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CHRONIC INFUSION OF ANGIOTENSIN RECEPTOR ANTAGONISTS IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS PREVENTS HYPERTENSION IN A RAT MODEL OF SLEEP APNEA

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Abstract

Sleep apnea is characterized by increased sympathetic activity and is associated with systemic hypertension. Angiotensin (Ang) peptides have previously been shown to participate in the regulation of sympathetic tone and arterial pressure in the hypothalamic paraventricular nucleus (PVN) neurons. We investigated the role of endogenous Ang peptides within the PVN to control blood pressure in a rat model of sleep apnea-induced hypertension. Male Sprague Dawley rats (250g), instrumented with bilateral guide cannulae targeting the PVN, received chronic infusion of Ang antagonists (A-779, Ang-(1-7) antagonist; losartan and ZD7155, AT_1 antagonists; PD123319, AT_2 receptor antagonist, or saline vehicle). A separate group received an infusion of the $GABA_A$ receptor agonist (muscimol) to inhibit PVN neuronal activity independent of angiotensin receptors. After cannula placement, rats were exposed during their sleep period to eucapnic intermittent hypoxia (IH; nadir 5% O_2; 5% CO_2 to peak 21% O_2; 0% CO_2) 20 cycles/hour, 7 hours/day, for 14 days while mean arterial pressure (MAP) was measured by telemetry. In rats receiving saline, IH exposure significantly increased MAP (+12±2 mmHg vs Sham −2±1 mmHg $P<0.01$). Inhibition of PVN neurons with muscimol reversed the increase in MAP in IH rats (MUS: −9±4 mmHg vs vehicle +12±2 mmHg; $P<0.01$). Infusion of any of the Ang antagonists also prevented the rise in MAP induced by IH (A-779: −5±1 mmHg, losartan: −9±4 mmHg, ZD7155: −11±4 mmHg and PD123319: −4±3 mmHg; $P<0.01$). Our results suggest that endogenous Ang peptides acting in the PVN contribute to IH-induced increases in MAP observed in this rat model of sleep apnea-induced hypertension.

Classification terms

hypothalamic-pituitary-adrenal regulation; peptides: anatomy and physiology; cardiovascular regulation

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Keywords

sympathetic activity; intermittent hypoxia; hypertension; renin-angiotensin system; angiotensin-(1-7)

Introduction

Chronic intermittent hypoxia (CIH), a characteristic of human sleep apnea, leads to sustained elevation of sympathetic nerve activity (SNA) and mean arterial pressure (AP). Although the mechanisms by which CIH augments AP are incompletely understood, activation of the sympathetic nervous system seems to be essential for the maintenance of hypertension (Sica *et al.*, 2000; Fletcher, 2003; Prabhakar *et al.*, 2005). Indeed, several methods of sympathetic blockade, including carotid body denervation, renal sympathectomy, adrenal demedullation and sympathetic nerve ablation prevent the rise in AP due to CIH exposure (Fletcher *et al.*, 1999; Fletcher, 1992a).

The hypothalamic paraventricular nucleus (PVN) is an important site regulating arterial pressure and sympathetic activity (Dampney, 1994; Tagawa & Dampney, 1999;). Inhibition of PVN neurons with nitric oxide donor (Allen, 2002; Horn *et al.*, 1994; Stern *et al.*, 2003) or γ-aminobutyric acid (GABA) receptor agonist (Kannan *et al.*, 1989; Allen, 2002; Silva *et al.*, 2005), reduces renal SNA and systemic AP. Conversely, activation of PVN neurons by microinjection of an excitatory amino acid (Kannan *et al.*, 1989) or $GABA_A$-receptor antagonist, increases arterial pressure and sympathetic activity in anesthetized (Chen *et al.*, 2003; Chen & Toney, 2003) and conscious (Kannan *et al.*, 1989) rats. Several studies have suggested that augmented discharge of PVN neurons may be involved in the sustained sympathoexcitation in pathological conditions including congestive heart failure (Zucker *et al.*, 2004), myocardial infarction (Zhang *et al.*, 2001; 2002), salt-sensitive hypertension (Weiss *et al.*, 2007), and genetic hypertension (Allen, 2002).

Angiotensin (Ang) peptides act as important neuromodulators in brain sites controlling sympathetic output and arterial pressure (Dampney *et al.*, 2002; Veerasingham & Raizada, 2003). Specifically in the PVN, iontophoretic application of Ang II and Ang-(1-7), two peptides produced by the renin-angiotensin system (RAS) cascade, increase the firing rate of neurons by acting on specific receptors (Ambuhl *et al.*, 1994; Cato & Toney, 2005). We have previously reported that both Ang II and Ang-(1-7) contribute to the maintenance of renal SNA and that microinjections of these peptides into the PVN increase sympathetic tone (Silva *et al.*, 2005). In this study we investigated the contribution of Ang II and Ang-(1-7) receptors in the PVN to hypertension developed during fourteen days of IH exposure.

Results

IH exposure and $GABA_A$ inhibition

Animals were subdivided after histological analysis of cannulae placement: those with injection sites located within or on the borders of the PVN were the experimental group; animals with injection sites outside the PVN were analyzed as a "control" group. Table 1 shows baseline values of MAP and HR before IH or Sham exposure for all experimental groups.

IH exposure significantly increased MAP in vehicle-treated rats compared to vehicle-treated Sham rats (IH: +12±2 mmHg vs Sham: −2±1 mmHg; $P<0.01$; Figure 1).

