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Similarities in development of substance P and somatostatin in peripheral sensory neurons: Effects of capsaicin and nerve growth factor

(dorsal root ganglion)

JOHN A. KESSLER AND IRA B. BLACK

Cornell University Medical College, Department of Neurology, Division of Developmental Neurology, 515 East 71st Street, New York, New York 10021

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ABSTRACT **Development of the two putative peptide neurotransmitters, substance P (SP) and somatostatin (SS), were compared in rat dorsal root ganglion (DRG) and spinal cord *in vivo*. The content of SS in the sixth cervical DRG increased 5-fold during the first 5 weeks of life, rising from 24 pg per ganglion at birth. SP content increased 4.5-fold during the first 5 weeks, from 56 pg per ganglion at birth. The developmental profiles for these two peptides were virtually parallel, suggesting that their respective neuronal populations developed in synchrony. Treatment with nerve growth factor (NGF) significantly increased the content of both SP and SS in the DRG and dorsal spinal cord. Conversely, treatment with capsaicin significantly decreased both SP and SS in the DRG and dorsal spinal cord. Consequently, experiments involving NGF or capsaicin treatment of sensory neurons must be interpreted with extreme care, because specificity is not limited to a single peptide phenotype. Although the mechanisms of action of NGF and capsaicin on SP and SS have not been defined, the similarity of the responses of the two peptides suggests that their development may be regulated by similar processes.**

Although the generation of neuronal specificity underlies normal organization and function of the nervous system, the individual traits that segregate neurons into common systems or families are largely unknown. The expression and development of neurotransmitter phenotypic characters represents one aspect of specificity that is relatively well defined, allowing detailed examination of neuronal subpopulations. In previous studies, we have examined the regulation of cholinergic and catecholaminergic development (for review see ref. 1), and more recently we have described factors that influence peptidergic maturation (2, 3). We have been particularly interested in examining factors that influence the development of neurons expressing widely divergent transmitter phenotypes.

The normal ontogeny of substance P-like immunoreactivity (henceforth termed SP) in the dorsal root ganglion (DRG) is dependent on the peripheral field of innervation (2, 3), mimicking target regulation of noradrenergic (1, 4) and cholinergic maturation (5–7). Moreover, the target–peptidergic interaction may be mediated by the trophic protein nerve growth factor (NGF), because NGF treatment prevents the inhibitory effects of target extirpation on SP development (2, 3). It is presently unclear, however, whether these regulatory processes are specific for the SP phenotype, or whether other peptidergic DRG neurons (and characters) are similarly regulated.

DRG neurons contain at least five putative peptide transmitters, which apparently reside in separate but comingling populations of type B neurons *in vivo* (8, 9). Of these peptides, the best characterized and studied are SP and somatostatin (SS). Like SP, SS in the DRG is highly localized to neuronal perikarya (9) and their projections to the substantia gelatinosa of the spinal cord (10). While SP appears to mediate transmission of nociceptive stimuli (11–13), the precise physiologic function of SS has not been defined. It appears, however, that SS exerts an inhibitory influence in the spinal cord, whereas SP is excitatory (14).

We have examined the development of these two neuropeptides in the neonatal rat DRG and spinal cord to compare the mechanisms regulating their respective neuronal populations. Our underlying goal was to determine whether the development of sensory neurons that express different putative transmitters is influenced by similar factors. This study demonstrates that SP and SS-like immunoreactivity develop synchronously in the DRG and spinal cord. Moreover, NGF treatment resulted in similar increases in SP and SS, whereas capsaicin administration caused similar decreases in SP and SS development. We conclude that the effects of capsaicin and NGF on sensory ganglia are not specific for a single peptide phenotype.

MATERIALS AND METHODS

Experimental Animals. Pregnant Sprague–Dawley rats (Charles River Breeding Laboratories) were housed in clear plastic and wire cages and were exposed to 540–810 lux of cool-white fluorescent illumination between 5 a.m. and 7 p.m. daily. Ralston Purina Lab Chow and water were offered ad lib. Litter sizes were adjusted when necessary so that each litter had between 12 and 14 pups.

