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Endothelin-1 Stimulation of Aldosterone and Zona Glomerulosa Ouabain-sensitive Sodium/Potassium-ATPase

Adali Pecci,[1] Eduardo N. Cozza,[1] Maura Devlin,[2] Celso E. Gomez-Sanchez and Elise P. Gomez-Sanchez[2]*

[1]Departamento de Quimica Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires and CONICET, Buenos Aires, Argentina and [2]Department of Internal Medicine and Research Service, Harry S. Truman Memorial Veterans Hospital, University of Missouri-Columbia, Columbia, MO, James A. Haley Veterans Hospital and University of South Florida College of Medicine, Tampa, FL, U.S.A.

Endothelin stimulates the cells of the zona glomerulosa of the adrenal gland and releases aldosterone. While it is a less potent aldosterone secretagogue than angiotensin II endothelin also potentiates the effects of angiotensin II on aldosterone biosynthesis. Two endothelin receptors have been cloned and are expressed in the adrenal zona glomerulosa. Intravenous infusion of endothelin at a rate of 80 ng/kg/min for 30 min into rats produced increases in blood pressure, adrenal content of aldosterone and stimulated the ouabain-sensitive sodium potassium ATPase in the zona glomerulosa, but not in the zona fasciculata, of the adrenal. The simultaneous infusion of the isopeptide specific endothelin receptor $_A$ (ET_A) antagonist BQ-123 blocked the pressor effects of endothelin, but did not alter the increase in aldosterone content of the zona glomerulosa or the ouabain-sensitive sodium potassium ATPase activity. Infusion of Sarafotoxin 6b, an ET_B agonist, also increased the aldosterone content of the adrenal and stimulated the ouabain-sensitive sodium potassium ATPase in the zona glomerulosa, further indicating that the effect of endothelin is probably mediated by ET_B or isopeptide non-specific endothelin receptor. The mechanism by which endothelin stimulates the sodium potassium ATPase is unclear as is the relation between a stimulated sodium potassium ATPase and the potentiation of angiotensin II effect on the adrenal.

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INTRODUCTION

Endothelins (ETs) are a 21-amino acid peptide family with powerful vasoconstrictor and pressor activity [1]. Sarofotoxins are 21-amino acid peptide venoms of the Israeli burrowing asp which share a high degree of structural and functional homology with ETs. Two different high affinity ET receptors have been described and cloned [2, 3]. The receptor with a high affinity for endothelin-1 and endothelin-2 (ET-1 and -2) and a low affinity for endothelin-3 and sarafotoxin has been called the isopeptide-specific or ET_A receptor [2]. The second receptor has a high affinity for all ETs and sarafotoxin and has been called the isopeptide non-specific receptor or the ET_B receptor [3]. High affinity, specific ET_A and ET_B receptors have been described in the whole adrenal gland and in the zona glomerulosa [2–5]. Binding [6] and autoradiographic [7] studies using [^{125}I]ET-1 show preferential binding to the zona glomerulosa.

ET-1 stimulates aldosterone production when incubated with cultured calf, and freshly dispersed rabbit or rat zona glomerulosa cells, with a potency less than that of angiotensin II (A-II) [5, 8–11]. ET-1 also potentiates both A-II- [12, 13] and ACTH-mediated [12, 14] stimulation of aldosterone production. The two adrenal actions of ET-1, the direct stimulation of aldosterone production, and the potentiation of A-II stimulation, seem to be exerted through different mechanisms, perhaps by acting through different ET-1 receptors [13].

The signalling system for ET-1 has been studied in many cell systems. ET-1 activates phospholipase C,

*Correspondence to E. P. Gomez-Sanchez.
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releasing diacylglycerol and the active inositol [1, 4, 5]-triphosphate, leading to activation of protein kinase C, and increasing cytosolic calcium levels, both by calcium release from intracellular stores and enhancement of its influx [15]. Ouabain has been found to inhibit both A-II- and ACTH-mediated stimulation of aldosterone production [16, 17]. In the present study we report that *in vivo* administration of ET-1 resulted in activation of the ouabain-sensitive Na^+/K^+-ATPase in the adrenal zona glomerulosa.

MATERIALS AND METHODS

Reagents

ET-1 was purchased from Peninsula Labs (Belmont, CA), BQ-123 [Cyclo (D-Asp-L-Pro-D-Val-L-Leu-D-Trp] from Peptides International (Louisville, KY) and ouabain, bovine serum albumin and other reagents from Sigma Chemicals (St Louis, MO). Stock solutions of ET-1 and BQ-123 were made in 0.1% acetic acid, and diluted with sterile physiologic saline just before use.

