Neurokinin Agonists and Antagonists

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INTRODUCTION

Substance P (SP), neurokinin A (NKA), and neurokinin B (NKB) are mammalian peptides characterized by the C-terminal sequence, Phe-X-Gly-Leu-Met-NH_2. The primary structures of these peptides are presented in TABLE 1 along with those of physalaemin (PHY), eledoisin (ELE), and kassinin (KAS), which are three tachykinins of nonmammalian origins. Neurokinins are present in the brain and in peripheral organs where they are known to mediate transmission of pain,[1] motility of smooth muscle,[2] distribution of peripheral blood flow,[3] and hormone and neurotransmitter secretion.[4,5] The neurokinins act through activation of three receptor types named NK-1 for SP, NK-2 for NKA, and NK-3 for NKB.[6]

The characterization of these receptors was made with pharmacological,[7] biochemical,[8] and histochemical[9] approaches. The pharmacological characterization required (a) the use of isolated smooth muscle preparations known to contain single or multiple receptor types and (b) the development of agonists selective for a single receptor type.

In this report, we analyze five vascular preparations that have been found to be useful for the study of neurokinin receptors and focus on two rabbit veins, the jugular[10] and vena cava (Nantel *et al.*, unpublished), which contain only the NK-1 type. The identification and the development of selective neurokinin receptor agonists are also described along with the use of selective NK-1 agonists for biochemical studies.

Another important step in receptor characterization is the utilization of selective antagonists. The search of such compounds is performed in various research centers and we are able to include here some recent data on new NK-3 receptor antagonists obtained in our laboratory.

PHARMACOLOGICAL PREPARATIONS

Sensitive pharmacological preparations are essential tools for receptor characterization. This is also the case for the three neurokinin receptor types that have been

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TABLE 1. Primary Structure of Neurokinins and Tachykinins

Name	Structure											
	0	1	2	3	4	5	6	7	8	9	10	11
Substance P		Arg-	Pro-	Lys-	Pro-	Gln-	Gln-	Phe-	Phe-	Gly-	Leu-	Met-NH_2
Neurokinin A		His-	Lys-	Thr-	Asp-	Ser-	Phe-	Val-	Gly-	Leu-	Met-NH_2	
Neurokinin B		Asp-	Met-	His-	Asp-	Phe-	Phe-	Val-	Gly-	Leu-	Met-NH_2	
Physalaemin		pGlu-	Ala-	Asp-	Pro-	Asn-	Lys-	Phe-	Tyr-	Gly-	Leu-	Met-NH_2
Eledoisin		pGlu-	Pro-	Ser-	Lys-	Asp-	Ala-	Phe-	Ile-	Gly-	Leu-	Met-NH_2
Kassinin	Asp-	Val-	Pro-	Lys-	Ser-	Asp-	Gly-	Phe-	Val-	Gly-	Leu-	Met-NH_2

studied on various isolated organs including the dog carotid artery (DCA),[11] the rabbit jugular vein (RJV),[10] and the rabbit vena cava (RVC), which contain only the NK-1 receptor; the rabbit pulmonary artery (RPA), which has only the NK-2 receptor type;[12] and the rat portal vein (RPV), which has only the NK-3 receptor type.[13] The pharmacological activities of neurokinins, tachykinins, and some neurokinin fragments on these preparations are presented in TABLE 2.

The data in TABLE 2 indicate that SP is the most active neurokinin on the DCA, the RJV, and the RVC, where it shows a pD_2 value higher than 8.6. On the DCA, NKA is more active than NKB, whereas NKA and NKB show similar affinities on the rabbit veins. The order of potency of the tachykinins is PHY > ELE > KAS on the DCA and PHY > ELE = KAS on the RJV and the RVC. All the fragments analyzed in TABLE 2 were found to have affinities lower than SP in the three NK-1 preparations.

