was either normalized or abolished. The decrease in sympathetic nerve activity occurred in spite of a drop in blood pressure of 6 to 53 mm-Hg. Figure 2 contains records illustrating the effects of antiarrhythmic doses of propranolol on neural activity. In the other experiment, propranolol not only failed to restore cardiac rhythm to normal but also failed to influence neural activity.

Another aspect of the correlation between sympathetic nerve activity and cardiac rhythm was observed in three animals that developed spontaneous ventricular arrhythmias before the administration of ouabain. In all three the arrhythmia was episodic and the occurrence and disappearance of several periods of arrhythmia were observed. The remarkable thing about these arrhythmias was their close association with changes in the activity in the cardiac sympathetic nerve fibers. Shortly before the arrhythmia, the normal rhythmic bursts of activity in the sympathetic nerves disappeared and were replaced by either randomly occurring bursts of higher amplitude or a continuous stream of activity similar to that produced by ouabain. This kind of neural activity persisted throughout the period of arrhythmia. When the neural activity reverted to normal, the cardiac rhythm also reverted to normal.

We recently reported (*9*) that ouabain has no significant effect on blood pressure in control animals but causes a large increase in blood pressure in baroreceptor denervated animals, and that these denervated animals are more susceptible to the arrhythmogenic effects of ouabain. To explain these findings (*9*) we postulated that the pressure rise and greater cardiotoxicity in denervated animals is due in large part to a ouabain-provoked increase in sympathetic nerve activity and that the absence of a pressor response and the lower cardiotoxicity in control animals is due to reflex inhibition of sympathetic activity. The present demonstration of the effects of ouabain on activity of the sympathetic nerves substantiates this postulate. In addition, the demonstration of the ability of propranolol to counteract this ouabain-induced increase in neural activity is in accord with the idea put forward by Standaert and colleagues (*10*) that neurodepression may be an important mechanism in the action of antiarrhythmic drugs.

In summary, these results provide direct evidence that the cardiac glycoside, ouabain, has effects on the sympathetic nervous system and substantiates the hypotheses that these neural effects are important in mediating or modifying the cardiovascular effects of this agent.

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References and Notes

1. R. D. Tanz, F. Kavaler, J. Roberts, *Factors Influencing Myocardial Contractility* (Academic Press, New York, 1967), section 7, pp. 489–496; section 8, pp. 563–577 and 579–589.
2. R. D. Tanz, *J. Pharmacol. Exp. Therap.* **144**, 205 (1964).
3. J. Roberts, S. Ehrreich, B. Levitt, *Fed. Proc.* **24**, 1421 (1965).
4. C. Mendez, J. Aceves, R. Mendez, *J. Pharmacol. Exp. Therap.* **131**, 191 (1961); Y. Abiko, *Jap. J. Pharmacol.* **13**, 160 (1963).
5. C. Mendez, J. Aceves, R. Mendez, *J. Pharmacol. Exp. Therap.* **131**, 199 (1961).
6. W. M. Daggett and M. L. Weisfeldt, *Amer. J. Cardiol.* **16**, 394 (1965).
7. Y. Abiko, *Jap. J. Pharmacol.* **13**, 305 (1963).
8. T. Cooper, V. L. Willman, C. R. Hanlon, *Dis. Chest* **45**, 284 (1964); V. L. Willman, T. Cooper, G. C. Kaiser, C. R. Hanlon, *Arch. Surg.* **91**, 805 (1965); J. F. Spann, Jr., E. H. Sonnenblick, T. Cooper, C. A. Chidsey, V. L. Willman, E. Braunwald, *Circ. Res.* **19**, 326 (1966).
9. R. A. Gillis and F. G. Standaert, *Pharmacologist* **10**, 220 (1968); J. A. Quest and R. A. Gillis, *Fed. Proc.* **28**, 607 (1969); R. A. Gillis, J. A. Quest, F. G. Standaert, *J. Pharmacol. Exp. Therap.*, in press.
10. F. G. Standaert, B. Levitt, J. Roberts, *Nature* **210**, 742 (1966); F. G. Standaert and J. Roberts, *Ann. N.Y. Acad. Sci.* **139**, 815 (1967); F. G. Standaert, B. Levitt, J. Roberts, A. Raines, *Eur. J. Pharmacol.* **6**, 209 (1969).
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22 July 1969

