British Journal of Pharmacology

DOI:10.1111/bph.13327
www.brjpharmacol.org

RESEARCH PAPER

Medial prefrontal cortex TRPV1 channels modulate the baroreflex cardiac activity in rats

D C Lagatta, N C Ferreira-Junior and L B M Resstel

Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP Brazil

Correspondence
Dr. Leonardo Resstel Barbosa Moraes, Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo, Bandeirantes Avenue 3900, 14049-900 Ribeirão Preto, São Paulo, Brazil.
E-mail: leoresstel@yahoo.com.br

Received
19 January 2015
Revised
18 August 2015
Accepted
27 August 2015

BACKGROUND AND PURPOSE

The ventral portion of the medial prefrontal cortex (vMPFC) comprises the infralimbic (IL), prelimbic (PL) and dorsopenducular (DP) cortices. The IL and PL regions facilitate the baroreceptor reflex arc. This facilitatory effect on the baroreflex is thought to be mediated by vMPFC glutamatergic transmission, through NMDA receptors. The glutamatergic transmission can be modulated by other neurotransmitters, such as the endocannabinoids, which are agonists of the TRPV1 receptor. TRPV1 channels facilitate glutamatergic transmission in the brain. Thus, we hypothesized that TRPV1 receptors in the vMPFC enhance the cardiac baroreflex response.

EXPERIMENTAL APPROACH

Stainless steel guide cannulae were bilaterally implanted into the vMPFC of male Wistar rats. Afterwards, a catheter was inserted into the femoral artery, for recording MAP and HR, and into the femoral vein for assessing baroreflex activation.

KEY RESULTS

Microinjections of the TRPV1 receptor antagonists capsazepine and 6-iodo-nordihydrocapsaicin (6-IODO) into the vMPFC reduced the cardiac baroreflex activity in unanaesthetized rats. Capsaicin microinjected into the vMPFC increased the cardiac baroreflex activity in unanaesthetized rats. When an ineffective dose of the TRPV1 receptor antagonist 6-IODO was used, the capsaicin-induced increase in the cardiac baroreflex response was abolished. The higher doses of capsaicin administered into the vMPFC after the ineffective dose of 6-IODO displaced the dose–response curve of the baroreflex parameters to the right, with no alteration in the maximum effect of capsaicin.

CONCLUSIONS AND IMPLICATIONS

The results of the present study show that stimulation of the TRPV1 receptors in the vMPFC increases the cardiac baroreceptor reflex response.

Abbreviations

2-AG, 2-arachidonylglycerol; 6-IODO, 6-iodo-nordihydrocapsaicin; AD, Alzheimer's disease; BP_{50}, medium blood pressure; G, average gain; IL, infralimbic cortex; MPFC, medial prefrontal cortex; MS, multiple sclerosis; P1, lower plateau; P2, upper plateau; PFC, prefrontal cortex; PL, prelimbic cortex; PTSD, post-traumatic stress disorder; vMPFC, ventral medial prefrontal cortex; ΔP, heart rate range

Tables of Links

TARGETS
GPCRs[a]
CB_1 receptor
Ligand-gated ion channels[b]
TRPV1 channels

LIGANDS	
2-AG	Capsaicin
6-IODO	Capsazepine
Anandamide (AEA)	Nitric oxide (NO)
	Phenylephrine

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b]Alexander *et al.*, 2013a, b).

Introduction

The prefrontal cortex (PFC) is a forebrain structure that is anatomically divided into the lateral prefrontal cortex and medial prefrontal cortex (MPFC) (Leonard, 1969).The ventral portion of the MPFC (vMPFC) comprises the prelimbic (PL), infralimbic (IL) and dorsopenducular (DP) cortices (Leonard, 1969; Groenewegen, 1988). The vMPFC is a limbic area, which is able to facilitate the autonomic and emotional responses that occur during stressful situations (Resstel *et al.*, 2006b; Lisboa *et al.*, 2010).

Some studies have demonstrated that the vMPFC modulates baroreflex activity, a neuronal reflex that is responsible for maintaining the BP at homeostatic levels (Resstel *et al.*, 2004; Resstel and Correa, 2006a; Ferreira-Junior *et al.*, 2013). Moreover, glutamatergic neurotransmission within the vMPFC has been shown to influence this autonomic function, because the NMDA receptor/NO pathway facilitates the bradycardic and tachycardic components of the baroreflex in rats (Resstel and Correa, 2006a; Ferreira-Junior *et al.*, 2013), indicating it has a role in the cardiac baroreflex activity.

In the CNS, the release of glutamate can be modified by other neurotransmitters, such as the endocannabinoids, anandamide (AEA) and 2-arachidonylglycerol (2-AG) (Hampson *et al.*, 1998). The vMPFC CB_1 receptors negatively modulate the release of glutamate (Auclair *et al.*, 2000). Previous experiments from our group showed that pharmacological antagonism of vMPFC CB_1 receptors increased the tachycardic baroreflex response, whereas facilitation of the vMPFC endocannabinoid neurotransmission by AEA decreased this response (Ferreira-Junior *et al.*, 2011). These results indicate that the vMPFC endocannabinoid system has an inhibitory effect on the cardiac baroreflex, possibly by diminishing the release of glutamate.

Furthermore, AEA was shown to increase neuronal firing in CB_1 receptor-knockout mice, suggesting that it can activate other receptors (Zygmunt *et al.*, 1999). In fact, AEA binds to transient receptor potential vanilloid type 1 (TRPV1) receptors (Caterina *et al.*, 1997; Rosenbaum and Simon, 2007; De Petrocellis and Di Marzo, 2010). Activation of the TRPV1 receptor controls calcium influx in the neurons (Di Marzo and Maccarrone, 2008), favouring the release of glutamate in several brain structures, such as the hypothalamus, striatum and substantia gelatinosa (Vaughan *et al.*, 2000; Cristino *et al.*, 2006; Xing and Li, 2007; Musella *et al.*, 2009; Yang *et al.*, 2011). In addition, TRPV1 channels are highly expressed in vMPFC neurons, being mainly located at the post-synaptic membrane (Toth *et al.*, 2005; Fogaca *et al.*, 2012).

Therefore, given that glutamatergic neurotransmission in the vMPFC enhances the cardiac baroreflex activity and that TRPV1 receptors facilitate the release of glutamate in several brain areas, we hypothesized that TRPV1 receptors in the vMPFC are able to intensify the cardiac baroreflex activity.

Methods

Ethical approval and animals

Experimental procedures were carried out following protocols approved by the Ethical Review Committee of the School of Medicine of Ribeirão Preto (Protocol number 127/2011), which complies with the Guiding Principles for Research Involving Animals and Human Beings of the American Physiological Society. All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010). A total of 70 Male Wistar rats weighing 230–270 g were used. Animals were housed in plastic cages in a temperature-controlled room at 25°C in the Animal Care Unit of the Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo. They were kept under a 12:00 h light–dark cycle (lights on between 6:00 h and 18:00 h) and had water and food *ad libitum*.

Animal preparation

Four days before the experiment, rats were anaesthetized with tribromoethanol (250 mg kg^{-1}, i.p., Sigma, St. Louis, Missouri, USA). After local anaesthesia with 2% lidocaine, the skull was surgically exposed, and stainless steel guide cannulae (26G) were bilaterally implanted into the vMPFC using a stereotaxic apparatus (Stoelting, Wood Dale, Illinois, USA). Stereotaxic coordinates for cannulae implantation into the vMPFC were selected from The Rat Brain Atlas of Paxinos and Watson (1997) and were: Antero-Posterior = +3.4 mm, Lateral = 2.6 mm from the medial suture and Vertical = −3.3 mm from the skull, with a lateral inclination of 24°. Cannulae were fixed to the skull with dental cement and one metal screw. After surgery,

animals were treated with a polyantibiotic preparation of streptomycins/penicillins (i.m., Pentabiotico®, Fort Dodge, Campinas, São Paulo, Brazil) to prevent infection and with the non-steroidal anti-inflammatory flunixine meglumine (s.c., Banamine®, Schering Plough, Cotia, São Paulo, Brazil) for analgesia.

One day before the experiment, rats were anaesthetized with tribromoethanol (250 mg kg^{-1}, i.p.), and a catheter (a 4 cm segment of PE-10 that was heat-bound to a 13 cm segment of PE-50, Clay Adams, Parsippany, New Jersey, USA) was inserted into the femoral artery, for recording BP. A second catheter was implanted into the femoral vein for the infusion of vasoactive substances. Both catheters were inserted under the skin and exteriorized on the animal's dorsum. After surgery, treatment with anti-inflammatory drugs was repeated.

Measurement of cardiovascular responses

The pulsatile arterial pressure of freely moving animals was recorded using an ML870 preamplifier (LabChart, USA) and an acquisition board (PowerLab, AD Instruments, USA) connected to a computer. Mean arterial pressure (MAP) and heart rate (HR) values were derived from pulsatile recordings and processed on-line.

Drug injection

The needles (33G, Small Parts, Miami Lakes, FL, USA) used for microinjection into the vMPFC were 1 mm longer than the guide cannulas and were connected to a 1 μL syringe (7002-H, Hamilton Co., Reno, NV, USA) through PE-10 tubing. The needle was carefully inserted into the guide cannula, and drugs were injected in a final volume of 200 nL over a 5 s period. After a 30 s period, the needle was removed and inserted into the second guide cannula for microinjection into the contralateral vMPFC.

Baroreflex assessment

The baroreflex was activated by phenylephrine (α_1 adrenoceptor agonist; 50 μg kg^{-1}; 0.34 mL min^{-1}) or SNP (NO donor; 50 μg kg^{-1}; 0.8 mL min^{-1}) infusion using an infusion pump (KD Scientific, Holliston, MA, USA). The phenylephrine or SNP infusion lasted 30–40 s and caused, respectively, an increase and decrease in BP (Alves *et al.*, 2009b).

