A comparison of bradykinin- and capsaicin-induced myocardial and coronary effects in isolated perfused heart of guinea-pig: involvement of substance P and calcitonin gene-related peptide release

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1 Bradykinin and capsaicin were compared for their ability to elicit functional effects and to release sensory neuropeptides from guinea-pig isolated perfused hearts.

2 Both bradykinin (10 μ M) and capsaicin (1 μ M) produced a marked increase in coronary flow, a large positive chronotropic effect and a significant reduction in contractile strength. These actions were associated with a marked release of substance P-like immunoreactivity (SP-LI) and calcitonin gene-related-like immunoreactivity (CGRP-LI). The percentage of the tissue content of SP-LI and CGRP-LI released by each agent was similar, although bradykinin was less effective than capsaicin. The ratio of SP-LI/CGRP-LI released by both agents was similar to that present in cardiac tissue.

3 Neuropeptide release could be evoked only once with capsaicin but at least four times with bradykinin. Also, functional responses to capsaicin underwent desensitization. After either *in vitro* or systemic capsaicin pretreatment, the release of SP-LI and CGRP-LI by bradykinin was reduced and the positive chronotropic effect of bradykinin was significantly reduced, while the increase in coronary flow and negative inotropic responses remained unchanged.

4 Pretreatment with indomethacin (10 μ M) strongly antagonized the release of SP-LI and CGRP-LI by bradykinin and reduced the increase in heart rate.

5 These findings suggest that activation by bradykinin (probably through indirect mechanisms) of capsaicin-sensitive sensory nerves in the heart, leads to a local release of sensory neuropeptides. These neuropeptides, in turn, could participate in determining the complex functional effects of this kinin on cardiac performance.

Introduction

Peripheral release of neuropeptides from capsaicin-sensitive sensory nerve endings leads to various motor, inflammatory and trophic effects (Holzer, 1988; Maggi & Meli, 1988). In guinea-pig isolated hearts perfused with capsaicin, myocardial and coronary effects coupled to release of substance P-like immunoreactivity (SP-LI) have also been described (Hoover, 1987). Neuropeptide-containing, capsaicin-sensitive sensory fibres are widely distributed in cardiac tissues of various mammalian species, including the guinea-pig (Papka *et al.*, 1984; Lundberg *et al.*, 1985; Uddman *et al.*, 1986; Houghland *et al.*, 1986; Saito *et al.*, 1986a).

In addition sensory neuropeptides, such as tachykinins and calcitonin gene-related peptide (CGRP) have also been found to produce powerful chronotropic, inotropic and vasodilator effects in cardiac preparations (Franco-Cereceda & Lundberg, 1985; Haas & Skofitsch, 1985; Holman *et al.*, 1986; Sigrist *et al.*, 1986; Saito *et al.*, 1986b).

Various data indicate that bradykinin, produced locally during inflammation, or following tissue damage, is capable of stimulating and/or sensitizing capsaicin-sensitive afferents (Juan & Lembeck, 1974;

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Kaufman *et al.*, 1980). Some of the peripheral effects of bradykinin, especially on iris smooth muscle preparations, are at least partially attributable to release of tachykinins (Bynke *et al.*, 1983; Ueda *et al.*, 1984; Hakanson *et al.*, 1987). However, thus far the potential involvement of neuropeptide release in the actions of bradykinin on heart tissues (for a review see Regoli & Barabé, 1980) has not been investigated. Recently, we have shown that infusion of bradykinin in guinea-pig isolated perfused hearts elicits a simultaneous release of SP-LI and CGRP-like immunoreactivity (CGRP-LI) from capsaicin-sensitive structures (Geppetti *et al.*, 1988). The aim of the present work was to investigate and to compare functional and neurochemical studies in the characteristics of the actions of capsaicin and bradykinin in guinea-pig isolated perfused hearts.

Methods

Functional studies

Male albino guinea-pigs (250–350 g) were used. Animals were killed by cervical dislocation and the heart rapidly removed, a cannula was inserted into the aorta immediately distal to the aortic valves and the preparation was transferred to a perfusion apparatus. Hearts were perfused under constant pressure at 37°C with oxygenated (96% O₂ and 4% CO₂) physiological salt solution of the following composition (mm): NaCl 118, KCl 4.7, CaCl₂ 2.5, MgCl₂ 0.54, NaH₂PO₄ 1.06, NaHCO₃ 24.5 and glucose 10. Coronary flow was detected with an electronic device (MARB Gx-84) and the electrocardiogram was recorded. Contractile function of the whole heart was assessed by means of a hook inserted in the cardiac apex connected to an isometric transducer under a resting tension of 2 g.

