Vasorelaxation in isolated bone arteries

Vasoactive intestinal peptide, substance P, calcitonin gene-related peptide, and bradykinin studied in pigs

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We assessed the effects of vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP), substance P (SP), and bradykinin in arteries (diameter ≈ 230 μm) isolated from cancellous bone from pigs. Arterial segments (2 mm long) were mounted on a myograph for measurement of isometric force development. After submaximal precontraction with norepinephrine, VIP (10^{-10}–10^{-7} M), CGRP (10^{-11}–10^{-7} M), SP (10^{-6} M), and bradykinin (10^{-11}–10^{-6} M) were added. 44 arterial segments (23 pigs) were investigated. VIP-, CGRP-, and bradykinin induced a concentration-dependent vasorelaxation, while SP mediated a transient relaxation. After mechanical removal of the endothelium, the effects of SP and bradykinin were completely abolished, while the relaxation to CGRP was still pronounced. This indicates that the effects of SP and bradykinin are mediated by the endothelium, while CGRP mainly mediates relaxation by a direct effect on vascular smooth muscle cells. The relaxations to CGRP and bradykinin were still significant after inhibition of nitric oxide synthase with 10^{-4} M N ω-nitro-L-arginine (L-NNA) and inhibition of prostaglandin synthesis with 10^{-5} M indomethacin, indicating the existence of an alternative vasorelaxing pathway. Our findings support the theory of a vasoregulatory role of neuropeptides in bone.

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During the last 2 decades, peptidergic nerves have been demonstrated in many organs, and their significance as vasoregulatory substances, sensory mediators, and endocrinologic and secretory modulators has been described. In bone tissue, Hohmann et al. (1986) were the first to describe a technique allowing direct immunohistochemical staining of neuropeptides. Bjurholm (1989) screened rat bone tissue for over 30 neuronally-related substances and found immunoreactivity to calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP), neuropeptide Y (NPY), substance P (SP), and tyrosine hydroxylase. Peptidergic nerves were most abundant in cancellous bone near the osteochondral junction of growth plates, and in the periosteum. Most immunoreactive nerves were found close to or within blood vessel walls, suggesting involvement in the regulation of intraosseous blood flow.

We assessed the possible roles of SP, CGRP, and VIP in bone vascular regulation. A recently introduced method for in vitro investigation of isolated bone arteries was used in order to allow control of neural, humoral, and metabolic interactions (Lundgaard et al. 1996). Earlier studies showed that bradykinin induces vasorelaxation via an effect on the endothelium in porcine bone arteries (Lundgaard et al. 1996). Therefore, bradykinin was included in the study to assess further the mechanisms underlying vasorelaxation in bone.

Animals and methods

Bone material and vessel dissection

Land race pigs of both sexes (aged 3–6 months, weighing 60–85 kg) were used. Immediately after killing, the distal femur was removed from the pigs. The condyles were sawn sagittally into 1–2 cm thick slices and transported to the laboratory in a physiological saline solution at 4 °C. From the bone specimen, arterial segments (about 2 cm long) with an approximate internal diameter of 250 μm were dissected from the epiphyseal cancellous bone (Lundgaard et al. 1996).

Before killing, the pigs had undergone various surgical experiments: 13 of 23 pigs underwent a 90-

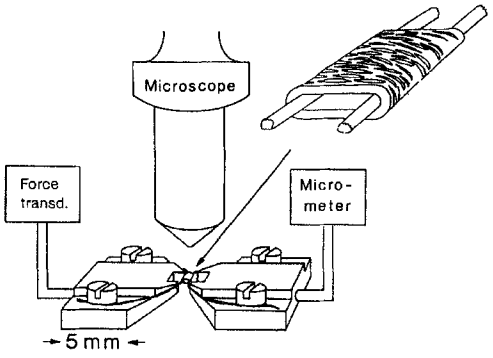


Figure 1. **Principle of the myograph.** Arterial segments (diameter ~ 230 μm) were isolated from epiphyseal cancellous bone and mounted as ring preparations. Kept at a predefined degree of stretch, the isometric force development in the vessel wall was recorded continuously.

minute heart operation and were killed by excision of the heart. The remaining 10 pigs had longer surgery (4–8 hours) before they were killed by intravenous injection of saturated potassium chloride. None of the pigs received atropine before or during operations.

