (see Halliwell and Adams 1982). For tests of the opiate antagonists, the usual protocol involved first applying the agonist (e.g., nociceptin), followed by washout with ACSF alone (“washout”), then adding naloxone or nBNI (to test their possible effects on membrane properties) followed by addition of nociceptin again but now together with the antagonist. In several neurons, we applied nBNI first (for 6-7 min), followed by addition of nociceptin in the presence of the \( \kappa \) antagonist. Similarly, in other studies we superfused the ORL-1 receptor antagonist, [Phε^IV(CH₂-NH)Gly³(NC(1-13)NH₂)] (ORLAN) first followed by nociceptin in the presence of the antagonist. At the end of some recordings, 2 mM Ba²⁺ was added to the superfuse to verify that \( I_{relax} \) relaxation currents were due to K⁺ currents.

We obtained nociceptin and ORLAN from Tocris Cookson (Ballwin, MO), naloxone from Sigma (St. Louis, MO), nBNI from Research Biochemicals International (Natick, MA), and TTX from Calbiochem-Novabiochem (San Diego, CA).

Quantification and statistics

All measurements are reported as means ± SE. We usually determined statistical significance by two-way ANOVA for repeated measures, followed by the Newman-Keuls post hoc test and simple main effects as warranted by significant group effects and interactions. For within-subject effects with at least 2 degrees of freedom, we used the more conservative Huynh-Feldt \( P \) value to reduce type 1 errors.

RESULTS

We recorded from a total of 45 CA1 HPNs. These neurons had an average resting membrane potential (RMP) of -68 ± 1 mV with a mean spike amplitude of 104 ± 3 mV (\( n = 28 \)).

Nociceptin alters I-V relationships

We generated I-V relationships to study the overall effects of nociceptin on steady-state membrane properties in the depolarized and hyperpolarized ranges (Fig. 1A). Superfusion of 0.5 and 1 µM nociceptin elicited an outward current in the depolarized range that reversed at -93 mV (mean). Figure 1B shows control-subtracted graphs generated from the I-V relationships, as described in METHODS, to isolate the current elicited by 0.1, 0.5, and 1 µM nociceptin. Superfusion of 0.1 µM nociceptin did not significantly (\( P > 0.5 \)) alter steady-state currents, although a slight outward current was seen at depolarized potentials (Fig. 1B, left panel; \( n = 4 \)). A higher concentration of nociceptin (0.5 µM; Fig. 1, middle panel; \( n = 6 \)) elicited a significant [\( F(7,35) = 10.118, P < 0.01 \)] outward current at membrane potentials of -51 to -65 mV and an inward current at potentials of -121 to -135 mV, with a reversal at -94 ± 7 mV. Similarly, 1 µM nociceptin (Fig. 1, right panel; \( n = 6 \)) induced a significant [\( F(7,35) = 43.228, P < 0.001 \)] outward current at potentials of -47 to -61 mV, an effect reversing at -93 ± 6 mV. Although 1 µM nociceptin also induced an inward current at hyperpolarized potentials (-117 to -131 mV), this was not significantly different compared with washout (perhaps because of persistence of effect or incomplete washout).

The theoretical reversal potential for K⁺ calculated by the Nernst equation, in ACSF containing 3.5 mM K⁺ and assuming an intracellular concentration of 150 mM K⁺, is -98 mV. The experimental reversal potentials we obtained (-94 and -93 mV with 0.5 and 1 µM nociceptin, respectively), were thus near the theoretical reversal potential for K⁺ ions. To further confirm the ionic nature of the nociceptin effect, we superfused Ba²⁺ at a concentration of 2 mM to nonspecifically block K⁺ conductances. In the presence of 2 mM Ba²⁺, nociceptin no longer affected steady-state current recordings generated with I-V protocols (Fig. 2, A and B). Comparison of the net effect of 0.5 µM nociceptin in the absence (\( n = 6 \)) or presence (\( n = 5 \)) of Ba²⁺ (Fig. 2C) indicated that Ba²⁺ prevented most of the nociceptin effect, consistent with involvement of K⁺ conductances.