Preparations were allowed to equilibrate for at least 60 min. Drug administration was by injection through a side arm of the perfusion apparatus immediately above the cannula. A volume of 0.5 ml of the required concentration of capsaicin or peptides was slowly injected over 2 min. Preliminary experiments indicated that no modification in cardiac and coronary parameters could be detected after administration of 0.5 ml of physiological salt solution. The injection of capsaicin vehicle (ethanol) produced a slight decrease in contractile force (about 10–15%) along with a slight and transient increase in coronary flow (less than 10%). In some experiments non-cumulative concentration-response curves were obtained by injecting increasing concentrations of neuropeptides at 30 min intervals. ECG, contractile function and coronary flow were continuously recorded on a three channel polygraph (MARB

3/50). Heart rate, at a fast speed of the recording system, was recorded at 2, 5, 10 and 15 min after drug injection.

Some experiments were performed in capsaicin-pretreated animals in which capsaicin desensitization was achieved by giving a total dose of 55 mg kg⁻¹ s.c. over two days according to Maggi *et al.* (1987a); the experiments were carried out five days after the last injection of capsaicin.

Sample collection and extraction

Fractions of the heart perfusate (approximately 6 ml) were taken every minute. Perfusates, collected in tubes containing enough acetic acid to give a final concentration of 2 N were freeze-dried. Atria and ventricles were dissected from the whole heart and then homogenized in 2 N acetic acid (1/10; w/v at 95°C) kept in a boiling water bath for 10 min and cooled at 4°C for 10 min. After centrifugation at 20,000 g for 30 min at 4°C, the supernatant was freeze-dried. All samples were then reconstituted with the assay buffer (0.1 M, pH 7.4, phosphate buffer containing 0.9% NaCl, 0.01% NaN₃ and 0.1% bovine serum albumin) and peptide content measured by radioimmunoassay (RIA).

CGRP-LI and SP-LI radioimmunoassays

After reconstitution in assay buffer, 100 µl of rat CGRP standard or samples were incubated with 100 µl of rabbit anti-human CGRP antiserum (RAS6012, Peninsula, CA, U.S.A.) for 48 h at 4°C; 100 µl of [¹²⁵I]-iodohistidyl-CGRP (Amersham, UK) was added and incubated for a further 48 h at 4°C. After the addition of 1 ml of buffer containing 7.5% polyethylene glycol, 1/200 goat anti-rabbit antiserum and 1/2000 normal rabbit serum-bound antigen was separated from free by centrifugation at 2000 g for 30 min at 4°C. The coefficient of variation was less than 10% for values between 20 and 300 pmol l⁻¹. The lower detection limit was 2.5 fmol per tube. Cross-reactivity of the antiserum was 100% for rat-CGRP I and human-CGRP.

SP-LI was measured as previously reported (Geppetti *et al.*, 1987). Briefly, SP-standard or samples were incubated overnight at 4°C with rabbit anti-SP antiserum and ¹²⁵I-Bolton and Hunter conjugated SP (Amersham, UK). Bound and free antigen were separated by double antibody precipitation. The sensitivity of the assay was 1.1 fmol per tube. The antiserum cross-reacts to 1% with NKA, 0.5% with NKB and less than 0.1% with physalaemin and eledoisin. Both anti-SP and anti-CGRP antiserum did not cross-react with bradykinin up to 100 µM.

Statistical analysis

All data in the text, Figures and Tables are given as the mean $\pm$ s.e.mean. Statistical analysis of the data was performed by means of Student's *t* test for paired or unpaired data, analysis of variance and Newman-Keuls test, when appropriate. A *P* value of less than 0.05 was considered to be significant.

Drugs

Drugs used were: bradykinin (Serva), calcitonin gene-related peptide (CGRP rat) (Peninsula), capsaicin (Serva), indomethacin (Sigma) and substance P (SP) (Serva).

Results

General

After 60 min equilibration, the resting performance of guinea-pig isolated perfused hearts was as follows: frequency of beat 248 ± 3 beats min^{-1} , force of contraction 2.7 ± 0.006 g and mean coronary flow 5.1 ± 0.1 ml min^{-1} ($n = 51$). Preliminary experiments indicated that such values remained stable for at least 2 h.

