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Role of bradykinin B_1 receptors in diabetes-induced hyperalgesia in streptozotocin-treated mice

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Abstract

Insulin-dependent diabetes mellitus (type-1 diabetes) is an inflammatory autoimmune disease associated with vascular permeability changes leading to many complications including nephropathy, retinopathy, hypertension, hyperalgesia and neuropathy. The bradykinin B_1 receptor was recently found to be upregulated during the development of the diabetes and to be involved in its complications. Kinins are known to be important mediators of a variety of biological effects including cardiovascular homeostasis, inflammation and nociception. In the present study, we studied the effect of the selective B_1 receptor agonist, des-Arg^9-bradykinin, and its specific antagonists, Ac-Lys-[D-β Nal^7, Ile^8]des-Arg^9-bradykinin (R-715) and Ac-Orn-[Oic^2, αMe Phe^5, D-β Nal^7, Ile^8]des-Arg^9-bradykinin (R-954), on diabetic hyperalgesia. Diabetes was induced in male CD-1 mice by injecting a single high dose of streptozotocin (200 mg kg^{-1}, i.p.) and the nociception was assessed using the hot plate and the tail flick tests, 1 week following the injection of streptozotocin. Our results showed that induction of diabetes by streptozotocin provoked a marked hyperalgesia in diabetic mice expressed as about 11% decrease in hot plate reaction time and 26% decrease in tail flick reaction time. Following acute administration of R-715 (200–800 μg kg^{-1}, i.p.) and R-954 (50–600 μg kg^{-1}, i.p.), this hyperalgesic activity was blocked and the hot plate and tail flick latencies of diabetic mice returned to normal values observed in control healthy mice. In addition, the acute administration of des-Arg^9-bradykinin (200–600 μg kg^{-1}, i.p.) significantly potentiated diabetes-induced hyperalgesia, an effect that was totally reversed by R-715 (1.6–2.4 mg kg^{-1}, i.p.) and R-954 (0.8–1.6 mg kg^{-1}, i.p.). These results provide a major evidence for the implication of the bradykinin B_1 receptors in the development of hyperalgesia associated with diabetes and suggest a novel approach to the treatment of this diabetic complication using the bradykinin B_1 receptor antagonists.

Keywords: Insulin-dependent diabetes mellitus; Hyperalgesia; Kinin; Bradykinin B_1 receptor; Des-Arg^9-bradykinin; Bradykinin B_1 receptor antagonist; R-715; R-954

1. Introduction

Chronic inflammatory and cardiovascular diseases that often lead to pain are of increasing importance for health care in aging populations. Pain is a normal physiological protective mechanism to avoid tissue damage. In the periphery, it is signalled by fine C and Aδ afferent fibres that respond to noxious stimuli (mechanical, heat, cold, chemical). Indeed, all tissues, with the exception of the neuropil of the central nervous system (CNS), are innervated by such afferent fibres. However, pain is not a uniform sensation, and the quality of pain as well as the initiation of protective responses is determined by many factors within the spinal cord and in higher brain structures involved in the integration and modification of nociceptive signals (Dray, 1997).

When significant tissue damage occurs, pain is often more persistent and is associated with inflammation. In these circumstances, hyperalgesia and tenderness around the inflamed region occur. Activation and sensitization of peripheral nociceptors by chemical mediators produced by tissue injury and inflammation partially accounts for this. However, in hyperalgesia, there is also facilitation of transmission at the level of the dorsal horn and the thalamus associated with changes in the central processing of pain signals, which allows signals generated by normally innocuous stimuli, such as gentle stroking, to be perceived as painful. In most cases, inflammation is a common and complex feature of clinical pain. The action of chemical mediators produced during inflammation is responsible for

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the multiplicity of events that occur, including hyperalgesia, alterations in cell phenotype, and the expression of new molecules (neurotransmitters, enzymes, ion channels, receptors) in the peripheral nervous system and the CNS (Levine et al., 1993; Dray, 1994).

Kinins have been known for some time to be important mediators implicated in a variety of biological effects including cardiovascular homeostasis, inflammation and nociception (for review, see Marceau et al., 1998; Calixto et al., 2000). They are probably the first mediators released in injured tissues from kininogens either by plasma kallikrein, which is activated early in the coagulation cascade, or tissue kallikrein, which is activated by proteases released at injured sites (Bhoola et al., 1992). Their production is critical for the initiation of pain and exaggeration of sensory signalling to produce hyperalgesia and allodynia. In addition, they promote many features of inflammation including an increase in blood flow and tissue oedema as well as the release of several mediators such as prostanoids and cytokines (Levine et al., 1993; Dray, 1994, 1995). Kinins mediate their biological effects by acting on two types of receptors, namely, B_1 and B_2. The B_2 receptors, which mediate many of the physiological effects of kinins, are constitutively expressed and involved in the acute phase of the inflammation and pain response. On the other hand, the B_1 receptors, usually absent in normal tissues, are highly induced and overexpressed during tissue injury and following treatment with inflammatory mediators like bacterial endotoxins and cytokines. They do not desensitize after agonist binding and participate in the chronic phase of the inflammation and pain response (Couture et al., 2001). The therapeutic value of intervention in the kallikrein–kinin system has not been fully explored. The known kinin receptors might be suitable pharmacological targets to treat chronic inflammatory and cardiovascular diseases and support new concepts of analgesic drug design through blockade of kinin receptors (Pesquero et al., 2000).

Diabetes mellitus is a term that describes a series of complex and chronic disorders characterized by symptomatic glucose intolerance due to defective insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes mellitus is associated with significant long-term sequelae, particularly damage, dysfunction and failure of various organs. The dysfunction of the vascular endothelium and the micro- and macrovascular permeability changes lead to many diabetic complications including nephropathy, retinopathy, neuropathy, hypertension and hyperalgesia (Steil, 1999).

