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Bradycardia elicited by microinjections of nociceptin/orphanin FQ into the intermediolateral cell column at T1–T2 in the rat

Vineet C. Chitravanshi and Hreday N. Sapru

Department of Neurological Surgery, MSB H-586, University of Medicine and Dentistry of New Jersey - New Jersey Medical School, 185 South Orange Ave., Newark, NJ 07103, USA

Abstract

Microinjections (30 nl) of nociceptin/orphanin FQ (N/OFQ) into the intermediolateral cell column (IML) at T1 and T2 levels of the spinal cord elicited bradycardia. The decreases in HR were 12.3 ± 2.9 , 17.3 ± 2.7 , 26.7 ± 3.1 , and 18.6 ± 3.4 beats/min in response to 0.075, 0.15, 0.62 and 1.25 mM concentrations, respectively. Maximally effective concentration of N/OFQ was 0.62 mM. No changes in BP were elicited by microinjections of N/OFQ into the IML at T1–T2. The bradycardic responses were completely blocked by prior microinjections of a N/OFQ receptor (NOP receptor) antagonist ([N-phe¹]-nociceptin-(1–13)-NH₂, 9 mM) into the IML at T1–T2. Blockade of myocardial β -1 adrenergic receptors also abolished the bradycardic responses elicited by microinjections of N/OFQ into the IML. It was concluded that activation of NOP receptors in right IML at T1–T2 by N/OFQ elicited bradycardic responses which were mediated via the sympathetic nervous system.

Keywords

atenolol; bradycardia; denopamine; IML; NOP receptor

Nociceptin/Orphanin FQ (N/OFQ) [18,19,25] is an endogenous ligand for a G-protein coupled receptor, named N/OFQ peptide (NOP) receptor (previously known as ORL1 receptor). N/OFQ has a high and selective affinity for NOP receptor and a very poor affinity for classical opioid receptors (μ , δ and κ receptors) [9,18]. Although NOP receptor shares a high sequence similarity with classical opioid receptors [4–6,13–14,20–22,24], it does not bind opioid peptides. Naloxone which is an antagonist at classical opioid receptors does not block the effects of NOP receptor agonists [4–5].

The sympathetic preganglionic neurons that provide innervation to the heart are located in the intermediolateral cell column (IML) of the thoracic cord at T1–T3 levels [30]. The presence of NOP receptors [2,24] and N/OFQ immunoreactivity [12,23,26], has been reported in the IML. Based on these reports [23–24,26] it was hypothesized that N/OFQ may elicit cardiac responses by activating NOP receptors in the IML. This hypothesis was tested in our above-mentioned model in which sympathetic preganglionic neurons providing innervation to the heart can be selectively stimulated [29].

Correspondence: H.N. Sapru, Ph.D., Department of Neurological Surgery, MSB H-586, UMDNJ - New Jersey Medical School, 185 South Orange Ave., Newark, N.J. 07103, USA, Telephone 973-972-5876, FAX 973-972-5986, Email sapru@umdnj.edu

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Experiments were done in adult male Wistar rats (Charles River Laboratories, Wilmington, MA, USA), weighing 300–350 g ($n = 48$). The experimental procedures were performed in accordance with the NIH guidelines for research involving animals. Additionally, protocols for animal use in this investigation were approved by the Institutional Animal Care and Use Committee of this university.

Details of general procedures used in this study have been described by us previously [8,15–16]. Briefly, the rats were anesthetized with urethane (1.2–1.4 gm/kg, i.v.; Sigma Chemicals, St. Louis, MO). Pulsatile arterial pressure (PAP), mean arterial pressure (MAP) and heart rate (HR) were monitored by standard techniques. The rats were artificially ventilated with room air and end-tidal CO₂ was maintained at 3.5–4%. Rectal temperature was monitored continuously and maintained at $37 \pm 0.5^\circ\text{C}$. All of the tracings were recorded on a polygraph (Grass Instruments, model 7D).