Effects of bradykinin and capsaicin on chronotropism, inotropism and coronary flow of guinea-pig isolated perfused hearts

Either bradykinin ($10 \mu\text{M}$) or capsaicin ($1 \mu\text{M}$) exerted similar, marked effects on myocardial and coronary function in the guinea-pig isolated perfused hearts. These concentrations were selected since they were near maximally effective in producing release of sensory neuropeptides (Geppetti *et al.*, 1988). Bradykinin ($10 \mu\text{M}$, $n = 9$, Figure 1a) produced a prompt and rapid increase in coronary flow (from 5.8 ± 0.3 to 8.1 ± 0.5 ml min^{-1} , $P < 0.05$), and a marked positive chronotropic effect (from 242 ± 5 to 285 ± 7 beats min^{-1} , $P < 0.05$) coupled to a significant reduction in contractile strength (from 3.1 ± 0.2 to 2.3 ± 0.1 g, $P < 0.05$).

Administration of capsaicin ($1 \mu\text{M}$, $n = 15$, Figure 1b) elicited a rapid increase in heart rate (from 245 ± 7 to 303 ± 10 beats min^{-1} , $P < 0.05$) and coronary flow (from 5.3 ± 0.2 to 7.1 ± 0.3 ml min^{-1} , $P < 0.05$) and a concomitant negative inotropic effect (force of contraction was reduced from 2.6 ± 0.1 to 2.0 ± 0.15 g, $P < 0.05$).

The myocardial and coronary effects of the two drugs peaked at about the second min from administration and returned to resting values, within 5–7 min (Figure 1).

Figure 1 Time-dependency of the effects of bradykinin (a) and capsaicin (b) on heart rate ($\circ$), coronary flow ($\square$) and contractile strength ($\triangle$) in guinea-pig isolated perfused hearts. Each value is the mean of at least 9 experiments; vertical bars show s.e.mean.

In some experiments the response to bradykinin or capsaicin was assessed several times (at 30 min intervals) in the same heart. With bradykinin similar responses (positive chronotropic effect, increase in coronary flow and negative inotropic effect) were elicited for at least four times without significant modification ($n = 5$). On the other hand, a second administration of capsaicin had no positive chronotropic effect, although a consistent increase in coronary flow was still observed in 5 out of 8 preparations (the mean increase in coronary flow was 0.95 ± 0.40 ml min^{-1} , as compared to 2.02 ± 0.48 at the first challenge, $P < 0.05$). Also a negative inotropic response was still evident at the second challenge with capsaicin (from 2.4 ± 0.2 to 1.8 ± 0.4 g, NS as compared to the effect of the first

dose). A third challenge with capsaicin did not produce any detectable effect on heart rate or coronary flow ($n = 4$), while a slight decrease in force of contraction was still evident (0.3 ± 0.01 g, $n = 4$; not significantly different from the effect of the vehicle alone, see methods section).

Effect of substance P or calcitonin gene-related-peptide on chronotropism, inotropism and coronary flow of isolated perfused guinea-pig hearts

Substance P (0.1 – $10 \mu\text{M}$, $n = 5$) in increasing non-cumulative concentrations produced a concentration-dependent increase in coronary flow without any significant inotropic or chronotropic effect (Figure 2, upper panel). At the highest concentration tested ($10 \mu\text{M}$) the increase in coronary flow was $2.07 \pm 0.24 \text{ ml min}^{-1}$ ($P < 0.01$); heart rate and force of contraction were 237 ± 10 and $240 \pm 9 \text{ beats min}^{-1}$ (NS) and 2.5 ± 0.1 and 2.3 ± 0.1 g (NS) before and after substance P, respectively.

On the other hand, CGRP produced a sharp and concentration-dependent (10 nM – $1 \mu\text{M}$; $n = 5$) increase in heart rate along with a marked increase in coronary flow (Figure 2, lower panel). A slight negative inotropic effect was also evident. At the

highest concentration tested ($1 \mu\text{M}$), CGRP increased coronary flow from 4.1 ± 0.3 to $5.7 \pm 0.4 \text{ ml min}^{-1}$ ($P < 0.05$), reduced the force of contraction from 2.6 ± 0.2 to 2.2 ± 0.3 g (NS) and increased heart rate from 245 ± 8 to $326 \pm 17 \text{ beats min}^{-1}$ ($P < 0.05$).

