anaerobic spore-forming bacterium; *Bifidobacterium bifidum* (DSM 20 215), an obligate anaerobic nonspore-forming bacterium; and *Escherichia coli* and *Pseudomonas denitrificans* (ATCC 13 867), as representatives of faculative anaerobic bacteria. The last two species were unable to grow in their normal medium for anaerobic growth (*7*) when it contained even traces of titanium(III) citrate. After complete oxidation of titanium(III), but still under anaerobic conditions, growth set in immediately. No inhibition due to titanium(IV) citrate was observed. In contrast, the first three anaerobic strains were clearly favored by the presence of titanium(III) citrate at concentrations up to $0.6 \times 10^{-3}M$ (*Methanobacterium* strain AZ) or $2 \times 10^{-3}M$ (*C. formicoaceticum* and *B. bifidum*) (Fig. 2). Some inhibition took place when the titanium(III) citrate concentration exceeded these values. In order to investigate a possible influence of titanium(IV) citrate on the growth of anaerobes, the ratio Ti^{3+}/Ti^{4+} was varied, while keeping constant the total titanium concentration. The slight growth inhibition observed for *C. formicoaceticum*, *B. bifidum*, and *Methanobacterium* strain AZ was found to be due to the amount of titanium(III) citrate in the medium, since the growth functions were the same as in Fig. 2. On the basis of these results, we assume that titanium(IV) citrate is biologically inert for the anaerobic and facultative anaerobic organisms investigated.

None of the organisms cited metabolize citrate to a significant extent. Thus the titanium(III) citrate complex remains intact during growth. In culturing organisms that utilize citrate, other possible Ti^{3+} ligands are tartrate (*4*), rhodanide (*8*), and oxalate (*9*). Even when the bacteria destroy the titanium(III) complex in the course of growth, its addition may still be helpful since it guarantees a medium absolutely free from oxygen at the start. Titanium(III) citrate may also be used whenever an oxygen-free water phase with high electron activity is required.

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7. For anaerobic culture of the facultative anaerobes, we added the following substrates to 1 liter of medium containing the necessary mineral salts and vitamins: *E. coli*, 10 g of glucose; and *P. denitrificans*, 2 g of potassium nitrate, 2.5 g of yeast extract (Difco), 5 g of tryptone (Difco), 1 g of glucose, and 5 g of sodium lactate.
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Vertebrate Central Nervous System: Same Neurons Mediate Both Electrical and Chemical Inhibitions

Abstract. *Identified goldfish medullary interneurons previously shown to inhibit the Mauthner cell electrically also produce a classical postsynaptic inhibition of that cell. Failure of active spike propagation in the processes of these interneurons underlies generation of the electrical component, and the resultant electrotonic terminal depolarization is sufficient to evoke inhibitory transmitter release.*

Although "electrical inhibition" has been postulated as a mechanism of neuronal interaction (*1*), electrical transmission that is purely inhibitory has been clearly demonstrated in only two instances. The first instance is that of the Mauthner cell (M-cell) in goldfish (*2*). This inhibition has been attributed to a failure of active spike propagation in the terminals of neurons projecting to the M-cell axon hillock through its surrounding axon cap. These endings therefore act as a "source" and create an external positivity, the so-called "extrinsic hyperpolarizing potential" (EHP) which is not seen during intracellular recordings and is the earlier part of the recurrent inhibition of the M-cell. More recently, we have observed (*3*, *4*) that the M-cell itself electrically inhibits adjacent neurons. This inhibition appears different from the previous one in that the inhibition is recorded intracellularly as a transmembrane hyperpolarization, named the "passive hyperpolarizing potential" (PHP). In fact, the difference is simply a matter of the location of the reference electrode, and both inhibitions depend upon a high extracellular resistivity in the axon cap which channels the action currents of the "presynaptic" neuron across the inhibited cell's membrane. In confirmation of this model, it was shown that (i) PHP neurons belong to the recurrent collateral network of the M-cell, (ii) their axons project within the axon cap toward its axon hillock, (iii) in turn, their activation (and that of no other neuron) generates the EHP (*4*). Since activation of the recurrent collateral system also evokes a powerful post synaptic chemical inhibition of the M-cell (*2*, *5*) we questioned whether the "peculiar" electrical inhibition and the more classical one could be mediated by the same interneurons. We report here that such is the case.

