Br. J. Pharmacol. (1988), **94**, 602–608

Comparison of the binding of radiolabelled neurokinin A and eledoisin in rat cortex synaptic membranes

[1]Alan C. Foster & Robert Tridgett

Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex, CM20 2QR

1 The binding of the ^{125}I-Bolton Hunter (BH) conjugates of neurokinin A and eledoisin to synaptic plasma membranes prepared from rat cerebral cortex was investigated.

2 Saturation analyses indicated that both radioligands labelled a similar number of binding sites, but [^{125}I]BH-eledoisin had a 7 fold higher affinity than [^{125}I]BH-neurokinin A.

3 An identical pharmacological profile was apparent for both radioligands and tachykinin peptides inhibited the binding in the order: neurokinin B > BH-eledoisin > kassinin > L-363,851, eledoisin > substance P, neurokinin A > physalaemin > DiMeC$_7$ > substance P methylester, indicating a profile consistent with the NK$_3$-subtype of tachykinin receptors.

4 The binding of [^{125}I]BH-neurokinin A and [^{125}I]BH-eledoisin was equally sensitive to inhibition by the guanosine triphosphate (GTP) analogue, guanyly-5'-(β-γ-imido) diphosphate.

5 These results indicate that [^{125}I]BH-neurokinin A and [^{125}I]BH-eledoisin appear to label a common site in rat cerebral cortex synaptic plasma membranes with the characteristics of an NK$_3$-receptor, and thus [^{125}I]BH-neurokinin A is not a selective radioligand for the NK$_2$-receptor.

Introduction

Multiple receptor types are thought to exist for the tachykinin family of neuropeptides. The original subdivision of SP-P and SP-E receptors (Lee *et al.*, 1982; Iversen, 1985) has been superceded by a scheme that recognises three receptor sub-types (Lee *et al.*, 1986; Iversen *et al.*, 1987). These are named NK$_1$-, NK$_2$- and NK$_3$-receptors, and have been defined on the basis of relative potencies of agonists (Lee *et al.*, 1986; Iversen *et al.*, 1987; McKnight & Maguire, 1987; Bristow *et al.*, 1987; see Table 1).

The definition of tachykinin receptor sub-types within the mammalian central nervous system (CNS) has relied almost exclusively on radioligand binding studies. Two pharmacologically distinct binding sites for radiolabelled tachykinins have been reported in CNS membrane preparations. Thus, substance P radiolabelled with [^{125}I]Bolton-Hunter reagent ([^{125}I]BH-substance P) or ^{3}H (Hanley *et al.*, 1980; Cascieri & Liang, 1983) and [^{3}H]-physalaemin (Mohini *et al.*, 1985) label sites which resemble NK$_1$-receptors and [^{125}I]BH-eledoisin (Cascieri & Liang, 1984; Beaujouan *et al.*, 1984), [^{3}H]-neurokinin B (Bergstrom *et al.*, 1987) and [^{125}I]BH [Asp^{5,6}, N-methyl-Phe^8] substance P (5-11) ([^{125}I]BH-senktide) (Laufer *et al.*, 1986) label sites with the pharmacological specificity of NK$_3$- receptors. Autoradiographical studies have further demonstrated the differential anatomical distributions within the CNS of these pharmacologically-distinct binding sites (Ninkovic *et al.*, 1984; Danks *et al.*, 1986; Beaujouan *et al.*, 1986). To date, NK$_2$-receptors have been reported to exist only in mammalian peripheral tissues, where they can be labelled by the radioligands [^{125}I]BH-neurokinin A and [^{125}I]BH-eledoisin (Buck *et al.*, 1984; Lee *et al.*, 1986).

Neurokinin A is the preferred tachykinin at NK$_2$-sites (Buck *et al.*, 1984; Lee *et al.*, 1986) and BH conjugation does not diminish the affinity of this peptide for NK$_2$-receptors (Lee *et al.*, 1986). In order to evaluate the possible existence of NK$_2$-receptors in the mammalian CNS, we have characterized the binding sites labelled by [^{125}I]BH-neurokinin A in synaptic membranes prepared from rat cerebral cortex and compared these with the sites labelled by [^{125}I]BH-eledoisin. A preliminary account of this work has appeared (Foster & Tridgett, 1986).

[1] Author for correspondence.