Nociceptin augments the M-current

Superfusion of 0.5-1 µM nociceptin enhanced \( I_M \) relaxation amplitudes. In the HPN recording shown in Fig. 3A, superfusion of 1 µM nociceptin enhanced \( I_M \) amplitudes to 229% of control, increased input conductance and induced an outward current (391 pA) at holding potential (- - -), with recovery on washout. The average of six such experiments with 1 µM nociceptin is shown in Fig. 3D: nociceptin significantly [\( F(2,10) = 15.798, P < 0.01 \); Newman-Keuls, \( P < 0.01 \) for control and wash] enhanced mean \( I_M \) amplitudes with recovery on wash out. In contrast, on average 0.1 µM nociceptin did not significantly (\( P > 0.5 \); \( n = 5 \)) enhance \( I_M \) amplitudes (Fig. 3B). An intermediate concentration (0.5 µM) significantly [\( F(2,30) = 13.832, P < 0.01 \); Newman-Keuls, \( P < 0.001 \) for control and wash; \( n = 16 \)] increased \( I_M \) relaxation amplitudes with recovery on washout (Fig. 3C). Because there are reports of ORL-1 desensitization (Connor et al. 1996a; Ma et al. 1997), in another set of five neurons we superfused 0.5 µM nociceptin twice with washout (mean 27 min) between applications. Both nociceptin applications increased \( I_M \) amplitudes similarly to 263 ± 94% of control for the first and 240 ± 59% of control for the second application (measured at the 15-mV step), suggesting a minimal tachyphylaxis over this time course.
Nociceptin-induced currents have an opiate receptor-mediated component

There are conflicting reports regarding opioid receptor mediation of nociceptin effects (Connor et al. 1996a; Knoflach et al. 1996; Nicol et al. 1996; Rossi et al. 1996; Wang et al. 1996; Zhang and Yu 1995). Therefore we superfused opiate antagonists to determine whether nociceptin effects in CA1 neurons could be mediated by an opiate mechanism (Fig. 4). We first performed I-V analysis in the presence of 4 μM naloxone, a broad spectrum opiate antagonist, to investigate a possible alteration of the nociceptin-induced steady-state currents. Although naloxone had no significant effect on the I-V curve analyzed over the entire voltage range, it significantly reduced $I_m$ at depolarized potentials and an inward current at the most hyperpolarized potential, with recovery on washout. Resting membrane potential (RMP) was $-70 \text{ mV}$. B: I-V curves derived from data like that shown in A, indicating the net steady-state currents induced by nociceptin (mean data pooled from multiple neurons). Superfusion of 0.1 μM nociceptin (left panel; $n = 4$) did not significantly alter membrane currents, although there was a slight outward current at depolarized potentials. A higher concentration of nociceptin (0.5 μM; $n = 6$; middle panel) induced a significant outward current reversing at $-94 \text{ mV}$. Similarly, 1 μM nociceptin (right panel; $n = 6$) induced an outward steady-state current reversing at $-93 \text{ mV}$. In this and subsequent figures, error bars = SE.

**Nociceptin-induced currents have an opiate receptor-mediated component**

We then investigated the effect of the specific κ-opioid receptor antagonist nBNI. As with naloxone, superfusion of 200–400 nM nBNI significantly [$F(1,5) = 20.79, P < 0.01$] inhibited the nociceptin-induced outward current in the depolarized range (Fig. 4, A, D, and E), while leaving the nociceptin-induced inward component unaffected. Figure 4, C and E, shows the nociceptin-induced components sensitive to naloxone and nBNI (currents elicited by nociceptin alone minus currents elicited by nociceptin in the presence of opioid antagonist). Thus isolating the naloxone- and nBNI-sensitive currents revealed two nociceptin effects: one sensitive and one insensitive to opiate antagonists.

