GABAergic modulation of noradrenaline release caused by blood pressure changes in the rat median preoptic area

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Experiments using in-vivo microdialysis methods were conducted to investigate whether blood pressure changes cause an alteration in the release of noradrenaline (NA) in the median preoptic nucleus (MnPO) and whether the γ-aminobutyric acid (GABA) receptor mechanism is involved in the modulation of the pressure response-induced alteration in the NA release. In urethane-anesthetized male rats, intravenous administration of metaraminol, an α-agonist, significantly produced an increase in dialysate NA concentration in the MnPO area accompanied by an elevation in the mean arterial pressure (MAP). Perfusion with GABA (10 μM) through the dialysis probe elicited a significant decrease in either MAP or the NA concentration in the MnPO area. Similar perfusion with either the $GABA_A$ receptor antagonist bicuculline (10 μM) or the $GABA_B$ receptor antagonist phaclofen (10 μM) caused a significant increase in both MAP and the NA release in the MnPO area. Either bicuculline or phaclofen administered together with the metaraminol further enhanced the metaraminol-induced MAP and NA release in the MnPO area. The degree of increases in the both MAP of the NA release was significantly greater in the bicuculline-treated group than in the phaclofen-treated group. These results suggest that the NA release in the MnPO area may be potentiated during an elevation in arterial pressure caused by the metaraminol injection and imply that the NA release may be mediated through $GABA_A$ receptors rather than $GABA_B$ receptors in the MnPO area. *NeuroReport* **28:485–491 Copyright © 2017 The Author(s). Published by Wolters Kluwer Health, Inc.**

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Introduction

The median preoptic nucleus (MnPO), a midline structure within the anteroventral third ventricle region, plays important roles in the regulation of cardiovascular function and body fluid balance [1–12]. The MnPO is densely innervated by noradrenergic nerve terminals arising from the brainstem [13,14]. The noradrenergic system in the MnPO participates in the regulation of cardiovascular and body fluid homeostasis. For example, inactivation of adrenoceptors or depletion of catecholamines in the MnPO area reduces angiotensin II (ANG II)-induced pressor and drinking responses [1–3]. Microinjection of noradrenaline (NA) into the MnPO produces an elevation in arterial pressure and bradycardia [7]. Reduction in body fluid volume enhances release [6] and turnover [12] of NA in the MnPO.

It has been shown that the MnPO contains γ-aminobutyric acid (GABA) neurons and terminals [15], and that GABA and its analogs influence the ANG II-induced pressor and drinking responses through the lamia terminalis along the anterior wall of the third ventricle [10,16]. Previous studies have indicated that the GABA receptor mechanism modulates the release of NA in the MnPO [17,18] and that the organum vasclosum of the lamina terminalis (OVLT), a circumventricular organ containing neurons that participate in the osmoregulation, exerts GABAergic inhibitory influences on the release of NA in the MnPO [17].

These findings led to the proposition that the noradrenergic system in the MnPO may be involved in the regulation of blood pressure response and that GABAergic receptor mechanisms may modulate the NA system. In an attempt to verify this proposition, we used intracerebral microdialysis methods to investigate the effects of intravenous administration of metaraminol, an α-agonist on the mean arterial pressure (MAP) and the release of NA in the MnPO area in male rats under urethane anesthesia. We also examined perfusion with GABA and its antagonists through a microdialysis probe on metaraminol-induced alterations in MAP and the NA release in the MnPO area.

Materials and methods

The experiment was conducted according to the guiding principles of the Physiological Society of Japan.