Myographic method

The arterial segments were threaded onto two stainless steel wires (40 μm in diameter) and mounted as ring preparations on a myograph for isometric force development (Mulvany and Halpern 1976, 1977) (Figure 1). A dual small vessel myograph was used (model 500; JP Trading, Aarhus, Denmark), enabling simultaneous investigation of 2 vessels. The test chamber contained 10 mL bicarbonate-buffered physiological saline solution (PSS), which was heated to 37 °C and bubbled continuously with 95% O_2 and 5% CO_2 to give a pH of 7.4–7.5. After an initial equilibration time of 30 min, the arteries were stretched stepwise to characterize the relation between the vessel circumference and the passive wall tension. The vessel size was normalized by defining L_{100} as the internal circumference the vessel would have if relaxed and exposed to a transmural pressure of 100 mmHg (Mulvany and Halpern 1977). The vessels were kept at $0.9 \times L_{100}$, since this degree of stretch allows maximal active force development (Lundgaard et al. 1996). Experiments were carried out by adding the agonist directly to the chamber and recording the isometric force development in the vessel wall.

Solutions and drugs

The physiological saline solution (PSS) had the following composition (in mmol/L): NaCl 119; KCl 4.7; $CaCl_2$ 2.5; $MgSO_4$ 1.17; $NaHCO_3$ 25; KH_2PO_4 1.18; ethylenediaminetetraacetic acid (EDTA) 0.026; glucose 5.5. Drugs used were: norepinephrine, bradykinin,N^{ω}-nitro-L-arginine (L-NNA), and N^{ω}-nitro-L-arginine methyl ester (L-NAME) (Sigma, St. Louis, MO, USA); human α calcitonin gene-related peptide, human/porcine/rat vasoactive intestinal peptide, and substance P (Peninsula Laboratories Inc., Belmont, California, USA); indomethacin (Merck, Darmstadt, Germany). After defrosting, the L-NNA and L-NAME solutions were placed in an ultrasound bath until the solution was clear (approximately 2 min).

Standard start

All arteries were stimulated 3 times with 10 μM norepinephrine for 2 min at 5-min intervals and then once for 5 min. This standard start was carried out prior to all protocols in order to mobilize the vessel. Contractions increased in the first stimulations, but after the third stimulation the same contraction level was reached repeatedly. The 5-min stimulation was used to evaluate the smooth muscle function. Only vessels with an active force development that would correspond to contraction against a pressure of at least 100 mmHg (Mulvany and Halpern 1977) were accepted for further experiments. To avoid adhesion of peptides, the chamber was coated with bovine albumin (0.1% in PSS for 10 min) before protocols were initiated.

Precontractions

Since earlier studies showed that norepinephrine can induce a stable contraction in bone arteries (Lundgaard et al. 1996), it was used to contract the arteries prior to stimulation with vasodilators. The arteries were stimulated cumulatively with norepinephrine (0.02–10 μM in 0.3 log-unit steps at 2-min intervals) in order to assess sensitivity and maximal effect. On the basis of the concentration-response curve, the norepinephrine concentration resulting in 80% of maximal contraction was estimated. This concentration was used to induce a submaximal contraction level in vasodilator studies. The 80% contraction level was chosen to ensure a submaximal level allowing vasodilators to induce their effect. Once chosen, the same concentration of norepinephrine was used for precontraction throughout the experiment.

Protocol I: Concentration-response curves for VIP, SP, and CGRP

After 6-min submaximal precontraction with norepinephrine, increasing concentrations of vasoactive intestinal peptide (VIP), substance P (SP) or calcitonin gene-related peptide (CGRP) were added cumulatively. VIP and SP were also added as single concentrations, since pilot studies indicated possible tachyphy-

