T cell lymphoma/leukemia in the United States, was reactive with serum from Japanese ATL patients (*13, 14*). Furthermore, patients with a form of ATL indistinguishable from Japanese ATL have been found in the West Indies. The serum of these patients is uniformly positive for HTLV antibodies (*15*). These observations indicate that HTLV is either identical to or closely related to ATLV and that the virus has a worldwide distribution.

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16. We thank H. Kanzaki for supplying umbilical cord blood specimens and K. Sugamura for helpful comments. Supported by grants-in-aid for cancer research from the Ministry of Education, Science, and Culture and the Ministry of Health and Welfare, Japan.

9 February 1982; revised 18 May 1982

Substance P: A Putative Sensory Transmitter in Mammalian Autonomic Ganglia

Abstract. *Repetitive presynaptic stimulation elicited slow membrane depolarization in neurons of inferior mesenteric ganglia from guinea pigs. This response was not blocked by cholinergic antagonists but was specifically and reversibly inhibited by a substance P analog, (D-Pro^2, D-Phe^7,D-Trp^9)-substance P, which also depressed the depolarization induced by exogenously applied substance P. The atropine-sensitive slow excitatory and slow inhibitory postsynaptic potentials evoked in neurons of rabbit superior cervical ganglia were not affected by the substance P analog. These and previous results provide strong support for the hypothesis that substance P or a closely related peptide is the transmitter mediating the slow depolarization. The latter may represent a sensory input from the gastrointestinal tract to neurons of the prevertebral ganglia.*

Synaptic transmission in autonomic ganglia involves several postsynaptic potentials with opposing polarities and time courses that range from a few milliseconds to several minutes (*1*). Acetylcholine, the classical transmitter in autonomic ganglia, acts on postsynaptic nicotinic and muscarinic receptors to give rise to fast and slow excitatory postsynaptic potentials (EPSP's), respectively. In a number of autonomic ganglia presynaptic stimulation evokes, in addition to cholinergic fast and slow excitatory potentials, a third excitatory potential that lasts for seconds or minutes. Since this slow depolarization is not blocked by cholinergic antagonists, it has been proposed that it is not mediated by acetylcholine (*2*).

Immunohistofluorescence, ultrastructural, biochemical, and electrophysiological studies (*3–8*) have implicated substance P (SP), an undecapeptide, as the transmitter mediating the slow, noncholinergic depolarization evoked in neurons of the guinea pig inferior mesenteric ganglia. Positive identification of SP as the transmitter in these ganglia has not been possible because of a lack of specific SP antagonists. Recently, several SP analogs have been developed that are antagonistic to SP in various tissues (*9*). We report here that SP analog (D-Pro^2,D-Phe^7,D-Trp^9)-SP (Pro, proline; Phe, phenylalanine; Trp, tryptophan), a differential antagonist of the effects of SP on smooth muscle (*9*), specifically and reversibly depresses noncholinergic transmissions in guinea pig inferior mesenteric ganglia.

Intracellular recordings were obtained from neurons of guinea pig inferior mesenteric ganglia and rabbit superior cervical ganglia (*5, 6, 10*). The ganglia were superfused with Krebs solution (*10*) to which appropriate concentrations of SP, SP analog, or other drugs were added.

Repetitive stimulation (30 Hz, 2 to 4 seconds) of hypogastric nerves elicited bursts of action potentials in the inferior mesenteric ganglia neurons. Most of these cells then underwent a slow depolarization (the noncholinergic depolarization). In some instances a prolonged hyperpolarization occurred subsequently (Fig. 1) (*6*). When applied in concentrations of 1 to 10 μM, the SP analog did not change the resting membrane potential or input resistance in 16 of 23 neurons tested (Fig. 1). In the remaining seven neurons, the analog caused a slow depolarization of several millivolts, accompanied in some instances by an increase in membrane resistance. The membrane potential generally returned to near the control level after continuous superfusion with SP analog for several minutes (Fig. 2).

The SP analog caused no measurable change in the amplitude of the initial fast EPSP's, whereas it reversibly depressed—in 13 of the 17 cells tested—the noncholinergic depolarization evoked by nerve stimulation. No appreciable change in the noncholinergic response was observed in the remaining four neurons. The depression of the depolarization induced by SP analog developed slowly; usually it reached a maximum 3 to 5 minutes after application of the substance was discontinued (Fig. 1). The amplitude of the depolarization gradually returned to the control level after the preparation was washed with Krebs solution for 15 to 30 minutes (Fig. 1).

