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New selective agonists for neurokinin receptors: pharmacological tools for receptor characterization

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The neurokinin system is replete with a multiplicity of endogenous ligands (substance P, neurokinin A, neurokinin B) and their receptors (NK_1, NK_2 and NK_3). Neurokinin receptors have previously been studied almost exclusively with natural – but non-selective – agonists. However, new selective agonists have now been developed. Domenico Regoli and colleagues *describe how such compounds have been successfully used* in vitro *to characterize the responses of peripheral organs to neurokinins, and* in vivo *to elucidate possible physiological roles of the neurokinin system.*

Since 1962, several peptides bearing the C-terminal sequence -Phe-X-Gly-Leu-Met.NH_2 have been identified in the salivary glands or the skin of lower species (octopods, amphibia)[1] and in the brain or spinal cord of various mammals[2,3].

The first mammalian peptide to be sequenced, substance P (SP), was extracted in 1970 from the bovine hypothalamus by Chang and Leeman[2]; more recently two other peptides were isolated in bovine or porcine spinal cords[3,4]. These neuropeptides were initially named neurokinins α and β by Kimura *et al.*[3], and later neurokinin A and B (NKA, NKB) following the recommendation of an IUPHAR committee[5]. This renaming was confirmed at the Montreal International Symposium on substance P and neurokinins[6].

The three peptides are expressed in neurons and are encoded by two genes, one of which produces SP alone or SP and NKA together (by variable RNA splicing), the other producing only NKB[4,7]. When released by appropriate stimuli in peripheral organs, these neuropeptides exert their effects by activating three receptor types, NK_1, NK_2 and NK_3. Of the natural agonists, SP has the highest affinity for NK_1, NKA for NK_2 and NKB for NK_3.

Studies on neurokinin receptors have faced two major problems: the use of biological assay organs that contain more than one receptor type; and the non-selectivity of the natural peptides that have been used as receptor agonists. Like other endogenous agents (e.g. the opioid peptides) neurokinins bind and activate at least three receptor types and may exert either excitatory or inhibitory activities. If two or more receptors are present together in a peripheral organ, two or three different simultaneous processes may contribute to the observed neurokinin effects, the interpretation of which in pharmacological terms is consequently nearly impossible. Following in the footsteps of the opioid system, considerable progress has now been made in the development of monoreceptor systems and selective agonists for neurokinins.

Pharmacological assay organs

As shown in Fig. 1, SP (1.6×10^{-10} M) and NKA (1.8×10^{-9} M) induce relaxation of the dog carotid artery contracted by noradrenaline (2.0×10^{-8} M) only when the endothelium is present. Since SP is the more potent agonist, the dog carotid artery preparation is considered to be an NK_1 receptor system. Both peptides are inactive in endothelium-denuded dog carotid artery whether or not it is contracted with noradrenaline.

In rabbit pulmonary artery SP (6.5×10^{-10} M) and NKA (4.4×10^{-9} M) evoke relaxations (short-lasting in the case of NKA) in tissues with endothelium, and contractions in those devoid of endothelium. Thus the rabbit pulmonary artery appears to contain two receptors: NK_1 on the endothelium and NK_2 on the muscle.

SP (6.5×10^{-10} M) and NKB (8.3×10^{-10} M) also potently contract the guinea-pig ileum and rat portal vein (SP, 6.5×10^{-7} M; NKB, 2.1×10^{-8} M). In the presence of atropine, responses of the guinea-pig ileum – particularly the response to NKB – are reduced while those of the rat portal vein are unchanged. The data shown in Fig. 1 and the results of structure–activity studies summarized in Table I demonstrate that the dog carotid artery and the rat portal vein are monoreceptor systems containing NK_1 and NK_3 receptors respectively.

The guinea-pig ileum is a multireceptor system whose two receptors – NK_1 present in the muscle and NK_3 present in the mesenteric plexus – have additive effects. The rabbit pulmonary artery is also a multireceptor system containing two receptors with opposing effects: the inhibitory NK_1 receptor in the endothelium and the stimulatory NK_2 receptor in the muscle. However, removal of the endothelium converts this preparation into a monoreceptor system of the NK_2 type[8].

