

## RESEARCH PAPER

The kinin B<sub>1</sub> receptor antagonist SSR240612 reverses tactile and cold allodynia in an experimental rat model of insulin resistanceJP Dias<sup>1</sup>, MA Ismael<sup>1</sup>, M Pilon<sup>1</sup>, J de Champlain<sup>1</sup>, B Ferrari<sup>2</sup>, P Carayon<sup>2</sup> and R Couture<sup>1</sup><sup>1</sup>Department of Physiology, Faculty of Medicine, Université de Montréal, Montréal, Québec, Canada and <sup>2</sup>Sanofi-Aventis R&D, Montpellier, France

**Background and purpose:** Diabetes causes sensory polyneuropathy with associated pain in the form of tactile allodynia and thermal hyperalgesia which are often intractable and resistant to current therapy. This study tested the beneficial effects of the non-peptide and orally active kinin B<sub>1</sub> receptor antagonist SSR240612 against tactile and cold allodynia in a rat model of insulin resistance.

**Experimental approach:** Rats were fed with 10% D-glucose for 12 weeks and effects of orally administered SSR240612 (0.3–30 mg kg<sup>-1</sup>) were determined on the development of tactile and cold allodynia. Possible interference of SSR240612 with vascular oxidative stress and pancreatic function was also addressed.

**Key results:** Glucose-fed rats exhibited tactile and cold allodynia, increases in systolic blood pressure and higher plasma levels of insulin and glucose, at 12 weeks. SSR240612 blocked tactile and cold allodynia at 3 h (ID<sub>50</sub> = 5.5 and 7.1 mg kg<sup>-1</sup>, respectively) in glucose-fed rats but had no effect in control rats. The antagonist (10 mg kg<sup>-1</sup>) had no effect on plasma glucose and insulin, insulin resistance (HOMA index) and aortic superoxide anion production in glucose-fed rats.

**Conclusions and implications:** We provide the first evidence that the B<sub>1</sub> receptors are involved in allodynia in an experimental rat model of insulin resistance. Allodynia was alleviated by SSR240612 most likely through a direct inhibition of B<sub>1</sub> receptors affecting spinal cord and/or sensory nerve excitation. Thus, orally active non-peptide B<sub>1</sub> receptor antagonists should have clinical therapeutic potential in the treatment of sensory polyneuropathy.

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**Keywords:** kinins; bradykinin; B<sub>1</sub> receptor; diabetes mellitus; insulin resistance; allodynia; polyneuropathy; SSR240612

**Abbreviations:** BK, bradykinin

## Introduction

The World Health Organization has estimated that there will be around 300 million patients with clinically diagnosed type II diabetes worldwide by the year 2025 (King *et al.*, 1998; Gorus *et al.*, 2004). The epidemic of diabetes is associated with a wide variety of long-term devastating macrovascular and microvascular complications that contribute to the increased mortality and morbidity (Feldman, 2003). Although several therapies are successfully used to control glycaemia with antidiabetic drugs and insulin, the major diabetic complications cannot be prevented and occur in 20–30% of type II diabetic patients which constitute 90% of the diabetic population (Feldman, 2003; Zimmet, 2003).

Recent evidence suggests a link between hyperglycaemia-induced oxidative stress and the kinin B<sub>1</sub> receptor in the development of these clinical complications (Couture and Girolami, 2004; El Midaoui *et al.*, 2005; Lungu *et al.*, 2007).

Kinins are pro-inflammatory mediators of pain, which are also thought to be involved in the central autonomic control of blood pressure and nociception (Couture and Lindsey, 2000; Couture *et al.*, 2001). These peptides acts on two G-protein-coupled receptors, a constitutive B<sub>2</sub> receptor and an inducible B<sub>1</sub> receptor. Bradykinin (BK) and Lys-BK or kallidin are the natural agonists for the prevailing B<sub>2</sub> receptors, while the kininase I metabolites des-Arg<sup>9</sup>-BK and des-Arg<sup>10</sup>-kallidin are the preferential agonists for B<sub>1</sub> receptors (Regoli *et al.*, 1998). The B<sub>1</sub> receptor is weakly expressed in healthy tissues and animals, but this receptor is upregulated and overexpressed in the presence of pro-inflammatory cytokines, bacterial endotoxins and hyperglycaemia-induced oxidative stress (Marceau *et al.*, 1998; deBlois and Horlick, 2001; Couture and Girolami, 2004). A role was suggested for B<sub>1</sub> receptors in vascular hyperpermeability, hyperalgesia,