Experiments were performed on goldfish (12 to 18 cm in length) paralyzed with Flaxedil (1 μg per gram of body weight) and perfused through the mouth with dechlorinated tap water. The preparation and basic electrophysiological techniques were similar to those described before (*4*). Simultaneous intracellular recordings or stimulation of the M-cell and PHP neurons, or both, were obtained (Fig. 1A) with micropipettes filled with either $3M$ KCl or $0.6M$ K_2SO_4. When necessary, these electrodes were coated with a silver paint which was grounded to avoid coupling artifacts. The presence of a PHP (Fig. $1B_1$) was used as the required criterion which established that the investigated presynaptic neurons unambiguously generated an electrical inhibition of the M-cell. In some experiments the post synaptic responses to directly evoked spikes in these interneurons were averaged on line with a Nicolet 1074 computer or were stored on tape for statistical analysis.

The inhibitory postsynaptic potential (IPSP) in the M-cell is often hard to detect as a potential change (*2*) because its equilibrium potential lies close to the resting potential. However, the recurrent collateral inhibition is associated with a marked increase in membrane conductance (*2*) and can, therefore, be demonstrated as a reduction in the test antidromic spike height when paired suprathreshold stimuli are applied to the spinal cord (Fig. $1B_2$). Under those conditions, a spike in a presynaptic PHP neuron did not produce any appreciable potential

change in the M-cell (Fig. $1B_3$). Nevertheless, a small measurable conductance change, measured as a 1 to 7 percent reduction in antidromic spike height [mean = 2.8 percent; standard deviation (S.D.) = 1.5 percent; N = 13], could also be detected when the spinal cord stimulus was preceded by a direct firing of the presynaptic neurons (Fig. $1B_4$).

Two other sets of experiments provided direct evidence that this change in membrane resistance reflected a chemically mediated IPSP. First, steady depolarizing currents which increase the amplitude of the full chemically mediated recurrent collateral IPSP (Fig. $1C_1$) allowed us to detect IPSP's evoked by activation of a single presynaptic neuron (Fig. $1C_2$) which, given their latency and size, were clearly "unitary" synaptic potentials, thus providing confirmation of our hypothesis. However, this technique was not used for a systematic study of these IPSP's because, given the low input resistance of the M-cell (*6*), even strong currents often failed to shift the membrane potential sufficiently.

Second, since the chemical component of the post synaptic inhibition produced in the M-cell by activation of the entire recurrent interneuronal pool is chloride-dependent (*2*), the same characteristic should be observed for an IPSP induced by stimulation of one single cell belonging to this network. After iontophoretic injection of Cl^-, both the full collateral IPSP (Fig. $1D_1$) and unitary inhibition (Fig. $1D_2$) were converted to depolarizing potentials. This effect could be reversed with time, but a large injection such as that used for Fig. $1D_1$ provided the best approach for the detection of stable unitary IPSP's which could then be studied in detail. In the absence of Cl^- injection no depolarizing responses were recorded from the M-cell after activation of a PHP cell, which rules out the possibility that the latter excite the M-cell.

The latency of the unitary IPSP was measured from the onset of the presynaptic spike to that of the IPSP; it ranged from 0.3 to 0.78 msec (mean = 0.51 msec; S.D. = 0.11; N = 33) and was thus in agreement with one synaptic delay at this temperature (*7*); its amplitude ranged widely from 0.13 mv to 8.7 mv (mean = 0.89 mv; S.D. = 1.27 mv; N = 39), which corresponded with 1.5 to 63 percent of the full recurrent IPSP amplitudes, the larger values rarely being encountered; its time to peak had a mean value of 0.89 msec (S.D. = 0.32; N = 20); the half-decay time ranged from 1.5 to 9 msec (mean = 3.52 msec; S.D. = 1.77; N = 29). In our experiments, only presynaptic stimuli above threshold for spike generation were sufficient to evoke detectable unitary IPSP's, and those produced by a given cell exhibited amplitude variations (Fig. 1E) which we found to fit a Poisson distribution (*8*).

