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Radioiodinated Substance P, Neurokinin A, and Eledoisin Bind Predominantly to NK1 Receptors in Guinea Pig Lung

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SUMMARY

In homogenates of guinea pig lung, binding of ^{125}I-Bolton-Hunter-labeled substance P (BHSP), Bolton-Hunter-labeled eledoisin (BHELE), and [^{125}I]iodohistidyl neurokinin A (INKA) was investigated. Equilibrium dissociation constants (derived from "cold" saturation experiments) for BHSP, INKA, and BHELE were 0.96 ± 0.15, 1.61 ± 0.26, and 1.98 ± 0.12 nM, respectively. Specific binding of all three radioligands was increased 2–3-fold by 10 μM phosphoramidon. The rank order of potency of unlabeled tachykinins in competing against BHSP was substance P (SP) > [Sar^9,Met(O$_2$)^{11}]-SP > SP methyl ester > neuropeptide γ > neurokinin A ≥ neurokinin B = kassinin ≥ eledoisin > scyliorhinin II ≫ neuropeptide K, indicating binding to sites with the general characteristics of NK1 receptors. Similar rank potency orders were observed for INKA and BHELE, showing binding to NK1 sites, rather than to NK2 or NK3 sites, which are labeled with high affinity by these radioligands in other tissues. For all radioligands, competition curves for SP and the NK1-selective agonist [Sar^9,Met(O$_2$)^{11}]-SP could be resolved into two components, representing high and low affinity binding sites. These were present in the approximate ratios 2:3 (for BHSP), 1:1 (for INKA), and 8:1 (for BHELE). Other agonist competition curves also yielded high and low affinity components. The data suggest that BHSP and INKA bind partly and BHELE predominantly to high affinity NK1 receptors. The nature of the low affinity site(s) could be another tachykinin receptor or a low affinity state of the NK1 receptor. Binding to a "classical" NK2 receptor is unlikely, because selective NK2 receptor antagonists and analogs were very weak competitors. Our data suggest that, in addition to the NK1 receptor, another type of tachykinin receptor may exist in this tissue. The inability to detect NK2 binding sites is strikingly at variance with functional studies.

The tachykinin peptides include SP, NKA, NKB, and the nonmammalian eledoisin, kassinin, physalaemin, scyliorhinin I, and scyliorhinin II (1–3). Recently, two mammalian amino-terminally extended forms of NKA, NPK and NPγ, have been described (4, 5), which share the common carboxyl terminus, Phe-X-Gly-Leu-Met-NH$_2$, characteristic of tachykinins. SP and NKA are colocalized with other peptides, including calcitonin gene-related peptide, in primary afferent nociceptive neurons in the respiratory tract (6–8) and other organs. SP and/or NKA immunoreactivity is found in close proximity to the respiratory epithelium, smooth muscle of airways, and bronchial glands (9, 10). In the mammalian respiratory tract, tachykinins induce tracheal and bronchial smooth muscle contraction, mucus secretion, and plasma extravasation (11–13). For this reason, tachykinin peptides may be implicated in the pathogenesis of asthma (14).

Currently accepted tachykinin receptor classification describes three homologous receptors, the SP-preferring NK1, the NKA-preferring NK2, and the NKB-preferring NK3 receptor (2, 15). The structures of these receptors have recently been determined (16–18). The endogenous tachykinins are somewhat receptor unselective, due to the common carboxyl terminus. Receptor classification has been assisted by the development of new selective analogs. These include the NK1-selective agonist [Sar^9,Met(O$_2$)^{11}]-SP, the NK3-selective agonist senktide, and new NK2-selective antagonists, such as L-659,877 (19), MDL 29,913 (20), and MEN 10207 (21, 22), which have defined subtypes of the NK2 receptor.

Considerable interest has centered on the nature of the tachykinin receptor mediating contraction of bronchial smooth muscle. Although this was first considered to be the NK2 receptor (23), recent data from a number of laboratories suggest that the receptor in guinea pig trachea may have unusual characteristics (24), and at one stage a novel "NK4" receptor was proposed (25). Tachykinin receptor characterization based on rank potency orders has been complicated in this tissue by the considerable metabolism of tachykinins (but not some analogs) by the enzyme enkephalinase (EC 3.4.24.11); thus,

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ABBREVIATIONS: SP, substance P; NKA, neurokinin A; BHSP, ^{125}I-Bolton-Hunter-substance P; INKA, [^{125}I]iodohistidyl-neurokinin A; BHELE, ^{125}I-Bolton-Hunter-eledoisin; Gpp(NH)p, 5′-guanylylimido diphosphate; NKB, neurokinin B; NPγ, neuropeptide γ; NPK, neuropeptide K.

