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Desensitization to Substance P-Induced Vasodilation *in vitro* Is Not Shared by Endogenous Tachykinin Neurokinin A

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Two mammalian tachykinins, substance P (SP) and neurokinin A (NKA), were measured by radioimmunoassay in canine cephalic blood vessels and tested for their vasoactivity *in vitro*. Levels of immunoreactive SP were approximately 2–3 times greater than those of immunoreactive NKA in common carotid, basilar, and middle cerebral arteries. Both endogenous tachykinins relaxed precontracted segments of common carotid and basilar arteries in a dose-dependent manner with an EC_{50} of 8.9×10^{-11} M and 7×10^{-10} M, respectively, when added cumulatively. Relaxation was endothelial dependent for both substances and not blocked or enhanced by pretreatment with indomethacin, propranolol, lithium chloride, or atropine. Neither SP nor NKA released ^{3}H-inositol phosphates from phospholipid membranes of canine carotid segments after preincubation with ^{3}H-inositol. SP but not NKA or the C-terminal fragments SP(4–11) caused desensitization to subsequent additions of itself but not to the relaxation induced by sodium nitroprusside, calcitonin gene-related peptide, or bradykinin. These studies demonstrate that at least 2 peptides derived from β-preprotachykinin are contained within cephalic blood vessels and that these products share similar vasoactive properties but differ in their ability to desensitize vascular tachykinin receptors.

The tachykinins—substance P (SP), neurokinin A (NKA), neurokinin B (NKB), kassinin, physalaemin, eledoisin—represent a family of peptides that possess similar biological activities and the common C-terminal amino acid sequence: Phe-X-Gly-Leu-Met-NH (Erspamer, 1980). To date, SP, NKA, and NKB have been found in mammalian tissues (von Euler and Gaddum, 1931; Kangawa et al., 1983; Maggio et al., 1983; Hunter and Maggio, 1984; Nawa et al., 1984a). Neurokinin A is a decapeptide that differs from the undecapeptide SP by containing the aliphatic amino acid valine instead of the aromatic amino acid phenylalanine at position 8 and by possessing a unique N-terminal sequence which shares only a lysine group in common with SP. β-Preprotachykinin, the tachykinin precursor, contains one copy each of the neuropeptides SP and NKA (Nawa et al., 1984b). Both SP and NKA promote contraction of intestinal smooth muscle, as well as vasodilation and extravasation of proteins from blood vessels by receptor-specific mechanisms (Couture et al., 1980; Hunter and Maggio, 1984; Regoli et al., 1984a–c; Gamse and Saria, 1985). More than one receptor site was hypothesized in order to explain the ability of eledoisin to contract guinea pig ileum previously desensitized to SP and to explain the differences in rank-order potencies of selective agonists and antagonists (Lee et al., 1982). Indeed, ligand binding studies suggest at least 3 binding sites in tissues (Cascieri and Liang, 1983; Buck et al., 1984; Shults et al., 1985). Some clues to a possible receptor-coupled mechanism were provided by experiments showing that SP increased the turnover of inositol phosphates within guinea pig ileum and hypothalamus (Mantyh et al., 1984).

The dog carotid artery has been useful for studying receptor-mediated tachykinin-induced relaxation of blood vessels (Couture et al., 1980; Regoli et al., 1984a–c, 1985). In this tissue, SP, NKA, physalaemin, and eledoisin all relaxed precontracted vessel segments in a concentration-dependent manner with the following order of potency: SP = physalaemin, NKA, eledoisin. Lee and Iversen have designated this potency pattern an SP-P response to distinguish it from those responses in which eledoisin and kassinin were substantially more potent (Lee et al., 1982). In order to better understand the mechanisms of tachykinin-induced vasodilation, we have addressed the following questions: Is NKA as well as SP present in canine cephalic blood vessels? Are receptor-mediated responses to SP and NKA the same in canine carotid vessels? Do both induce desensitization in this tissue? Might tachykinin second-messenger mechanisms involve cleavage of inositol phosphates from phosphatidylinositol and polyphosphatidylinositol in membranes of vascular tissue?

Materials and Methods

SP and NKA radioimmunoassays. The development and radioimmunoassay (R14) for SP have been described previously for the F_2 antisera (Liu-Chen et al., 1983). Production of antisera to NKA was accomplished by immunizing adult male rabbits previously sensitized with Bortedella pertussis with a succinylated hemocyaninin/1-ethyl, 3-(3-dimethylaminopropyl)-carbodiimide/His[1]-NKA conjugate. Characterization of NKA antiserum #4 revealed that it bound 30% of added ^{125}I-tyr-NKA trace at 1:300,000 dilution. Half-maximal displacement of ^{125}I-tyr-NKA by unlabeled synthetic NKA occurred at 60 fmol/tube. At this antibody dilution, SP cross-reactivity was 0.000075% and neurokinin B was 12%; no cross-reactivity was observed to met-enkephalin. Interassay variation at 50 fmol/tube is 8% and log-logit transformation of the assay data shows linearity from 85 to 20% of bound trace (18–200 fmol/tube).

Tissues were homogenized in 2 *N* acetic acid containing 0.01 M mer-

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captoethanol at 0°C. The dried extracts were reconstituted in 0.1 M PBS (pH 7.4) and then subjected to RIA. NKA levels were the same when tissues were extracted in either boiling water or 2 *N* acetic acid. SP values were significantly higher when 2 *N* acetic acid was used (unpublished data). Consequently, 2 *N* acetic acid was used for all extractions.

