Glycinergic Inputs to Cardiac Vagal Neurons in the Nucleus Ambiguus Are Inhibited by Nociceptin and \( \mu \)-Selective Opioids

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Venkatesan, Priya, Sunit Baxi, Cory Evans, Robert Neff, Xin Wang, and David Mendelowitz. Glycinergic inputs to cardiac vagal neurons in the nucleus ambiguus are inhibited by nociceptin and \( \mu \)-selective opioids. J Neurophysiol 90: 1581–1588, 2003. First published May 21, 2003; 10.1152/jn.01117.2002. Most parasympathetic activity regulating heart rate originates from preganglionic cardiac vagal neurons within the nucleus ambiguus. Little is known regarding the modulation of glycinergic transmission to these neurons. However, the presence of \( \mu \)-opioid receptors and opioid-receptor-like (ORL,) receptors within the ambiguus, together with the presence of endogenous ligands for both receptor types in the same area, suggests opioids may modulate synaptic transmission to cardiac vagal neurons. This study therefore examined the effects of endomorphin-1 and endomorphin-2 (the \( \mu \)-selective endogenous peptides), DAMGO (a synthetic, \( \mu \)-selective agonist), and nociceptin (the ORL,selective endogenous peptide) on spontaneous glycinergic inhibitory postsynaptic currents (IPSCs) in rat cardiac parasympathetic neurons. All four of the opioids used in this study decreased spontaneous IPSCs. At concentrations of 100 \( \mu \)M, the amplitude of the IPSCs was reduced significantly by nociceptin (56.6%), DAMGO (46.5%), endomorphin-1 (45.1%), and endomorphin-2 (26.8%). IPSC frequency was also significantly reduced by nociceptin (61.1%), DAMGO (69.9%), and endomorphin-1 (40.8%) but not endomorphin-2. Lower concentrations of nociceptin and DAMGO (10–30 \( \mu \)M) also effectively decreased IPSC amplitude and frequency. The inhibitory effects of DAMGO were blocked by d-Phe-Cys-Tyr-d-Trp-Orn-Thr-Pen-Thr-NH\(_2\) (C-TOP; 10 \( \mu \)M), a selective \( \mu \)-receptor antagonist. Neither nociceptin nor DAMGO inhibited the postsynaptic responses evoked by exogenous application of glycine or affected TTX-insensitive glycinergic mini-IPSCs. These results indicate that \( \mu \)-selective opioids and nociceptin act on preceding neurons to decrease glycinergic inputs to cardiac vagal neurons in the nucleus ambiguus. The resulting decrease in glycinergic transmission would increase parasympathetic activity to the heart and may be a mechanism by which opioids induce bradycardia.

INTRODUCTION

A major inhibitory neurotransmitter on motoneurons within the mammalian CNS is thought to be glycine, which operates via the activation of distinct postsynaptic receptors (GlyRs) gating chloride channels (Danober et al. 2000; Donato and Nistri 2000; Werman et al. 1967). Studies show GlyRs are expressed in the telencephalon, including in the hippocampus as well as areas in the spinal cord (Birinyi et al. 2001; Lewis and Faber 1996) and brain stem (Frostholm and Rotter 1985; Malosio et al. 1991; Zarbin et al. 1981), including rostral ventrolateral medulla neurons (Wu et al. 1997), hypoglossal motoneurons (Donato and Nistri 2000; Eggers et al. 2000; O’Brien and Berger 1999), the pontine reticular formation (Stevens et al. 1996), and the substantia gelatinosa (Kiyosawa et al. 2001).