earlier studies without appropriate enzyme inhibition may have yielded misleading information. The potency of the endogenous tachykinins in contracting respiratory smooth muscle is markedly enhanced by the enkephalinase inhibitors phosphoramidon and thiorphan (13, 26, 27). Other functional studies indicate that mucus secretion is mediated by NK1 receptors (11), whereas plasma extravasation in the lung may be a mixed NK1/NK3 receptor-mediated response (12, 28). Thus, functional studies suggest multiple tachykinin receptors in the respiratory tract.

Receptors can also be characterized using radioligand binding. BHSP binds to NK1 receptor sites in the central nervous system and most peripheral tissues (15). INKA labels NK2 receptors in hamster bladder (29) and rat vas deferens (30). However, in sections of guinea pig lung, both BHSP and INKA bind to NK1 and not to NK2 sites (31). The aim of this study was to make a detailed characterization of tachykinin receptors in the guinea pig lung, in the presence of the enkephalinase inhibitor phosphoramidon, using conventional homogenate binding techniques. A preliminary report of this study has been presented (32).

Experimental Procedures

Materials. Tachykinin peptides were purchased from Auspep (Melbourne, Australia) or Peninsula Laboratories. Standard solutions (0.5 mM) were stored frozen in 0.01 M acetic acid containing 1% β-mercaptoethanol (except for scyliorhinin II, which was stored in 0.01 M acetic acid alone). ^{125}I-Bolton-Hunter reagent and INKA were purchased from Amersham. The radioligands BHSP and BHELE were prepared and purified by reverse phase high performance liquid chromatography, as previously described (33). Specific activity of all radioligands was 1900–2100 Ci/mmol. Chymostatin, leupeptin, bacitracin, and phosphoramidon were purchased from Sigma Chemical Co. MDL 29,913 (cyclo[Gln-Trp-Phe-Gly-Leu-Met]) and MDL 28,564 (Asp-Ser-Phe-Val-Gly-Leu-CH_2-NH-Leu-NH_2) were generous gifts from Dr. S. L. Harbeson and Dr. S. H. Buck, Marion Merrell Dow Research Institute (Cincinnati, OH). MEN 10207 (Asp-Tyr-D-Trp-Val-D-Trp-D-Trp-Arg-NH_2) was a gift from Dr. C. A. Maggi, A. Menarini Pharmaceuticals (Florence, Italy), and [Lys^5,MeLeu^9,Nle^{10}]-NKA(4–10) was a gift from Dr. S. Lavielle, Pierre et Marie Curie University (Paris, France). L-659,877 (cyclo[Gln-Trp-Phe-Gly-Leu-Met]) was purchased from Cambridge Biochemicals (UK). All other reagents were of analytical grade.

Binding assays. English short-haired guinea pigs were killed by cervical dislocation and exsanguinated. The lungs were quickly removed, snap-frozen in liquid nitrogen, and stored at −80° until required. Membranes were prepared (using one whole lobe of lung) as previously described (33) and finally suspended in incubation buffer, which consisted of 50 mM Tris (pH 7.4, 25°) containing 3 mM $MnCl_2$, 0.02% bovine serum albumin, and peptidase inhibitors.

Initially, the effect of peptidase inhibitors on BHSP, INKA, and BHELE binding was determined by preincubating membrane suspension (3–4% tissue, based on original wet weight) in the presence and absence of phosphoramidon (1 nM to 10 μM), enalapril (1 μM), leupeptin (4 μg/ml), chymostatin (4 μg/ml), or bacitracin (40 μg/ml). Radioiodinated tachykinin (~50 pM) was then added to all tubes, and the reaction was allowed to proceed for 15, 30, and 45 min for BHSP, INKA, and BHELE, respectively. Nonspecific binding was defined in replicate tubes with 1 μM unlabeled SP, NKA, or eledoisin, respectively. In subsequent studies with BHSP and INKA, phosphoramidon (10 μM), leupeptin (4 μg/ml), and chymostatin (4 μg/ml) were included. For experiments with BHELE, phosphoramidon (10 μM) and chymostatin (4 μg/ml) were used.

