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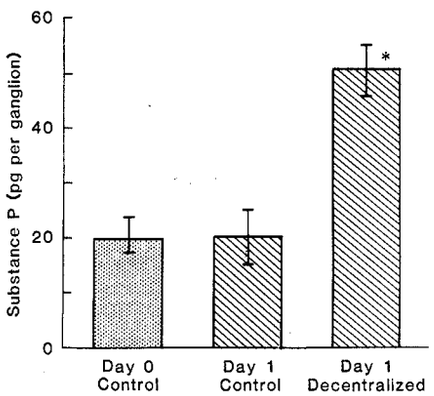
Substance P in Principal Sympathetic Neurons: Regulation by Impulse Activity

Abstract. *Regulation of the putative peptide neurotransmitter substance P was examined in the superior cervical sympathetic ganglion of the neonatal rat. Surgical decentralization (denervation) of the superior cervical ganglion increased ganglion substance P content. In cultured ganglia, the amount of substance P increased more than 50-fold after 48 hours, and this rise was dependent on protein and RNA synthesis. Veratridine prevented the increase in substance P in vitro, and tetrodotoxin blocked the veratridine effect; this suggests that sodium influx and membrane depolarization prevent substance P elevation. Immunohistochemical analysis of cultured ganglia indicated that substance P was present in the perikarya of principal sympathetic neurons and in ganglionic nerve processes. Transsynaptic impulses, through the mediation of postsynaptic sodium influx, may decrease substance P in sympathetic neurons.*

Traditional concepts of neuronal specificity and brain function have been dramatically altered by recent work suggesting that peptides act as neurotransmitters (*1*, *2*). It is now apparent that peptidergic neurons are distributed throughout the neuraxis and that peptides and well-recognized transmitters, such as norepinephrine, may coexist in the same neurons (*2*). In order to examine the functional implications of these observations, we studied peptidergic expression and metabolism in a relatively simple neuronal structure, the rat superior cervical sympathetic ganglion (SCG).

Traditional teaching maintains that sympathetic ganglion neurons use only norepinephrine or acetylcholine as transmitters and that nerves innervating ganglia are cholinergic (*3*). However, the recent demonstration of putative peptide transmitters in sympathetic ganglia (*4*, *5*) suggests that the biochemical organization of the sympathetic nervous system is more complicated. For example, the undecapeptide substance P has been detected in ganglion nerve fibers (*4*), and application of substance P to sympathetic neurons elicits membrane depolarization and neuronal discharge (*6*). Moreover, since the peptide is released from ganglia by a high potassium stimulus in a calcium-dependent manner, substance P appears to subserve a physiologic role in sympathetic ganglia (*7*).

Recently, we found that surgical decentralization (denervation) of the SCG in the adult rat, or pharmacological blockade of transmission, increased sub-



