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Changes in paw oedema triggered via bradykinin B_1 and B_2 receptors in streptozotocin-diabetic rats

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Abstract

The present study investigated hind paw oedema mediated by bradykinin B_1 and B_2 receptors in streptozotocin-diabetic rats. Paw oedema induced by intraplantar (i.pl.) injection of bradykinin or the selective bradykinin B_2 receptor agonist, Tyrosine^8-bradykinin ([Tyr^8]bradykinin) (both 3 nmol/paw), was significantly reduced at 4 weeks after streptozotocin treatment (34 ± 8% and 40 ± 7%). At 6 weeks after streptozotocin, when paw oedema caused by substance P or prostaglandin E_2 (both 10 nmol/paw) was unchanged, inhibition of bradykinin B_2 receptor-mediated oedema was maximal (66 ± 6% and 72 ± 2%, for bradykinin and [Tyr^8]bradykinin, respectively). The selective bradykinin B_1 receptor agonist, [des-Arg^9]bradykinin (100 nmol/paw), induced only slight paw oedema in non-diabetic controls. Responses to [des-Arg^9]bradykinin were markedly enhanced 8 weeks after streptozotocin (from 0.09 ± 0.01 to 0.38 ± 0.05 ml), less so at 10 weeks (0.22 ± 0.03 ml), and returning to basal values at 12 weeks (0.11 ± 0.03 ml). Treatment with insulin protamine zinc (1–3 U/day/7 weeks, s.c.) did not reverse the inhibition of responses to [Tyr^8]bradykinin or the potentiation of responses to [des-Arg^9]bradykinin seen at 8 weeks. Thus, streptozotocin-induced diabetes induces long-lasting alterations in oedematogenic responsiveness to kinins in the rat, characterized by marked reduction of oedema involving activation of bradykinin B_2 receptors, associated with enhancement of bradykinin B_1 receptor-mediated oedema. © 2001 Published by Elsevier Science B.V.

Keywords: Bradykinin B_1 receptor; Bradykinin B_2 receptor; Streptozotocin; Diabetes; Paw oedema (Rat); Insulin

1. Introduction

Generated in plasma and peripheral tissues, especially in response to tissue trauma or infection, bradykinin and other related endogenous kinins are a group of peptides, which not only participate in blood pressure control, but exert important actions in inflammation and pain (for review see: Regoli and Barabé, 1980; Bhoola et al., 1992; Marceau and Bachvarov, 1998; Calixto et al., 2000). Indeed, kinins seem to be implicated in several physiopathologies including asthma, allergy, rheumatoid arthritis, endotoxic shock and acute pancreatitis (for review see: Calixto et al., 2000).

Most actions of kinins are mediated by activation of two specific G protein-coupled transmembrane receptors, denoted as B_1 and B_2. Both bradykinin receptor types have been well characterized at the pharmacological, genetic and structural levels (Regoli and Barabé, 1980; Eggerix et al., 1992; Hess et al., 1994; McEachern et al., 1991; Pesquero et al., 1996, Marceau and Bachvarov, 1998). The bradykinin B_2 receptors are expressed largely in a constitutive fashion, and seem to underlie most of the physiological responses to kinins and mediate several early (acute phase) inflammatory events (Marceau and Bachvarov, 1998). The bradykinin B_1 receptors, on the other hand, are not normally present in non-traumatised tissues, but may be upregulated under some special conditions, particularly related to chronic inflammatory processes and activation of the cytokine network (Marceau et al., 1998). More recently, the roles of both bradykinin B_1 and B_2 receptors have also begun to be probed by means of gene knockout technology. In this regard, pronounced changes in respon-

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siveness to nociceptive and pro-inflammatory stimuli have been detected in mice lacking bradykinin B_1 (Pesquero et al., 2000) or B_2 receptors (Boyce et al., 1996).

Diabetes, a disease currently affecting over 120 million people worldwide (Seidell, 2000), is caused either by insulin deficiency due to pancreatic β-cell dysfunction or destruction, or by failure of target organs to respond to this hormone. The diabetic condition is associated with multiple changes of vascular structures, as well as interference with production of vasoactive substances (Fortes et al., 1983; Morrish et al., 1991; Giannattasio et al., 1999). Onset of diabetes seems to be related to functional alterations of the endothelium, an action which contributes directly to the progress and complexity of the disease (Hopfner and Gopalakrishnan, 1999; Chan et al., 2000). Several lines of evidence have suggested the involvement of the kallikrein–kinin system in the pathogenesis of diabetes (Garcia Leme et al., 1973; Wang et al., 1998, Cloutier and Couture, 2000). Indeed, different symptoms of diabetes in mice can be offset by treatment with antagonists of bradykinin B_1 and B_2 receptors (Zuccollo et al., 1996). Streptozotocin, a nitrosamine that produces insulitis and selective destruction of pancreatic β-cells, has been widely employed to induce insulin-dependent (type I) diabetes in rats and mice. This model of experimental diabetes has been of paramount importance regarding current knowledge about this disease (for review see: Chan et al., 2000).

In the present study, we investigated the time-related influence of streptozotocin-induced type I diabetes on the rat paw oedema formation, induced by selective bradykinin B_1 and B_2 receptors agonists.