Although it was established earlier (*4*) that PHP-exhibiting neurons are those which generate the electrical inhibition, it remained to be demonstrated that the same cell mediates the chemical inhibition as well. Thus, responses to direct stimulation of identified PHP neurons were recorded successively in the axon cap and in the M-cell. In Fig. $2A_1$, the presynaptic neuron had a PHP of 1.8 mv; direct stimulation of this cell produced a

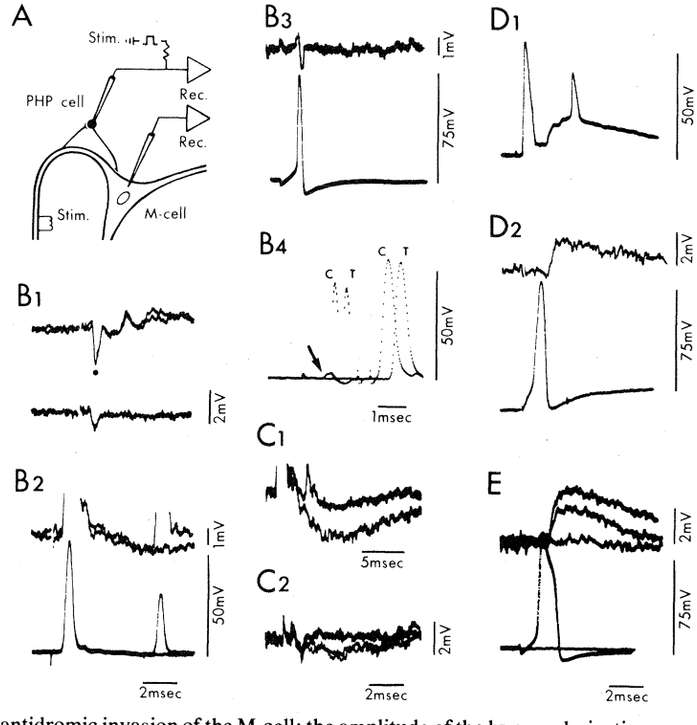

Fig. 1. Chemically mediated postsynaptic inhibition produced in the M-cell by individual presynaptic PHP neurons. (A) Schematic diagram of the experimental set up. (B_1 to B_4) Demonstration that activity in a PHP neuron evokes a conductance change in the M-cell. (B_1) The spinal cord was stimulated above threshold for M-cell antidromic invasion (several superimposed sweeps in each record). Upper trace: this stimulus evoked a PHP in a medullary neuron. Lower trace: corresponding field potential outside that cell. (B_2) Intracellular recordings from the M-cell during paired stimulation of the spinal cord. The antidromic spikes were not followed by an obvious IPSP; the first stimulus did, however, evoke a recurrent collateral inhibition, its underlying conductance change being seen as a reduction of the second spike's height (traces with and without a second stimulus). (B_3) Simultaneous recording from the M-cell (upper trace) and from the same PHP neuron as in B_1 (lower trace); a transmembrane current pulse directly applied to the latter evoked a spike and no synaptic response in the M-cell (the biphasic potential in the upper trace is an artifact caused by cross talk between recording electrodes). (B_4) Effect of the same presynaptic stimulus as in B_3 on M-cell antidromic spike height. The spike that occurred on presentation of both stimuli (*T*) was smaller than that following spinal cord stimulation alone (*C*). (The arrow indicates the pickup, caused by electrode coupling, of the differentiated presynaptic impulse.) Insert: peak of both control (*C*) and test (*T*) spikes as recorded at high gain (each of the illustrated spikes represents the computed average of four successive trials, and calibrations do not pertain to the insert). (C_1 and C_2) Disclosure of unitary hyperpolarizing IPSP's by M-cell depolarization. In both, two successive sweeps recorded at resting potential are superimposed with two others which were obtained while the M-cell was depolarized by a steady transmembrane current. (C_1) Full recurrent collateral IPSP following antidromic invasion of the M-cell; the amplitude of the hyperpolarization was increased by the applied depolarizing current. (C_2) A spike in an adjacent PHP neuron (not illustrated) produced no evident response in the M-cell at resting potential, but a unitary hyperpolarizing IPSP was revealed during the constant depolarization of the cell. (D_1 and D_2) Chloride dependence of the unitary IPSP. Recordings were made after iontophoretic injection of Cl^- in the M-cell. (D_1) A spinal stimulus produced an M-cell antidromic action potential followed by a depolarizing collateral IPSP which generated a second but smaller spike. (D_2) Direct activation of an adjacent PHP neuron (lower trace) evoked a unitary depolarizing IPSP in the M-cell (upper trace). (E) Further properties of unitary depolarizing IPSP. In two out of three trials a current pulse fired a PHP neuron (lower trace); simultaneous recording from the adjacent M-cell (upper trace) shows amplitude variations of the induced depolarizing IPSP's. M-cell recordings were obtained with K_2SO_4 microelectrodes in B_1 to B_4 and C_1 and C_2, and with KCl microelectrodes in D_1 and D_2 and E. In the M-cell recordings of B_3 and C_2, as well as in the extracellular recording of Fig. $2A_2$ there are transient artifacts due to cross talk between the microelectrodes. High-gain a-c recordings in B_1, C_1, and C_2, and upper traces of B_2, B_3, D_2, and E. Low-gain d-c recordings in lower traces of B_2, B_3, D_2, E, and in B_4 and D_1. In this and in Fig. 2, positivity is upwards and the PHP is indicated by a closed circle.

