dure was developed for the identification and separation of amines and polyamines (*17*) and has proved to be reliable (*1*, *18*).

Results of the analysis of the electrophoretograms of the eye and head extracts are shown in Fig. 2; the largest amount of transported radioactivity is contained in the putrescine fraction. The protein precipitate fraction was analyzed for radioactive content by liquid scintillation counting and was found to contain insignificant amounts of activity.

Since putrescine and its related polyamine metabolites (*19*), spermidine and spermine, are found in relatively high amounts in the nervous system (*20*) of all animals studied so far, the demonstration of axonal transport of putrescine is of special significance. We have demonstrated that axonal transport of putrescine is probably not dependent on the integrity of the neurotubules. Although we have shown the axonal transport of exogenously administered putrescine, this does not mean necessarily that putrescine axonal transport is a normal physiological phenomenon.

H. A. FISCHER
Neurochemical Research Group, Max Planck Institute for Brain Research, Frankfurt/M., Germany
E. SCHMATOLLA
Neuropathology Division, Max Planck Institute for Brain Research, Frankfurt/M., Germany

References and Notes

1. H. A. Fischer, H. Korr, N. Seiler, G. Werner, *Brain Res.* **39**, 197 (1972).
2. S. H. Barondes and F. E. Samson, *Neurosci. Res. Program Bull.* **5**, 307 (1967); B. Grafstein, in *Advan. Biochem. Psychopharmocol.* **1**, 11 (1969); F. O. Schmitt and F. E. Samson, *Neurosci. Res. Program Bull.* **6**, 113 (1969); P. Weiss, *Symp. Int. Soc. Cell Biol.* **8**, 3 (1969).
3. J. S. Elam, J. M. Goldberg, N. S. Radin, B. W. Agranoff, *Science* **170**, 458 (1970); S. H. Barondes, *Acta Neuropathol.* **5** (Suppl.), 97 (1971).
4. H. Rahmann, *Z. Mikrosk. Anat. Forsch.* **78**, 289 (1968).
5. N. Miani, *J. Neurochem.* **10**, 859 (1963).
6. L. Casola, G. A. Davis, R. A. Davis, *ibid.* **16**, 1037 (1969); S. C. Bondy, *Exp. Brain Res.* **13**, 135 (1971).
7. L. B. Geffen, C. Hunter, R. A. Rush, *J. Neurochem.* **16**, 469 (1969); T. Hökfelt and A. Dahlström, *Z. Zellforsch.* **119**, 460 (1971).
8. S. Ochs, J. Johnson, M.-H. Ng, *J. Neurochem.* **14**, 317 (1967).
9. E. J. Herbst and U. Bacharach, *Ann. N.Y. Acad. Sci.* **171**, 691 (1970).
10. E. Schmatolla and H. A. Fischer, *Exp. Brain Res.*, in press.
11. H. A. Fischer, N. Seiler, G. Werner, *J. Label. Compounds* **7**, 175 (1971).
12. H. A. Fischer, H. Korr, H. Thiele, G. Werner, *Naturwissenschaften* **58**, 101 (1971).
13. G. G. Borisy and E. W. Taylor, *J. Cell Biol.* **34**, 525 (1967); *ibid.*, p. 535.
14. N. K. Gonatas and E. Robbins, *Protoplasma* **59**, 377 (1965).
15. H. Wiśniewski and R. D. Terry, *Lab. Invest.* **17**, 577 (1967); R. E. Hinkley and R. S. Green, *J. Neurobiol.* **2**, 97 (1971).
16. R. Peters and J. E. Vaughn, *J. Cell Biol.* **32**, 113 (1967); D. Bodian, E. C. Melby, Jr., N. Taylor, *J. Comp. Neurol.* **133**, 113 (1968).
17. F. G. Fischer and H. Bohn, *Hoppe-Seyler's Z. Physiol. Chem.* **308**, 108 (1957).
18. H. A. Fischer, J. M. Schröder, N. Seiler, *Z. Zellforsch.* **128**, 393 (1972).
19. H. Tabor and C. W. Tabor, *Pharmacol. Rev.* **16**, 245 (1964).
20. N. Seiler and M. Wiechmann, *Hoppe-Seyler's Z. Physiol. Chem.* **348**, 1285 (1967); N. Seiler and R. Askar, *J. Chromatogr.* **62**, 121 (1971).
21. We thank I. Kränzlein for the preparation of the specimens and autoradiographs, and L. Ostertag for help in grain counting.

