Vol. 119, No. 2, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 15, 1984 Pages 689-693

REDUCED GLANDULAR KALLIKREIN-LIKE ACTIVITY IN THE ANTERIOR PITUITARY OF THE NEW ZEALAND GENETICALLY HYPERTENSIVE RAT

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Received January 16, 1984

Summary: Anterior pituitaries of New Zealand genetically hypertensive and normotensive rats were compared for their content of glandular kallikrein-like activity. Anterior pituitary homogenates were assayed for their ability to cleave a chromogenic peptide substrate for glandular kallikrein (H-D-val-leu-arg-p-nitroanilide) at pH 8.0. Anterior pituitaries of both male and female New Zealand genetically hypertensive rats contained significantly less glandular kallikrein-like activity than their normotensive counterparts (-55% for females and -31% for males).

Recently, we have detected proteases resembling glandular kallikrein in the neurointermediate and anterior lobes of the rat pituitary (1,2). These proteases release kinins from kininogen and cleave chromogenic peptide substrates for glandular kallikrein at pH 8.0, and are sensitive to inhibition by aprotinin and resistant to soybean trypsin inhibitor. Previously, pituitaries of Dahl rats susceptible to salt-induced hypertension have been reported to have reduced levels of "trypsin-like" proteases (3,4). These proteases cleave tosyl-arginine-methyl-ester (TAME) at pH 8.0, and are also sensitive to aprotinin and resistant to soybean trypsin inhibitor.

TAME is a substrate for glandular kallikreins (5); thus, the trypsin-like proteases in Dahl rat pituitaries may correspond to the kallikrein-like proteases we detected in the rat pituitary. Indeed, we have recently found that pituitaries from Dahl salt-sensitive rats exhibit a reduction in kallikrein-like activity (unpublished observations). The purpose of this study was to determine if another hypertensive rat strain exhibits alterations in

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Abbreviations: S-2266, H-D-val-leu-arg-p-nitroanilide; S-2251, H-D-val-leu-lys-p-nitroanilide; TAME, tosyl-arginine methyl ester.

0006-291X/84 $1.50

pituitary kallikrein-like activity. We report that anterior pituitaries of New Zealand genetically hypertensive rats have reduced concentrations of glandular kallikrein-like activity.

MATERIALS AND METHODS

Animals: Male and female New Zealand genetically hypertensive or normotensive rats (3-5 months old) were obtained from a colony maintained at this institution by Philip G. Baer (for details on the origin and maintenance of this colony, see 6). Rats were routinely weighed and their blood pressures measured using tail cuff sphygmography.

Tissue preparation: Rats were anesthetized with Nembutal, weighed, and transcardially perfused with 0.9% NaCl to remove blood from the tissues prior to dissection. After opening the cranial vault and removing the brain, the neurointermediate lobe was removed with a pin, and the anterior pituitary was removed with a scoop. Individual lobes were placed in microcentrifuge tubes containing 100 µl of 0.9% NaCl, 0.5% deoxycholic acid, and homogenized with a micro-ultrasonic cell disruptor (Kontes, Vineland, NJ; 4.3 watts for 10 sec in an ice bath, repeated twice). The protein content of the homogenates was determined using the method of Lowry et al. (7).

Kallikrein assay: Pituitary homogenates were measured for their ability to cleave H-D-val-leu-arg-p-nitroanilide (S-2266; a chromogenic peptide substrate for glandular kallikrein) (8,9), or H-D-val-leu-lys-p-nitroanilide (S-2251; a chromogenic peptide substrate for plasmin) (9); the substrates are manufactured by Kabi Diagnostia, Stockholm, Sweden, and were purchased from Helena Laboratories, Beaumont, Texas. Reactions were conducted at 37°C in 0.1 M Tris-HCl (pH 8.0) and were started by the addition of S-2266 or S-2251 to give final concentrations of 200 µM or 300 µM, respectively. Reactions were stopped by heating 5 min at 100°C; p-nitroaniline released was measured at 546 nm after conversion to a purple azo dye (1). Control reactions consisted of pituitary homogenates or substrate incubated alone; absorbance at 546 nm in controls was subtracted from that in samples.

RESULTS

Male hypertensive rats exhibited a 27% reduction in body weight, a 22% reduction in total anterior pituitary protein, and a 55 mmHg elevation in systolic blood pressure compared to male normotensive rats (Table 1). The

TABLE 1
BODY WEIGHTS, TOTAL ANTERIOR PITUITARY PROTEIN, AND SYSTOLIC BLOOD PRESSURE OF NEW ZEALAND GENETICALLY HYPERTENSIVE OR NORMOTENSIVE RATS

Sex and Strain	n	Body Weight (g)	Anterior Pituitary Total Protein (mg)	Systolic Blood Pressure (mmHg)
male, normotensive	6	401 ± 14	1.62 ± 0.07	137 ± 4
male, hypertensive	6	291 ± 18*	1.26 ± 0.05*	192 ± 13*
female, normotensive	5	256 ± 7	1.66 ± 0.08	137 ± 7
female, hypertensive	5	233 ± 10	1.51 ± 0.10	183 ± 3*

Values are the mean ± SE. * $P<0.01$ vs. normotensives of same sex; t-test.