Synaptic Current at the Squid Giant Synapse

Abstract. *Transmission in the giant synapse of squid was studied by measuring synaptic currents in the voltage-clamped postsynaptic giant axon. These currents varied linearly with the axon's membrane potential, and showed an intercept on the voltage axis at, or near, the sodium equilibrium potential. The intercept shifted in seawater containing less sodium by even more than the shift in the sodium equilibrium potential. It is concluded that the transmitter at this synapse causes a significant change in the sodium conductance only.*

At the frog neuromuscular junction, acetylcholine increases the conductance of the postsynaptic membrane to both sodium and potassium ions (*1*, *2*). It has recently been found that under voltage-clamp conditions the sodium and potassium conductance changes caused by a quantum of acetlycholine have different time courses and that procaine affects only the change on sodium conductance (*3*). This evidence suggests that acetylcholine may open separate channels for sodium and potassium ions in the postsynaptic membrane. At the squid giant synapse, the transmitter is probably not acetylcholine but glutamate (*4*). It is of interest to know whether a neurotransmitter other than acetylcholine also opens separate sodium and potassium channels in postsynaptic membranes.

Experiments designed to answer this question were performed during the summer of 1968 at the Marine Biological Laboratory in Woods Hole, Massachusetts. The stellate ganglion with about 3 cm of distal giant axon (averaging about 500 μ in diameter) and about 2 cm of presynaptic nerve was dissected from the squid *Loligo pealii* as described originally by Bullock (*5*). The preparation was mounted in a shallow bath through which flowed cooled, artificially oxygenated seawater, and the preparation was kept at 8° to 12°C. The presynaptic nerve was electrically stimulated either extracellularly or with an intracellular microelectrode. Because of the multiplicity of excitatory pathways, particular care was taken to excite only that presynaptic nerve which formed a giant synapse with the postsynaptic axon (*6*). The potential across the postsynaptic membrane was monitored by a microelectrode inserted into the postsynaptic axon in the same region. An enameled silver wire (50 μ in diameter), the end of which had been scraped clean of enamel for about 1 mm, was mounted on a micromanipulator so that it could be threaded into the open end of the postsynaptic giant axon. The postsynaptic membrane potential could be maintained at any desired level by an electronic feedback circuit which supplied or received the necessary current through the wire, a technique rather similar to that of Hagiwara and Tasaki (*7*) and Takeuchi and Takeuchi (*7*). As the wire was being

inserted, action potentials were elicited in the giant axon either by stimulation of the presynaptic nerve or by passing current through the wire to stimulate the giant postsynaptic axon directly so that the action potential at the tip of the wire could be observed. After the wire had been inserted 1 to 1.5 cm, the action potential was generally up to its maximum amplitude (100 to 125 mv). No further change in amplitude occurred as the wire was moved on into the synaptic region except when the membrane was damaged by being pushed by the wire tip during the entry. In contradistinction to the frog neuromuscular junction (*1, 8*) there was no difference between action potentials synaptically or directly elicited. This suggested that the transmitter equilibrium potential might be close to the sodium equilibrium potential. At the frog neuromuscular junction, synaptically activated action potentials are distorted by the synaptic "shunt" which tends to pull the action potential toward an effective synaptic equilibrium potential of -10 to -20 mv. In four experiments with the squid synapse, such as shown in Fig. 1, an antidromic action potential in the postsynaptic nerve was made to collide with a synaptic potential at different points in its time course. No change was seen in the amplitude of these action potentials. This technique is possibly less sensitive here than at the frog neuromuscular junction where the ratio of the maximum conductance associated with synaptic activity to the membrane conductance of the electrically excitable component causing the action potential may be greater than in the squid.