Both the chronotropic and vasodilator-responses to substance P and CGRP had a time-course similar to that of capsaicin ($1 \mu\text{M}$) or bradykinin ($10 \mu\text{M}$), peaking about 2–3 min from administration and rapidly declining within 5–10 min.

In hearts from capsaicin-pretreated animals, both substance P ($10 \mu\text{M}$) and CGRP ($1 \mu\text{M}$) elicited myocardial and coronary responses similar to those observed in control hearts ($n = 4$; data not shown).

Effects of bradykinin after acute or chronic treatment with capsaicin

To investigate further whether activation of capsaicin-sensitive sensory fibres could be involved in the effects of bradykinin, experiments were performed in hearts obtained from animals desensitized to capsaicin five days earlier (see methods section for details) or in hearts pre-exposed *in vitro* to a bolus injection of capsaicin ($1 \mu\text{M}$), 30 min before the addition of bradykinin ($10 \mu\text{M}$).

Figure 2 Concentration-dependent effect of substance P (SP, upper panels) and calcitonin gene-related peptide (CGRP, lower panels) on heart rate (●), coronary flow (■) and contractile strength (▲) in guinea-pig isolated perfused hearts. Each value is the mean of at least 5 experiments; vertical bars show s.e.mean.

Figure 3 Effect of bradykinin on heart rate, contractile strength and coronary flow in hearts obtained from control animals (open column), animals systematically pretreated with capsaicin (55 ng kg^{-1} , s.c.) five days before experiment (solid columns), and in hearts previously (30 min) challenged *in vitro* with capsaicin ($1 \mu\text{M}$) (hatched column). Each point is the mean of at least 6 experiments; s.e.mean shown by vertical bars.

* $P < 0.05$ as compared to response obtained in hearts from control animals.

Both the *in vitro* and systemic capsaicin desensitization produced a significant reduction in the increase in heart rate by bradykinin (44 ± 6 , 25 ± 6 and 25 ± 5 beats min^{-1} in control hearts, $n = 9$, and after *in vitro*, $n = 6$, or chronic pretreatment, $n = 9$, with capsaicin, respectively, $P < 0.05$). On the other hand the effect of bradykinin on coronary flow and cardiac inotropism were not significantly altered by capsaicin pretreatment (Figure 3).

In some experiments ($n = 4$) the effects of capsaicin ($1 \mu\text{M}$) were assessed in hearts previously challenged four times with bradykinin ($10 \mu\text{M}$). The

amplitude of the responses to capsaicin on coronary flow and cardiac performance were not significantly different from those in control hearts (data not shown).

Bradykinin and capsaicin-induced release of SP-LI and CGRP-LI in guinea-pig isolated perfused hearts

SP-LI and CGRP-LI in the 6 hearts examined were 7.71 ± 0.88 and 19.2 ± 1.8 pmol per whole heart, respectively. The content of SP-LI and CGRP-LI in the atria and ventricles of control and capsaicin-pretreated guinea-pigs are shown in Table 1. In both atria and ventricles the ratio of SP-LI/CGRP-LI was approximately 0.4. The systemic capsaicin pretreatment depleted SP-LI by 94–80% and CGRP-LI by 87–65%.

The infusion of either capsaicin or bradykinin evoked a marked and concentration-related increase of both SP-LI and CGRP-LI in the perfusate (Table 2). On a molar basis, the outflow of SP-LI induced by bradykinin ($10 \mu\text{M}$) or capsaicin ($1 \mu\text{M}$) was about 32% and 41% of the CGRP-LI released by the same concentrations of both drugs, i.e. very close to the SP-LI/CGRP-LI ratio in heart (see above). Furthermore, both capsaicin and bradykinin released a similar amount of either SP-LI or CGRP-LI as a percentage of their respective tissue content (Table 2). When bradykinin ($10 \mu\text{M}$) or capsaicin ($1 \mu\text{M}$) was perfused 30 min after a challenge *in vitro* with capsaicin ($1 \mu\text{M}$), neither agent produced a measurable release of SP-LI or CGRP-LI. Likewise, neither capsaicin nor bradykinin released SP-LI or CGRP-LI in hearts excised from animals pretreated *in vivo* with capsaicin (Table 2).