For several years, the NK-1 receptor has been studied on the DCA despite some limitations; in fact, SP exerts an indirect inhibitory effect on this tissue because the DCA responds to neurokinins with an endothelium-dependent relaxation. The NK-1 receptor is present on endothelial cells and its activation brings about the release of the endothelium-derived relaxing factor (EDRF),[11] a potent relaxant of smooth muscle fibers believed to be nitric oxide.[14] The effect of SP on the DCA is therefore the result of two processes, namely, the stimulus-secretion (release of EDRF) and a subsequent stimulus-relaxation process (inhibition of vascular tonus). When suspended *in vitro*, the artery has no intrinsic tension and it must first be precontracted with noradrenaline (NA) (2.8×10^{-8} M). SP then acts as a physiological antagonist of the NA-elicited contraction. Because the tissue may be not contracted to its maximum, it is then possible that the affinities of agonists may be overestimated. This is suggested by the results shown in FIGURE 1, where maximal contraction of the DCA obtained with a high dose of NA (1.2×10^{-5} M) causes a rightward shift in the SP relaxation curve and a drop in the relative affinity from a pD_2 value of 10.0 to 9.53. There is also a decrease of the maximal effect, as if SP could not completely eliminate the contraction induced by the high dose of NA. Furthermore, because the DCA cannot be relaxed by 100%, the evaluation of agonist intrinsic activities (α^E) is extremely difficult, if not impossible, if we also consider that the maximal relaxation of a given tissue may depend more on the state of its endothelium than on the nature or concentration of the agonist used.

On the other hand, the two rabbit veins respond to neurokinins with concentration-dependent contractions that are evoked by the stimulation of smooth muscle receptors because they occur equally well in the absence or in the presence of the endothelium. The observed effect is thus direct and results from a stimulus-contraction process in which the endothelium may play no role. The two veins then permit a precise evaluation of the apparent affinity (pD_2) and the intrinsic activity (α^E),[10] according to the classical receptor theory.[15]

On the RPA (TABLE 2), NKA is the most active neurokinin, followed by NKB and SP. The order of affinity of the tachykinins is ELE > KAS > PHY. NKA(4–10) is the most active fragment with an affinity even higher than that of NKA or ELE. The heptapeptide and hexapeptide fragments of SP have also been found to be more active than SP.

The RPV shows a high sensitivity to NKB, which is the neurokinin that shows the highest affinity ($pD_2 = 7.68$) on this tissue, whereas NKA is over 10 times less active than NKB and SP is almost inactive ($pD_2 = 5.82$). The tachykinins have the following order of affinity: KAS > ELE > PHY. Among the fragments, both SP(6–11) and NKA(4–10) are more active than their corresponding peptides.

TABLE 2. Pharmacological Activities of Neurokinins, Tachykinins, and Fragments on Vascular Smooth Muscle Preparations[a]

	DCA		RJV			RVC			RPA			RPV		
	pD_2	RA	pD_2	RA	α^E	pD_2	RA	α^E	pD_2	RA	α^E	pD_2	RA	α^E
Substance P	10.00	100	8.83	100	1.0	8.63	100	1.0	6.13	100	1.0	5.82	100	1.0
Neurokinin A	9.40	25	7.65	7	0.9	7.31	5	1.0	8.22	12,303	1.4	6.45	427	3.0
Neurokinin B	8.90	8	7.84	10	0.7	7.20	4	1.0	7.45	2089	1.4	7.68	7244	2.5
Physalaemin	10.00	100	8.88	112	1.1	8.88	162	1.1	6.02	78	1.0	6.18	229	2.0
Eledoisin	9.50	32	8.43	43	1.0	7.72	12	1.0	8.22	12,303	1.4	7.11	1950	3.5
Kassinin	9.20	16	8.48	45	0.9	7.78	14	1.0	7.66	3388	1.4	7.20	2399	3.5
SP(4–11)	9.67	47	8.62	61	1.0	8.52	78	1.0	6.23	126	0.7	5.87	112	2.5
SP(5–11)	9.30	20	8.20	26	0.9	8.08	24	1.0	6.53	251	0.9	5.54	52	2.0
SP(6–11)	8.64	4	8.41	38	0.9	7.57	9	1.0	5.71	38	0.9	6.30	281	2.3
NKA(4–10)	8.62	4	7.40	4	0.9	6.75	1	1.0	8.52	24,547	1.1	6.79	933	2.5

[a]Terms—pD_2: −log of the molar concentration of agonist causing 50% of the maximal response; RA: relative affinity expressed in percentage of that of SP; α^E: intrinsic activity expressed as a fraction of that of SP.