Bioassay. Dogs were anesthetized and exsanguinated via the femoral artery. Common carotid arteries or brains were removed and placed in Krebs-Henseleit solution containing 120 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 10 mM D-glucose, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, and 25 mM $NaHCO_3$. The Krebs solution was aerated with O_2:CO_2 (95:5) to maintain pH at 7.4. The carotid arteries were cleaned, taking care not to rub the intimal surfaces together. The basilar artery was carefully dissected from the brain under a stereomicroscope. The arachnoid was removed, and the arteries were cut into 5 mm segments. Carotid rings 4 mm wide or basilar segments were mounted on L-shaped wires attached to Grass FT.03C force-displacement transducers. Isometric contractions were recorded on a Grass model 7D polygraph. The mounted tissues were placed in 10 ml tissue baths containing Krebs solution maintained at 37°C and continuously aerated. A maximum of 7 vessel segments could be studied simultaneously. Carotid artery rings were gradually adjusted to a resting tension of 2 *g* during a 2–3 hr equilibration. Basilar segments were adjusted to a tension of 2.5 *g*. To study relaxation, carotid rings were contracted with 2.5×10^{-7} M noradrenaline, and basilar segments were contracted with 2.1×10^{-6} M $PGF_{2\alpha}$. When a stable tension was reached, peptides were added cumulatively to the tissue bath. After each experiment sodium nitroprusside (1 μM) or papaverine (100 μM) was added to produce maximum relaxation in the carotid and basilar rings, respectively. Atropine, propranolol, or indomethacin was added to precontracted segments, which were allowed to stabilize for periods of up to 90 min prior to the addition of peptide.

In experiments to examine tachyphylaxis and cross-desensitization, the protocol was as follows: the substance to be tested was added to a precontracted vessel segment in a cumulative manner to achieve a concentration at least 10 times above the maximum response (10^{-7} M). When indicated, desensitization was induced by a single dose. The tissue was then incubated for approximately 45 min. The vessel was washed with 10 ml Krebs buffer and allowed to stand for 40 min and then washed at 20 min intervals. Additional washes were performed at equal intervals when indicated. A 3 wash protocol was followed unless otherwise indicated. Following contraction with noradrenaline (2×10^{-7} M), the vessel was allowed to stabilize for an additional 40 min before obtaining a cumulative response curve.

Removal of the carotid endothelium was achieved by gently pulling the vessel over a small roll of filter paper. The basilar endothelium was removed by rubbing segments over a roughened hypodermic needle. Endothelium was demonstrated by silver nitrate staining *en face* by a method modified from Poole (1958) and Zand (1982). Vessels were cut open and pinned flat in silicone-filled petri dishes, fixed for 20 min in 3% glutaraldehyde, rinsed in 5% glucose, stained with 0.1% silver nitrate for 30 sec (carotid) or 1 min (basilar), washed with 5% glucose, treated with 3% cobalt bromide/1% ammonium bromide for 3 min, washed in 5% glucose, and fixed in 3% glutaraldehyde under a high-intensity lamp for several hours. Rings were then rinsed with water and examined as a wet mount under a light microscope.

Arteries were used within 48 hr after procurement and stored overnight at 4°C in Krebs solution without loss of reactivity. Data are expressed as percentage relaxation relative to the maximum relaxation produced by sodium nitroprusside or papaverine and reflect the mean ± SEM of at least 3 determinations. EC_{50} values (concentration of agonist producing 50% of maximal response) were determined by interpolation from log dose–response curves.

^{3}H-inositol studies. Segments of carotid arteries were incubated as previously described (Derian and Moskowitz, 1986). Briefly, arteries were obtained aseptically, cleaned of adventitia, and cut into 1 cm segments. Segments were maintained in Dulbecco's modified Eagle's medium with 5% calf serum for 16–24 hr. Segments were then prelabeled with 20 μCi ^{3}H-inositol for 24 hr and incubated with lithium chloride for 10 min prior to incubation with SP. Some segments were also incubated with bradykinin as a positive control. Total ^{3}H-inositol phosphates, as well as individual fractions (^{3}H-inositol mono-, bis-, and trisphosphates), were measured as described previously. This experiment was repeated 3 times.

Materials. Noradrenaline, prostaglandin $F_{2\alpha}$, papavarine, sodium nitroprusside, and silver nitrate were purchased from Sigma (St. Louis, MO). SP and NKA were obtained from Peninsula Labs (Belmont, CA). All other chemicals were reagent grade. Stock solutions (1 mM) of SP and NKA were prepared in 0.01 *N* acetic acid. Aliquots were stored at −70°C.

Peptides were diluted in acid–saline (0.9% NaCl/0.01 *N* acetic acid) and added to the tissue bath in 100 μl volumes. Stock solutions of atropine and propranalol (10 mM) were prepared in acid–saline. Indomethacin was stored in 100% dimethylsulfoxide at 4°C. A 0.1 M stock solution of noradrenaline was prepared in distilled water containing 10% ascorbic acid. Aliquots were stored at −20°C. Noradrenaline was diluted in modified Krebs solution containing 5.95 mM EDTA, and 100 μl was added to the tissue bath. Prostaglandin $F_{2\alpha}$ was dissolved in methanol (2.1 mM), stored at −20°C, and diluted in acid–saline. Concentrated solutions of papaverine (0.1 M) and sodium nitroprusside (10 mM) were also stable at −20°C.

Table 1. SP and NKA in canine cephalic vascular tissues

Vessel	SP/NKA	NKA (fmol/g, wet weight)	SP
Middle cerebral artery			
1	2.93	40	117
2	3.03	38	115
3	1.64	120	197
4	2.32	83	195
Mean	2.48		
Basilar artery			
1	2.60	48	125
2	1.91	328	628
3	1.95	155	303
4	2.43	141	342
Mean	2.34		
1 Carotid	3.14	7	22

Results

The presence of 2 endogenous tachykinins was measured by RIA in canine cephalic arteries. Immunoreactive SP and NKA were assayed in middle cerebral and basilar arteries over concentration ranges 38–120 and 48–328 fmol/gm wet weight, respectively. Levels were lower in common carotid vessels (Table 1).