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Surgical procedures

A total of 52 male Wistar rats weighing 260–320 g were used for the experiments. The animals were anesthetized with urethane (1.4–1.6 g/kg, intraperitoneally). The level of anesthesia was determined by monitoring the response to toe or tail pinch, and further injections of anesthetic were administered as necessary. A trachea cannula was inserted. The femoral artery and vein were then catheterized to allow continuous monitoring of MAP and administration of a peripherally active vasoconstrictor metaraminol, respectively. The animals were placed in a stereotaxic frame. The skull was exposed and small holes were drilled to allow implantation of a guide cannula. A concentric dialysis probe (AI-1-12-1; Eicom Co., Kyoto, Japan) whose tip had a 1.0-mm-long semipermeable membrane (0.2 mm outside diameter) was used. The microdialysis probe guide cannula (AI-12–1; Eicom Co.) was lowered to a coordinate that was 1 mm dorsal to the MnPO as the probe assembly protrudes 1 mm below the ventral tip of the guide cannula when inserted. The stereotaxic coordinates of the guide cannula for the MnPO were 0.3 mm posterior to the Bregma, 0.0 mm lateral to the midline, and 6.6 mm ventral to the cortical surface. The guide cannula was then fixed to the skull with dental cement and small stainless-steel screws. The rectal temperature was maintained at 37–38°C using a heating pad and an infrared lamp during the dialysis experiment.

Microdialysis

Microdialysis in the MnPO area and measurement of NA were performed using procedures described in our previous studies [6,9,11,17–19]. Briefly, after the implantation of the guide cannula, the dialysis probe was inserted into the implanted guide cannula. The probe was perfused continuously at a rate of 2 μl/min using a microinfusion pump (EP-60; Eicom Co.) and a gas-tight syringe (Hamilton Co., Reno, Nevada, USA) with Ringer's solution (147 mM NaCl, 4 mM KCl, 2.3 mM $CaCl_2$). The samples were collected every 20 min into plastic tubes. Five to six hours after the beginning of the perfusion, stable basal NA levels in the dialysates were obtained.

NA analysis

The dialysate were analyzed for concentrations of NA using high-performance liquid chromatography (EP-10; Eicom Co.) with electrochemical detection (ECD-100; Eicom Co.). A mobile phase consisting of 0.1 M sodium acetate, 0.1 M citric acid, 0.75 mM sodium 1-octanesulfonate, 0.3 M EDTA, and 20% methanol (pH 3.9) was used to elute the monoamine from a reverse-phase column (3.0 × 100 mm SC-3ODs column; Eicom Co.). The graphite working electrode was set at +450 mV versus a Ag/AgCl reference electrode and the flow rate was 0.5 ml/min.

Administration of metaraminol

The α-agonist metaraminol (Aldrich, St. Louis, Missouri, USA) was dissolved in isotonic saline and injected into the femoral vein. To maintain elevated blood pressure for 20 min, the drug was administered continuously at a rate of 0.5 μg/min using a microinjection pump. The drug application was separated by 80 min.

Treatment with GABA and its antagonists

GABA (Sigma, St. Louis, Missouri, USA), L-bicuculline methiodide (Sigma), a $GABA_A$ receptor antagonist, and phaclofen (Tocri Neuramin, Bristol, UK), a $GABA_B$ receptor antagonist, were dissolved in Ringer's solution. As we have previously found that perfusion with GABA, bicuculline, or phaclofen at a concentration of 10 μM alters the release of NA in the MnPO area [17,18], this concentration was used for the application of the antagonists in this study. Following the collection of four stable baseline dialysate samples (80 min), the drugs were applied for 20 min directly into the MnPO through the dialysis probe. The antagonist applications were separated by 80 min.

Histology

At the termination of each experiment, the animal was then killed with an overdose of urethane and perfused through the heart with isotonic saline to clear blood, which was followed by 10% formalin for fixation. The brain was removed and stored in the formalin saline before being cut on a freezing microtome at 50 m in transverse sections. Sections were mounted on glass slides and stained with neutral red for microscope examination. The stereotaxic coordinates for the sites of dialysis probe were determined with reference to the atlas of Paxinos and Watson [20].

