Neurochem Res (2013) 38:2063–2071
DOI 10.1007/s11064-013-1114-0

ORIGINAL PAPER

Enhanced Assymetrical Noradrenergic Transmission in the Olfactory Bulb of Deoxycorticosterone Acetate-Salt Hypertensive Rats

Tamara Abramoff · María J. Guil · Vanina P. Morales · Sandra I. Hope · Celeste Soria · Liliana G. Bianciotti · Marcelo S. Vatta

Received: 20 February 2013 / Revised: 10 July 2013 / Accepted: 13 July 2013 / Published online: 26 July 2013

Abstract The ablation of olfactory bulb induces critical changes in dopamine, and monoamine oxidase activity in the brain stem. Growing evidence supports the participation of this telencephalic region in the regulation blood pressure and cardiovascular activity but little is known about its contribution to hypertension. We have previously reported that in the olfactory bulb of normotensive rats endothelins enhance noradrenergic activity by increasing tyrosine hydroxylase activity and norepinephrine release. In the present study we sought to establish the status of noradrenergic activity in the olfactory bulb of deoxycorticosterone acetate (DOCA)-salt hypertensive rats. Different steps in norepinephrine transmission including tyrosine hydroxylase activity, neuronal norepinephrine release and uptake were assessed in the left and right olfactory bulb of DOCA-salt hypertensive rats. Increased tyrosine hydroxylase activity, and decreased neuronal norepinephrine uptake were observed in the olfactory bulb of DOCA-salt hypertensive rats. Furthermore the expression of tyrosine hydroxylase and its phosphorylated forms were also augmented. Intriguingly, asymmetrical responses between the right and left olfactory bulb of normotensive and hypertensive rats were observed. Neuronal norepinephrine release was increased in the right but not in the left olfactory bulb of DOCA-salt hypertensive rats, whereas non asymmetrical differences were observed in normotensive animals. Present findings indicate that the olfactory bulb of hypertensive rats show an asymmetrical increase in norepinephrine activity. The observed changes in noradrenergic transmission may likely contribute to the onset and/or progression of hypertension in this animal model.

Keywords DOCA-salt hypertension · Noradrenergic transmission · Norepinephrine release · Norepinephrine uptake · Olfactory bulb · Tyrosine hydroxylase

Introduction

Diverse studies have focused on the role of the central nervous system (CNS) in the development and/or maintenance of hypertension. It has been clear for many years that the CNS is essential for the short-term regulation of blood pressure, but, current evidence strongly supports that it also contributes to its long-term control [1]. Various brain areas have been implicated in the chronic management of blood pressure particularly in pathophysiological situations like hypertension [2, 3].

The deoxycorticosterone acetate (DOCA)-salt rat is an animal model of hypertension characterized by neurohormonal activation and volume expansion that lead to elevated blood pressure [4]. The genetic component of hypertension is absent so this model is useful for the understanding of causes resulting from hypervolaemia, hyperaldosteronism and high salt intake [4]. Although the DOCA-salt model has been

Tamara Abramoff and María J. Guil have contributed equally to the present study.

T. Abramoff · M. J. Guil · V. P. Morales · S. I. Hope · C. Soria · M. S. Vatta (✉)
Cátedra de Fisiología e Instituto de la Química y Metabolismo del Fármaco (IQUIMEFA-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, Piso 7, 1113AAD-CABA Buenos Aires, Argentina
e-mail: mvatta@ffyb.uba.ar

L. G. Bianciotti
Cátedra de Fisiopatología e Instituto de Inmunología, Genética y Metabolismo (INIGEM-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

extensively studied, the underlying mechanisms of its origin and development still remain to be fully elucidated. It has been proposed that factors contributing to DOCA-salt hypertension are divided into neural and non-neural components [4]. Diverse studies show that the ablation of the area postrema or the AV3V area of Brody prevents the increase in blood pressure in DOCA-salt rats [5, 6]. Brain catecholamines are impaired, being brainstem norepinephrine (NE) turn-over decreased, whereas cardiac and renal NE turn-over as well as plasma NE levels are increased [2].

The olfactory bulb (OB) is an extension of the rostral telencephalon (4 % of the total mass of the rat brain) and communicates with areas closely implicated in the central regulation of the cardiovascular function [7–9]. It sends numerous neural projections to different areas of the midbrain and forebrain, including the hypothalamus [8, 9]. The OB communicates with the dorsal medulla, specifically with the nucleus tractus solitari that integrates the information of afferent carotid endings from sinus baroreceptor fibers. Various studies show that OB ablation modifies NE, dopamine and monoamine oxidase activity in the brain stem [9–11]. Although these studies support a close relationship between the OB and the cardiovascular system, the strength of this relationship is presently unknown.

Anatomical asymmetry between the right and left OB has been reported [12]. In different animal species the existence of brain asymmetry including NE levels in the hypothalamus has been reported [13]. In accordance, studies from our laboratory show that NE transmission is asymmetrically modified in the anterior hypothalamus of DOCA-salt hypertensive rats (unpublished data). Although brain functional asymmetry has been reported, its physiological relevance remains elusive.

On these bases, we sought to evaluate noradrenergic transmission in the right and left OB of normotensive as well as DOCA-salt hypertensive rats. Present findings show that NE transmission is significantly enhanced in the OB of DOCA-salt functional asymmetry.

Experimental Procedures

Animals and Experimental Design

Male Sprague–Dawley rats weighing 100–130 g (Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires) were used in the experiments. Rats were housed four per cage under a 12-h light/dark cycle and fed with a standard chow diet and water ad libitum. All experiments were performed following the recommendations of the Guide for the Care and Use of Laboratory Animal (NIH Publication N85-23, 1985, revised 1996) and all experimental protocols were approved by the Institutional Animal Care and Use Committee of the Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.

DOCA-salt hypertension was induced by weekly subcutaneous injections of 30 mg/kg DOCA (MP Biomedicals, CA, USA) in sesame seed oil as vehicle and 1 % NaCl administration in the drinking water for 5 weeks. Control animals were injected with vehicle and given tap water. Rats were weekly weighed and had blood pressure recorded by tail plethysmography (ADInstruments PowerLab 8/30 and NIBP Controller ML125). Other set of animals received only DOCA or saline (1 % NaCl).

