Vasoconstrictive action of neuropeptide Y in bone

The porcine tibia perfused in vivo

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The hemodynamic effects of neuropeptide Y (NPY) and norepinephrine (NE) in bone were studied by infusion into the nutrient artery of an in vivo and in situ perfused tibia in 19 pigs. NPY and NE caused elevation of the perfusion pressure and decline in intraosseous pressure, which was evidence of intraosseous vasoconstriction. The study suggests that NPY, along with NE, acts as a sympathetic neurotransmitter in the control of vascular tone in bone.

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It has been known for more than a century that bone is innervated (Gros 1846, Hurrel 1937). Sympathetic nerve fibers follow the course of arteries and pass through the cortex at arterial inlets. Norepinephrine (NE), the classical sympathetic neurotransmitter, is known to exert vasoconstriction in bone (Brinker et al. 1990). Consequently, sympathetic denervation of extremities increases bone blood flow (Trotman and Kelly 1963). Immunohistochemical screening of bone tissue has revealed the presence of neuropeptide Y (NPY) co-localized with NE in vascular nerve fibers, predominantly in the epiphyseal marrow, periosteum, and along the osteochondral junction at the growth plates (Bjurholm 1989). Sympathetic nerve stimulation is known to elicit the co-release of NE and NPY (Lundberg et al. 1982, 1986). Immunoreactivity to NPY is reduced dramatically by guanethedine-induced sympathectomy in rat periosteum, which is evidence of the presence of NPY in sympathetic nerve terminals (Hill and Elde 1991).

Osteoblastic cell lines have been shown to possess receptors for NPY, which downregulates the production of cAMP stimulated with parathyroid hormone and NE. This indicates interactions between local neuroendocrine peptides and systemic circulating hormones in physiological events in the skeleton, e.g., calcium homeostasis (Bjurholm 1989).

Heterotopic bone, induced by demineralized allogeneic bone matrix in rats, is gradually supplied with nerves containing NPY during the development of the ossicle, which is presumably coincident with angiogenesis. A burst of NPY containing nerve fibers has been observed at the time of bone marrow maturation. Hence NPY may be involved in the process of immunological maturation (Bjurholm 1989). Therefore vasoregulation may be an important function of NPY in bone tissue.

We investigated the vascular effects of neuropeptide Y in bone. The purposes of the study were to investigate the hemodynamic effect of NPY on bone vascular smooth muscle in vivo: to compare the vascular effects of NPY and norepinephrine in bone since both agents are co-localized in vascular nerves.

Animals and methods

Danish Landrace pigs of both sexes, weighing 60–76 kg, were used. The investigations complied with the Danish Law on Animal Experimentation and were approved by the Danish Ministry of Justice. The nutrient artery of one tibia was exposed, cannulated, and perfused at a constant rate with autologous blood via a pulsatile perfusion pump during continuous registration of the bone perfusion pressure (Figure 1). Intraosseous pressure was measured bilaterally through bone cannulae, and the central hemodynamics was checked. The NE and NPY neurotrans-