For "cold" saturation experiments (34), membranes were incubated with a constant amount (~50 pM) of BHSP, INKA, or BHELE and increasing concentrations of the corresponding unlabeled peptide (SP, NKA, or eledoisin, respectively). Incubation times were 15, 30, and 45 min for BHSP, INKA, and BHELE, respectively. In competition studies, 8–12 concentrations of unlabeled tachykinin or analog were coincubated with approximately 50 pM BHSP, INKA, or BHELE. Nonspecific binding was defined as before.

All binding reactions were terminated by filtration over Whatman GF/B glass fibers and washing with 5 × 3 ml of 50 mM Tris buffer, pH 7.4 at 4°, containing 3 mM $MnCl_2$. Filters were presoaked overnight at 4° in 0.1% polyethyleneimine (BHSP) or 0.5% bovine serum albumin (INKA and BHELE). Filter-bound radioactivity was counted in a Packard Minaxi Auto γ counter, at 79% efficiency.

Data analysis. Raw dpm were processed by the computer program EBDA (34), in conjunction with the nonlinear iterative curve-fitting program LIGAND (35). The F test was used to determine whether the data fitted a multiple-site model rather than a single-site model (35). A p value of <0.05 was considered to be statistically significant. Data are expressed as the geometric mean ± the approximate standard error (35) or the arithmetic mean ± standard error.

Results

Effect of peptidase inhibitors on binding of BHSP, INKA, and BHELE. Chymostatin, leupeptin, and bacitracin had minimal effect on specific binding of BHSP, INKA, and BHELE. The endopeptidase inhibitor phosphoramidon increased specific binding of all three radioligands, in a concentration-dependent manner (Fig. 1). Phosphoramidon (10 μM) was included in all subsequent experiments. Chymostatin, leupeptin, and enalapril did not alter specific binding, and bacitracin increased nonspecific binding. Chymostatin (4 μg/ml) and leupeptin (4 μg/ml) were also included, as a precaution against possible destruction of unlabeled tachykinins in competition studies.

Fig. 2 shows the time courses of specific binding (in the presence of 10 μM phosphoramidon) to guinea pig lung membranes for the three radioligands. Equilibrium was reached after 10, 30, and 45 min for BHSP, INKA, and BHELE, respectively, and was stable for at least 20 min thereafter.

Saturation studies. In "cold" saturation studies, specific binding of BHSP and INKA was saturable at approximately 5 nM, whereas BHELE binding appeared to saturate above 5 nM (Fig. 3A). Scatchard plots of saturation data for all three radioligands were generally linear, showing specific binding to

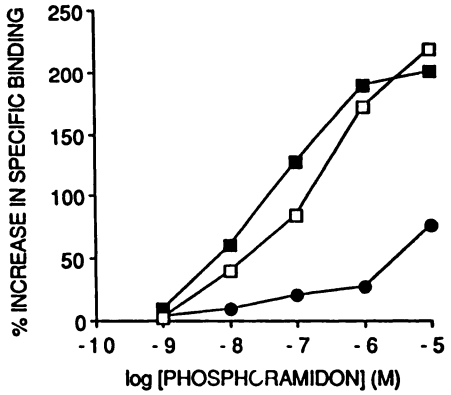

Fig. 1. Effect of phosphoramidon on the binding of BHSP (●), INKA (■), and BHELE (□) to guinea pig lung membranes. One representative curve is shown for each radioligand. Each *point* represents the percentage increase in specific binding (duplicate determinations) at each concentration of phosphoramidon, relative to incubations in the absence of phosphoramidon.

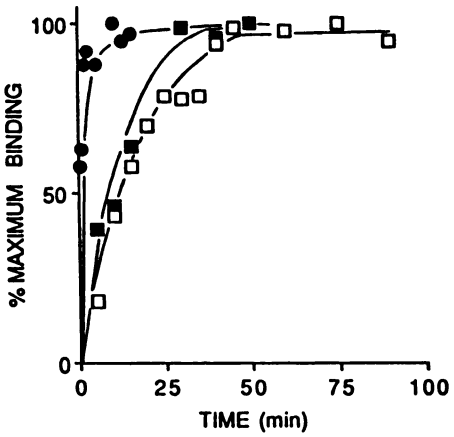

Fig. 2. Representative association time-courses of specific BHSP (●), INKA (■), and BHELE (□) binding to guinea pig lung membranes. Binding is expressed as a percentage of maximum binding at approximately 50 pM of each radioligand. Points at 35 and 40 min for BHSP and INKA are superimposed.