The SP analog's depressant effect was related to its concentration. At concentrations of 1 and 10 μM, SP analog depressed the amplitude of the noncholinergic response 14 percent ($P < .05$, paired Student's t-test; $N = 6$) and 42 percent ($P < .01$; $N = 7$), respectively. At 50 μM, SP analog completely abolished the depolarization in less than 5 minutes in two cells tested; the effect was reversed after a 30-minute wash with Krebs solution. An initial augmentation of the depolarization was observed in 4 of the 13 neurons to which SP analog was applied; the enhancement was generally small (< 10 percent), and it was consistently followed by a much larger depression of the response. The prolonged hyperpolarization that follows the noncholinergic depolarization continued to be present when the application of SP analog attenuated or blocked the depolarization (Fig. 1).

Next we determined the effects of SP analog on membrane depolarization induced by exogenously applied SP. Membrane depolarization was elicited by ex-

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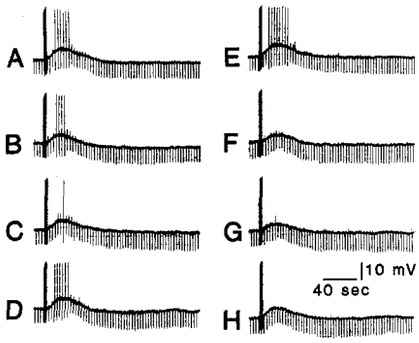

Fig. 1. Antagonistic effects of 1 and 10 μM SP analog on the noncholinergic depolarization elicited in a guinea pig inferior mesenteric ganglion cell. Repetitive stimulation (30 Hz, 4 seconds) of hypogastric nerves elicited a burst of action potentials (initial vertical bar) followed by a slow depolarization succeeded in turn by a even longer hyperpolarization. Downward deflections represent hyperpolarizing electrotonic potentials used to monitor changes in input resistance of the cell in question. (A) Control response. Several off-spikes are seen at the peak of noncholinergic depolarization. The spikes in this and subsequent recordings are attenuated because of the frequency response limitation of the pen recorder employed. (B) Response 5 minutes after the superfusion of SP analog (1 μM). The amplitude of the noncholinergic depolarization was depressed in this case by about 30 percent. (C and D) Responses 10 minutes (C) and 25 minutes (D) after washing with Krebs solution. (E) Response 60 minutes after washing with Krebs solution. (F) Response 5 minutes after superfusion with SP analog (10 μM). The noncholinergic depolarization was suppressed by about 50 percent with respect to the response shown in (E). (G) Response 5 minutes after wash. The noncholinergic depolarization remained attenuated. (H) Response 30 minutes after wash. The noncholinergic depolarization had recovered to near the control level.

ogenous application of SP (*5*). Superfusion for 3 to 5 minutes with SP analog (1 to 10 μM) effectively suppressed the depolarization in five of the eight ganglion cells investigated. A large membrane depolarization evoked by 1 μM SP was accompanied by intense neuronal discharges; after the membrane potential had returned to near the resting level, application of SP analog (10 μM) caused a slow depolarization in this neuron which gradually subsided in the continuous presence of the analog (Fig. 2). At the end of a 5-minute superfusion with SP analog, application of SP elicited only a small depolarization; a large depolarization could again be elicited by SP after a 20-minute wash with Krebs solution.

Luteinizing hormone–releasing factor (LRF), a peptide that has been implicated as the transmitter mediating the noncholinergic transmission in bullfrog sympathetic ganglia (*11*), caused membrane depolarization in neurons of the guinea pig inferior mesenteric ganglia (*5*). The depolarizing effect was not appreciably affected by SP analog.

We further determined the specificity of the action of SP analog by evaluating its effect on slow EPSP's and slow inhibitory postsynaptic potentials (IPSP's) in neurons of rabbit superior cervical ganglia. These potentials are known to be sensitive to atropine, a muscarinic antagonist (*1*). In these experiments *d*-tubocurarine (50 μM) was used to suppress the initial fast EPSP.

Repetitive stimulation (10 to 20 Hz, 1 to 3 seconds) of the cervical sympathetic nerve trunk elicited a monophasic slow EPSP in eight neurons (cell 1 in Fig. 3); in two other neurons this stimulation caused a biphasic response consisting of an initial slow IPSP followed by a slow EPSP (cell 2). Application of the SP analog (10 μM) caused no significant change in resting membrane potential or input resistance in any of the cells. The amplitude of the slow EPSP's was not measurably altered by the SP analog in five of eight cells (cell 1) and was reduced by less than 10 percent in the other three. In the two cells in which repetitive stimulation elicited a biphasic response, both the slow IPSP's and slow EPSP's were not measurably affected by

