LHRH in present-day birds, reptiles, and teleosts might represent the ancestral LHRH molecule that occurred in the ancient osteichthyes and has persisted for about 400 million years. A single mutation in the "parent" molecule might have occurred in a lineage common to amphibians and mammals after its divergence from the common stock from which the extant birds and reptiles arose, and this mutant molecule may have given rise to the mammalian-type analog in extant amphibians and mammals (Fig. 3). In spite of the physicochemical differences in vertebrate immunoreactive LHRH, the biologically active region of the molecule has clearly been conserved during evolution since we have shown (*12*) that when it is purified by affinity chromatography, the hormone from all species stimulates the release of luteinizing hormone from sheep anterior pituitary cells in culture.

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Cerebral Norepinephrine: Influence on Cortical Oxidative Metabolism in situ

Abstract. *Unilateral lesion of the locus coeruleus and the resultant norepinephrine depletion in the ipsilateral cerebrum alters the relationship between cerebral metabolic demands and local delivery of oxygen and substrates. This effect of norepinephrine depletion is demonstrated by slower recovery of the redox ratio of cytochrome* a,a_3 *during increased metabolic demands induced by local cortical stimulation.*

Monoamines, norepinephrine in particular, are putative central nervous system (CNS) neurotransmitters or modulators. Various physiological functions of the CNS ranging from attentiveness and learning to regulation of muscle tone may be influenced by central noradrenergic mechanisms (*1*). Several states of CNS dysfunction, including mania and depression, appear to be associated with altered metabolism of norepinephrine (*2*). Many of the pharmacologic agents that profoundly affect CNS function, such as antidepressant drugs, amphetamine, and cocaine, are thought to mediate their effects by modifying central norepinephrine metabolism (*3*). Furthermore, cerebral blood flow and cerebrovascular permeability may be regulated by central noradrenergic neurons originating in the locus coeruleus (LC) (*4*). However, it is not known whether this represents a direct effect of noradrenergic neurons on cerebral vasculature or a secondary phenomenon due to an alteration of cerebral metabolic demands.

We have studied the question of whether norepinephrine has an influence on oxidative energy metabolism of the cerebral cortex. We took advantage of the fact that most, if not all, of the noradrenergic supply of the mammalian cerebral cortex emanates from a compact nucleus of noradrenergic cells in the ipsilateral brainstem, the nucleus LC (*1*, *5*). Discrete lesion of the LC offers the opportunity to ascertain the effects of selective cerebral cortical norepinephrine depletion. In view of the evidence that LC neurons are most active during periods of stress and increased alertness and, conversely, are inactive during periods of drowsiness or depressed cortical functioning (*1*, *6*), we elected to study the metabolic effects of LC lesions under conditions of increased cerebral activity induced by direct electrical stimulation of the cerebral cortical surface. To accomplish this, we required a signal of oxidative metabolic activity that allowed kinetic interpretation in intact cerebral cortical tissue. The development of dual wavelength reflection spectrophotometry of changes in the redox ratio of cytochrome a,a_3 offered this ability, because the cytochrome redox ratio has been shown to vary with the tissue respiratory state (*7*).

Experiments were performed on male Wistar rats (250 to 350 g). Unilateral LC lesions were made on either side by local injection of 6-hydroxydopamine as described previously (*8*) with minor modifications. Littermate rats without lesions served as controls. Two weeks later, the rats were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally), tracheotomized, paralyzed with curare, and artificially respired with a mixture of 30 percent oxygen and 70 percent nitrogen. Holes, approximately 5 mm in diameter, were drilled in the skull on either side over the frontoparietal cortex leaving the dura intact. Cytochrome a,a_3 redox ratio changes were monitored within fields 3 mm in diameter on either cortex. In half of the rats, recordings were first made on the side ipsilateral to the LC lesion. The cortical surface was stimulated (2-second trains, 20 Hz, 0.5-msec square-wave pulse duration) through forked electrodes 2 mm apart positioned extradurally at the center of the 3-mm optical field. A stainless steel electrode, referenced to neck muscle, was used to monitor shifts in the cortical steady potential.

