Renal Medullary Adenylate Cyclase in Drug-Induced Nephrogenic Diabetes Insipidus (37101)

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Feeding rats with an experimental drug, 2-amino-4,5-diphenyl thiazol-HCl (Bax 439), produces a syndrome characterized by a striking decrease in concentrating ability of the kidney, while diluting ability is unchanged (1).

Total tissue solutes are slightly decreased in the papilla and not changed in the medulla. Administration of exogenous antidiuretic hormone (ADH) does not affect urine concentration. These findings indicate a decreased water permeability of collecting ducts and/or a defect in the cellular action of ADH.

It is now a generally accepted view that adenosine-3′,5′-cyclic-monophosphate (cyclic AMP) plays a key role in the cellular action of ADH in the mammalian kidney (2, 3). ADH stimulates the formation of cyclic AMP in tubular cells, and cyclic AMP increases water permeability of lumenal plasma membrane, which forms the limiting barrier to resorption of water along the osmotic gradient across collecting ducts (4). In the present study we investigated the activity of renal medullary adenylate cyclase in animals with nephrogenic diabetes insipidus induced by Bax 439 and the response of adenylate cyclase to ADH.

Methods and Materials. Six 150–160 g specific pathogen-free Sprague–Dawley male rats were given Bax 439[1] by gavage for 4 days, and 6 additional similar rats were used as pair-fed controls. All animals were housed in individual metabolic cages. Urine output was measured daily, and the concentrating ability was assessed by a 16-hr dehydration test which was performed on the fourth day. The 3 drug-treated rats with the lowest urine osmolalities and their respective controls were used for adenyl cyclase assays.

The animals were decapitated, and the kidneys were quickly removed and chilled in ice-cold isotonic medium. The right kidney was bisected. Half was fixed in formalin for light microscopy, and half of the right kidney along with the entire left kidney was used for adenylate cyclase studies. The medullary tissue was dissected from cortex of the 3 drug-treated and 3 control animals and was pooled in 2 groups. The adenylate cyclase (washed 600*g* sediment of the homogenate) was prepared from each group as described in previous reports (5). In a similar way cortical adenylate cyclase was prepared from superficial cortical slices of the same kidneys. The adenylate cyclase activity was assayed as described previously (5). Adenylate cyclase was stimulated by synthetic (8-arginine)-vasopressin[2] and a counterpart to specific hormonal stimulation of adenylate cyclase by ADH, we used stimulation with fluoride, an anion which stimulates adenylate cyclase from many multicellular organisms (6).

Results. Animals treated with Bax 439 developed a concentrating defect characterized by increased urinary output and a markedly decreased maximal concentrating ability (Table I).

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[1] Bax 439 was donated by Baxter Laboratories, Inc., Morton, Grove, Illinois.

[2] Synthetic (8-arginine) vasopressin was a gift of Drs. Schwartz and Walter from Mt. Sinai Medical School, New York.

TABLE I.

Group (No. of animals)	Urine vol. (ml/24 hr)	Max. concn. (mOsm/kg)	Adenylate cyclase activity (pmole cyclic-AMP/min/mg protein) Medulla Basal	Medulla $10^{-6}M$ ADH	Medulla 10 mM NaF	Cortex Basal	Cortex 0.1 mg/ml PTH	Cortex 10 mM NaF
Drug-treated	23.9[a]	821	41.4	91.8	121.4	2.5	21.7	32.2
(3)	±2.8	±24	±2.4	±5.6	±5.3	±.4	±1.2	±3.2
Control	9.2	2318	43.7	145.6	104.3	3.2	19.5	46.1
(3)	±.4	±212	±2.6	±.8	±2.0	±.4	±1.5	±3.5
P[b]	<.025	<.005	>.5	<.05	<.05	>.2	>.5	>.5

[a] All values are the mean ± standard error.
[b] Values derived from group t tests.

Mild histologic changes were present after the 4 days of treatment in the inner stripe of the outer medulla and outer portions of the inner medulla of 2 of the 3 drug-treated animals. These changes included focal interstitial mononuclear cell infiltration, occasional protein and loose cellular casts in the collecting ducts and the presence of rare mitotic figures in a given tissue section. Some of the epithelial cells lining the collecting ducts were reduced in height. The nuclei of these cells were large and pale and contained an increased number of very prominent nucleoli. These nuclear changes were also present in some epithelial cells lining the loops of Henle. Control animals showed no detectable lesions. These findings were compatible with those of a sequential morphologic study of this drug (7).