Method used to evaluate baroreflex activity

Baroreflex curves were constructed, matching MAP variations with HR responses. Paired values for variations in MAP (ΔMAP) and HR (ΔHR) were plotted to create sigmoid curves for each rat, which were used to determine baroreflex activity (Resstel *et al.*, 2004). To analyse bradycardic and tachycardic responses separately, HR values matching 10, 20, 30 and 40 mmHg of MAP changes were calculated (Alves *et al.*, 2009a; Crestani *et al.*, 2009). Values were plotted to create linear regression curves for each rat, and their slopes were compared to determine changes in baroreflex gain.

Drugs

The following drugs were used: two TRPV1 receptors antagonists (capsazepine and 6-iodo-nordihydrocapsaicin (6-IODO); Tocris, Westwoods Business Park Ellisville, MO, USA); capsazepine was dissolved in 10% DMSO in saline (0.9% NaCl), and 6-IODO were dissolved in DMSO 100%. A TRPV1 receptor agonist (capsaicin; Tocris, Westwoods Business Park, Ellisville, MO, USA) was dissolved in 10% DMSO in saline (0.9% NaCl). Phenylephrine–HCl (Sigma, St. Louis, MO, USA) and SNP (Sigma, St. Louis, MO, USA) were dissolved in saline (0.9% NaCl). Tribromoethanol (Sigma, St. Louis, MO, USA) and urethane (Sigma, St. Louis, MO, USA) were dissolved in distilled water. The solutions were prepared immediately before use and were kept on ice and protected from the light during the experimental sessions.

Experimental protocols

All groups of animals used in our study received three sets of phenylephrine or sodium nitroprusside (SNP) infusion to determine control values of baroreflex activity. Posteriorly, the first group received microinjections of 200 nL of 10% DMSO dissolved in saline (0.9% NaCl); the second group received microinjections of 200 nL of capsazepine (1, 10 or 100 nmol, (Aguiar *et al.*, 2009)); the third group received microinjections of 200 nL of 6-IODO (0.3, 3 or 30 nmol, (Aguiar, 2009)); the fourth group received microinjections of 200 nL of capsaicin (0.01, 0.1 or 1 nmol, (Terzian *et al.*, 2009)); the fifth group received a bilateral microinjection of 200 nL of 6-IODO (0.3 nmol) and 5 min later capsaicin (1, 3 and 10 nmol). In all experimental groups, phenylephrine and SNP infusion was repeated 10 and 60 min after the bilateral vMPFC microinjection.

Histological procedure

At the end of the experiments, the rats were anaesthetized with urethane (1.25 g kg^{-1}, i.p.), and 200 nL of 1% Evan's blue dye was bilaterally injected into the vMPFC as a marker of injection sites. The chest was surgically opened, the descending aorta occluded, the right atrium severed and the brain perfused with 10% formalin through the left ventricle. Brains were post-fixed for 24 h at 4°C, and 40 μm sections were cut with a cryostat (CM-1900, Leica, Wetzlar, Germany). The actual placement of the injection needles was verified in serial sections, according to the Rat Brain Atlas of Paxinos and Watson, 1997.

Data analysis

Baseline cardiovascular values before and after pharmacological treatment into the vMPFC were compared using Student's *t*-test. Baroreflex activity was analysed using sigmoid curves which were characterized as five parameters: (i) P1 (beats min^{-1}) lower heart rate plateau and P2 (beats min^{-1}) upper heart rate plateau; (ii) heart rate range (beats min^{-1}), difference between upper and lower plateau levels (ΔP); and (iii) average gain (G, beats min^{-1} mmHg^{-1}), which is the average slopes of the nonlinear curves; the medium blood pressure (BP50) which is the value of MAP when 50% of the HR is altered. Significant differences among sigmoid curves or linear regression parameters were analysed using one-way ANOVA followed by the Dunnett's *post hoc* test. The slope of

linear regression curves (Δ HR vs. Δ MAP) before, 10 and 60 min after microinjection of each treatment was determined, and results were analysed to detect alterations in cardiac baroreflex gain using one-way ANOVA followed by Dunnett's *post hoc* test. Results of statistical tests where $P < 0.05$ were considered significant.

Results

Figure 1 shows a representative photomicrograph of a vMPFC coronal section and diagrammatic representations of the vMPFC microinjection sites of all experimental groups used in this study.

Effects of bilateral microinjection of vehicle, DMSO 10%, into the vMPFC on cardiac baroreflex activity

Microinjection of vehicle DMSO 10% did not alter the basal levels of MAP (before = 103 ± 2.56; after = 101 ± 2.59 mmHg; $t = 0.49$; $P > 0.05$) and HR (before = 376 ± 16; after = 373 ± 15 beats min^{-1}; $t = 0.18$; $P > 0.05$). The tachycardic (before = −1.60 ± 0.09 and after = −1.61 ± 0.09; $F_{(2,14)} = 0.34$; $P > 0.05$) and bradycardic (before = −1.82 ± 0.16 and after = −1.72 ± 0.13; $F_{(2,14)} = 0.41$; $P > 0.05$) responses were also not altered (data not shown). The sigmoid curve parameters were not affected either (data not shown).

Effects of bilateral microinjection of the TRPV1 receptor antagonist, capsazepine, into the vMPFC on the cardiac baroreflex activity

Capsazepine 1 nmol 200 nL^{-1}, bilaterally microinjected in the vMPFC ($n = 6$), did not affect the baseline MAP or HR and failed to alter the linear regression slope of both the bradycardic and tachycardic responses (Figure 2). It was also unable to affect the sigmoid curve parameters (Table 1).

Microinjection of capsazepine 10 nmol 200 nL^{-1} ($n = 6$) did not affect the baseline MAP or HR. However, the linear regression slope of the bradycardic and tachycardic responses was significantly reduced ($P < 0.05$) (Figure 2). Apart from BP50, the parameters of the sigmoid curve were also reduced (Table 1).

Moreover, administration of capsazepine 100 nmol 200 nL^{-1} ($n = 6$) did not alter the baseline MAP or HR but it also decreased the linear regression slope of both the bradycardic and tachycardic responses ($P < 0.05$) (Figure 2). The nonlinear regression parameters (G, P1, P2, ΔP and BP50) were also affected (Table 1).

The linear regression slope of the curves 60 min after the microinjection of capsazepine 10 nmol 200 nL^{-1} and 100 nmol 200 nL^{-1} (Figure 2), as well as the sigmoid curve parameters returned to basal levels (Table 1).

Effects of bilateral microinjection of the TRPV1 receptor antagonist, 6-iodo-nordihydrocapsaicin (6-IODO), into the vMPFC on the cardiac baroreflex activity

Microinjection of 6-IODO 0.3 nmol 200 nL^{-1} ($n = 6$) did not affect the basal values of either the MAP or HR. It was also unable to alter the linear regression curve slopes of the bradycardic and tachycardic responses (Figure 3). The sigmoid curve parameters were not affected (Table 2).

The bilateral microinjection of 6-IODO 3 nmol 200 nL^{-1} ($n = 6$) into the vMPFC did not affect the baseline MAP and HR. However, the slope of the regression line of the bradycardic and tachycardic components of the baroreflex was significantly reduced (Figure 3). Almost all of the nonlinear regression curve parameters were also reduced (Table 2).

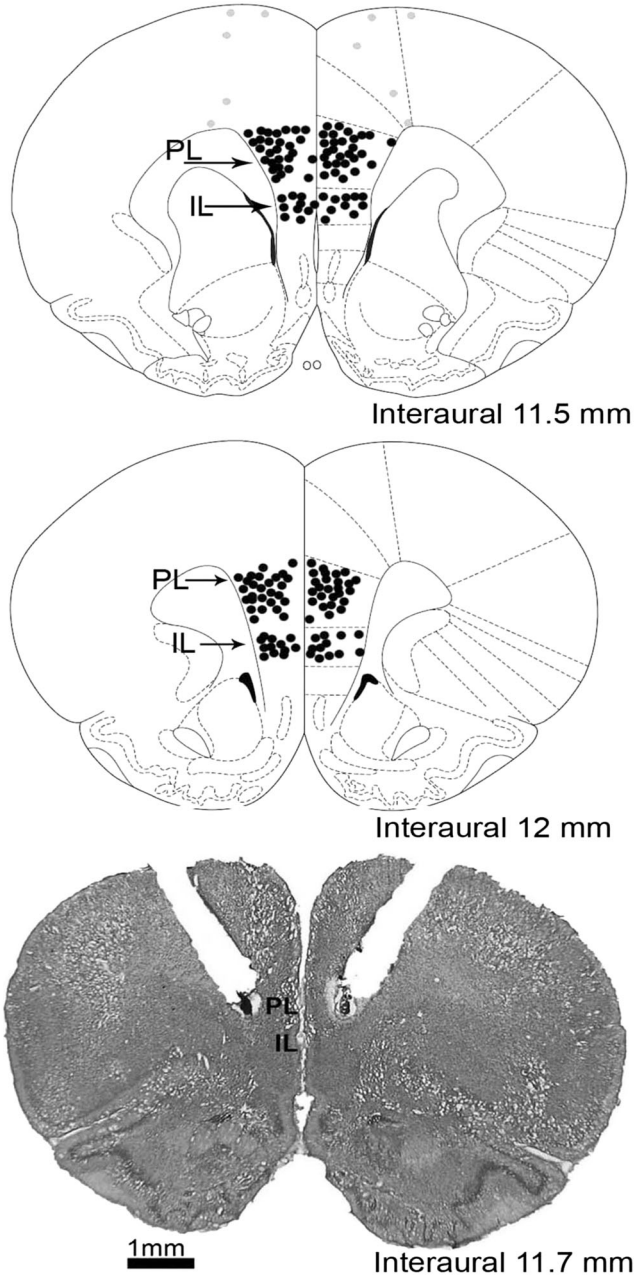

Figure 1

Photomicrograph of the ventral portion of the medial prefrontal cortex (vMPFC) coronal section and diagrammatic representations with the microinjection sites of vehicle, capsaicin, capsazepine and 6-IODO into (dark circles) and structures surrounding (grey circles) of the vMPFC, based on the Rat Brain Atlas of Paxinos and Watson.