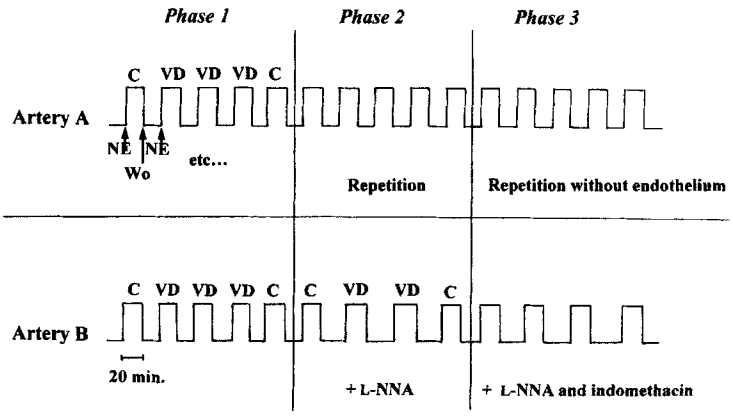


Figure 2. **Study design. Protocol II: Inhibition of vasodilatory mechanisms.** Two arteries were mounted in each study. NE: submaximal contraction with norepinephrine. Wo: wash out with physiological saline solution. C: time control contraction. VD: addition of a vasodilator (CGRP, bradykinin or SP) to the precontracted artery. Phase 1: addition of CGRP (10^{-9}–10^{-7} M), bradykinin (10^{-11}–10^{-6} M), and SP (10^{-6} M) in randomized order. Artery A: vasodilator studies were repeated (phase 2), and then repeated once more after removal of the endothelium (phase 3). Artery B: studies on bradykinin and CGRP were repeated after inhibition with 10^{-4} M L-NNA (phase 2), and after further inhibition with 10^{-5} M indomethacin (phase 3).

laxis on cumulative stimulation. Between each vasodilator study, the vessels were allowed to rest in PSS for 20 min. To minimize possible interactions, the peptides were added in randomized order in each experiment. The intervals between the cumulative steps were estimated after evaluation of the effect profiles of the vasodilators in pilot studies. VIP (10^{-10}–10^{-7} M) was added in half log-steps at 3-min intervals or as a single concentration of 10^{-7} M after 24 min of precontraction (time corresponding to the addition of 10^{-7} M in the concentration-response experiment). SP (10^{-11}–10^{-6} M) was added in one log-unit steps at 2-min intervals or as a single concentration of 10^{-6} M after 16 min of precontraction. CGRP (10^{-11}–10^{-7} M) was added in one log-unit steps at 4-min intervals. Time controls were submaximal contractions sustained for 28 min. These were interposed between the vasodilator studies.

Protocol II: Inhibition of vasodilatory mechanisms

Endothelium. Stimulations with SP (10^{-6} M) and CGRP (10^{-9}–10^{-7} M) were repeated to reveal possible tachyphylaxis. The vasorelaxing effects were assessed before and after removal of the endothelium to reveal whether the effect was mediated by the endothelium (Figure 2, Artery A). Before removal of the endothelium, bradykinin (10^{-11}–10^{-6} M) was used to verify endothelial function, since previous studies have shown that bradykinin induces endothelium-mediated relaxation in porcine bone arteries (Lundgaard et al. 1996). The endothelium was removed mechanically by passing a human hair through the vessel lumen and rubbing the luminal surface. Reduced force development was generally observed after this procedure. Vessels were excluded from further experiments if the contraction level was reduced more than 50% after removal of the endothelium.

Nitric oxide and prostaglandin synthesis. The possible roles of nitric oxide (NO) and prostaglandins as mediators of CGRP- and bradykinin-induced responses were investigated. The effects of CGRP and bradykinin were assessed after inhibition of NO synthesis with 10^{-4} M N^{ω}-nitro-L-arginine (L-NNA) or 10^{-4} M N^{ω}-nitro-L-arginine methyl ester (L-NAME), and further inhibition of the prostaglandin synthesis with 10^{-5} M indomethacin (an inhibitor of the cyclooxygenase) (Figure 2, Artery B). The arteries were incubated with the inhibitors for approximately 20 min before experiments were carried out.

Calculations

The following parameters were derived from the measurement of isometric force (F) exerted by the arterial segment on the force transducer:

1. Active force (F_{active}) was defined as the change in force induced by addition of an agonist to an artery resting in PSS exerting only passive force, $F_{active} = F_{total} - F_{passive}$.
2. Active tension (T_{active}) was calculated as active force divided by twice the vessel length.

Sensitivity to the vasodilators was expressed as IC_{50}, defined as the agonist concentration (in mol/L) resulting in half-maximum relaxation. pD_2 was calculated as the negative logarithm to IC_{50}. Graphpad Prism (Graphpad Software Inc. version 2.0, San Diego, USA) was used for these calculations. In 4 concentration-response experiments it was not possible to estimate IC_{50}: in 2 vessels, the relaxations were too small to allow curve-fitting and in 2 vessels, the relaxations did not follow a sigmoidal curve. Therefore, these four sets of data were omitted.

Vasorelaxing effect was expressed as the relative reduction in wall tension, compared to the precontraction level. The relative fall in tension in the time control (mean of controls carried out before and after the

vasodilator study, Figure 2) was subtracted to give the actual relaxing effect.