To again ensure that desensitization to nociceptin was not a confound in these experiments, another five neurons were exposed to nociceptin only once, in the presence of nBNI. Such nBNI pretreatment significantly [ANOVA between subjects; $F(1,42) = 9.075, P < 0.01$; measured at $-48$ to $-79 \text{ mV}$] blunted the nociceptin-induced outward steady-state current,
indicating that the lack of effect of nociceptin was not due to desensitization caused by repeated applications.

**Opiate antagonists block nociceptin M-current effects**

Using the $I_M$ protocol, 4 μM naloxone inhibited both the nociceptin-induced $I_M$ increase and the outward holding steady-state current (Fig. 5A). Mean data pooled from a different set of five neurons (Fig. 5B) showed that nociceptin (0.5 μM, $n = 3$; 1 μM, $n = 2$) significantly [F(4,16) = 24.107, $P < 0.001$; Newman-Keuls, $P < 0.05$ for all conditions] increased $I_M$ relaxation amplitudes with recovery on washout. In the same neurons, naloxone (4 μM) alone did not significantly alter $I_M$ but prevented the nociceptin enhancement of $I_M$ amplitudes (Fig. 5B). Although naloxone alone did not affect $I_M$, it induced a slight inward current in the depolarized range (Fig. 5C), perhaps due to block of opiate receptors activated constitutively or by endogenous opioids.

Because of the high structural homology between ORL-1 and $\kappa$ receptors, and because nociceptin and dynorphin had similar effects on $I_M$, we tested nBNI, the specific $\kappa$ receptor antagonist. Superfusion of 200 nM nBNI alone did not alter $I_M$ amplitudes nor change the holding current, but blocked the nociceptin-induced $I_M$ increase and outward holding current (Fig. 6A). Subsequent application of 2 mM Ba$^{2+}$ induced an inward current and totally blocked $I_M$, verifying the involvement of K$^+$ channels. Figure 6B shows the average of six experiments like that shown in Fig. 6A. As before, 0.5 μM nociceptin significantly [F(4,20) = 9.825, $P < 0.05$; Newman-Keuls, $P < 0.001$ for all conditions] increased mean $I_M$ amplitudes. Superfusion of 200 nM nBNI alone ($n = 6$) did not
either a linear ($Y = A + B \cdot X$) or a polynomial regression ($A = A + B_1 \cdot X + B_2 \cdot X^2$) fit.

significantly ($P > 0.05$) alter $I_M$ but blocked the nociceptin-induced $I_M$ increase. In another set of five neurons, nociceptin was superfused only once to eliminate the possibility of tachyphylaxis: we pretreated slices with 200–400 nM nBNI first, followed by 0.5 µM nociceptin together with nBNI. In the presence of nBNI, 0.5 µM nociceptin did not significantly ($P > 0.5$) alter $I_M$ relaxation amplitudes (Fig. 6C).

**ORL-1 antagonist blocks nociceptin-induced steady-state current near rest, but not $I_M$**

Recently, Guerrero et al. (1998) identified a nociceptin receptor antagonist, ORLAn. In CA1 HPNs, superfusion of 1–2 µM ORLAn alone induced steady-state currents (Fig. 7A, *left panel*). In the continued presence of ORLAn, 0.5 µM nociceptin had little effect near rest. For the net nociceptin-induced steady-state currents in the presence of ORLAn, we show the current induced by nociceptin alone (Fig. 7A, *right panel*); notice that ORLAn prevented most of the nociceptin-induced current near rest. Because ORLAn had both agonist and antagonist actions in CA1 neurons, it might be characterized as a partial agonist.