TABLE 2

S-2266 and S-2251 CLEAVAGE BY ANTERIOR PITUITARY HOMOGENATES OF NEW ZEALAND GENETICALLY HYPERTENSIVE OR NORMOTENSIVE RATS

Sex and Strain	n	pmol p-nitroaniline released/mg protein/min S-2266 Cleavage	S-2251 Cleavage
male, normotensive	6	72 ± 5	-
male, hypertensive	6	50 ± 4*	-
female, normotensive	5	1198 ± 237	231 ± 45
female, hypertensive	5	544 ± 35*	91 ± 5*

Values are the mean ± SE. *$P < 0.01$ vs. normotensives of same sex; t-test. Anterior pituitary homogenates were incubated for either 30 min (female homogenates) or 120 min (male homogenates) at 37°C in 0.1 M Tris-HCl (pH 8.0) containing either H-D-val-leu-arg-p-nitroanilide (S-2266, 200 μM) or H-D-val-leu-lys-p-nitroanilide (S-2251, 300 μM). The reaction was stopped by heating 5 min at 100°C; p-nitroaniline released was measured at 546 nm after conversion to a purple azo dye.

body weights and total anterior pituitary protein of female hypertensive rats were 9% less than those of female normotensive rats, and systolic blood pressure was elevated by 46 mmHg (Table 1).

The specific activity of S-2266 cleavage by anterior pituitary homogenates from male and female hypertensive rats was reduced by 31% and 55%, respectively, relative to normotensive rats of the same sex (Table 2). The ratio of total anterior pituitary kallikrein-like activity (pmol S-2266 hydrolyzed/min) to body weight (g) was 27% lower in male hypertensive rats than male normotensive rats (0.22 ± 0.01 vs. 0.30 ± 0.02), and was 57% lower in female hypertensive rats than female normotensive rats (3.60 ± 0.13 vs. 8.36 ± 1.60); both of these differences were statistically significant ($p < 0.01$; t-test).

In confirmation of previous results (2), female anterior pituitaries contained over 10 times more kallikrein-like activity than male anterior pituitaries, and S-2266 was cleaved over 5 times faster than S-2251 (Table 2).

DISCUSSION

These results demonstrate that the anterior pituitaries of male and female New Zealand genetically hypertensive rats contain reduced glandular kallikrein-like activity. Pituitaries of Dahl rats susceptible to salt-

induced hypertension have also been reported to have reduced concentrations of trypsin-like proteases (3,4), and we have observed less glandular kallikrein-like activity in pituitaries of Dahl salt-sensitive rats (unpublished observations). Thus, two different strains of rats susceptible to hypertension exhibit alterations in pituitary proteolytic activity.

The function of the glandular kallikrein-like activity in the rat pituitary is unknown. However, proteases which appear to process precursors of nerve-growth factor and epidermal growth factor in the mouse submaxillary gland are closely related to glandular kallikrein (10,11), and the structure of the glandular kallikrein gene family suggests a general function in the processing of biologically active peptides (12). We have previously speculated that the neurointermediate pituitary kallikrein-like activity participates in the processing of pro-opiomelanocortin (the ACTH, MSH and endorphin precursor) (1). The anterior pituitary kallikrein-like activity was speculated to process an uncharacterized growth factor or a related substance (2).

In relation to hypertension, it should be noted that the pituitary may be the source of novel substances regulating fluid and electrolyte balance. Experiments in nephrectomized rats and dogs have indicated that an unidentified pituitary factor may stimulate the secretion of aldosterone (13,14), a potent regulator of salt excretion. Indeed, a novel aldosterone-stimulating peptide was recently isolated from human urine and localized in the anterior pituitary using immunohistochemistry (15). In addition, a novel substance selectively stimulating renal growth has been detected in pituitary extracts (16,17). However, it is clear that the anterior pituitary kallikrein-like activity is not simply correlated with blood pressure. Male and female New Zealand genetically hypertensive rats have similar elevations in blood pressure even though the anterior pituitaries of hypertensive females have seven times more kallikrein-like activity than the anterior pituitaries of normotensive males. Any role of these proteases in the development of hypertension must involve other sex-dependent factors.

Alternatively, the reduction in kallikrein-like activity may reflect a response to metabolic abnormalities associated with an established hypertension or a coincidental genetic difference unrelated to hypertension. Study of pituitary glandular kallikrein-like activity in other experimental models of hypertension should further illuminate the association of these enzymes with hypertension.

ACKNOWLEDGMENTS

Supported in part by NIH grants HL-18579, HL-23548 and 5 T32 HL 07339-03. We thank Mrs. Pam Blank for typing this manuscript.

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