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Short communication

The dogfish peptides scyliorhinin I and scyliorhinin II bind with differential selectivity to mammalian tachykinin receptors

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The dogfish intestinal, linear tachykinin scyliorhinin I (SCYI) and cyclic tachykinin scyliorhinin II (SCYII) bound with differential selectivity to mammalian tachykinin, membrane receptor sites. SCYI bound with highest affinity to NK-1 sites in rat submandibular gland ($K_I = 0.9$ nM) and to NK-2 sites in hamster urinary bladder ($K_I = 2$ nM) whereas SCYII bound with highest affinity to NK-3 sites in rat cerebral cortex ($K_I = 2.5$ nM). These results suggest that SCYI is a dual NK-1/NK-2 tachykinin receptor agonist while SCYII is an NK-3 selective tachykinin receptor agonist.

Tachykinin; Neurokinin; Peptide receptors; (Receptor binding)

1. Introduction

The tachykinins are a family of mammalian and non-mammalian peptides possessing the C-terminus Phe-X-Gly-Leu-Met-NH_2. Three major mammalian tachykinins have been identified: substance P (SP), neurokinin A (NKA) (substance K), and neurokinin B (NKB) (neuromedin K) (Harmar, 1984) (table 1). Recent receptor binding studies have been indicated the existence of three distinct types of tachykinin receptors (Quirion, 1985; Buck and Burcher, 1986; Regoli et al., 1987). One of these receptor types (NK-1) exhibits preferential affinity for SP, one (NK-2) exhibits preferential affinity for NKA, and one (NK-3) exhibits preferential affinity for NKB. Pharmacological assessment of biological responses to the tachykinins has generally been consistent with this receptor classification scheme (Jacoby et al., 1986; Wormser et al., 1986; Regoli et al., 1987; Bristow et al., 1987).

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TABLE 1

Amino acid sequences of some tachykinin peptides.

SP	H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
NKA	H-His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2
NKB	H-Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH_2
PHYS	pGlu-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met-NH_2
ELE	pGlu-Pro-Ser-Lys-Asp-Ala-Phe-Ile-Gly-Leu-Met-NH_2
KAS	H-Asp-Val-Pro-Lys-Ser-Asp-Gln-Phe-Val-Gly-Leu-Met-NH_2
SCYI	H-Ala-Lys-Phe-Asp-Lys-Phe-Tyr-Gly-Leu-Met-NH_2
SCYII	┌Cys-Pro-Asp-Gly-Pro-Asp-Cys-Phe-Val-Gly-Leu-Met-NH_2 └Lys-Ser-Asn-Ser-Pro-Ser-H

Two new tachykinins with smooth muscle contractile activity have recently been identified in extracts of the dogfish intestine (Conlon et al., 1986). One of these is a linear decapeptide, scyliorhinin I (SCYI) (table 1), and the other is the first naturally occurring cyclic tachykinin to be discovered, scyliorhinin II (SCYII) (table 1). SCYI and SCYII share considerable structural homology with NKA and NKB, and with the non-mammalian tachykinins eledoisin (ELE), kassinin (KAS) and physalaemin (PHYS) (table 1). We have now examined the receptor binding affinity of SCYI and SCYII at tachykinin NK-1, NK-2 and NK-3 receptors.

2. Materials and methods

SCYII, SCYII-(5-18), and SCYII-(7-18) were synthesized by solid phase methods. All other peptides were purchased from commercial sources. Receptor binding studies were carried out as previously described using filtration techniques and 0.1 nM ^{125}I-Bolton-Hunter-SP (BHSP) to label tachykinin NK-1 sites in rat submandibular gland crude membranes, 0.1 nM [^{125}I]iodohistidyl^{1}-NKA (INKA) to label NK-2 sites in hamster urinary bladder crude membranes, or 0.1 nM ^{125}I-Bolton-Hunter-ELE (BHELE) to label NK-3 sites in rat cerebral cortex crude membranes (Buck and Burcher, 1986; citations therein). $MnCl_2$ at a concentration of 2 mM was included in all binding assays.

3. Results

In crude membranes from rat submandibular gland, specific BHSP binding to NK-1 receptors amounted to 5000 c.p.m./mg tissue and was 80% of total binding. This binding was inhibited in the rank order (K_I; mean (nM) ± S.E.M. of 3-5 determinations) SP (0.5 ± 0.1) > SCYI (0.9 ± 0.1) > NKA (50 ± 5) > SCYII (5-18) (135 ± 25) > septide ([pGlu^{6}-Pro^{9}]SP-(6-11)) (315 ± 65) > NKB (340 ± 70) > SCYII (440 ± 70) > SCYII-(7-18) (4800 ± 1200) > senktide ([sucAsp^{6}mePhe^{8}]SP-(6-11)) (> 10000).

In crude membranes from hamster bladder, specific INKA binding to NK-2 receptors amounted to 3000 c.p.m./mg tissue and was 90% of total binding. This binding was inhibited in the rank order NKA (0.8 ± 0.2) > SCYI (2 ± 0.2) > NKB (16 ± 4) > SCYII-(5-18) (70 ± 17) > SP (130 ± 15) > SCYII (500 ± 40) > SCYII-(7-18) (900 ± 85) > septide/senktide (> 10000 each).

