Neural Factors Contribute to Atherogenesis

Abstract. *Electron microscopic evidence of early atherogenic changes in the aorta and coronary arteries was obtained in normally fed, conscious, unrestrained rats receiving electrical stimulation in the lateral hypothalamus for periods of up to 62 days. Hypertension and hypercholesterolemia were not etiologic factors. In view of recent observations concerning neuropsychological mechanisms in human ischemic heart disease, the findings raise the possibility that the human central nervous system has a role in the development of atherosclerotic lesions.*

In recent years there has been a growing awareness of the role of behavioral responses to psychosocial stimuli in human ischemic heart disease (*1–3*). Considerable evidence has accumulated which implicates neuropsychological mechanisms as important risk factors. Indeed, epidemiologic data suggest that these may have a greater impact than factors which are now accepted as being of consequence, such as hyperlipidemia, hypertension, and cigarette smoking (*4*). Inasmuch as ischemic heart disease is almost always associated with underlying atherosclerosis of the coronary arteries, there is the implication of a possible role for neural factors in atherogenesis. Despite this, surprisingly little work has been performed with experimental animals to determine the role of neurogenic mechanisms in the evolution of arterial lesions.

We have developed an animal model for exploring the relationship of neuroexcitation to morphological, metabolic, biochemical, and physiological events which have been described during atherogenesis. This report is a preliminary description of ultrastructural changes in the aortas and coronary arteries of rats receiving electrical brain stimulation for periods ranging up to 2 months. Some plasma lipid data and measurements of blood pressure are also presented.

Seventeen male rats of the Sprague-Dawley strain, weighing approximately 200 g each, were implanted stereotactically in the lateral hypothalamic area with bipolar, insulated, stainless steel electrodes (*5*). Miniaturized electronic self-powered stimulators, designed and constructed in this laboratory (*6*), were implanted subcutaneously and connected to the electrodes. After a 7-day recovery period, stimulation was initiated with a preset program which delivered 125-hertz, bipolar square waves, with a pulse duration of 0.5 msec for positive and negative phases, in 33-second trains at 70-minute intervals. Current intensities ranged from 100 to 250 μa, although for each animal the level remained constant throughout the experiment. All animals were individually housed, confined to their cages but otherwise unrestrained. Dietary regimes consisted of free access to Purina rat chow (4.5 percent crude fat) (Ralston Purina Co., St. Louis, Missouri) and tap water.

Selected groups of animals were killed at periods ranging from 4 to 62 days after the onset of stimulation. Plasma cholesterol concentrations were measured (*7*) before the onset of stimulation and at the conclusion of the experiment in 15 of the rats. Blood pressures were determined in eight conscious animals by the tail-cuff method (*8*) before the animals were killed. With the animals under sodium pentobarbital anesthesia (3 mg per 100 g of body weight), perfusion of the heart and aorta with half-strength Karnovsky's medium (*9*) was performed at end-diastolic pressure (80 mm-Hg) and tissues were processed for electron microscopy (*10*). Six blocks from the abdominal aorta and the same number from the left descending coronary artery at different levels were sectioned for each animal and examined with a Siemens 1A Elmiscope. The brains were removed and processed for histological localization of electrode tips (*11*). Controls were treated identically except that nonfunctional stimulators were connected to their electrodes.

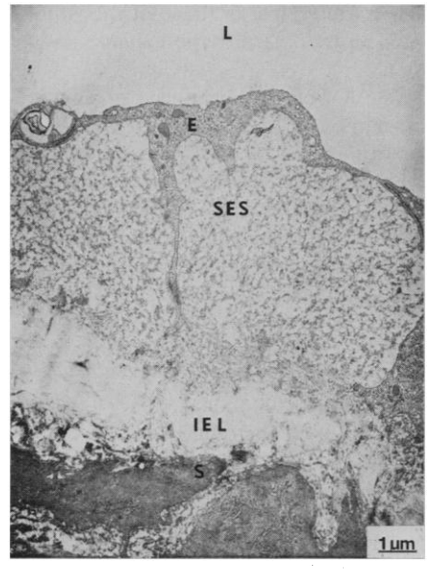

Fig. 1. Section of abdominal aorta in rat stimulated for 62 days. Abbreviations: *L*, lumen; *E*, endothelial cell; *SES*, subendothelial space; *IEL*, internal elastic lamina; and *S*, smooth muscle cell. Note the vacuolated structure at the junction of two endothelial cells (top left) and the marked widening of the subendothelial space, which contains granular electronopaque material resembling plasma proteins.