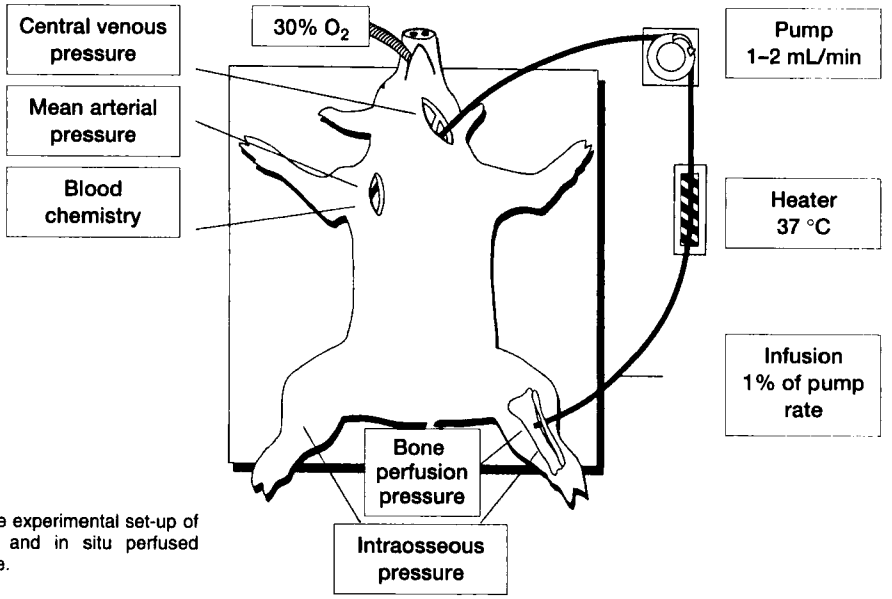


Figure 1. The experimental set-up of the in vivo and in situ perfused porcine tibiae.

mitters were infused in incremental concentration steps into the tibial nutrient artery. 2 groups were studied, the NPY group comprised 11 pigs, the NE group an additional 8 pigs.

Preparation and pressure recordings

After premedication with midazolam 0.25 mg/kg bw (Dormicum, Roche, Switzerland) and i.v. injection of methomidate chloride 2.5 mg/kg bw (Hypnodil, Janssen, Belgium), the pigs were intubated orally and ventilated mechanically with a Siemens Servo ventilator (Siemens-Elema, Sweden) with atmospheric air and oxygen (30%) (6–8 L/min, 12–14 cycles/min). Anesthesia was maintained by a continuous infusion of ketamine 8 mg/kg bw/h (Ketalar, Parke-Davis, U.S.A.), pancuronium 0.06 mg/kg bw/h (Pavulon, Organon, The Netherlands), midazolam 0.15 mg/kg bw/h (Dormicum, Roche, Switzerland), and pethidine 0.55 mg/kg bw/h (Petidin, DAK, Denmark). The animals received ampicillin 1 g/4h (Ampicillin, Nycomed, Norway), starting after the induction of anesthesia, and were then heparinized (Heparin, LEO, Denmark) with an initial dose of 400 IU/kg followed by 100 IU/kg bw/h. Intraosseous pressure (IOP) was registered in both tibiae through Radner bone cannulae (OD 2.0 mm, ID 1.4 mm) inserted percutaneously into the proximal metaphyseal cancellous bone. 17G polyethylene catheters (Viggo, Sweden) were inserted into the brachial artery for measurement of systemic arterial pressure and into the left jugular vein for measurement of the central venous pressure. All catheters were perfused with heparinized saline (1–2 mL/h).

Core temperature was monitored and adjusted with a radient heater during the experiment. Arterial gases (PaO_2, $PaCO_2$, pH) (ABL, Radiometer, Denmark), as well as plasma sodium and potassium levels (KNA, Radiometer, Denmark), were monitored intermittently and kept within the normal range.

All pressures were recorded with a liquid-filled electromanometric system (Siemens-Elema, Mingograf 805, Sweden).

Operative technique

One tibia was approached via a longitudinal anterior incision. The foramen of the nutrient artery was located posterior to the lateral tibial ridge at the junction of the proximal and the middle thirds of the diaphysis. By dissection along the popliteal artery and the anterior tibial artery, the tibial nutrient artery was exposed and catheterized with a 20G, 1.1 × 150 mm polyethylene catheter (Viggo, Sweden) through the anterior tibial artery. The nutrient artery was immediately supplied with blood from the left carotid artery by a constant volume peristaltic infusion pump (Masterflex, Cole-Parmer, U.S.A.). The collaterals and muscular branches from the nutrient artery were ligated and the preparation was checked with arteriography (Utravist 370 mg J/mL, Schering, Germany). The peristaltic infusion pump had a frequency rate of 72 ± 1.4 (mean ± SEM) per minute and the flow rate to the osseous vascular bed was set to produce an initial tibial bone perfusion pressure (BPP) equal to the mean arterial pressure (MAP). To maintain the temperature of the blood, the catheter

from the left carotid artery to the nutrient artery of tibia was coiled and positioned in a water bath at 37 °C. Bone perfusion pressure was monitored through a 3-way stop-cock inserted in the perfusion circuit between the water bath and the nutrient artery of the tibia, close to the site of cannulation (Figure 1).