After 5 weeks, animals were euthanized by decapitation between 9:00 and 12:00 h. Brains were quickly removed and OB immediately dissected according to Palkovits and Brownstein [14]. Hearts were also removed and weighed, and the left and right ventricles dissected and weighed.

Assays

TH Activity

OB were homogenized in 500 µl of buffer (5 mM KH_2PO_4 and 0.2 % Triton X-100, pH 7.0). After saving an aliquot for protein determination, samples were centrifuged at 10,000*g* for 10 min at 4 °C and TH activity was determined according to Reinhard et al. [15]. Briefly, an aliquot of the supernatant was incubated for 20 min at 37 °C with 50 mM HEPES (pH 7.0), containing 15 nmol L-tyrosine (0.5 µCi ^{3}H[3, 5] L-tyrosine from PerkinElmer Life and Analytical Sciences, MA, USA), 420 mM β-mercaptoethanol, 1,000 U catalase, and 0.75 mM 6-methyl-tetrahydrobiopterin. Reaction was stopped by the addition of 1 ml of 7.5 % (w/v) activated charcoal suspension in 1 N HCl. The final mixture was vortexed and centrifuged at 500*g* for 10 min followed by supernatant separation to measure ^3H_2O. Blank values were obtained by omitting 6-methyl-tetrahydrobiopterin from the mixture. Recovered ^3H_2O was determined by usual scintillation counting methods. Results were expressed as dpm/µg protein ± SEM.

Neuronal NE Uptake

Norepinephrine uptake was assessed as previously described with minor modifications [16]. Briefly, OB were pre-incubated in a Dubnoff incubator for 30 min at 37 °C in Krebs bicarbonate solution, pH 7.4, and bubbled with a gas mixture (95 % O_2 and 5 % CO_2) under continuous shaking. NE stores were labelled with 2.5 µCi/ml [^{3}H]-NE (PerkinElmer Life and Analytical Sciences, MA, USA) for 5 min followed by three consecutive washes (10 min each) with cold Krebs. Monoamine-oxidase activity and extraneuronal NE uptake were inhibited by the addition of 50 µM pargyline and

100 μM hydrocortisone, respectively. Tissues were homogenized, an aliquot was saved for protein measurement and [^{3}H]-NE was determined by usual scintillation counting methods. Results were expressed as dpm/μg protein ±SEM.

Neuronal NE Release

Neuronal NE release was measured as previously reported with minor modifications [16]. OB of control and DOCA-salt were submitted to the same experimental procedures as described for NE uptake except that before the last wash, 10 μM desipramine was added in order to inhibit neuronal NE uptake. Tissues were incubated for 30 min and seven consecutive samples of the incubation medium were collected every 5 min. [^{3}H]-NE was measured in the incubation medium by conventional scintillation counting methods. Results were expressed as the area under the curve corresponding to 30 min (dpm/μg protein).

Western Blot Assay

Tissues were homogenized in lysis buffer (20 mM Tris–Cl$^-$ pH: 7.4, 1 mM PMSF, 5 mM EDTA, 25 mM NaF, 1 % Triton X-100, 1 % protease inhibitor cocktail). Following centrifugation for 20 min at 4 °C, an aliquot was withdrawn from the supernatant for protein measurement. The remaining sample was mixed with LAEMMLI buffer (62.5 mM Tris–Cl pH: 6.8, 2 % SDS, 5 % B-ME, 10 % glycerol, 4 % bromophenol blue), boiled for 5 min and then subjected to SDS-PAGE gel at 100 V for 2 h and 30 min. The gels were then transferred to PVDF membranes (GE Healthcare, Amersham Biosciences, UK) at 100 V for 75 min. The membranes were blocked overnight at 4 °C in blocking solution (5 % non fat powder milk in Tris-buffered saline containing 0.1 % Tween 20 [TBS-T]) and the gels were stained overnight at 4 °C with Coomassie Blue. After washing with TBS-T, transfers were incubated with anti-TH monoclonal-antibody (1/2,000 overnight at 4 °C) (TH-Ab, from Sigma, MO, USA), TH phospho-Ser-19, 31 and 40 (1/1,000 overnight at 4 °C) (TH-S19P, TH-S31P and TH-S40P; from Chemicon, CA, USA), NET (1/500 overnight at 4 °C) (NET-Ab, from Chemicon, CA, USA), actin (1/2,000, 1 h at room temperature) (Sigma, MO, USA) and anti-mouse or anti-rabbit peroxidase conjugated antibody (Piece, IL, USA) (1/5,000, 1 h at room temperature). After the final wash with TBS-T bands were visualized by using Super Signal West Femto kit (Pierce, IL, USA). Bands were analyzed by densitometry and results expressed as relative densitometric units × 100 ± SEM.

Statistical Analysis

Results were expressed as mean ± SEM. The statistical analysis was performed by the ANOVA followed by the simple effects analysis and Bonferroni post test. p values of 0.05 or less were considered statistically significant.

Results

As expected systolic blood pressure and the left ventricular weight ratio (left ventricular weight relative to total body weight) were significantly elevated in DOCA-salt rats (159 ± 2 and 2.65 ± 0.06 mmHg, respectively) compared with control rats (115 ± 2 and 2.02 ± 0.07 mmHg, respectively). However, no changes were observed in either body weight or relative right ventricular weight between the two groups (data not shown). These findings validate the animal model used in the study. On the other hand, DOCA only and salt only groups did not show modifications in blood pressure (data not shown).

In order to evaluate noradrenergic activity, neuronal NE release, TH activity and expression and the expression of NE transporter were studied in the right and left OB of DOCA-salt rats.

TH activity was similar in the right and left OB of all normotensive groups (control, DOCA only and salt only) (Fig. 1.). However, TH activity was significantly augmented in both OB of DOCA-salt rats, the increase being more prominent in the right OB (Fig. 1b). These findings reveal that TH activity displays an asymmetrical behaviour in hypertensive rats. Total TH protein content assessed by Western blot showed that total TH protein was similar in both OB of normotensive rats (Fig. 2.). In DOCA-salt rats, although total TH level was augmented in both OB hemi portions as compared to normotensive animals, it was higher in the right OB (Fig. 2b). The phosphorylated forms of TH were assessed to evaluate whether DOCA-salt-induced enzymatic stimulation correlated with phosphorylation events which are known to enhance TH activity. Results showed that TH-S19P increased only in the right OB of DOCA-salt rats, whereas TH-S31P and TH-S40P augmented in both OB, with a slight increase, but not significant, in the right compared to the left (Fig. 3).