Statistics

Values are given as mean (SEM). Results were analyzed for possible differences between groups by the Student's two-tailed t-test, probability levels of 5% being considered significant. N = number of vessels (a maximum of 2 vessels was isolated from each pig). The paired t-test was used in comparisons between repeated agonist effects in the same vessel.

Results

Of 53 arteries which were mounted on the myograph, 9 arteries were excluded from experiments: in 2 arteries, the maximal force development corresponded to less than 100 mmHg; in 5 arteries, a stable contraction level could not be achieved; in 2 arteries, the contraction level was reduced more than 50% after removal of the endothelium. The remaining 44 vessels (23 pigs) were investigated. Internal diameter (at L_{100}) was 230 (11) μm (range: 117–404 μm). Prior to removal of the endothelium, all arterial segments relaxed to bradykinin, indicating preserved function of the endothelium.

Baseline

Neither removal of the endothelium nor inhibition of NO and prostaglandin synthesis affected the baseline. Thus, there seemed to be no important basal release of NO, vasodilatory prostaglandins or other endothelium-mediated vasodilators in these arteries.

Precontractions

The maximal tension induced with norepinephrine was 3.52 (0.28) N/m, suggesting that the function of the vascular smooth muscle was intact (n 44 vessels, 23 pigs). The submaximal contractions (measured after 6-min contraction) corresponded to 85 (1)% of maximal contraction (n 44 vessels, 23 pigs). This was achieved by stimulation with 0.25–1.5 μM norepinephrine (mean of all vessels was 0.71 μM). To describe the contraction curve, the natural logarithm to the tension was plotted against the natural logarithm to time and a linear relationship was found. The contraction curve could thus be described by the slope of a line. In 5 vessels, the tension level rose during the contraction period (range: 2–9% of the precontraction level), but generally an obvious fall in tension level was observed. The tension fall varied considerably between vessels (range: 1–35% of the precontraction range). However, when comparing the time controls

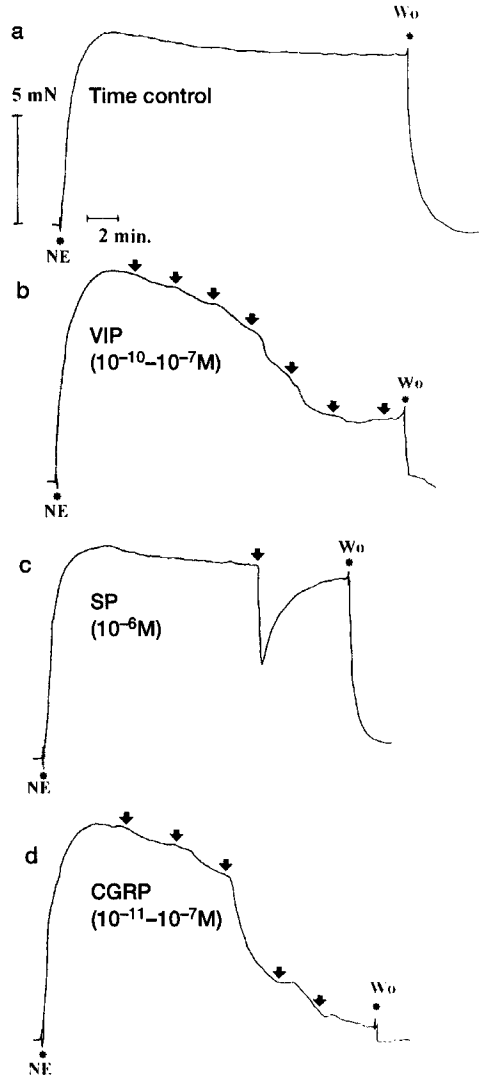
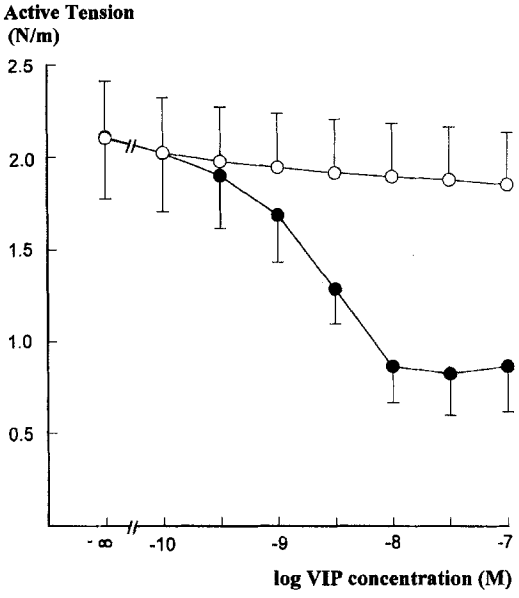