© The Macmillan Press Ltd 1988

Table 1 Tachykinin receptor subtypes and pharmacology

Old nomenclature	SP-P	SP-E (SP-K)	SP-N
New nomenclature[1]	NK_1	NK_2	NK_3
Order of agonist potency	SP > Phys > NKA ≥ NKB ≥ Ele ≥ Kas	NKA ≥ NKB ≥ Kas ≥ Ele ≫ SP > Phys	NKB > Ele ≥ Kas > NKA = Phys = SP
Most selective ligand	SPOME	NKA?	Senktide

Abbreviations: SP: substance P; Phys: physalaemin; NKA: neurokinin A; NKB: neurokinin B; Ele: eledoisin; Kas: Kassinin; SPOME: substance P methyl ester; Senktide: succinyl-[Asp^6, Me-Phe^8] substance P_{6-11}.
[1] This nomenclature for tachykinin receptor sub-types was agreed by the participants of a satellite symposium on 'Substance P and Neurokinins' held in Montreal, Canada in July 1986 as part of the XXX International Congress of Physiological Sciences (see also Buck & Burcher, 1986).

Methods

Preparation of rat cerebral cortex synaptic plasma membranes

Synaptic plasma membranes were prepared by a modification of the method of Jones & Matus (1974). Male Sprague-Dawley rats were killed by decapitation and their cerebral cortices dissected and placed in ice cold 0.32 M sucrose, 1 mM potassium phosphate buffer (pH 7.5), 0.1 mM EDTA (solution A). All further procedures were carried out at 4°C. The tissue was weighed and homogenized with a teflon-glass homogenizer in 10 volumes (weight/volume) of solution A followed by centrifugation at 1000 *g* for 10 min. The supernatant (S_1) was decanted and the pellets resuspended in a small volume of solution A and recentrifuged at 1000 *g* for 10 min. The supernatant was added to S_1 and then centrifuged at 17000 *g* for 20 min. The supernatant was discarded and the pellet (P_2) resuspended in 20 vol (with respect to original tissue weight) of 5 mM Tris-acetate buffer, pH 8.0 (solution B). The suspension was lysed by stirring on ice for 60 min and then centrifuged at 50000 *g* for 30 min. The lysed P_2 pellet was suspended in 1.2 M sucrose buffered with 5 mM Tris-acetate (pH 7.4) and 15 ml placed in the bottom of Beckman Ultraclear Ultracentrifuge tubes. This was carefully overlayed with 15 ml of 0.9 M sucrose (containing 5 mM Tris-acetate, pH 7.4, solution C), followed by 8 ml of solution B. The tubes were placed in a Beckman SW28.1 swing out rotor and centrifuged at 83000 *g* for 90 min in a Beckman L8-M Ultracentrifuge. Synaptic plasma membranes (SPM) were collected from the 0.9/1.2 M sucrose interface, diluted with solution C and centrifuged at 50000 *g* for 30 min. The final SPM pellets were suspended in a small volume of solution C (approx. 10 mg membrane protein per ml) and stored at −80°C.

Binding assays

For routine binding assays, a mixture of rat cerebral cortex SPM (50 μg protein per 150 μl), 50 mM Tris-acetate buffer (pH 7.4), 1 mM $MnCl_2$, 50 μg ml^{-1} chymostatin and 0.5 nM of either [^{125}I]BH-eledoisin or [^{125}I]BH-neurokinin A was prepared. Compounds under investigation (dissolved in either 0.1 M acetic acid or dimethylsulphoxide (DMSO)) were added to polypropylene assay tubes in a volume of 1.5 μl. Tubes used to determine the level of total and non-specific binding also received an equivalent volume of vehicle, which affected specific radioligand binding by < 10% compared to controls. The incubation was initiated by the addition of 150 μl of SPM-buffer-radioligand mixture and continued for 60 min at room temperature (20°C). The incubation mixture was filtered through Whatman GF/C filter strips (presoaked in a solution of 0.3% polyethylenimine and 0.5% Triton X-100) using a Brandell Cell Harvester and washed with 6 ml of ice cold 50 mM Tris-acetate (pH 7.4), 1 mM $MnCl_2$. Filters were placed in gamma vials and radioactivity measured in an LKB Clinigamma counter. Non-specific binding for both [^{125}I]BH-eledoisin and [^{125}I]BH-neurokinin A was determined by the inclusion of a 5 μM final concentration of eledoisin or neurokinin A, respectively.