Glycine receptors have also been reported within the nucleus tractus solitarius (NTS) (Fukushima et al. 1998; Kubo and Kihara 1987; Nunez-Abades et al. 1990; Talman and Robertson 1989) and the nucleus ambiguus (Chitravanshi et al. 1991), which are two medullary areas important for central cardiovascular regulation. Glycine itself is mainly found in the caudal brain stem (Stevens et al. 1996), where it is a significant determinant of neuronal activity (Curtis et al. 1971; Koch and Friauf 1995; Werman et al. 1967; Yoshimura and Nishi 1995). Endogenous glycine is thought to modulate the excitability of respiratory neurons and affect respiratory patterns (Bracci et al. 1996; Paton and Richter 1995; Shao and Feldman 1997), to be involved in spinal antinociception (Kiyosawa et al. 2001) and play a role in controlling premotor circuitry within the medial pontine reticular formation (Stevens et al. 1996).

Most parasympathetic activity regulating heart rate and cardiac function originates from specific preganglionic cardiac neurons within the nucleus ambiguus (Cheng and Powley 2000; Mendelowitz 1999; Wang et al. 2001a,b). These neurons are intrinsically silent and recent studies (Mendelowitz and Kunze 1991) have identified three synaptic inputs, including glutamatergic (Neff et al. 1998b), cholinergic (Mendelowitz 1998), and GABAergic (DiMicco et al. 1979; Wang et al. 2001a,b) pathways to these neurons. The glutamatergic and GABAergic pathways to the nucleus ambiguus are well established, originating from the NTS (Mendelowitz 1998; Neff et al. 1998a; Wang et al. 2001a,b), a medullary area that receives cardiorespiratory sensory inputs. Ionophoretic application of glycine reduces activity of NTS neurons (Bennett et al. 1987) and microinjections of glycine into both the NTS (Cassell et al. 2000; Kubo and Kihara 1987) and the nucleus ambiguus (Chitravanshi et al. 1991) causes an increase in heart rate (possibly due to reduced parasympathetic outflow to the heart). Microinjections of strychnine into the medial area of the NTS decrease heart rate in the rat (Kubo and Kihara 1987), indicating that endogenous glycinergetic activity is involved in the central control of heart rate.

Little is known, however, regarding the modulation of glycinergetic inputs to neurons that control heart rate. A major
Determinant of glycinergic activity may be opioids, since a reduction in glycinergic inhibitory postsynaptic currents (IPSCs) and glycine responses is seen after administration of morphine in the spinal cord (Gruol and Smith 1981; Werz and Macdonald 1982) and the NTS (Fukushima et al. 1998) and with DAMGO (a synthetic, μ-receptor selective opioid) in the substantia gelatinosa (Grudt and Henderson 1998) and the periaqueductal area (Min et al. 1996). Studies in rats show that systemic administration of morphine (Randich and Gebhart 1992) causes hypotension and bradycardia, while microinjections of morphine-like drugs into the nucleus ambiguus also produces a pronounced bradycardia (Wu and Martin 1983). Bilateral cervical vagotomy attenuates opioid-evoked bradycardia (Randich and Gebhart 1992), indicating that increased vagal activity is at least partially responsible for the reduction in heart rate. There is a high distribution of μ-opioid receptors (Ding et al. 1996; Sim and Childers 1997; Xia and Haddad 1991) and ORL₁ receptors (Houtani et al. 2000; Mollereau and Mouledous 2000; Mollereau et al. 1994; Neal et al. 1999a; Sim and Childers 1997) within the nucleus ambiguus and NTS. In addition, μ-opioid receptor-like immunoreactivity has been localized to the dendrites and cell bodies of efferent neurons in the ambiguus (Nomura et al. 1996), including those of cardiac premotor neurons (Aicher 2001). Mapping studies detailing the ambiguus (Nomura et al. 1996), including those of cardiac vagal neurons using these procedures has been previously reported (2002). 