Receptor characterization

Various agonists have been used to characterize neurokinin receptors in pharmacological and biochemical assays; recent data is summarized in Table I. This Table compares relative affinities of neurokinins, tachykinins, neurokinin fragments and a few selective analogues in the three monoreceptor systems described in Fig. 1 and also in the isolated organs most commonly used in the study of the pharmacology of tachy- and neurokinins (guinea-pig ileum, rat duodenum, hamster urinary bladder).

In dog carotid artery (NK_1 monoreceptor system), the rank orders of agonist potencies are: SP > NKA > NKB; physalaemin > eledoisin > kassinin; [Sar^9, $Met(O_2)^{11}$]SP is a selective agonist, while [Nle^{10}]NKA(4–10) and

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[MePhe^7]NKB are almost inactive. Both SP and NKA fragments are weak agonists, less active than their respective precursors. This pattern of agonist activity is very similar to that found with ^{125}I-Bolton–Hunter-SP in binding assays on rat cerebral synaptosomes[9], but differs markedly from that measured in the multireceptor system of the guinea-pig ileum, where (1) the differences between the various neurokinins are not so marked as in the other two systems; (2) kassinin is more active than physalaemin; and (3) some of the SP fragments show very high affinities. In addition, differences between selective agonists are not as great as in the other two systems. Treatment of the tissue with atropine moderately reduces the effect of NKB but markedly reduces that of the NK_3-selective agonist, [MePhe^7]NKB (Ref. 10), suggesting that the NK_3 receptor is primarily involved in the release of acetylcholine from the myenteric plexus[11].

In the endothelium-denuded rabbit pulmonary artery (NK_2 monoreceptor system)[8] NKA and eledoisin are the most potent neuro- and tachykinins, followed by kassinin and NKB. SP fragments are weak, while NKA fragments are very active, NKA(4–10) being twice as potent as NKA. Of the selective agonists, only [Nle^{10}]NKA(4–10) shows significant activity (50% of that of NKA).

Patterns of relative pharmacological activities of the various groups of agonists in the rabbit pulmonary artery are similar to those measured with [^{3}H]NKA on rat duodenal membranes[12], the only exceptions being that NKA(4–10) and eledoisin have only 78% and 27% of the activity of NKA in the biochemical assay. In general, the correspondence between the biological and biochemical data is very good. Thus, rank orders of agonist activities for NK_2 receptors are as follows: NKA > NKB > SP; eledoisin ≥ kassinin > physalaemin; [Nle^{10}]NKA(4–10) is a selective agonist, while [MePhe^7]-NKB and [Sar^{9}Met(O$_2$)^{11}]SP are practically inactive. Relative activities of the same compounds measured in the multireceptor system of the rat duodenum indicate a fairly high affinity of NKB, kassinin and, in particular, [MePhe^7]NKB, which suggests the presence in the rat duodenum of high levels of NK_3 receptors, with lower levels of NK_2 receptors.