The ability of compounds to inhibit the binding of [^{125}I]BH-eledoisin or [^{125}I]BH-neurokinin A was determined with 10 concentrations of test compound. Inhibitor affinity values (*Ki*) and Hill coefficients (NH) were calculated by an iterative curve fitting programme using RS1 (Bolt, Beranek & Newman Inc.) for both radioligands. Saturation analyses were carried out over a concentration range of 0.01–10 nM, and at concentrations > 0.7 nM [^{125}I]BH-eledoisin and [^{125}I]BH-neurokinin A were supplemented with the respective unlabelled BH-conjugated peptide. Equilibrium dissociation constants (K_D) and maximum number of binding sites

Table 2 Inhibition of [^{125}I]BH-neurokinin A and [^{125}I]BH-eledoisin binding to rat cerebral cortex synaptic membranes by tachykinins

	[^{125}I]*BH-neurokinin A*		[^{125}I]*BH-eledoisin*	
	K_i(nM)	*NH*	K_i(nM)	*NH*
Neurokinin B	0.66 (0.45, 0.96)	0.91 ± 0.05	0.54 (0.42, 0.69)	0.83 ± 0.02
BH-eledoisin	3.59 (2.02, 6.36)	1.07 ± 0.12	1.15 (0.98, 1.35)	1.04 ± 0.30
Kassinin	14.4 (13.5, 15.3)	1.08 ± 0.13	12.1 (10.4, 14.1)	1.17 ± 0.06
L-363,851	20.1 (17.9, 22.5)	0.74 ± 0.25	23.1 (18.5, 28.9)	0.80 ± 0.23
Eledoisin	20.8 (18.1, 23.9)	1.12 ± 0.21	20.9 (17.6, 24.7)	1.00 ± 0.21
Substance P	35.1 (27.8, 44.3)	0.81 ± 0.03	40.0 (32.9, 48.7)	0.76 ± 0.09
Neurokinin A	42.6 (34.2, 52.9)	0.77 ± 0.06	35.3 (28.9, 43.1)	0.89 ± 0.14
Physalaemin	81.3 (69.8, 94.7)	0.87 ± 0.14	53.7 (35.8, 80.6)	1.19 ± 0.32
$DiMeC_7$	182.3 (104.5, 318.1)	1.07 ± 0.21	137.8 (117.2, 162.1)	1.01 ± 0.19
Substance P methylester	>1000	—	>1000	—

K_i values are expressed as the geometric mean (−s.e.mean, +s.e.mean) and Hill coefficients (NH) as the mean ±s.e.mean of 3–4 separate determinations.

(B_{max}) were determined by the iterative curve fitting programme mentioned above.

Protein assay

Membrane protein was determined by the method of Lowry *et al.* (1951).

Materials

[^{125}I]BH-eledoisin and [^{125}I]BH-neurokinin A (specific activities = 2200 Ci mmol^{-1}) were supplied by Dupont–New England Nuclear (Boston, U.S.A.) and the purity checked by h.p.l.c. analysis. All other peptides were purchased from Bachem and the purity and concentrations of stock solutions used were routinely checked by amino acid analysis.

Results

Preliminary experiments revealed that the specific binding of both [^{125}I]BH-eledoisin and [^{125}I]BH-neurokinin A reached equilibrium by 60 min at room temperature and was optimal in the presence of 1 mM $MnCl_2$.

Saturation analyses

A comparison of K_D and B_{max} values for [^{125}I]BH-eledoisin and [^{125}I]BH-neurokinin A was made by incubating rat cerebral cortex SPM with the radiolabelled tachykinins over a concentration range of 0.01–10 nM. As illustrated in the Eadie-Hofstee plots of Figure 1, both radioligands exhibited a single binding component. Kinetic values were: [^{125}I]BH-eledoisin: K_D = 0.63 (0.51, 0.78) nM (geometric mean (−s.e.mean, +s.e.mean)), B_{max} = 47.5 ± 4.0 fmol mg^{-1} protein (n = 4); [^{125}I]BH-neurokinin A: K_D = 4.35 (3.45, 5.47) nM, B_{max} = 49.5 ± 13.9 fmol mg^{-1} protein (n = 3). Thus, both radioligands had similar B_{max} values but [^{125}I]BH-eledoisin had a 7 fold higher affinity than [^{125}I]BH-neurokinin A.