Statistical analysis

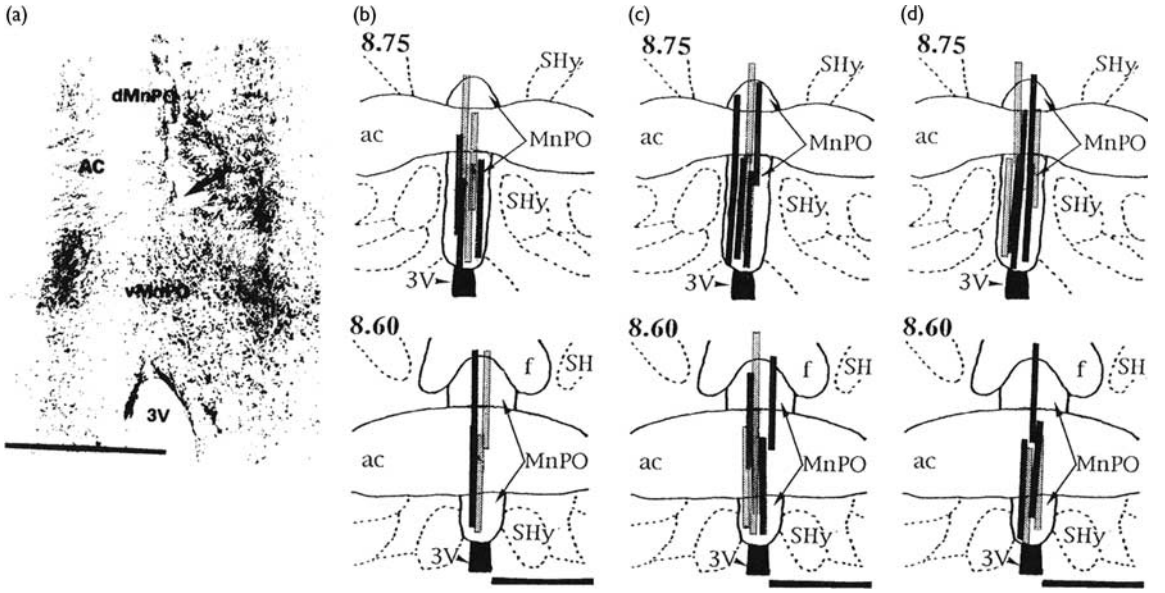
Changes in MAP and the concentrations of NA in brain dialysate were expressed as a percent of the basal level calculated from the last four samples before the first perfusion with the antagonist or the first intravenous injection of metaraminol. Data are presented as mean ± SEM. The results were analyzed by one-way repeated-measures analysis of variance and a subsequent *t*-test. A *P* value of less than 0.05 was required for significance.

Results

The dialysis probe placements

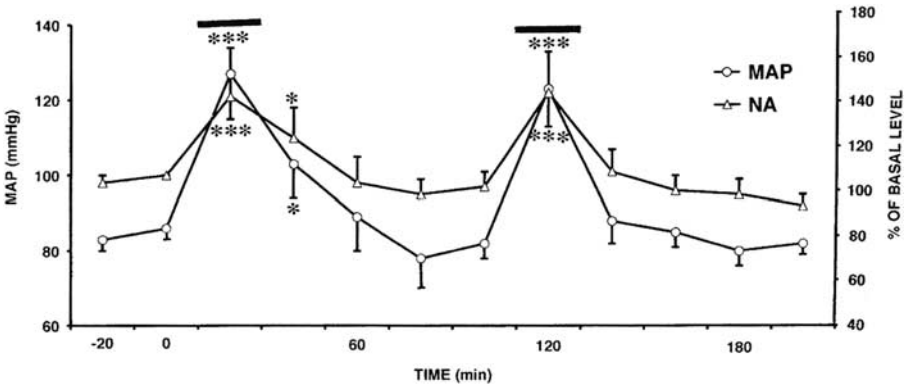
Histological analysis from the rat brains showed that 49 out of 52 rats tested had the probe placement in the region of the MnPO (Fig. 1). The data from the remaining three rats with the probe placement outside the MnPO were not included in the analysis.

Fig. 1



Locations of the dialysis probes. (a) A photograph from neutral red-stained coronal section shows the location of the probe placement (arrow). (b–d) Vertical bars on schematic illustrations (8.75 and 8.60 mm anterior to the interaural line) indicate the locations of the 1-mm long tip the dialysis probe in the region of the median preoptic nucleus (MnPO). (b) Closed and stippled bars show the probe locations of rats that received a repeated intravenous injection of metaraminol ($n = 6$) and γ-aminobutyric acid in the MnPO area ($n = 5$), respectively. (c) Closed and stippled bars indicate the probe placements of rats that received of the bicuculline perfusion combined with the metaraminol injection ($n = 8$) and the bicuculline perfusion alone ($n = 5$), respectively. (d) Closed and stippled bars indicate the probe placements of rats that received the phaclofen perfusion combined with the metaraminol injection ($n = 8$) and the bicuculline perfusion alone ($n = 5$), respectively. 3V, third ventricle; ac, anterior commissure; dMnPO, dorsal median preoptic area; f, fornix; SHy, septohypothalamic nucleus; vMnPO, ventral median preoptic nucleus. Horizontal scale bars indicate 1 mm.

