and *p*H 5–insoluble cortical proteins (Table 1) suggests that no one protein subfraction was preferentially affected. This is also indicated by our finding that the specific activity of insoluble cortical proteins (that is, those contained in the 50,000*g* sediment) was decreased during SCD to the same extent as the soluble proteins. In order to confirm this suggestion, the *p*H 5–soluble proteins were further fractionated (*8*) by disc electrophoresis on polyacrylamide gels (*9*). The unstained gels, 12.5 mm in diameter and 10 cm long, were cut into 40 cross-sectional slices, and the radioactivity of each slice was measured (*10*). Duplicate gels were stained, and specific activity was estimated by comparing the radioactivity in each slice with the dye intensity of the corresponding area of a stained gel. Although the analysis was limited by the assumption that the intensity was directly proportional to protein concentration and by the low radioactivity in each slice, there was no indication of preferential incorporation into any of the protein bands.

The primary event in spreading depression is believed to be the loss of intracellular potassium and the rise of the extracellular potassium (*11*). Other biochemical alterations have also been reported. Concentrations of glucose, glycogen, and creatine phosphate fall, while the amounts of lactate, citrate, inorganic phosphate, and total ninhydrin-positive substances increase (*12*). These changes are enhanced by simultaneous induction of ischemia and indicate a generalized alteration in the oxidative metabolism of cortical cells, possibly through a stimulation of the sodium-potassium pump. Similar biochemical events have been reported in brain slices during electrical stimulation (*13*) and in media of high potassium concentration (*14*). These observations suggest that the effect of spreading cortical depression on incorporation of labeled leucine into protein is a nonspecific, secondary consequence of the diversion of energy sources or amino acids (or both) into different metabolic pathways.

Our results confirm those of Ruščák (*2*) and extend them to conscious, freely moving animals. The findings suggest the design of experiments to detect changes in the synthesis of particular proteins as a result of more specific alterations in neural activity such as learning and memory. Several groups have reported experiments showing that unilateral spreading cortical depression appears to restrict learning to the normal hemisphere (*4*, *15*). This experimental system might therefore be used to detect possible biochemical alterations in a "trained" hemisphere relative to the depressed hemisphere.

GUDRUN S. BENNETT
GERALD M. EDELMAN
Rockefeller University, New York 10021

References and Notes

1. For a complete description of SCD see reviews by W. H. Marshall, *Physiol. Rev.* **39**, 239 (1959) and S. Ochs, *Int. Rev. Neurobiol.* **4**, 1 (1962).
2. M. Ruščák, *Physiol. Bohemoslov.* **13**, 16 (1964).
3. We thank Dr. S. I. Chorover, M.I.T., for the procedure for implanting cranial cannulas.
4. J. Bures, in *Second Conference on the Central Nervous System and Behavior*, M. A. B. Brazier, Ed. (Josiah Macy, Jr. Foundation, New York, 1959), p. 207.
5. After freezing the separated left and right hemispheres, we pried away the brainstem along a crack which consistently developed during the freezing procedure and extended anteriorly and ventrally from the choroid fissure into the recesses of the third ventricle. This led to the operational definition of "brainstem" as thalamus, hypothalamus, and midbrain, and "cortex" as the remaining tissue. Thus "cortex" contained the entire telencephalon including the basal ganglia.
6. The scintillation fluid consisted of 50 g of naphthalene, 500 ml of dioxane, 70 ml of Liquifluor (Pilot Chemicals, Inc., Watertown, Mass.) brought to 1 liter with toluene. The liquid scintillation counter (model 574) is from Packard Instrument Co., Downers Grove, Ill.
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
8. G. S. Bennett and G. M. Edelman, *ibid* **243**, 6234 (1968).
9. B. J. Davis, *Ann. N.Y. Acad. Sci.* **121**, 404 (1964).
10. J. A. Boyd and H. K. Mitchell, *Anal. Biochem.* **14**, 441 (1966).
11. F. J. Brinley, Jr., *Int. Rev. Neurobiol.* **5**, 183 (1963); B. Grafstein, in *Brain Function*, M. A. B. Brazier, Ed. (Univ. of California Press, Berkeley, 1963), p. 87.
12. M. Ruščák, *J. Neurochem.* **7**, 305 (1961); *Physiol. Bohemoslov.* **11**, 192 (1962); *ibid.* **12**, 562 (1963); ——— and E. Macejová, *ibid.*, p. 570; J. Křivánek, *J. Neurochem.* **6**, 183 (1961).
13. F. Orrego and F. Lipmann, *J. Biol. Chem.* **242**, 665 (1967).
14. P. J. Heald, *Phosphorus Metabolism of Brain* (Pergamon, New York, 1960).
15. I. S. Russell and S. Ochs, *Brain* **86**, 37 (1963); D. J. Albert, *Neuropsychologia* **4**, 49 (1966).
16. Supported by NSF grant GB 6546 and NIH grant AM 04256.

