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Induction of bradykinin B_1 receptors *in vivo* in a model of ultra-violet irradiation-induced thermal hyperalgesia in the rat

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1 The role of bradykinin B_1 receptors in the thermal hyperalgesia following unilateral ultra-violet (u.v.) irradiation of the hindpaw of rats has been investigated.

2 In non-irradiated (naive) animals the B_1 receptor agonist des-Arg^9-bradykinin and bradykinin (BK) (up to 1 μmol kg^{-1} i.v.) had no effect on withdrawal latency to a noxious heat stimulus when administered 60 min before testing.

3 Following exposure of one hindpaw to strong u.v. irradiation the withdrawal latency of the u.v.-treated paw to radiant noxious heat fell by a maximum of 50% after 48 h. There was no reduction in latency in the contralateral paw.

4 des-Arg^9-BK (1-100 nmol kg^{-1} i.v.) administered 24 h after u.v. exposure caused a further dose-dependent fall (50 $\pm$ 4% reduction from saline injected animals at 100 nmol kg^{-1} i.v.) in withdrawal latency in the u.v.-treated paw when measured 60 min after injection. The withdrawal latency of the contralateral paw was also reduced but to a lesser extent following des-Arg^9-BK (100 nmol kg^{-1} i.v.) with a maximum reduction of 19 $\pm$ 3%.

5 Bradykinin also induced a further reduction in withdrawal latency (33 $\pm$ 5% reduction at 1 μmol kg^{-1}) although it was not as effective as des-Arg^9-BK. Bradykinin did not reduce the withdrawal latency in the contralateral paw.

6 The hyperalgesic action of both des-Arg^9-BK (10 nmol kg^{-1} i.v.) and bradykinin (100 nmol kg^{-1} i.v.) were antagonized by the B_1 receptor antagonist, des-Arg^9,Leu^8-BK (200 nmol kg^{-1} i.v.) but not by the B_2 receptor antagonist, HOE 140 (0.5 μmol kg^{-1} i.v.).

7 The results suggest that in conditions of inflammatory hyperalgesia bradykinin B_1 receptors are induced both locally and distant to the inflamed area, activation of which leads to further thermal hyperalgesia. In addition, in these conditions bradykinin appears to act predominantly via B_1 receptors, presumably after degradation to des-Arg^9-BK.

Keywords: Bradykinin; B_1 receptors; inflammation; nociception

Introduction

The acute activation of sensory neurones by kinins in normal animals has been shown to be mediated through the bradykinin B_2 receptor (Dray *et al.*, 1988; Steranka *et al.*, 1989; Rang *et al.*, 1991).

Recently we have shown that in two persistent inflammatory models in rats the accompanying thermal and mechanical hyperalgesia is reversed by the bradykinin (BK) B_1 receptor antagonist des-Arg^9,Leu^8-BK (Perkins *et al.*, 1992; 1993). In contrast, the potent B_2 antagonist HOE 140 (D-Arg[Hyp^3, Thi^5, D-Tic^7, Oic^8]BK) (Hock *et al.*, 1991) was only weakly active in these models (Perkins *et al.*, 1992; 1993) suggesting a different pharmacology for bradykinin under these pathophysiological conditions. In support of this was the finding that des-Arg^{9}BK could induce a small exacerbation of the hyperalgesia particularly in the model of ultra-violet (u.v.) irradiation-induced thermal hyperalgesia (Perkins *et al.*, 1992; 1993). This study was the first demonstration *in vivo* that in inflammatory hyperalgesia there was the induction of a bradykinin B_1 receptor and further suggested that the accompanying kinin contribution to the maintenance of this hyperalgesia was predominantly via the activation of B_1 and not B_2 receptors.

That study, however, relied mainly on the use of bradykinin B_1 and B_2 antagonists rather than agonists. We have extended that previous study in order to establish the pharmacology of the hyperalgesic actions of des-Arg^9-BK and bradykinin as well as the time course of the induction of the B_1 receptor following u.v. irradiation. In addition, in view of recent findings suggesting that there can be expression of bradykinin B_1 receptors systemically following a localised inflammatory insult (Farmer *et al.*, 1991), we have explored the possibility of induction of bradykinin B_1 receptors mediating hyperalgesia distant to the u.v.-treated area.

