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Scyliorhinin-I and -II induce reciprocal hindlimb scratching in mice: differentiation of spinal and supraspinal neurokinin receptors in vivo

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Scyliorhinin-I amide (SCY-I) (selective for NK-1 and NK-2 receptors) or scyliorhinin-II amide (SCY-II) (selective for NK-3 receptors) were injected either spinally (i.t.; intrathecally) or supraspinally (i.c.v.; intracerebroventricularly) to mice. Following i.c.v. administration, SCI-I and SCY-II produced potent, dose-related reciprocal hindlimb scratching about equipotently (ED_{50} = 0.05 and 0.08 nmol, respectively). However, following i.t. administration, only SCY-I elicited greater than 50% response (ED_{50} = 0.07 nmol). Reciprocal hindlimb scratching is a behavioral response that has not been associated previously with neurokinins. These results might provide the first functional in vivo correlate for the differential localization of neurokinin receptor types within the mammalian central nervous system.

Substance P and the neurokinins (mammalian tachykinins), are polypeptides that share a common C-terminal sequence (Phe-X-Gly-Leu-Met-NH_2) and a similar spectrum of biological activities mediated through distinct neurokinin (NK) receptors designated NK-1, NK-2 and NK-3 [2, 3, 9, 14]. In both humans and rats, two separate genes encode for the precursors of substance P and neurokinin A (designated PPT-I; preprotachykinin gene I) or neurokinin B (PPT-II) [1]. Although all three NK receptor types have been demonstrated in mammalian brain and spinal cord [e.g., 7, 8, 22], it is unclear whether all three types of NK receptors, particularly NK-3, are present in sufficient densities in either brain or spinal cord [15] to have functional significance in both or whether it is possible to differentiate, functionally, between spinal and supraspinal NK receptor types. Although substance P, neurokinin A (NKA) and neurokinin B (NKB) are the putative endogenous ligands for the NK receptor types, they lack the required receptor-type selectivity for this analysis [3, 5]. Likewise, the previous lack of NK-3-selective iodinated radioligands has hampered the direct visualization of NK-3 receptors in tissues containing multiple NK receptor types (e.g., [^{125}I]Bolton-Hunter-eledoisin lacks sufficient receptor selectivity [3] and although the substance P analog senktide is highly NK-3-selective, the iodinated form has high non-specific binding [13]).

Mussap and Burcher [15] recently demonstrated that the iodination with Bolton-Hunter reagent of the dogfish disulfide-bridge octadecapeptide scyliorhinin-II (SCY-II) [6] (Fig. 1), which has NK-3 receptor selectivity and affinity similar to NKB [4], resulted in a novel, selective radioligand for NK-3 receptors in the mammalian central nervous system. The present study used SCY-II to examine the possible functional differentiation of NK receptor types. Specifically, SCY-II or scyliorhinin-I (SCY-I), a linear decapeptide having NK-1 and NK-2 receptor selectivity and affinity similar to that of substance P and NKA, were administered into the brains or spinal cords of mice. We report a novel behavioral effect elicited by both SCY-I and SCY-II, namely atropine-insensitive reciprocal hindlimb scratching (RHS – alternately scratching first with one hindlimb and then the other) and, using this novel behavioral response, the apparent differentiation of spinal and supraspinal neurokinin receptors. These results appear to provide a functional in vivo correlate for the localization of neurokinin receptor types.

Male, virus-free albino CD-1 mice, 18–25 g (Charles River Laboratories; Kingston Facility, Stoneridge, NY) were group housed 8–10 per plastic cage and maintained in a climate controlled room on a 12 h light/dark cycle (lights on at 07.15 h). Food and water were available ad libitum up to 1 h prior to test. Each animal was tested

