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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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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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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

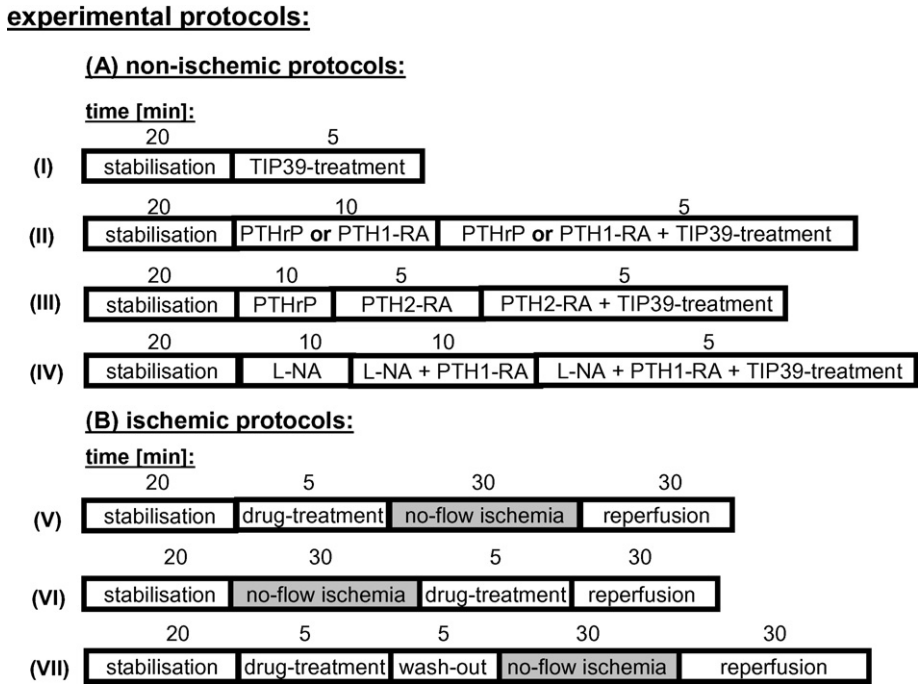

Fig. 1 – Overview of the different protocols used in this study. *Abbreviations*: TIP39: tuberoinfundibular peptide of 39 residues; PTHrP: Ile^5-PTHrP(1–36); PTH1-RA: PTHrP(7–34); PTH2-RA: Ile^5-Trp^{23}-Tyr^{36}-PTHrP(1–34); L-NA: L-nitro arginine. All experiments under non-ischemic conditions were performed in the presence of phenylephrine and atenolol.

(30 min)). The proteins were precipitated by trichloroacetic acid. Afterwards the probes were dotted on nitrocellulose membranes and incubated for 2 h with bovine serum albumin (2%) to inhibit non-specific binding. Then the membranes were incubated with the first antibody (polyclonal rabbit anti-PTHrP; Merck, Darmstadt, Germany) at 4 °C overnight. The membranes were incubated for 2 h with the second antibody afterwards (anti-rabbit gtxRb IgG; Chemicon, Hofheim, Germany). These were conjugated with alkaline phosphatase. Thereafter, the nitroblue tetrazolium reaction (substrates: nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate; product: dark-blue nitroblue tetrazolium formazan) was used to visualize the proteins. Spots were scanned and quantified electronically (Image Quant; Molecular Dynamics, Krefeld, Germany).

2.4. *Bioactive substances*

The following substances were used during the experiments: Ile^5-Trp^{23}-Tyr^{36}-PTHrP(1–36) [Ile^5-PTHrP; 100 nmol/l Bachem, Torrance, USA], TIP39 [100 nmol/l; Bachem, Torrance, USA], Nω-nitro-L-arginine (L-NA) [100 μmol/l; Sigma–Aldrich, Munich, Germany], Trp^{23}-Tyr^{36}-PTHrP(1–36) [PTH-2 receptor-antagonist (PTH-2RA) [26] 100 nmol/l; Bachem, Torrance, USA], PTHrP(7–34) [PTH-1 receptor antagonist; PTH-1RA; 100 nmol/l; Bachem, Torrance, USA] [27], phenylephrine [PE; 10 μmol/l; Sigma–Aldrich, St. Louis, USA], atenolol [At; 10 μmol/l; Sigma–Aldrich, St. Louis, USA], bradykinin [100 nmol/l, Merck, Darmstadt, Germany]. The concentration used in these experiments is based on the previously performed concentration–response curve [26].

2.5. *Statistics*

Quantitative results are expressed as means ± standard error of the means (S.E.M.). In experiments with more than two groups, an analysis of variance (ANOVA) was used for statistical comparison, with a Student–Newman–Keul's test for post hoc analysis. In cases in which only two groups were compared, conventional *t*-tests were performed. A *p*-value < 0.05 was considered as a significant difference between groups.

3. Results

The experiments described in this study can be subdivided into two parts. The effect of TIP39 on coronary resistance was analyzed (i) under non-ischemic conditions and (ii) in the post-ischemic heart. The latter one was done to study the effect of the endothelial-dependent release of PTHrP on the TIP39 effects.

