Research Paper

Melanocortin 3/4 receptors in paraventricular nucleus modulate sympathetic outflow and blood pressure

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New findings

- **What is the central question of this study?**
  The paraventricular nucleus (PVN) is important in regulating sympathetic activity and blood pressure. This study addresses whether activation of melanocortin 3/4 receptors in the PVN modulates sympathetic activity and blood pressure, and whether intracellular signalling involves the cAMP–protein kinase A pathway.
- **What is the main finding and its importance?**
  Activation of melanocortin 3/4 receptors in the PVN increases sympathetic outflow and blood pressure via the cAMP–protein kinase A pathway. Melanocortin 3 receptors in the PVN exert a tonic excitatory effect on sympathetic activity. Melanocortin 3/4 receptors in the PVN are a putative therapeutic target to inhibit sympathetic activity.

Central melanocortin 3/4 receptors (MC3/4Rs) are known to regulate energy balance. Activation of MC3/4Rs causes a greater increase in the firing activity of the PVN neurons in obese Zucker rats than in lean Zucker rats. The present study was undertaken to determine the roles of MC3/4Rs in the hypothalamic paraventricular nucleus (PVN) in modulating the sympathetic activity and blood pressure and its downstream pathway. Renal sympathetic nerve activity (RSNA) and mean arterial pressure (MAP) were recorded in anaesthetized rats. Microinjection of the MC3/4R agonist melanotan II (MTII) into the PVN increased the RSNA and MAP. The MC3/4R antagonist agouti-related peptide (AgRP) or SHU9119 decreased the RSNA and MAP, but the MC4R antagonist HS024 had no significant effect on the RSNA and MAP. The effects of MTII were abolished by pretreatment of the PVN with AgRP, SHU9119, the adenylate cyclase inhibitor SQ22536 or the protein kinase A inhibitor Rp-cAMP, and substantially attenuated by HS024. Microinjection of SQ22536 alone into the PVN had no significant effect on the RSNA and MAP, but Rp-cAMP caused significant decreases in the RSNA and MAP. Furthermore, MTII increased the cAMP level in the PVN. These results indicate that activation of MC3/4Rs in the PVN increases the sympathetic outflow and blood pressure via the cAMP–protein kinase A pathway. Melanocortin 3 receptors in the PVN may exert a tonic excitatory effect on sympathetic activity.

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Melanocortins (MCs) are peptides derived by proteolytic cleavage from pro-opiomelanocortin, and include α-, β- and γ-melanocyte-stimulating hormones (MSHs) and ACTH. Five types of melanocortin receptors (MCRs) have been identified, of which MC3R and MC4R are predominantly expressed in the brain, while MC1R, MC2R and MC5R are in the peripheral tissues. Moderate MC3R and high MC4R expressions are found in the

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hypothalamic paraventricular nucleus (PVN; Roselli-Rehfuss *et al.* 1993; Mountjoy *et al.* 1994; Siljee-Wong, 2011). Melanocortin neurons in the PVN exert a tonic inhibition of feeding behaviour (Giraudo *et al.* 1998).

Intracerebroventricular administration of the MC3/4R agonist melanotan II (MTII) produced a dose-dependent sympathetic activation affecting renal and lumbar beds and brown adipose tissue, which was blocked by an MC3/4R antagonist SHU9119 (Haynes *et al.* 1999). Chronic intracerebroventricular infusion of SHU9119 reduced heart rate (HR) in both normotensive rats and spontaneously hypertensive rats, while lowering mean arterial pressure (MAP) to a greater extent in spontaneously hypertensive rats than in normotensive rats (da Silva *et al.* 2008). Obese subjects with MC4R impairment due to functional *MC4R* mutations showed an inverse relationship between obesity and muscle sympathetic nerve activity, suggesting that central sympathetic outflow to the vasculature might depend on functional melanocortinergic pathways (Sayk *et al.* 2010).

The PVN is an important integrative site in the control of sympathetic outflow and cardiovascular activity via its projections to the rostral ventrolateral medulla and the intermediolateral column of spinal cord (Li *et al.* 2006*a*,*b*; Ferguson *et al.* 2008; Kc & Dick, 2010). It has been found that the PVN regulates cardiovascular reflexes, such as the baroreflex (Rossi *et al.* 2010; Crestani *et al.* 2010), the chemoreflex (Olivan *et al.* 2001), the cardiac sympathetic afferent reflex (Xu *et al.* 2011*a*,*b*) and the adipose afferent reflex (Shi *et al.* 2012). The PVN is involved in long-lasting excessive sympathetic activation, which participates in the pathogenesis of hypertension and the progression of organ damage (Fisher & Fadel 2010; Grassi *et al.* 2010). However, it is unknown whether the MC3/4Rs in the PVN are involved in the regulation of sympathetic activity and blood pressure.