typical monophasic positive-going unitary EHP in the axon cap (Fig. 2A$_2$). The same presynaptic stimulus evoked a large monosynaptic depolarizing IPSP in the M-cell (Fig. 2A$_3$); again the EHP was not recorded intracellularly (*2, 4*), but superposition of the potentials recorded inside and outside the cell indicate that the two inhibitions overlap in time (Fig. 2A$_4$).

The model for one PHP-exhibiting neuron generating both electrical and chemical inhibitions is shown in Fig. 2B. It is based on the following assumptions: (i) as can be inferred from the similarity in time course of the EHP and the spike in the presynaptic cell (Fig. 2A$_2$), the latter fails to invade the terminals (*2, 4*) (in Fig. 2B the active spike-generating membrane is represented by E_e and R_a, and the terminal region by R_p), thereby producing an action current (i_e) sufficient to generate an extracellular positivity in the axon cap (R_2) and, consequently, to hyperpolarize the M-cell's axon hillock and soma (R_1). (ii) Although the spike does not actively propagate to the endings the remaining passively conducted terminal depolarization is, nevertheless, above threshold (*9*) for inhibitory transmitter release. (iii) The presynaptic neurons which have been shown previously with intracellular dye injections to project into the axon cap (*4*), terminate upon the M-cell's axon hillock and soma, being most likely the unmyelinated club endings described by Nakajima (*10*).

A possible objection to this conclusion is that PHP neurons are electrotonically coupled (*11*) with one cell directly evoking an EHP and activating others which produce the IPSP. Such coupling could also account for the large amplitude unitary IPSP's occasionally observed. However, we did not find evidence for this coupling during recordings from 32 pairs of PHP neurons. Specifically, a spike in one PHP cell, whether evoked synaptically (Fig. 3A$_1$) or directly (Fig. 3A$_2$) did not bring an adjacent one to firing level, although the recorded cells were in close proximity, as indicated by the position of the recording electrodes and by the similarity of the M-cell field recorded just outside the two (Fig. 3A$_3$). Thus, the postsynaptic responses are necessarily due to spikes in one cell alone. On the other