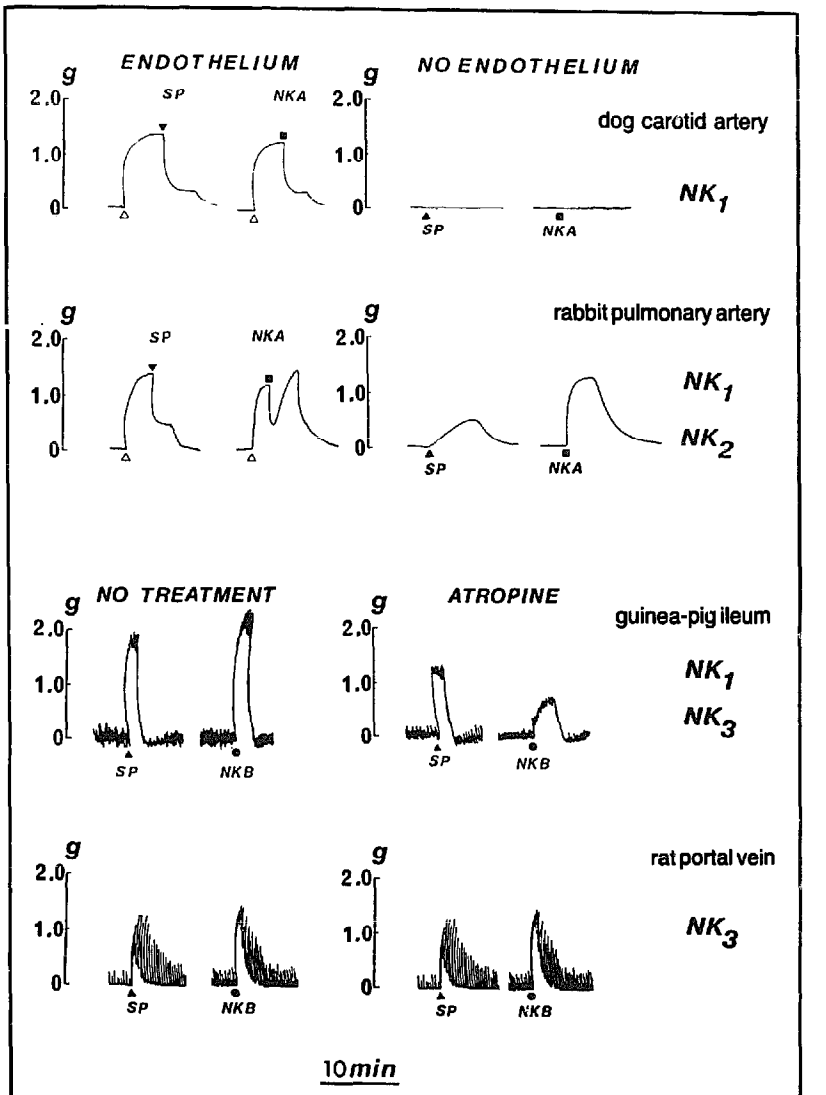

Fig. 1. Typical responses to neurokinins in four isolated organs, dog carotid artery and rabbit pulmonary artery, which were used with or without endothelium, and guinea-pig ileum and rat portal vein, which were used without treatment or after application of atropine 4.0 × 10^{-6} M.

Patterns of activities in the NK_3 system as measured in the biological assays of the rat portal vein[8] or with the ligand ^{125}I-BH-eledoisin (on rat cortical synaptosomes)[9] indicate that NKB and kassinin are the most active neuro- and tachykinins. Activities of fragments are negligible and, among the selective agonists, [MePhe^7]-NKB shows high activity, while the other two are inactive. Thus relative agonist potencies of NK_3 receptors are as follows: NKB > NKA > SP; kassinin > eledoisin > physalaemin; [MePhe^7]NKB is a selective agonist.

In hamster urinary bladder, NKA is the most potent neurokinin and some of the NKA fragments show extremely high affinities, particularly NKA(3–10). Among the selective agonists [Nle^{10}]NKA(4–10) is more active than [MePhe^7]NKB. These data suggest that the hamster urinary bladder has a fairly large population of NK_2 receptors and also a lower population of NK_3 receptors.

A variety of isolated organs has been investigated to determine which types of neurokinin receptor contribute to the biological

TABLE I. Relative affinities of neurokinins, tachykinins and related peptides

Peptide	NK_1			NK_2			NK_3		
	guinea-pig ileum[a]	dog carotid artery	^{125}I-BH-SP	rat duodenum	rabbit pulmonary artery[b]	[^{3}H]NKA	hamster urinary bladder	rat portal vein	^{125}I-BH-eledoisin
Neurokinins									
SP	100	100	100	2	0.8	3.5	2	1.7	4
NKA	42	25	0.5	100	100	100	159	6	6
NKB	72	8	0.03	85	17	22	100	100	100
Tachykinins									
Physalaemin	182	100	26	2	0.6	0.8	5	3	9
Eledoisin	269	32	0.6	30	100	27	72	27	36
Kassinin	372	16	0.06	60	28	30	501	33	86
Neurokinin fragments									
SP(4–11)	331	47	1.3	1	1	0.2	1	1.5	
SP(5–11)	214	20	0.7	2	2		0.4	0.7	
NKA(3–10)		12		107	79		1148	13	
NKA(4–10)	10	4		93	200	78	282	13	
Selective agonists									
[Sar^9,Met(O$_2$)^{11}]SP	135	282	31	3	0	0.4	0	0	0.1
[Nle^{10}]NKA(4–10)	16	<0.1	<0.01	63	49	83	78	0	0.3
[MePhe^7]NKB	96	<0.1	<0.01	275	0.1	0.05	9	417	282

Relative affinities are expressed in percent of SP (NK_1), NKA (NK_2) or NKB (NK_3).
[a]No treatment; [b]devoid of endothelium
Data taken from Refs 8–10, 20

effects[11] (see Table II). Based on the assumption that the organ response to selective agonists is indicative of the existence and function of a particular receptor, many of the tissues analysed in Table II (e.g. guinea-pig ileum) contain two or even three receptor types. An interesting finding is the presence of NK_2 receptors in the autonomic nerves of the rat[13] and rabbit vas deferens[11] where they facilitate the release of neurotransmitters, in particular noradrenaline. Rabbit and rat vas deferens are both NK_2 monoreceptor systems[11]. Human urinary bladder (the detrusor muscle) is also a pure NK_2 system[11].

