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Endotoxin sensitization to kinin B_1 receptor agonist in a non-human primate model: haemodynamic and pro-inflammatory effects

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1 Although endotoxaemia induces kinin B_1 receptors in several animal models, this condition is not documented in primates. This study examined the up-regulation of haemodynamic and pro-inflammatory responses to the B_1 agonist des-Arg[10]-kallidin (dKD) in a non-human primate model.

2 Green monkeys (*Cercopithecus aethiops St Kitts*) received lipopolysaccharide (LPS; 90 μg kg^{-1}) or saline intravenously. After 4 h, anaesthetized monkeys were canulated *via* the carotid artery to monitor blood pressure changes following intra-arterial injections of dKD or the B_2 agonist bradykinin (BK). Oedema induced by subcutaneous kinin administration was evaluated as the increase in ventral skin folds in anaesthetized monkeys injected with captopril at 4 h to 56 days post-LPS.

3 LPS increased rectal temperature but did not affect blood pressure after 4 h. dKD reduced blood pressure (E_{max}: 27$\pm$4 mmHg; EC_{50}: 130 pmol kg^{-1}) and increased heart rate (E_{max}: 33 b.p.m.) only after LPS. In contrast, the dose-dependent fall in blood pressure with BK was comparable in all groups. The selective B_1 antagonist [Leu[9]]dKD (75 ng kg^{-1} min^{-1}, intravenously) abolished responses to dKD but not BK.

4 dKD injection induced oedema dose-dependently (2.4$\pm$0.1 mm at 150 nmol) only following LPS (at 4 h to 12 days but not 56 days). In contrast, BK-induced oedema was present and stable in all monkeys. Co-administration of [Leu[9]]dKD (150 nmol) significantly reduced oedema induced by dKD (50 nmol).

5 These results suggest LPS up-regulation of B_1 receptor effects in green monkeys. This non-human primate model may be suitable for testing new, selective B_1 antagonists with therapeutic potential as anti-inflammatory agents.

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Abbreviations: BK, bradykinin; dKD, des-Arg[10]-kallidin; [Leu[9]]dKD, [Leu[9]]des-Arg[10]-kallidin; LPS, lipopolysaccharide; s.e.mean standard error of the mean

Introduction

B_1 receptors for kinins are considered potential therapeutic targets for the development of novel therapies aimed at controlling complications of septicemia and inflammation (Ahluwalia & Perretti, 1999; Marceau *et al*., 1998). Responses mediated by kinin B_1 receptors are up-regulated from a null level following administration of bacterial lipopolysaccharide (LPS) or inflammatory cytokines in rabbits, rats and pigs (Marceau *et al*., 1998). In these models, systemic stimulation of B_1 receptors reduces blood pressure. Induction followed by local stimulation of B_1 receptors promotes oedema and hyperalgesia associated with chronic inflammation in the rat skin and joints (Ahluwalia & Perretti, 1999; Belichard *et al*., 2000). In human isolated tissues, B_1 receptors appear to follow a similar pattern of up-regulation with injury or immune activation (Marceau *et al*., 1998). However, current evidence suggesting B_1 receptor up-regulation in primates *in vivo* is limited. A recent study documented enhanced B_1 receptor mRNA expression and B_1 receptor-dependent chemotaxis in peripheral T lymphocytes isolated from patients with active multiple sclerosis as compared to non-affected individuals (Prat *et al*., 1999). Thus, the aim of this study was to determine whether bacterial lipopolysaccharide (LPS) administration enhances the sensitivity to a selective B_1 agonist in a non-human primate model, namely green monkeys. Acute regulation of blood pressure and oedema induction by B_1 receptors were chosen as relevant endpoints in this model because of the potential of B_1 antagonists in the treatment of cardiovascular and inflammatory disorders.

Methods

The present studies were conducted on male green monkeys (*Cercopithaecus aethiops St Kitts*) at the Caribbean Primates Ltd. experimental farm (St Kitts, West Indies). Procedures were reviewed and accepted by the Animal Care Commit-

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tees of the CR-CHUM (Montreal, Canada) and of Caribbean Primates Ltd. (St Kitts, West Indies). Animals weighing 6.0 ± 0.5 kg ($n=67$) were anaesthetized (50 mg ketamine kg^{-1}) and pretreated with a single intravenous injection of LPS (90 μg kg^{-1}) or saline (1 ml) *via* the saphenous vein.

Blood pressure studies

At 4 h after pre-treatment, monkeys were anaesthetized again as described above, for haemodynamic measurements. Pressure data were acquired using a pressure transducer (Harvard Apparatus) connected to a Macintosh PowerBook G3 running the BioPac data acquisition software. The left common carotid artery was canulated and the changes in mean blood pressure were monitored in response to intra-arterial bolus injections of vehicle (100 μl saline), dKD (1–10 000 pmol kg^{-1}) or BK (1–1000 pmol kg^{-1}). All injections (saline or peptide), which were given at 1–3 min intervals, were immediately followed by saline (300 μl) to ensure delivery of the bolus. In a subset of LPS-treated animals, the haemodynamic response to dKD (1 nmol kg^{-1}) and BK (0.1 nmol kg^{-1}) were measured during and 5 min after a continuous intravenous infusion of the B_1 selective antagonist [Leu^9]dKD (75 nmol kg^{-1} min^{-1} *via* the saphenous vein). In these animals, rectal temperature was measured immediately after induction of anaesthesia, i.e. before and at 4 h after LPS or saline injection.