Fig. 2



Effects of repeated intravenous injections of metaraminol on the mean arterial pressure (MAP) and extracellular noradrenaline (NA) in the MnPO area ($n = 6$). In this and subsequent figures NA, the NA values are expressed as the percentage of the sample obtained immediately before the first perfusion. Results in this and subsequent figures are shown as mean ± SEM. Closed horizontal bars indicate the period of the metaraminol injection. $^{*}P < 0.05$, $^{***}P < 0.001$ compared with the value taken immediately before the first metaraminol injection (the basal control level; 0 min).

Changes in MAP and the NA release in the MnPO to intravenous metaraminol

Basal concentrations (immediately before the first drug administration) of NA in 20-min dialysate samples from the MnPO area were 14.9 ± 0.5 pg/40 µl dialysate ($n = 46$). The first intravenous injection of metaraminol elicited significant increases in the NA release that accompanied 42.0 ± 6.9 mmHg (range: 28–52 mmHg) elevation in MAP ($n = 6$, Fig. 2).

The effects of repeated injections of metaraminol on MAP and the NA release in the MnPO area (Fig. 2) were examined. In

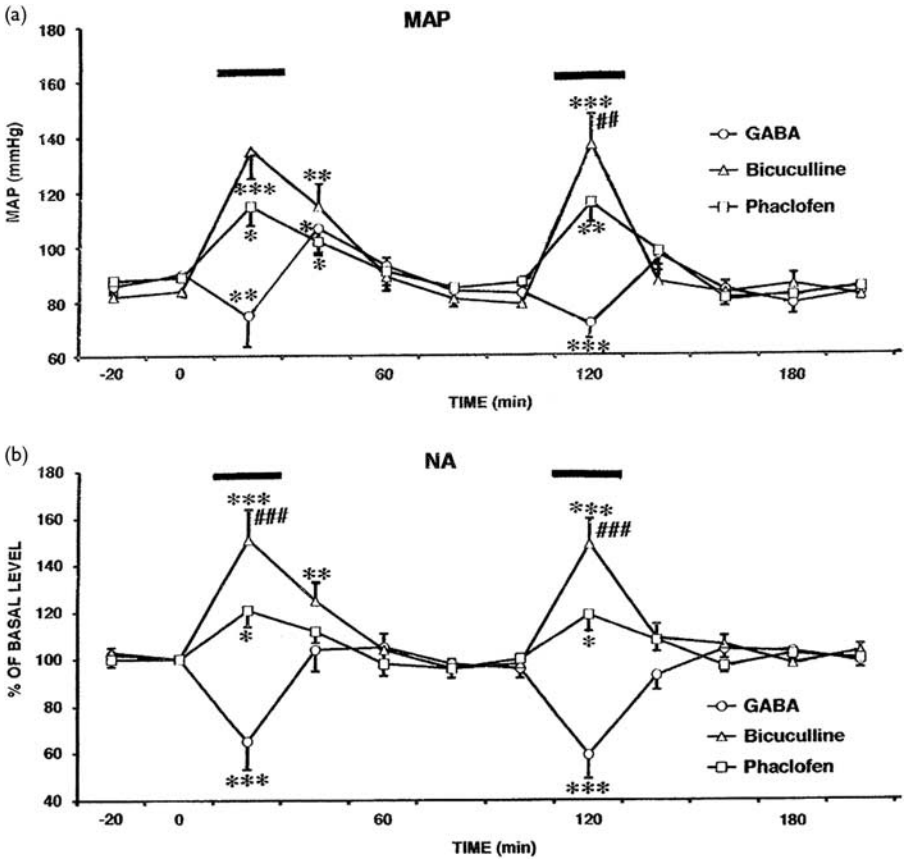
both the first and the second injections, metaraminol injected intravenously led to a significant increase in MAP and the NA release in the MnPO area ($n=6$). There were no significant differences between the first and the second treatments in the degree of the elevation in MAP and the release of NA. Vehicle (isotonic saline) perfusions did not cause any changes in MAP and the NA release in the MnPO area in response to the metaraminol injection ($n=4$, data not shown).

