which are undoubtedly due to C-H stretching modes associated with deoxyribose or ribose groups as well as to C-H stretching modes associated with the base residues. In this respect, they are in almost exact agreement with the Raman spectrum of solid calf thymus DNA (*11*), which also shows two intense broad C-H stretching bands at 2900 and 2950 cm^{-1}. Plane deformation modes of C-H and N-H in the base residues also give rather strong peaks in the tunneling spectrum between 1300 and 1500 cm^{-1}.

Characteristic modes due to the symmetric and antisymmetric stretching vibrations of the PO_2^- ion are identified at $\sim$ 1050 cm^{-1} and 1180 to 1240 cm^{-1}, respectively, in the infrared spectrum. The tunneling spectra show fairly strong peaks at $\sim$ 1065 cm^{-1} and $\sim$ 1258 cm^{-1}, which is quite consistent with the infrared identification, although this should be considered tentative since other modes may contribute in this region.

The tunneling spectra of both DNA and tRNA show modes of medium intensity between 1600 and 1700 cm^{-1}. These can reasonably be assigned to C=O, C=N, and C=C double bond stretching and NH_2 deformation vibrations of the base residues.

These results demonstrate that the solution doping technique should be applicable to any soluble organic compound. The inelastic tunneling spectra are comparable in information content to infrared or Raman spectra and have great potential as a new analytic tool—especially where only microgram quantities of sample are available.

Important questions remain for further detailed investigation. What is the effect of the vacuum dehydration and cooling on the vibrational spectra of macromolecules? Are the macromolecules damaged by the evaporation of the Pb top electrode? A comparison of the tunneling spectra with the infrared and Raman spectra suggests that frequency shifts due to surface adsorption on the alumina and due to the evaporated Pb electrode are small in large classes of compounds (*3, 4*). However, there are other compounds that undergo surface reactions (*2, 7, 12*). Can general rules be formulated to predict this behavior?

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13. We thank H. Hansma for help in obtaining and preparing biological compounds. We thank M. G. Simonsen, J. E. Coleman, and D. J. Scalapino for helpful suggestions and discussions. Supported by NSF grants GH-37239 and GB-13344 and AEC contract AT-(40-1)-3105.

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Peptide Regulation of Bursting Pacemaker Activity in a Molluscan Neurosecretory Cell

Abstract. *Vasopressin and related peptides (10^{-9} to 10^{-6} molar) induced bursting pacemaker potential activity and altered the current-voltage relations of the membrane in a specific molluscan neurosecretory cell. These effects long outlasted the period of application of the peptides. Sensitivity of the cell to these peptides was primarily localized on the axon hillock region. The observed effects do not resemble conductance changes evoked by conventional neurotransmitters, but rather suggest a membrane regulatory role for these peptides, and thus may be indicative of a new form of information transfer in the nervous system.*

The presence of a seasonal rhythm in the electrophysiology and biochemistry of a specific neurosecretory cell in the land snail, *Otala lactea* (*1, 2*), has prompted us to investigate the effects of vertebrate peptide hormones as putative neurohormones mediating this rhythm. We report here that lysine-vasopressin (LVP) and related peptides have potent and specific regulatory effects on the electrophysiology of this neurosecretory cell. Results demonstrate that specific peptides can regulate electrical activity of neurons in a different manner than conventional synaptic transmitters; this may represent a new form of neuronal modulation.

Specimens of *Otala lactea* were obtained from Scozzaro, Inc. (Brooklyn, New York) and were maintained in well-aerated plastic containers in a dormant, semidormant, or activated state, according to methods previously described (*2, 3*). In a typical experiment the fused ganglia were removed from the snail and pinned to Sylgard in snail saline (*4*), and the protective sheaths covering the large nerve cells were carefully excised. One or two micropipettes filled with 3*M* KCl were placed in identified cells (*1*), and the electrical activity of these cells was recorded by using conventional techniques. The LVP and related peptides (*5*) were made up as 1 m*M* stock solutions in distilled water. Each stock solution was either diluted for bath application or placed in a micropipette to be iontophoresed (as the cation). Acetylcholine (ACh) was treated in a similar fashion.

