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Short communication

Does the median preoptic nucleus contribute to sympathetic hyperactivity in spontaneously hypertensive rats?

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ABSTRACT

The present study sought to determine the involvement of median preoptic nucleus (MnPO) in the regulation of the cardiovascular function and renal sympathetic activity in normotensive (NT) and spontaneously hypertensive rats (SHR). MnPO inhibition evoked by Muscimol (4 mM) nanoinjections, elicited fall in MAP and renal sympathoinhibition in NT-rats. Surprisingly, in SHRs these responses were greater than in NT-rats. These results demonstrated, for the first time that MnPO was involved in the tonic control of sympathetic activity in NT and SHRs. Furthermore, our data suggest the MnPO involvement in the increased sympathetic outflow and consequent arterial hypertension observed in SHRs.

1. Introduction

Multiple evidences in the literature point to the central nervous system (CNS) participation in the development and maintenance of the hypertension (Blanch et al., 2013; Toney et al., 2010). Moreover, studies have shown evidence of an increase in sympathetic activity in many hypertension models (Oliveira-Sales et al., 2014; Toney et al., 2010). In rats with renovascular hypertension or spontaneously hypertensive rats (SHR), increase in sympathetic tone and plasma concentration of noradrenaline were observed (Linz et al., 2015; Oliveira-Sales et al., 2014). Together these studies suggest direct role of sympathetic nervous system (SNS) in the development and maintenance of this pathology.

Previous experimental investigations demonstrated the involvement of MnPO in the ingestive behavior, endocrine and cardiovascular adjustments induced by acute changes in volume or composition of the extracellular fluid compartment (EFC; (McKinley and Johnson, 2004; Pedrino et al., 2009). Lesion that encompasses the MnPO region reduced sodium (Na^+) ingestion evoked by systemic sodium depletion (De Luca et al., 1992; Gardiner et al., 1986), and vasopressin secretion in response to hyperosmolarity (Mangiapane et al., 1983; McKinley et al., 2004). Overall, these studies illustrated the important role of MnPO in body fluid homeostasis. However, the participation of MnPO in the tonic control of SNS in normovolemic condition remains to be clarified. Thus, we tested the hypothesis that, besides the involvement of the MnPO in reflex responses induced by acute changes in the EFC, this nucleus may also be involved in the tonic autonomic and cardiovascular regulation. Moreover, we investigated the participation of MnPO in the increase in sympathetic nerve activity and arterial blood pressure (ABP) in SHR.

2. Methods

2.1. Animals

Male Wistar normotensive (NT) rats and SHRs weighing 250–350 g were used. The animals were housed individually in stainless steel cages in a room with controlled temperature (23 ± 2 °C), water and food *ad libitum*. Lights were on from 7:00 am to 7:00 pm. The animals were provided by the *Universidade Federal de Goiás* (UFG). The protocols used in this work were approved by the ethics committee of the UFG (protocol number: 34/12) and performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Surgical procedures

The rats were anesthetized with urethane (1.2 g/kg, i.v.; Sigma-Aldrich, St. Louis, MO, USA) and the femoral vein and artery were

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catheterized for drug administration and recording of ABP, respectively. Tracheostomy was performed to reduce airway resistance. The rats were positioned in a stereotaxic apparatus for craniotomy and instrumented for recording the renal sympathetic nerve activity (RSNA).

2.3. ABP and electrocardiogram recording

In order to record the ABP, the arterial catheter was connected to a pressure transducer that was attached to a Bridge Amplifier (FE221; ADInstruments, Colorado Springs, CO, USA). The pulsatile ABP was recorded continuously with a PowerLab System (ADInstruments, Colorado Springs, CO, USA). The mean arterial pressure (MAP) was calculated from the pulsatile signal using the LabChart program (Chart v7.3.7, ADInstruments, Colorado Springs, CO, USA).

