# Renin activity in rat choroid plexi: Effects of water-deprivation and hypovolemia[1]

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*Summary.* Choroid plexus contains components of a renin-angiotensin system that could regulate hydration. Hypovolemia or 48 h water deprivation did not change choroidal renin-like activity; but under identical assay conditions, brain perfusion with cold isotonic sucrose containing EDTA and PMSF increased enzyme activity 3–4-fold.

Choroid plexus (CP) contains considerable amounts of renin, angiotensin and angiotensin converting enzyme[2–4]. It has been suggested that a CP renin-angiotensin system (RAS) autoregulates CP function[2]. Two previous publications[5,6] have discussed whether choroid renin activity varies with pertubations in hydration. One report contained data showing that CP renin activity was equivalent in homozygous and heterozygous Brattleboro rats as well as in Long-Evans controls[5]; the other indicated decreased activity in CP from nephrectomized, hemorrhaged rats[6]. The aim of our experiments was to analyze CP renin activity in rats made hypovolemic and thirsty by polyethylene glycol injections[7], and by water deprivation for 48 h.

*Materials and methods.* Lateral ventricular CP was obtained from male Sprague-Dawley rats (333 ± 43 g SD). In one protocol, rats were kept with food and with or without water for 48 h. Our experience has been that rats dehydrated in this manner increase their plasma renin activity 4-fold, and plasma vasopressin levels 7-fold; they drink about 10 ml water during the first h when it becomes available[8].

The other protocol consisted of rats injected s.c. with polyethylene glycol (compound 20-M, Union Carbide, 20% dissolved in isotonic saline, 15 ml/kg) to induce hypovolemia[7]; isotonic saline (15 ml/kg s.c.) was given to control rats. Rats in this protocol were used 6 h after s.c. injection. Polyethylene glycol gradually sequesters iso-osmotic fluid, thus this protocol has been used to simulate hypovolemia[7]. Sodium and water are retained by the kidneys and hematocrit increases about 6%; elevated water intake will occur if water is available[7].

15 min after each rat was anesthetized with pentobarbital (45 mg/kg i.p.), the thorax was opened both as a sham procedure before harvesting CP, and in preparation for a cardiac perfusion of ice-cold sucrose solution containing 50 mM EDTA and 0.1 mM phenylmethanesulfonyl fluoride (PMSF) to remove blood from CP. After opening the thorax, the abdominal aorta was clamped, the right atrium lanced and the perfusion continued for 10 min. The pH of the isotonic perfusate was adjusted to 7.2–7.3. Alternate control and experimental rats were killed for tissue collection in all experiments to minimize variability. CP was harvested from 7–10 rats per group.

CP was rinsed with homogenizing solution (artificial CSF[9] containing 10 mM EDTA, and 10 µl dimercaprol + 30 µl 8-hydroxyquinoline [New England Nuclear] per 1.5 ml) to eliminate CSF contamination. Tissue was homogenized in 1.5 ml of this solution (pH 7.2–7.3) and aliquots (3 × 0.4 ml) were frozen until assayed; blanks (homogenizing solution) were also frozen.

Frozen homogenates and blanks were thawed at 4 °C. Excess tetradecapeptide substrate (0.5 µg/ml, Beckman) and maleate buffer (pH 6.0) containing 0.1 mM PMSF were added to all samples. Samples were incubated for 1 h at 37 °C. Protein[10] and angiotensin I generation were measured (New England Nuclear kits). Apparent renin activity of CP was corrected by subtracting the blanks (thawed homogenizing solution, buffer, tetradecapeptide, but no tissue).

*Results and discussion.* CP apparent renin activity after water deprivation and polyethylene glycol induced hypovolemia is given in the table. No change ($p > 0.05$) in enzyme activity was related to hydration status. These data do not support the possibility that CP renin is utilized to form angiotensin II in response to dehydration or hypovolemia, even though the peptide does clearly affect salt/water balance by central mechanisms[2–4]. Although Ganten et al.[7] found a significant decrease in CP renin activity in nephrectomized rats after bleeding, data in the present experiments indicate that the gradually-developing hypovolemia associated with the PG model[7] does not alter CP renin activity. Reasons for diverse results with the 2 models of hypovolemia remain to be elucidated.