Methods

The thermal hyperalgesia induced by the u.v. irradiation protocol previously described (Perkins *et al.*, 1992; 1993) approached the maximum that could be obtained and was not, therefore suitable for studying the pharmacology of agonists which might be expected to produce further hyperalgesia.

Therefore, in these experiments rats (female Sprague Dawley 100 g) received one instead of two unilateral exposures to u.v.A light (90 s, intensity maximum 365 nm, 69 mwatt cm^{-2}). On each day for the next four days, different groups of u.v.-treated rats (typically four) were placed in a transparent perspex box with a thin glass floor. The time taken to withdraw each hind paw following a heat stimulus, applied to the underside of the paw was measured. The heat stimulus was a focused radiant heat beam provided by a light bulb. The hind paws were exposed to the heat stimulus in a random manner so that there was no consistent order of testing between ipsilateral and contralateral paws and no rat had both paws exposed to heat immediately after each other. The withdrawal latencies of both paws were then measured at various times after the u.v. exposure. Drugs were administered intravenously, typically 60 min prior to

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measurement of withdrawal latency and antagonists were co-administered with agonists.

All drugs except the B_1 and B_2 agonists and antagonists were obtained from Sigma. Bradykinin was synthesized at Sandoz, Basel. des-Arg^9-BK and des-Arg^9,Leu^8-BK were obtained from Bachem A.G. (D-Arg[Hyp^3, Thi^5, D-Tic^7, Oic^8]BK) (HOE 140) was synthesized by Dr A. Hallett, The Sandoz Institute for Medical Research, London.

Statistical analysis

Results are expressed as mean latency (s) ± s.e.mean in the case of the determination of time courses. Drugs effects were expressed as the % reduction in withdrawal latency following drug administration, compared with the pre-drug measurement in the same animal. For the time course measurement data were analysed by Dunnett's test for multiple comparison, otherwise one way analysis of variance followed by paired Student's *t* test (for comparison of post- and pre-drug treatment values) or unpaired Student's *t* test (for comparison between drug and vehicle treated groups).

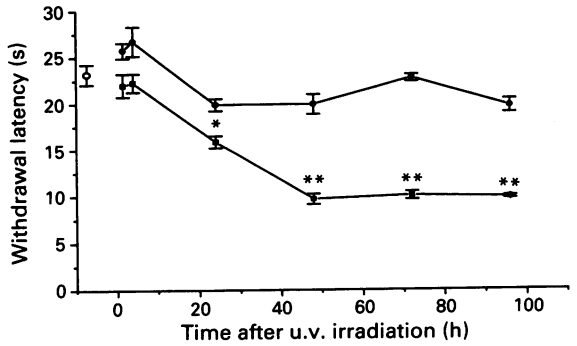

Figure 1 Time course (h) of foot withdrawal latency (s, means ± s.e. mean, $n = 4$) to a thermal stimulus in the ipsilateral (solid squares) and contralateral (solid circles) hindpaws of rats following u.v. irradiation of the ipsilateral paw. The open circle shows the withdrawal latency of naive rats. There was a significant reduction in paw withdrawal latency by 24 h, which persisted throughout the period of measurement to 96 h. There was no hyperalgesia in the contralateral paw. $^{**}P<0.01$ and $^{*}P<0.05$ denote significant differences compared with naive animals.

Results

Naive animals

In non-u.v. treated (naive) rats neither des-Arg^9-BK nor bradykinin had any effect when injected 60 min before testing at up to 1 μmol kg^{-1} i.v. Additionally, as has been shown previously, neither HOE 140 nor des-Arg^9,Leu^8-BK had any effect on withdrawal latency when administered to naive animals (data not shown and Perkins *et al.*, 1992).

Time course of hyperalgesia

On the day after the u.v. exposure there was reddening of the treated hind paw but no evidence of skin damage. Mild inflammation was evident and was characterized by erythema but there was no evidence of swelling of the digits or foot pad. There was some mild blistering of the glabrous skin without signs of weeping or abrasions, the blistering lasting for 2–3 days. Animals appeared to groom normally and did not exhibit any obvious signs of distress while being observed or when being handled. There was no weight loss at any stage following u.v. treatment.