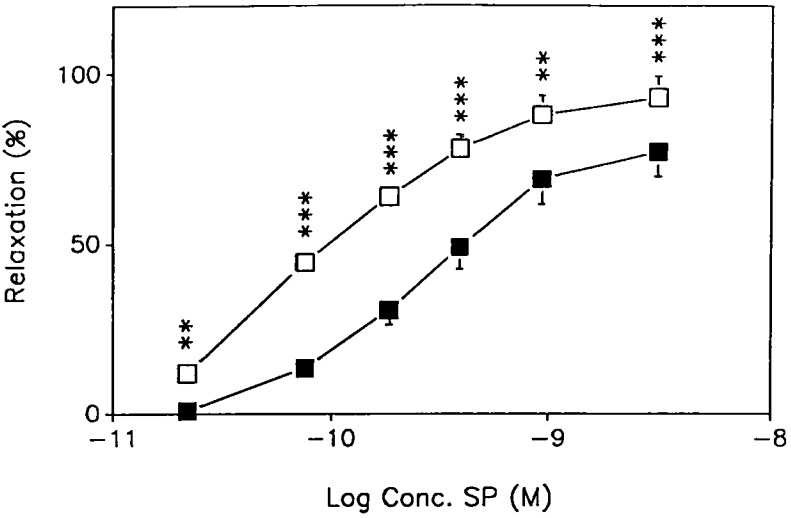

FIGURE 1. Concentration-response curve of substance P on the dog carotid artery previously contracted with noradrenaline—2.8×10^{-8} M (□) or 1.2×10^{-5} M (■). Ordinate: Relaxation expressed in percentage. Abscissa: Log of the molar concentration of SP. Each point represents the average from seven determinations and the vertical bars represent the standard error; **: $p < 0.01$; ***: $p < 0.001$.

DEVELOPMENT OF SELECTIVE AGONISTS

Even though the neurokinins and tachykinins were useful in the characterization of their respective receptors, it was found that they were not selective enough for biochemical or physiological studies. Among the natural neurokinins, SP is the most selective, having 100 to 1000 times more activity on the NK-1 than on the NK-2 or NK-3 systems (TABLE 2). NKA is at most 10 times more active on the RPA (NK-2) than on the two rabbit veins (NK-1), but it has a lower affinity than on the DCA, the other NK-1 preparation. The affinity of NKB on the NK-3 system of the rat portal vein ($pD_2 = 7.68$) is lower than its affinity on the DCA ($pD_2 = 8.90$) or on the RJV ($pD_2 = 7.84$) and it is almost equal to that of the RPA ($pD_2 = 7.45$). This has prompted the search for more selective agonists that are active on only one neurokinin receptor type.

NK-1 Selective Agonists

Results obtained with the selective compounds as well as with other analogues of the three neurokinins that led to the identification of selective agonists are summarized in TABLE 3. Early work on neurokinin selective agonists was purely empirical and centered on the SP molecule. It was first reported that the methylation of SP led to NK-1 receptor selectivity: SP-OMe was found to be active on the DCA and inactive on the RPA and the RPV.[16] However, SP-OMe was metabolically unstable: to improve the stability and affinity for the NK-1 receptor, Gly^9 was replaced by Sar.[17] The compound, [Sar^9]SP, has a high affinity on the DCA, is inactive on the RPA, and

has a low activity on the RPV. The oxidation of Met[11] to Met(O_2) increased the selectivity without decreasing the affinity for the NK-1 receptor of the DCA. Indeed, [Sar[9],Met(O_2)[11]]SP is selective for the NK-1 receptor, being very active on the DCA and inactive on the other two preparations (TABLE 3). The size of the selective agonist was then decreased to that of a hexapeptide, [Arg[6],Sar[9],Met(O_2)[11]]SP(6–11), without a major loss of affinity.[18] Acetylation of the N-terminal amine of this hexapeptide to prevent histamine release from mast cells[19] kept its affinity and selectivity for the NK-1 receptor.

NK-2 Selective Agonists

As shown earlier in TABLE 2, it was found that NKA(4–10) was much more active than NKA on the NK-2 receptor system of the RPA. Replacement of the Met[10] with Nle lowered the peptide affinity on the NK-2 receptor by 40%, but almost abolished the activity on NK-1 and NK-3 receptor systems.[17] Also, elongation of the peptide chain at the level of Gly[8] by one carbon gave a compound, [βAla[8]]NKA(4–10), that was more active than NKA on the RPA (pD_2 = 8.60), but that showed little activity on the DCA[20] and some decrease on the RPV (TABLE 3).