Inflammation studies

Kinin-induced oedema was evaluated by the ventral skin fold assay (Sciberras *et al*., 1987) in a separate subset of anaesthetized monkeys injected with captopril (1 mg kg^{-1} 30 min before assay). A single subcutaneous injection of dKD, BK or the vehicle (2 mM amastatin in 100 μl Ringer's lactate) was given in the ventral area and the increase in thickness of skin folds was monitored for 30–45 min using a calibrated caliper. The results were expressed as the difference between the skin fold thickness before and after the subcutaneous injection. Captopril and amastatin were used to reduce degradation of kinins at the carboxyl- and amino-terminus, respectively. The time course after LPS for oedema induction by dKD (50 nmol) was determined at 4, 24 and 48 h, 12 and 56 days after LPS administration. BK (100 nmol) was used as a positive control. In following experiments, the dose-response relationship for dKD (1–150 nmol)-induced oedema was determined at 24 h post-LPS. A dose-response curve was obtained in each monkey studied by injecting peptides at different sites in the ventral area. To document inhibition of oedema formation by a B_1 receptor antagonist, [Leu^9]dKD (150 nmol) was co-injected or not with dKD (50 nmol) at 24 h post-LPS. Rectal temperature was measured in anaesthetized animals before and up to 56 days after LPS injection. Animals were used only once throughout these studies.

Drugs

Ketamine hydrochloride, LPS, amastatin and captopril were from Sigma (MO, U.S.A.). All peptides were from Phoenix Pharmaceuticals (CA, U.S.A.).

Statistics

Values are presented as mean $\pm$ standard error of the mean (s.e.mean). In oedema studies, the pre-injection thickness of the skin folds was subtracted from the values after subcutaneous challenge. Curve fitting and EC_{50} calculations were obtained using the Delta Graph 4.0 software for Apple Computers. Data were compared by two-way analysis of variance followed by unpaired, one tail Student's *t*-test with Bonferroni correction. $P<0.05$ was considered statistically significant.

Results

LPS injection induced a significant increase in rectal temperature after 4 h (38.5 ± 0.2°C; $n=15$) as compared to pre-LPS values (37.7 ± 0.2°C; $P<0.05$) or values in saline-treated monkeys (data not shown). Rectal temperature remained elevated at 24 h (38.4 ± 0.3°C; $n=6$) but not 48 h after LPS (37.7 ± 0.2°C; $n=8$). At 4 h, LPS pretreatment did not affect mean blood pressure (112 ± 10 mmHg; $n=12$) as compared to saline-treated animals (112 ± 10 mmHg; $n=5$).

Blood pressure regulation by kinins

Administration of dKD at 4 h dose-dependently reduced blood pressure in monkeys pretreated with LPS (EC_{50}: 130 pmol kg^{-1}) but had a marginal effect in saline-pretreated animals (Figure 1a). In contrast, BK dose-dependently reduced blood pressure in monkeys pretreated with saline or LPS, with no difference between experimental groups (Figure 1b). There was no evidence that the maximal effect of BK was attained at the highest dose administered (1 nmol kg^{-1}). Figure 2 shows a blood pressure tracing obtained at 4 h in an LPS-treated animal. The maximal hypotensive response was attained at 21 ± 3 s in response to dKD (1 nmol kg^{-1}) and 17 ± 2 s for BK (1 nmol kg^{-1}, not significantly different from dKD). At these time points, dKD and BK decreased blood pressure and increased heart rate similarly (control values for heart rate: 157 ± 3 and 153 ± 4 b.p.m., respectively; Figure 3). However, these effects were longer lasting with dKD (>1 min) than BK ($\leqslant 1$ min) (Figure 3). Continuous intravenous infusion of the selective B_1 antagonist [Leu^9]dKD (75 nmol kg^{-1} min^{-1}) did not affect blood pressure (2 ± 3 mmHg increase at 1 min). However, [Leu^9]dKD infusion abolished the response to dKD (1 nmol kg^{-1}) but not BK (0.1 nmol kg^{-1}) (Figure 4). In contrast, these doses of dKD and BK were equipotent in reducing blood pressure at 5 min after the end of [Leu^9]dKD infusion in the same animals (Figure 4).