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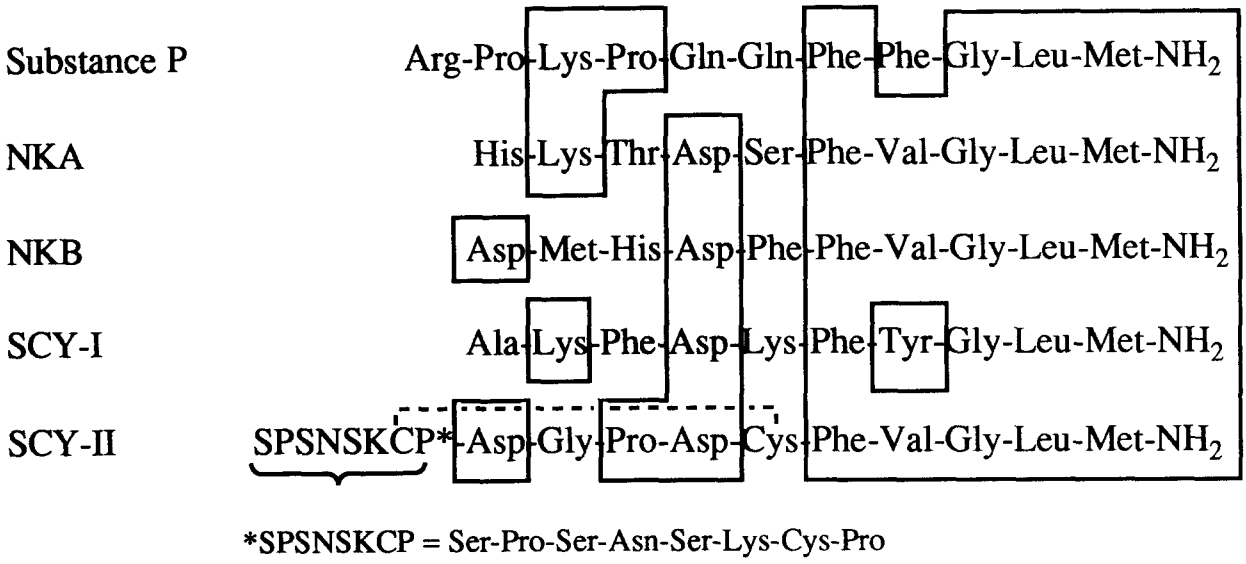

Fig. 1. Amino acid sequence homology between the dogfish peptides scyliorhinin-I (SCY-I) and scyliorhinin-II (SCY-II) with the mammalian tachykinins.

once. All testing was performed in accordance with the recommendations and policies of the National Institutes of Health (NIH) and Johnson & Johnson Guidelines for the care and use of laboratory animals. The procedure was similar to that described by Scott et al. [18]. Scyliorhinin-I amide or scyliorhinin-II amide (Peninsula Laboratories, Inc., Belmont, CA), atropine sulfate, naloxone hydrochloride, or pirenzepine hydrochloride (commercial sources) were dissolved in double distilled water and injected in a volume of 5 μl either i.c.v. (intracerebroventricularly) according to the method of Haley and McCormick [10] or i.t. (intrathecally) according to the method of Hylden and Wilcox [12]. Following injection, each mouse was immediately placed into a large glass jar (14 cm diameter; 2 mice per jar) containing a thin layer of ground wood chips. The mice were observed for 5 min for the occurrence of a single RHS response. The results are expressed as the percentage of the mice that demonstrated RHS within the 5 min observation period. The ED_{50} value (dose of agonist that elicited RHS in 50% of the mice tested) and 95% confidence limits were determined from the linear regression analysis of probit plots.

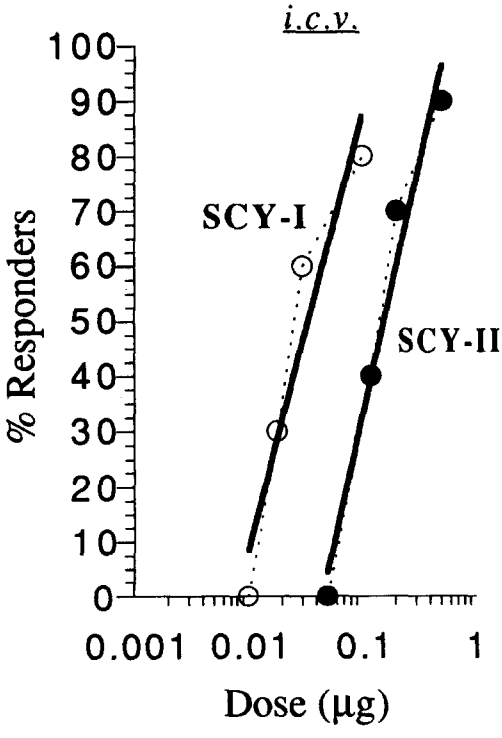

Fig. 2. Elicitation of reciprocal hindlimb scratching response following the supraspinal (i.c.v.) administration of scyliorhinin-I (SCY-I; ○) or scyliorhinin-II (SCY-II; ●) to mice (n = 10 per dose).