Four hours after u.v. treatment the withdrawal latency of the ipsilateral paw remained unchanged from that of naive animals, but at 24 h, the latency fell significantly from 22 ± 1 s (mean ± s.e.mean, $n = 4$) to 15.9 ± 0.7 s ($n = 8$, see Figure 1). There was then a further fall in latency to 10.8 ± 0.7 s ($n = 4$) by 48 h after which the latency remained fairly constant up to 96 h (see Figure 1). In the contra-lateral paw there was no significant reduction in latency at any time point.

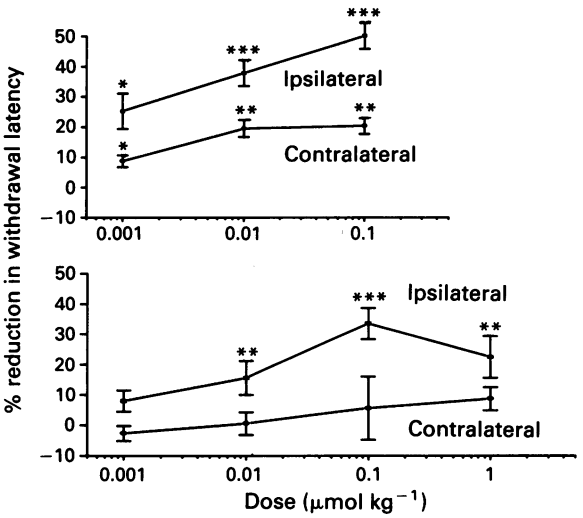

Figure 2 Withdrawal latencies (% reduction from vehicle controls, mean ± s.e.mean, $n = 4$) for both the ipsilateral (u.v.-treated paw, solid squares) and contralateral (solid circles) hindpaws of rats following intravenous injections (μmol kg^{-1}) of des-Arg^9-bradykinin (BK) (a) and bradykinin (b). There was a significant reduction in withdrawal latency in both paws after all doses of des-Arg^9-BK (a). Bradykinin (b) was less effective in reducing latency and only in the ipsilateral paw. $^{***}P<0.001$, $^{**}P<0.01$ and $^{*}P<0.05$ denote significant differences compared with vehicle injected animals.

Effect of bradykinin receptor agonists and antagonists

Ipsilateral paw 24 h after u.v.-irradiation, des-Arg^9-BK, injected 60 min before testing, caused a dose-dependent reduction in withdrawal latency with a 25 ± 6% ($P<0.05$, $n = 4$) reduction in withdrawal latency at 1 nmol kg^{-1} i.v. rising to 50 ± 4% at 100 nmol kg^{-1} i.v. (see Figure 2). Bradykinin also produced a reduction in latency although it was less efficacious than des-Arg^9-BK producing a 33 ± 5% ($P<0.001$, $n = 4$) reduction at 100 nmol kg^{-1} with no further reduction at 1 μmol kg^{-1} i.v. (see Figure 2).

Co-administration of the B_1 antagonist, des-Arg^9,Leu^8-BK (200 nmol kg^{-1} i.v.), antagonized the hyperalgesic action of both des-Arg^9-BK (10 nmol kg^{-1} i.v.) and bradykinin (100 nmol kg^{-1} i.v.) (see Figure 3). Co-administration of the B_2 antagonist, HOE 140 (0.5 μmol kg^{-1} i.v.) neither prevented the hyperalgesic action of des-Arg^9-BK nor bradykinin in the ipsilateral paw (see Figure 3).

Contralateral paw Bradykinin had no significant effect on the withdrawal latency of the contralateral paw at doses from 1 nmol kg^{-1} i.v. to 1 μmol kg^{-1} i.v. In contrast, des-Arg^9-BK produced a small but significant reduction in withdrawal latency of 9 ± 2% ($P<0.05$, $n = 4$) in the contra-lateral paw at 1 nmol kg^{-1} i.v. increasing to a reduction of 19.5 ± 3% ($P<0.01$, $n = 4$) at 10 nmol kg^{-1} i.v. with no further increase at 100 nmol kg^{-1} i.v. (see Figure 2).

