process of new synthesis of neurosecretory granules.

3) This method of emptying the neurosecretory axons within a very short, experimentally controlled period of time offers many advantages for the study of the dynamic aspects of the neurosecretory process.

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5. It is assumed here that neurosecretory granules are formed in the perikaryon. However, these experiments offer no direct evidence concerning this question.
6. This work was supported by NIH grant NB-04887.
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23 September 1966

Neurosecretory Cell: Capable of Conducting Impulse in Rats

Abstract. *Electric stimulation of the rat neurohypophysis in situ evoked the unit action potentials in the supraoptic nucleus. Compound and unit action potentials were recorded from the isolated neurohypophysis after electric stimulation. The application of solutions high in potassium or devoid of sodium made the neurohypophysis inexcitable. Mammalian neurosecretory cells have the neuronal properties of generation and conduction of action potentials.*

As discussed by Bern and Yagi (*1*), the question of whether mammalian neurosecretory cells have the ability to generate and conduct action potentials has not yet been solved. The mammalian neurohypophysis contains a very small number of nerve fibers; these range from sympathetic nerve fibers running with the posterior median hypophysial artery into the neurohypophysis (*2*), to the nerve fibers running within the hypothalamo-neurohypophysial tract originating in hypothalamic nuclei other than the supraoptic and paraventricular nuclei which send the neurosecretory fibers into the neurohypophysis. Moreover, not all neurons in the supraoptic and paraventricular nuclei are neurosecretory ones (*3*). Because of this anatomical situation, the possibility that the recorded impulses from supraoptic or paraventricular nuclei (*4*) and from the stalk of the neural lobe (*5*) might be derived from non-neurosecretory cells has not been excluded. In the present study we aim to indicate the capability of impulse conduction of rat neurosecretory cells by recording the antidromically conducted impulses from the supraoptic nucleus of the hypothalamus after electric stimulation of the neural lobe. The experiment on the isolated neurohypophysis was also conducted to examine the excitable nature of the membrane of neurosecretory cells.

For the in vivo experiments, the areas of the hypothalamus and hypophysis of five female rats anesthetized by intraperitoneal injection of urethane (1.5 g per kilogram body weight) were exposed from the ventral side with minimum bleeding. The rat was placed on its back, and artificial respiration was employed after an intramuscular injection of about 0.6 mg of tubocurarine chloride. With this procedure, locations of recording and stimulating electrodes can be identified under the dissecting microscope (*6*). For the in vitro experiments, 11 albino rats (body weight, 160 to 280 g) of both sexes were anesthetized and decapitated for removal of the neural lobe. The isolated neural lobe and a small piece of the attached stalk were placed in a vessel containing about 10 ml of Locke's solution. The solution was kept at 37°C and aerated continuously.

A stimulating current of spike shape (duration, 0.07 msec) was applied by an Aika E-1 stimulator attached to a pair of bipolar electrodes of silver wire, insulated by a glass capillary of about 0.3 mm OD. Bipolar electrodes of the same type were used for recording compound action potentials in vitro. Unipolar recording of unit action potentials was made with a silver microelectrode, similar to that used by Yagi and Bern (*7*), which was connected to an amplifier through a cathode follower. The action potentials were amplified by an EB-10 amplifier and displayed on a BO-207 cathode ray oscilloscope.

Single-unit action potentials could be induced in the supraoptic nucleus by stimulating the neural lobe in vivo (Fig. 1B). Some units, however, did not respond to the stimulation. The duration of unit spikes was 1 to 2 msec. A rough estimation of the velocity of antidromic conduction along the hypothalamo-neurohypophysial tract was 1 m/sec. This figure is comparable to the values of 0.5 m/sec in fish hypothalamic neurosecretory fibers (*8*), 1 m/sec in fish caudal neurosecretory fibers (*9*), and 0.6 to 1.4 m/sec in the cat hypothalamo-neurohypophysial tract (*5*). Our estimate includes errors arising from regarding the latent time between the stimulus and the spike potential as conduction time and from assuming the surface distance to be the length of the fiber. Changes in spontaneous firing frequencies were observed in these units after an infusion of hypertonic or hypotonic solution. The presence of antidromic responses indicates that the observed unit action

