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Adrenalectomy Blocks the Compensatory Increases in UT-A1 and AQP2 in Diabetic Rat Kidney

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Abstract. In normal rats we showed that glucocorticoids participate in the downregulation of UT-A1 protein abundance in the inner medullary tip and in lowering of basal and vasopressin-stimulated facilitated urea permeability in terminal IMCDs. To examine the relevance of this response to a rat model of human disease, we studied rats with uncontrolled diabetes mellitus (DM) induced by streptozotocin (STZ), since these rats have increased corticosterone production and urea excretion. We found that at 3 days of DM, UT-A1 protein abundance is downregulated in the inner medullary tip compared to pair-fed control rats, while DM for more than 7 days caused an increase in UT-A1. To test whether adrenal steroids could be a mechanism contributing to the latter increase, we studied adrenalectomized rats (ADX), ADX rats given STZ to induce diabetes (ADX + STZ), and ADX + STZ rats receiving exogenous aldosterone or dexamethasone. In contrast to control rats, UT-A1 protein abundance was not increased by prolonged DM in the ADX rats. Aquaporin 2 (AQP2) was not increased in the inner medullas of 10-day DM rats either. However, UT-A1 protein abundance was significantly reduced in the inner medullary tips from both diabetic aldosterone-treated (40 ± 2%) and dexamethasone-treated (43 ± 2%) ADX rats compared to diabetic ADX rats without steroid replacement. AQP2 was unaffected by steroid hormone treatments. Thus, both mineralocorticoids and glucocorticoids downregulate UT-A1 protein abundance in rats with uncontrolled diabetes mellitus for 10 days. These results suggest that: 1) the increase in UT-A1 observed in DM is dependent upon having adrenal steroids present; and 2) adrenal steroids are not sufficient to enable the compensatory rise in UT-A1 to a steroid-deficient diabetic animal.

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Introduction

Patients with uncontrolled type I diabetes mellitus often experience an inability to concentrate urine properly. This polyuria is caused by a persistent osmotic diuresis due to glucosuria and can result in serious volume depletion. The kidneys compensate for this ongoing osmotic diuresis, in part, by altering key medullary transport proteins in order to minimize the effects of the progressive decline of urine concentrating ability (Nejsum et al., 2001; Kim et al., 2003). The urea transporter UT-A1 is one of these key transport proteins. We previously reported that at 3 days of diabetes, UT-A1 protein abundance is decreased (Klein et al., 1997). However, at 10 to 21 days of diabetes, UT-A1 protein is increased in abundance (Kim et al., 2003). Diabetes mellitus is characterized by both increased glucocorticoid production and increased urea excretion (Almdal et al., 1990; Almdal et al., 1992; Atchley et al., 1933; Freyse et al., 1996; Kim et al., 2005). We began to investigate the possible role of glucocorticoids in mediating the decrease in UT-A1 protein in 3-day DM animals by looking at UT-A1 in adrenalectomized (ADX) animals that had been made diabetic. We observed that in the absence of glucocorticoids, UT-A1 did not decrease (Klein et al., 1997). Administering exogenous glucocorticoids restored the reduction in UT-A1 abundance. However, it was not known whether the subsequent increase in UT-A1 at longer duration of diabetes was also dependent upon glucocorticoids. Therefore, the first goal of the present study was to determine if the increase in UT-A1, or of aquaporin 2 (AQP2), that accompanies a longer duration of diabetes was dependent upon glucocorticoids.

Table 1. Physiologic parameters of the diabetic ADX rats

	ADX	*n*	ADX + DM	*n*	ADX + DM + Aldo	*n*	ADX + DM + Dex	*n*
Blood glucose (mg/dl)	92 ± 6	9	249 ± 16	11	263 ± 15	16	285 ± 10	26
Urine volume (ml)	28 ± 3	18	102 ± 25	11	202 ± 53	10	218 ± 46	8
Urine OsM (mOsm/kg H_20)	1133 ± 42	23	942 ± 83	16	788 ± 90	11	820 ± 38	18
Weight gain (g)	178 ± 15		140 ± 16		169 ± 42		121 ± 5	

We previously showed that aldosterone also causes a decrease in UT-A1, similar to the effect of glucocorticoids, and that aldosterone's effect occurs through the mineralocorticoid receptor (Gertner et al., 2004). Therefore, the second goal of the present study was to determine whether mineralocorticoids were necessary for the UT-A1 or AQP2 changes that are part of the response to diabetes.

