hand, it should be kept in mind that since these cells exhibit PHP's they are coupled at their terminals through the extracellular space, which is further confirmed by their pickup of the recurrent collateral EHP (*4*). However, any effect of this coupling would be minimal in the case of one single presynaptic spike since the unitary EHP's were never greater than 1 mv, and furthermore, it would rather hyperpolarize the adjacent terminals, as with the M-cell (Fig. $2A_4$).

It follows from the above considerations that both electrical and chemical inhibitions are brought about by one interneuron which is otherwise functionally the same as others mediating postsynaptic chemical inhibition in the vertebrate central nervous system (*12*). Thus, it can be speculated that electrical inhibition will be observed in instances where the axons of such common interneurons lie at least in part in a region of increased extracellular resistance. In addition, the system described here appears to be a good model allowing the study of the input-output relations of a vertebrate inhibitory central synapse.

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The Renin-Angiotensin System and Thirst: A Reevaluation

Abstract. *Systemic injections of renin that stimulate substantial amounts of drinking in nephrectomized rats can produce plasma renin activities that fall well above the physiological range. Furthermore, increases in plasma renin activities that occur in rats with intact kidneys during experimental hypotension appear to be too low to provide the basis for the observed elevations in water intake. These findings question the contribution of the renin-angiotensin system to thirst under normal physiological conditions.*

Hypovolemia is an effective stimulus for renin secretion from the kidneys (*1, 2*) and for thirst in rats (*2–6*). It is well known that renin acts on its substrate (angiotensinogen, a plasma globulin) to release the decapeptide, angiotensin I (AI), which is then rapidly hydrolyzed, especially by pulmonary converting enzymes, to the octapeptide, angiotensin II (AII). Fitzsimons (*7*) has postulated that AII may serve an important role in mediating thirst following intravascular fluid losses or other disturbances of the circulation because (i) infusions of renin or of AII provoke water consumption by rats, in proportion to the administered dose (*8, 9*), and (ii) treatments which produce cardiovascular hypotension, such as ligation of the inferior vena cava or administration of the β-adrenergic agonist, isoproterenol, stimulate renin secretion and elicit thirst in rats, effects which are abolished by bilateral nephrectomy (*2, 6, 8, 10–12*). Our work disputes the implications of these studies. We now report that plasma renin activity resulting from dipsogenic doses of exogenous renin is much higher than that produced by any of the common experimental treatments which are presumed to elicit thirst by stimulating renin secretion.

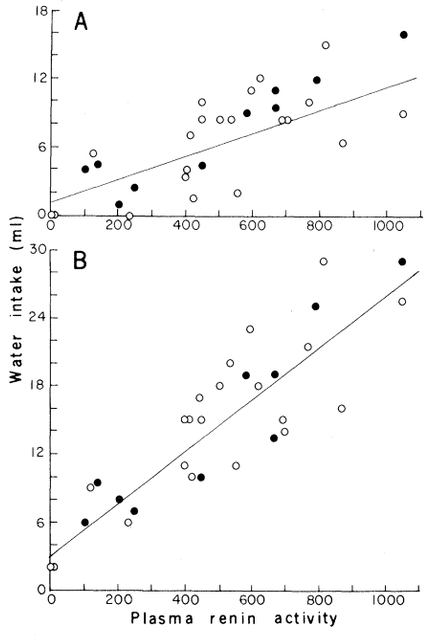

Fig. 1. Water intakes of nephrectomized rats (A) 1 hour or (B) 3 hours after exogenous renin administration (0 to 6 units, intraperitoneally), in relation to their plasma renin activities (in nanograms per milliliter, per 90 minutes) estimated 3 hours after renin was given (○). Each point represents a single animal. Regression equations are: for (A), $y = 0.010x + 1.1$; for (B), $y = 0.023x + 3.0$. For additional animals, SQ20881 (3 mg/kg, intraperitoneally) was given 10 minutes before renin administration (●).