2. Materials and methods

2.1. Rat paw oedema

The procedures used were similar to those described previously (Campos and Calixto, 1995). Experiments were performed with non-fasted male Wistar rats (160–180 g; $n = 308$) housed at 22 ± 2°C with a 12/12 h light–dark cycle (lights on at 06:00). Under light anesthesia with 2,2,2 tribromoethanol (0.12 g/kg), the animals received a 0.1 ml intraplantar (i.pl.) injection, into the right hind paw, of bradykinin (3 nmol/paw), [Tyr^8]bradykinin (3 nmol/paw) or [des-Arg^9]bradykinin (100 nmol/paw) in phosphate-buffered saline (PBS, composition in mmol/l: NaCl 137, KCl 2.7 and phosphate buffer 10). The left hind paw received a similar 0.1 ml injection of PBS alone and was used as control. Paw volume (below the ankle joint) was measured with a plethysmometer (Ugo Basile, Milan, Italy) at several time points (10, 20, 30, 60 and 120 min). Paw oedema is expressed (in ml) as the difference between the volumes of right and left paws. The animals were always pretreated with the angiotensin-converting enzyme inhibitor captopril (5 mg/kg, s.c.), 1 h before any experiment, in order to retard the degradation of kinins (Corrêa and Calixto, 1993). In some experiments, hind paw oedema was induced by i.pl. injection of substance P (10 nmol/paw) or prostaglandin E_2 (10 nmol/paw) and evaluated 30, 60, 120 and 240 min after injection of these inflammatory agents, as described before. The doses of kinin agonists used in the present work were chosen based on those in our previous publications (Campos et al., 1995, 1996).

2.2. Induction of diabetes

Diabetes was induced as described by Wang et al. (1998) with minor modifications. Briefly, the animals (160–180 g) received a single injection of streptozotocin (60 mg/kg, i.p.) diluted in 0.05 M citrate buffer (pH 4.5) at different intervals (4, 6, 8, 10 and 12 weeks) before the experiments. The onset of diabetes was confirmed by assessing glucose levels in blood samples collected from the tail vein, 1 week after treatment with streptozotocin. Only animals with blood glucose levels ≥ 250 mg/dl, 1 week after streptozotocin treatment (about 70%), were used in the experiments. Determination of blood glucose levels (and body weight) was repeated periodically in several (but not all) animals, 6, 8, 10 and 12 weeks after streptozotocin injection, in the week of the experiments. Different groups of rats were used to assess the oedematogenic responses to each agonist. Age-matched control rats received citrate buffer vehicle alone. In a separate group of experiments, the animals were treated with insulin protamine zinc (1–3 U/rat/day, s.c.) once a day for 7 consecutive weeks, according to the procedures described before (Longhurst, 1991; Courteix et al., 1996). The treatment was initiated 1 week after streptozotocin injection and the control animals were similarly treated with saline. Oedema was induced by the i.pl. injection of the selective B_2 [Tyr^8]bradykinin (3 nmol/paw) or B_1 [des-Arg^9]bradykinin (100 nmol/paw) receptor agonists, and the oedema was evaluated as described before. In another set of experiments, the effect of transitory hyperglycemia on kinin-induced oedema was evaluated in animals treated with dextrose (0.028 mmol/kg, i.p.), 25 min before kinin injection. The procedures reported were carried out in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigations of experiments in conscious animals, as recommended by the International Association for Studies on Pain (Zimmerman, 1983). Each animal was used only once and received a single i.pl. injection of oedematogenic substance.

2.3. Drugs

The following drugs were used: [des-Arg^9]bradykinin, [Tyr^8]bradykinin, bradykinin, substance P, prostaglandin E_2, streptozotocin (all from Sigma, St. Louis, USA). Dextrose (Merck, Hamburg, Germany). Insulin protamine zinc was kindly supplied by Eli-Lilly (São Paulo, Brazil).

Table 1
Time-dependent changes in blood glucose levels and body weights of vehicle- or streptozotocin-treated animals

Interval of time		Blood glucose (mg/dl)	Body weight (g)
0 day	Vehicle-treated	88 ± 6.5	179 ± 2.2
	Streptozotocin-treated	91 ± 4.1	184 ± 2.0
1 week	Vehicle-treated	90 ± 3.4	198 ± 3.1
	Streptozotocin-treated	360 ± 2.1[a]	174 ± 7.2[a]
6 weeks	Vehicle-treated	91 ± 2.4	280 ± 6.5
	Streptozotocin-treated	398 ± 2.4[a]	171 ± 8.1[a]
8 weeks	Vehicle-treated	89 ± 3.0	302 ± 4.0
	Streptozotocin-treated	401 ± 4.8[a]	153 ± 3.3[a]
	Insulin-treated	112 ± 3.2[a,b]	289 ± 5.5[a,b]
10 weeks	Vehicle-treated	101 ± 2.5	312 ± 6.2
	Streptozotocin-treated	396 ± 8.4[a]	224 ± 7.5[a]
12 weeks	Vehicle-treated	94 ± 2.0	388 ± 2.0
	Streptozotocin-treated	312 ± 9.5[a]	301 ± 3.4[a]

$n = 10–12$ animals/group.
[a] $P < 0.05$ (versus vehicle-treated animals).
[b] $P < 0.05$ (versus streptozotocin-treated animals).

