J. Steroid Biochem. Molec. Biol. Vol. 39, No. 2, pp. 181–184, 1991
Printed in Great Britain

0960-0760/91 $3.00 + 0.00
Pergamon Press plc

ONTOGENY OF DEXAMETHASONE BINDING AND SODIUM POTASSIUM ATPASE ACTIVITY IN EXPERIMENTAL MURINE POLYCYSTIC KIDNEY DISEASE*

MALCOLM R. OGBORN[1]† and JOHN F. S. CROCKER[2]

[1]Department of Pediatrics and Child Health, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba and [2]Department of Pediatrics, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada

(Received 31 May 1990; received for publication 3 May 1991)

Summary—The induction of polycystic kidney disease (PKD) by glucocorticoids in newborn mice behaves as a "threshold" trait, with prevalence of PKD varying in different inbred strains after exposure to an inducing steroid. C3H mice (low threshold for PKD) demonstrated greater specific dexamethasone binding than DBA mice (high threshold) on the second day of life. Treatment with methylprednisolone acetate (MPA), a cyst-inducing steroid, down regulated dexamethasone binding earlier than in DBA mice. C3H mice demonstrated greater whole kidney homogenate Na-K ATPase activity than DBA mice within 24 h of MPA injection. Specific renal glucocorticoid binding may be a regulator of threshold for murine glucocorticoid induced PKD. Our findings support *in vitro* evidence that glucocorticoid induced Na-K ATPase activity during critical periods of nephron development is an important regulatory point of this model.

INTRODUCTION

MacDonald *et al.* [1] have reported that induction of polycystic kidney disease (PKD) by neonatal hydrocortisone acetate injection in differing inbred strains of mice fits a "threshold" model of disease, with the prevalence and severity of PKD varying in a reproducible fashion between strains. This variation fits a mathematical model which predicted that PKD occurred when a certain heritable, strain-specific threshold value of accumulated liability factors was exceeded.

The interaction of a steroid molecule with a specific receptor [2] is a limiting random event in glucocorticoid induction of PKD. The operation of random factors is inherent to the model of MacDonald *et al.* [1]. We hypothesized that the difference in the prevalence and the degree of glucocorticoid-induced cyst formation between inbred strains of mice might arise from a difference in the ontogeny of specific renal glucocorticoid receptors during early post-natal life. Also, based upon findings of a relationship between cyst formation and glucocorticoid induction of Na-K ATPase in serum free metanephric murine organ culture [3], we hypothesized that this would be associated with a difference in the ontogeny of induction of renal Na-K ATPase by glucocorticoids.

MATERIALS AND METHODS

C3H and DBA mice, identified as having low and high thresholds for PKD, respectively [1], were purchased from Jackson Laboratories, MA, and kept in the animal care quarters of the Izaak Walton Killam Hospital for Children. Animals were kept under conditions in accordance with the standards of the Canadian Council on Animal Care. All procedures were approved by the Dalhousie University Committee on Live Animal Use. [1,2,4-^{3}H]Dexamethasone (47 Ci/mmol) was purchased from Amersham, IL. Methyl prednisolone acetate (MPA) was purchased from The Upjohn Company of Canada, Don Mills, Ontario.

Protein determinations were by the brilliant Coomassie blue method [4] using a Biorad

*Presented in part to the *Annual Scientific Meeting of the Canadian Society of Clinical Investigation*, Edmonton, Alberta, 22–25 September (1989) and the Society of Pediatric Research, Anaheim, California, 7–10 May (1990).

†To whom correspondence should be addressed at: AE102, Health Sciences Centre, 840 Sherbrook Street, Winnipeg, Manitoba, R3A 1S1.

kit with bovine serum globulin as a standard (Biorad, Richmond, CA). All other chemicals and reagents were purchased from Sigma Chemical Co., St Louis, MO, or BDH Chemicals, Toronto, Ontario. A preliminary study to test MPA as a cyst-inducing agent produced a distribution of cysts in 40 animals of each strain on the 5th post-natal day that was consistent with the predictions of the model of MacDonald *et al.* [1], who used hydrocortisone acetate.