Table 1 Substance P-like immunoreactivity (SP-LI) and calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) (pmol g^{-1}) in hearts pretreated *in vitro* or *in vivo* with capsaicin

		Right atrium	Left atrium	Right ventricle	Left ventricle
<i>In vitro</i> pretreatment					
SP-LI	Vehicle	14.7 ± 1.8	11.7 ± 0.9	5.1 ± 0.9	3.4 ± 0.4
	Capsaicin	12.9 ± 1.3	10.4 ± 1.7	5.1 ± 0.9	2.9 ± 0.5
<i>In vivo</i> pretreatment					
SP-LI	Vehicle	15.1 ± 1.6	12.9 ± 0.9	6.2 ± 1.0	4.8 ± 0.9
	Capsaicin	$0.9 \pm 0.2^*$	$0.7 \pm 0.1^*$	$0.9 \pm 0.1^*$	$1.0 \pm 0.2^*$
CGRP-LI	Vehicle	34.5 ± 3.1	28.2 ± 1.9	16.8 ± 1.2	12.1 ± 1.3
	Capsaicin	$4.5 \pm 1.2^*$	$5.2 \pm 0.9^*$	$5.1 \pm 0.7^*$	$4.3 \pm 1.0^*$

In vitro pretreatment: hearts excised from control guinea-pigs mounted in the perfusion apparatus and challenged with injection of capsaicin ($1 \mu\text{M}$) or vehicle.

In vivo pretreatment: hearts excised from animals pretreated, five days before, with capsaicin (55 mg kg^{-1} s.c. on two consecutive days) or vehicle.

Each value is the mean $\pm$ s.e.mean from at least 4 experiments.

* $P < 0.01$.

Table 2 Substance P-like immunoreactivity (SP-LI) and calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) outflow induced by bradykinin or capsaicin from guinea-pig isolated perfused hearts: experiments were performed in hearts excised from (A) control animals; (B) animals pretreated *in vivo* with capsaicin (55 mg kg⁻¹ s.c. on two consecutive days) and (C) control animals challenged *in vitro* with capsaicin (1 μ M) 30 min before drug administration

	SP-LI (fmol 4 min ⁻¹)	% of tissue content	CGRP-LI (fmol 4 min ⁻¹)	% of tissue content
A				
Basal	19 $\pm$ 1	—	37 $\pm$ 3	—
Bradykinin 1 μ M	50 $\pm$ 5*	0.6	161 $\pm$ 19*	0.8
Bradykinin 10 μ M	181 $\pm$ 21**	2.3	565 $\pm$ 98**	2.7
Capsaicin 0.1 μ M	524 $\pm$ 59**	6.8	n.m.	n.m.
Capsaicin 1 μ M	1152 $\pm$ 156**	15.1	2761 $\pm$ 263**	14.2
B				
Bradykinin 10 μ M	12 $\pm$ 2	—	15 $\pm$ 2	—
Capsaicin 1 μ M	16 $\pm$ 2	—	19 $\pm$ 3	—
C				
Bradykinin 10 μ M	14 $\pm$ 1	—	23 $\pm$ 3	—
Capsaicin 1 μ M	18 $\pm$ 1	—	27 $\pm$ 4	—

Each value is the mean $\pm$ s.e.mean from at least 4 experiments.

n.m. = not measured.

* $P < 0.05$ as compared to basal values; ** $P < 0.01$ as compared to basal values.

At its second administration, bradykinin (10 μ M) evoked a release, although this was much reduced, of both SP-LI and CGRP-LI, and at the third and fourth injection of bradykinin, release of SP-LI and CGRP-LI was still detectable (Figure 4). After four challenges with bradykinin (10 μ M), capsaicin (1 μ M) was still able to evoke a marked increase of SP-LI and CGRP-LI outflow (Figure 4).

Effect of indomethacin on functional and neurochemical actions of bradykinin

Since many of the biological actions of bradykinin are mediated by prostaglandin release (Regoli & Barab, 1980) we investigated whether blockade of tissue prostaglandin synthesis by indomethacin could influence either the release of neuropeptides by bradykinin or its functional effects.

As shown in Figure 5, in the presence of indomethacin (10 μ M) the release of both SP-LI and CGRP-LI by bradykinin was markedly reduced (by 83% and 88% respectively, $n = 5$). Indomethacin alone did not evoke neuropeptide release.