Effects of perfusion with the GABA or its antagonists on the metaraminol-induced changes in MAP and the NA release in the MnPO

Perfusion with GABA through the dialysis probe (Fig. 1b) elicited a significant reduction in either MAP [$n=5$; $F(1,8)=20.003$, $P<0.01$ for the first injection; $F(1,8)=29.673$, $P<0.001$ for the second injection) or the NA release in the MnPO area ($n=5$; $F(1,8)=36.486$, $P<0.001$ for the first injection; $F(1,8)=39.418$, $P<0.001$ for the second injection; Fig. 3]. In both the first and the second GABA administrations, no significant changes in the amounts of MAP and the NA release compared with the control level (immediately before the GABA administration) in 20-min dialysate samples were observed (Fig. 3).

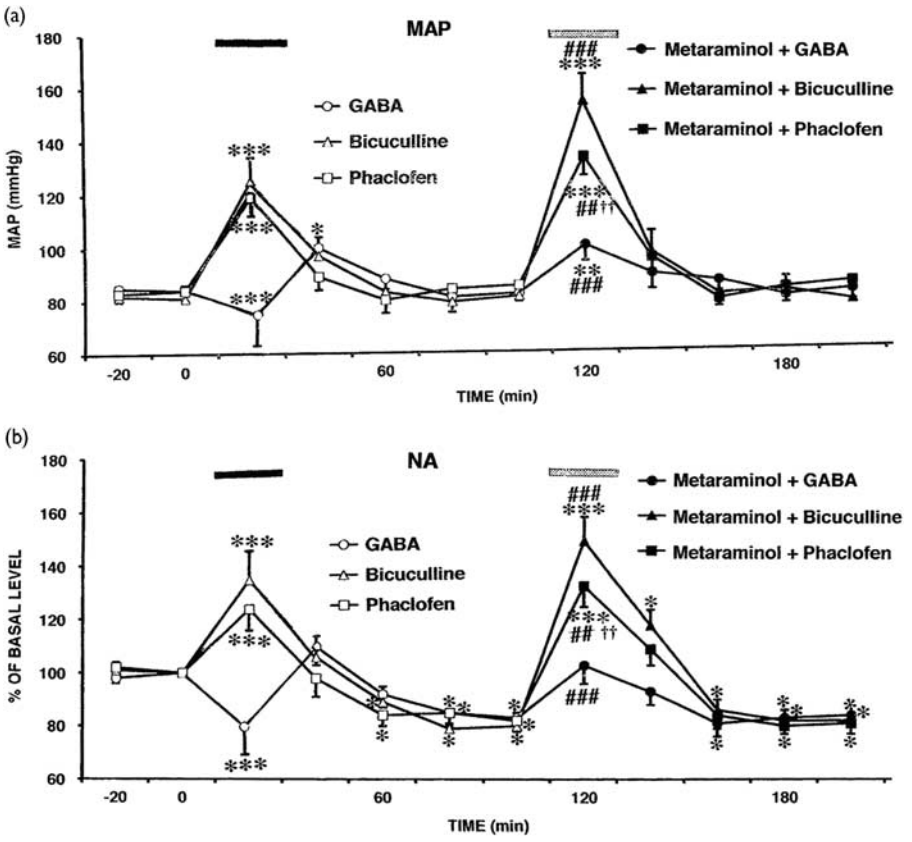
Perfusion with bicuculline (Fig. 1c) significantly enhanced both MAP [$n=5$, $F(1,8)=38.203$, $P<0.001$ for the first injection; $F(1,8)=40.114$, $P<0.001$ for the second injection; Fig. 3a] and the release of NA in the MnPO area [$n=5$, $F(1,8)=36.486$, $P<0.001$ for the first injection; $F(1,8)=39.418$, $P<0.001$ for the second injection; Fig. 3b]. Perfusion with phaclofen (Fig. 1d) also produced a significant elevation in both MAP [$n=5$, $F(1,8)=14.625$, $P<0.05$ for the first injection; $F(1,8)=21.974$, $P<0.01$ for the second injection; Fig. 3a] and the NA release [$n=5$, $F(1,8)=13.670$, $P<0.05$ for the first injection; $F(1,8)=14.449$, $P<0.01$ for the second injection; Fig. 3b]. The effects of repeated perfusions with the GABA antagonists on MAP and the NA release in the MnPO area were also examined. In either the bicuculline-treated or the phaclofen-treated group, there were no significant differences between the first and the second treatments in the number of changes in