In crude membranes from rat cerebral cortex, specific BHELE binding to NK-3 receptors amounted to 250 c.p.m./mg tissue and was 75% of total binding. This binding was inhibited in the

TABLE 2

Selectivity at tachykinin receptor binding sites. In this tabulation, the larger the K_I/K_I ratio, the greater the receptor selectivity. The hyphens for senktide indicate K_I values too large for an accurate ratio calculation. IC_{50} values were calculated with a computer program (LIGAND) from an 8-10 point inhibition curve. K_I was assumed to be equal to IC_{50} since 0.1 nM ligand is at least one order of magnitude less than any reported K_D for the three tachykinin ligands.

Selectivity:	NK-1		NK-2		NK-3	
K_I/K_I:	NK-2/ NK-1	NK-3/ NK-1	NK-1/ NK-2	NK-3/ NK-2	NK-1/ NK-3	NK-2/ NK-3
SP	260	130	0.004	0.5	0.008	2
NKA	0.02	0.86	63	54	1	0.02
NKB	0.05	0.004	21	0.09	227	11
SCYI	2	106	0.45	48	0.009	0.02
SCYII	1	0.006	0.88	0.005	176	200
SCYII-(5-18)	0.5	0.03	2	0.05	36	18
SCYII-(7-18)	0.19	0.0003	5	0.002	3200	600
Septide	> 32	21	< 0.03	< 0.67	0.05	> 1.5
Senktide	–	< 0.00001	–	< 0.0001	> 10000	> 10000

rank order senktide (1.0 ± 0.2) > NKB (1.5 ± 0.3) = SCYII-(7-18) (1.5 ± 0.2) > SCYII (2.5 ± 0.5) > SCYII-(5-18) (3.8 ± 1) > NKA (43 ± 5) > SP (65 ± 10) > SCYI (95 ± 10) > septide (6700 ± 700).

4. Discussion

Our data indicate that SCYI possesses highest affinity for tachykinin NK-1 and NK-2 receptors. The affinity at each of these receptors is essentially identical to that of the putative mammalian endogenous ligand at each, SP and NKA, respectively. In contrast, SCYI has 100-fold lower affinity for NK-3 receptor sites relative to NK-1 sites and 48-fold lower affinity for NK-3 receptor sites relative to NK-2 sites (table 2). At NK-1 receptors, SCYI has at least 100-fold greater affinity than septide which does not possess the NK-1 receptor affinity expected from the EC_{50} of 2.5 nM in the guinea-pig ileum (Wormser et al., 1986). Rather than the NK-1 selectivity ratio (EC_{50} NK-3/EC_{50} NK-1) of 520 reported for septide in this tissue, we obtained a binding selectivity ratio (K_I NK-3/K_I NK-1) of 21. Lee et al. (1986) observed a similar low NK-1 selectivity for septide at tachykinin receptor binding sites. Thus, septide is not as selective for NK-1 receptors over NK-3 receptors as is SP (ratio = 130) of SCYI (ratio = 100). However, septide has much less affinity for NK-2 receptors than SP or SCYI (table 2).

SCYII is an NK-3 selective tachykinin with 176- and 200-fold greater affinity for NK-3 receptors than for NK-1 and NK-2 receptors, respectively. Removal of N-terminal residues from SCYII has little effect on its affinity for NK-3 sites, but SCYII-(5-18) is less NK-3 selective (K_I NK-1/K_I NK-3 = 36; K_I NK-2/K_I NK-3 = 18) and SCYII-(7-18) is more NK-3 selective (K_I NK-1/K_I NK-3 = 3200; K_I NK-2/K_I NK-3 = 600) than SCYII. SCYII has essentially the same affinity for NK-3 receptors as NKB, but SCYII is substantially more NK-3 selective than NKB. However, the synthetic peptide senktide (Wormser et al., 1986) is clearly the most NK-3 selective peptide known in that it has at least 10000-fold greater affinity for NK-3 receptors than for either of the other two types (table 2).

SCYII is the only naturally occurring tachykinin having an Asp residue in the position corresponding to position 5 of SP that has very low affinity for NK-2 receptors. NKA, NKB, ELE, KAS and SCYI all possess substantially more NK-2 affinity than SCYII. The low NK-2 affinity is most likely due to an unfavorable conformational constraint imposed by the cyclic structure in SCYII. The greater NK-3 selectivity of SCYII-(7-18) is consistent with the hypothesis of Schwyzer (1987) that NK-3 receptor selectivity is enhanced by greater overall net charge (e.g. SCYII-(7-18) lacks the positively charged Lys^6 residue of SCYII). SCYI, on the other hand, contains two Lys residues that would result in a more favorable interaction with the anionic fixed-charge compartment of the plasma membrane and greater access to NK-1 and NK-2 receptors (Schwyzer, 1987).

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