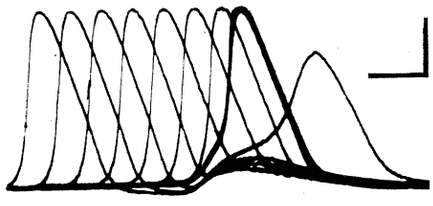

Fig. 1. The effect of synaptic transmission on antidromic action potentials in the postsynaptic axon. The record was obtained with a microelectrode in the synaptic region of the postsynaptic axon. The presynaptic nerve was stimulated once every 5 seconds and an antidromic action potential was directly elicited in the postsynaptic axon at different times before or after each presynaptic stimulus. In this illustration successive trials were recorded on the same film frame so that the synaptic responses were superimposed and appear darker. Antidromic action potentials precede or appear on the synaptic responses. When the antidromic action potentials preceded the synaptic potentials by less than 2 msec, synaptic potentials without action potentials were recorded. Calibration: 49 mv and 1 msec.

When the postsynaptic membrane was clamped at a fixed potential, a record was made of the current which was passed through the intra-axonal wire to maintain that potential steady during synaptic activity (Fig. 2). The amplitude of the synaptic current varied as the membrane potential was clamped at different levels and the relationship between the two variables was linear. The results obtained from one such experiment are plotted in Fig. 3. At membrane potentials between -40 mv and $+80$ mv (inside, relative to an outside ground), the normal voltage clamp ionic currents of the nonsynaptic membrane begin to turn on and increase with further depolarizations. The effect of these currents is to mask the decreasing synaptic currents to the point where the measurement of their amplitudes in this region was relatively inaccurate. In two experiments, reversed synaptic currents were recorded at membrane potentials more positive than $+80$ mv and the points were found to lie on a line extrapolated from the linear relationship between synaptic current and membrane potential in the region -150 to -40 mv. If it is assumed that the synaptic current is carried only by sodium and potassium ions as at the frog neuromuscular junction (*1, 2*), the following linear equation relating synaptic current to membrane potential can be written:

$$I_s = \Delta g_{Na} (E_m - E_{Na}) + \Delta g_K (E_m - E_K)$$

where I_s denotes synaptic current, Δg_{Na} is the change in sodium conductance and Δg_K the change in potassium conductance caused by the transmitter, E_m is the membrane potential, and E_{Na} and E_K are the sodium and potassium equilibrium potentials, respectively. From the voltage intercept of the line in Fig. 3, it can be seen that the synaptic current would be zero when the membrane potential is $+55$ mv. At nine synapses the mean value of this intercept was $+45 \pm 3.6$ mv (mean $\pm$ S.E.). The sodium equilibrium potential has been measured in squid giant axons and found to range from $+45$ to $+59$ mv in fresh axons with an average of $+50$ mv (*9*). In Fig. 3, I_s is zero when E_m is $+55$ mv, which is at, or very close to, E_{Na}. In two experiments, when the sodium concentration was reduced by replacing 50 percent of the sodium in the seawater with tris chloride, the synaptic current intercept shifted 24 and 36 mv in the direction of a reduced E_{Na}; this is more than the shift of 16 to 18 mv that would be predicted from the Nernst relation. The fact that it takes a long time (15 to 30 minutes) for changes in the medium or additions of toxins to be reflected in the activity of this synaptic preparation may underlie this discrepancy. Moore and Adelman (*9*) observed a decline in E_{Na} of 0.125 mv/min in resting squid axons. In the 15 to 30 minutes between the observations in normal and reduced sodium, E_{Na} could be expected to fall by at least 2 to 4 mv. Furthermore, the increased surface-to-volume ratio in the vicinity of the synapse (where the postsynaptic axon tapers to its soma) may result in a higher rate of decline in E_{Na} than reported for the cylindrical portion of the axon. In any case it is difficult to see how these observations could be explained in terms of an appreciable change in g_K in the postsynaptic membrane.