The spectrophotometric procedure is based on the fact that reduced cytochrome a,a_3 absorbs more light (reflects less) at 605 nm than does its oxidized form. The cerebral cortex was illuminated at 605 nm and also at 590 nm. Reflectance at the latter wavelength provided a reference compensation for changes in light scattering, blood volume and hemoglobin saturation. The voltage record of the reference (590 nm) reflection was subtracted from that of the 605-nm reflection to provide the more accurate cytochrome a,a_3 signal. Changes in cytochrome a,a_3 redox ratios and the compensation signal were displayed on a chart recorder. Because this signal at 590 nm is near the isobestic point for hemoglobin oxygenation-deoxygenation shifts, it has been considered indicative of blood volume changes within the optical field (*7*). Records from the cerebral cortex ipsilateral to the LC lesion were compared to those from the contralateral cortex of the rats with lesions and to

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those from the control rats without lesions.

At the end of the recording, the rats were decapitated and bilateral cerebral cortical samples were obtained that were immediately frozen in liquid nitrogen and stored at −60°C until they were assayed for their norepinephrine content by a radioenzymatic method (*9*). The lesion was considered successful if cortical norepinephrine ipsilateral to the lesion was less than one-third that of the contralateral side. In nine rats where the LC lesion was considered successful, cortical norepinephrine was 40.2 ± 5.8 (mean ± standard error) and 184.3 ± 15.4 ng/g, ipsilateral and contralateral to the LC lesion, respectively. Cortical norepinephrine in six samples from the control group of rats was 193.3 ± 20.6 ng/g.

When the cerebral cortical surface was stimulated, there was a transient decrease in light absorption at 605 nm which signals a transient decrease in the ratio of reduction to oxidation of cytochrome a,a_3. The level of oxidized cytochrome a,a_3 reached its peak at approximately 6 seconds after the stimulation and recovered back to baseline within approximately 20 to 30 seconds. These rates were similar in both cerebral hemispheres of control rats (Fig. 1A), and were similar to the rate reported previously in rat and cat (*10*). Responses similar in direction and kinetics were also recorded from the contralateral cerebral cortex of rats with unilateral LC lesions (Fig. 1, B and C). However, in norepinephrine-depleted cortices ipsilateral to the LC lesion, a significant change in these responses was noted. When cytochrome responses of approximately equal magnitude were compared, the rate of oxidation was similar but the rate at which the cytochrome became re-reduced was slowed. This slowing of re-reduction was evident whether the lesion of the LC was on the right or the left (Fig. 1, B and C). Since extensive investigations in vitro and in vivo have related respiratory chain redox states to the rates of adenosine diphosphate (ADP) and adenosine triphosphate (ATP) production (*11*), it appears that the recovery to baseline redox ratio, and therefore the recovery of the ATP concentration, is slowed in the absence of norepinephrine in vivo.

Another difference between hemispheres of rats with lesions of the LC was observed in the reference signal trace. Stimulation was accompanied by an increase in reference voltage in the hemisphere contralateral to the LC lesion whereas no change was recorded in the norepinephrine-depleted hemisphere (Fig. 1D). To the extent that the reference signal indicates blood volume (*7, 12*), it is demonstrated that the increase in blood volume that usually accompanies stimulation of rat cerebral cortex is lost when norepinephrine was absent.

These results indicate that the destruction of noradrenergic input to the cerebral cortex influences cerebral oxidative metabolism in situ. The slow recovery after activity in the norepinephrine-depleted cerebral cortex could be the result of a direct effect within the respiratory chain, such as a decreased ability to rephosphorylate ADP. Another possibility is that the norepinephrine influence on cerebral oxidative metabolism is mediated through an effect on cerebral circulation. Our results showing lack of blood volume increase in the norepinephrine-depleted cortex is consistent with this hypothesis. It may be, for example, that provision of substrate for re-reduction is inadequate since it is known from work in vitro that decreases in substrate can result in oxidative changes in the respiratory chain (*11*). A third possible explanation for the slowed re-reduction of cytochrome a,a_3 in the norepinephrine-depleted hemisphere is that the stimulus for increased turnover in the respiratory chain (that is, ADP) remains available longer because of prolonged extracellular K^+ activity after the electrical stimulation [for example, see (*13*)]. Such an effect may be due to altered cerebrovascular permeability to water and ions brought about by altered central norepinephrine metabolism (*4*).