2.4. Statistical analysis

The results are presented as the means ± S.E.M. for six animals. The percentages of inhibition are reported as means ± S.E.M. of inhibitions obtained for each individual experiment, relative to the respective time-matched non-diabetic control group, at 20 min after injection of the peptides. Statistical comparison of the data was performed

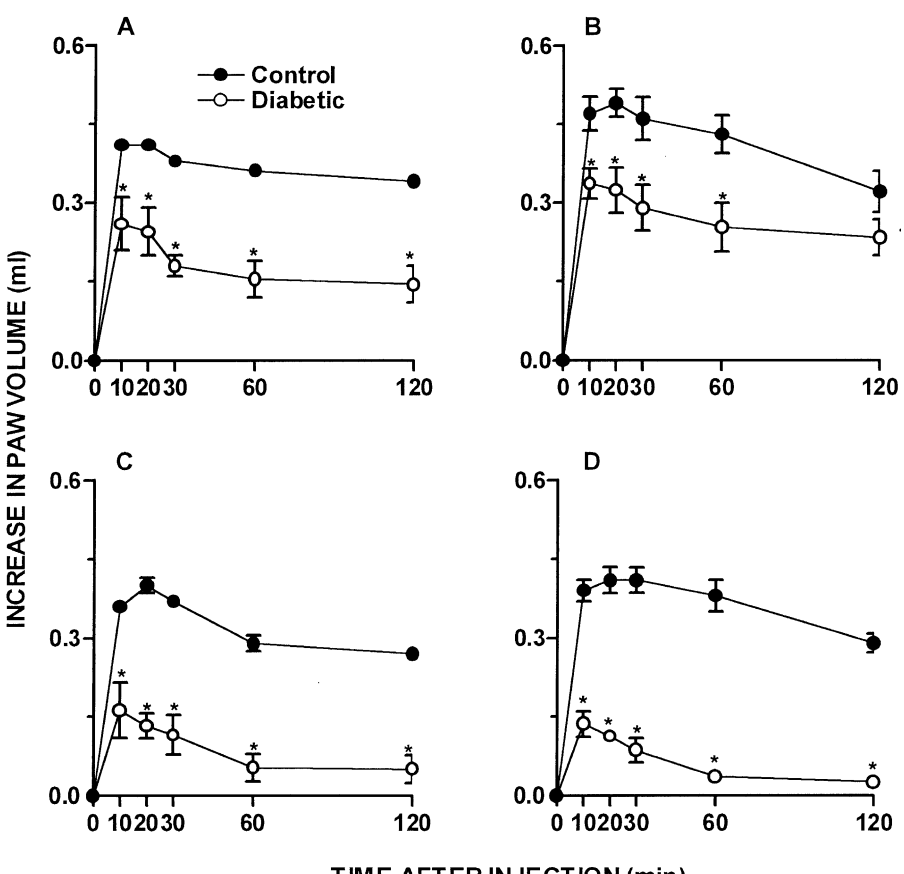

Fig. 1. Rat paw oedema caused by intraplantar injection of bradykinin (panels A and C) or [Tyr[8]]bradykinin (panels B and D) (both 3 nmol/paw) in control (●) and streptozotocin-diabetic animals (○) at 4 (panels A and B) or 6 weeks (panels C and D) after induction of diabetes. Values represent the differences between volumes (in ml) of PBS- and drug-injected paws. Each point represents the mean ± S.E.M. for six rats. In some cases, the error bars are hidden within the symbols. Significantly different from vehicle-pretreated animals: * $P < 0.05$ (two-way ANOVA followed by Bonferroni's test).

using one-way analysis of variance (ANOVA), followed by Dunnett's test, or with a two-way ANOVA followed by Bonferroni's test, as indicated. *P* values of less than 0.05 were considered significant.

3. Results

The treatment of animals with streptozotocin produced, within 1 week, a sustained increase in blood glucose levels, associated with a severe decrease in body weight (see Table 1). As described previously (Campos and Calixto, 1995; Campos et al., 1995), the i.pl. injection of bradykinin or the selective bradykinin B_2 receptor agonist [Tyr^8]bradykinin (each at 3 nmol/paw) produced a marked increase in rat hind paw volume, with peak responses of 0.44 ± 0.04 and 0.40 ± 0.01 ml, respectively, 20 min after injection. The results depicted in Fig. 1A and B show that paw oedema induced by i.pl. injection of bradykinin or [Tyr^8]bradykinin (both at 3 nmol/paw) was significantly