The rats were placed in a prone position in a stereotaxic instrument (model 1430) with a rat spinal unit attachment (model 980) (David Kopf Instruments, Tujunga, CA, USA) and the dorsal surface of the spinal cord from C7 to T5 was exposed [29]. Microinjections were made as described previously using a picospritzer (General Valve Corp, Fairfield, NJ, USA) and four barreled glass micropipettes (tip size 20–40 μm) [7–8,15–16,28–29]. All of the solutions for the microinjections were freshly prepared in aCSF. The volume of all microinjections was 30 nl. The duration of microinjection was 10 sec. Controls for microinjections consisted of aCSF. The coordinates for the IML at T1–T2 were: 0.8–1 mm lateral to the midline and 0.8–1.1 mm deep from the dorsal surface of the spinal cord.

Typical sites in the IML, where L-Glu and N/OFQ were microinjected, were marked by a microinjection of diluted India ink contained in one of the barrels of the glass micropipette used for microinjections. The details of perfusion of the animals, tissue fixation, and cutting and preparation of sections (30 μm) have been described previously [8,15–16].

For statistical analyses, the means and standard error of the means (SEM) were calculated for maximum changes in heart rate (HR) in response to microinjections of N/OFQ. Comparisons of changes in HR elicited by different concentrations of N/OFQ (Sigma Chemicals) were made by using a one-way analysis of variance followed by Tukey-Kramer multiple comparison test. Comparisons of the maximum decreases in HR elicited by N/OFQ before and after the microinjections of NOP receptor antagonist, [N-Phe¹]-nociceptin-(1–13)-NH₂ (Phoenix Pharmaceuticals, Belmont, CA) [3], were made by using paired t-test. In all cases, the differences were considered significant at $p < 0.05$.

Baseline HR and MAP were 426 ± 9.8 beats/min and 110 ± 2.7 mmHg, respectively in the urethane-anesthetized rats ($n = 48$).

In each rat, right IML at T1 and T2 was selected at random for microinjections. As mentioned earlier, the IML site was identified by microinjections of L-Glu (5 mM); tachycardic responses (36.9 ± 4.1 bpm), with no accompanying changes in BP, were observed. Microinjections of aCSF at these sites did not elicit any response. The interval between the microinjections of L-Glu and other agents was 5 min. N/OFQ in different concentrations (0.075, 0.15, 0.62, and 1.25 mM) was microinjected into the right IML ($n = 12$). No more than 3 concentrations of N/OFQ were microinjected in random order in each rat and the interval between these microinjections was at least 30 min. The decreases in HR elicited by the afore-mentioned concentrations of N/OFQ were 12.3 ± 2.9 , 17.3 ± 2.7 , 26.7 ± 3.1 and 18.6 ± 3.4 beats/min, respectively (Fig. 1A). No changes in BP were elicited by microinjections of N/OFQ. Since the responses to 0.62 mM were greater than other concentrations, this concentration was selected for other experiments. The onset and duration of HR responses to 0.62 mM

concentration were 12.8 ± 2.7 sec and 5.31 ± 0.4 min, respectively. At T1–T2, microinjections of either L-Glu (5 mM) or N/OFQ (0.62 mM) outside IML (e.g., 0.5–0.6 mm lateral to the midline and 2.0–2.2 mm deep from the dorsal spinal surface) did not elicit any cardiovascular response. Intravenous injections of the same dose of N/OFQ (i.e., 0.62 mM, 30 nl) in another group of rats ($n = 12$) elicited no changes in HR.

In another group of rats ($n = 12$), N/OFQ (0.62 mM) was microinjected into IML 3 times, at 30 min intervals. The decreases in HR in response to these 3 consecutive microinjections of N/OFQ were 26.7 ± 3.1 , 25 ± 3.4 , and 27.5 ± 5.5 bpm, respectively; the differences between the bradycardic responses to repeated microinjections of N/OFQ were not statistically significant ($p > 0.05$).