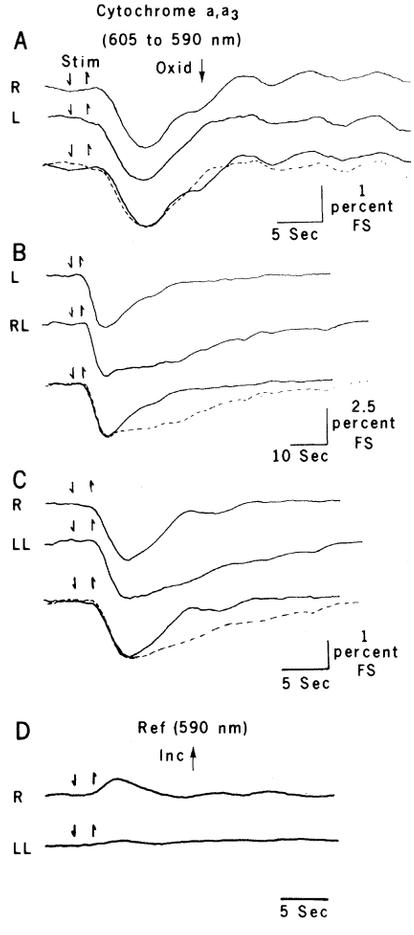

Fig. 1. Effect of unilateral LC lesion on the transient response of cytochrome a,a_3 (A to C) and blood volume (D) to direct cortical stimulation (2-second trains at 20 Hz, 0.5-msec pulse duration). The period of stimulation is marked by half-arrows above each tracing. In (A to C) a downward deflection indicates oxidation of cytochrome a,a_3 (see text). The vertical coordinate is measured in percentage of full scale (*FS*) deflection. Full scale is based upon zero being the condition with no sample light with full reference illumination and 100 percent being the condition at rest previous to stimulation with equal light at the sample and reference wavelengths. In (D) an upward deflection means increased voltage required to compensate for decreased reflectance at 590 nm, indicating increased blood volume (see text). (A) Traces from a control rat without a lesion. The upper trace shows a stimulus-evoked response from the right cerebral cortex (*R*, right control) which is similar to the middle trace taken from the left control hemisphere (*L*, left control). The lower trace shows the upper two traces superimposed. (B) Traces from a rat with a lesion in the right LC. The upper trace is from the left cerebral cortex (*L*, left control), while the middle trace is from the right cortex ipsilateral to the lesion (*RL*, right lesion). The lower trace shows the traces of left control and right lesion superimposed and illustrates the marked decrease in the rate of re-reduction of cytochrome a,a_3 in the norepinephrine-depleted cortex. (C) Results similar to (B) except that the LC lesion is on the left side (*R*, right control, *LL*, left lesion). (D) The 590-nm reflectance voltage obtained simultaneously from the same rat depicted in (C). Note the transient increase in blood volume induced by stimulation of the right (*R*, right control) cerebral cortex contralateral to the lesion and the lack of a similar increase in the norepinephrine-depleted cortex ipsilateral to the lesion (*LL*).

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Tyrosine Hydroxylase Immunoreactivity in Familial Dysautonomia

Abstract. *Tyrosine hydroxylase antigen was localized immunohistochemically in sympathetic neurons from human autopsy tissue. The reaction persists in paraffin-embedded tissue, and the method is applicable to archival specimens. Increased amounts in this antigen per cell may partially compensate for decreased numbers of sympathetic neurons in familial dysautonomia.*

Tyrosine hydroxylase (TH) antigen was detected immunocytochemically in neurons of human sympathetic ganglia and found to be increased in the disease familial dysautonomia (FD). The antigen was detectable unchanged in control tissue embedded for 11 years in paraffin after fixation in formalin. Thus, the technique could be used in retrospective studies. The TH-immunoreactivity (TH-IR) as measured by the peroxidase-anti-peroxidase (PAP) technique (*1*) does not indicate enzymatic activity but, when all staining factors are kept constant and with substrates in excess, the quantity of reaction product is determined by the amount of TH protein present. Studies of rat PC12 pheochromocytoma in which TH enzymatic activity was manipulated over long periods indicated corresponding changes in TH-IR.