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nephropathy, retinopathy, neuropathy and cardiovascular complications associated with type I diabetes (Cloutier and Couture, 2000; Campos *et al.*, 2001, 2005; Mage *et al.*, 2002; Simard *et al.*, 2002; Abdouh *et al.*, 2003; Gabra and Sirois 2003; Ongali *et al.*, 2004; Lawson *et al.*, 2005; Gabra *et al.*, 2006). Recently, our laboratory reported an increased density of B<sub>1</sub> receptor binding sites in the brain, spinal cord and peripheral tissues of rats treated with glucose (10% in drinking water) for a period of 4 and 12 weeks (El Midaoui *et al.*, 2005; Lungu *et al.*, 2007). This experimental rat model presents several hallmarks of type II diabetes such as greater plasma levels of glucose and insulin, arterial hypertension, insulin resistance and increases in the production of superoxide anion (marker of oxidative stress) in the heart and aorta (El Midaoui and de Champlain, 2002, 2005; El Midaoui *et al.*, 2003, 2005). Further studies have shown sensory abnormalities, namely tactile and cold allodynia, after 4 weeks of treatment with glucose (Lungu *et al.*, 2007).

The primary objective of this study was to determine whether the newly orally active non-peptide and selective B<sub>1</sub> receptor antagonist SSR240612 (Gougat *et al.*, 2004) can reverse tactile and cold allodynia in glucose-fed rats, a model of insulin resistance. The possibility that the acute anti-allodynic effect of SSR240612 could be attributed to a correction of pancreatic function or oxidative stress was also addressed.

## Methods

### *Animal model*

Young male Sprague–Dawley rats (50–75 g, Charles River, Quebec, Canada) were housed two per cage, under controlled conditions of temperature (23°C) and humidity (50%), on a 12 h light–dark cycle and allowed free access to normal chow diet (Charles River Rodent no. 5075) and tap water (control rats) or 10% D-glucose (Sigma-Aldrich Canada, Oakville, Ontario, Canada) in the drinking water. All research procedures and the care of the animals were in compliance with the guiding principles for animal experimentation as enunciated by the Canadian Council on Animal Care and were approved by the Animal Care Committee of our University.

Systolic arterial blood pressure was measured by tail-cuff photoplethysmography (Harvard Apparatus Ltd, Kent, UK) and registered using MacLab/8 system. For each measurement, three individual readings were averaged (El Midaoui *et al.*, 2005).

### *Behavioural testing*

Behavioural signs representing tactile and cold allodynia were assessed with the rats placed on a wire mesh floor beneath an inverted plastic cage (20 × 10 × 10 cm). The rats were allowed to acclimatize for about 15 min or until explorative behaviour ceased.

### *Tactile allodynia*

Tactile allodynia was assessed by measuring the hind paw-withdrawal threshold to a calibrated series of six von Frey

filaments (2, 4, 6, 8, 10 and 15 g) (Stoelting, Wood Dale, IL, USA) using the up-down method of Chaplan *et al.* (1994). Starting with the filament that has the lowest force (2 g), the filament was applied perpendicularly to the mid-plantar surface with sufficient force to cause the filament to buckle slightly. Brisk withdrawal or paw flinching was considered as positive response. Each filament was applied five times to each paw (for 6–8 s per stimulation, with an interstimulus interval of 1–2 min). Minimum recording of five positive responses (50%) out of 10 stimulations for both paws was considered to be the threshold (in grams). Absence of a response (less than five withdrawals) prompted use of the next graded filament of increasing weight. Maximal withdrawal threshold in control rats was fixed at 15 g.

### *Cold allodynia*

Cold allodynia was assessed using the acetone drop method described by Choi *et al.* (1994). With the rats under the same conditions of testing, an acetone bubble formed at the end of a standard plastic syringe was placed to the plantar surface of the hind paws. Acetone was applied five times to each paw at intervals of 3–5 min. Normal rats either ignore the stimulus or occasionally respond with a small and brief withdrawal. Allodynic rats respond with a prompt and intense paw withdrawal or escape behaviour to acetone application. The frequency of paw withdrawal was expressed as a percentage (the number of paw withdrawals/number of trials × 100).