3.1. *Experiments under non-ischemic conditions*

First, we investigated whether TIP39 influences the coronary resistance. Hearts were treated with TIP39 (100 nmol/l) for 5 min after an initial stabilization period. Under these conditions, TIP39 had no influence on the coronary resistance (pre-treatment [(–) TIP39]: 8.9 ± 1.1 mmHg/(ml min) to post-treatment (post-treatment [(+) TIP39]: 8.9 ± 0.6 mmHg/(ml min)). Second, we repeated these experiments but increased the coronary resistance prior to the application of

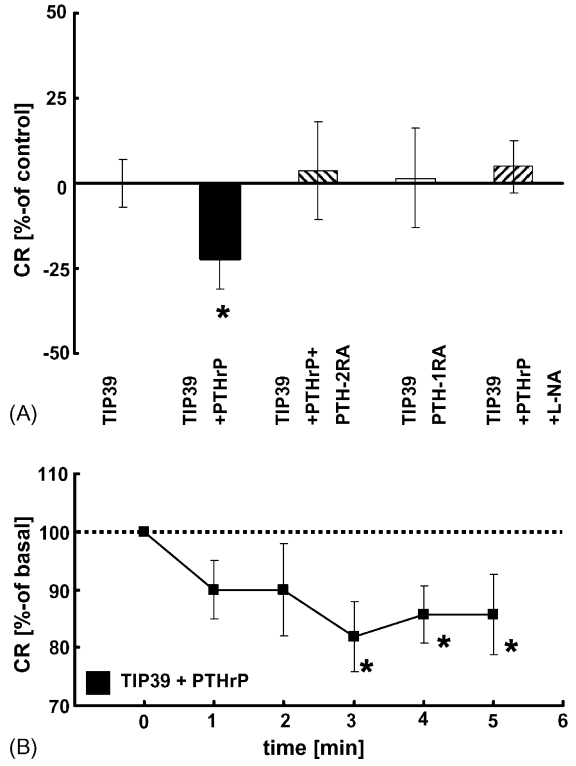

Fig. 2 – Effect of TIP39 on coronary resistance under non-ischemic conditions. (A) Changes in the coronary resistance (CR) 5 min after addition of TIP39 (100 nmol/l). Data are means ± S.E.M. (each $n = 6$; $^{*}p < 0.05$ vs. pre-treatment). For abbreviations used see Fig. 1. (B) Time dependent effect of TIP39 on the coronary resistance. Data are means ± S.E.M. from $n = 6$ experiments.

TIP39. This was done by addition of the α-adrenoceptor agonist phenylephrine (PE) in the co-presence of the of the β-adrenoceptor antagonist atenolol (At). Again, TIP39 had no influence on coronary resistance in this preparation (pre-constriction PE/At: 14.1 ± 1.8 mmHg/(ml min); post-treatment with TIP39 13.4 ± 1.3 mmHg/(ml min); $n = 6$ [Fig. 2A, column 1]). All subsequently performed experiments on non-ischemic hearts were performed under these conditions, namely in the co-presence of PE and At.

We next examined the influence of PTH-1r stimulation on the vasoactive effect of TIP39. In order to address this question, the hearts were pretreated with Ile^5-PTHrP for 10 min (protocol II) before the application of TIP39 was performed. Within the first 5 min after application of Ile^5-PTHrP the heart rate (HR) transiently increased by 22 ± 7% ($p < 0.05$ controls versus pre-treatment, $n = 6$). HR was normalized within 10 min (7 ± 4%; n.s. versus pre-treatment, $n = 6$). Renewed application of Ile^5-PTHrP at this time did not exert a positive chronotropism again (data not shown). Once the success of the PTH-1r desensitization was proved, hearts were now exposed again to TIP39. In contrast to the formerly described experiments without receptor desensitization by Ile^5-PTHrP, TIP39 reduced coronary resistance by about 22% compared to controls in a time-dependent manner (control (−) TIP39: 14.1 ± 1.6 mmHg/(ml min), treated (+) TIP39: 11.0 ± 1.0 mmHg/(ml min), $p < 0.05$ controls versus treated) (Fig. 2A (column 2) and B).

In a next step we investigated whether the vasoactive effect of TIP39 under these conditions depends on the activation of the PTH-2r. Hearts were pre-treated again with Ile^5-PTHrP. Then the potent PTH-2 receptor antagonist (PTH-2RA) Trp^{23}-Tyr^{36}-PTHrP was added (protocol III). In the presence of the PTH-2RA, TIP39 was unable to reduce the coronary resistance any more (control (−) TIP39: 8.6 ± 1.5 mmHg/(ml min), (+) TIP39: 8.9 ± 1.3 mmHg/(ml min); $n = 6$) (Fig. 2A, column 3).