Experimental animals. Their general health remained good and their weight gain was similar to that of the controls. Daily consumption of food and water was not recorded, although frequent observations disclosed no deviations of feeding or drinking behavior from that of nonexperimental subjects. During periods of stimulation there was a restiveness accompanied by hurrying and scurrying about the cage, whisker twitching, and rapid, shallow respirations. Immediately after stimulation, for approximately 10 seconds, biting and chewing movements occasionally occurred. Accommodation of these behavioral activities over the 62-day period was slight. Between stimulations, no unusual behavior was observed. Blood pressure did not exceed 120 mm-Hg in any of the animals in which it was measured.

In 6 of 15 subjects, despite maintenance on a "normal" diet, plasma cholesterol concentrations increased to 5 to 24 percent above prestimulation values. However, neither the rise in cholesterol concentration nor the magnitude of increase was correlated with the number of days of stimulation.

Electron microscopic examination disclosed similar features in the aorta and the coronary artery. The endothelium was severely altered. There were large vacuoles (2 to 6 μm in diameter) which enclosed detached membrane fragments and contained granular material with the appearance of fluid. Intercellular junctions were sometimes partially separated because the plasma membranes of adjacent cells had undergone degeneration at the junction. Increased numbers of pinocytotic vesicles were also observed. The latter two changes were similar to those described by Robertson and Khairallah (*12*) in rats injected with angiotensin II. Endothelial cells were frequently edematous with increased numbers of mitochondria and dilatation of rough endoplasmic reticulum.

Still more striking was the widening of the subendothelial space (SES), which occasionally exceeded one cell width in thickness (Fig. 1). Within the SES there was an accumulation of electron-opaque granular material with the appearance of plasma protein. Also present in the SES, near the internal elastic lamina, were mi-

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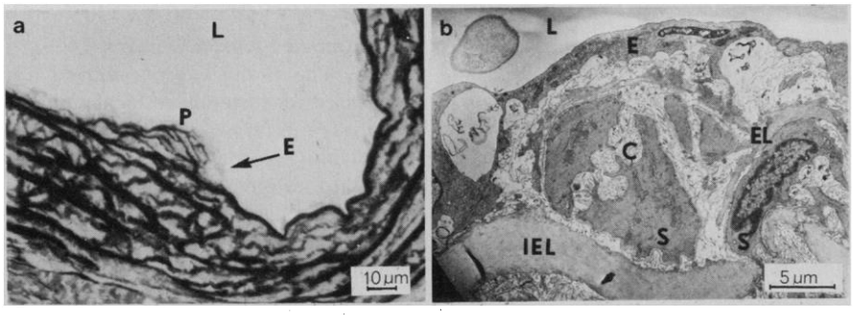

Fig. 2. (a) Araldite-embedded section (2 μm thick) of the abdominal aorta of a rat that received lateral hypothalamic stimulation for 62 days; toluidine-blue stain. Focal proliferation of smooth muscle cells can be seen forming a small plaque (*P*). Within the plaque are reduplicated strands of elastica which are separated by smooth muscle cells (light spaces). Covering the plaque are faintly outlined endothelial cells (*E*), some of which show vacuolated changes. Abbreviation: *L*, lumen. (b) Electron micrograph of a section comparable to that in (a). Elevation of the endothelium (*E*) with lifting from the basement membrane and widening of the subendothelial space are seen. At top left is an endothelial vacuole containing membrane fragments. The subendothelial space contains granular electron-opaque material, collagen (*C*) fibers, strands of elastic tissue (*EL*), and proliferating smooth muscle cells (*S*). Abbreviations: *L*, lumen; *IEL*, internal elastic lamina.

crofibrils 80 to 100 Å in diameter and small islands of elastic tissue, closely associated with the lamina. In animals stimulated for longer periods (30 days), mature collagen fibers were present beneath the endothelium.

The internal elastic lamina was frequently interrupted by extensions of smooth muscle cells from the media into the SES. Usually, these extensions, as well as smooth muscle cells themselves, were located in regions of the SES where widening and accumulation of plasma protein–like material was considerable. Smooth muscle cells in their entirety were observed in the SES as early as the eighth day, and in some cases encroached on the lumen forming small "plaques" (Fig. 2).

