Br. J. Pharmacol. (1988), **94**, 288–290

Simultaneous release by bradykinin of substance P- and calcitonin gene-related peptide immunoreactivities from capsaicin-sensitive structures in guinea-pig heart

[1]Pierangelo Geppetti, *Carlo Alberto Maggi, **Francesca Perretti, Stefania Frilli & **Stefano Manzini

Institute of Internal Medicine and Clinical Pharmacology, University of Florence, *Department of Pharmacology, Menarini Pharmaceuticals and **Department of Pharmacology, Malesci Pharmaceuticals, Florence, Italy

Both bradykinin and capsaicin infusion evoked a marked increase in the outflow of substance P- (SP-LI) and calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) from guinea-pig isolated, perfused heart. After acute exposure to capsaicin *in vitro*, or in hearts taken from animals pretreated *in vivo* with capsaicin, bradykinin failed to induce any release. The positive chronotropic effect of bradykinin was reduced after acute capsaicin administration. The effect of bradykinin in the guinea-pig heart could be mediated, at least partly, by release of neuropeptides from peripheral endings of capsaicin-sensitive sensory neurones.

Introduction It is well known that bradykinin stimulates sensory fibres at both the somatic and the visceral level (Juan & Lembeck, 1974; Kaufman *et al.*, 1980). Further, there is evidence that in certain systems the local effects of bradykinin (motor and inflammatory) are dependent upon neuropeptide release from peripheral endings of capsaicin-sensitive neurones (Butler & Hammond, 1980; Hakanson *et al.*, 1987). However, direct neurochemical evidence of release of substance P (SP) or calcitonin gene-related peptide (CGRP) from peripheral endings of primary sensory neurones, in response to bradykinin, is still lacking.

It has recently been shown that elevated amounts of SP-like immunoreactivity (SP-LI) is released by capsaicin from guinea-pig isolated, perfused heart (Hoover, 1987). This study investigates the outflow of SP-LI and CGRP-like immunoreactivity (CGRP-LI) from guinea-pig isolated perfused heart upon infusion of bradykinin.

[1] Author for correspondence at: Institute of Internal Medicine and Clinical Pharmacology, V. le Morgagni 85, 50134 Florence, Italy.

Methods Hearts from albino guinea-pigs (250–350 g) were rapidly removed, cannulated through the aorta and perfused under constant pressure (coronary flow, $5.7 \pm 0.4\,ml\,min^{-1}$) with oxygenated physiological salt solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 0.54, NaH_2PO_4 1.06, $NaHCO_3$ 24.5 and glucose 10. Electrocardiogram, contractile strength and coronary flow were continuously recorded. After equilibration (60 min), a volume of 0.5 ml of 1 μM capsaicin or 10 μM bradykinin was infused over 2 min through a side arm of the perfusion apparatus. Desensitization by capsaicin was carried out by giving a total dose of $55\,mg\,kg^{-1}$, s.c. in 2 days (Maggi & Meli, 1987) and experiments were done 5 days after the last injection.

One min fractions of heart perfusate, collected in tubes containing enough acetic acid to give a final concentration of 2 N, were freeze-dried, reconstituted with 0.1 M, pH 7.4 phosphate buffer and measured by radioimmunoassay. Heart tissues were homogenized in boiling 2 N acetic acid (1/10; w/v) and after centrifugation at 20,000 *g* for 20 min, the supernatant was freeze-dried. SP-LI radioimmunoassay was performed as described elsewhere (Geppetti *et al.*, 1987). CGRP-LI was measured by incubating experimental samples or samples containing rat-CGRP standards for 48 h at 4°C with an anti human-CGRP rabbit antiserum (Peninsula, CA) that demonstrates 100% cross-reactivity towards rat-CGRP. ^{125}I-labelled human-CGRP (Amersham), was added and incubated for a further 48 h at 4°C. Separation of bound from free antigen was by double antibody precipitation. The lower detection limit was 2.5 fmol per tube. Bradykinin up to 10 μM did not cross-react with either antiserum. All peptides were from Peninsula, CA.