As catecholamine biosynthesis is tightly related to the release of the amine, neuronal NE release was assessed [17–19]. Results showed that NE secretion was increased in the right but not in the left OB of DOCA-salt rats as compared to control animals (Fig. 4).

Basal neuronal NE uptake was lower in the left than in the right OB of control rats (Fig. 5b). Animals receiving DOCA or salt alone showed no differences as compared to control group (Fig. 5a). However DOCA-salt hypertensive rats showed diminished NE uptake in both OB hemi portions, although the reduction was more pronounced in the right OB (Fig. 5b). Neuronal NE uptake is dependent on NET density at the neuronal plasmatic membrane, thus a decrease in neuronal NE uptake may be attributed to enhanced NET

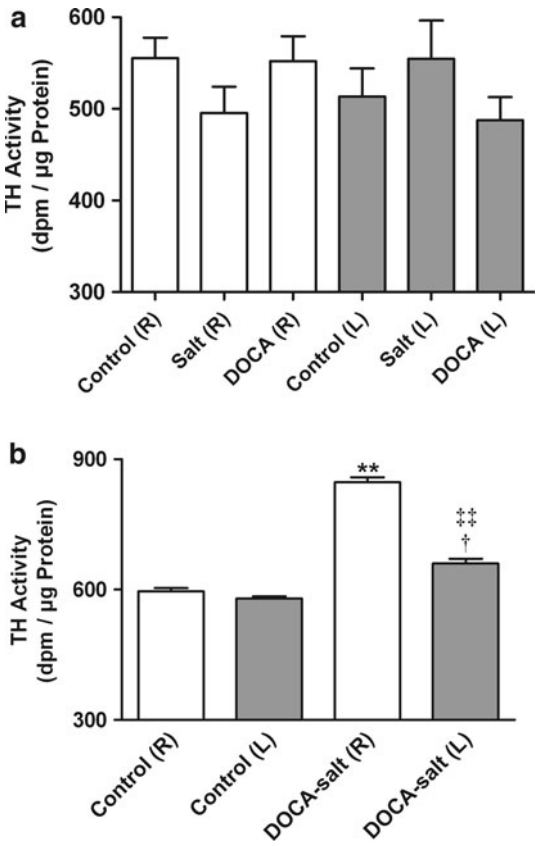


Fig. 1 Tyrosine hydroxylase (TH) activity in the *right* (*open bars*) and *left* (*filled bars*) olfactory bulb (OB) of normotensive groups (control, DOCA only and salt only) (**a**), and control and DOCA-salt hypertensive rats (**b**). Th activity was assessed as described in "Experimental procedures" section and expressed as dpm/µg protein ± SEM. $^{**}p < 0.01$ versus control right OB; $^{\dagger}p < 0.05$ versus control left OB; $^{\ddagger\ddagger}p < 0.01$ versus DOCA-salt right OB. Number of animals: 4–8 in each experimental group