The role of NOP receptors in mediating the responses to N/OFQ was tested in another group of rats ($n = 7$). In this group, microinjections of L-Glu (5 mM) into the right IML elicited tachycardic responses (28.6 ± 7.3 bpm) ($p < 0.01$) (Fig. 1B, a). When HR returned to baseline levels, a 5 min interval was allowed. Microinjections of aCSF (30 nl) at the same site did not elicit any cardiovascular response (Fig. 1B, b). Five min later, microinjections of N/OFQ (0.62 mM) at the same site elicited a decrease in HR (28.6 ± 3.4 bpm) ($p < 0.01$) (Fig. 1B, c). When the HR returned to baseline levels, an interval of 30 min was allowed when an NOP receptor antagonist, [N-phe¹]-nociceptin-(1–13)-NH₂ (9 mM), was microinjected at the same site. The antagonist by itself did not elicit significant changes in HR compared to the baseline values (Fig. 1B, d); HR values before and after the microinjection of the NOP receptor antagonist were 405.7 ± 3.7 and 415 ± 4.1 bpm ($p > 0.05$). An interval of about 5 min was allowed after the microinjection of the antagonist when N/OFQ (0.62 mM) was microinjected again at the same site. At this time, N/OFQ failed to elicit bradycardic responses (Fig. 1B, e); HR values before and after the microinjection of N/OFQ were 407.9 ± 4.1 and 407.1 ± 3.6 bpm, respectively ($p > 0.05$). The differences in the N/OFQ-induced decreases in HR before (28.6 ± 3.4 bpm; Fig. 1B, c) and after (0.71 ± 0.71 bpm; Fig. 1B, e) the microinjection of the NOP receptor antagonist were highly significant ($p < 0.001$). After an interval of 60 min, bradycardic responses to microinjections of N/OFQ (0.62 mM) showed some recovery but it was not complete; a decrease of 20.7 ± 1.7 bpm (Fig. 1B, f) was observed as compared to a decrease of 28.6 ± 3.4 bpm before the microinjection of the NOP receptor antagonist (Fig. 1B, c) ($p > 0.05$). A typical tracing showing the blockade of N/OFQ responses by NOP receptor antagonist is shown in Fig. 2. The right IML site was identified by a microinjection of L-Glu (5 mM); an increase in HR was observed (Fig. 2A). When the HR returned to baseline levels, an interval of 5 min was allowed when aCSF was microinjected at the same site; no change in HR was observed (Fig. 2B). After an interval of 5 min, microinjection of N/OFQ (0.62 mM) at the same site elicited a decrease in HR (Fig. 2C). Thirty min after the recovery of HR to baseline levels, NOP receptor antagonist, [N-phe¹]-nociceptin-(1–13)-NH₂ (9 mM), was microinjected into the same site; no significant change in HR was elicited (Fig. 2D). Five min after the microinjection of NOP receptor antagonist, N/OFQ (0.62 mM) was again microinjected at the same site. The bradycardic responses to microinjection of N/OFQ were completely blocked by prior microinjection of NOP receptor antagonist (Fig. 2E). The bradycardic responses to microinjection of N/OFQ (0.62 mM) into the IML showed some recovery within 60 min. (Fig. 2F). In these experiments, microinjections of aCSF, L-Glu, N/OFQ or NOP receptor antagonist into the right IML at T1–T2 did not elicit any changes in MAP.

The effect of beta-adrenergic receptor blockade on N/OFQ-induced decreases in HR was studied in another group of rats ($n = 5$). Microinjections of L-Glu (5 mM) into the right IML elicited an increase in HR (54 ± 4 bpm) (Fig. 3A). When the HR returned to baseline levels, microinjection of aCSF at the same site elicited no change in HR. Within 5 min, microinjection of N/OFQ (0.62 mM) at the same site elicited a decrease in HR (34 ± 2.4 bpm) (Fig. 3B). After an interval of 30 min, atenolol (10 µg/kg; β -1 adrenergic receptor antagonist) was injected

intravenously (i.v.); a decrease in HR (48 ± 2 bpm) was observed (Fig. 3C). In preliminary experiments, atenolol blocked the responses to denopamine ($40 \mu\text{g/kg}$, i.v.). Denopamine has been reported to be a β -1 adrenergic receptor agonist [31]. Within 10–15 min, while atenolol-induced bradycardia persisted, N/OFQ (0.62 mM) was microinjected into the IML; the responses to N/OFQ were blocked by atenolol (Fig. 3D). About 60 min later when the baseline HR showed about 80% recovery, N/OFQ (0.62 mM) was microinjected into the IML again; the responses showed incomplete recovery (Fig. 3E).