Experimental evidence suggests that diabetes upregulates bradykinin B_1 receptors as a consequence of the overproduction of cytokines and of the oxidative stress effect of hyperglycemia (Rabinovitch, 1998; Yerneni et al., 1999). Other pharmacological studies suggest that the B_1 receptor intervenes in the pathogenesis of streptozotocin-induced diabetes in mice as bradykinin B_1 receptor antagonists could normalize glycemia and renal function (Zuccollo et al., 1996, 1999). In addition, it has been recently demonstrated that these antagonists were able to inhibit the increase in plasma extravasation associated with diabetic mice (Simard et al., submitted for publication). The Streptozotocin model is the most commonly used to study the cardiovascular and neuropathic complications of type-1 diabetes. Streptozotocin is an antibiotic extracted from *Streptomyces acromogens*, which is selectively toxic for pancreatic islet β-cells. The decomposition products of streptozotocin alter the cellular membrane proteins so that they are no longer recognized as self and thus initiating an autoimmune inflammatory process associated with cytokines (Wilson and Leiter, 1990; Lukić et al., 1998) resulting in the destruction of pancreatic β-islets. In addition, streptozotocin can alter DNA in such a manner that a previously silent gene is expressed or a normal protein is altered by point mutation (Wilson and Leiter, 1990). The objective of the present study was to investigate the role of bradykinin B_1 receptors in hyperalgesia associated with the development of diabetes induced by streptozotocin in mice. The effects of the selective bradykinin B_1 receptor agonist des-Arg^9-bradykinin (Regoli et al., 1998, 2001) and its specific antagonists Ac-Lys-[D-β Nal^7, Ile^8]des-Arg^9-bradykinin (R-715) and Ac-Orn-[Oic^2, αMe Phe^5, D-β Nal^7, Ile^8]des-Arg^9-bradykinin (R-954) (Neugebauer et al., 2002) were studied on the hyperalgesic response in diabetic mice.

2. Materials and methods

2.1. Animals

Male CD-1 mice weighing between 25 and 30 g (Charles River Breeding Laboratory, St. Constant, PQ, Canada) were used. The mice were housed four by cage with free access to food and water. They were maintained under conditions of standard lighting (alternating 12-h light/dark cycle), temperature (22 ± 0.5 °C) and humidity (60 ± 10%) with food and water available ad libitum. Animals were used only once in a given experiment. Experiments were conducted between 1000 and 1800 h. All experiments were carried out in accordance with the recommendations of the International Association for the Study of Pain (IASP) Committee for Research and Ethical Issues Guidelines, and were approved by the Animal Care Committee of University of Sherbrooke.

2.2. Drugs

Streptozotocin (Pharmacia & Upjohn, Mississauga, ON, Canada) was dissolved in saline at a pH of 4.5 and administered to mice i.p. The bradykinin-related peptides, des-Arg^9-bradykinin, R-715 and R-954, were synthesized by Dr. Witold Neugebauer in the Institute of Pharmacology of Sherbrooke, School of Medicine, University of Sherbrooke, Canada. They were dissolved in saline and administered to mice i.p.

2.3. Methods

2.3.1. Induction of type-1 diabetes

Insulin-dependent diabetes mellitus was induced in mice using streptozotocin. Male CD-1 mice received a single high i.p. dose of streptozotocin (200 mg kg^{-1}) (McEvoy et al., 1984). The induction of diabetes was confirmed by measuring the blood glucose level 96 h after streptozotocin administration (Katovich et al., 1995; Chakir and Plante, 1996). Blood was withdrawn from the retro-orbital sinus of mice with a 50-μl heparinized capillary tube. Blood glucose levels were determined with an automatic analyzer (Glucometer Elite XL, Bayer, Toronto, ON, Canada) using glucose oxidase/potassium ferricyanide reagents strips. The glucometer provides readings that are accurate within ± 1 mmol l^{-1} from 1.1 to 33.3 mmol l^{-1}. The diabetic animals used in our study had a blood glucose level higher than 20 mmol l^{-1} while the normal value is from 5 to 8 mmol l^{-1} (Chakir and Plante, 1996; Plante et al., 1996). The rate of induction of diabetes was 86%.

2.3.2. Assessment of nociception

While nociception is defined as the normal electrophysiological response of peripheral sensory organs to noxious (tissue-damaging) stimuli, mediated by C and Aδ nociceptors, hyperalgesia is an exaggerated response to the same stimuli evoked by a hypersensitisation of peripheral nociceptors and a central facilitation of pain transmission at the level of the dorsal horn neurones and thalamus (Rang et al., 1999). Pain was measured in both healthy and diabetic mice using two types of thermal nociceptive tests.

2.3.2.1. The hot plate test. A hot plate test derived from that of Eddy and Leimbach (1953) was used. A Plexiglas cylinder (20 × 14 cm) was used to confine the mouse to the anodized heated surface (275 × 263 mm) of the apparatus (IITC Hot Plate Analgesia Meter, Life Science, California, USA). The plate was adjusted to a temperature of 55 ± 0.5 °C. When the pain threshold is reached, the animal starts to react by licking its hind paw or to jump, and the reaction time is recorded with a built-in timer, with a maximum cutoff time of 30 s to avoid tissue damage. Mice with latency value between 10 and 15 s were selected.

2.3.2.2. The tail flick test. The tail flick test of D'amour and Smith (1941) modified for mice was used. The mice were habituated in a Plexiglas cylindrical mouse restrainer (4 cm in diameter and 8 cm long), 15 min daily for 1 week before starting the experiments. To measure the latency of the tail flick response, mice were gently placed in the restrainer and the tail put in the tail groove of the apparatus (Model IITC 336 Paw/Tail Stimulator Analgesia Meter, Life Science). The tail flick response was elicited by applying radiant heat from a halogen bulb lamp (150 W) to the dorsal surface of the animal tail. The radiant light was focussed on a blackened spot in the mid-region of the animal's tail (2–3 cm from the tip of the tail) and the latency between the application of the stimulation light and the flicking of the animal's tail was recorded. When the animal flicks its tail, its exposes a photocell in the apparatus immediately below the tail and the instrument is automatically stopped and the time is automatically recorded. A cutoff time of 10 s was used to prevent blistering. The intensity of radiation was set at 40 to provide a pre-drug tail flick response of 4–5 s.