The slope of the relation between the synaptic current and postsynaptic membrane potential usually gave a conductance of about 0.1 mmho. It is interesting to note that this is the input resistance of a semi-infinite cable (extending to infinity in only one direc-

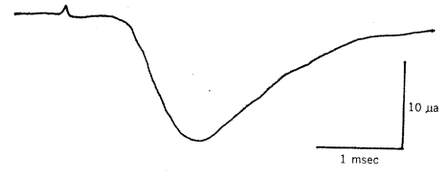

Fig. 2. Synaptic current in a postsynaptic axon clamped at -70 mv (traced from an *X-Y* plotter record).

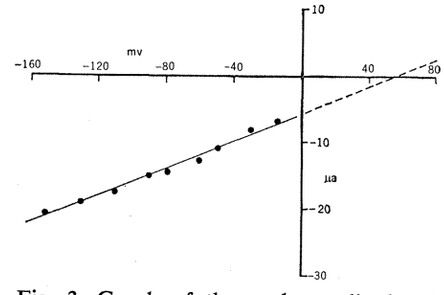

Fig. 3. Graph of the peak amplitude of synaptic currents against the clamped membrane potentials at which they were recorded. The line through the points has been extrapolated to give a voltage intercept.

tion) with the characteristics of a squid axon 500 μ in diameter. This would appear to represent a biological example of an optimal match (equality) of source to load for maximum power transfer.

The observation that the transmitter equilibrium potential in this synapse is at, or close to, E_{Na} suggests that the synaptic conductance change is due predominantly to a change in sodium conductance. This contrasts markedly with the effect of acetylcholine at the frog neuromuscular junction where an increase in both sodium and potassium conductance occurs. Here, because the increases in conductance occur in much the same time (*3*), the effective transmitter equilibrium potential is −10 to −20 mv (*1*, *2*).

One implication of some significance which may be derived from these results is that different excitatory neurotransmitters in the central nervous system may have different effects on ionic conductances in postsynaptic membranes. A transmitter which increased only sodium conductance in a postsynaptic membrane would be more effective in synaptic transmission than one which increased sodium and potassium conductance equally, that is, a quantum of transmitter which increased only sodium conductance would produce more depolarization than would be obtained if both sodium and potassium conductances were increased by about the same amount as at cholinergic neuromuscular junctions. The finding of the rather selective effect on sodium conductance of the transmitter at the squid giant synapse is of particular interest in connection with the hypothesis that the sodium and potassium channels may be separately activated by transmitters (*3*).

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References and Notes

1. P. Fatt and B. Katz, *J. Physiol.* (*London*) **115**, 320 (1951).
2. A. Takeuchi and N. Takeuchi, *J. Neurophysiol.* **22**, 395 (1959); *J. Physiol.* (*London*) **154**, 52 (1960).
3. P. W. Gage and C. M. Armstrong, *Nature* **218**, 363 (1968).
4. R. Miledi, *J. Physiol.* (*London*) **192**, 379 (1967); J. S. Kelly and P. W. Gage, unpublished observations.
5. T. H. Bullock, *J. Neurophysiol.* **11**, 343 (1948).
6. In addition to the excitatory synaptic transmission between giant pre- and postsynaptic axons, there are additional excitatory pathways provided by small fibers in the presynaptic nerve trunk which make synaptic contact near the soma of the postsynaptic ax → [J. Z. Young, *Phil. Trans. Roy. Soc. London Ser. B* **229**, 465 (1939); S. H. Bryant, *J. Gen. Physiol.* **42**, 609 (1959); R. Miledi and C. R. Slater, *J. Physiol.* (*London*) **184**, 473 (1966)]. Preliminary experiments showed a number of complications if the "accessory pre-fibers" were stimulated also.
7. S. Hagiwara and I. Tasaki, *J. Physiol.* (*London*) **143**, 114 (1958); A. Takeuchi and N. Takeuchi, *J. Gen. Physiol.* **45**, 1181 (1962).
8. J. Del Castillo and B. Katz, *J. Physiol.* (*London*) **125**, 546 (1954); *Progr. Biophys.* **6**, 121 (1956).
9. J. W. Moore and W. J. Adelman, Jr., *J. Gen. Physiol.* **45**, 77 (1961).
10. Supported by NIH grant NB 03437.