Surgical Procedures. *Dissection of the DRG.* Ganglia were dissected as described (2). The sixth cervical (C_6) ganglia were removed for study.

Dissection of the spinal cord. The spinal cord was dissected as described (3) for separate examination of dorsal and ventral areas of cervical spinal cord.

Radioimmunoassays for SP and SS. SP-like immunoreactivity (henceforth termed SP) and SS-like immunoreactivity (henceforth termed SS) were measured by radioimmunoassay. Details of the SP (2, 3) and SS (15) assays have been described.

Preparation of NGF. β-NGF was prepared from adult male mouse salivary glands by the method of Mobley *et al.* (16).

Capsaicin Treatment. Capsaicin solutions were prepared according to Jansco *et al.* (17). Neonates were injected on day 1 to day 5 of life with capsaicin (18) at 50, 100, 200, 200, and 400 mg/kg. When necessary, respiration was assisted manually for up to 5 min after injection of 1-day-old rats. Controls were injected with equal volumes of the solvent.

Protein Determination. Total soluble protein was measured by the method of Lowry *et al.* (19).

Statistics. Data were analyzed by Student's *t* test.

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Abbreviations: SP, substance P; SS, somatostatin; DRG, dorsal root ganglion; C_6, sixth cervical; NGF, nerve growth factor.

RESULTS

Development of SS and SP in the DRG. To define the normal developmental profiles for SS and SP, the C_6 ganglia from rats of different ages were examined (Fig. 1). SP increased over a 4.5-fold range during the first 5 weeks of life, rising from a mean ± SEM of 56 ± 8 pg per ganglion at birth. SS, by contrast, increased over a 6-fold range during the first 5 weeks, rising from 24 ± 5 pg per ganglion at birth. The major increase in ganglion SP occurred during the first 12 days, whereas SS increased relatively uniformly over the initial 5 weeks of life. Both peptides tended to plateau at approximately 5 weeks, with only a small rise thereafter. In contrast, total ganglion protein increased less than 3-fold during the first 5 postnatal weeks, resulting in a 50% rise in specific SP content and almost a 100% increase in specific SS content.

Effects of NGF Treatment. NGF treatment stimulates development of SP in the neonatal rat DRG (2) and in the dorsal spinal cord to which the DRG projects (3). To determine whether somatostatin development is also stimulated by NGF administration, neonates were treated with the factor, and C_6 ganglion and spinal cord SP (Fig. 2 *Upper*) and SS (Fig. 2 *Lower*) were assayed 2 days later. NGF treatment resulted in a dramatic rise in DRG SP to 155% of control values (Fig. 2 *Upper*). Treatment resulted in a similar increase in SP in the dorsal spinal cord (157%), but not in the ventral spinal cord (Fig. 2 *Upper*). NGF administration produced strikingly similar changes in the development of SS. SS content increased to 198% in the DRG and 149% in the dorsal spinal cord (Fig. 2 *Lower*). NGF failed to alter SS in the ventral cord, reproducing effects observed with SP. Consequently, NGF increased both SP and SS in the neonatal DRG and selectively in the dorsal spinal cord, to which the DRG projects.

