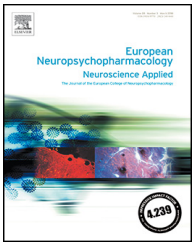
European Neuropsychopharmacology (2019) 29, 672–680



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$GABA_A$ but not $GABA_B$ receptors in the lateral hypothalamus modulate the tachycardic response to emotional stress in rats

Lucas Gomes-de-Souza[a,b], **Ricardo Benini**[a,b], **Willian Costa-Ferreira**[a,b], **Carlos C. Crestani**[a,b,*]

[a] *Laboratory of Pharmacology, School of Pharmaceutical Sciences, São Paulo State University (UNESP), Rodovia Araraquara-Jau Km 01 (Campus Universitário), Campus Ville, 14800-903 Araraquara, SP, Brazil*
[b] *Joint UFSCar-UNESP Graduate Program in Physiological Sciences, São Carlos, SP, Brazil*

Received 28 October 2018; received in revised form 5 February 2019; accepted 2 March 2019

KEYWORDS
Restraint stress;
Hypothalamus;
GABAergic neurotransmission;
Blood pressure;
Heart rate;
Sympathetic activity

Abstract
The lateral hypothalamus (LH) has been described as one of the hypothalamic areas involved in the behavioral and physiological responses triggered by aversive stimuli. Previous studies indicated involvement of the LH in cardiovascular responses to stress. Despite this evidence, the local neurochemical mechanisms involved in LH control of stress responses is still poorly understood. Therefore, in the present study, we investigated the role of GABAergic neurotransmission within the LH in cardiovascular responses induced by an acute session of restraint stress in rats. For this, we evaluated the effect of bilateral microinjection of selective antagonists of either $GABA_A$ or $GABA_B$ receptors into the LH on arterial pressure increase, heart rate (HR) increase and reduction in tail skin temperature induced by restraint stress. We found that microinjection of the selective $GABA_A$ receptor antagonist SR95531 into the LH decreased the increase in HR caused by restraint stress, but without affecting the increase in arterial pressure increase or the reduction in tail skin temperature. Conversely, LH treatment with the selective $GABA_B$ receptor antagonist CGP35348 did not affect the restraint-evoked cardiovascular changes. These

* Corresponding author at: Laboratory of Pharmacology, School of Pharmaceutical Sciences, São Paulo State University (UNESP), Rodovia Araraquara-Jau Km 01 (Campus Universitário), Campus Ville, 14800-903 Araraquara, SP, Brazil.
E-mail address: crestani@fcfar.unesp.br (C.C. Crestani).

https://doi.org/10.1016/j.euroneuro.2019.03.001
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findings indicate that GABAergic neurotransmission in the LH, acting through activation of local $GABA_A$ receptors, plays a facilitatory role in the tachycardic response observed during aversive threats.
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Introduction

Physiological adjustments play a prominent role in survival and welfare by maintaining homeostasis during aversive situations (Crestani, 2016; Sterling, 2012; Ulrich-Lai and Herman, 2009). Changes in autonomic activity evoke immediate responses during stress, including increased arterial pressure and heart rate (HR); redistribution of blood flow, including reduced cutaneous and visceral perfusion and increased blood flow to the skeletal muscles; and modulation of baroreflex activity (Crestani, 2016; Dampney, 2015; Dampney et al., 2008). Increased sympathetic activity reduces cutaneous blood flow (Blessing, 2003), which in turn decrease skin temperature during aversive threats (Busnardo et al., 2019; Vianna and Carrive, 2005).

Cardiovascular responses during aversive threats are triggered by overlapping circuits in the central nervous system (CNS) (Dampney, 2015; Fontes et al., 2011; Myers, 2017; Ulrich-Lai and Herman, 2009). The lateral hypothalamus (LH) is a complex diencephalic area which has been found to be activated by several aversive stimuli (Briski and Gillen, 2001; Cullinan et al., 1995; Furlong et al., 2014; Motta and Canteras, 2015). Accordingly, the LH has been linked to the central network that regulates the stress responses (Myers, 2017; Ulrich-Lai and Herman, 2009). Regarding the cardiovascular changes, initial studies provided functional evidence of a role of this hypothalamic area in control of arterial pressure response evoked by conditioned aversive stimuli (Iwata et al., 1986; LeDoux et al., 1988). The influence of the LH in the control of cardiovascular responses to unconditioned aversive threats was reported only recently (Deolindo et al., 2013). In this sense, acute bilateral LH neurotransmission inhibition, caused by local treatment with the nonselective synaptic blocker $CoCl_2$, enhanced tachycardia evoked by an acute session of restraint stress, but without affecting the pressor response (Deolindo et al., 2013). This effect was mimicked by local treatment with a selective NMDA glutamatergic receptor antagonist, thus indicating the involvement of local glutamatergic neurotransmission in LH control of cardiovascular responses to restraint stress (Deolindo et al., 2013). However, despite reports that the control of physiological and behavioral responses by the LH reflects a balance of local excitatory and inhibitory inputs (Stanley et al., 2011), the role of local inhibitory neurochemical mechanisms in LH control of stress responses has never been evaluated.