Materials and Methods

Animal Preparation

All animal protocols were approved by the Emory University Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (100–150 g, Charles River Laboratories, Wilmington, MA), received free access to water and standard rat chow (Purina) containing 23% protein. Rats were adrenalectomized as previously described (Klein et al., 1997) and received 1/4 normal saline to drink thereafter. After 14 days, some rats received aldosterone replacement (12 μg/rat/day) via a 14-day mini-pump (Alzet, Durect). Some rats received daily dexamethazone injections (1 or 15 μg/100 g b.w.) for the next 14 days. Half of each of these groups were given 40 mg/kg streptozotocin by tail vein injection to induce diabetes. After 24 hours, blood from a tail cut was assessed for blood glucose levels. After the animals were shown to be diabetic for 10 days, they were killed by decapitation and their blood, urine, and kidneys collected.

Sample Preparation

Following sacrifice, rat kidneys were dissected into inner medullary tip and base, and outer medulla. Tissues were placed into ice-cold isolation buffer (10 mM triethanolamine, 250 mM sucrose, pH 7.6, 1 μg/ml leupeptin, and 2 mg/ml PMSF), homogenized; then, SDS was added to a final concentration of 1% for Western analysis of the total cell lysate (Kim et al., 2003, 2004, 2005). Total protein in each sample was measured by a modified Lowry method (Bio-Rad DC protein assay reagent, Bio-Rad, Richmond, CA). Urine was collected for osmolality (vapor pressure osmometer, Wescor, Logan, UT). Blood glucose was monitored using a One Touch Profile glucometer (Lifescan, Inc., Milpitas, CA).

Western Analysis

Western analysis was performed as previously described (Naruse et al., 1997; Klein et al., 1997, 1999; Kato et al., 2000; Fröhlich et al., 2004). Briefly, rat inner medullary tissue was homogenized in ice-cold isolation buffer and brought to 1% SDS for Western analysis of total cell lysate (Naruse et al., 1997; Klein et al., 1997; Timmer et al., 2001). Proteins were size-separated by SDS-PAGE on Laemmli gels, electroblotted to PVDF membranes, and incubated with primary antibody overnight at 4°C. After washing, the blot was incubated with fluorescently labeled secondary antibody and visualized with the LICOR IR imaging scanner. Parallel gels were stained with Coomassie blue to verify uniformity of gel loading. Laser densitometry was used to quantify the intensity of the resulting bands on western blots.

Statistics

All data are presented as mean ± SE. To test for statistical significance between 2 groups, we used a Student's t-test. To test more than 2 groups, we used an analysis of variance, followed by Fisher's least significant difference (protected *t*-test) (Snedecor and Cochran, 1980) to determine which groups were significantly different. The criterion for statistical significance was $P < 0.05$.

Results

Diabetic Adrenalectomized Rats

The adrenalectomized 10-day diabetic rats had significantly higher blood glucose levels than the adrenalectomized control animals (Table 1). While not as robust as the control animals, the adrenalectomized animals gain weight at approximately the same rate as the control animals if they are given saline to drink (*data not shown*). The adrenalectomized animals that were given STZ were more fragile than control animals with DM: while not losing weight over the 10 days of DM, they failed to gain at the same rate as the non-diabetic ADX rats. The urine volumes for the last 24 hours were higher for the diabetic rats than the controls, regardless of whether the animals were given aldosterone or dexamethasone, and the urine osmolalities were lower due to the large volume of urine (Table 1). The urine showed clear evidence of glucosuria when tested by a urine dipstick (Multistix 10SG, Bayer, Elkhart, IN).