The first experiment was designed to determine whether injections of renin that stimulated thirst in rats produced plasma renin activities (PRA) that fell within the physiological range. Male albino rats (250 to 350 g) of the Sprague-Dawley strain (Zivic-Miller, Pittsburgh) were housed and tested in individual wire-mesh cages. Twenty-one rats were anesthetized with ether and bilaterally nephrectomized, to remove the principal endogenous source of renin. Three hours later, they were given 0, 0.5, 1, 2, 4, or 6 units of hog renin per 100 g of body weight, intraperitoneally, in order to produce elevated PRA. Water intakes were measured every 15 minutes for the following 3 hours. (Food was absent during this time.) Then, all rats were decapitated, and blood was collected from the neck into chilled EDTA-treated plastic tubes, which were centrifuged at 5°C. Plasma was removed and kept frozen until PRA were estimated with a radioimmunoassay for AI (New England Nuclear). Plasma renin activities were calculated as the amount of AI generated by renin from endogenous renin substrate per milliliter of plasma during incubation of the sample for 90 minutes at 37°C (the *p*H was adjusted with a buffer at 6.0) (*13*).

Rats began drinking within 5 to 10 minutes after being injected with renin and drank about half of their ultimate 3-hour intakes within the first hour. As expected (*8*), water intakes were proportional to the administered dose of hog renin ($r = .82$; $P < .001$). Plasma renin activities also were found to increase in proportion to the dose of renin that was given ($r = .80$; $P < .001$). Figure 1 (open circles) depicts cumulative water intakes by individual nephrectomized animals after 1 hour and after 3 hours, and correlates these values with their PRA estimated 3 hours after renin administration.

There are clear linear relationships between PRA and water consumption, over a wide range of values (Fig. 1, A and B, $r = .69$ and .84, respectively; both P's $< .001$). However, it must be emphasized that the PRA found in all of these animals are exceptionally high. Indeed, while PRA fell between 150 and 250 ng/ml per 90 minutes 3 hours after 1 unit of hog renin per 100 g of body weight was administered, peak values of 400 to 600 ng/ml per 90 minutes have been observed 15 minutes after the injection (*14*). In contrast, six rats maintained on a sodium-deficient diet for 2 weeks showed PRA of only 15 to 25 ng/ml per 90 minutes (increased above baseline values from ten rats of 5 to 10 ng/ml per 90 minutes; $P < .001$). Plasma renin activities as high as 250 to 400 ng/ml per 90 minutes were found in eight rats 6 hours and 16 hours after combined ligation of the inferior vena cava and subcutaneous injection of 5 ml of 30 percent polyethylene glycol (PEG; Carbowax, 20-M) solution, but this preparation elicits more drinking with fluid retention than any other experimental procedure known (*6*). If these PRA represent the maximal values that can be generated acutely from renal renin in rats, then the administration of more than 1 unit of hog renin per 100 g evidently produces higher PRA in rats than can be obtained from endogenous renin. It is noteworthy that such high doses were used to establish the phenomenon of renin-induced drinking (*8*) and have been common in later investigations by most other laboratories. Those experiments, therefore, can no longer be used to support the popular hypothesis that the renin-angiotensin system contributes significantly to thirst during normal physiological conditions.

The regression equations in Fig. 1 indicate that increments in PRA of 100 ng/ml per 90 minutes are required for each milliliter of water that is consumed in a 1-hour period, and that increments of 43.5 ng/ml per 90 minutes are required for each milliliter of water consumed in a 3-hour period (*15*). Given these values, the renin-angiotensin system appears to contribute very little to the water intakes observed after caval ligation (3 to 6 ml in 3 hours, with PRA stable at 25 to 50 ng/ml per 90 minutes) (*2, 6, 8, 14*), hypovolemia (4 to 6 ml in 3 hours, with PRA gradually increasing to 45 to 70 ng/ml per 90 minutes) (*2, 5*), or isoproterenol treatment (6 to 9 ml in 1 hour, with PRA peaking at 250 to 300 ng/ml per 90 minutes 30 minutes after the injection) (*10, 14*), the three experimental conditions that have been used most commonly in studies of