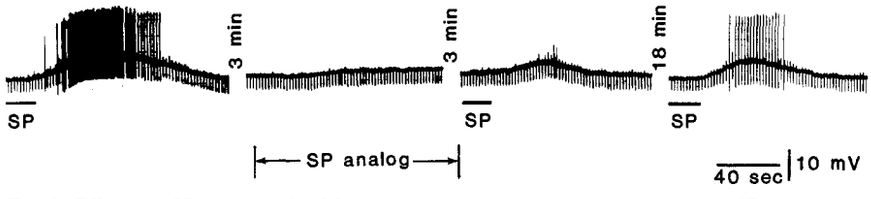

Fig. 2. Effects of SP analog (10 μM) on membrane depolarization induced by SP (1 μM) in a guinea pig inferior mesenteric ganglion cell. The tracings constitute a continuous recording interrupted at intervals marked in minutes. Downward deflections represent hyperpolarizing electrotonic potentials. Application of SP caused a large depolarization and repetitive neuronal discharges. The analog, applied for 5 minutes, caused a slow membrane depolarization. In the continuous presence of the analog the membrane depolarization gradually subsided and returned to near the resting level. At the end of the 5-minute application the response to superfusion of SP was reduced by approximately 50 percent. After 18 minutes of washing with Krebs solution, SP again caused a large depolarization. SP was applied for 20 seconds as indicated by the horizontal bars.

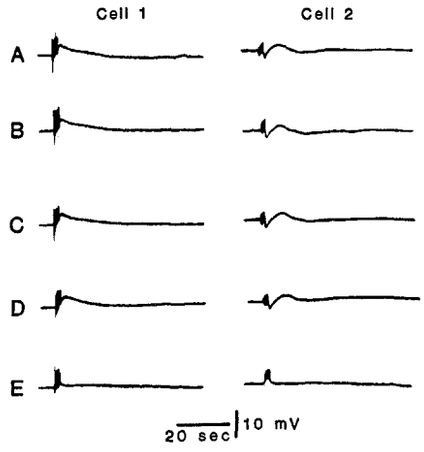

Fig. 3. Effects of SP analog (10 μM) and atropine (1 μM) on slow membrane potentials elicited from two rabbit superior cervical ganglion cells. D-Tubocurarine (50 μM) was present in the perfusing Krebs solution to suppress the initial fast EPSP (initial dark tracings present in all the records). In cell 1 repetitive stimulation (30 Hz, 2 seconds) elicited only a monophasic, slow depolarization (slow EPSP), whereas in cell 2 repetitive stimulation evoked an initial hyperpolarization (slow IPSP) followed by a depolarization (slow EPSP). (A) Control responses; (B) 5 minutes after superfusion with SP analog; (C) 5 minutes after washing; (D) 10 minutes after washing; (E) 5 minutes after exposure to atropine. Addition of SP analog did not affect the slow responses of the cells, whereas atropine readily and completely eliminated the slow responses.

the analog (cell 2). The slow potentials recorded from all these cells were abolished by atropine (Fig. 3E).

These experiments demonstrate that the noncholinergic depolarization elicited in neurons of guinea pig inferior mesenteric ganglia can be reversibly depressed by an SP analog. It was previously reported that the SP analog employed in this study is a specific SP antagonist since it abolishes the stimulating action of SP, but not of histamine or acetylcholine, on ileum smooth muscle and since it inhibits SP-induced, but not acetylcholine-induced, vasodilation in dental pulp (*9*). Antagonism of the noncholinergic transmission by the SP analog appears to be specific because (i) the depolarization induced by SP but not by LRF was antagonized by the analog, (ii) the three cholinergic ganglionic potentials were not affected by the analog, and (iii) the depressant effect of this compound on the noncholinergic depolarization was concentration-dependent. The compound in question may be a partial agonist since it caused in some neurons a slow depolarization similar to that induced by SP (*5*) and since in a few cells the noncholinergic response was initially augmented. This partial agonistic action

of the SP analog is consistent with specificity of its action on the SP receptor. In this context, it is pertinent to point out that another SP analog, (D-Pro^2,D-Trp^{7,9})-SP, has a partial agonistic effect on taenia coli isolated from guinea pigs (*12*).

