Fetal uninephrectomy in male sheep alters the systemic and renal responses to angiotensin II infusion and AT1R blockade

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Singh RR, Moritz KM, Wintour EM, Jefferies AJ, Iqbal J, Bertram JF, Denton KM. Fetal uninephrectomy in male sheep alters the systemic and renal responses to angiotensin II infusion and AT1R blockade. Am J Physiol Renal Physiol 301: F319–F326, 2011. First published May 4, 2011; doi:10.1152/ajprenal.00139.2011.—Fetal uninephrectomy (uni-x) at 100 days of gestation results in compensatory nephrogenesis in the remaining kidney, resulting in a 30% reduction in total nephron number in male sheep. Recently, we showed that uni-x males at 6 mo of age have elevated arterial pressure, reduced renal blood flow (RBF), glomerular filtration rate (GFR), and low plasma renin levels (Singh R, Denton K, Bertram J, Jefferies A, Head G, Lombardo P, Schneider-Kolsky M, Moritz K. J Hypertens 27: 386–396, 2009; Singh R, Denton K, Jefferies A, Bertram J, Moritz K. Clin Sci (Lond) 118: 669–680, 2010). We hypothesized this was due to upregulation of the intrarenal renin-angiotensin system (RAS). In this study, renal responses to ANG II infusion and ANG II type 1 receptor (AT1R) blockade were examined in the same 6-mo-old male sheep. Uni-x animals had reduced levels of renal tissue and plasma renin and ANG II. Renal gene expression of renin, and gene and protein levels of AT1R and AT2R, were significantly lower in uni-x animals. In response to graded ANG II infusion, sham animals had the expected decrease in conscious RBF and GFR. Interestingly, the responses were biphasic in uni-x sheep, with GFR initially decreasing, but then increasing at higher ANG II doses (34% ± 7%; P/group x treatment < 0.001), due to a paradoxical decrease in renal vascular resistance (P/group < treatment < 0.001). In response to AT1R blockade, while GFR and RBF responded similarly between groups, there was a marked increase in sodium excretion in uni-x compared with sham sheep (209 ± 35 vs. 25 ± 12%; P < 0.001). In conclusion, in 6-mo-old male sheep born with a single kidney, these studies demonstrate that this is a low-renin form of hypertension, in which responses to ANG II are perturbed and the intrarenal RAS is downregulated.

hypertension; nephron number; losartan; ANG II; intrarenal RAS; fetal programming

REDUCED NEPHRON ENDOWMENT is associated with an increased risk of developing chronic kidney and cardiovascular diseases, particularly hypertension in adulthood (6). Many experimental studies have shown an association between a nephron deficit from birth to development of hypertension in adult offspring that experienced perturbations such as maternal dietary protein restriction (17, 35, 51) or elevations in the levels of maternal stress hormones, glucocorticoids, in utero (9, 42, 46). However, most prenatal perturbations also cause fetal growth restriction (17, 24, 48), which can independently predispose to cardiovascular disease in developmental programming models (5, 18, 25, 50). This makes it difficult to appreciate the contribution of just a nephron deficit and the role of the kidney in the development of hypertension in these models.

To investigate the direct association between a congenital nephron deficit and development of hypertension, our group has established a model of fetal uninephrectomy (uni-x) in sheep (12, 30). Development of the permanent (metanephric) kidney in sheep (29) is very similar to that in humans (45), with both species completing nephrogenesis before birth. This is different from the rat, where nephrogenesis is ongoing after birth and final nephron complement can be altered by the postnatal environment (47). We have previously reported that fetal uni-x in male sheep results in a 30% reduction in nephron number rather than 50% due to compensatory nephrogenesis in the remaining kidney (12), low plasma renin levels, elevated arterial pressure, and reduced glomerular filtration rate (GFR) and renal blood flow (RBF) at 6 mo of age (40, 41). Recently, we reported, that uni-x sheep have delayed but exaggerated renal sodium excretion and a reduced ability to suppress plasma renin activity (PRA) in response to an acute period of saline loading (41). This finding prompted the hypothesis that the intrarenal renin-angiotensin system (RAS) is upregulated in uni-x animals. In the current study, we tested this hypothesis by investigating the renal and cardiovascular responses of the same animals used in the previous study (41), to acute doses of intravenously infused ANG II and to ANG II type 1 receptor (AT1R) blockade by losartan. In addition, basal expression of components of the intrarenal RAS was determined.