It has been reported that the MC3/4R agonist MTII in the nucleus tractus solitarii rapidly increases the phosphorylation of both extracellular signal-regulated kinase 1/2 and cAMP response element-binding protein in a dose-dependent manner (Sutton *et al.* 2005). Activation of the MC4R induces expression of brain-derived neurotrophic factor in rat cultured astrocytes through the cAMP–protein kinase A (PKA) pathway (Caruso *et al.* 2012). However, the melanocortin signalling pathway involved in the sympathetic activation is not well understood. The present study was designed to determine the roles of the MC3/4R in the PVN in the regulation of sympathetic outflow and blood pressure and its downstream signalling pathway.

Methods

Experiments were carried out on male Sprague–Dawley rats weighing between 350 and 400 g. The procedures were approved by the Experimental Animal Care and Use Committee of Nanjing Medical University and complied with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996). Each rat was anaesthetized with urethane (800 mg kg^{-1}, I.P.) and α-chloralose (40 mg kg^{-1}, I.P.). Supplemental doses of anaesthetic agents were administered intravenously to maintain an adequate depth of anaesthesia during the experiment. Adequate depth of anesthesia was assessed by the absence of corneal reflexes and paw withdrawal response to a noxious pinch. Supplemental doses of anaesthetic agents were administered at one-tenth of the initial dose when necessary. The rats were ventilated with room air using a rodent ventilator (683; Harvard Apparatus Inc., Holliston, MA, USA). The right carotid artery was cannulated for continuous recording of MAP and HR.

Recording of renal sympathetic afferent activity

A retroperitoneal incision was made and the left renal sympathetic nerve isolated. The nerve was cut distally to eliminate its afferent activity and placed on a pair of silver electrodes immersed in warm mineral oil. Renal sympathetic afferent activity (RSNA) was amplified with an AC/DC differential amplifier (3000; A-M Systems Inc., Sequim, WA, USA) with a low-frequency cut-off at 60 Hz and a high-frequency cut-off at 3000 Hz, and integrated at a time constant of 1.0 s. The raw RSNA, integrated RSNA, MAP and HR were simultaneously recorded on a PowerLab data acquisition system (8SP; ADInstruments, Bella Vista, NSW, Australia). The background noise level was determined after section of the central end of the nerve, and was subtracted from the RSNA value (Chen *et al.* 2011).

Microinjection into PVN

The PVN co-ordinates were 1.8 mm caudal to bregma, 0.4 mm lateral to the mid-line and 7.9 mm ventral to the dorsal surface. The bilateral PVN microinjections were carried out with two glass micropipettes (about 50 μm tip diameter) and completed within 1 min (50 nl for each side of the PVN). At the end of the experiment, the same volume of Evans Blue (2%) was injected into the microinjection site for histological identification in most rats except the rats used for the cAMP measurement to avoid disturbing the results of enzyme-linked immunosorbent assay. The rat was excluded from data analysis if the distance between the centre point of microinjection and the boundary of the PVN was less than 0.15 mm (Chen *et al.* 2011).

Measurement of cAMP level in PVN

The rats were killed with an overdose of pentobarbital (100 mg kg^{-1}, I.V.). The brains were removed and quickly frozen in liquid nitrogen and stored at −70°C until they were sectioned. Coronal sections of the brain were made with a cryostat microtome (Leica CM1900-1-1; Wetzlar, Hessen, Germany) at the PVN level, which was determined on the coronal sections according to the atlas of Paxinos & Watson, (2005). The thickness of the PVN sections used for cAMP analysis was 450 μm. The PVN areas were punched out with a 15-gauge needle (inner diameter 1.5 mm). The punched tissues were homogenized in lysis buffer. The total protein in the homogenate was extracted and measured using a protein assay kit (BCA; Pierce, Santa Cruz, CA, USA). The cAMP levels of the PVN were determined by an enzyme immunoassay kit (R&D Systems Inc., Minneapolis, MN, USA) following the manufacturer's instructions (Caruso *et al.* 2012).