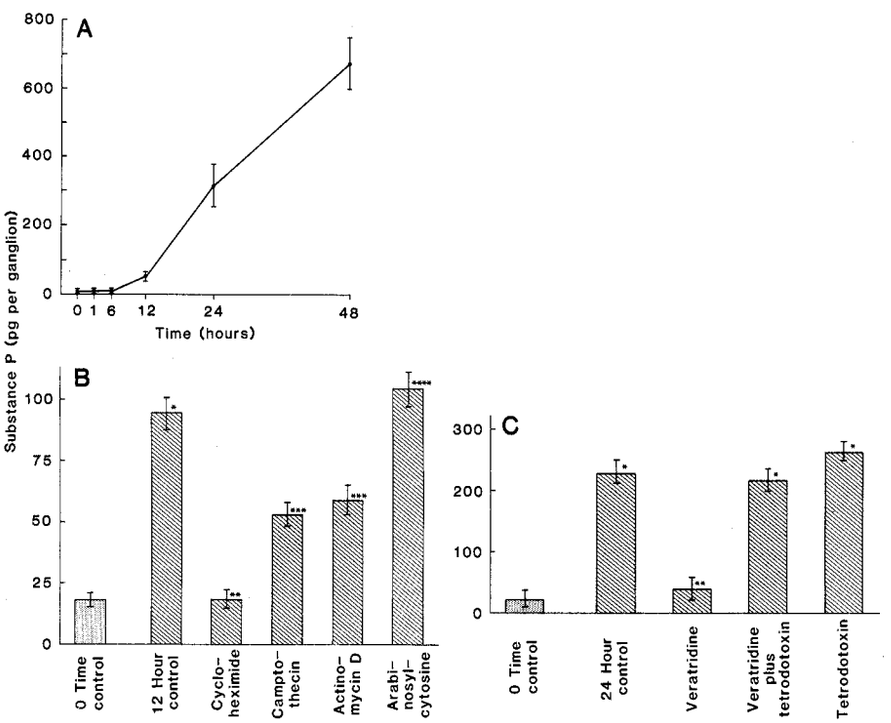


Fig. 1 (left). Effects of ganglion decentralization in the neonate. Unilateral surgical denervation of the SCG was performed on the day of birth. The denervated and contralateral control ganglia were examined 36 hours later for substance P content. Values of substance P are expressed as means ± standard error (S.E.) for eight animals. *Differs from control at $P < .001$. Fig. 2 (right). (A) Time course of ganglion substance P accumulation in culture. Ganglia were placed on filter paper rafts in Ham's nutrient mixture F12 with 10 percent fetal calf serum, penicillin (50 U/ml), and streptomycin (50 μg/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 examined after varying times in culture for substance P content. Substance P values are expressed as means ± S.E. for eight animals. (B) Effects of metabolic inhibitors. Ganglia were cultured in the presence of cycloheximide (2 μg/ml), camptothecin (2 μg/ml), actinomycin D (1 μg/ml), or arabinosylcytosine (2.4 μg/ml). After 12 hours, ganglia were examined for substance P content; $N = 8$. *Differs from 0 time control at $P < .001$. **Differs from 12 hour control at $P < .001$. ***Differs from both 0 time control and 12 hour control at $P < .002$. ****Differs from 0 time control at $P < .001$. (C) Effects of membrane depolarization. Ganglia were cultured in the presence of veratridine ($2 \times 10^{-4} M$), tetrodotoxin ($10^{-7} M$), or both. After 24 hours, ganglia were examined for substance P content; $N = 8$. *Differs from 0 time control at $P < .001$. **Differs from 24 hour control at $P < .001$.

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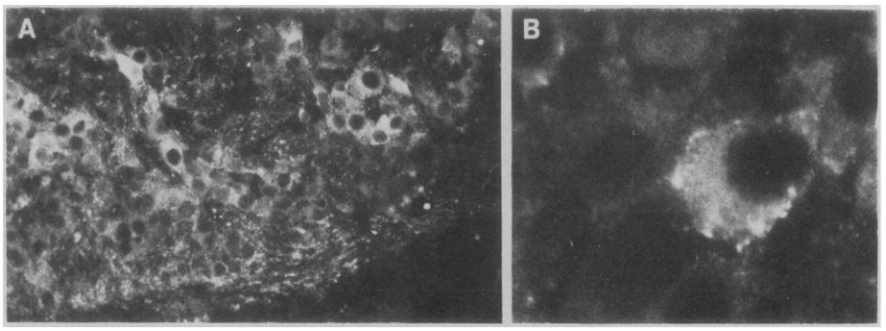

Fig. 3. Demonstration of substance P in SCG cultured for 48 hours with added nerve growth factor (100 ng/ml). Ganglia fixed in 4 percent buffered paraformaldehyde were embedded in polyethylene glycol. Sections (10 μm) were stained with rabbit antiserum to substance P at a dilution of 1:500 and processed for fluoroisothiocyanate immunofluorescence. Control ganglia incubated with serum from nonimmunized rabbits were negative. (A) Substance P immunoreactivity was observed in most perikarya and in many beaded fibers coursing through the ganglia. The intensity of staining varied, with a few cells intensely fluorescent (×200). (B) Substance P immunofluorescence had a granular appearance that filled the cytoplasm and left the nuclei clear. Apparent fluorescent boutons were observed impinging on the soma of many substance P–containing ganglion neurons (×1000).

stance P–like immunoreactivity (subsequently termed substance P) in the ganglion (*8*). Conversely, pharmacological stimulation of sympathetic activity reduced ganglion substance P (*8*). These observations suggested that presynaptic impulse activity decreases substance P through a transsynaptic process.