2.4. Experimental protocol

In both the hot plate and the tail flick tests, pretreatment latencies were determined three times with an interval of 24 h starting 3 days before the injection of streptozotocin or saline, and the mean was calculated in order to obtain stable pre-drug response latency. On day 7 following the injection of streptozotocin, the selective bradykinin B_1 receptor agonist des-Arg^9-bradykinin and/or its specific antagonists R-715 and R-954 were given i.p. and the effect of their acute administration on nociception were determined at different time intervals. The mice were divided into the following groups: (i) control group, treated with saline; (ii) group treated with streptozotocin (200 mg kg^{-1}, i.p., once); (iii) group treated with streptozotocin + R-715 (200–800 μg kg^{-1}, i.p.); (iv) group treated with streptozotocin + R-954 (50–600 μg kg^{-1}, i.p.); (v) group treated with streptozotocin + des-Arg^9-bradykinin (200–600 μg kg^{-1}, i.p.); (vi) group treated with streptozotocin + des-Arg^9-bradykinin (400 μg kg^{-1}, i.p.) + R-715 (1.6–2.4 mg kg^{-1}, i.p.); (vii) group treated with streptozotocin + des-Arg^9-bradykinin (400 μg kg^{-1}, i.p.) + R-954 (0.8–1.6 mg kg^{-1}, i.p.); and (viii, ix and x) groups received only R-715 or R-954 or des-Arg^9-bradykinin, respectively. Each group was made of 6–10 mice.

The effect of the selected drugs on nociception was determined by converting the hot plate and tail flick responses from latencies into Maximum Percent Effect (MPE) according to the following equation (Bhargava and Zhao, 1996):

$$(\%\ \mathrm{MPE}) = \frac{(\mathrm{Posttreatment\ latency} - \mathrm{Pretreatment\ latency})}{(\mathrm{Cutoff\ time} - \mathrm{Pretreatment\ latency})} \times 100.$$

2.5. Statistical analysis

Data are expressed as mean (% MPE) ± S.E.M. and analysis of variance (ANOVA) followed by the "Student–Newman–Keuls multiple comparisons test" were performed using GraphPad Instat, version 2.01 (GraphPad Software, San Diego, CA, USA). $P < 0.05$ was considered significant. ID_{50}, the dose that inhibit hyperlagesia in diabetic mice by 50% in the hot test plate and the tail flick tests, was estimated at time 20 min, which correspond to the

maximal effect observed at each of the five doses used for both tested drugs. The ID_{50} values were calculated using SigmaPlot, version 5.0 (SPSS Science, Chicago, IL, USA) based on a curve-fit using GraphPad Prism (GraphPad Software) between the dose of the drug and % inhibition of hyperalgesic activity.

3. Results

3.1. Streptozotocin-induced hyperalgesia

Seven days following the injection of streptozotocin, a marked hyperlagesia developed in diabetic mice. The MPE in the hot plate test was established at $0.76 \pm 0.09\%$ and $-11.51 \pm 0.67\%$ in control and streptozotocin-diabetic mice, respectively (Fig. 1A). The tail flick test also revealed an MPE of $0.53 \pm 0.08\%$ and $-26.41 \pm 0.90\%$ in control and diabetic mice, respectively (Fig. 1B). Both increased hyperalgesic activities were stable over time (60 min) (Figs. 2A and 4A).

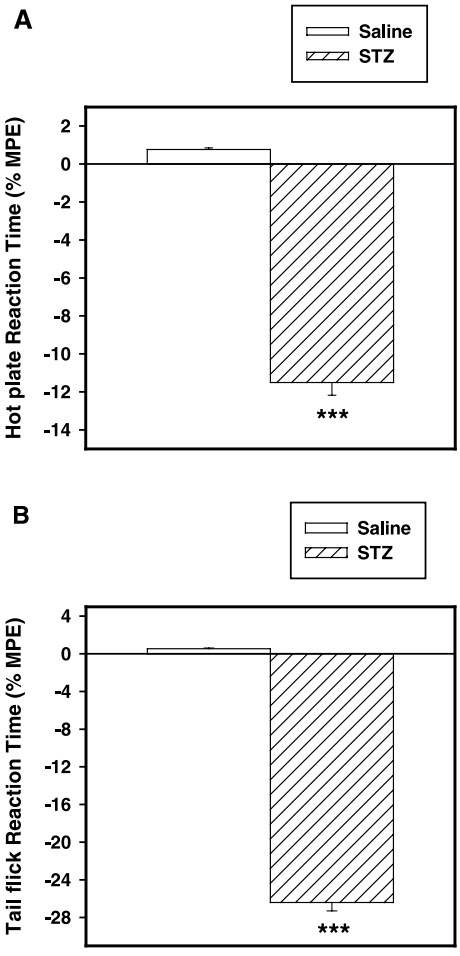

Fig. 1. Evaluation of the nociceptive activity in control versus streptozotocin-diabetic mice using the hot plate (A) and tail flick (B) tests. Diabetes was induced in CD-1 mice using streptozotocin (STZ; 200 mg kg^{-1}, i.p.). On day 7 following the induction of the disease, the hot plate test or the tail flick test was carried out. Data are expressed as mean (% MPE) ± S.E.M. ($n = 10–14$). MPE = Maximum Percent Effect. n = number of animals. ***$P < 0.001$, significantly different from the saline group.

3.2. The hot plate test

Administration (i.p.) of increasing doses of R-715 (100–800 μg kg^{-1}) or R-954 (50–600 μg kg^{-1}) did not affect the nociceptive threshold (baseline) in control healthy mice (data not shown). Conversely, R-715 produced an inhibition of the hyperalgesic activity observed in diabetic mice, which was dose- and time-dependent (Fig. 2A,B).