Pro-inflammatory effects of kinins

The mean thickness of ventral skin folds in naïve monkeys was 2.3 ± 0.2 mm ($n=50$). This parameter did not change significantly after LPS (data not shown). Administration of vehicle slightly increased skin fold thickness (by 0.5–1.0 mm), with no difference between experimental groups (Figure 5). In LPS- but not in saline-pretreated monkeys, dKD (50 nmol) induced a time-related increase in skin fold

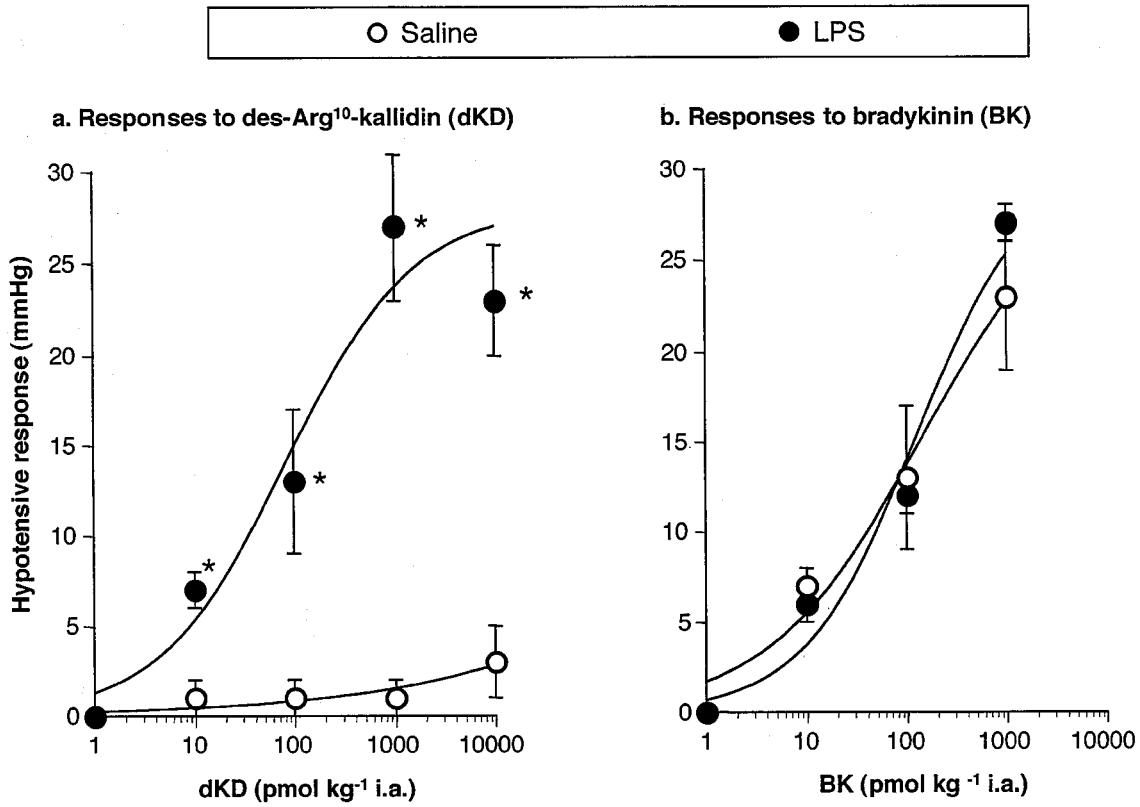

Figure 1 Dose-dependent decrease in mean arterial blood pressure in response to intra-arterial injections of (a) dKD or (b) BK in green monkeys anaesthetized 4 h after intravenous injection of LPS (90 μg kg^{-1}) or saline vehicle. *Significantly different ($P<0.05$) from response in saline-pretreated monkeys ($n=5-6$ per group).

