[Frontiers in Bioscience E2, 1250-1253, June 1, 2010]

The (pro)renin receptor and the mystic HRP –Is there a role in cardiovascular disease?

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1. ABSTRACT

In 2002, Nguyen *et al.* cloned the (pro)renin receptor [(P)RR]. Two years later, Suzuki, Ichihara and colleagues provided a concept to inhibit the (P)RR through HRP. This decapeptide mimics a sequence of the prorenin prosegment and functions thereby as a decoy peptide. They showed that HRP prevented diabetic nephropathy in rodents and ameliorated renal and cardiac damage in spontaneously hypertensive rats. We tested HRP and the human renin inhibitor aliskiren in transgenic rats overexpressing the human renin and angiotensinogen genes (dTGR). Only aliskiren, but not HRP, was able to ameliorate target organ damage in this model. HRP had also no effect on target organ damage in renovasular hypertensive rats. *In vitro* studies showed that HRP did not inhibit (pro)renin binding and signaling. More confusing was the fact that HRP bound to cells lacking (P)RR on their surface. We believe that HRP does not act as a competitive antagonist for the (P)RR and promotes its action via an alternative mechanism. Elucidating this mechanism could offer further opportunities, in terms of (pro)renin research.

2. INTRODUCTION

Renin the rate-limiting enzyme in the renin-angiotensin system (RAS) cleaves angiotensinogen into angiotensin I. For decades, researchers hunted for a receptor, which binds renin and its inactive precursor, prorenin (1). In 2002, Nguyen *et al.* cloned a receptor termed (Pro)Renin Receptor (P)RR, which is able to bind both renin and prorenin. The (P)RR is a 35 kDa single-transmembrane receptor that is able to activate intracellular signaling, and surprisingly, (P)RR-bound prorenin becomes enzymatically active as a result of a conformational change without cleavage of the prosegment (2). The degree of homology between human, rat and, mice *(P)RR* is about 95% for the nucleotide sequence and over 80% at the amino-acid level, indicating an extremely conserved protein. Unlike other components of the RAS, the *(P)RR* gene is highly conserved among species, and *(P)RR* orthologues are found in species as far from mammals as C.elegans and Drosophila, which express some of the RAS components, but not for hemodynamic functions (3,4). The highest homology is in the trans-membrane and

cytoplasmic regions pointing to an important function of this fragment of (P)RR (5,6). Therefore, the (P)RR may be involved in other action beside the modulation of the RAS. Furthermore, (P)RR also exists as a soluble receptor s(P)RR. The truncated 28 kDa form occurs after furin cleavage and provides the molecular basis for these additional functions (7).

3. IS THE FUNCTION OF THE (P)RR NOT ONLY RELATED TO THE RAS, BUT ALSO TO THE VACUOLAR ATPASE?

Up today the generation of (P)RR null mice was not successful, possibly because *(P)RR*-/- embryonic stem (ES) cells do not form chimeras after blastocyst injection (Michael Bader, personal communication). *(P)RR* deletion in C. elegans and zebrafish yield embryos that die before the end of embryogenesis, thus supporting an essential cellular function for (P)RR (8). Interestingly, a truncated part of the (P)RR composed of the trans-membrane and cytoplasmic domains of (P)RR co-purifies with the vacuolar H^+-ATPase (V-ATPase) (9). It is tempting to speculate that an interplay between this of part of the (P)RR and v-ATPase is essential for various biological processes. The v-ATPase plays an essential role in controlling cellular and intracellular vesicle pH (10). The gene coding this (P)RR is called *ATP6ap2* (ATPase associated protein 2). Zebrafish embryo mutants for v-ATPase subunits, and for *(P)RR/ATP6AP2,* display similar phenotypes, suggesting a possible link between the two proteins (9). More evidence for a functional link between the two proteins derives from a recent study by Advani *et al.* who showed that (P)RR co-localized with the v-ATPase in the apical villi of intercalated cells of the distal nephron and that inhibition of v-ATPase with bafilomycin impaired ERK activation induced by (pro)rennin (11).

4. CAN WE BLOCK RENIN OR PRORENIN BINDING TO (P)RR WITH EXISTING COMPOUNDS?

Suzuki, Ichihara, and colleagues identified a sequence within the prosegment and proposed that a decapeptide that they called HRP blocks binding of prorenin to the (P)RR (12, 13). The whole concept is based on competitive binding of HRP to the (P)RR. Per definition, a competitive antagonist is a receptor antagonist that binds to a receptor but does not activate the receptor. The antagonist will compete with available agonist for receptor binding sites on the same receptor. Given in a sufficient high dosage, the antagonist will displace the agonist from the binding sites, resulting in a lower frequency of receptor activation.

Here comes the first controversy. Ichihara *et al.* used in their *in vivo* studies HRP doses ranging from 7 µg/kg/d up to 1 mg/kg/d (13-16). They used the same doses in rats and mice despite an about 10-fold difference in blood volume. Beside the question why different doses were used in different models, the major issue arises if at all these doses of a peptide are sufficient for competitively blocking a receptor. It is well known that peptides are rapidly degraded by peptidases in the blood.

