Biochem. J. (1988) 252, 545–551 (Printed in Great Britain)

Solubilization and characterization of substance P-binding sites from chick brain membranes

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Sites binding monoiodinated-Bolton–Hunter-reagent-labelled substance P were solubilized from 1-day-old-chick brain membrane by using non-ionic detergents (1% digitonin/1% n-octyl glucoside) and a high concentration of NaCl (0.5 M). The solubilized preparation retained the pharmacological properties of the high-affinity binding sites found in the native membrane. The high density of specific binding sites (~ 2 pmol of binding sites/mg of protein) suggests that the chick brain membranes may be a useful source for the purification of the substance P-binding sites.

INTRODUCTION

Substance P (SP) is an undecapeptide (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2) belonging to a family of biologically active peptides (tachykinins) that share a common conserved *C*-terminal sequence (Ersparmer, 1981). SP has a broad spectrum of effects which may be mediated through high-affinity receptor sites on peripheral tissues and the central nervous system (Too & Hanley, 1984).

Despite the large body of information obtained from binding studies *in vitro* using labelled SP, very little is known of the molecular nature of this receptor. One of the fundamental approaches to the understanding of the biochemistry of the SP receptor is to solubilize it in a functional state, a prerequisite for the purification of the receptor. Earlier attempts by various workers to solubilize the receptor in a functional state have met with some success, although in low yield (Maruyama, 1986; Payan *et al.*, 1986). In the present paper we report the characterization of a novel enriched source of SP-binding sites and their solubilization in high yield with retention of the pharmacological properties seen in the membrane-bound state. The resulting preparation is stable to freezing and prolonged storage, thus enabling further characterization and purification.

MATERIALS AND METHODS

Monoiodinated Bolton–Hunter reagent was obtained from Amersham International; SP, SK, NK, Phy, Ele, and Kass were purchased either from Bachem or Cambridge Research Biochemicals; bacitracin, soya-bean and chicken-egg trypsin inhibitors, benzamidine hydrochloride, leupeptin, chymostatin, PEI, Triton X-100 and Lubrol PX were obtained from Sigma Co.; digitonin was from BDH; CHAPS and deoxycholate were obtained from Pierce Co. GF/B filters were obtained from Whatman Co.

Preparation of ^{125}I-BHSP

Monoiodinated Bolton–Hunter reagent (1 mCi) was flushed with N_2 to evaporate off the solvent. A portion (20 μl) of a solution (0.2 mg/ml) of substance P in 0.1 M-sodium borate, pH 8.5, was then added to the reagent. The reaction was allowed to proceed for 60 min on ice and the mixture subsequently injected on to a C_{18} μBondapak column (Waters). The products were eluted with a linear gradient of from 5 to 95% acetonitrile in 0.1% trifluoroacetic acid in 40 min. Aliquots (2 μl) of each fraction (1 ml/fraction) were collected and the radioactivity (γ-radiation) counted. The radioactivity profile obtained was compared with that of the elution profile of monoiodinated ^{125}I-BHSP. The fractions containing monoiodinated ^{125}I-BHSP were pooled, and mercaptoethanol (5 mM) was added to prevent oxidation of the peptide during storage. The radioligand was used within 2 months after preparation and was stored at −80 °C. The specific radioactivity of the radioligand as shown in each experiment was calculated from the radioactivity after correction for decay. The radiopurity after 2 months was greater than 70%.

Preparation of membranes

Chicks (1 day old) were decapitated. The brains (minus cerebella) were rapidly frozen in liquid N_2 and stored at −80 °C until use.

To obtain membranes, frozen brains were first thawed in 5 vol. (v/v) of ice-cold Tris/HCl (50 mM, pH 7.5) buffer containing 10% (w/v) sucrose and proteinase inhibitors (0.01% bacitracin, 0.002% soya-bean trypsin inhibitor, 0.002% chicken-egg trypsin inhibitor and 1 mM-benzamidine hydrochloride), followed by several homogenizations with a Brinkman Polytron PT-10 instrument at maximal setting. The homogenates were centrifuged at 1086 *g* ($r_{av.}$ 10.8 cm) for 20 min, and the resulting supernatants were re-centrifuged at 9770 *g* ($r_{av.}$ 10.8 cm) for another 20 min. The pellets were then resuspended by homogenization at setting 6 on the

Abbreviations used: SP, substance P; SK, substance K; NK, neuromedin K; Phy, physalaemin; Ele, eledoisin; Kass, kassinin; ^{125}I-BHSP, monoiodinated-Bolton–Hunter-reagent-labelled substance P; PEI, polyethyleneimine; PEG, poly(ethylene glycol); NEM, *N*-ethylmalaeimide; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]propanesulphonic acid.