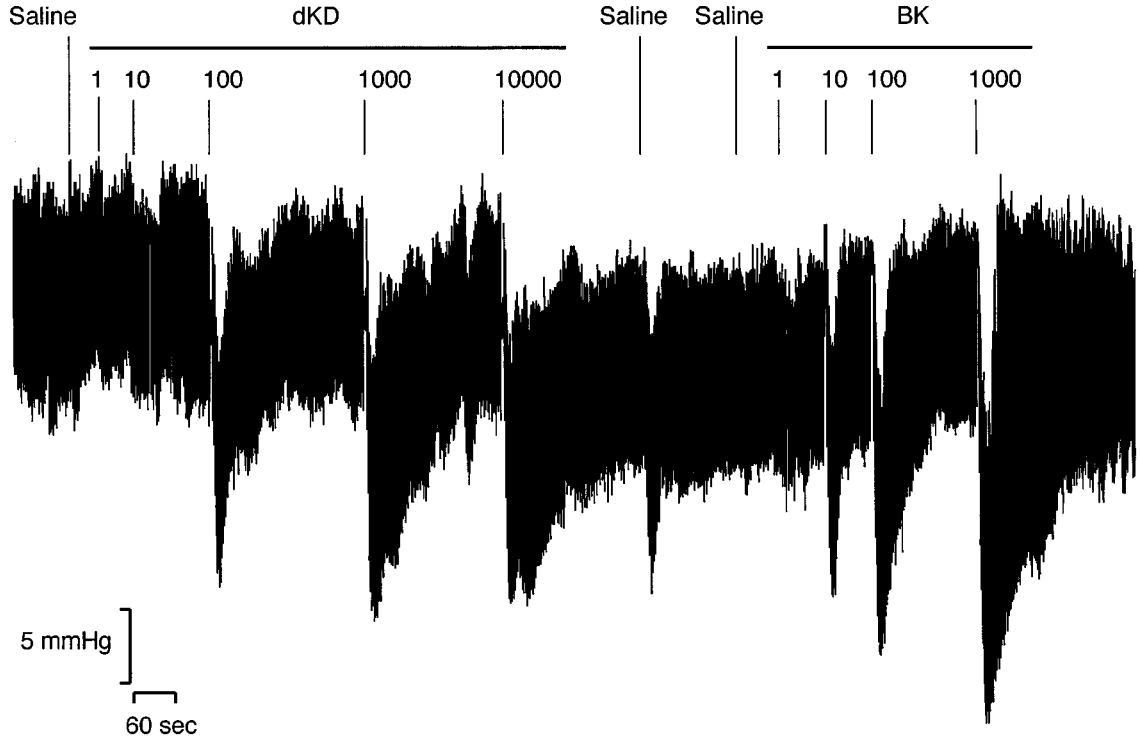

Figure 2 Representative tracing of blood pressure changes in response to intra-arterial bolus injection of saline vehicle (100 μl), des-Arg^{10}-kallidin (dKD) or bradykinin (BK) in anaesthetized green monkeys. Animals were anaesthetized 4 h after intravenous injection of LPS (90 μg kg^{-1}).

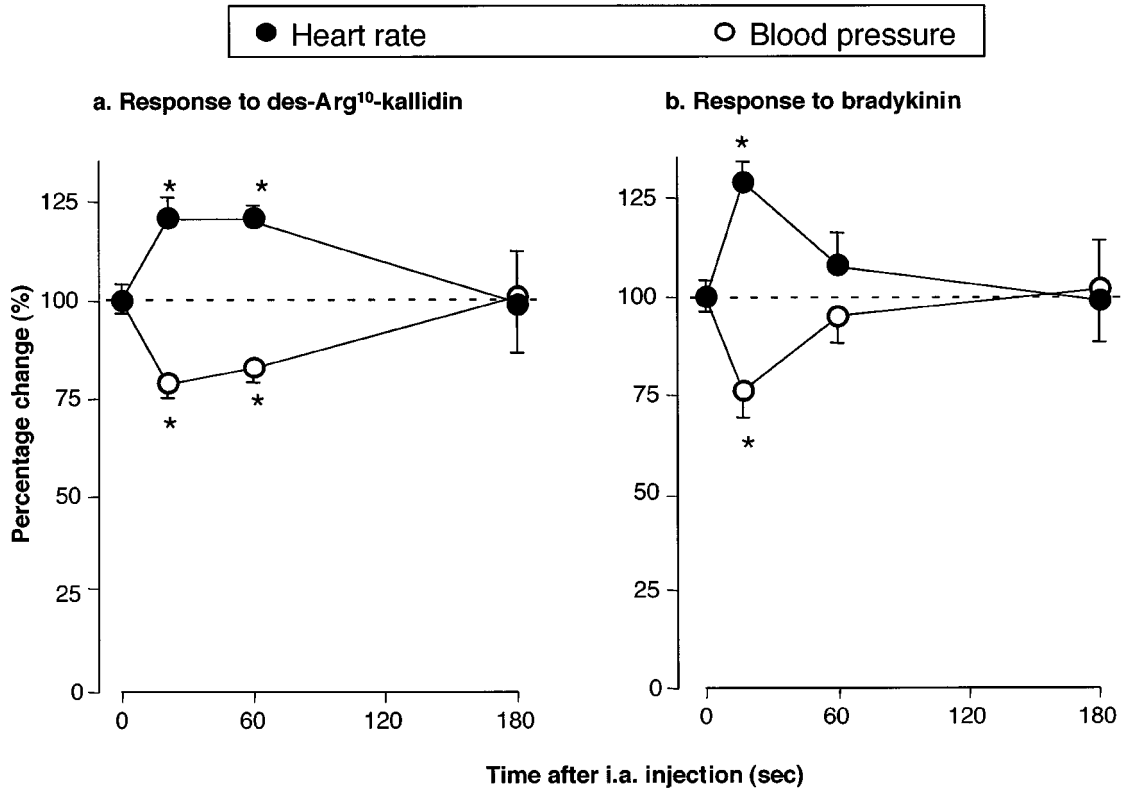

Figure 3 Time course for the decrease in mean arterial blood pressure and increase in heart rate in response to intra-arterial injection of (a) dKD (1 μmol kg^{-1}) or (b) BK (1 μmol kg^{-1}) in green monkeys. Heart rate before dKD or BK injection was 157 ± 3 and 153 ± 4 b.p.m., respectively. Animals were anaesthetized 4 h after intravenous injection of LPS (90 μg kg^{-1}). * Significantly different ($P < 0.05$) from pre-injection values ($n = 6$ per group).