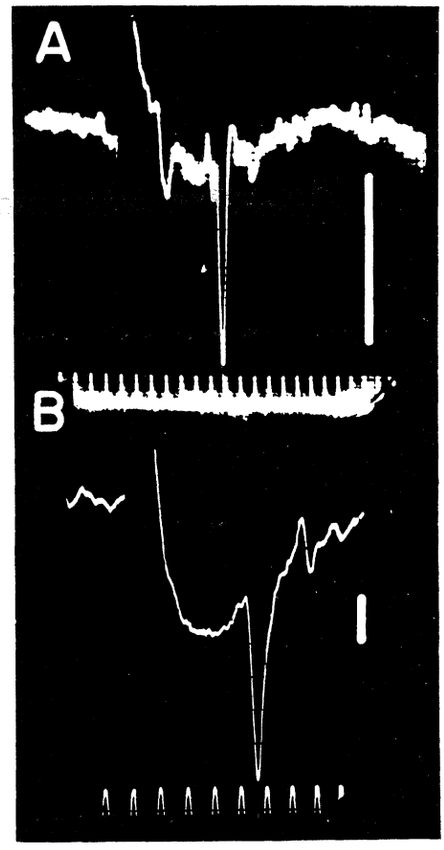

Fig. 1. Unit action potentials recorded from the isolated neural lobe (A) and from the supraoptic nucleus (B). (A) An electric shock of 55 volts was applied to the stalk region of the neurohypophysis in vitro. Vertical calibration indicates 50 μv. (B) An example of impulses conducted antidromically along the hypothalamo-neurohypophysial tract. Stimulation of 15 volts was given to the neural lobe in situ. Vertical line represents 100 μv. Time marks are equal to 1 msec.