The IML sites where microinjections of N/OFQ elicited bradycardic effects were marked with India ink (30 nl) in 10 rats. Composite diagrams of the sites of microinjections are shown in Fig. 4.

The main finding in this study was that activation of NOP receptors in the IML by direct microinjections of N/OFQ elicited bradycardia. Maximum responses were elicited at 0.62 mM concentration; at higher concentration (1.25 mM), the responses were smaller. This type of bell-shaped concentration-response curve has been consistently observed with N/OFQ and other peptides [7–8,15–16]. It is possible that at very high concentrations of N/OFQ, the NOP receptors are desensitized. There are no reports in the literature in which the effects of microinjections of N/OFQ into the IML were tested. However, our results are consistent with known inhibitory effect of N/OFQ on sympathetic preganglionic neurons [17]. Inhibition of sympathetic preganglionic neurons in the IML is expected to decrease sympathetic input to the heart resulting in bradycardia. This conclusion was confirmed by the observation that the blockade of myocardial β -1 adrenergic receptors by intravenously injected atenolol blocked the sympathoinhibitory effects of N/OFQ as well as sympathoexcitatory effects of L-Glu microinjections into the IML on the heart. At the cellular level, the mechanisms of N/OFQ-induced inhibition of IML neurons may include inhibition of adenylate cyclase [18,25], activation of potassium channels [1,11,17], and/or inhibition of calcium channels [10].

Concentrations of N/OFQ microinjections into the IML that elicited bradycardic responses did not elicit a response when injected intravenously indicating that leakage, if any, of N/OFQ from the microinjection site into the peripheral circulation was not responsible for the observed responses. The sites at which N/OFQ elicited bradycardic responses were restricted to IML.

The bradycardic responses to microinjections of N/OFQ into the IML were mediated via NOP receptors because, microinjection of a specific antagonist for NOP receptors [3] at this site, abolished N/OFQ responses. The lack of responses to N/OFQ after the microinjections of the NOP receptor antagonist was not due to tachyphylaxis because repeated microinjections of N/OFQ (0.62 mM) into the IML at 30 min intervals did not exhibit tachyphylaxis. In the concentrations used, the NOP receptor antagonist did not exert any nonspecific effects because it did not alter responses to another opioid receptor agonist, endomorphin-2 [15,28]. Microinjections of the NOP receptor antagonist by itself elicited no appreciable increases in HR suggesting that NOP receptors in the IML are not tonically active in the rat.

In summary, the data presented demonstrate that activation of NOP receptors on the neurons located in the IML, by N/OFQ, at T1–T2 results in bradycardia. This effect of N/OFQ can be explained by inhibition of neurons in the IML that provide sympathetic innervation to the heart [29–30] and subsequent decrease in the activity of sympathetic nerves innervating the heart. HR is controlled by a complex neuronal circuitry in the brain. The sympathetic input to the heart is provided by the IML located in upper thoracic levels while the parasympathetic innervation to this organ is provided primarily by the nucleus ambiguus [27]. Different circuits controlling the heart may be activated in different physiological or pathophysiological conditions. The physiological or pathophysiological situations which N/OFQ inhibits IML neurons to elicit bradycardia remain to be elucidated.