Analog signals of the electrocardiogram (ECG), obtained through electrodes positioned in the forelimbs, were amplified 1000 times and filtered between 100 and 1000 Hz (ECG100C; ADInstruments, Colorado Springs, CO, USA). The heart rate (HR) was calculated as instantaneous frequency of the ECG signal (Chart v7.3.7, ADInstruments, Colorado Springs, CO, USA).

2.4. Renal sympathetic nerve activity recording

For recording the RSNA, the renal nerve was located with the assistance of the microscope, carefully dissected and placed on a pair of bipolar silver electrodes coupled to amplifier (P511 AC, Grass Technologies; Warwick, USA Bridge). The RSNA was amplified 20,000 times and filtered between 30 and 1000 Hz. To quantify the noise of the signal obtained at the end of the experiment, ganglionic blocker hexamethonium (30 mg/kg, iv., Sigma-Aldrich, St. Louis, MO, USA) was administered. The RSNA signal was rectified and integrated (resetting every 1 s; Chart 7 v7.3.7; ADInstruments, Colorado Springs, CO, USA).

2.5. Nanoinjections into MnPO

NT rats and SHRs were placed in ventral decubitus on a stereotaxic apparatus and the craniotomy was performed for positioning of the glass micropipette that was coupled to a syringe forming a pressure nanoinjection system. Then, 100 nl of saline (NaCl; 150 mM) and 4 mM muscimol ($GABA_A$ agonist; Sigma–Aldrich, St. Louis, MO, USA) were nanoinjected into MnPO, according to the following coordinates: 0.6 mm rostral to the bregma, at a depth of 7.1 mm below the dorsal surface of the brain, modified from: (Paxinos and Watson, 1998). As a negative control, saline 150 mM and 4 mM muscimol (100 nl) were nanoinjected directly into the third ventricle at the following coordinates: 0.0 mm rostral to the bregma, at a depth of 7.1 mm below the dorsal surface of the brain. At the end of the experiment, 100 nl of a solution of Evans Blue (4%; Sigma-Aldrich, St. Louis, MO, USA) were nanoinjected at the same site of previous nanoinjections for further histological confirmation.

2.6. Histology

At the end of the experiments the animals were perfused with a (NaCl; 150 mM) saline solution, followed by 10% formaldehyde (LabSynth, Itapira, SP, Brazil). Then, the brain was removed and fixed in the same formaldehyde solution and subsequently stored for a period of 48 h in 30% sucrose solution. The brains were dissected into 40 μm coronal sections with the aid of a freezing microtome (Leica, Wetzlar, Germany). To determine the sites of nanoinjections into MnPO, the sections obtained from this hypothalamic region were stained using neutral red.

2.7. Statistical analysis

Statistical analysis and graph confections were done using GraphPad Prism software (v 5.1). The baseline values were compared between the groups using an unpaired Student's *t*-test. The autonomic and cardiovascular effects induced by saline and muscimol nanoinjections into the MnPO and third ventricle were analyzed by a one-way ANOVA, followed by the Newman–Keuls test. Value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Histological analysis

Fig. 1A shows photomicrograph of a coronal section of the forebrain of a representative site with 4% Evans blue nanoinjection. Analysis of the spread of dye nanoinjected at the end of the experiment showed that the drug injection sites were confined to the region that included the MnPO (Fig. 1B).

3.2. Participation of the median preoptic nucleus in the tonic control of cardiovascular and sympathetic parameters in NT and SHRs

Both, NT ($n = 6$) and SHR ($n = 6$) exhibited a similar body weight (278.6 ± 9.8 *vs.* 287.8 ± 18.7) and HR baseline values (367.5 ± 15.1 bpm *vs.* 378.2 ± 15.7 bpm). The baseline RSNA values of SHR and NT rats were 0.0790 ± 0.0185 a.u. and 0.0457 ± 0.0091 a.u., respectively. As expected, the baseline MAP in SHR (124.0 ± 0.7 mm Hg) was higher than in NT rats (108.4 ± 2.6 mm Hg).