GABA has been shown to be a dominant neurotransmitter in the hypothalamus (Decavel and Van Den Pol, 1990). Accordingly, inhibitory control of LH neurons by local GABAergic (inter)neurons has been described previously (Burt et al., 2011; Konadhode et al., 2015). GABAergic inputs from limbic structures involved in the control of stress-evoked responses was also reported within the LH (Gritti et al., 1994; Jennings et al., 2013). Despite these findings, the role of LH GABAergic neurotransmission in the control of stress responses has not been investigated. Therefore, this study aimed to evaluate the hypothesis that GABAergic neurotransmission within the LH is involved in the control of cardiovascular responses evoked by an acute session of restraint stress in rats. To test this hypothesis, animals received intra-LH bilateral microinjections of selective $GABA_A$ or $GABA_B$ receptor antagonists.

Experimental procedures

Animals

Twenty male Wistar rats weighing 240–260 g (60-days-old) were used. Animals were obtained from the animal breeding facility of the São Paulo State University (UNESP) (Botucatu, SP, Brazil), and were housed in plastic cages in a temperature-controlled room at 24 °C in the Animal Facility of the Laboratory of Pharmacology, School of Pharmaceutical Sciences – UNESP. They were kept under a 12:12 h light-dark cycle (lights on between 7:00 am and 7:00 pm) with free access to water and standard laboratory food. Housing conditions and experimental procedures were approved by the Ethical Committee for Use of Animals of the School of Pharmaceutical Sciences/UNESP (approval # 61/2015), which complies with Brazilian and international guidelines for animal use and welfare.

Surgical preparation

Five days before the trial, rats were anesthetized with tribromoethanol (250 mg/kg, i.p.). After scalp anesthesia with 2% lidocaine, the skull was exposed and stainless-steel guide cannulas (26 G, 13 mm-long) were bilaterally implanted into the LH at a position 1 mm above the site of injection, using a stereotaxic apparatus (Stoelting, Wood Dale, IL, USA). Stereotaxic coordinates for cannula implantation into the HL were: antero-posterior = +6.2 mm from interaural line; lateral = 1.8 mm from the medial suture; dorso-ventral = −7.6 mm from the skull (Paxinos and Watson, 1997). Cannulas were fixed to the skull with dental cement and one metal screw. After surgery, the animals received a poly-antibiotic containing streptomycins and penicillins (560 mg/ml/kg, i.m.) to prevent infection and the non-steroidal anti-inflammatory flunixin meglumine (0.5 mg/ml/kg, s.c.) for post-operation analgesia.

One day before the trial, rats were anesthetized with tribromoethanol (250 mg/kg, i.p.), and a catheter (Clay Adams, Parsippany, NJ, USA) filled with a solution of heparin (50 UI/ml, Hepamax-S®, Blausiegel, Cotia, SP, Brazil) diluted in saline (0.9% NaCl) was inserted into the abdominal aorta through the femoral artery for cardiovascular recording. Catheter was tunneled under the cutaneous

and exteriorized on the animal's dorsum. After surgery, the non-steroidal anti-inflammatory flunixin meglumine (0.5 mg/ml/kg, s.c.) was administered for post-operation analgesia. The animals were kept in individual cages during the postoperative period and cardiovascular recording.

Blood pressure and heart rate recording

The catheter implanted into the femoral artery was connected to a pressure transducer (DPT100, Utah Medical Products Inc., Midvale, UT, USA). Pulsatile arterial pressure was recorded using an amplifier (Bridge Amp, ML224, ADInstruments, Australia) and an acquisition board (PowerLab 4/30, ML866/P, ADInstruments, NSW, Australia) connected to a personal computer. Mean arterial pressure (MAP) and HR values were derived from the pulsatile arterial pressure recording.

