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Triamcinolone Activation of Renal Ammonia Production (39587)

T. C. WELBOURNE, P. PHENIX, C. THORNLEY-BROWN, AND C. J. WELBOURNE

Department of Medicine, University of Montreal; and Institute of Clinical Research, 110 Pine Avenue West, Montreal, Quebec, Canada

Chronic metabolic acidosis leads to activation of the mitochondrial glutaminase 1 pathway (1, 2) resulting in a marked increase in renal ammonia production. This homeostatic response to acidosis appears to be mediated by the adrenal glands since plasma corticosterone levels are elevated in response to acid loading (3) while activation of the mitochondrial pathway does not occur in adrenalectomized animals (3, 4). Consequently, corticosteroids appear to play the key role in the adaptive increase in renal ammonia production. If this is so, administering exogenous glucocorticoids should elicit a renal response, via activation of the mitochondrial pathway, which resembles the response seen during acid loading. This report demonstrates that triamcinolone activates the mitochondrial pathway and results in a pattern of ammonium excretion similar to that observed in acidosis.

Materials and methods. Twenty-four male Sprague–Dawley rats weighing between 350 and 450 g were divided into two groups. One group was studied *in vivo* while the second group provided kidneys for studies on ammonia production by the isolated perfused kidneys.

In vivo study. Twelve rats were housed, one per cage, in a metabolic cage for the collection of 24-hr urine samples, and maintained on Purina rat chow and tap water *ad libitum*. After 3 control days, six of the rats were given triamcinolone (Sigma), 1 mg kg^{-1} day^{-1} in two divided doses by im injection of the hormone suspended in 0.5 ml of 0.9% sodium chloride; six other rats received 0.5 ml of 0.9% sodium chloride. Following 3 days of treatment, the injections were suspended and 24-hr urine samples were collected for a subsequent 2-day period. Daily urine samples were collected, preserved, and analyzed as already described (5). Urinary and plasma sodium and potassium concentrations were determined by flame photometry; plasma total CO_2 was measured manometrically on a Natelson microgasometer (6) while urine pH was monitored on an Orion pH meter.

In vitro study. Six rats were administered triamcinolone, 1 mg kg^{-1} for a 24-hr period prior to the isolation and perfusion of their kidneys (2, 7); six others were sham-treated and employed as controls. The kidneys were perfused with an artificial plasma solution (7) and dextran T-110 (Pharmacia) was satured with 95% O_2:5% CO_2 and adjusted to pH 7.4 Carrier L-glutamine at an initial concentration of 1 m*M* and tracer amounts of L-[μ-^{14}C]glutamine (sp act 127 μCi/mole, New England Nuclear) were the sole exogenous substrates. The perfusion circuit, normally opened to the atmosphere, was converted to a closed system by the modification introduced by Trimble and Bowman (8). Kidneys were perfused for 60 min after which the PO_2 of the perfusion media, measured by a Gilson oxygraph, was always in excess of 200 mm Hg. Concentrations of ammonia, glutamine, glucose, and radioactive CO_2 were measured at 15-min intervals; changes in substrate uptake, glutamine, product formation ammonia, $^{14}CO_2$, and glucose were linear over the perfusion period.

Analysis. Ammonia and glutamine were measured as previously published (2, 9); glucose was determined enzymatically using a modification of the glucose oxidase method (10). Radioactive CO_2 was liberated with 6 *N* HCl and completely trapped in hyamine hydroxide in the center well of a microdiffusion chamber after 90 min. All analyses were performed in triplicate. Calculations of the rates of glutamine uptake and ammonia and glucose production have been described (2, 11). The conversion of L-glutamine to CO_2 was calculated from the

appearance of $^{14}CO_2$ as formulated below.

$$\mu\text{mole L-glutamine}, t = 0 \times \text{cpm } {}^{14}CO_2, t = 60/$$
$$\text{cpm}, t = 0 \text{ L-[}^{14}\text{C]glutamine} = \mu\text{mole glutamine to } CO_2$$

Statistics. All data were analyzed using the Student's *t* test.

Results. Figure 1 presents the influence of a single injection of triamcinolone upon 24-hr ammonium excretion measured at 4-hr intervals. Excretion level increased 365% during the first 4-hr period, peaked at 8 hr, and thereafter declined. Cumulatively, excretion rate was 1329 μmole 24 hr^{-1} with the hormone compared to 628 μmole 24 hr^{-1} for control giving only a twofold overall increase. Consequently, triamcinolone was administered at 12-hr intervals, 1 mg kg^{-1} 24 hr^{-1}. As a result (Fig. 2), 24-hr ammonium excretion rose to greater than threefold during the first day, to fourfold after the second day, and to greater than fourfold on the third day. With cessation of triamcinolone, excretion levels promptly dropped to pretreatment values after 48 hr. In comparison, rats receiving ammonium chloride increase their excretion levels four- to five-fold over a several day period (12, 13); withdrawing the acid load causes a sharp fall off in the rat with ammonium excretion returning to control values after 2 days (13, 14). Clearly, triamcinolone can induce a pattern of ammonium excretion similar to that observed with acid loading despite the absence of an acid stimulus.

