potentially a highly specific assay for detection of a humoral response to leprosy infection and, for the first time, represents a direct method for studying such a response without complications due to previous exposure to environmental mycobacteria or BCG vaccination. It will be of particular interest to apply such a test to detection of the very early stages of leprosy infection. Early detection and treatment of leprosy has the potential benefits of reducing the incidence of deformities due to the disease and also of reducing the transmission of leprosy in the community.

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22. We thank S. Levery for the gas-liquid chromatography and mass spectroscopy, and J. P. Harnish, R. A. Miller, S. Estrada-Parra, S. Dissanayake, F. Quesada-Pascual, and E. Garcia-Ortigoza for assistance with the collection of serums and clinical characterization of the patients. Supported in part by the Immunology of Leprosy component of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases and by the Rockefeller Foundation Program for Research on Great Neglected Diseases. Armadillo tissue infected with *M. leprae* was obtained through the National Institutes of Health.

28 December 1982; revised 10 June 1983

Substance P and Somatostatin Regulate Sympathetic Noradrenergic Function

Abstract. *Peptidergic-noradrenergic interactions were examined in explants of rat sympathetic superior cervical ganglia and in cultures of dissociated cells. The putative peptide transmitters substance P and somatostatin each increased the activity of the catecholamine-synthesizing enzyme tyrosine hydroxylase after 1 week of exposure in culture. Maximal increases occurred at 10^{-7} molar for each peptide, and either increasing or decreasing the concentration reduced the effects. Similar increases in tyrosine hydroxylase were produced by a metabolically stable agonist of substance P, while a substance P antagonist prevented the effects of the agonist. The data suggest that the increased tyrosine hydroxylase activity was mediated by peptide interaction with specific substance P receptors and that peptides may modulate sympathetic catecholaminergic function.*

The demonstration of putative peptide neurotransmitters in sympathetic ganglia (*1*, *2*) suggests that the biochemical organization of the autonomic nervous system is considerably more complicated than previously thought. For example, the peptide neurotransmitters substance P (SP) and somatostatin (SS) are present in mammalian autonomic ganglia (*1*, *2*), and electrophysiologic evidence suggests that they subserve a physiologic role in the sympathetic system (*3*, *4*). Repetitive stimulation of presynaptic nerves to sympathetic ganglia elicits not only a burst of action potentials, but also slow depolarization which is insensitive to cholinergic antagonists (*5*). Iontophoretic application of SP to sympathetic neurons mimics this noncholinergic, slow excitatory postsynaptic potential (EPSP) (*3*, *4*). Moreover, the slow EPSP elicited by repetitive stimulation is blocked by antagonists of SP (*4*). Finally, SP is released from sympathetic ganglia by a high potassium stimulus in a calcium-dependent manner (*3*). These observations suggest that SP release elicits the slow EPSP in sympathetic neurons. Although the function of peptide transmitters in autonomic ganglia is unclear, the prolonged nature of this electrophysiologic response suggests that they may subserve modulatory functions.

We studied the influence of SP and SS on catecholaminergic function in autonomic ganglia and found that both peptides regulate noradrenergic phenotypic characters in sympathetic neurons in culture.

To examine peptidergic influences on sympathetic, noradrenergic function, we studied the effects of SP and SS on the catecholamine-synthesizing enzyme tyrosine hydroxylase (TOH) in the rat sympathetic superior cervical ganglion (SCG) in vitro. TOH, the rate-limiting enzyme in catecholamine synthesis (*6*), is localized in the principal neurons of sympathetic ganglia, and is a convenient index of noradrenergic function. Two separate tissue culture techniques were used, SCG organ transplants and dissociated cell cultures of the SCG.

First we found that cultures rapidly metabolized exogenous peptide introduced into the medium. However, addition of a mixture of bacitracin and captopril, potent peptidase inhibitors, reduced this loss to less than 10 percent of added SP and 35 percent of added SS after 24 hours. Consequently, peptidase inhibitors were routinely added to the culture medium. The medium was also changed every other day to maintain the original peptide concentrations.

Addition of bacitracin and captopril to the medium significantly increased TOH activity both in ganglion explants (33 percent increase) and in dissociated cell cultures after 1 week (29 percent increase) (Fig. 1A). Moreover, addition of SP led to further significant 40 percent and 34 percent increases in explant and cell cultures, respectively; addition of SS led to 41 and 30 percent increases, respectively (Fig. 1). By contrast, the peptides had no effect on TOH activity in ganglion homogenates, excluding a direct effect on the TOH enzyme or radiochemical catalytic assay (data not shown). Consequently, SP and SS apparently increased TOH activity through processes that transpired in culture and not through direct interaction with the apo- or holoenzyme.