MATERIALS AND METHODS

Animals. Experiments were performed in accordance with the guidelines for animal ethics of the National Health and Medical Research Council of Australia. Merino ewes carrying male fetuses underwent surgery at 100 days postconception where uni-x (n = 6) or sham surgery (n = 6) was performed, as previously reported (30), following which animals were allowed to recover from surgery and then returned to the farm. Only male fetuses were used in this study and studied as offspring. Following birth, lambs remained with their mothers on pasture until weaned at 16 wk of age. At 6 mo of age, mean arterial pressure (MAP) and heart rate (HR) were measured in conscious animals housed in metabolic cages using an indwelling carotid arterial catheter as previously described (10). Lambs were also instrumented with chronic bladder catheters, and GFR and RBF were determined by clearance methods (GFR, chromium-EDTA; RBF, PAH) as previously described (41). Animals were meal-fed and given water ad libitum while in the laboratory; thus sodium intake was similar in both groups as animals were all given the same quantity of food, which was eaten in its entirety. Experiments commenced following 1 wk of acclimatization.

Renal function in response to ANG II infusion or AT1R blockade. All experiments were performed in conscious animals during which MAP, HR, GFR, RBF, urine flow (UF), and sodium excretion (UNaV)
were measured. Measurements were performed in the following order on 3 separate days, with 2–3 days between each study: 1) time control studies were performed during which renal and cardiovascular measurements were recorded on a 7-h period (these data have been previously reported) (41); 2) graded doses of ANG II (0.2, 0.4, 0.8 μg·kg⁻¹·h⁻¹ iv) were infused over 120 min (per dose) with renal function determined over the final 90 min (per dose); and 3) a dose of losartan (1.9 mg·kg⁻¹·h⁻¹ iv), previously shown by us to block the effects of the highest dose of ANG II used in this study (11), was infused over 120 min and renal function was determined over the final 90 min. Basal measurements were obtained over 2 h on each day before ANG II infusion or AT1R blockade.

**Determination of renal and systemic components of RAS.** Three weeks following the completion of all experiments, animals were humanely euthanized (pentobarbital, Lethabar). A 0.5-cm slice taken from one-half of the right kidney, in the transverse plane, was homogenized, and RNA was extracted for determining gene expression of angiotensinogen, renin, and AT1R and AT2R by real-time PCR as previously described (9). The frozen slice was further subdivided into cortex and medulla (inner and outer combined) to determine tissue levels of renin and ANG II and for Western blot analysis. Protein extraction and Western blot analysis were performed as previously described (19), using rabbit polyclonal anti-AT1 (40 kDa, 1:500) and anti-AT2 (44 kDa, 1:500) antibodies (Alomone Laboratories). Membranes were stripped and reprobed with anti-α-smooth muscle actin (α-SMA, 1:1,000). Image J software (Fujifilm, Minato, Tokyo, Japan) was used to determine band densities for AT1R and AT2R in the cortex and medulla which were normalized to band densities of α-SMA. Tissue and plasma levels of renin and ANG II were determined via radioimmunoassay (Prosearch International, Malvern, Australia).

**Statistical analysis.** Values are presented as means ± SE, with the level of significance set at *P* < 0.05. Absolute data and percent change from the basal period in response to ANG II or losartan infusion data are reported. A one-way repeated ANOVA was used to examine the effect of group (sham vs. uni-x) and drug treatment, with Bonferroni post hoc analysis. An unpaired *t*-test was used to determine the differences between the sham and uni-x groups for the analysis of components of the RAS. Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA).

**RESULTS**

Gestation length and birth weight were not different between the groups. As reported previously, at the time of cull at 6 mo of age, body weight (sham: 39 ± 3 kg; uni-x: 35 ± 3 kg) and total kidney weight (sham 2 kidneys): 73 ± 6 g; uni-x (1 kidney): 79 ± 6 g) between the treatment groups were similar (41).