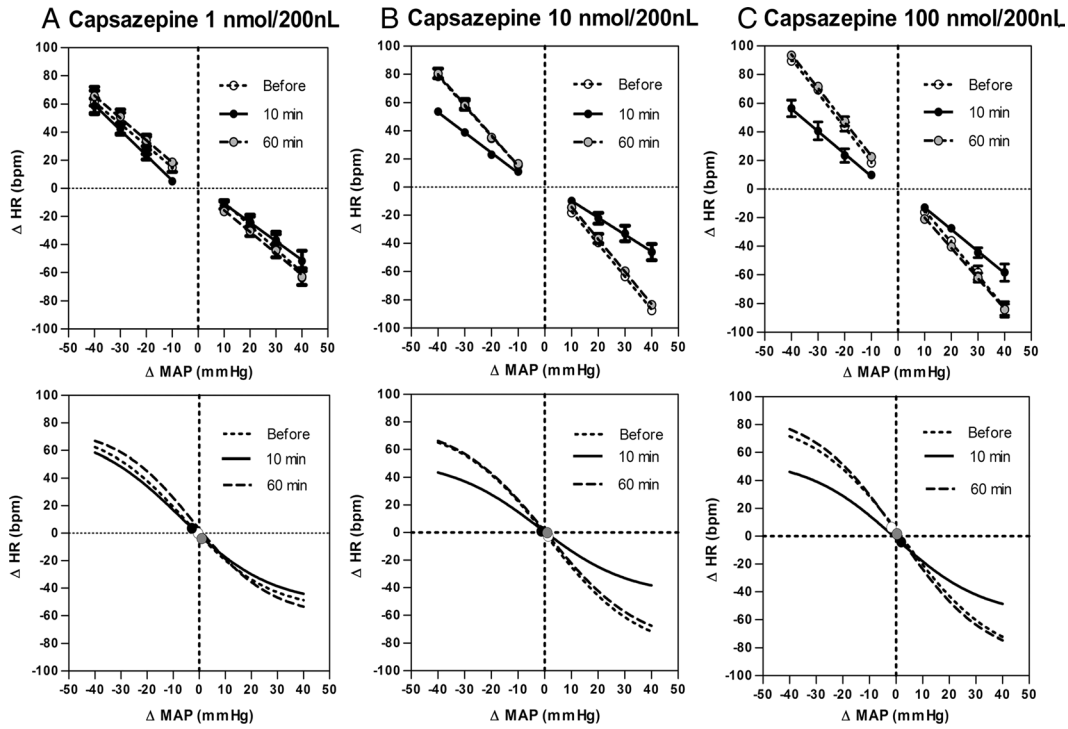

Figure 2

(A, B C, higher) Regression curves correlating the responses of ΔMAP and ΔHR before, 10 min and 60 min after bilateral microinjection of capsazepine into the vMPFC. Correlation r^2 values for bradycardic regression curves were 0.70, 0.97 and 0.89 for data recorded before; 0.59, 0.64 and 0.80 for data recorded 10 min after and 0.77, 0.95 and 0.92 for data recorded 60 min after microinjection of capsazepine 1 (A), 10 (B) or 100 nmol/200 nL (C) into the vMPFC. Correlation r^2 values for tachycardic regression curves were 0.58, 0.90 and 0.96 for data recorded before; 0.85, 0.91 and 0.71 for data recorded 10 min after; and 0.73, 0.93 and 0.96 for data recorded 60 min after microinjection of capsazepina into the vMPFC. (A, B and C, lower) Sigmoid curves correlating mean arterial pressure (ΔMAP) and heart rate (ΔHR) before ($r^2 = 0.91$; 0.97; 0.96) and 10 min ($r^2 = 0.93$; 0.92; 0.91) and 60 min ($r^2 = 0.87$; 0.96; 0.97) after bilateral microinjection of 1 nmol ($n = 6$; A), 10 nmol ($n = 6$; B), or 100 nmol ($n = 6$; C) capsazepine into the vMPFC. Values are means ± SEM. bpm, beats min^{-1}. The circles in the sigmoidal curves represent the BP_{50}.

Table 1

Sigmoidal curve parameters generated before, 10 and 60 min after bilateral microinjection of 1, 10 or 100 nmol capsazepine into the vMPFC

Group	G (beats min^{-1} $mmHg^{-1}$)	P1 (beats min^{-1})	P2 (beats min^{-1})	ΔP (beats min^{-1})	BP_{50} (mmHg)
Capsazepine 1 nmol	$F_{(2,17)} = 0.19$	$F_{(2,17)} = 0.65$	$F_{(2,17)} = 0.51$	$F_{(2,17)} = 0.54$	$F_{(2,17)} = 0,95$
Before	−1.68 ± 0.95	−61 ± 6	67 ± 8	130 ± 13	0,97 ± 2,18
10 min	−1.37 ± 0.07	−54 ± 7	65 ± 5	120 ± 8	−3.59 ± 2,82
60 min	−1.84 ± 0.12	−63 ± 6	77 ± 13	137 ± 14	−1,35 ± 1,96
Capsazepine 10 nmol	$F_{(2,17)} = 56.65$	$F_{(2,17)} = 37.64$	$F_{(2,17)} = 30.68$	$F_{(2,17)} = 79,66$	$F_{(2,17)} = 0,14$
Before	−1.80 ± 0.04	−87 ± 2	79 ± 3	166 ± 4	1,81 ± 0,69
10 min	−1.09 ± 0.05*	−46 ± 6*	54 ± 2*	101 ± 5*	−1.49 ± 1,77
60 min	−1.73 ± 0.06	−83 ± 3	81 ± 3	164 ± 4	−1,41 ± 0,87
Capsazepine 100 nmol	$F_{(2,17)} = 49.65$	$F_{(2,17)} = 8.79$	$F_{(2,17)} = 13,91$	$F_{(2,17)} = 53,37$	$F_{(2,17)} = 0,68$
Before	−1.91 ± 0.06	−84 ± 5	85 ± 5	173 ± 6	0,50 ± 1,50
10 min	−1.23 ± 0.07*	−56 ± 6*	57 ± 6*	115 ± 5*	0,50 ± 1,56
60 min	−2.08 ± 0.06	−95 ± 8	90 ± 4	178 ± 4	−1,58 ± 1,84

Values are means ± SEM; $n = 6$ for 1 nmol, 10 nmol and 100 nmol capsazepine. G, average gain; P1, lower HR plateau; P2, upper HR plateau; Range, ΔP.

*$P < 0.05$, significant difference from values before capsazepine administrations, one-way ANOVA followed by Dunnett's *post hoc* test.

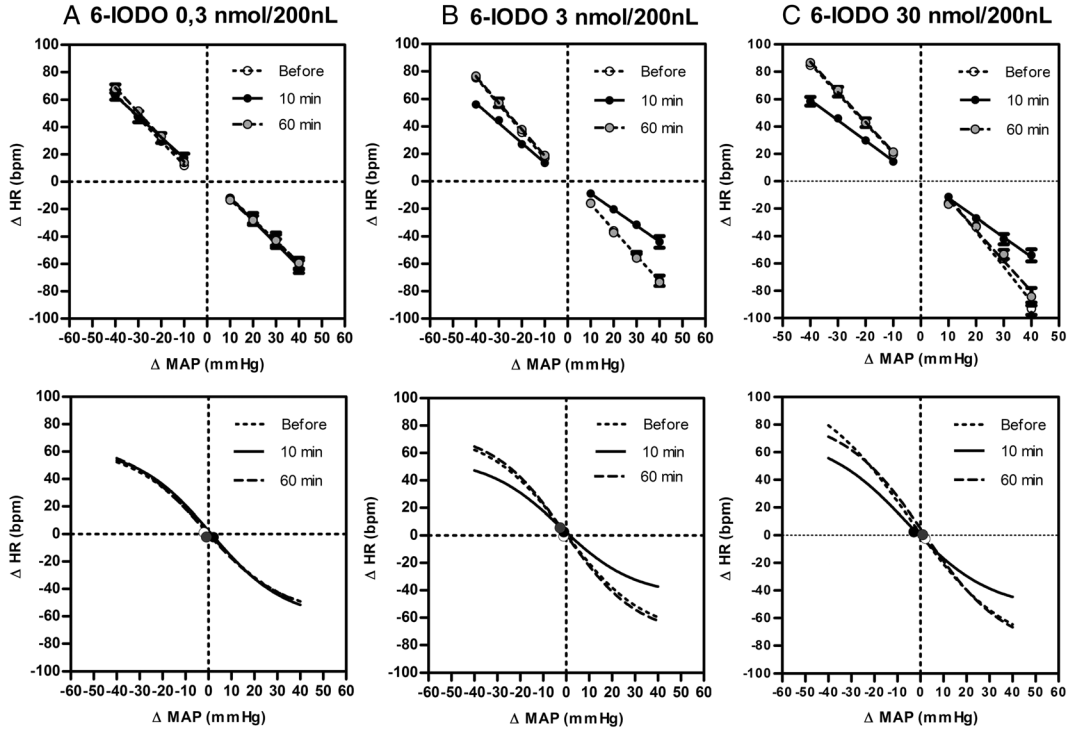

Figure 3

(A, B and C, higher) Regression curves correlating the responses of ΔMAP and ΔHR before, 10 min and 60 min after bilateral microinjection of 6-IODO into the vMPFC. Correlation r^2 values for bradycardic regression curves were 0.80, 0.93 and 0.93 for data recorded before; 0.89, 0.82 and 0.82 for data recorded 10 min after; and 0.83, 0.95 and 0.87 for data recorded 60 min after microinjection of 6-IODO 0.3 (A), 3 (B) or 30 nmol (C) into the vMPFC. Correlation r^2 values for tachycardic regression curves were 0.89, 0.93 and 0.93 for data recorded before; 0.87, 0.82 and 0.82 for data recorded 10 min after; and 0.94, 0.94 and 0.87 for data recorded 60 min after microinjection of capsazepine into the vMPFC. (A, B and C, lower) Sigmoid curves correlating mean arterial pressure (ΔMAP) and heart rate (ΔHR) before (r^2 = 0.94; 0.92; 0.95) and 10 min (r^2 = 0.96; 0.96; 0.95) and 60 min (r^2 = 0.91; 0.96; 0.93) after bilateral microinjection of 0.3 nmol (n = 6; A), 3 nmol (n = 6; B), or 30 nmol (n = 7; C) 6-IODO into the vMPFC. Values are means ± SEM. bpm, beats min^{-1}. The circles in the sigmoidal curves represent the BP50.