In order to show that the observed TIP39 effect requires indeed a desensitization of the PTH-1r, we performed additional experiments using the N-terminal truncated peptide PTHrP(7–34) (protocol II). This peptide binds to the PTH-1r, but does not activate the receptor. As expected, PTHrP(7–34) did not influence heart rate, contractility or vessel tone (data not shown). Hearts were pre-treated with PTHrP(7–34). TIP39 did not change coronary resistance under these conditions (control (−) TIP39: 7.9 ± 1.1 mmHg/(ml min), treated (+) TIP39: 8.1 ± 1.2 mmHg/(ml min)) (Fig. 2A, column 4).

Finally, we investigated whether the vasodilatory effect of TIP39 is nitric oxide (NO)-dependent. NO formation was attenuated by addition of $N\omega$-nitro-L-arginine (L-NA) (protocol IV). Hearts were initially stabilized, and then L-NA was added for 10 min before Ile^5-PTHrP was given to desensitize the PTH-1r. Inhibition of endogenous NO-formation by L-NA reduced the coronary flow (basal: 4.5 ± 0.6 ml/min, L-NA/Ile^5-PTHrP: 3.6 ± 0.5 ml/min). The application of TIP39 had no influence on coronary resistance in the presence of L-NA (Ile^5-PTHrP/L-NA (−) TIP39: 14.1 ± 1.8 mmHg/(ml min), Ile^5-PTHrP/L-NA (+) TIP39: 14.8 ± 1.1 mmHg/(ml min)) (Fig. 2A, column 5).

3.2. *Experiments under post-ischemic conditions*

As the former experiments indicated that pharmacological desensitization of the PTH-1r is required for induction of a vasoactive effect of TIP39, we now addressed the question, whether the ischemia-dependent release of PTHrP is sufficient to desensitize the PTH-1r. Increased release of PTHrP was confirmed. As shown in Fig. 3, the amount of PTHrP in the effluent increased at the beginning of reperfusion. All subsequently described experiments show the coronary resistance during the reperfusion period after a 30 min no-flow ischemia.

In order to make sure that TIP39 is present at the beginning of the reperfusion, the peptide was given 3 min before starting the no-flow ischemia (Fig. 1, protocol V). TIP39 showed no vasodilatory effect in the pre-ischemic period, consistent with the former experiments (pre-treatment: 8.4 ± 1.3 ml/min; post-treatment: 8.5 ± 1.2 ml/min). During reperfusion, TIP39 was not added to the perfusate. Nevertheless, the coronary resistance in the hearts pre-treated with TIP39 remained lower compared to untreated control hearts (Fig. 4A). At the end of reperfusion the difference between the TIP39-treated hearts to the non-treated hearts amounted to about 28% (control (−) TIP39: 10.0 ± 2.0 mmHg/(ml min), (+) TIP39: 7.2 ± 1.4 mmHg/(ml min)). This effect of TIP39 on the reperfused rat heart was specific for the coronary resistance, as the other parameters under investigation (heart rate and left ventricular developed pressure) did not change (Table 1).

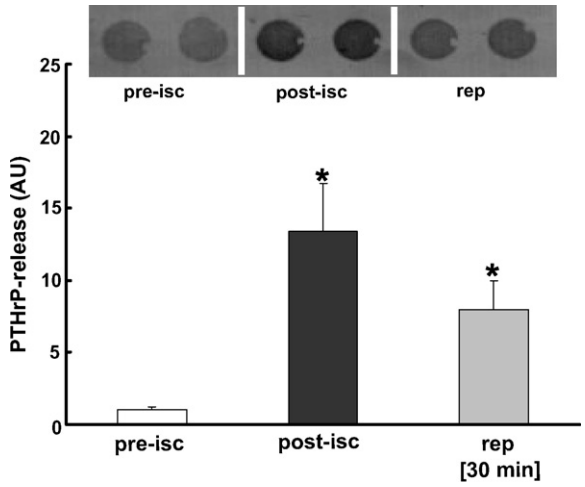

Fig. 3 – PTHrP release into the effluent. Effluents were collected before the onset of ischemia (pre-isc), at the beginning of reperfusion (post-isc), and at the end of the reperfusion (rep 30 min). Samples were collected; the proteins precipitated and spot on a dot-blot membrane. Quantification of PTHrP in the effluent was performed by incubation with a PTHrP antibody and visualization with an alkaline coupled secondary antibody. Data are means ± S.E.M. ($n = 6$; $^{*}p < 0.05$ vs. pre-isc). The insert on the top shows a representative dot blot.

In order to test whether the TIP39-dependent effect on the post-ischemic coronary resistance depends on PTH-2r activation, hearts were treated with TIP39 (100 nmol/l) and the PTH-2RA (100 nmol/l) prior to ischemia. The previously observed TIP39 effect, namely a reduction of the coronary resistance in the reperfusion period, was completely abolished when the PTH-2RA was present during ischemia together with TIP39 (Fig. 4B).

