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Oxygen-derived radicals stimulate renin release of isolated juxtaglomerular cells

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Abstract We assessed effects of reactive oxygen metabolites on renin release of juxtaglomerular cells (JG-cells) prepared in primary culture from mouse kidneys. Renin activity was measured in culture supernatants and cells. Basal renin release was increased by incubation of JG-cells with xanthine/xanthine oxidase from 26 ± 1% up to 58 ± 3% of total activity. This increase was slightly inhibited by superoxide dismutase, and was eliminated by addition of catalase, implicating H_2O_2 as an intermediate product in the stimulatory cascade. H_2O_2 applied exogeneously dose-dependently stimulated renin release up to 55 ± 2%; this effect was also prevented by catalase. We propose that reactive oxygen metabolites stimulate renin release in isolated JG-cells. This could have important implications in inflammatory kidney diseases.

Key words: Renin; Oxygen radical; Superoxide dismutase; Juxtaglomerular cell; Catalase; Hydrogen peroxide

1. Introduction

Reactive oxygen metabolites are produced by a variety of cells, including circulating polymorphonuclear neutrophils and resident cells in various organs. In kidneys these cells resemble glomerular and various vascular cells [1,2]. There is now general agreement that reactive oxygen metabolites such as O_2^- and H_2O_2 are continuously produced in vivo, e.g. by endothelial cells [3,4] or by activated macrophages [5]. However, formation of reactive oxygen metabolites is enhanced in inflammatory kidney diseases like glomerulonephritis or interstitial nephritis, or in post-ischemic acute renal failure [6–8]. These diseases are frequently associated with enhanced plasma levels of renin, contributing to arterial hypertension [9,10]. In view of the close vicinity of reactive oxygen metabolites-producing cells to the juxtaglomerular apparatus, the source for renin, we investigated whether reactive oxygen metabolites influence renin release of juxtaglomerular cells (JG-cells). Therefore, we investigated the renin release of isolated mouse JG-cells prepared in primary culture after exposure to the O_2^- generating xanthine/xanthine oxidase reaction and to exogeneously applied H_2O_2. Furthermore, we studied the influence of the O_2^- and H_2O_2-removing enzymes superoxide dismutase and catalase on renin release.

2. Materials and methods

2.1. Chemicals

Melittin, xanthine, xanthine oxidase (XOD), H_2O_2, superoxide dismutase (SOD), catalase, trypsin, forskolin, and Trypan blue were obtained from Sigma (Munich, FRG), collagenase from Boehringer (Mannheim, FRG), and Percoll solution from Pharmacia (Freiburg, FRG). XOD was from bovine milk with a specific activity of 1 U/mg protein; SOD was from bovine erythrocytes with a specific activity of 3,570 U/mg protein, and catalase was from bovine liver with a specific activity of 40,000 U/mg protein. All drugs were diluted in buffer I of the following composition: 130 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 10 mM glucose, 20 mM sucrose, 10 mM HEPES (Boehringer, Mannheim, FRG). Culture medium (RPMI 1640) was supplemented with 100 U/ml penicillin, 0.66 U/ml insulin, 100 μg/ml streptomycin, and 2% dialyzed fetal bovine serum (Gibco BRL, Eggenstein, FRG).

2.2. Preparation and primary culture of mouse JG-cells

Preparation of mouse (strain C57Bl6) JG-cells was performed according to Kurtz et al. [11]. Briefly, kidneys were removed, decapsulated, minced, and incubated in buffer I supplemented with 0.25% trypsin and 0.1% collagenase. Single cells were obtained by sifting the cell suspension over a 22 μm mesh and further separated by centrifugation in iso-osmotic Percoll solution. Four apparent bands were obtained. For primary culture of JG-cells, the cells of the band III with the highest specific renin activity were used. These cells were suspended in culture medium and distributed in 50 μl portions into 96-well plates (Falcon, New Yersey, USA) and incubated at 37°C. Renin immunostaining was carried out as described recently by Della Bruna et al. [12]. Almost all cells stained positively for renin when the specific antiserum against mouse renin was used. When normal rabbit serum instead of the specific antiserum was used as control, no cells stained positively.