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Thyrocalcitonin: Evidence for Physiological Function

Abstract. *A calcium salt given by stomach tube in modest amounts, such as might be ingested in a normal meal, produced hypercalcemia in acutely thyroidectomized fasted rats, whereas in rats with intact thyroid glands the same dose of calcium had little or no detectable effect, presumably because of release of thyrocalcitonin. Thyrocalcitonin apparently protects against hypercalcemia during feeding after deprivation.*

The mammalian thyroid gland, by virtue of its ability to release the hypocalcemic polypeptide, thyrocalcitonin (*1*), can protect against hypercalcemia produced in the laboratory by artificial procedures, such as parenteral administration of a calcium salt (*2*), injection of parathyroid hormone (*3*), or treatment with massive doses of vitamin D (*4*). Nevertheless, there has been justified skepticism concerning the physiological significance of thyrocalcitonin, because the experimental evidence for protection against hypercalcemia by the thyroid gland has been based entirely on results obtained under circumstances never encountered in normal life. Furthermore, the experiments of Bronner *et al.* (*5*) had indicated that even at high rates of calcium absorption from the gut, the concentrations of calcium in the serum of normal and thyroidectomized rats did not differ significantly. We now report our results on the feeding (by stomach tube) after fasting of ordinary amounts of calcium to thyroidectomized animals and animals with intact thyroid glands. In related experiments two other groups obtained ambiguous results (*6*).

A modest dose of calcium intragastrically produced marked hypercalcemia persisting at least 2 hours after the gavage in thyroidectomized rats, whereas the increase in the concentration of calcium in the serum in sham-operated rats was minimal and short-lived (Fig. 1). In these young male albino rats the parathyroid glands had been transplanted to the cervical strap muscles at 48 days of age. One week later the concentration of calcium in the serum was analyzed after the rats were fasted overnight, with 9 mg/100 ml or more serving as an indication that the transplanted parathyroid glands were functional. Rats meeting this criterion were divided randomly into four groups on the following day, and food (Purina laboratory chow) was withheld for 24 hours. Some of the rats were thyroidectomized by blunt dissection (without cautery) under ether anesthesia and others underwent sham operation. Calcium (15 mg per 100 grams of body weight; as a 1 percent solution of calcium chloride) was given by stomach tube immediately after the operation. Control rats, some thyroidectomized and some with thyroid gland intact, were given an equal volume of 0.9 percent sodium chloride by the same route. Blood samples for calcium analysis were obtained by cardiac puncture at 60 minutes and 120 minutes after gavage. The concentration of calcium in serum was determined in the Technicon Autoanalyzer (*7*) within 2 hours after the blood was collected. Since the concentrations of calcium in the serum of thyroidectomized rats and normal rats given sodium chloride were not significantly different, they are presented as combined means in Fig. 1, and represent the control situation.

Similar experiments were conducted with acutely thyroparathyroidectomized rats (without cautery), because a separate experiment (not shown) demonstrated that removal of the parathyroid glands did not impair the ability of the thyroid gland to protect against hypercalcemia after oral ingestion of a calcium load. In 13 experiments involving 280 rats the amounts of calcium admin-