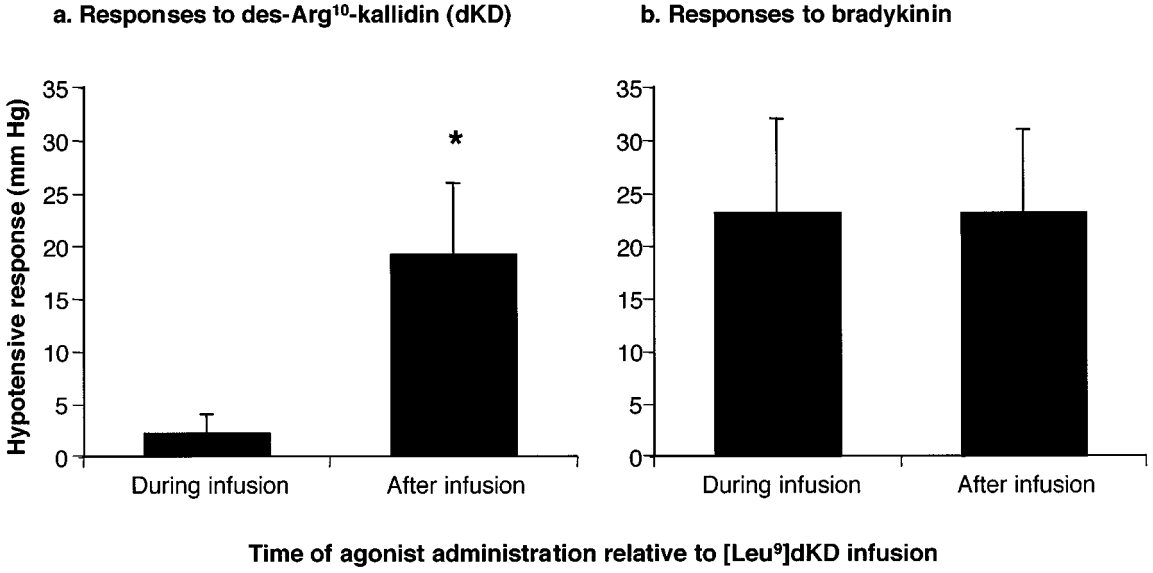

Figure 4 Decrease in mean arterial blood pressure in response to intra-arterial injection of (a) dKD (1 nmol kg^{-1}) or (b) BK (100 pmol kg^{-1}) during intravenous infusion of [Leu^9]dKD (75 nmol kg^{-1} min^{-1}) or 5 min after the end of [Leu^9]dKD infusion in green monkeys. Animals were anaesthetized 4 h after intravenous injection of LPS (90 μg kg^{-1}). * Significantly different ($P < 0.05$) from response obtained during [Leu^9]dKD infusion ($n = 6$ per group).

thickness with a maximum at 10–20 min (Figure 5). Responses to dKD were weaker at 4 h as compared to 24 h. Similar dKD responses were observed at 24 and 48 h and 12 days post-LPS (data at 48 h not shown), suggesting that maximal induction of B_1 sensitivity was reached within 24 h post-LPS. In contrast, BK (100 nmol) induced comparable oedema in all experimental groups including saline-pretreated animals. At 56 days post-LPS,