Glucocorticoid binding assay

Dexamethasone binding was measured in 581 animals [mean number measured in each treatment group: 32 ± 7 (SD)]. Animals were killed by cervical dislocation on the 1st day of post-natal life (baseline group) or on the 2nd to 5th post-natal days, 1 to 4 days after an i.m. injection of 200 mg/kg of MPA (treatment group) or 0.9% saline (control group). One kidney was removed, chilled in TTESM buffer (TES 10 mM, sucrose 250 mM, EDTA 1.5 mM, monothioglycerol 12 mM, sodium molybdate 5 mM, pH = 7.4 at 25°C), minced and frozen in liquid nitrogen. Tissue was thawed to 4°C, homogenized in 0.7 ml of TTESM buffer for 30 s in a Kontes Teflon–glass grinder, centrifuged at 240,000 *g* for 45 min, and the supernatant collected and refrozen [5]. 100 μl Of cytosol were added to glass tubes to which [^{3}H]dexamethasone in ethanol–benzene (9:1 v/v) had been added and carrier solvent evaporated to give a final concentration of 50 nM. 1 h Into the incubation, 50 μl was removed and added to tubes containing a final concentration of 100 μM unlabelled dexamethasone (2000 fold excess over labelled hormone) to determine non-specific binding. Both sets of tubes were incubated for a further 4 h at 4°C. Preliminary studies found little specific binding before 1 h of incubation, whereas maximal binding occurred by 4 h. This system was adopted to guarantee the same quantity of free labelled hormone in both total and non-specific binding assays. Bound hormone was isolated by the method of Luttge *et al.* [6]. 25 μl Of the incubation mixtures were layered onto Sephadex LH-20 gel beds in Kontes Miniflex 10 cm columns with a nominal void volume of 1 ml. Specific glucocorticoid binding was eluted with TTESM buffer at 1–1.5 void volumes. Column clean up was performed with TTESM with 30% ethanol followed by TTESM alone. Dexamethasone binding assays were only accepted as comparable if a measurement of free labelled steroid in the incubation mixture was within 5% of the target concentration of 50 nM.

Na-K ATPase assay

Na-K ATPase activity was measured in 652 animals [mean number measured in each treatment group: 36 ± 10 (SD)]. Activity was measured by an adaptation of the method of Avner *et al.* [3]. The kidney not used for dexamethasone binding was minced in 20 mM Tris/200 mM sucrose buffer (pH = 7.0) at 4°C, snap frozen and stored in liquid nitrogen. 30 μl Of homogenized kidney were added to duplicate 1 ml cuvettes containing a 100 mM NaCl, 10 mM KCl, 2.5 mM $MgCl_2$, 1 mM disodium ATP, 1 mM phosphoenolpyruvate, 30 mM Imidazole–HCl (pH = 7.3, 37°C), 0.15 mM NADH, 50 μg/ml lactate dehydrogenase and approx. 30 μg/ml pyruvate kinase with or without 2.5 mM ouabain. One cuvette of each pair contained 2.5 mM ouabain. Enzyme activity was calculated as the difference in rates of oxidation of NADH between the ouabain inhibited and ouabain free mixtures at 37°C, determined by changing absorbence at 340 nm in a Pye Unicam L550 spectrophotometer. The enzyme kinetics were determined by analysis on a simple linear regression model built within Lotus Symphony (Version 1.0). Quality control was monitored by the use of a purified canine renal Na-K ATPase (Sigma Chemical Co.).

Statistical analysis

The results of comparisons of means between various experimental groups at each post-natal age were analysed by independent *t*-tests using the Systat statistical package (Systat, Inc., Evanston, IL). Relationships between the glucocorticoid receptor status and Na-K ATPase activity were studied by linear regression using the same package.

RESULTS

Measurement of cytosolic dexamethasone binding revealed that there was greater dexamethasone binding in saline treated C3H animals compared to DBA animals on the 2nd day of post-natal life (44.1 fmol/mg protein vs 26 fmol/mg protein: $P = 0.011$), one day after the injection treatment. The second was the tendency for cytosolic binding to decrease after MPA treatment in both strains, which is consistent with the predictions of other work on steroid receptor physiology [7]. This down regulation