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References

- [1]. Brailoiu GC, Lai CC, Chen CT, Hwang LL, Lin HH, Dun NJ. Sympathoinhibitory action of nociceptin in the rat spinal cord. *Clin. Exp. Pharmacol. Physiol* 2002;29:233–237. [PubMed: 11906490]
- [2]. Bunzow JR, Saez C, Mortrud M, Bouier C, Williams JT, Low M, Grandy DK. Molecular cloning and tissue distribution of a putative member of the rat opioid receptor gene family that is not mu, delta or kappa opioid receptor type. *FEBS. Lett* 1994;347:284–288. [PubMed: 8034019]
- [3]. Calo G, Guerrini R, Bigoni R, Rizzi A, Marzola G, Okawa H, Bianchi C, Lambert DG, Salvadori S, Regoli D. Characterization of [N-Phe¹]-nociceptin-(1–13)-NH₂, a new selective nociceptin receptor antagonist. *Brit. J. Pharmacol* 2000;129:1183–1193. [PubMed: 10725267]
- [4]. Calo G, Guerrini R, Rizzi A, Salvadori S, Regoli D. Pharmacology of nociceptin and its receptor: a novel therapeutic target. *Brit. J. Pharmacol* 2000;129:1261–1283. [PubMed: 10742280]
- [5]. Calo G, Bigoni R, Rizzi A, Guerrini R, Salvadori S, Regoli D. Nociceptin/orphanin FQ receptor ligands. *Peptides* 2000;21:935–947. [PubMed: 10998527]
- [6]. Chen Y, Fan Y, Liu J, Mestek A, Tian M, Kozak CA, Yu L. Molecular cloning, tissue distribution and chromosomal localization of a novel member of the opioid receptor gene family. *FEBS Lett* 1994;347:279–283. [PubMed: 8034018]
- [7]. Chitravanshi VC, Sapru HN. Mechanism of cardiovascular effects of nociceptin microinjected into the nucleus tractus solitarius of the rat. *Am. J. Physiol* 2005;288:R1553–R1562.
- [8]. Chitravanshi VC, Sapru HN. Microinjections of nociceptin into the nucleus ambiguus elicit tachycardia in the rat. *Brain Res* 2005;1051:199–204. [PubMed: 16000194]
- [9]. Civelli O, Nothacker HP, Reinscheid R. Reverse Physiology: discovery of the novel neuropeptide, orphanin FQ/nociceptin. *Crit. Rev. Neurobiol* 1998;12:163–176. [PubMed: 9847053]
- [10]. Connor M, Yeo A, Henderson G. The effect of nociceptin on Ca²⁺ channel current and intracellular Ca²⁺ in the SH-SY5Y human neuroblastoma cell line. *Brit. J. Pharmacol* 1996;118:205–207. [PubMed: 8735615]
- [11]. Connor M, Vaughan CW, Chieng B, Christie MJ. Nociceptin receptor coupling to a potassium conductance in rat locus coeruleus neurones in vitro. *Brit. J. Pharmacol* 1996;119:1614–1618. [PubMed: 8982509]
- [12]. Dun NJ, Dun SL, Hwang LL. Nociceptin-like immunoreactivity in autonomic nuclei of the rat spinal cord. *Neurosci. Lett* 1997;234:95–98. [PubMed: 9364506]
- [13]. Fukuda K, Kato S, Mori K, Nishi M, Takeshima H, Iwabe N, Miyata T, Houtani T, Sugimoto T. cDNA cloning and regional distribution of a novel member of the opioid receptor family. *FEBS Lett* 1994;343:42–46. [PubMed: 8163014]
- [14]. Henderson G, McKnight AT. The orphan opioid receptor and its endogenous ligand-nociceptin/orphanin FQ. *Trends Pharms. Sci* 1997;18:293–300.
- [15]. Kasamatsu K, Chitravanshi VC, Sapru HN. Depressor and bradycardic responses to microinjections of endomorphin-2 into the nucleus tractus solitarius are mediated via ionotropic glutamate receptors. *Am. J. Physiol* 2004;287:R715–R728.
- [16]. Kawabe T, Chitravanshi VC, Kawabe K, Sapru HN. Cardiovascular effects of adrenocorticotropin microinjections into the rostral ventrolateral medullary pressor area of the rat. *Brain Res* 2006;1102:117–126. [PubMed: 16797500]
- [17]. Lai CC, Wu SY, Chen CT, Dun NJ. Nociceptin inhibits rat sympathetic preganglionic neurons in situ and in vitro. *Am. J. Physiol* 2000;278:R592–R597.
- [18]. Meunier JC. Nociceptin/orphanin FQ and the opioid receptor-like ORL1 receptor. *Eur. J. Pharmacol* 1997;340:1–15. [PubMed: 9527501]
- [19]. Meunier JC, Mollereau C, Toll L, Suaudeau C, Moisand C, Alvinerie P, Butour JL, Guillemot JC, Ferrara P, Monsarrat B, Mazargull H, Vassaart G, Parmentier M, Costentin J. Isolation and structure of the endogenous agonist of opioid receptor-like ORL₁ receptor. *Nature* 1995;377:532–535. [PubMed: 7566152]