UT-A1 and AQP2

Unlike the increases observed in adrenally-intact 10-day diabetic rats (up 51 ± 4% in IM tip; 46 ± 1% in IM base, Figs. 1 and 2, respectively, and Kim et al., 2003), there was no increase in UT-A1 in the adrenalectomized rats in either IM tip (Fig. 1) or IM base (Fig. 2). AQP2 was slightly but significantly decreased in the IM base of the diabetic rat. The apparent decrease in AQP2 in the IM tip failed to reach statistical significance (Fig. 3). When dexa-

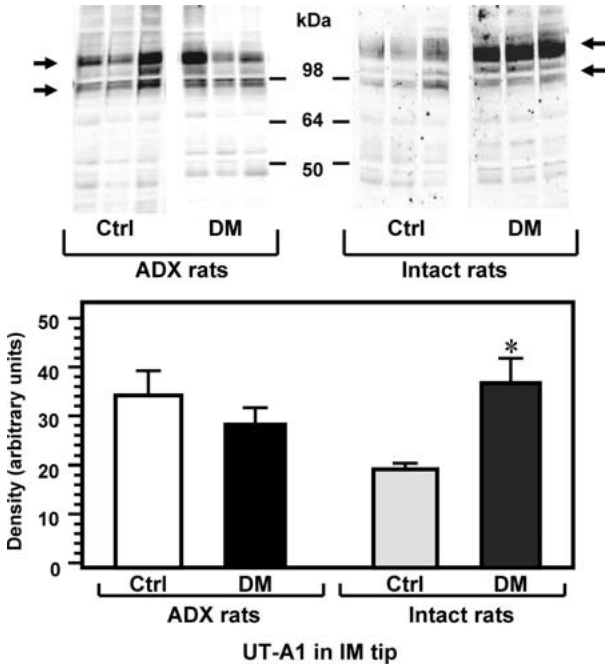

Fig. 1. In the inner medullary (IM) tip of adrenalectomized (*ADX*) rats, UT-A1 is not increased by 10 days of diabetes mellitus (*DM*). In contrast, UT-A1 is significantly increased by 10 days of diabetes in adrenally-intact (*Intact*) rats. The western blots (*top panel*) show representative samples of IM tips (3 per group) of a total of >20 animals per group, combining 6 cohorts of animals. Arrows indicate the prominent UT-A1 glycoprotein forms at 117 and 97 kDa. The *bottom panel* shows bar graphs summarizing the densitometric data from all cohorts of ADX rats (*left bars*) and intact rats, without (*grey bar, ctrl*) or with diabetes (*black bar, DM*). Data are presented as mean ± standard error; * = $P < 0.05$.

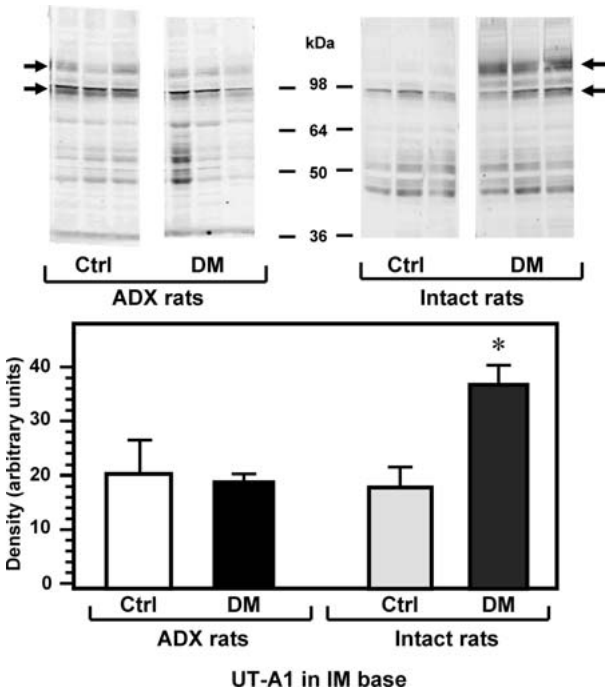

Fig. 2. In the inner medullary (IM) base of adrenalectomized (*ADX*) rats, UT-A1 is not increased by 10 days of diabetes mellitus (*DM*). In contrast, UT-A1 is significantly increased by 10 days of diabetes in adrenally-intact (*Intact*) rats. The western blots (*top panel*) show representative samples of IM bases (3 per group) of a total of >20 animals per group combining 6 cohorts of animals. Arrows indicate the prominent UT-A1 glycoprotein forms at 117 and 97 kDa. The *bottom panel* shows bar graphs summarizing the densitometric data from all cohorts of ADX rats (*left bars*) and intact rats, without (*grey bar, ctrl*) or with diabetes (*black bar, DM*). Data are presented as mean ± standard error; * = $P < 0.05$.

methasone was given back to the adrenalectomized rats, the amount of UT-A1 present in the IM tip was decreased 43 ± 2% ($P < 0.05$, $n = 12$, Fig. 4).