Table 2

Sigmoidal curve parameters generated before, 10 and 60 min after bilateral microinjection of 0.3, 3 or 30 nmol 6-IODO into the vMPFC

Group	G (beats min^{-1} mmHg^{-1})	P1 (beats min^{-1})	P2 (beats min^{-1})	ΔP (beats min^{-1})	BP_{50} (mmHg)
6-IODO0.3 nmol	$F_{(2,17)}$ = 2.51	$F_{(2,17)}$ = 0.11	$F_{(2,17)}$ = 0.81	$F_{(2,17)}$ = 0.48	$F_{(2,17)}$ = 0,40
Before	−1.34 ± 0.06	−60 ± 4	64 ± 4	123 ± 4	−1,30 ± 1,63
10 min	−1.49 ± 0.06	−62 ± 5	63 ± 2	125 ± 2	1,25 ± 1,39
60 min	−1.47 ± 0.04	−59 ± 4	68 ± 3	127 ± 2	−0,13 ± 0,63
6-IODO 3 nmol	$F_{(2,17)}$ = 83.75	$F_{(2,17)}$ = 21.66	$F_{(2,17)}$ = 22.20	$F_{(2,17)}$ = 32.37	$F_{(2,17)}$ = 0,37
Before	−1.75 ± 0.04	−72 ± 4	76 ± 3	148 ± 5	−0,22 ± 1,63
10 min	−1.17 ± 0.04*	−44 ± 4*	56 ± 2*	100 ± 5*	−2,24 ± 1,53
60 min	−1.84 ± 0.04	−73 ± 3	77 ± 2	150 ± 4	−0,53 ± 0,53
6-IODO 30 nmol	$F_{(2,20)}$ = 33.64	$F_{(2,20)}$ = 8.54	$F_{(2,20)}$ = 32.35	$F_{(2,20)}$ = 16.76	$F_{(2,20)}$ = 0,84
Before	−1.80 ± 0.05	−85 ± 7	85 ± 3	170 ± 9	1,03 ± 2,10
10 min	−1.38 ± 0.05*	−54 ± 4*	59 ± 3*	112 ± 6*	−0,47 ± 1,67
60 min	−1.893 ± 0.04	−84 ± 6	87 ± 2	171 ± 8	0,48 ± 1,62

Values are means ± SEM; n = 6 for 0.3 nmol, n = 6 for 3 nmol and n = 7 for 30 nmol 6-IODO. G, average gain; P1, lower HR plateau; P2, upper HR plateau; Range, ΔP.
*P < 0.05, significant difference from values before 6-IODO administration, one-way ANOVA followed by Dunnett's *post hoc* test

This same dose of 6-IODO (3 nmol 200 nL^{-1}) was used to verify if the PL or IL TRPV$_1$ receptors equally modulate baroreflex activity. Microinjection of 6-IODO 3 nmol into the PL area ($n = 6$) resulted in a decreased linear regression slope of bradycardic (before = −2.00 ± 0.14; after = −1.38 ± 0.10; $F_{(2,17)} = 8.88$; $P < 0.05$) and tachycardic (before = −2.02 ± 0.17; after = −1.31 ± 0.13; $F_{(2,17)} = 7.54$; $P < 0.05$) responses of the baroreflex. Administration of 6-IODO 3 nmol into the IL region ($n = 6$) was able to reduce the linear regression slope of bradycardic (before = −1.87 ± 0.11; after = −1.16 ± 0.12; $F_{(2,17)} = 14.96$; $P < 0.05$) and tachycardic (before = −1.97 ± 0.11; after = −1.45 ± 0.08; $F_{(2,17)} = 8.96$; $P < 0.05$) components of the baroreflex. The non-linear regression parameters were also decreased by the microinjection of 6-IODO in both PL and IL regions (data not shown).

When microinjected into the structures surrounding the vMPFC 6-IODO 3 nmol did not alter the basal levels of MAP (before = 101 ± 3.57; after = 102 ± 2.21 mmHg; $t = 0.49$; $P > 0.05$) and HR (before = 367 ± 15; after = 367 ± 13 beats min^{-1}; $t = 0.02$; $P > 0.05$). The reflex tachycardia (before = −1.70 ± 0.22; after = −1.65 ± 0.21; $F_{(2,14)} = 0.02$; $P > 0.05$) and bradycardia (before = −1.60 ± 0.24 and after = −1.61 ± 0.20; $F_{(2,14)} = 0.22$; $P > 0.05$) were not changed by the drug (not shown). The sigmoid curve parameters were not altered either (not shown).

Furthermore, 6-IODO 30 nmol 200 nL^{-1} ($n = 7$) did not affect baseline MAP or HR. However, it did reduce the slope of linear regression of both the bradycardic and tachycardic responses (Figure 3). The parameters of the sigmoid curve (G, P1, P2 and ΔP) were also decreased (Table 2).

The slope of the linear regression curves 60 min after the microinjection of either 6-IODO 3 nmol or 30 nmol (Figure 3), as well as the non-linear regression curve parameters returned to normal levels (Table 2).

Effects of the bilateral microinjection of the TRPV1 receptors agonist, capsaicin, into the vMPFC on the cardiac baroreflex activity

Capsaicin 0.01 nmol 200 nL^{-1} ($n = 6$) did not alter the basal levels of the MAP or HR. In addition, it was unable to change the slope of the linear regression for the bradycardic and tachycardic components of the baroreflex (Figure 4), as well as the sigmoid curve parameters (Table 3).

Likewise, microinjection of capsaicin 0.1 nmol 200 nL^{-1} ($n = 6$) did not change the basal values of the MAP and HR. Nevertheless, it was able to increase the slope of the bradycardic and tachycardic responses (Figure 4). The parameters of the non-linear regression curve were also increased 10 min after the microinjection of this drug, at this dose (Table 3).

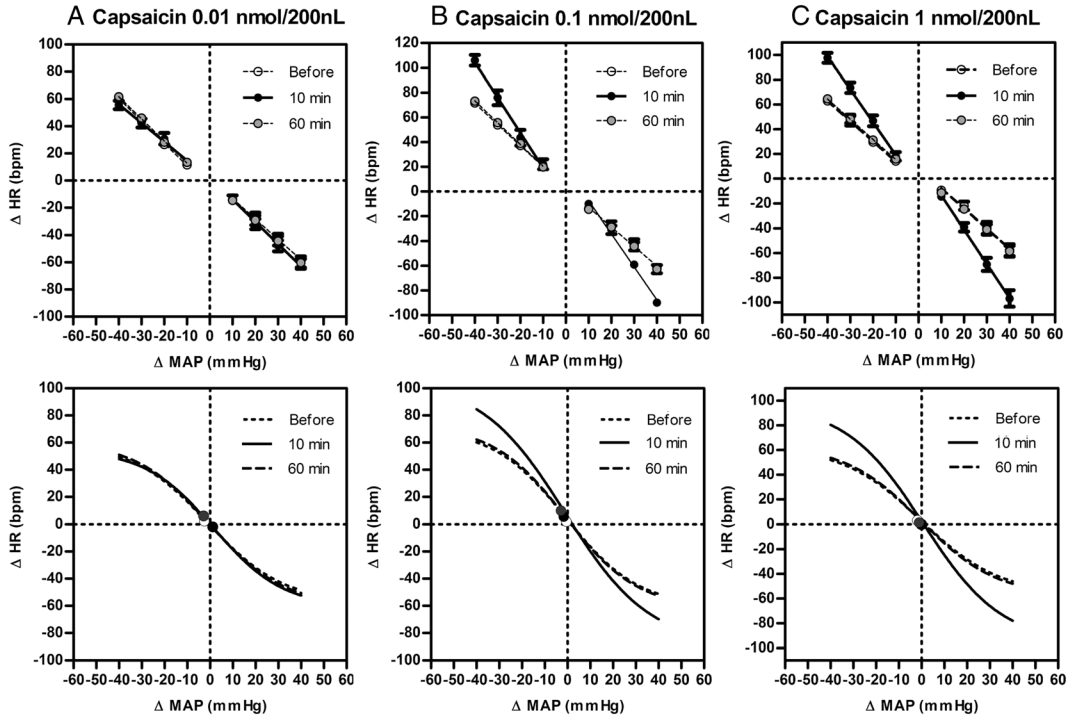

Figure 4

(A, B and C, higher) Regression curves correlating the responses of ΔMAP and ΔHR before, 10 min and 60 min after bilateral microinjection of capsaicin into the vMPFC. Correlation r^2 values for bradycardic regression curves were 0.83, 0.83 and 0.84 for data recorded before; 0.85, 0.96 and 0.91 for data recorded 10 min after; and 0.85, 0.89 and 0.87 for data recorded 60 min after microinjection of capsaicin 0.01 (A), 0.1 (B) or 1 nmol (C) into the vMPFC. Correlation r^2 values for tachycardic regression curves were 0.97, 0.87 and 0.90 for data recorded before; 0.82, 0.87 and 0.93 for data recorded 10 min after; and 0.97, 0.93 and 0.93 for data recorded 60 min after microinjection of capsazepina into the vMPFC. (A, B and C, lower) Sigmoid curves correlating mean arterial pressure (ΔMAP) and heart rate (ΔHR) before ($r^2 = 0.92$; 0.93; 0.93) and 10 min ($r^2 = 0.92$; 0.89; 0.96) and 60 min ($r^2 = 0.95$; 0.96; 0.96) after bilateral microinjection of 0.01 nmol ($n = 6$; A), 0.1 nmol ($n = 6$; B) or 1 nmol ($n = 5$; C) of capsaicin into the vMPFC. Values are means ± SEM. bpm, beats min^{-1}. The circles in the sigmoidal curves represents the BP50.