### *Thermal hyperalgesia*

The development of thermal hypersensitivity associated with neuropathic pain was measured using the paw-withdrawal latency according to the method described by Hargreaves *et al.* (1988), with minor modifications. Rats were placed (not restrained) within a Plexiglass enclosure on a transparent glass floor and allowed to acclimatize for 20–30 min. An infrared beam that constitutes the noxious heat source was moved beneath the plantar surface of the hind paw. Thermal nociceptive threshold was defined as the latency (seconds) between the heat stimulus (46°C) onset and the paw withdrawal using a feedback-controlled shut-down unit. A cut-off time of 33 s was used to avoid tissue damage. Each paw was tested three times alternatively at minimum intervals of 3 min between stimulation to avoid sensitization of the hind paw. Mean values of each paw were used as the thermal threshold.

### *Measurement of plasma glucose and insulin and insulin resistance*

At the end of protocol, overnight fasted rats were anaesthetized with CO<sub>2</sub> inhalation and blood was rapidly collected from cut carotid arteries and immediately transferred into a chilled tube containing no more than 10 IU heparin per ml of blood collected (Thorell and Lanner, 1973). The plasma was separated from blood cells by centrifugation and kept frozen at –20°C for the later measurement of glucose with a glucometer (Elite, Bayer Inc., Toronto, Canada) and insulin by radioimmunoassay (rat insulin RIA kit, Linco Research, St Charles, MO, USA) using 100 µl of plasma. The Homeostasis Model Assessment

(HOMA) was used as an index of insulin resistance and calculated with the following formula: (insulin ( $\mu\text{U ml}^{-1}$ )  $\times$  glucose (mM)/22.5) (Matthews *et al.*, 1985).

#### Superoxide anion measurement

Superoxide anion production was measured in isolated thoracic aortic rings using the lucigenin-enhanced chemiluminescence method as described previously (Munzel *et al.*, 1995). Briefly, 2–5 mg aortic tissue were pre-incubated in Krebs-HEPES buffer (saturated with 95% O<sub>2</sub> and 5% CO<sub>2</sub>, at room temperature during 30 min) and then transferred to a glass scintillation vial containing 5  $\mu\text{M}$  lucigenin for the determination of basal O<sub>2</sub><sup>-</sup> levels. The chemiluminescence was recorded in duplicate every minute for 6 min at room temperature by a liquid scintillation counter (Wallac 1409, Turku, Finland). Background counts were determined from vessel-free incubation media and subtracted from the readings obtained with vessels. Lucigenin counts were expressed as c.p.m. mg<sup>-1</sup> of dry weight of tissue.

#### Experimental protocols

**Effect of orally administered SSR240612 on allodynia.** SSR240612 was administered orally to rats fed D-glucose for 12 weeks, which displayed consistent symptoms of sensory abnormalities. The effects of four doses (0.3, 3.0, 10 and 30 mg kg<sup>-1</sup>) of SSR240612 were determined on allodynia at 1, 3, 6, 24, and 48 h post-gavage. Values were compared to baseline values (time 0) measured on three subsequent days beforehand and to vehicle values. A single dose of B<sub>1</sub> receptor antagonist was randomly administered to a rat and the experimenter was unaware of the dose of antagonist.

Rats were randomly divided into five groups as follows: Group 1 ( $n=6$ ): Glucose + vehicle; Group 2 ( $n=6$ ): glucose + SSR240612 (0.3 mg kg<sup>-1</sup>); Group 3 ( $n=12$ ): glucose + SSR240612 (3.0 mg kg<sup>-1</sup>); Group 4 ( $n=13$ ): glucose + SSR240612 (10 mg kg<sup>-1</sup>); Group 5 ( $n=11$ ): glucose + SSR240612 (30 mg kg<sup>-1</sup>). SSR240612 was also administered to two groups of age-matched control rats at the dose of 10 mg kg<sup>-1</sup> ( $n=6$ ) and 30 mg kg<sup>-1</sup> ( $n=6$ ) and the impact on sensory threshold to tactile and cold stimulation was measured at 1, 3, 6, 24 and 48 h post-gavage.

**Effect of orally administered SSR240612 on pancreas function and oxidative stress.** SSR240612 was administered orally at the dose of 10 mg kg<sup>-1</sup> to rats treated for 12 weeks with glucose ( $n=8$ ). Rats were fasted overnight and killed by CO<sub>2</sub> asphyxia at 6 h post-gavage, and then the blood and thoracic aorta were collected for the measurement of plasma levels of glucose and insulin and the determination of superoxide anion, respectively. Data were compared to age-matched control rats ( $n=8$ ) and glucose-fed rats treated with the vehicle by gavage 6 h earlier ( $n=8$ ). Blood pressure and allodynia were measured in those rats the preceding day to ascertain that glucose-fed rats displayed hypertension and allodynia.