From these results, it is evident that future studies on neurokinin biological interactions, particularly the characterization of receptors should be carried out with selective agonists; monoreceptor systems should be used for the evaluation of potential antagonists. As pointed out by Leslie[14] in his recent review on opioids, *'receptor heterogeneity within a tissue may seriously confound measurement of antagonist pA_2 values, such that the calculated value reflects a weighted average of the pA_2 values at two different receptors. Since antagonist pA_2 values are used as a primary means of receptor classification, measurement of "intermediate" values may result in the erroneous identification of novel types of receptors'.*

This applies to substance P and related peptides, because nearly all the antagonists that have been reported since 1982 have been tested in the guinea-pig ileum or in other multiple receptor systems and against natural and non-selective tachy- and neurokinins (see Refs 15 and 16 for reviews). Data so far obtained with antagonists are therefore of little use for neurokinin receptor characterization. The classification of receptors into NK_1, NK_2 and NK_3 is almost exclusively based on agonist potencies[8,9,17]. This is obviously not sufficient; potent, competitive and selective antagonists are needed to corroborate the three neurokinin receptor hypothesis. Furthermore, results obtained in bioassays should be complemented with binding assays since, as shown in Table I, these assays discriminate fairly well between the three different receptor types. More selective ligands should be prepared, using for instance some of the compounds analysed below, as exemplified by a recent study[18] in which the NK_3-selective agonist senktide has been labelled and used successfully for binding assays.

TABLE II. NK receptor types in different tissues

Isolated organ	NK_1	NK_2	NK_3
Dog carotid artery	+		
Guinea-pig ileum (not treated)	+	+	+
Guinea-pig ileum (atropine treated [4.0×10^{-6} M])	+	+	
Guinea-pig urinary bladder	+	+	+
Rabbit pulmonary artery		+	
Rat duodenum	+	+	+
Guinea-pig trachea	+	+	
Hamster urinary bladder		+	+
Rabbit mesenteric vein	+	+	
Rat vas deferens (electrically stimulated prostatic section)		+	
Rat vas deferens (non-stimulated epidydimal section)		+	
Rabbit vas deferens (electrically stimulated prostatic section)		+	
Rat urinary bladder	+	+	
Dog urinary bladder	+	+	
Human urinary bladder		+	
Rat portal vein			+

+: Organ responds to receptor-selective agonists [Sar^9,Met(O$_2$)^{11}]SP (NK_1), [Nle^{10}]-NKA(4–10) (NK_2) or [MePhe^7]NKB (NK_3).
Data based on Ref. 11.

Selective agonists for neurokinin receptors

In a systematic analysis of the C-terminal methionine residue in the three neurokinins we found that oxidation of methionine in SP increased its selectivity for NK_1 receptors while selectivity for the NK_2 receptors was increased by the isosteric replacement of methionine with Nle (norleucine) in NKA. In a parallel study, it was observed that DiMeC$_7$ ([pGlu^5,MePhe^8,Sar^9]SP(5–11)), a metabolically stable SP fragment[19], maintained good activity on the

NK_3 receptor but was inactive on the NK_2 and very weak on the NK_1 receptor system[20].

In an attempt to identify the chemical change that confers upon $DiMeC_7$ such NK_3 selectivity, the following three compounds were prepared and tested: [MePhe^7]NKB(4–10), which was found to have selectivity for the NK_3 receptor; [Sar^8]NKB(4–10), which was found to be selective for the NK_1 receptor; and [MePhe^7, Sar^8]-NKB(4–10) (Ref. 20). Further work on selective neurokinin agonists has recently been published and is summarized in Table III.