Maximal inhibition was observed after 20 min with all doses of R-715. Complete inhibition (99%, $P < 0.0001$; back to normal values in control mice) was reached after 20 min at a dose of 400 μg kg^{-1}, whereas lower dosages (100 and 200 μg kg^{-1}) at that same time did significantly inhibit (21% and 60%, respectively; $F = 143.49$, $P < 0.0001$) diabetes-mediated hyperlagesia. Inhibition by high doses (≥ 400) of R-715 was reduced by half at 40 min, and completely receded after 60 min (Fig. 2A). Lower doses followed a similar course. The R-715 ID_{50} at the time of the maximal inhibition (20 min) was estimated at 172 ± 2 μg kg^{-1}.

To further support the role of the B_1 receptor in diabetes-induced hyperlagesia, des-Arg^9-bradykinin was exogenously administered (200, 400 and 600 μg kg^{-1} SPSS, i.p.) to both groups of mice. In control mice, des-Arg^9-bradykinin did not induce significant changes in nociceptive response (data not shown). Conversely, the des-Arg^9-bradykinin potentiated by 34–98% ($F = 28.78$, $P < 0.0001$) the hyperalgesic response in streptozotocin-treated mice. At all doses, the effect was maximal at 20 min post-injection and receded after 40 min. Co-administration of des-Arg^9-bradykinin, at the selected mid-dose of 400 μg kg^{-1}, and R-715, at selected doses of 1.6, 2.0 and 2.4 mg kg^{-1}, reversed the potentiating effect of des-Arg^9-bradykinin on streptozotocin-induced hyperalgesia and produced a marked shift ($F = 151.50$, $P < 0.0001$) in the hot plate latencies to values equivalent to those recorded in the control healthy mice (Fig. 2B).

The more potent and stable analogue to R-715, R-954, was also administered in the same model. R-954, at half the dose (200 μg kg^{-1}) of R-715, abolished streptozotocin-mediated hyperalgesia after 20 min (Fig. 3A). Lower dosages (50 and 100 μg kg^{-1}) still significantly ($F = 169.55$, $P < 0.0001$) inhibited the hyperalgesic activity by 33% and 61%, respectively, after 20 min (maximal inhibition), its effectiveness slowly decreasing but over a longer period of time (up to 50 min). With higher doses (>200), the R-954 inhibitory potency was reduced by half at 45 min and completely receded after 60 min (Fig. 3A). Lower doses followed a similar course. The R-954 ID_{50} at the time of the maximal inhibition (20 min) was estimated at 78 ± 3 μg kg^{-1}.

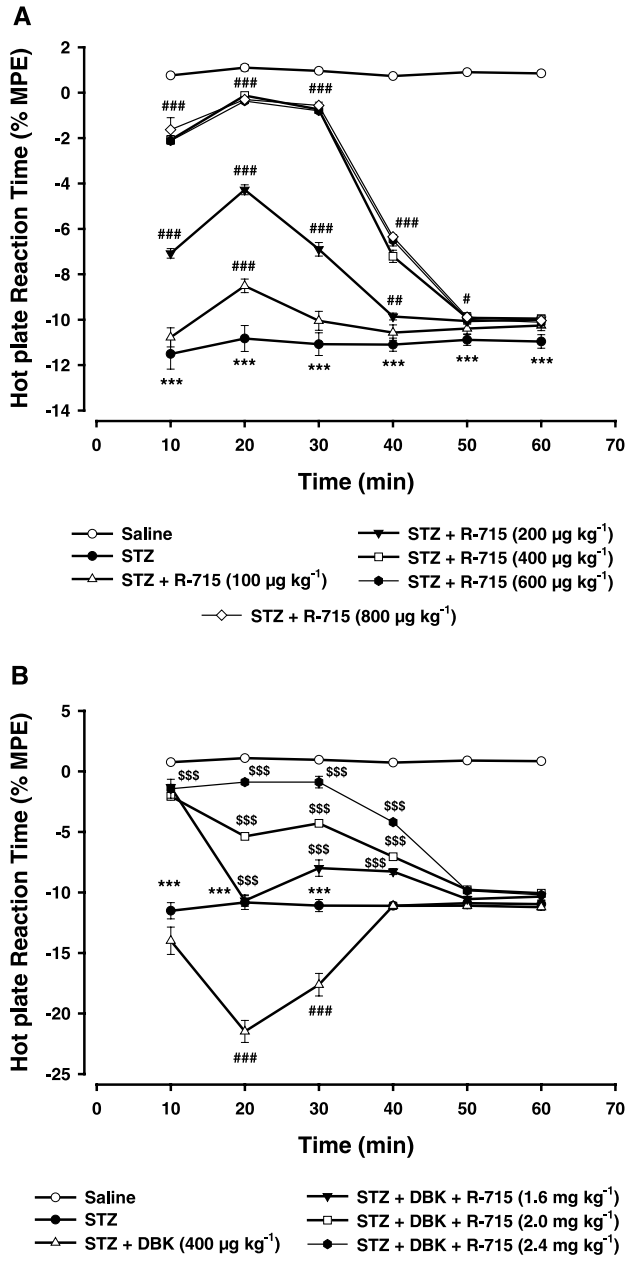

Fig. 2. Effect of single acute administration of R-715 (A) or its combined administration with des-Arg^9-bradykinin (B) on nociception in streptozotocin-diabetic mice in the hot plate test. Diabetes was induced in CD-1 mice using streptozotocin (STZ; 200 mg kg^{-1}, i.p.). On day 7 following the induction of the disease, mice were injected with R-715 (100–800 μg kg^{-1}, i.p.) (A), des-Arg^9-bradykinin (DBK; 400 μg kg^{-1}, i.p.) (B) or a combination of DBK and R-715 (1.6–2.4 mg kg^{-1}, i.p.) (B). The hot plate test was carried out at different time intervals (10–60 min) following injections. Data are expressed as mean (% MPE) ± S.E.M. (n = 7–12). MPE = Maximum Percent Effect. n = number of animals. ***P < 0.001, significantly different from the saline group; $^{\#}P$ < 0.05, $^{\#\#}P$ < 0.01 and $^{\#\#\#}P$ < 0.001, significantly different from the STZ group; and $^{\$\$\$}P$ < 0.001, significantly different from the STZ/DBK group.