Dose-response relationships were examined by culturing explants in the presence of different peptide concentrations (Fig. 1B). Both SP and SS exerted maximal effects at $10^{-7}M$. Diminishing concentrations below $10^{-7}M$ reduced the stimulatory action on TOH activity, with no effect at $10^{-9}M$. Similarly, increasing concentrations above $10^{-7}M$ reduced the effect on TOH, with loss of action at $10^{-4}M$. Submaximal responses at concentrations above $10^{-7}M$ may reflect a variety of effects, including receptor desensitization or down regulation, as defined for other transmitters (*7*). Although

these possibilities are made more likely by the demonstration of specific SP receptors (*8*), a number of ambiguities complicate such interpretations in our experiments.

First, these studies were performed in the presence of bacitracin and captopril which in themselves increased TOH activity. Addition of these peptidase inhibitors to assay tubes containing ganglion homogenates did not elevate TOH activity, excluding a direct effect on the enzyme or assay. However, bacitracin and captopril may have influenced TOH activity by a number of mechanisms including (i) prevention of degradation of peptides released by sympathetic ganglia, (ii) prevention of degradation of TOH itself, (iii) prevention of degradation of cofactors (or their enzymes) for TOH, and (iv) interaction with sympathetic neurons by a process unrelated to peptidase inhibition. The recent development of antagonists and agonists of SP actions helped us clarify those points.

The amino acid substitutions (*9*) in [pGlu^5, MePhe^8, Sar^9]SP-(5–11) (Sar-SP), an agonist of SP, protect against peptidase degradation (*10*). Consequently, we were able to examine the effects of Sar-SP in the absence of bacitracin or captopril. Sar-SP significantly increased TOH activity in explants and cell culture with maximal increases at $10^{-6}M$ (Fig. 1C). Higher concentrations had lesser effects on TOH activity, reproducing the observations with SP itself. The maximal effect for Sar-SP ($10^{-6}M$) occurred at a tenfold larger dose than for SP ($10^{-7}M$), suggesting either a difference in agonist potency or even partial metabolic degradation of Sar-SP. Regardless, it is clear that the peptide-mediated increase in TOH activity did not require bacitracin or captopril.