Tail cutaneous temperature measurement

Tail cutaneous temperature was recorded using a thermal camera (IRI4010, Infra Red Integrated Systems Ltd., Northampton, UK). The analysis was performed using a software for thermographic analysis, and temperature was represented by color intensity variations (Busnardo et al., 2019; Vianna and Carrive, 2005). For image analysis, the temperature was measured on five points along the animal's tail, and the mean value was calculated for each recording (Adami et al., 2017; Gomes-de-Souza et al., 2016).

Drugs and solutions

SR95531 (selective $GABA_A$ receptor antagonist) (Tocris, Westwoods Business, Park Ellisville, MO, USA), CGP35348 (selective $GABA_B$ receptor antagonist) (Tocris), tribromoethanol (Sigma–Aldrich, St. Louis, MO, USA) and urethane (Sigma–Aldrich) were dissolved in saline (NaCl 0.9%). Flunixin meglumine (Banamine®; Schering-Plough, Cotia, SP, Brazil) and the poly-antibiotic preparation (Pentabiotico®; Fort Dodge, Campinas, SP, Brazil) were used as provided.

Drug microinjection into the lateral hypothalamus

The needles (33 G, Small Parts, Miami Lakes, FL, USA) used for microinjection into the LH were 1 mm longer than the guide cannulas and were connected to a 2 μL syringe (7002-KH, Hamilton Co., Reno, NV, USA) using a PE-10 tubing (Clay Adams, Parsippany, NJ, USA). Needles were carefully inserted into the guide cannulas without restraining the animals, and drugs were injected in the final volume of 100 nL (Crestani et al., 2009; Deolindo et al., 2013).

Restraint stress

Each rat was placed in a plastic cylindrical restraint tube (diameter 6.5 cm, length 15 cm), ventilated by holes (1 cm diameter) that made up approximately 20% of the tube surface. Restraint lasted 60 min (Deolindo et al., 2013), and immediately after the end of the stress exposure rats were returned to their home cages. Each rat was submitted to only one session of restraint in order to avoid habituation.

Experimental protocols

Animals were brought to the experimental room in their own cages. Animals were allowed at least 60 min to adapt to the experimental room conditions, such as sound and illumination, before the experiment was commenced. The experimental room was temperature-controlled (24 °C) and acoustically isolated from the other rooms.

Independent sets of animals received bilateral microinjections into the LH of the selective $GABA_A$ receptor antagonist SR95531 (0.01 nmol/100 nL, $n = 6$), the selective $GABA_B$ receptor antagonist CGP35348 (10 nmol/100 nL, $n = 7$) or vehicle (saline, 100 nL, $n = 7$). The drug dosage was selected according to the Ki value of the drug (Heaulme et al., 1986; Olpe et al., 1990), as well as the dosage used in previous studies that performed intra-hypothalamic microinjections (Zhong et al., 2008; Zhou et al., 2014). Ten minutes after LH pharmacological treatment, the animals underwent a 60 min session of restraint stress.

Cardiovascular recording began at least 30 min before the onset of restraint and was performed throughout the session of stress. The tail cutaneous temperature was measured 10, 5 and 0 min before the restraint for baseline values, and at 10, 20, 40 and 60 min during restraint (Busnardo et al., 2019; Gomes-de-Souza et al., 2016). Each animal received a single pharmacological treatment and was submitted to one session of restraint.

Histological determination of the microinjection sites

At the end of experiments, rats were anesthetized with urethane (250 mg/ml/200 g body weight, i.p.) and 100 nL of 1% Evan's blue dye was injected into the brain as a marker of the injection site. Then, the chest was surgically opened, the descending aorta occluded, the right atrium severed and the brain perfused with 0.9% NaCl followed by 10% formalin through the left ventricle. Afterwards, the brain was removed and post-fixed in 10% formalin for at least 48 h at 4 °C. Serial 40 μm-thick sections of the LH region were cut using a cryostat (CM1900, Leica, Wetzlar, Germany). The actual placement of the microinjection needles was determined according to the rat brain atlas of Paxinos and Watson (1997) by analyzing the serial sections under a light microscopy.