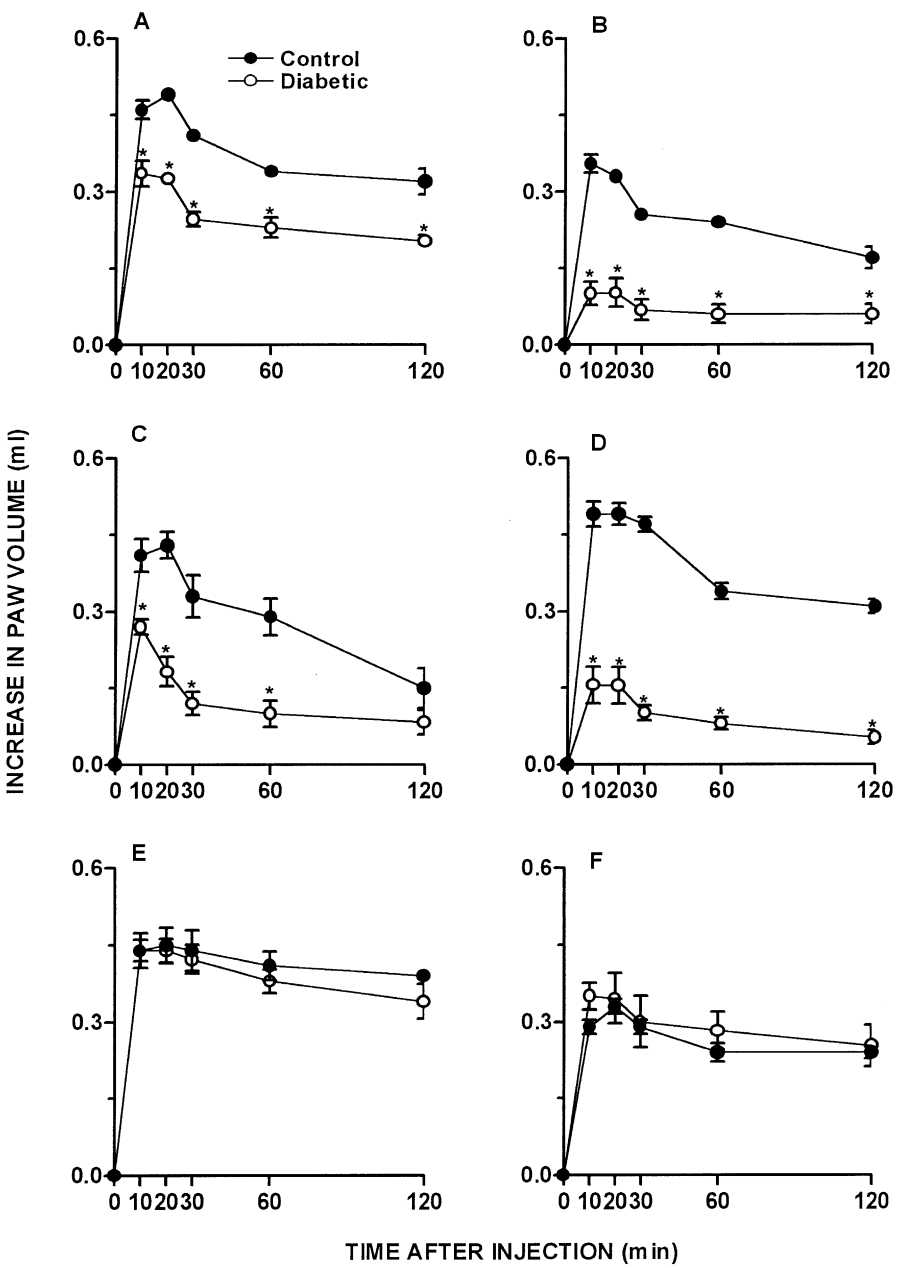

Fig. 2. Rat paw oedema caused by intraplantar injection of bradykinin (panels A, C and E) or [Tyr^8]bradykinin (panels B, D and F) (both 3 nmol/paw) in control (●) and streptozotocin-diabetic animals (○) at 8 (panels A and B), 10 (panels C and D) or 12 weeks (panels E and F) after induction of diabetes. Values represent the differences between volumes (in ml) of PBS-and drug-injected paws. Each point represents the mean ± S.E.M. for six rats. In some cases, the error bars are contained within the symbols. Significantly different from vehicle-pretreated animals: $^*P < 0.05$ (two-way ANOVA followed by Bonferroni's test).

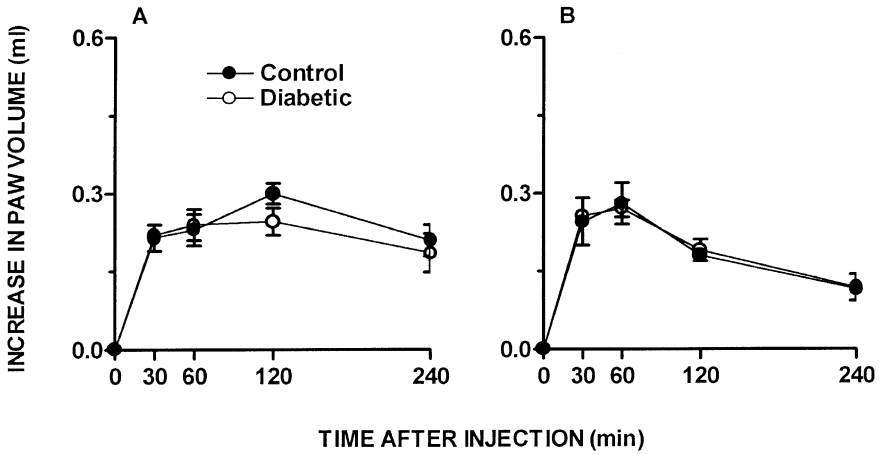

Fig. 3. Rat paw oedema caused by intraplantar injection of substance P (panel A) or prostaglandin E_2 (panel B) (both 10 nmol/paw) in control (●) and streptozotocin-diabetic animals (○) at 6 weeks after induction of diabetes. Values represent the differences between volumes (in ml) of PBS-and drug-injected paws. Each point represents the mean ± S.E.M. for six rats. In some cases, the error bars are hidden within the symbols.