This hyperalgesic action of des-Arg^9-BK was antagonized by des-Arg^9,Leu^8-BK (200 nmol kg^{-1} i.v., $n = 4$) but not by HOE 140 (0.5 μmol kg^{-1} i.v.) (see Figure 3).

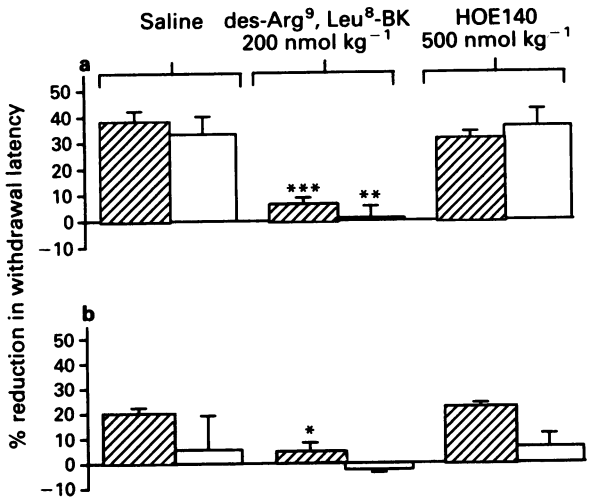

Figure 3 Percentage reduction in withdrawal latency (mean ± s.e. mean, $n = 4$) for the ipsilateral (a) and contralateral (b) hindpaws following administration of des-Arg^9-bradykinin (10 nmol kg^{-1} i.v., shaded columns) and bradykinin (BK, 100 nmol kg^{-1} i.v., open columns). The B_1 receptor antagonist, des-Arg^9,Leu^8-BK co-administered (200 nmol kg^{-1} i.v.) with the agonist significantly reduced the response to both des-Arg^9-BK and bradykinin. The B_2 antagonist, HOE 140 (500 nmol kg^{-1} i.v.) co-administered in the same way did not reduce the response to either agonist. $***P<0.001$, $**P<0.01$ and $*P<0.05$ denote significant differences compared with respective saline injected animals.

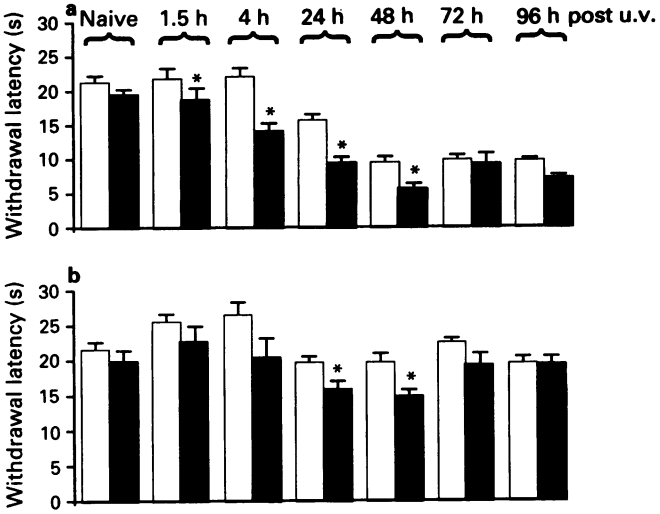

Figure 4 Withdrawal latencies (s, mean ± s.e.mean, $n = 4$) of ipsilateral (a) and contralateral (b) hindpaws of rats showing the time course of induction of des-Arg^9-bradykinin (BK)-induced hyperalgesia. In both (a) and (b) the open columns represent the withdrawal latency preceding administration of des-Arg^9-BK (10 nmol kg^{-1} i.v.) and the black columns the withdrawal latency 60 min after des-Arg^9-BK injection. In naive animals des-Arg^9-BK had no effect. Following u.v. exposure to the ipsilateral paw there was a reduction in withdrawal latency signifying hyperalgesia only in the ipsilateral paw. des-Arg^9-BK was able to produce a further reduction in withdrawal latency 1.5–4 h after u.v. exposure in the ipsilateral paw (a) and 24 h later in the contralateral paw (b). $*P<0.05$ denote significant differences compared with respective saline injected animals.