Chemicals

Melanotan II (MTII; an MC3/4R agonist), Ac-Nle-c(Asp-His-D-2-Nal-Arg-Trp-Lys)-NH_2 (SHU9119; an MC3/4R antagonist) and cyclic (AcCys^3,Nle^4,Arg^5,D-Nal^7,Cys-NH_2^{11}) α-MSH-(3–11) (HS024; a selective MC4R antagonist) were purchased from Bachem (Bubendorf, Switzerland). Agouti-related protein (83–132)-amide (AgRP; an MC3/4R antagonist) was obtained from Phoenix Pharmaceuticals Inc. (Burlingame, CA, USA). Dibutyryl-cAMP (db-cAMP; a cAMP analogue), 9-(tetrahydro-2-furanyl)-9H-purin- 6-amine (SQ22536; an adenylyl cyclase inhibitor) and rp-adenosine-3′,5′-cyclic monophosphothionate (Rp-cAMP; a PKA inhibitor) were purchased from Sigma Chemical Co. (St Louis, MO, USA). The chemicals were dissolved in normal saline. The doses were selected according to previous reports (Ludvig & Moshe, 1989; Giraudo *et al.* 1998; Kask *et al.* 1998; Datta & Prutzman 2005; Singru *et al.* 2007; Dar, 2011) and our preliminary studies.

Experimental protocols

The rats were randomly divided into 12 groups ($n = 6$ for each). The PVN microinjections were carried out for administration of saline, three doses of MTII (0.05, 0.1 or 0.2 nmol), AgRP (0.4 nmol), SHU9119 (0.4 nmol), HS024 (1 nmol), db-cAMP (1 nmol), SQ22536 (2 nmol), Rp-cAMP (1 nmol) or MTII (0.2 nmol) pretreated with AgRP, SHU9119, HS024, SQ22536 or Rp-cAMP. Melanotan II was administered 8 min after the pretreatment.

To exclude the possibility that the dose of HS024 was not high enough to block the MC4R completely, the effects of three doses of HS024 (0.2, 1.0 and 5.0 nmol) were determined ($n = 6$ for each group). Melanotan II was administered 8 min after saline or HS024 pretreatment.

In two additional groups of rats ($n = 6$ for each), the samples were quickly collected for the measurement of cAMP level in the PVN 8 min after the PVN microinjection of saline or MTII. In addition, microinjection of a high dose of MTII (0.2 nmol) into the anterior hypothalamic area, which is adjacent to the PVN, was carried out to exclude the possibility that the effects were caused by diffusion to this site ($n = 6$).

Statistical analysis

The RSNA and MAP values were determined by averaging 2 min of the maximal responses and compared with the values before the microinjection. For the rats in pretreatment groups, the RSNA and MAP changes induced by MTII were determined by averaging 2 min of the maximal responses to MTII and compared with the values before MTII. All data are expressed as means ± SEM. Comparisons between two observations in the same rats were assessed by Student's paired *t* test. One-way or two-way ANOVA was used, followed by Bonferroni test for *post hoc* analysis when multiple comparisons were made. Correlation analysis was performed using a linear regression to determine the correlation between the dose of MTII and the changes in RSNA and MAP. A value of $P < 0.05$ was considered statistically significant.

Results

Dose and time effects of MC3/4R agonist

Microinjection of three doses of the MC3/4R agonist MTII into the PVN significantly increased the RSNA and MAP in a dose-dependent manner. There was a significant positive correlation between the dose of MTII and the changes in RSNA and MAP (Fig. 1*A*). The responses to the high dose of MTII reached their maximum within 10 min and lasted at least 30 min (Fig. 1*B*). Melanotan II had no significant effect on the HR. Representative recordings show that the PVN microinjection of a high dose of MTII increased the RSNA and MAP (Fig. 2). In six rats, microinjection of the same dose of MTII into the anterior hypothalamic area, which is adjacent to the PVN, had no significant effects on the RSNA (+0.9 ± 0.5%, $P = 0.531$) and MAP (−0.1 ± 0.4 mmHg, $P = 0.868$).

Effects of MC3/4R antagonist

Microinjection of the MC3/4R antagonist AgRP or SHU9119 into the PVN significantly decreased the RSNA

and MAP, while the selective MC4R antagonist HS024 had no significant effect on the RSNA and MAP (Fig. 3). In the rats subjected to the microinjection of MTII, pretreatment with AgRP, SHU9119 or HS024 caused similar changes in the baseline RSNA and MAP to the microinjection of AgRP, SHU9119 or HS024 alone (Table 1). Pretreatment with AgRP or SHU9119 in the PVN abolished the RSNA and MAP enhancement responses to MTII, while HS024 substantially attenuated the MTII responses (Fig. 3). These chemicals had no significant effect on the HR.

Effects of different doses of selective MC4R antagonist

Three doses of the selective MC4R antagonist HS024 had no significant effect on the baseline RSNA and MAP, but attenuated the MTII-induced increases in the RSNA and MAP. A high dose of HS024 (1 nmol) caused greater inhibitory effects on the RSNA and MAP responses to MTII than a low dose of HS024 (0.2 nmol), but a very high dose of HS024 (5 nmol) failed to augment the inhibitory effects further (Fig. 4),

Effects of cAMP analogue and adenylyl cyclase inhibitor

The PVN microinjection of db-cAMP, a cAMP analogue, increased the RSNA and MAP. An adenylyl cyclase inhibitor, SQ22536, had no significant effect on the RSNA and MAP but abolished the MTII-induced RSNA and MAP enhancement responses (Fig. 5).