Data analysis

Data were expressed as mean ± SEM. Basal values of MAP, HR and tail cutaneous temperature before and after LH pharmacological treatment were compared using the Student's *t*-test. Time-course curves of cardiovascular and cutaneous temperature changes were analyzed using the two-way ANOVA, with treatment as a main factor and time as a repeated measure, followed by Bonferroni's *post hoc*

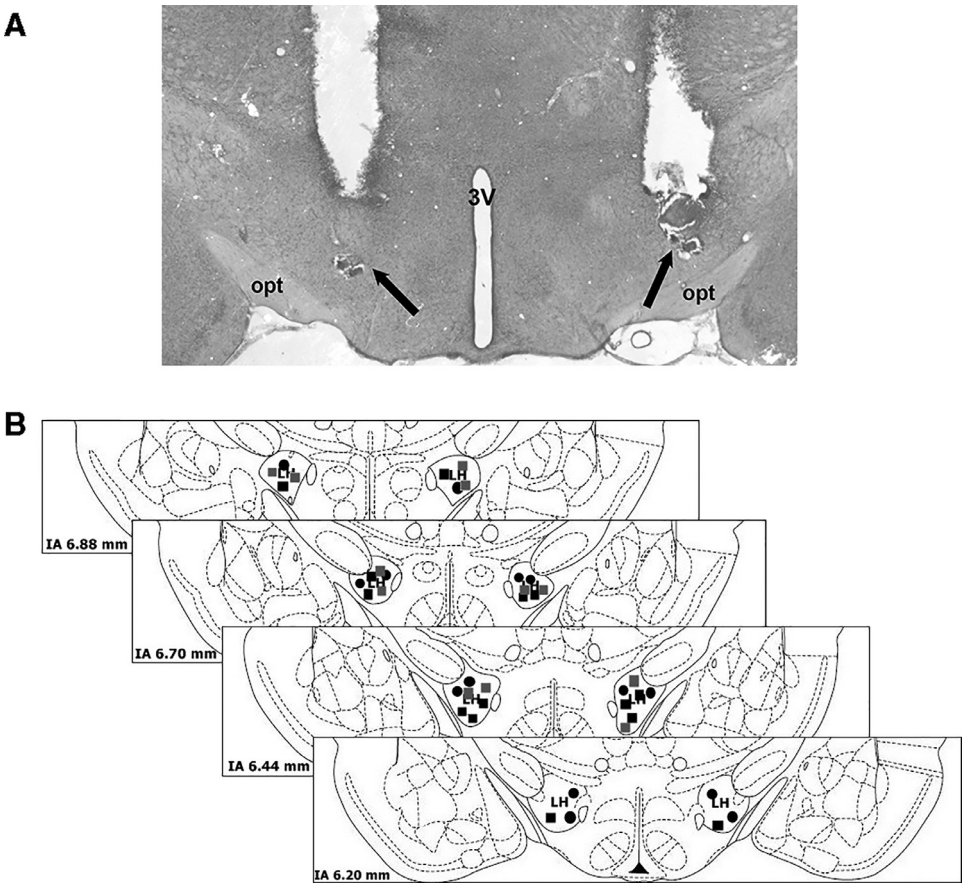

Fig. 1 (A) Photomicrograph of a coronal brain section from a representative rat showing bilateral injection sites into the lateral hypothalamus (LH). (B) Diagrammatic representation based on the rat brain atlas of Paxinos and Watson (1997) indicating the microinjection sites of vehicle (black circles), the selective $GABA_A$ receptor antagonist SR95531 (grey squares) and the selective $GABA_B$ receptor antagonist CGP35348 (black squares) into the LH. IA-interaural coordinate; opt - optic chiasm.

test. The mean of the values during the first 10 min of the restraint session (onset), and during the remaining restraint stress period (plateau) were also calculated. These values were compared using the two-way ANOVA, with treatment as a main factor and time (onset vs plateau) as a repeated measure, followed by Bonferroni's *post hoc* test. Results of statistical tests with $P < 0.05$ were considered statistically significant.

Results

Diagrammatic representations showing the bilateral injection sites into the LH of all animals used in the present study are presented in Fig. 1. A photomicrograph of a coronal brain section depicting microinjection sites into the LH of a representative animal is also presented in Fig. 1.