NK-3 Selective Agonists

The search for an NK-3 selective agonist started with the observation that DiMeC7 shows low activity on NK-1 and NK-2 systems.[21] The chemical features of DiMeC7 were reproduced in the sequence NKB(4–10) and its activity on the NK-3 receptor was found to be fairly good, whereas activity on NK-1 did not change. Activity on the NK-3 receptor was further improved by the elimination of Sar[8]: the

TABLE 3. Pharmacological Activities of Neurokinin Receptor Selective Agonists[a]

	DCA		RPA		RPV	
	pD_2	RA	pD_2	RA	pD_2	RA
Substance P	10.0	100	6.13	100	5.82	100
SP-OMe	9.60	40	inactive		inactive	
[Sar[9]]SP	10.4	240		0.1	5.80	98
[Sar[9],Met(O_2)[11]]SP	10.5	282	inactive		inactive	
[Arg[6],Sar[9],Met(O_2)[11]]SP(6–11)	10.1	126	inactive		inactive	
Ac-[Arg[6],Sar[9],Met(O_2)[11]]SP(6–11)	10.2	174	inactive		inactive	
Neurokinin A	9.40	100	8.22	100	6.45	100
NKA(4–10)	8.62	17	8.52	200	6.77	219
[Nle[10]]NKA	7.64	4.4	8.01	62	6.49	110
[Nle[10]]NKA(4–10)	7.00	0.1	7.64	26		< 0.1
[βAla[8]]NKA(4–10)	6.71	0.5	8.60	240	6.13	48
Neurokinin B	8.90	100	7.45	100	7.68	100
NKB(4–10)	8.70	63	7.95	316	6.67	10
[pGlu[6],MePhe[8],Sar[9]]SP(5–11)(DiMeC7)	7.50	4	inactive		6.35	5
[MePhe[7],Sar[8]]NKB(4–10)	7.61	5	inactive		7.03	22
[MePhe[7]]NKB(4–10)	7.09	2	inactive		7.58	79
[MePhe[7]]NKB	7.15	2	5.24	1	8.30	417

[a]Terms are defined in the footnote to TABLE 2.

hexapeptide [MePhe^7]NKB(4–10) was one of the first NK-3 selective agonists to be identified,[22] together with senktide.[23] Activity on NK-3 and selectivity were further improved by replacing Val^7 with MePhe on the full NKB molecule: [MePhe^7]NKB is a compound with an activity 4 times higher than NKB on the RPV and with a low affinity for the NK-1 and NK-2 receptor sites,[17] as shown in TABLE 3.

Uses of Selective Agonists

Selective agonists have been used first to identify other tissues that contain only one neurokinin receptor type (TABLE 4). This has been the case for the two rabbit veins presented earlier and also for some human tissues. Indeed, the RJV and the RVC respond to the NK-1 selective agonist [Sar^9,Met(O$_2$)^{11}]SP, but show little sensitivity to [Nle^{10}]NKA(4–10) and [MePhe^7]NKB. On the other hand, human bronchus (HB) and human urinary bladder (HmUB) are 10 times more sensitive to NKA than to other neurokinins. In fact, the NK-2 selective agonist [Nle^{10}]NKA(4–10) is active in both preparations, whereas the NK-1 and NK-3 selective agonists have no or very little effects (TABLE 4). These findings suggest that both HB and HmUB contain only NK-2 receptors.[24] The human ileum was also characterized by Maggi *et al.*[25] using different selective agonists and it was shown to be a pure NK-2 receptor preparation.

Other organs currently used in pharmacological studies of neurokinin receptors include, for instance, the guinea pig ileum (GPI), which is considered an NK-1 preparation when treated with atropine;[26] the rat duodenum (RD), which is used as an NK-2 system;[27] and the hamster urinary bladder (HUB), which was initially used for identification of the NK-3 site.[8] As shown in TABLE 4, the GPI responds to all three selective agonists and therefore possesses NK-1, NK-2, and NK-3 receptor sites. As to the NK-3 sites, these were shown by Laufer *et al.*[26] to promote the release of acetylcholine from intramural nerves: in fact, treatment of the tissue with atropine abolishes the response of the GPI to [MePhe^7]NKB (not shown), but not to NK-1 and NK-2 selective agonists. The rat duodenum, which has been used as an NK-2 preparation, responds to both [Nle^{10}]NKA(4–10) and [MePhe^7]NKB, indicating that both NK-2 and NK-3 receptors may be present. The same is true for the HUB, which has both NK-2 and NK-3 functional sites, because it responds to NK-2 and NK-3 selective agonists. In conclusion, three of the preparations analyzed in TABLE 4 (GPI, RD, and HUB) contain more than one receptor type and are not suited for pharmacologic studies directed to characterization of neurokinin receptors or of new neurokinin-related peptides, both agonists and antagonists. For further discussion on this point, see references 2 and 28.