Effects of PKA inhibitor

The PVN microinjection of Rp-cAMP, a PKA inhibitor, decreased the RSNA and MAP and abolished the MTII-induced RSNA and MAP enhancement responses (Fig. 5).

Effects of MC3/4R agonist on cAMP level in the PVN

Microinjection of MTII into the PVN significantly increased the cAMP level in the PVN compared with the microinjection of saline (197.1 ± 10.6 *versus* 143.3 ± 9.2 pg mg^{-1} protein, $P = 0.003$).

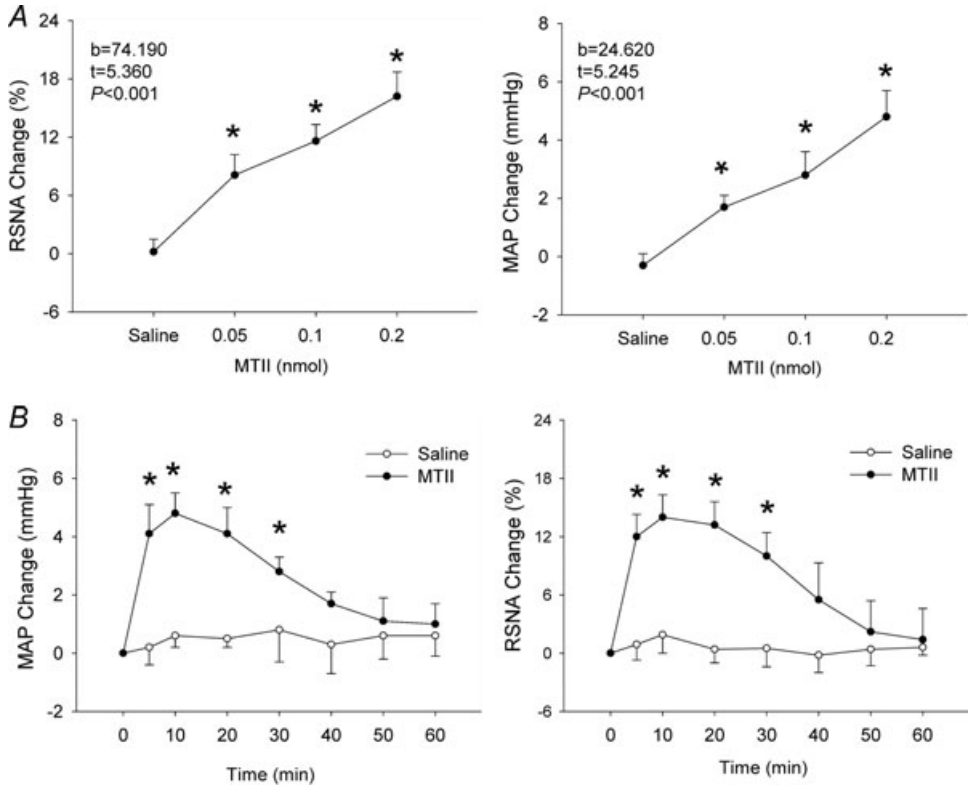

Figure 1. Dose and time effects of melanotan II (MTII) in the paraventricular nucleus (PVN)
A, effects of the PVN microinjection of saline or three doses of MTII (0.05, 0.1 and 0.2 nmol) on the renal sympathetic nerve activity (RSNA) and mean arterial pressure (MAP). *B*, time effects of the PVN microinjection of saline or the high dose of MTII (0.2 nmol) on the RSNA and MAP. Abbreviations: b, β coefficient (standardized regression coefficient); and t, *t* value, which is used to test the significance of the regression coefficient. Values are means ± SEM. $^*P < 0.05$ *versus* saline. $n = 6$ for each group.

