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Effect of Chronic Alterations in Sodium Intake on Prostaglandin Release from Isolated Perfused Rabbit Kidney (41013)

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Abstract. Bioassayable PGE_2 like material released from isolated perfused rabbit kidneys was assayed in superfusion cascade, and the response to bradykinin and angiotensin II quantitated in a dose-dependent manner. Chronic high-sodium intakes produced qualitatively and quantitatively similar responses compared to normal diets when kidneys were stimulated with BK or A_{II}. Low Na^+ diets increased sensitivity to BK or A_{II} with a shift of the dose–response curves (DRC) to the left with the same maximum response. No difference in *in vitro* metabolism of [^{14}C]arachidonic acid by microsomal preparations of cortex and medulla from Na^+-loaded and Na^+-depleted rabbits could be detected. These results suggest that the shift of the DRC to the left for angiotensin II and BK may be related to increased sensitivity for arachidonic acid release in response to the peptides and may be responsible for the observed increase in PGE_2 urinary excretion observed in chronic salt depletion in rabbits.

Previous studies on the relationship between sodium balance and renal prostaglandin biosynthesis have been somewhat confusing and remain controversial. The observations in the literature may relate in part to the varied animal models used, in part to the nature of the studies, that is whether acute or chronic sodium depletion or expansion and to as yet undefined differences in experimental design. Chronic sodium depletion in the rat has been associated with an increase in renal PGE_2 content. Conversely chronic salt loading has been associated with impaired PG release from the perfused rat kidneys (2) and with decrease in intrarenal PGE_2 (1). In the rabbit a low-sodium diet has been shown to be associated with an increase in urinary PGE_2 while a high-sodium diet produced a fall in urinary PGE_2 (3). Since urinary PGE_2 levels are felt to reflect renal production and release (4) then the sodium balance in these rabbits was affecting renal PGE_2 biosynthesis. On the other hand, Lifschitz *et al.* (5) could find no significant changes in urinary PGE_2 levels in the face of large alterations in urinary sodium excretion occurring with chronic high- and low-salt diets. We therefore determined the effects of bradykinin and angiotensin II in PGE_2 biosynthesis in the perfused rabbit kidney. In these experiments the kidneys were removed from sodium-loaded rabbits and from sodium-depleted rabbits. These experiments have the advantage that the responses of the kidney reflect the interaction between peptide hormone angiotensin II or bradykinin and a putative cell surface receptor leading to prostaglandin biosynthesis. Thus the kidneys are devoid of the systemic influence of the renin–angiotensin system, the renal nerves, circulating catecholamines, and circulating kinins.

Materials and methods. New Zealand male white rabbits weighing 2.0 to 3 kg were placed on regular lab chow with free access to water, regular lab chow, and drinking water containing 1% saline and 5% sucrose (salt loaded), and synthetic diet containing <0.01% Na^+ (Ralston Purina, St. Louis, Mo.) and free access to water (salt depleted). The animals were maintained on one of these diets for 6–7 days and then sacrificed and the kidneys were removed and perfused at constant flow with Krebs–Hensleit bicarbonate buffer, pH 7.5. The perfusion was kept constant at 12 ml/min to eliminate any perfusion rate-dependent prostaglandin release (6). At the time of sacrifice a sample of urine was taken for analysis of sodium and potassium. Prostaglandins were determined by the di-

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rect parallel bioassay technique in a superfusion cascade system (7) using the chick rectum and rat fundal stomach strip as assay organs. These tissues were rendered more specific and sensitive by use of various receptor blockers infused directly over the assay organs, as previously described (8). Dose–response curve to angiotensin II and bradykinin were carried out after standardization with authentic PGE_2 supplied by Upjohn Company, Kalamazoo, Michigan.

Results. Figure 1 shows the dose-dependent release of PGE_2 from the perfused kidneys of salt-loaded and salt-depleted rabbits in response to angiotensin II. The salt-loaded rabbits had a mean urinary Na^+ of 183.5 ± 80 meq/liter, while the salt depleted rabbits had a mean urinary Na^+ of 8.3 ± 5.3 meq/liter.

The maximum response obtained from both the salt-loaded and salt-depleted rabbits was the same, 400 ng E_2. However, there was a marked shift of the dose–response curve to the left with half-maximal stimulation of PGE_2 release occurring at 15 and 300 ng AII in salt-depleted and salt-loaded, respectively (n = 7). Figure 2 shows dose–response curve to bradykinin which again showed a marked shift to the left and the maximum value of PGE_2 released was 450 ng (data not shown). The

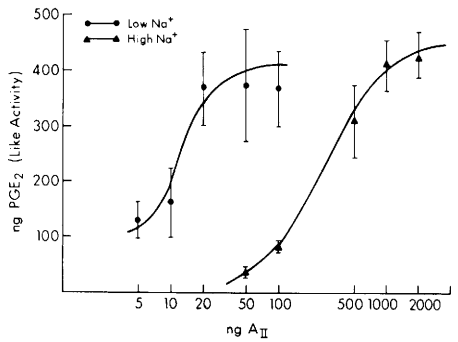

FIG. 1. Dose-dependent release of bioassayable PGE_2 from perfused rabbit kidneys in response to angiotensin II (AII). Assay tissues were chick rectum and rat fundal stomach strip standardized to authentic PGE_2. (●——●), DRC from Na^+-depleted animals (▲——▲) DRC from salt-loaded animals (n = 7). The numbers represent the amounts of prostaglandins released above baseline.