Table 3

Sigmoidal curve parameters generated before, 10 and 60 min after bilateral microinjection of 0.01, 0.1 or 1 nmol capsaicin into the vMPFC

Group	G (beats min^{-1} mmHg^{-1})	P1 (beats min^{-1})	P2 (beats min^{-1})	Range (beats min^{-1})	BP_{50} (mmHg)
Capsaicin 0.01 nmol	$F_{(2,17)} = 1.95$	$F_{(2,17)} = 0.12$	$F_{(2,17)} = 2.27$	$F_{(2,17)} = 0.22$	$F_{(2,17)} = 0.13$
Before	−1.32 ± 0.06	−58 ± 3	60 ± 2	119 ± 9	−1.33 ± 1.52
10 min	−1.48 ± 0.07	−61 ± 4	55 ± 3	116 ± 2	1.88 ± 1.31
60 min	−1.42 ± 0.05	−60 ± 4	61 ± 2	122 ± 4	−1.20 ± 1.10
Capsaicin 0.1 nmol	$F_{(2,17)} = 1.00$	$F_{(2,17)} = 30.82$	$F_{(2,17)} = 35.89$	$F_{(2,17)} = 58.50$	$F_{(2,17)} = 0.83$
Before	−1.62 ± 0.06	−61 ± 3	71 ± 3	133 ± 3	−0.20 ± 1.50
10 min	−1.76 ± 0.09	−89 ± 3*	196 ± 4*	196 ± 6*	−0.98 ± 0.61
60 min	−1.70 ± 0.05	−62 ± 3	73 ± 3	135 ± 4	−1.11 ± 1.13
Capsaicin 1 nmol	$F_{(2,14)} = 33.69$	$F_{(2,14)} = 17.33$	$F_{(2,14)} = 37.37$	$F_{(2,14)} = 35.34$	$F_{(2,14)} = 0.46$
Before	−1.25 ± 0.06	−57 ± 5	63 ± 3	120 ± 6	−1.05 ± 0.79
10 min	−1.96 ± 0.09*	−96 ± 7*	97 ± 4*	194 ± 9*	0.72 ± 1.05
60 min	−1.38 ± 0.05	−58 ± 4	64 ± 3	123 ± 5	−0.75 ± 1.24

Values are means ± SEM; $n = 6$ for 0.01 nmol, $n = 6$ for 0.1 nmol, and $n = 5$ for 1 nmol capsaicin. G, average gain; P1, lower HR plateau; P2, upper HR plateau; Range, ΔP.
*$P < 0.05$, significant difference from values before capsaicin administration, one-way ANOVA followed by Dunnett's *post hoc* test.

Once more, the injection of capsaicin 1 nmol 200 nL^{-1} ($n = 5$) did not modify the basal levels of the MAP and HR. However, it did increase the slope of the linear regression curve of both the bradycardic and tachycardic components of the baroreceptor reflex (Figure 4). The sigmoid curve parameters were also increased by the microinjection of capsaicina 1 nmol 200 nL^{-1} (Table 3).

The slope of the linear regression curves 60 min after the microinjection of capsaicin 0.1 nmol and 1 nmol (Figure 4), as well as the non-linear regression curve parameters returned to normal levels (Table 3).

Afterwards, the vMPFC was pretreated with 6-IODO 0.3 nmol 200 nL^{-1} (ineffective dose) ($n = 5$), and 5 min later the animals received the microinjection of capsaicin 1 nmol 200 nL^{-1}. This protocol was used to confirm that capsaicin activated the TRPV1 receptors. This pretreatment with 6-IODO 0.3 nmol abolished the increased baroreflex activity caused by capsaicin, as observed from the linear regression analysis of the bradycardic and tachycardic reflex responses (Figure 5). It also inhibited the alteration in the sigmoid curve parameters induced by capsaicin (Table 4).

Posteriorly, the vMPFC was pretreated with 6-IODO 0.3 nmol, and 5 min later capsaicin 3 nmol was microinjected ($n = 6$). The linear regression curve slope of the tachycardic response was significantly increased (Figure 5). However, the change in the bradycardic reflex slope was not significant (Figure 5). The sigmoid curve parameters were augmented after the microinjection of 6-IODO and capsaicin 3 nmol (Table 4).

Additionally, the vMPFC was pretreated with 6-IODO 0.3 nmol and 5 min later capsaicin 10 nmol was microinjected ($n = 5$). The slope of the tachycardic and bradycardic components of the baroreflex activity was increased (Figure 5). The sigmoid curve parameters were significantly increased after the microinjection of the drugs into this area (Table 4).

The slope of the linear regression curves 60 min after the microinjection of 6-IODO 0.3 nmol and capsaicin 3 nmol or 10 nmol (Figure 5), as well as the non-linear regression curve parameters had returned to basal levels (Table 4).

Based on these results, dose–response curves were plotted. Such curves correlate the doses of capsaicin before (0.01, 0.1 and 1 nmol) and after (1, 3 and 10 nmol) the vMPFC pretreatment with 6-IODO 0.3 nmol with the sigmoid curve parameters, the slope of the bradycardic and tachycardic components. The medium effective-concentration (EC_{50}) of the curves related to the gain (G), P1, P2, the ΔP, as well as to the slope of the bradycardic and tachycardic responses were shifted towards the right (Figure 6). Moreover, the maximum response level with regard to the slope of the cureves for G, P1, P2, the ΔP, bradycardic and tachycardic reflex was not altered (Figure 6).

Discussion

In the present work we assumed that the vMPFC TRPV1 receptors have a facilitatory role on baroreflex activity. Therefore, we primarily tested the effect of the TRPV1 receptor antagonist, capsazepine. This compound was able to reduce the bradycardic and tachycardic responses, suggesting that such receptors increase the cardiac parasympathetic and sympathetic outflow during baroreflex stimulation (Head and McCarty, 1987). Nevertheless, capsazepine has also been shown to block voltage-gated calcium channels (Docherty *et al.*, 1997). Thus, in order to confirm the action of capsazepine was mediated by its effect on TRPV1 receptors, we used 6-IODO, a more selective TRPV1 antagonist (Appendino *et al.*, 2003). Similarly to capsazepine, 6-IODO also reduced the baroreflex response, and the effective dose of this compound was lower than that of capsazepine,

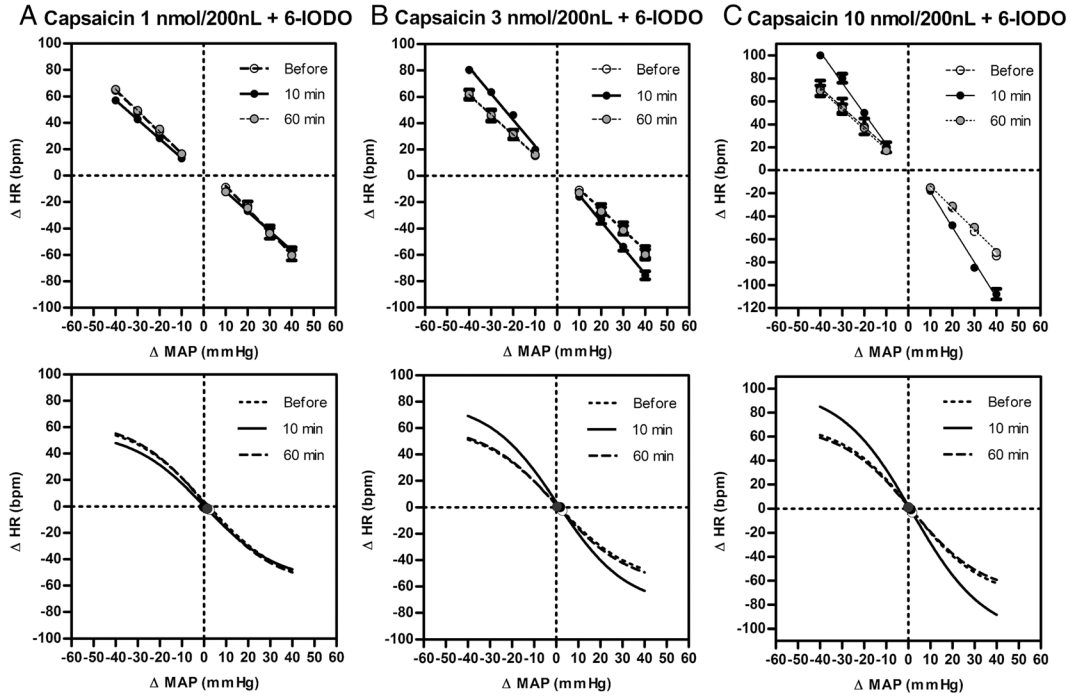

Figure 5

(A, B and C, higher) Regression curves correlating the responses of ΔMAP and ΔHR before, 10 min and 60 min after bilateral microinjection of capsaicin into the vMPFC. Correlation r^2 values for bradycardic regression curves were 0.84, 0.82 and 0.94 for data recorded before; 0.84, 0.92 and 0.95 for data recorded 10 min after; and 0.90, 0.86 and 0.95 for data recorded 60 min after microinjection of 6-IODO 0.3 nmol previously to capsaicin 1 (A), 3 (B) or 10 nmol (C) into the vMPFC. Correlation r^2 values for tachycardic regression curves were 0.97, 0.87 and 0.90 for data recorded before; 0.82, 0.87 and 0.93 for data recorded 10 min after and 0.97, 0.93 and 0.93 for data recorded 60 min after microinjection of capsaicin and 6-IODO into the vMPFC. (A, B and C, lower) Sigmoid curves correlating mean arterial pressure (ΔMAP) and heart rate (ΔHR) before (r^2 = 0.95; 0.94; 0.95) and 10 min (r^2 = 0.97; 0.97; 0.95) and 60 min (r^2 = 0.97; 0.95; 0.97) after bilateral microinjection of 6-IODO 0.3 nmol, previously to capsaicin 1 nmol (n = 5; A), 3 nmol (n = 6; B) or 10 nmol (n = 5; C) into the vMPFC. Values are means ± SEM. bpm, beats min^{-1}. The circles in the sigmoidal curves represent the BP_{50}.

corroborating previous data from the literature, which demonstrated that 6-IODO is more potent than capsazepine (Appendino *et al.*, 2003; Aguiar, 2009; Aguiar *et al.*, 2009).