Materials and methods

Slice preparation

In an initial surgery, Sprague-Dawley rats (postnatal days 4–7) were anesthetized with halothane and hypothermia and received a right thoracotomy. The heart was exposed, and 0.05 ml rhodamine (XRITC, Molecular Probes) was injected into the pericardial sac to retrogradely label cardiac vagal neurons. The selective labeling of cardiac vagal neurons using these procedures has been previously described (Mendelowitz and Kunze 1991). On the day of the experiment (2–4 days later), the animals were anesthetized with halothane and hypothermia and killed by rapid cervical dislocation. The hindbrain was rapidly removed and placed in ice-cold buffer (0–2°C) of the following composition (in mM): 140 NaCl, 5 KCl, 2 CaCl₂, 25 glucose, and 10 HEPES and oxygenated with 100% O₂. Slices of medulla (500–600 μm in thickness) were cut using a vibratome. Slices were mounted in a perfusion chamber and submerged in perfusate of the following composition (in mM): 125 NaCl, 3 KCl, 2 CaCl₂, 5 glucose, 26 NaHCO₃, and 5 HEPES, oxygenated with a 95% O₂–5% CO₂ gas mixture. The osmolarity of both solutions was 285–290 mOsm, and the pH was maintained between 7.35 and 7.4.

Electrophysiological recording

Individual cardiac neurons were identified by the presence of the fluorescent tracer (Mendelowitz and Kunze 1991) and imaged with differential contrast optics, infrared illumination, and infrared-sensitive video detection cameras to visually guide and position the patch pipette onto the surface of the identified neuron. Pipettes were made with a puller (Narishige, Japan); filled resistances were 2–4 MΩ in the bath. The electrode solution contained (in mM) 150 KCl, 4 MgCl₂, 2 EGTA, 2 Na-ATP, 5 lidocaine N-ethyl bromide (QX-314), and 10 HEPES; pH 7.3. With this pipette solution, the Cl⁻ current activated was recorded as an inward current. The spontaneous glycinergic IPSCs were isolated using 100 μM picrotoxin to prevent GABAergic currents and 50 μM 5-cyano-7-nitro quinoxaline-2-3(1H, 4H)-dione (CNQX) to prevent non-N-methyl-d-aspartate (NMDA) glutamatergic currents. At the end of most experiments, the specific glycine antagonist strychnine (10 μM) was used to block the glycinergic IPSCs. The pipette was advanced until obtaining a seal over 1 GΩ between the pipette tip and the membrane of the identified neuron. The membrane under the pipette tip was then ruptured with a brief suction to obtain whole cell patch-clamp configuration, and the cell voltage was clamped at a holding potential of ~80 mV. The effects of endomorphin-1, endomorphin-2, DAMGO (10–100 μM), and nociceptin (1–100 μM) on spontaneous glycinergic IPSCs were examined at this holding potential. A separate series of similar experiments tested the effects of a selective δ-opioid receptor agonist, DPLPE (100 μM), on spontaneous glycinergic IPSCs. Drugs were applied after recording 60 s of control events; only one concentration of drug was used per neuron (2 neurons per slice). In other experiments, the selective μ-opioid agonist D-Phe-Cys-Tyr³-Trp⁴-Orn⁵-Thr⁶-Asp⁷ (C-TOP: 10 μM) was used in conjunction with an effective concentration of DAMGO. These experiments were performed in fresh slices that had not been previously exposed to opioid agonists. A parallel series of experiments was performed in other slices to examine the effects of the selective δ-opioid receptor agonist, DPLPE (100 μM), on spontaneous glycinergic IPSCs. Drugs were applied after recording 60 s of control events; only one concentration of drug was used per neuron (2 neurons per slice). In other experiments, the selective δ-opioid agonist DPLPE (100 μM) was used in conjunction with an effective concentration of DAMGO to ensure the lack of contribution of δ-receptors to the inhibitory effects of DAMGO. In a separate series of experiments, under conditions of synaptic blockade with TTX (10 μM), CNQX (50 μM), and picrotoxin (100 μM), exogenous glycine (10 μM) was puffed onto the recording cell (using a patch pipette positioned 15–20 μm from the cell and a WPI Pneumatic Picopump) in the absence and presence of opioid (100 μM) to determine if the postsynaptic current evoked by exogenous glycine was altered. Under these conditions, ≥15 control responses were obtained before drug application; again, each neuron was used only once. To further establish the effects of opioids on cardiac vagal neurons, spontaneous, glycinergic, TTX-insensitive, miniature synaptic events (mIPSCs) were recorded in a solution of the following composition (in mM): 105 NaCl, 23 KCl, 2 CaCl₂, 5 glucose, 26 NaHCO₃, 5 HEPES, in the presence of TTX (1 μM), picrotoxin (100 μM), and CNQX (50 μM). At the end of 6 of 12 experiments, the glycine antagonist strychnine (10 μM) was used to eliminate the mIPSCs. Analysis of events was performed using MiniAnalysis (Synaptosoft, version 4.3.1) with the minimal acceptable amplitude of events set at 6–15 pA. The responses to exogenous glycine were analyzed in Clampfit. Results are presented as mean ± SE percentage of control and statistically compared with Student’s t-test (for significance of difference, *P < 0.05).