Dose-response investigations

The pharmacologic experiments were performed by infusion of agonists intraarterially through a Y-connector placed close to the entry in the tibia by means of a Harvard microinfusion pump (Harvard 11, U.S.A.) set to deliver at a flow rate 1/100 of that of the peristaltic roller pump. The agonists were infused continuously for 5 min at each concentration step, after which the tibial bone perfusion pressure was allowed to return to baseline. Stable baseline recordings were required for 10 min (wash-out period) before the next infusion of a concentration of an agonist. The tibial bone perfusion pressure at baseline immediately before infusion of an agonist was recorded and so was the maximal bone perfusion pressure, obtained during agonist infusion at each infusion step.

Both NPY and NE were dissolved in isotonic saline with 0.05% serum albumin. NPY (Peninsula Laboratories, U.S.A.) was studied in concentrations ranging from 10^{-10} to 10^{-6} M and NE (Sigma, U.S.A.) was studied in the range 10^{-8}–10^{-4} M in arterial blood.

All data were expressed as the mean ± SEM. Statistical analysis employed ANOVA for repeated measurements as well as the Student's paired *t*-test.

Results

The bone perfusion pressure increased within 10 sec after injection of NPY, with a peak response 1–3 min after injection. The pressure returned to baseline within 1–15 min. The effect was marginally significant at a threshold concentration of 10^{-8} M and significant at concentrations of 10^{-7} M and 10^{-6} M (Figure 2). The maximal pressure increase was seen at 10^{-7} M (63 ± 17 percent). The baseline pressure after washout was reset to a slightly higher level, i.e., it shifted slightly upwards before infusion of 10^{-6} M NPY. The vasoconstrictive response to NE started 5–30 sec after injection. The peak pressure was reached within 1–2 min after injection, while the pressure normalized within 5–30 min after washout. The pressure increased by increasing concentrations of NE (Figure 3). The increase was marginally significant at 10^{-7} M and significant at levels of 10^{-6} M–10^{-4} M. The maximal response to NE was obtained at 10^{-4} M (105 ± 26 percent). The effect of NPY was first discerned at a concentration of 10^{-9} M. Thus, on a molar basis, NPY was more potent than NE. However, the vasoconstrictive effect, as reflected by the maximal effect, was greater for NE.

The applied doses of NPY and NE caused no systemic side-effects.

The intraosseous pressure showed a delayed response (5–10 sec) to the injection of test substance, as compared to the recorded bone pressures. NPY caused a decrease in intraosseous pressure (ANOVA; $P < 0.05$). NE also produced a significant decline in pressure (ANOVA; $P < 0.0001$). The decrease was significant in the NPY study at a concentration of 10^{-7} M and in the NE study at concentrations of 10^{-7} M or greater. In the NPY- experiments, the greatest drop was observed at 10^{-7} M (26 ± 0.07 percent), and in the NE study at 10^{-4} M (58 ± 0.06 percent) (Figure 2). An inverse linear correlation was found between intraosseous pressure and the concentration of NPY (r 0.7834, $P < 0.05$) and NE (r –0.9223, $P < 0.05$). The corresponding pressure of the normoperfused contralateral tibia was unchanged in both studies.