Angiotensin receptor blockade

Chronic blockade of Ang(1-7) receptors with A-779 prevented an increase in MAP in the IH exposed rats so that MAP was not different from Sham exposed rats on any day (Figure 2A). Heart rate was also not different between groups (Figure 2B). The AT_2 receptor antagonist also prevented an increase in MAP in the IH exposed group but MAP still tended to be higher in the compared to Sham rats receiving PD123319 (Figure 2C). The AT_2 receptor blocker also tended to decrease HR and HR was significantly lower than during the control period on day 14 in both groups (Figure 2D). Figure 3 shows MAP and HR in Sham and IH rats receiving chronic infusion of the AT_1 receptor blockers, losartan (Figure 3A and 3B) or ZD7155 (Figure 3C and 3D). Both antagonists reduced MAP in the IH and Sham exposed rats compared to vehicle treated rats but did not change HR. In the rats receiving the AT_1 receptor blockers, MAP was not different between Sham and IH rats.

Similar to the AT_1 receptor antagonists, the $GABA_A$ receptor agonist, muscimol, prevented the increase in MAP and decreased HR in IH rats and tended to lower MAP in the Sham exposed rats so that MAP was not different between SHAM and IH rats treated with muscimol (Figure 4).

Figure 5 shows MAP and HR at day 7 and 14 in Sham and IH exposed rats receiving chronic infusion of saline, A-779, losartan, ZD7155 or PD123319 bilaterally into the PVN. IH exposure induced a gradual increase in MAP peaking at day 14, as shown in Figure 1. Chronic treatment with A-779 and ZD7155 lowered MAP compared to vehicle treatment at day 7 (A-779: −3±1 mmHg and ZD7155: −6±1 mmHg vs vehicle: +3±1 mmHg; $P<0.01$) and all angiotensin antagonists lowered MAP even more by day 14 completely preventing the increase in MAP observed in the vehicle group at day 14 (A-779: −5±1 mmHg, losartan: −9±4 mmHg, ZD7155: −11±4 mmHg and PD123319: −4±3 mmHg vs vehicle: +12±2 mmHg; $P<0.01$). HR did not change in any group, but ZD7155 infusion induced a significant increase in HR compared to the other angiotensin antagonists (ZD7155: +21±9 bpm vs A-779: −14±6 bpm, losartan: −12±8 bpm and PD123319: −18±4 bpm; $P<0.01$; at day 14).

Angiotensin antagonist infusion into sites completely outside PVN borders had no effect on the IH exposure-induced rise in MAP or in HR demonstrating that the effects of Ang blockade are due to antagonist-receptor interactions within the PVN (Supplemental Figure 1A). Examples of the injection sites considered within and out of the PVN are illustrated in Supplemental Figure 1B

The activity data for the two groups indicated a tendency for all of the IH exposed rats independent of antagonist treatment to move less during the dark hours (awake) and more during the light hours (sleep/exposure time) but due to the between animal variability, there were no significant differences in activity between groups or within groups over time (data not shown). Therefore the differences in MAP and HR between groups do not appear to be due to different levels of activity.

Discussion

The main findings in this study were 1) PVN neurons appear to modulate IH-induced increases in AP in rats; 2) angiotensin-(1-7) receptors along with both AT_1 and AT_2 angiotensin II receptors are necessary for this PVN-dependent increase in AP; 3) both AT_1 and AT_2 receptors appear to regulate AP in Sham rats while Ang(1-7) receptors only contribute to AP control after IH.

Furthermore, the effect of muscimol to lower AP and HR in Sham as well as in IH rats reflects that PVN neurons are normally active and control AP (Decavel & Van den Pol, 1990; Allen, 2002; Silva *et al.*, 2005). This also indicates that $GABA_A$ mediated inhibition is functional following IH exposure. Thus the increased AP following IH is more likely due to exaggerated excitatory mechanisms in the PVN rather than to loss of $GABA_A$ receptor function.

Sleep apnea is characterized by recurrent brief cessations in breathing during sleep which repeatedly reduces arterial blood oxygen saturation (hypoxia) accompanied by small increases in circulating CO_2 (Wolk *et al.*, 2003; Prabhakar *et al.*, 2007). Although hypoxia is thought to be the primary stimulus for inducing hypertension in sleep apnea patients and IH models, combining hypoxia with hypercapnia more profoundly stimulates the sympathetic nervous system (Morgan *et al.*, 1995; Fletcher, 2001; Xie *et al.*, 2001; Cutler *et al.*, 2004). Cutler et al showed that sympathetic activation stimulated by hypoxia combined with either hypercapnia or eucapnia increased sympathetic activity more than hypocapnic hypoxia (Cutler *et al.*, 2004). Our model of IH similarly combined increased inhaled CO_2 during hypoxia periods to maintain eucapnia in the face of the hypoxia-induced hyperventilation (Snow *et al.*, 2008) to more closely mimic the changes in arterial gases and sympathetic activation experienced in sleep apnea.

The mechanisms by which IH exposure leads to hypertension are not completely understood, but activation of the sympathetic nervous system appears necessary (Fletcher *et al.*, 1992a; Fletcher *et al.*, 1992b; Fletcher, 2003). Several studies have suggested hypoxia stimulation of carotid body chemoreceptors (Smith *et al.*, 1996; Kara *et al.*, 2003; Narkiewicz & Somers, 2003) leads to sustained reflex sympathetic activation (Narkiewicz & Somers, 2003; Prabhakar *et al.*, 2007) with eventual resetting of sympathetic tone and arterial pressure to higher set points (Narkiewicz *et al.*, 1998). This is supported by the observation that IH leads to sensitization and long-term facilitation of the carotid body responses to hypoxia (Peng *et al.*, 2003) and enhanced sympatho-excitatory responses to chemoreflex activation after IH exposure (Greenberg *et al.*, 1999; Braga *et al.*, 2006; Dick *et al.*, 2007).

