Hawaiian Islands (Published under Patronage, Honolulu, 1913), pp. 336–365.
9. S. Carlquist [*Brittonia* **18**, 317 (1966)] uses a similar geographic comparison to show that species of *Tetraplasandra* with large flowers and fruits are derived from species with small flowers and fruits.
10. G. L. Stebbins, Jr. [*Evol. Biol.* **1**, 137 (1967)] discusses the probable genetic basis for the near irreversibility of hypogyny-epigyny and similar evolutionary trends.
11. Examples are *Schefflera* and *Hedera*. See H. Harms, in A. Engler and K. Prantl, *Die Natürlichen Pflanzenfamilien*, Teil III, Abt. 8 (1894), p. 8; N. V. Pervukhina, *Bot. Zh.* **38**, 188 (1953).
12. *Heracleum*. See, for example, E. Liehr, *Mitt. Inst. Allgem. Bot. Hamburg* **6**, 365 (1927); V. G. Aleksandrov and N. V. Pervukhina, *Akad. Nauk SSSR, Tr. Bot. Inst.*, ser. 7, **3**, 5 (1952).
13. V. Grant, *Evolution* **4**, 179 (1950).
14. S. Carlquist, *Quart. Rev. Biol.* **41**, 263 (1966); *Evolution* **20**, 433 (1966).
15. Supported in part by grants from the Smithsonian Research Foundation (to R.H.E.) and the NSF (to C.C.T.). Drawings by C. Reinecke. We thank J. Morgan and C. Warner for microtechnical assistance, H. Robinson and W. R. Philipson for reading the typescript.

29 May 1969

Cardiac Sympathetic Nerve Activity: Changes Induced by Ouabain and Propranolol

Abstract. *A study of the effects of ouabain and propranolol on the spontaneous activity in the preganglionic sympathetic nerves to the cat heart showed that ouabain can produce both an inhibition and a stimulation of the spontaneous activity in sympathetic nerves. The inhibition appears to be reflex in nature and is not present when the buffer nerves are sectioned. The stimulation is correlated with the development of cardiac arrhythmias and is antagonized by propranolol.*

The role of the sympathetic nervous system in the cardiac actions of digitalis drugs has been studied by many investigators, but unanimity of opinion on it has not been forthcoming; indeed, controversy seems to describe best the present state of affairs (*1*). Thus, according to some, the sympathetic nervous system is activated by these drugs and the effects of this activation contribute to the contractile (*2*) and arrhythmogenic responses (*3*). According to others, the sympathetic nervous system is inhibited by the cardiac glycosides and the effects of this inhibition modify the heart rate (*4*), refractory period (*5*), and contractile responses (*6*); two of these authors (*6, 7*) reported that digitalis inhibits efferent discharges in the pre- and postganglionic stellate nerve fibers. Finally, some investigators maintain that these substances are not dependent on the sympathetic nervous system for their actions (*8*).

I have recently obtained direct evidence that ouabain can both inhibit and stimulate the adrenergic nervous system; the response is dependent on the functional state of the cardiovascular reflexes, and the stimulant effect is antagonized by propranolol. This report presents evidence for these effects of ouabain and propranolol on the activity in the preganglionic sympathetic nerves to the heart.