1 February 1972

Low Internal Conductivity of Aplysia Neuron Somata

Abstract. *The internal conductivity of* Aplysia *neuron somata was measured by passing constant current pulses across a calibrated four-electrode array. The intracellular medium is less than one-tenth as conductive as seawater. The low conductivity probably results from structured cell water since ions are present in quantity and do not appear to be bound.*

Most living cells are so small that a direct measurement of the electrical conductivity of the internal medium is not possible. The most notable exception is the giant axon of the squid, for which values of axoplasmic conductance (the inverse of resistance) between 0.7 and 1 times that of seawater have been obtained (*1*). In addition, the internal conductance of frog muscle fibers has been reported to be one-half to one-third that of saline (*2*). Schwan and Li have measured the conductivities of packed tissues at ultrahigh frequencies and report that mammalian skeletal and heart muscle, liver, kidney, and lung tissues all have internal conductivities considerably less than that of the external medium (*3*). For red blood cells Pauly and Schwan have shown (*4*) that the internal conductivity was less than half that expected from the known concentration and mobility of the internal ions, even when allowance was made for the volume concentration of hemoglobin.

We have recently reported a new technique for the measurement of intracellular conductivity in which a single metal microelectrode at a current frequency of 100 khz is used (*5*). With this method it is possible to measure the conductivity of a thin layer of fluid at the tip of the electrode. We found that, although the conductivity of squid axoplasm is near to that of seawater, the internal conductivity of *Aplysia* neuronal somata is only about 5 percent of that of the external medium. In the study reported here we have measured the internal conductivity of *Aplysia* neurons by an entirely independent method as a test of both the technique used and the conclusions reached in our earlier study.

This method was developed by Li *et al.* (*6*) for measurement of the resistivity of brain tissue. Four electrodes were fixed with dental cement in a linear array. The electrodes were approximately equidistant, and the separation between the outermost electrodes was about 500 μm. Constant current pulses were passed between the outer two electrodes, and the voltage drop was recorded between the center two electrodes. Since the distance between electrodes is fixed and the current was constant, the voltage recorded is a function only of the resistance of the medium between the recording electrodes.

In order to use this technique within single cells we made arrays of glass-insulated platinum metal microelectrodes (*7*), each coated with a layer of platinum black. The individual electrodes had a diameter of about 1 μm at the tip and were about 12 μm in diameter at the point where the insulation began, approximately 15 μm from the tip. The array was calibrated in dilutions of artificial seawater of known conductivity, with all recordings made against a Ag-AgCl reference wire; there is a linear relationship between conductivity and concentration over this range (*5*). In different experiments the current pulses used varied between 1 and 10 μa and their durations varied between 0.5 and 1.0 msec. As long as there was a good coating of platinum black on the electrodes, they remained stable at low frequencies (usually six pulses per minute). If the voltage changed with time, the electrode arrays were discarded or replatinized.

These arrays were used to measure the internal conductivity in three different identified neurons of *Aplysia*: the giant cell located in the left pleural ganglion and cells R_2 and L_6 [terminology from (*8*)] in the visceral ganglion. These cells may be in excess of 800 μm in diameter in large animals. Ganglia were removed from the animal, pinned to paraffin in a Lucite chamber, and maintained under flowing seawater and controlled temperature. The connective