reduced as early as 4 weeks after streptozotocin treatment (34 ± 8% and 40 ± 7% inhibition relative to normoglycemic controls, respectively). The decrease of oedematogenic responses mediated by the activation of bradykinin B_2 receptors was maximal after 6 weeks of treatment with streptozotocin (66 ± 6% and 72 ± 2% inhibition, for bradykinin and [Tyr^8]bradykinin, respectively, Fig. 1C and D). The inhibition of bradykinin- and [Tyr^8]bradykinin-induced oedema remained significant at 8 and 10 weeks of diabetes, but was no longer evident at 12 weeks (Fig. 2).

On the other hand, paw oedema, in response to i.pl. injection of substance P or prostaglandin E_2 (both 10 nmol/paw), in diabetic rats was unchanged at 6 weeks after streptozotocin treatment. The responses obtained were: 0.28 ± 0.04 and 0.27 ± 0.02 ml for substance P and 0.23 ± 0.03 and 0.24 ± 0.03 ml for prostaglandin E_2, in control and diabetic rats, respectively (Fig. 3). In addition, transitory hyperglycemia induced by the treatment of animals with dextrose (0.028 mmol/kg, i.p.), which raised blood glucose levels to 298 ± 11 mg/dl, failed to affect the paw oedema induced by the selective B_2 receptor agonist, [Tyr^8]bradykinin (0.38 ± 0.03 and 0.41 ± 0.035 ml, for control and dextrose-treated animals, respectively).

In naive normoglycemic animals, i.pl. injection of the selective B_1 receptor agonist, [des-Arg^9]bradykinin, caused a very slight increase in paw oedema formation (0.09 ± 0.01 ml). In diabetic animals, 8 weeks after streptozotocin injection, however, i.pl. injection of [des-Arg^9]bradykinin (100 nmol/paw) resulted in pronounced paw oedema, corresponding to a 4-fold increase in peak paw volume when compared to that in saline-treated non-diabetic control animals (Fig. 4). The increased responsiveness to the selective bradykinin B_1 receptor agonist was maximal 8

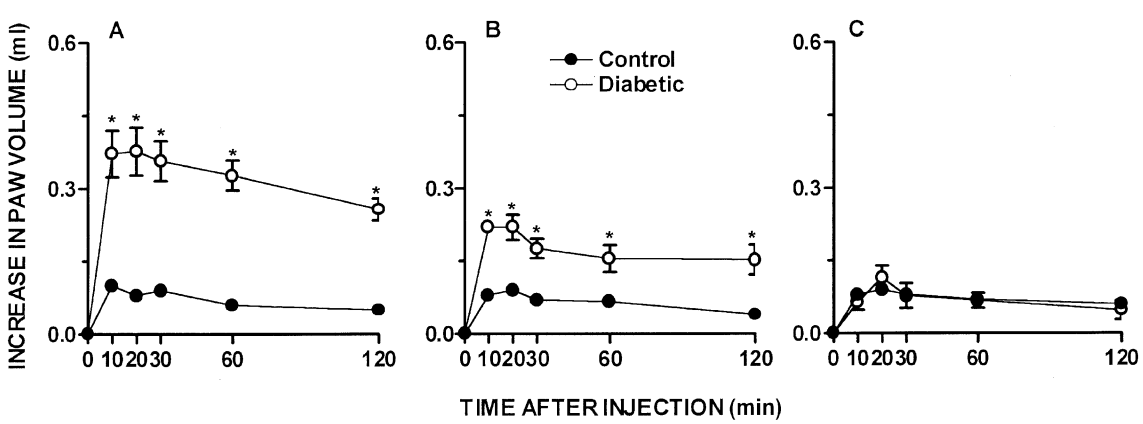

Fig. 4. Rat paw oedema caused by intraplantar injection of [des-Arg^9]bradykinin (100 nmol/paw) in control (●) and streptozotocin-diabetic animals (○) at 8 (panel A), 10 (panel B) or 12 weeks (panel C) after induction of diabetes. Values represent the differences between volumes (in ml) of PBS-and drug-injected paws. Each point represents the mean ± S.E.M. for six rats. In some cases, the error bars are hidden within the symbols. Significantly different from vehicle-pretreated animals: $^{*}P < 0.05$ (two-way ANOVA followed by Bonferroni's test).