The hypothalamic PVN is one of the major premotor neuron cell groups contributing to sympathetic control of the cardiovascular system and arterial pressure (Dampney, 1994). The PVN modulates SNA and AP responses to chemoreflex activation through both direct projections to the sympathetic preganglionic neurons in the spinal cord and via indirect synaptic relays with the rostral ventrolateral medulla (RVLM) and connections with the nucleus tractus solitarii (NTS) (Badoer, 2001; Olivan *et al.*, 2001). Olivan et al demonstrated that bilateral electrolytic lesion of the PVN attenuated pressor responses to chemoreflex activation in awake rats (Olivan *et al.*, 2001). In addition, blockade of neurotransmission in the PVN in anesthetized rats attenuated renal and phrenic nerve responses to peripheral chemoreflex activation (Reddy *et al.*, 2005) while peripheral chemoreflex activation increased Fos expression in PVN neurons (Cruz *et al.*, 2008). Thus the PVN appears to be an integral part of the central neuronal circuitry processing the sympathoexcitatory component of the peripheral chemoreflex (Olivan *et al.*, 2001; Reddy *et al.*, 2005; Cruz *et al.*, 2008).

Previous studies indicate RAS peptides importantly modulate sympathetic output and that a dysfunctional brain RAS participates in the pathogenesis of some forms of hypertension (Dampney *et al.*, 2002; Veerasingham & Raizada, 2003). Immunocytochemical studies have identified both Ang II (Egli *et al.*, 2000) and Ang-(1-7) (Block *et al.*, 1988), as well as AT_1, AT_2 (Wright & Harding, 1994; Allen *et al.*, 1998; Shelat *et al.*, 1998; McKinley *et al.*, 2003) and Mas receptors (Becker *et al.*, 2007) in the PVN. Iontophoretic application of Ang II or

Ang-(1-7) has also been shown to increase firing in PVN neurons (Ambuhl *et al.*, 1994; Becker *et al.*, 2007) and we reported previously that Ang II and Ang-(1-7) administered into the PVN increase RSNA and AP (Silva *et al.*, 2005). Others have shown that AT_1 receptors in the PVN regulate both baseline AP (Zhu *et al.*, 2002; Silva *et al.*, 2005) and the augmented AP and RSNA observed during central hyperosmolar stimulation (Chen & Toney, 2001) or when PVN neurons are disinhibited (Chen & Toney, 2003). Thus angiotensin receptors appear to modulate PVN neuronal activity under basal and excited conditions.

In pathophysiological states with elevated sympathetic discharge from the PVN such as congestive heart failure (Shelat *et al.*, 1998; Zhang *et al.*, 2001; 2002; Zucker *et al.*, 2004) and hypertension (Weiss *et al.*, 2007; Allen, 2002), both overexpression of AT_1 receptors (Zucker, 2002; Zucker *et al.*, 2004) and downregulation of inhibitory nNOS (Zhang *et al.*, 2001) and $GABA_A$ (Zhang *et al.*, 2002) pathways have been reported. Very recently, IH exposure was shown to be associated with reduced nNOS expression in the PVN and augmented AT_1 expression in circumventricular organ (CVO) neurons projecting to the PVN (Weiss *et al.*, 2007). The incomplete blood-brain barrier of the CVO (Wright & Harding, 1994) would allow PVN neurons to be stimulated during IH by Ang peptides produced locally or delivered by neuronal projections from the CVO which are stimulated by the cyclic hypoxia (Weiss *et al.*, 2007).

In this study, we observed that the Ang-(1-7) receptor blocker did not affect AP in Sham rats while AT_1 receptor antagonists in the PVN significantly reduced and the AT_2 receptor antagonist tended to reduce AP in Sham rats. These findings corroborate previous demonstrations that AT_1 receptors in the PVN contribute to AP control under normal conditions through tonic activation (Zhu *et al.*, 2002; Silva *et al.*, 2005) and further suggesting that AT_2 receptors in the PVN may also play a role in tonic AP control.

AT_1 and AT_2 receptors are 34% homologous and have high affinity for Ang II (Stoll & Unger, 2001). Although most brain areas express only one subtype, a few regions, including the PVN, express both (McKinley *et al.*, 2003). The PVN has a predominance of AT_1 receptors, with especially high densities in the cardiovascular and sympathetic regulatory parvicellular area (Blair *et al.*, 1996; Allen *et al.*, 1998; Badoer, 2001; McKinley *et al.*, 2003). Thus cardiovascular actions exerted by Ang II in this region may be mostly mediated by AT_1 receptors but this study and our previous work (Silva *et al.*, 2005) suggest AT_2 receptors in the PVN can also mediate a significant portion of Ang II effects slightly different from those exerted by AT_1 receptors as shown by the greater fall in AP at day 14 in the Sham rats receiving the AT_1 receptor antagonist.

Strong staining for Mas in both parvi and magnocellular portions of the PVN (Becker *et al.*, 2007), are consistent with Ang-(1-7) receptors also regulating sympathetic tone originating in the PVN. Additionally, direct activation of Ang-(1-7) receptors in the PVN increases the firing rate of PVN neurons (Santos *et al.*, 2003). Thus the effects on arterial pressure of the different antagonists may have occurred through blockade of unique receptors which each separately contribute to AP regulation. Alternatively there may be functional interactions between the receptors as shown in previous demonstrations of interactions between these receptor subtypes. For example, it has been demonstrated that the excitatory effects produced by Ang II, Ang III and Ang-(1-7) in the PVN can be blocked by an AT_2 receptor antagonist (Felix et al., 1991). In addition, the excitatory effects produced by Ang-(1-7) in PVN neurons can be blocked by AT_1 or AT_2 receptor antagonists (Ambuhl et al., 1992) and a functional interaction between Ang-(1-7) and AT_1 receptors has been demonstrated in other brain regions (Von Bohlen und Halbach et al., 2000). Thus the simplest explanation for the current findings seems to be that following 14 days of IH exposure, multiple

angiotensin receptors on PVN neurons contribute to the vasomotor response in conscious rats.