Once more, the co-administration of des-Arg^9-bradykinin and R-954 was assessed. While des-Arg^9-bradykinin raised the MPE values in streptozotocin mice (as mentioned above), smaller doses of R-954 (0.8, 1.2 and 1.6 mg kg^{-1}), compared to R-715, also abolished the hyperalgesic activity induced by exogenous des-Arg^9-bradykinin and

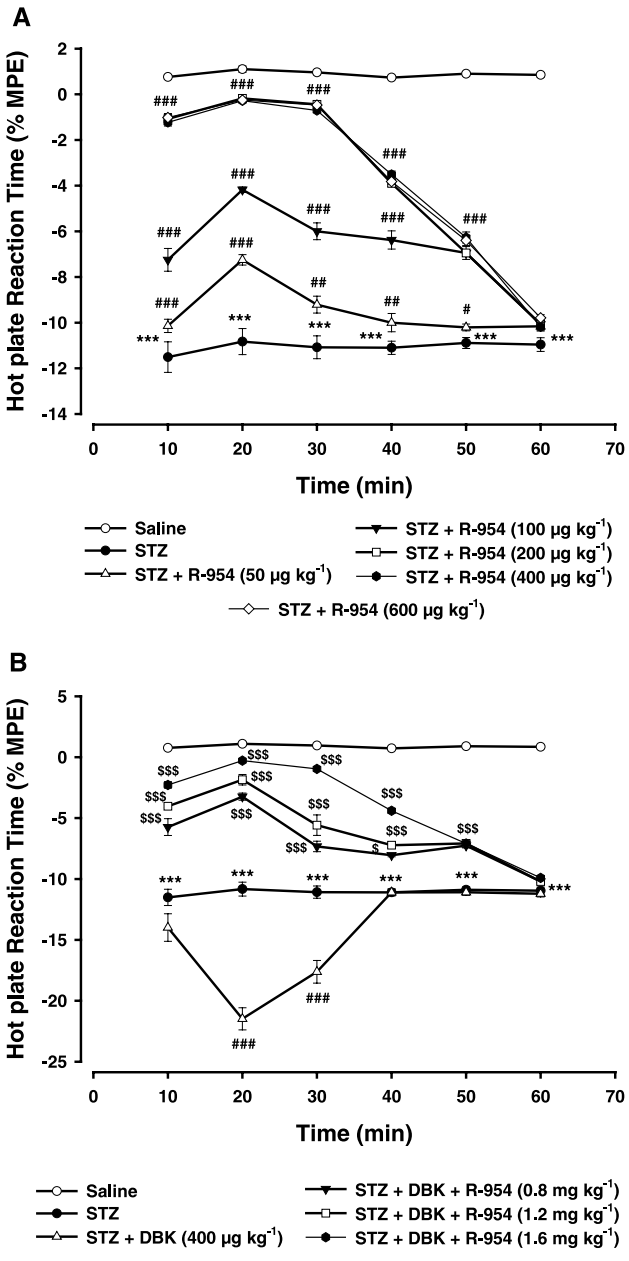

Fig. 3. Effect of acute administration of R-954 (A) or its combined administration with des-Arg^9-bradykinin (B) on nociception in streptozotocin-diabetic mice in the hot plate test. Diabetes was induced in CD-1 mice using streptozotocin (STZ; 200 mg kg^{-1}, i.p.). On day 7 following the induction of the disease, mice were injected with R-954 (50–600 μg kg^{-1}, i.p.) (A), des-Arg^9-bradykinin (DBK; 400 μg kg^{-1}, i.p.) (B) or a combination of DBK and R-954 (0.8–1.6 mg kg^{-1}, i.p.) (B). The hot plate test was carried out at different time intervals (10–60 min) following injections. Data are expressed as mean (% MPE) ± S.E.M. (n = 7–12). MPE = Maximum Percent Effect. n = number of animals. ***P < 0.001, significantly different from the saline group; $^{\#}P$ < 0.05, $^{\#\#}P$ < 0.01 and $^{\#\#\#}P$ < 0.001, significantly different from the STZ group; and $^{\$\$\$}P$ < 0.001, significantly different from the STZ/DBK group.