Time course of induction of B_1 response

The time course of the induction of the response to des-Arg^9-BK following u.v. irradition was determined for both the ipsi- and contra-lateral paws; 1.5 h after u.v. exposure des-Arg^9-BK (10 nmol kg^{-1} i.v.) caused a small but significant reduction in withdrawal latency from 22 ± 1 s to 19 ± 1 s ($P<0.05$, $n = 4$) in the ipsi-lateral paw (see Figure 4). By 4 h there was a much more marked hyperalgesic effect of des-Arg^9-BK the withdrawal latency falling to 14 ± 0.8 s ($n = 4$) (see Figure 4). The hyperalgesic action of des-Arg^9-BK was clearly present for up to 48 h post-u.v. and then became erratic in that there was no significant effect at 72 h but a small, though significant, reduction in withdrawal latency at 96 h post u.v. exposure (see Figure 4).

In the contralateral paw des-Arg^9-BK produced significant hyperalgesia (20-22% reduction in latency) only at the 24 and 48 h points (Figure 4).

Discussion

The data presented here show that following an inflammatory insult des-Arg^9-BK is able to induce thermal hyperalgesia both in the inflamed area and in the contralateral paw. A previous study, with B_1 and B_2 receptor antagonists, had provided evidence that B_1 receptor-mediated effects were a predominant factor in the hyperalgesia observed following u.v.-irradiation (Perkins *et al.*, 1993).

The reduction of des-Arg^9-BK-induced hyperalgesia by a B_1, but not a B_2, receptor antagonist suggests that this action of des-Arg^9-BK is due to the induction of B_1 receptors which are not normally present, as des-Arg^9-BK is inactive in naive animals. In addition, it appears that the action of bradykinin in inducing thermal hyperalgesia is also via a B_1 receptor. Bradykinin is rapidly broken down after systemic administration with over 90% being degraded to des-Arg^9-BK and other metabolites after a single passage through the lungs (see Regoli & Barabe, 1980). It is likely, therefore, that the bradykinin-induced hyperalgesia was due to the production of des-Arg^9-BK.

The presence of contralateral hyperalgesia induced by des-Arg^9-BK may imply that u.v. irradiation of one paw leads to a systemic induction of B_1 receptors. This is consistent with a recent study by Farmer *et al.* (1991) showing that following antigen-induced arthritis in a knee joint of the rabbit there was induction of B_1 receptors on the aorta. In view of the evidence *in vitro* linking the induction of B_1 receptors with inflammatory mediators (Toda *et al.*, 1987; Bouthillier *et al.*, 1987; Cahill *et al.*, 1988; deBlois *et al.*, 1988; 1989; D'Orleans-Juste *et al.*, 1989; Drapeau *et al.*, 1991), one explanation for this contralateral hyperalgesia is that the circulating cytokines produced during inflammation are sufficient to induce the formation of the B_1 receptor.

The time course of the induction of the B_1 response is consistent with that observed in studies *in vitro* where responsiveness to des-Arg^9-BK increases over a 6 h period (Regolie & Barabe 1980; Bouthillier *et al.*, 1987; deBlois *et al.*, 1989). With respect to the contralateral side it is not possible to determine whether the apparent delay in induction of the des-Arg^9-BK-induced hyperalgesia is a real phenomenon or is a consequence of the smaller hyperalgesic response. In either case, if the B_1 receptor induction is dependent on inflammatory mediators such as cytokines then the reduced degree of des-Arg^9-BK-induced hyperalgesia in this paw may reflect lower levels of these mediators in areas distant to the u.v.-inflamed paw.