Familial dysautonomia is an autosomal recessive disease which appears in infancy and is characterized by motor, sensory, and autonomic abnormalities (*2*). Clinical, biochemical, pharmacologic, and anatomic data indicate diminution in the numbers of sympathetic neurons (*3*). Superior cervical sympathetic ganglia (SCSG) from nine controls and nine FD patients (*3*) were stored in paraffin after formalin fixation (Table 1). Sections (10 μm) of this tissue were subjected to simultaneous processing in the same reagents to ensure that the only variable was the amount of TH antigen present; the sections were stained as described (*4*). Rabbit antibodies to TH antigen purified from human pheochromocytoma (*5*) have been used to demonstrate catecholaminergic neurons in human brain, adrenal medullary cells, and sympathetic axons (*4*).

Control sympathetic neurons exhibited varied staining intensity (Fig. 1A), with a few showing virtually no reaction product. Variation in catecholamine content of sympathetic neurons has been demonstrated by induced histofluorescence (*6*). In some species, adrenergic (A) and dopaminergic (DA) neurons are present in addition to the predominating noradrenergic (NA) neurons (*7*). If human ganglia are similar, variability in TH-IR might be anticipated since amounts of TH appear to be higher in A and DA neurons than in NA neurons (*8*). Neurons without TH-IR possibly contain noncatecholamine transmitters, as has been suggested for other species (*9*).

Within FD neurons, skeins of fine fibrils stained more intensely than in controls. Fibrils extended into the axons where they formed spirals around one another. Axons in FD were wider than in controls, and the number of fibrils was greater. In every FD patient, almost all SCSG neurons stained with greater intensity than controls (Fig. 1B). Mean densitometer readings of TH/PAP end product in 50 neurons of each of the patients and controls appear in Fig. 1C. Mean absorbancy of the controls was 0.39 ± 0.05. In FD it was 0.80 ± 0.15 ($P < .01$). Significant difference persisted if the two older controls were omitted. Individual patients differed sig-

Table 1. Ages and causes of death of FD patients and controls. All FD patients had complex clinical histories including episodes of hypoxia and hypotension. Pharmacology: during vomiting crises six patients were treated for brief periods (usually a few days and not just prior to death) with chlorpromazine (a) and one had received this drug over a long period (b). One patient was occasionally treated with methacholine (c). Two patients received no neuropharmacologic agents. No relation was found between the form of therapy and TH-IR in neurons. Except as indicated by diagnosis, controls were apparently in good health until their deaths.

Code number	Age (years)	Period of block storage (years)	Pharmacology	Cause of death
		Patients		
54436	1	11	0	Respiratory failure
UA 49 73	4	6	0	Aspiration pneumonia
N 71 73	9	6	a, c	Aspiration of vomitus
F 2909	15	1	a	Respiratory failure
UA 178 72	16	7	a	Aspiration pneumonia
RI 74 106	17	5	a	Aspiration pneumonia
UA 113 77	18	2	a	Cardiac arrest
F 2560	28	3	b	Aspiration of vomitus
UA 17 76	33	3	a	Renal failure
		Controls		
54632	1	11	0	Werdnig-Hoffman disease
F 2596	1	3	0	Drowned
57839	3	3	0	Fanconi syndrome
F 2615	14	3	0	Barbiturate poisoning
F 2920	25	1	0	Gunshot
F 2585	28	3	0	Car crash
B 76-1656	36	3	0	Gunshot
B 2919	50	1	0	Gunshot
58379	57	1	0	Myocardial infarct

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