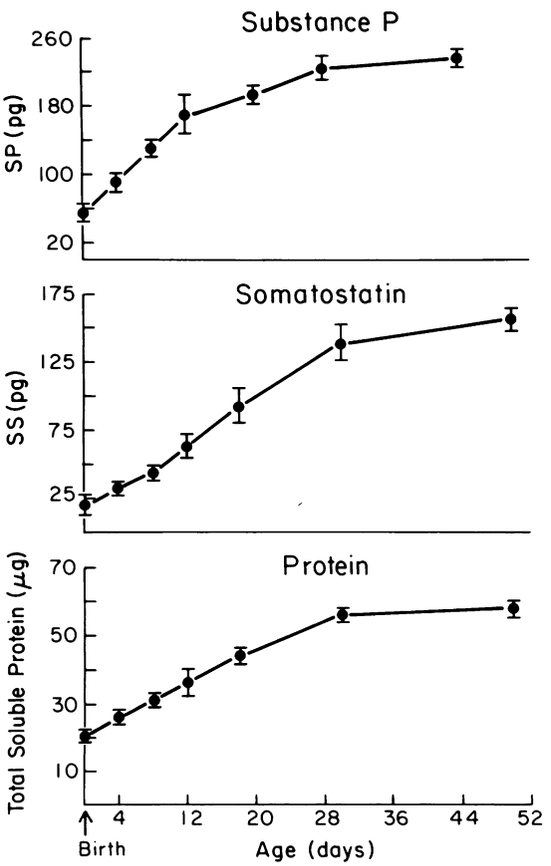

FIG. 1. Postnatal development of SP, SS, and total protein in the DRG. The C_6DRG was removed from animals at different ages and examined for SP and SS content and total soluble protein. Each point represents eight animals. SP and SS are expressed as mean pg per ganglion (±SEM); protein is expressed as mean μg per ganglion (±SEM).

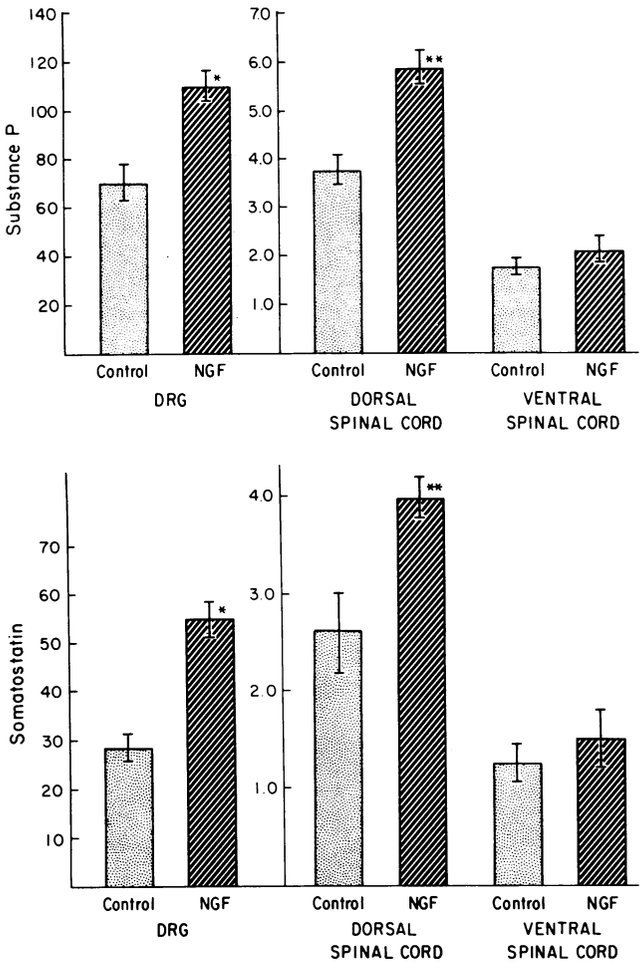

FIG. 2. Effects of NGF on SP (*Upper*) and SS (*Lower*) in the C_6DRG and spinal cord. Neonates were injected subcutaneously with 100 μl of either NGF (10^4 units) or control buffer. For each group, $n = 8$. Two days later the C_6DRGs and spinal cords were examined for SP content. (*Upper*) Ganglion SP is expressed as mean pg per ganglion, ±SEM; spinal cord SP is expressed as mean ng per mg of protein, ±SEM. *, Differs from respective control at $P < 0.001$. **, Differs from respective control at $P < 0.005$. (*Lower*) Ganglion SS is expressed as mean pg per ganglion, ±SEM; spinal cord SS is expressed as mean ng per mg of protein, ±SEM. *, Differs from respective control at $P < 0.001$. **, Differs from respective control at $P < 0.05$.

Effects of Capsaicin Treatment. Capsaicin administration depletes SP in the dorsal spinal cord and dorsal nerve roots of neonatal (20, 21) and adult (18) rats. To determine whether this effect is specific for SP, neonates were treated with capsaicin, and both SP (Fig. 3 *Upper*) and SS (Fig. 3 *Lower*) were assayed in the DRG and spinal cord. Capsaicin administration decreased SP content in the DRG to 50%, while SP in the dorsal spinal cord was reduced to 42% of normal (Fig. 3 *Upper*). In contrast, SP content in the ventral spinal cord was not significantly altered after treatment.