Cell 11 from dormant (or semidormant) snails either was electrically inactive (Fig. 1, A1), or exhibited spontaneous activity characterized by a beating pattern of spikes at a fairly constant frequency (Fig. 1, B1, B2, and D). The current-voltage relations of the membranes of these cells were linear (Fig. 1, A2), and changes in membrane potential by injections of transmembrane current could not produce bursting pacemaker potential (BPP) activity (for example, see Fig. 1, A1, control). Bath application of $10^{-9}M$ LVP rapidly induced BPP activity (Fig 1, A1), and altered the current-voltage relation of the membrane from linear to nonlinear (Fig. 1, A2) (*6*). Identical results were obtained with various similar peptides (10^{-9} to $10^{-6}M$), including arginine-vasopressin, homolysine-vasopressin, 8-L-homonorleucine-vasopressin, and oxytocin. The effects of these peptides were dose-dependent and long-lasting, and prolonged washing (about 1 to 4 hours) in peptide-free saline was required to restore the cell's membrane properties to control values (Fig. 1, A1 and A2). Higher concentrations of LVP (and related peptides) caused

sustained depolarization of the membrane potential (and high-frequency spike activity). Under these conditions, injection of hyperpolarizing current was necessary to reveal the augmented underlying BPP activity. The response of cell 11 to the active peptides was remarkably specific, since these peptides were without effect on ten other identified molluscan neurons (*7*). Furthermore, bath application of a variety of other peptides did not change the membrane properties of cell 11 (*8*).

Bursting pacemaker potential activity was induced by iontophoresis of LVP directly onto the surface of cell 11. This response, which developed within seconds (Fig. 1, B1), far outlasted the period of application of the iontophoretic current (Fig. 1, B2). The activating effect of LVP on the cell was also observed with solutions containing five times the normal Mg^{2+} concentration, which reduces the likelihood that the LVP effect was presynaptically mediated. Iontophoresis of LVP onto various parts of cell 11 revealed that the sensitivity to this peptide was located primarily on the external surface of the axon and axon hillock. Of 15 cells tested, 13 responded exclusively to application of LVP in the axonal region, while in 2 cells the soma was also sensitive to LVP. Intracellular injection of LVP by iontophoresis or pressure was without effect. In contrast, ACh responses were always elicited from both soma and axonal regions.

The membrane mechanisms underlying the LVP effect are unlike those associated with responses to conventional neurotransmitters. For example, in cell 11, ACh produces a transient depolarization of the membrane (Fig. 1, C and D3) associated with an increase in membrane conductance (Fig. 1, C). As expected from theoretical considerations (*9*), this potential change increased as the cell's membrane was hyperpolarized (Fig. 1, D3); the reversal potential of this response was estimated to be +10 mv by extrapolation. In contrast, iontophoresis of LVP onto a cell whose membrane potential was hyperpolarized (by current injection) to potentials beyond −70 mv did not produce an observable potential (or conductance) change (Fig. 1, D2). However, after removal of the hyperpolarizing current [so that the cell was returned to the membrane potential range over which the BPP could be expressed (*6*)], it was clear that BPP activity had in fact been induced by the LVP (Fig. 1, D2). It should be noted that hyperpolarization of the membrane potential alone did not induce BPP activity (Fig. 1, D1). Thus, in contrast to the transient membrane conductance changes produced by ACh and other conventional transmitters (*10*), the peptide interaction with this cell (at relatively lower concentrations) induced a prolonged regulatory effect on a specific membrane property (the BPP).

Vasopressin and oxytocin are elaborated from the vertebrate posterior pituitary directly into the circulatory system, which conveys them to appropriate target organs (*11*). Aside from its well-described effects on the kidney and smooth muscle, vasopressin has been implicated in the release of adrenocorticotrophic hormone from the anterior pituitary (*12*) and has been proposed as a putative neurotransmitter mediating recurrent inhibition in the supraoptic neurosecretory system (*13*). The results described in this report demonstrate that these peptides have specific effects on a molluscan neurosecretory cell at concentrations comparable to those present in mammalian plasma (*11*). It is possible that either these or similar peptide-like substances may naturally exist in the snail (*14*). The results obtained with this invertebrate system may thus serve as a model to describe a new form of cellular communication in the nervous system whereby peptides may modulate electrical activity. The induction (or potentiation) of BPP activity in this system would serve to increase the frequency of action potentials, which would presumably increase the output of neurosecretory product from this cell's terminals. The involvement of these or other peptide-like substances in the natural regulation of the electrophysiology of this cell and its metabolic consequences remain to be elucidated.

