k'_4 (*10*). In contrast to the result of Gibson (*17*), we find that k'_4 decreases with increasing DPG.

The results of our calculation show that (i) the major kinetic effects of different quaternary conformations and of phosphate binding are reproduced sufficiently well by considering simply quaternary constraints and preferential binding; (ii) the inequivalence of subunits ($\alpha \neq \beta$) is important in analyzing deoxygenation data in the presence of IHP; (iii) the observed increase in the "overall" deoxygenation rate in the presence of IHP largely results from the increase in the rate of dissociation of the second molecule, and this is related to the switchover to the deoxy quaternary conformation at this stage of the reaction; (iv) the calculated values of $\tilde{k}_4$, $\tilde{k}^{\beta}_{22}$, and the binding constants K_{oxy} and K_{deoxy} for HPT, DPG, and IHP are in agreement with previously determined values (*10, 11, 17, 18*); and (v) our value of k^{α}_{22} is somewhat lower than that determined by Olson *et al.* (*18*). While we believe that k_3 is well determined in this analysis, the values of k_1 and k_2 can only be considered to be estimates. Our results point to the need for an accurate set of data at various DPG (or IHP) levels from O_2 binding experiments and from experiments where significant quantities of intermediates are produced (*19*). The successful use of the master equation formulation to predict the kinetics of hemoglobin reactions suggests the possible use of this approach to the kinetics of other cooperative proteins.

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Negative Resistance Characteristic Essential for the Maintenance of Slow Oscillations in Bursting Neurons

Abstract. *Voltage clamping giving step commands reveals a steady-state negative resistance characteristic in the current-voltage curves of* Aplysia *bursting neurons. This is observed below spike threshold in the unstable range through which the membrane potential slowly oscillates. The negative resistance characteristic underlies this instability and shapes the rapid depolarization-hyperpolarization phase of the cycle. When bursting cells are converted to silent cells (by cooling) the negative resistance is abolished; conversely, when normally silent cells are made to burst (by warming) a negative resistance develops. The presence of negative resistance thus enables the bursting cell to oscillate, whereas its absence precludes such oscillations.*

Bursting neurons of the marine mollusk *Aplysia californica* show a remarkable voltage instability within their range of oscillation. It is impossible, by injecting steady currents, to stabilize the membrane potential of a bursting cell between the extremes of its cycle. The membrane potential will come to a steady value only at the top of the range (with the cell firing steadily), or near the most hyperpolarized point. Studies by Carnevale and Wachtel (*1*) demonstrated the presence of a steady inward current which depolarizes a bursting cell, and a transient outward current which, triggered by the depolarization, temporarily hyperpolarizes the neuron. However, the simple combination of these reciprocating membrane currents does not explain the voltage instability of these cells. Such a model would be expected to stabilize at any potential within the oscillating range if one injected sufficient hyperpolarizing current to counter the depolarizing influence.

In order to elucidate the mechanisms specifically underlying the oscillations, we took advantage of the fact that the cycle can be reversibly blocked by cooling (*2*). When the temperature of the ganglion is reduced from 22° to 10°C, the intrinsic region of voltage instability is often eliminated, and a stable resting potential is established near the top of the oscillation range. To explore fully the essential features distinguishing warm bursting cells from cells silenced by cooling, we used the voltage clamp technique to examine the current-voltage (*I-V*) relation of the same cell under both temperature conditions. Intracellular recordings were made from abdominal ganglion bursting cells (L_2 to L_6) by using two KCl-filled microelectrodes and standard techniques (*3*). Our voltage clamp was designed to study small currents (0.1 na) below spike threshold. Axonal charging currents prohibited the study of events shorter than 100 msec (*4*), but this response time was sufficient to register the slow currents underlying the oscillation.

We adjusted the voltage clamp to hold the cell at the least negative point in the cycle of oscillation (usually between −25 and −30 mv). When the clamp was activated, the holding current achieved a steady inward value

after a period of 15 seconds to 2 minutes. We then applied a series of hyperpolarizing commands. Next, the cell was cooled and clamped to the same holding potential. In this case little or no holding current was required. (This finding is consistent with the stable resting potential seen in cooled cells.) The hyperpolarizing commands were then repeated.

The currents resulting from a typical experiment are shown in Fig. 1A for two different temperatures (10° and 22°C). In the cooled cell, hyperpolarizing commands cause inward currents, which is the response one would expect from a passive resistance. However, at 22°C hyperpolarizing commands produce incremental outward currents, which develop with a rise time of 1 to 2 seconds and thereafter remain constant. For hyperpolarizing commands greater than 15 mv (at 22°C), the net response is inward current, although the slowly developing outward current component is clearly present. This can be seen by comparison with the cold-cell currents.