Figure 3. **Traces showing (a) time control and the effects of VIP, SP, and CGRP in a bone artery precontracted with norepinephrine.** VIP (b) and CGRP (d) induced concentration-dependent vasorelaxation. The relaxation induced by SP (c) was transient. The effect of SP was quick in onset, while responses to VIP and CGRP were slower. NE: submaximal precontraction with norepinephrine. Wo: wash out with physiological saline. (Vessel diameter at L_{100} was 225 μm).

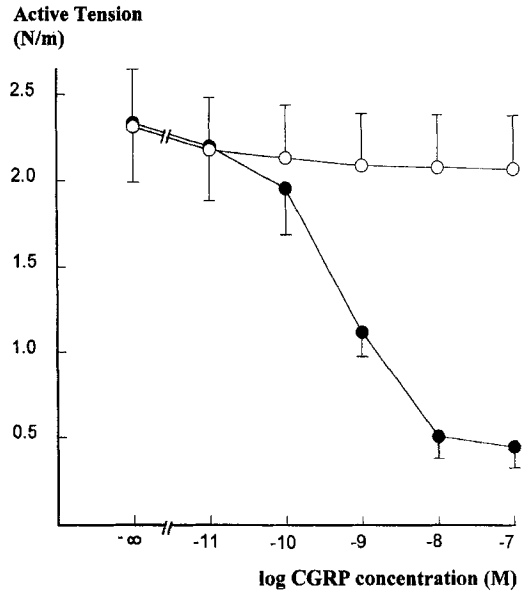
in the same vessel, there was no significant change in the slope of the curve with time. Thus, for each vessel the time control could be used as an estimated predictive baseline in vasodilator studies (Figure 3).

When the submaximal contraction was repeated, the precontraction level (after 6-min contraction) was unaltered as compared to the first stimulation ($p = 0.2$). After removal of the endothelium the precontraction level was reduced to 69 (5)% (range: 53–

Figure 4. Effects (mean ± SEM) of cumulative concentrations of VIP and CGRP after submaximal precontraction with norepinephrine.



Effect of 10^{-10}–10^{-7} M VIP (●) and the time control contraction (○), n 8.



Effect of 10^{-11}–10^{-7} M CGRP (●) and the time control contraction (○), n 15.

90)% of the maximal contraction level (p = 0.005, n 9 vessels, 9 pigs).

In arteries with preserved endothelium, addition of L-NNA did not change the precontraction level (p = 0.19), while the precontraction level was slightly but definitely increased after further inhibition with indomethacin (3.97 (0.83) N/m, compared to 3.79 (0.79) N/m, p = 0.0006, n 6 vessels, 6 pigs).

Vasoactive intestinal peptide

VIP induced concentration-dependent vasorelaxation (Figures 3 and 4). In one vessel, there was no response to VIP stimulation. In the remaining arterial segments, the maximal relaxing effect was 47 (11)% of the precontraction level (range: 7–92%, n 7 vessels, 7 pigs) and pD_2 was 8.8 (0.14) ($IC_{50} = 1.6 \times 10^{-9}$ M). When added as a single concentration of 10^{-7} M, all arteries relaxed to VIP. The relaxation was 55 (9)% of the precontraction level (range: 30–91%, n 7 vessels, 7 pigs). Thus, there was no significant difference between the maximal relaxing effect when comparing cumulative addition with single-dose addition of VIP (p = 0.4).

Substance P

All relaxations induced by SP were transient (Figure 3). When SP (10^{-11}–10^{-6} M) was added cumulatively, only 6 of 14 vessels relaxed. These relaxations were induced within the concentration range of 10^{-10} M–10^{-8} M, and the relaxations ranged from 2 to 58% of the precontraction level. When 10^{-6} M SP was added as a single concentration, relaxation was induced in 19 of 21 vessels. The mean relaxing effect was 33 (4)% of the precontraction level (range: 6–59%, n 19 vessels, 13 pigs). This finding suggests that small concentrations of SP may induce tachyphylaxis, resulting in lack of response when SP is added cumulatively.