Perfusion with indomethacin produced significant reduction in heart rate (from 222 $\pm$ 17 to 200 $\pm$ 16 beats min⁻¹, $P < 0.05$) while coronary flow and contractile strength were unaffected ($n = 6$). In the presence of indomethacin, functional responses to bradykinin were greatly changed as compared to those observed in control hearts: the increase in

heart rate was significantly reduced (22 $\pm$ 6 compared to 44 $\pm$ 6 beats min⁻¹ in controls, $P < 0.05$); the negative inotropic effect was virtually abolished (0.2 $\pm$ 0.07 g compared to 0.8 $\pm$ 0.1 g in controls, $P < 0.05$) and the increase in coronary flow was almost doubled (4.3 $\pm$ 0.5 ml min⁻¹ compared to 2.4 $\pm$ 0.4 ml min⁻¹ in controls, $P < 0.01$).

Discussion

In guinea-pig isolated perfused hearts both bradykinin and capsaicin produced qualitatively similar functional and neurochemical effects. Both agents evoked a marked increase in coronary flow and heart rate, at the same time as reducing contractile force. These effects were paralleled by the release of sensory neuropeptides (SP-LI and CGRP-LI) suggesting that a local release from sensory nerve endings could be involved in mediating the functional responses to these agents.

It has already been established that stimulation by bradykinin of sensory afferents of cardiac origin is relevant to the initiation of reflex cardiovascular responses (Kaufmann *et al.*, 1980). Our data indicate that activation of these fibres by bradykinin also evokes effects *in situ* due to a local release of neuropeptides. Thus the dual 'sensory-efferent' function of these fibres, as defined by Szolcsanyi (1984) and con-

Figure 4 Substance P-like immunoreactivity (SP-LI) and calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) outflow from guinea-pig isolated perfused heart, elicited by four consecutive challenges with bradykinin ($10 \mu\text{M}$) (open bar, $\square$). At the end capsaicin ($1 \mu\text{M}$) (solid bars, $\blacksquare$) was administered. Each challenge was performed at 30 min interval. Horizontal arrows ($\leftrightarrow$) indicate an interval of 25 min. Each bar represent a 1 min fraction, and is the mean of at least 5 vertical lines show s.e.mean experiments; Drugs were slowly infused over 2 min.

* $P < 0.05$ as compared to respective predrug values.

Figure 5 Influence of indomethacin ($10 \mu\text{M}$) on bradykinin ($10 \mu\text{M}$)-induced substance P-like immunoreactivity (SP-LI) and calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) outflow from guinea-pig isolated perfused hearts. Indomethacin was perfused 15 min before, during bradykinin infusion and in the following 15 min. Horizontal arrows ($\leftrightarrow$) indicate an interval of 13 min. Indomethacin itself did not elicit neuropeptide release. Each bar represents a 1 min fraction, and is the mean of at least 5 experiments: vertical lines show s.e.mean. Bradykinin was slowly infused over 2 min.

* $P < 0.05$ as compared to respective predrug values.

firmed in various tissues and test systems mainly by the use of capsaicin (Maggi & Meli, 1988; Holzer, 1988) appears to be a specific feature of these fibres which follows their activation either by exogenous or endogenous agents.

However some important differences may exist in the mechanisms for the release of neuropeptides by bradykinin or capsaicin. Thus at the maximal dose used, the amount of SP-LI and CGRP-LI released is about five times greater with capsaicin than with bradykinin. Second, neuropeptide release can be evoked repeatedly with bradykinin but only once with capsaicin and finally, after challenge with capsaicin, bradykinin is no longer able to produce release of neuropeptide. These data can be explained on the basis of the hypothesis advanced by Hakanson *et al.* (1987) that at least two releasable neuropeptide pools are present in sensory nerve endings, i.e.: a minor one that can be rapidly released and refilled and which is sensitive both to bradykinin and capsaicin, and a second pool that is capsaicin-sensitive but bradykinin-resistant, which refills the former pool and is not replenishable on a short term basis. However, an additional factor that might be involved is that exposure to capsaicin may produce certain irreversible modifications in the membrane and/or intraneuronal structures of sensory nerve endings that render the nerve refractory to successive stimulations. The observation that acute exposure to capsaicin produces a slight, and not significant, reduction in tissue content of SP-LI confirms that the bulk of neuropeptides contained in the nerve endings are in a non-releasable store (at least by capsaicin), as previously suggested in other preparations (Maggi *et al.*, 1987b; Hakanson *et al.*, 1987).