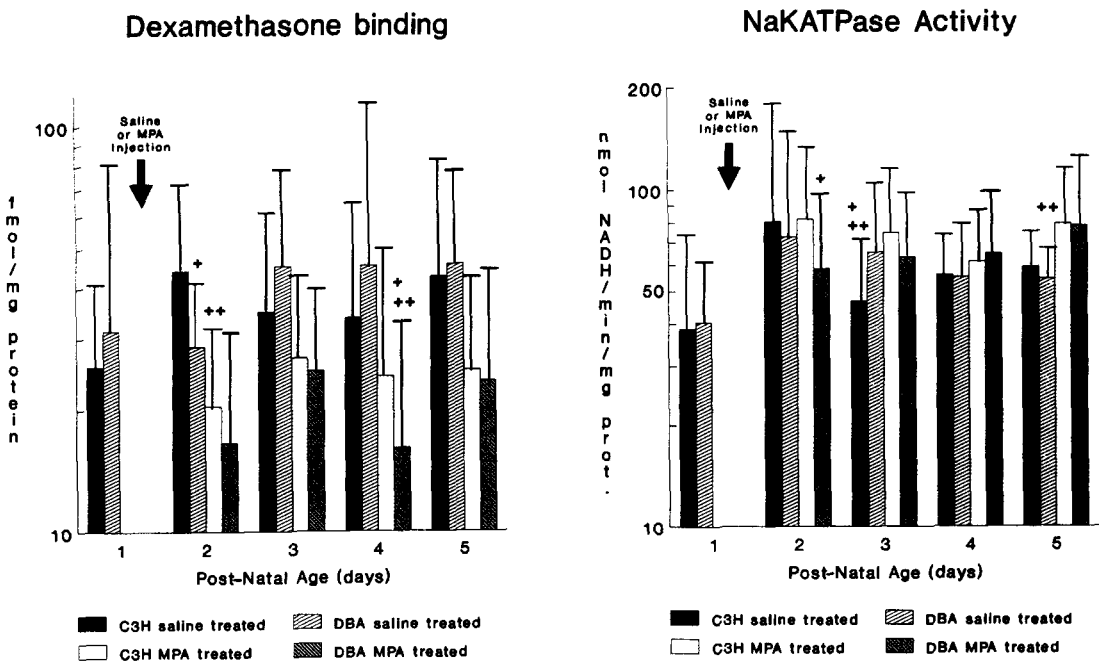

Fig. 1. Ontogeny of dexamethasone binding and Na-K ATPase activity in C3H and DBA mice after neonatal saline or MPA injection. Bars represent mean results with error bars representing SD. $^{+}$Significantly different from other strain with same treatment and age ($P < 0.05$). $^{++}$Significantly different from saline treated animals of same strain and age ($P < 0.05$).

was evident in the MPA treated C3H mice on the 2nd day of post-natal life, but DBA mice did not have significantly lower levels until the 4th post-natal day.

MPA treated C3H mice demonstrated greater Na-K ATPase activity on the 2nd post-natal day compared to DBA mice (81.9 vs 58.3 nmol NADH/min/mg protein: $P = 0.018$). Interestingly, the activity in the MPA treated C3H mice was not significantly greater than saline treated mice of the same strain (81.9 vs 80.4 nmol NADH/min/mg protein); homogenate protein was greater in the MPA group (2.3 vs 2.9 mg/ml), implying that the degree of induction is associated with a general trophic response. Saline treated C3H mice demonstrated a fall in Na-K ATPase activity on the 3rd post-natal day to levels that were significantly less than saline treated DBA mice (46.7 vs 65.4 nmol NADH/min/mg protein: $P = 0.038$). After MPA treatment, corrected Na-K ATPase activity in 3-day-old C3H mice was significantly greater than the saline treated group (74.6 vs 46.6 nmol NADH/min/mg protein: $P = 0.005$).

Although there was a trend towards an inverse relationship between cytosolic glucocorticoid binding and Na-K ATPase in both strains, a significant linear relationship could not be established in either strain.

DISCUSSION

Our results indicate that C3H mice, with a low threshold for induction of PKD, demonstrate higher levels of renal Na-K ATPase immediately after post-natal MPA injection than DBA mice, which are relatively resistant to such induction. Our findings also suggest that a factor in this response may be a difference in the ontogeny of specific dexamethasone binding. The availability of the steroid receptors that this binding may represent may be influenced by the previous hormonal milieu of the animal or, in pre-natal life, of the mother. Material and fetal steroid metabolism are intimately related and tightly regulated in mammalian species, particularly the mouse [8, 9]. Ellis *et al.* [7] have demonstrated a reciprocal relationship between murine renal Na-K ATPase and glucocorticoid receptors. They further demonstrated that previous glucocorticoid exposure appeared to down regulate the availability of such receptors which is consistent with our results [10].

Our studies did not demonstrate a difference in the absolute amount of induction of Na-K ATPase, suggesting that this is not a determinant of the PKD threshold. The timing of that induction relative to other events in the developing metanephros may be a more important factor. Furuse *et al.* [11] have demonstrated that

renal tubular differentiation and growth in normal metanephric tissue from the mouse is associated with a steady increase in Na-K ATPase activity. Previous work by Avner *et al.* [3] has shown that Na-K ATPase activity is a limiting factor in cyst formation in serum free organ culture using a model with hydrocortisone induction of cysts.

Activity of Na-K ATPase might reasonably be thought to oppose cyst formation by favouring the egress of fluid and electrolytes from the nephron [12]. It seems likely, therefore, that activity of the enzyme is part of transduction of a signal that regulates the proliferation of renal tubular cells that is a common feature of models of PKD. Glucocorticoids stimulate increased activity of the Na + –H + antiporter in some renal tubular cells [13], a property shared with the potent renal mitogen epidermal growth factor (EGF) [14]. EGF has been implicated in human and murine PKD [15, 16]. Glucocorticoids have been shown to enhance expression of EGF receptors in other cell systems [17]. Such an effect warrants further study in this model.

