Hypotensive action of parathyroid hormone preparations on rats and dogs

(synthetic parathyroid hormone/vasodilation/perfused kidney/perfused hindlimb/helical aorta strip)

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ABSTRACT  Bovine parathyroid extract and two commercial preparations containing the first 34 amino acids of synthetic bovine parathyroid hormone [bPTH(1–34)] produced dose-related hypotension in anesthetized rats. Dogs were 10 times more sensitive to the two bPTH(1–34) preparations than were rats. Propranolol, phentolamine, atropine, and promethazine did not affect the hypotensive action of bPTH(1–34) in rats and dogs. bPTH(1–34) decreased perfusion pressure in rat hindlimbs perfused in situ with Ringer’s solution and was a vasodilator in dog kidneys perfused in vitro with Ringer’s solution. Helical strips of rabbit aorta were also relaxed by bPTH(1–34). We conclude that the direct vasodilatory action of bPTH preparations represents an intrinsic property of parathyroid hormone and that the hypotensive effect of this hormone is produced by part or all of the first NH₂-terminal 34 amino acids.

The antiuretic action of exogenous parathyroid preparations in the South American lungfish (Leptodactyulus paradoxa) was described in a previous report (1). Because renal function in lungfish is significantly affected by systemic blood pressure (2, 3), we suspected that the antiureasis resulting from administration of parathyroid extracts might be related to a hypotensive action of this hormone. A vasodilatory action of parathyroid extract and the peptide containing the first 34 amino acids of synthetic bovine parathyroid hormone [bPTH(1–34)] has been reported in dogs (4, 5). This hypotensive action of parathyroid hormone (PTH) has not been confirmed in any other vertebrate species. Furthermore, it was not clear whether the hormone is itself hypotensive or whether its action is mediated through other endogenous vasoactive substances.

In a recent series of studies, we observed the vasodepressor actions of synthetic bPTH(1–34) in the following vertebrates: the South American lungfish, the bullfrog, the water snake Natrix fasciata, and the domestic chicken (6). In the present studies we investigated the specific hypotensive actions of bPTH in the laboratory white rat and the mongrel dog. A dose-related vasodepressor response was demonstrated with two preparations of synthetic bPTH(1–34). In both rats and dogs, the hypotensive action of bPTH(1–34) was not inhibited by α- or β-adrenergic, cholinergic, or histaminergic blocking agents. Furthermore, vasodilation could be demonstrated by using dog kidneys in vitro and rat hindlimbs perfused in situ with mammalian Ringer’s solutions. Helical strips of rabbit aorta were also relaxed by bPTH(1–34). On the basis of these results we propose that mammalian PTH has an intrinsic direct vasodilatory action on the vascular system. The exact mechanism of this action remains to be demonstrated.

MATERIALS AND METHODS

Three types of experiments were performed. Dogs and rats were used to determine the depressor effect of bPTH preparations in vivo. To test the vasodilatory effects of the synthetic bPTH(1–34) in vitro, dog kidneys and rabbit aortic strips were perfused. In addition, rat hindlimbs were perfused in situ with bPTH(1–34). Synthetic bPTH(1–34) was used in the experiments in vitro and in situ. bPTH (Eli Lilly) and preparations of bPTH(1–34) from Beckman Bioproducts (Palo Alto, CA) and Peninsula Laboratories (San Carlos, CA) were tested in the experiments in vitro. The Eli Lilly extract contained 100 USP units/ml as determined by the standard in vivo dog assay for hypercalcemic activity. bPTH(1–34) from Beckman Bioproducts and Peninsula Laboratories contained 6000 and 10,000 international units (IU)/mg, respectively, according to the in vitro assay for adenylate cyclase activation in the rat renal cortex. The synthetic peptides were dissolved in physiological saline to give a concentration of 1000 IU/ml. When physiological saline was tested, no significant effects were observed in any of our experimental preparations.