Polytron for 5 s in 10 vol. (v/v) of Tris/HCl (50 mM, pH 7.5), containing 120 mM-NaCl, 300 mM-KCl, 10 mM-EDTA. The homogenates were incubated on ice for 30 min with occasional agitation and subsequently centrifuged at 40000 *g* ($r_{av.}$ 10.8 cm) for 20 min. The pellets obtained were then resuspended in 10 vol. (v/v) of ice-cold Tris/HCl (50 mM, pH 7.5) and re-centrifuged at 40000 *g* for 20 min. This washing procedure was repeated twice more and the pellets were finally resuspended in 1 vol. (v/v) of ice-cold Tris/HCl (50 mM, pH 7.5). Aliquots (1 ml) were centrifuged at 14000 *g* (4 °C) in a Beckman bench-top centrifuge for 20 min. The supernatants were discarded, and the pellets frozen in liquid N_2 and stored at −80 °C for no longer than 1 month.

Solubilization procedure

Membrane-bound binding sites were solubilized by resuspending the frozen membranes in sodium Tricine (50 mM, pH 7.5, 22 °C) to final concentrations at 6–8 mg of protein/ml. The suspensions were preincubated with 10 mM-$MnCl_2$, 0.002% soya-bean trypsin inhibitor, 0.002% chicken-egg trypsin inhibitor, 1 mM-benzamidine hydrochloride and 0.5 M-NaCl, for 30 min at 30 °C. The suspension was then placed on ice for 5–10 min before incubating with a combination of detergents (1% n-octyl glucoside and 1% digitonin; protein/detergent ratio, 1:3, w/w). Solubilization was carried out at 4 °C with constant and gentle agitation for 30 min, and the suspensions were centrifuged at 105000 *g* ($r_{av.}$ 63.4 cm) for 60 min at 4 °C. The resulting supernatants were collected, pooled, and then subjected to filtration on a 22 μm-pore-size filter. The filtrates were used for soluble-binding-site assay as described below.

Binding assays

Membranes were preincubated with 50 mM-sodium Tricine supplemented with 0.004% bacitracin, 0.004% bovine serum albumin, 0.0002% chymostatin, 0.0004% leupeptin, 10 mM-$MnCl_2$ for 30 min at 22 °C. Unlabelled peptides and ^{125}I-BHSP were then added and the incubations terminated after 30 min at 22 °C by filtration through GF/B filters presoaked in 0.5% PEI (> 3 h at 22 °C), followed by four washes with ice-cold sodium Tricine buffer (50 mM, pH 7.5). The filters were then measured for radioactivity with 70% counting efficiency.

Soluble binding sites were assayed similarly at protein concentrations of 0.1–0.6 mg/ml.

Protein concentrations were determined by the mini-Lowry method (Markwell *et al.*, 1978), after precipitation with 9.2% (w/v) trichloroacetic acid. BSA was used as the standard.

To evaluate the effects of thiol reagent (NEM) and high temperature on the binding of ^{125}I-BHSP to soluble and membrane preparations, the membranes were first thawed in sodium Tricine (pH 7.5, 50 mM), and portions (4–7 mg of protein/ml) were either incubated with NEM (10 mM) at 32 °C for 30 min or boiled for 15 min. The incubations were terminated by centrifugation at 10000 *g* for 30 min at 4 °C. The pellets were then resuspended in 1 ml of Tris/HCl, pH 7.5 (50 mM) and re-centrifuged for 15 min at 10000 *g* (22 °C). The final pellets were resuspended in 0.6 ml of sodium Tricine, and triplicate 50 μl portions were taken for membrane binding. The remaining membrane suspensions were solubilized as described above.