15 July 1968; revised 28 October 1968

Adrenergic Blood Pressure Responses in the Shark

Abstract. *Both nurse and lemon sharks recovered slowly from the pressor effect of epinephrine and from the depressor effect of isoproterenol. The recovery time increased with the dose of epinephrine. Grading of dosage and the use of dibenzyline revealed that these species exhibit alpha and beta adrenergic vascular responses in a manner qualitatively similar but quantitatively dissimilar to that for mammals.*

The pressor response of the shark to epinephrine has been described as prolonged, with accompanying desensitization to subsequent doses (*1*). Little else is known about the nature of the pressor response in sharks. We have studied such responses in small (1 to 6 kg) nurse sharks *Ginglymostoma cirratum* and lemon sharks *Negaprion brevirotris* (*2*). *d*-Tubocurarine (3.0 mg/kg) was injected into the caudal vein. The animal was then restrained in a supine position and anesthetized by perfusion of tricaine methanesulfonate (1:10,000 in seawater) across the gills. The gills were continuously perfused with fresh seawater throughout the experimental period. A deep incision was made through the ventral surface of the tail which was then reflected dorsally exposing the caudal artery and vein, and polyethylene cannulas were inserted in these vessels. Arterial blood pressure was recorded with a Statham P23 DC transducer and a Grass model 79-2 polygraph. Drugs were diluted in an elasmobranch solution (Nicholl) (*3*) and administered by way of the venous cannula.

No differences in response attributable to sex or species were observed among 19 lemon sharks and 17 nurse sharks. Control arterial systolic pressures ranged from 20 to 36 mm-Hg, diastolic pressures from 16 to 30 mm-Hg, and pulse pressures from 3 to 7 mm-Hg. Control heart rates ranged from 16 to 44 beats per minute.

When initial doses of epinephrine chloride (100 to 1000 μg/kg) were administered, mean arterial pressure increased by 10 to 20 mm-Hg. This pressor response decreased only slightly during the subsequent 3-hour period. During the peak pressor response, the administration of additional epinephrine or norepinephrine did not elicit further pressor responses. When the initial doses of epinephrine were below 10 μg/kg, blood pressure returned to that of the control within 60 minutes after administration. Doses of epinephrine below 0.5 μg/kg failed to elicit a pressor response.

In mammals low doses of epinephrine will often elicit a depressor response. Doses of epinephrine as low as 0.0001 μg/kg were administered to