Systemic parameters

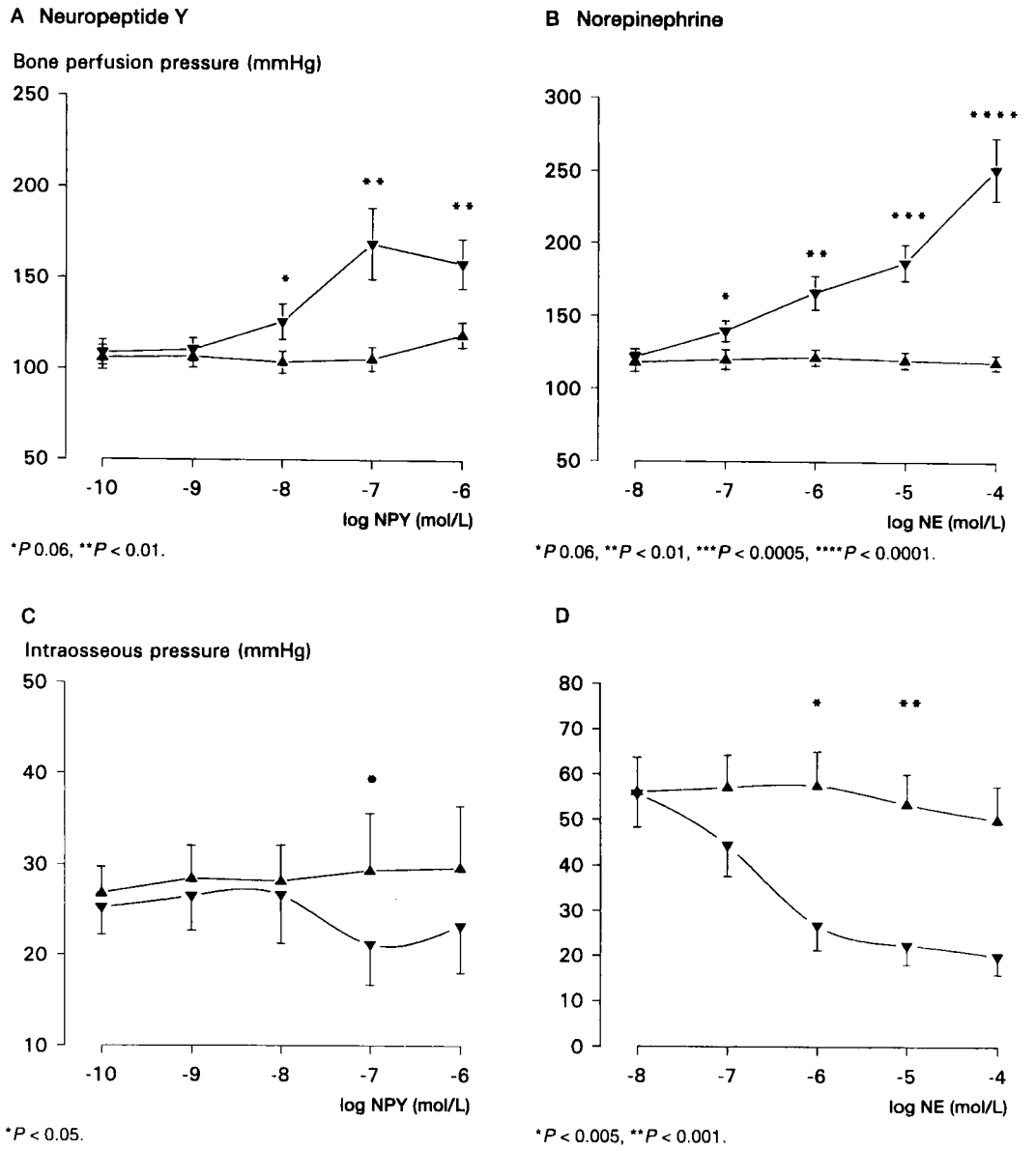
No changes in central pressures, arterial gases, blood chemistry, or core temperature were observed during the experiments.