Drugs and chemicals

All drugs were purchased from Sigma Chemicals (St. Louis, MO). TTX was dissolved in acetate buffer, while glycine was prepared in slice perfusate; all opioids were dissolved in H₂O and stored at −20°C until the day of use. Opioids were added into the recording chamber by changing the perfusion line to the one containing the drug.
RESULTS

Effects of nociceptin and μ-selective opioids on spontaneous glycinergic IPSCs

Glycinergic IPSCs were isolated for study and were abolished by strychnine (10 μM), demonstrating these events were due to activation of glycine receptors (control frequency 4.28 ± 0.45; frequency with strychnine 0.23 ± 0.14, n = 5).

All four opioids significantly inhibited glycinergic activity in cardiac vagal neurons (see Fig. 1). Nociceptin was the most effective agonist used in this study. At concentrations of 100, 30, and 10 μM, nociceptin significantly (P < 0.05) decreased the average IPSC amplitude by 56.6 ± 4.4% (responses ranged from 39.1 to 73.7%; n = 7), 47.3 ± 8.1% (responses ranged from 18.7 to 72.9%; n = 6), and 46.5 ± 6.1% (responses ranged from 27.4 to 66.7%; n = 6), respectively (see Fig. 1A). Application of DAMGO also significantly reduced the IPSC amplitude at 100 μM [by 46.5 ± 6.1% (responses ranged from 19.4% to 67.8%; n = 6)] and at 30 μM [by 28.0 ± 7.6% (responses ranged from 9.5 to 61.9% with 2 nonresponders; n = 9); Fig. 1B]. Endomorphin-1 and endomorphin-2 both effectively reduced IPSC amplitude at only 100 μM (by 45.1 ± 8.6% (responses ranged from 23.1 to 72.2%; n = 7) and by 26 ± 7.5% (responses ranged from 7.3 to 64.7%; n = 9), respectively (Fig. 1, C and D). The holding current in these neurons did not change on application of the opioid.

Nociceptin also significantly (P < 0.05) reduced the frequency of the IPSCs at 100 μM by 61.1 ± 12.8% (responses ranged from 35.3 to 93.1%; n = 7) and at 30 μM by 68.3 ± 13.9% (responses ranged from 7.5 to 94.6%; n = 6), and was again, the most effective agonist used here (see Fig. 1A). DAMGO was also effective at these concentrations; addition of 100 μM DAMGO caused a 69.9 ± 13.0% inhibition (responses ranged from 7.14 to 92.7% with one nonresponder; n = 7) and 30 μM caused a 49.3 ± 8.8% inhibition (responses ranged from 19.4 to 75%; n = 9) of the frequency of the spontaneous IPSCs (P < 0.05 for the above values; see Fig. 1B). The endomorphins proved less effective than either nociceptin or DAMGO in this study. Only the top concentration of endomorphin-1 (100 μM) significantly reduced the IPSC frequency, causing a 40.8 ± 12.1% inhibition (responses ranged from 13.6 to 80% with 1 nonresponder; n = 7; P < 0.05), while endomorphin-2 did not significantly reduce the IPSC frequency at any concentration (see Fig. 1, C and D). Lower concentrations of opioids that did not produce significant inhibition, as well as these responses, are shown in Fig. 1.