Infusions

Sprague–Dawley (SD), inbred strains of the Dahl salt sensitive (SS/jr) and salt resistant (SR/jr), and CHBB-Thom rats weighing 230–270 g consuming a normal salt chow (0.3% sodium chloride) were used. SD, SSR/jr and SR/jr rats were anesthetized with 5% isoflurane, maintained at 2.5% for the surgical implantation of the femoral arterial and venous catheters for blood pressure measurements and the infusion of test solutions, and then at 1.5–2% for the duration of the experiment. All animals remained anesthetized throughout the infusions. After a stabilizing period of at least 20 min, saline or BQ-123 (200 ng/kg-min) was infused for 30 min, followed by saline, ET-1 at 80 ng/kg-min or BQ-123 for an additional 45 min. The infusions in the CHBB-Thom rats were as described previously [18]. At the end of the infusions, the adrenal glands were removed, placed in ice-cold saline, cleaned of fat and connective tissues, and separated into capsules (predominately glomerulosa) and cores (fasiculata/reticularis and medulla). Zones corresponding to the same adrenal pair were homogenized separately in isolation medium [5 mM Tris–HCl buffer (pH = 7.0) containing 250 mM mannitol, 5 mM EDTA and 30 mM histidine]. Microsomal and cytosolic fractions were prepared by centrifugation as described elsewhere [19]. Aortas were also removed after the infusions, washed with ice-cold saline, cut into 1–2 mm pieces and cleaned of surrounding tissues by three strokes in a loose fitting glass–Teflon homogenizer in saline, which eliminated the fat, connective and other adherent tissue without affecting the aorta wall. Clean pieces of aorta were then homogenized in a Polytron homogenizer in the isolation medium.

Ouabain-sensitive Na^+/K^+-ATPase activity

Enzyme activity was measured in adrenal capsule and core and aorta homogenates, and in microsomal fractions as described previously [20]. Briefly, an enzyme source was incubated in 1 ml of incubation medium containing 100 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 3 mM ATP, 5 mM sodium azide and 30 mM histidine, pH 7.4. After incubation at 37°C, the reaction was stopped by adding 0.3 ml trichloracetic acid, followed by 1 ml of ice-cold water. After 10 min in an ice-water bath, samples were centrifuged at 3000 rpm for 10 min and inorganic phosphate was measured in supernatants by the ascorbic acid/ammonium molybdate method [21]. Na^+/K^+-ATPase activity was calculated as the difference between incubations with and without 1 mM ouabain. Proteins were measured by the Bradford method (Bio-Rad, Hercules, CA). Aldosterone was measured in cytosols by direct ELISA or RIA using a monoclonal antibody [22]. Statistical comparisons were made by ANOVA using the Statview SE computer program (Abacus Concepts Inc, Berkeley, CA). Differences were considered significant at $P < 0.05$.

RESULTS

The time-course for the activity of ouabain-sensitive Na^+/K^+-ATPase in adrenal zona glomerulosa homogenates of SD rats was linear up to 20 min, and an incubation time of 15 min was used for all subsequent experiments. A linear relationship was also obtained with increasing amounts of protein (100–300 μg) and all measurements were done on samples within this range.

Effects of ET-1 infusion on adrenal ouabain-sensitive Na^+/K^+-ATPase

The i.v. infusion of ET-1 in SD rats provoked a significant increase in ouabain-sensitive Na^+/K^+-ATPase activity in homogenates of adrenal capsule

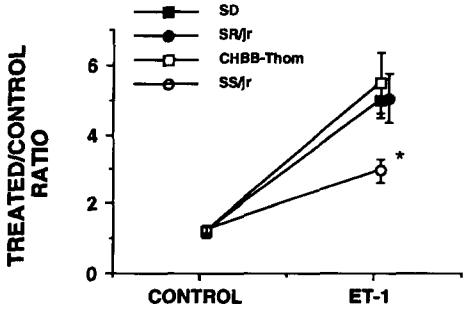

Fig. 1. Na^+/K^+-ATPase of zona glomerulosa homogenates of SD CHBB-Thom. Dahl SS/jr and Dahl SR/jr rats infused with ET-1. Results are expressed as the ratio of treated over control. *$P < 0.05$ in comparison to the 3 other groups. The difference between stimulated and control were significantly different in all groups ($P < 0.05$ for the SS/jr rats and $P < 0.01$ for the other groups).