Fig. 3



Effects of repeated perfusions with γ-aminobutyric acid (GABA) ($n=5$, circles), bicuculline ($n=5$, triangles), or phaclofen ($n=5$, squares) through the dialysis probe on mean arterial pressure (MAP) (a) and the noradrenaline (NA) release in the median preoptic nucleus area (b). Closed horizontal bars indicate the period of the perfusion with the drugs. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared with the immediately before the first perfusion of each drug (0 min). $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ compared with the corresponding value in the phaclofen perfusion.

Fig. 4



Effects of perfusion with γ-aminobutyric acid (GABA) ($n=5$, circles), bicuculline ($n=8$, triangles), or phaclofen ($n=8$, squares) together with the intravenous injection of metaraminol on the metaraminol injection alone in the amount of mean arterial pressure (MAP) (a) and noradrenaline (NA) release in the median preoptic nucleus area (b). Closed and stippled horizontal bars show the period of the administration of the drugs. Stippled horizontal bars indicate the period of the perfusion with GABA, bicuculline, or phaclofen combined with the metaraminol injection. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared with the basal control level (0 min). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ compared with the corresponding value in the metaraminol alone. $^{\dagger\dagger}P<0.01$ compared with the corresponding value in the metaraminol + bicuculline group.

MAP and the NA release (Fig. 3). Vehicle (Ringer's solution) perfusions did not cause any change in MAP and the NA release in the MnPO area in the drug application ($n=4$; data not shown).

Perfusion with bicuculline significantly enhanced the amount of the increase in either MAP [$n=8$; $F(1,12)=41.812$, $P<0.001$; Figs 2 and 4a] or the NA concentration in the MnPO area [$n=8$, $F(1,12)=43.051$, $P<0.001$, Figs 2 and 4b] in response to the metaraminol injection. Similar perfusion with phaclofen also significantly potentiated the amount of the metaraminol-evoked MAP [$n=8$, $F(1,12)=27.738$, $P<0.01$; Figs 2 and 4a] and the release of NA in the MnPO area [$n=8$, $F(1,12)=27.740$, $P<0.01$; Figs 2 and 4b].

A comparison between the effects of the GABA antagonists on the metaraminol-induced changes in MAP and the NA release

In an attempt to determine whether the influences in MAP and the NA release resulting from the metaraminol injection were mediated by $GABA_A$ or $GABA_B$ receptors, the difference between the degree of the change in the condition combining the metraminol injection with the antagonist application and the total amount of the change in the metaraminol injection alone was calculated in each animal, and compared. In MAP, the differences in the amount in the bicuculline-treated and phaclofen-treated groups were 29.8 ± 7.6 and $12.8\pm4.6\%$, respectively (Figs 2 and 4a). The difference in the amount was significantly greater in the bicuculline-treated group than in the phaclofen-treated group [$F(1,14)=26.748$, $P<0.01$]. In the NA release, the differences in the amount in the bicuculline-treated and phaclofen-treated groups were 15.3 ± 4.3 and $4.1\pm1.2\%$, respectively (Figs 2 and 4b). The difference in the amount was significantly greater in the bicuculline-treated group than in the phaclofen-treated group [$F(1,14)=21.980$, $P<0.01$].

Discussion

In the present study, we found that the release of NA in the MnPO area is increased during the elevation in

arterial pressure caused by intravenous injections of the α-agonist metaraminol, suggesting that the increased NA release in the MnPO area may because of activation of the peripheral baroreceptors in response to the increase in arterial pressure. Previous findings have been shown that the neural projections from the A1 noradrenergic region of the ventrolateral medulla to the MnPO may transmit the peripheral baroreceptor information [8,11]. Thus, the present results lead to the proposition that the noradrenergic inputs from the A1 region may be modulated by GABAergic receptor mechanisms in the MnPO.