**Basal cardiovascular and renal function obtained over 2 h before either ANG II infusion or AT1R blockade.** Uni-x animals had a significantly higher conscious basal MAP compared with sham animals (~10 mmHg; *P* < 0.001) (Figs. 1 and 2). However, HR was similar between the groups (sham: 86 ± 2 beats/min; uni-x: 87 ± 3 beats/min). Basal GFR (~25%; *P* < 0.001) and RBF (~30%; *P* < 0.001) were significantly lower in the uni-x compared with the sham animals (Figs. 1 and 2). UF was similar between the groups (Figs. 1 and 2), but UNaV was significantly reduced (~37%; *P* < 0.01) in uni-x animals compared with sham animals. Reduction in both filtered load (~25%; *P* < 0.01) and fractional excretion of sodium (FENa; ~45%; *P* < 0.01) contributed to the reduction in UNaV in the uni-x sheep. Plasma sodium levels were not different between the groups (sham: 136 ± 3 mmol/l; uni-x: 138 ± 3 mmol/l). These basal measurements, made over a 2-h period, did not differ significantly from those determined during the 7-h time control study, the data for which have been previously reported (41).

**Cardiovascular and renal responses to ANG II infusion.** MAP increased in a dose-dependent manner in response to ANG II infusion (*P* < 0.001) (Fig. 1), but the percent increase was lower in uni-x sheep (*P* < 0.001, *P*<sub>group × treatment</sub> = 0.05). RVR increased progressively in sham animals, whereas RVR increased initially but then decreased in uni-x sheep (*P* < 0.001, *P*<sub>group × treatment</sub> < 0.001, *P*<sub>group × treatment</sub> < 0.001) (Fig. 1). RBF decreased in both groups at the lowest dose of ANG II, but this fall was significantly less in uni-x animals compared with sham (*P* = 0.01) (Fig. 1). However, at the higher doses of ANG II, uni-x animals had an increase in RBF compared with sham (*P* < 0.001; all). Similarly, a decrease in GFR was observed at the lowest dose (*P* < 0.05) while GFR increased at the higher doses of ANG II (*P* < 0.001, all) in the uni-x animals (Fig. 1). In contrast, RBF and GFR decreased in the sham animals at all doses of ANG II. The filtration fraction increased in both groups at the lowest dose of ANG II and while it remained elevated at the higher doses in the sham group, it progressively declined in the uni-x animals with increasing doses of ANG II (*P*<sub>group × treatment</sub> < 0.001). This was more pronounced in the sham group (*P*<sub>group × treatment</sub> < 0.001; all) (Fig. 1). While the lowest dose of ANG II, UF, UNaV, and FENa decreased by a similar extent in both groups, at the highest ANG II dose these increased above basal levels in the uni-x but remained below basal levels in the sham sheep.

**Cardiovascular and renal responses to AT1R blockade.** In response to losartan, MAP decreased in both groups, but the fall was greater in the uni-x animals (sham: 9 ± 2%; uni-x: 14 ± 1% (*P*<sub>group × treatment</sub> = 0.02) (Fig. 2). RVR decreased in both groups following losartan infusion, but the extent of this decrease was greater in the uni-x group compared with the sham animals (sham: 20 ± 2%; uni-x: 26 ± 2% (*P*<sub>group × treatment</sub> < 0.001) (Fig. 2). RBF (~15%), GFR (~10%), and UF (~65%) all increased in response to losartan similarly in both groups (Fig. 2). The filtration fraction decreased but not significantly in both groups (sham: 3 ± 1%; uni-x: 6 ± 2% (*P*<sub>group × treatment</sub> = 0.2) (Fig. 2). UNaV and FENa increased by a greater extent in the uni-x compared with the sham group in response to losartan (UNaV, sham: 25 ± 12%; uni-x: 209 ± 15% (*P*<sub>group × treatment</sub> = 0.003; FENa, sham: 16 ± 11%; uni-x: 197 ± 19% (*P*<sub>group × treatment</sub> < 0.001) (Fig. 2).