Table 1. Effect of ET-1 infusion on adrenal Na^+/K^+-ATPase activity

Fractions	Ouabain-sensitive Na^+/K^+-ATPase (nmol phosphate/μg protein) Control	ET-1	BQ-123	BQ123 + ET-1
Homogenates				
Capsules	0.14 ± 0.009	0.67 ± 0.043*	0.13 ± 0.007	0.64 ± 0.045*
Cores	0.081 ± 0.004	0.079 ± 0.003	0.071 ± 0.004	0.079 ± 0.006
Microsomes				
Capsules	0.48 ± 0.024	2.54 ± 0.02**	0.436 ± 0.025	2.31 ± 0.15**
Cores	0.27 ± 0.025	0.29 ± 0.018	0.25 ± 0.031	0.29 ± 0.013

Data are mean ± SEM of triplicates from 2 different experiments.
*$P < 0.01$ with respect to control homogenate, **$P < 0.001$ with respect to control microsomes from capsules (ANOVA).

(Fig. 1), but not in adrenal cores (Table 1). Similar increases were seen in Dahl SR/jr and CHBB-Thom rats, and the relative increase in enzyme activity was significantly less in the Dahl SS/jr rats (Fig. 1). Coinfusion of BQ-123 did not affect the ET-1 increase in ouabain-sensitive Na^+/K^+-ATPase (Table 1). Microsomal ouabain-sensitive Na^+/K^+-ATPase from zona glomerulosa, but not from zona fasciculata/reticularis, was also increased by ET-1 and ET-1 plus BQ-123, and was not affected by BQ-123 infused alone (Table 1). Infusion of the ET_B receptor-agonist Sarafotoxin 6b (S6b) [4] in SD rats (80 ng/kg/min) also increased the activity of capsule homogenate Na^+/K^+-ATPase, with the ratio of treated to control being 2.5 ± 0.3 (mean ± SEM, $n = 5$, $P < 0.02$).

Effects of aldosterone production

The infusion of ET-1 increased aldosterone concentration in zona glomerulosa cytosols of SD rats from 12.2 ± 1.8 to 28.4 ± 3.4 ng/capsule ($n = 6$ rats, $P < 0.05$) and to 22.2 ± 1.9 ng/capsule by infusion with ET-1 + BQ-123 ($n = 6$ rats, $P < 0.05$). The infusion of BQ-123 alone did not statistically affect the control value ($n = 6$ rats, 10.2 ± 1.2 ng/capsule) (Fig. 2)

Effects on aortic Na^+/K^+-ATPase

The infusion of ET-1 produced a slight, but significant, increase in rat aortic Na^+/K^+-ATPase activity from 180 ± 12 to 340 ± 23 pmol phosphate/μg protein ($P < 0.05$, $n = 5$ rats each group). Infusion of BQ-123 + ET-1 increased levels to 327 ± 11 pmol phosphate/μg protein ($P < 0.05$ with respect to controls, $n = 6$). BQ-123 infused alone had no effect (172 ± 12 pmol phosphate/μg protein, $n = 4$). The increase over control of the Na^+/K^+-ATPase activity in the capsules of rats infused with S6b was 2.5 ± 0.3 (mean ± SEM, $n = 5$, $P < 0.02$, ANOVA).

Effects on mean Blood Pressure (BP)

ET-1 provoked a sizeable increase in mean BP in SD rats, which was blocked by co-infusion with BQ-123 (Fig. 3) Infusion of BQ-123 alone did not affect mean BP. The relative elevation of BP by ET-1 in the Dahl SS/jr rat was slightly higher than in the SD and SR/jr.

DISCUSSION

ET-1 infusion increases the adrenal zona glomerulosa content of aldosterone [18]. BQ-123, an ET_A receptor antagonist, had no effect on the levels of aldosterone in adrenal cytosol or on the increase in aldosterone production induced by the infusion of

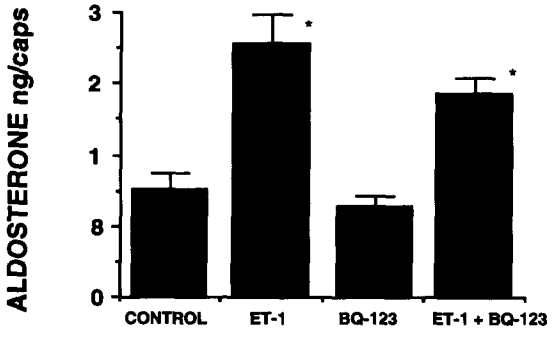

Fig. 2. Aldosterone content of the zona glomerulosa of SD rats infused with vehicle, ET-1, BQ-123, and ET-1 + BQ-123. *$P < 0.05$.