#### Statistical analysis

Data are expressed as mean  $\pm$  s.e. mean of values obtained from  $n$  rats in each group. Statistical analysis of data and the

doses of SSR240612, which inhibited allodynia by 50% (ID<sub>50</sub>), were calculated with GraphPad Prism (version 4.00) software. ID<sub>50</sub> value was determined from sigmoid dose-response on maximal values with 95% confidence intervals. Statistical differences were evaluated with Student's *t*-test on unpaired (between groups) or paired (within the same group) samples. Multiple comparisons were analysed using one- or two-way analysis of variance (ANOVA), followed by the Bonferroni or Dunnett post-test accordingly. Probability values (*P*) less than 0.05 were considered to be statistically significant.

#### Drugs and reagents

The selective non-peptide B<sub>1</sub> receptor antagonist SSR240612 ((2*R*)-2-[(3*R*)-3-(1,3-benzodioxol-5-yl)-3-[(6-methoxy-2-naphthyl)sulphonylamino]propanoyl]amino]-3-(4-[(2*R*,6*S*)-2,6-dimethylpiperidinyl]methyl]phenyl)-*N*-isopropyl-*N*-methylpropanamide fumarate) (Gougat *et al.*, 2004) was synthesized at Sanofi-Aventis R&D (Montpellier, France). SSR240612 was dissolved in dimethylsulphoxide (0.5%), and then ethanol (5%) and Tween-80 (5%) were added in this sequence. The solution was completed in distilled water. The drug was administered orally by gavage in a volume of 1 ml by 100 g of body weight. D-Glucose was purchased from Sigma-Aldrich Canada.

## Results

#### Baseline parameters in D-glucose-fed rats

As shown in Table 1, plasma levels of glucose and insulin as well as resting systolic blood pressure were significantly increased in rats fed with 10% D-glucose for a period of 12 weeks when compared to age-matched control rats. However, the same treatment with D-glucose had no significant effect on the body weight. Data of Table 1 also show values of tactile and cold allodynia in glucose-fed rats when compared to baseline values obtained in control rats. The thermal nociceptive threshold as determined by the paw-withdrawal

**Table 1** Baseline values in rats treated with glucose for 12 weeks

Parameters	Glucose-fed rats	Control rats
Weight (g)	501.9 $\pm$ 6.4 $n=48$	535.4 $\pm$ 23.5 $n=7$
Plasma glucose (mmol l <sup>-1</sup> )	6.2 $\pm$ 0.3** $n=48$	4.1 $\pm$ 0.2 $n=7$
Plasma insulin (ng ml <sup>-1</sup> )	1.3 $\pm$ 0.3*** $n=8$	0.40 $\pm$ 0.06 $n=8$
Systolic blood pressure (mm Hg)	155.7 $\pm$ 1.6*** $n=48$	124.6 $\pm$ 4.8 $n=7$
Tactile allodynia (g)	5.8 $\pm$ 0.2*** $n=48$	11.4 $\pm$ 1.1 $n=7$
Cold allodynia (%)	75.1 $\pm$ 3.4*** $n=48$	37.0 $\pm$ 3.8 $n=7$
Thermal nociception (s)	9.3 $\pm$ 0.4 $n=30$	9.3 $\pm$ 0.8 $n=7$