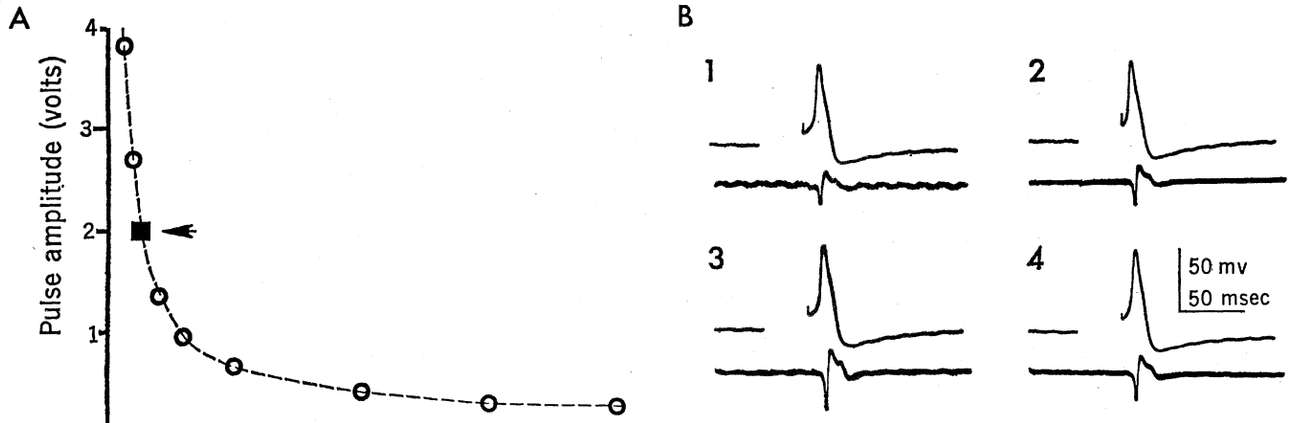

Fig. 1. Measurement of the internal conductivity of the giant cell in the left pleural ganglion of *Aplysia* with a four-pole electrode array. (A) Calibration of the electrode in dilutions of artificial seawater (open circles). A constant current pulse of 2 μa was passed between the outer two electrodes of the array. The pulse duration was 0.5 msec, and the repetition rate was six pulses per minute. The ordinate shows the amplitude of the voltage change recorded across the center two electrodes on a high-impedance a-c amplifier as a function of the seawater dilution (abscissa). The closed square marked by an arrow represents the pulse amplitude obtained when the array was inserted into the cell. (B) Action potentials recorded from each of the five electrodes in the cell. The upper trace in each case is from the glass micropipette (filled with 3*M* KCl) recorded through a high-impedance d-c amplifier. The resting potential was — 57 mv. The lower traces show the action potentials recorded through the two recording (*1* and *2*) and two stimulating (*3* and *4*) metal electrodes. These recordings were made through the same a-c amplifier used for the pulses, but in this case with one electrode at a time recorded against the preparation ground. The voltage calibration applies only to the upper traces. The temperature in (A) was 23°C, and the spikes in (B) were recorded at 12°C.

tissue capsule was slit with a razor blade so as to expose the soma. A glass pipette was also inserted in all cells to accurately measure membrane and action potentials. Action potentials were initiated by the passage of current from the glass pipette through a standard bridge circuit and were recorded through each of the electrodes of the array in order to document that the exposed tips were in fact all within the cell.

The results from a typical experiment are shown in Fig. 1. The curve in Fig. 1A is the electrode calibration in dilutions of seawater. The solid square represents the value obtained when the array was inserted into the pleural giant neuron. In this case the conductivity in the cell corresponds to that of a solution containing 93 percent (by volume) distilled water and 7 percent seawater. Figure 1B shows the action potentials recorded through each of the five microelectrodes in the cell.

A total of six neurons were studied in which it was possible to record action potentials on all five electrodes. The internal conductivity of these neurons was, on the average, equivalent to that of a solution containing 6 percent seawater (range, 2 to 10 percent). Six other cells were examined in addition; in these cells there was sufficient damage that spikes could not be elicited, although all five electrodes appeared to be in the cell. These neurons had an average internal conductivity equivalent to that of a solution containing 7.5 percent seawater (range, 5 to 10 percent). The results from all 12 cells are in close agreement with the conclusions obtained in our earlier experiments with a single metal electrode (*5*).

There are several factors, in addition to a low internal conductivity, which might contribute to the large voltage drop recorded in the cell on these small current pulses. The lines of current flow could be distorted by the cell either as a result of its small size or because of anisotropy resulting from cell membranes. In addition, the high resistance could be a resistance arising primarily across the intracellular membranes. We cannot exclude these possibilities completely but believe all of these to be unlikely explanations for our results. We have tested the effect of sample size by studying drops of fluid smaller than these neurons on a Ag-AgCl wire loop. Under these circumstances the amplitudes of the voltage pulses are similar to those of larger quantities of the same solutions. Anisotropy resulting from intracellular membranes is not so likely in a spherical cell and should not be the same on multiple penetrations. Therefore, the consistency of our results in different cells argues against anisotropy in current pathways. The resistance of at least some intracellular membranes is very low (*9*). Furthermore, the values obtained for intracellular conductivity with this technique are in agreement with those that we obtained earlier with the single electrode where the measurement depends only on the composition of the small layer of fluid at the electrode tip.

