PEPTIDES 28 (2007) 878–886

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Vasodilatory effect of tuberoinfundibular peptide (TIP39): Requirement of receptor desensitization and its beneficial effect in the post-ischemic heart

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ARTICLE INFO

Article history:
Received 19 July 2006
Received in revised form
11 December 2006
Accepted 12 December 2006
Published on line 20 December 2006

Keywords:
Vasodilatory peptides
Coronary system
Nitric oxide
Parathyroid hormone
Parathyroid hormone-related protein

ABSTRACT

Tuberoinfundibular peptide of 39 residues (TIP39) is a member of the parathyroid hormone (PTH) family and a highly specific ligand of the PTH-receptor type 2 (PTH-2r). Recent studies have shown vasoactive properties of TIP39 in the kidney. This effect was stronger after desensitization of the parathyroid hormone-receptor type 1 (PTH-1r). The aims of our study were three-fold: (1) to investigate the influence of TIP39 on coronary resistance (CR), (2) to investigate a possible cross-talk between vascular PTH-receptors in the cardiovascular system, and (3) to investigate whether the endogenously released PTHrP during ischemia induces such a desensitizing effect. Experiments were performed on isolated rat hearts that were perfused with a constant pressure (Langendorff mode) and the coronary flow was determined. Under basal conditions, TIP39 showed no influences on CR. However, TIP39 reduced the CR by approximately 22% after pre-treatment of the hearts with a PTH-1r agonist. This TIP39 effect was abolished either by co-administration of a PTH-2r antagonist or by inhibition of nitric oxide (NO) formation. In an ischemia-reperfusion model endogenously released PTHrP desensitized the PTH-1r and pre-ischemic addition of TIP39 reduced post-ischemic CR by about 28%. Again, this effect was completely abolished in the presence of the PTH-2r antagonist or the PTH-1r-antagonist or by inhibition of NO formation. However, no effect was observed when TIP39 was washed-out prior to ischemia or if the treatment with TIP39 was restricted to the reperfusion. Furthermore, a pre-ischemic application of the NO-dependent vasorelaxant bradykinin provoked a similar effect on the post-ischemic CR than TIP39. In conclusion, a NO-dependent vasodilatory effect of TIP39 was demonstrated if the PTH-1r is desensitized by either exogenously applicated PTHrP peptides or endogenously released PTHrP.

1. Introduction

A new member of the parathyroid hormone (PTH) family has recently been described, namely tuberoinfundibular peptide of 39 residues (TIP39) [34]. Other members of this family are the eponymous peptide PTH and parathyroid hormone-related peptide (PTHrP). Based on its amino acid composition, TIP39 has only a poor homology to PTH and PTHrP [35], but its secondary and tertiary structure shows a stronger similarity [25,33].

Peptides that belong to this peptide family activate common receptors, namely the PTH-receptor type 1 (PTH-1r) and the PTH-receptor type 2 (PTH-2r) [10]. Both receptors are G-protein coupled receptors and members of the same

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0196-9781/$ – see front matter © 2007 Published by Elsevier Inc.
doi:10.1016/j.peptides.2006.12.010

subgroup of the superfamily of heptahelical receptors. Both PTH-receptors share a homology of 52% [34]. Furthermore, both receptors are widely expressed in the mammalians, including the cardiovascular system (CVS) (PTH-1r [31]; PTH-2r [32]). The PTH-1r can be activated by PTH as well as by PTHrP [11]. Several signal transduction pathways are described for this receptor (e.g. cAMP/PKA or PLC). Furthermore, this receptor can rapidly be desensitized [1,5,22]. Initial studies showed the ability of PTH but not PTHrP to activate also the PTH-2r [10]. However, recent investigations discovered TIP39 as the natural and high affinity ligand of the PTH-2r [35]. PTH is a less potent activator of downstream signaling pathways (e.g. cAMP accumulation or increasing intracellular calcium) compared to TIP39 [1,3,7]. TIP39 shows no interaction with the classical PTH-1r [35].

Whereas PTH is released by the parathyroid glands as an endocrine factor, PTHrP acts as a paracrine or autocrine factor. It can also act as an intracrine factor. Among pathophysiological circumstances, such as humoral hypercalcemia of malignancy, PTHrP is released as an endocrine factor, too [30]. The expression and release of this peptide is described for many tissues and cells, even for the CVS [28]. Within the CVS, PTHrP is a very strong vasodilator of coronary vessels, and exerts a positive chronotropism and under specific circumstances also a positive inotropism [9,28]. Under conditions of pressure overload, PTHrP contributes to the progression of cardiac hypertrophy [28,38]. Some effects of PTHrP in the CVS can be mimicked by PTH, i.e. the chronotropic and vasodilative response. In contrast, the positive inotropic response seems to be specific for PTHrP. Our studies have shown that microvascular endothelial cells release PTHrP in a mechanosensitive manner or under hypoxic or ischemic conditions, respectively [2,27].