FIG. 1. Representative traces showing spontaneous glycinergic inhibitory postsynaptic currents (IPSCs) in a cardiac vagal neuron, before and after the addition of (A) nociceptin (30 μM), (B) DAMGO (30 μM), (C) endomorphin-1 (100 μM), and (D) endomorphin-2 (100 μM). Next to each trace is a typical plot showing amplitude and frequency changes with opioid addition. Dose-response histograms summarize the effects of increasing concentrations of opioids on the spontaneous glycinergic activity. Drug effectiveness from this study is as follows: nociceptin > DAMGO > endomorphin-1 > endomorphin-2.
Effect of DPLPE on spontaneous glycinergic IPSCs

A high concentration of the selective δ-agonist DPLPE (100 μM) failed to significantly alter either the amplitude (16.9 ± 14.7%; n = 6) or the frequency (−20.1 ± 32.8%; n = 6) of the spontaneous IPSCs.

Effect of C-TOP and naltrindole on DAMGO-mediated inhibition of spontaneous IPSCs

To determine if the inhibition of spontaneous activity observed after opioid administration was directly due to an action on μ-opioid receptors, the selective μ-receptor antagonist C-TOP (10 μM) was applied 10 min before and during application of DAMGO (100 μM). These experiments were performed in fresh slices that had not been previously exposed to opioid agonists. DAMGO was the μ-selective opioid of choice in this series of experiments as it was shown to produce greater inhibitory effects on spontaneous activity than the endorphins. C-TOP effectively prevented the inhibitory effects of DAMGO on both the amplitude (Fig. 2A; 10.8 ± 7.1% inhibition, n = 7) and the frequency (Fig. 2B; 10.7 ± 14.6% inhibition, n = 7) of the spontaneous, glycinergic IPSCs. This confirmed that the inhibitory actions of DAMGO in these experiments were mediated via a direct action on μ-opioid receptors. To rule out the possibility that δ-receptors contributed to the responses to high concentrations of DAMGO, the above experiments were repeated in separate, previously unused slices, using the selective δ-antagonist naltrindole (10 μM) in conjunction with DAMGO (100 μM). Naltrindole failed to prevent the significant inhibitory effects of DAMGO on either the amplitude (37.9 ± 11.5% inhibition, n = 6) or the frequency (44.1 ± 9.7% inhibition, n = 6) of the spontaneous glycinergic IPSCs, indicating that the effects of DAMGO were not mediated via the δ-receptor.

Effects of opioids on responses to exogenously applied glycine

The observed decrease in IPSC amplitude with opioid application could reflect an action on presynaptic opioid receptors, leading to changes in neurotransmitter release, and/or an action on postsynaptic receptors located on the recording neuron itself, leading to a decrease in the glycinergic response. To test this, the effects of DAMGO and nociceptin on responses to exogenously applied glycine were examined. Application of glycine (10 μM) induced a postsynaptic current that was not significantly reduced by either 100 μM nociceptin (Fig. 3A; 3.1 ± 4.3% inhibition, n = 5) or by 100 μM DAMGO (Fig. 3B; 3.8 ± 5.3% inhibition, n = 5). (The responses to exogenous glycine were larger than the observed spontaneous IPSCs yet were still at submaximal levels, since greater concentrations of glycine evoked larger responses). The lack of effect of these opioids on postsynaptic glycinergic currents suggests their actions may be mediated by receptors located presynaptically.