Results in the table show a marked, significant ($p < 0.01$) increase in CP renin activity related to brain perfusion. The reason for this rapid increase is not clear. Inactive renin might be converted to an active form but Hirose et al.[11] did not detect inactive renin in nonperfused CP from hog brain. Stabilization of renin, or protection from catabolism are possible, but speculative, explanations for increased enzyme activity. Cryoactivation should be considered; however, this process is usually inhibited by protease inhibitors[12] and seems unlikely.

The only difference between protocols in our experiments was that some choroid plexi were perfused with an ice-cold, isotonic solution of sucrose containing angiotensinase and converting enzyme inhibitors at pH 7.2–7.3. Once removed from the brain, all tissue samples (perfused and nonperfused) were treated identically. We suggest the hypothesis that the increased enzyme activity may be due to calcium chelation produced by the large amount of EDTA in the perfusate. Further work will be required to elucidate the precise mechanism for the large and rapid increase in apparent renin activity of choroid plexi after brain perfusion. It is not known whether large, rapid changes in enzyme activity occur in vivo, but our data raise this possibility. Despite our concerted efforts to standardize experimental conditions, renin activity in choroid plexi was not responsive to changes in hydration.

Renin activity in lateral ventricular choroid plexi of 48 h water deprived (48 h WD) and hypovolemic (PG) rats[a]

| | Renin activity (ng/mg protein/h ± SE) | | | |
|---|---|---|---|---|
| | Control | 48 h WD | Saline control | PG |
| Perfused | 148 ± 9 | 188 ± 21 | 174 ± 31 | 200 ± 34 |
| Not perfused | 48 ± 4 | 64 ± 6 | 39 ± 3 | 51 ± 4 |

[a] Renin activities were determined in choroid plexi (CP) after cardiac perfusion with isotonic solutions of sucrose and inhibitors of angiotensinase and converting enzyme, and in choroid plexi removed without cardiac perfusion. Normally hydrated rats were used as controls for 48 h WD rats. Saline injected rats were used as controls for polyethylene glycol (PG) induced hypovolemia. N = 3–5 experiments/group. Pooled choroids from 7 to 10 rats were used per experiment. Assays were done in triplicate. 2 × 4 analysis of variance revealed a strong effect associated with cardiac perfusion ($p < 0.01$), but no effect related to hydration status ($p > 0.05$).

1 Acknowledgment. These experiments were supported by grant HLB-19320 from the National Institutes of Health, USA.
2 Inagami, T., Okamura, T., Hirose, S., Clemens, D.L., Celio, M.R., Takii, Y., and Yokosawa, H., in: The renin angiotensin system in the brain, p.64. Eds D. Ganten, M. Printz, M. Phillips and B. Scholkens. Springer, Heidelberg 1982.
3 Hirose, S., Ohsawa, T., Murakami, K., and Inagami, T., in: The renin angiotensin system in the brain, p.53. Eds D. Ganten, M. Printz, M. Phillips and B. Scholkens. Springer, Heidelberg 1982.
4 Changaris, D.G., Severs, W.B., and Keil, L.C., J. Histochem. Cytochem. *26* (1978) 593.
5 Hoffman, W.E., Ganten, U., Schelling, P., Phillips, M.I., Schmid, P.G., and Ganten, D., Neuropharmacology *17* (1978) 919.
6 Ganten, D., Fuxe, K., Phillips, M.I., Mann, J.F.E., and Ganten, U., Front. Neuroendocr. *5* (1978) 61.
7 Severs, W.B., Summy-Long, J., and Daniels-Severs, A.E., Am. J. Physiol. *226* (1974) 340.
8 Severs, W.B., Kapsha, J.M., Klase, P.A., and Keil, L.C., Pharmacology *15* (1977) 254.
9 Merlis, J.K., Am. J. Physiol. *131* (1940) 67.
10 Bradford, M.M., Analyt. Biochem. *72* (1976) 248.
11 Hirose, S., Yokosawa, H., Inagami, T., and Workman, R.J., Brain Res. *191* (1980) 489.
12 Osmond, D.H., and Loh, A.Y., Lancet *1* (1978) 102.