The TRPV1 receptors trigger calcium influx (Miyamoto *et al.*, 2009), which may lead to the synthesis of NO (Miyamoto *et al.*, 2009). NO can increase glutamate release at presynaptic neurons (Brenman *et al.*, 1996; Cheah *et al.*, 2006). Because the vMPFC NMDA/NO signalling pathway enhances the baroreflex response (Ferreira-Junior *et al.*, 2013), it is possible that activation of the TRPV1 receptor could stimulate NO synthesis and glutamate release, facilitating the baroreceptor reflex response.

A possible source of glutamate input to the vMPFC is the thalamus, which stimulates the PL and IL during cardiovascular events associated with contextual fear conditions (Groenewegen, 1988; Powell *et al.*, 1990; Pirot *et al.*, 1994). Beyond that, the vMPFC reciprocally connects to medullary areas involved in baroreflex and autonomic control, such as the caudal ventrolateral medulla, the rostral ventrolateral medulla and the nucleus of the solitary tract (Verberne and Owens, 1998; Owens *et al.*, 1999; Fisk and Wyss, 2000; Owens and Verberne, 2000). Thus, we propose that TRPV1 channels are involved in the stimulation of these projections by increasing glutamate release in the vMPFC, during baroreflex stimulation.

In addition, it was observed that 6-IODO abolished the cardiac baroreflex alterations evoked by capsaicin. Therefore, by exploring the competitive properties of these compounds, we confirmed that the increase in the cardiac baroreflex response evoked by capsaicin was due to activation of the vMPFC TRPV1 receptors. Moreover, microinjections of higher doses of capsaicin (3 and 10 nmol) into the area, after 6-IODO, was able to enhance baroreflex activity to levels similar to those observed with lower doses of capsaicin (0.1 and 1 nmol) in the absence of 6-IODO. The medium EC_{50} corresponds to the agonist dose that is able to reach 50% of the maximum response (Wyllie and Chen, 2007). Through analysis of EC_{50} values, we observed that 6-IODO displaced the capsaicin dose–response curve to the right, with no changes in the maximum response, which is suggestive of competitive antagonism (Colquhoun *et al.*, 1979; Wyllie and Chen, 2007), and indicates that capsaicin and 6-IODO are binding to the same site.

It is possible that AEA activates CB_1 receptors (Devane *et al.*, 1992) as well as TRPV1 receptors (Zygmunt *et al.*, 1999; Smart and Jerman, 2000). Furthermore, it has been demonstrated that in the PL region the endovanilloid and endocannabinoids systems interact to modulate anxiety-like behaviour in rats (Fogaca *et al.*, 2012), suggesting the existence of an opposing physiological relationship between these systems in the vMPFC. Previous experiments from our group showed that baroreflex activity is enhanced CB_1 receptors in the are antagonized (Ferreira-Junior *et al.*, 2011). In

Table 4

Sigmoidal curve parameters generated before, 10 and 60 min after bilateral microinjection of 0.3 nmol of 6-IODO previously to 1, 3 or 10 nmol capsaicin into the vMPFC

Group	G (beats min^{-1} mmHg^{-1})	P1 (beats min^{-1})	P2 (beats min^{-1})	Range (beats min^{-1})	BP_{50} (mmHg)
6-IODO 0,3 + Capsaicina 1	$F_{(2,14)} = 2.66$	$F_{(2,14)} = 0.15$	$F_{(2,14)} = 3.66$	$F_{(2,14)} = 2.02$	$F_{(2,14)} = 0.92$
Before	−1.36 ± 0.06	−60 ± 6	65 ± 3	125 ± 5	1.06 ± 1.83
10 min	−1.34 ± 0.03	−57 ± 3	57 ± 2	114 ± 4	0.49 ± 0.98
60 min	−1.48 ± 0.04	−60 ± 4	65 ± 2	125 ± 4	0.19 ± 1.59
6-IODO 0,3 + Capsaicina 3	$F_{(2,17)} = 27.06$	$F_{(2,17)} = 6.30$	$F_{(2,17)} = 12.40$	$F_{(2,17)} = 14.09$	$F_{(2,17)} = 0.83$
Before	−1.38 ± 0.06	−58 ± 4	62 ± 4	119 ± 6	1.02 ± 0.66
10 min	−1.93 ± 0.06*	−76 ± 3*	80 ± 2*	156 ± 3*	0.87 ± 0.43
60 min	−1.47 ± 0.05	−60 ± 4	62 ± 3	122 ± 6	0.39 ± 1.05
6-IODO 0,3 + Capsaicina10	$F_{(2,14)} = 20.90$	$F_{(2,14)} = 34.51$	$F_{(2,14)} = 12.11$	$F_{(2,14)} = 36.31$	$F_{(2,14)} = 0.61$
Before	−1.79 ± 0.10	−74 ± 2	71 ± 7	146 ± 6	2.92 ± 1.80
10 min	−2.29 ± 0.05*	−108 ± 5*	100 ± 2*	207 ± 5*	1.94 ± 101
60 min	−1.69 ± 0.04	−72 ± 3	69 ± 4	149 ± 6	0.96 ± 1.15

Values are means ± SEM; $n = 5$ for 1 nmol, $n = 6$ for 3 nmol and $n = 10$ for 1 nmol capsaicin after 0.3 nmol of 6-IODO. G, average gain; P1, lower HR plateau; P2, upper HR plateau; range, ΔP.
*$P < 0.05$, significant difference from values before capsaicin and 6-IODO administration, one-way ANOVA followed by Dunnett's *post hoc* test.

contrast, blockade of the vMPFC TRPV1 receptors induced a reduction in the baroreflex response, suggesting that they are acting through the same pathway inside the area to modulate the baroreflex cardiac activity.

During stressful situations, there is an alteration in the set point of the baroreflex activity, which allows the HR and MAP to increase concomitantly (Crestani *et al.*, 2010). The vMPFC has been suggested to subserve this cardiovascular adjustment (Resstel *et al.*, 2006b; Tavares *et al.*, 2009; Lisboa *et al.*, 2010). In addition, vMPFC TRPV1 channels advocate an enhancement in the HR and MAP in animals subjected to contextual fear conditioning (Terzian *et al.*, 2013). The results of the present study show that these receptors increase the tachycardic response to baroreflex stimulation. Therefore, it is possible that vMPFC TRPV1 receptors are involved in the baroreflex alterations during defensive reactions, which lead to a concomitant increase in MAP and HR, characteristic of autonomic responses to aversive situations. However, we found that the TRPV1 receptors can also intensify the bradycardic response, which seems to be inhibited during stressful situations (Tavares *et al.*, 2009). Nevertheless, there are other brain structures that can be activated during emotional stress, which are able to decrease the baroreflex parasympathetic activity, such as the lateral hypothalamus (Kiss, 2007; Crestani *et al.*, 2009), enabling the HR and MAP to increase during stressful situations.

The vMPFC is also engaged in the emotional and cognitive components of pain (Mohr *et al.*, 2005; Wiech *et al.*, 2006). Giordano and co-workers (Giordano *et al.*, 2011) demonstrated an increased TRPV1expression in the PL and IL regions of neuropathic mice (Giordano *et al.*, 2011). Painful stimuli modify the basal levels of MAP, HR and sympathetic activity, revealing a cardiovascular component in the pain response (Fazalbhoy *et al.*, 2012). In addition, the bradycardic reflex is suppressed in rats that had undergone nerve injury (Gemes *et al.*, 2009). Together with our results, these findings raise the possibility that the vMPFC TRPV1 channels are involved in the enhancement of the tachycardic response during painful conditions and could be involved in the changes in the baroreflex activity induced by chronic pain.