Nevertheless, several groups including ours have tried to repeat the protective actions of HRP. Recently, Reudelhuber *et al.* failed to demonstrate an effect of HRP in prorenin-transgenic mice (17). Wenzel *et al.* tested the concept of (P)RR blockade with HRP in ischemic clipped kidneys. However, in their study HRP also did not ameliorate renal injury (18). We tested HRP and the human renin inhibitor aliskiren in transgenic rats overexpressing the human renin and angiotensinogen genes (dTGR) (19). In dTGR, the renin gene is under the control of its own promoter. DTGR rats exhibit high human renin, 10-fold higher human prorenin levels, and 3-5 fold increased circulating and local angiotensin II levels. To our surprise only aliskiren, but not HRP, was able to ameliorate target organ damage in this model. In dTGR rats, we took care to apply both human and rat HRP sequences since presumably in the dTGR, the endogenous rat (P)RR is operative in these animals. We wondered whether the lack of HRP potency might have been caused by species difference between the human ligands (human renin and prorenin) and the rat (P)RR. Therefore, we tested HRP in renovascular hypertensive rats, which solely depend on rat components (20). Nevertheless, also in this model a rat HRP did not ameliorate target organ damage.

We cannot absolutely exclude the possibility that HRP is a blocker for prorenin *in situ*ations, where prorenin is elevated, but renin and angiotensin are suppressed. Nevertheless, the data of Ichihara *et al.* in SHR rats argue against this hypothesis. They showed that HRP treatment reduced local cardiac Ang II levels leading to reduced cardiac hypertrophy and fibrosis (21). Current notions suggest that cardiac Ang II depends to a large extent on renal renin that is released in its active form and is taken up by the heart and thus initiates local Ang II generation (22, 23). Susic *et al.* also treated SHR rats with HRP. They described an amelioration of left ventricular hypertrophy, an improved left ventricular function, cardiac collagen content and a decrease in serum creatinine levels under high salt condition. Surprisingly, under low salt HRP decreased left ventricular mass only, while all other parameters were not improved (24).

Could (pro)renin be more relevant in the brain or could its interaction with the (P)RR be the key to increased renin activity in the CNS? The Raizada's group showed that renin stimulation via the (P)RR involved MAP kinase phosphorylation and was able to inhibit neuronal activity *in vitro*. The effect could be reversed with HRP. However, this is rather unexpected since activation of the RAS is usually associated with neuronal excitation, and it has potentially interesting consequences (25).

Taken together the conflicting data, we believe that HRP efficacy *in vivo* depends on an undefined mechanism, but not on competitive antagonism for the (P)RR.

In vitro results have even increased the confusion. Several groups using different methods such as inhibition of binding of radiolabeled prorenin and ERK 1/2 activation in the presence of HRP, found no inhibitory effects, even at HRP concentrations as high as 10 μM (19, 26, 27). Others have reported that HRP can inhibit not only prorenin binding but also renin binding to recombinant PRR and have also reported that HRP stimulates ERK1/2 by itself, which pinpoints to a partial agonist concept (28, 29). Altogether, it is difficult to understand how HRP could totally inhibit ERK1/2 phosphorylation in some *in vivo* studies when it could itself induce ERK 1/2 phosphorylation. Another unsolved conceptual issue is why HRP would not be effective in high renin models when it could inhibit renin binding *in vitro*.

At the moment, there remains skepticism concerning the mode of action of HRP, and the discrepancy between the *in vitro* and the *in vivo* data cannot be explained at this time.

5. SHOULD WE BLOCK THE (P)RR?

In the light of (*P)RR* gene deletion experiments in mice and zebrafish leading to an embryonic phenotype, it is quite questionable whether a complete (P)RR blockade might be favorable. From the cardiovascular point of view, one could imagine that blockade of the (P)RR could ameliorate target organ damage. Given that the (P)RR might also be involved in basic cell biological processes, the risk for side-effects seems to be rather high. The only known mutation in the (*P)RR* gene in humans leads (P)RR protein lacking amino acid 100 until 132. Patients with this the (*P)RR* mutation develop epilepsia with mental retardation without obvious cardiovascular problems (30).

6. PERSPECTIVES

The (P)RR is more complex than expected in several ways. It is a multifunctional protein existing in different molecular forms, and even its cellular localization is intriguing. Currently, we still know very little about the structure of this protein because recombinant PRR is very difficult to generate in native form and about its other cell functions beyond serving as a (pro)renin receptor. It may well be that the soluble 28 kDa (P)RR has an own function, while the transmembrane cytoplasmic part of the (P)RR has a completely different one rather linked to v-ATPase function. Therefore, studies with tissue-specific (*P)RR* knock-out mice are desperately needed to elucidate the role of the (P)RR in development, physiology and pathophysiology.

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Key Words : (Pro)renin receptor, HRP, cardiovascular disease, v-ATPase, Review

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