In the IM base, UT-A1 abundance in the dexamethasone-treated diabetic animal was not statistically different from the diabetic rats that did not receive any corticosteroid (Fig. 4). AQP2 levels in the IM tip were unaffected by treatment of the adrenalectomized animal with dexamethasone (Fig. 5).

Aldosterone treatment caused a 40 ± 2% decrease in the UT-A1 in the IM tip, but not in the IM base (Fig. 6). As was seen with dexamethasone, AQP2 was unchanged in either IM tip or base of the adrenalectomized animals in response to aldosterone replacement (Fig. 7).

Discussion

The major finding in this study is that steroid hormones are necessary for the increases in UT-A1 and AQP2 seen at 10 days of diabetes. The fact that adrenalectomy causes a loss of DM-dependent UT-A1 and AQP2 accumulation suggests that mineralocorticoids and/or glucocorticoids are necessary for the diabetic response. Alternatively the adrenalectomy may have already caused a maximal increase in UT-A1 that could not be further increased above adrenalectomized control levels. Since the DM-dependent increases in UT-A1 and AQP2 are part of a compensatory response by the kidney to decrease the loss of solute and water (Nejsum et al., 2001; Kim et al., 2003), this finding suggests that one or both steroid hormones play a role in stimulating this compensatory response.

Administering either glucocorticoids or mineralocorticoids alone to adrenalectomized diabetic rats decreased the abundance of the UT-A1 in the IM tip (Figs. 4 and 6). This result is similar to what occurs in non-diabetic adrenalectomized normal rats given either glucocorticoids or mineralocorticoids alone (Naruse et al., 1997; Gertner et al., 2004). Thus, these results are somewhat surprising, as our hypothesis was that replacing either glucocorticoids or mineralocorticoids would have enabled the compensatory response. This suggests that the

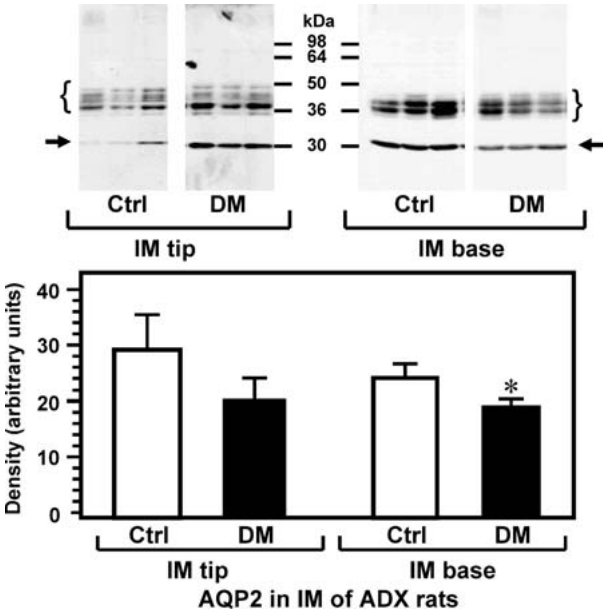

Fig. 3. In adrenalectomized (*ADX*) rats, aquaporin 2 (*AQP2*) is not increased by 10 days DM. The same protein samples from the UT-A1 analysis were subjected to a western analysis of AQP2 content. Shown is a western blot with representative samples from inner medullary (*IM*) tip (*left*) and base (*right*) (3 per group) of a total of >20 animals per group combining 6 cohorts of animals. Arrows indicate the prominent AQP2 forms at 35–50 kDa (glycosylated) and 29 kDa (unglycosylated). Below are bar graphs summarizing the densitometric data from all cohorts in the ADX rats without (control, *ctrl.,white bar*) or with diabetes (*DM, black bar*). Data are presented as mean ± standard error; * = $P < 0.05$.