Nanoinjections of saline (150 mM NaCl) did not change MAP (NT: Δ 0.4 ± 0.2 mm Hg/Δ% 0.4 ± 0.2%; SHR: Δ0.2 ± 0.4 mm Hg/Δ% 0.3 ± 0.4%), HR (NT: Δ 0.7 ± 0.9 bpm/Δ% 0.2 ± 0.3%; SHR: Δ 1.1 ± 1.4 bpm/Δ% 0.2 ± 0.4%) and RSNA (NT: Δ% 0.4 ± 0.2%; SHR: Δ% −0.2 ± 1.3%; Fig. 2A, C, D and E).

The pharmacological inhibition of the MnPO changed the cardiovascular and autonomic parameters in both NT and SHR. Fig. 2A and B represent typical representative tracings of cardiovascular and autonomic changes caused by nanoinjections into the MnPO of NT and SHR, respectively.

The inhibition of MnPO promoted fall in MAP of NT rats (Δ −17.0 ± 1.2 mm Hg/Δ% −15.4 ± 1.1%). However, this response was strikingly greater ($p < 0.05$) in SHR (Δ −31.6 ± 5.0 mm Hg/Δ% −26.8 ± 3.9%; Fig. 2A, B and C). The range of negative chronotropy in response to muscimol nanoinjections into MnPO were observed in NT (Δ −55.4 ± 12.3 bpm/Δ% −14.3 ± 3.5%; Fig. 2A and D) and in SHR (Δ −18.3 ± 7.2 bpm/Δ% −4.8 ± 2.0%; Fig. 2B e D).

Inhibition of MnPO neurons by muscimol resulted in renal sympathoinhibition in both groups. This pharmacologic blockade resulted in the decrease of RSNA; an effect that elicited greater ($p < 0.05$) impact in SHR (Δ% −54.4 ± 6.2%; Fig. 2B and E) than in NT (Δ% −37.5 ± 3.94%; Fig. 2A and E).

3.3. Muscimol nanoinjections into the third ventricle of NT and SHRs

The saline nanoinjections in third ventricle did not promotes changes in MAP, HR and RSNA (Δ −0.2 ± 0.8 mm Hg/Δ% −0.2 ± 0.8% *vs.* Δ1.3 ± 0.7 mm Hg/Δ% 1.1 ± 0.6%; Δ 0.7 ± 0.3 bpm/Δ% 0.2 ± 0.1% *vs.* Δ −0.1 ± 2.4 bpm/Δ% 0.2 ± 0.9%; Δ%1.7 ± 0.1% *vs.* Δ%1.6 ± 0.4%) in NT ($n = 4$) and SHR ($n = 4$), respectively. Muscimol nanoinjections into the third ventricle did not modify any parameters in NT(MAP: Δ 0.5 ± 1.6 mm Hg/Δ% 0.4 ± 1.5%; HR: Δ 1.4 ± 1.5 bpm/Δ% 0.5 ± 0.5%; RSNAΔ% 0.5 ± 0.8%;)and SHR (MAP: Δ −1.8 ± 0.4 mm Hg/Δ% −1.3 ± 0.3%; HR:Δ −0.4 ± 2.1 bpm/Δ% −0.1 ± 0.6%; RSNA: Δ% −1.6 ± 1.0%). These results demonstrate that the responses observed were not mediated by unspecific action of muscimol in neuronal structures around third ventricle.