A low internal conductivity must reflect one of three possible conditions: (i) a lack of ions, (ii) a binding of ions to cellular macromolecules, or (iii) a structuring of water such that the ions are restricted in their ability to carry electrical current. Sato *et al.* (*10*) have found the internal ionic concentrations of *Aplysia* neurons to be similar to those of other cells, and Brown and his co-workers (*11, 12*) have shown with ion-specific electrodes that the internal activities of K^+ and Cl^- are not very different from those that would be expected from the known internal concentrations. Therefore, ions are present and are not bound. By elimination we attribute the low conductivity of *Aplysia* neurons to an extensive structuring of water probably resulting from hydrogen-bond formation with polar and charged groups of cellular macromolecules.

MARTIN M. HOVEY
ANTHONY F. BAK
DAVID O. CARPENTER
Laboratory of Neurophysiology, National Institute of Mental Health, Bethesda, Maryland 20014

References

1. K. S. Cole, *Membranes, Ions, and Impulses* (Univ. of California Press, Berkeley, 1968).
2. W. Hartree and A. V. Hill, *Biochem. J.* **15**, 379 (19
3. H. P. Schwan and K. Li, *Proc. Inst. Radio Eng.* **41**, 1735 (1953).
4. H. Pauly and H. P. Schwan, *Biophys. J.* **6**, 621 (1966).
5. D. O. Carpenter, M. M. Hovey, A. F. Bak, *Int. J. Neurosci.* **2**, 35 (1972).
6. C. L. Li, A. F. Bak, L. O. Parker, *Exp. Neurol.* **20**, 544 (1968).
7. M. L. Wolbarsht, E. F. MacNichol, Jr., H. G. Wagner, *Science* **132**, 1309 (1960).
8. W. T. Frazier, E. R. Kandel, I. Kupfermann, R. Waziri, R. E. Coggeshall, *J. Neurophysiol.* **30**, 1288 (1967).
9. B. Katz, *Proc. Roy. Soc. Ser. B Biol. Sci.* **135**, 506 (1948).
10. W. R. Loewenstein and Y. Kanno, *J. Cell Biol.* **22**, 565 (1964).
11. J. Sato, G. Austin, H. Yai, J. Maruhashi, *J. Gen. Physiol.* **51**, 321 (1968).
12. A. M. Brown, J. L. Walker, Jr., R. B. Sutton, *ibid.* **56**, 559 (1970).
13. D. L. Kunze and A. M. Brown, *Nature* **229**, 229 (1971).

23 March 1972

Melanophore Stimulating Hormone: Release Inhibition by Ring Structures of Neurohypophysial Hormones

Abstract. *Tocinamide and tocinoic acid, ring structures of oxytocin, are potent inhibitors of the release of melanophore stimulating hormone from the rat and hamster pituitary in vitro. Tocinamide is effective at concentrations as low as* 10^{-14}M *on the mammalian pituitary. These peptides do not affect release of the hormone on the frog* (Rana pipiens) *pars intermedia, but they do inhibit release in the bullfrog* (Rana catesbeiana) *and the toad* (Bufo marinus). *The specificity of the peptides on inhibition of the hormone is demonstrated by the fact that oxytocin, lysine vasopressin, and pressinoic acid and pressinamide (ring structures of the vasopressins) do not show such inhibitory activity. Hypothalamic extracts of either the frog* (Rana pipiens) *or the rat inhibit release of the hormone from pituitaries of either species. The inhibitory effects of tocinamide and tocinoic acid, like that of hypothalamic extracts, are reversible.*

The release of melanophore stimulating hormone (MSH) from the vertebrate pars intermedia can be inhibited by the hypothalamus (*1*). Direct neurosecretory (*2*) and an adrenergic (*3*) innervation of pars intermedia cells have been suggested as the morphological basis for this inhibitory control of hormone release. It has been suggested that both inhibitory (*4*) and stimulatory (releasing) (*5*) factors of hypothalamic origin regulate pars intermedia function. The chemical structure of an MSH release inhibiting factor (MRIF) has been reported (*6*) to be L-Pro-L-Leu-Gly-NH_2, the side chain of oxytocin. We have found (*7*) that this synthetic crystalline tripeptide failed to inhibit the in vitro release of MSH from either rat or frog pituitaries, whereas hypothalamic extracts of these species were effective inhibitors of MSH release. However, we have found that tocinoic acid (*8*)