Previous studies have demonstrated that the Ang peptides play an excitatory role in the PVN (Ambuhl *et al.*, 1994; Cato & Toney, 2005). Although acute blockade of Ang-(1-7) and AT_2 receptors in normotensive anesthetized rats dramatically reduced renal SNA in a previous study, it did not affect AP (Silva *et al.*, 2005). In the present study, AP did not change significantly in Sham animals receiving A-779 or PD123319, but both antagonists prevented AP increases in the IH rats and the AT_2 antagonist tended to decrease AP in the Sham rats below that in the IH rats throughout the exposure period suggesting that acute and chronic effects of Mas and AT_2 receptor activation may differ. In a previous study in anesthetized rats, blockade of AT_1 receptors in the RVLM increased sympathetic activity under hypoxic conditions (Sheriff *et al.*, 2006) and Ang-(1-7) attenuated the pressor effect of Ang II in the anterior hypothalamus (Hocht *et al.*, 2006). Our results in conscious rats suggesting an excitatory role for both Ang II and Ang-(1-7) in the PVN following IH suggest the effect of central angiotensins on sympathetic activity might depend upon the balance between tonic excitatory and inhibitory effects on sympathetic premotor neurons (Dampney *et al.*, 2007). This balance may be altered in pathophysiological conditions such as chronic IH since only blockade of AT_1 receptors decreased AP in Sham animals and IH rats below baseline. Together, these observations suggest that under normal conditions, AT_1 and perhaps AT_2 receptors mediate tonic excitation in PVN neurons to modulate AP. However, after IH exposure, both Ang-(1-7) and Ang II receptors in the PVN participate in generating the increased AP. Although only direct measures of sympathetic activity can confirm the suggested changes in Ang regulation of sympathetic discharges following exposure to IH, the differential effects of the antagonists combined with previous studies (Zhu *et al.*, 2002; Silva *et al.*, 2005) suggest that the different angiotensin receptor types all regulate AP but through different mechanisms and under different conditions.

Perspectives

Our results suggest sympathetic premotor neurons in the PVN importantly mediate increases in AP induced by IH in rats. Although other mechanisms may be recruited to participate in the systemic pressor response, activation of angiotensin receptors in the PVN appears necessary for increased AP in this rat model of simulated sleep apnea. Clinical studies also suggest the RAS is activated in sleep apnea. Therefore, the ability of the Ang-(1-7) antagonist to lower arterial pressure in the IH rats and not in the Sham rats suggests this might be an important target for targeted pharmacological interventions.

Experimental Procedures

Experiments were performed in male Sprague-Dawley rats (250 to 300 g, Harlan Sprague Dawley). All animal protocols were reviewed and approved by the institutional animal care and use committee of the University of New Mexico Health Sciences Center and conform to National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Telemeter implantation and PVN cannula placement

Rats were instrumented with blood pressure telemetry devices (DSI model PA-C40, Arden Hills, MN) under isofluorane (2%) anesthesia. The catheter portion was inserted into the abdominal aorta through the femoral artery and the transmitter fixed in the peritoneal cavity, allowing 24 hour recordings of mean arterial pressure (MAP) and heart rate (HR).

After 5 days of recovery, two 30-gauge microinjection needles connected to a primed osmotic minipump (Alzet model 2002) were inserted bilaterally into the paraventricular

nucleus (PVN) using a stereotaxic apparatus and cemented into place. The coordinates used were 1.8 mm posterior and 0.5 mm lateral to the bregma, at a depth of 7.8 mm below dura (Silva *et al.*, 2005). Rats were allowed to recover for 2 more days, before the initiation of exposure protocols.

Eucapnic Intermittent Hypoxia Protocol

Rats were housed in Plexiglas boxes with free access to food and water and exposed to either intermittent hypoxia with CO_2 supplementation to maintain eucapnia (IH; nadir 5% O_2; 5% CO_2 to peak 21% O_2; 0% CO_2 every 90 seconds) or room air (Sham; same environmental conditions as IH, however rats were constantly maintained at 21% O_2; 0% CO_2). The gas composition inside the chambers was controlled by timed solenoid switches operating 7 hr/day during the rats' sleep period for 14 days as described previously (Allahdadi *et al.*, 2005). The chosen frequency of apneas was used to mimic moderate sleep apnea, and the reduction in breathing oxygen levels was sufficient to induce hypoxia without promoting arousal after acclimatization. During the 14 days of IH exposure, MAP and HR were recorded continuously and osmotic minipumps delivered either vehicle or drugs bilaterally into the PVN (0.5 µl/hr). The drugs administered were: A-779 (Ang-(1-7) antagonist, 2 nmol/hr); losartan (AT_1 receptor antagonist, 2 nmol/hr, a kind gift from Dr. Robson Santos); ZD7155 (AT_1 receptor antagonist, 0.2 nmol/hr); PD123319 (AT_2 receptor antagonist, 2 nmol/hr).The doses of drugs administered were based on the ability of the same doses to acutely lower either blood pressure or renal sympathetic nerve activity in similarly prepared anaesthetized rats (Silva *et al.*, 2005). Additionally, we compared the effects of Ang antagonists with those evoked by muscimol (20 pmol/hr), a powerful $GABA_A$ agonist known to reduce MAP when injected acutely into the PVN. NaCl 0.9 % was used as vehicle since all administered drugs were prepared in saline solution. At the end of all protocols, rats were deeply anesthetized with sodium pentobarbital (150 mg/kg i.p.) and brains fixed (paraformaldeheyde 4% and sucrose 30%) to stain for histological identification of injection sites, as described previously (Silva *et al.*, 2005) (Supplemental Figure 1).