**Systemic and renal RAS.** Uni-x animals had significantly reduced PRA (~38%; *P* < 0.001) (Fig. 3A) and levels of plasma ANG II (~35%; *P* < 0.001) (Fig. 3B) compared with the sham animals. Furthermore, uni-x animals had significantly lower levels of renin in the kidney cortex (~37%) but not the medulla compared with the sham group (Fig. 3C). A corresponding decrease in renal cortical ANG II (~41%) but not medullary ANG II was observed in the uni-x kidneys (Fig. 3D). Whole kidney mRNA expression of angiotensinogen was significantly upregulated in the uni-x animals (~60%; *P* < 0.01) (Fig. 4A), whereas renin was reduced (~40%; *P* < 0.001) (Fig. 4B). Uni-x animals had reduced levels of the AT1R (~43%; *P* < 0.001) and AT2R mRNA (~90%; *P* < 0.001) in whole kidney homogenate compared with the sham group (Fig. 4).
Similarly, uni-x animals had significantly lower protein levels of AT1R in the kidney cortex ($P < 0.05$) (Fig. 5A) and medulla ($P = 0.02$) (Fig. 5B) and reduced AT2R in the kidney cortex ($P < 0.001$) (Fig. 5C) and medulla ($P = 0.05$) (Fig. 5D) compared with the sham group.

**DISCUSSION**

The current study demonstrates that renal and cardiovascular responses to ANG II infusion or blockade are markedly altered in 6-mo-old male sheep that had undergone uninephrectomy as fetuses. Despite reduced expression of ANG II and AT1R in the kidney, AT1R blockade exposed some contributions of the intrarenal RAS to the promotion of sodium reabsorption and blood pressure elevation in the uni-x sheep. Responses to exogenous ANG II infusion were paradoxical, with renal vasodilation and an increase in GFR observed in the uni-x sheep at the higher doses. In combination, these data suggest that regulation of renal function by the RAS is perturbed in young sheep with a congenital nephron deficit. Importantly, the elevation in blood pressure and reduction in renal function observed following fetal uni-x are independent of low birth weight and direct changes to other organ systems, factors that
confounded the conclusion in other models that a reduction in nephron number is the sole contributor to the development of hypertension (4, 5). While we have previously shown that fetal uni-x results in compensatory nephrogenesis in the remaining kidney (12), our follow-up studies have shown that this compensation is not enough to prevent the development of renal impairment and resultant hypertension in animals in postnatal life (40). We have previously shown that uni-x animals have a reduced ability to maintain sodium homeostasis, which contributes to an elevation in plasma volume in these animals (41) and drives the initial increase in cardiac output and blood pressure elevation in male sheep at 6 mo of age (40), thus strongly suggesting that the hypertension in this model is directly associated with the reduction in nephron endowment.

Furthermore, these uni-x male sheep exhibit a delayed but exaggerated natriuresis and diuresis and a reduced suppression of plasma renin activity in response to an acute period of saline loading (50 ml·kg⁻¹·30 min⁻¹) (41). The renal RAS plays a major role in blood pressure regulation and sodium homeostasis (22), and we hypothesized that the impairment in suppression of the RAS in response to an acute saline load indicated an upregulation of the intrarenal RAS as observed in low-renin hypertension in humans (31). Contrary to this hypothesis, the present study shows that the systemic and intrarenal RAS are downregulated in the uni-x sheep at 6 mo of age. In other models of programmed hypertension, up- and downregulation of all components of the intrarenal RAS have been reported (21). This is likely due to the multiplicity of models employed, and the sex and age at which offspring were studied. However, often a biphasic response is observed, when more than one age point is studied (21). For example, Connors et al. (8) have shown that renal renin expression was decreased in early gestation, increased in late gestation, and not different at 1 yr of age in female sheep exposed to prenatal betamethasone.

The current study was only performed in male offspring, and there is evidence that the expression of the RAS is altered differentially, influenced by age, sex, and model of study. There is also clear evidence that sex differences exist in the fetal programming of adult disease, with the females being relatively protected (16, 21, 38). Initial studies performed in this model were conducted in female sheep only and showed that uni-x female sheep had normal plasma renin levels at 6 mo of age, with levels being reduced at 12 mo of age, which is in contrast to observations in male sheep in the present study (30). Furthermore, arterial pressure responses to ANG II infusion were not altered in uni-x female sheep at 12 mo of age; however, renal responses were not examined, and hence we do not have any information whether the intrarenal RAS is altered in female uni-x sheep and whether it is different from male sheep (30). Taken together, these observations do indicate that the response to fetal uni-x is different between the sexes. Previous studies have demonstrated sex differences in expression of components of the RAS and in responses to angiotensin-converting enzyme inhibition in models of programmed hypertension as well (15, 44, 49). Therefore, future studies are needed to establish the ontogeny of the intrarenal RAS from the time of fetal uninephrectomy to adulthood to elucidate the contribution of the renal RAS to the relative risk of future cardiovascular disease in both males and females.