Both tachykinins were biologically active when tested for their ability to relax cephalic vessels *in vitro*. When added to precontracted segments, both SP and NKA produced a concentration-dependent relaxation. The threshold for SP in carotid artery was $>10^{-12}$ M and the EC_{50} approximately 8.9×10^{-11} M. The threshold for NKA in carotid artery was approximately 10^{-10} M, with an EC_{50} of 7×10^{-10} M. Maximum responses were achieved at 10^{-8} and 10^{-7} M for SP and NKA, respectively. Relaxation was unchanged by the prior addition of propranalol, atropine, indomethacin, or lithium chloride. Carotid relaxation to both SP and NKA approached 80% of the response achieved with sodium nitroprusside (Fig. 1). Relaxation in basilar artery was approximately 60 and 70% of maximum for both tachykinins. The time course for the relaxation following a single application of SP or NKA did not differ in general, both sustained for longer than 20 min in carotid segments. Some vessels gradually regained tension without any additional manipulation. NKA- and SP-induced relaxation in the basilar artery differed from that in the carotid in being of much shorter duration, with tension returning to baseline sooner (Fig., 2*A*, *B*). There is no explanation for this difference at the present time.

Both endogenous tachykinins dilated blood vessels by endothelial-dependent mechanisms. Removing the endothelium completely abolished the relaxation responses to SP and NKA

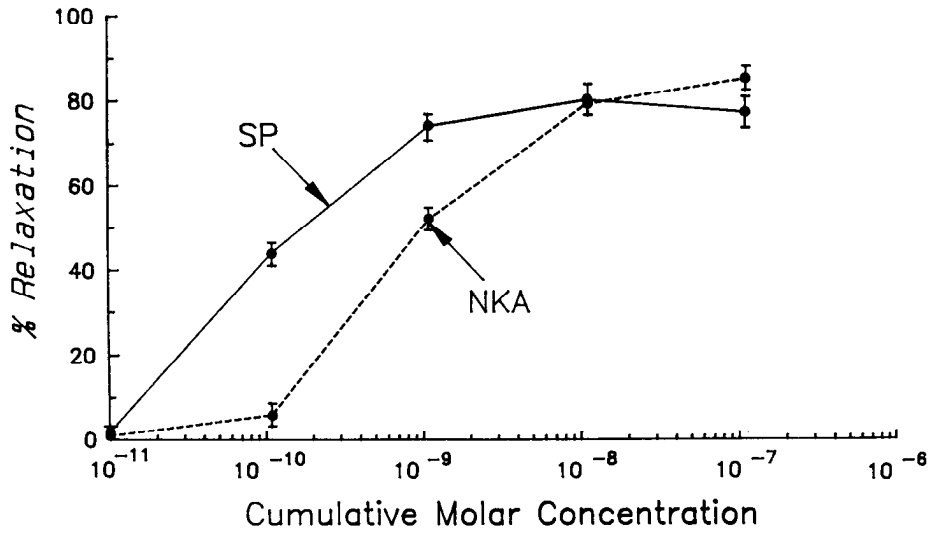


Figure 1. Dose responses to the cumulative addition of SP or NKA in the canine carotid artery. SP and NKA were added to carotid rings previously contracted with noradrenaline. The decrease in tension is expressed as a percentage of the maximum relaxation elicited by adding sodium nitroprusside to the same segment. Values represent means ± SEM of 6 experiments (11 rings) for SP and 5 experiments (10 rings) for NKA.

in both basilar and carotid arteries (Fig. 2*C*). However, even small islands of remaining endothelium appeared sufficient to elicit some relaxation.

Substance P significantly increased the EC_{50} and decreased the maximum response to a second administration of itself (homologous desensitization) in carotid and basilar arteries at concentrations above threshold (Fig. 3*A*). Representative tracings are shown in Figure 4. Dose–response curves constructed from single applications of peptide (data not shown) closely resembled responses shown in Figure 1. Some of the desensitization resulted from residual peptide since increasing the number of washes between the first and second peptide application (e.g., from 3 to 9) significantly attenuated the response. However, extensive washing with more than 1000 volumes did not abolish SP-induced desensitization. Furthermore, the conditioned buffers from 3 or more washes failed to relax precontracted segments not previously exposed to SP. Higher thresholds and reduced maximum responses to the second tachykinin were observed by using larger desensitizing single concentrations of SP, whereas tachyphylaxis was not observed with subthreshold doses. Vessels became more unresponsive as the length of exposure between the first and second application increased from 10 to 40 min. In preliminary experiments, the canine basilar artery responded to SP in a similar manner.

Applications of SP at concentrations sufficient to desensitize did not decrease responses of pretreated vessels to nitroprusside, calcitonin gene-related peptide (CGRP), or bradykinin. The C-terminal octapeptide did not desensitize to subsequent additions of itself or SP except at 10^{-10} M. Multiple additions of SP-(5–11) did not induce tachyphylaxis. As shown in Figure 5, the threshold and EC_{50} for both C-terminal fragments were higher than for the parent molecule. SP was the only tachykinin tested that induced cross-desensitization to NKA (Fig. 3*B*). Neither kassinin nor eledoisin reduced the relaxation response in carotid segments to the addition of SK; physalaemin did so but at a single concentration only. By contrast, neither physalaemin nor NKA desensitized vessels to the subsequent addition of SP. Furthermore, desensitization to NKA was not observed following vessel pretreatment with the peptidase inhibitor bacitracin (1 U/ml).