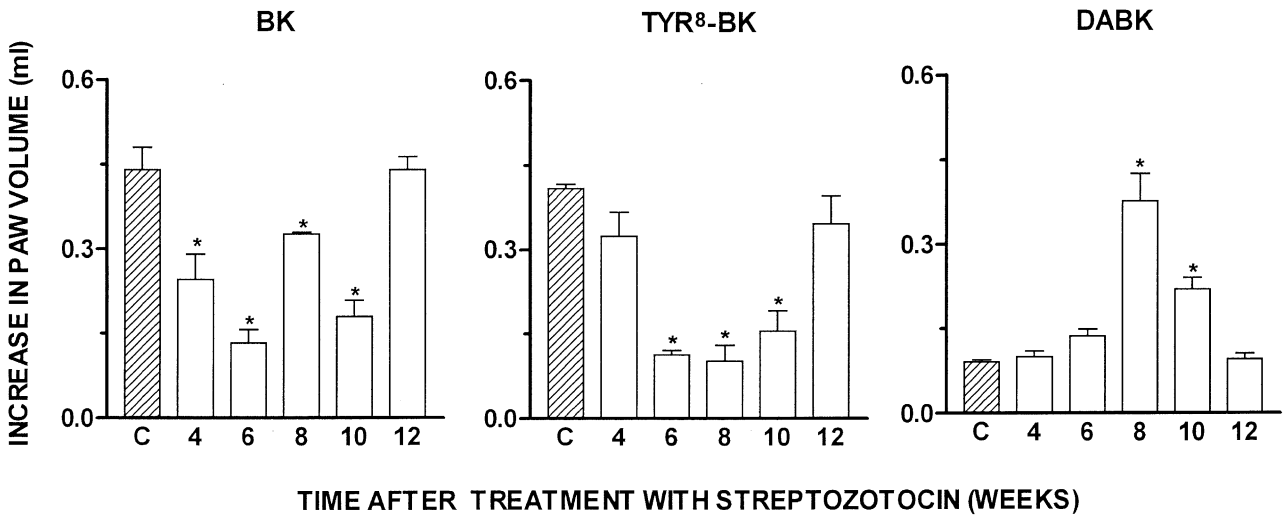

Fig. 5. Time-dependent alterations of rat hindpaw volume in response to intraplantar injection of bradykinin (3 nmol/paw, panel A), [Tyr^8]bradykinin (3 nmol/paw, panel B) or [des-Arg^9]bradykinin (100 nmol/paw, panel C) in vehicle-treated (hatched columns; pooled controls) or streptozotocin-diabetic animals (open columns). Values represent the differences between volumes (in ml) of PBS-and drug-injected paws at 20 min. Each column represents the mean ± S.E.M. for six rats. Significantly different from vehicle-pretreated animals: $^*P < 0.05$ (one-way ANOVA followed by Dunnett's test).

weeks after streptozotocin treatment (0.38 ± 0.05 ml, Fig. 4A), being reduced 10 weeks after treatment with streptozotocin (0.22 ± 0.03 ml, Fig. 4B), returning to basal values at 12 weeks (0.11 ± 0.03 ml, Fig. 4C) ($P > 0.05$).

The results depicted in Fig. 5 reveal significant differences in the time-courses of changes in peak oedematogenic responses to bradykinin, [Tyr^8]bradykinin and [des-Arg^9]bradykinin in streptozotocin-induced diabetic animals. Whereas the inhibition of bradykinin-induced oedema was maximal at 6 weeks, was partially reversed at 8 weeks and reinstated again at 10 weeks (66 ± 6%, 33.2 ± 11% and 57 ± 6% inhibition, respectively), the oedema caused by [Tyr^8]bradykinin was depressed to similar extents at 6, 8 and 10 weeks (72 ± 2%, 69.3 ± 9% and 58 ± 6% inhibition, respectively). In contrast, [des-Arg^9]bradykinin-induced oedema was unchanged up to 6 weeks, was markedly increased at 8 weeks and had partially subsided at 10 weeks. Oedematogenic responses to all three kinins returned to basal control levels 12 weeks after streptozotocin treatment.

Chronic treatment of diabetic animals with daily injections of insulin protamine zinc for 7 weeks, starting at 1 week after streptozotocin injection, failed to restore the oedematogenic responses to kinins to normal values. Peak paw oedemas obtained in insulin-treated and non-treated 8-week diabetic animals, in response to i.pl. injections of