Previous studies, in which B_2 and B_1 agonists have been used with respect of activation of nociceptors, have all been performed in animals with no persistent inflammatory condition. BK but not des-Arg^9-BK has been shown to activate polymodal nociceptors in a number of circumstances. These range from direct recording *in vitro* of C-fibre nociceptor activity and bradykinin-evoked nociceptive reflexes (Griesbacher & Lembeck 1987; Lang *et al.*, 1990), to recording *in vivo* of bradykinin mediated excitation of nociceptors in skin, skeletal muscle, joints and visceral organs (Fock & Mense, 1976; Berkely *et al.*, 1988; 1990; Mizumura *et al.*, 1990). In

man it has been shown that bradykinin but not des-Arg^9-BK produces pain when applied to an acutely produced blister base and this pain was inhibited by a B_2 but not a B_1 antagonist (Whalley *et al.*, 1987).

It is not possible, from these experiments, to determine whether the B_1 receptor-mediated hyperalgesia is due to an induction of B_1 receptors on the nociceptive terminal itself with consequent direct activation by des-Arg^9-BK or whether there is an indirect action of des-Arg^9-BK via the release of sensitizing agents from other cell types. des-Arg^9-BK has been shown to increase the synthesis and release *in vitro* of inflammatory mediators such as prostacyclin (Toda *et al.*, 1987; Cahill *et al.*, 1988). and interleukin-1 (IL-1) (Tiffany & Burch 1989; Burch *et al.*, 1989). In the case of IL-1 release this has been shown to occur from macrophage cell lines (Tiffany & Burch 1989; Burch *et al.*, 1989), a cell type which would be expected to increase in numbers in inflamed areas. In addition, there may be further interactions between des-Arg^9-BK and inflammatory mediators such as the synergism that has been demonstrated between des-Arg^9-BK and IL-1 in increasing prostaglandin E_2 formation in human fibroblasts (Lerner & Modeer, 1991).

In summary, the data presented here provide further evidence that in persistent inflammatory conditions in the rat bradykinin B_1 receptors are expressed, activation of which produces significant hyperalgesia. This expression of B_1 receptors is not restricted to the area of inflammation occuring in the contralateral paw. Whether the B_1 receptor-induced hyperalgesia is a direct or indirect action on the nociceptive neurone remains to be elucidated.

References

BERKELY, K.J., HOTTA, H., ROBBINS, A. & SATO, Y. (1990). Functional properties of afferent fibres supply reproductive and other organs in pelvic area of female rat. *J. Neurophysiol.*, **63**, 256–272.

BERKELY, K.J., ROBBINS, A. & SATO, Y. (1988). Afferent fibres supplying the uterus in the rat. *J. Neurophysiol.*, **59**, 142–163.

BOUTHILLIER, J., DEBLOIS, D. & MARCEAU, F. (1987). Studies on the induction of pharmacological responses to des-Arg^9-bradykinin *in vitro* and *in vivo*. *Br. J. Pharmacol.*, **92**, 257–264.

BURCH, R.M., CONNOR, J.R. & TIFFANY, C.W. (1989). The kallikrein-kininogen-kinin system in chronic inflammation. *Agents Actions*, **27**, 258–260.

CAHILL, M., FISHMAN, J.B. & POLGAR, P. (1988). Effect of des arginine^9-bradykinin and other bradykinin fragments on the synthesis of prostacyclin and the binding of bradykinin by vascular cells in culture. *Agents Actions*, **24**, 224–231.

DEBLOIS, D., BOUTHILLIER, J. & MARCEAU, F. (1988). Effect of glucocorticoids, monomines and growth factors on the spontaneously developing responses of the rabbit isolated aorta to des-Arg^9-bradykinin. *Br. J. Pharmacol.*, **93**, 969–977.

DEBLOIS, D., BOUTHILLIER, J. & MARCEAU, F. (1989). Pharmacological modulation of the up-regulated responses to des-Arg^9-bradykinin *in vivo* and *in vitro*. *Immunopharmacology*, **17**, 187–198.

D'ORLEANS-JUSTE, P., DE NUCCI, G. & VANE, J.R. (1989). Kinins act on B_1 or B_2 receptors to release conjointly endothelium-derived factor and prostacyclin from bovine aortic endothelial cells. *Br. J. Pharmacol.*, **96**, 920–926.

DRAPEAU, G., BEBLOIS, D. & MARCEAU, F. (1991). Hypotensive effects of Lys-des-Arg^9-bradykinin and metabolically protected agonists of B_1 receptors for kinins. *J. Pharmacol. Exp. Ther.*, **259**, 997–1003.