Effects of opioids on glycinergic miniature synaptic events

Glycinergic miniature synaptic events (mIPSCs) were studied in the absence and presence of DAMGO and nociceptin to further determine the location of the recruited opioid receptors.
control amplitude 33.5 ± 5.7 pA, mean test amplitude 37.6 ± 6.9 pA; mean control frequency 1.23 ± 0.4, mean test frequency 1.28 ± 0.4) or 100 μM DAMGO (mean control amplitude 46.5 ± 5.3 pA, mean test amplitude 46.3 ± 7.4 pA; mean control frequency 2.5 ± 0.52, mean test frequency 2.67 ± 0.5; see Fig. 4, n = 6 for all). The results indicate that the inhibitory effects of DAMGO and nociceptin are mediated via μ-receptors and ORL₁ receptors not located on the presynaptic terminals.

Discussion

Mendelowitz and Kunze (1991) have previously described the methods used to selectively identify cardiac vagal neurons. Rhodamine (XRITC) injected into the pericardial sac is absorbed by preganglionic cardiac motoneuron nerve terminals located at the base of the heart (without surgical penetration of the heart) and transported retrogradely to their soma in the medulla. Control injections of XRITE into the thoracic cavity, but outside of the pericardial sac, do not label any neurons in the medulla (Mendelowitz and Kunze 1991). Similarly, in other control experiments, XRITE injected intravenously also does not label any neurons in the medulla except for a few neurons in the area postrema, an area devoid of the blood-brain barrier. This study uses these techniques to identify a mechanism by which opioids evoke a vagally mediated bradycardia. The results from this study demonstrate that cardiac vagal neurons in the rat nucleus ambiguus receive a glycnergic input. μ-Opioid receptor-selective agonists and nociceptin, acting at μ-opioid receptors and ORL₁ receptors, respectively, inhibit this glycnergic activity.

Glycnergic mechanisms have been previously implicated in central cardiovascular regulation (Cassell et al. 2000; Chitravanshi et al. 1991; Kubo and Kihara 1987; Nunez-Abades et al. 1990; Talman and Robertson 1989; Wessberg et al. 1983). Microinjections of glycine into the nucleus ambiguus cause an increase in heart rate (Chitravanshi et al. 1991), demonstrating the presence of functional glycine receptors within this area. This study supports this idea and shows the presence of spontaneous inhibitory glycnergic currents in cardiac vagal neurons within the ambiguus.

Since the strength of glycnergic inhibition governs the output of many motor systems (Bracci et al. 1996) and glycine-receptor deficits are linked to several human pathologies (Andrew and Owen 1997; Rajendra et al. 1994), it is possible that factors governing glycnergic transmission to cardiac vagal neurons could have a strong influence on motor control of the heart. In this study, nociceptin, an ORL₁ agonist, and μ-selective opioids, such as DAMGO, endomorphin-1, and endomorphin-2, all have a strong inhibitory effect on glycnergic currents in cardiac vagal neurons in the nucleus ambiguus. The
The suppression of spontaneous inhibitory glycinergic inputs to cardiac vagal neurons may be one mechanism by which opioids act centrally to evoke bradycardia. The lack of effect of DPLPE in this study indicates that glycinergic transmission to cardiac vagal neurons is not influenced by δ-agonists and that any centrally evoked cardiac responses by δ-opioids may be acting via a separate pathway.