The effect of triamcinolone upon ammonium excretion is apparently not dependent upon potassium depletion since urinary potassium levels are not significantly elevated nor does hypokalemia occur (Table I). Nor is ammonium excretion secondary to an augmentation in a putative sodium for hydrogen exchange since urinary sodium increases while hydrogen ion excretion tends to decrease. As a result of triamcinolone's stimulation of ammonia production excess, acid is eliminated in the form of ammonium causing metabolic alkalosis, plasma total

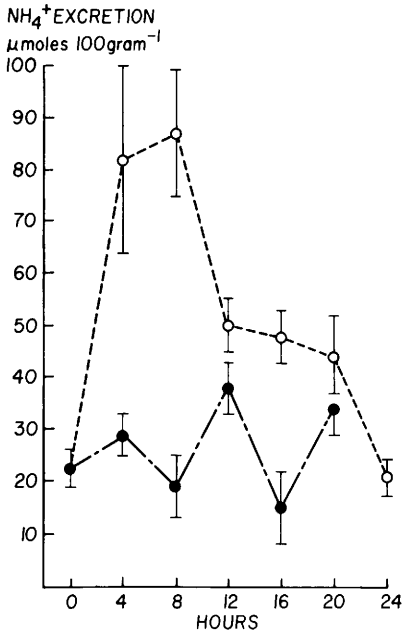

FIG. 1. Influence of a single daily injection of triamcinolone 1 mg kg^{-1}, upon ammonium excretion collected at 4-hr intervals. Points are means ± SE from six sham (closed circles) and six hormone-injected, (open circles) rats.

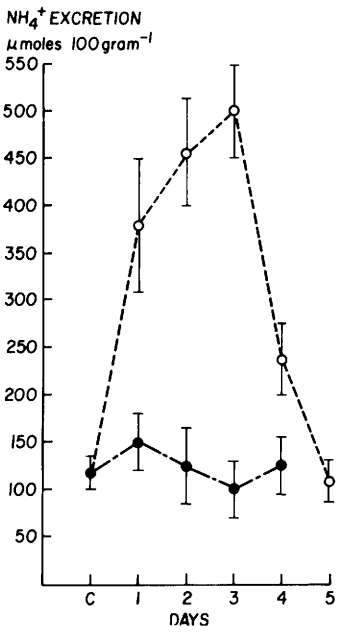

FIG. 2. Influence of triamcinolone, 1 mg kg^{-1}, administered as a divided dose at 12-hr intervals on 24-hr ammonium excretion over a 3-day period. Results are means ± SE from six rats in control (closed circles) and six hormone-injected (open circles) rats.

TABLE I. INFLUENCE OF TRIAMCINOLONE UPON URINE AND PLASMA ELECTROLYTES.

	Urine				Plasma	
Day	Volume (ml)	pH (units)	Na^+ (mEq)	K^+ (mEq)	HCO_3 (mEq)	K^+ (mEq)
Control	17[b]	6.81	1.48	2.83	25.8	4.2
(6)[a]	±3	±0.10	±0.33	±0.73	±2.3	±0.5
1[c]	32[d]	6.94	3.18[d]	3.56	33.4[d]	5.6[d]
(6)	±4	±0.07	±0.72	±0.56	±3.6	±0.7
2[c]	30[d]	6.94[d]	1.96	2.28	32.8[d]	4.5
(6)	±6	±0.05	±0.42	±0.12	±1.9	±0.4
3[c]	21	7.04[d]	1.25	2.59	36.5[d]	4.1
(6)	±5	±0.11	±0.25	±0.22	±4.1	±0.6

[a] Number of rats.
[b] Mean ± SE.
[c] Triamcinolone 1 mg kg^{-1} per day.
[d] Significantly different from control ($P < 0.05$).

CO_2 = 33.4 m*M*. The high level of ammonium excretion is therefore all the more extraordinary in view of the limitations placed upon ammonia excretion by the systemic alkalosis and near neutral urine pH.