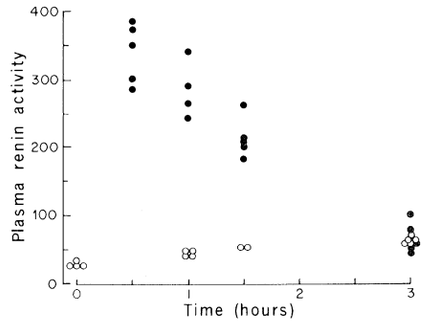

Fig. 2. Plasma renin activities (in nanograms per milliliter, per 90 minutes) in rats made hypovolemic by the administration of PEG (5 ml of 20 percent solution, subcutaneously) 4 hours prior to the 3-hour drinking test. Some rats were pretreated with SQ20881 (3 mg/kg, intraperitoneally) 10 minutes before the drinking test (•); some were not (○). Each point represents a single animal.

"extracellular thirst." Thus, some other stimulus for thirst must be generated by those treatments, such as a baroreceptor signal resulting from cardiovascular hypotension (*16*). In this regard, note that mean blood pressure, measured at the carotid artery in pentobarbital-anesthetized rats, drops to 60 to 80 mm-Hg almost immediately after caval ligation or isoproterenol treatment (*11, 14*). Since mean arterial pressure in nephrectomized rats falls below 40 to 60 mm-Hg after these treatments (*14*), the fact that drinking is also attenuated may not be due to removal of the stimulus for thirst but to an impairment in the capacity for drinking behavior.

The contribution of the renin-angiotensin system to thirst has also been questioned recently by Lehr *et al.* (*17*). They found that administration of SQ20881, an enzyme inhibitor that blocks the conversion of AI to AII (*18*), did not abolish thirst in rats during hypovolemia; in fact, it increased water intakes in such treated animals. We have confirmed those findings using Lehr's testing procedure, but we believe that the elevated intakes paradoxically reflect unusually high activity of the renin-angiotensin system. Hypovolemia was produced in 34 rats by injecting 5 ml of 20 percent PEG solution subcutaneously (*5*). In 19 rats, 3 mg of SQ20881 per kilogram was administered intraperitoneally 230 minutes later. Water intakes during a subsequent 3-hour test session (between 4 and 7 hours after the PEG treatment) were found to be 19.5 ± 0.9 ml (mean ± standard error) ($N = 5$), significantly elevated above the intakes of rats given either the PEG treatment (8.5 ± 0.3 ml, $N = 5$, $P < .001$) or the converting enzyme inhibitor alone (1.1 ± 0.7 ml, $N = 6$, $P < .001$). More than 80 percent of this water was consumed in the first 90 minutes, yet no urine was excreted during the 3-hour test period. Osmotic dilution of body fluids presumably inhibited further water intake despite mean plasma volume deficits of 13.6 percent [estimated from increases in plasma protein concentration (*5*)] (*6, 19*). Their PRA were comparable to those found in the control PEG-treated rats, whereas the PRA from PEG-treated rats that were killed at shorter intervals after treatment with SQ20881 were considerably elevated during the first half of the test period (Fig. 2).

By blocking the formation of AII, the converting enzyme inhibitor may have increased PRA by precipitating marked hypotension (*1, 2*) or by removing the feedback inhibition of renin secretion by AII (*20*). However, there are three reasons for believing that AII formation was not eliminated throughout the 3-hour test period. First, PRA did not remain elevated after treatment with SQ20881, but decayed progressively (Fig. 2). Second, after 3 mg of SQ20881 per kilogram were given to PEG-treated rats arterial pressure decreased maximally (by 60 to 80 mm-Hg) within 30 seconds and rapidly returned to normal within 3 to 5 minutes. Finally, injection of SQ20881 had no effect on water intake of ten nephrectomized rats given 1, 2, 4, or 6 units of renin per 100 g of body weight, intraperitoneally (Fig. 1, closed circles). These findings are consistent with previous reports that inhibition of the converting enzyme by SQ20881 is relatively short-lived (*18*), and indicate that the hyperreninemia induced by the inhibitor outlasts its antagonism of AII formation.