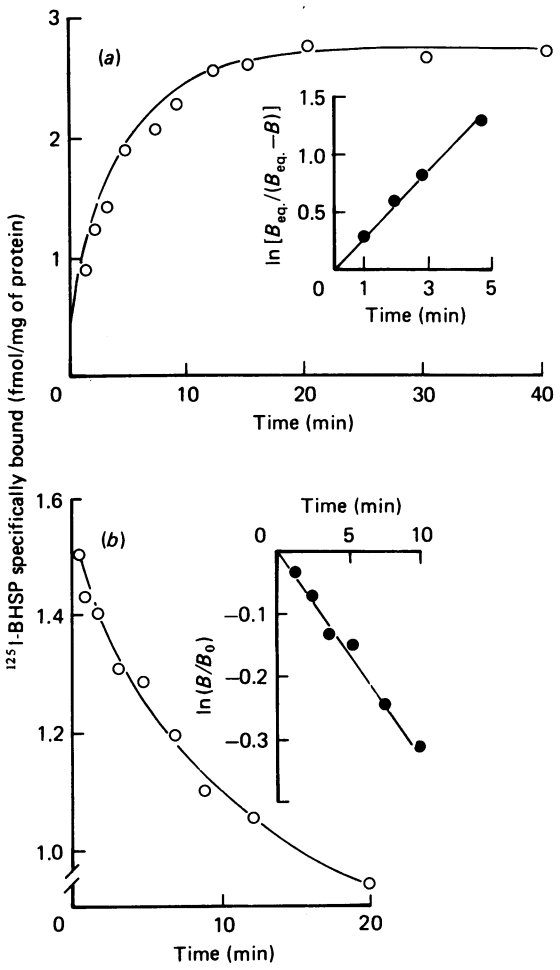

Fig. 1. Association and dissociation time course of ^{125}I-BHSP binding to chick brain membrane

(*a*) Association time course. Membrane (0.38 mg of protein/ml) was preincubated with inhibitors in the presence of 10 mM-$MnCl_2$ for 30 min. Radioligand (20 pM, ~ 20000 c.p.m.) was then added and the incubation terminated by filtration at various times as indicated. Non-specific binding was determined simultaneously for all the time points. The results obtained from specific binding were plotted as $\ln[B_{eq.}/(B_{eq.}-B)]$ versus time (inset), where $B_{eq.}$ is the concentration of ligand bound at equilibrium and B is that bound at the time indicated. (*b*) Dissociation time course. The results obtained from specific binding were plotted as $\ln(B/B_0)$ versus time (inset), where B_0 is the amount of ligand bound before dissociation. Both association and dissociation time courses were carried out at 22 °C. Each point was the mean for triplicate determinations, with an S.D. of less than 10%, and the experiment was repeated three times.

RESULTS

Pretreatment of filters

Pretreatment of the GF/B filters with 0.5% PEI greatly reduced the non-specific binding of radioligand (2% of added radioactivity as compared with 46% binding in the absence of pretreatment). More importantly, there was no displaceable site on the pretreated filters with a high concentration of unlabelled P (10^{-4} M). There were no significant differences in the amount of

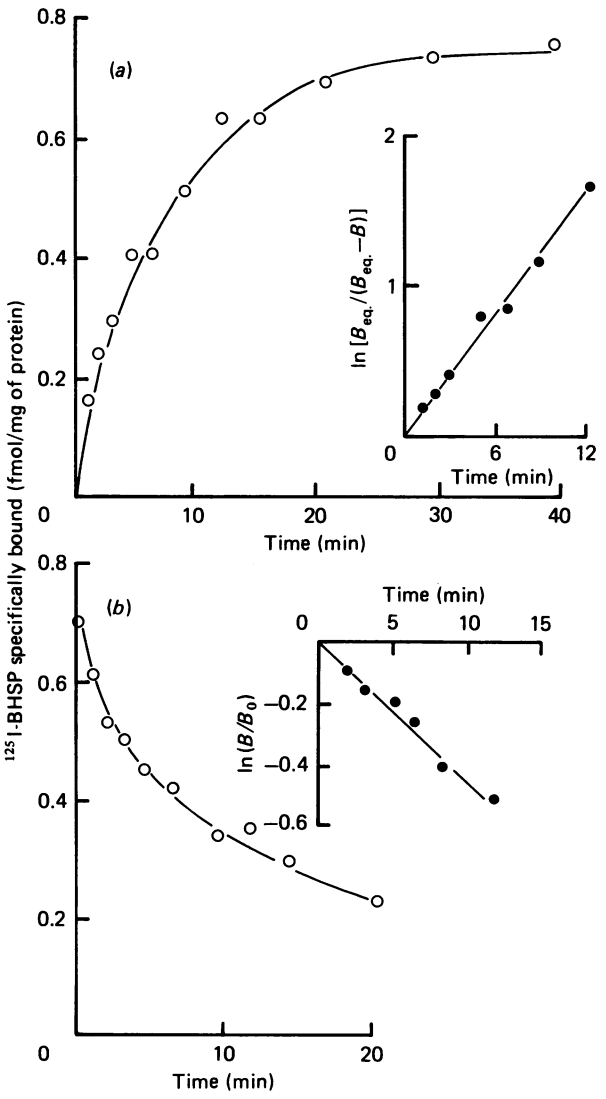

Fig. 2. Association and dissociation time courses of ^{125}I-BHSP binding to solubilized binding sites from chick brain membrane