The responses of SS to capsaicin administration paralleled those of SP. After capsaicin, SS contents in the DRG and dorsal spinal cord were significantly reduced to 66% and 53% of controls (Fig. 3 *Lower*). Capsaicin treatment lowered SS in the

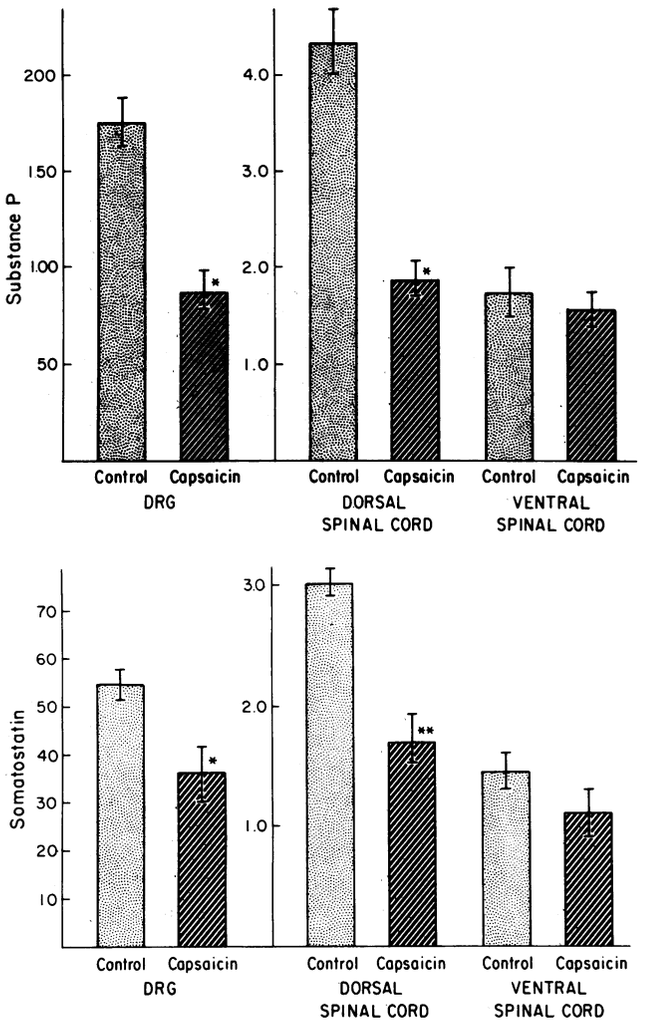

FIG. 3. Effects of capsaicin on SP (*Upper*) and SS (*Lower*) in the C_6DRG and spinal cord. Neonates were injected with capsaicin for 5 days (50, 100, 200, 200, 400 mg/kg of body weight) or control buffer. For each group, $n = 8$. On day 10 the C_6 dorsal root ganglia and spinal cords were examined for SP and SS content. (*Upper*) Ganglion SP is expressed as mean pg per ganglion, ±SEM; spinal cord SP is expressed as mean ng per mg of protein, ±SEM. *, Differs from respective control at $P < 0.001$. (*Lower*) Ganglion SS is expressed as mean pg per ganglion, ±SEM; spinal cord SS is expressed as mean ng per mg of protein, ±SEM. *, Differs from respective control at $P < 0.025$. **, Differs from respective control at $P < 0.005$.

ventral spinal cord by 24%, but this decrease was not statistically significant. The magnitude of the capsaicin-induced changes in SS in the DRG was highly reproducible in multiple experiments. Decreases in SS in the dorsal spinal cord were somewhat more variable among experiments, presumably reflecting differences in dissection of the tissue. Regardless, it was clear that capsaicin prevented the normal developmental increases of both SP and SS in the DRG and dorsal spinal cord.