Renal ammonia production by isolated perfused kidneys from rats administered triamcinolone for 24 hr is shown in Table II. Ammonia production increased 348% ($P < 0.001$) and glutamine uptake 232% ($P < 0.001$), while the rise in the ammonia produced per glutamine uptake ratio, 1.32 to 1.97, indicates a shift in glutamine utilization from predominant γ-glutamyl-transferase to the more efficient mitochondrial glutaminase I–glutamate dehydrogenase pathway (15, 16). Consistent with shift to the mitochondrial pathway is the increase in the rate of glucose and $^{14}CO_2$ production, the carbon end products of glutamine utilization via the mitochondrial pathway. Glucose production rose from 2.1 ± 0.4 to 8.9 ± 1.6 μmole hr^{-1} ($P < 0.01$), while the micromoles of glutamine converted to CO_2 increased from 3.0 ± 0.8 to 11.1 ± 2.5 μmole hr^{-1} ($P < 0.01$).

Discussion. Ammonium excretion is markedly elevated by triamcinolone injection (Fig. 2) as the result of activation of the mitochondrial glutaminase I pathway (Table II). Stimulation of renal ammonia production is not secondary to tubular ion exchanges or to potassium depletion (Table I). Rather, the activation occurs promptly (Fig. 1), a finding consonant with an effect of corticoids upon the inner mitochondrial membrane's permeability to glutamine. An

TABLE II. EFFECT OF TRIAMCINOLONE ON AMMONIA PRODUCTION AND PATHWAYS OF GLUTAMINE (Gln) UTILIZATION.

	NH_3 production	Gln uptake	Glucose production	$^{14}CO_2$[a]
Control	22.9[b]	17.4	2.1	3.0
(6)	±1.7	±0.6	±0.4	±0.8
Triamcinolone (6)[c]	79.8[d]	40.5[d]	8.9[d]	11.1[d]
	±3.0	±1.4	±1.6	±2.5

[a] Micromoles glutamine converted to $^{14}CO_2$ per hour.
[b] Mean ± SE.
[c] Triamcinolone administered 1 mg kg^{-1} to six intact rats and their kidneys perfused as described in Methods.
[d] Significantly different from control kidney ($P < 0.01$).

increase in substrate glutamine availability within the inner compartment could adequately explain both the renal response to exogenous glucocorticoids as well as the acidosis.

Endogenous glucocorticoids appear to play the key role in the normal response to acidosis since acid loading elevates systemic corticosterone levels (3), while in the absence of the adrenals acidosis does not activate the mitochondrial pathway (3, 4). In fact, pH changes appear to have relatively minor, if any, direct effect to enhance ammonia production (13). Consequently, potassium depletion was suggested as an intermediary factor in ammonia production activation. Yet, the adaptive increase in ammonia production occurs despite maintenance of potassium levels (12) while glucocorticoids can stimulate ammonia production

(Table II) causing metabolic alkalosis in the absence of alterations in potassium homeostasis (17).

Summary. Administering triamcinolone, 1 mg kg^{-1} 24 h^{-1}, results in increased ammonium excretion which resembles that observed during acid loading. This increase cannot be attributed to tubular ion exchanges or potassium depletion but, rather, is due to triamcinolone's effect upon renal ammonia production. The three- to fourfold increase in ammonia, glucose, and $^{14}CO_2$ production is consistent with glucocorticoid mediation of the mitochondrial glutaminase I activation.

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1. Damian, A. C., and Pitts, R. F., Amer. J. Physiol. **218,** 1249 (1970).
2. Welbourne, T. C., Amer. J. Physiol. **226,** 544 (1974).
3. Welbourne, T. C., Endocrinology (1976).
4. Welbourne, T. C., Amer. J. Physiol. **226,** 555 (1974).
5. Welbourne, T. C., Amer. J. Physiol. **224,** 796 (1973).
6. Natelson, S., Amer. J. Pathol. **21,** 1153 (1951).
7. Welbourne, T. C., Canad. J. Physiol. Pharmacol. **50,** 883 (1972).
8. Trimble, M. E., and Bowman, R. H., Amer. J. Physiol. **225,** 1057 (1973).
9. Welbourne, T. C., Proc. Soc. Exp. Biol. Med. (1976).
10. Huggett, A. St. F., and Nixon, D. A., Lancet **2,** 368 (1957).
11. Welbourne, T. C., Amer. J. Physiol. **226,** 549 (1974).
12. Rector, F. C., Jr., Seldin, D. W., and Copenhaver, J. H., J. Clin. Invest. **34,** 29 (1955).
13. Balagura-Baruch, S., *in* "The Kidney" (C. Rouiller and A. F. Muller, eds.), Vol. 3, p. 295. Academic Press, London (1971).
14. Dies, F., and Lotspeich, W. D., Amer. J. Physiol. **212,** 61 (1967).
15. Phenix, P., and Welbourne, T. C., Amer. J. Physiol. **228,** 1289 (1975).
16. Welbourne, T. C., Med. Clin. North Amer. **59,** 629 (1975).
17. Grollman, A. P., and Gamble, J. L., Jr., Amer. J. Physiol. **196,** 135 (1959).

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