All data are mean ± s.e. mean. Statistical analysis was performed with Student's *t* test on unpaired data

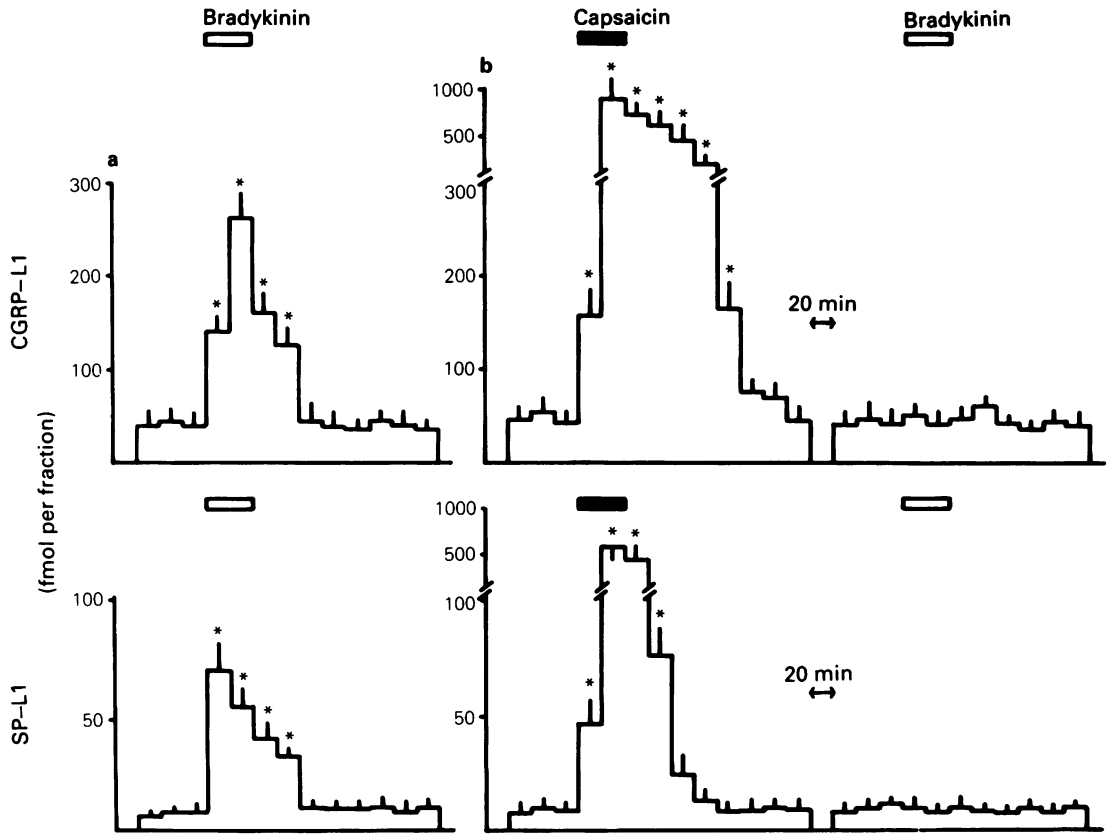

Figure 1 Effect of the infusion (0.5 ml over 2 min) of bradykinin 10 μM ($n = 6$) or capsaicin 1 μM ($n = 4$) on substance P-like (SP-LI) and calcitonin-gene-related peptide-like (CGRP-LI) outflow from guinea-pig isolated, perfused heart. Thirty min after exposure to capsaicin, bradykinin (10 μM) failed to evoke any further neuropeptide release ($n = 4$). * Significantly different from basal values, $P < 0.01$.

or (SP-LI and CGRP-LI outflow) with the analysis of variance and Newman-Keuls test.

Results Preliminary experiments using various concentrations indicated that 10 μM bradykinin and 1 μM capsaicin induced a marked increase in the outflow of SP-LI and CGRP-LI ($n = 6$, data not shown). Therefore these doses were chosen for further experiments. Infusion of either bradykinin or capsaicin markedly increased the outflow of SP-LI and CGRP-LI (Figure 1). In hearts pre-exposed to capsaicin, bradykinin failed to evoke any further release of sensory neuropeptides (Figure 1b). Likewise, no release of SP-LI and CGRP-LI by bradykinin was observed in hearts excised from guinea-pigs pretreated with capsaicin ($n = 4$, data not shown). SP-LI and CGRP-LI concentrations in cardiac tissue were 7.71 ± 0.8 and 19.2 ± 1.8 pmol g^{-1}, respectively. On a molar basis, capsaicin and bradykinin were capable of releasing about 14% and 2.5%, respectively of the SP-LI and CGRP-LI contained in the guinea-pig heart.

Injection of bradykinin ($n = 6$) induced a $32 \pm 6.8\%$ and $18 \pm 2.2\%$ increase of coronary flow and heart rate, respectively, while contractile strength was reduced by $26 \pm 2.5\%$. Thirty min after the *in vitro* capsaicin pretreatment ($n = 6$) (which itself increased by $35 \pm 6\%$ and $28 \pm 5\%$ coronary flow and heart rate, respectively, and reduced the force of contraction by $31 \pm 7\%$) the positive chronotropic effect of bradykinin was significantly lowered; the increase in heart rate was 44.6 ± 5.1 and 25 ± 3.4 beats min^{-1} in control ($n = 6$) and capsaicin-pretreated hearts ($n = 6$), respectively ($P < 0.05$). The effects of bradykinin on coronary flow and cardiac inotropy were unaffected by previous exposure to capsaicin *in vitro*.