Discussion

The circulating concentration of NPY in normal human blood has been found to be 25.8 ± 2.6 pM, i.e., several hundred times lower than that required to evoke vasoconstriction in pig bone. However, an in vivo human experiment demonstrated vasoconstriction in the splanchnic circulation at a blood concentration of 260 pM NPY (Ahlborg et al. 1992a). Plasma concentrations in this range have been found during exercise in humans (Ahlborg et al. 1992b) and in chronic hypoxia in rats (Cheng et al. 1992). Although these data suggest that circulating NPY under certain circumstances may play a physiological role, the local NPY concentration at the receptor site after nerve stimulation undoubtedly is far greater than that in circulating blood. Presumably this local release is the most physiologically relevant.

In bone, NPY elicits its maximal response at higher concentrations than in other organs (Corder et al. 1987, Ahlborg et al. 1992a, b). This may be due to variations in vascular sensitivity to NPY in differ-

Figure 2. Bone perfusion pressure and intraosseous pressure by concentrations of neuropeptide Y (A and C) or norepinephrine (B and D). Values are mean (SEM). –▲– is pressure before infusion and –▼– maximal pressure at infusion.



ent organs. Thus, circulation in the mesenterium and kidneys exhibits a higher sensitivity than in skeletal bone. Differences in mode of administration, i.e., bolus injection or continuous infusion (Corder et al. 1987), and differences between species may also play a role. However, the potency of NPY found in bone in the present investigation is in accordance with in vitro findings in arteries of rats, cats, rabbits, and pigs, even though the studies vary considerably with respect to species and experimental preparation employed (Ahlborg et al. 1992a, b).

The vascular activity in bone is known to be controlled by local regulatory mechanisms (autoregulation), vasoactive metabolites, circulating vasoconstrictors, and neural regulation. The present study in pigs verifies that the infusion of norepinephrine into the tibial nutrient artery enhances the bone perfusion pressure, as previously shown in vitro and in vivo (Brinker et al. 1990, Davis and Wood 1991). However, our threshold concentration of NE seems higher

than that observed in previous studies on bone and other organs (Corder et al. 1987, Davis and Wood 1991). The discrepancies may be ascribed to variations in mode of administration and tissue sensitivity.

It is established that NE is the principal catecholamine transmitter in sympathetic nerve terminals in the smooth muscle of skeletal blood vessels. The postsynaptic receptors mediating the excitatory responses of arterial vasoconstriction in bone belong to the alpha-receptor group. NE is known to have specificity for $alpha_1$ and $alpha_2$ receptors in various organs, including bone (Davis and Wood 1991). Interestingly, the vasoconstriction in cats, elicited by the injection of NPY, has been shown to be resistant to alpha-adrenergic receptor blockade (Lundberg and Tatemoto 1982). It is not known whether the role of NPY in sympathetic neurotransmission in the vascular bed of bone is to potentiate the noradrenergic response by modulating the presynaptic release of norepinephrine or to act directly as a vasoconstrictor.

Many studies have been done on the hemodynamics of the bone marrow circulation (Shaw 1963, Morris and Kelly 1980, Bünger et al. 1981). The registration of intraosseous pressure has exposed many methodological and interpretational problems, relating to species, age and size, site of measurement, the influence of bone blood flow and the venous outflow resistance from bone (Bünger et al. 1981, Tøndevold and Bülow 1984). Intraosseous pressure measured by bone cannulation reflects the pressure in a small hematoma at the needle tip. This pressure mainly depends on the venous pressure in bone. Both NPY and NE caused significant lowering of the intraosseous pressure. The decrease in intraosseous pressure observed with NE confirms previous observations (Shaw 1963). Presumably, the decrease in intraosseous pressure can be explained by a decrease in bone blood flow, since the arterial resistance increases because of arteriolar vasoconstriction. It is not known whether NPY has a direct effect on venous outflow resistance in bone (Luu et al. 1992). The intraosseous veins have no muscular coat but, theoretically, NPY may modulate venous tone at the outlet from bone.

The doses of NPY and NE used in our experiment had no side-effect on systemic blood pressure. However, Lundberg et al. (1982) found slight bradycardia and long-lasting enhancement of systemic arterial blood pressure in cats after intraarterial injection of 100 pmol/kg.