These voltage clamp results were used to derive a pair of *I-V* plots as shown in Fig. 1B. In the *I-V* curve of the warm cell there is a well-defined region of negative slope (negative resistance), while the curve obtained when the neuron is cooled is linear, with positive slope. This negative resistance characteristic (NRC) apparently causes the steady inward holding currents that were seen by Carnevale and Wachtel (*1*) at all voltages in the oscillating range. In the unclamped cell, the NRC precludes the maintenance of a stable potential and manifests itself as a regenerative depolarization which accelerates the membrane potential beyond firing threshold.

Referring to the transient outward current (*1*) and considering this negative resistance property, we can now postulate the sequence of events underlying the slow oscillation. Starting at the bottom (hyperpolarized extreme) of the cycle, a neuron slowly depolarizes because of the decay of the outward current. As a result of the NRC, the depolarization causes the buildup of inward current in a regenerative manner. By this action, the depolarization accelerates and drives the membrane potential beyond firing threshold, resulting in a burst of action potentials. However, this depolarization activates the transient outward current, which slows and eventually overcomes the depolarization and terminates the burst. Once the cell begins to repolarize, this action is accelerated because of the degeneration of the inward current. This gives rise to the distinctive hyperpolarizing "swoop" which drives the cell toward the bottom of the cycle (*5*). The cell reaches this maximum hyperpolarized value from which it depolarizes as the transient outward current begins to decay. The cycle then begins anew.

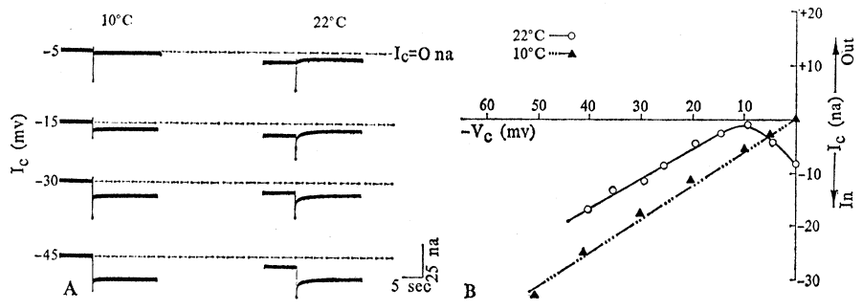

Fig. 1. Voltage clamp data from cell L_3 (which bursts at 22°C when unclamped but is silenced at 10°C). (A) Currents (I_c) resulting from step commands from the clamp holding potential ($V_c = 0$) to increasingly hyperpolarized values, at 10° and 22°C. Inward currents are downward. Zero clamp current is indicated by a dashed line. At 10°C, the holding current before the step commands is zero. At 22°C, there is a steady inward holding current. The holding potential was the same for both temperatures and corresponds to a membrane potential of −30 mv. After a step command, there is a brief capacitive transient (seen here as a vertical line). The currents stabilized within 2 seconds at 10°C and 5 seconds at 22°C. (B) Current-voltage plot of voltage clamp data. Steady-state currents are measured 15 seconds after the step command. At 22°C a steady inward holding current is present throughout, and a region of negative slope (negative resistance) is clearly seen. Both the inward holding current and the negative resistance are abolished at 10°C.

In addition to elucidating the details of the cycle, we explored the intermediate state between the linear resistance property of the cooled cell and the negative resistance property of the warm cell capable of oscillating. We found a gradual transition between the two states. For example, at about 15°C the *I-V* curves for cell L_3 are of positive slope throughout, but have a distinctly nonlinear nature which is reminiscent of "anomalous rectification" (that is, the slope resistance decreases with hyperpolarization) (*6*). This finding led us to conjecture that "nonbursting" neurons which exhibit anomalous rectification at 22°C might take on an NRC and a bursting rhythm if warmed up. In confirmation of this conjecture, we often found that the giant cell R_2, which shows distinct anomalous rectification at 22°C (*7*), becomes a bursting cell when warmed to 25° to 28°C, and this is accompanied by the conversion of anomalous rectification to a negative resistance. A related study has shown that R_2 and other nonbursting *Aplysia* neurons can be stimulated to burst by applying the convulsant drug pentylenetetrazol (*8*). These drug-induced oscillations are invariably accompanied by negative resistance (*9*). Thus, in *Aplysia* neurons, the existence of slow oscillations is correlated with the presence of negative resistance under a variety of conditions.

Our findings suggest that these oscillations result from an interplay of two distinct electrophysiological mechanisms: (i) a regenerative inward current which underlies the essential instability (negative resistance) of membrane potential in the range of the oscillation and inexorably drives the membrane potential toward a voltage well beyond spike threshold and (ii) an outward current which follows the regenerative depolarization, in turn temporarily overwhelms the negative resistance, and thus drives the membrane potential in the hyperpolarized direction (*5*, *7*). There is a strong correlation between the presence of negative resistance and the ability of the unclamped cell to oscillate. Moreover, this NRC can be seen to underlie the essential instability of membrane potential within the oscillating range. In situations where normally stable neurons can be converted to oscillators, the causal factor is clearly the development of a negative resistance region. Thus, the NRC appears to be the most critical determinant of the cyclic behavior of these bursting neurons.