Using Met(O_2) as the C-terminal residue and replacing Gly9 with Sar (sarcosine), a potent and selective NK_1 receptor agonist was obtained that was threefold more active than SP on the NK_1 and inactive on the NK_2 and NK_3 receptor systems. The same modifications were made in the C-terminal fragments SP(4–11) and SP(6–11), with additional changes at the amino end to prevent metabolic degradation and improve affinity. It was thereby possible to reduce the size of the compound to that of an hexapeptide, while maintaining potent activity on the NK_1 receptor, and full selectivity. This hexapeptide (Ac-[Arg^6,Sar^9,Met(O_2)^{11}]SP(6–11)) is of particular interest because: (1) it is protected from degradation at both the N and C termini and possibly also from the action of some endopeptidases by the presence of Sar9: and (2) it does not cause release of histamine or (possibly) prostaglandin, but is a potent activator of NK_1 receptors and therefore stimulates the release of EDRF (endothelium-dependent relaxing factor). It appears therefore to have potential as a vasodilatator.

TABLE III. Selective neurokinin receptor agonists

Receptor	Compound	Biological activity					
		dog carotid artery		rabbit pulmonary artery		rat portal vein	
		pD_2	RA[c]	pD_2	RA	pD_2	RA
NK_1	[Sar^9,Met(O_2)^{11}]SP	10.45	282	inactive		inactive	
	[β-Ala^4,Sar^9,Met(O_2)^{11}]-SP(4–11)	10.35	224		0.1		0.1
	Ac-[Arg^6,Sar^9,Met(O_2)^{11}]-SP(6–11)	10.24	174	inactive		inactive	
	Septide[a]	9.69	49	5.20	0.1	5.58	0.8
	SP-OMe	9.60	40	inactive		inactive	
NK_2	NKA(4–10)	8.62	4	8.52	199	6.79	13
	[Nle^{10}]NKA(4–10)	7.00	0.4	7.90	48		<1
NK_3	[β-Asp^4,MePhe^7]-NKB(4–10)	7.02	0.1	5.14	0.08	7.56	76
	[MePhe^7]NKB	7.15	0.14	5.24	0.1	8.30	417
	Senktide[b]	6.39	0.02	5.46	0.2	7.61	85
	[Cys^{2,5}]NKB	9.05	11	6.12	0.8	8.12	272

[a][pGlu^6,Pro^9]SP(6–11); [b]Succ-[Asp^6,MePhe^8]SP(6–11); [c]relative affinity of agonists calculated in percent of SP for the dog carotid artery, of NKA for the rabbit pulmonary artery and of NKB for the rat portal vein.

pD_2: The −log of the concentration of compound producing 50% of the maximal response. It measures apparent affinity.

For comparison the activity of SP-OMe and septide, two NK_1 selective agonists described by Watson *et al.*[22] and by Laufer *et al.*[23], are also shown in Table III; these compounds are however less active than SP *in vitro* and SP-OMe is almost inactive *in vivo*, because of its rapid degradation.

The C-terminal fragment NKA-(4–10) is very potent at the NK_2 receptor but still maintains too high an affinity for the NK_3 system to be described as NK_2-selective (Table I). Further selectivity has been achieved by replacing Met10 with Nle (Table III)[21]. Work is under way in various laboratories

Primary structures of agonists for neurokinin receptors

Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met(O_2).NH_2	[Sar^9,Met(O_2)^{11}]SP
β-Ala-Gln-Gln-Phe-Phe-Sar-Leu-Met(O_2).NH_2	[β-Ala^4,Sar^9,Met(O_2)^{11}]SP (4–11)
Ac-Arg-Phe-Phe-Sar-Leu-Met(O_2).NH_2	Ac[Arg^6,Sar^9,Met(O_2)^{11}]SP (6–11)
pGlu-Phe-Phe-Pro-Leu-Met.NH_2	[pGlu^6,Pro^9]SP(6–11) (Septide)
Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-OMe	SP-OMe
succinyl-Asp-Phe-MePhe-Gly-Leu-Met.NH_2	Succ-[Asp^6,MePhe^8]SP(6–11) (senktide)
Asp-Met-His-Asp-Phe-Phe-MePhe-Gly-Leu-Met.NH_2	[MePhe^7]NKB
β-Asp-Phe-Phe-MePhe-Gly-Leu-Met.NH_2	[β-Asp^4,MePhe^7]NKB (4–10)
Asp-Cys-His-Asp-Cys-Phe-Val-Gly-Leu-Met.NH_2	[Cys^{2,5}]NKB
Asp-Ser-Phe-Val-Gly-Leu-Met.NH_2	NKA(4–10)
Asp-Ser-Phe-Val-Gly-Leu-Nle.NH_2	[Nle^{10}]NKA (4–10)
Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met.NH_2	Substance P
pGlu-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met.NH_2	Physalaemin
His-Lys-Thr-Asp- Ser-Phe-Val-Gly-Leu-Met.NH_2	Neurokinin A
pGlu-Pro-Ser-Lys-Asp-Ala-Phe- Ile-Gly-Leu-Met.NH_2	Eledoisin
Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met.NH_2	Neurokinin B
Asp-Val-Pro-Lys-Ser-Asp-Gln-Phe-Val-Gly-Leu-Met.NH_2	Kassinin