Experiments In Vivo. (i) Dogs. Mongrel dogs of both sexes, weighing between 6 and 15 kg, were anesthetized with an intravenous injection of 30 mg of sodium pentobarbital per kg of body weight. The femoral artery and vein were cannulated with polyethylene tubing PE 200. The trachea was also intubated, but the animals were allowed to breathe spontaneously. The arterial blood pressure was determined with a Statham pressure transducer and recorded on a Grass polygraph. Mean arterial blood pressure (MAP) was calculated for the periods before and after hormonal injection. Changes in MAP were recognized as the differences between the MAP before injection and the lowest MAP after bPTH or bPTH(1–34) injection. Several bolus injections of bPTH were given to each dog. The MAP was allowed to return to the resting level between injections. Each injection was followed by a wash of 2 ml of 0.9% saline. bPTH(1–34) from both Beckman Bioproducts and Peninsula Laboratories was tested in these anesthetized dogs at doses of 1, 2, 4, and 10 IU/kg of body weight. The various doses were given in random orders.

(ii) Rats. Sprague–Dawley rats of both sexes, weighing between 100 and 200 g, were anesthetized with sodium pentobarbital and the right carotid artery and jugular vein were cannulated with polyethylene tubing PE 50. bPTH was administered and blood pressure was recorded in the anesthetized animals as described above for dogs. The doses of bPTH(1–34) used were 10, 20, 40, and 100 IU/kg.

Abbreviations: PTH, parathyroid hormone (parathyrin); bPTH(1–34), amino acid residues 1–34 of bovine PTH; IU, international units; MAP, mean arterial blood pressure.

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(iii) Pharmacological antagonist studies. Rats and dogs were prepared as outlined above. The effects of propranolol, phentolamine, atropine, and promethazine on the depressor action of PTH-(1-34) was established, an agonist was injected in an effective dose. The specific antagonist was then given slowly. As soon as the MAP stabilized, the same dose of antagonist was given again. If the agonists’ actions were blocked, a test dose of bPTH-(1-34) was then given immediately. In dogs, the test dose of bPTH-(1-34) was 5 IU/kg and in rats, 40 IU/kg. Iso- proterenol (0.1 μg/kg) was the β-adrenergic agonist used and propranolol (2 mg/kg) was the antagonist. Norepinephrine (0.5 μg/kg) served as the α-adrenergic agonist and phentolamine (2 mg/kg), the antagonist. Atropine (2 mg/kg) was used to block the action of methacholine (1 μg/kg). The antihistamine promethazine (2 mg/kg) was used to inhibit the depressor effect of histamine (1 μg/kg). Each agonist—antagonist combination was tested in 5 dogs and 12 rats.

Experiments In Vitro. (i) Dog kidney perfusion. Mongrel dogs of both sexes, weighing approximately 14 kg, were anesthetized with sodium pentobarbital (30 mg/kg, intravenous). A midline incision was made in the abdomen and the kidneys were visualized. A clamp was then placed across the renal artery and vein near the descending aorta to ensure that a maximal length of the renal artery remained with the kidney. All vessels to the kidney were sectioned and the freed kidney was placed in cold heparinized lactate Ringer’s solution at 4°C. Cannulae were inserted into the renal artery (arteries), and the kidney was perfused with cold heparinized Ringer’s solution. All accessory renal arteries were ligated. If the kidney was properly flushed after 10 min of perfusion (as determined by a blanched appearance), it was connected to a pulsatile pressure kidney preparation apparatus (Waters MOX 100 Tm renal transport system). If the kidney was not successfully flushed, it was not used. The flow rate through the kidney was determined by measuring the length of time required to deliver 20 ml of perfusate through the kidney. Flow rate measurements were made at 1-min intervals. If a stable flow rate was seen after 1½ to 2 hr, the kidney was considered acceptable for testing the action of bPTH-(1-34). Kidneys in which flow rates failed to stabilize were also discarded.

When a steady baseline flow rate was established, bolus injections of bPTH-(1-34) at doses of 10, 50, 100, and 200 IU were made into the arterial perfusion. A second dose of bPTH-(1-34) was not given until the kidney recovered from the previous injection. In total, four kidneys were successfully used to study the action of bPTH-(1-34).