Bilateral microinjection of either the selective $GABA_A$ receptor antagonist SR95531 (0.01 nmol/100 nL, $n=6$) or the selective $GABA_B$ receptor antagonist CGP35348 (10 nmol/100 nL, $n=7$) into the LH did not alter the baseline values of MAP, HR and tail cutaneous temperature; when compared with animals locally treated with vehicle (100 nL, $n=7$) (Table 1). However, analysis of the time-course curves indicated that restraint stress evoked increase in MAP (time factor: $F_{(35,595)} = 21.11$, $P < 0.0001$) and HR (time factor: $F_{(35,595)} = 13.42$, $P < 0.0001$), and decreased the tail skin temperature (time factor: $F_{(6102)} = 11.8$, $P < 0.0001$) (Fig. 2). Analysis also indicated main effect of the LH pharmacological treatments (treatment factor: $F_{(2,17)} = 3.60$, $P=0.049$) and treatment *x* time interaction ($F_{(70,595)} = 1.60$, $P=0.002$) for the HR values (Fig. 2). Nevertheless, analysis did not indicate effect of treatment (MAP: $F_{(2,17)} = 1.24$, $P=0.313$; skin temperature: $F_{(2,17)} = 0.68$, $P=0.515$) or treatment x time interaction (MAP: ($F_{(70,595)} = 0.78$, $P=0.897$); skin temperature: $F_{(12,102)} = 1.35$, $P=0.202$) for the MAP and tail skin temperature responses (Fig. 2). *Post-hoc* analysis of the HR response revealed that LH treatment with SR95531 ($P=0.039$), but not with CGP35348 ($P=0.875$), decreased the restraint-evoked tachycardic response (Fig. 2).

Analysis of the mean responses during the onset (10 first minutes) and plateau period of restraint stress indicated main effect of treatment ($F_{(2,34)} = 6.69$, $P=0.003$) and time ($F_{(1,34)} = 4.85$, $P=0.034$), but without treatment *x* time interaction ($F_{(2,34)} = 0.62$, $P=0.540$) for the HR values (Fig. 2). Nevertheless, analysis did not indicate effect of either treatment (MAP: $F_{(2,34)} = 2.97$, $P=0.065$; skin temperature: $F_{(2,34)} = 1.22$, $P=0.307$) or time (MAP: $F_{(1,34)} = 2.54$, $P=0.120$; skin temperature: $F_{(1,34)} = 2.79$,

Table 1 Basal parameters of mean arterial pressure (MAP), heart rate (HR) and tail skin temperature before and after pharmacological treatment of the lateral hypothalamus (LH) with the selective $GABA_A$ receptor antagonist SR95531 ($n=6$), the selective $GABA_B$ receptor antagonist CGP35348 ($n=7$) or vehicle (control, $n=7$).

	MAP (mmHg)		HR (bpm)		Tail skin temperature (°C)	
Groups	before	after	before	after	before	after
Control	97 ± 5 *t = 0.2, P = 0.78*	99 ± 4	362 ± 2 *t = 0.6, P = 0.55*	389±3	29 ± 0.6 *t = 0.1, P = 0.85*	29 ± 0.4
SR95531	111 ± 2 *t = 0.6, P = 0.51*	113 ± 2	375 ± 9 *t = 2.2, P = 0.05*	404 ± 9	30 ± 0.7 *t = 1.2, P = 0.2*	29 ± 0.1
CGP35348	108 ± 2 *t = 0.5, P = 0.61*	106 ± 2	413 ± 1 *t = 0.1, P = 0.88*	409 ± 2	28 ± 0,5 *t = 0.1, P = 0.88*	28 ± 0,6

Mean ± SEM, Student's *t*-test.

$P=0.104$), or treatment *x* time interaction (MAP: $F_{(2,34)} = 0.17$, $P=0.840$; skin temperature: $F_{(2,34)} = 0.47$, $P=0.627$) for the MAP and tail skin temperature responses (Fig. 2). *Post-hoc* analysis of the HR response revealed that LH treatment with SR95531 (onset: $P=0.016$, plateau: $P=0.047$), but not with CGP35348 (onset: $P=0.192$, plateau: $P=0.994$), decreased the restraint-evoked tachycardic response during both onset and plateau period of the restraint stress session (Fig. 2).

Discussion

Our results provide the first evidence supporting the involvement of GABAergic neurotransmission within the LH in the control of stress responses. Specifically, we observed that bilateral microinjection of the selective $GABA_A$ receptor antagonist SR95531 into the LH decreased the HR increase during restraint stress, but without affecting the pressor response or the reduction in tail cutaneous temperature. Nevertheless, LH treatment with the selective $GABA_B$ receptor antagonist CGP35348 did not affect the restraint-evoked cardiovascular changes evaluated in the present study.