- [20]. Meunier J, Mouldous L, Topham CM. The nociceptin (ORL1) receptor: molecular cloning and functional architecture. *Peptides* 2000;21:893–900. [PubMed: 10998522]
- [21]. Mollereau C, Mouldous L. Tissue distribution of the opioid receptor-like (ORL1) receptor. *Peptides* 2000;21:907–917. [PubMed: 10998524]
- [22]. Mollereau C, Parmentier M, Mailleux P, Butour JL, Moisand C, Chalon P, Caput D, Vassart G, Meunier JC. ORL1, a novel member of the opioid receptor family. *FEBS Lett* 1994;341:33–38. [PubMed: 8137918]
- [23]. Neal CR Jr, Mansour A, Reinscheid R, Nothacker HP, Civelli O, Watson SJ. Localization of orphanin FQ (Nociceptin) peptide and messenger RNA in the central nervous system of the rat. *J. Comp. Neurol* 1999;406:503–547. [PubMed: 10205026]
- [24]. Neal CR Jr, Mansour A, Reinscheid R, Nothacker HP, Civelli O, Akil H, Watson SJ. Opioid receptor-like (ORL1) receptor distribution in the rat central nervous system: comparison of ORL1 receptor mRNA expression with ¹²⁵I-[¹⁴Tyr]-orphanin FQ binding. *J. Comp. Neurol* 1999;412:563–605. [PubMed: 10464356]
- [25]. Reinscheid RK, Nothacker HP, Bourson A, Ardati A, Henningsen RA, Bunzow JR, Grandy DK, Langen H, Monsma FJ Jr, Civelli O. Orphan FQ: a neuropeptide that activates an opioid-like G-protein-coupled receptor. *Science* 1995;270:792–794. [PubMed: 7481766]
- [26]. Riedl M, Shuster S, Vulchanova L, Wang J, Loh H, Elde R. Orphanin FQ/nociceptin-immunoreactive nerve fibers parallel those containing endogenous opioids in rat spinal cord. *Neuroreport* 1996;7:1369–1372. [PubMed: 8856677]
- [27]. Sapru HN. Glutamate circuits in selected medullo-spinal areas regulating cardiovascular function. *Clin Exp Pharmacol Physiol* 2002;29:491–496. [PubMed: 12010197]
- [28]. Shah N, Chitravanshi VC, Sapru HN. Cardiovascular responses to microinjections of nociceptin into a midline area in the commissural subnucleus of the nucleus tractus solitarius of the rat. *Brain Res* 2003;984:93–103. [PubMed: 12932843]
- [29]. Sundaram K, Murugaian J, Sapru HN. Cardiac responses to the microinjections of excitatory amino acids into the intermediolateral cell column of the rat spinal cord. *Brain Res* 1989;482:12–22. [PubMed: 2565136]
- [30]. Ter Horst GJ, Van den BA, Homminga SA, Hautvast RW, Rakhorst G, Mettenleiter TC, De Jongste MJ, Lie KI, Korf J. Transneuronal viral labelling of rat heart left ventricle controlling pathways. *Neuroreport* 1993;4:1307–1310. [PubMed: 8260610]
- [31]. Yokoyama H, Yanagisawa T, Taira N. Details of mode and mechanism of action of denopamine, a new orally active cardiotonic agent with affinity for beta 1-receptors. *J Cardiovasc Pharmacol* 1988;12:323–331. [PubMed: 2464105]

Fig. 1

Fig 1.