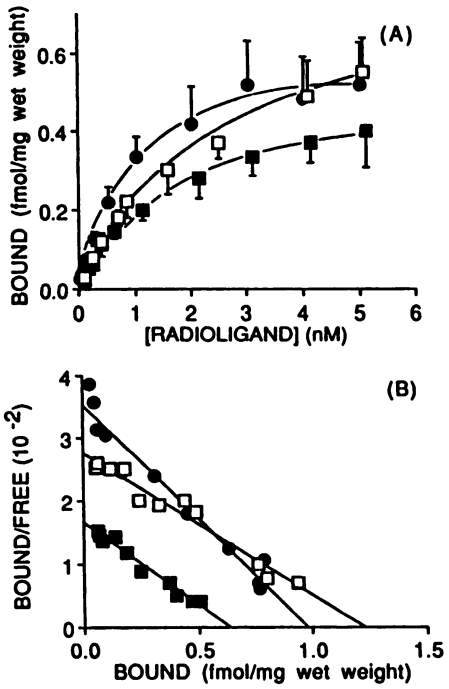

Fig. 3. A, Saturation curves of BHSP (●), INKA (■), and BHELE (□) binding to homogenates of guinea pig lung. Each *point* is the mean ± standard error of four determinations at each concentration of radioligand. B, Representative Scatchard plots of data, showing linear relationship between bound/free and bound radioligand.

a single class of sites (Fig. 3B). (Although several saturation experiments showed evidence of two sites, LIGAND analysis failed to give statistically significant two-site fits.) Table 1 shows the binding constants obtained. BHSP bound with greater affinity than INKA and BHELE. The mean B_{max} values for the three radioligands were not significantly different (analysis of variance). Hill coefficients (n_H) were close to unity, showing that no cooperativity was involved in binding (Table 1).

One-site analysis of agonist competition studies. The binding characteristics of BHSP, INKA, and BHELE to guinea pig lung homogenates were further examined in competition

TABLE 1

Binding constants from cold saturation studies for BHSP, INKA, and BHELE binding to membranes from guinea pig lung

Values for equilibrium dissociation constant (K_D) and maximum density of binding sites (B_{max}) were generated by LIGAND. Hill coefficients (n_H) were generated by EBDA and are the arithmetic mean ± standard error of four experiments.

Radioligand	K_D	B_{max}	n_H
	nM	*fmol/mg of wet weight*	
BHSP	0.96 ± 0.15	0.65 ± 0.07	0.98 ± 0.01
INKA	1.61 ± 0.26	0.51 ± 0.05	0.99 ± 0.01
BHELE	1.98 ± 0.12	0.79 ± 0.17	0.99 ± 0.01

studies using endogenous tachykinins and analogs. Table 2 shows the slope factors (pseudo-Hill coefficients) and LIGAND values for equilibrium dissociation constants (K_D values) of competitors for binding of the three radioligands, using a one-site binding model. The order of potency of agonists for BHSP binding was SP > [Sar^9,Met(O$_2$)^{11}]-SP > SP methyl ester > NPγ > NKA ≈ NKB ≈ kassinin > eledoisin > scyliorhinin II > NPK. An almost identical rank potency order was observed against INKA binding, SP ≫ [Sar^9,Met(O$_2$)^{11}]-SP > NPγ ≈ SP methyl ester ≈ NKA ≥ NKB > kassinin ≥ eledoisin > scyliorhinin II ≥ NPK, suggesting that both BHSP and INKA labeled sites with the general characteristics of NK1 receptors. There is a strong correlation between the pK_D (−log K_D) values for tachykinins and analogs for BHSP and INKA binding sites (r = 0.98) (Fig. 4). A slightly different potency order was observed against BHELE, i.e., SP ≥ [Sar^9,Met(O$_2$)^{11}]-SP > eledoisin ≥ NKA ≈ NPγ ≈ SP methyl ester ≥ kassinin > NPK ≈ NKB ≥ scyliorhinin II, although the binding was essentially to an NK1 site. Inhibition constants against INKA and BHELE binding were always lower than the corresponding data with BHSP binding (Table 2; Fig. 5), a phenomenon we have previously observed when radioligands bind to "nonpreferred" receptors (33, 36, 37).

Two-site analysis of agonist competition studies. In contrast to our saturation data, competition data for several tachykinins could be resolved into distinct high and low affinity components (Table 2). Against BHSP binding, a two-site model yielded a significantly better ($p < 0.01$) fit for SP, [Sar^9,Met(O$_2$)^{11}]-SP, and eledoisin competition data, compared with a one-site model. Super-high affinity and lower affinity sites were present in the ratio of approximately 2:3 for all three competitors.