We also asked if ORLAn could alter $I_M$ relaxation amplitudes and block the nociceptin enhancement of $I_M$. Interestingly, 1 µM ORLAn itself increased $I_M$ and induced an outward holding current (Fig. 7B), an effect similar to that of nociceptin. However, addition of nociceptin in the continued presence of ORLAn elicited a further enhancement of $I_M$, suggesting lack of antagonism by ORLAn. Washout of both ORLAn and nociceptin returned $I_M$ relaxation amplitudes to control levels. A second application of 0.5 µM nociceptin again increased $I_M$ amplitudes (not shown). On average ORLAn alone and nociceptin with ORLAn both significantly [$F(2,12) = 16.884, P < 0.001$; Newman-Keuls; $P < 0.05$ for all cases] increased $I_M$ amplitudes (Fig. 7C).

In HPNs, the voltage-dependent cationic conductance termed $I_Q$ (Halliwell and Adams 1982) or $I_0$, is seen as a slow inward current relaxation with hyperpolarizing voltage steps to potentials more negative than −65 mV. In the presence of 2 mM Ba$^{2+}$ to isolate $I_Q$, nociceptin had little effect on this current. Subsequent superfusion of 1 mM Cs$^+$ inhibited the inward current, verifying $I_Q$ identity (Fig. 8A). In the same cells where nociceptin increased $I_M$ amplitudes (see Fig. 6B), nociceptin did not significantly ($P > 0.05$) alter $I_Q$ (Fig. 8B), although there was a slight increase at −123 mV (to 111% of control; $n = 6$).

**DISCUSSION**

Our studies have shown that 1) nociceptin augmented Ba$^{2+}$-sensitive currents that reversed near the equilibrium potential for K$^+$; 2) naloxone and nBNI inhibited most of the nociceptin current at depolarized potentials but not that in the hyperpolarized range; 3) the ORL-1 antagonist ORLAn blocked the nociceptin currents near RMP; 4) nociceptin increased $I_M$ relaxation amplitudes; 5) naloxone and nBNI, but not ORLAn, prevented the enhancement of $I_M$; and 6) nociceptin had little effect on $I_Q$. These data suggest that nociceptin can postsynaptically modulate the excitability of HPNs through both ORL-1 and κ-like opiate receptors linked to different K$^+$ channels.

**Nociceptin-evoked currents**

The I-V relationships from CA1 HPNs showed that nociceptin currents reversed near −98 mV in 3.5 mM K$^+$, suggesting...
that they are carried by K⁺ ions. Furthermore, Ba²⁺ inhibited the nociceptin effect, further indicating involvement of K⁺ conductances. Other studies have found that nociceptin hyperpolarized neurons by activating an inwardly rectifying K⁺ conductance in locus coeruleus (Connor et al. 1996a) and dorsal raphe (Vaughan and Christie 1996). Similarly, nociceptin hyperpolarized mouse CA3 HPNs by activating an inwardly rectifying K⁺ channel current coupled to a G-protein, and this effect was blocked by Ba²⁺ (Ikeda et al. 1997). In our hands, a low (100 nM) concentration of nociceptin had little effect on steady-state currents or $I_M$ amplitudes, whereas a slightly higher concentration (0.5 μM) elicited a marked effect.

FIG. 5. Naloxone blocks the nociceptin augmentation of $I_M$. A: representative current recordings: superfusion of 0.5 μM nociceptin (Noc; 6 min) increased $I_M$ to 167% of control and induced an outward holding current (220 pA) with recovery on washout (8 min). Application of 4 μM naloxone (Nal; 10 min) did not alter $I_M$ amplitude but elicited an inward holding current. However, naloxone pretreatment blocked the effects of a 2nd application of nociceptin (Noc + Nal; 6 min).

- - - , control holding current; holding potential was 248 mV; RMP was 271 mV.

B: pooled data: $I_M$ relaxation amplitudes averaged over 5 voltage steps in various drug conditions, taken from 5 HPNs. Pretreatment with 4 μM naloxone blocked the nociceptin increase of $I_M$.

C: control-subtracted steady-state current showing the inward steady-state current induced by naloxone alone.