Experiments did not show that receptor occupancy was coupled to release of inositol phosphates from prelabeled membranes. Neither SP nor NKA released ^{3}H-inositol phosphates (measured as ^{3}H-inositol 1-phosphate, ^{3}H-inositol 1,4-bisphosphate, or inositol 1,4,5-trisphosphate) from phospholipid membranes of canine carotid vascular segments when added at concentrations of 100 pM–1 μM and tested at 1, 10, or 30 min (data shown for 10 min point only) (Fig. 6). A significant release of all 3 fractions was observed after the addition of bradykinin. In other experiments, SP did not inhibit the bradykinin-induced cleavage of inositol phosphates from these tissues.

Discussion

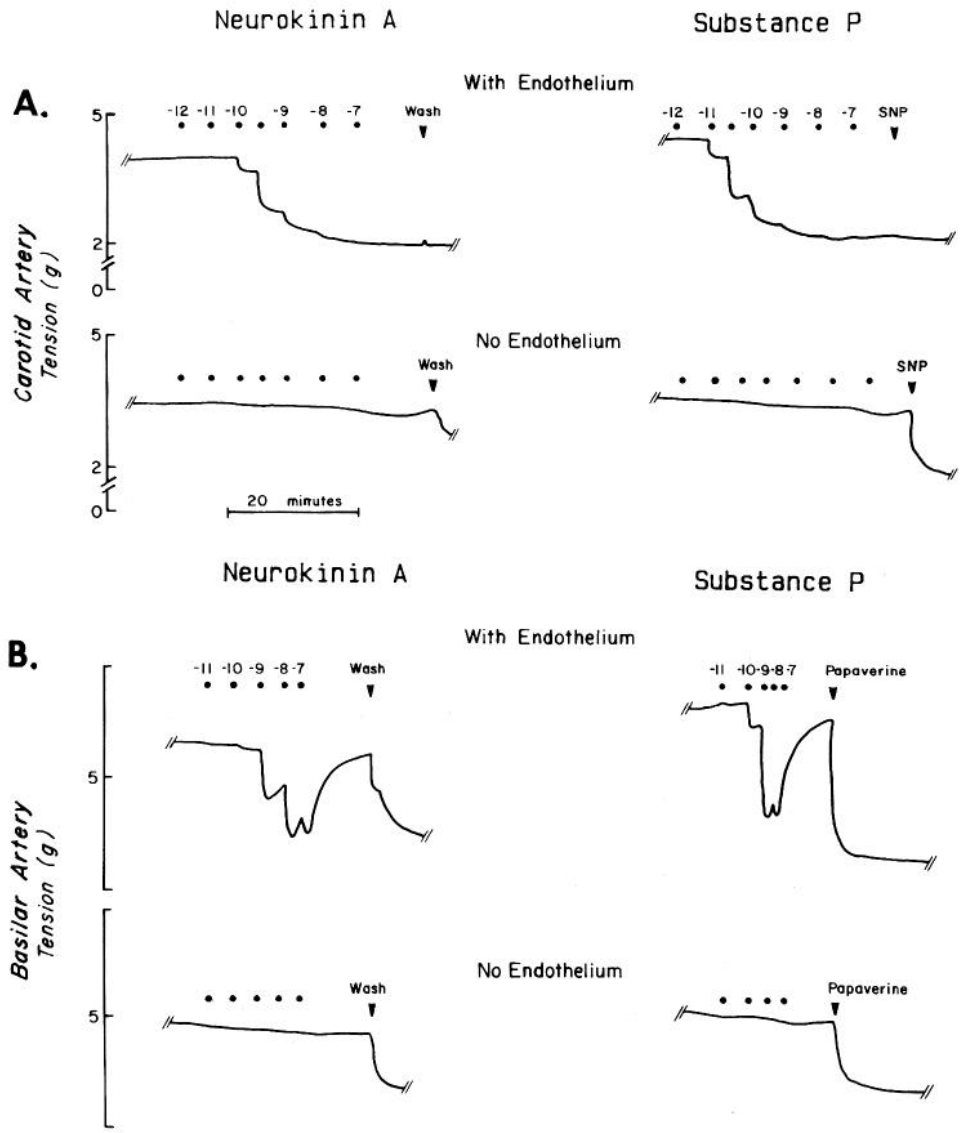
These studies provide evidence for the existence of both NKA and SP within blood vessels and establish that both endogenous tachykinins relax precontracted blood vessels *in vitro* by endothelial-dependent mechanisms. Receptor-mediated release of ^{3}H-inositol phosphates was not observed in isotopically labeled membranes from canine cephalic arteries following the addition of either tachykinin. The absence of this response distinguishes tachykinin receptors in blood vessels from those in other tissues.

Presence and origin of endogenous tachykinins

Previous work with cat and rat cephalic vessels established that most iSP resides within perivascular sensory axons arising from the trigeminal and upper cervical sensory ganglia (Liu-Chen et al., 1983; Norregaard and Moskowitz, 1985). Unilateral lesions of trigeminal, but not sympathetic, ganglia reduce iSP levels within intra- and extracranial cephalic blood vessels by more than 50% and significantly decrease the density of perivascular axons containing iSP as determined by immunohistochemistry. Preliminary data in the cat also indicate that iNKA levels decrease significantly in pial arteries following unilateral trigeminal ganglionectomy (Saito et al., 1987). Since we observed that iNKA and iSP apparently exist in proportions similar to one another within canine cephalic arteries, and others have localized NKA to a population of SP-containing dorsal root ganglia cells (Dalsgaard et al., 1985), there is the strong likelihood that iNKA and iSP coexist within perivascular sensory axons.