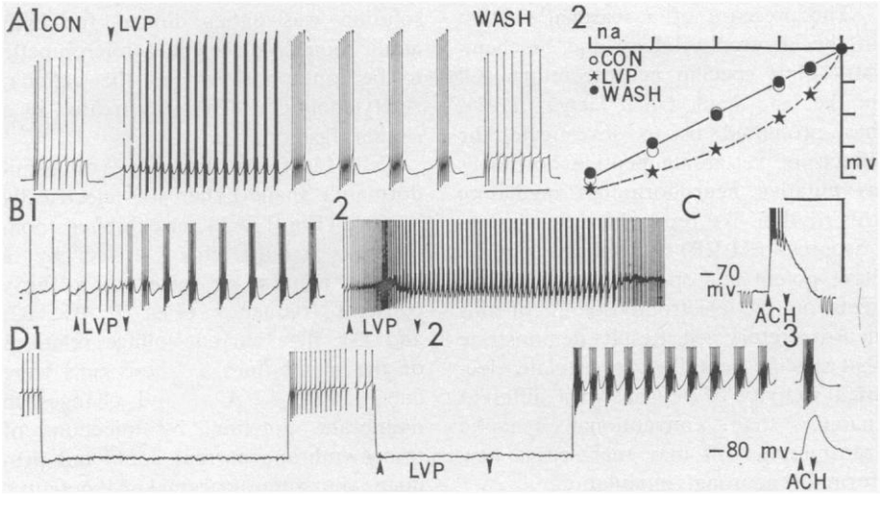

Fig. 1. Effects of LVP on the membrane properties of cell 11. Membrane potential traces of cell 11's taken from dormant and semidormant snails. (A1) Resting membrane potential, −48 mv; *CON*, control. Injection of depolarizing current (during the period marked by a bar beneath the trace) does not produce BPP activity. Bath application of $10^{-9}M$ LVP (at arrow) induces BPP rhythm. The first half of the trace is recorded at one-quarter of the speed of the last half. Washing in LVP-free saline for 1 hour (*WASH*) restores the cell's membrane properties. (A2) Current-voltage relations of the cell before, during, and after treatment with LVP. The marks on the axes represent 1-na and 10-mv intervals. The origin is the threshold for firing (−42 mv). The LVP reversibly induces marked nonlinearity in the curve. (B1) Iontophoresis of LVP leads to BPP activity. The LVP was ejected between the arrows by using a 4-na cationic current. The resting membrane potential was −46 mv. (B2) Effects of LVP (iontophoresed between arrows) long outlast the application period. (C) Bath application of $10^{-5}M$ ACh depolarizes the membrane potential and increases membrane conductance. Downward deflections are voltage responses to constant-current pulses (1 na). (D1) Sustained hyperpolarization of a cell does not induce BPP activity once the hyperpolarizing current is removed. The resting membrane potential was −48 mv. (D2) Iontophoresis of LVP (between the arrows) when the cell is hyperpolarized does not cause an observable voltage response, but on removal of the hyperpolarizing current well-developed BPP activity is evident. (D3) Similar hyperpolarization of the cell increases the size of the depolarizing ACh response. Calibration: 40 mv; 12 seconds in A1, B1, and D1 to D3; 48 seconds in A1 and B2; 6 minutes in C.

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4. Snail saline consisted of (in m*M*): 80 NaCl, 4 KCl, 10 $CaCl_2$, 5 $MgCl_2$, and 10 tris(hydroxymethyl)aminomethane hydrochloride (*p*H 7.8).
5. Lysine-vasopressin, arginine-vasopressin, and oxytocin were obtained from Sigma Chemical Co. (St. Louis, Mo.). Homolysine-vasopressin and 8-L-homonorleucine-vasopressin were generous gifts of M. Bodanszky (Case Western Reserve University, Cleveland, Ohio). Pressinamide was obtained from V. du Vigneaud (Cornell University, Ithaca, N.Y.). Lysine-vasopressin without its glycinamide terminal was a gift of D. Fisher (National Institutes of Health).
6. Cell 11's obtained from active snails typically exhibit BPP activity and nonlinear membrane current-voltage relations (*1*, *2*). In such active cells, LVP and related peptides also cause increases in both the BPP amplitude and the nonlinearity of membrane properties. The BPP is thought to be due to a K^+ conductance that is dependent on time and voltage [D. Junge and C. Stephens, *J. Physiol. (Lond.)* **235**, 155 (1973)] coupled to a high resting Na^+ conductance [D. O. Carpenter, in *Neurobiology of Invertebrates* (Plenum, New York, 1973), p. 38; H. Gainer, *Brain Res.* **39**, 403 (1972)]. The BPP is also directly associated with the presence of nonlinear current-voltage relations over a restricted range of membrane potentials (−35 to −70 mv) [D. S. Faber and M. R. Klee, *Nat. New Biol.* **240**, 29 (1972); M. R. Klee, D. S. Faber, W.-D. Heiss, *Science* **179**, 1133 (1973); H. Wachtel and W. A. Wilson, in *Neurobiology of Invertebrates* (Plenum, New York, 1973), p. 59]. Although BPP activity can be induced in these cells by manipulation of the external concentration of divalent cations (*3*), we think it highly unlikely that similar events underlie the LVP effect, which can be obtained at $10^{-9}M$.
7. The peptides that were effective in inducing BPP activity in cell 11 were without effect on identified neurons 9, 10, and 12 (*1*) in the *Otala* ganglion and cells R_2, R_{14}, R_{3-13}, L_{2-6}, L_7, L_{11}, and LPG in the ganglia of *Aplysia californica*. Only R_{15} (*Aplysia*), which is similar in its physiological and biochemical properties to cell 11 [F. Strumwasser, *Physiologist* **16**, 9 (1973)], was affected by the active peptides [in a manner similar to that described for the active cell 11 (*5*)]. For *Aplysia* nomenclature see W. T. Frazier, E. R. Kandell, I. Kupfermann, R. Waziri, R. E. Coggeshall, *J. Neurophysiol.* **30**, 1288 (1967).
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14. In recent experiments we have isolated a peptide fraction from the snail brain which is similar to LVP in its effect on cell 11, and is eluted in the same fraction as LVP after chromatography on Sephadex G-25 (M. Ifshin, H. Gainer, J. L. Barker, in preparation).