Against INKA binding, SP, [Sar^9,Met(O$_2$)^{11}]-SP, NKB, and NPγ gave significantly better ($p < 0.05$) two-site fits. The ratio of super-high to lower affinity sites was 1:1 for SP and [Sar^9,Met(O$_2$)^{11}]-SP and 9:1 for NKB and NPγ. Against BHELE binding, SP, [Sar^9,Met(O$_2$)^{11}]-SP, and scyliorhinin II also gave significantly better ($p < 0.05$) two-site fits. High affinity BHELE binding sites greatly outnumbered low affinity sites (9:1).

Antagonist competition studies. MDL 29,913 was the most potent antagonist tested but was a very weak competitor, compared with agonists. Inhibition K_D values were 2.1, 1.2, and 1.5 μM against BHSP, INKA, and BHELE binding, respectively (Table 2). A single-site model was the most suitable to describe competition data for MDL 29,913 against the binding of all three radioligands. The structurally similar antagonist L-659,877 yielded inhibition K_D values of >10, 7.9, and 3.3 μM for BHSP, INKA, and BHELE, respectively; however, these values

TABLE 2

Competition by tachykinins and analogs against BHSP, INKA, and BHELE binding in guinea pig lung membranes

Values for equilibrium dissociation constant (K_D) were generated by LIGAND and are the geometric mean ± standard error (approximate) of three to six curves analyzed simultaneously (35). Slope factors were generated by EBDA and are the arithmetic mean ± standard error. K_D values for the high (H) and low (L) affinity components are shown where competition data yielded significantly better ($p < 0.05$) two-site fits. The percentages of high and low affinity sites are shown in parentheses.

Competitor	BHSP		INKA		BHELE	
	Slope factor	K_D	Slope factor	K_D	Slope factor	K_D
		nM		*nM*		*nM*
SP	0.87 ± 0.09	0.012 ± 0.004 (H) (40) 0.72 ± 0.13 (L) (60)	0.40 ± 0.04	0.0004 ± 0.0013 (H) (45) 0.035 ± 0.020 (L) (55)	0.93 ± 0.11	0.08 ± 0.01 (H) (71) 689 ± 191 (L) (29)
[Sar^9,Met(O$_2$)^{11}]-SP	0.97 ± 0.05	0.43 ± 0.0013 (H) (37) 15.1 ± 4.01 (L) (63)	0.67 ± 0.03	0.009 ± 0.004 (H) (48) 0.47 ± 0.11 (L) (52)	0.86 ± 0.04	0.08 ± 0.02 (H) (89) 10 ± 11 (L) (11)
SP methyl ester	0.87 ± 0.05	3.69 ± 0.62	0.69 ± 0.13	0.93 ± 0.17	0.81 ± 0.05	1.29 ± 0.12
NPγ	0.88 ± 0.04	6.07 ± 1.8	0.71 ± 0.06	0.63 ± 0.13 (H) (89) 18 ± 23 (L) (11)	0.92 ± 0.03	1.15 ± 0.12
NKA	0.84 ± 0.04	17.9 ± 4.6	0.83 ± 0.09	1.19 ± 0.11	0.84 ± 0.14	1.11 ± 0.02
NKB	0.76 ± 0.07	20.9 ± 3.4	0.73 ± 0.06	1.14 ± 0.32 (H) (89) 86 ± 115 (L) (11)	0.65 ± 0.03	3.53 ± 0.68
Kassinin	0.85 ± 0.01	21.3 ± 0.79	0.89 ± 0.02	3.22 ± 0.70	0.97 ± 0.06	1.62 ± 0.13
Eledoisin	0.83 ± 0.13	4.7 ± 4.7 (H) (42) 74 ± 49 (L) (58)	0.72 ± 0.06	4.06 ± 1.00	1.03 ± 0.09	0.97 ± 0.07
Scyliorhinin II	0.90 ± 0.07	42.2 ± 6.8	0.72 ± 0.03	7.55 ± 2.00	0.91 ± 0.01	0.63 ± 0.13 (H) (85) 18 ± 23 (L) (15)
NPK	1.15 ± 0.10	90.1 ± 2.1	0.90 ± 0.12	9.76 ± 1.3	1.03 ± 0.03	3.37 ± 0.24
[MePhe^7]-NKB	—[a]	—	—	—	0.78 ± 0.11	17 ± 31
[Pro^7]-NKB	—	—	—	—	0.97 ± 0.09	285 ± 33
Senktide	ND[b]	>10,000	—	—	0.92 ± 0.07	1,060 ± 140
[Lys^5,MeLeu^9,Nle^{10}]-NKA(4–10)	0.61 ± 0.06	549 ± 127	0.92 ± 0.04	55.6 ± 8.9	0.84 ± 0.07	55.9 ± 12.5
MDL 29,913	1.32 ± 0.05	2,060 ± 330	1.12 ± 0.04	1,180 ± 89	0.96 ± 0.10	1,470 ± 310
MEN 10207	0.91 ± 0.20	2,936 ± 554	0.76 ± 0.15	1,970 ± 51	ND	>10,000
L-659,877	ND	>10,000	0.73 ± 0.04	7,900 ± 520	0.85 ± 0.01	3,270 ± 880