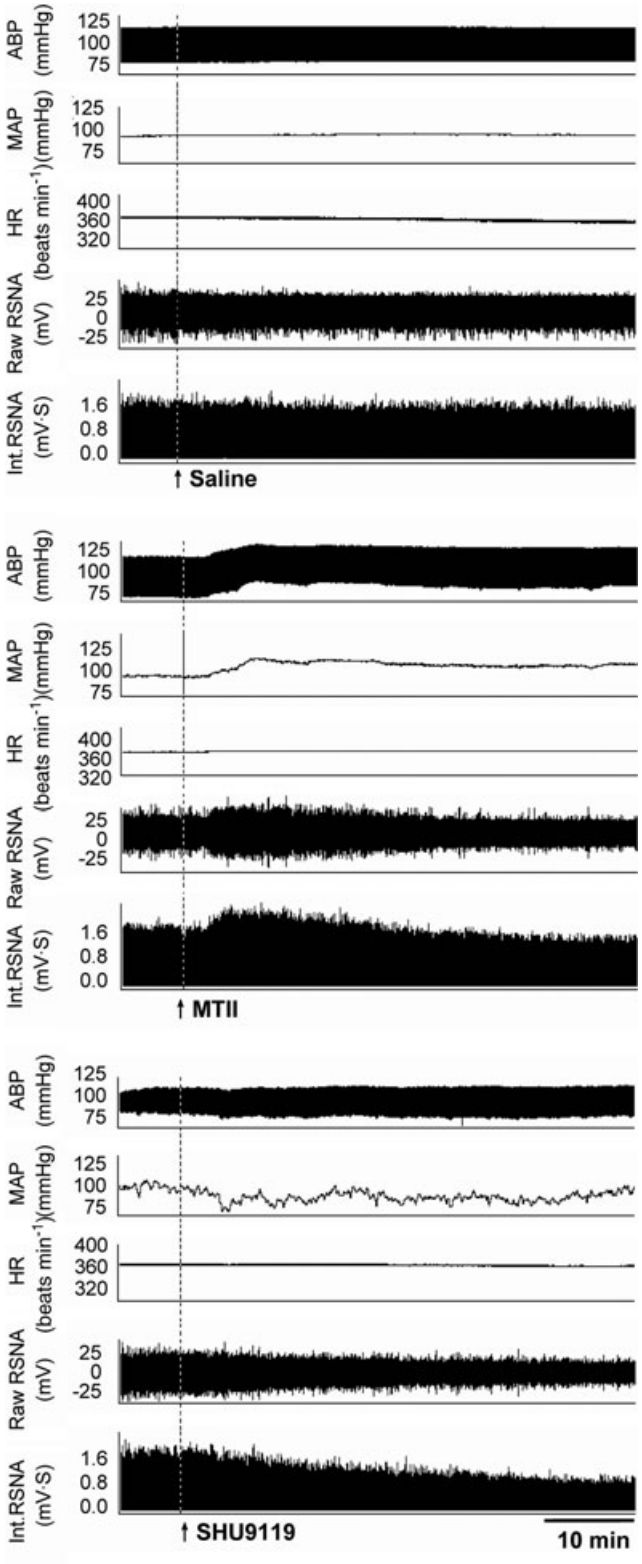

Figure 2. Representative recordings showing the effects of microinjection into the PVN of saline, MTII (0.2 nmol) or SHU9119 (0.4 nmol) on the RSNA and MAP
Abbreviation: ABP, arterial blood pressure; HR, heart rate; and Int. RSNA, integrated RSNA.

Discussion

Central MC3/4Rs are known to participate in the regulation of food intake and energy metabolism (Mountjoy, 2010). The MC3/4Rs are expressed in the PVN (Roselli-Rehfuss *et al.* 1993; Mountjoy *et al.* 1994; Siljee-Wong, 2011). Agouti-related peptide is an endogenous selective antagonist for MC3Rs and MC4Rs (Voisey *et al.* 2003; Yang, 2011), which binds to MC3Rs and MC4Rs using orthosteric and allosteric binding (Yang *et al.* 1999). The allosteric site is located in the extracellular loop and the orthosteric site is located in the transmembrane region (Oosterom *et al.* 2001; Yang *et al.* 2003). Projections from AgRP-containing cell bodies in the arcuate nucleus were distributed in the PVN (Légrádi & Lechan, 1999).