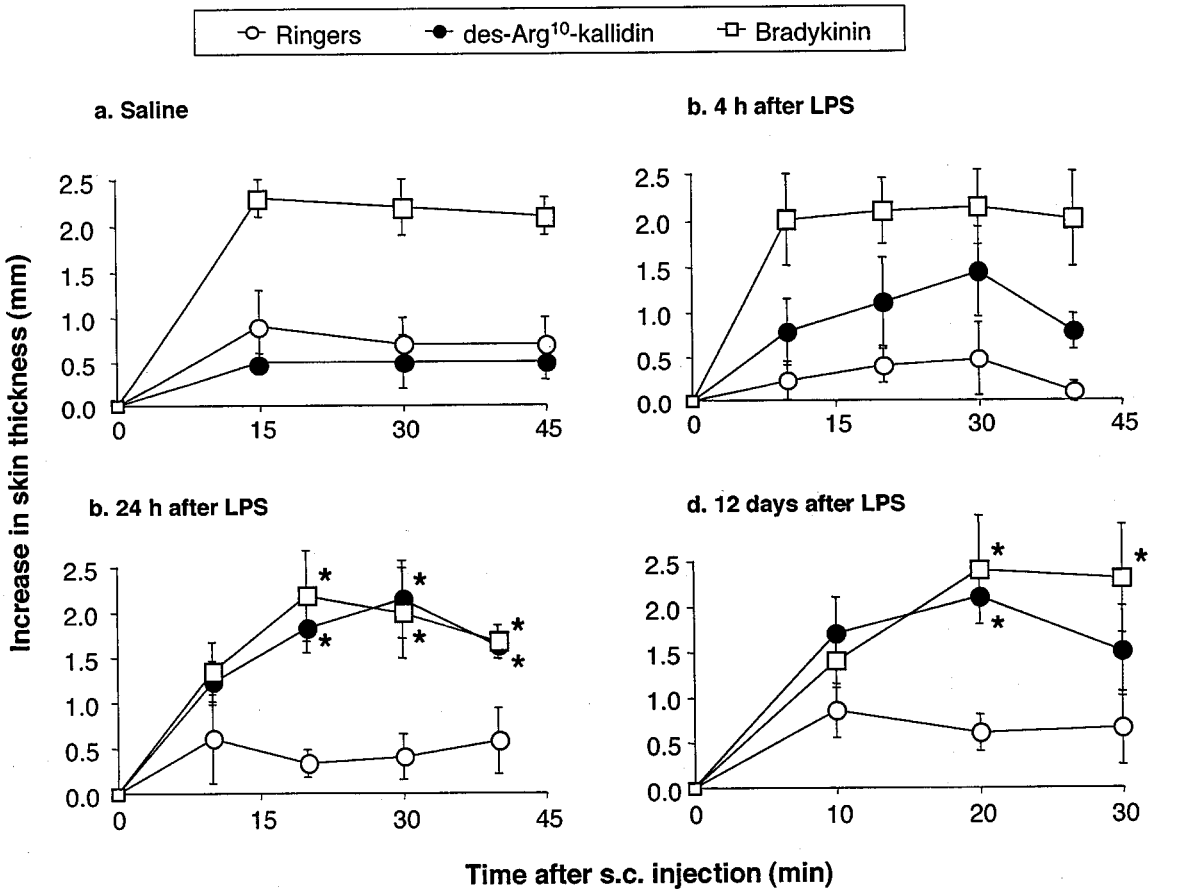

Figure 5 Oedema development in response to sub-cutaneous injections of Ringer's lactate vehicle (100 μl), dKD (75 nmol) or BK (100 nmol) in ventral skin of anaesthetized green monkeys at (a) 24 h following pre-treatment with saline (1 ml; $n=7$), or at (b) 4 h ($n=3$) (c) 24 h ($n=6$) and (d) 12 days ($n=8$) following pre-treatment with LPS (90 μg kg^{-1}). * Significantly different ($P<0.05$) from response induced by vehicle.

oedema formation was observed in response to BK (2.1 ± 0.6 mm within 20 min post-challenge) but not dKD (0.9 ± 0.5 mm) as compared to vehicle (0.8 ± 0.6 mm; $n=8$). At 24 h post-LPS, maximal oedema in response to dKD (within 20 min post-challenge) was dose-dependent (Figure 6) and the effect induced by dKD (50 nmol) was significantly reduced by co-injection of [Leu^9]dKD (150 nmol) (Figure 7).

Discussion

A growing body of evidence suggests a role for kinin B_1 receptors in immunopathology, particularly in promoting chronic inflammatory disorders (Marceau *et al.*, 1998). In contrast with kinin B_2 receptors, current evidence suggests that B_1 receptor-mediated cardiovascular or inflammatory responses are normally low or absent in humans, rabbits and rats (Ahluwalia & Perretti, 1999; Marceau *et al.*, 1998; Prat *et al.*, 1999). To date, constitutive B_1 receptor expression has been documented in vascular tissues of dogs (Belichard *et al.*, 1996; Staszewska-Woolley & Woodman, 1991), the central nervous system of spontaneously hypertensive rats (Emanueli *et al.*, 1999), the hindlimb and pulmonary vasculature in cats (Santiago *et al.*, 1995; Seyedi *et al.*, 1997) and human ocular tissues (Ma *et al.*, 1996) and T

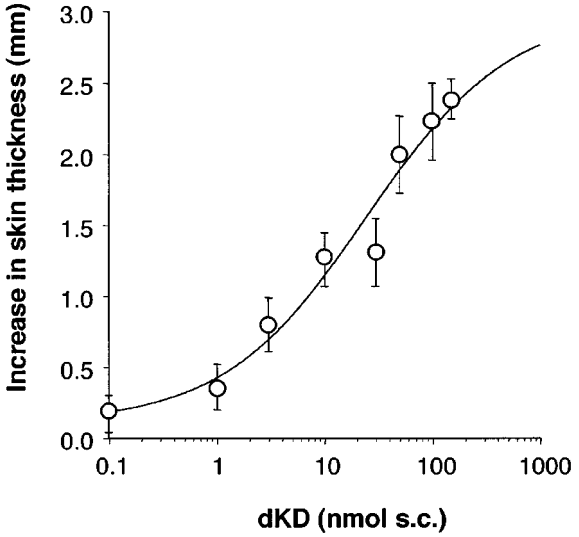

Figure 6 Dose-response relationship for maximal oedema development in response to subcutaneous injection of dKD (1–150 nmol) in ventral skin of green monkeys anaesthetized 4 h after pre-treatment with LPS (90 μg kg^{-1}; $n=7$).

lymphocytes (Prat *et al.*, 1999). Factors implicated in B_1 receptor induction include bacterial endotoxin, pro-inflam-

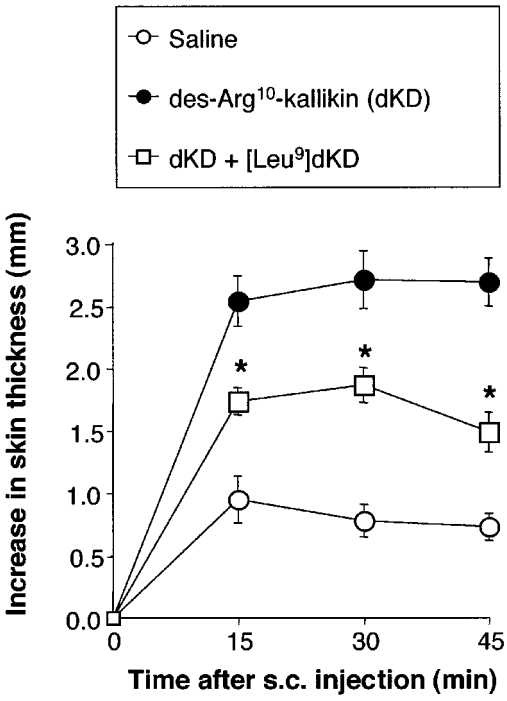

Figure 7 Inhibition by [Leu^9]dKD (150 nmol) of oedema induced by subcutaneous injection of dKD (50 nmol) in green monkeys. [Leu^9]dKD was co-injected with dKD. * Significantly different ($P<0.05$) from response without [Leu^9]dKD ($n=7$).