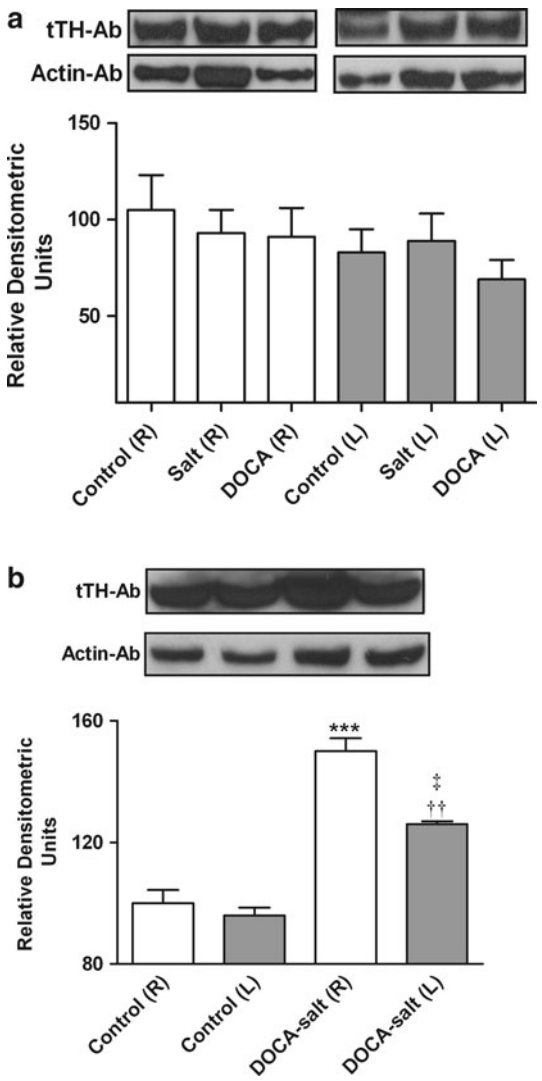


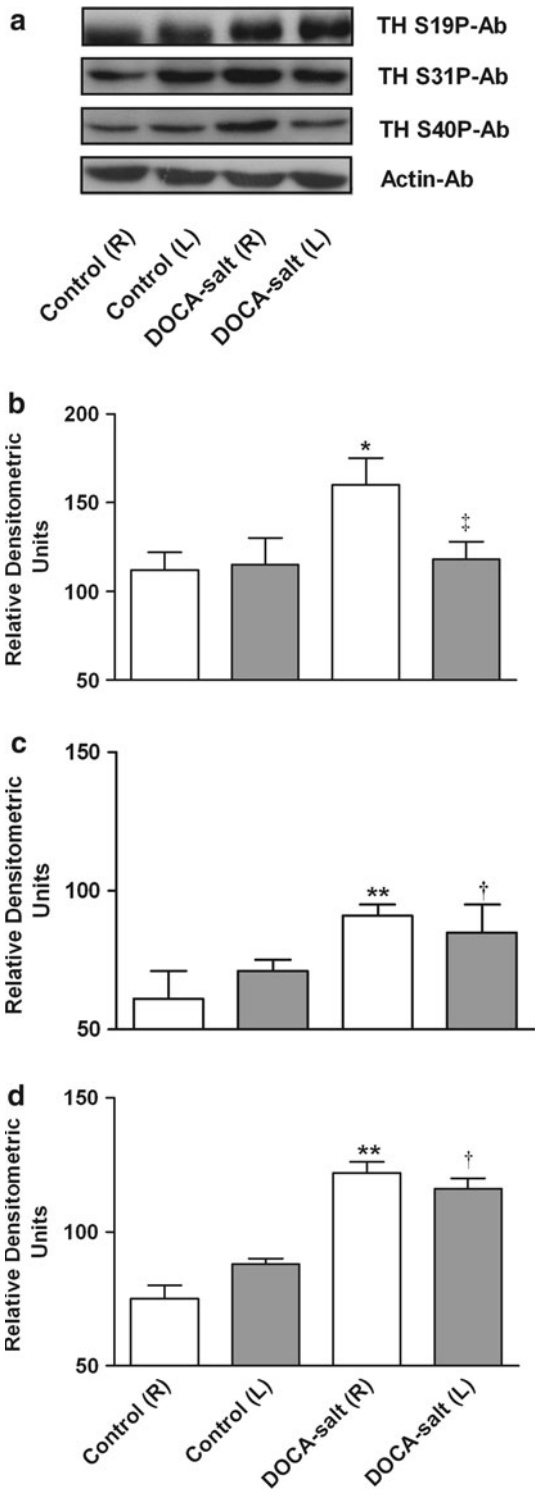
Fig. 2 The protein expression of tyrosine hydroxylase (tTH) in the *right* (*open bars*) and *left* (*filled bars*) olfactory bulb (OB) of control, DOCA only and salt only (**a**), and control and DOCA-salt hypertensive rats (**b**). Total TH expression was determined by Western blot as detailed in "Experimental procedures" section and expressed as relative densitometric units × 100 ± SEM. $^{***}p < 0.001$ versus control right OB; $^{\dagger\dagger}p < 0.01$ versus control left OB; $^{\ddagger}p < 0.05$ versus DOCA-salt right OB. The immunoblot shown is representative of four independent experiments. Number of animals: 3–4 in each experimental group

internalization [20]. In order to evaluate the correlation between NET and neuronal NE uptake, NET content was assessed by Western blot. Results showed that two bands at 80 and 50 kDa were expressed in normotensive and DOCA-salt rats (Fig. 6). The glycosylated form of the transporter (80 kDa) but not the non-glycosylated (50 kDa) form mediates NE transport [20, 21]. In all normotensive animals (control, DOCA only and salt only), glycosylated NET expression was lower in the left OB than in the right OB (Fig. 6). In hypertensive animals it was diminished in both OB hemi portions, but the reduction was more pronounced in the right OB (Fig. 6b right). Conversely, 50 kDa NET was increased in both OB of DOCA-salt rats being it higher in the left than in the right OB (Fig. 6c, right).

Discussion

The present study shows that noradrenergic activity was significantly enhanced in the OB of DOCA-salt hypertensive rats and further it exhibited an asymmetrical behaviour. These findings support an excitatory role of the sympathetic activity in this telencephalic region. The catecholaminergic activity in the OB is mediated by NE in nerve endings originated in the Locus coeruleus and by dopamine located in neuronal somas of the olfactory glomerular layer [10, 22]. Although NET mRNA was reported in the mouse OB, its physiological significance remains elusive [23].

Vascular and cardiac physiological changes induce modifications in the neurochemistry of various neurotransmitters including NE in the OB [10]. Although a few



◀ **Fig. 3** The expression of tyrosine hydroxylase (TH) phosphorylated forms at S19P (**b**), S31P (**c**) and S40P (**d**) in the *right* (*open bars*) and *left* (*filled bars*) olfactory bulb (OB) of normotensive (Control) and DOCA-salt hypertensive rats were determined Western blot as detailed in "Experimental procedures" section and expressed as relative densitometric units × 100 ± SEM. **a** Representative blots. $^{*}p < 0.05$ and $^{**}p < 0.01$ versus control right OB; $^{\dagger}p < 0.05$ versus control left OB; $^{\ddagger}p < 0.05$ versus DOCA-salt right OB. The immunoblot shown is representative of four independent experiments. Number of animals: 4 in each experimental group

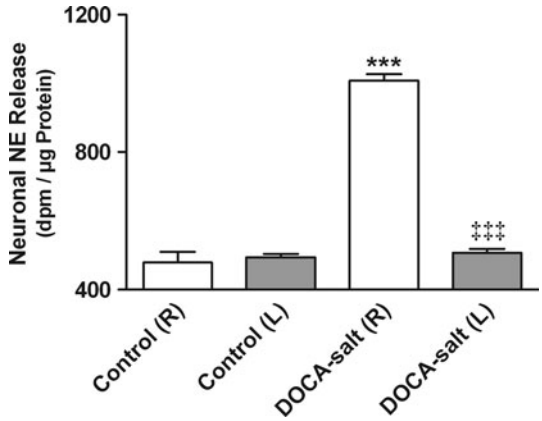


Fig. 4 Neuronal norepinephrine (NE) release in the *right* (*open bars*) and *left* (*filled bars*) olfactory bulb (OB) of normotensive (Control) and DOCA-salt hypertensive rats was assessed as detailed in "Experimental procedures" section and expressed as dpm/µg protein ± SEM. $^{***}p < 0.001$ versus control right OB; $^{\ddagger\ddagger\ddagger}p < 0.001$ versus DOCA-salt right OB. Number of animals: 6 in each experimental group

studies have been performed, they strongly support that the OB is involved in the regulation of cardiovascular function [8]. Changes in blood pressure and heart rate occur following OB removal [8, 9]. Moreover, OB integrity is crucial for the CNS to trigger a normal sympathoexitatory response to diverse physiological stimuli [24, 25]. Animals with bilateral olfactory bulbectomy, a validated animal model of depression, show functional changes associated with the impairment of neurotransmitters like NE, serotonin, acetylcholine, glutamate and GABA [9, 10]. In addition, olfactory bulbectomy results in augmented NE and MAO activity in different brain stem areas [26]. The increase in NE correlates with enhanced blood pressure in this model [9, 10]. A recent study shows that cervical stimulation activates alpha adrenoceptors on the adrenergic fibbers in the OB leading to venous constriction [27]. All these findings and our present results strongly support a clear relationship between the OB catecholaminergic activity and the regulation of the blood pressure. In addition, preliminary results from in our laboratory show that, bilateral olfactory bulbectomy performed in hypertensive DOCA-salt rats, induced a changes in cardiovascular parameters (unpublished data).