Much evidence has been presented for a co-localization of both peptides in central and peripheral sensory nerve endings and a dense network of fibres containing both SP-LI and CGRP-LI, has been demonstrated in cardiac tissues (Papka *et al.*, 1984; Houghland *et al.*, 1986; Lundberg *et al.*, 1985; Mulderry *et al.*, 1985; Saito *et al.*, 1986a).

It is interesting to note, that the percentage of tissue SP-LI or CGRP-LI released by each agent is fairly similar (14% and 2.5% for capsaicin and bradykinin, respectively). Further, the ratio of SP-LI and CGRP-LI released by capsaicin (0.41) or bradykinin (0.32) is similar to that found in atrial and ventricular tissues (range 0.36–0.45). Taken together, these findings strongly suggest that SP-LI and CGRP-LI are co-released from sensory nerve endings. In addition, it is interesting to note that the effects of capsaicin and bradykinin on heart could be mimicked by substance P (increase in coronary flow) and CGRP (positive chronotropic effect and increase in coronary flow). The possibility that other neuropeptides may also be released from sensory nerve

endings to participate in mediating functional responses must also be considered. For example, neurokinin A has been reported to be colocalized with SP and CGRP in sensory structures and to exert a negative inotropic effect in the isolated perfused heart (Lundberg *et al.*, 1985). This latter effect, which is observed with both bradykinin and capsaicin, is not mimicked by SP and CGRP. Regarding the positive chronotropic response, our data with capsaicin, substance P and CGRP confirm results obtained in other cardiac preparations (Franco-Cereceda & Lundberg, 1985; Lundberg *et al.*, 1985) and further substantiate the hypothesis that CGRP is a major neurotransmitter in non-adrenergic non-cholinergic cardioaccelerator nerves (Saito *et al.*, 1986a).

An important difference between the functional effects of bradykinin and of capsaicin is that after previous exposure to capsaicin, while all functional effects of capsaicin undergo desensitization, those of bradykinin do not, with the exception of part of its positive chronotropic effect. Thus it is likely that in the isolated perfused heart the overall action of bradykinin will be the resultant not only of the stimulation of sensory nerve endings but also of a direct effect on its own receptor(s) on the coronary vasculature (Toda, 1977; Regoli & Barabé, 1980; Beny *et al.*, 1987), and release of prostaglandin (Goldberg *et al.*, 1976; Warren *et al.*, 1987) and/or histamine (Johnson & Erdos, 1973). It is important to emphasize that, to obtain detectable release of sensory neuropeptides we used a high concentration of bradykinin. In addition, since the peptide was injected into the coronary vasculature, all structures of the heart would be exposed. In view of the above, it is likely that in our experimental conditions there was activation of all bradykinin-dependent mechanisms. Further studies are necessary to investigate whether localized application of low concentrations of bradykinin could selectively act by releasing neuropeptides from sensory nerve endings.

It is interesting that most of the bradykinin-induced release of SP-LI and CGRP-LI is strongly reduced in the presence of indomethacin and this release may therefore be due to an indirect activation of sensory fibres through the action of prostanoids. Indeed, in various tissues prostaglandins have been reported to be endogenous activators of afferent fibres (Yanagisawa *et al.*, 1986; Longhurst & Dittman, 1987; Maggi *et al.*, 1988). In this context, it is worth noting that the effect of indomethacin on the positive chronotropic effect of bradykinin is similar to that of capsaicin desensitization.

In conclusion our findings indicate that in heart tissues, bradykinin can activate (mostly through prostaglandin release) capsaicin-sensitive sensory nerve fibres. The consequent release of neuropeptides

may produce at least part of the complex functional effects of this kinin on cardiac performance. Thus bradykinin might activate both the sensory (Kaufman *et al.*, 1980) and the local peripheral (this study) functions of the sensory nerves, giving rise to local and systemic cardiovascular responses. It is interesting to note that during myocardial ischaemia in addition to bradykinin, other chemical or physical agents, such as anoxia, hypoxia or prostaglandins

(Yanagisawa *et al.*, 1986; Longhurst & Dittman, 1987), could produce further activation of sensory nerve fibres. Therefore the 'sensory-efferent' function of these fibres might play an important role in the pathophysiology of ischaemic cardiac diseases.

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