In response to blockade of endogenous ANG II with losartan, the fall in MAP was greater in the uni-x sheep, whereas MAP increased less in the uni-x group in response to ANG II infusion; these findings are dichotomous. This is in contrast to observations in male sheep in the present study (30). In response to blockade of endogenous ANG II with losartan, the fall in MAP was greater in the uni-x sheep, whereas MAP increased less in the uni-x group in response to ANG II infusion; these findings are dichotomous. This is in contrast to observations in male sheep in the present study (30).

Fig. 2. Cardiovascular and renal responses before (basal) and during losartan infusion in sham (●) and uni-x (○) in male sheep (n = 6/group) at 6 mo of age. Values are means ± SE. P values represent the results of a 1-way repeated measures ANOVA with factors group (g, sham or uni-x; t, treatment before and during losartan) and their interaction (gt). Abbreviations are defined as in Fig. 1.
previously demonstrated that uni-x sheep at 6 mo of age have cardiac if not vascular hypertrophy (40). The mechanisms underlying the attenuated pressor response to ANG II infusion are likely multifactorial. The uni-x sheep had lower basal levels of ANG II, which might mean that the uni-x sheep were exposed to lower levels of ANG II; however, this difference is unlikely to be an important factor at the highest dose of ANG II when the greatest difference in MAP response between the groups was observed. A reduction in cardiac functional reserve is a more likely explanation as uni-x sheep at 6 mo of age have a reduced cardiac response to dobutamine challenge (40). Thus we suggest that the attenuated MAP response to ANG II infusion was likely due to a lesser increase in cardiac output. Finally, alterations in the indirect actions of ANG II on the sympathetic nervous system or/and other hormone systems may also make a contribution to these responses.

In response to AT1R blockade, a similar increase in RBF and GFR, but a greater decrease in RVR was observed in the uni-x compared with sham animals. Since the increase in RBF in response to losartan was similar between the groups, the greater decrease in RVR was due to the greater fall in MAP in the uni-x animals. The greater decrease in MAP in response to AT1R blockade in the uni-x animals, despite reduced systemic and renal ANG II levels, suggests a contribution of other vascular beds to the elevated arterial pressure in the uni-x animals. All components of the RAS are expressed in the fetal kidney at the time uni-x has been performed (7). It is possible that removal of a kidney may reduce the levels of circulating

Fig. 3. Effect of fetal uninephrectomy on systemic and intrarenal expression of renin and ANG II. Plasma renin activity (PRA; A), plasma ANG II (B), renin content in kidney cortex and medulla (C), and ANG II content in kidney cortex and medulla (D) are shown. Values are means ± SE. Open bars, sham, n = 6; filled bars, uni-x, n = 6. ***P < 0.001 compared with sham. In C and D, P values are from 2-way ANOVA with factors group (sham or uni-x), kidney zone (cortex and medulla), and their interaction.

Fig. 4. Relative mRNA expression of angiotensinogen (A’ogen; A), renin (B), ANG II receptor type 1 (AT1R; C), and ANG II receptor type 2 (AT2R; D) in kidneys of male sheep at 6 mo of age. **P < 0.01, ***P < 0.001 compared with sham.
RAS in the fetus. It is not known whether other vascular beds undergo increases in the local RAS to compensate for this loss, but it is possible.

Surprisingly, despite downregulation of the intrarenal RAS and the fall in arterial pressure, in response to AT1R blockade a marked increase in FENa occurred in the uni-x sheep. The increase in sodium excretion in response to losartan unmasks a contribution of the intratubular RAS to sodium reabsorption in the uni-x animals. A likely explanation for this finding is that the intratubular RAS was upregulated, such that tubular sodium reabsorption was enhanced by ANG II in the uni-x. While ANG II levels within the cortex of the uni-x animals were reduced, the levels in the medulla were similar to those of the sham animals, supporting the possibility that the RAS in the collecting ducts may be enhanced. A generalized systemic blockade of AT1R has altered/blunted sympathetic tone associated with AT1R inhibition and its contribution to sodium excretion need further investigation in our model of uni-x.