2.3. Experimental design

JG-cells were stimulated with melittin, forskolin, xanthine, XOD, or H_2O_2 between the 20th and 40th hour of culturing after their plating. At that time the cells were washed, and fresh buffer with the drugs or their vehicles was added. The influence of SOD or catalase on basal and stimulated renin release was tested by adding 0.1 μM SOD or 100 U/ml catalase to the incubation medium during the stimulation periods. At the end of the stimulation periods the supernatants and the cells were harvested. Renin activity in terms of its ability to generate angiotensin I from angiotensinogen was detected both in the supernatants and in the cells by radioimmunoassay using a commercially available kit (Sorin, Saluggia, Italy) as previously described [11]. For quantitative comparison of data, renin release rates were calculated as the percentage of extracellular renin activity compared to the total (intracellular + extracellular) renin activity. In each experiment, 4 separate wells treated identically with melittin, xanthine, XOD, or H_2O_2 were tested for viability at the end of the stimulation period by exclusion of Trypan blue. Viability of the cells after the incubation periods in terms of exclusion of Trypan blue was preserved.

2.4. Statistics

Data in Figs. 1–3 are presented as means ± S.E. of n experiments, each experiment representing the mean of five replicate culture wells. Statistical significance of differences was calculated using Students t-test for unpaired data. For multiple comparison of data, Bonferroni's correction was applied. Differences were considered significant at an error probability of $P < 0.05$.

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Abbreviations: JG-cells, juxtaglomerular cells; SOD, superoxide dismutase; XOD, xanthine oxidase.

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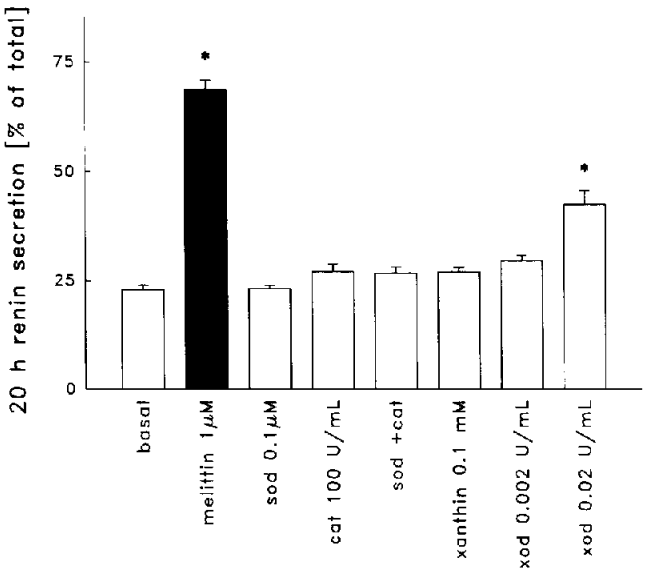

Fig. 1. Bar graph showing the influence of melittin (1 μM, solid bar), of superoxide dismutase (SOD, 0.1 μM), of catalase (cat, 100 U/ml), of xanthine (0.1 mM), and of xanthine oxidase (xod, 0.002 and 0.02 U/ml) on basal renin release in cultured mouse juxtaglomerular cells. Renin release was calculated as a percentage of extracellular renin activity compared to total renin activity after 20 h of incubation with the respective substances. $P < 0.05$ vs. basal renin release. Data are means ± S.E. of three independent experiments, each representing 5 replicate wells.

3. Results

3.1. Effects of melittin and forskolin on renin release of JG-cells

Renin release of JG-cells, determined as the percentage of extracellular renin activity compared to the total renin activity after 20 h of incubation, was 26 ± 1% of total renin activity under basal, unstimulated conditions. Stimulation of the cells with melittin (0.1–10 μM) or forskolin (1–100 μM) concentration-dependently increased the renin release up to 82 ± 3% (data obtained with melittin 1 μM shown in the solid columns of Figs. 1–3).