DRAY, A., BETTANEY, J., FORSTER, P. & PERKINS, M.N. (1988). Activation of a bradykinin receptor in peripheral nerve and spinal cord in the neonatal rat *in vitro*. *Br. J. Pharmacol.*, **95**, 1008–1010.

FARMER, S.G., MCMILLAN, B.A., MEEKER, S.N. & BURCH, R.M. (1991). Induction of vascular smooth muscle bradykinin B_1 receptors *in vivo* during antigen arthritis. *Agents Actions*, **34**, 191–193.

FOCK, S. & MENSE, S. (1976). Excitatory effects of 5-hydroxytryptamine, histamine and potassium ions on muscular group IV afferent units: a comparison with bradykinin. *Brain Res.*, **105**, 459–467.

GRIESBACHER, T. & LEMBECK, F. (1987). Effect of bradykinin antagonists on bradykinin-induced plasma extravasation, venoconstriction, prostaglandin E_2 release, nociceptor stimulation and contraction of the iris sphincter muscle in the rabbit. *Br. J. Pharmacol.*, **92**, 333–340.

HOCK, F.J., WIRTH, K., ALBUS, U., LINZ, W., GERHARDS, H.J., WIEMER, G., ST. HENKE, G., BREIPOHL, G., KONIG, W., KNOLLE, J. & SCHOLKENS, B.A. (1991). Hoe 140 a new potent and long acting bradykinin-antagonist: *in vitro* studies. *Br. J. Pharmacol.*, **102**, 769–774.

LANG, E., NOVAK, A., REEH, P.W. & HANDWERKER, H.O. (1990). Chemosensitivity of fibre afferents from rat skin *in vitro*. *J. Neurophysiol.*, **63**, 887–901.

LERNER, U.H. & MODEER, T. (1991). Bradykinin B_1 and B_2 receptor agonists synergistically potentiate interleukin-1-induced prostaglandin biosynthesis in human gingival fibroblasts. *Inflammation*, **15**, 427–436.

MIZUMURA, K., MINAGAWA, M., TSUJII, Y. & KUMAZAWA, T. (1990). The effects of bradykinin agonists and antagonists on visceral polymodal receptor activities. *Pain*, **40**, 221–227.

PERKINS, M.N., CAMPBELL, E.A., DAVIS, A. & DRAY, A. (1992). Anti-nociceptive activity of bradykinin B_1 and B_2 antagonists in two models of persistent hyperalgesia in the rat. *Br. J. Pharmacol.*, **107**, 237P.

PERKINS, M.N., CAMPBELL, E.A. & DRAY, A. (1993). Anti-nociceptive activity of the bradykinin B_1 and B_2 receptor antagonists, desArg^{9}Leu^{8}Bk and HOE 140, in two models of persistent hyperalgesia in the rat. *Pain*, **53**, 191–197.

REGOLI, D. & BARABE, J. (1980). Pharmacology of bradykinin and related kinins. *Pharmacol. Rev.*, **32**, 1–46.

RANG, H.P., BEVAN, S. & DRAY, A. (1991). Chemical activation of nociceptive peripheral neurones. *Br. Med. Bull.*, **47**, 534–548.

STERANKA, L.R., FARMER, S.G. & BURCH, R.M. (1989). Antagonists of B_2 bradykinin receptors. *FASEB. J.*, **3**, 2019–2025.

TIFFANY, C.W. & BURCH, R.M. (1989). Bradykinin stimulates tumor necrosis factor and interleukin 1 release from macrophages. *FEBS Lett.*, **247**, 189–192.

TODA, N., BIAN, K., AKIBA, T. & OKAMURA, T. (1987). Heterogeneity in mechanisms of bradykinin action in canine isolated blood vessels. *Eur. J. Pharmacol.*, **135**, 321–329.

WHALLEY, E.T., CLEGG, S., STEWART, J. & VAVREK, R.J. (1987). The effect of kinin agonists and antagonists on the pain response of the human blister base. *Arch. Pharmacol.*, **336**, 652–655.

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