Tachykinin-induced vasodilation

The vasodilations induced by SP and NKA share some similarities but also differ in important ways. Both peptides relax basilar and carotid arteries at threshold and half-maximal re-



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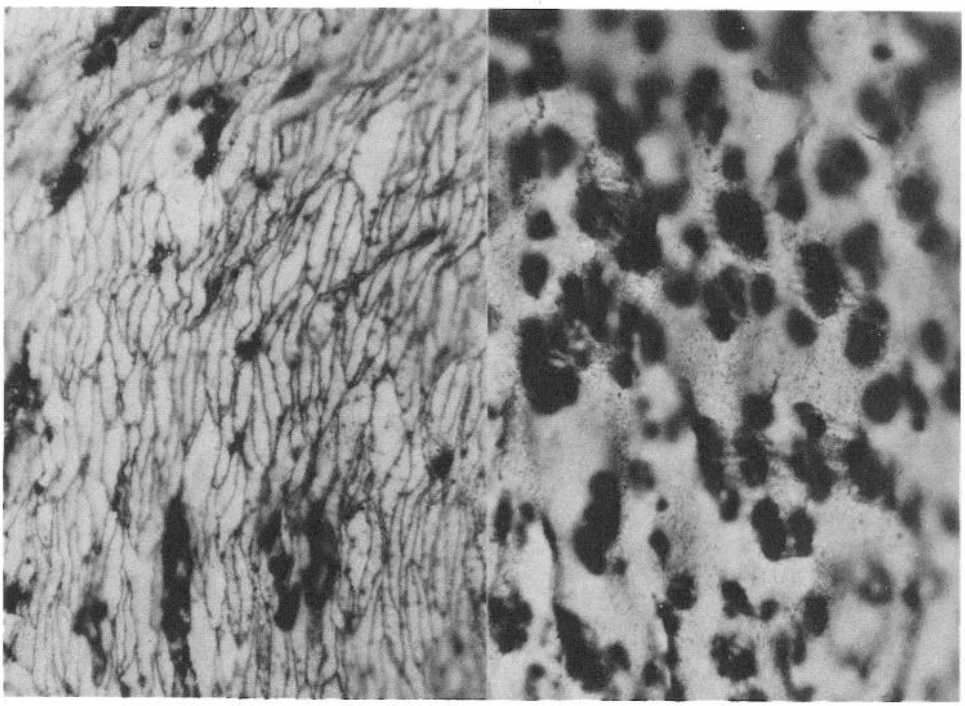

Figure 2. Representative tracings showing responses to the application of SP or NKA in carotid (*A*) and basilar (*B*) arteries with and without an intact endothelium. Precontracted vessels were relaxed with NKA, washed 4 times, recontracted, and then relaxed with SP. Carotid and basilar segments were precontracted with noradrenaline and $PGF_{2\alpha}$, respectively. As indicated, vessels were relaxed maximally with papaverine (10^{-4} M) or sodium nitroprusside (10^{-6} M). *C,* Silver stain showing an intact (*left*) and denuded (*right*) endothelium from basilar artery.