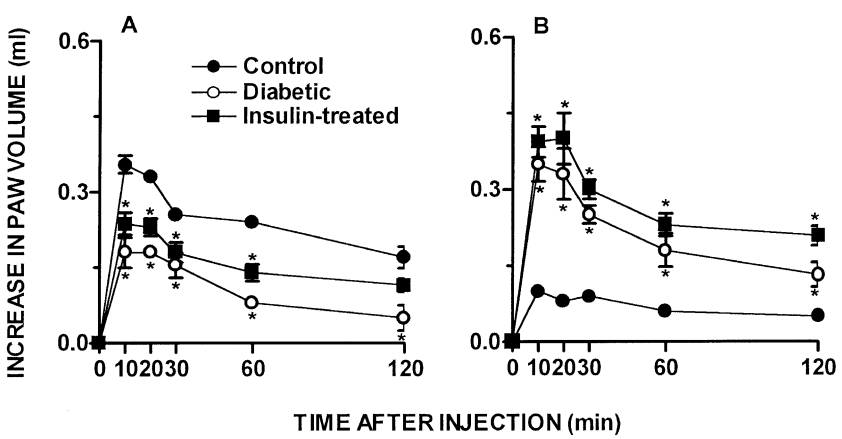

Fig. 6. Effect of chronic treatment with insulin protamine zinc (■ 1–3 U/day/rat/7 weeks) on paw oedema induced by [Tyr^8]bradykinin (3 nmol/paw, panel A) or [des-Arg^9]bradykinin (100 nmol/paw, panel B) in diabetic rats. Control responses (●) and responses obtained in streptozotocin-diabetic animals (○). Values represent the differences between volumes (in ml) of PBS- and drug-injected paws. Each point represents the mean ± S.E.M. for six rats. In some cases, the error bars are hidden within the symbols. Significantly different from vehicle-pretreated animals: $^*P < 0.05$ (two-way ANOVA followed by Bonferroni's test).

[Tyr^8]bradykinin, were 0.18 ± 0.05 and 0.23 ± 0.02 ml, whereas responses to [des-Arg^9]bradykinin were 0.33 ± 0.05 and 0.40 ± 0.05 ml, respectively (Fig. 6).

4. Discussion

We now investigated the time-related alterations in oedematogenic responsiveness of the hind paw of streptozotocin-induced diabetic rats to stimulation of bradykinin B_1 and B_2 receptors. The results reveal substantial and distinct changes in oedema triggered by bradykinin B_1 and B_2 receptors in this experimental model of type I diabetes.

Our results demonstrate that the treatment of animals with streptozotocin results in a marked decrease of the paw oedema responses mediated by bradykinin B_2 receptors. This effect seems to be selective for kinins as, at 6 weeks after streptozotocin injection, when oedema induced by bradykinin or the selective bradykinin B_2 receptor agonist, [Tyr^8]bradykinin, was maximally depressed, oedematogenic responses to substance P or prostaglandin E_2 were unchanged relative to those of non-diabetic controls. A similar decrease in bradykinin B_2 receptor-mediated vasoconstriction triggered by bradykinin has recently been reported in tail arteries obtained from 8-week streptozotocin-treated rats (Wang et al., 1998).

Several putative mechanisms could underlie the reduction of bradykinin B_2 receptor-mediated oedema formation in streptozotocin diabetic animals, including down-regulation of bradykinin B_2 receptor expression and alterations in signal transduction mechanisms associated with these receptors in the appropriate cells/tissues, among others. A 1.8-fold increase in bradykinin B_2 receptor density has been reported in renal cortex of 12-week diabetic rats (Tschöpe et al., 1999a), whereas bradykinin B_2 receptor expression in myocardium was unchanged in these animals (Tschöpe et al., 1999b). However, to the best of our knowledge, no studies have yet assessed the influence of diabetes on bradykinin B_2 (or B_1) receptor expression in tissues/cells (e.g. endothelial cells, nociceptive sensory fibres mediating neurogenic inflammation) implicated in bradykinin-induced enhancement of vascular permeability and oedema. Another possibility is the uncoupling of bradykinin B_2 receptors from phospholipase C-dependent stimulation of protein kinase C, a phenomenon which results in substantial depression of bradykinin-induced contraction in glomeruli and mesangial cells, isolated from rats 1 week after streptozotocin treatment (Ouardani et al., 1997). On the other hand, it is also widely accepted that vascular changes during diabetes are associated with altered prostaglandin formation, at least in part via decreased phospholipase A_2 activity (Subbiah and Deitemeyer, 1980; Hagen et al., 1985). In this regard, many inflammatory effects of bradykinin are critically dependent on stimulation of phospholipase A_2, as well as cyclooxygenase-1 and cyclooxygenase-2, with the consequent formation of prostanoids such as prostaglandin E_2 (for review see: Calixto et al., 2000). Furthermore, bradykinin B_2 receptor-mediated enhancement of vascular permeability, and hence oedema, is also directly related to stimulation of sensory fibres and release of oedematogenic neuropeptides such as substance P, neurokinin A and calcitonin gene-related peptide (Geppetti, 1993; Campos and Calixto, 2000). Since insulin-dependent (type I) diabetes has been shown to reduce tissular neuropeptide levels and/or release (Garrett et al., 1995; Nemeth et al., 1999), this may explain why we found decreased oedematogenic responses to bradykinin and [Tyr^8]bradykinin, but not to exogenous substance P or prostaglandin E_2 in 6-week diabetic rats. Thus, the treatment with streptozotocin might affect neuropeptide release from sensory fibres, without alteration of the responses evoked by exogenous neuropeptides. Finally, the streptozotocin-induced inhibition of bradykinin B_2 receptor-mediated responses may be related, at least in part, to the impairment of nitric oxide (NO)-mediated vascular events, as type I diabetes is associated with a reduced NO production, as well as impairment of the reactivity of vascular endothelium to NO (Cellek et al., 1999; Chan et al., 2000).