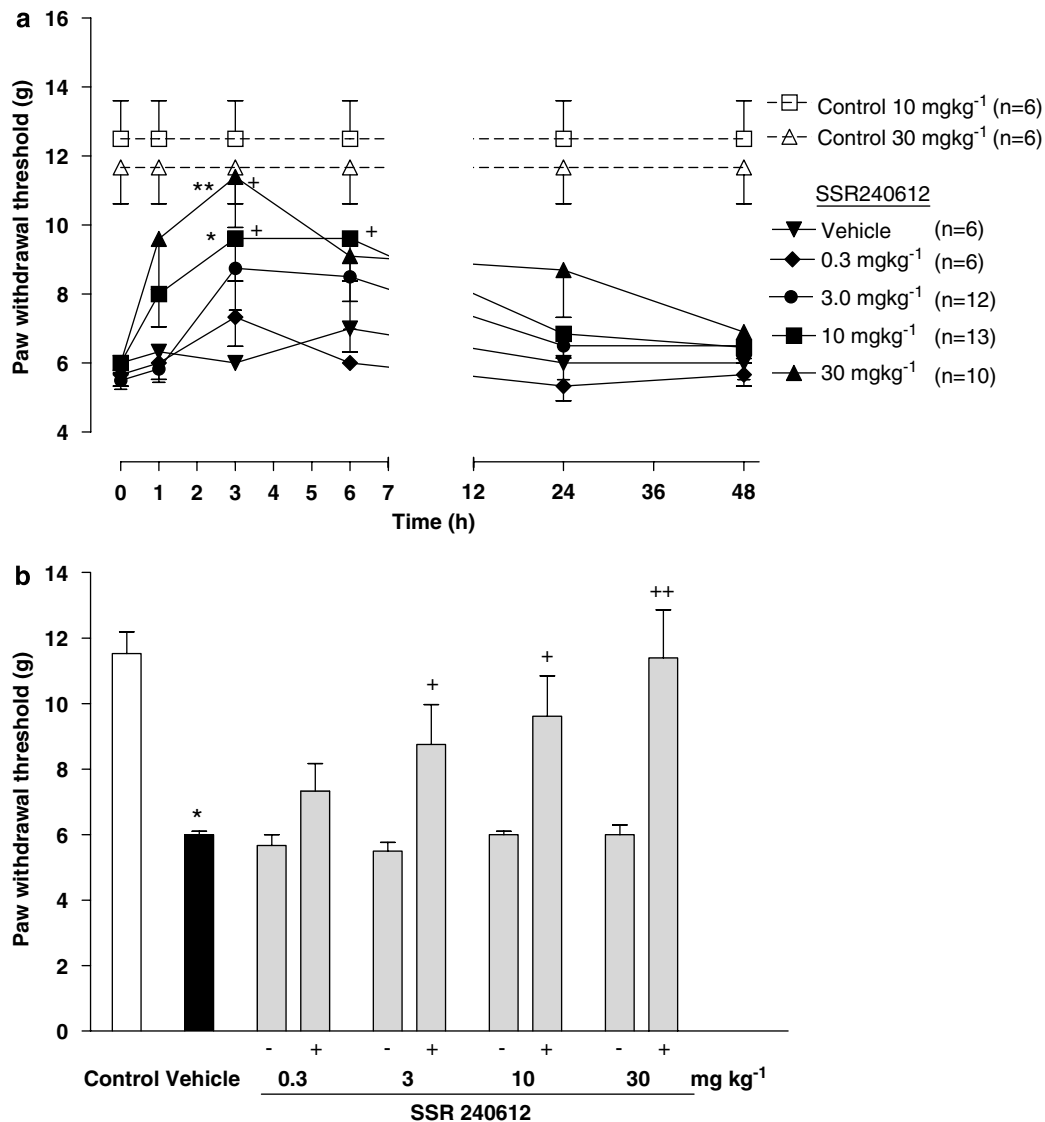
Values represent the mean  $\pm$  s.e. mean of ( $n$ ) rats. Statistical comparison to control rats is indicated by \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

reflex latency to a noxious thermal stimulus was not significantly different between 12 weeks glucose-fed rats and control rats (Table 1).