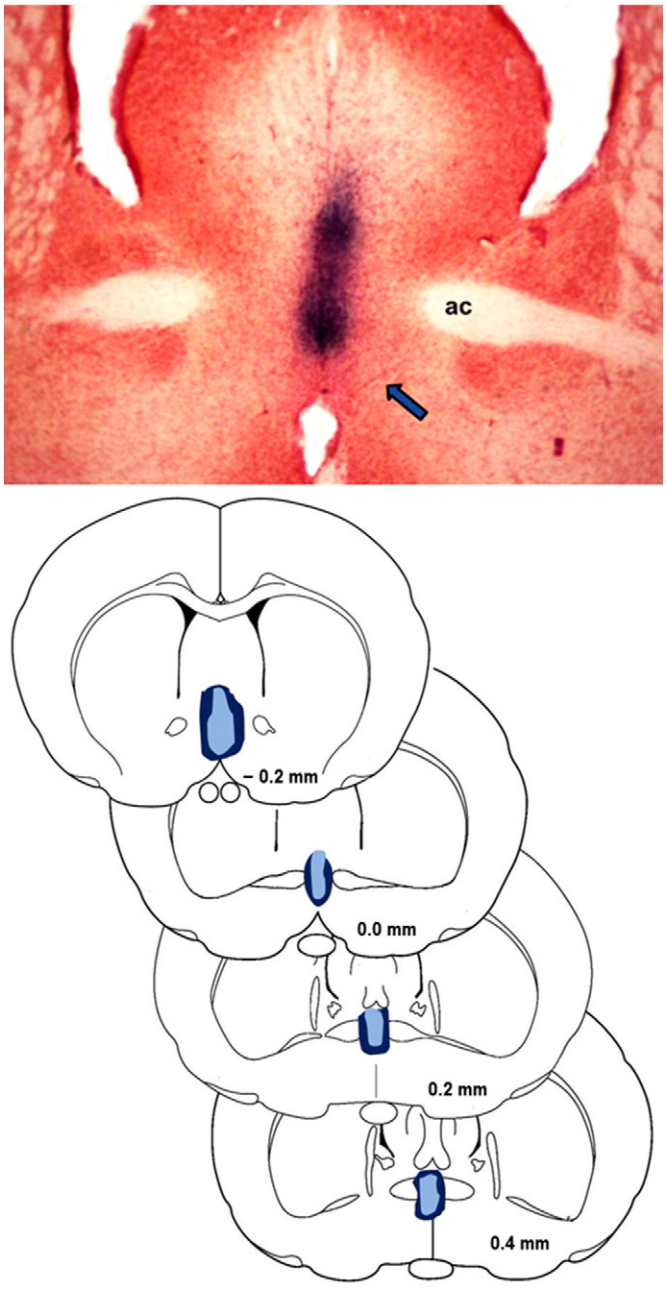

Fig. 1. (A) Photomicrograph of a coronal brain section from one representative rat, showing the nanoinjection site into the Median Preoptic Nucleus (MnPO; arrow). ac, anterior commissure. (B) Four sequential coronal sections showing the approximate spreading of the drugs nanoinjections into the MnPO. Dark blue area: maximal spreading. Light blue area: minimum spreading.

4. Discussion

The MnPO have been described as an integral part of the blood fluid and endocrine control (Llewellyn et al., 2012; Pedrino et al., 2009; Stocker and Toney, 2005). Despite evidences highlighting the importance of the MnPO in hydroelectrolytic balance, the participation of this nucleus in tonic regulation of ABP and sympathetic nerve activity remains unknown. In the present study, we have demonstrated the key role performed by the MnPO in the RSNA tonic control and sympathetic hyperactivity observed in SHR. Therefore, these results strongly indicate that the MnPO is a supramedullary area responsible for maintaining the renal sympathetic hyperactivity and consequent hypertension observed in SHR.

The renal territory is crucial to the hydroelectrolytic balance as the kidneys are involved in corporal excretion of excess plasma sodium (Antunes-Rodrigues et al., 2004; Brody and Johnson, 1980; Pedrino et al., 2008; Toney and Stocker, 2010; Yasuda et al., 2000; Da Silva et al., 2013). The renal blood flow is thinly regulated by the renal sympathetic nerves and. Hormonal factors such as atrial natriuretic peptide, oxytocin and vasopressin (Amaral et al., 2014; Pedrino et al., 2008).