Tavares and colleagues demonstrated that the PL and IL areas have an opposing role in the cardiac response of rats subjected to restraint stress (Tavares *et al.*, 2009). Moreover, the pressor response related to the chemoreflex stimulation is facilitated by the PL but not by the IL in rats (Granjeiro *et al.*, 2011). Such differences between these areas in their effects on autonomic control could be explained by their different patterns of projections. For instance, the IL densely innervates the NTS of the brain stem, central, medial, basomedial and cortical amygdaloid nuclei, while the PL sends projections to the ventral tegmental area and basolateral nucleus of the amygdala (Fisk and Wyss, 2000; Vertes, 2004). Thus, we investigated if the TRPV1 in these subareas could differently modulate baroreflex activity. Microinjection of 6-IODO into either the PL or IL regions showed that these TRPV1 receptors similarly modulate the cardiac baroreflex response. Nonetheless, our results are consistent with other data showing no difference in the modulation of cardiac baroreflex activity by either PL or IL CB_1 receptors (Ferreira-Junior *et al.*, 2011). In addition, the PL and IL commonly connect to other brain regions that regulate autonomic activity, such as several nuclei of the thalamus, periaqueductal grey matter, the bed nucleus of the stria terminalis and lateral hypothalamus (Fisk and Wyss, 2000; Vertes, 2004). Such structures could work as a relay for projections from the vMPFC, suggesting a possible explanation for

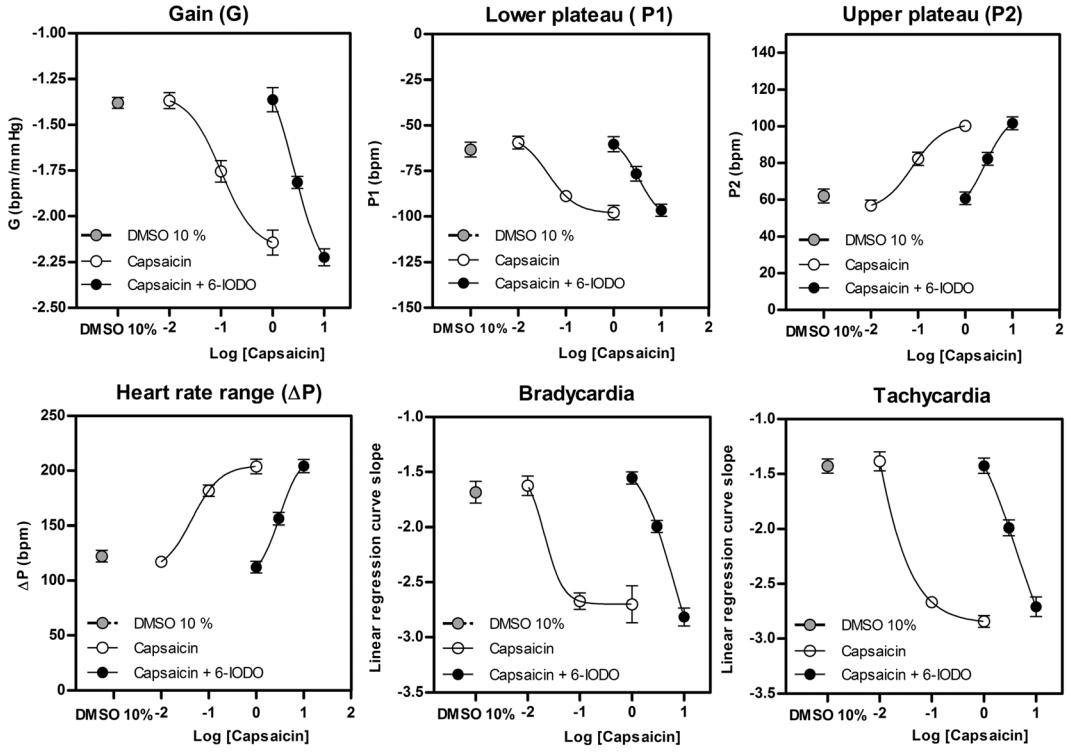

Figure 6

Correlation between increasing doses of capsaicin before (0.01, 0.1 and 1 nmol) and after (1, 3 and 10 nmol) the pretreatment of the vMPFC with 6-IODO 0.3 nmol and the alterations in the non-linear regression (G, P1, P2, ΔHR) and linear regression (bradycardia and tachycardia) parameters. Correlation values of the regression curves were: G ($r^2 = 0{,}86$; gl = 14); P1 ($r^2 = 0{,}81$; gl = 17); P2 ($r^2 = 0{,}85$; gl = 17); ΔHR ($r^2 = 0{,}89$; gl = 17); Bradycardia ($r^2 = 0{,}78$; gl = 14) and tachycardia ($r^2 = 0{,}95$; gl = 14) for the curves plotted before and G ($r^2 = 0{,}91$; gl = 14); P1 ($r^2 = 0{,}71$; gl = 17); P2 ($r^2 = 0{,}80$; gl = 17); ΔHR ($r^2 = 0{,}88$; gl = 17); bradycardia ($r^2 = 0{,}93$; gl = 14) and tachycardia ($r^2 = 0{,}90$; gl = 14) for the curves plotted after the pretreatment with 6-IODO.

the lack of difference between the roles played by the PL and IL TRPV1 channels in the baroreflex function.

It has been demonstrated that autonomic and cardiovascular activities are impaired in psychiatric and neurodegenerative disorders, such as posttraumatic stress disorder (PTSD), multiple sclerosis (MS) and Alzheimer's disease (AD) (Sanya *et al.*, 2005; Hughes *et al.*, 2007; Femminella *et al.*, 2014). Moreover, the concentration of AEA has been reported to be lowere in the vMPFC of animals subjected to an MS model (Cabranes *et al.*, 2005). Furthermore, there is cortical atrophy and prefrontal cortex dysfunction in PTSD/AD patients (Koenigs and Grafman, 2009; Kulijewicz-Nawrot *et al.*, 2012). Therefore, the present study adds information for a better understanding of the cardiovascular symptoms associated with these disorders.

In conclusion, the present study demonstrates that the PL/ILTRPV1 receptors facilitate the cardiac baroreflex activity.

Acknowledgements

The authors wish to thank Camargo, L.H.A. and Mesquita O. for technical help. Davi C. Lagatta has a Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) fellowship; Nilson C. Ferreira-Junior has a Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) fellowship. The present research was also supported by the Fundação de Apoio ao Ensino, Pesquisa e Assistência do Hospital das Clínicas daFMRP-USP (FAEPA) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Author contributions

D. C. L. and L. R. conceived and designed this research; D.C. L. and N. C. F-J. performed the experiments; D.C. L. and L. R. analysed the data; D.C. L., N. C. F. –J. and L. R. interpreted the results of experiments; D. C. L. prepared the figures and drafted the manuscript; D. C. L. and L. R. edited and revised the manuscript; L. R. approved the final version of the manuscript.

Conflict of interests

None.

References

Aguiar DC (2009). Anxiolytic effects of 6-iodonordihydrocapsaicin, a TRPV1 antagonist, injected into the medial prefrontal cortex of rats. Eur Neuropsychopharmacol 19: 597–598.

Aguiar DC, Terzian AL, Guimaraes FS, Moreira FA (2009). Anxiolytic-like effects induced by blockade of transient receptor potential vanilloid type 1 (TRPV1) channels in the medial prefrontal cortex of rats. Psychopharmacology (Berl) 205: 217–225.

Alexander SP, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013a). The concise guide to PHARMACOLOGY 2013/14: G protein-coupled receptors. Br J Pharmacol 170: 1459–1581.

Alexander SP, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013b). The concise guide to PHARMACOLOGY 2013/14: ligand-gated ion channels. Br J Pharmacol 170: 1582–1606.

Alves FH, Crestani CC, Resstel LB, Correa FM (2009a). Insular cortex alpha1-adrenoceptors modulate the parasympathetic component of the baroreflex in unanesthetized rats. Brain Res 1295: 119–126.

Alves FH, Crestani CC, Resstel LB, Correa FM (2009b). N-methyl-D-aspartate receptors in the insular cortex modulate baroreflex in unanesthetized rats. Auton Neurosci 147: 56–63.

Appendino G, Harrison S, De Petrocellis L, Daddario N, Bianchi F, Schiano Moriello A *et al.* (2003). Halogenation of a capsaicin analogue leads to novel vanilloid TRPV1 receptor antagonists. Br J Pharmacol 139: 1417–1424.

Auclair N, Otani S, Soubrie P, Crepel F (2000). Cannabinoids modulate synaptic strength and plasticity at glutamatergic synapses of rat prefrontal cortex pyramidal neurons. J Neurophysiol 83: 3287–3293.

Brenman JE, Christopherson KS, Craven SE, McGee AW, Bredt DS (1996). Cloning and characterization of postsynaptic density 93, a nitric oxide synthase interacting protein. J Neurosci 16: 7407–7415.

Cabranes A, Venderova K, de Lago E, Fezza F, Sanchez A, Mestre L *et al.* (2005). Decreased endocannabinoid levels in the brain and beneficial effects of agents activating cannabinoid and/or vanilloid receptors in a rat model of multiple sclerosis. Neurobiol Dis 20: 207–217.

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997). The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389: 816–824.

Cheah JH, Kim SF, Hester LD, Clancy KW, Patterson SE 3rd, Papadopoulos V *et al.* (2006). NMDA receptor-nitric oxide transmission mediates neuronal iron homeostasis via the GTPase Dexras1. Neuron 51: 431–440.

Colquhoun D, Dreyer F, Sheridan RE (1979). The actions of tubocurarine at the frog neuromuscular junction. J Physiol 293: 247–284.

Crestani CC, Deolindo MV, Alves FH, Resstel LB, Correa FM (2009). Non-N-methyl-d-aspartate glutamate receptors in the lateral hypothalamus modulate cardiac baroreflex responses in conscious rats. Clin Exp Pharmacol Physiol 36: 1079–1085.

Crestani CC, Tavares RF, Alves FH, Resstel LB, Correa FM (2010). Effect of acute restraint stress on the tachycardiac and bradycardiac responses of the baroreflex in rats. Stress 13: 61–72.

Cristino L, de Petrocellis L, Pryce G, Baker D, Guglielmotti V, Di Marzo V (2006). Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor potential vanilloid type 1 receptors in the mouse brain. Neuroscience 139: 1405–1415.

De Petrocellis L, Di Marzo V (2010). Non-CB1, non-CB2 receptors for endocannabinoids, plant cannabinoids, and synthetic cannabimimetics: focus on G-protein-coupled receptors and transient receptor potential channels. J Neuroimmune Pharmacol 5: 103–121.

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G *et al.* (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258: 1946–1949.

Di Marzo V, Maccarrone M (2008). FAAH and anandamide: is 2-AG really the odd one out? Trends Pharmacol Sci 29: 229–233.

Docherty RJ, Yeats JC, Piper AS (1997). Capsazepine block of voltage-activated calcium channels in adult rat dorsal root ganglion neurones in culture. Br J Pharmacol 121: 1461–1467.