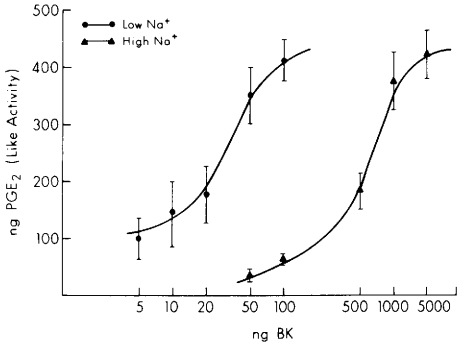

FIG. 2. Dose-dependent release of bioassayable PGE_2 in response to bradykinin in (BK), (●——●), DRC from Na^+-depleted rabbits (▲——▲) DRC from salt-loaded rabbits (n = 7).

dose-dependent release of PGE_2 from kidneys in response to bradykinin and angiotensin II obtained from rabbits on normal rabbit chow was no different from high-salt rabbits perhaps reflecting the high Na^+ content of normal rabbit chow (0.4%). (Marion Feeds, St. Louis, Mo.). During these experiments there were no qualitative or quantitative differences in perfusion pressure in response to bradykinin or angiotensin II. In addition we could not detect the release of thromboxane A_2 in our bioassay system (below sensitivity of system).

Because of these marked differences in PGE_2 biosynthesis we prepared microsomes (100,000g pellet) from cortex and medulla (8) of salt-depleted or salt-loaded rabbits and incubated them with [^{14}C]-arachidonic acid to determine if we could demonstrate any differences in an arachidonic acid metabolism in the two groups of rabbits. These incubations were carried out for 5, 10, 15 min at 37° in 100 mM phosphate buffer, pH 7.6, in the presence of 1.2 mM L-epinephrine to determine initial rates of conversion to PGE_2 in an attempt to determine if there were any changes in cyclooxygenase activity. The protein content of microsomes was determined by method of Lowry (9). Figure 3 shows that the *in vitro* capacity of cortex and medullary cyclooxygenase to metabolize exogenous arachidonate to prostaglandins was identical in Na^+-depleted and Na^+-loaded rabbits. In these experiments there were no added pyridine

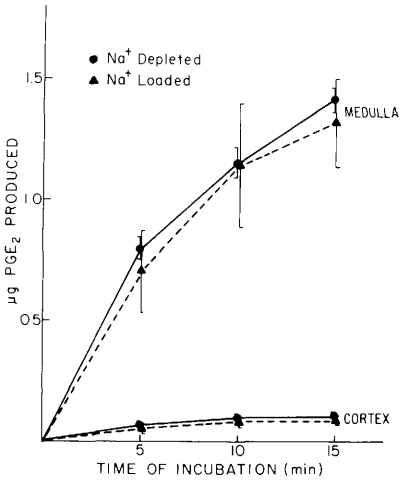

FIG. 3. Metabolism of [^{14}C]arachidonic acid (55 mCi/mmole Amersham Searle) by 100,000*g* pellet of cortex and medulla prepared from Na^+-loaded and Na^+-depleted rabbits. Incubations carried out for 5, 10, 15 min in presence of 500,000 cpm [^{14}C]arachidonic acid and 1.2 m*M* L-epinephrine. At the respective times, the reaction was stopped by addition of 2 *N* formic acid lowering pH from 3.0 to 3.5. Lipids were then extracted with equal volumes ethyl acetate twice and then chromatographed on Silica Gel G plates in solvent system benzene:dioxane:acetic acid (60:30:5). [^{14}C]PGE_2 zones counted liquid scintillation counter. Results are expressed as micrograms of PGE_2 per milligram of microsomal protein (n = 5).

nucleotides, thus the 9-keto-reductase activity could not be expressed.

Discussion. There are several potential explanations for this enhanced prostaglandin biosynthesis. First, they may involve changes in receptor affinity or number (10, 11). Since the maximum response to bradykinin or angiotensin II was the same in both sodium-loaded and sodium-depleted kidney the changes in sensitivity could not be explained entirely on changes in receptor number. These studies do not exclude alterations in affinity of receptor or receptors for bradykinin or angiotensin. However, since bradykinin and angiotensin are felt to interact at different receptors then we would have to postulate similar qualitative changes in receptor affinity. Second, Weber *et al.* (3) have shown changes in PGE_2-9-ketoreductase with salt balance which could explain the observation seen. However, while a decrease in 9-keto-reductase activity would explain in part the differences in sensitivity, one would have to invoke another mechanism to explain similar maximum responses. A third possibility is that salt balance determines the amount of arachidonic acid released from endogenous phospholipids and available for metabolism through the cyclooxygenase. Further experiments would have to be undertaken to evaluate this possibility.

Recently experiments (12) have shown that indomethacin administration markedly attenuates the natriuretic and kaliuretic response of immersion-induced central volume expansion in sodium-depleted but not sodium-replete man. Thus the possibility exists that sodium balance may determine the sensitivity of the renal PGE_2 biosynthesis to circulating angiotensin II and kinins. Thus at the same level of circulating kinins or angiotensin II in a state of salt depletion more renal PGE_2 would be produced. In addition, Stahl *et al.* (13) have shown stimulation of rabbit renal PGE_2 biosynthesis in slices by dietary sodium restriction which is consistent with our observation in the perfused kidney experiments. Our experiments would thus suggest that one explanation for this stimulation of PGE_2 biosynthesis would be increased arachidonic acid release from endogenous phospholipids.

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