Pharmacological specificity

A number of tachykinin peptides and derivatives were tested as inhibitors of [^{125}I]BH-eledoisin and [^{125}I]BH-neurokinin A binding to rat cerebral cortex SPM and K_i and Hill coefficients determined (Figure 2, Table 2). The pharmacological specificity of the binding sites labelled by both radioligands was virtually identical. Of the mammalian tachykinins,

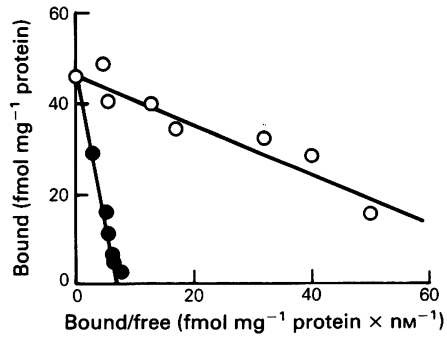

Figure 1 Eadie-Hofstee plots of [^{125}I]BH-neurokinin A and [^{125}I]BH-eledoisin binding to rat cortex synaptic plasma membranes. Values for the specific binding of [^{125}I]BH-neurokinin A (●) and [^{125}I]BH-eledoisin (○) are from a single experiment which was repeated 2–3 further times with similar results (see text for averaged K_D and B_{max} values).

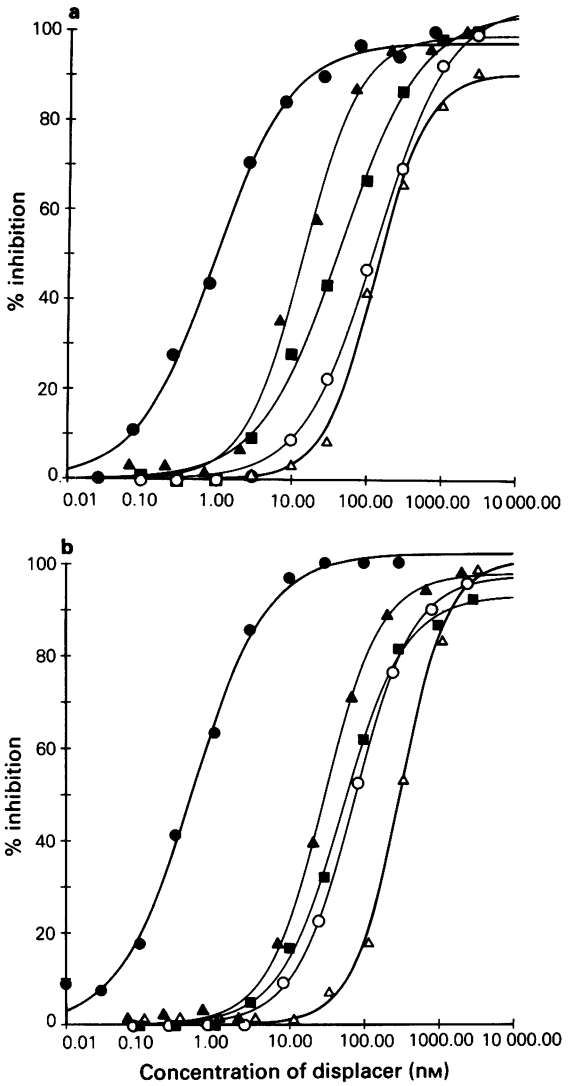

Figure 2 Inhibition by various tachykinins of [^{125}I]BH-neurokinin A and [^{125}I]BH-eledoisin binding to rat cerebral cortex synaptic plasma membranes. Values are percentage inhibition of specific binding and curves were fitted using an iterative curve-fitting procedure as described in the text. Experiments were carried out at a radioligand concentration of 0.5 nM. At this concentration, the absolute levels of specific binding for [^{125}I]BH-neurokinin A and [^{125}I]BH-eledoisin were 12.6 ± 1.0 and 32.1 ± 4.0 fmol mg^{-1} protein, respectively, representing 67.1 ± 3.2 and 84.3 ± 1.7% of the total binding, respectively ($n = 5$). Each curve is from a single experiment which was repeated 2–3 further times with similar results (see Table 2 for averaged K_i and Hill coefficients). (a) [^{125}I]BH-neurokinin A; (b) [^{125}I]BH-eledoisin. (●) Neurokinin B; (▲) eledoisin; (○) physalaemin; (■) neurokinin A; (△) DiMeC$_7$.