Deolindo et al. (2013) reported that acute bilateral LH neurotransmission inhibition caused by local treatment with the nonselective synaptic blocker $CoCl_2$ enhanced tachycardia evoked by restraint stress without affecting the pressor response. This effect was mimicked by LH treatment with a selective NMDA receptor antagonist (Deolindo et al., 2013), thus supporting the role of local glutamatergic neurotransmission. Taken together, these results provided evidence of an inhibitory role of the LH in the control of the cardiac responses to stress, which is mediated, at least partly, by local glutamatergic neurotransmission. The results reported in the present study provide further evidence of the inhibitory role of the LH in the control of the tachycardic response to stress. Indeed, we identified that antagonism of local inhibitory neurotransmission (i.e., GABAergic neurotransmission), which increase local neuronal activation (Jones et al., 2011), decreased the restraint-evoked HR increase. More importantly, the results reported in the present study provide the first evidence of activation of GABAergic neurotransmission within the LH during aversive threats, which is involved in the processing of stress responses via activation of the $GABA_A$ receptor. In this sense, since the enhanced tachycardia observed following the nonselective synaptic blockade within the LH by local microinjection of $CoCl_2$ is mimicked by local treatment with a glutamate receptor antagonist (Deolindo et al., 2013), it seems that GABA release within the LH during stress modulates the effects mediated by activation of local glutamatergic inputs rather than mediates the LH control of stress-evoked responses. In this sense, similar to the control of behavioral responses (Stanley et al., 2011), the role of the LH in stress-evoked cardiovascular responses seems to be the resulted of the balance in activation of glutamatergic and GABAergic inputs to the LH.

The LH has been linked to the central network that regulates the cardiovascular responses to stress by connecting limbic structures, such as the hippocampus and the central amygdala, to effector structures in the hypothalamus, brainstem and sympathetic neurons in the intermediolateral column (Myers, 2017). In this sense, GABAergic inputs within the LH were identified from limbic structures such as the preoptic area, the anterior hypothalamic area (AHA) and the bed nucleus of the stria terminalis (BNST) (Gritti et al., 1994; Jennings et al., 2013). Accordingly, all of these regions contain a high density of GABA-producing neurons (Boudaba et al., 1996; Cullinan et al., 1993; Okamura et al., 1990; Radley et al., 2009), and most projections for other limbic structures are GABAergic (Boudaba et al., 1996; Myers et al., 2014; Roland and Sawchenko, 1993). Previous studies reported that activation of either glutamate receptors within the BNST or angiotensinergic AT_1 receptors within the AHA during restraint stress enhanced restraint-evoked cardiovascular responses (Adami et al., 2017; Kubo et al., 2001). Therefore, it is possible that control of restraint-evoked cardiovascular responses demonstrated in the present study by LH $GABA_A$ receptors is part of the neural circuitry related to BNST and AHA control, since activation of GABAergic inputs from the BNST and AHA might inhibit neuronal activation within the LH, which in turn facilitates cardiovascular responses. On the other hand, recent studies have reported that local neurotransmission inhibition in preoptic nuclei (that is, medial and lateral) enhanced restraint-evoked tachycardia, thus indicating an inhibitory role (Duarte et al., 2017; Fassini et al., 2017). Although these findings preclude the idea of an involvement of LH GABAergic neurotransmission in networks related to preoptic area control of cardiovascular responses to stress, the possible role of local excitatory neurochemical