USE OF NK-1 SELECTIVE AGONISTS AS LABELS

Biochemical characterization of NK-1 receptors in rat brain homogenates has been carried out with radiolabeled ^{125}I-Bolton-Hunter-SP.[29,30] However, results obtained in pharmacological studies on isolated organs have suggested that SP may not be a very selective compound because it acts (although weakly) on NK-2 and NK-3 functional sites.[2,7,28] After the identification of NK-1 selective agonists, some of those compounds were labeled with either ^{125}I or ^{3}H to better characterize the NK-1 binding site.

Results obtained in our laboratory with three ligands are presented in TABLE 5 by

TABLE 4. Pharmacological Activities of Neurokinins and Selective Agonists on Rabbit Veins and Human Smooth Muscle Preparations[a]

	NK-1						NK-2						NK-3	
	RJV		RVC		GPI		HB		HmUB		RD		HUB	
	pD_2	RA	pD_2	RA	pD_2	RA	pD_2	RA	pD_2	RA	pD_2	RA	pD_2	RA
Substance P	8.83	100	8.63	100	8.78	100	6.30	3	6.00	3	6.50	2	5.57	2
Neurokinin A	7.65	7	7.31	5	8.40	42	7.83	100	7.60	100	8.22	100	7.40	159
Neurokinin B	7.84	10	7.20	4	8.64	72	6.05	2	6.53	9	8.15	85	7.20	100
[Sar^{9},Met$(O_2)^{11}$]SP	8.86	107	8.60	93	8.91	135	inactive		5.65	1	6.67	3	inactive	
[Nle^{10}]NKA(4–10)	5.88	0.1	5.37	0.1	7.99	16	6.97	14	6.60	10	8.02	63	7.09	78
[MePhe^{7}]NKB	6.23	0.3	5.63	0.1	8.76	96	inactive		5.19	0.4	8.66	275	6.14	9

[a]Terms are defined in the footnote to TABLE 2 and in the text.

showing the relative affinity of neurokinins and selective agonists to inhibit three putative NK-1 ligands, mainly, ^{125}I-BH-SP, ^{3}H-[Sar^{9},Met(O$_2$)^{11}]SP, and ^{125}I-BH-[Sar^{9},Met(O$_2$)^{11}]SP, from rat brain membranes. The three ligands gave very similar results: in fact, the order of the affinity of neurokinins was found to be SP > NKA > NKB and, of the selective agonists, only [Sar^{9},Met(O$_2$)^{11}]SP showed high affinity, with the other two being almost inactive. These results are similar to those observed in the dog carotid artery, that is, the NK-1 receptor system. They show that ^{125}I-BH-SP is selective for the NK-1 receptor and is equivalent to the other two selective ligands. Furthermore, ^{125}I and ^{3}H can both be used with success because no differences were observed either in the affinity of the ligand or in the order of the affinity of nonlabeled peptides in competition studies. The iodinated ligands have the advantage of being more suitable for autoradiographic studies, whereas the tritiated ligand (which has the same structure as the natural peptide) shows much lower nonspecific binding (Tousignant *et al.*, unpublished).

NK-1 binding sites were also characterized on guinea pig ileum membranes using ^{3}H-[Sar^{9},Met(O$_2$)^{11}]SP as a ligand. A comparison of the affinities of neurokinins and analogues obtained in competition studies performed on rat brain and guinea pig ileum membranes is presented in FIGURE 2. The two sets of data fit a straight line and show a good correlation: indeed, the correlation coefficient is positive and significant ($r = 0.95$, $p < 0.001$), suggesting that the NK-1 binding site that is present on the rat brain is the same as that of the guinea pig ileum.

Furthermore, the biological activities of neurokinins, tachykinins, SP fragments, and selective agonists observed on the rabbit jugular vein are compared with binding affinities of the same compounds evaluated in competition studies on rat brain membranes using as ligand either ^{125}I-BH-SP or ^{125}I-BH-[Sar^{9},Met(O$_2$)^{11}]SP. With both ligands, a positive and significant correlation between binding affinities and biological activities is observed (FIGURE 3). Correlation coefficients of 0.91 ($p < 0.001$) and 0.82 ($p < 0.001$) are observed, respectively, with ^{125}I-BH-SP and ^{125}I-BH-[Sar^{9},Met(O$_2$)^{11}]SP. These findings strongly support the interpretation that NK-1 binding sites characterized in binding studies are very similar, possibly identical to the functional sites that are responsible for the biological responses of the RJV to substance P and related peptides.