(*a*) Association time course. Solubilized preparation (0.13 mg of protein/ml) was preincubated with inhibitors in the presence of 10 mM-$MnCl_2$ for 30 min. Radioligand (90 pM) was then added and the incubation terminated at the various times indicated. (*b*) Dissociation time course. Each point was the mean for triplicate determinations, with an S.D. of less than 10%, and the experiment was repeated three times.

^{125}I-BHSP bound by the soluble binding sites when assayed either by filtration through pretreated filters or by employing PEG precipitation (results not shown). Thus the former method was adopted to compare the characteristics of binding of ^{125}I-BHSP to the membrane-bound and soluble binding sites.

General properties of ^{125}I-BHSP binding

The binding of ^{125}I-BHSP to both the membrane-bound (Fig. 1*a*) and soluble binding sites (Fig. 2*a*) were time-dependent. The binding to membrane-bound binding sites reached a plateau in about 12 min, and that to soluble sites in 25 min, and they remained unchanged for

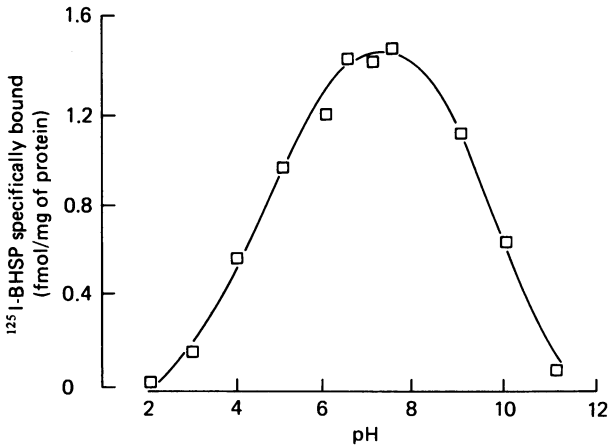

Fig. 3. pH-dependence of ^{125}I-BHSP binding to membrane

Frozen membrane was thawed and then suspended in 1 ml of Tricine buffer at the test pH. The membrane was allowed to equilibrate with the buffer for at least 15 min before centrifugation at 10000 *g* for 20 min. The pellet was then resuspended with the buffer and used directly for binding assays. HCl was used to adjust the pH of the Tricine buffer to below 4 and NaOH to adjust the pH to above 4. Each point was the mean of triplicates, with S.D. less than 10%, and the experiment was repeated three times at 22 °C.

up to 40 min. Thus incubation times of 30 and 35 min at 22 °C were adopted for routine analyses of membrane-bound and soluble binding sites respectively.

Within the time courses used, there was no detectable degradation of ^{125}I-BHSP by either the soluble or membrane bound sites (results not shown), as analysed by t.l.c. by the method of Lee *et al.* (1981).

The pH effect on the binding to membrane-bound sites showed a broad optimum between pH 6.5 and 8 (Fig. 3).

Membrane when extracted with either Triton X-100, Lubrol PX, CHAPS, deoxycholate or cholate at a concentration of 1% did not show any specific binding to ^{125}I-BHSP. Digitonin or n-octyl glucoside when used individually yielded extracts that bind ^{125}I-BHSP. However, the soluble extracts showed higher specific activities for the binding of ^{125}I-BHSP when the detergents were used as a mixture. The specific binding to the soluble sites remained unchanged even when filtered through a 0.22 μm-pore-size Millipore filter.

Treatment of membranes with NEM reduced the specific binding of ^{125}I-BHSP (17±4.4% of control; mean±S.E.M. for three separate experiments). Boiling for 15 min abolished specific membrane binding. Soluble preparations prepared from NEM-pretreated membranes showed little specific binding activity (0.9±0.6% of control; mean±S.E.M. for three separate experiments), and there was no observable specific binding activity when extraction was from membranes previously boiled for 15 min. The concentrations of protein extracted under these different conditions were similar (results not shown).

Storage at 4 °C for up to 4 days did not affect soluble binding significantly. The soluble binding sites retained activity after freezing in liquid N_2 and could be stored thereafter at −70 °C for as long as 2 months.