In sharp contrast to the decrease of bradykinin B_2 receptor-mediated oedema, we demonstrated that streptozotocin treatment causes a pronounced increase of oedematogenic responses to kinin B_1 receptor stimulation with a selective agonist ([des-Arg^9]bradykinin). The effect only became apparent and actually peaked (4-fold increase) at 8 weeks of diabetes, was partially reversed at 10 weeks and had entirely disappeared by 12 weeks. Despite the distinct time-course, this new and interesting finding fits in well with the recent demonstration that rats given streptozotocin display marked cardiovascular responses to intrathecal [des-Arg^9]bradykinin at 3 weeks of diabetes (Cloutier and Couture, 2000). Thus, diabetes can be included among the various stimuli capable of up-regulating kinin B_1 receptors in vivo, which already include tissue trauma, endotoxins, Freund's adjuvant, many cytokines and bacillus Calmette-Guérin, among others (Marceau et al., 1998; Campos et al., 1997, 1998). The increased oedema, induced through kinin B_1 receptor activation in streptozotocin-treated rats, might be related to the immunoregulatory role of some cytokines, especially interleukin-1β, which is implicated in the pathogenesis of insulin-dependent diabetes (Herold et al., 1996; Rabinovitch, 1998). Indeed, we have shown that i.pl. interleukin-1β or tumor necrosis factor injections into the paw, or systemic treatment of animals with endotoxin of *Escherichia coli* or bacillus Calmette-Guérin are all effective to increase the B_1-selective agonist [des-Arg^9]bradykinin-mediated oedema formation in the rat (Campos et al., 1996, 1997, 1998). Furthermore, oedema triggered by [des-Arg^9]bradykinin in the paw of rats, treated with endotoxin of *E. coli*, depends to a great extent on stimulation of capsaicin-sensitive sensory fibres and release of tachykinins and calcitonin gene-related peptide (Campos and Calixto, 2000; Ferreira et al., 2000).

Earlier studies by our group have shown that [des-Arg^9]bradykinin(100 nmol)-induced rat paw oedema in LPS-treated rats seems to involve the activation of B_1, but not B_2 kinin receptors (Campos et al., 1996). This conclusion is based on consistent experimental evidence of our group, demonstrating that [des-Arg^9]-bradykinin (100 nmol/paw)-mediated oedematogenic responses are significantly inhibited by B_1 selective receptor antagonists (des-Arg^9-[Leu^8]-bradykinin and NPC17765), while B_2 antagonists (HOE 140 and NPC 17731) had no effect. The same profile has been found in other models of functional B_1 receptor up-regulation, including the complete desensitisation with B_2 agonists (Campos and Calixto, 1995; Campos et al., 1995), after treatment with BCG (Campos et al., 1997) or with the pro-inflammatory cytokines IL-1β and TNFα (Campos et al., 1998). Therefore, in all the studies mentioned, the oedema formation induced by intraplantar injection of [des-Arg^9]bradykinin is likely to have been mediated by activation of B_1 and not of B_2 receptors. Streptozotocin-diabetic rats display increased plasma levels of high and low molecular weight kininogens and bradykinin, as well as of plasma prekallikrein activity (Tschöpe et al., 1999b; Rothschild et al., 1999). It is therefore plausible to speculate that long-term enhanced activation of the plasma kallikrein–kinin system, during streptozotocin-induced diabetes, may play a key role in the desensitization of bradykinin B_2 receptors and up-regulation of B_1 receptors controlling vascular permeability and oedema in the rat hind paw. Further studies should examine this issue more closely. Another aspect which remains unresolved is what mechanisms operate to restore the bradykinin B_2 and B_1 receptor-mediated oedematogenic responsiveness of 12-week diabetic rats back to normal non-diabetic levels, despite the maintenance of a 3-fold increase in blood glucose levels.

An unexpected finding was that long-term treatment of streptozotocin-diabetic animals with insulin for 7 weeks (starting at 1 week after streptozotocin injection), at a dose regimen sufficient to fully reverse the hyperglycemia and body weight loss, entirely failed to counteract the changes in oedema triggered by selective agonists of bradykinin B_2 and B_1 receptors at 8 weeks. The same doses and schedule of insulin treatment used in the present study have been shown to completely restore nitric oxide-mediated neurotransmission in anococcygeus muscle of streptozotocin-diabetic rats (Way and Reid, 1995). Insulin treatment has also been shown to reverse in vitro the decrease in bradykinin-induced contractions of mesangial cells isolated from 1-week streptozotocin-diabetic rats (Ouardani et al., 1997), as well as in vivo, the increase in plasma kininogen levels seen in 12-week diabetic animals (Rothschild et al., 1999). On the other hand, as seen from our current study, treatment with insulin also failed to counteract the decrease in pain threshold of diabetic rats to radiant heat in the tail-flick model (Lee and McCarthy, 1992). The reason for such discrepancies remain unclear, and additional studies are required to clarify this point. In addition, it is so far not possible to determine if the alterations of kinin-mediated oedematogenic responses are due to the diabetic state, or if they are related to a particular effect of streptozotocin.

Taken together, the results of the present study clearly demonstrate substantial and long-lasting changes, in opposite directions, of oedematogenic responses to selective bradykinin B_2 and B_1 receptor agonists in the paw of rats treated with streptozotocin. These findings reinforce the view indicating that kinins seem to have an important role during inflammatory disorders, and they also might contribute towards a better understanding of the effects played by kinins in the pathophysiology of streptozotocin-induced diabetes.

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