[a] —, not investigated.
[b] ND, negligible inhibition of binding at 10 μM.

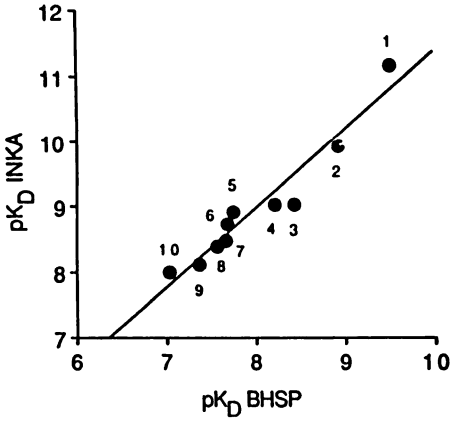

Fig. 4. Correlation between the pK_D (−log K_D) of tachykinins and analogs in competing for BHSP and INKA binding in guinea pig lung (one-site analysis). The *line* was fitted by linear regression, with a correlation coefficient of 0.98. Competitors are as follows: *1*, SP; *2*, [Sar^9,Met(O$_2$)^{11}]-SP; *3*, SP methyl ester; *4*, NPγ; *5*, NKA; *6*, NKB; *7*, kassinin; *8*, eledoisin; *9*, scyliorhinin II; and *10*, NPK.

are interpolated, because binding of BHSP, INKA, and BHELE was inhibited by only 50–60% at the highest concentration used (10 μM). The NK2 subtype-selective analog MDL 28,564 produced negligible inhibition at up to 20 μM.

Effect of Gpp(NH)p on BHSP binding. The nonhydrolyzable GTP analog Gpp(NH)p completely inhibited the binding of BHSP at a concentration of approximately 100 μM (K_i, inhibition constant, 114 ± 32 nM; slope factor, 0.36 ± 0.02; three experiments). This observation suggests that the receptors labeled by radioiodinated SP in guinea pig lung are linked to a guanine nucleotide-binding regulatory protein.

Discussion

Binding of radioligands to NK1 receptors in guinea pig lung. In this study, we find that the most potent competitors against BHSP, INKA, and BHELE binding in guinea pig lung are SP and the NK1-selective agonist [Sar^9,Met(O$_2$)^{11}]-SP. This strongly suggests binding to NK1 receptors. We have previously shown that the NK2-preferring INKA and NK3-preferring BHELE bind to receptors other than NK2 and NK3 receptors in peripheral tissues, because radioligands derived from endogenous tachykinins are not highly selective (33, 57) (see Ref. 15). However, our competition binding data suggest the existence of additional binding sites or receptors (see below). The high affinity site is likely to be the NK1 receptor, whereas the identity of the lower affinity site(s) is unclear. Possibilities for the low affinity site(s) include differential affinity states of the NK1 receptor, "classical" NK2 receptors or subtypes of the NK2 receptor, NK3 receptors, or a novel lung-specific tachykinin receptor. The level of binding for the three radioligands was highly dependent on the presence of the enkephalinase inhibitor phosphoramidon. This finding is in agreement with functional studies showing that enkephalinase inhibitors increase the potency of tachykinins in contracting respiratory smooth muscle (13, 28, 38, 39) and stimulating mucus secretion (40). The enzyme appears to be located mainly in the respiratory epithelium, and removal of the epithelium or inclusion of phosphoramidon leads to comparable increases in the potency of tachykinins (13, 28).