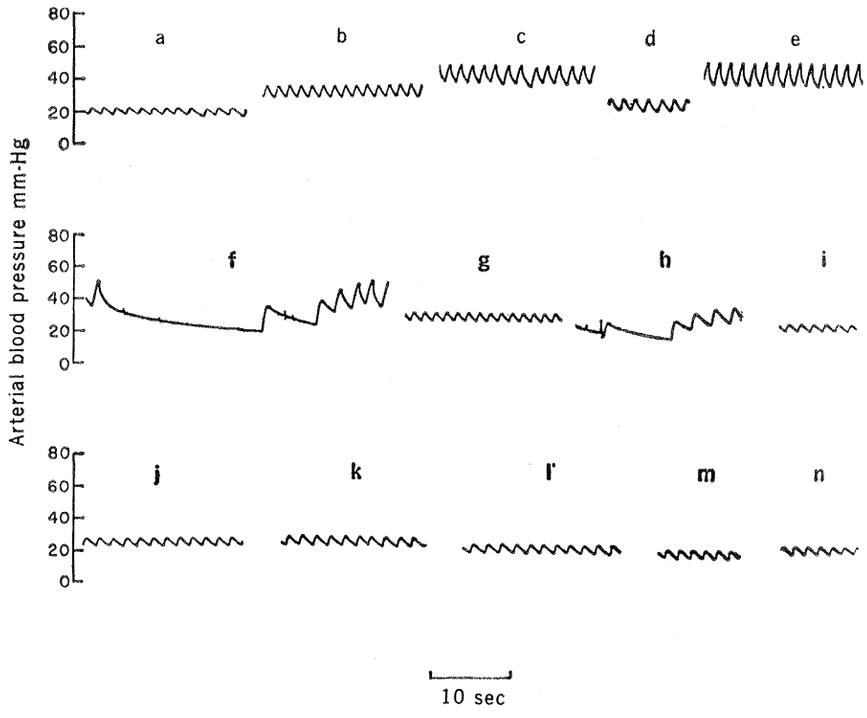

Fig. 1. Response of nurse shark to epinephrine (EPI) and isoproterenol (ISO) when EPI was administered first (doses are as micrograms per kilogram; unless otherwise indicated dose was administered while blood pressure was at the immediately preceding level): (*a*) control; (*b*) EPI, 1.0 (returned to control level 7 minutes after administration); (*c*) EPI, 10.0; (*d*) 33 minutes after *c*; (*e*) EPI, 100; (*f*) EPI 1000 (administered 9 minutes after *e*); (*g*) 3 minutes after *f*; (*h*) EPI, 1000; (*i*) 1.5 minutes after *h*; (*j*) 8 minutes after *h*; (*k*) ISO, 25; (*l*) ISO, 250 (administered 5 minutes after *k*; (*m*) EPI, 1000; (*n*) 3 minutes after *m*.

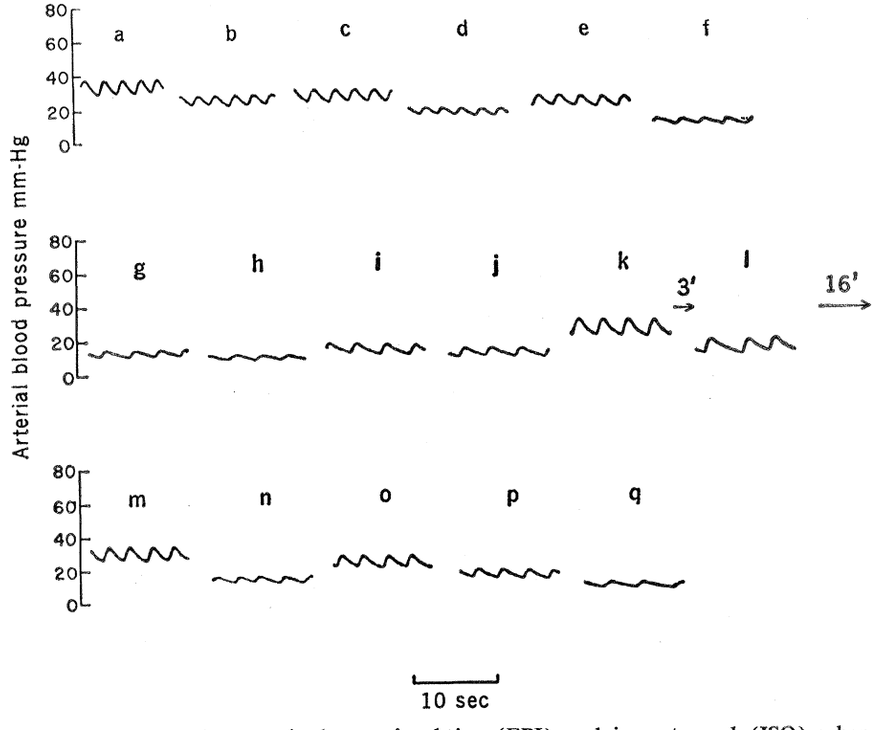

Fig. 2. Response of nurse shark to epinephrine (EPI) and isoproterenol (ISO) when ISO was administered first (doses are as micrograms per kilogram; unless otherwise indicated, dose was administered while blood pressure was at the immediately preceding level): (*a*) control; (*b*) ISO, 1.0; (*c*) 13.5 minutes after *b*; (*d*) ISO 3.0; (*e*) 25 minutes after *d*; (*f*) ISO 10.0; (*g*) 14 minutes after *f*; (*h*) ISO, 30; (*i*) EPI, 10.0 (administered 5 minutes after *h*); (*j*) 6 minutes after *i*; (*k*) EPI, 100; (*l*) ISO, 30; (*m*) EPI, 100; (*n*) 37 minutes after *m*; (*o*) EPI, 100; (*p*) 9.5 minutes after *o*; (*q*) ISO, 100.