Within the media, the smooth muscle cells were separated from each other by collagen fibers, which appeared to be more abundant than in control animals. Basement membrane material surrounding the smooth muscle cells also seemed to be increased. In addition, enhanced metabolic activity was indicated by the presence of large numbers of mitochondria, dilatation of rough endoplasmic reticulum, and numerous pinocytotic vesicles along the cell periphery which imparted a bubbly appearance to the plasma membrane margin. The adventitia was not remarkable.

Control animals. There were no unusual feeding, drinking, or behavioral activities as a result of electrode implantation alone. In four animals in which measurements were made, plasma cholesterol concentrations declined 4 to 12 percent. Blood pressures ranged from 80 to 110 mm-Hg.

Electron microscopic examination disclosed some endothelial vacuoles in the control animals. These were much less frequent than in experimental rats. In other respects the fine-structural details were not remarkable and conformed to those described by Buck (*13*).

Electrode tips were located in an area of the lateral hypothalamus specified by modified de Groot coordinates (*5*): AP = 5.0 to 5.2, L = 1.5 to 2.0, and V = 9.0 to 9.5. This region lies at the caudal border of the dorsomedial nucleus, between the fornix and the medial border of the internal capsule and superior to the optic tract.

The behavioral activities described above are typical of those of rats stimulated in the lateral hypothalamic region (*14*).

The normal blood pressures recorded in eight experimental animals were similar to those of the controls and indicate that the area of stimulation in this experiment is different from that in which pressor responses may be elicited in the rat. The latter lies in the posterior aspect of the hypothalamus (*15*).

Elevated plasma cholesterol concentrations in some of the stimulated rats were due not to any special dietary regimes but to functional biliary obstruction. Both the obstruction and the resulting hypercholesterolemia have been shown to be induced by electrical stimulation of the lateral hypothalamic area (*16*).

Ultrastructural features similar to those observed in the experimental animals have been described by investigators studying early phases of atherogenesis (*17–19*). Since a variety of methods were used to induce lesions in these studies, it is difficult to speculate on a common mechanism for the results in the literature and those reported here.

In our animals, hypertension did not appear to be a factor since several stimulated rats with developing lesions had normal blood pressures. For a similar reason it can be concluded that the plasma cholesterol concentration itself is not an etiologic factor since it was normal in many animals with advanced arterial changes.

Theories of atherogenesis are still emerging, and it is not known whether the precursor of the clinically important fibrous plaque is an early lesion known as the fatty streak, a mural microthrombus, or an edematous state of the intima in which plasma proteins, including lipoproteins, accumulate (*20*). These conditions are not mutually exclusive. Smooth muscle cells have been unequivocally identified in human fatty streaks by Geer *et al.* (*21*) and in fibrous plaques by Haust *et al.* (*22*), and their role in lipid uptake and connective tissue elaboration has been demonstrated by these investigators. Consequently, smooth muscle cells present in the intima focally as "cushions" (*23*), diffusely as a function of age (*20*), or migrating from the media (*24*) may respond to an accumulation of plasma proteins by lipid imbibition (*20*, p. 69), proliferation, and synthesis of collagen, elastic fiber proteins, and proteoglycans (*23, 25*). This sequence of events may well account for the presence of lipids and formed elements in advanced lesions despite the lack of a primary role for plasma cholesterol in atherogenesis (*26*).

The possible influence of the nervous system itself in bringing about such alterations, which are of particular importance as early events, has not previously been explored. There is some experimental evidence supporting the role of the diencephalon in increasing atherogenicity due to special diets (*27*) or in producing "hypertrophy" of small intramyocardial arteries (*28*). The results reported here, however, demonstrate that sustained electrical stimulation of the lateral hypothalamic area in normally fed, conscious, unrestrained rats can produce morphological changes of the aorta and major coronary arteries which are representative of those believed to be involved in the early phases of both human and experimental atherogenesis. We have previously shown that chronic stimulation of autonomic nerves directly supplying the aorta can produce advanced lesions in the form of fibrocalcific arteriosclerosis (*29*). Taken together, these results in an atherogenesis-resistant species raise the possibility that neural factors may be of considerable importance in human atherogenesis, even in

the absence of coexisting hypercholesterolemia or hypertension.