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Growth Hormone Responses to Melatonin in Man

Abstract. *Oral administration of 1 gram of melatonin caused rapid and significant elevations of serum human growth hormone in eight out of nine normal male subjects. The mean (± standard error of the mean) of the peak response by growth hormone was 22.9 ± 4.6 microunits per milliliter. The increased secretion of growth hormone may be due to interaction of melatonin with hypothalamic serotoninergic receptors.*

Attempts have been made to show that melatonin, a pineal substance, is responsible, at least in part, for the antigonadotrophic and antigrowth effects exerted by the pineal gland. Evidence obtained in rats suggests that melatonin acts via the hypothalamus to block luteinizing hormone (LH) secretion and ovulation at critical periods (*1*). We have shown that melatonin blocks the increase in growth hormone (GH) in rats stimulated by 5-hydroxy-L-tryptophan (*2*) and also suppresses the human GH responses to insulin-induced hypoglycemia (*3*). In the latter studies (*3*) we noted a significant elevation in human serum GH before the induction of hypoglycemia. We thus investigated the question of whether melatonin could exert an early stimulatory effect on GH secretion. The serum GH response to orally administered melatonin was measured in ten healthy male volunteers from whom informed consent had been obtained. All tests on the subjects were begun at 8:30 a.m., after the subjects had fasted overnight. The melatonin was administered orally in two doses of 500 mg (in capsule form) each, 30 minutes apart, in order to duplicate conditions of the GH inhibition studies (*3*). Blood samples were collected every 15 minutes for 3 hours via an indwelling catheter in an antecubital vein. Serum GH was measured by means of the double antibody radioimmunoassay technique of Mollinatti *et al.* (*4*), and the results are expressed in microunits of the World Health Organization international reference for human GH radioimmunoassay.

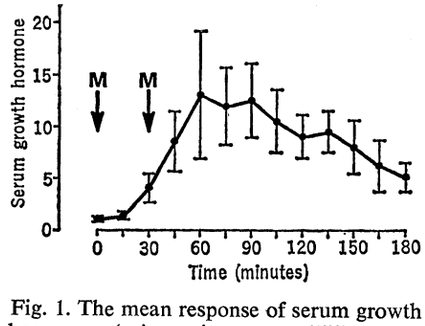

Fig. 1. The mean response of serum growth hormone (microunits per milliliter) to oral melatonin (2 × 500 mg at points indicated by *M*) in nine normal male subjects. The vertical lines represent the standard error of the mean.

The mean serum GH responses after melatonin administration are shown in Fig. 1. One subject in whom the serum GH was elevated at the start of the test was eliminated from the study. Of the other subjects only one failed to exhibit a GH rise above 10 μunit/ml in response to the melatonin. The mean (± the standard error of the mean) of the individual peak GH responses was 22.9 ± 4.6 μunit/ml.

The effects of melatonin administration on the serum levels of human GH are in the direction opposite to that which might have been expected in the light of its ability to block GH stimulation (*2*, *3*). We postulate that the ability of melatonin to increase serum GH levels in man is due to interaction with hypothalamic serotonin receptor sites mediating in the release of GH from the pituitary gland. It is suggested that once melatonin has occupied the serotonin receptors (displacing any serotonin present) it acts as a competitive inhibitor of serotonin and consequently blocks GH stimulation via serotoninergic pathways (*2*, *3*). This proposal is in accord with the observation of Anton-Tay *et al.* (*5*) that administration of melatonin to rats causes an increase in serotonin in the midbrain and hypothalamus which these workers suggested may possibly follow inhibition of serotonin release or metabolism by melatonin.

The mode of action that we have proposed for melatonin has some precedent in the known actions of D-lysergic acid diethylamide (LSD). LSD has a high affinity for serotonin receptors and enhances the actions of serotonin on uterine contractions at low doses but inhibits serotonin action at higher doses. The ability of melatonin to increase GH release in man would thus be analogous to the former (stimulatory) effect of LSD.

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