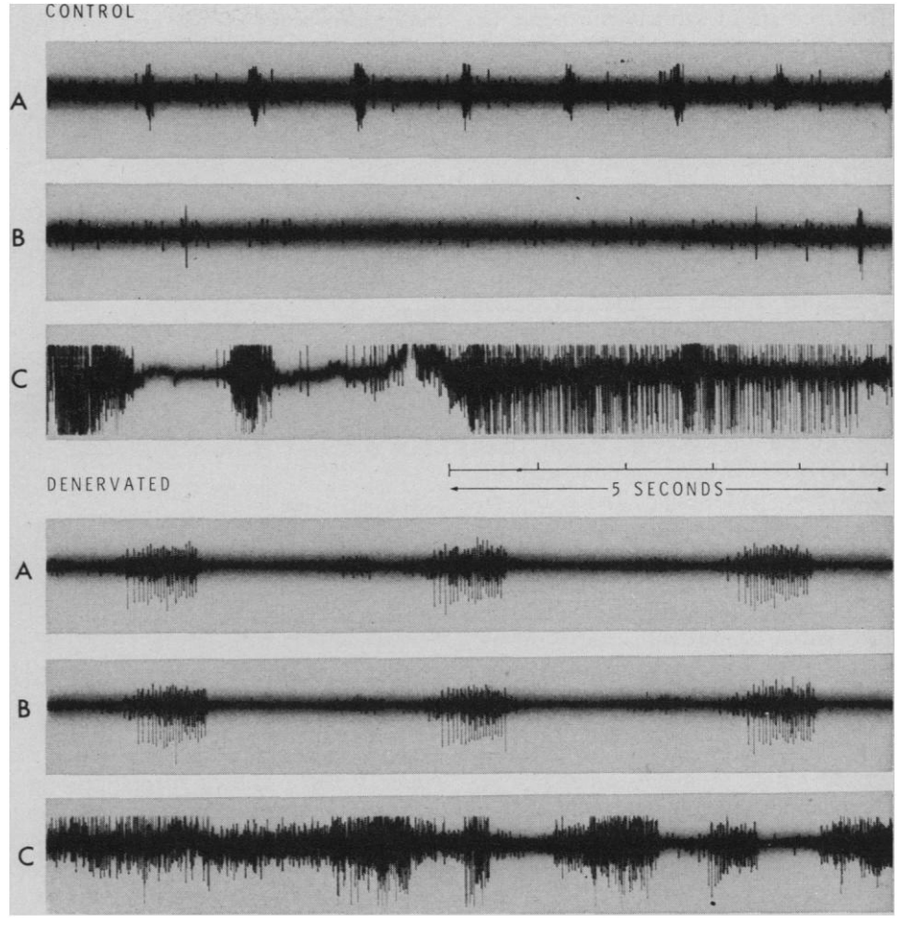
Experiments were carried out in cats decerebrated during ether anesthesia by a stereotaxically placed electrolytic lesion that transected the brain stem in the midcollicular plane. Denervation of the reflexogenic areas was performed in some animals by bilateral section of the carotid sinus and vagus nerves. All animals were ventilated mechanically with 97 percent oxygen and 3 percent carbon dioxide, and their rectal temperatures were maintained between 37° and 38.5°C with an infrared lamp. Femoral blood pressure and lead I or II of the electrocardiogram (ECG) were recorded continuously. Spontaneously occurring activity in the preganglionic nerves to the right stellate ganglion was recorded with bipolar platinum electrodes. The nerves from the ganglion to the heart were not cut; that these nerves innervated the myocardium was evidenced by the fact that their stimulation consistently increased heart rate. Ouabain was administered in single doses of 20, 50, or 100 μg per kilogram of body weight by injection through a cannula in the femoral vein.

The normal activity in these nerves can best be described as bursting and rhythmical. As shown in the A records (controls) of Fig. 1, the bursts of activity occur with a constant interval and frequency in each experiment and in synchrony with inspiration. The effects of ouabain on this activity in control animals (carotid sinus and vagus nerves intact) depend upon the time after injection and the size of the dose. Inhibition of the spontaneous sympathetic activity is the initial effect. It is produced by doses as low as 20 μg/kg and often is the only effect produced by these low doses. After doses in the range of 40 to 100 μg/kg, the initial inhibitory effect is greater, but as time after the injection increases, the spontaneous neural activity returns and increases far above the predrug control. In the inhibitory phase the sympathetic nerve activity is reduced in amplitude or abolished (Fig. 1, control B). The inhibition begins within 1 to 5 minutes, is maximum from 4 to 9 minutes after administration of the drug, and lasts 5 to 20 minutes, depending on the dose. It is immediately followed by a stimulatory phase (Fig. 1, control C) in which the bursts of activity reappear, increase in intensity and duration, and become arrhythmic. In addition, neural activity between the bursts begins and becomes increasingly intense. In a final stage, the burst and the interburst activities merge into a continuous stream of high-intensity neural activity; ventricular arrhythmias occur and the animal dies. After doses of 100 μg/kg the inhibitory phase is transient, or sometimes absent, and excitation is the predominant effect. In the 32 experiments performed, a biphasic response was observed in 23, inhibition only in four (in which only small doses were given), and stimulation only in five (in which only large doses were administered).

The changes in neural activity induced by ouabain do not seem to be related to changes in blood pressure. That is, the entire pattern may be observed in animals in which there is no appreciable change in blood pressure; in animals in which changes do occur, there is no consistent correlation between changes in blood pressure and changes in neural activity. Cutting the sympathetic nerve distal to the electrode does not change the neural activity or the effects of ouabain on this activity.

Denervation of the reflexogenic areas in the carotid sinuses and aortic arch prevents the inhibitory effect of ouabain on this neural activity (Fig. 1, denervated). The administration of 20 μg/kg of ouabain has no effect on the ongoing sympathetic discharge. Larger doses produce an increase in neural activity which is greater and occurs sooner after injection of the drug than doses of the same size produce in control animals; a dose of at least 50 μg/

Fig. 1. Effects of ouabain on sympathic nerve electrical activity. The upper three panels refer to the control animal (intact buffer nerves), and the lower three to the denervated animal (sectioned buffer nerves). Panels marked A are control recordings; those marked B, the recordings obtained 5 minutes after administration of 20 μg of ouabain per kilogram; and those marked C, recordings obtained 8.5 minutes after 80 μg (control) and 14 minutes after 60 μg (denervated) of ouabain per kilogram.



kg is required to produce stimulation. The experiment illustrated in the lower part of Fig. 1 demonstrates the failure of a dose of 20 μg/kg to affect activity in the cardiac sympathetic nerves and the action of a larger dose to increase neural traffic in these nerves. This pattern of response was observed in 22 of 26 experiments. In three of the four remaining experiments, there was no change in sympathetic activity and in the fourth a brief phase of inhibition preceded the stimulation. The increased neural activity was usually initiated at a time when the blood pressure was significantly higher than the level of the control.