TIP39 was first investigated by Usdin and colleagues in bovine hypothalamus preparations [35]. The release mechanisms and the physiological or pathophysiological relevance are still under investigation. Initially, it was found that TIP39 contributes to the release of several hypothalamic hormones, e.g. corticotropin-releasing factor, antidiuretic hormone or vasoactive intestinal peptide [37]. It may also modify nociception [9] or the complex regulation of anxiety and depression [12]. The wide distribution of the PTH-2r and of the TIP39 expression outside the CNS suggests that TIP39 may have physiological side effects in other tissues as well [4,8,26,36]. Endothelial cells, vascular smooth muscle cells, and the myocardium express PTH-2r and TIP39 [4,8,26,36]. This observation leads to the question whether TIP39 has vasoactive properties. Eichinger et al. [4] demonstrated such properties for the first time in rat renal preparations (isolated renal vessels). They showed a concentration dependent vasodilatation mediated by TIP39 in renal vessels pre-constricted by phenylephrine [4]. More importantly, they documented a stronger vasodilatation, under conditions of desensitization of the PTH-1r. These results suggest a possible cross-talk between both PTH-receptors.

In our own investigations we demonstrated for the first time inotropic actions of TIP39 in rat hearts [26]. TIP39 seems to influence the contractility of the rat heart in two different and contrary ways. A positive inotropism mediated by an activation of the nitric oxide signaling pathway and a negative inotropism via a yet unknown pathway. An activation of the PTH-2r seems to be involved in both effects.

Since vasodilatory properties of the two other peptide-family members, namely PTH and PTHrP, have been shown in the coronary system and TIP39 exerts vasoactive properties in the kidney, the question arises whether TIP39 has any effect on the coronary flow as well and whether this effect depends on a desensitization of the classical PTH-1r or on NO formation.

2. Materials and methods

2.1. Experimental animals

Female rats (Wistar–Hannover) with a body weight of 220 ± 25 g (age: about 16 weeks) were used in the experiments. All animal studies were performed in accordance with guidelines described in the *NIH Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH, Publication no. 85-23, revised 1996). The animals were kept under standardized conditions of temperature, humidity and light. They had free access to standard diet and drinking water ad libitum.

2.2. Isolated, perfused hearts (Langendorff mode)

After rats were anaesthetized by diethyl ether and killed by breaking the neck, hearts were rapidly excised and the aorta was cannulated and connected via a 16 gauge needle with a Langendorff-perfusion system for retrograde perfusion. During the experiments hearts were mounted in a temperature-controlled chamber (37 °C) with humidified air. Hearts were perfused with oxygenated saline medium with a temperature of 37 °C [composition of the perfusate (mmol/l): 124.0 NaCl, 2.7 KCl, 0.4 NaH_2PO_4, 1.0 $MgCl_2$, 1.8 $CaCl_2$, 24.0 $NaHCO_3$ and 5.0 glucose, gassed with carbogen (95% O_2 + 5% CO_2), pH 7.4]. During the stabilization period (20 min), the perfusion pressure was adjusted to 50 mmHg and was held constant thereafter. Diastolic pressure was adjusted to 12 mmHg at the same time. Afterwards hearts were perfused according to the protocols specified in Fig. 1. The perfusion pressure was measured by a pressure transducer connected to the perfusion line just before the heart. The coronary flow [ml/min] was determined by collecting the effluents. Coronary resistance was calculated as perfusion pressure divided by the coronary flow per minute [mmHg/(ml min)]. The heart rate (HR), systolic and diastolic pressure was determined by a balloon inserted into the left ventricle and connected with a pressure transducer. The left ventricular developed pressure (LVDP) was calculated as the amplitude of the diastolic pressure and the peak systolic pressure and taken as readout for contractility. Each experimental group consisted of six hearts ($n = 6$).

2.3. Dot-blot analysis of PTHrP in coronary effluent

The effluent was collected at different time-points (basal, pre-ischemic, directly after the start of reperfusion (post-ischemic), and at the end of the reperfusion period