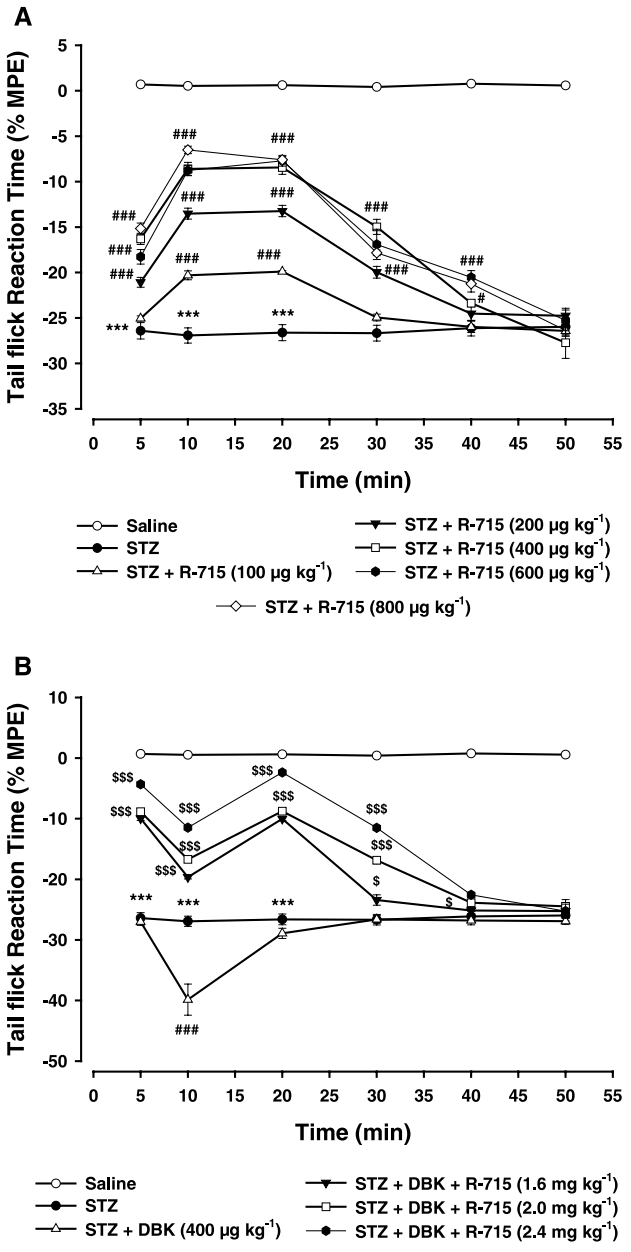

Fig. 4. Effect of single acute administration of R-715 (A) or its combined administration with des-Arg^9-bradykinin (B) on nociception in streptozotocin-treated diabetic mice in the tail flick test. Diabetes was induced in CD-1 mice using streptozotocin (STZ; 200 mg kg^{-1}, i.p.). On day 7 following the induction of the disease, mice were injected with R-715 (100–800 µg kg^{-1}, i.p.) (A), des-Arg^9-bradykinin (DBK; 400 µg kg^{-1}, i.p.) (B) or a combination of DBK and R-715 (1.6–2.4 mg kg^{-1}, i.p.) (B). The tail flick test was carried out at different time intervals (10–60 min) following injections. Data are expressed as mean (% MPE) ± S.E.M. (n = 7–12). MPE = Maximum Percent Effect. n = number of animals. ***P < 0.001, significantly different from the saline group; $^{\#}P$ < 0.05 and $^{\#\#\#}P$ < 0.001, significantly different from the STZ group; and $^{\$}P$ < 0.05 and $^{\$\$\$}P$ < 0.001, significantly different from the STZ/DBK group.

returned the hot plate latencies almost to normal values observed in control mice (F = 205.07, P < 0.0001) (Fig. 3B). The maximal inhibitory effect was observed at 20 min and later decreased over time until 60 min.

3.3. The tail flick test

Administration of increasing doses of R-715 (< 800 µg kg^{-1}) or R-954 (< 600 µg kg^{-1}) had no significant effect on nociception in the tail flick test in nondiabetic mice (data

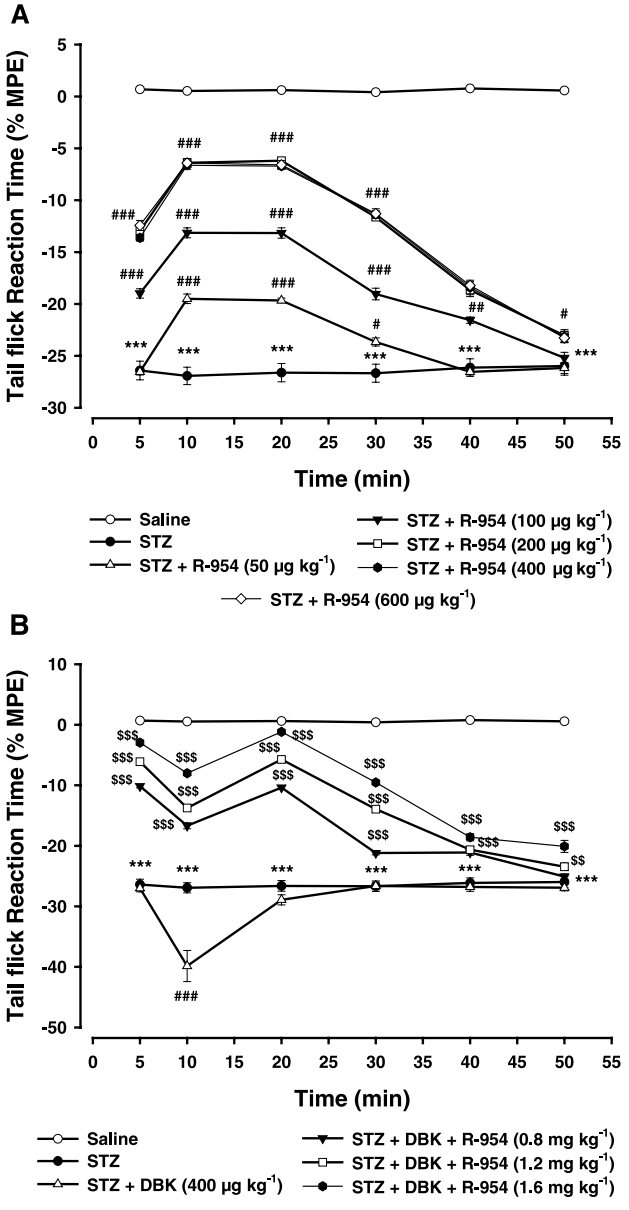

Fig. 5. Effect of single acute administration of R-954 (A) or its combined administration with des-Arg^9-bradykinin (B) on nociception in streptozotocin-treated diabetic mice using the tail flick test. Diabetes was induced in CD-1 mice using streptozotocin (STZ; 200 mg kg^{-1}, i.p.). On day 7 following the induction of the disease, mice were injected with R-954 (50–600 µg kg^{-1}, i.p.) (A), des-Arg^9-bradykinin (DBK; 400 µg kg^{-1}, i.p.) (B) or a combination of DBK and R-954 (0.8–1.6 mg kg^{-1}, i.p.) (B). The tail flick test was carried out at different time intervals (10–60 min) following injections. Data are expressed as mean (% MPE) ± S.E.M. (n = 7–12). MPE = Maximum Percent Effect. n = number of animals. ***P < 0.001, significantly different from the saline group; $^{\#}P$ < 0.05, $^{\#\#}P$ < 0.01 and $^{\#\#\#}P$ < 0.001, significantly different from the STZ group; and $^{\$\$}P$ < 0.01 and $^{\$\$\$}P$ < 0.001, significantly different from the STZ/DBK group.

not shown). Conversely, both drugs attenuated the hyperalgesic activity observed in diabetic mice in a dose- and time-dependent manner (Fig. 4A,B).