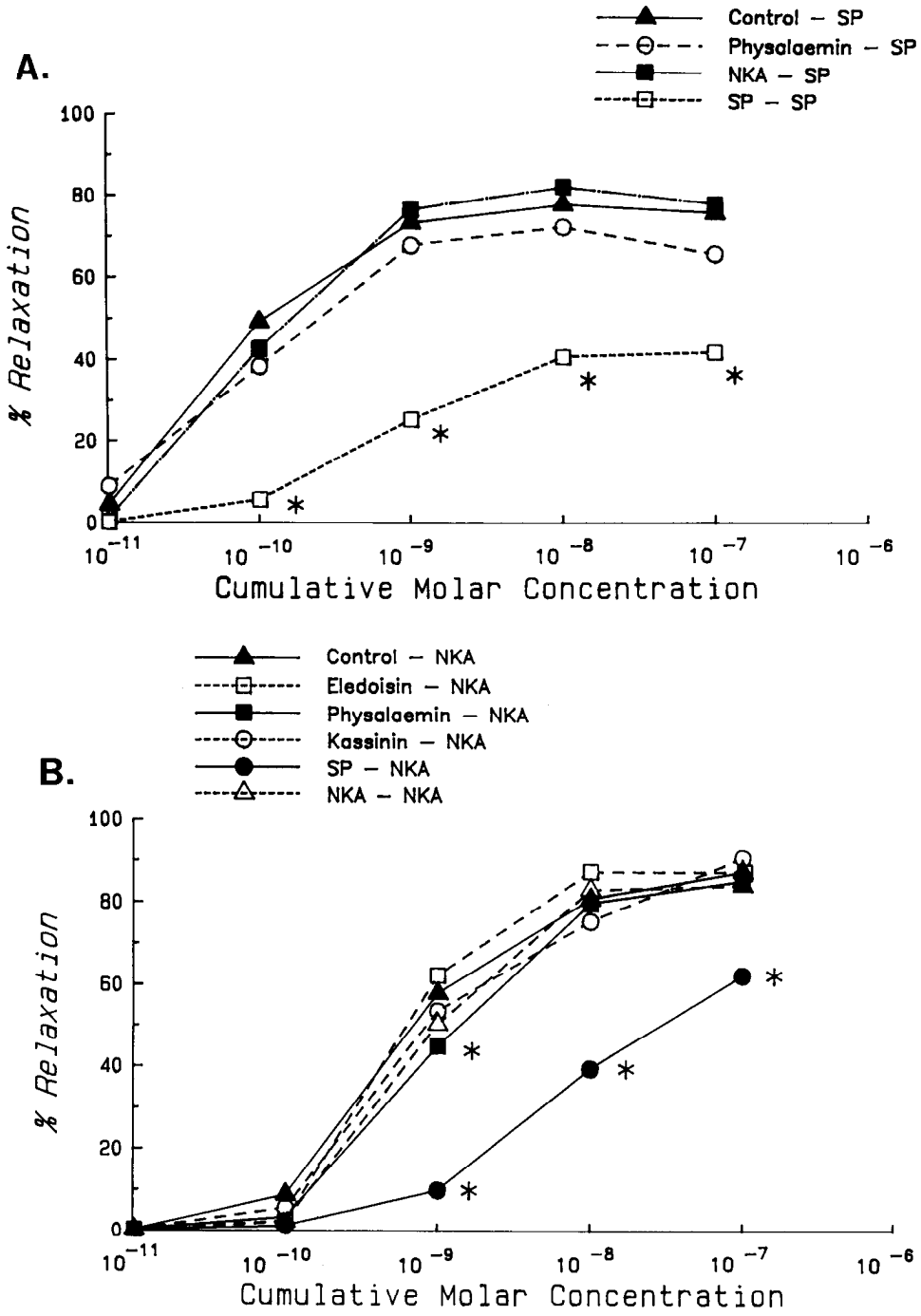

Figure 3. Tachyphylaxis to SP- but not to physalaemin- or NKA-induced relaxation in the canine carotid artery. *A*, Cumulative response curves for SP in rings previously relaxed with SP (□), NKA (■), physalaemin (○), or acid–saline vehicle (△). *B*, Cumulative response curves for NKA in rings previously relaxed with NKA (△), SP (●), eledoisin (□), physalaemin (■), kassinin (○), or acid–saline vehicle (▲). Segments were exposed to cumulative concentrations of SP, NKA, eledoisin, physalaemin, kassinin, or acid–saline over 44 min, washed 3 times, and contracted and stabilized an additional 40 min before a second cumulative response curve was obtained. Each point in *A* represents the mean ± SEM of 6 experiments (11–12 rings), except for those in which physalaemin was added ($n = 2$). Values in *B* represent means ± SEM of 5 experiments (9–10 rings), except those in which eledoisin, physalaemin, or kassinin was added ($n = 2$). *Asterisk* denotes differences from control ($p < 0.01$).

sponses in picomolar concentrations. As judged by these responses, SP is approximately 10 times more potent than NKA. NKA, like SP (Furchgott, 1984; D'Orleans-Juste et al., 1985) requires an intact endothelium to produce maximum dilation, and the dilation achieved at highest agonist concentration for both is 70–80% of that achieved with either sodium nitroprusside or papaverine.

These data also indicate that tachykinin receptors that mediate vasodilation are expressed on the endothelial surface. Other peptides sharing a similar mechanism include bradykinin and vasopressin (Furchgott, 1984; Katusic et al., 1984). Since both vasodilation and plasma extravasation accompany sensory nerve stimulation, and both responses can be partially attenuated by specific receptor blockers (Lembeck et al., 1982), it may inferred that neuropeptides released from perivascular sensory fibers do gain access to this receptor site.

Endothelial dependent relaxation

Many agonists (e.g., bradykinin, vasopressin, ACh), including the tachykinins, have been found to relax blood vessels in an endothelial-dependent manner since the first report by Furchgott and Zawadski (1980). The general mechanism proposed is that an agonist reacts with its specific receptor on the endothelium (with the exception of calcium ionophore A 23187), and this interaction causes the synthesis or release of endothelial-dependent relaxing factor (EDRF). EDRF then stimulates smooth

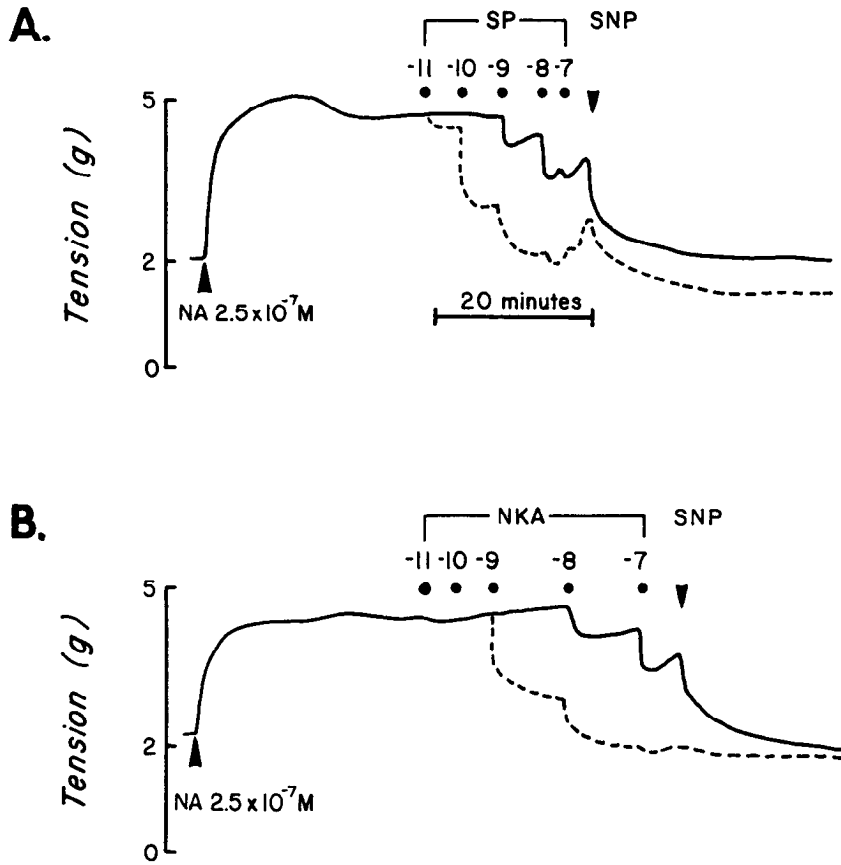

Figure 4. Representative tension tracings illustrating homologous and heterologous desensitization induced by SP in carotid artery. *A,* SP cumulative relaxation in a ring previously exposed to substance P (*solid line*) or an unsensitized control ring exposed to vehicle only (*dashed line*). *B,* NKA cumulative relaxation in a ring previously desensitized with with SP (*solid line*) or unsensitized control ring exposed to vehicle only.