Both SCY-I and SCY-II produced a dose-related RHS response by the i.c.v. route (Fig. 2) with SCY-I slightly, but not significantly ($P > 0.05$) more potent than SCY-II (Table I). Higher doses elicited excessive grooming behavior. The RHS response had a rapid onset (4–5 min) and lasted for more than 30 min after the injection.

SCY-I also produced a dose-related RHS response by the i.t. route (Fig. 3, Table I). Atropine, naloxone, or pirenzepine did not block the RHS response when administered i.c.v. or i.t. concurrently with SCY-I (Table II). The doses of atropine (0.1 μg) and pirenzepine (0.1 μg) were 10 times the amount previously shown to completely block pilocarpine-induced RHS [17, 18]. Critically, i.t. administration of SCY-II failed to produce 50% RHS at doses up to 2 nmol, more than 25-fold the ED_{50}

TABLE I

ED_{50} VALUES (95% CONFIDENCE INTERVALS) AND RELATIVE POTENCY (rp) FOR THE ELICITATION OF RECIPROCAL HINDLIMB SCRATCHING IN MICE (n = 10–20 PER DOSE) ADMINISTERED SCYLIORHININ-I (SCY-I) OR SCYLIORHININ-II (SCY-II) INTRACEREBROVENTRICULARLY (i.c.v.) OR INTRATHECALLY (i.t.)

	i.c.v.		i.t.	
	μg/5 μl	nmol	μg/5 μl	nmol
SCY-I	0.06 (0.02–0.26)	0.05 (0.02– 0.21)	0.08 (0.03– 0.20)	0.07 (0.02– 0.16)
SCY-II	0.15 (0.10– 0.23)	0.08 (0.05– 0.12)	> 4.0*	> 2.2*
rp (II/I)	2.5	1.6	> 50.0*	> 31.4*

*Significantly different ($P < 0.05$ from i.c.v., relative potency analysis).

by the i.c.v. route and 30-fold the i.t. ED_{50} of SCY-I (Table I).

The observation of centrally mediated RHS is a novel finding with scyliorhinins and, in general, not a common drug-induced effect. Pilocarpine was reported to cause RHS through activation of muscarinic receptors [18], but the failure of atropine and pirenzepine to block the behavior in the present case suggests a different mechanism of action for SCY-I-induced RHS. In addition, the inability of naloxone to block this behavior rules out binding to spinal opioid receptors.

Autoradiographic data suggest that all three neurokinin receptor types are present in rat brain, but that NK-2 or NK-3 receptors are present to a lesser extent in the spinal cord [16, 22]. The results of the present study demonstrate a functional correlate between the anatomical distribution of neurokinin receptor types and an in vivo response. These findings could have implications for a wide variety of biological effects believed to be mediated through neurokinin receptor-related mechanisms. For example, neurokinin-containing sensory neurons have been associated with the transmission of noxious afferent information involved in acute pain or neurogenic inflammatory processes [e.g., 19].

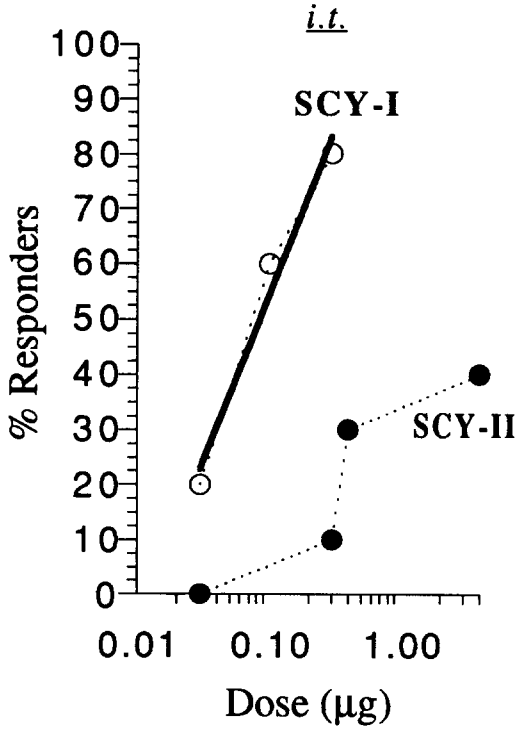

Fig. 3. Elicitation of reciprocal hindlimb scratching response following the spinal (i.t.) administration of scyliorhinin-I (SCY-I; ○) or scyliorhinin-II (SCY-II; ●) to mice (n = 10 per dose).