Maximal inhibition was observed after 10 min at either doses of R-715. The maximal inhibition (76%, $P<0.0001$) was observed at a dose of 800 μg kg^{-1} whereas lower dosages (100, 200, 400 and 600 μg kg^{-1}) at 10 min significantly inhibit diabetes-mediated hyperlagesia by 24%, 50%, 68% and 67%, respectively ($F=121.89$, $P<0.0001$; Fig. 4A). Calculated ID_{50} at $T=10$ min is 143 ± 4 mg kg^{-1}. The inhibition at all doses remained stable for an additional 10 min (up to 20 min). It decreased thereafter over time and activity ceased after 50 min (Fig. 4A).

The administration of des-Arg^9-bradykinin (200, 400 or 600 μg kg^{-1}) in control mice did not induce significant changes in the tail flick test (data not shown). In streptozotocin mice, the potentiating effect of des-Arg^9-bradykinin (25 to 48%) was maximal after 10 min at all three doses ($F=341.20$, $P<0.0001$) and receded after 20 min (Fig. 4B). Co-administration of des-Arg^9-bradykinin, at the selected mid-dose of 400 μg kg^{-1}, and R-715, at selected doses of 1.6–2.4 mg kg^{-1}, reversed by 27–57% ($P<0.0001$) the potentiating effect of des-Arg^9-bradykinin on streptozotocin-induced hyperalgesia observed at $T=10$ min (Fig. 4B). The inhibitory effect of R-715 was more potent at 5 and 20 min post-injection since des-Arg^9-bradykinin at these time points did not significantly potentiate the streptozotocin-induced hyperlagesia (Fig. 4B). Finally, the effect of all three doses of R-715 receded after 40 min (Fig. 4B).

R-954 (200 mg kg^{-1} and above) significantly inhibited by 76% ($P<0.0001$) streptozotocin-induced hyperalgesia after 10 min (Fig. 5A). Lower dosages (50 and 100 μg kg^{-1}) still significantly ($F=147.28$, $P<0.0001$) inhibited the hyperalgesic activity by 28% and 51%, respectively, after 10 min. The inhibitory effect remained stable and receded after 20 min to cease at 50 min (Fig. 5A). The R-954 ID_{50} at the time of the maximal inhibition (10 min) was estimated at 72 ± 2 μg kg^{-1}.

Co-administration of des-Arg^9-bradykinin and R-954 exhibited the same profile of effects as observed with R-715 except that R-954 was more potent and lasted longer, up to 50 min (Fig. 5B).

4. Discussion

Recent studies have demonstrated that the bradykinin B_1 receptors play a significant role in the pathogenesis of experimental diabetes and the development of its complications (Zuccollo et al., 1996, 1999; Simard et al., submitted for publication). However, there was no direct evidence for their involvement in the hyperalgesia induced in diabetic animals. In the present study, we showed that streptozotocin-diabetic mice developed a marked hyperalgesia. This hyperalgesic effect was inhibited by single acute i.p. administration of the recently developed specific bradykinin B_1 receptor antagonists, R-715 (Regoli et al., 1998, 2001) and R-954 (Neugebauer et al., 2002). In addition, acute i.p. treatment with the selective bradykinin B_1 receptor agonist, des-Arg^9-bradykinin, significantly potentiated the diabetes-induced hyperalgesia, an effect that was totally reversed by both R-715 and R-954.

The inducible bradykinin B_1 receptors were shown to participate in the chronic phase of the inflammatory and pain response (Dray and Perkins, 1993, 1997; Dray, 1997). B_1 receptors elicit persistent responses and signalling that are subject to very limited desensitization and receptor internalization with very low ligand dissociation (Couture et al., 2001). In addition, upon long-term agonist exposure, the B_1 receptor is upregulated (Faussner et al., 1999). Chronic activation of B_1 receptors is likely to be amplified by the accumulation of des-Arg^9-bradykinin (the metabolite resulting from the degradation of bradykinin) at the site of inflammation because the half-life of des-Arg^9-bradykinin is 4- to 12-fold longer than that of bradykinin (Décarie et al., 1996a,b; Marceau et al., 1998). Upregulation of carboxypeptidase M (kininase I, the enzyme responsible for the metabolism of bradykinin to des-Arg^9-bradykinin) may also account for the increasing endogenous level of des-Arg^9-kinin metabolites and bradykinin B_1 receptor agonists in inflammation (Schremmer-Danninger et al., 1998). Moreover, a synergistic interaction appears to exist between bradykinin B_1 receptor ligands and interleukin-1β to enhance the expression of B_1 receptors (Phagoo et al., 1999). The induction of B_1 receptor by cytokines is controlled by mitogen-activated protein kinase (MAP kinase) and by the transcriptional nuclear factor kappa B (NF-κB) (Campos et al., 1999).