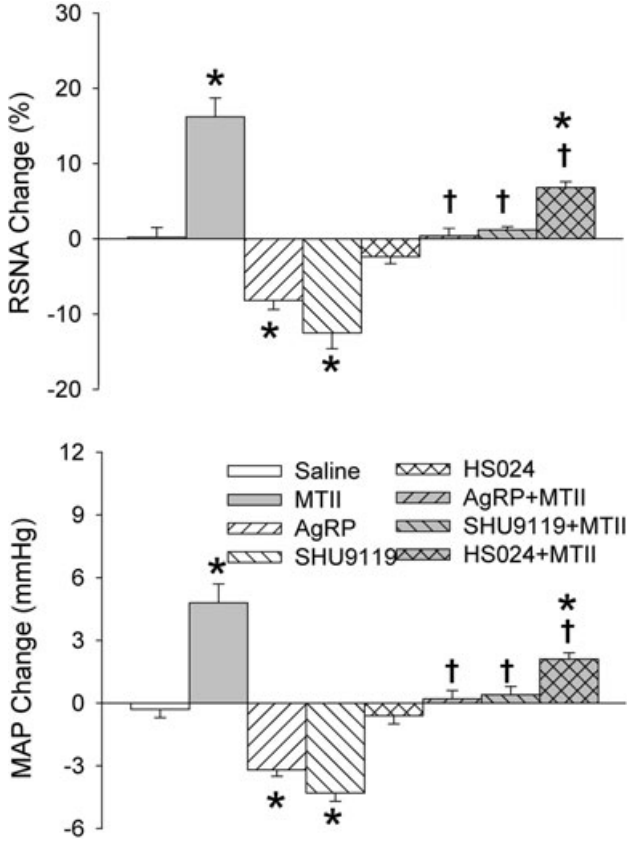

Figure 3. Effects of microinjection into the PVN of saline, MTII (0.2 nmol), agouti-related peptide (AgRP; 0.4 nmol), SHU9119 (0.4 nmol), HS024 (1 nmol) and MTII after pretreatment with AgRP, SHU9119 or HS024 on the RSNA and MAP
Melanotan II was administered 8 min after the microinjection of the MC3/4R antagonists (pretreatment). The changes in RSNA and MAP in response to MTII were calculated from new baseline values measured after the microinjection of the pretreatment drugs. Values are means ± SEM. $^{*}P < 0.05$ *versus* saline; and $\dagger P < 0.05$ *versus* MTII. $n = 6$ for each group.

Table 1. Effects of paraventricular nucleus (PVN) pretreatment with agouti-related peptide (AgRP), SHU9119 or HS024 on baseline renal sympathetic nerve activity (RSNA), mean arterial pressure (MAP) and heart rate

Parameter	Saline	AgRP	SHU9119	HS024	SQ22536	Rp-cAMP
RSNA (%)						
Before	100	100	100	100	100	100
After	100.2 ± 0.7	91.8 ± 0.6*	87.5 ± 1.4*	97.6 ± 0.9	96.0 ± 1.9	92.9 ± 1.0*
Change	0.2 ± 0.7	−8.2 ± 1.2*	−12.5 ± 1.4*	−2.4 ± 0.9	−4.0 ± 1.9	−7.1 ± 1.0*
MAP (mmHg)						
Before	86.9 ± 3.4	90.6 ± 2.4	88.8 ± 3.4	91.9 ± 2.8	91.5 ± 3.3	89.4 ± 3.1
After	86.6 ± 3.3	87.5 ± 2.6	84.5 ± 3.4	91.2 ± 3.1	90.3 ± 3.2	87.1 ± 3.3
Change	−0.2 ± 0.4	−3.2 ± 0.3*	−4.3 ± 0.3*	−0.7 ± 0.4	−1.2 ± 0.2	−2.3 ± 0.3*
Heart rate (beats min^{-1})						
Before	340 ± 10	348 ± 19	340 ± 13	326 ± 18	353 ± 16	326 ± 12
After	339 ± 11	343 ± 20	337 ± 15	325 ± 18	352 ± 15	322 ± 14
Change	−1 ± 1	−5 ± 4	−2 ± 2	−1 ± 1	−1 ± 1	−4 ± 2

The RSNA values before PVN pretreatment were set at 100%. Values are expressed as means ± SEM. *$P < 0.05$ *versus* saline. $n = 6$ for each group.