Based on limited electrophysiological and anatomical experiments, studies have demonstrated that the involvement of MnPO in cardiovascular and endocrine regulation is due to its inputs to magnocellular and parvocellular regions of the paraventricular nucleus (PVN) (Llewellyn et al., 2012; Stocker and Toney, 2005). Recently, Llewellyn et al. (2012) demonstrated that that the activation of MnPO increases the activity of PVN neurons. Taken together, these results indicate that the MnPO could be a source of excitatory inputs to the sympathetic premotor neurons of the PVN (Llewellyn et al., 2012).

Hence, it is conceivable that MnPO could modulate, through its PVN connections, the pathways that are involved in sympathetic outflow, and consequently the cardiovascular function. Based on these evidences (Allen, 2002; Takeda et al., 1991), it is conceivable that the inhibition of MnPO could decrease the activity of PVN and RVLM neurons which subsequently result in decreases in the RSNA and MAP. In fact, in the present study, we observed that the inhibition of the MnPO caused decrease in the RSNA and BP in NT rats. Moreover, we also observed that inhibition of MnPO potentiated hypotension and renal sympathoinhibition in SHR, indicate hyperactivity of the nucleus in this hypertension model. To the best of our knowledge, no other study has demonstrated that MnPO could play a central role in the increase of RSNA and consequent hypertension observed in SHR.

Recent investigations from our laboratory indicating the participation of the MnPO in the ABP control. Silveira et al. (2014) showed that the blockaded of MnPO promoted hypotension in NT rats. Consistent with these results, we have demonstrated that inhibition of the MnPO promotes hypotension in both normotensive and hypertensive rats. Moreover, the present study has advanced on current knowledge, demonstrating, for the first time in the literature, that the inhibition of MnPO induces renal sympathoinhibition, thereby indicating the involvement of this nucleus in the tonic regulation of sympathetic nerve activity.

The MnPO also receives afferences of the regions that participate in cardiovascular modulation, such as catecholaminergic neurons of the Nucleus of the Solitary Tract (NTS; A2 neurons) and ventrolateral medulla (*A*1 and *C*1 neurons; (Tucker et al., 1987). Moreira et al. (2009) demonstrated the importance of the NTS in the maintenance of hypertension in SHR. In this study, the authors showed that lesion of the commissural NTS and Anteroventral Third Ventricle region (AV3V region) reduced the BP of the SHRs (Moreira et al., 2009). The MnPO receives dense projections from the NTS. Hence, both electrolytic lesions of the NTS and pharmacological blockade of the MnPO promote decrease in MAP (which is more expressive in the hypertensive animals). It is not unreasonable to suppose that the MnPO hyperactivity in SHR originate, at least partly, from the NTS.

In summary, the present results show evidence of MnPO neurons participation in the tonic control of BP, RSNA and consequently, in the maintenance of hypertension in SHR. However, further studies are required to elucidate the pathways and neurotransmitters in the MnPO involved in the control of sympathetic nerve activity.