TABLE 5. Relative Affinity of Neurokinins and Selective Agonists to Inhibit Various NK-1 Selective Ligands on Rat Brain Membranes[a]

	Radioactive Ligand					
	^{3}H-[Sar^{9},Met(O$_2$)^{11}]SP		^{125}I-BH-[Sar^{9},Met(O$_2$)^{11}]SP		^{125}I-BH-SP	
Peptide	$-\log K_i$	RA	$-\log K_i$	RA	$-\log K_i$	RA
Neurokinins						
SP	9.19	100	9.21	100	9.11	100
NKB	5.25	0.01	5.57	0.02	6.35	0.2
NKA	6.95	0.6	7.20	1	7.18	1
Selective Agonists						
[Sar^{9},Met(O$_2$)^{11}]SP	8.50	10	9.00	62	8.60	31
[MePhe^{7}]NKB	4.53	0.002	4.40	0.002	4.51	0.002
[βAla^{8}]NKA(4–10)	inactive		4.46	0.002	4.12	0.002

[a]Terms are defined in the footnote to TABLE 2; $-\log K_i$: $-\log$ of the concentration of peptide that inhibits 50% of the bound ligand.

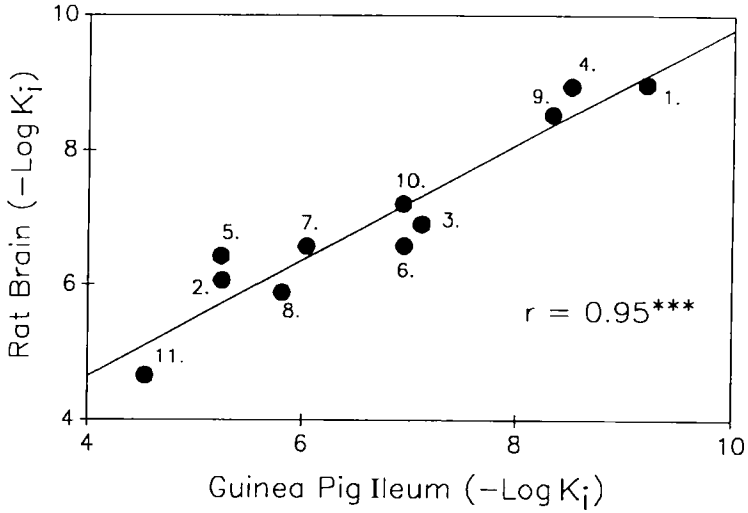

FIGURE 2. Log-log plot of the binding affinities of neurokinins and analogues on rat brain and guinea pig ileum membranes. Key: (1) SP, (2) NKB, (3) NKA, (4) PHY, (5) ELE, (6) SP(4–11), (7) SP(5–11), (8) SP(6–11), (9) [Sar^9,Met(O$_2$)^{11}]SP, (10) Ac-[Arg^6,Sar^9,Met(O$_2$)^{11}]SP(6–11), and (11) [MePhe^7]NKB. Ordinate: $-\text{Log } K_i$ of peptides evaluated by their ability to inhibit the ligand ^{3}H-[Sar^9,Met(O$_2$)^{11}]SP on guinea pig ileum membrane. Abscissa: $-\text{Log } K_i$ of peptides evaluated by their ability to inhibit the ligand ^{3}H-[Sar^9,Met(O$_2$)^{11}]SP on rat brain membrane. ***: $p < 0.001$.

NK-3 ANTAGONISTS

To obtain new selective NK-2 agonists suitable for labeling, Val^7 of [βAla^8]NKA(4–10) was replaced with a Tyr. [Tyr^7,βAla^8]NKA(4–10), although weak as an agonist on the NK-2 receptor, was found to be an antagonist on the rat portal vein, that is, the NK-3 receptor system.

Until now, only weak and possibly nonselective antagonists for the NK-3 receptor have been reported.[31] The observation in the preceding paragraph therefore prompted a careful analysis of new NK-3 antagonists derived from the sequence of [βAla^8]NKA(4–10) and [βAla^8]NKB(4–10). Several compounds were prepared and tested.[32] The pharmacological activities of six putative NK-3 receptor antagonists, evaluated on four selective monoreceptor systems [mainly, the rat portal vein (NK-3), the rabbit pulmonary artery (NK-2), the dog carotid artery, and the rabbit jugular vein (NK-1)], are presented in TABLE 6.