In order to show that the endogenous release of PTHrP during ischemia is responsible for the induction of the post-ischemic effect of TIP39, we used the PTH-1RA, PTHrP(7–34) (100 nmol/l). The antagonist was given in combination with TIP39 prior to ischemia (Fig. 1; protocol V). Under these conditions the PTH-1r activation and subsequent desensitization by ischemia-dependent release of PTHrP is attenuated by the PTH-1RA. No TIP39-dependent effect on the coronary resistance during reperfusion was found under these experimental conditions (Fig. 4C).

In the next set of experiments the question was addressed whether the observed effect of TIP39 on coronary resistance requires TIP39 during ischemia or whether the effect is induced by our pre-ischemic treatment. Therefore, hearts exposed to TIP39 prior to ischemia as before but the peptide was washed out before starting the ischemic period (Fig. 1, protocol VII). In the reperfusion period there were no differences between the non-treated (control (–) TIP39) and the treated (treated (+) TIP39) groups under these conditions (Fig. 5). It was further investigated whether TIP39 acts during the initial reperfusion period. Therefore, TIP39 (100 nmol/l) was administrated directly at the start of the reperfusion (Fig. 1, protocol VI). TIP39 had no acute or long-lasting effects

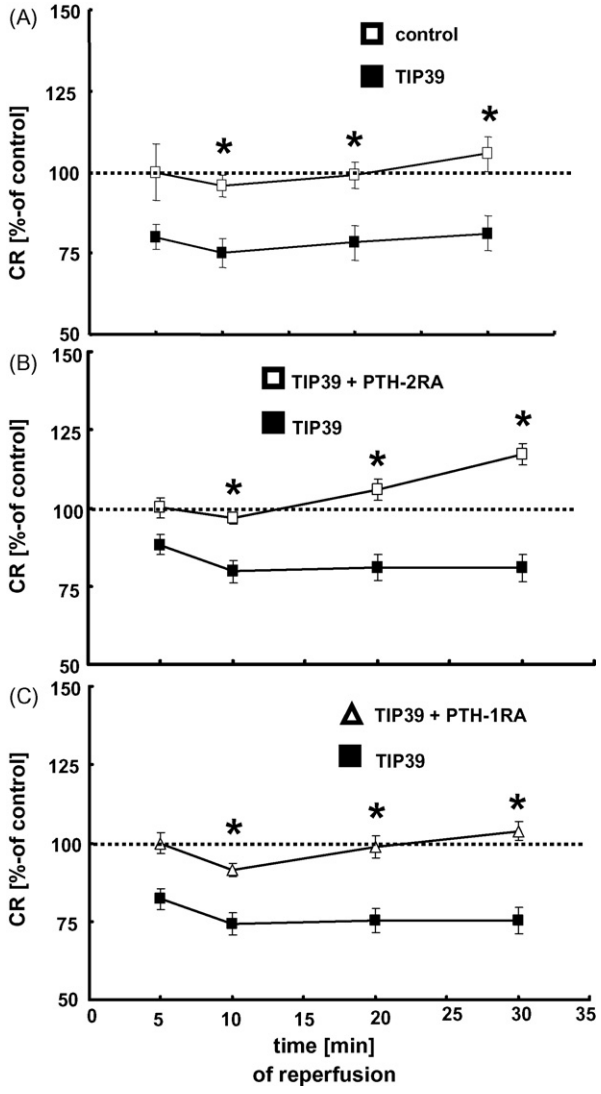

Fig. 4 – Effect of TIP39 on the post-ischemic coronary resistance (CR) with TIP39 present during ischemia. (A) The development of coronary resistance during reperfusion in hearts treated with TIP39 during ischemia compared to untreated controls. (B) The development of coronary resistance during reperfusion in hearts treated with TIP39 during ischemia compared to hearts treated with TIP39 in the co-presence of a PTH-2R antagonist (PTH-2RA). (C) The development of coronary resistance during reperfusion in hearts treated with TIP39 during ischemia compared to hearts treated with TIP39 in the co-presence of a PTH-1R antagonist (PTH-1RA). Data are means ± S.E.M. from $n = 6$ each, $^{*}p < 0.05$ vs. TIP39).

when given exclusively during reperfusion (Fig. 6). Both experiments that are described in Figs. 5 and 6 show that TIP39 must be present during ischemia to induce this effect.

Subsequently we addressed the question whether the post-ischemic effect of TIP39 is also NO-dependent as found for the aforementioned pre-ischemic effect. L-NA was added in combination with TIP39 directly prior to ischemia for 5 min. During the ischemic period both substances were present in

Table 1 – The effect of TIP39 on the contractile performance of the hearts

	LVDP (mmHg)	HR (bpm)	dP/dt_{max} (mmHg/s)	dP/dt_{min} (mmHg/s)
Pre-ischemic				
C ($n = 6$)	114 ± 7	236 ± 9	7044 ± 229	5520 ± 726
TIP39 ($n = 6$)	107 ± 2	250 ± 11	6634 ± 127	5167 ± 409
	$p < 0.05$	n.s.	$p < 0.05$	n.s.
0 min reperfusion				
C ($n = 6$)	93 ± 7	201 ± 25	4898 ± 899	3890 ± 969
TIP39 ($n = 6$)	95 ± 5	243 ± 19	5705 ± 483	4291 ± 1833
	n.s.	n.s.	n.s.	n.s.