The exploration of hormone action, electrolyte transport, growth factor activity and cell proliferation in renal cysts offers great potential for gaining knowledge of the regulation of growth and development during cyst formation. This comparative model, using mice with high and low thresholds for induction of PKD by glucocorticoids, should prove a powerful and increasingly relevant tool for the study of this important group of human disorders.

Acknowledgements—We gratefully acknowledge the technical assistance of Susanna McCarthy in animal care and Na-K ATPase assays, and Janice Martin and Sanjay Sareen in preparation of this manuscript. This work was supported by operating grants from the Kidney Foundation of Canada.

REFERENCES

1. MacDonald A. T. J., Crocker J. F. S., Digout S. C., McCarthy S. C., Blecher S. R. and Cole D. E. C.: Glucocorticoid induced polycystic kidney disease—a threshold trait. *Kidney Int.* **37** (1990) 901–908.
2. Scheidereit C., Krauter P., Von Der Ahe D., Janich S., Rabenau O., Cato A. C. B., Suske G., Westphal H. M. and Beato M.: Mechanism of gene regulation by steroid hormones. *J. Steroid Biochem.* **24** (1986) 19–24.
3. Avner E. D., Sweeney W. P., Finegold D. N., Piesco N. P. and Ellis D.: Sodium potassium ATPase activity mediates cyst formation in metanephric organ culture. *Kidney Int.* **28** (1985) 447–455.
4. Bradford M. M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt. Biochem.* **72** (1976) 248–254.
5. Sherman M. R., Pickering L. A., Rollwagen F. M. and Miller L. K.: Mero-receptors: proteolytic fragments of receptors containing the steroid binding site. *Fedn Proc.* **37** (1978) 167–173.
6. Luttge W. G., Emadian S. M., Densmore C. L. and Slatick R. S.: Efficacy of five methods for the bound-free separation of gluco- and mineralocorticoids from type I, II, and III receptors found in Hepes- and Tris-buffered mouse brain cytosol. *Steroids* **43** (1984) 429–443.
7. Ellis D., Sothi T. D., Curthoys N. P., Ballou B. and Avner E. D.: Regulation of glucocorticoid receptors and Na-K ATPase activity by hydrocortisone in proximal tubular cells. *In Vitro Cell. Dev. Biol.* **24** (1988) 811–816.
8. Michaud N. J. and Burton A. F.: Maternal-fetal relationships in corticosteroid metabolism. *Biol. Neonate* **32** (1977) 132–137.
9. Tye L. M. and Burton A. F.: Variation in the pattern of metabolism of corticosteroids in fetal mouse tissues. *Life Sci.* **26** (1980) 35–39.
10. Ellis D., Sothi T. D. and Avner E. D.: Glucocorticoids modulate renal glucocorticoid receptors and Na-K ATPase activity. *Kidney Int.* **32** (1987) 464–471.
11. Furuse A., Bernstein J., Welling L. W. and Welling D. J.: Renal tubular differentiation in mouse metanephric culture II Na-K-ATPase activity. *Pediat. Nephr.* **3** (1989) 273–279.
12. Katz A. I.: Renal Na-K-ATPase: its role in tubular sodium and potassium transport. *Am. J. Physiol.* **242** (1982) F207–F219.
13. Bidet M., Merot J., Tauc M. and Poujeol P.: Na + –H + exchanger in proximal cells isolated from kidney II. short term regulation by glucocorticoids. *Am. J. Physiol.* **253** (1987) F945–F951.
14. Pouysségur J., Franci A., L'Allemain G., Magnaldo I., Paris S. and Sardet C.: Genetic approach to structure, function and regulation of Na + /H + antiporter. *Kidney Int.* **32** (Suppl.) (1987) 5144–5149.
15. Das K., Gabow P. W. and Wilson P. D.: Abnormal growth factor response by human adult polycystic disease epithelia *in vitro*. *Kidney Int.* **35** (1989) 310.
16. Moscowitz D. W., Bonar S. L., Marcus M. D., Clayman R. V. and Avner E. D.: Epidermal growth factor content of human and mouse renal cysts. *Clin. Res.* **37** (1989) 497A.
17. Kyakumoto S., Kurokawa R. and Ota M.: Effect of glucocorticoid on epidermal growth factor in human salivary gland adenocarcinoma cell line HSG. *Biochim. Biophys. Acta* **1053** (1990) 204–212.