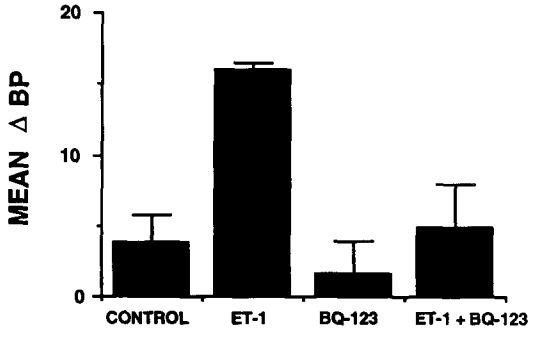

Fig. 3. Delta mean arterial blood pressure after the infusion of vehicle, ET-1, BQ-123, and ET-1 + BQ-123.

ET-1, suggesting that this ET-1 effect is mediated by ET_B receptors. This is in agreement with our previous observation that S6b also increases aldosterone production *in vitro* [4].

ET-1 infused *in vivo* activates ouabain-sensitive Na^+/K^+-ATPase in adrenal capsules and aorta. The activity of the Na^+/K^+-ATPase is localized mainly in the microsomal fraction. BQ-123 did not affect the basal level or the ET-1-induced increase in the enzyme, suggesting this action of ET-1 is exerted through the ET_B receptor. The effect of the ET_B receptor agonist S6b in activating the adrenal ouabain-sensitive Na^+/K^+-ATPase supports this conclusion. ET-1-mediated increase in the zona glomerulosa Na^+/K^+-ATPase was similar in SD, Dahl SR/jr and CHBB-Thom rats, but was significantly lower in the Dahl SS/jr (Fig. 4). The significance of the lower activity of the adrenal Na^+/K^+-ATPase after ET-1 infusion in the Dahl SS/jr rat in comparison to the other strains is not yet clear.

ET infusion provoked a large and sustained increase in blood pressure which was almost completely blocked by co-infusion of BQ-123, suggesting that the pressor effect was exerted through the ET_A receptors. It also increased the ouabain-sensitive Na^+/K^+-ATPase in the rat aorta, albeit to a lesser extent than in the adrenal gland, an effect which was not inhibited by the co-infusion of the ET_A antagonist BQ-123. The mechanism for the increase in Na^+/K^+-ATPase is not clear, since ET_A, but not ET_B, receptors are found in vascular smooth muscle [2]. The ET_B receptor in the endothelium mediates the initial transient vasodilation preceding the sustained vasoconstriction produced by the infusion of ET in these and other experiments [1, 23, 24]. The increase in aortic Na^+/K^+-ATPase may also be mediated at the level of the endothelial ET_B receptor. ET-1 has been reported to inhibit the Na^+/K^+-ATPase in intact renal tubular epithelial cells [25], so that the inhibitory effect may be mediated through a different receptor or may be a cell specific response to ET-1.

The role of the activation of Na^+/K^+-ATPase in the adrenal zona glomerulosa is unclear. Na^+/K^+-ATPase activity is greater in the zona glomerulosa than the zona fasciculata. While subpressor doses of A-II have been shown to stimulate vascular Na^+/K^+-ATPase in rats [26], stimulation with A-II, ACTH or potassium has been shown to have no effect on the adrenal Na^+/K^+-ATPase [27]. Ouabain inhibits the A-II and ACTH-mediated increases in aldosterone production [16, 17]. A-II-mediated stimulation of aldosterone secretion is potentiated by ET-1 [12, 13] and as part of the mechanism of action of A-II there is an intracellular alkalinization due to activation of Na^+-H^+ exchange, resulting in an increase in intracellular sodium [28, 29]. Stimulation of the Na^+/K^+-ATPase by ET-1 would decrease intracellular sodium. Thus, the mechanism by which endothelin potentiates the A-II stimulation of aldosterone secretion is not clear, but it is possible that a greater sodium gradient than normal created by the activation of the Na^+/K^+-ATPase would allow faster intracellular alkalinization by A-II [28, 29].

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