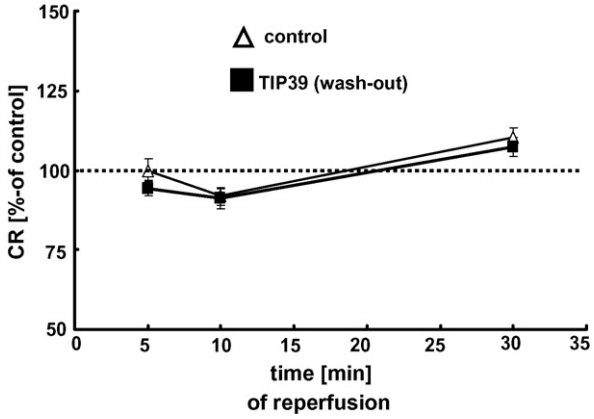

Fig. 5 – Effect of TIP39 on post-ischemic coronary resistance with TIP39 added prior to ischemia and washed out before no-flow ischemia was started (TIP39 wash-out). Data are means ± S.E.M. from $n = 6$ each, n.s. vs. untreated controls.

the coronary system. In the pre-ischemic period TIP39 did not alter coronary resistance, but significantly reduced left ventricular developed pressure (LVDP) and +dP/dt, by $-24.7 \pm 10.8\%$ and $-11.7 \pm 1.9\%$, respectively ($p < 0.05$, $n = 6$). However, in the co-presence of L-NA (100 μmol/l) TIP39 was unable to decrease coronary resistance during reperfusion (Fig. 7).

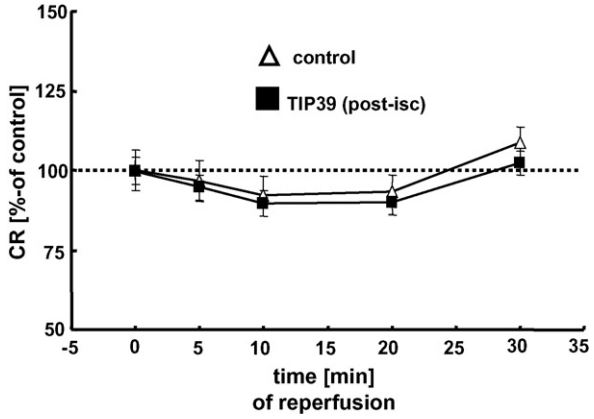

Fig. 6 – Effect of TIP39 on the post-ischemic coronary resistance with TIP39 present after ischemia. Data are means ± S.E.M. from $n = 6$ each, n.s. vs. untreated controls.

These results led us assume, that the long-lasting effect of TIP39 is NO-dependent. To investigate whether NO-release per se exerts such an effect, we repeated the initial ischemic experiment and used bradykinin instead TIP39 to induce NO formation. Hearts were treated according to protocol V (Fig. 1). As expected, the pre-ischemic application of bradykinin caused a decrease in the coronary resistance (data not shown). However, in the reperfusion period bradykinin decreased the coronary resistance in a similar and long lasting manner than TIP39 (Fig. 8).

4. Discussion

Our results demonstrate for the first time vasodilatory properties of TIP39 and a cross-talk between the PTH-1r and the PTH-2r in the coronary system. The conclusion is based on our finding, that TIP39 has no vasoactive properties under basal conditions but after desensitization of the PTH-1r. Receptor desensitization was performed in our experiments either by exogenously added PTHrP (Ile^5-PTHrP) or by stimulation of endogenously release of PTHrP during ischemia. TIP39 increased coronary flow under such conditions in a NO-dependent way. These results suggest that the PTH-1r inhibits the coupling of PTH-2r to the NO pathway. Our conclusion that the observed TIP39 effects on coronary resistance are PTH-2r-dependent comes from the following observations: (i) TIP39 is a highly selective ligand for this receptor, (ii) the effects are attenuated in the co-presence of the highly specific receptor antagonist Trp^{23}-Tyr^{36}-PTHrP(1–36), and (iii) the effects are NO-dependent, a pathway well known to be activated by PTH-2r activation.

Endothelial cells display the highest expression of the PTH-2r outside the brain [36]. They are the source for NO formation and the release of other vasorelaxing factors, like endothelium-derived hyperpolarizing factor (EDHF) or PTHrP. Our own investigations have shown that endothelial cells strongly express TIP39 mRNA [26]. Although not proven at present one may speculate that endothelial cells release TIP39 as a local factor that acts in a paracrine or autocrine manner. Such a release could be demonstrated for PTHrP, too [28]. Eichinger et al. [4] reported about a TIP39-dependent vasodilation in renal vessels pre-constricted by phenylephrine even under basal conditions. Such an effect was not observed in the coronary vessels that were analyzed in this study. Nevertheless, these authors found that in renal vessels pre-treated with PTHrP, TIP39-dependent vasodilation increased by about 60% compared to the basal situation.