A: Concentration-response for HR. Microinjections (30 nl) of different concentrations of N/OFQ (0.075, 0.15, 0.62 and 1.25 mM) into the IML at T1–T2 elicited decreases in HR ($n = 12$). The responses to 0.62 mM were significantly ($* p < 0.05$) greater compared to other concentrations. B: Blockade of nociceptin responses. Microinjections of L-Glu (5 mM) into the right IML at T1–T2 elicited tachycardic responses ($n = 7$) (Fig. 1B,a). At the same site, microinjections of aCSF (30 nl) did not elicit any response (Fig. 1B,b). Microinjections of N/OFQ (0.62 mM) at the same site elicited a decrease in HR (Fig. 1B,c). After an interval of 30 min, microinjection of an NOP receptor antagonist, [N-phe¹]-nociceptin-(1–13)-NH₂ (9 mM) elicited no significant HR responses (Fig. 1B,d). An interval of about 5 min was allowed when N/OFQ (0.62 mM) was microinjected again at the same site; at this time, N/OFQ failed to elicit

bradycardic responses (Fig. 1B,e). The difference in N/OFQ-induced decreases in HR before (Fig. 1B,c) and after (Fig. 1B,e) the microinjection of the NOP receptor antagonist was highly significant (* $p < 0.001$). After an interval of 60 min, the bradycardic responses to microinjections of N/OFQ showed some recovery but it was incomplete (Fig. 1B,f).

Fig. 2

Fig. 2.

Tracing showing the N/OFQ response and its blockade. In all panels, top trace: pulsatile arterial pressure (PAP, mmHg); 2nd trace: mean arterial pressure (MAP; mmHg), 3rd trace: heart rate (HR; beats/min). A: Identification of the IML at T2; microinjection of L-Glu (5 mM) into the right IML elicited an increase in HR with no concomitant change in PAP and MAP. B: Five min later, microinjection of aCSF (30 nl) at the same site elicited no HR or BP responses. C: After an interval of 5 min, microinjection of N/OFQ (0.62 mM) at the same site decreased HR. D: An interval of 30 min was allowed after the HR returned to basal levels when an NOP receptor antagonist ([N-Phe¹]-nociceptin-(1–13)-NH₂; 9 mM), was microinjected at the same site; no significant changes in HR and BP were elicited. E: Five min later, microinjection of the same dose of N/OFQ failed to elicit a bradycardic response. F: Sixty min later, the bradycardic response to microinjection of N/OFQ (0.62 mM) at the same site showed some recovery but it was not complete.

Fig 3.

Blockade of N/OFQ responses by atenolol. Microinjections of L-Glu (5 mM) into the right IML at T1–T2 elicited tachycardic responses ($n = 5$) (Fig. 3A). Microinjections of N/OFQ (0.62 mM) at the same site elicited a decrease in HR (Fig. 3B). After an interval of 30 min, intravenous injection of atenolol (10 μ g/kg) elicited a decrease in HR (Fig. 3C). The bradycardic responses to subsequent microinjection of N/OFQ (0.62 mM) at the same site were almost completely blocked ($*p < 0.001$) (Fig. 3D). After an interval of 60 min, the bradycardic responses to microinjections of N/OFQ showed some recovery but it was incomplete (Fig. 3E).

Fig 4.

Drawings of coronal sections of the spinal cord showing microinjection sites as dark spots ($n = 10$); each spot represents the site of microinjection in one animal. The sites were located in the IML at T1 (A) and T2 (B) levels (5 sites at each level). The location of the sites was 0.8 – 1 mm lateral to midline and 0.8–1.1 mm deep from the dorsal spinal surface. Abbreviations: CC: central canal; IML: intermediolateral cell column of the spinal cord.