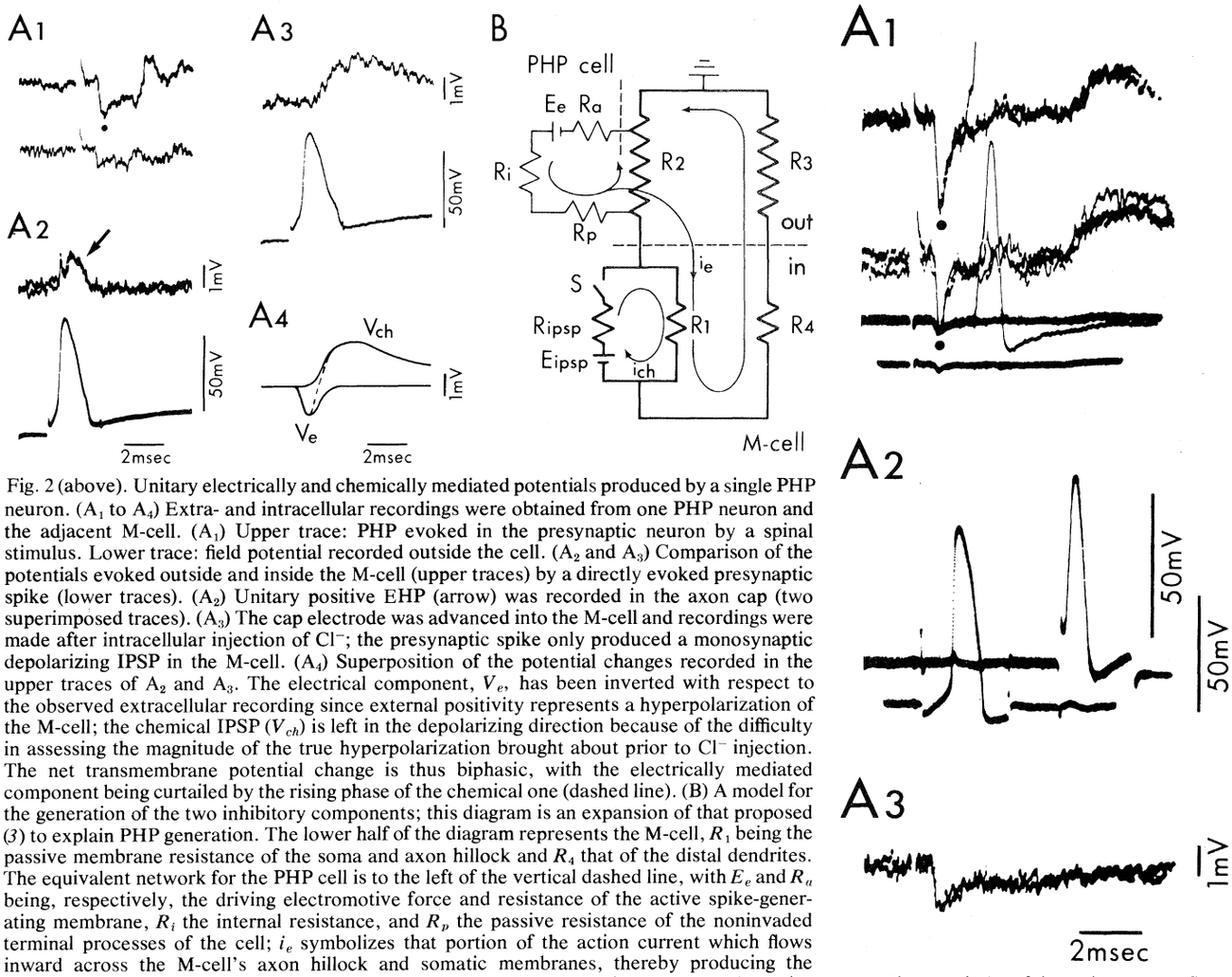

Fig. 2 (above). Unitary electrically and chemically mediated potentials produced by a single PHP neuron. (A$_1$ to A$_4$) Extra- and intracellular recordings were obtained from one PHP neuron and the adjacent M-cell. (A$_1$) Upper trace: PHP evoked in the presynaptic neuron by a spinal stimulus. Lower trace: field potential recorded outside the cell. (A$_2$ and A$_3$) Comparison of the potentials evoked outside and inside the M-cell (upper traces) by a directly evoked presynaptic spike (lower traces). (A$_2$) Unitary positive EHP (arrow) was recorded in the axon cap (two superimposed traces). (A$_3$) The cap electrode was advanced into the M-cell and recordings were made after intracellular injection of Cl^-; the presynaptic spike only produced a monosynaptic depolarizing IPSP in the M-cell. (A$_4$) Superposition of the potential changes recorded in the upper traces of A$_2$ and A$_3$. The electrical component, V_e, has been inverted with respect to the observed extracellular recording since external positivity represents a hyperpolarization of the M-cell; the chemical IPSP (V_{ch}) is left in the depolarizing direction because of the difficulty in assessing the magnitude of the true hyperpolarization brought about prior to Cl^- injection. The net transmembrane potential change is thus biphasic, with the electrically mediated component being curtailed by the rising phase of the chemical one (dashed line). (B) A model for the generation of the two inhibitory components; this diagram is an expansion of that proposed (*3*) to explain PHP generation. The lower half of the diagram represents the M-cell, R_1 being the passive membrane resistance of the soma and axon hillock and R_4 that of the distal dendrites. The equivalent network for the PHP cell is to the left of the vertical dashed line, with E_e and R_a being, respectively, the driving electromotive force and resistance of the active spike-generating membrane, R_i the internal resistance, and R_p the passive