Current evidence indicates that type-1 diabetes is due to an autoimmune response associated with overproduction of cytokines, including interleukin-1β and tumor necrosis factor-α (TNF-α), which leads to the destruction of pancreatic islet β-cells (Rabinovitch, 1998). These cytokines are believed to be implicated in the induction of the B_1 receptors (see above). In addition, hyperglycemia and the resulting oxidative stress can also activate the NF-κB (Yerneni et al., 1999), which is also involved in the induction of B_1 receptor expression (Marceau et al., 1998). Pharmacological evidence further suggests that the bradykinin B_1 receptor intervenes in the pathogenesis of streptozotocin-induced diabetes in mice. To that effect, Zuccollo et al. (1996) demonstrated that bradykinin B_1 receptor antagonists normalize glycemia and renal function. When administered with streptozotocin, they reversed the elevation of blood glucose level and prevented the renal abnormalities, including increased urine volume and increased excretion of protein, nitrite and kallikrein. Also, it has been established that B_1 receptors are overexpressed in the stomach of diabetic mice since the sensitivity of the stomach fundus to des-Arg^9-bradykinin was substantially increased in these animals (Pheng et al., 1997). Further-

more, it has been reported that B_1 receptor plays a determinant role in the increased vascular permeability associated with diabetes as B_1 receptor antagonists could inhibit the enhanced permeability as measured by extravasation of Evans blue dye in several mouse tissues (liver, pancreas, duodenum, ileum, kidney) (Simard et al., 2002). More recently, the bradykinin B_1 receptor was reported to be induced in kidneys and spinal cord of rats treated 3 weeks earlier with streptozotocin (Mage et al., 2002; Cloutier and Couture, 2000).

Regarding the pain process, recent immunohistochemical studies have shown the basal expression of B_1 receptors in sensory ganglia as well as in central and peripheral nerve terminals of sensory neurones (Aδ and C-fibres) in the rat (Wotherspoon and Winter, 2000). However, bradykinin B_1 receptors agonists neither affected nociception in normal rats nor in acute models of inflammation (Dray and Perkins, 1997), or caused second messenger activation, neuropeptide release or electrophysiological events in sensory neurones under control or inflammatory conditions (Dray et al., 1992). On the other hand, pharmacological antagonists of the bradykinin B_1 receptor induced analgesia only in animal models of persistent inflammatory mechanical and thermal hyperalgesia (Perkins et al., 1993, 1995; Rupniak et al., 1997; Poole et al., 1999; Bélichard et al., 2000) or of persistent visceral pain (Jaggar et al., 1998). Furthermore, in a rat model of neuropathic hypersensitivity following peripheral nerve injury, treatment with bradykinin B_1 receptor antagonists had an analgesic effect, 14 days after the injury (Levy and Zochodne, 2000). Nevertheless, it has been recently demonstrated that the hyperalgesia induced by Freund's adjuvant was reduced in bradykinin B_1 receptor-knockout mice (Ferreira et al., 2001). However, Pesquero et al. (2000) reported that under normal non-inflamed conditions, bradykinin B_1 receptor-deficient mice proved to more resistant to pain in behavioural tests of chemical and thermal nociception. With this exception, B_1 receptors are mainly involved in persistent inflammatory pain and still the potential role of B_1 receptors in the control of acute pain needs further studies.

Moreover, Couture and Lindsey (2000) showed that activation of bradykinin B_1 receptors in streptozotocin-pretreated rats caused changes in the thermonociceptive threshold in the rat tail flick test. The bradykinin B_1 receptor agonist des-Arg^9-bradykinin—administered intrathecally—did not affect the nociceptive threshold in control rats. However, the agonist induced a biphasic response in the animals, 24 h after treatment with streptozotocin; an initial hyperalgesic response was noted 1 min after the injection, followed by a secondary antinociceptive effect 6 min after the B_1 agonist (Couture and Lindsey, 2000). The biphasic response was completely blocked by B_1 receptor antagonists, with the hyperalgesic effect also blocked by substance P antagonists, nitric oxide synthase (NOS)-inhibitors and cyclooxygenase-2 inhibitors, suggesting that central B_1 receptor activation is associated with the release of substance P and the production of nitric oxide and prostaglandins (Couture and Lindsey, 2000).

Our results showed that streptozotocin-treated animals developed a well-defined hyperalgesia that was evaluated in two types of thermal noxious tests, the hot plate test (supraspinal) and the tail flick test (spinal). The diabetes-induced hyperalgesia was abolished by the specific bradykinin B_1 receptor antagonists, R-715 and R-954. In addition, the selective bradykinin B_1 receptor agonist, des-Arg^9-bradykinin, significantly increased this hyperalgesic activity induced by diabetes, an effect that was almost completely reversed by R-715 and R-954. This exaggeration of sensory signaling and pain sensation could be attributed to the overexpression of the bradykinin B_1 receptors during the development of diabetes and to the increase in the endogenous level of des-Arg^9-bradykinin. Several mechanisms related to the upregulation of bradykinin B_1 receptors could explain this diabetic hyperalgesia. The first potential mechanism that contributes to the hyperalgesia associated with diabetes is the upregulation of bradykinin B_1 receptors in sensory neurones (Petersen et al., 1998). The direct effect of kinins on sensory neurones to release substance P, calcitonin gene-related peptide (CGRP), neurokinin A and other nociceptive neurotransmitters could be sensitized by the action of prostaglandins or other mediators released from other cells by the activation of kinin receptors. An alternative mechanism could be the induction of B_1 receptors on cells other than the sensory neurones (macrophages, fibroblasts or endothelial cells) which may be responsible for releasing mediators (prostaglandins, cytokines and nitric oxide) that sensitize or activate the nociceptors (Dray and Perkins, 1997). It has been also suggested by Walker et al. (1995) that stimulation of sympathetic nerves by kinins causes the release of neuropeptides, prostanoids, sympathetic transmitters or other mediators that sensitize nociceptive nerve terminals, leading to hyperalgesia. Furthermore, the increase in vascular permeability induced by the bradykinin B_1 receptors is believed to enable the extravasation of blood constituents that might have a sensitizing effect on pain perception.

In conclusion, the bradykinin B_1 receptor participates in the chronic phase of inflammation and of somatic and visceral pain, and is likely to play a strategic role in diseases with a strong immune component such as rheumatoid arthritis, multiple sclerosis, septic shock and diabetes. Our results provide major evidence for the involvement of the bradykinin B_1 receptors in the development of inflammatory hyperalgesia associated with diabetes and suggest a novel approach to the treatment of this diabetic complication using bradykinin B_1 receptor antagonists.

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