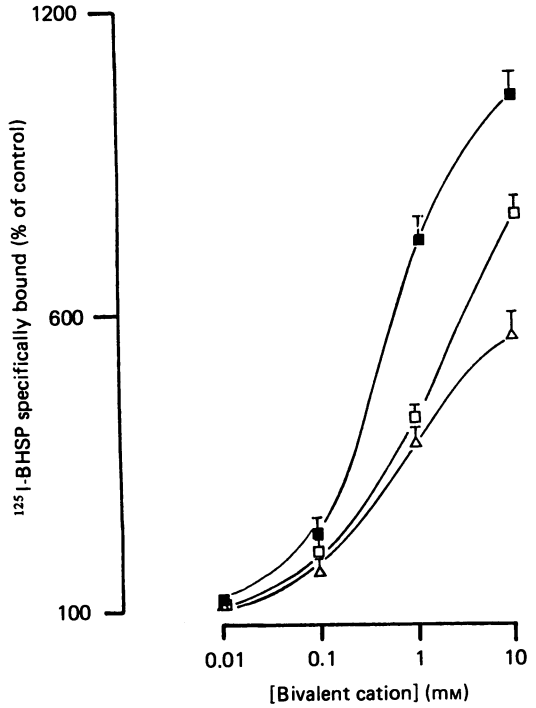

Fig. 4. Effects of bivalent cations on ^{125}I-BHSP (20 pM) binding to chick brain membranes

Mn^{2+} (■) was the most potent bivalent cation (as $MnCl_2$) in stimulating the specific binding to ^{125}I-BHSP and Ca^{2+} (△) was the least potent. Mg^{2+} (□, 10 mM) was intermediate between the above two bivalent cations in stimulating specific binding. In the absence of bivalent cations, the specific binding was 180 c.p.m. compared with 1737 c.p.m. (10 mM-$MnCl_2$), 1365 c.p.m. (10 mM-$MgCl_2$) and 795 c.p.m. (10 mM-$CaCl_2$). The results in the Figure were expressed as means ± S.E.M. for three separate experiments.

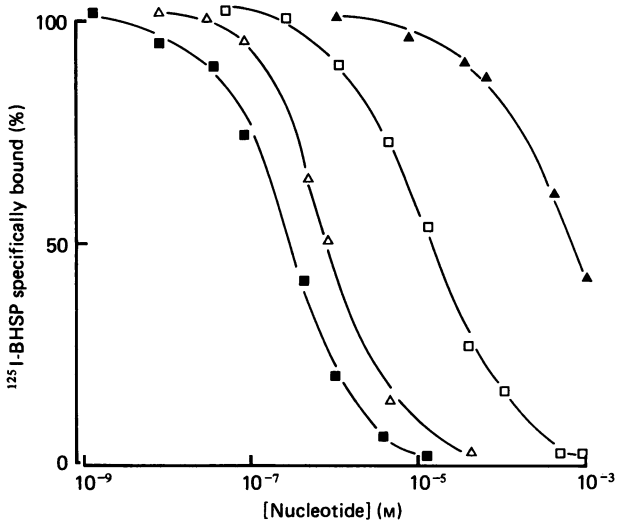

Fig. 5. Effects of guanine and adenine nucleotides on ^{125}I-BHSP (20 pM) binding to chick membrane in the absence of 10 mM-$MnCl_2$

The potency in displacing specific ^{125}I-BHSP binding was: guanosine 5′-[βγ-imido]triphosphate (■) > guanosine 5′-[β-thio]diphosphate (△) > GTP (□) < ATP (▲). Data represent the means of triplicates, with S.D. less than 10%; the experiment was repeated four times.

Table 1. Inhibition of ^{125}I-BHSP binding to membrane and soluble binding sites by nucleotides

The results are expressed as means ± S.E.M. for the number of experiments shown in parentheses. Abbreviations used: ND, not determined; GMP-PNP, guanosine 5′-[βγ-imido]triphosphate; GDPβS, guanosine 5′-[β-thio]diphosphate.