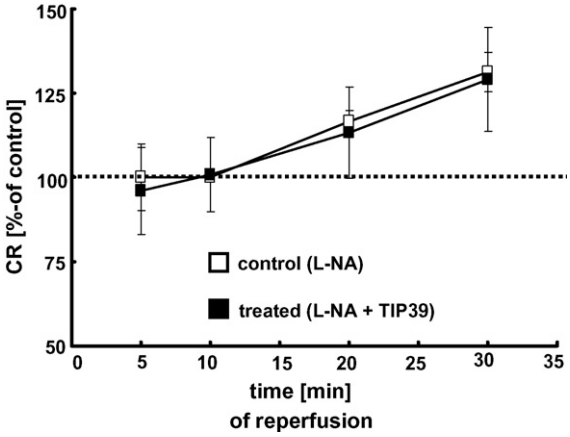

Fig. 7 – Effect of L-nitro arginine (L-NA) on the coronary resistance and the changes evoked by TIP39 during ischemia, respectively. Data are means ± S.E.M. from $n = 6$ each, n.s. vs. untreated L-NA alone.

From these former results a cross-talk between the two PTH-receptors has been proposed. To prove such a cross-talk we desensitized the PTH-1r either by addition of exogenous PTHrP or by the stimulation of endogenous PTHrP-release during ischemia. Our results suggest that the increased release, stimulated by ischemia, and the accumulation of PTHrP during this period was sufficient to desensitize the PTH-1r. A desensitization of the PTH-1r either by PTHrP or PTH has been described by several authors in various vascular models [4,17,18,21,22]. Our results obtained with PTHrP(7–34) as a PTH-1r antagonist, demonstrated that binding to the PTH-1r alone is insufficient to induce a TIP39-dependent effect. Our assumption is in accordance with results of Nickols et al. [20]. The mechanism by which the PTH-1r undergoes receptor desensitization and internalization are controversially discussed (e.g. phosphorylation, PKC- and/or cAMP/PKA-pathways, contribution of β-arrestin) [6,16,29]. Recent studies have shown, that β-arrestin 2 not only leads to internalization of the receptor, but down-regulates the activity of the coupled G-protein. Therefore, downstream signals such as cAMP accumulation are decreased [6]. Keeping this in mind, cAMP may trigger receptor desensitization and this would explain why PTHrP(7–34) in unable to induce such an effect, because it is lacking the adenylylcyclase-activating domain.

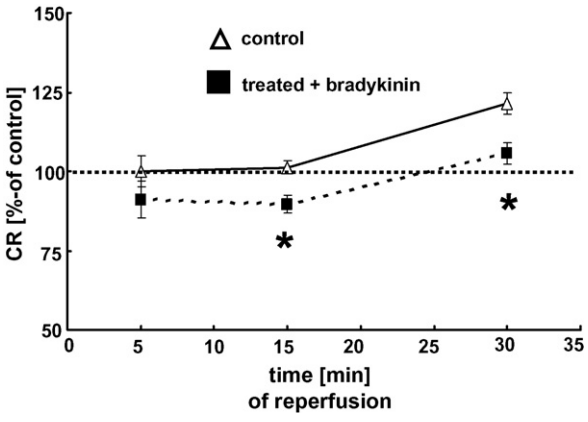

Fig. 8 – Effect of bradykinin on the post-ischemic coronary resistance (CR) with bradykinin present during ischemia. Data are means ± S.E.M. from $n = 6$ each, $^{*}p < 0.05$ vs. untreated controls.

The vasodilating effect of TIP39 displayed an acute and a long-lasting part. Eichinger et al. observed a long lasting effect in renal vessels of the rat, too [4]. From our experiments using L-NA as an inhibitor of endogenous NO-formation we conclude, that the vasodilating effect of TIP39 is NO-dependent. This hypothesis is further highlighted by the additional finding that bradykinin induced a similar effect. These results are in accordance with experiments of Parratt et al. [23,24], who identified NO as a trigger of bradykinin-dependent preconditioning. Such a protective effect of NO was also suggested by Laude et al. [13,14]. Some authors have suggested, that the protective effect of NO on post-ischemic hearts is related to an interaction between blood cells and the endothelium (platelets aggregation or neutrophil adhesion) [15]. However, our experiments were performed in a blood free system. Therefore, it is oversimplified to restrict the NO-effect to such interactions. This notion is further supported by the work of Muscari et al. [19].

5. Conclusion

In summary our data indicate long lasting vasodilatory effects mediated by TIP39 on the coronary resistance that may be of relevance in the post-ischemic myocardium. Furthermore, the data indicate an interaction between both PTH-receptor subtypes in the heart. Ischemia-dependent endogenously released PTHrP is desensitizes the PTH-1r. This enables the activation of PTH-2r by TIP39. PTH-2r activation dilates vessels in a NO-dependent manner. The data confirm in the myocardium a similar mechanism than that previously shown for the renal perfusion. Whether endothelial-derived TIP39 can exert such effects requires future studies. The present study shows, nevertheless, that endogenous PTHrP can be release in sufficient amounts to desensitize the vascular bed to an extent that allows PTH-2r coupling.