The findings in which perfusion with either bicuculline or phaclofen applied alone to the MnPO dialysis site elicits an increase in dialysate NA concentrations suggest the possibility that endogenous GABA acting on both $GABA_A$ and $GABA_B$ receptor mechanisms may inhibit tonically the basal level of NA release in the MnPO area, which are in agreement with the results of previous investigations [17,18]. Our data imply that the increase in the NA release may be mediated at least in part by the reduction of GABAergic inhibitory inputs caused by enhanced arterial pressure. A microdialysis study has reported that a part of the efferent projections from the OVLT may exert the GABAergic inhibitory influence on the release of NA in the MnPO area through predominantly $GABA_B$ receptor mechanisms [18]. Previous observations in several lines have shown that the MnPO receives GABAergic afferent projections from the subfornical organ (SFO), a circumventricular structure lacking a normal blood–brain barrier [4], plays important roles in pressor and drinking responses caused by circulating ANG II [21]. Thus, it is possible that the NA release in the MnPO area may be suppressed by the GABAergic inputs from the SFO through $GABA_A$ receptor mechanisms.

Our results in this study indicate that the amount of antagonist-induced NA release is much greater in the bicuculline-treated group than in the phaclofen-treated group, suggesting that the GABAergic inhibitory effect on the NA release in the MnPO area may be mediated through $GABA_A$ receptors rather than $GABA_B$ receptors in response to an elevation in arterial pressure. Thus, it is tempting to speculate that the difference in the GABA receptor types in the MnPO area may contribute, in part, toward the functional role and/or the responsiveness of NA neurons to several stimuli. Indeed, the GABAergic inhibitory influence from the OVLT on the NA release in the MnPO area is mediated through $GABA_B$ receptors [18].

Several reports have shown that the noradrenergic system in the MnPO plays vital roles in eliciting the pressor and drinking responses caused by ANG II [1,2,10]. Previous investigations have indicated that activation of GABA receptor mechanisms influences the ANG II-induced responses [10,16]. Microdialysis findings have suggested the participation of GABAergic receptor mechanisms in the enhanced NA release in the MnPO area caused by hypovolemia following a subcutaneous injection of polyethylene glycol [17]. Therefore, these data and the present results offer the possibility that the GABAergic system in the MnPO area may be involved in the control of cardiovascular function and body fluid balance by altering the release of NA.

It has been postulated that the interaction between the angiotensinergic and catecholaminergic systems in the MnPO is important for initiating dipsogenic and cardiovascular responses [1,2]. The MnPO receives angiotensinergic inputs from the SFO [22] that are deemed essential for generating these responses to ANG II [4,9,23]. Activation of the SFO increases the excitability of MnPO neurons through ANG II receptors [23] and the NA release in the MnPO area [9], indicating the attribution of the angiotensinergic pathways from the SFO to the control of NA release in the MnPO. The MnPO also receives afferent projections including GABAergic from the OVLT [18], a site known to contain neurons that participate in the osmoregulation [5]. It has been shown that neurons within the MnPO have glutamate (Glu) receptors like those in other regions of the brain [24,25] and that Glu receptors in the MnPO modulate glutamatergic and GABAergic inputs from the SFO [24]. Our recent findings have shown that *N*-methyl-D-asparatate and non-*N*-methyl-D-asparatate Glu receptor mechanisms in the MnPO participate in the noradrenergic regulatory system for body fluid balance [19]. These experimental observations and our data raise the hypothesis that the GABAergic receptor mechanisms in the MnPO area may play important roles in the convergence or integration for the various afferent signals that modulate the cardiovascular response, body fluid volume, and plasma osmolality.

Conclusion

The present study shows that the NA release in the MnPO area is enhanced during an elevation in arterial pressure induced by an intravenous administration of metaraminol and the enhanced NA release may be mediated in part through $GABA_A$ receptors rather than $GABA_B$ receptors in the MnPO area.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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