to obtain compounds more active and selective for the NK_2 receptor than those analysed in Table III.

Further improvements in NK_3 selectivity were obtained by N-terminal protection of [MePhe^7]-NKB(4–10) with β-Asp. Affinity of NK_3 selective agonists was considerably increased by using the whole NKB sequence: indeed, [MePhe^7]NKB is four times more active than NKB and is much more selective[21]. Another compound, senktide, has also been demonstrated to be a selective NK_3 receptor agonist[24]: this compound shows good selectivity, when assayed in the three mono-receptor systems and is as active as [β-Asp^4,MePhe^7]NKB(4–10). Other NK_3-selective agonists, one of which is analysed in Table III, have recently been reported[9]. The cyclic analogue of NKB is fairly potent on the rat portal vein, but is not as selective as the others since it demonstrates high affinity for the dog carotid artery.

Possible roles of neurokinins and their receptors

The actions of neurokinin-selective agonists will eventually need to be tested *in vivo*, to elucidate the functional roles of these peptides and their receptors. However, the complexity of an *in vivo* response is much greater than that of any *in vitro* assay organ, even a multireceptor one, since, when injected at therapeutic doses, neurokinins can simultaneously affect cardiovascular, respiratory, gastrointestinal, exocrine, endocrine and other functions. Because of the lack of selectivity of the natural peptides, whose release occurs together with that of co-localized classical neurotransmitters, the final effect is controlled by the receptor type and number in target organs. In such a situation, the exogenous application of a selective agonist may provide the ideal tool for studying receptor function, perhaps even better than that of a selective antagonist, since agonists induce physiological effects that are more easily measured than those of antagonists. Data obtained in recent studies support the above considerations: some of these data are summarized schematically in Table IV.

Because of the implication of SP in pain transmission at the spinal cord level[25], selective agonists (e.g. NK_1-selective [β-Ala^4,Sar^9,Met-(O$_2$)^{11}]SP(4–11) and NK_3-selective [β-Asp^4,MePhe^7]NKB(4–10)) were administered intrathecally in conscious restrained rats and the reaction time to a noxious radiant heat stimulus was measured[26]. The NK_1-selective compound reduced reaction time and thus appeared to facilitate nociceptive transmission, while NKB and the NK_3-selective agonist had the opposite effect and acted as analgesics. The effect of NKB was not affected by intrathecal idazoxan (an α_2-adrenoceptor antagonist) or by [Thi^{5,8},D-Phe^7]bradykinin (a bradykinin B_2 antagonist), but was blocked by naloxone, and has therefore been attributed to the local release of endogenous opioids[26]. Naloxone is inactive against other analgesic agents such as noradrenaline and bradykinin[26].

These observations provide an explanation for the biphasic (hyperalgesic/analgesic) effect of SP, described several years ago[27] and attributed to a postsynaptic excitatory action followed by a reflex compensatory analgesia mediated through opioids. They also raise the interesting question as to whether NK_3-selective agonists could represent a new class of analgesics, whose action is to promote the release of endogenous opioids. Finally, the finding that selective NK_1 agonists are hyperalgesics leaves the way open for another class of analgesics, the NK_1 antagonists.

Another recent study which used selective agonists to investigate the role of neurokinin receptors in the regulation of the cardiovascular system (R. C. *et al.*, unpublished) confirmed the results of previous studies, in which SP was shown to be the most active neurokinin in producing hypotension in rats[28]. When administered i.v. in urethane-anesthetized rats, the selective NK_1 receptor agonist [β-Ala^4,Sar^9,Met(O$_2$)^{11}]-SP(4–11) was found to be more potent and longer acting than SP; it did not produce any major change of heart rate, except perhaps for a short-lasting and rather modest compensatory tachycardia. Thus NK_1 receptors appear to be primarily involved in the hypotensive effect of SP, which is brought about by peripheral vasodilatation.