On repeated stimulation with a single concentration of 10^{-6} M SP, the relaxation was 22 (5)% of the precontraction level (range: 4–43%, n 6 vessels, 6 pigs). This was not significantly different from the initial response to SP (p = 0.06). However, the effect of SP was totally abolished after removal of the endothelium (n 6 vessels, 6 pigs), demonstrating that SP induces vasorelaxation through an effect on the endothelium.

Calcitonin gene-related peptide

CGRP induced concentration-dependent vasorelaxation (Figures 3 and 4). Of 15 investigated arteries (9 pigs), one vessel did not respond to CGRP. The maximal relaxing effect in the remaining 14 vessels was 65 (7)% of the precontraction level (range: 14–99%) and pD2 was 9.1 (0.14) ($IC_{50} = 7.9 \times 10^{-10}$ M).

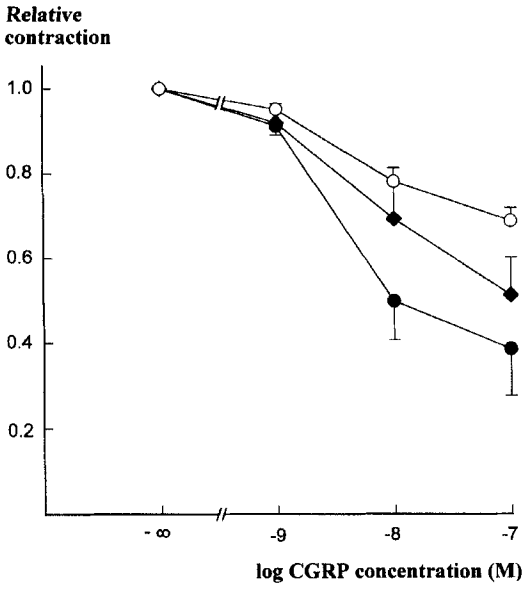


Figure 5. **Effect (mean ± SEM) of CGRP before and after removal of the endothelium.** Relative contraction of 1 equals the time control contraction. Effect of 10^{-9}–10^{-7} M CGRP (●, n 6), on repeated stimulation (✦, n 6), and after removal of the endothelium (○, n 5). The vasorelaxing effect of CGRP was still pronounced after removal of the endothelium.

When the arteries were stimulated repeatedly with CGRP, no reduction in the effect of CGRP was found (area under curve, p 0.08, n 6 vessels, 6 pigs) (Figure 5). After removal of the endothelium, the effect of CGRP was still significant (p = 0.006, n 5 vessels, 5 pigs) (Figure 5), indicating that CGRP exerts its effect partly through a vasorelaxing pathway which is not dependent on endothelial function.

In arteries with preserved endothelium, inhibition with L-NNA reduced the effect of CGRP (area under curve, p = 0.0003), while further addition of indomethacin had no significant effect on the response to CGRP (p = 0.06, n 6 vessels, 6 pigs) (Figure 6). After addition of both inhibitors, the effect of CGRP was still pronounced (p = 0.006). Thus NO and prostaglandin synthesis do not seem to be the prime pathways of CGRP-induced relaxation.

Bradykinin

Bradykinin elicited a concentration-dependent relaxation in all arteries. As shown previously (Lundgaard et al. 1996), this response was totally abolished after removal of the endothelium.

After inhibition with L-NNA, no significant reduction in pD_2 or the maximal effect was observed (p =