3.2. Influence of xanthine/XOD on renin release of JG-cells

To investigate the influence of O_2^- on renin release, JG-cells were exposed to the O_2^- generating xanthine/XOD reaction. Control experiments revealed that xanthine alone had no influence on renin release. XOD in a concentration of 0.002 U/ml was also without influence on renin release, whereas 0.02 U/ml had a stimulatory effect. SOD and catalase, enzymes removing O_2^- and H_2O_2, were without influence on basal renin release (Fig. 1).

In the presence of its substrate, XOD dose-dependently stimulated renin release (Fig. 2). Addition of SOD only slightly inhibited the stimulation of renin release. However, the H_2O_2-removing enzyme catalase completely eliminated the stimulation induced by xanthine and 0.002 U/ml XOD, and markedly suppressed renin release stimulated by xanthine and 0.02 U/ml XOD, implicating H_2O_2 as an intermediate product in the stimulatory cascade (Fig. 2). Therefore, we investigated the effect of exogeneously applied H_2O_2 on renin release.

3.3. Effect of exogeneously applied H_2O_2 on renin release of JG-cells

When the cells were stimulated with H_2O_2 for only 2 h, no effect on renin release was detectable. However, when stimulated for 20 h, H_2O_2 dose-dependently increased renin release (Fig. 3). This stimulation was also prevented in the presence of catalase, providing further evidence that H_2O_2 or a consecutively generated reaction product was responsible for stimulation of renin release. SOD and catalase were without effect on renin release stimulated by melittin or forskolin (data not shown).

4. Discussion

In the vascular wall, numerous sources for O_2^- production exist, such as electron transport [13], prostaglandin metabolism [14], or a protein kinase C stimulated process [15]. Furthermore, the vascular endothelium contains xanthine dehydrogenase, an enzyme which can be converted to O_2^- generating XOD [16]. Stimulants responsible for this conversion include reperfusion/ischemia [17] and inflammatory processes [18], pathological influences which frequently affect the kidney. At this time, it is not known whether JG-cells themselves are producing O_2^-. However, cells in close vicinity to JG-cells have been identified as a source for O_2^- production [1,2]. In this study, we made use of a cell-independent O_2^- generating system, the xanthine/XOD reaction. In the presence of its substrate, XOD rapidly generates O_2^- [19,20]. Once O_2^- is formed, it can readily be removed by anti-oxidant defence mechanisms such as SOD enzymes, which convert O_2^- into O_2 and H_2O_2 [2]. High levels of SOD activity have been found in human kidney tissue, particularly in the tubules [21]. However, an excess of SOD-activity, in relation to the activity of H_2O_2-removing enzymes, can result