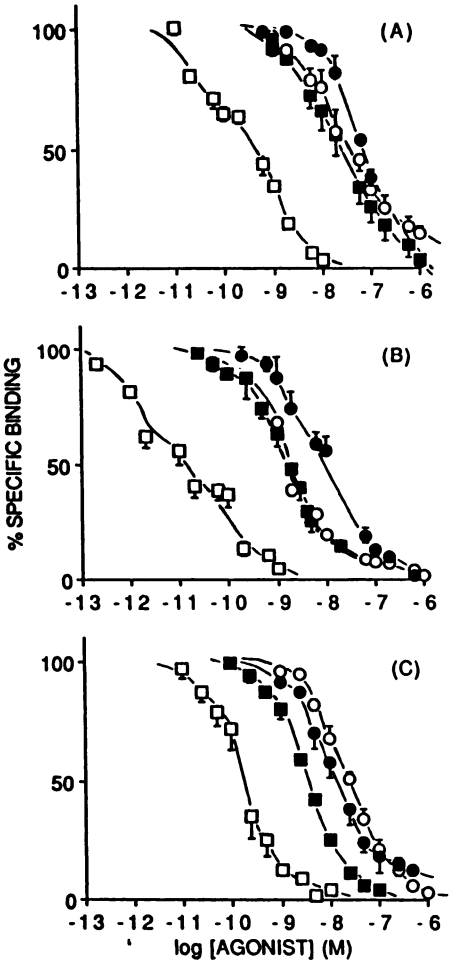

Fig. 5. Competition curves for SP (□), NKA (■), NKB (○), and NPK (●) against BHSP (A), INKA (B), and BHELE (C) binding to homogenates of guinea pig lung. Each *point* represents the mean ± standard error of three to six determinations.

There were 10–40-fold differences in K_D values for all competitors against BHSP, INKA, and BHELE binding. However, there was a strong correlation between the affinities of tachykinins and analogs for BHSP and INKA binding, suggesting that these radioligands bind to similar populations of sites. We have previously observed that some competitors yield lower IC_{50} values (concentration of competitor that inhibits binding by 50%) when competing against unselective radioligands at nonpreferred receptors, compared with IC_{50} values produced at preferred receptors (33, 57). For all radioligands, competition curves for SP and the selective NK1 agonist [Sar^9,Met(O$_2$)^{11}]-SP could be resolved into two components, representing high and low affinity binding sites. These data could be interpreted as a super-high and (normally) high state of the NK1 receptor or, less likely, as a high and a low affinity state of the NK1 receptor. The nonhydrolyzable GTP analog Gpp(NH)p, at a concentration of 100 μM, completely inhibited BHSP binding. This indicates that NK1 receptors in guinea pig lung are coupled to a guanine nucleotide-binding regulatory protein. Morishima *et al.* (41) reported a super-high affinity binding site for [^{3}H]SP in rat brain, which was sensitive to GTP and analogs. However, if the high and low affinity sites for BHSP represent GTP-regulated states of the NK1 receptor, then all agonists would have produced biphasic competition profiles.

NK1 receptors are found in several different locations in the respiratory tract. Although NKA and NKA-related peptides are more potent than SP and NK1-selective agonists in contracting bronchial smooth muscle, recent studies, including those from our laboratory, have demonstrated the existence of NK1 receptors mediating contraction of respiratory smooth muscle in the guinea pig (28, 42–44), although not in the human (13, 45). Our binding data support earlier autoradiographic studies, where dense binding of BHSP has been observed on airway smooth muscle, with binding also found on tracheal glands and the smooth muscle of large pulmonary blood vessels (31, 46, 47). It is not clear whether these differentially located NK1 receptors are a biochemically homogeneous population. Preliminary evidence for different NK1 receptor subtypes in rat brain and submandibular gland has been presented (48). Two distinct groups of NK1 receptors in lung might explain our finding of high and low affinity binding sites.

Binding of radioligands to other tachykinin receptors in guinea pig lung. Numerous functional studies have demonstrated that NKA is a more potent contractile agent than SP in bronchial smooth muscle, suggesting the existence of NK2 receptors on smooth muscle in the guinea pig respiratory tract (13, 23, 24, 43). In the present study, the inability of INKA to detect binding sites corresponding to NK2 receptors is in striking contrast to these functional studies. Recent evidence from several laboratories, based on the use of new selective NK2 antagonists and analogs, suggests that NK2 subtypes may exist. Our observation that INKA binds (at least partly) to NK1 receptors confirms our previous findings with INKA in sections of guinea pig lung (31), although INKA binds with high affinity to classical NK2 receptors in, for example, hamster, rat, and dog urinary bladder and rat vas deferens (30, 33, 49, 50). The present study strongly suggests that the tachykinin receptors mediating bronchial smooth muscle contraction are different from the classical NK2 receptors in the aforementioned tissues, in that they do not appear to be able to interact with INKA. This may be due to steric hindrance produced by the large iodine atom on the amino-terminal histidine.