In these studies I noted a correlation between the ouabain-induced increase in sympathetic activity and the occurrence of ventricular tachycardia; in many experiments this arrhythmia developed shortly after the neural activity became most intense. This observation posed the intriguing question of whether the arrhythmia was related to the activation by ouabain of the sympathetic nerves. In an attempt to answer this question, I performed 11 experiments in which an antiarrhythmic drug, *d,l*-propranolol (1.5 to 6.0 mg/kg) was administered intravenously to restore the cardiac electrical activity to normal. In ten of these experiments the ventricular rhythm was converted to a normal sinus rhythm and in all of these the sympathetic nerve activity

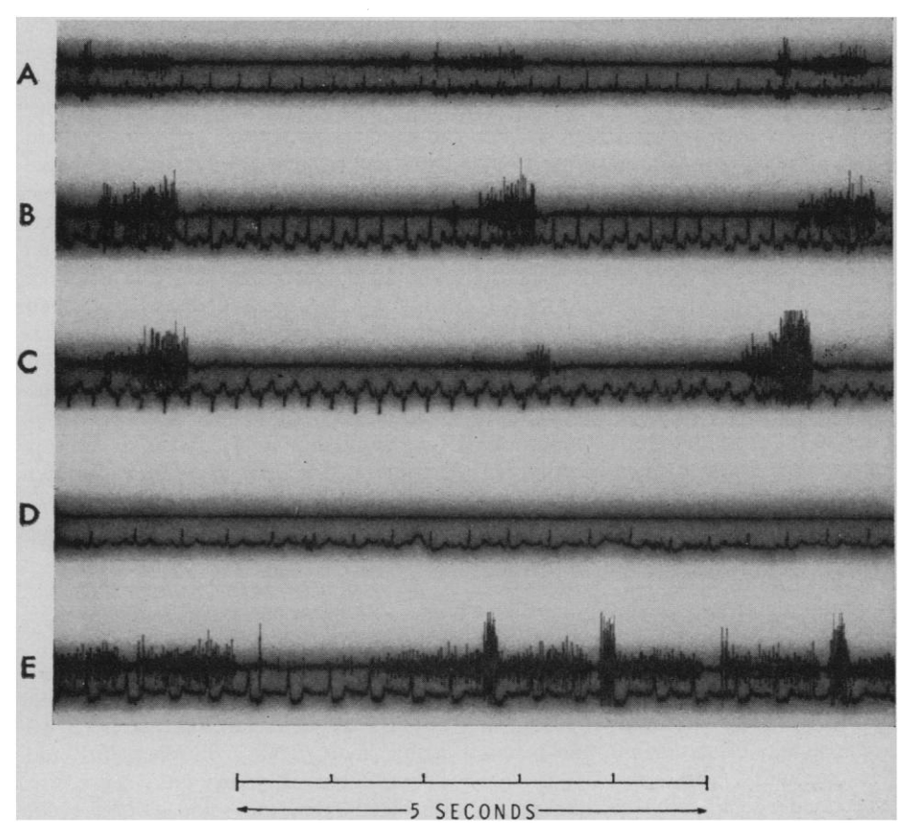


Fig. 2. Effects of ouabain and propranolol on sympathetic nerve electrical activity (upper trace) and on electrocardiogram (lower trace). Panel A, control recordings; panels B and C, recordings obtained at 4 and 7 minutes after administration of 50 μg of ouabain per kilogram, respectively; panel D, recordings obtained 3 minutes after record of panel C and 2 minutes after 2 mg of propranolol per kilogram; and panel E, records obtained 9 minutes after record of panel D and 2 minutes after an additional 20 μg of ouabain per kilogram.

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.

RICHARD A. GILLIS
Department of Pharmacology, Georgetown University Schools of Medicine and *Dentistry, Washington, D.C. 20007*

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).
11. I express my appreciation to Professor Frank G. Standaert for his guidance and aid in the preparation and execution of this study. The technical assistance of Harold Thibodeaux is gratefully acknowledged. Supported by grants from PHS (NB-07651, FR 5360, and FR 5306) and a grant from the Washington Heart Association.

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