(ii) Isolated rabbit aortas. New Zealand White rabbits of either sex weighing approximately 1 kg received daily injections of reserpine (0.3 mg/kg) for 7 days to deplete catecholamine stores. On the 8th day, the animals were stunned by a blow to the back of the head and exsanguinated. After thoracotomy, a section of the thoracic aorta between the aortic arch and the diaphragm was removed and placed in oxygenated Ringer’s solution of the following composition (mM): NaCl, 154; KCl, 5.4; CaCl2, 2.4; NaHCO3, 6.0; and glucose, 11.0. The aortas were cleaned of all fat and connective tissue and cut into helical strips according to the method of Furchgott (7). The aortic strip was then arranged in a tissue holder assembly and placed in a tissue chamber. The free end of the aorta was attached to a Grass FT 03 force displacement transducer by a silk thread. Isometric contractions were recorded on a Grass model 79 polygraph. Before exposure to drugs, each aorta preparation was equilibrated for 60 min under a resting tension of 2 g. During the equilibration period, the aortic strips were washed every 15 min. The solution in the tissue chambers was continuously oxygenated with a gas mixture of 95% O2 and 5% CO2 and maintained at 37°C.

After the equilibration period, vasoconstriction was induced by using the α-adrenergic agonist methoxamine (100 μM). When a steady tension was achieved, a cumulative dose–response curve for the vasodilatory effect of bPTH-(1-34) was obtained. bPTH-(1-34) was added to the tissue bath to reach concentrations of 5, 10, and 20 IU/ml. The entire dose–response curve was obtained within 45 min, a period during which the contractile effect of methoxamine was not altered.

Experiments In Situ with Perfused Rat Hindlimbs. Male Sprague–Dawley rats weighing between 200 and 300 g were anesthetized with sodium pentobarbital (50 mg/kg, intravenous). After a midline incision had been made, the abdominal aorta below the kidneys was isolated and cannulated with polyethylene tubing. Through this cannula the hindlimbs and the posterior part of the body were perfused with the aid of a Buchler polysalt pump with a modified Krebs–Henseleit solution of the following composition (mM): NaCl, 115.3; KCl, 4.6; CaCl2, 1.8; NaHCO3, 22.1; KH2PO4, 1.1; MgSO4, 1.1; and glucose, 11.1. In addition, dextran (2%), heparin (5000 IU/liter), and ascorbic acid (38 mg/liter) were added to this perfusion medium. The medium was continuously aerated with a gas mixture of 95% O2 and 5% CO2 and maintained at room temperature (approximately 25°C). The perfusion pressure was recorded with a Statham pressure transducer connected to the arterial cannula with a T connector. When the cannulation was completed and perfusion had begun, the inferior vena cava was severed, allowing free outflow of the perfusate. The rat was then killed by intracardiac injection of sodium pentobarbital. The entire procedure from cannulation to outflow of perfusate required less than 2 min.

The flow was adjusted to increase the perfusion pressure to approximately 70 mm Hg (1 mm Hg = 133 Pa). After a 30-min equilibration period, methoxamine (1 mM) was added to the perfusate to increase the perfusion pressure to 150 mm Hg. While the preparation was under the vasoconstricting effect
RESULTS

The dose-related responses of dogs and rats to bPTH are shown in Fig. 1. In both species, increased doses of all bPTH preparations produced increasing depressor responses. bPTH-(1–34) from both Beckman Bioproducts and Peninsula Laboratories had very similar effects in dogs. 1 IU/kg lowered the MAP by 10 mm Hg and 10 IU/kg, by approximately 40 mm Hg. Rats appeared to be 10 times less sensitive to the bPTH preparations. Eli Lilly bovine parathyroid extract and Peninsula bPTH-(1–34) had almost superimposable dose–response curves. In both species, low doses of bPTH lowered the MAP for approximately 2 min, but the effects of high doses lasted for 20 min or longer in some animals.