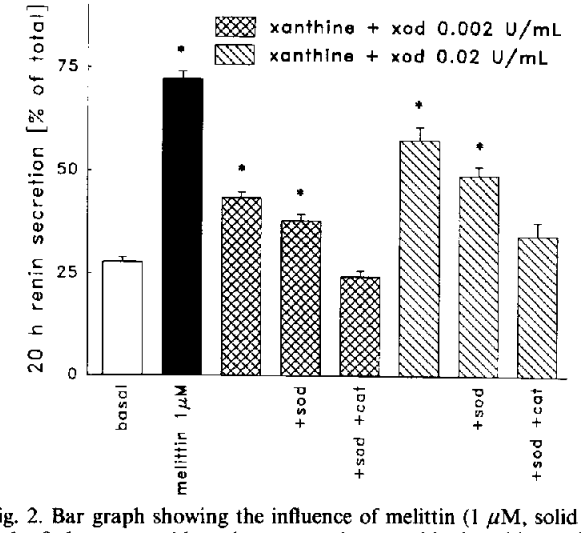

Fig. 2. Bar graph showing the influence of melittin (1 μM, solid bar) and of the superoxide anion generating xanthine/xanthine oxidase (XOD) reaction on basal renin release in cultured juxtaglomerular cells. Renin release was calculated as a percentage of extracellular renin activity compared to total renin activity after 20 h of incubation with the respective substances. Renin release was dose-dependently stimulated by XOD (0.002 U/ml, cross-hatched bars, and 0.02 U/ml, striped bars) in the presence of its substrate xanthine (0.1 mM). Superoxide dismutase (SOD, 0.1 μM) slightly inhibited renin release, while addition of catalase (cat, 100 U/ml) abolished or markedly inhibited renin release. $P < 0.05$ vs. basal renin release. Data are means ± S.E. of four independent experiments, each representing 5 replicate wells.

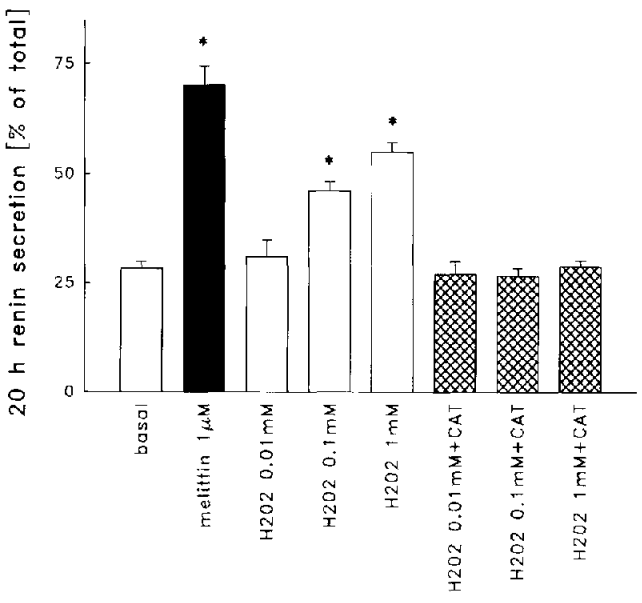

Fig. 3. Bar graph showing the influence of melittin (1 μM, solid bar) and of exogenously applied hydrogen peroxide (H_2O_2) on basal renin release in cultured juxtaglomerular cells. Renin release was calculated as a percentage of extracellular renin activity compared to total renin activity after 20 h of incubation with the respective substances. Renin release was dose-dependently stimulated by H_2O_2 (0.01–1 mM). Addition of catalase (cat, 100 U/ml, cross-hatched bars) prevented the stimulation of renin release. $P < 0.05$ vs. basal renin release. Data are means ± S.E. of three independent experiments, each representing 5 replicate wells.

in the production of agents with even more powerful oxidizing capacity. For example, in the presence of transition metals, H_2O_2 can be reduced to generate hydroxyl radicals, which can attack cell membranes by setting off free radical chain reactions [2]. The latter mechanism helps to interpret the effects of SOD and catalase in this study. While SOD had only minor influence on renin release, the stimulation of renin release was completely eliminated by catalase. Thus, it is conceivable that H_2O_2 was produced as intermediate product. H_2O_2 or further reaction products such as hydroxyl radicals might then stimulate renin release. In line with this interpretation, H_2O_2 applied exogeneously dose-dependently stimulated renin release to a degree similar to that of the xanthine/XOD reaction. The mechanism by which reactive oxygen metabolites stimulate renin release remains to be determined. A likely explanation is that reactive oxygen metabolites attack the cell membrane of JG-cells, leading to release of renin stored in intracellular granules.

In summary, we report for the first time the effects of O_2^- and H_2O_2 on renin release in JG-cells. The data presented demonstrate stimulation of renin release after activation of O_2^- production by the xanthine/XOD reaction. The preventive effect of the H_2O_2-removing enzyme catalase and the stimulatory action of exogenously applied H_2O_2 provide evidence for a role of H_2O_2 or a consecutively formed reaction product in this process. Free radical-induced stimulation of renin release may have important implications in inflammatory kidney diseases with increased plasma renin levels.

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