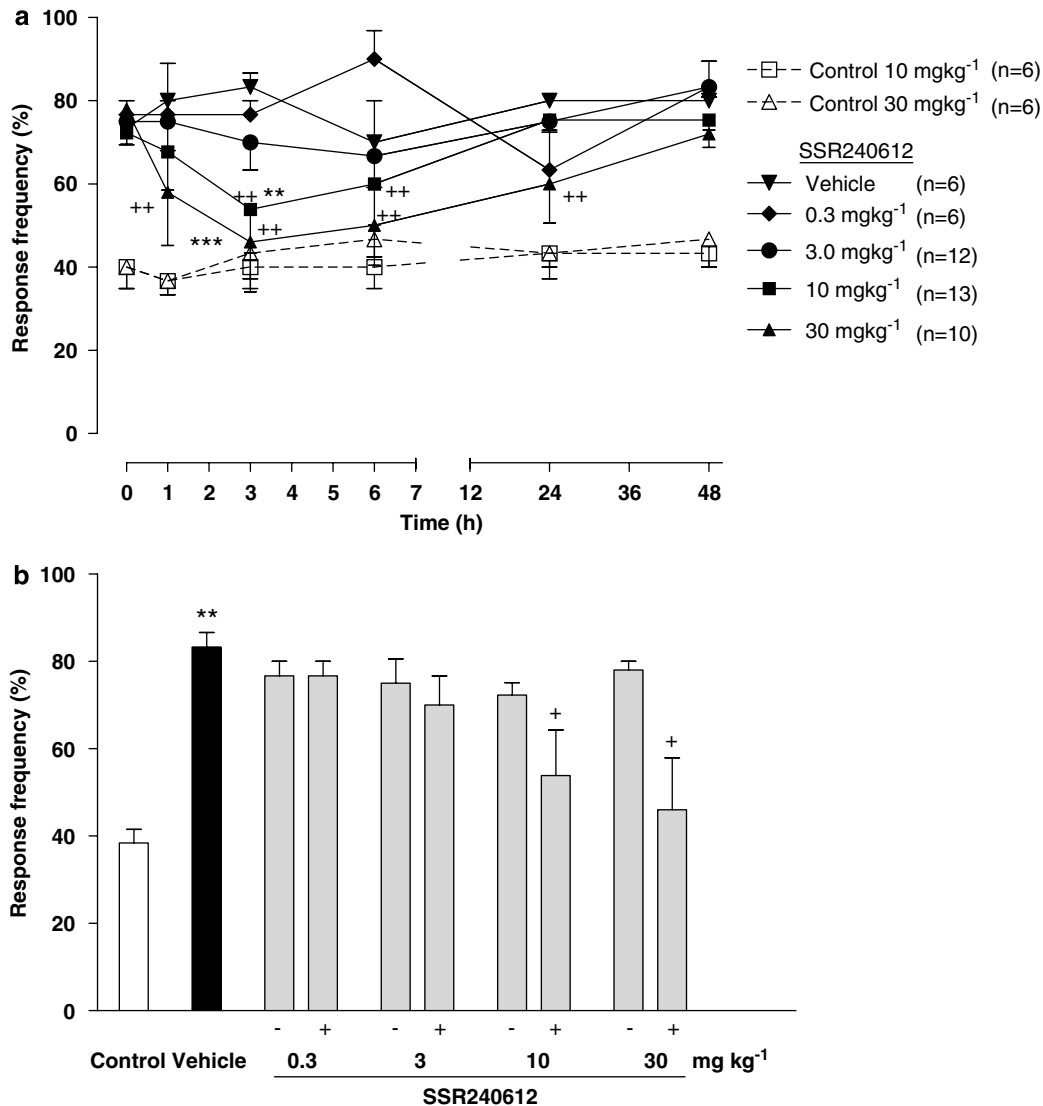
*Effect of orally administered SSR240612 on allodynia*

The oral administration of SSR240612 blocked dose-dependently tactile allodynia ( $ID_{50} = 5.5$  ( $4.0-7.6$ )  $mg\ kg^{-1}$ ) in glucose-fed rats although the doses of  $0.3\ mg\ kg^{-1}$  did not reach statistical significance (Figure 1). The dose of  $3\ mg\ kg^{-1}$  blocked at 3 h post-administration when compared to pre-administration values only (paired Student's *t*-test). Doses of 10 and  $30\ mg\ kg^{-1}$  blocked tactile allodynia at 3 h when compared to vehicle values (two-way ANOVA and post-hoc Bonferroni test) or pre-administration values (paired Student's *t*-test) (Figures 1a and b). At the highest dose of SSR240612, tactile allodynia was completely blocked and the

paw-withdrawal threshold was not significantly different from that measured in control rats. In contrast, doses of 10 and  $30\ mg\ kg^{-1}$  SSR240612 did not affect paw-withdrawal threshold to tactile stimulation from 1 to 48 h post-administration in control rats when compared to pre-administration values (Figure 1a). Cold allodynia was blocked by SSR240612 ( $ID_{50} = 7.1$  ( $5.9-8.4$ )  $mg\ kg^{-1}$ ) administered orally in glucose-fed rats (Figure 2). Doses of 10 and  $30\ mg\ kg^{-1}$  blocked significantly at 3 h when compared to vehicle values (two-way ANOVA and post-hoc Bonferroni test) and from 3 to 6 h and 1-24 h post-administration respectively when compared to pre-administration values (one-way ANOVA and post-hoc Dunnett test) (Figures 2a and b). Whereas the inhibition of cold allodynia did not seem to be complete at  $30\ mg\ kg^{-1}$ , the residual response frequency was not significantly different from that measured in control rats at 3 h post-administration. In control rats, the



**Figure 1** Time-course effect (a) and maximal effect (b) of SSR240612 ( $0.3, 3, 10$  and  $30\ mg\ kg^{-1}$ , p.o.) on tactile allodynia (paw-withdrawal threshold, in grams) in rats fed with glucose (10% in drinking water) for 12 weeks. Effects of SSR240612 ( $10$  and  $30\ mg\ kg^{-1}$ , p.o.) are also shown in age-matched control rats. Data are means  $\pm$  s.e.mean of (*n*) rats in each group. Statistical comparison to vehicle (\* in a), control (\* in b) or pre-administration values (time 0) (+) is indicated by \* +  $P < 0.05$ ; \*\* +  $P < 0.01$ .