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REFERENCES

[1] Castro M, Dicker F, Vilrdaga JP, Krasel C, Bernhartdt M, Lohse MJ. Dual regulation of the parathyroid hormone (PTH)/PTH-related peptide receptor signalling by protein kinase C and beta-arrestins. Endocrinology 2002;143: 3854–65.

[2] Degenhardt H, Jansen J, Schulz R, Sedding D, Braun-Dullaeus R, Schlüter K-D. Mechanosensitive release of parathyroid hormone-related peptide from coronary

endothelial cells. Am J Physiol Heart Circ Physiol 2002;283:H1489–96.
[3] Della Penna K, Kinose F, Sun H, Koblan KS, Wang H. Tuberoinfundibular peptide of 39 residues (TIP39): molecular structure and activity for parathyroid hormone 2 receptor. Neuropharmacology 2003;44:141–53.
[4] Eichinger A, Fiaschi-Taesch N, Massfelder T, Fritsch S, Barthelmebs M, Helwig JJ. Transcript expression of the tuberoinfundibular peptide (TIP)39/PTH2 receptor system and non-PTH1 receptor-mediated tonic effects of TIP39 and other PTH2 receptor ligands in renal vessels. Endocrinology 2002;143:3036–43.
[5] Ferrari SL, Behar V, Chorev M, Rosenblatt M, Bisello A. Endocytosis of ligand-human parathyroid hormone receptor 1 complexes is protein kinase C-dependent and involves beta-arrestin2. Real-time monitoring by fluorescence microscopy. J Biol Chem 1999;274:29968–75.
[6] Ferrari SL, Bisello A. Cellular distribution of constitutively active mutant parathyroid hormone (PTH)/PTH-related protein receptors and regulation of cyclic adenosine 3′,5′-monophosphate signalling by beta-arrestin 2. Mol Endocrinol 2001;15:149–63.
[7] Goold CP, Usdin TB, Hoare SR. Regions in rat and human parathyroid hormone (PTH) 2 receptors controlling receptor interaction with PTH and with antagonist ligands. J Pharmacol Exp Ther 2001;299:678–90.
[8] Hansen IA, Jakob O, Wortmann S, Arzberger T, Allolio B, Blind E. Characterization of the human and mouse genes encoding the tuberoinfundibular peptide of 39 residues, a ligand of the parathyroid hormone receptor family. J Endocrinol 2002;174:95–102.
[9] Jansen J, Gres P, Umschlag C, Heinzel FR, Degenhardt H, Schlüter KD, et al. Parathyroid hormone-related peptide improves contractile function of stunned myocardium in rats and pigs. Am J Physiol Heart Circ Physiol 2003;284: H49–55.
[10] Juppner H, Abou-Samara AB, Freemann M, Kong XF, Schipani E, Richards J, et al. A G protein-linked receptor for parathyroid hormone and parathyroid hormone-related peptide. Science 1991;254:1024–6.
[11] Juppner H, Abou-Samra AB, Uneno S, Gu WX, Potts JT, Serge GV. The parathyroid hormone-like peptide associated with humoral hypercalcemia of malignancy and parathyroid hormone bind to the same receptor on the plasma membrane of ROS 17/2.8 cells. J Biol Chem 1988;263: 8557–60.
[12] LaBuda CJ, Dobolyi A, Usdin TB. Tuberoinfundibular peptide of 39 residues produces anxiolytic and antidepressant actions. Neuroreport 2004;15:881–5.
[13] Laude K, Favre J, Thuillez C, Richard V. NO produced by endothelial NO synthase is a mediator of delayed preconditioning-induced endothelial protection. Am J Physiol Heart Circ Res 2003;284:H2053–60.
[14] Laude K, Thuillez C, Richard V. Peroxynitrite triggers a delayed resistance of coronary endothelial cells against ischemia-reperfusion injury. Am J Physiol Heart Circ Physiol 2001;283:H1418–23.
[15] Lefer DJ, Scalia R, Campbell B, Nossuli T, Hayward R, Salamon M, et al. Peroxynitrite inhibits leukocyte-endothelial cell interactions and protects against ischemia-reperfusion injury in rats. J Clin Invest 1997;99:684–91.
[16] Malecz N, Bambino T, Bencsik M, Nissenson RA. Identification of phosphorylation sites in the G protein-coupled receptor for parathyroid hormone. Receptor phosphorylation is not required for agonist-induced internalisation. Mol Endocrinol 1998;12:1846–56.
[17] Massfelder T, Stewart AF, Endlich K, Soifer N, Judes C, Helwig JJ. Parathyroid hormone-related protein detection and interaction with NO and cyclic AMP in the renovascular system. Kidney Int 1996;50:1591–603.
[18] Mok LL, Ajiwe E, Martin TJ, Thompson JC, Cooper CW. Parathyroid hormone-related protein relaxes rat gastric smooth muscle and shows cross-desensitization with parathyroid hormone. J Bone Miner Res 1989;4:433–9.