For comparison, data obtained with NKA(4–10) and NKB(4–10) have also been shown. The two natural fragments act as full agonists in the four preparations (see pD$_2$ values in TABLE 6): their activities are very similar because the two compounds differ only by the residue in position 5, which is Ser in NKA(4–10) and Phe in NKB(4–10). Replacement of Gly^8 by βAla and of Val^7 by an aromatic residue—Tyr, Phe, MePhe, or Trp—leads to fairly active NK-3 antagonists. In fact, a pA$_2$ value of 6.93 is observed with [Tyr^7,βAla^8]NKA(4–10) on the NK-3 system (RPV), whereas the compound is an agonist on the other three tissues and, when applied at high concentrations, also on the RPV. Elimination of the Met in position 10 as in [Tyr^7,βAla^8]NKA(4–9) leads to a marked decrease in all activities. When Phe is used

instead of Tyr in position 7 as in [Phe^7,βAla^8]NKA(4–10), the compound maintains a good antagonist affinity (pA_2 = 6.92) and loses some of its agonistic effect on the RPV. However, [Phe^7,βAla^8]NKA(4–10) is not selective because it also acts as an antagonist on the RPA (NK-2) with a pA_2 of 7.04. Replacement of Val^7 with Trp^7 leads to an increase of antagonist affinity on the RPV (pA_2 value of 7.46) and to a weak agonistic effect on the other preparations. [Trp^7,βAla^8]NKA(4–10) also stimulates the RPV, but only at concentrations 50 times or higher than those needed for antagonism.

Some analogues of [βAla^8]NKB(4–10) were also prepared and the results are shown in TABLE 6. Both [Tyr^7,βAla^8]NKB(4–10) and [MePhe^7,βAla^8]NKB(4–10) act as antagonists on the RPV with a residual agonistic activity and they act as full

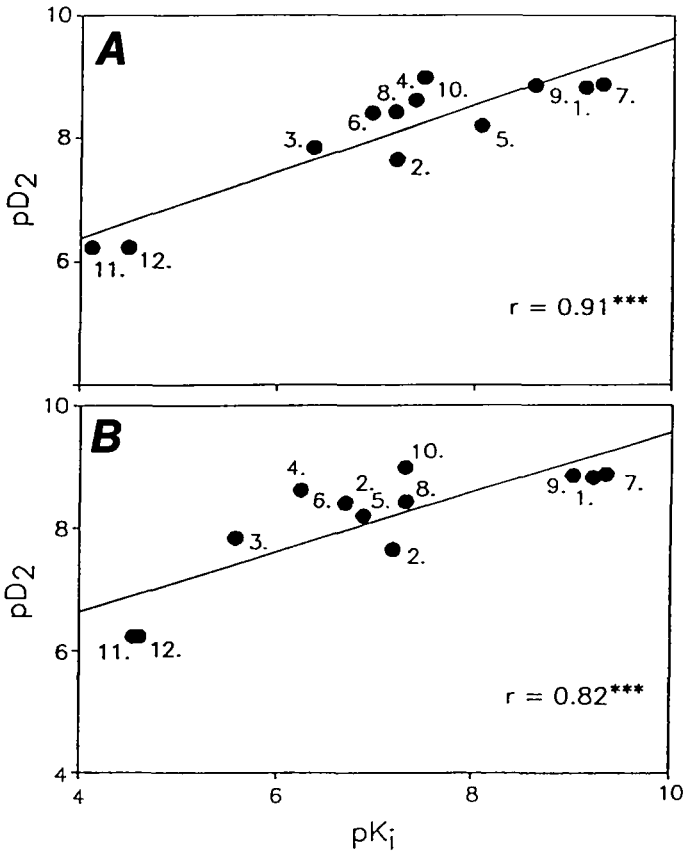

FIGURE 3. Log-log plot of the biological activities and binding affinities of neurokinins and analogues on the rabbit jugular vein and rat brain membranes. Key: (1) SP, (2) NKA, (3) NKB, (4) SP(4–11), (5) SP(5–11), (6) SP(6–11), (7) PHY, (8) ELE, (9) [Sar^9,Met(O$_2$)^{11}]SP, (10) Ac-[Arg^6,Sar^9,Met(O$_2$)^{11}]SP(6–11), (11) [βAla^8]NKA(4–10), and (12) [MePhe^7]NKB. Ordinate: −Log of the molar concentration of peptide at the ED_{50} (pD_2) in the biological assay. Abscissa: −Log of K_i (pK_i) of peptides evaluated by their ability to inhibit ^{125}I-Bolton-Hunter-SP (in *A*) or ^{125}I-Bolton-Hunter-[Sar^9,Met(O$_2$)^{11}]SP (in *B*) from rat brain membranes. ***: $p < 0.001$.