muscle synthesis of guanylate cyclase (Rapoport and Murad, 1983), which in turn induces relaxation in vascular smooth muscle. EDRF is extremely labile; estimates of its half-life vary from 6 sec (Griffith et al., 1984) to 20–40 sec (Forstermann et al., 1985). The chemical structure of EDRF is unknown, but it can be distinguished from prostacyclin because cyclooxygenase inhibitors fail to block its production. It can only be detected by bioassay. Many studies have attempted to characterize EDRF pharmacologically (see reviews by Furchgott, 1984; Peach et al., 1985). Hemoglobin, methylene blue, nordihydroguiaretic acid (NDGA), eicosatetraynoic acid (ETYA), metyrapone, SKF 525, calcium-channel blockers, and several antioxidants are some of the many agents that inhibit endothelium-dependent relaxation to a variety of agonists. Superoxide dismutase and Cu^{2+} have recently been shown to decrease the breakdown of EDRF released by bradykinin (Gryglewski et al., 1986). Because NDGA and ETYA inhibit EDRF, and arachidonic acid can induce EDRF, it has been proposed that EDRF may be a metabolite (through a non-cyclooxygenase pathway). Other evidence does not support this hypothesis. For example, several fatty acids

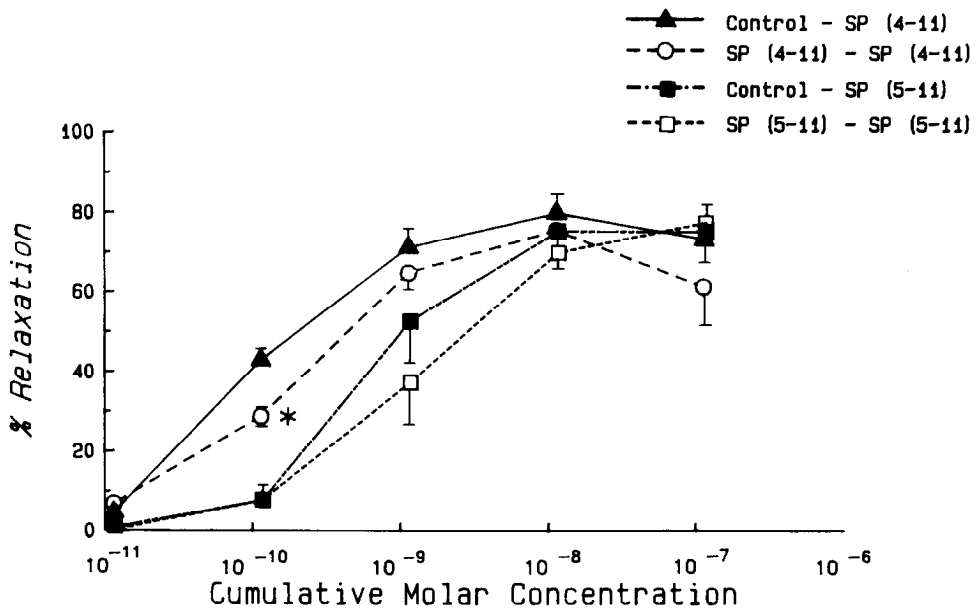

Figure 5. Repeated applications of either SP-(4–11) or SP-(5–11) did not cause desensitization in canine carotid arteries. Cumulative dose responses were performed as described in Figure 3. No significant differences were determined in the responses of those arteries previously relaxed with peptide versus those pretreated with vehicle only, except those denoted by *asterisk* ($p < 0.05$).

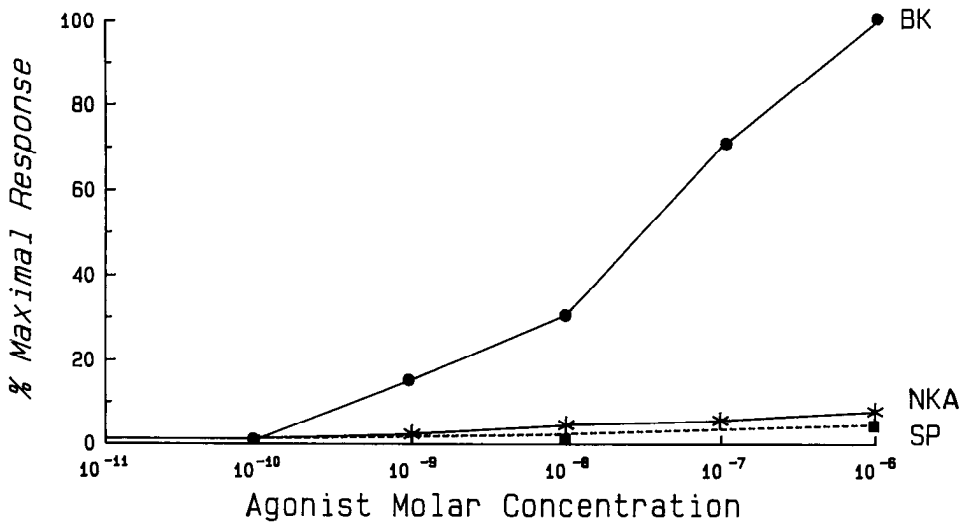

Figure 6. Release of ^{3}H-inositol phosphates from canine carotid artery by bradykinin (*BK*) is not observed following the addition of SP or NKA. Carotid segments were incubated with ^{3}H-inositol and lithium chloride as described. Segments were then exposed to BK (10 pM–1 μM), SP or NKA (100 pM–10 μM) for 10 min, after which the 3 cleaved sugar phosphates (IP_1, IP_2, and IP_3) were resolved by ion-exchange chromatography using Dowex-1F resin from trichloroacetic acid extracts of tissues as previously described. The data shown represent total ^{3}H-inositol phosphate released and are expressed as a percentage of that response observed in vehicle-treated segments (mean of 4 vessels).