The present study did not address the question of which NK receptor type(s) mediate the RHS response. The recent autoradiographic data [16] needs to be considered in the context of other studies in terms of NK-1, NK-2 and NK-3 receptor localization [e.g., 20] and possible species differences [e.g., 11 and 20]. In the spinal cord, the NK-2 sites are the least abundant and the number of NK-3 sites appears to be intermediate between the other two types. The NK-1 receptors are most concentrated in the dorsal and ventromedial borders of the dorsal horn, the intermediolateral nucleus of the thoracic cord and the phrenic motor nucleus in the cervical ventral horn; NK-2 receptors are mostly found along the dorsal and ventromedial borders of the dorsal horn, in a narrow band connecting the two lateral horns of the thoracic cord, around the central canal of the lumbar, and sacral segments and lamina IX of the cervical ventral horn; NK-3 receptors are most dense in the dorsal border of the dorsal horn, with moderate amounts in the lateral horn of the thoracic cord and around the central canal of lumbar and sacral segments [22]. Hence, by their differential distribution, each NK receptor type could mediate sensory, autonomic or motor functions [reviewed in 11]. With regard to sensory input, intrathecal administration of NK-1-selective agonists to rats mimics the nociceptive effects of tachykinin release from pri-

TABLE II

TEST FOR ANTAGONISM OF SCYLIORHININ-INDUCED RECIPROCAL HINDLIMB SCRATCHING (RHS) IN MICE, INDICATED AS PERCENT OF MICE (n = 10–20 PER DOSE) RESPONDING.

Each antagonist was administered concurrently with SCY-I (0.5 μg/5 μl i.c.v. or 0.3 μg/5 μl i.t.) or pilocarpine (2.0 μg/5 μl).

	RHS
i.c.v.	
Atropine (0.1)*	0%
Pirenzepine (0.1)	0%
Naloxone (10)	0%
SCY-I	100%
SCY-I + atropine (0.1)	100%
SCY-I + pirenzepine (0.1)	90%
SCY-I + naloxone (10)	100%
i.t.	
SCY-I	90%
SCY-I + atropine (0.1)	90%
SCY-I + pirenzepine (0.1)	90%
SCY-I + naloxone (10)	100%
Pilocarpine	90%
Pilocarpine + atropine (0.1)	0%
Pilocarpine + pirenzepine (0.1)	0%
Pilocarpine + naloxone (10)	90%

*Dose of antagonist, expressed as μg/5 μl.

mary sensory nerve terminals in the dorsal horn of the spinal cord, consistent with the enhanced expression of the PPT-I gene (mRNA levels) following painful stimuli, and NK-3 receptor activation results in antinociception (possibly by the spinal release of an endogenous opioid peptide). Whether the elicitation of RHS relates to the sensory, or other, aspects of the NK receptors cannot be determined from the present study.

Pilocarpine, and other muscarinic agonists, are among a relatively limited number of compounds that produce RHS [18]. Whether pilocarpine and the scyliorhinins induce this behavior through independent pathways or whether they share one or more common steps is not presently known. Pilocarpine-induced RHS is blocked by the neurokinin antagonists [D-Pro^2,D-Trp^{7,9}]-SP and [D-Pro^2,D-Trp^{6,8},Nle^{10}]-NK [18], but bombesin- or somatostatin-induced RHS is not [21].

In summary, this study provides evidence for the functional differentiation of spinal and supraspinal neurokinin receptor types in mice and correlates these receptor types to a behavioral response. These results also appear to provide a practical method of evaluating the potential in vivo receptor-type selectivity of neurokinin-like agonists and antagonist analogs.

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