L-Cys-L-Tyr-L-Ile-L-Gln-L-Asn-L-Cys-OH

the ring of oxytocin (*9*), is a potent inhibitor of MSH release from the rat pars intermedia, and we have proposed that it (or a closely related structure) may be a more likely candidate as the natural MRIF in vertebrates. We now provide additional data on the MRIF-like activity of various ring compounds related to the neurohypophysial hormones in a number of vertebrate species.

Rats (Sprague-Dawley), hamsters (*Mesocricetus auratus*), toads (*Bufo marinus*), and frogs [*Rana pipiens* and *Rana catesbeiana* (bullfrog)] were used. The amount of MSH released into the incubation medium from isolated pituitaries or pars intermedia is determined (*10*) by photoreflectance methods described originally for the frog skin bioassay for MSH (*11*). The MRIF-like activity of various neurohypophysial hormone structures is reported as the percentage of inhibition of MSH release, compared to the control release taken as 100 percent, or in some cases, simply as the percentage of darkening. Student's *t*-test was used to determine statistical significance in all experiments.

Tocinoic acid inhibits MSH release from the rat pituitary at concentrations as low as 10^{-10} g/ml (*7*). We now report that tocinamide (the ring structure of oxytocin terminating in a carboxamide group)

L-Cys-L-Tyr-L-Ile-L-Gln-L-Asn-L-Cys-NH_2

inhibits MSH release at even lower concentrations (Fig. 1). In the mammal pituitary, this compound is about equally effective at any of the various concentrations employed. However, in the hamster pituitary, tocinamide at equivalent doses is less effective in inhibiting MSH release (Table 1). Both the amide and the acid inhibit MSH release from the toad (*Bufo marinus*) pituitary and are variable in their MRIF-like activity on the bullfrog pituitary. In *Rana pipiens*, neither the acid, as reported previously (*7*), nor the amide inhibits MSH release (Table 1).

The specificity of tocinamide and tocinoic acid inhibition of MSH release from the mammalian pituitary is demonstrated by the fact that we have found that neither oxytocin, lysine vasopressin, nor pressinoic acid

L-Cys-L-Tyr-L-Phe-L-Gln-L-Asn-L-Cys-OH

or its amide, pressinamide—the ring structures of the vasopressins (*12*)—possesses any MRIF-like activity in vitro. These structures are similarly ineffective in preventing MSH release from

Table 1. Demonstration in vitro of the MRIF-like activity of tocinamide and tocinoic acid. Twelve pituitaries (hamster) or four pars intermedia (amphibian) were incubated at each concentration. Each value represents the percentage inhibition of MSH release as compared to the control release taken as 100 percent; N.S., not significant. These results are representative of numerous experiments which have provided similar data.

Tocinamide			Tocinoic acid		
Concentration (g/ml)	Inhibition of control (%)	*P*	Concentration (g/ml)	Inhibition of control (%)	*P*
		Hamster			
10^{-8}	33 ± 3.1	< .01	10^{-5}	36 ± 2.8	< .01
10^{-10}	30 ± 3.0	< .01	10^{-6}	32 ± 3.6	< .01
12^{-12}	24 ± 2.9	< .05			
		Bufo marinus			
10^{-7}	41 ± 2.5	< .001	10^{-5}	37 ± 2.4	< .01
10^{-9}	27 ± 3.4	< .05	10^{-6}	31 ± 1.7	< .01
10^{-11}	25 ± 2.9	< .05			
		Rana catesbeiana			
10^{-5}	70 ± 1.6	< .001	10^{-6}	45 ± 2.5	< .01
10^{-6}	20 ± 4.6	N.S.	10^{-8}	0 ± 2.4*	N.S.
10^{-9}	13 ± 3.3	N.S.			
		Rana pipiens			
10^{-5}	0 ± 2.1*	N.S.	10^{-5}	0 ± 2.8	N.S.
10^{-7}	0 ± 1.7	N.S.	10^{-6}	0 ± 1.8	N.S.

* Zero inhibition represents experimental groups where release of MSH was equal to or greater than that of the control.