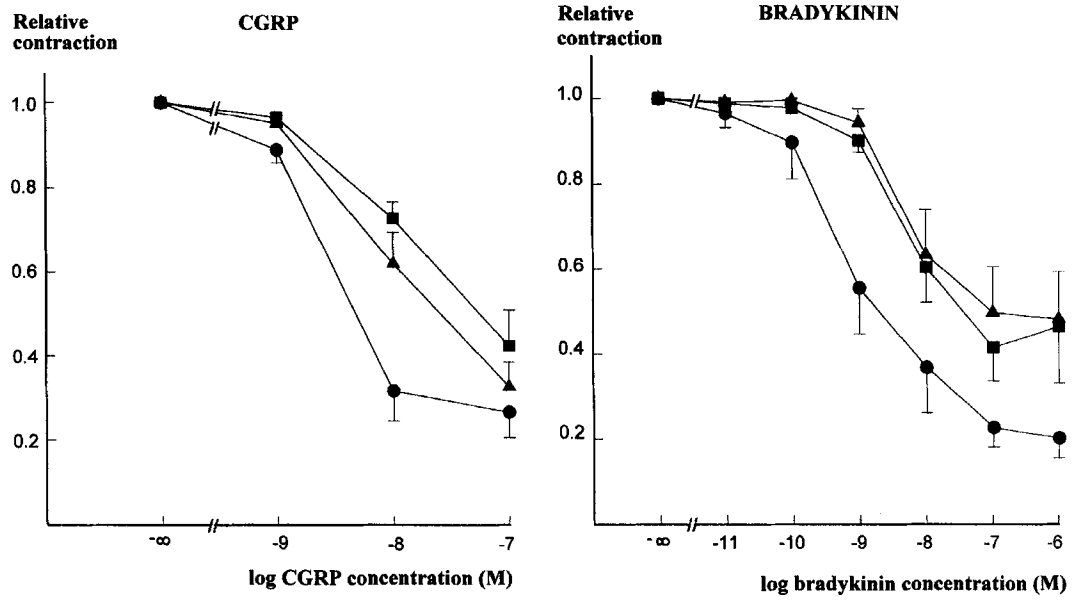


Figure 6. **Effect (mean ± SEM) of CGRP and bradykinin before and after inhibition with 10^{-4} M L-NNA and 10^{-5} M indomethacin.** Relative contraction of 1 equals the time control contraction. Effect of 10^{-9}–10^{-7} M CGRP and 10^{-11}–10^{-6} M bradykinin (●), after inhibition of NO synthesis with 10^{-4} L-NNA (■), and further inhibition of prostaglandin synthesis with 10^{-5} M indomethacin (▲), n 6 in all groups.

0.06 for both tests, n = 6 vessels, 6 pigs) (Figure 6). Further inhibition with indomethacin had no effect on pD_2 or on the maximal effect of bradykinin (p = 0.9 and p = 0.6, respectively, n 6 vessels, 6 pigs) (Figure 6). Moreover, when 10^{-4} M L-NAME was used to inhibit the NO synthase, neither pD_2 nor the maximal effect of bradykinin was affected (p = 0.2 in both tests, n 5 vessels, 5 pigs).

Discussion

Bone perfusion is influenced by neural, humoral, and metabolic parameters. In this study, we documented vasorelaxing effects of exogenous VIP, CGRP, SP, and bradykinin in isolated bone arteries.

VIP induced concentration-dependent relaxation with a pD_2 of 8.7. Compared to the study by Edvinsson et al. (1989), who found a pD_2 of 6.8 in isolated rat femoral arteries, VIP thus seems to be quite potent in bone arteries. In bone tissue, Lindblad et al. (1995) demonstrated a concentration-dependent vasodilatory effect of VIP in an in vivo perfused porcine tibia, whereas Hohmann et al. (1986) found reduced bone perfusion after i.v. infusion of VIP ($1\ g \times kg^{-1} \times min^{-1}$) in piglets. This may be due to the systemic administration of VIP in the experiment by Hohmann et al. In that study, a systemic vasodilatory effect was observed which may have led to redistribution of cardiac output, resulting in a relative reduction in bone perfusion. In another study, Hohmann et al. showed that VIP stimulates bone resorption in mouse calvaria with half-maximal effect at 25 nM (Hohmann et al. 1983). This is approximately the same concentration found to induce half-maximum relaxation in our studies, and it suggests that VIP may affect several physiological processes in the same concentration range.

We found the vasorelaxing effect of SP to be mediated via the endothelium. Furthermore, our data indicate desensitization upon continued exposure to SP. These findings are consistent with previous observations in other vascular beds (Zawadzki et al. 1981, Furchgot 1984, Mione et al. 1990).

CGRP induced concentration-dependent vasorelaxation in agreement with findings in the in situ in vivo perfused tibia model (Lindblad et al. 1995) as well as other vascular beds (Brain et al. 1985, Edvinsson et al. 1989, Mione et al. 1990, Prieto et al. 1991). In the present study, we found that only a small part of the CGRP-induced relaxation was endothelium-mediated, since relaxation by CGRP was pronounced after removal of the endothelium. This indicates that the response to CGRP is mediated mainly by a direct effect on the vascular smooth muscle. Reports on CGRP show both an endothelium-dependent pathway (Brain et al. 1985, Grace et al. 1987, Prieto et al. 1991) and an endothelium-independent pathway (Edvinsson et al. 1985, Brizzolara and Burnstock 1991, Prieto et al. 1991, Samuelson and Jernbeck 1991). This may imply variations between different vascular beds and species. The relaxation to CGRP was still significant after inhibition of both nitric oxide and prostaglandin synthesis, indicating the existence of an alternative vasorelaxing pathway.