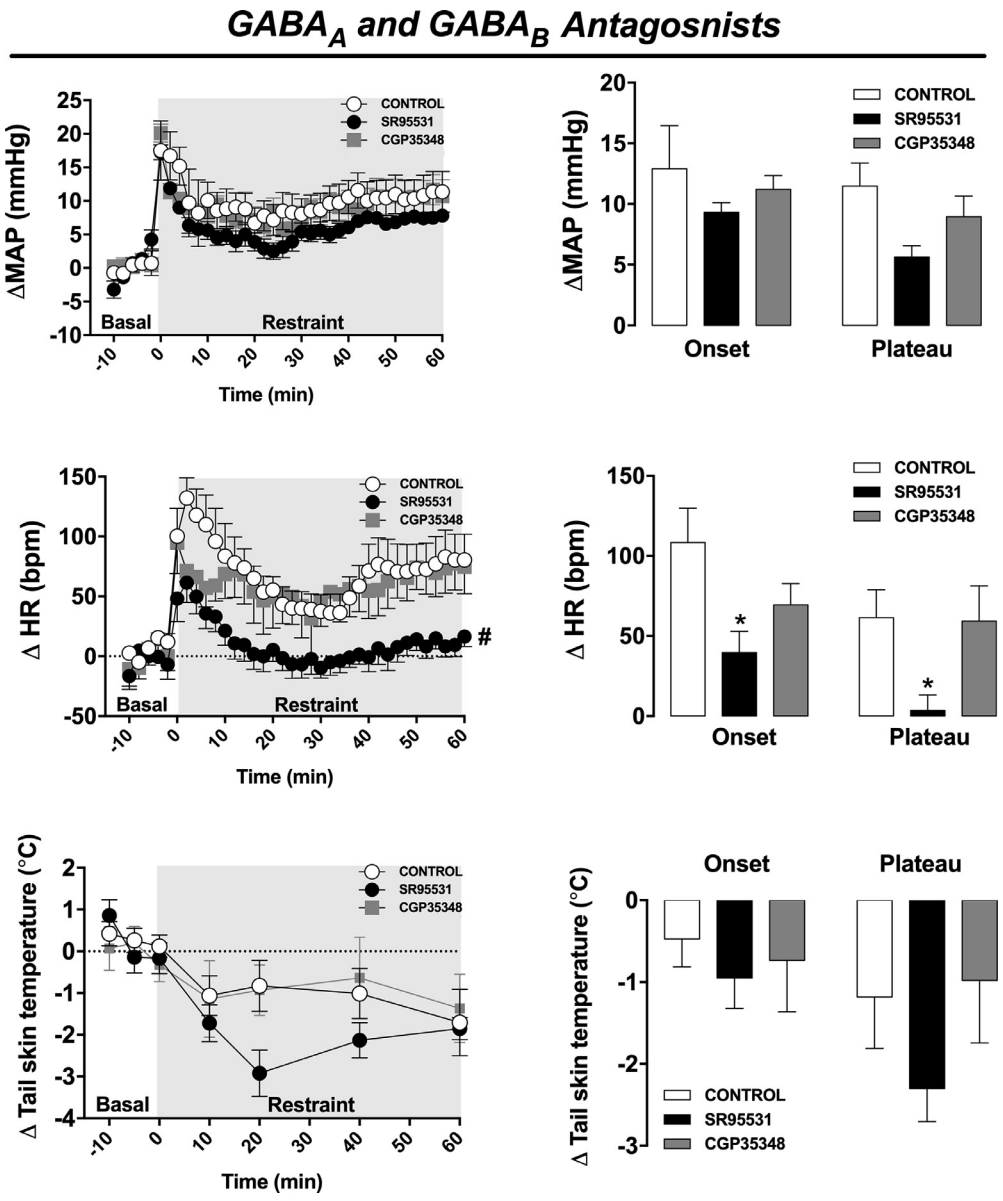

Fig. 2 Effect of lateral hypothalamus (LH) treatment with selective $GABA_A$ and $GABA_B$ receptor antagonists in cardiovascular changes evoked by acute restraint stress. (Left) Time course curves of changes in mean arterial pressure (ΔMAP), heart rate (ΔHR), and tail skin temperature (Δ tail skin temperature) induced by restraint stress in animals that received bilateral microinjection of vehicle (control, 100 nL; $n = 7$ ○), the selective $GABA_A$ receptor antagonist SR95531 (0.01 nmol/100 nL; $n = 6$ ●) or the selective $GABA_B$ receptor antagonist CGP35348 (10 nmol/100 nL; $n = 7$ ●) into the LH. Shaded area indicates the period of restraint. Circles represent the mean and bars the standard error of the mean (SEM). # $P < 0.05$ over the whole restraint period compared to vehicle-treated animals, two-way ANOVA followed by Bonferroni's *post hoc* test. (Right) Mean ΔMAP, ΔHR and Δ tail temperature during onset (10 first minutes) and plateau phase of restraint stress in animals subjected to LH treatment with vehicle (white bars, $n = 7$), SR95531 (black bars, $n = 6$) or CGP35348 (grey bars, $n = 7$). Columns represent the mean and bars the SEM. * $P < 0.05$ versus vehicle-treated group, two-way ANOVA followed by Bonferroni's *post hoc* test.

mechanisms in control of cardiovascular responses to stress by preoptic nuclei has never been reported. Despite these findings, further studies are necessary to elucidate the central network related to LH GABAergic neurotransmission control of stress-evoked cardiovascular responses.