Further evidence against the possibility of classical NK2 receptors representing the lower affinity site in our study is our finding that the recently described NK2 classical subtype-selective antagonists L-659,877 and MDL 29,913 (19, 51) were extremely weak competitors, although they have high affinity for INKA binding sites in hamster bladder (20, 49). Similarly, here the NK2 receptor-preferring NPK and the NK2-selective analog [Lys^5,MeLeu^9,Nle^{10}]-NKA(4–10) had very low affinity for BHSP binding sites. In contrast, [Lys^5,MeLeu^9,Nle^{10}]-NKA(4–10) has high affinity for NK2 receptors in rat duodenum (52), and NPK is approximately equipotent with NKA and NPγ in competing for INKA binding in homogenates of hamster bladder and rat vas deferens, fundus, and bladder (20, 53).[1] Thus, apart from NKA and NPγ, all NK2-preferring or NK2-selective agonists and antagonists show negligible affinity for tachykinin binding sites in guinea pig lung. In the isolated guinea pig hilus bronchus, we find that NPγ is considerably more potent than NKA and NKB but that all three agonists are antagonized by MDL 29,913 (pA_2, 7.0 against NKA) (43, 44).

The possibility that a novel type of tachykinin receptor

[1] C. J. Mussap and E. Burcher, unpublished data.

(designated NK4) might exist in guinea pig trachea was first proposed by McKnight *et al.* (25). Suggestions that McKnight's data can be explained by a combination of NK1 and NK2 receptors have been advanced (24). The NK4 receptor, which may represent the lower affinity component in our study, could in fact be a subtype of the NK2 receptor. Other workers (20–22, 51) have developed selective antagonists (e.g., MEN 10207) that are weak at classical NK2 receptors (including hamster trachea) and prefer the subtype found in, for example, rabbit pulmonary artery, guinea pig trachea, bovine stomach, and SKLKB82#3 cells (which express the recently cloned NK2 (substance K) receptor from bovine stomach). However, MEN 10207 was a very weak competitor in our study, although it has a pA_2 of 7.2 in functional studies in guinea pig bronchus (22). MEN 10297-preferring NK2 receptor subtypes may, therefore, exist in this tissue, although they are not detectable using these three iodinated ligands.

The low affinity component of binding, detected in competition experiments with BHELE, might possibly represent the NK3 receptor. The rank potency order of tachykinins for inhibition of BHELE binding suggested binding to NK1 receptors, as for BHSP and INKA. Thus, the present study supports previous conclusions that BHELE is an unselective radioligand. BHELE binds with high affinity to NK3 receptors in rat brain (54, 55, 56), but in peripheral tissues BHELE has been shown to bind to NK2 sites in rat, mouse, and hamster bladder and rat duodenum (33, 57) and to NK1 sites in guinea pig bladder and rat salivary gland (33, 36). Our linear Scatchard plots show BHELE binding to a single class of sites. However, competition curves for SP, [Sar^9,$Met(O_2)^{11}$]-SP, and the NK3-preferring scyliorhinin II against BHELE could be resolved into two sites. The more numerous, high affinity component probably represents NK1 receptors. The lower affinity sites may be of the NK3 type, on the basis of the biphasic inhibition by scyliorhinin II (58). However, evidence against a population of lung NK3 sites is the extreme weakness of the highly NK3-selective senktide as a competitor against BHELE and BHSP binding and the lack of specific binding of ^{125}I Bolton-Hunter-scyliorhinin II in guinea pig lung homogenates (56).

Conclusion. In conclusion, the present radioligand binding studies suggest that the guinea pig lung contains a mixed tachykinin receptor population, of which a high affinity NK1-like site appears to form a large proportion. Whether the other binding site is a lower affinity form of the NK1 receptor, a subtype of the NK2 receptor, or a new class of tachykinin receptor has yet to be determined. The NK1, NK2, and NK3 tachykinin receptors have now been cloned (16–18, 59, 60), and recent reports suggest that the human tracheal NKA receptor (18) has some differences from NK2 receptors in other species (59, 60). This "human" receptor could be similar to the NK2 receptor mediating contraction of human (45) and guinea pig (43) bronchial smooth muscle and could, perhaps, be one of the low affinity binding sites described here in guinea pig lung.

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