The reported vasoactive effects of bradykinin are diverse. A vasoconstrictive effect has been demonstrated in basilar arteries isolated from dogs (Yen and Lai 1992). However, bradykinin is generally known as a potent vasodilator and both endothelium dependent and endothelium-independent pathways have been found, implying species variation (Cherry et al. 1982). We previously showed that bradykinin mediates a reproducible and endothelium-dependent relaxation in isolated porcine bone arteries (Lundgaard et al. 1996). In the present study, neither L-NNA nor L-NAME affected the response to bradykinin, indicating that the effect is not mediated via release of NO from the endothelium. After further inhibition of the cyclooxygenase, the vasorelaxing effect of bradykinin was still pronounced. This finding suggests that bradykinin-induced vasorelaxation in bone may be mediated by release of an endothelium-derived factor that is neither NO nor a vasodilatory prostaglandin. A known alternative pathway for endothelium-mediated vasorelaxation is release of endothelium-derived hyperpolarizing factor (EDHF). This is a still unidentified diffusible substance, which contributes to endothelium-dependent vasorelaxation by opening potassium channels in the underlying vascular smooth muscle (see Garland et al. 1995 for review). In pilot studies, we demonstrated a vasorelaxing effect of pinacidil, an ATP-sensitive K^+-channel opener, in bone arterial segments. Pinacidil induced concentration-dependent vasorelaxation, which was reproducible and could be blocked by glibenclamide, an ATP-sensitive K^+-channel blocker (own data). Thus, K_{ATP} channels are present in the isolated bone arteries. However, after inhibition with glibenclamide (3 μM), the vasorelaxing effect induced by bradykinin seemed to be unaffected (n 2). Therefore, ATP-sensitive potassium channels are probably not involved in the bradykinin response. Nevertheless, other potassium channels may be responsible for the bradykinin response in bone arteries, since the types of potassium channels involved in the endothelium-dependent hyperpolarization differ among blood vessels and species (Standen et al. 1989, Brayden et al. 1991, Garland et al. 1995).

Our data indicate that NO may be of minor vasoregulatory importance in porcine bone. This is consistent with findings in the perfused tibia in dogs (Brinker et al. 1990) and earlier studies on arteries isolated from porcine bone (Lundgaard et al. 1996). In the present study, the arteries were investigated in vitro and this should be taken into consideration. Thus, it could be argued that, during in vitro investigations, depletion of substrate for NO synthesis (i.e., L-arginine) may occur and this could explain the apparent insignificance of NO synthase inhibition. Moran and Wood described depletion of L-arginine, which resulted in lack of response to acetylcholine after one hour of perfusion in the perfused tibia model (Moran and Wood 1992). We did, however, consider this aspect in earlier studies and found that addition of L-arginine in concentrations of 1, 5 or 10 mM had no effect on the response to acetylcholine (Lundgaard et al. 1996). Moreover, arteries isolated from rabbit and human bone relax readily to acetylcholine after more than 6 hours of mounting, indicating sufficient stores of L-arginine in the preparation (Brogaard Hansen et al. 1996, Lundgaard et al. 1997). Therefore, NO seems to be of minor importance in vasoregulation in porcine bone.

Modulation of the vascular resistance may be only one aspect of the physiological significance of neuropeptides in bone. VIP, CGRP, and bradykinin can influence bone resorptive processes (Hohman et al. 1983, Gustafson and Lerner 1984, D'Souza et al. 1986, Roos et al. 1986, Zaidi et al. 1987). Since the neuropeptides are most abundant in areas with high mineral turnover and in connection to blood vessel walls (Bjurholm 1989), the peptides may play an integrating role in bone physiology.

In conclusion, we have demonstrated that CGRP, SP, VIP, and bradykinin can relax porcine bone arteries. This finding indicates that release of neuropeptides from perivascular nerves may modulate vascular tone in bone tissue. Unlike Brinker et al. (1990), we found that bone vessels are susceptible to direct vasodilation. VIP, SP, CGRP, and bradykinin were all found to be potent vasodilators. Therefore, we suggest that blockade of vasoconstriction by neuropeptides is an important vasodilatory mechanism in bone.

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