Statistical Analysis

All data are expressed as mean ± SEM. MAP and HR values are expressed as the average of a 12 hour period to monitor diurnal variation in AP. Additional data expressing 24 hour average is shown in Figure 5. Differences between groups were evaluated using two-way repeated measures ANOVA followed by Bonferroni post-hoc test. Differences were considered significant for $P<0.05$.

Research Highlights

- Blocking AT_1-, AT_2- or mas-receptors in the PVN prevents IH-induced hypertension
- Blocking AT_1- but not mas-receptors in the PVN lowered blood pressure in Sham rats
- Mas-receptors only appear to regulate blood pressure in pathological conditions
- Angiotensin receptors in the PVN are necessary for IH to increase arterial pressure

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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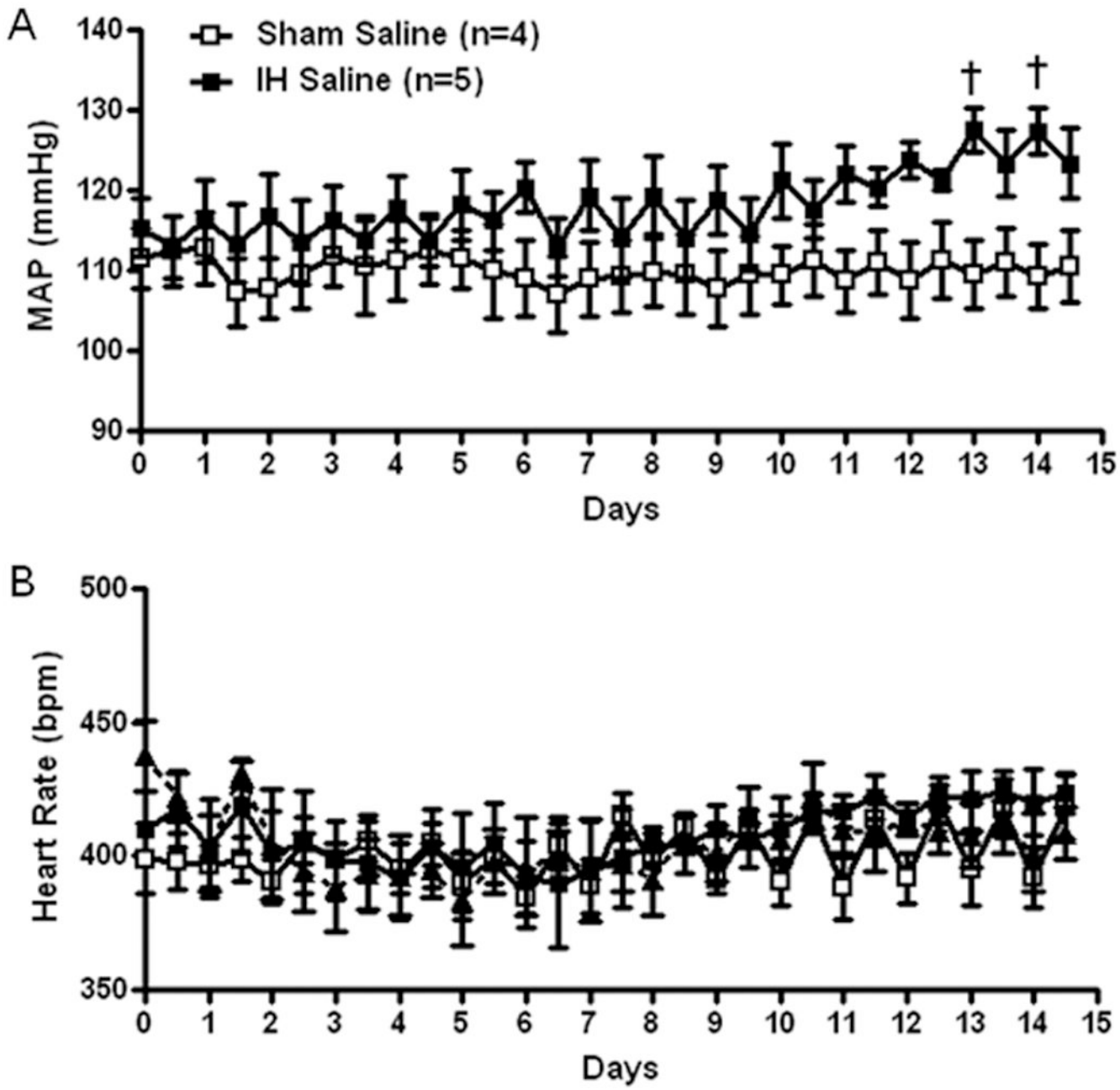

Figure 1.

Grouped data showing 12 hour average recordings of mean arterial pressure (MAP, A) and heart rate (B) in Sham and IH exposed rats, chronically infused with saline (200 nL/hr) into the PVN. Daytime MAP and heart rate correspond to X-axis whole numbers while night time MAP and heart rate corresponds to values plotted between numbers.