Fazalbhoy A, Birznieks I, Macefield VG (2012). Individual differences in the cardiovascular responses to tonic muscle pain: parallel increases or decreases in muscle sympathetic nerve activity, blood pressure, and heart rate. Exp Physiol 97: 1084–1092.

Femminella GD, Rengo G, Komici K, Iacotucci P, Petraglia L, Pagano G *et al.* (2014). Autonomic dysfunction in Alzheimer's disease: tools for assessment and review of the literature. J Alzheimers Dis 42: 369–377.

Ferreira-Junior NC, Fedoce AD, Alves FH, Correa FM, Resstel LB (2011). Medial prefrontal cortex endocannabinoid system modulates baroreflex activity through CB1 receptors. Am J Physiol Regul Integr Comp Physiol 302: 876–885.

Ferreira-Junior NC, Fedoce AG, Alves FH, Resstel LB (2013). Medial prefrontal cortex N-methyl-D-aspartate receptor/nitric oxide/cyclic guanosine monophosphate pathway modulates both tachycardic and bradycardic baroreflex responses. J Neurosci Res 91: 1338–1348.

Fisk GD, Wyss JM (2000). Descending projections of infralimbic cortex that mediate stimulation-evoked changes in arterial pressure. Brain Res 859: 83–95.

Fogaca MV, Aguiar DC, Moreira FA, Guimaraes FS (2012). The endocannabinoid and endovanilloid systems interact in the rat prelimbic medial prefrontal cortex to control anxiety-like behavior. Neuropharmacology 63: 202–210.

Gemes G, Rigaud M, Dean C, Hopp FA, Hogan QH, Seagard J (2009). Baroreceptor reflex is suppressed in rats that develop hyperalgesia behavior after nerve injury. Pain 146: 293–300.

Giordano C, Cristino L, Luongo L, Siniscalco D, Petrosino S, Piscitelli F *et al.* (2011). TRPV1-dependent and -independent alterations in the limbic cortex of neuropathic mice: impact on glial caspases and pain perception. Cereb Cortex 22: 2495–2518.

Granjeiro EM, Scopinho AA, Correa FM, Resstel LB (2011). Prelimbic but not infralimbic cortex is involved in the pressor response to chemoreflex activation in awake rats. Exp Physiol 96: 518–527.

Groenewegen HJ (1988). Organization of the afferent connections of the mediodorsal thalamic nucleus in the rat, related to the mediodorsal–prefrontal topography. Neuroscience 24: 379–431.

Hampson AJ, Bornheim LM, Scanziani M, Yost CS, Gray AT, Hansen BM *et al.* (1998). Dual effects of anandamide on NMDA receptor-mediated responses and neurotransmission. J Neurochem 70: 671–676.

Head GA, McCarty R (1987). Vagal and sympathetic components of the heart rate range and gain of the baroreceptor-heart rate reflex in conscious rats. J Auton Nerv Syst 21: 203–213.

Hughes JW, Dennis MF, Beckham JC (2007). Baroreceptor sensitivity at rest and during stress in women with posttraumatic stress disorder or major depressive disorder. J Trauma Stress 20: 667–676.

Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG (2010). Animal research: reporting in vivo experiments: the ARRIVE guidelines. Br J Pharmacol 160: 1577–1579.

Kiss A (2007). Immobilization induced fos expression in the medial and lateral hypothalamic areas: a limited response of hypocretin neurons. Ideggyogy Sz 60: 192–195.

Koenigs M, Grafman J (2009). Posttraumatic stress disorder: the role of medial prefrontal cortex and amygdala. Neuroscientist 15: 540–548.

Kulijewicz-Nawrot M, Verkhratsky A, Chvatal A, Sykova E, Rodriguez JJ (2012). Astrocytic cytoskeletal atrophy in the medial prefrontal cortex of a triple transgenic mouse model of Alzheimer's disease. J Anat 221: 252–262.

Leonard CM (1969). The prefrontal cortex of the rat. I. Cortical projection of the mediodorsal nucleus. II. Efferent connections. Brain Res 12: 321–343.

Lisboa SF, Reis DG, da Silva AL, Correa FM, Guimaraes FS, Resstel LB (2010). Cannabinoid CB1 receptors in the medial prefrontal cortex modulate the expression of contextual fear conditioning. Int J Neuropsychopharmacol 13: 1163–1173.

McGrath J, Drummond G, McLachlan E, Kilkenny C, Wainwright C (2010). Guidelines for reporting experiments involving animals: the ARRIVE guidelines. Br J Pharmacol 160: 1573–1576.

Miyamoto T, Dubin AE, Petrus MJ, Patapoutian A (2009). TRPV1 and TRPA1 mediate peripheral nitric oxide-induced nociception in mice. PLoS One 4: 7596–7606.

Mohr C, Binkofski F, Erdmann C, Buchel C, Helmchen C (2005). The anterior cingulate cortex contains distinct areas dissociating external from self-administered painful stimulation: a parametric fMRI study. Pain 114: 347–357.

Musella A, De Chiara V, Rossi S, Prosperetti C, Bernardi G, Maccarrone M *et al.* (2009). TRPV1 channels facilitate glutamate transmission in the striatum. Mol Cell Neurosci 40: 89–97.

Owens NC, Sartor DM, Verberne AJ (1999). Medial prefrontal cortex depressor response: role of the solitary tract nucleus in the rat. Neuroscience 89: 1331–1346.

Owens NC, Verberne AJ (2000). Medial prefrontal depressor response: involvement of the rostral and caudal ventrolateral medulla in the rat. J Auton Nerv Syst 78: 86–93.

Pawson AJ, Sharman JL, Benson HE, Faccenda E, Alexander SP, Buneman OP *et al.* (2014). The IUPHAR/BPS Guide to PHARMACOLOGY: an expert-driven knowledgebase of drug targets and their ligands. Nucl Acids Res 42 (Database Issue): D1098–D1106.

Paxinos G, Watson C (1997). The Rat Brain in Stereotaxic Coordinates, 2nd edn. Academic Press: Sydney, Australia.

Pirot S, Jay TM, Glowinski J, Thierry AM (1994). Anatomical and electrophysiological evidence for an excitatory amino acid pathway from the thalamic mediodorsal nucleus to the prefrontal cortex in the rat. Eur J Neurosci 6: 1225–1234.

Powell DA, Buchanan SL, Gibbs CM (1990). Role of the prefrontal–thalamic axis in classical conditioning. Prog Brain Res 85: 433–465 discussion 436–465.

Resstel LB, Correa FM (2006a). Medial prefrontal cortex NMDA receptors and nitric oxide modulate the parasympathetic component of the baroreflex. Eur J Neurosci 23: 481–488.

Resstel LB, Fernandes KB, Correa FM (2004). Medial prefrontal cortex modulation of the baroreflex parasympathetic component in the rat. Brain Res 1015: 136–144.

Resstel LB, Joca SR, Guimaraes FG, Correa FM (2006b). Involvement of medial prefrontal cortex neurons in behavioral and cardiovascular responses to contextual fear conditioning. Neuroscience 143: 377–385.

Rosenbaum T, Simon SA (2007). TRPV1 receptors and signal transduction. Frontiers in Neuroscience, chapter 5.

Sanya EO, Tutaj M, Brown CM, Goel N, Neundorfer B, Hilz MJ (2005). Abnormal heart rate and blood pressure responses to baroreflex stimulation in multiple sclerosis patients. Clin Auton Res 15: 213–218.

Smart D, Jerman JC (2000). Anandamide: an endogenous activator of the vanilloid receptor. Trends Pharmacol Sci 21: 134.

Tavares RF, Correa FM, Resstel LB (2009). Opposite role of infralimbic and prelimbic cortex in the tachycardiac response evoked by acute restraint stress in rats. J Neurosci Res 87: 2601–2607.

Terzian AL, Aguiar DC, Guimaraes FS, Moreira FA (2009). Modulation of anxiety-like behaviour by Transient Receptor Potential Vanilloid Type 1 (TRPV1) channels located in the dorsolateral periaqueductal gray. Eur Neuropsychopharmacol 19: 188–195.

Terzian AL, Dos Reis DG, Guimaraes FS, Correa FM, Resstel LB (2013). Medial prefrontal cortex Transient Receptor Potential Vanilloid Type 1 (TRPV1) in the expression of contextual fear conditioning in Wistar rats. Psychopharmacology (Berl) 231: 149–157.

Toth A, Boczan J, Kedei N, Lizanecz E, Bagi Z, Papp Z *et al.* (2005). Expression and distribution of vanilloid receptor 1 (TRPV1) in the adult rat brain. Brain Res Mol Brain Res 135: 162–168.

Vaughan CW, Connor M, Bagley EE, Christie MJ (2000). Actions of cannabinoids on membrane properties and synaptic transmission in rat periaqueductal gray neurons in vitro. Mol Pharmacol 57: 288–295.

Verberne AJ, Owens NC (1998). Cortical modulation of the cardiovascular system. Prog Neurobiol 54: 149–168.

Vertes RP (2004). Differential projections of the infralimbic and prelimbic cortex in the rat. Synapse 51: 32–58.

Wiech K, Kalisch R, Weiskopf N, Pleger B, Stephan KE, Dolan RJ (2006). Anterolateral prefrontal cortex mediates the analgesic effect of expected and perceived control over pain. J Neurosci 26: 11501–11509.

Wyllie DJ, Chen PE (2007). Taking the time to study competitive antagonism. Br J Pharmacol 150: 541–551.

Xing J, Li J (2007). TRPV1 receptor mediates glutamatergic synaptic input to dorsolateral periaqueductal gray (dl-PAG) neurons. J Neurophysiol 97: 503–511.

Yang L, Fujita T, Jiang CY, Piao LH, Yue HY, Mizuta K *et al.* (2011). TRPV1 agonist piperine but not olvanil enhances glutamatergic spontaneous excitatory transmission in rat spinal substantia gelatinosa neurons. Biochem Biophys Res Commun 410: 841–845.

Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sorgard M, Di Marzo V *et al.* (1999). Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 400: 452–457.