sharks. At no time was an unequivocal depressor response observed. However, the relationship between the pressor and depressor effects of epinephrine in the sharks was unusual (Fig. 1). A maximum pressor response was obtained with 100 μg of epinephrine per kilogram. During the peak response, a dose of 1000 μg/kg was administered. Acardia was followed by a return of heart action and the original pressor level was restored. Within 3 minutes after injection, a depressor response occurred. A subsequent dose of epinephrine resulted in a return to control pressure levels. Further doses of epinephrine or isoproterenol did not elicit either a pressor or a depressor response. In one of the animals, a maximum mean pressor response of 17 mm-Hg was obtained with 100 μg of epinephrine per kilogram. Eleven subsequent doses of 100 μg of epinephrine per kilogram were administered causing a gradual decrease from the peak pressor level. After the eleventh dose, the mean pressure had decreased 12 mm-Hg. A final dose of epinephrine (1000 μg/kg) resulted in a return to control pressure. Subsequent doses of isoproterenol (100 μg/kg and 1000 μg/kg) had no effect on the blood pressure. At the point at which no further effect could be elicited with either epinephrine or isoproterenol, the blood pressure had returned to control value observed prior to treatment.

The depressor response to isoproterenol was prolonged and refractory to subsequent doses once a maximum decrease in blood pressure was reached (Fig. 2). The maximum pressor response to epinephrine was only that sufficient to return the pressure to the control level. The administration of epinephrine during maximum pressor response caused a depressor effect. In animals first treated with isoproterenol, the dose of epinephrine required to produce a depressor response was less than that required in animals that were not treated in such a manner. Subsequent to the last dose shown in Fig. 2 (isoproterenol, 100 μg/kg), doses of isoproterenol (100 μg/kg) and epinephrine (100 μg/kg) were administered with no response being elicited.

Dibenzyline (1.0 mg/kg) caused a depressor response of about 50 percent of the control blood pressure. This was followed by a 50 percent increase in pressure over control level and then a return to the depressor level of 50 percent of control. Epinephrine (10 μg/kg) had no effect; but 1000 μg of epineph-

rine per kilogram resulted in a further decrease in blood pressure.

Mammalian studies beginning with that of Alquist (*4*) have led to the classical concept of alpha and beta receptors for the reaction of animals to catecholamines. Usually, stimulation of alpha receptors causes vasoconstriction, and stimulation of beta receptors causes vasodilatation. Epinephrine will stimulate both receptors; isoproterenol stimulates only the beta receptors. Epinephrine and isoproterenol elicited similar responses in the species of sharks studied. However, the depressor response to epinephrine was not obtained until after a maximum pressor response occurred. In mammals, the depressor response to epinephrine precedes the pressor effect.

The epinephrine-induced depressor effect in sharks was unusual. The response was only to lower blood pressure to the control value. The only circumstance in which epinephrine elicited a decrease in blood pressure to below control values was in the animals first treated with dibenzyline, an alpha-receptor blocking agent. This observation suggests that the depressor effect is mediated in part, at least, by a beta-like receptor. Similarly, blockade of the pressor response may result from blockade of alpha-like receptors.

The prolonged pressor response to epinephrine may be a reflection of a deficiency of inactivation mechanisms. However, amine oxidase and *O*-methyltransferase are present in shark tissues (*5*), and sharks have chromaffin cells containing epinephrine and norepinephrine (*6*). Apparently, then, epinephrine is not foreign to the cardiovascular system of the animals studied.

The effect of epinephrine on heart rate was small. Large doses did have a negative chronotropic effect, confirming previous observations (*7*). However, the isolated heart of *Squalus acanthius* reacts to epinephrine and norepinephrine with positive inotropic and chronotropic effects (*6, 8*).

Thus, in both lemon and nurse sharks the basic qualitative reactions to epinephrine and isoproteronol suggest the presence of alpha- and beta-like receptor areas.