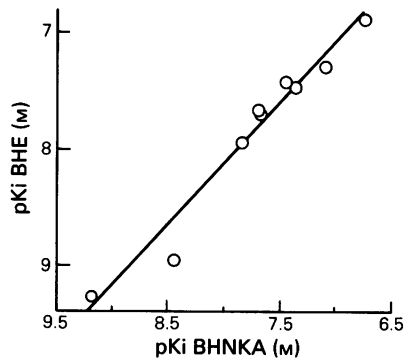

Figure 3 Correlation between pKi values for inhibition of [^{125}I]BH-neurokinin A (BHNKA) and [^{125}I]BH-eledoisin (BHE) binding to rat cerebral cortex synaptic plasma membranes. pKi values were calculated from the average K_i values in Table 2. The line was fitted by linear regression and had a slope of 1.06. The correlation coefficient was 0.98.

neurokinin B had the highest affinity, being 40–100 times more potent than neurokinin A and substance P. Amongst the other naturally-occurring tachykinins, eledoisin and kassinin were 3–7 times more potent than physalaemin. Of the synthetic analogues, BH-eledoisin was more potent than eledoisin itself and the conformationally-restrained hexapeptide substance P analogue *p*Glu-Phe-Phe-(R)-Gly[ANC-2]Leu-Met-NH_2 (L-363,851; Cascieri *et al.*, 1986) was active. [*p*Glu^5, MePhe^8, Sar^9]-substance P(5-11) (DiMeC$_7$) was a weak inhibitor of both radioligands and the NK_1-selective agonist substance P methyl-ester had a $K_i > 1000$ nM. In general the Hill coefficients for the inhibition of [^{125}I]BH-neurokinin A and [^{125}I]BH-eledoisin binding were close to 1.

An excellent correlation was obtained between the K_i values of the compounds tested against [^{125}I]BH-eledoisin and [^{125}I]BH-neurokinin A (Figure 3), giving a correlation coefficient of 0.98.

Inhibition by a non-hydrolysable GTP analogue

The binding of both [^{125}I]BH-eledoisin and [^{125}I]BH-neurokinin A was inhibited to a similar extent by addition of the non-hydrolysable GTP analogue guanyly-5′-(β-γ-imido)diphosphate (Gpp-(NH)p; Figure 4) with IC_{50} values of 199 (192, 207) μM and 196 (162, 238) μM, respectively ($n = 3$).

Discussion

The studies presented here indicate that [^{125}I]BH-neurokinin A and [^{125}I]BH-eledoisin appear to bind to a common site in rat cerebral cortex synaptic

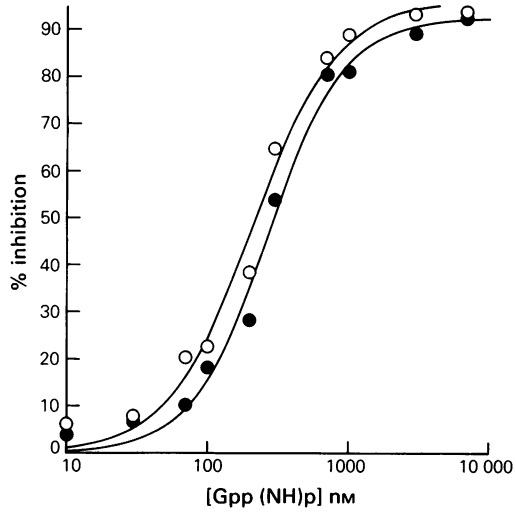

Figure 4 Inhibition by guanyly-5'-(β-γ-imido)diphosphate (Gpp(NH)p) of [^{125}I]BH-neurokinin A and [^{125}I]BH-eledoisin binding to rat cerebral cortex synaptic plasma membranes. Values are percentage inhibition of specific [^{125}I]BH-neurokinin A (●) and [^{125}I]BH-eledoisin (○) binding and curves were fitted using an iterative curve fitting procedure as described in the text. The curves are from a single experiment which was repeated twice with similar results (see text for averaged IC_{50} values).

membranes which has the characteristics of an NK_3-receptor. That a common site is labelled by these radioligands was evident on the basis of three criteria. Firstly, the B_{max} values for [^{125}I]BH-neurokinin A and [^{125}I]BH-eledoisin were similar, although the latter ligand has a 7 fold higher affinity. Secondly, the ability of various tachykinins to inhibit the binding of both radioligands indicated an identical pharmacological specificity and an excellent correlation was obtained for K_i values against the binding of [^{125}I]BH-neurokinin A and [^{125}I]BH-eledoisin. Thirdly, the guanine nucleotide Gpp(NH)p inhibited the binding of both radioligands with a similar IC_{50} value.

