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Relationships between neurokinin receptor-expressing interstitial cells of Cajal and tachykininergic nerves in the gut

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Abstract

The so-called interstitial cells of Cajal (ICC) are distributed throughout the muscle coat of the alimentary tract with characteristic intramural location and species-variations in structure and staining. Several ICC sub-types have been identified: ICC-DMP, ICC-MP, ICC-IM, ICC-SM. Gut motility is regulated by ICC and each sub-type is responsible for the electrical activities typical of each gut region and/or muscle layer. The interstitial position of the ICC between nerve endings and smooth muscle cells has been extensively considered. Some of these nerve endings contain tachykinins. Three distinct tachykinin receptors (NK1r, NK2r and NK3r) have been demonstrated by molecular biology. Each of them binds with different affinities to a series of tachykinins (SP, NKA and NKB). In the ileum, SP-immunoreactive (SP-IR) nerve fibers form a rich plexus at the deep muscular plexus (DMP), distributed around SP-negative cells, and ICC-DMP intensely express the SP-preferred receptor NK1r; conversely a faint NK1r-IR is detected on the ICC-MP and mainly after receptor internalization was induced by agonists. ICC-IM are never stained in laboratory mammals, while those of the human *antrum* are NK1r-IR. RT-PCR conducted on isolated ileal ICC-MP and gastric ICC-IM showed that these cells express NK1r and NK3r. Colonic ICC, except those in humans, do not express NK1r-IR, at least in resting conditions. Outside the gut, NK1r-IR cells were seen in the arterial wall and exocrine pancreas. In the mouse gut only, NK1r-IR is present in non-neuronal cells located within the intestinal villi, so-called *myoid cells*, which are c-kit-negative and α-smooth muscle actin-positive. Immunohistochemistry and functional studies confirmed that ICC receive input from SP-IR terminals, with differences between ICC sub-types. In the rat, very early after birth, NK1r is expressed by the ICC-DMP and SP by the related nerve varicosities. Studies on pathological conditions are few and those on mutant strains practically absent. It has only been reported that in the inflamed ileum of rats the NK1r-IR ICC-DMP disappear and that at the peak of inflammatory conditions ICC-MP are NK1r-IR. In the ileum of mice with a mutation in the W locus, ICC-DMP were seen to express c-kit-IR but not NK1-IR, and SP-IR innervation seems unchanged. In summary, there are distinct ICC populations, each of them under a different tachykininergic control and, likely, having different functions. Further studies are recommended at the aim of understanding ICC involvement in modulating/transmitting tachykininergic inputs.

Keywords: alimentary tract • interstitial cells of Cajal • ICC • neurokinin receptors • NKr • substance P • SP • immunohistochemistry

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The interstitial cells of Cajal of the alimentary tract

The so-called interstitial cells of Cajal (ICC) are distributed throughout the smooth muscle coat of the alimentary tract. These highly branched cells occur in networks associated with the muscular plexuses of the enteric nervous system. Both in humans and laboratory mammals, ICC have a characteristic intramural distribution specific to the different regions of the gut and species-variations in structure and staining have also been reported [1–12]. Consequently, several ICC sub-types have been identified.

One population of ICC (the ICC at the myenteric plexus of Auerbach, called ICC-MP or ICC-AP or IC-MY) is located at the ganglionated myenteric plexus level all along the gut length; another ICC population is located intramuscularly and exists as two ICC sub-types: those located within muscle bundles are called intramuscular ICC or ICC-IM, and those located in the septa dividing muscle bundles are called ICC-SEP. A third ICC population (the ICC-SMP or ICC-SM) is distributed along the submucosal border of the circular muscle layer of the gastric antrum and the colon. Finally, a fourth ICC population (the ICC-DMP) is present in the small intestine, distributed between the inner and the outer portions of the circular muscle layer at the level of the deep muscular plexus (DMP). All the ICC sub-types have in common to be closely associated with nerve endings, with each other and with smooth muscle cells.

ICC identification by c-kit labeling

Several specific molecules are expressed by ICC. One of them is the c-kit receptor [13], which is expressed by all the ICC sub-types in most of the animal species and, therefore, has a great importance since it allows easy recognition of ICC either under light or electron microscope [10]. For this reason, c-kit labelling is widely used to study both normal and pathological specimens and also to follow ICC differentiation during prenatal life. To understand the roles played by ICC in regulating gut motility it is of critical importance to gain knowledge about neurotransmitter receptors on ICC. The NK1 receptor may be of particular interest [14–18].

ICC innervation

The interstitial position of the ICC between nerve endings and smooth muscle cells, firstly stressed by Cajal himself, has been extensively considered [19]. Interestingly, although all ICC are intimately associated with nerve endings, important differences in the frequency of these contacts among the ICC sub-types, both in laboratory mammals and humans, are present [2, 4]. For example, the ICC-MP have a lower number of contacts with nerve endings with respect to the ICC-DMP, to the colonic ICC-SMP and to the gastric ICC-IM and ICC-SEP.