The LH is a heterogenous area containing distinct cell types, including excitatory and inhibitory amino acids; as well as neuropeptides, being orexin and melanin-concentrating hormone (MCH) predominantly found in the LH (Berthoud and Münzberg, 2011; Stuber and Wise, 2016). Orexin neurons project to hypothalamic and brainstem autonomic regions, as well as to sympathetic neurons in the spinal cord (Carrive, 2017). Previous studies reported decreased cardiovascular responses to several psychological stressors in orexin knockout mice, as well as following systemic injection of orexin receptor antagonists (Beig et al., 2015; Furlong et al., 2009; Kayaba et al., 2003). The facilitatory influence of orexin in stress-evoked

cardiovascular responses precludes the idea of an involvement in the effects reported here, since disinhibition of orexin neurons evoked by LH treatment with the $GABA_A$ receptor antagonist is expected to increase, rather than decrease, cardiovascular responses to restraint stress. This is further supported by evidence that orexin neurons are not involved in cardiovascular responses evoked by restraint stress (Furlong et al., 2009). Regarding the MCH, despite reports that MCH receptor antagonists decrease stress-induced hyperthermia (Smith et al., 2009, 2006), its specific role in cardiovascular responses during aversive threats has never been documented. However, observations that intracerebroventricular administration of MCH decreased blood pressure and HR in non-stressed animals (Messina and Overton, 2007) suggests that the facilitatory influence of LH GABAergic neurotransmission in restraint-evoked tachycardic response might be mediated by inhibition of local MCH neurons. In addition to neuropeptides, we cannot exclude the prominent role of neurons expressing amino acids, since in addition to identification of the abundant expression of glutamatergic and GABAergic neurons within the LH, markers of these amino acids were identified in orexin and MCH neurons (Stuber and Wise, 2016). Despite these findings, further studies are necessary to elucidate the local neuronal population involved in control of stress-evoked cardiovascular responses.

Both the sympathetic and parasympathetic nervous system are involved in cardiovascular responses during aversive threats (Crestani, 2016). Regarding the HR increase, it was previously reported that antagonism of muscarinic cholinergic receptors facilitated, while β_1-adrenoceptor blockade decreased this response (Carrive, 2006; Dos Reis et al., 2014), thus indicating coactivation of both sympathetic and parasympathetic tone to the heart during stress. In this sense, there is anatomic evidence of projections from the LH to brainstem regions containing parasympathetic neurons, such as the dorsal motor nucleus and the nucleus ambiguus (Luiten et al., 1987; ter Horst et al., 1984). Accordingly, previous studies indicated that the inhibitory control of HR response to restraint stress by NMDA glutamate receptors within the LH was mediated by facilitation of parasympathetic tone to the heart (Deolindo et al., 2013). Therefore, the facilitatory influence of LH $GABA_A$ receptor in restraint-evoked tachycardia might be mediated by inhibition of facilitatory drive to parasympathetic neurons in the brainstem. However, considering that the LH connects directly, and indirectly via regions in the ventrolateral medulla, to sympathetic neurons in the intermediolateral column (Allen and Cechetto, 1992; Saper et al., 1976), we cannot exclude the possibility that control by LH GABAergic neurotransmission is also mediated by inhibition of inhibitory drive to sympathetic neurons.

In summary, the findings reported in the present study reveal the involvement of GABAergic neurotransmission within the LH in control of cardiovascular changes observed during aversive threats. Specifically, our results provide evidence that activation of $GABA_A$, but not $GABA_B$, receptors within the LH plays a facilitatory role in stress-evoked tachycardia, but without affecting the arterial pressure increase and the sympathetically-mediated cutaneous vasoconstriction.

Contributors

L.G.S. and C.C.C. conceived and designed this research; L.G.S., R.B. and W.C.F. performed the experiments and analysed the data; L.G.S., R.B. and W.C.F. and C.C.C. interpreted the results of experiments; L.G.S. prepared the figures and drafted the manuscript; L.G.S. and C.C.C. edited and revised the manuscript; C.C.C. approved the final version of the manuscript.

Conflict of interest

The authors declare no conflicts of interest.

Role of funding source

Funding for this study was provided by FAPESP (grants # 2015/05922-9 and 2017/19249-0) and CNPq (grant # 456405/2014-3), and Scientific Support and Development Program of School of Pharmaceutical Sciences (UNESP). LGS and WCF are FAPESP PhD fellow (2016/05028-9 and 2016/05218-2, respectively), RB is a CAPES PhD fellow and CCC is a CNPq research fellow (process # 305583/2015-8). The FAPESP, CAPES and CNPq had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

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