This possibility is further strengthened by evidence for the role of neuropsychological mechanisms in human ischemic heart disease (*30*). Such mechanisms would of necessity be mediated through the nervous system. Whether they would act directly by affecting the vascular wall through neural transmission or indirectly by elaborating arteriopathic substances (such as angiotensin II) in extravascular sites, can only be answered by future investigation.

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31. The technical assistance of L. Kiu is gratefully acknowledged. This work was supported by grant HL-18085 from the National Heart, Lung and Blood Institute.

29 September 1977

Pentobarbital: Differential Postsynaptic Actions on Sympathetic Ganglion Cells

Abstract. *The frog sympathetic ganglion has been used as a model to elucidate the cellular mechanism of barbiturate anesthesia. Anesthetic concentrations of pentobarbital markedly reduced the fast nicotinic excitatory postsynaptic potential while having no effect on the slow excitatory postsynaptic potential or slow inhibitory postsynaptic potential, even though all three synaptic potentials depend on the presynaptic release of acetylcholine. A similar differential effect was seen for nicotinic and muscarinic responses to exogenously applied agonists, while the depolarizing action of γ-aminobutyric acid (GABA) was enhanced. These results indicate that pentobarbital has remarkably selective actions on the sympathetic ganglion and further indicate that blockade of ganglionic transmission by anesthetic concentrations of pentobarbital can be entirely explained by a postsynaptic action. The present results strengthen the concept that pentobarbital anesthesia results from a postsynaptic blockade of central excitatory synapses which increase sodium conductance coupled with a postsynaptic enhancement of GABA-mediated synaptic inhibition.*

It is well documented that barbiturates have a remarkably selective action on synaptic transmission, and it is generally agreed that alterations in synaptic function play a crucial role in producing general anesthesia. However, the exact site at which these drugs act remains controversial. On the one hand there is evidence that barbiturates have presynaptic effects which could lead to the blockade of transmitter release (*1, 2*), while on the other hand there is considerable evidence that barbiturates act postsynaptically to block excitatory transmitter action (*3–7*). Moreover, the preservation or augmentation of synaptic inhibition in the presence of barbiturates both in the vertebrate central nervous system (*8*) and invertebrate systems (*9*) suggests that a block of inhibitory transmitter release is not occurring. This differential action of barbiturates also indicates a striking selectivity of barbiturates for certain types of synapses. The sympathetic ganglion is a particularly useful preparation to examine the selectivity of barbiturate action, since a variety of synaptic potentials can be recorded (*10, 11*). In addition, insight can be gained into the relative importance of pre- and postsynaptic sites of action.

Changes in membrane potential of frog (*Rana catesbeiana*) paravertebral sympathetic neurons were recorded by the sucrose gap technique from the ninth or tenth postganglionic branch (*12*). Initial studies confirmed that transmission through the frog sympathetic ganglion was much more sensitive to barbiturates than was axonal conduction (*13*). Thus a 50 percent block of synaptic transmission occurred at a concentration of 100 μM pentobarbital, while a 50 percent block of axonal conduction required approximately a 20-fold increase in concentration. Examination of the relative sensitivity of the various synaptic potentials indicated an additional highly selective action. Figure 1A shows that pentobarbital severely depresses the fast excitatory postsynaptic potential (EPSP) but has no effect on the slow inhibitory postsynaptic potential (IPSP) or slow EPSP. A 50 percent reduction in the fast EPSP occurred at a concentration of 100 μM ($N = 8$). To reduce the slow potentials to a similar extent required increasing the concentration approximately tenfold. This differential sensitivity, although less pronounced, was seen with a number of other general anesthetics including ketamine, chloral hydrate, chloralase, ether, and halothane. The convulsant barbiturate 5-(2-cyclohexylideneethyl)-5-ethyl barbituric acid also had a selective action. These results complement other research on the effect of anesthetics on ganglionic discharges (*14*).

A comparison of the action of pentobarbital was also made on the depolarization produced by γ-aminobutyric acid (GABA), which in mammalian ganglia results from an increase in chloride conductance (*15*); β-alanine, which weakly mimics GABA in sympathetic ganglia (*16*); and carbachol. Whereas pentobarbital (100 μM) reduced the amplitude of the carbachol response an average of 80 percent ($N = 6$), the amplitude of the GABA and β-alanine responses was actually increased and prolonged (Fig. 1B). This effect on GABA responses is consistent with observations on central neurons that GABA-mediated synaptic inhibition (*8*) and the action of exogenously applied GABA (*4, 7*) are prolonged by

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