The existence of an NK_3-receptor for tachykinins is evident from both functional and radioligand binding experiments. Laufer *et al.* (1985) proposed that a novel tachykinin receptor was present on cholinergic neurones of the guinea-pig myenteric plexus, for which neurokinin B was the preferred agonist. The relative potencies of neurokinin B, neurokinin A, substance P, physalaemin, eledoisin and kassinin at this receptor did not correspond to the classical 'SP-E' receptor profile, but correlated with the pharmacological characteristics of [^{125}I]BH-eledoisin binding to rat CNS membranes. Subsequent studies led to the development of succinyl-[Asp^6, Me-Phe^8] substance P_{6-11} (senktide), a potent and selective NK_3-receptor agonist in the guinea-pig ileum (Wormser *et al.*, 1986), and revealed that [^{125}I]BH-senktide labelled sites in rat cerebral cortex with an NK_3-receptor profile (Laufer *et al.*, 1986). Recently, contractile responses of the rat portal vein to tachykinins have been shown to be mediated by an NK_3-receptor (McKnight & Maguire, 1987). The binding site labelled by both [^{125}I]BH-neurokinin A and [^{125}I]BH-eledoisin in the present study is clearly of the NK_3-type. Thus, neurokinin B was the most potent displacer followed by kassinin and eledoisin, whereas neurokinin A, substance P and physalaemin were weaker. An involvement of NK_1-receptors was ruled out by the inactivity of substance P methylester, and the ratio of potencies between neurokinin B and neurokinin A were not compatible with an NK_2-receptor profile. In agreement with previous reports, BH-conjugation of eledoisin increased the potency of this peptide (Cascieri *et al.*, 1985) and the conformationally-restricted substance P analogue L-363,851 was a potent inhibitor (Cascieri *et al.*, 1986). Thus, in rat cerebral cortex synaptic membranes [^{125}I]BH-neurokinin A labels sites with the pharmacological specificity of an NK_3-receptor.

Four previous studies have examined the binding of [^{125}I]BH-neurokinin A in the mammalian CNS (Mantyh *et al.*, 1984; Shults *et al.*, 1985; Quirion & Dam, 1985; Crossman *et al.*, 1987). The autoradiographical distribution of [^{125}I]BH-neurokinin A binding sites is distinct from that of [^{125}I]BH-substance P (Mantyh *et al.*, 1984; Shults *et al.*, 1985; Quirion & Dam, 1985), and is similar to the pattern obtained with [^{125}I]BH-eledoisin (Danks *et al.*, 1986; Beaujouan *et al.*, 1986), suggesting that in such experiments [^{125}I]BH-neurokinin A and [^{125}I]BH-eledoisin label a common binding site. However, Mantyh *et al.* (1984) and Quirion & Dam (1985) indicate that binding sites for [^{125}I]BH-neurokinin A were observed in the substantia nigra, an area devoid of binding sites for [^{125}I]BH-eledoisin (Danks *et al.*, 1986; Beaujouan *et al.*, 1986). This may indicate that [^{125}I]BH-neurokinin A labels a distinct population of sites in this brain region, however, the autoradiographical experiments of Crossman *et al.* (1987) and our own preliminary studies comparing [^{125}I]BH-neurokinin A and [^{125}I]BH-eledoisin binding in serial rat brain sections (Foster & Tridgett, unpublished observations), have indicated a lack of binding sites in the substantia nigra. Quirion & Dam (1985) also reported some characteristics of [^{125}I]BH-neurokinin A binding to homogenates of guinea-pig brain. Although the K_D and B_{max} values observed were similar to those obtained in the present study, the pharmacological characteristics

were quite different, with the relative order of potencies being neurokinin A > eledoisin > kassinin > neurokinin B > substance P, physalaemin. The reason for the discrepancies between the present study and that of Quirion & Dam (1985) is unclear, but could be related to differences in species, brain area, membrane preparation and assay conditions. However, it seems possible that under certain conditions and in particular brain regions [^{125}I]BH-neurokinin A labels sites that are not common to those labelled by [^{125}I]BH-eledoisin and do not resemble NK_3-receptors.