The inhibitory action of presynaptic activity on ganglion substance P is unusual, since impulse flow causes sympathetic activation and induces a number of noradrenergic substances, such as the biosynthetic enzymes tyrosine hydroxylase and dopamine β-hydroxylase (*9*). To further characterize this apparently paradoxical response of substance P, we performed studies in tissue culture, in which we used the SCG from neonatal rats, since the ganglion at this stage is more conveniently maintained in vitro.

In initial studies in vivo, we had found that the response of ganglion substance P in the neonate paralleled that in the adult. Thirty-six hours after SCG decentralization on day 1 of life, radioimmunoassay (*10*) revealed a 148 percent increase in substance P above that found in contralateral control ganglia (Fig. 1).

To characterize the regulation of substance P in greater detail, we explanted ganglia to organ culture dishes and cultured them for various periods in Ham's nutrient mixture F12 with 10 percent fetal calf serum (*11*). Initial studies indicated that nerve growth factor did not substantially alter the observations made in vitro, so that nerve growth factor was not routinely added to the medium. The amount of substance P increased dramatically in cultured ganglia (Fig. 2A); although the amount was unchanged for the first 6 hours in culture, it increased more than 5-fold by 12 hours, more than 20-fold by 24 hours, and then 50-fold after 48 hours.

To determine whether the striking elevation in ganglion substance P required protein, RNA, or DNA synthesis, we cultured the ganglia for 12 hours in the presence of appropriate metabolic inhibitors (Fig. 2B). Cycloheximide completely abolished the increase in substance P, an indication that protein synthesis is necessary for the increase. By contrast, arabinosylcytosine, an inhibitor of DNA synthesis, had little effect, whereas camptothecin and actinomycin D, inhibitors of RNA synthesis, partially blocked the increase in substance P. These observations suggest that protein synthesis, and to a lesser extent RNA synthesis, is required for the increase in substance P in vitro.

Since preganglionic impulse activity decreased the amount of substance P in vivo, the effects of membrane depolarization were examined in culture. Veratridine, which increases sodium influx by binding to sodium channels (*12*), prevented the rise in substance P (Fig. 2C). Addition of tetrodotoxin, which antagonizes the effects of veratridine on sodium ions (*13, 14*), blocked the actions of veratridine on substance P, but tetrodotoxin alone had no effect (Fig. 2C). The inhibitory effects of veratridine on substance P elevation thus appear to be specifically related to the increased sodium ion flux. Although it is not yet clear whether the resulting decrease in substance P reflects decreased synthesis, increased catabolism, or increased neuronal release of the peptide, our observations suggest that transsynaptic impulses, mediated by postsynaptic sodium influx, are responsible for the decrease in substance P in sympathetic neurons.

Previous studies have demonstrated rare substance P–containing fibers in the SCG (*4*), but their origin has been unclear (*4, 7, 15*). The remarkable increase of SP in cultured ganglia afforded an optimal opportunity to localize substance P by immunohistochemical methods. Examination of the ganglia after 48 hours in culture revealed striking substance P–like immunofluorescence in most of the principal neuron perikarya and in varicose fibers coursing within the ganglia (Fig. 3). Staining was also observed in boutons apposed to substance P–containing perikarya.

Our studies suggest that the apparent putative transmitter of a neuron, as revealed by immunohistochemical analysis, may depend on the functional state of the neuron. Normal changes in physiological stimuli may qualitatively alter neurotransmitter phenotype or alternatively, may transform immunologically unreactive molecules, such as precursors, into immunoreactive peptide. Negative immunohistochemical data may simply reflect unfavorable physiological conditions. Thus, neurotransmitter expression and metabolism may represent a dynamic, changing process that reflects the physiologic state of the neuron.

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