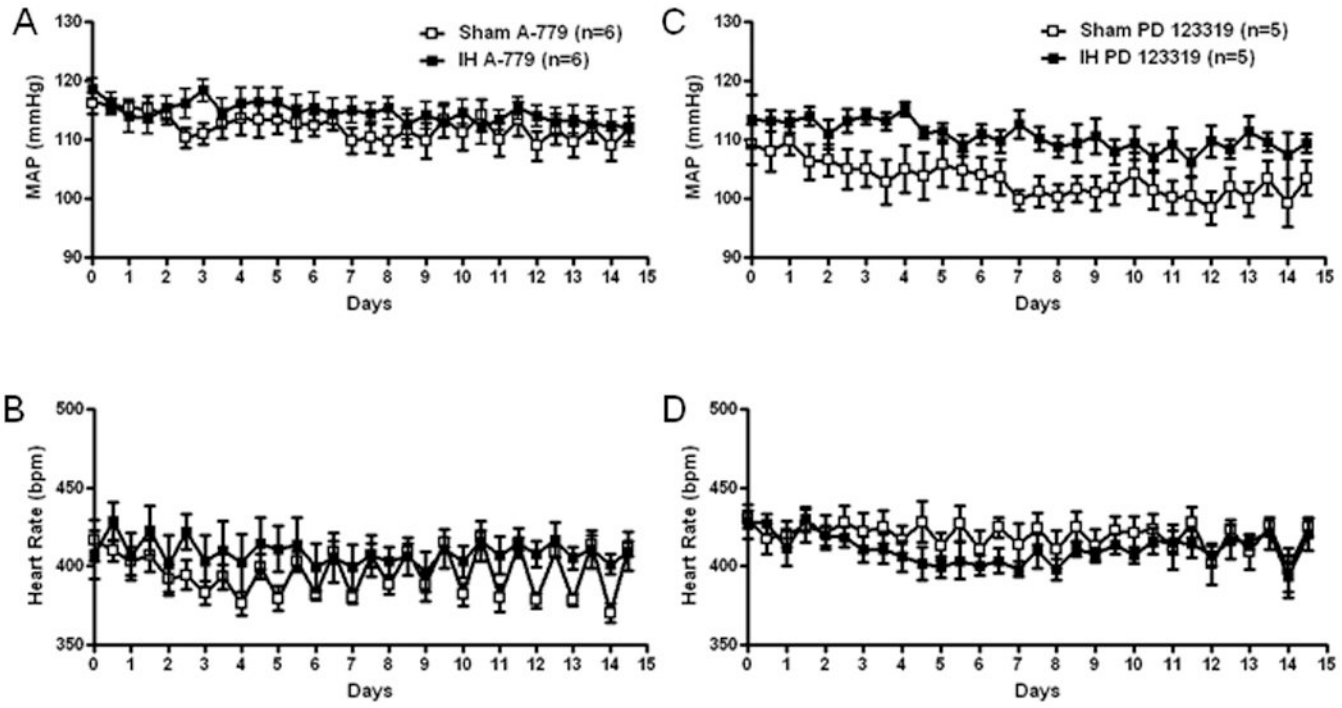

Figure 2.

Grouped data showing 12 hour average recordings of mean arterial pressure (MAP) and heart rate (HR) in Sham and IH exposed rats chronically infused with A-779 (A and B, 2 nmol/h) or PD 123319 (C and D, 2 nmol/h) into the PVN. Daytime MAP (A and C) and heart rate (B and D) correspond to X-axis whole numbers while nighttime MAP and heart rate correspond to values plotted between numbers.