resistance of the noninvaded terminal processes of the cell; i_e symbolizes that portion of the action current which flows inward across the M-cell's axon hillock and somatic membranes, thereby producing the electrical inhibition. The i_e returns to the active site through R_4 and the distal extracellular resistance R_3. The remainder of the action current flows back through the extracellular resistance of the axon cap, R_2, the high value of which was shown to underlie the generation of the described electrical inhibitory mechanisms (*4*). In addition, the release of transmitter by the PHP cell, which depends upon terminal depolarization, produces an inhibitory Cl^- dependent current, i_{ch}, in the M-cell; the analog for this additional chemical inhibitory process is represented by the network in parallel with R_1 (E_{ipsp}, driving force for i_{ch}; R_{ipsp}, resistance of the activated inhibitory channels; S, switch illustrating the opening of these channels). Fig. 3 (right). Simultaneous intracellular recordings from a pair of PHP neurons (A$_1$). A spinal stimulus evoked a PHP in both adjacent cells and a subsequent spike in the upper cell (upper first and third traces). (A$_2$) Spikes were also produced in each cell by depolarizing current pulses. Neither of these action potentials could generate a spike in the neighboring neuron. (A$_3$) The same field potential was obtained outside the two cells after a spinal stimulation. (A$_3$ and upper two traces in A$_1$, high-gain a-c recording; A$_2$ and lower two traces in A$_1$, low-gain a-c recordings.)

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.

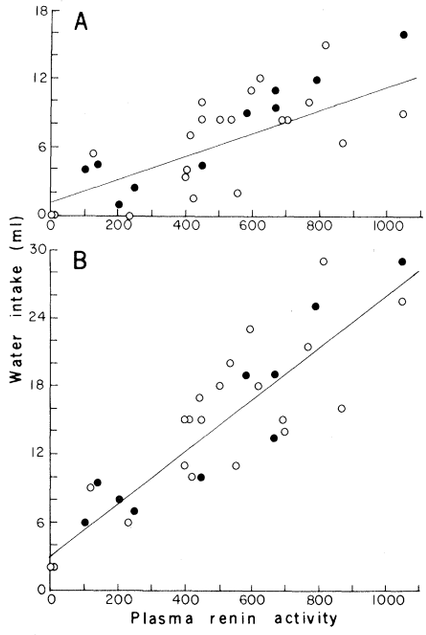

Fig. 1. Water intakes of nephrectomized rats (A) 1 hour or (B) 3 hours after exogenous renin administration (0 to 6 units, intraperitoneally), in relation to their plasma renin activities (in nanograms per milliliter, per 90 minutes) estimated 3 hours after renin was given (○). Each point represents 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.