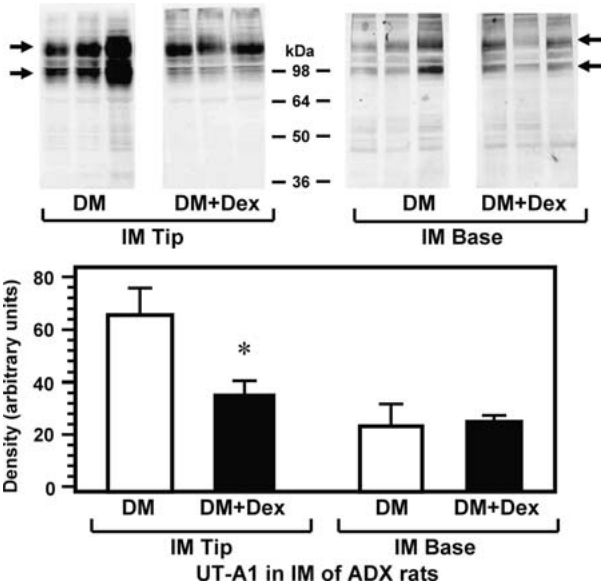

Fig. 4. UT-A1 in the inner medullary (*IM*) tip is decreased by treatment with dexamethasone. Adrenalectomized (*ADX*) rats were given daily injections of dexamethasone (*Dex)* beginning 3 days prior to making them diabetic (*DM*) by injection of streptozotocin as described in Methods. Shown is a western blot of representative samples (3 per group) of a total of 12 animals per group combining 3 cohorts of animals. Shown is UT-A1 in the IM tip (*left*) and IM base (*right*). Arrows indicate the prominent UT-A1 glycoprotein forms at 117 and 97 kDa. Below are bar graphs summarizing the densitometric data from all cohorts. Data are presented as mean ± standard error; * = $P < 0.05$.

compensatory response may require both steroid hormones. Another possibility is that physiologic variation in one or both steroid hormones may be necessary for the compensatory response. If changes in the levels of glucocorticoids and/or mineralocorticoids are important for the compensatory response, then the fixed replacement dosages that we administered to the adrenalectomized diabetic rats would not have reproduced the results in adrenally intact diabetic rats.

We previously showed that AQP2 is increased in the IM tip and base of 10-day DM rats (Kim et al., 2003). This increase did not occur in the adrenalectomized diabetic rats, and in fact, AQP2 decreased in the IM base. Thus, the compensatory increase in AQP2 may also require one or both steroid hormones. Neither administration of glucocorticoids nor mineralocorticoids alone restored the compensatory increase in AQP2 that occurs at 10 days of diabetes.

In summary, diabetes for 10 days results in an increase in UT-A1 and AQP2 protein abundances in the inner medulla (Kim et al., 2003). These increases do not occur in adrenalectomized diabetic rats. Neither administration of glucocorticoids nor mineralocorticoids alone restored the increase in UT-A1

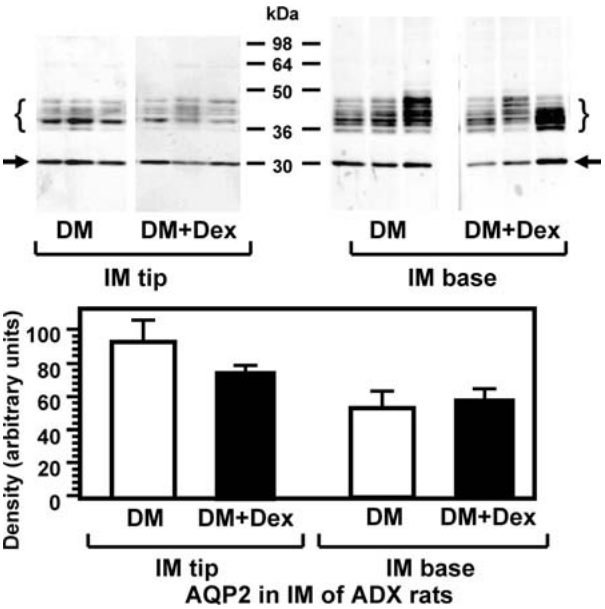

Fig. 5. Aquaporin 2 (*AQP2*) is unaffected by dexamethasone treatment. The same protein samples from the UT-A1 analysis were subjected to a western analysis of AQP2 content. The left hand blots show the AQP2 in the inner medullary (*IM*) tip; the right hand panel shows the IM base. Below is a bar graph summarizing the densitometric data from all cohorts. Data are presented as mean ± standard error.