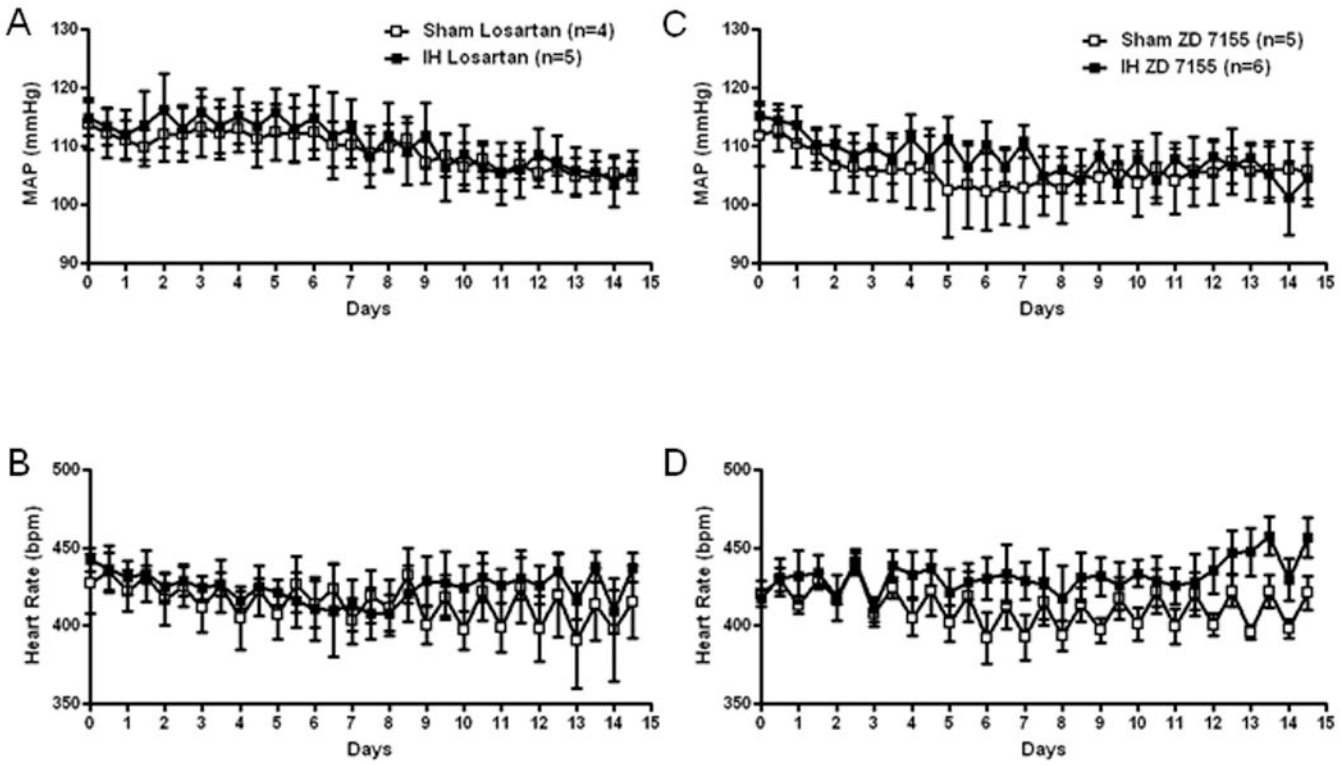

Figure 3.

Grouped data showing 12 hour average recordings of mean arterial pressure (MAP, A and C) and heart rate (B and D) in Sham and IH exposed rats chronically infused with losartan (A and B, 2 nmol/h) or ZD 7155 (C and D.0.2 nmol/h) into the PVN. Daytime MAP and heart rate correspond to X-axis whole numbers while nighttime MAP and heart rate corresponds to values plotted between numbers.

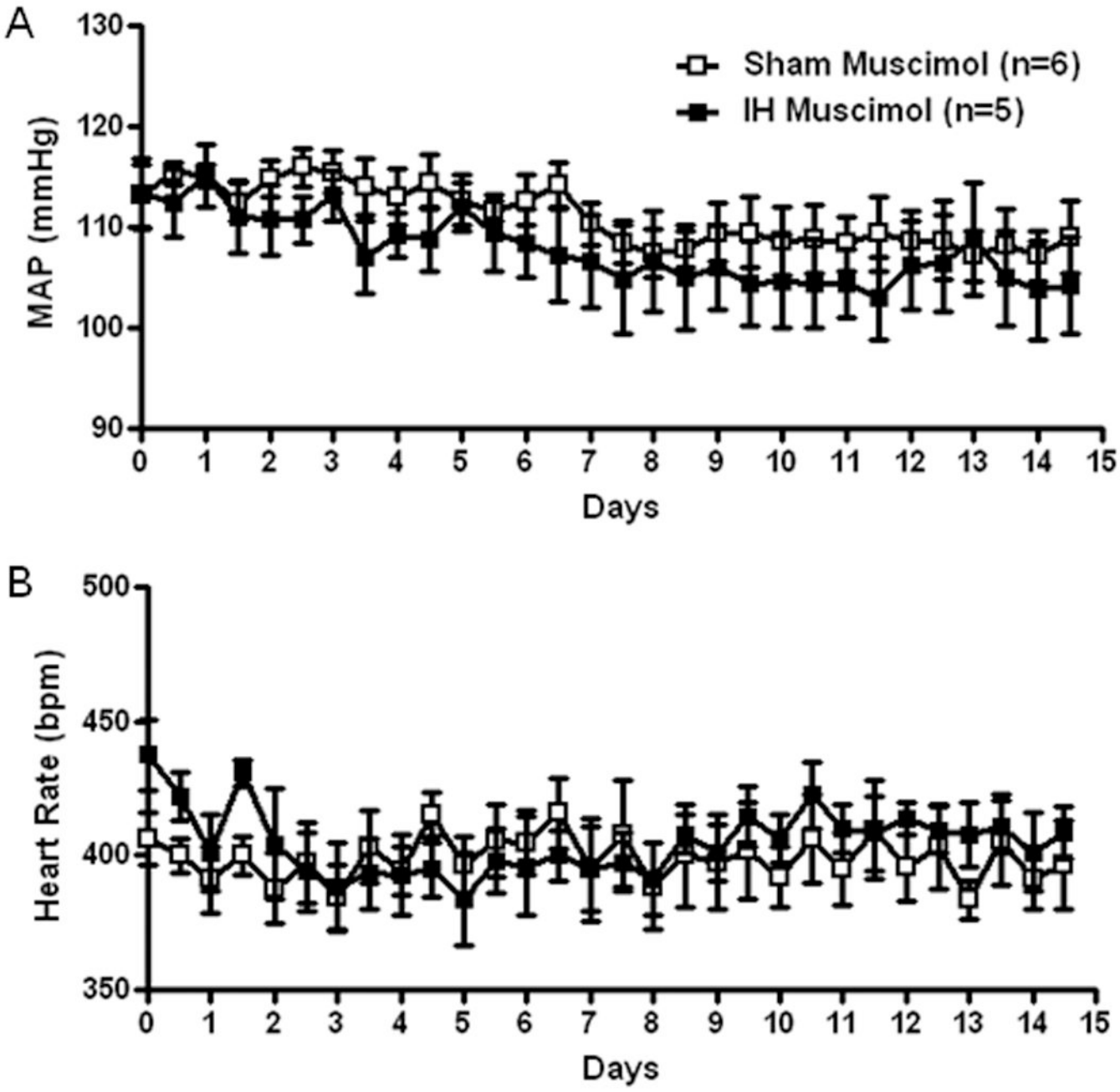

Figure 4.