TABLE 6. Pharmacological Evaluation of Putative NK-3 Receptor Antagonists on Four Isolated Vessels[a]

	RPV			RPA			DCA		RJV		
	pA_2	pD_2	α^E	pA_2	pD_2	α^E	pA_2	pD_2	pA_2	pD_2	α^E
NKA(4–10)	In.	6.79	1.0	In.	8.52	1.0	In.	8.62	In.	7.40	0.9
NKB(4–10)	In.	6.67	1.0	In.	7.95	1.0	In.	8.70	In.	6.07	0.8
[Tyr^7,βAla^8]NKA(4–10)	6.93	5.66	1.0	In.	6.75	1.0	In.	7.12	In.	6.70	0.9
[Tyr^7,βAla^8]NKA(4–9)	5.46	4.89	0.4	5.85	4.90	0.6	In.	6.50	In.	6.28	0.8
[Phe^7,βAla^8]NKA(4–10)	6.92	ND	0.5	7.04	6.29	1.0	In.	7.25	In.	6.99	1.1
[Trp^7,βAla^8]NKA(4–10)	7.46	5.73	1.0	In.	6.31	1.0	In.	6.50	In.	6.86	0.5
[Tyr^7,βAla^8]NKB(4–10)	6.96	5.41	0.6	In.	5.40	0.8	In.	7.35	In.	7.75	0.8
[MePhe^7,βAla^8]NKB(4–10)	7.26	5.41	0.5	In.	5.97	1.1	In.	6.18	In.	6.24	0.7

[a]Terms are defined in the footnote to TABLE 2 and in the text; pA_2: −log of the concentration of antagonist that reduces the effect of a double dose of agonist to that of a single one; In. = inactive; ND = not determined.

agonists on the other three preparations. The antagonist affinity of [MePhe^7,βAla^8]NKB(4–10) is fairly good (pA_2 value of 7.26) and the compound is less active as an agonist than [Trp^7,βAla^8]NKA(4–10) on the RPA, DCA, and RJV. Further studies are needed to improve antagonist affinity, eliminate residual agonistic effects, and improve selectivity for the NK-3 receptor.

CONCLUSIONS

The neurokinins exert a wide variety of central and peripheral effects including transmission of pain, vasodilation, plasma extravasation, and contraction of smooth muscle cells of the cardiovascular (arteries, veins), pulmonary, urinary, and gastrointestinal systems. The neurokinins act on three receptors, NK-1, NK-2, and NK-3. Each receptor has been characterized pharmacologically on isolated vascular smooth muscle preparations by the use of neurokinins, tachykinins, and fragments. The dog carotid artery, the rabbit jugular vein, and the rabbit vena cava have been found to contain only receptors of the NK-1 type. The responses of both rabbit veins to neurokinins are direct and result in contractions elicited by the activation of receptors that may be localized on smooth muscle cells. The relaxation of the dog carotid artery in response to neurokinins is indirect because the NK-1 receptor is present on the endothelium, where it mediates the release of the endothelium-derived relaxing factor. The rabbit pulmonary artery and the rat portal vein have, respectively, NK-2 and NK-3 receptor type.

Because the naturally occurring neurokinins were not selective for one receptor only, selective agonists for a single receptor type were developed and tested on the isolated organ preparations. Selective agonists also permitted the characterization of neurokinin receptors on human tissues. The human bronchus, urinary bladder, and ileum were found to contain only NK-2 receptors.

Biochemical studies with radiolabeled NK-1 selective agonists suggest that the NK-1 binding site in the central nervous system and in peripheral organs is the same entity. In addition, a positive correlation was found between the binding affinities and the biological activities of neurokinins and their analogues when measured on the NK-1 receptor on the rat brain and rabbit jugular vein. These findings support

the interpretation that the biological effects of substance P are the result of the coupling of the peptide with the NK-1 receptor type.

NK-3 receptor antagonists were developed by substituting the Val^7 of [βAla^8]NKA(4–10) or of [βAla^8]NKB(4–10) with aromatic amino acids (Phe, MePhe, Tyr, or Trp). [Trp^7,βAla^8]NKA(4–10) and [$MePhe^7$,βAla^8]NKB(4–10) were found to exert fairly good antagonist activities on the NK-3 system of the rat portal vein (pA_2 values > 7.0) and weak agonistic activities on NK-1 and NK-2 preparations.

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