In the present study two different aspects are to be considered in the analysis of results: one is the increase in noradrenergic activity observed in the OB of hypertensive DOCA-salt rats, whereas the other is the asymmetrical behaviour displayed by OB hemi portions in this animal model.

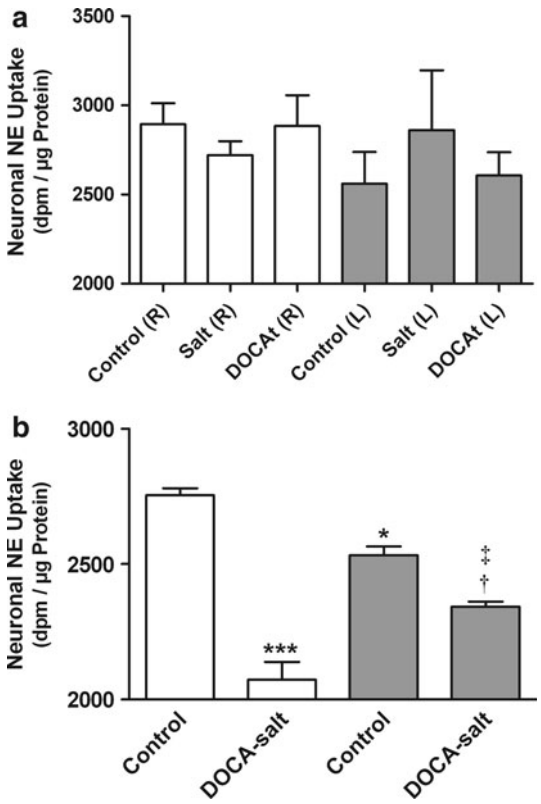


Fig. 5 Neuronal norepinephrine (NE) uptake in the *right* (*open bars*) and *left* (*filled bars*) olfactory bulb (OB) of control, DOCA only and salt only (**a**), and control and DOCA-salt hypertensive rats (**b**). Neuronal NE uptake was determined as detailed in "Experimental procedures" section and expressed as dpm/µg protein ± SEM. $^{*}p < 0.05$ and $^{***}p < 0.001$ versus control right OB; $^{\dagger}p < 0.05$ versus control left OB; $^{\ddagger}p < 0.05$ versus DOCA-salt right OB. Number of animals: 4–6 in each experimental group

In the OB of DOCA-salt rats, TH activity as well as total enzyme and its phosphorylated forms were augmented. In addition, neuronal NE release was also enhanced. However, NET activity was diminished as supported by a reduction in the glycosylated form and an increase in the non-glycosylated form of the transporter. We found that these responses (the activity and expression of TH, neuronal NE release as well as NET activity and expression) exhibited an asymmetrical behaviour in hypertensive DOCA-Salt rats as compared with normotensive animals. These findings support the hypothesis that NE transmission is augmented in the OB of DOCA-salt rats being higher in the right hemi portion. To our knowledge, this is the first report to show a relationship among the OB, noradrenergic transmission and the hypertensive DOCA-Salt model, and furthermore an asymmetry in these responses.

Changes in TH activity were reported in other regions and areas of the CNS in different animal model of hypertension including the DOCA-salt model [28–30]. The presence of TH mRNA and protein was shown in rat OB periglomerular neurons [22, 31]. TH catalyzes the rate-limiting step in catecholamine biosynthesis and its activity is tightly controlled by diverse mechanisms [17–19]. Regulation of TH activity involves short- and long-term mechanisms, including feedback inhibition, allosteric regulation and phosphorylation that take place following the enzyme gene transcription [17–19]. Phosphorylation of serine residues by diverse protein kinases is considered the major mechanism of TH activation [17–19]. The N-terminal region of the enzyme contains four serine residues (Ser-8, 19, 31 and 40) which phosphorylate in response to diverse physiological stimuli, leading to a more active but less stable form of the enzyme [17, 18]. While changes in Ser-8 phosphorylation occur only in dividing cells and are not involved in TH activity regulation, phosphorylation of Ser-40 substantially increases the enzyme activity by blunting the catecholamine-mediated inhibitory feedback mechanism [17, 19]. Phosphorylation of Ser-31 results in a modest increase in TH activity whereas phosphorylation of Ser-19 produces a more open enzyme conformation which enhances the phosphorylation rate of Ser-40, thus leading to increased TH activity [17, 19]. Present results show that DOCA-salt rats showed increased phosphorylation at TH Ser-40 in the right and left OB, whereas at TH Ser-19 only in the right OB. In addition, decreased neuronal NE uptake consistent with diminished glycosilated (80 kDa) and increased non-glycosilated (50 kDa) NET content was found in the OB hypertensive rats. NET cell surface expression and activity are dependent, at least in part, on the transporter N-glycosylation [20, 21]. When neuronal NE release was further analyzed an increase in the OB of DOCA-salt rats was found. Since NE reuptake is responsible for TH inactivation through a negative feedback mechanism, an imbalance between NET activity and neuronal NE release may contribute to affect NE turnover in pathophysiological situations like hypertension [32, 33]. A few reports in the literature address the steps of noradrenergic transmission studied herein in the brain of DOCA-salt hypertensive rats. Rylett et al. [28] observed that TH activity is increased in the whole brain, whereas Nagaoka and Lovenberg [29] showed that it is reduced in the hypothalamus. Conversely, it was also shown that NE release in the anterior and posterior hypothalamus as well as in the A2 region of the nucleus tractus solitari is similar in normotensive and hypertensive rats [34]. Other studies report that NE turn-over is decreased in the hypothalamus and pons medulla of DOCA-salt rats [35]. In the present study, noradrenergic activity is clearly enhanced in the OB of hypertensive rats supporting the hypothesis that this telencephalic region plays a regulatory role in this animal model of hypertension. Nevertheless, whether enhanced noradrenergic activity in the OB triggers hypertension or contributes to its maintenance is presently unknown.