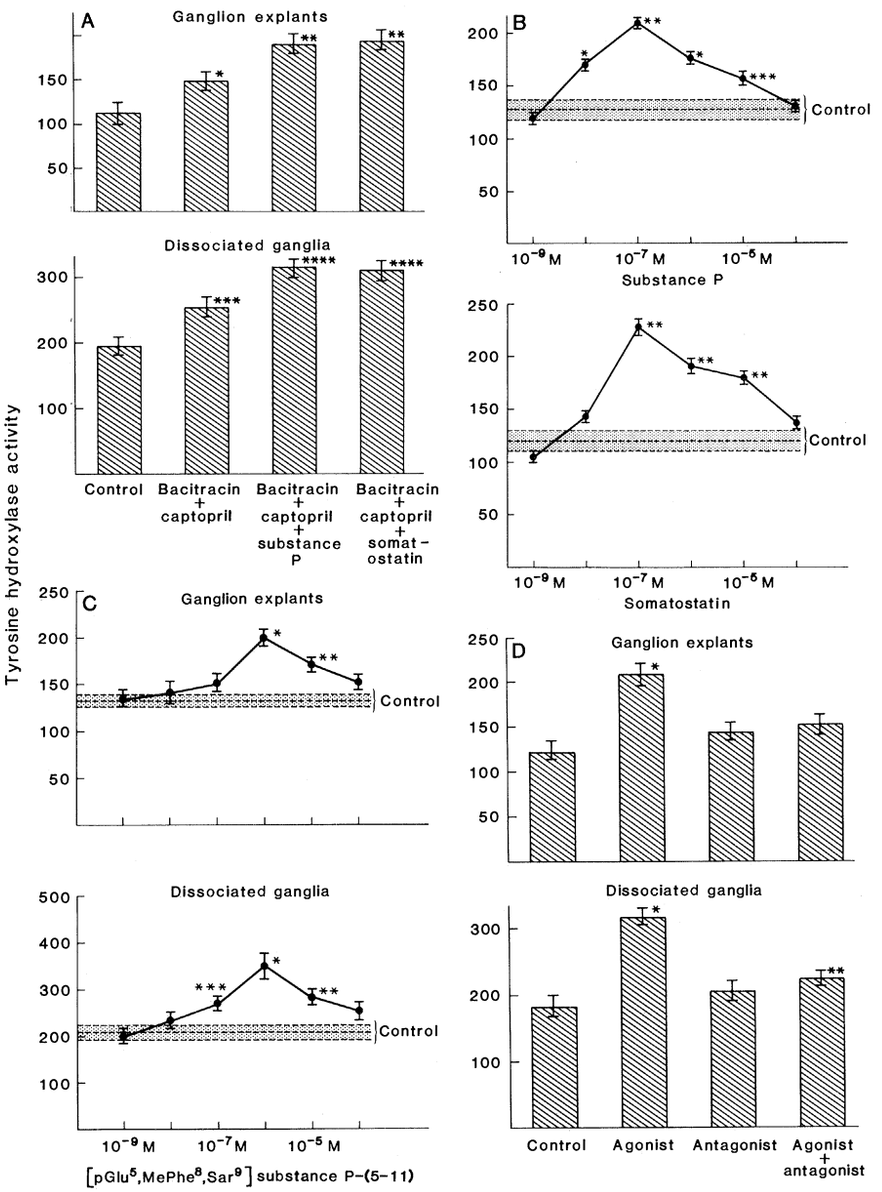

Fig. 1. (A) Effects of SP and SS on tyrosine hydroxylase in the rat superior cervical ganglion in vitro. Ganglia were removed from neonates and explanted to the bottom of collagen-coated culture dishes in a defined medium consisting of Ham's nutrient mixture F_{12} with transferrin (100 μg/ml), putrescine (100 μ*M*), insulin (5 μg/ml), progesterone (20 n*M*), selenium (30 n*M*), penicillin (50 U/ml), streptomycin (50 μg/ml), and nerve growth factor (100 ng/ml). Cultures were maintained at 37°C in an atmosphere of 95 percent air and 5 percent CO_2 at nearly 100 percent relative humidity. Ganglia were also dissociated in trypsin (0.3 percent for 30 minutes), plated at a density of 10,000 to 12,000 neurons per dish, and grown under the same conditions as the explants. After 1 week, bacitracin (2 mg/ml in explants; 0.5 mg/ml in dissociated cells), captopril (5 m*M* in explants; 1.5 m*M* in dissociated cells), SP ($10^{-7}M$), and SS ($10^{-7}M$) were added, and 1 week later cultures were examined for content of TOH. Neuron numbers were counted in the dissociated ganglia, and there were no significant differences among the groups. Tyrosine hydroxylase is expressed as mean product (picomoles) per ganglion per hour ± standard error of the mean ($N = 8$ for each group). Probabilities were as follows (unless stated otherwise), P values reflect comparisons with controls: (*) $P < 0.05$, (**) $P < 0.01$, and different from the group that received bacitracin plus captopril at $P < 0.05$, (***) $P < 0.025$, (****) $P < 0.005$, and from bacitracin plus captopril group at $P < 0.05$. (B) Effects of different doses of SP or SS. Ganglia were explanted and grown for 1 week, and then different doses of SP or SS were added to the medium. Bacitracin (2 ng/ml) and captopril (5 m*M*) were added to all groups including the control group. One week later the ganglia were examined for content of TOH, which is expressed as mean picomoles of product per ganglion per hour ± S.E.M. The shaded area represents the mean control value ± S.E.M. ($N = 10$). Probabilities: (*) $P < 0.01$, (**) $P < 0.005$, and (***) $P < 0.05$. (C) Effects of an SP analog and agonist. Ganglion explants and dissociated cells were grown for 1 week as described for (A). Different doses of [pGlu^5, MePhe^8, Sar^9]SP-(5–11) were then added to the medium, and 1 week later cultures were examined for content of TOH. Numbers of neurons in the dissociated ganglia did not differ significantly among the groups. Tyrosine hydroxylase is expressed as mean picomoles of product per ganglion per hour ± S.E.M. The shaded area represents the mean control value ± S.E.M. ($N = 10$). Probabilities: (*) $P < 0.01$, (**) $P < 0.03$, and (***) $P < 0.05$. (D) Effects of combined treatment with an SP antagonist and an agonist. Ganglion explants and dissociated cells were grown for 1 week, and were then treated with the SP agonist [pGlu^5, MePhe^8, Sar^9]SP-(5–11) ($10^{-6}M$), the SP antagonist [D-Pro^2, D-Trp^7, D-Phe^9]SP ($10^{-5}M$), or both. One week later the cultures were examined for content of TOH, which is expressed as mean picomoles of product per ganglion per hour ± S.E.M. Numbers of neurons in the dissociated ganglia did not differ significantly among the groups ($N = 10$). Probabilities: (*) Different from all other groups at $P < 0.01$ and (**) $P < 0.05$.

To determine whether the increase resulted from peptide interaction with a specific SP receptor, we used a specific receptor antagonist [D-Pro^2, D-Phe^7, D-Trp^9]-SP (Phe-SP) (*11*). The SP agonist (Sar-SP) increased ganglion TOH by approximately 70 percent in both explants and dissociated cell cultures (Fig. 1D), reproducing the effects of authentic SP. Addition of Phe-SP blocked the effect of Sar-SP, suggesting that elevation of TOH activity by Sar-SP is mediated by interaction with specific SP receptors. Addition of antagonist alone had no significant effect.