matory cytokines (e.g., interleukin-1) as well as intracellular pathways regulating the cell response to environmental stress (e.g., the p38 MAP kinase and NF-κB pathways) (Bastian *et al*., 1998; Larrivee *et al*., 1998; Marceau *et al*., 1998; Phagoo *et al*., 1999; Prat *et al*., 1999; Sardi *et al*., 1998; Schanstra *et al*., 1998; Tsukagoshi *et al*., 1999). In turn, B_1 receptor stimulation may activate further these same pathways (Campos *et al*., 1999; Naraba *et al*., 1998; Schanstra *et al*., 1998; Tsukagoshi *et al*., 1999). B_1 receptors may also interact with cytokines to promote further B_1 receptor up-regulation (Phagoo *et al*., 1999). The role of B_1 receptors during haemodynamic disorders associated with septicaemia is still unclear (Siebeck *et al*., 1996). However, studies in rodent models of inflammation have provided fairly convincing evidence implicating B_1 receptors as mediators of chronic oedema and hyperalgesia in the skin and joints (Ahluwalia & Perretti, 1999). By virtue of its selective induction at sites of inflammation, the B_1 receptor is considered a potential therapeutic target for the control of tissue response to immune activation or mechanical injury, with minimal secondary effects in non-affected tissues. To date, however, there is no primate model of *in vivo* B_1 receptor sensitization. Moreover, it remains unclear whether B_1 agonists are pro-inflammatory in these species, although the enhanced B_1 receptor-mediated migration shown by T lymphocytes from patients with active multiple sclerosis (Prat *et al*., 1999) support this hypothesis.

Functional studies of human B_1 receptor sensitization in the cardiovascular system come in large part from *in vitro* studies with cultured cells or incubated smooth muscle preparations (Marceau *et al*., 1998). To our knowledge, the present study in green monkeys provides the first evidence that endotoxin up-regulates B_1 receptor-mediated responses in primates *in vivo*, a condition associated with increased haemodynamic and pro-inflammatory responses to a selective B_1 agonist. This interpretation is based on three lines of observations. (1) dKD induced significant, dose-dependent haemodynamic or inflammatory responses only in LPS-pretreated monkeys. (2) The increased sensitivity to kinins was specific for dKD (responses to BK were significant in control monkeys and unaffected after LPS). (3) The selective B_1 antagonist dLKD inhibited the responses to dKD. Although supportive molecular evidence has not been obtained in monkeys, the present functional evidence is compatible with the notion that LPS increased tissue expression of the B_1 receptor gene in sensitized tissues, as shown in rats and rabbits (Marceau *et al*., 1999; Marin-Castano *et al*., 1998; McLean *et al*., 1999). Beside LPS, several other conditions known to up-regulate sensitivity to B_1 agonists are also associated with increased B_1 receptor mRNA levels (Bastian *et al*., 1998; Belichard *et al*., 1999; Phagoo *et al*., 1999; Prat *et al*., 1999; Tsukagoshi *et al*., 1999). Notably, increased B_1 gene expression and biological activity were recently observed in a model of inflammatory hyperalgesia induced by zymosan injection in the plantar tissue of the rat (Belichard *et al*., 2000). Based on current data (Davis *et al*., 1996; Marceau *et al*., 1998; McLean *et al*., 2000), candidate cell types expressing functional B_1 receptors in LPS-treated monkeys include endothelial cells, vascular smooth muscle cells, fibroblasts, mast cells and peripheral sensory neurons.

The haemodynamics effects of B_1 receptor agonists are complex and may involve the modulation of cardiac contractility and vasodilatation following the release of prostaglandins or nitric oxide depending on the species and vascular bed. In conscious instrumented dogs under ganglionic blockade, infusion of des-Arg^9-BK or BK reduces mean arterial pressure and coronary vascular resistance without changing cardiac contractility (Belichard *et al*., 1996). In contrast, both cardiac contractility and arterial pressure are reduced (even under ganglionic blockade) in response to B_1 receptor stimulation in rabbits anaesthetized following LPS pretreatment (Audet *et al*., 1997). In anaesthetized monkeys however, kinin-induced tachycardia was evident, an effect that was significantly more sustained with the B_1 agonist. These differences may depend on the species or experimental conditions such as the type of anaesthesia used. Possible mechanisms for kinin-induced tachycardia in monkeys include reflex sympathetic activation in response to the fall in blood pressure, and pre-synaptic stimulation of catecholamine release as shown in rats, guinea-pigs and cats following stimulation with a B_2 agonist (Kurz *et al*., 1997; Loro *et al*., 1998; Seyedi *et al*., 1997) or a B_1 agonist (DeWitt *et al*., 1994). Vascular B_1 receptors coupled to nitric oxide release mediate relaxation in bovine (Drummond & Cocks, 1995), porcine (Pruneau *et al*., 1996) and human (Su *et al*., 2000) coronary arteries, rabbit carotid arteries (Pruneau & Belichard, 1993) and cat pulmonary arteries (DeWitt *et al*., 1994). Alternatively, B_1 receptors coupled to prostaglandin release mediate vascular relaxation in rabbit mesenteric arteries (deBlois & Marceau, 1987), rabbit coeliac arteries (Ritter *et al*., 1989), dog mesenteric veins (Toda *et al*., 1987) and rat coronary arteries (McLean *et al*., 1999). Proposed mechanisms for kinin-induced vasorelaxation also include the