SORELL L. SCHWARTZ*
U.S. Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland
JOSEPH F. BORZELLECA
Department of Pharmacology, Medical College of Virginia, Richmond

References and Notes

1. B. R. Lutz and L. C. Wyman, *Biol. Bull.* **62**, 17 (1932); J. C. Mott, in *Physiology of Fishes*, M. E. Brown, Ed. (Academic Press, New York, 1957), vol. 1, p. 102.
2. These studies were carried out at the Lerner Marine Laboratory of the American Museum of Natural History, Bimini, Bahamas.
3. L. J. Hale, *Biological Laboratory Data* (Wiley, New York, 1958), p. 92.
4. R. P. Alquist, *Amer. J. Physiol.* **153**, 586 (1948).
5. E. G. Trams, presented at the meeting of the Shark Research Panel, American Institute of Biological Sciences, Washington, D.C., 26 April 1968.
6. E. Ostlund, *Acta Physiol. Scand.* **31** (Suppl.), 112 (1954).
7. J. W. Burger and S. E. Bradley, *J. Cell. Comp. Physiol.* **37**, 389 (1951).
8. Ostlund also reported that epinephrine "sometimes" had an initial inhibitory effect on the isolated dogfish heart which could be blocked by atropine.
9. We thank R. Mathewson, director of the Lerner Marine Laboratory, and his staff for technical advice and guidance. From the Bureau of Medicine and Surgery, Navy Department, Research Task No. MR005.04-0112. Supported by contract 104-037 from the Office of Naval Research.
* Present address: Department of Pharmacology, Georgetown University, Schools of Medicine and Dentistry, Washington, D.C.

11 October 1968; revised 20 November 1968

Sodium Current in Ventricular Myocardial Fibers

Abstract. *Membrane currents were measured in thin bundles of dog ventricular myocardium under voltage-clamp conditions. A rather large initial inward current which had an equilibrium potential at about + 55 millivolts could be recorded. When the external sodium concentration was reduced, the equilibrium potential for this current was shifted by the amount predicted theoretically for a current carried solely by sodium ions. The size of the sodium inward current (I_{Na}) was largely dependent on the preceding membrane potential. The I_{Na} was completely inactivated if the membrane potential was as low as − 45 millivolts. Sodium ions are the main carrier of charge during the rapid depolarization phase of the action potential.*

There is evidence suggesting that in cardiac Purkinje fibers, as in squid axon (*1*), the rapid depolarization phase of the action potential depends on a specific permeability increase of the membrane for sodium ions (*2*). The application of the voltage-clamp method to frog atrial (*3*) and mammalian ventricular (*4*) myocardial fibers showed that movement of sodium ions down their electrochemical gradient into the fibers might also be the basic mechanism for rapid depolarization in these excitable tissues. The experiments reported here were performed to obtain information about the sodium current in mammalian myocardial fibers.

Thin ventricular trabeculae or very small papillary muscles (diameter, 0.5 mm or less; length, 5 mm or more) were isolated from dog heart. The preparations were pulled through tightly fitting holes in two rubber membranes which divided a chamber into three compartments. The middle compartment was continuously perfused with isotonic sucrose solution, and the two outer compartments were perfused with Tyrode's solution (*5*). In one of the outer compartments only about 1 mm of the fiber was exposed. When a voltage was applied between the two outer compartments, current flowed between these compartments through intracellular pathways of the fiber bundle, owing to the high resistivity of the sucrose solution and the relatively low intracellular resistance. The current flow through the membrane resulted in a homogeneous depolarization in the small portion of fiber exposed to Tyrode's solution. The membrane potential in this fiber portion was recorded between an intracellular and an extracellular microelectrode filled with 3*M* KCl, and the potential was controlled by a negative feedback circuit which used a Philbrick P85A operational amplifier. The gain and frequency response of the system were sufficient to produce a stepwise membrane-potential change within 0.5 msec. Contractions of the same fiber portion were measured with a force-displacement transducer (Grass FTO3C). Contraction, membrane voltage, and membrane current were recorded simultaneously on an oscilloscope (Tektronix 565). There are advantages and limitations to this kind of voltage-clamp method (*6*).

The data recorded were the membrane current and the contraction in response to depolarizing voltage-clamp steps (Fig. 1). The resting potential (−77 mv) was identical with the holding potential from which rectangular voltage steps of increasing amplitudes were applied to the membrane of the fiber bundle. Immediately after all potential steps, a surge of capacitative current flowed. At potential levels up to − 64 mv, constant outward current was recorded throughout the depolarizing pulse. At potentials beyond − 64 mv, however, the capacita-