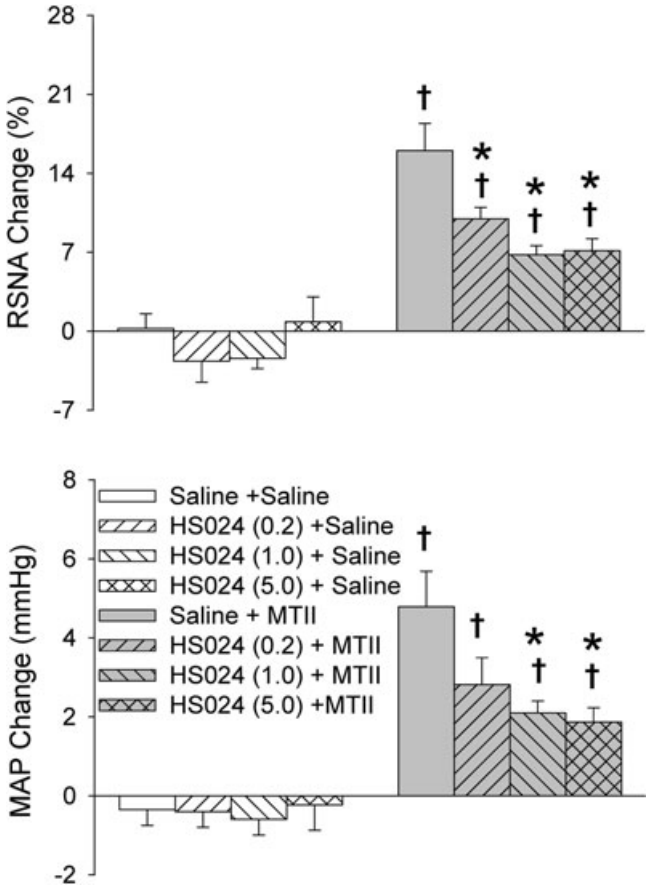

Figure 4. Effects of microinjection into the PVN of saline or three doses of HS024 (0.2, 1.0 and 5.0 nmol) on the RSNA and MAP, as well as the RSNA and MAP responses to MTII (2 nmol)
Saline or MTII was administered 8 min after the microinjection of HS024 or saline (pretreatment). The changes in RSNA and MAP in response to MTII were calculated from new baseline values measured after the microinjection of the pretreatment drugs. Values are means ± SEM. *$P < 0.05$ *versus* saline + MTII; and †$P < 0.05$ *versus* the rats with the same pretreatment followed by saline. $n = 6$ for each group.

In the present study, injection of the exogenous MC3/4R agonist MTII into the PVN increased the RSNA and MAP, which were blocked by the MC3/4R antagonist AgRP or SHU9119, and were substantially attenuated by the selective MC4R antagonist HS024. Agouti-related peptide or SHU9119 in the PVN decreased the baseline RSNA and MAP, but HS024 had no significant effects on the RSNA and MAP. The results indicate that the sympathoexcitatory and pressor effects of MTII in the PVN are mediated by both MC3Rs and MC4Rs in the PVN. Activation of MC3Rs or MC4Rs in the PVN contributes to the regulation of sympathetic outflow and blood pressure. Melanocortin 3 receptors in the PVN may be involved in the tonic control of sympathetic activity and blood pressure in the normal state. Unfortunately, a selective MC3R antagonist is not available to date. HS024 is a cyclic MSH analogue, and a potent and selective MC4R antagonist (Kask *et al.* 1998). All three doses of HS024 had no significant effect on the baseline RSNA and MAP, but did attenuate the MTII-induced increases in the RSNA and MAP. However, the highest dose of HS024 (5 nmol) failed to cause greater inhibitory effects than 1 nmol of HS024. These results suggest that the higher doses of HS024 used in the present study were sufficient to block MC4Rs completely, which is supported by previous studies (Kask *et al.* 1998; Giuliani *et al.* 2006, 2007; Caruso *et al.* 2012). Possible non-specific effects of exogenous SHU9119 on the RSNA and MAP can be excluded because application of the endogenous MC3/4R antagonist AgRP had similar effects to those of SHU9119.

Microinjection of MTII into the PVN increased the cAMP level in the PVN. Pretreatment with the adenylyl cyclase inhibitor SQ22536 or the PKA inhibitor Rp-cAMP in the PVN completely abolished the effects of the MC3/4R agonist MTII. These results indicate that the downstream cAMP–PKA pathway is important in MC3/4R signalling to regulate sympathetic activity and blood pressure, which is supported by the previous finding

that MC4R activation in astrocytes induces expression of brain-derived neurotrophic factor through the cAMP–PKA–cAMP response element-binding protein pathway (Caruso *et al.* 2012).

An interesting question is whether the changes of cAMP level and PKA activity are involved in tonic control of sympathetic activity and blood pressure in the physiological state. Dibutyryl-cAMP is a membrane-permeable cAMP analogue, and treatment with db-cAMP stimulates PKA activity (Valenti *et al.* 2011). Microinjection of db-cAMP into the PVN increased the RSNA and MAP, whereas the PKA inhibitor Rp-cAMP decreased the RSNA and MAP. However, no significant effects on the RSNA and MAP were found after the PVN microinjection of the adenylyl cyclase inhibitor SQ22536. The results suggest that cAMP is not responsible for tonic control of sympathetic activity and blood pressure in normal rats, but the increased cAMP level in the PVN may be involved in sympathetic activation in some cardiovascular diseases.