Acknowledgements

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References

Ahlborg G, Weitzberg E, Sollevi A, Lundberg J M. Splanchnic and renal vasoconstrictor and metabolic responses to neuropeptide Y in resting and exercising man. Acta Physiol Scand 1992a; 145 (2): 139–49.

Ahlborg G, Weitzberg E, Lundberg J M. Splanchnic and renal vasoconstriction during neuropeptide Y infusion in healthy humans. Clin Physiol 1992b; 12 (2): 145–53.

Bjurholm A. Neuroendocrine peptides in bone. Thesis, Karolinska Institutet, Stockholm, Sweden 1989.

Brinker M R, Lippton H L, Cook S D, Hyman A L. Pharmacological regulation of the circulation of bone (published erratum appears in J Bone Joint Surg Am 1990 Sep; 72(8):1270). J Bone Joint Surg Am 1990; 72 (7): 964–75.

Bünger C, Sørensen S S, Djurhuus J C, Lucht U. Intraosseous pressures in the knee in relation to simulated joint effusion, joint position, and venous obstruction. An experimental study in growing dogs. Scand J Rheumatol 1981; 10 (4): 283–8.

Cheng J T, Chen C F, Shum A Y, Wang J Y, Chen H I. Increase of plasma neuropeptide Y-like immunoreactivity following chronic hypoxia in the rat. Neurosci Lett 1992; 140 (2): 211–4.

Corder R, Lowry P J, Withrington P G. The actions of the peptides, neuropeptide Y and peptide YY, on the vascular and capsular smooth muscle of the isolated, blood-perfused spleen of the dog. Br J Pharmacol 1987; 90 (4): 785–90.

Davis T R, Wood M B. The effects of ischemia on long bone vascular resistance. J Orthop Res 1991; 9 (6): 883–9.

Gros M. La disposition des nerfs des os. Bull Soc Anat Paris 1846; 16: 273–84.

Hill E L, Elde R. Distribution of CGRP-, VIP-, D beta H-, SP-, and NPY-immunoreactive nerves in the periosteum of the rat. Cell Tissue Res 1991; 264 (3): 469–80.

Hurrel D J. The nerve supply of bone. J Anat 1937; 72: 54–61.

Lundberg J M, Tatemoto K. Pancreatic polypeptide family (APP, BPP, NPY and PYY) in relation to sympathetic vasoconstriction resistant to alpha-adrenoceptor blockade. Acta Physiol Scand 1982; 116 (4): 393–402.

Lundberg J M, Terenius L, Hökfelt T, Martling C R, Tatemoto K, Mutt V, Polak J, Bloom S, Goldstein M. Neuropeptide Y (NPY)-like immunoreactivity in peripheral noradrenergic neurons and effects of NPY on sympathetic function. Acta Physiol Scand 1982; 116 (4): 477–80.

Lundberg J M, Hemsen A, Fried G, Theodorsson Norheim E, Lagercrantz H. Co-release of neuropeptide Y (NPY)-like immunoreactivity and catecholamines in newborn infants. Acta Physiol Scand 1986; 126 (3): 471–3.

Luu T N, Chester A H, O Neil G S, Tadjkarimi S, Yacoub M H. Effects of vasoactive neuropeptides on human saphenous vein. Br Heart J 1992; 67 (6): 474–7.

Morris M A, Kelly P J. Use of tracer microspheres to measure bone blood flow in conscious dogs. Calcif Tissue Int 1980; 32 (1): 69–76.

Shaw N E. Observations on the intramedullary blood-flow and marrow-pressure in bone. Clin Sci 1963; 24: 311–8.

Trotman N M, Kelly W D. The effect of sympathectomy on blood flow to bone. JAMA 1963; 183: 121–2.

Tøndevold E, Bülow J. Microsphere determined regional bone blood flow in conscious dogs at rest and long time muscular exercise. Bone-Circulation 1984; 175–7.