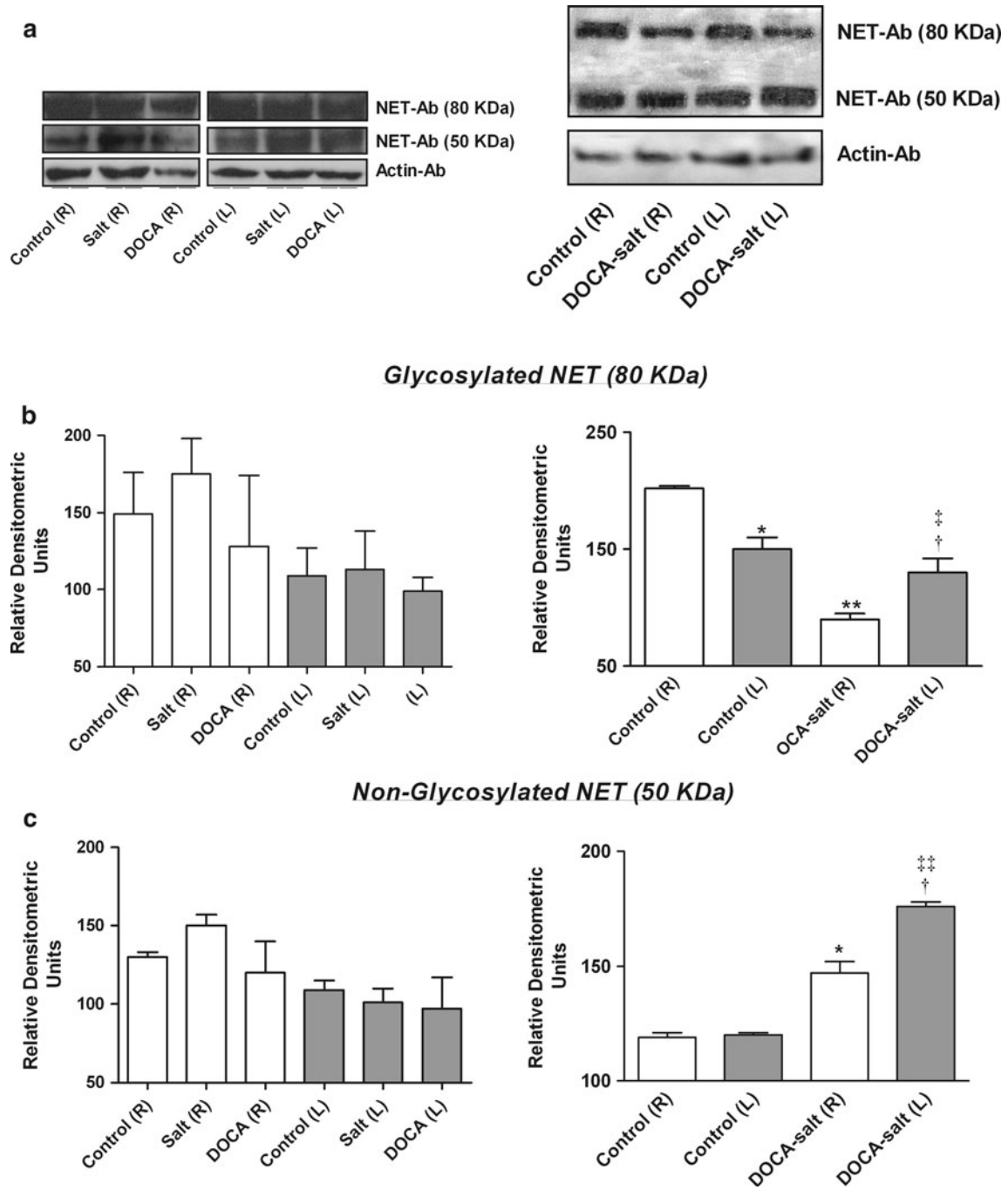


Fig. 6 Neuronal norepinephrine transporter (NET) expression was assessed in the *right* (*open bars*) and *left* (*filled bars*) olfactory bulb (OB) of control, DOCA only and salt only (*left panel*), and control and DOCA-salt hypertensive rats (*right panel*). **a**, Representative blots. The expression of glycosylated NET (80 kDa) (**b**) and non glycosylated NET (50 kDa) (**c**) levels was determined by Western blot as detailed in "Experimental procedures" section and expressed as relative densitometric units × 100 ± SEM. $^{*}p < 0.05$ and $^{**}p < 0.01$ versus control right OB; $^{\dagger}p < 0.05$ versus control left OB; $^{\ddagger}p < 0.05$ and $^{\ddagger\ddagger}p < 0.01$ versus DOCA-salt right OB. The immunoblot shown is representative of four independent experiments. Number of animals: 3–4 in each experimental group

However, it is well established that noradrenergic nerve endings present in the OB originate in the LC which shows enhanced NE turn-over and changes in the spontaneous firing rate of neurons in hypertension [36–38]. Direct evidence suggests that enhanced sympathetic nerve activity plays a crucial role in DOCA-salt hypertension. Plasma catecholamines are elevated during the development of this animal model (2–6 weeks) [4, 39, 40]. Ganglionic blockade elicits a greater decrease in arterial pressure in early and established DOCA-salt hypertension [41]. Furthermore,

peripheral sympathectomy prevents hypertension and the increase in plasma NE in this model [42].

The significance of the asymmetry in noradrenergic activity observed between the right and left OB of DOCA-salt rats is unknown. However, anatomical asymmetry between both OB hemi portions was previously reported in rats; it was found that the OB and the outer stratum are larger on the right side [12]. A recent interesting study reports an asymmetrical descending cardiovascular pathway in the dorsomedial hypothalamus evidenced by the observation that the activation of the right portion causes higher heart rate and tail vascular conductance as compared with the left hemi portion [42].