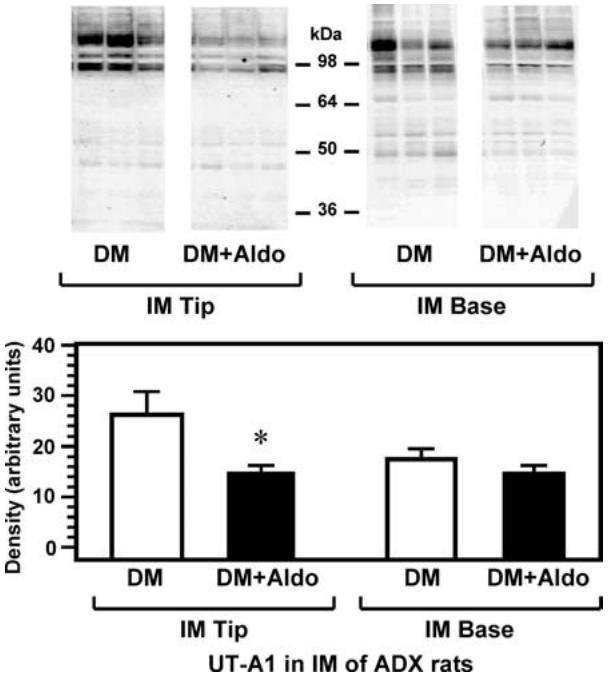

Fig. 6. UT-A1 is decreased in the inner medullary (*IM*) tip, but not the IM base, by treatment with aldosterone. Adrenalectomized (*ADX*) rats were given a constant infusion of aldosterone via minipump beginning 3 days prior to making them diabetic (*DM*) by injection of streptozotocin as described in Methods. Shown is a western blot of representative samples (3 per group) of a total of 11 - 14 animals per group combining 3 cohorts of animals. On the left are blots showing UT-A1 in the IM tip and on the right are the IM base samples. Arrows indicate the prominent UT-A1 glycoprotein forms at 117 and 97 kDa. Below are bar graphs summarizing the densitometric data from all cohorts. *White bar:* diabetes mellitus (DM), *black bar:* DM + aldosterone replacement. Data are presented as mean ± standard error; * = $P < 0.05$.

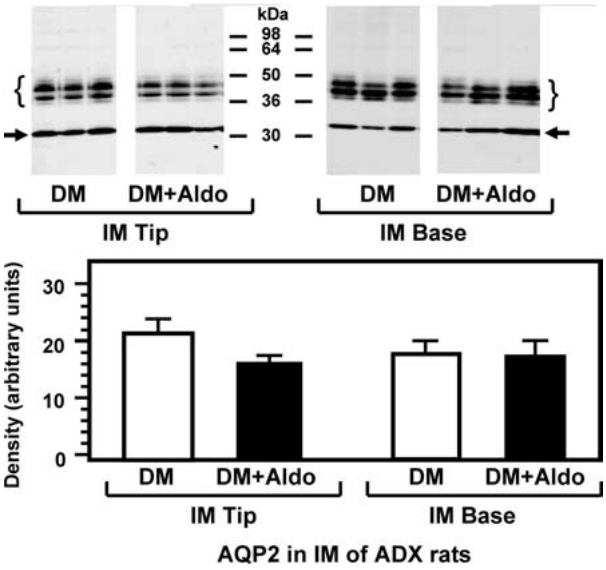

Fig. 7. Aquaporin 2 (AQP2) is unaffected by aldosterone treatment. The same protein samples from the UT-A1 analysis were subjected to a western analysis of AQP2 content. On the left are blots showing the AQP2 in the inner medullary (IM) tip and on the right are the IM base samples. Below is a bar graph summarizing the densitometric data from all cohorts. *White bar:* diabetes mellitus (DM), *black bar:* DM + aldosterone replacement. Data are presented as mean ± standard error.

or AQP2 that occurs at 10 days of diabetes. Since the increases in UT-A1 and AQP2 that occur at 10 days of diabetes are part of a compensatory response by the kidney to decrease the loss of solute and water (Bardoux et al., 2001; Kim et al., 2003), we conclude that one or both steroid hormones, or the physiologic variation in their levels following the induction of diabetes with streptozotocin, play a role in stimulating the compensatory increases in UT-A1 and AQP2 proteins in the rat inner medulla.

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