[19] Muscari C, Bonafe F, Gamberini C, Giordano E, Tantini B, Fattori M, et al. Early preconditioning prevents the loss of endothelial nitric oxide synthase and enhances its activity in the ischemic/reperfused rat heart. Life Sci 2004;74: 1127–37.
[20] Nickols GA, Nickols MA, Helwig JJ. Binding of parathyroid hormone and parathyroid hormone-related protein to vascular smooth muscle of rabbit renal microvessels. Endocrinology 1990;126:721–7.
[21] Nyby MD, Hino T, Berger ME, Ormsby BL, Golub MS, Brickman AS. Desensitization of vascular tissue to parathyroid hormone and parathyroid hormone-related protein. Endocrinology 1995;136:2497–504.
[22] Okano K, Wu S, Huang X, Pirola CJ, Juppner H, Abou-Samra AB, et al. Parathyroid hormone (PTH)/PTH-related protein (PTHrP) receptor and its messenger ribonucleic acid in rat aortic vascular smooth muscle cells and UMR osteoblast-like cells: cell-specific regulation by angiotensin-II and PTHrP. Endocrinology 1997;135:1093–9.
[23] Parratt JR, Vegh A, Papp JG. Bradykinin as an endogenous myocardial protective substance with particular reference to ischemic preconditioning—a brief review of the evidence. Can J Physiol Pharmacol 1995;73:837–42.
[24] Parratt JR, Vegh A, Zeitlin IJ, Ahmad M, Oldroyd K, Kazala K, et al. Bradykinin and endothelial-cardiac myocyte interactions in ischemic preconditioning. Am J Cardiol 1997;80:124A–31A.
[25] Piserchio A, Usdin TB, Mierke DF. Structure of tuberoinfundibular peptide of 39 residues. J Biol Chem 2000;275:27284–90.
[26] Ross G, Engel P, Abdallah Y, Kummer W, Schlüter K-D. Tuberoinfundibular peptide of 39 residues: a new mediator of cardiac function via nitric oxide production in the rat heart. Endocrinology 2005;146:2221–8.
[27] Schlüter K-D, Katzer C, Frischkopf K, Wenzel S, Taimor G, Piper HM. Expression, release, and biological activity of parathyroid hormone-related peptide from coronary endothelial cells. Circ Res 2000;86:946–51.
[28] Schlüter KD, Piper HM. Cardiovascular actions of parathyroid hormone and parathyroid hormone-related peptide. Cardiovasc Res 1998;37:34–41.
[29] Sneddon WB, Magyar CE, Willick GE, Syme CA, Galbiati F, Bisello A, et al. Ligand-selective dissociation of activation and internalization of the parathyroid hormone (PTH) receptor: conditional efficacy of PTH peptide fragments. Endocrinology 2004;145:2815–23.
[30] Suva LJ, Winslow GA, Wettenhall RE, Hammonds RG, Moseley JM, Diefenbach-Jagger H, et al. A parathyroid hormone-related protein implicated in malignant hypercalcemia: cloning and expression. Science 1987;237:893–6.
[31] Urena P, Kong XF, Abou-Samra AB, Juppner H, Kronenberg HM, Potts Jr JT, et al. Parathyroid hormone (PTH)/PTH-related peptide receptor messenger ribonucleic acids are widely distributed in rat tissues. Endocrinology 1993;133:617–23.
[32] Usdin TB, Bonner TI, Harta G, Mezey E. Distribution of parathyroid hormone-2 receptor messenger ribonucleic acid in rat. Endocrinology 1996;137:4285–97.
[33] Usdin TB, Dobolyi A, Ueda H, Palkovits M. Emerging functions for tuberoinfundibular peptide of 39 residues. Trends Endocrinol Metab 2003;14:14–9.

[34] Usidin TB, Gruber C, Bonner TI. Identification and functional expression of a receptor selectively recognizing parathyroid hormone, the PTH2 receptor. J Biol Chem 1995;270:15455–8.
[35] Usdin TB, Hoare SR, Wang T, Mezey E, Palkovits M. TIP39: a new neuropeptide and PTH2-receptor agonist from hypothalamus. Nat Neurosci 1999;2:941–3.
[36] Usdin TB, Wang T, Hoare SR, Mezey E, Palkovits M. New members of the parathyroid hormone/parathyroid hormone receptor family: the parathyroid hormone 2 receptor and tuberoinfundibular peptide of 39 residues. Front Neuroendocrinol 2000;4:349–83.
[37] Ward HL, Small CJ, Murphy KG, Kennedy AR, Ghatei MA, Bloom SR. The actions of tuberoinfundibular peptide on the hypothalamo-pituitary axes. Endocrinology 2001;142: 3451–6.
[38] Wenzel S, Schorr K, Degenhardt H, Frischkopf K, Kodja G, Wiesner RJ, et al. TGF-beta(1) downregulates PTHrP in coronary endothelial cells. J Mol Cell Cardiol 2001;33: 1181–90.