Present findings agree with previous reports showing that the right brain region is more related to the control of the cardiovascular function than the left [42, 43]. Patients with essential hypertension show higher NE release in the right jugular vein than in the left [32]. Furthermore, stimulation of the right sinoatrial node triggers higher tachycardia than the activation of the left node [44, 45]. These findings support that the heart rate is controlled by the right sympathetic nervous system. An asymmetrical sympathetic response was reported in the insular cortex of Sprague–Dawley rats and Macaca fascicularis monkeys, where the right hemi portion evokes a higher response that the left [43, 46]. This study further supports that the heart rate and blood pressure are regulated by the right hemi portion given that this brain area contains neurons involved in baroreflex control [13, 43]. Moreover, diverse biological noxa increase NE in the right side [13, 47]. Mice injected with lipopolysaccharides exhibit sympathoexitation induced by NE release in the right region [47]. In addition, rodents show higher monoamine content in the right parietal cortex than in the left cortex [13]. Patients with essential hypertension without renal stenosis show asymmetry in renal sympathetic activity, where the right kidney evokes a higher discharge than the left [48]. Most studies suggest that in all forms of hypertension, NE release is enhanced in right areas supporting the hypothesis that the brain control of the cardiovascular function is asymmetrical.

Present findings show that asymmetrical noradrenergic transmission impairment occurs in the OB of DOCA-salt hypertensive rats mainly in the right hemi portion. TH activity, expression and phosphorylated forms as well as neuronal NE release were increased whereas neuronal NE uptake was diminished likely by NET internalization.

The association between the OB and mood-related diseases like depression is well documented [10]. Diverse studies show a bidirectional relationship between depression and cardiovascular diseases, but the underlying mechanisms are presently unknown [49, 50]. A close connection between the OB and the limbic system-related areas involved in cardiovascular regulation exists. Taken together, previous and present findings provide rational basis to support the existence of an interaction between the asymmetric changes in the noradrenergic neurochemistry of the OB and the cardiovascular system, and further a possible association with mood disorders as depression.

Our study further advances in the knowledge that catecholaminergic neurotransmission is impaired in the OB of DOCA-salt rats. To our knowledge the present study is the first to report alterations in noradrenergic transmission in the OB of DOCA-salt hypertensive rats. Although brain catecholamines are implicated in DOCA-salt hypertension, the present study does not allow us to conclude whether noradrenergic changes in the OB cause hypertension or result from the hypertensive state.

Acknowledgments This work was supported by grants from the Universidad de Buenos Aires (UBACyT), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT).

References

1. Orlov SN, Mongin AA (2007) Salt-sensing mechanisms in blood pressure regulation and hypertension. Am J Physiol Heart Circ Physiol 293:H2039–H2053
2. Oparil S, Chen YF, Bereck KH, Calhoun DA, Wyss JM (1995) The role of the central nervous system in hypertension. In: Laragh JH, Brenner RH (eds) Hypertension: pathophysiology, diagnosis, and management. Raven, New York, pp 713–740
3. Kasparov S, Teschemacher AG (2008) Altered central catecholaminergic transmission and cardiovascular disease. Exp Physiol 93:725–740
4. Yemane H, Busauskas M, Burris SK, Knuepfer MM (2010) Neurohumoral mechanism in deoxycortiocosterone acetate (DOCA)-salt hypertension in rats. Exp Physiol 95:51–55
5. Buggy J, Fink GD, Haywood JR, Johnson AK, Brody MJ (1978–1979) Interruption of the maintenance phase of established hypertension by ablation of the anteroventral third ventricle (AV3V) in rats. Clin Exp Hypertens 1:337–353
6. Bruner CA, Mangiapane ML, Fink GD, Webb RC (1988) Area postrema ablation and vascular reactivity in deoxycorticosterone-salt-treated rats. Hypertension 11:668–673
7. Cain DP (1974) The role of the olfactory bulb in limbic mechanisms. Psychol Bull 81:654–671
8. Kawasaki H, Watanabe SH, Ueki SH (1980) Changes in blood pressure and heart rate following bilateral olfactory bulbectomy in rats. Physiol Behav 24:51–56
9. Song C, Leonard BE (2005) The olfactory bulbectomized rats as a model of depression. Neurosci Biobehav Rev 29:627–647
10. Kelly JP, Wrynn AS, Leonard BE (1997) The olfactory bulbectomized rats as a model of depression: an update. Pharmacol Ther 74:299–316
11. Masini CH, Holmes P, Freeman K, Maki A, Edwards G (2004) Dopamine overflow is increased in bulbectomized rats: an in vivo microdialysis study. Physiol Behav 81:111–119
12. Heine O, Galaburda AM (1986) Olfatory asymmetry in the rat brain. Exp Neurol 91:392–398
13. Ramirez M, Prieto I, Vivex F, De Gasparo M, Alba F (2004) Neuropeptides neuropeptidases and Brain asymmetry. Curr Protien Pept Sci 6:497–506
14. Palkovits M, Brownstein MJ (1988) Maps and guide to microdissection of the rat brain. Elsevier, New York