DISCUSSION

The study of neural ontogeny has been facilitated by the availability of molecular markers specific for different neuronal subpopulations (22, 23). For example, we recently demonstrated that SP is a specific and convenient biochemical index of growth and development of DRG neurons. However, insights gained from the study of SP-containing neurons might not be applicable to other subpopulations of sensory neurons. The development of a different DRG neuropeptide, somatostatin, was therefore examined for comparison with SP ontogeny.

The developmental profiles for SP and SS were similar, suggesting that their respective neuronal populations develop contemporaneously. The striking similarity in their ontogenetic patterns implies that critical regulatory events may occur simultaneously for the two peptides. Moreover, development of both SP and SS was stimulated by NGF treatment, suggesting that both SP- and SS-containing neurons possess NGF receptors. Because NGF increased protein *specific* SP and SS content, the trophic molecule may selectively regulate both putative transmitters. Consequently, SS may be added to the growing list of putative transmitters affected by NGF (2, 24). Moreover, our studies provide insight into mechanisms of NGF action. Because NGF increased both putative transmitters, it is probable that the factor did not elicit expression of a *specific* peptidergic phenotype, but rather stimulated development of the transmitter normally expressed by receptive neurons. This contention is entirely consistent with observations in sympathetic neurons, in which NGF increases cholinergic *and* noradrenergic characters but does not elicit a specific choice between the two transmitters (24, 25). Thus NGF can stimulate development of any one of a variety of neurotransmitters, presumably depending upon prespecified neuronal phenotypes. These effects may result from an increase in the amount of transmitter *per neuron*, an increase in the number of neurons, or a combination of both effects. In developing sympathetic ganglia, NGF increases both neuron number and the amount of transmitter biosynthetic enzyme per neuron (24). It it is not yet clear whether NGF has analogous effects in neonatal sensory ganglia.

Developmental increases of both SP and SS were diminished after capsaicin treatment. Administration of capsaicin to neonates is known to produce degeneration of a population of type B DRG neurons and results in a 70% reduction in the number of unmyelinated fibers of the saphenous nerve, a major afferent trunk in the lower limb (26). Moreover, capsaicin treatment releases SP from the spinal cord and depletes SP from the substantia gelatinosa and dorsal roots (18, 20, 21). These observations have fostered the contention that capsaicin may specifically affect SP. The present study, however, indicates that capsaicin decreases DRG and spinal SS as well as SP. Consequently, capsaicin cannot be regarded as specific for SP. This contention is buttressed by the observation that capsaicin superfusion of adult rat spinal cord produces a 4-fold increase in SS release, as well as a 9-fold increase in SP release (27). It is not yet clear whether other peptidergic neurons are also sensitive to capsaicin and whether, consequently, an even larger subpopulation of peptidergic neurons is sensitive to the agent.

The similarities in SP and SS developmental profiles and in responses to NGF and capsaicin suggest that the respective neuronal subpopulations may be subject to common developmental influences. Additionally, the commonality of responses raises the possibility that SP and SS are not, in fact, localized to *separate* populations, but rather coexist in the *same* neurons. Certainly, coexistence of two putative peptide transmitters in the same neuron has been demonstrated previously (8). However, careful immunohistochemical studies of sensory ganglia have failed to detect SP and SS within the same neuronal perikarya (8, 9). Nevertheless, the results of at least one *in vitro* study suggest that the same neurons may elaborate either SP or SS, depending upon culture conditions (28). Consequently, the degree of overlap of SP- and SS-containing neuronal populations has yet to be fully defined.

Although the issue of localization remains to be resolved, the present study demonstrates that the two peptide phenotypes are similarly influenced by NGF and capsaicin. Consequently,

experiments involving NGF or capsaicin must be interpreted with extreme care, because SP, SS, and possibly other peptides may be altered. Clearly, specificity of effects is limited. More generally, our work raises the possibility that a number of common mechanisms may govern development of a variety of peptidergic phenotypes in the nervous system. Further, the present studies, in conjunction with previous work, indicate that a single molecular species, NGF, may influence development of neurons utilizing a variety of transmitters, including SS and SP, as well as catecholamines and acetylcholine.

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