In conclusion, [^{125}I]BH-neurokinin A and [^{125}I]BH-eledoisin label a common binding site in rat cerebral cortex synaptic membranes which has the pharmacological specificity expected of an NK_3-receptor. Thus, [^{125}I]BH-neurokinin A is not a selective ligand for the NK_2-receptor despite the fact that neurokinin A is the preferred tachykinin for this receptor type. The NK_2-receptor profile of [^{125}I]BH-neurokinin A binding to membranes prepared from mammalian peripheral organs (Buck *et al.*, 1984; Lee *et al.*, 1986) is presumably due to an absence (or very low density) of NK_3-receptors in such tissues. The identification of NK_2-receptors in the mammalian CNS must await a selective radioligand for this receptor sub-type.

References

BEAUJOUAN, J.C., TORRENS, Y., SAFFROY, M. & GLOWINSKI, J. (1986). Quantitative autoradiographic analysis of the distribution of binding sites for [^{125}I]Bolton Hunter derivatives of eledoisin and substance P in the rat brain. *Neuroscience*, **18**, 857–876.

BEAUJOUAN, J.C., TORRENS, Y., VIGER, A. & GLOWINSKI, J. (1984). A new type of tachykinin binding site in the rat brain characterised by specific binding of a labelled eledoisin derivative. *Mol. Pharmacol.*, **26**, 248–254.

BERGSTROM, L., TORRENS, Y., SAFFROY, M., BEAUJOUAN, J.C., LAVIELLE, S., CHASSAING, G., MARGAT, J.L., GLOWINSKI, J. & MARQUET, A. (1987). ^{3}H-neurokinin B and ^{125}I-Bolton Hunter eledoisin label identical tachykinin binding sites in the rat brain. *J. Neurochem.*, **48**, 125–133.

BRISTOW, D.R., CURTIS, N.R., SUMAN-CHAUHAN, N., WATLING, K.J. & WILLIAMS, B.J. (1987). Effects of tachykinins on inositol phospholipid hydrolysis in slices of hamster urinary bladder. *Br. J. Pharmacol.*, **90**, 211–217.

BUCK, S.H. & BURCHER, E. (1986). Neurokinin binding site nomenclature – definition. *Trends Pharmacol. Sci.*, **7**, 437.

BUCK, S.H., BURCHER, E., SHULTS, C.W., LOVENBERG, W. & O'DONOHUE, T.L. (1984). Novel pharmacology of substance K-binding sites: A third type of tachykinin receptor. *Science*, **226**, 987–989.

CASCIERI, M.A., CHICCHI, G.G., FREIDINGER, R.M., COLTON, C.D., PERLOW, D.S., WILLIAMS, B., CURTIS, N.R., MacKNIGHT, A.T., MAGUIRE, J.J., VEBER, D.F. & LIANG, T. (1986). Conformationally constrained tachykinin analogs which are selective ligands for the eledoisin binding site. *Mol. Pharmacol.*, **29**, 34–38.

CASCIERI, M.A., CHICCHI, G.G. & LIANG, T. (1985). Demonstration of two distinct tachykinin receptors in rat brain cortex. *J. Biol. Chem.*, **260**, 1501–1507.

CASCIERI, M.A. & LIANG, T. (1983). Characterization of the substance P receptor in rat brain cortex membranes and the inhibition of radioligand binding by guanine nucleotides. *J. Biol. Chem.*, **258**, 5158–5164.

CASCIERI, M.A. & LIANG, T. (1984). Binding of [^{125}I]Bolton-Hunter conjugated eledoisin to rat brain cortex membranes – evidence for two classes of tachykinin receptors in the mammalian central nervous system. *Life Sciences*, **35**, 179–184.

CROSSMAN, A.R., FARMERY, S.M. & STUART, A.M. (1987). The autoradiographic localisation of neurokinin A binding sites in primate brain. *Br. J. Pharmacol., Proc. Suppl.*, **90**, 259P.

DANKS, J.A., ROTHMAN, R.B., CASCIERI, M.A., CHICCHI, G.G., LIANG, T. & HERKENHAN, M. (1986). A comparative autoradiographic study of the distributions of substance P and eledoisin binding sites in rat brain. *Brain Res.*, **385**, 273–281.