Discussion Infusion of bradykinin produced a release of SP-LI and CGRP-LI from guinea-pig, isolated, perfused heart, that was no longer observed when the same dose of bradykinin was administered 30 min after an infusion of capsaicin. Bradykinin also failed to evoke release of SP-LI and CGRP-LI in hearts taken from animals pretreated with capsaicin. These observations strongly indicate that SP-LI and CGRP-LI are released by bradykinin from capsaicin-sensitive structures, that is, the terminals of primary sensory neurones.

Previous investigations on rabbit ocular structures had suggested that effects of bradykinin are mediated through SP release from trigeminal neurones (Butler & Hammond, 1980; Hakanson *et al.*, 1987). Our findings provide for the first time, direct neurochemical evidence that exposure to bradykinin induces release of SP-LI; we have also demonstrated that CGRP-LI is simultaneously released. Both capsaicin (Hoover 1987) and bradykinin (present paper) induce marked effects on coronary blood flow, heart rate and heart contractility. A variety of direct and indirect mechanisms have been proposed to be involved in the effects of bradykinin (Regoli & Barabe', 1980). The present data suggest that the release of neuropeptides from capsaicin-sensitive sensory neurones could play a prominent role in the positive chronotropic effect of bradykinin. Bradykinin is thought to be produced in the heart during ischaemia (Kimura *et al.*, 1973) and is capable of stimulating cardiac afferent C-fibres (Kaufman *et al.*, 1980). Release of CGRP-LI from guinea-pig isolated perfused heart has been recently shown following anoxia (Franco-Cereceda *et al.*, 1987). The ability of bradykinin to release sensory neuropeptides from capsaicin-sensitive sensory neurones suggests that the local 'efferent' function (Maggi & Meli, 1988) exerted by these neurones in certain pathophysiological conditions (such as ischaemia) could be mediated, at least in part, by the action of endogenous bradykinin on their peripheral terminals.

This work was in part supported by CNR, Rome, Progetto Finalizzato 'Chimica Fine e Secondaria' grant no. 85.0019.15. We thank Ms M.K. Lokken for revision of the English text.

References

BUTLER, J.M. & HAMMOND, B.R. (1980). The effects of sensory denervation on the response of the rabbit eye to prostaglandin E_1, bradykinin and substance P. *Br. J. Pharmacol.*, **69**, 495–502.

FRANCO-CERECEDA, A., SARIA, A. & LUNDBERG, J.M. (1987). Ischemia and changes in contractility induce release of CGRP but not neuropeptide Y from isolated perfused guinea-pig heart. *Acta Physiol. Scand.*, **131**, 319–320.

GEPPETTI, P., MAGGI, C.A., ZECCHI-ORLANDINI, S., SANTICIOLI, P., MELI, A., FRILLI, S., SPILLANTINI, M.G. & AMENTA, F. (1987). Substance P-like immunoreactivity in capsaicin-sensitive structures of the rat thymus. *Regulat. Pept.*, **18**, 312–329.

HAKANSON, R., BEDING, B., EKMAN, M., WAHLESTEDT, C. & SUNDLER, F. (1987). Multiple tachykinin pools in sensory nerve fibres in the rabbit iris. *Neuroscience*, **4**, 943–950.

HOOVER, D.B. (1987). Effects of capsaicin on release of substance P-like immunoreactivity and physiological parameters in isolated perfused guinea-pig heart. *Eur. J. Pharmacol.*, **141**, 489–492.

JUAN, H. & LEMBECK, F. (1974). Action of peptides and other algesic agents on paravascular pain receptors of the isolated perfused rabbit ear. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **283**, 151–164.

KAUFMAN, M.P., BAKER, D.G., COLERIDGE, H.M. & COLERIDGE, J.C.G. (1980). Stimulation by bradykinin of afferent vagal C-fibers with chemosensitive endings in the heart and aorta of the dog. *Circ. Res.*, **46**, 476–484.

KIMURA, E., HASHIMOTO, K., FUKUWARA, S. & HAYAKAWA, H. (1973). Changes in bradykinin level in coronary sinus blood after the experimental occlusion of a coronary artery. *Am. Heart J.*, **85**, 635–647.

MAGGI, C.A. & MELI, A. (1988). The sensory-efferent function of capsaicin-sensitive sensory neurons. *Gen. Pharmacol.*, **19**, 1–43.

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

(Received January 18, 1988
Accepted February 29, 1988)