FIG. 6. κ-Opioid receptor antagonist nBNI blocks the nociceptin-induced augmentation of $I_M$. A: $I_M$ relaxations recorded from a representative HPN. Superfusion of 0.5 μM nociceptin (Noc; 8 min) elicited an outward holding current of 475 pA and increased $I_M$ to 222% of control, with recovery on washout (17 min). Superfusion of 200 nM nBNI alone for 12 min did not alter $I_M$, nor change the holding current (---). In the presence of nBNI, subsequent application of nociceptin (0.5 μM; 7 min) had no effect. Superfusion of 2 mM Ba²⁺ for 8 min totally blocked the current relaxation, indicating it was due to $I_M$.

Holding potential was 244 mV; RMP was 266 mV.

B: pooled data: mean $I_M$ amplitudes over 5 hyperpolarizing steps; 0.5 μM nociceptin significantly increased $I_M$ with recovery on wash out. On average, nBNI alone (200 nM, n = 6) had no effect on $I_M$. However, in the presence of nBNI, nociceptin (0.5 μM) did not alter $I_M$ (Noc + nBNI). C: mean $I_M$ amplitudes from a separate set of 5 HPNs. In these experiments, 200–400 nM nBNI was superfused 1st, then 0.5 μM nociceptin added together with nBNI. Once again, nociceptin in the presence of nBNI had no significant effect on $I_M$. 
throughout the voltage range tested. Superfusion of 1 mM nociceptin did not elicit an effect beyond that with 0.5 mM, indicating that the maximum effect was reached with 0.5 mM nociceptin. Therefore nociceptin has a fairly steep dose-response relationship with an apparent EC50 probably falling between 0.1 and 0.5 mM.

The nociceptin effect at depolarized membrane potentials, where I_M is activated, was mostly inhibited by naloxone and nBNI. Several studies have found that naloxone did not inhibit nociceptin effects, in dorsal raphe nucleus (Vaughan and Christie 1996), Xenopus laevis oocytes coexpressing ORL-1 with potassium channel subunits (Matthes et al. 1996), and periaqueductal gray neurons (Vaughan et al. 1997). Similarly, in our study naloxone and nBNI did not alter the nociceptin current seen at hyperpolarized potentials, suggesting that a nonopioid mechanism may be involved in this voltage range (see Fig. 4).

We used a recently identified nociceptin antagonist to investigate specific ORL-1 mediated effects in CA1 HPNs.

FIG. 7. Putative nociceptin antagonist [Phe^1Ψ(CH₂-NH)Gly^2]NC(1–13)NH₂ (ORLAn) inhibits some nociceptin effects but is a partial agonist in HPNs. A, left panel: control-subtracted plot averaged from 7 neurons showing that superfusion of 1–2 mM ORLAn alone elicited steady-state currents throughout the voltage range tested. In the presence of ORLAn, 0.5 mM nociceptin had little effect, especially near rest. Right panel: comparison of the nociceptin-induced current in the presence of ORLAn (■; derived from left panel) with the nociceptin-induced current in normal media (Noc alone; taken from Fig. 2, middle panel). Nociceptin had a limited effect in the presence of ORLAn. B: ORLAn increased I_M relaxation amplitudes. Superfusion of 1 mM ORLAn (6 min) elicited an outward holding current and increased I_M relaxation amplitudes. In the continued presence of ORLAn, 0.5 mM nociceptin (Noc; 5 min) still caused an outward holding current and increased I_M relaxation amplitude, with recovery on washout (23 min). C: I_M relaxation amplitudes averaged from 7 cells. Both 1–2 mM ORLAn alone and 0.5 mM nociceptin in the presence of ORLAn increased I_M relaxation amplitudes.