**Figure 2** Time-course effect (a) and maximal effect (b) of SSR240612 (0.3, 3, 10 and 30 mg kg<sup>-1</sup>, p.o.) on cold allodynia (paw-withdrawal response frequency) in rats fed with glucose (10% in drinking water) for 12 weeks. Effects of SSR240612 (10 and 30 mg kg<sup>-1</sup>, p.o.) are also shown in age-matched control rats. Data are means  $\pm$  s.e. mean of (*n*) rats in each group. Statistical comparison to vehicle (\* in a), control (\* in b) or pre-administration values (time 0) (+) is indicated by +  $P < 0.05$ ; ++  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

administration of 10 and 30 mg kg<sup>-1</sup> SSR240612 had no significant effect on the response frequency to cold stimulation (Figure 2a).

#### Effect of orally administered SSR240612 on pancreas function and oxidative stress

As shown in Table 2, plasma levels of glucose and insulin as well as insulin resistance (HOMA index) and the basal aortic superoxide anion production were significantly increased in glucose-fed rats in comparison with control rats. These values were not significantly affected when SSR240612 (10 mg kg<sup>-1</sup>) was given orally 6 h earlier.

## Discussion

The present study strongly suggests a role for the inducible kinin B<sub>1</sub> receptor in glucose-induced allodynia, a manifesta-

tion of sensory polyneuropathy. The B<sub>1</sub> receptor antagonist SSR240612 is orally active and reversed dose- and time-dependently tactile and cold allodynia in an experimental rat model of insulin resistance. Because the compound was found to be inactive on basal sensory function in control rats, we concluded that SSR240612 was not interfering with normal pain pathways and was not associated with non-specific effects. The lack of effect of SSR240612 in control rats was consistent with the absence of B<sub>1</sub> receptors.

Earlier studies performed in glucose-fed rats for a period of 4 weeks have shown significant increases in systolic blood pressure and plasma levels of insulin and glucose along with insulin resistance, and an overproduction of basal superoxide anion in cardiovascular tissues (El Midaoui and de Champlain, 2002, 2005; El Midaoui *et al.*, 2003, 2005). Tactile and cold allodynia occurred, however, after 4 weeks of treatment with glucose and arterial blood pressure was significantly higher at 12 weeks when compared to 4 weeks

**Table 2** Effects of SSR240612 (10 mg kg<sup>-1</sup>) given orally 6 h before killing in rats treated with glucose for 12 weeks

Parameters	Glucose-fed rats + vehicle, n = 8	Glucose-fed rats + SSR240612, n = 8	Control rats, n = 8
Plasma glucose (mmol l <sup>-1</sup> )	6.3 ± 0.4**	6.2 ± 0.3**	4.6 ± 0.4
Plasma insulin (ng ml <sup>-1</sup> )	1.7 ± 0.5***	1.9 ± 0.3***	0.6 ± 0.07
HOMA index	9.4 ± 2.4***	10.6 ± 1.8***	2.4 ± 0.3
Aortic superoxide anion (c.p.m. mg <sup>-1</sup> )	1500 ± 91*	1537 ± 106*	1239 ± 36

Values represent the mean ± s.e. mean of (n) rats. Statistical comparison to control rats is indicated by \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001.

of treatment (Lungu *et al.*, 2007). The present study confirms the elevation of arterial blood pressure and the occurrence of allodynia at 12 weeks. Our data in glucose-fed rats are consistent with the values of arterial hypertension generally measured in patients and animal models of types II diabetes (Hwang *et al.*, 1987; Dai and McNeill, 1995; Schrier *et al.*, 1996; Song *et al.*, 2005).

In 12-week glucose-fed rats, B<sub>1</sub> receptor mRNA and B<sub>1</sub> receptor binding sites were significantly increased in the spinal cord and several peripheral tissues (aorta, liver, lung, kidney) (Lungu *et al.*, 2007).  $\alpha$ -Lipoic acid (thioctic acid), a radical scavenger and powerful antioxidant (Packer *et al.*, 2001), prevented or attenuated the development of arterial hypertension, allodynia, the upregulation of B<sub>1</sub> receptors, insulin resistance, hyperglycemia, hyperinsulinemia and the oxidative stress in the heart and aorta in glucose-fed rats (El Midaoui and de Champlain, 2002; El Midaoui *et al.*, 2003, 2005; Lungu *et al.*, 2007). These data highlight a possible link between oxidative stress, the induction of B<sub>1</sub> receptors and the development of sensory abnormalities.