In both rats and dogs, the depressor responses did not seem to be mediated through the α- and β-adrenergic, cholinergic, or histaminergic receptors. In dogs (Fig. 2A), propranolol, atropine, phentolamine, and promethazine almost completely blocked the action of isoproterenol, methacholine, norepinephrine, and histamine, respectively. In each instance, however, the depressor response to Peninsula bPTH-(1–34) was undiminished. Although the blockade of the same agonists by the respective antagonists was not as complete in the rats, less than 50% of the agonistic action was evident after the administration of antagonists in each case. Once again, the action of Peninsula bPTH-(1–34) was unaffected (Fig. 2B).

In the four successful dog kidney perfusion experiments, increased doses of Peninsula bPTH-(1–34) caused increasing vasodilation. The results of one such experiment are given in Fig. 3. In that experiment, bolus injection of 10 IU of bPTH-(1–34) had no effect; 50 IU produced increased flow through the kidney for 4 min. A greater response, lasting 9 min, was seen with 100 IU of bPTH-(1–34). Although a still higher dose (200 IU) did not produce a greater increase in flow, the responses
to the hypercalcemic effect of PTH. By definition, in a dog, 10 USP units of PTH per kg of body weight is required to raise plasma calcium by 1 mg/100 ml 16 hr after injection. We would, therefore, not expect any significant change in plasma calcium levels seconds after injection of 1 or 2 USP units/kg. Furthermore, in perfused organ studies, the calcium level of the perfusion fluid is constant. In rats, both bPTH and two synthetic bPTH-(1-34) preparations produced increasing hypotension with increased doses. This shows that the vasodepression is not due to a common contamination in the three different bovine PTH preparations. According to the manufacturer (Peninsula Laboratories), the synthetic bPTH-(1-34) preparation is pure. With two different solvent systems in thin-layer chromatography and in electrophoresis, only one spot was observed. Dogs are 10 times more sensitive than rats to both synthetic bPTH-(1-34) preparations; 1-2 IU/kg of body weight produced clear-cut vasodepression. This is equivalent to about 0.1 µg of the hormone per kg of body weight in a dog. This would give a blood level within the range recorded for humans and cattle. These studies further demonstrated that the vasodepressor property of the bPTH molecule resides in the first 34 NH2-terminal amino acids of the hormone. It is interesting that tachyphylaxis was not observed in either rats or dogs: repeated injections of bPTH preparations administered to the same animal produced reproducible responses.

The studies with blocking agents show that in both species bPTH-(1-34) does not work by releasing histamine or acetylcholine or by modifying the α-adrenergic, β-adrenergic, acetylcholinergic, or histaminergic receptors. This is an important observation in terms of showing the specific vasodepressor property of PTH.

The fact that bPTH-(1-34) caused vasodilation in the dog kidney and rat hindlimb perfused with Ringer’s solution provides clear evidence that bPTH-(1-34) is itself vasodilatory. The relaxation of rabbit aortic strip by bPTH-(1-34) further substantiates such a notion. In both perfused hindlimb and rabbit aortic strip preparations, an α agonist was used to produce vasoconstriction first. Because studies in vivo on the dog and the rat show that α-adrenergic receptors are not involved in the action of bPTH-(1-34), the effects of bPTH-(1-34) in these two preparations are due to bPTH-(1-34) itself and are not due to inhibition of the α-adrenergic system. Furthermore, in the dog kidney perfusion, only Ringer’s solution was used. bPTH-(1-34), however, still produced clear-cut vasodilation.

Both the dog kidney and rat hindlimb systems are more sensitive to bPTH-(1-34) than the rabbit aortic strip. This suggests that the main vasodilatory receptors for bPTH-(1-34) reside in the resistance vessels, i.e., the arterioles and venules. The rabbit thoracic aorta, being a capacitance vessel, responds poorly to the administration of bPTH-(1-34).

In conclusion, bPTH and bPTH-(1-34) are vasodilatory. This property is intrinsic in the molecule itself. The ability to lower blood pressure resides in the first 34 NH2-terminal amino acids. We believe that this present series of studies provides the definitive proof of the direct vasodilatory action of PTH.