	IC_{50} (M)	
Nucleotide Sites ...	Membrane-bound	Soluble
ATP	$6.9(\pm 1.2)\times 10^{-4}$ (3)	$1.2(\pm 0.3)\times 10^{-5}$ (4)
GTP	$1.8(\pm 1.0)\times 10^{-5}$ (4)	$1.0(\pm 0.5)\times 10^{-7}$ (4)
GMP-PNP	$2.1(\pm 0.1)\times 10^{-7}$ (3)	$5.0(\pm 0.3)\times 10^{-8}$ (4)
GDPβS	$6.7(\pm 1.2)\times 10^{-7}$ (3)	ND

Effects of bivalent cations of ^{125}I-BHSP binding

With membrane assays the inclusion of bivalent-metal cations was found to increase the amount of specifically bound ^{125}I-BHSP (Fig. 4). Mn^{2+} was found to be the most potent cation, followed by Mg^{2+}, the least potent being Ca^{2+} (all as chloride salts).

Effects of guanine and adenine nucleotides

The specific binding of ^{125}I-BHSP to both soluble and membrane-bound binding sites was sensitive to the presence of nucleotide (Fig. 5), with the most potent analogue being guanosine 5′-[βγ-imido]triphosphate, the non-metabolized analogue of GTP. The adenine nucleotides were much less active. All of the guanine nucleotides at high concentrations were found to abolish specific binding of ^{125}I-BHSP completely. The IC_{50} (concentration causing 50% of maximum inhibition) values of these nucleotides were given in Table 1. These nucleotides were in all cases more potent in inhibiting the binding of ^{125}I-BHSP to the soluble than to the membrane-bound sites.

Kinetic binding data

The association time courses for the binding of ^{125}I-BHSP to the membrane-bound and soluble sites are shown in Figs. 1(*a*) and 2(*a*) respectively. The binding was performed at 22 °C in the presence of 10 mM-$MnCl_2$, pH 7.4. Under these conditions the non-specific binding (defined as that in the presence of 1 μM unlabelled SP) was about 12% of the total bound. The non-specific binding reached a maximal in about 5 min (results not shown), whereas the specific binding reached a plateau in about 12 min and 25 min for the membrane-bound and soluble sites respectively (Figs. 1*a* and 2*a*). As the total binding did not exceed 10% of the amount of ^{125}I-BHSP added, and there was no degradation of the radioligand detectable by t.l.c. (results not shown), the data were linearized according to the pseudo-first-order reaction (Kitabgi *et al.*, 1977). The slopes of the linearized data (insets to Figs. 1*a* and 2*a*) were equal to the $K_{ob.}$ (see below; L is ligand concentration, k_1 is the association constant and k_{-1} is the dissociation rate constant) and were determined to be 0.24 min^{-} and 0.139 min^{-1} for membrane-bound (Fig. 1*a*) and soluble binding sites

Table 2. Inhibition of specific binding to membrane and soluble sites

The binding of ^{125}I-BHSP was assayed at a concentration of 20 pM for membrane and 90 pM for soluble preparation. IC_{50} values were defined as the concentrations of peptides required to inhibit 50% of specific binding and were expressed as means ± S.E.M. for the numbers of experiments show in parentheses. Abbreviations and symbols: ND, not determined; *h*, Hill coefficient.

	Inhibition of ^{125}I-BHSP binding			
Sites ...	Membrane-bound		Soluble	
Peptide	IC_{50}	*h*	IC_{50}	*h*
SP	2.6±1.1 (6)	1.00	4.6±0.1 (4)	0.98
SK	446.0±41.0 (4)	0.95	237.0±40.0 (4)	0.95
NK	2250.0±250.9 (4)	0.87	2100.0±255.0 (4)	0.92
Kass	262.0±76.0 (3)	0.98	ND	
Phy	19.7±7.1 (3)	1.05	ND	
Ele	365.0±55.0 (3)	0.90	ND	