15. Reinhard JF, Scmith GK, Nicho CA (1986) A rapid and sensitive assay for tyrosine-3 mono-oxygenase based upon the release of $^{3}H_2O$ and absorption of [^{3}H] tyrosine by charcoal. Life Sci 55:257–279
16. Vatta MS, Presas M, Bianciotti LG, Zarrabeitia V, Fernández BE (1996) B and C types natriuretic peptides modulate norepinephrine uptake and release in the rat hypothalamus. Regul Pept 65:175–181
17. Kumer SC, Vrana KE (1996) Intrincate regulation of tyrosine hydroxylase activity and gene expression. J Neurochem 67:443–462
18. Flatmark T, Almas B, Ziegler MG (2002) Catecholamine metabolism: an update on key biosynthetic enzymes and vesicular monoamine transporters. Ann NY Acad Sci 971:69–75
19. Dunkley PR, Bobrovskaya L, Graham ME, von Nagy-Felsobuki M, Dickson PW (2004) Tyrosine hydroxylase phosphorylation: regulation and consequences. J Neurochem 91:1025–1043
20. Ramamoorthy S, Melikian HE, Qian Y, Blakely RD (1988) Biosynthesis, N-glycosylation, and surface trafficking of biogenic amine transporter proteins. Method Enzymol 296:347–370
21. Jayanthi LD, Devadoss JS, Ramamoorthy S (2004) Regulated Internalization and phosphorylation of the native norepinephrine transporter in response to phorbol esters. J Biol Chem 279: 19315–19326
22. Baker H, Kawano T, Margolis FL, Joh TH (1983) Transneuronal regulation of tyrosine hydroxylase expression in olfactory bulb of mouse and rat. J Neurosci 3:69–78
23. Dluzen DE, Park J-H, Kim K (2002) Modulation of olfactory bulb tyrosine hydroxylase and catecholamine transporter mRNA by estrogen. Mol Brain Res 108:121–128
24. Moffitt JA, Grippo AJ, Holmes PV, Johnson AK (2001) Cardiovascular excitatory reflexes are blunted following bilateral olfactory bulbectomy in the rat (abstract). FASEB J 15:A807
25. Moffitt JA, Grippo AJ, Holmes PV, Johnson AK (2002) Olfactory bulbectomy attenuates cardiovascular sympathoexcitatory reflexes in rats. Am J Physiol Heart Circ Physiol 283:H2575–H2583
26. Pohorecky LA, Zigmond MJ, Heimer L, Wurtman RJ (1969) Olfactory bulb removal: effects on brain norepinephrine. Proc Natl Acad Sci USA 62:1052–1055
27. Shiba K, Machida T, Uchida S, Hotta H (2009) Sympathetic neural regulation of olfactory bulb blood flow in adult and aged rats. Auton Neurosci 147:75–79
28. Rylett P, Dean HG, Lee MR (1976) Brain tyrosine hydroxylase activity and systolic blood pressure in rats treated with either deoxycorticosterone and salt or angiotensin. J Pharm Pharmacol 28:559–562
29. Nagaoka A, Lovenberg W (1977) Regional changes in the activities of aminergic biosynthetic enzymes in the brains of hypertensive rats. Eur J Pharmacol 43:297–306
30. Moura E, Afonso J, Serrao MP, Vieira-Coelho A (2008) Effect of clonidine on tyrosine hydroxylase activity in adrenal medulla and brain of spontaneously hypertensive rats. Basic Clin Pharmacol Toxicol 104:113–121
31. Biffo S, Marti E, Fasolo A (1992) Carnosine, nerve growth factor receptor and tyrosine hydroxylase expression during the ontogeny of the rat olfactory bulb. J Chem Neuroanat 5:51–62
32. Esler MD, Jennings GL, Lambert GW (1988) Release of noradrenaline into the cerebrovascular circulation in patients with primary hypertension. J Hypertens 6(Suppl):S494–S496
33. Bönisch H, Brüss M (2006) The norepinephrine transporter in physiology and disease. Handb Exp Pharmacol 175:485–524
34. Meldrum MJ, Westfall TC (1986) Comparison of norepinephrine release in hypertensive rats: I. Hypothalamic and brainstem tissues. Clin Exp Hypertens A 8:201–219
35. Fujita T, Sato Y (1984) The antihypertensive effect of taurine in DOCA-salt rats. J Hypertens 2(Suppl 3):S563–S565
36. Olpe HR, Berecek K, Jones RSG, Steinmann MW, Sonnenberg Ch, Hofbauer KG (1985) Reduced activity of locus coeruleus neurons in hypertensive rats. Neurosci Lett 61:25–29
37. Ye S, Ozgur B, Campese VM (1997) Renal afferent impulses, the posterior hypothalamus, and hypertension in rats with chronic renal failure. Kidney Int 51:722–727
38. Kvetnansky R, Sabban EL, Palkovits M (2009) Catecholaminergic system in stress: structural and molecular genetic approaches. Physiol Rev 89:535–606
39. Reid JK, Zivin JA, Kopin I (1975) Central and peripheral adrenergic mechanism in the development of deoxycorticosterone-saline hypertension in rats. Circ Res 37:569–579
40. Bouvier M, Champlain J (1986) Increased basal and reactive plasma norephinephrine and epinephrine levels in awake DOCA-salt hypertensive rats. J Auton Nerv Syst 15:191–195
41. Fink GD, Johnson RJ, Galligan JJ (2000) Mechanisms of increased venous smooth muscle tone in desoxycorticosterone acetate-salt hypertension. Hypertension 35:464–469
42. Xavier CH, Nalivaiko E, Beif MI, Menenzes GB, Cara DC, Campagnole-Santos ML, Fontes AP (2009) Functional asymmetry in the descending cardiovascular pathways from dorsomedial hypothalamic nucleus. Neuroscience 164:1360–1368
43. Zhang Z, Oppenheimer SM (1997) Characterization, distribution and lateralization of baroreceptor-related neurons in the rat insular cortex. Brain Res 760:243–250
44. Kamosinska B, Nowicki D, Szulczyl P (1989) Control of the heat rate by sympathetic nervous in cats. J Auton Nerv Syst 26: 241–249
45. Furukawa Y, Wallick DW, Martin PJ, Levy MN (1990) Chronotropic and dromotropic responses to stimulation of intracardiac sympathetic nerves to sinoatrial or atrioventricular nodal region in anesthetized dogs. Circ Res 66:1391–1399
46. Zhang ZH, Dougherty PM, Oppenheimer SM (1998) Characterization of baroreceptor-related neurons in the monkey insular cortex. Brain Res 796:303–306
47. Dong J, Mrabet O, Moze E, Li K, Neveu PJ (2003) Lateralization and catecholaminergic neuroimmunomodulation: Prazosin and alpha1/alpha2-adrenergic receptor antagonist, suppresses interleukin-1 and increases interleukin-10 production induced by lipopolysaccharides. NeuroImmunoModulation 10:163–168
48. van Onna M, Houver AJ, Kroon AA, Wierema TK, Koster D, van Engelshoven JM, de Leeuw PW (2003) Asymmetry of renal blood flow in patients with moderate to severe hypertension. Hypertension 41:108–113
49. Davis J, Fujimoto RY, Juarez DT, Hodges KA, Asam JK (2008) Major depression associated with rates of cardiovascular disease state transitions. Am J Manag Care 14:125–129
50. Grippo AJ, Johnson AK (2009) Stress, depression and cardiovascular dysregulation: a review of neurobiological mechanisms and the integration of research from preclinical disease models. Stress 12:1–21