FIG. 8. Nociceptin does not affect the hyperpolarization-activated Q-current. A: current traces from an HPN recorded in the presence of 2 mM Ba²⁺ to isolate I_Q. Superfusion of 1 mM nociceptin did not affect the I_Q relaxation amplitude. Further superfusion of 1 mM Cs⁺ abolished the inward relaxation, demonstrating involvement of I_Q. Holding potential was −60 mV; RMP (in Ba²⁺) was −62 mV. B: mean I_Q amplitudes of 6 HPNs exposed to 0.5 mM nociceptin (same neurons as in Fig. 6B). Although nociceptin slightly increased I_Q the augmentation was not significant (P > 0.05). The I_Q relaxation was obtained by applying several hyperpolarizing steps (−15-mV increments) from a −63-mV holding potential (see METHODS).

The nociceptin effect at depolarized membrane potentials, where I_M is activated, was mostly inhibited by naloxone and nBNI. Several studies have found that naloxone did not inhibit nociceptin effects, in dorsal raphe nucleus (Vaughan and Christie 1996), Xenopus laevis oocytes coexpressing ORL-1 with potassium channel subunits (Matthes et al. 1996), and periaqueductal gray neurons (Vaughan et al. 1997). Similarly, in our study naloxone and nBNI did not alter the nociceptin current seen at hyperpolarized potentials, suggesting that a nonopioid mechanism may be involved in this voltage range (see Fig. 4).

We used a recently identified nociceptin antagonist to investigate specific ORL-1 mediated effects in CA1 HPNs.
et al. (1997) reported that ORLAn prevented the nociceptin-induced contractions of guinea pig ileum. In the presence of ORLAn, most of the nociceptin-induced steady-state current near RMP was blocked in CA1 HPNs. However, superfusion of ORLAn alone elicited an effect similar to that with nociceptin, suggesting that it may act as a partial agonist. Interestingly, Butour et al. (1998) reported that ORLAn acted as an agonist in transformed CHO cells expressing the human ORL-1 receptor. These studies indicate that ORLAn may need further characterization and also suggest possible multiple ORL-1 receptor types (see Nociceptin augments $I_M$).

Our findings indicate that nociceptin inhibits CA1 HPNs by concomitantly activating at least two conductances: an opiate receptor-mediated outward current (most probably $I_{Na}$) at depolarized potentials and an ORL-1-mediated current near rest. A possible candidate for this latter ORLAn-sensitive current might be either the inward rectifier (Ikeda et al. 1997) or a voltage-independent $K^+$ current (so-called “resting” current), that are both sensitive to $Ba^{2+}$, although a more complete characterization of this current is needed. Interestingly, we reported that somatostatin also inhibits HPNs by activating both $I_M$ and a voltage-insensitive $K^+$ current (Schweitzer et al. 1998). In rat hippocampus, $I_Q$ (or $I_h$) is a mixed Na/$K^+$ inwardly rectifying conductance activated at hyperpolarized membrane potentials. We found that nociceptin (like somatostatin) did not significantly alter $I_Q$, indicating that this conductance is unlikely to account for the nociceptin current at hyperpolarized potentials.

**Nociceptin augments $I_M$**

The nociceptin increase in $I_M$ relaxation amplitudes was blocked by naloxone and nBNI, suggesting that nociceptin may interact with a $κ$ opiate receptor. Still, naloxone inhibition of nociceptin effects is controversial. The original studies that identified nociceptin/orphanin FQ (Meunier et al. 1995; Reichscheid et al. 1995) showed that nociceptin shares a high homologous sequence with dynorphin A but did not interact with the opiate receptors. However, there are some reports suggesting that the nociceptin system may interact with the opioid system. Thus Zhang and Yu (1995) reported that dynorphin inhibited forskolin-stimulated cyclic AMP through ORL-1 receptors expressed in Xenopus oocytes. Nociceptin-induced analgesia was blocked by naloxone in mice and rats (Rossi et al. 1996, 1998). In a microdialysis study of rat striatum, nociceptin, suggesting that it may act as a partial agonist. In accord with this idea, ORLAn alone increased $I_M$ amplitudes but did not prevent the nociceptin enhancement of $I_M$. Earlier studies characterizing the ORL-1 receptor have suggested splice variants that differ in conformation (Halford et al. 1995; Wang et al. 1994). Similarly, Rossi et al. (1996) have suggested that $κ_3$ agonist naloxone benzoylhydrazone inhibited nociceptin reduction of Ca$^{2+}$ currents in rat dorsal root ganglia (Abdulla and Smith 1997). Interestingly, $σ$ receptors also have been implicated in some nociceptin effects (Kobayashi et al. 1997).