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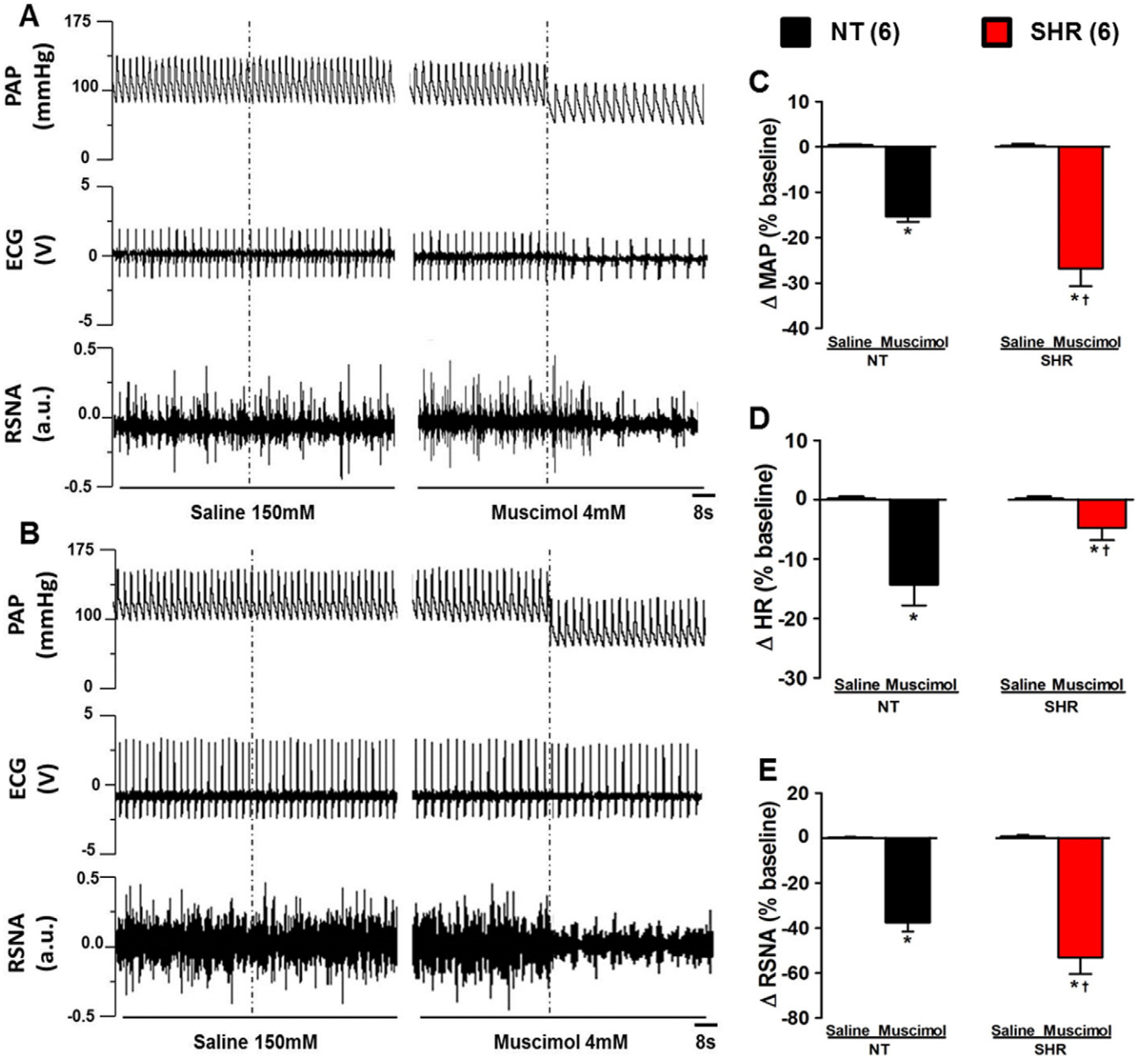

Fig. 2. Representative tracing showing the changes in pulsatile arterial pressure (PAP); electrocardiogram (ECG); renal sympathetic nerve activity (RSNA) and integrate of RSNA (a.u.) induced by nanoinjections of saline (NaCl; 150 mM) and muscimol ($GABA_A$ agonist; 4 mM) into MnPO in normotensive rats (A) and hypertensive (B) rats. Maximal responses in mean arterial pressure (Δ% MAP, C), heart rate (Δ% HR, D) and renal sympathetic nerve activity (Δ% RSNA; E) promotes by saline and muscimol nanoinjections in NT and SHR. * different from the saline nanoinjections; ǂ different from the NT rats; $p < 0.05$.

Moreover, all authors have contributed sufficiently in this study to be included as authors. There are no conflicts of interest.

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