Recently, several attempts have been made to elucidate the ionic basis of these oscillations. Although different

ionic flux mechanisms have been suggested, all the models attempt to explain the basis of the oscillations in terms of the dynamics of a single current source having no regenerative characteristics (*2, 5, 10*). Our voltage clamp results suggest, however, that any ionic model must take into account two distinct current sources and incorporate the negative resistance characteristic.

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Lesch-Nyhan Syndrome: Evidence for Abnormal Adrenergic Function

Abstract. *Subjects with the Lesch-Nyhan syndrome (hypoxanthine-guanine phosphoribosyltransferase deficiency with self-mutilation) exhibit an apparently unique pattern of adrenergic dysfunction characterized by elevated plasma dopamine β-hydroxylase activity and an absence of pressor response to acute sympathetic stimulation. Patients with a partial deficiency of hypoxanthine-guanine phosphoribosyltransferase without self-mutilation do not exhibit these abnormalities of adrenergic function.*

The Lesch-Nyhan syndrome is an X-linked disorder of purine metabolism characterized by hyperuricemia, an excessive production of uric acid, and profound neurological dysfunction which includes spasticity, mental retardation, choreoathetosis, and a compulsive type of self-mutilation (*1*). These patients have a virtually complete deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT) (*2*). Subjects with a less severe deficiency of this enzyme (partial HGPRT deficiency) exhibit a clinical syndrome characterized by hyperuricemia, an excessive production of uric acid, and gout (*3*). Despite occasional neurologic dysfunction in this latter group of patients, self-mutilation does not occur.

The pathogenesis of the neurological and behavioral manifestations in some patients with HGPRT deficiency remains unclear. The development of self-mutilation and "sham rage" in the laboratory animal after administration of caffeine, theophylline, and toxic doses of amphetamine (*4*) led to speculation that the Lesch-Nyhan syndrome might result from a functional alteration in one of the neurologic pathways susceptible to these agents. In the present study we have shown that HGPRT-deficient patients with self-mutilation and severe neurologic dysfunction exhibit a reproducible and apparently unique pattern of adrenergic dysfunction.

The activity of dopamine β-hydroxylase (DBH) in plasma has been proposed as an index of sympathetic nervous system activity (*5*). This enzyme, which catalyzes the conversion of dopamine to norepinephrine, is present in the synaptic vesicles of postganglionic neurons. At the time of discharge, DBH is released into the synaptic cleft along with the neurotransmitter norepinephrine (*6*). Laboratory and clinical data suggest that circulating DBH activity might serve as a quantitative index of adrenergic function (*7*).

Ten children with HGPRT deficiency were studied. Six subjects, 8 to 14 years in age, from five different families manifested the classical Lesch-Nyhan syndrome with choreoathetosis, mental retardation, and self-mutilation. The HGPRT activity in erythrocytes was assayed by a radiochemical method (*3*); a unit is defined as 1 nmole of product per hour per milligram of protein. The HGPRT activity of these six patients ranged from 0.01 to 1.31 units, with a mean and standard deviation (S.D.) of 0.32 ± 0.37 unit. Four subjects ranging in age from 18 months to 16 years exhibited HGPRT deficiency without evidence of self-mutilation. Three of these patients had the partial enzyme defect with no evidence of neurologic dysfunction; erythrocyte HGPRT activity ranged from 0.97 to 1.19 units (mean ± S.D., 1.05 ± 0.10 unit). The fourth patient, whose erythrocyte HGPRT activity was 0.95 unit, a level similar to that of the other patients with the partial enzyme defect, exhibited mental retardation without self-mutilation. Thirty-four controls, without known neurologic disease or neuroblastoma, were randomly selected from the pediatric wards and outpatient clinics. These subjects ranged in age from 1 month to 18 years and had normal HGPRT activity (90 ± 10 units).

Random samples of peripheral venous blood were collected in chilled tubes containing heparin and immediately centrifuged; the plasma was assayed for DBH activity (*8*). The presence of endogenous inhibitors of DBH was excluded (*9*). Since plasma DBH activity is thought to increase progressively with age throughout the developmental years (*10*), the control data were expressed as a 95 percent confidence band for DBH activity with respect to age.

Plasma DBH activity for all groups is depicted in Fig. 1. The four HGPRT-deficient subjects without self-mutilation exhibited plasma DBH activity appropriate for their respective ages. In addition, three randomly selected patients (ages 5 to 14) with cerebral palsy and normal HGPRT activity also exhibited normal plasma DBH activity. In marked contrast, the patients with the Lesch-Nyhan syndrome uniformly exhibited DBH activity well above the 95 percent confidence limit ($P < .001$).

Despite the elevated plasma DBH activity, none of the subjects with the Lesch-Nyhan syndrome exhibited visceral manifestations of adrenergic overactivity such as hypertension, tachycardia, or mydriasis. For this reason we chose to study a clinical index of adrenergic responsiveness. The cold