Grouped data showing 12 hour average recordings of mean arterial pressure (MAP, A) and heart rate (B) in Sham and IH exposed rats chronically infused with muscimol (20 pmol/h) into the PVN. Daytime MAP and heart rate correspond to X-axis whole numbers while nighttime MAP and heart rate corresponds to values plotted between numbers.

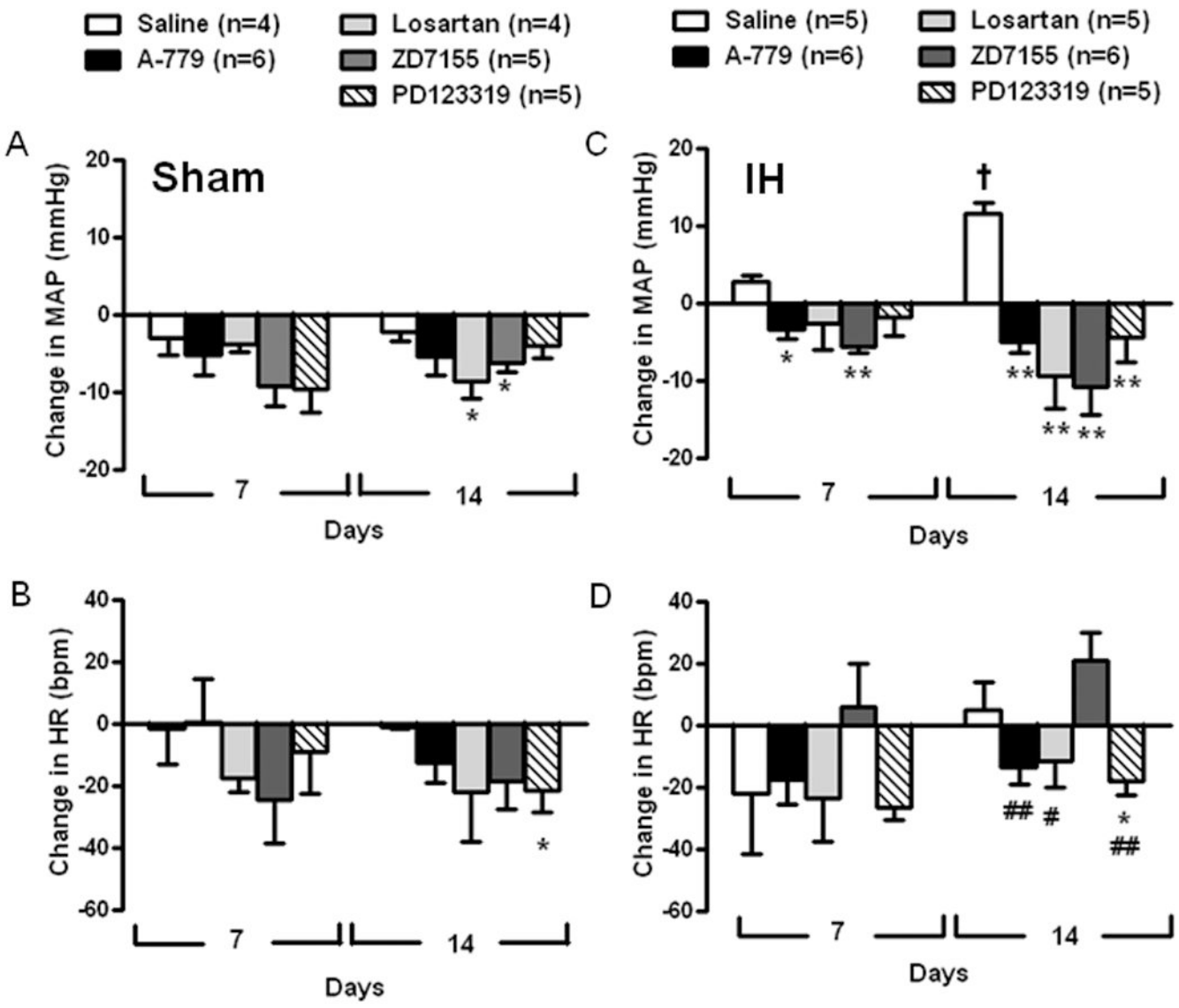

Figure 5.

Grouped data showing the change in mean arterial pressure (MAP) and heart rate (HR) on days 7 and 14 of Sham exposed rats (A and B) and IH exposed rats (C and D) chronically infused with saline (200 nL/h), A-779 (2 nmol/h), losartan (2 nmol/h), ZD7155 (0.2 nmol/h) or PD123319 (2 nmol/h) into the PVN. Values are expressed as the averages of the change from starting MAP for each rat. **P*<0.05 and ***P*<0.01 compared to saline; #*P*<0.05 and ##*P*<0.01 compared to IH ZD7155; †*P*<0.01 for IH saline on day 14 compared to day 7.

Table 1

Basal values for MAP and HR before IH or sham xposure with drug infusion into the PVN.

Experimental Group	n	MAP (mmHg)	HR (bpm)
Sham Saline	4	111 ± 4	400 ± 11
IH Saline	5	113 ± 4	415 ± 4
Sham A-779	6	116 ± 1	408 ± 10
IH A-779	6	118 ± 2	419 ± 12
Sham Losartan	4	113 ± 4	431 ± 16
IH Losartan	5	115 ± 3	437 ± 8
Sham ZD7155	5	112 ± 4	427 ± 7
IH ZD7155	6	114 ± 3	423 ± 3
Sham PD123319	6	106 ± 4	428 ± 6
IH PD123319	5	113 ± 4	428 ± 9
IH Muscimol	5	113 ± 3	433 ± 7

Values are expressed as mean ± SEM.