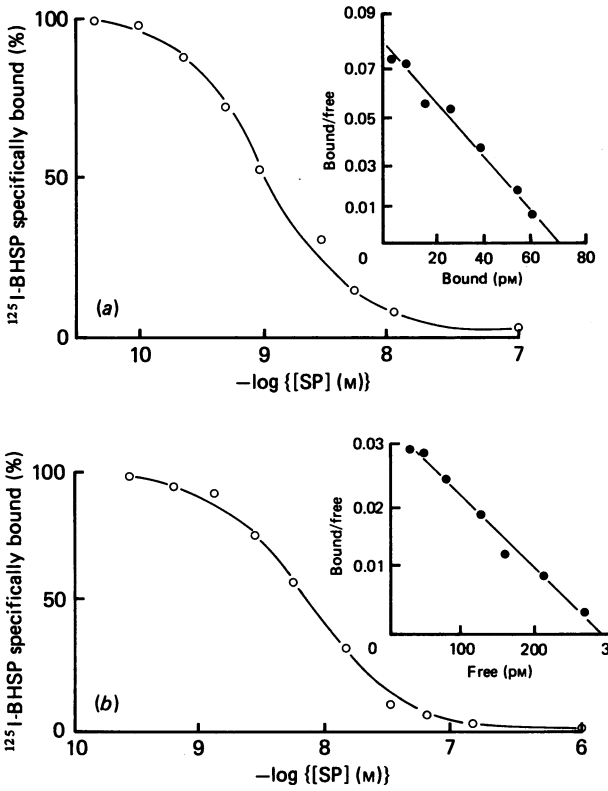

Fig. 6. Displacement of specific ^{125}I-BHSP binding

(*a*) Displacement of specific binding to membrane (0.42 mg of protein/ml). The IC_{50} was 1.1 nM. A Scatchard plot (inset) of the binding data showed a single class of binding sites with an apparent K_d of 0.84 nM. $B_{max.}$ was calculated to be 171 fmol of sites/mg of protein. (*b*) Displacement of specific binding to solubilized sites. The IC_{50} was 5.1 nM. A Scatchard plot (inset) of the data showed an apparent single class of sites with a K_d of 8.46 nM. $B_{max.}$ was calculated to be 2153 fmol of binding sites/mg of protein. The experiment was repeated three additional times.

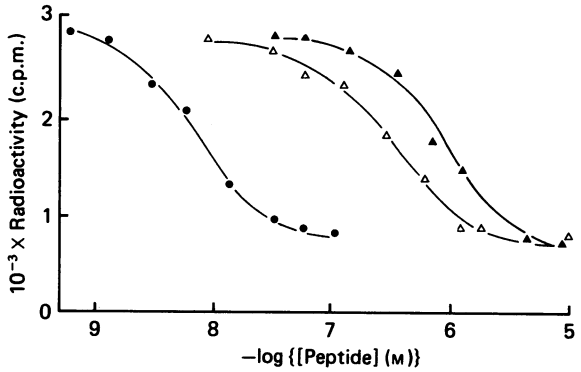

Fig. 7. Displacement of ^{125}I-BHSP binding to solubilized binding sites by SP (●), SK (△) and NK (▲)

Radioligand (70 pM) was added into each sample in a final volume of 0.5 ml. The total binding accounted for 2997 c.p.m., and the non-specific binding, as defined by 1 μM-SP, for 973 c.p.m.

(Fig. 2*a*) respectively. Since $[(L)k_1+k_{-1}]$ is equal to $K_{ob.}$, the association constants (k_1) for the membrane-bound and soluble sites were calculated to be 1.0×10^{10} min^{-1}·M^{-1} and 1.1×10^{9} min^{-1}·M^{-1} respectively.

The specific binding to both the membrane-bound and soluble binding sites were reversible, as on reaching equilibrium the addition of 1 μM-SP caused the dissociation of bound ^{125}I-BHSP (Figs. 1*b* and 2*b*). The dissociation when linearized according to the equation for first-order kinetics yielded straight lines with slopes (k_{-1}) of 0.039 min^{-1} and 0.042 min^{-1} for the membrane-bound and soluble sites. The equilibrium dissociation constants ($K_d = k_{-1}/k_1$) were 3.9×10^{-12} M and 3.8×10^{-11} M for the membrane-bound and soluble sites respectively.

Competitive inhibition of ^{125}I-BHSP binding

Unlabelled SP competed for the binding of ^{125}I-BHSP potently (Table 2). Scatchard plots of these data yielded equilibrium constants of 0.84±0.05 nM ($n = 4$), and 8.0±0.7 nM ($n = 4$), with maximal binding ($B_{max.}$) of 183±53 ($n = 4$) and 1798±354 ($n = 4$) fmol/mg of protein for the binding to membrane-bound and soluble sites respectively (Figs. 6*a* and 6*b*).

The competitive inhibition of ^{152}I-BHSP by various tachykinins are shown in Table 2. The potency of inhibition was in the order of (SP > Phy > Kass > Ele > SK > NK. The soluble receptor showed a similar order of potency to the membrane-bound sites (Fig. 7 and Table 2).