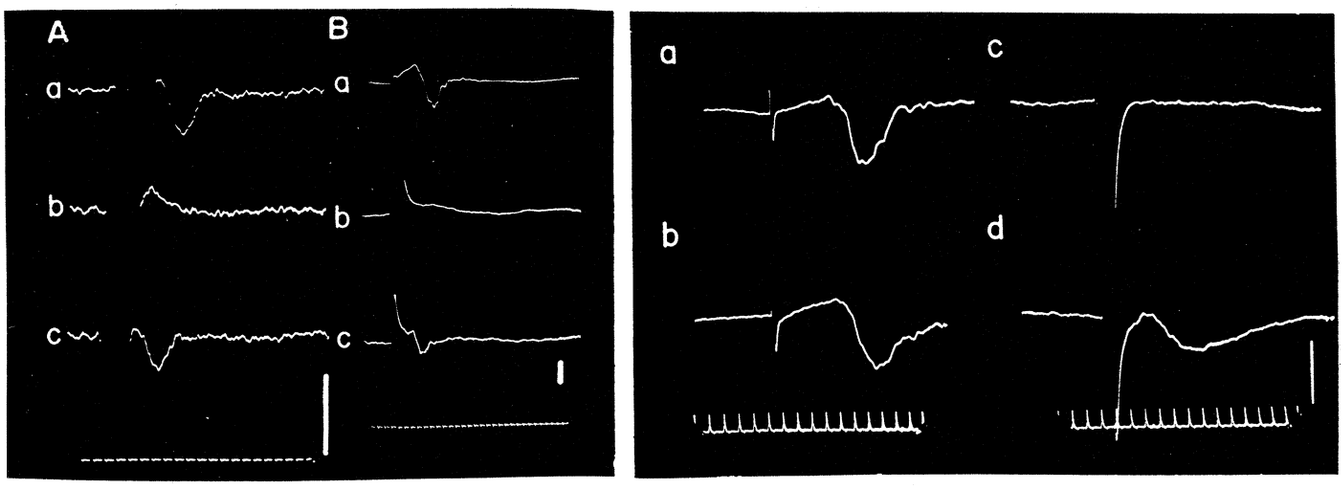

Fig. 2 (left). Effects of high potassium solution (A) and sodium-free solution (B) on the compound action potentials recorded from the rat neural lobe in vitro. A*a* and A*c* record the effects of Locke's solution (stimulus intensity, 50 volts) before and after an application of high-potassium solution (56 mmole/liter) in which no action potential was evoked by 70 volts stimulation (A*b*). B*a* and B*c* were recorded before and after treatment with sodium-free solution in B*b*. Stimulus intensity was 5 volts in B*a* and 50 volts in both B*b* and B*c*. Each unit of the time mark represents 1 msec, and the vertical bar indicates 50 μv. Fig. 3 (right). Compound action potentials of the neural lobe in vitro. (*a*) Action potential in Locke's solution; (*b*) maintenance of excitability in the modified Locke's solution (NaCl : 104 mmole/liter, KCl : 5.6 mmole/liter, sucrose : 100 mmole/liter); (*c*) action potential abolished in the high-potassium solution (NaCl, 104 mmole/liter; KCl, 56 mmole/liter); (*d*) restoration of the excitability in Locke's solution. Stimulus intensities are 10, 20, 100, and 100 volts for *a*, *b*, *c*, and *d*, respectively. Vertical calibration indicates 50 μvolt. Each unit of time mark equals 1 msec.

potentials were derived exclusively from the neurons in the supraoptic nucleus which send their axons into the neural lobe. If we assume that the observed unit action potentials are derived from the non-neurosecretory neurons in the supraoptic nucleus (whose axons have not been proved histologically to terminate in the neural lobe), they could be some sort of secretomotor fibers, judging from their response to osmotic stimuli. This possibility, however, is unlikely because no structural evidence has ever been found for the presence of secretomotor fiber in the neural lobe (*10*). The present results, therefore, reasonably lead us to conclude that the mammalian neurosecretory cells are capable of impulse generation and conduction.

The unit (Fig. 1A) and compound (Figs. 2 and 3) action potentials of the electrically stimulated neurohypophysis were recorded by a unipolar microelectrode and a bipolar electrode, respectively. The compound action potential disappeared (Fig. 2 A*b*) after application of a solution in which the potassium concentration was raised to ten times as much as that of the normal Locke's solution. Excitability of the neurohypophysis was observed (Fig. 2 A*c*) after the potassium-rich medium was replaced by normal Locke's solution. It was observed that under these experimental conditions, excitability could usually be maintained for more than an hour. The action potential was maintained in the solution containing low sodium and low potassium concentrations (Fig. 3*b*), whereas the successive application of the solution of low sodium and high potassium abolished the action potential (Fig. 3*c*). The high potassium concentration, rather than the low sodium concentration in the medium rich in potassium, was the cause of the loss of action potential.

The effect of sodium-free Locke's solution, wherein NaCl was replaced by 308 mmole/liter of sucrose, can be seen in Fig. 2 B*b*. Fig. 2 B*b* shows that the excitability was abolished in the absence of sodium. The neural lobe in vitro requires the low potassium and high sodium concentrations to respond to electric stimulations. Although the movement of the tissue during exchange of the medium made it difficult to continue the recording from the same single unit, no unit spikes were ever observed in solutions high in potassium or devoid of sodium. The time course of the response is very short, therefore the observed responses probably cannot be attributed to the glial cells (pituicytes), because the electric response of glial cells in tissue culture is reported to be of much longer duration (*11*). Accordingly, the disappearance of electric responses in media high in potassium or devoid of sodium suggests that neurosecretory cells have the same membrane activities as non-neurosecretory neurons. Our results reasonably lead us to conclude that the mammalian neurosecretory cells have full neuronal function along with their glandular function.

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13 June 1966