release of endothelial hyperpolarizing factor(s) (Mombouli *et al*., 1992). In dogs and LPS-treated rabbits, inhibition of prostaglandin or nitric oxide production does not elicit a marked reduction in maximal hypotensive response to B_1 receptor stimulation (Audet *et al*., 1997; Belichard *et al*., 1996). The duration of the hypotension is reduced, however, by cyclo-oxygenase inhibition in rabbits (Audet *et al*., 1994; Drapeau *et al*., 1991), suggesting a significant haemodynamic role for prostaglandins. Based on these data, it is difficult to speculate on the possible role of the cyclo-oxygenase and nitric oxide pathways in B_1 receptor-mediated hypotensive responses in monkeys, although a role for reduced cardiac output can be ruled out.

The present results are consistent with observations made in rat models of oedema development (Campos *et al*., 1996; 1997; de Campos *et al*., 1998). At 24 h following systemic administration of LPS in rats, intra-plantar administration of des-Arg^9-BK promotes paw oedema (EC_{50}: 42 nmol), an effect blocked by co-injection of des-Arg^9[Leu^8]-BK (IC_{50}: 134 nmol) (Campos *et al*., 1996). Injection of rats with *Mycobacterium bovis bacillus Calmette-Guerin* results in long-term sensitization to B_1 agonists in the paw oedema assay (for up to 75 days) (Campos *et al*., 1997). Although no live bacteria were used in monkeys, B_1 sensitization in the skin was sustained for up to 12 days (but less than 56 days) following LPS. In contrast, the pyrogenic response to LPS lasted less than 2 days. Together, these data suggest a slow rate of receptor turnover *in vivo*, a feature consistent with known characteristics of B_1 receptors which include lack of ligand-induced receptor desensitization and sequestration (Faussner *et al*., 1999). A role for prostaglandins in the present model is likely. Pro-inflammatory prostaglandins were implicated in mediating B_1 receptor-induced oedema in rats (Campos & Calixto, 1995; Campos *et al*., 1996). Moreover, several studies showed B_1 receptor activation of the cyclo-oxygenase pathway in vascular cells or fibroblasts (Lerner & Modeer, 1991; Marceau *et al*., 1998). New, potent, peptide and non-peptide antagonists of B_1 receptors are being developed, with inflammation-associated hyperalgesia as a primary target condition (Altamura *et al*., 1999; Galoppini *et al*., 1999; Gobeil *et al*., 1999; Horlick *et al*., 1999). The observation that dKD mediates oedema in green monkeys supports the assumption that B_1 receptor antagonists may be effective anti-inflammatory tools in humans. In non-human primates, B_1 receptor-induced oedema may offer a simple and ethical surrogate assay for B_1-mediated hyperalgesia.

Several studies provided evidence that the structure-relationship of B_1 antagonists is species-dependent (MacNeil *et al*., 1997; Nsa Allogho *et al*., 1998; Wohlfart *et al*., 1997). Notably, peptide B_1 antagonists such as [Leu^8]-des-Arg^9-BK act as partial agonists on the murine B_1 receptor (MacNeil *et al*., 1997; Nsa Allogho *et al*., 1998). In hexamethonium-treated dogs, administration of [Leu^8]des-Arg^9-BK (25 μg kg^{-1}) elicits a significant fall in blood pressure (Belichard *et al*., 1996). In LPS-treated monkeys, however, we observed no blood pressure change with infusion of a larger dose of the extended peptide antagonist [Leu^9]dKD, as previously reported in rabbits (Marceau *et al*., 1998). In the skin fold assay, dKD-induced oedema was partially inhibited by [Leu^9]dKD (150 nmol), i.e. at a dose comparable to the IC_{50} of des-Arg^9[Leu^8]-BK (134 nmol) in suppressing des-Arg^9-BK-induced oedema in LPS-treated rats (Campos *et al*., 1996). Collectively, these data suggest that the partial inhibition of oedema observed with [Leu^9]dKD in monkeys was due to incomplete blockade of B_1 receptors rather than partial agonist activity of [Leu^9]dKD.

In summary, LPS administration to green monkeys increased from a null level their sensitivity to a B_1 receptor agonist in a blood pressure regulation assay and an oedema formation assay. Comparatively, responses to the B_2 receptor agonist BK were not affected. A selective B_1 receptor antagonist inhibited the effects of dKD. Taken together with the previous results showing an association between increased B_1 receptor mRNAs and biological activity, the present data suggest that LPS up-regulates B_1 receptor expression in green monkeys. This non-human primate model may be suitable for testing new, selective B_1 antagonists with therapeutic potential as anti-inflammatory agents in a species with a high degree of genetic homology with humans.

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