DISCUSSION

In the present study we have adopted a methodology which used PEI pretreated filters to trap soluble and membrane-bound binding sites. Different methods have been developed for separating soluble receptor-bound ligand from free ligand. These include gel filtration, equilibrium dialysis, PEG or $(NH_4)_2SO_4$ precipitation, charcoal separation, affinity adsorption and filtration through PEI-pretreated glass filters (Bruns *et al.*, 1983). We have examined PEG and alcohol precipitation in addition to filtration through glass filters pretreated with PEI. The latter method was used because it was reliable, rapid, gave low non-specific binding and could be used for comparing the characteristics of ^{125}I-BHSP binding to these two different preparations directly.

In addition to low temperature, we have included proteinase inhibitors in the preparation of membranes to reduce the possibility of receptor degradation. The combination of proteinase inhibitors used in the binding assays (chymostatin, leupeptin and bacitracin) inhibited the degradation of radioactive SP in this tissue and in others (Lee *et al.*, 1983; Burcher *et al.*, 1986).

The sites labelled by ^{125}I-BHSP in chick brain membranes displayed binding properties similar to those reported in the mammalian brain (Cascieri *et al.*, 1985; Torrens *et al.*, 1985; Lee *et al.*, 1986). The binding was time-dependent, reversible, saturable and of high affinity. The competitive inhibition by various tachykinins suggests that the binding was to the 'SP-P' subclass of tachykinin receptor (Lee *et al.*, 1982). The dissociation constant (K_d) determined from equilibrium studies (0.84 nM) was different from that determined kinetically (0.0039 nM). A similar discrepancy between the K_d as determined kinetically (0.034 nM) and that by equilibrium (0.39 nM) studies has been reported previously by others (Torrens *et al.*, 1986). The origin of the large discrepancy observed in our studies is unclear and may be indicative of a greater preference for the binding of Bolton–Hunter-iodinated SP over native SP. However, the K_d determined from equilibrium studies was similar to that reported by others using authentic [^{3}H]SP (Lee *et al.*, 1983; Perrone *et al.*, 1983).

A combination of non-ionic (digitonin and octyl glucoside) detergents was found to extract binding sites from the chick brain membrane effectively. Attempts to extract binding sites with either ionic and zwitterionic detergents were unsuccessful, even though as much as 40% of the membrane proteins were extracted. With the non-ionic detergents we have solubilized binding sites with high specific activities ($\sim$ 1.8 pmol/mg protein), and the preparations were stable with little loss of binding activity when stored at 4 °C. The binding of ^{125}I-BHSP to the solubilized preparations were also time-dependent, reversible and saturable. However, the dissociation constant for the binding was different, at least an order of magnitude less potent than the binding to membrane-bound sites (Fig. 6). The order of the potency of inhibition by the three mammalian tachykinins (Maggio, 1985) was identical with that observed with membrane-bound sites (Table 2). Thus solubilization with the combination of these non-ionic detergents did not alter the pharmacological characteristics of the binding sites.

A number of plasma-membrane receptors for hormones, drugs and neurotransmitters are coupled to guanine-nucleotide-regulatory proteins (Spiegel, 1987). The 'SP-P' subclass of tachykinin receptors is no exception, as it has been shown in a variety of tissues to be coupled to guanine-nucleotide-regulatory proteins (Lee *et al.*, 1983; Too & Hanley, 1984; Maruyama, 1986; Sharma & Musacchio, 1987). The solubilized 'SP-P'-type binding sites were sensitive to nucleotides (Table 1), suggesting a direct coupling of guanine-nucleotide-binding proteins to the binding sites in complexes. At present the identity of the nucleotide-binding proteins is unknown. With the successful solubilization in high yield of the binding sites/guanine-nucleotide-binding complexes, it will be possible to isolate and identify the nucleotide binding proteins along with the binding sites.

Pretreatment of the chick brain membrane with NEM dramatically reduced specific binding of ^{125}I-BHSP. Consistent with this observation, the 'SP-P' subtype of tachykinin receptors in other tissues has been shown to be sensitive to a variety of thiol reagents (Sharma & Musacchio, 1987; Too *et al.*, 1988). These observations suggest the presence of reactive thiol residues located near, or at, the binding sites.

In the present study we have shown that chick brain membranes have high-affinity binding sites for ^{125}I-BHSP similar to those observed in mammalian brain membrane. Furthermore, these binding sites have been solubilized with high specific binding activities and retained the basic pharmacological characteristics of the equivalent membrane-bound sites. Thus the protocol used here may permit the solubilization of the ^{152}I-BHSP binding site in functional state and will enable further biochemical characterization and eventual purification.

H.-P.T. was a recipient of a Proctor and Gamble Fellowship during this research.

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Received 27 July 1987/3 December 1987; acccepted 9 February 1988