0014-4754/83/040429-02$1.50+0.20/0


## Influence of hypothyroidism on adrenal dopamine-beta-hydroxylase in the developing rat

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*Summary.* In young rats rendered hypothyroid from birth, adrenal dopamine-beta-hydroxylase activity increases more rapidly than in the control animals.

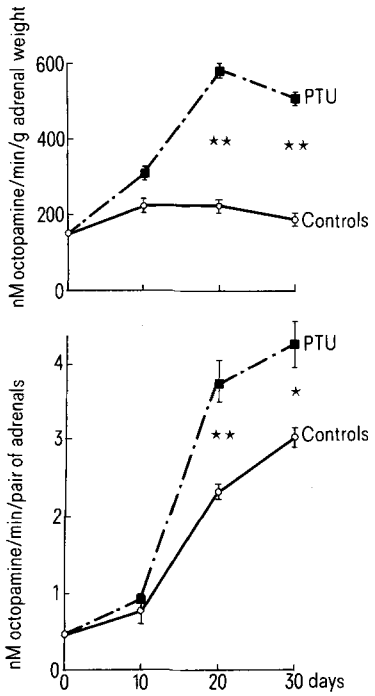
In a previous paper[1] we described the influence of the thyroid status of young rats on the catecholamine (CA) content of their adrenals, together with the activity of tyrosine hydroxylase (TH), the enzyme which catalyzes the initial and rate-limiting step in CA biosynthesis. In the present experiment, we attempted to explain the influence of neonatal hypothyroidism on the activity of another enzyme of CA biosynthesis, i.e. dopamine-beta-hydroxylase (DBH; EC 1-14-17-1). DBH catalyzes the conversion of dopamine to norepinephrine[2].

*Materials and methods.* Young rats of both sexes were rendered hypothyroid from birth by daily administration of propylthiouracil (PTU; 50 mg/day in 4 ml of water) to their mother by gastric intubation. Control animals received no treatment. The animals were studied from 0 to 30 days of postnatal life. They were killed between 09.00 and 11.00 h and the adrenals were rapidly dissected out and weighed; the glands were homogenized in ice-cold 0.1 M phosphate buffer pH 7.4 containing 0.13% triton X 100. The homogenate was mixed for 20 min at 4 °C and centrifuged for 90 min at 7000×g and the supernatant was assayed for enzyme activity. 2–4 pairs of adrenals were used for each determination. DBH activity was measured according to Kato et al.[3,4]. The following concentrations of reagents in the final incubation mixture were used: pargyline: 1 mM; $CuSO_4$: 10 µM; N-ethylmaleimide: 30 mM. The results are expressed as nM of octopamine formed per min. The statistical analysis of the results was performed according to the U-test of Mann and Whitney.

*Results.* The body weight, adrenal weight and the relative weight of the adrenals are given in the table. From 10 days both body and adrenal weights were lower in the PTU-treated rats than in their controls. Furthermore, the eye-opening age was retarded by the PTU (19 days instead of 15 days in the controls).

The evolution of DBH activity is reported in the figure. The enzymatic activity is expressed both per pair of adrenals and per g of adrenal weight. In the control group, the enzymatic activity remained constant from 0 to 30 days if it is calculated per unit of adrenal weight, and increased per pair of glands. In the hypothyroid rats, using either way of expressing the results, DBH activity increased from 0 to 20 days and remained nearly constant between 20 and 30 days. At 20 and 30 days, the activity was 1.4–2.7 times higher in the hypothyroid rats than in the control animals.

*Discussion.* It has already been clearly established that PTU given p.o. to a suckling female rat results in the clinical signs of hypothyroidism in the pups, with a very low level of plasmatic thyroxin[5–7]. The different results we obtained concerning the growth and differentiation of our animals show that PTU induces a clearly identifiable state of hypothyroidism. In this respect, the variations in DBH




Evolution of adrenal dopamine-beta-hydroxylase activity in young rats. PTU, hypothyroid animals. Means ± SEM. Statistical significance between treated animals and controls: * $p \leq 0.05$; ** $p \leq 0.01$. Number of determinations per group; 0 day: 8; 10 days: 5 and 7; 20 days: 8 and 8; 30 days: 9 and 14.