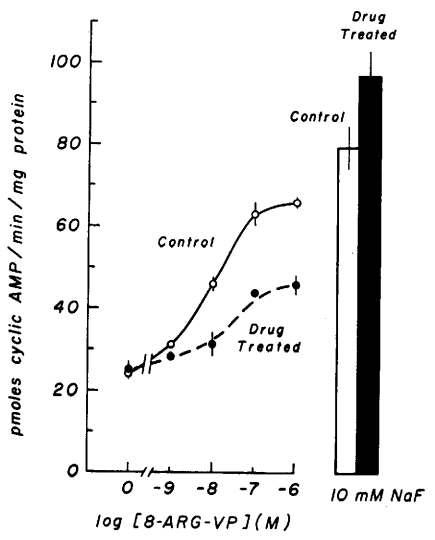

FIG. 1. Response of renal medullary adenylate cyclase to (8-arginine)-vasopressin and to sodium fluoride.

The basal activity of renal medullary adenylate cyclase (without addition of stimulating agents) was the same in drug-treated and control animals and adenylate cyclase stimulated by the addition of 10 mM sodium fluoride was greater in drug-treated than in control animals. The stimulatory response to 10^{-6} M ADH was markedly reduced in drug-treated animals which developed nephrogenic diabetes insipidus, while the response of cortical adenylate cyclase to PTH showed no difference between animals treated with Bax 439 and untreated controls (Table I). The response of renal medullary adenylate cyclase from drug-treated animals was decreased in all concentrations of ADH used, but the half maximum response to the ADH (assessed from graphic analysis) (3) was identical with controls (Fig. 1).

Discussion. Oral administration of Bax 439 caused a syndrome in rats characterized by a marked increase in urinary output, decrease of maximal ability to concentrate urine under conditions of dehydration or after administration of exogenous ADH. Histologic changes were very mild after 4 days of treatment compared to longer terms of drug administration (7). No apparent structural changes such as basement membrane thickening, changes in plasma membranes or amyloid deposits (8) were found which would form

an obvious mechanical hindrance for the access of ADH to the tubular cells or form a hindrance for osmotic water flow.

Adenylate cyclase studies *in vitro* indicate that the response to ADH of adenylate cyclase from the medulla of animals with a drug-induced concentrating defect is markedly decreased, while the basal activity of the enzyme and nonhormonal stimulation with fluoride is not diminished. The response to ADH is reduced at all concentrations of hormone but the one-half maximum response was not changed. This suggests that the treatment with drug did not change the affinity of the receptor for ADH but either decreased the total number of receptor sites or impaired the coupling between the hormone receptor and catalytic units of the adenylate cyclase complex. The normal response of cortical adenylate cyclase to parathyroid hormone (PTH) indicates that the drug acts specifically on medullary adenylate cyclase or else that due to the handling of the drug, it is more concentrated in the medullary tissue.

The question arises whether the reduced response of ADH-stimulated renal medullary adenylate cyclase could explain resistance of the drug-treated animals in ADH *in vivo*. It is conceivable that only a certain portion of cyclic AMP formed under the influence of ADH reaches and acts on the lumenal plasma membrane, whereas a substantial amount of cyclic AMP is quickly broken down through the action of cyclic AMP phosphodiesterase or leaks out of the cell. In such a case, only a slight decrease in the total capacity of the ADH-sensitive adenylate cyclase system to form cyclic AMP could cause a partial or total defect in the antidiuretic response to ADH *in vivo*. This view is supported by the findings that lithium (9) or vasopressinoic acids (10) which reduce the response of renal medullary adenylate cyclase *in vitro* block the renal response to ADH in the whole animal (10, 11).

It is also possible that the decrease in response of renal medullary adenylate cyclase to ADH *in vitro* is not relevant to the final antidiuretic response, and that the drug-induced defect in the cellular action of ADH resides in steps which are subsequent to the formation of cyclic AMP.

Summary. In rats with drug-induced nephrogenic diabetes insipidus the response of renal medullary adenylate cyclase to ADH *in vitro* is markedly diminished. It is suggested that this defect in hormone-dependent cyclic AMP formation could be the underlying cause of the ADH resistance as found *in vivo*.

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