About half of the 10 to 12 ml of extra water that was consumed by PEG-treated rats after administration of SQ20881 may be attributed to the increased PRA, both because those PRA would be expected to elicit that much drinking (Fig. 1B) and because bilateral nephrectomy abolishes this effect (*17*). Thus, while earlier results of this report question the contribution of the renin-angiotensin system to thirst under normal physiological conditions, these findings indicate that sufficiently high levels of AII can be generated pharmacologically from endogenous renin to elicit substantial drinking in rats. Similarly, unusually pronounced secretions of renin are found in certain pathophysiological circumstances in man, such as malignant hypertension associated with chronic renal failure, and thirst may well be at-

tributed to the extraordinarily high levels of AII that are then observed (*21*). The mechanism by which AII stimulates thirst during these extreme conditions remains to be elucidated (*22*).

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Hypoxanthine-Guanine Phosphoribosyltransferase Mutant Glioma Cells: Diminished Monoamine Oxidase Activity

Abstract. *A defective capability of cultured rat glioma cells to reutilize purine bases (hypoxanthine-guanine phosphoribosyltransferase deficiency) was associated with a reduced capacity to oxidatively deaminate serotonin and tryptamine. The mutant glioma cells were also more sensitive to the cytotoxic effects of serotonin than were normal cells.*

The Lesch-Nyhan syndrome (*1*) is a rare X-linked recessive form of cerebral palsy characterized by an excessive production of uric acid, hyperuricemia, and a severe deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT) (*2*). Patients with this disease exhibit neurological dysfunction which includes choreoathetosis, spasticity, mental retardation, and a compulsive self-mutilation of the lips and fingers. Patients with a less severe deficiency of HGPRT also exhibit an excessive production of uric acid and a hyperuricemia, as well as gouty arthritis, but only a portion of them manifest mild neurological dysfunction (*3*).

Rockson *et al.* (*4*) have shown that the plasma of patients with the Lesch-Nyhan syndrome has elevated activity of the enzyme dopamine-β-hydroxylase, which catalyzes the conversion of dopamine to norepinephrine. In general, however, the lack of neurologic material from affected individuals and the absence of an animal model for this disease make it difficult to study the biochemical sequence responsible for the neurological manifestations present in Lesch-Nyhan patients.

Clonal cell lines derived from nervous tissue and grown in vitro represent homogeneous populations of neural cells which are easy to manipulate in large numbers. Neuroblastoma cells deficient in HGPRT in culture are a potentially useful model system for identifying secondary metabolic abnormalities accompanying this mutation, for example, an increased intracellular concentration of the putative neurotransmitter glycine (*5*). Recent findings that suggest a neurochemical role for glial cells—that is, the metabolism of biogenic amines and acetylcholine (*6*)—make these cells a potentially useful model system as well. We now report that glioma cells deficient in HGPRT exhibit decreased activity of monoamine oxidase (MAO) and an enhanced response to the cytotoxicity of serotonin.

Rat glioma clone C6 (*7*) and HGPRT-deficient clones selected from mutagenized (C6-16) and nonmutagenized ($C1_2$) C6 cells have been described (*8*). Cells were cultured in monolayer at 37°C in sealed plastic culture flasks purged with a mixture of 10 percent CO_2 and 90 percent air, in Dulbecco's modified Eagle's medium supplemented with 10 percent fetal calf serum and 2 m*M* L-glutamine (*5*). All cultures were negative when tested for mycoplasma contamination (*9*).

The HGPRT activity of wild-type clone C6 represents 202 nmole of inosinic acid formed per hour per milligram of protein (*8*). The decreased HGPRT activity in the mutant clones (< 1 percent of normal) is associated with an increased intracellular concentration of 5-phosphoribosyl-l-pyrophosphate, a small increase in the overall rate of the early steps of de novo purine synthesis (measured by [^{14}C]formate incorporation into α-*N*-formylglycinamide ribonucleotide), and a greatly diminished ability to incorporate guanine, but not adenine, into soluble nucleotides (*8*). These characteristics are essentially the same as those observed in fibroblasts and lymphoblasts derived from Lesch-Nyhan patients (*10*, *11*).

Monoamine oxidase activity toward serotonin and tryptamine in normal and HGPRT-deficient glioma clones was determined as described (*12*) (Table 1).