besides arachidonate can induce EDRF, NDGA may be acting as an antioxidant instead of a lipoxygenase inhibitor (Griffith et al., 1984), and other lipoxygenase inhibitors such as BW 755 are ineffective. Griffith and others have suggested that EDRF may be a lactone, ketone, aldehyde, epoxide, free radical-, or hydroperoxy fatty acid. As indicated previously, endothelial-dependent relaxation suggests that vascular permeability may be the primary physiological consequence of such a mechanism (Moskowitz, 1984).

Endothelial-dependent relaxation elicited by SP was reported *in vitro* by Zawadski et al. (1981) and *in vivo* by Angus et al. (1983). D'Orleans-Juste et al. (1985) also examined the role of the endothelium for SP, physalaemin, eledoisin, kassinin, and other agents. Substance P is unusual compared to other endothelial-relaxing agents because continued exposure produces a desensitization to its activity (Couture et al., 1980; Zawadski et al., 1981; Regoli et al., 1984a–c; Peach et al., 1985). We have provided evidence that the relaxation produced by NKA is also endothelium dependent. In this respect, NKA is similar to the other tachykinins but, unlike SP, does not produce detectable desensitization.

Receptor desensitization

SP and NKA differ in their ability to cause tachyphylaxis in blood vessels. Differences in receptor occupancy rates seem unlikely to explain these differences inasmuch as NKA-induced desensitization was never observed, even after 10 times the amount of peptide required to elicit a maximum response was added. By contrast, SP concentrations above threshold were sufficient to induce desensitization. Assuming that the prolonged incubation times employed did not obscure the demonstration of desensitization for a peptide with rapid K_{off} rates, explanations based on the greater SP receptor affinity and lower K_{off} rates seem unlikely. As noted above, differences in desensitization responses between the 2 peptides were not quantitative but appeared to be all-or-none phenomena.

The potencies of the smooth muscle relaxing agents nitroprusside, CGRP, or an endothelial-dependent relaxing substance such as bradykinin were unaffected by SP pretreatment. Hence, the mechanism points more to events surrounding the agonist–receptor interaction than to a secondary effect common to metabolic and physiological processes that relax vascular smooth muscle directly or via the endothelium. Furthermore, desensitization was not observed following the addition of other tachykinins. For example, pretreatment with eledoisin or kassinin did not modify the relaxation caused by NKA. The protonated N-terminal region may be important for the development of SP-induced desensitization, as previously reported for angiotensin II. In those studies, a correlation was found between the degree of amino terminal protonation and the ability of analogs to induce desensitization (Pavia et al., 1977). To strengthen such an argument for the SP response, further studies are planned using synthetic peptides that incorporate the N-terminal sequence of SP into NKA and other tachykinins.

Whether SP-induced desensitization has any physiological relevance for blood vessels, however, remains to be determined. Desensitization to SP, but not NKA, was reported in rabbit iris sphincter muscle (Fujiwara et al., 1985). In this model, electrical transmural stimulation caused a tachykinin-induced slow contraction that did not desensitize with repeated electrical stimulation but was attenuated by adding an SP antagonist. Since the direct addition of SP caused desensitization whereas NKA did not, the suggestion was made that NKA released from trigeminal nerve endings mediated this response.

The differences in desensitization among SP and other tachykinins suggest the possibility that more than a single receptor mechanism mediates tachykinin-induced vasodilation in canine carotid artery. Compatible with a single-receptor model, SP pretreatment decreased the EC_{50} for both itself and NKA to the same extent (Fig. 4, *A, B*). The lack of desensitization to repeated NKA administration, however, could also suggest the existence of a separate population of NKA receptors that must also recognize SP (crossed desensitization to NKA followed SP pretreatment). As noted below, the number of receptor subtypes might best be determined by ligand binding experiments and by second-messenger responses. Estimates of the affinities for the tachykinin antagonists, however, might prove most useful in this bioassay.

As in the case of the β-receptor (Sibley and Lefkowitz, 1985), desensitization may be associated with the uncoupling, sequestration, and translocation of receptors from plasma membrane, which reportedly involve receptor phosphorylation or phosphorylation of the receptor-coupled regulatory proteins. The increase in half-maximal and maximum responses upon rerelaxation may have resulted from some of these events. Further clarification requires ligand binding studies and the discovery of a second messenger. Unfortunately, ligand binding is made difficult by the low density of tachykinin receptors in vessels

(M. A. Moskowitz and T. Liang, unpublished observations), perhaps because the single-layered endothelium comprises only a small percentage of the total mass. Furthermore, AMP is unlikely to serve as a second messenger in vascular tissue since no tachykinin has yet been shown to stimulate adenylate cyclase activity reliably. Similarly, the results of the present studies do not support a phosphatidylinositol coupling mechanism for the tachykinins in dog carotid artery. A lack of tachykinin responsiveness has also been observed in primary cultures of endothelial cells from aorta and brain microvessels (Moskowitz and Derian, 1986). Unlike the guinea pig ileum, salivary glands, and brain, the SP-P type response in dog carotid artery is probably not coupled to mechanisms involving phosphatidylinositol lipids in endothelium, at least as determined by ^{3}H-inositol phosphate release.

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