In this study, the degree of inhibitory responses achieved varied among the opioids used. The range of potencies from these experiments was as follows: nociceptin = DAMGO > endomorphin-1 > endomorphin-2. These results differ from those obtained in studies examining opioid influence on GABAergic activity to cardiac vagal neurons (Venkatesan et al. 2002a,b). In those studies, nociceptin and endomorphin-2 had similar potencies and were the only ligands found to decrease GABAergic inputs to these neurons, while DAMGO and endomorphin-1 had no effect at all. Such differences are also reported from other studies examining the effects of these opioids on acute pain (Przewlocka et al. 1999), the cardiovascular system (Czapla et al. 2000), and G protein activation (Narita 1998; Narita et al. 2000). These studies found DAMGO has greater efficacy in these models than either endomorphin-1 or endomorphin-2. A possible explanation is that the endomorphins may act only as partial agonists in certain species or certain areas. The differences between responses produced by endomorphin-1 and endomorphin-2 may also be explained by their differing affinities for μ-receptor subtypes. Other studies examining the antinociceptive activity of endomorphin-1 and endomorphin-2 (Kamei et al. 2000; Sakurada et al. 2000) and the actions of DAMGO on the hypothalamo-pituitary adrenal axis (Eisenberg 1993) have found that, in these test paradigms, endomorphin-2 acts via μ1-opioid receptors while DAMGO and endomorphin-1 possibly act at μ2-receptors. The results from this study indicate a prevalence of ORL1 receptors and μ2-receptors whose activation reduces glycinergic inputs to cardiac vagal neurons, with comparatively fewer μ1-receptors within the same area. However, there is, as yet, no commercially available μ2-selective antagonist to determine this. The concentration of C-TOP (the selective μ-antagonist) used in this study has been used in other studies examining the effects of high concentrations of μ-selective agonists (Ammer and Schulz 1993; Lippl et al. 2001). The possibility of δ-receptors contributing to the inhibitory effects of DAMGO in this study was ruled out by the use of the selective δ-antagonist naltrindole, which did not prevent the significant inhibitory effects of DAMGO on the spontaneous IPSCs.

The use of a selective ORL1 antagonist would also have been useful in this study, to determine the direct effects of nociceptin on these receptors. However, the few “antagonists” available have disadvantages to their usage, displaying either agonist or partial agonist activity (Emmerson and Miller 1999; Kapusta et al. 1999; Siniscalchi et al. 1999) or are nonselective for the ORL1 receptor (Schlicker and Morari 2000). A potent, nonpeptidyl selective ORL1 receptor antagonist (J-113397) has been discussed in another study (Ozaki et al. 2000) but is not yet commercially available.

In these experiments, the exact location of opioid action is not known. Neither nociceptin nor DAMGO had any effect on the responses to exogenous application of glycine, indicating these agonists do not affect postsynaptic glycinergic function. In addition, neither nociceptin nor DAMGO had an effect on the amplitude or frequency of TTX-insensitive glycinergic mIPSCs, indicating that opioids do not act at the presynaptic terminal and may be acting at the cell bodies or dendrites of the glycinergic neurons or even neurons precedent to those glycinergic neurons.

The results from this study may have significant clinical implications. Experiments performed on rodents show that glycine antagonists may prolong the analgesic effects of opioids and attenuate their dependence and tolerance syndromes, when systemically administered in combination over several days (Martinez et al. 2002; Quartaroli et al. 2001). However, as seen from the results of this study, this may prove detrimental, suppressing the glycinergic influence on parasympathetic neurons and further increasing the level of bradycardia seen. Another study has shown that a compensatory increase in sympathetic activity occurs in dogs after a few days of exposure to opioids (Napier et al. 1998). Although the overall bradycardic effects of opioids remains throughout the exposure, this increase in sympathetic activity may have severe cardiovascular consequences if the opioid-mediated vagal opposition were suddenly withdrawn; the degree of severity may depend on the preceding level of parasympathetic activity to the heart that would be augmented by supplemental glycinergic antagonism.

In summary, this study demonstrates that cardiac vagal neurons located in the nucleus ambiguous receive glycinergic inputs. Nociceptin and μ-opioid receptor selective agonists act on ORL1 receptors and μ-opioid receptors to decrease spontaneous glycinergic neurotransmission to cardiac vagal neurons. There may be a greater number of μ2-receptors involved in this response, compared with μ1-receptors, accounting for the greater effectiveness of DAMGO and endomorphin-1 over endomorphin-2. The resulting increase in parasympathetic outflow to the heart from the opioid-mediated reduction in glycinergic transmission to cardiac vagal neurons may be one mechanism by which nociceptin and μ-selective opioids act centrally to evoke a bradycardia. This phenomenon should be taken into account when considering glycinergic antagonists as potential enhancers of opiate analgesics.

DISCLOSURES

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REFERENCES