We do not know whether the increased TOH activity represented an increased number of enzyme molecules or activation of a preexistent enzyme. Nevertheless, our studies suggest that SP and SS may regulate noradrenergic metabolism, possibly through the mediation of SP receptors and the slow, noncholinergic EPSP. In the rat SCG, where SP (*12*, *13*) and SS (*3*) are localized primarily within the noradrenergic sympathetic neurons and in intraganglionic collateral processes (*14*, *15*), these peptides may subserve a feedback autoregulatory mechanism. By contrast, in the guinea pig inferior mesenteric ganglion where SP is localized in sensory fibers (*1*, *12*), sensory peptide may influence autonomic neurons, thus allowing environmental stimuli to regulate sympathetic noradrenergic function.

Our finding that peptide interactions with sympathetic neurons may modulate autonomic functions suggests that pharmacologic manipulation of peptide systems may offer a novel therapeutic approach to disorders involving autonomic neurons, such as hypertension. In a different perspective, the changes in TOH activity in sympathetic neurons in vitro provide a convenient and quantitative biochemical assay for peptidergic agonist or antagonist activity. Our observations also reinforce the concept that neurotransmitter interactions play an integral role in the maintenance and regulation of neurotransmitter phenotypic characters. Analogous transmitter interactions are well documented in the adrenal gland and elsewhere in the nervous system (*13*, *16*). Consequently, neurotransmitter phenotypic expression is a dynamic process that reflects the physiologic state and milieu of the neuron.

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27 January 1983; revised 4 April 1983

Antibodies to Human T-Cell Leukemia Virus Membrane Antigens (HTLV-MA) in Hemophiliacs

Abstract. *Along with homosexual men, Haitians, and intravenous drug abusers, hemophiliacs are at high risk of contracting acquired immunodeficiency syndrome (AIDS). An earlier study revealed that 36 percent of a group of the AIDS patients had antibodies to cell membrane antigens associated with the human T-cell leukemia virus (HTLV-MA), whereas only 1.2 percent of matched asymptomatic homosexual controls had these antibodies. In the present experiments, serum samples from 172 asymptomatic hemophiliacs were examined for the presence of antibodies to HTLV-MA. Such antibodies were detected in 5 to 19 percent of the hemophiliacs examined from four geographical locations, but in only 1 percent or less of laboratory workers, normal blood donors, donors on hemodialysis, or donors with chronic active hepatitis.*

Hemophiliac patients, most of whom receive infusions of blood clotting preparations, become exposed to products from thousands of blood donors. Such hemophiliacs may also have abnormal T-lymphocyte profiles (*1*). Along with homosexual men with multiple partners, Haitians, and intravenous drug abusers, hemophiliacs represent one of the major risk groups for developing the acquired immunodeficiency syndrome (AIDS) (*2*).

Most researchers suspect that AIDS may be caused, at least in part, by an infectious agent (*3*). Such an agent would presumably be transmitted only with great difficulty, and in the case of intravenous drug abusers and hemophiliacs it could perhaps be transmitted by blood or blood products. Among the many candidate etiologic agents that have received research attention is the human T-cell leukemia virus (HTLV) (*4*). We recently reported that AIDS patients have substantially increased rates of exposure to HTLV; this conclusion was based on our finding, by means of indirect membrane immunofluorescence (IMI) and radioimmunoprecipitation (RIP), an antibody to HTLV-infected cells in the blood of AIDS patients (*5*). Independently, Gallo and co-workers reported that HTLV had been isolated from one AIDS patient and proviral sequences had been found in two (*6*).

Using the same procedures that we described earlier (*5*), we examined serum samples from 172 hemophiliacs that were asymptomatic, two hemophiliacs with AIDS, and one with severe lymphadenopathy. Of the 172 asymptomatic hemophiliacs, 45 were from Atlanta, Georgia; 41 were from Birmingham, Alabama; 39 were from Los Angeles, California; and 47 were from New York City (see Table 1). Only one sample was examined from each individual. Except in the case of the New York hemophiliacs and the control blood donors, all the serum samples were collected in late 1982 or early 1983. Thirty-nine of the samples from New York hemophiliacs were collected in 1978 and 1979; the remaining eight samples were collected in 1976, 1977, or 1981. Also examined were serum samples from 47 healthy workers at the hospitals or laboratories where HTLV-producer cell cultures or tissue samples from patients were handled (the Harvard School of Public Health Laboratory, the Centers for Disease Control, the University of Buffalo, and the University of Alabama Medical