The finding that the noncholinergic transmissions of cells of guinea pig inferior mesenteric ganglia are specifically inhibited by an SP analog should be considered in the light of the observations that nerve fibers of these ganglia exhibit SP immunoreactivity (*3*), that SP immunoreactivity can be released by solutions high in potassium in a calcium-dependent manner (*7*), and that the membrane depolarization induced by exogenous SP resembles the noncholinergic depolarization elicited synaptically (*5, 6*). All this evidence strongly supports the hypothesis that SP or a closely related peptide is the transmitter responsible for the generation of the noncholinergic depolarization. Furthermore, the demonstration that SP-containing fibers in the inferior mesenteric ganglia originate from sensory neurons in the dorsal root ganglia (*4, 7, 8*) raises the possibility that SP is involved in the transmission of sensory signals from the gastrointestinal tract to the spinal cord as well as to the prevertebral ganglia (*3*); this provides a mechanism for a peripheral reflex regulation of the gastrointestinal activity. Finally, the evidence indicating that SP acts as a sensory transmitter in autonomic ganglia is consistent with its proposed function as a transmitter released from primary afferent fibers in the spinal cord (*13*).

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10. Inferior mesenteric ganglia with their left and right hypogastric nerves and superior cervical ganglia with their cervical sympathetic trunks were rapidly removed from male guinea pigs and rabbits, respectively. The ganglia were continuously superfused with a Krebs solution of the following composition (m*M*): NaCl, 117; KCl, 4.6; $CaCl_2$, 2.5; $MgCl_2$, 1.2; $NaHCO_3$, 25; NaH_2PO_4, 1.2; and glucose, 11.5. The solution was equilibrated with 95 percent O_2 and 5 percent CO_2 and maintained at 35° to 36°C. Microelectrodes filled with 3*M* KCl and having a tip resistance of 30 to 50 megohms were used for intracellular recording and stimulating. Presynaptic stimulation was accomplished by the suction electrode method, in which the hypogastric nerves or the cervical sympathetic trunks were drawn into a small glass pipette for orthodromic stimulation. The potential changes were recorded on a Tektronix oscilloscope and on a Gould Brush pen recorder (model 2200). The figures were reproduced from the tracings of the pen recorder. The peptides, substance P, (D-Pro^2,D-Phe^7,D-Trp^9)-SP and LRF were purchased from Peninsula Laboratories.
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16 February 1982; revised 26 April 1982

Rabbit Hepatic Progesterone 21-Hydroxylase Exhibits a Bimodal Distribution of Activity

Abstract. *Progesterone 21-hydroxylase activity varies extensively among liver microsomes prepared from individual New Zealand White (NZW) rabbits. The 21-hydroxylase activities are distributed between two groupings that differ by more than tenfold in mean activity. Both male and female animals are represented in the two groupings. However, females exhibited the higher activity more frequently than males. The 21-hydroxylation of progesterone is catalyzed by one of the liver microsomal cytochrome P-450 isozymes, form 1, and these differences in activity are suggestive of differences in the occurrence of this isozyme among NZW rabbits.*

The product of the 21-hydroxylation of progesterone is deoxycorticosterone (DOC), a hormone that affects sodium retention. This reaction is catalyzed by a microsomal form of cytochrome P-450 which occurs most prominently in the adrenal cortex where DOC serves as a precursor for the formation of other adrenal steroid hormones (*1*). Plasma concentrations of DOC increase in humans during pregnancy. However, this has been attributed to the extra-adrenal formation of DOC from circulating progesterone, a hormone that is secreted in large amounts during the latter part of pregnancy (*2, 3*). We have recently identified a specific form of rabbit liver microsomal cytochrome P-450, form 1, that catalyzes this reaction (*4*). Form 1 is one of several forms of cytochrome P-450 that metabolize a wide variety of endogenous and exogenous compounds including drugs and carcinogens as well as steroid hormones (*5*). Many factors are known to alter the expression of the multiple forms of cytochrome P-450 and thereby alter the metabolism of drugs, carcinogens, and steroid hormones (*6*). In the case of progesterone, differences in the expression of cytochrome P-450 form 1 could affect the extent of DOC formation from progesterone. Since DOC can affect sodium retention, increases in the production of DOC from progesterone in the liver could exacerbate hypertensive conditions that arise frequently during pregnancy. In this report, we describe extensive differences in the initial rate of hepatic microsomal 21-hydroxylation among New Zealand White rabbits. These variations are suggestive of differences in the occurrence of cytochrome P-450 form 1 among these animals.

For this study, New Zealand White rabbits (12 males and 11 virgin females, 3 to 4 kg) were purchased from commercial rabbitries in the San Diego area. The animals were given continuous access to food and water. The food was then withdrawn, and after a 24-hour fast the animals were killed by intravenous administration of lethal doses of sodium pentobarbital. Microsomes were prepared (*7*), suspended in 50 m*M* potassium phosphate, *p*H 7.4, containing 20 percent glycerol, and stored at −60°C. Protein and cytochrome P-450 concentrations were determined as described by Bensadoun and Weinstein (*8*) and by Omura and Sato (*9*), respectively.

Each 1.0-ml reaction mixture con-

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