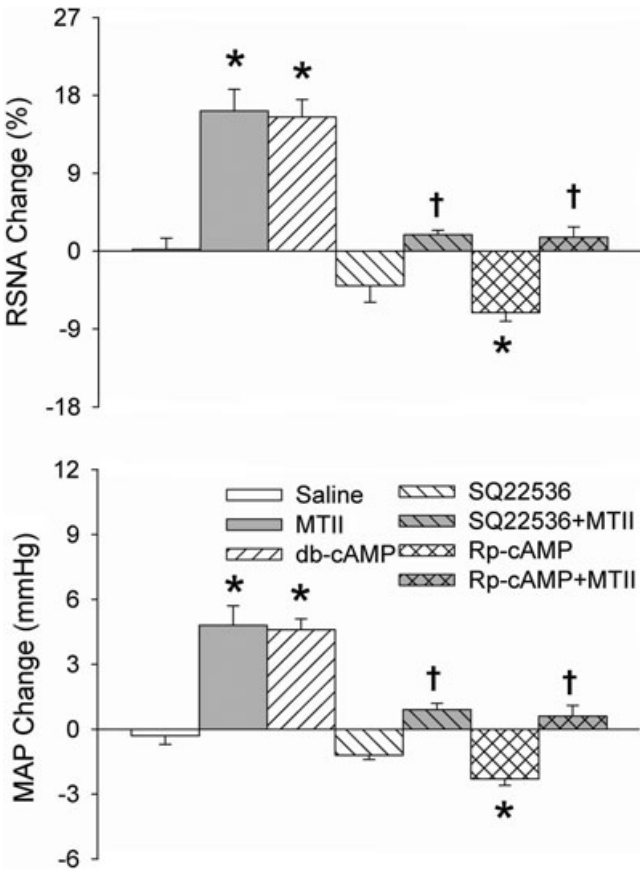

Figure 5. Effects of microinjection into the PVN of the cAMP analogue db-cAMP (1 nmol), the adenylyl cyclase inhibitor SQ22536 (2 nmol), the protein kinase A (PKA) inhibitor Rp-cAMP (1 nmol) and MTII after microinjection of SQ22536 or PKA inhibitor Rp-cAMP on RSNA and MAP
Melanotan II was administered 8 min after the microinjection of SQ22536 or Rp-cAMP (pretreatment). The changes in RSNA and MAP in response to MTII were calculated from new baseline values measured after the microinjection of the pretreatment drugs. Values are means ± SEM. $^*P < 0.05$ *versus* saline; and $\dagger P < 0.05$ *versus* MTII. $n = 6$ for each group.

It is generally accepted that the baroreceptor reflex operates as a feedback control system to provide compensation for transient changes in blood pressure (Averill, 2000). Afferent inputs from baroreceptors, chemoreceptors, trigeminal receptors and subsets of cardiopulmonary receptors with vagal afferents increase cardiovagal activity and decrease HR (Chapleau & Sabharwal, 2011). A recent study in our laboratory has shown that baroreceptor denervation and vagotomy enhances the RSNA and MAP responses to epicardial application of capsaicin or PVN microinjection of angiotensin II in rats (Gan *et al.* 2011). It is noteworthy that the present study was carried out in intact rats, without baroreceptor denervation and vagotomy. There was a possibility that the RSNA and MAP changes induced by chemicals in the PVN might be attenuated by the secondary effects of the baroreflex.

Central melanocortins are involved in obesity and hypertension. It has been found that a high-fat diet increases endogenous activity of the central MC3/4Rs and that MC3/4Rs appear to play an important role in linking increased blood pressure with diet-induced obesity (Dubinion *et al.* 2010). Chronic central administration of the MC3/4R antagonist SHU9119 caused a greater reduction in blood pressure in spontaneously hypertensive rats than in Wistar–Kyoto rats despite marked increases in food intake, weight gain and insulin resistance (da Silva *et al.* 2008). Insulin activates a melanocortin-dependent pathway to the PVN that increases glutamatergic drive to the rostral ventrolateral medulla and alters cardiovascular function (Ward *et al.* 2011). Chronic hypothalamic MC3/4R activation raises arterial pressure despite reduced food intake, whereas MC3/4R inhibition causes marked weight gain without raising arterial pressure, suggesting that intact hypothalamic MC3/4Rs may be necessary for excess weight gain to raise arterial pressure (Kuo *et al.* 2003). Melanotan II or α-MSH produced a significantly greater increase in the firing activity of the PVN presympathetic neurons in obese Zucker rats than in lean Zucker rats, which was abolished by the MC3/4R antagonist SHU9119 (Ye & Li, 2011). The roles and mechanisms of the MC3/4Rs in the PVN in the pathogenesis of obesity and hypertension need further investigation.

In conclusion, MC3/4R activation in the PVN increases the sympathetic outflow and blood pressure via the cAMP–PKA pathway. Endogenous MC3Rs in the PVN may exert a tonic excitatory effect on sympathetic activity.

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