Similar to our observations, acute losartan treatment has been shown to increase GFR and sodium excretion in rats with reduced renal mass; however, blood pressure was unaltered (3), which is in contrast to our findings. In contrast, chronic treatment with losartan in the rat has been shown to lower arterial pressure, without altering GFR (27), indicating that the chronic and acute effects of losartan on the systemic circulation and renal function are likely different and need to be further investigated in the model of reduced nephron endowment.

MAP responses to ANG II were blunted in the uni-x animals, in contrast to studies in some models of reduced nephron endowment that have shown an increased sensitivity to ANG II (20, 37). Aside from methodological considerations, such as species differences and anesthetized vs. conscious measurements, it is also possible that since basal ANG II levels are lower in uni-x animals, infusion of graded ANG II doses would not have increased the circulating levels of ANG II in uni-x.
animals compared with sham. Renal responses to exogenous ANG II infusion were strikingly different between the uni-x and sham sheep. ANG II was administered systemically, as the sheep were not set up for direct intrarenal infusions. Thus the results must be interpreted with the understanding that ANG II-induced increases in MAP will contribute indirectly to the renal responses. However, with this in mind, the lowest dose of ANG II caused only minor changes in arterial pressure, and this response was not different between the groups. Under these circumstances at the lowest dose of ANG II, GFR and RBF declined and RVR increased to a lesser degree in the uni-x animals, which is in accord with our finding of lower AT1R expression in the uni-x kidney. However, in response to higher ANG II doses, associated with increases in arterial pressure, paradoxically renal vasodilation was observed and this was likely a predominant pregglomerular effect as GFR increased in the uni-x animals. In contrast, in the sham animals ANG II infusion elicited a reduction in GFR and RBF at the low dose, with no further reductions observed at higher doses, which is similar to the response seen in normotensive males (28). It is likely that the renal responses to ANG II at these higher doses were offset via renal autoregulatory responses in the sham animals (32) but suggest failure of these mechanisms in response to the increase in arterial pressure in uni-x animals. Studies in preterm infants, which are purported to have a reduced nephron endowment (34), have shown an altered trajectory for maturation of renal function (1), suggesting the tight coupling between glomerular and tubular function normally observed in the adult is absent in the preterm infant, likely due to tubuloglomerular feedback not being fully functional (1, 2). Additional studies are needed to determine whether autoregulatory mechanisms are altered following fetal uni-x.

**Conclusion.** The present study explored the contribution of the intrarenal RAS, which is usually upregulated in the setting of low-renin hypertension. We observed that both the systemic and intrarenal components of the RAS are suppressed in these young uni-x male sheep, although changes in specific renal compartments are differentially affected as functional evidence of an ANG II-mediated enhancement of sodium reabsorption was observed in response to acute AT1R blockade. In addition to the upregulation in sodium channels and transporters reported previously in these uni-x animals (41), the intrarenal RAS is likely contributing to the increased sodium reabsorption at this age. Furthermore, renal autoregulatory mechanisms may not be intact in the remnant kidney as observed by paradoxical vasodilation in response to higher doses of ANG II in the face of increased systemic pressure. Likely alterations in myogenic mechanisms associated with changes in renal vascular structure and/or a resetting of the tubuloglomerular feedback operating point may be contributing to this and need further exploration. Adaptive changes in the RAS and/or in other, as yet unexplored systems within the remnant kidney may occur in the short term to maximize renal functional capacity but as a consequence the fragile glomerular capillaries may be subjected to marked fluctuations in glomerular pressure. An inability to maintain tight control of glomerular pressure likely becomes maladaptive in adulthood, contributing to disease progression. Thus these studies may help explain why individuals born with a reduced renal mass, including preterm and growth-restricted infants and children born with only one kidney (39), are at risk of adult renal and cardiovascular disease. Future studies are required to understand the adaptations that the kidney undergoes in early childhood as the kidney matures if we are to limit the adverse impact of commencing life with a reduced nephron endowment.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

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