Several reports have suggested that ORL-1 receptors may undergo acute and homologous desensitization (Connor et al. 1996a; Ma et al. 1997). However, we did not detect desensitization of nociceptin enhancement of $I_M$ amplitudes. Furthermore, the averaged nociceptin-induced steady-state current was not significantly different between two successive applications. Similarly, the nociceptin-induced decrease of Ca$^{2+}$ currents in dissociated hippocampal neurons did not desensitize after repeated applications of nociceptin (Knoflach et al. 1996).

**Possible functional role for nociceptin in CA1**

As noted in the preceding paper, several groups have reported that dynorphin inhibits LTP, the cellular model of learning and memory, in guinea pig hippocampus (Terman et al. 1994; Wagner et al. 1993; Weisskopf et al. 1993). Similarly, nociceptin inhibits LTP in rat CA1 hippocampus (Yu et al. 1997) and reduces voltage-sensitive calcium currents (required for CA1 LTP) in dissociated rat pyramidal neurons (Knoflach et al. 1996). In behavioral studies, both nociceptin and dynorphin (via $κ$ receptors) diminish spatial learning in rats (Sandin et al. 1997, 1998). Thus endogenous nociceptin, by activating an outward current carried by two different $K^+$ conductances, could play a role in preventing or regulating LTP induction. Conversely, dynorphin and/or nociceptin might enhance or mediate long-term depression (LTD). This possibility is supported by our recent data showing a novel form of LTD in CA1 that is blocked by naloxone (Francesconi et al. 1997). Studies in progress should help determine whether the opiate receptors and ion channels involved in this LTD are the same as those mediating dynorphin and nociceptin effects.

In an immunohistochemical study, Anton et al. (1996) found evidence for postsynaptic ORL-1 localization in the hilus of the dentate. The hilar mossy cells and somatostatin containing neurons are believed to play a role in epilepsy and seizures (for review see McNamara 1994). A recent study also showed nociceptin immunoreactivity in the hippocampus (Mitsuma et al. 1998). It is possible that nociceptin is co-localized with somatostatin or is located in hilar interneurons, where it could serve as a brake to inhibit seizures, as postulated for somatostatin (Tallent and Siggins 1997). Indeed, nociceptin inhibits epileptiform activity in CA3 neurons in slices treated with Mg$^{2+}$-free ACSF (M. K. Tallent, unpublished observations). Nociceptin and somatostatin have similar actions in CA1 pyramidal neurons: augmentation of $I_M$ amplitudes and induction of an outward current at rest. Somatostatin and nociceptin also both have inhibitory presynaptic actions in the hippocampus (Tallent and Siggins 1997; Yu et al. 1997).
These and other studies show that nociceptin markedly inhibits neurons by a variety of mechanisms, including activation of $I_{K}$ and an inwardly rectifying $K^{+}$ conductance (Ikeda et al. 1997), reduction of various $Ca^{2+}$--currents (Abdulla and Smith 1997; Connor et al. 1996b), and reduction of synaptic transmission (Yu et al. 1997). These effects are consistent with an anticonvulsive function. Our findings that nociceptin can augment at least two $K^{+}$ conductances, one active at depolarized potentials ($I_{K}$) and the other near rest and at more hyperpolarized potentials, suggest that endogenous nociceptin could provide a powerful inhibitory influence over a wide range of membrane potentials. This influence may serve to clamp the membrane potential at rest and provide an intrinsic braking mechanism against neuronal hyperexcitability.

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