FOSTER, A.C. & TRIDGETT, R. (1986). Tachykinin receptors in rat cerebral cortex: [^{125}I]Bolton-Hunter-labelled-eledoisin and neurokinin A label a common site. *Br. J. Pharmacol. Proc. Suppl.*, **89**, 744P.

HANLEY, M.R., SANDBERG, B.E.B., LEE, C.M., IVERSEN, L.L., BRUNDISH, D.E. & WADE, R. (1980). Specific binding of ^{3}H-substance P to rat brain membranes. *Nature*, **286**, 810–812.

IVERSEN, L.L. (1985). Multiple receptors for tachykinins. In *Tachykinin Antagonists*. 8th Eric K. Fernstrom Symposium. ed. Hakansen, R. & Sundler, F. pp. 291–304. Amsterdam: Elsevier.

IVERSEN, L.L., FOSTER, A.C., WATLING, K.J., McKNIGHT, A.T., WILLIAMS, B.J. & LEE, C.M. (1988). Multiple receptors and binding sites for tachykinins. In *Substance P and Neurokinins*, ed. Henry, J.L. New York: Springer Verlag (in press).

JONES, D.H. & MATUS, A.I. (1974). Isolation of synaptic plasma membrane from brain by combined flotation-sedimentation density gradient centrifugation. *Biochim. Biophys. Acta*, **356**, 276–287.

LAUFER, R., GILON, C., CHOREV, M. & SELINGER, Z. (1986). Characterization of a neurokinin B receptor site in rat brain using a highly selective radioligand. *J. Biol. Chem.*, **261**, 10257–10263.

LAUFER, R., WORMSER, U., FRIEDMAN, Z.Y., GILON, C., CHOREV, M. & SELINGER, Z. (1985). Neurokinin B is a preferred agonist for a neuronal substance P receptor and its action is antagonised by enkephalin. *Proc. Natl. Acad. Sci., U.S.A.*, **82**, 7444–7448.

LEE, C.M., CAMPBELL, N.J., WILLIAMS, B.J. & IVERSEN, L.L. (1986). Multiple tachykinin binding sites in peripheral tissues and brain. *Eur. J. Pharmacol.*, **130**, 209–217.

LEE, C.M., IVERSEN, L.L., HANLEY, M.R. & SANDBERG, B.E.B. (1982). The possible existence of multiple receptors for substance P. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **318**, 281–287.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurements with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.

MANTYH, P.W., MAGGIO, J.E. & HUNT, S.P. (1984). The autoradiographic distribution of kassinin and substance K binding sites is different from the distribution of substance P binding sites in rat brain. *Eur. J. Pharmacol.*, **102**, 361–364.

McKNIGHT, A.T. & MAGUIRE, J.J. (1987). Comparison of the receptor for tachykinins in the vas deferens and portal vein of the rat. *Br. J. Pharmacol. Proc. Suppl.*, **90**, 103P.

MOHINI, P., BAHOUTH, S.W., BRUNDISH, D.E. & MUSACCHIO, J.M. (1985). Specific labelling of rat brain substance P receptor with [^{3}H]physalaemin. *J. Neuroscience*, **5**, 2078–2085.

NINKOVIC, M., BEAUJOUAN, J.C., TORRENS, Y., SAFFROY, M., HALL, M.D. & GLOWINSKI, J. (1984). Differential localization of tachykinin receptors in rat spinal cord. *Eur. J. Pharmacol.*, **106**, 463–464.

QUIRION, R. & DAM, T-V. (1985). Multiple tachykinin receptors in guinea pig brain. High densities of substance K (neurokinin A) binding sites in the substantia nigra. *Neuropeptides*, **6**, 191–204.

SHULTS, C.W., BUCK, S.H., BURCHER, E., CHASE, T.N. & O'DONOHUE, T.L. (1985). Distinct binding sites for substance P and neurokinin A (substance K): co-transmitters in rat brain. *Peptides*, **6**, 343–345.

WORMSER, U., LAUFER, R., HART, Y., CHOREV, M., GILON, C. & SELINGER, Z. (1986). Highly selective agonists for substance P receptor subtypes. *EMBO Journal*, **5**, 2805–2808.

(Received October 6, 1987
Revised January 9, 1988
Accepted January 15, 1988)