The original observation regarding the inhibition of allodynia by SSR240612 in this model of insulin resistance is consistent with the acute inhibitory effects of peptide B<sub>1</sub> receptor antagonists on tactile allodynia and mechanical hyperalgesia in various animal models of nerve injury and persistent inflammation (Perkins *et al.*, 1993; Davis and Perkins, 1994; Bélichard *et al.*, 2000; Fox *et al.*, 2003; Yamaguchi-Sase *et al.*, 2003; Calixto *et al.*, 2004; Ferreira *et al.*, 2005). Although the cellular mechanism by which SSR240612 inhibits allodynia is still unknown, it could not simply reflect a correction of pancreatic function or an antioxidative property of SSR240612 because the higher plasma levels of glucose and insulin, insulin resistance and the higher production of vascular superoxide anion remained unaffected by the dose of SSR240612 (10 mg kg<sup>-1</sup>), which caused significant inhibition of allodynia. This supports a direct inhibition of B<sub>1</sub> receptors affecting spinal cord and/or sensory nerve excitation.

Thermal hyperalgesia was inhibited by peptide B<sub>1</sub> receptor antagonists in experimental rodent models of type I and type II diabetes (Gabra *et al.*, 2006) and was absent in streptozotocin-induced type I diabetic B<sub>1</sub> receptor knock-out mice (Gabra *et al.*, 2005). The thermal nociceptive threshold was not affected in glucose-fed rats using the plantar heat stimulation, which confirms a previous study using the tail-flick test in the same experimental model (Lungu *et al.*, 2007). Thermal hyperalgesia is mediated by myelinated A $\delta$  and unmyelinated primary afferent neurons, which include both peptidergic and non peptidergic C-fibres (Handwerker and Kobal, 1993; Yeomans and Proudfit, 1996). Whereas

C-fibres are thought to be involved in the transmission of warm sensations, A $\delta$ -fibres are stimulated by cold stimulus (Pierau and Wurster, 1981). On the contrary, tactile allodynia is a central phenomenon mediated by large myelinated A $\beta$ -fibres (Woolf *et al.*, 1992; Shortland *et al.*, 1997; Pitcher and Henry, 2000). Thus, sensory abnormalities measured in glucose-fed rats which is primarily a non-genetic model of insulin resistance may affect sensory A $\beta$  and A $\delta$  fibres but not polymodal C-fibres, at least in the early stages of the disease. This statement is supported by personal unpublished findings showing that the destruction of afferent C-fibres with capsaicin in neonates had no impact on the development of arterial hypertension and cold/tactile allodynia in adult glucose-fed rats. It is also worth noting that capsaicin-sensitive C-fibres are not required in the development of tactile allodynia in the rat model of diabetes induced by streptozotocin (Khan *et al.*, 2002). The fact that afferent C-fibres are unlikely to be affected in our pre-diabetic model of insulin resistance may also account for the lack of thermal hypoalgesia that occurs in prolonged diabetic neuropathy (Calcutt *et al.*, 1998, 2004). Transient thermal hyperalgesia and subsequent progressive thermal hypoalgesia occur in diabetic rats secondary to exaggerated flux through the polyol pathway that leads to depletion of ciliary neurotrophic factor (Calcutt *et al.*, 2004). Nitrosative stress, increased aldose reductase activity and the loss of intra-epidermal nerve fibres were also found to contribute to thermal hypoalgesia during the progression of the disease (Drel *et al.*, 2006; Vareniuk *et al.*, 2007). Thus, small sensory nerve fibre degeneration, the metabolic and neurotrophic abnormalities associated with thermal hypoalgesia are unlikely to occur in our model of insulin resistance.

## Conclusion

The present findings provide the first pharmacological evidence that the inducible B<sub>1</sub> receptor is involved in tactile and cold allodynia occurring in chronically glucose-fed rats, an experimental model of insulin resistance. Therefore, selective kinin B<sub>1</sub> receptor antagonists, especially the new orally active non-peptide antagonist SSR240612, might represent drugs of potential interest for the treatment of sensory polyneuropathy.

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## Conflict of interest

The authors state no conflict of interest.

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