The intravenous injection of the NK_3-selective agonist, [β-Asp^4,MePhe^7]NKB(4–10) (6.5 nmol kg^{-1}), was followed by a rapid decrease of heart rate, accompanied by hypotension especially after high doses (32.5 nmol kg^{-1}). Both effects lasted for several minutes. Treatment with methylatropine prevented the bradycardia but only slightly reduced the hypotension. These findings suggest that NK_3 receptors may play a functional role in the regulation of heart rate by promoting the release of acetylcholine from the vagus, similar to their actions in the myenteric plexus[12]. Furthermore, NK_3 receptors in peripheral vessels are probably involved in vasodilatation or plasma extravasation which may account for the atropine-resistant hypotensive effect. The hypotensive effect appears to be independent of NK_1 receptor activation since its desensitization with SP failed to affect the response.

NK_2 receptor agonists, when administered i.v., induce tachycardia that lasts for several minutes and a short lasting decrease of blood pressure. Tachycardia is prevented by treating the rats with propranolol (1 mg kg^{-1}). NK_2 receptors appear therefore to facilitate the release of noradrenaline from the cardiac sympathetic nerves as they do in the rat vas deferens *in vitro*[13].

Two other physiological functions of neurokinins, the stimulation of salivary secretion and the increase of vascular permeability, have recently been investigated with neurokinin-selective agonists. The NK_1-selective compound, [Pro^9,Met(O$_2$)^{11}]SP has been found to be a very potent promoter of salivary secretion[29] and to exert a

TABLE IV. Results obtained *in vivo* with neurokinins and agonists selective for neurokinin receptors

	Receptor type			
	NK_1	NK_2	NK_3	Ref.
Analgesia[a]	–	–	+	25
Hyperalgesia[a]	+	–	–	25
Hypotension[b]	+	–	–	27
Bradycardia[b]	–	–	+	27
Tachycardia[b]	–	+	–	27
Salivary secretion[b]	+	–	–	29
Capillary permeability[c]	+	–	+	30

Agonists were administered [a]intrathecally, [b]i.v. or [c]s.c.
+: Receptor(s) involved in the biological effect.

prolonged effect, while the other two selective agonists, [Nle^{10}]-NKA(4–10) and [MePhe^{7}]NKB, are completely inactive. This suggests that salivary secretion is mediated exclusively by NK_1 receptors.

Capillary permeability, evaluated in rats by the extravasation of Evans blue, involves both NK_1 and NK_3 receptors, since NK_1-selective agonists ([β-Ala^{4},Sar^{9}, Met(O$_2$)^{11}]SP(4–11)) are more active than other selective compounds such as [β-Asp^{4},MePhe^{7}]-NKB(4–10) (Ref. 30).

Some of these actions observed in isolated organs and in animals (Table IV) have recently been confirmed in humans. Thus, experiments by Evans *et al.*[31] indicate that NK_1 receptors are involved in the cardiovascular effects of SP, while NK_2 mediate bronchoconstriction by NKA.

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Formation of toxic metabolites from drugs and other xenobiotics by glutathione conjugation

Peter J. van Bladeren

Conjugation with glutathione, usually regarded as a detoxification pathway for xenobiotics, can give rise to the formation of compounds that are at least as reactive as the parent electrophiles. Reactions with cellular constituents such as DNA may then result in toxicity. Peter van Bladeren *describes the chemical rules governing this process which have been extensively investigated in the last few years, and explains why the occurrence of activation via glutathione conjugation can now be predicted fairly accurately.*

Conjugation with glutathione is an important detoxification reaction for electrophilic xenobiotics. Such electrophiles are most often formed in the body by other drug-metabolizing enzymes, and may be responsible for a number of adverse effects ranging from liver necrosis to allergenic reactions.

In general the conjugation is catalysed by the glutathione *S*-transferases, a group of enzymes found in almost every species and in most organs of mammals[1]. However, all substrates for the enzymatic reaction will to some extent also react spontaneously with glutathione.

Despite its primary detoxifying role, it has recently become apparent that glutathione conjugation of some compounds results in their transformation into more reactive and thus usually more toxic derivatives. In principle, two mechanisms could account for this: (1) the conjugate exerts its effect via (reversible) interaction with a receptor; or (2) conjugation transforms, or is a step in the transformation of, a

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