Several neurotransmitters have been identified in these nerve endings by immunohistochemistry, both under light and electron microscope [16, 20–26]. Some of them are excitatory (substance P [SP] and acetylcholine [Ach]) and others inhibitory (vasointestinal polypeptide [VIP] and nitric oxide [NO]) transmitters. Importantly, immunohistochemical studies demonstrated the presence of receptors on ICC for some of these neurotransmitters [14–18, 27–30] and functional studies confirmed that these cells are one of their targets [30–33]. No information is available on a possible sensory innervation of these cells, but this possibility cannot be excluded and deserves further attention.

Roles of the ICC sub-types

Physiological studies have shown that gut motility is fundamentally regulated by ICC and that ICC sub-types are differently responsible for the electrical activities typical of each gut region and/or muscle layer [7, 19, 26, 31, 32, 34–39].

The ICC-MP, which are distributed throughout the entire gut, and the colonic ICC-SMP are responsible for pacemaker activity [9, 38, 40]. The presence of nerve endings close to ICC-MP has been interpreted as a neural control of the slow-wave activity [41]. ICC-IM, which show a large number of nerve ending contacts, have been proposed to be intermediaries in neurotransmission [9, 30–32, 35, 39, 42–47]. Functional investigations performed on the stomach of mutant mouse strains lacking one or more ICC sub-types and of dystrophic mice (*mdx* mice) showed that the ICC-SEP generate the secondary regenerative component of slow waves [40,

48–51]. ICC-DMP have been suggested to be involved in mediation of NO-dependent neurotransmission [52] and were also seen to produce slow waves, which, however, are of a different character from those produced by the ICC-MP [53]. Recently, these cells have been demonstrated to be part of the intestinal stretch receptor [26, 38].

Neurokinin receptors

Three distinct G-protein-coupled tachykinin receptors (NK1r, NK2r and NK3r) have been demonstrated by molecular biology [54, 55]. Each of them binds with different affinities to a series of neuropeptides called tachykinins, including SP, neurokinin A (NKA) and neurokinin B (NKB). In particular, NK1r, NK2r and NK3r have the highest affinity to SP, NKA and NKB, respectively [56–58]. Moreover, in the ileum of mouse, rat and guinea-pig, species-dependent variations in the affinity of these receptors have been demonstrated by pharmacology [56, 57, 59, 60] and in amino acid sequence by molecular biology [61].

The availability of specific antibodies [62, 63] has allowed identifying the sites of localization of NK1r, NK2r and NK3r in the gut of several mammals [14, 15, 17, 29, 63–67] and have shown a region-specific distribution. NK1r- and NK3r-immunoreactivity (IR) was detected in both submucous and myenteric neurons. NK1r-IR was also detected in non-neuronal cells. *i.e.* in some of the ICC populations and, in the mouse only, in the *myoid cells* of the villi (see later). Despite the pharmacological hypothesis of the potent excitatory action exerted in the small intestine by SP, smooth muscle cells were found to express NK2r but not NK1r. This fact was explained by the existence at the level of the longitudinal muscle layer of a NK1r subtype, the so-called septide preferring receptor [68], not identifiable with available antibodies, and at the circular muscle layer with the possibility that contractions due to SP could be mediated through ICC activation [14, 15]. Recently [67, 69], NK1r-IR presence in the intestinal smooth muscle cells was demonstrated in the guinea pig, but only after incubation with agonists. Moreover, NK2r-IR has been detected in nerve terminals of guinea-pig and rat ileum [14, 63], a result that was not predicted by pharmacology. The absence of NK2r-IR in nerve varicosities in the mouse suggests that in this species, at variance with rat and guinea-pig, the NK2r does not play a presynaptic role.

Substance P

The tachykinins are a family of closely related peptides which act *via* three distinct tachykinin receptors [70]. In the muscle coat of the gut, the most highly represented tachykinins are SP and NKA where they act as excitatory transmitters causing contraction of the muscle directly to both muscle layers or, indirectly, through the activation of enteric neurons [71]. Obviously, the tachykinin actions depend on the specific distribution of the neurokinin receptors [56, 57, 72].

It has to be noted that SP-containing nerves in the *muscularis externa* are primarily excitatory motor neurons and morphological and functional evidence indicates that they are located in the enteric plexuses, capable to synthesize and store tachykinins [73, 74]. Indeed, in laboratory mammals, SP-IR was detected in myenteric and submucous neurons and in varicose nerve fibers [73, 74] which are distributed in the mucosa, submucosa and muscle layers, especially in the circular muscle. In the ileum they form a rich plexus at the DMP closely associated with SP-negative cells (Fig. 1A). Thin, varicose SP-IR nerve fibers have also been described in the human stomach [29, 75], but, at variance with the intestine, all the gastric areas, especially the *fundus,* have a low density of these nerve fibers [75].

SP-IR intensity and networks formed by SP-IR nerve fibers were found to be different according to the various plexuses and animal species [27]. As an example, SP-IR intensity at the DMP is similar to that observed at the MP in the mouse and rat ileum and higher than that in the guinea pig MP; SP-IR nerve fibers at the DMP are more numerous than those at the MP in all animal species. Moreover, at the MP, both in the rat and guinea pig, the SP-IR network has large meshes, the varicose SP-IR axons are few, oriented parallel or obliquely to the longitudinal muscle layer and run single. In the guinea-pig, the SP-IR DMP network has either large or small meshes, all made of numerous varicose fibers oriented parallel to the

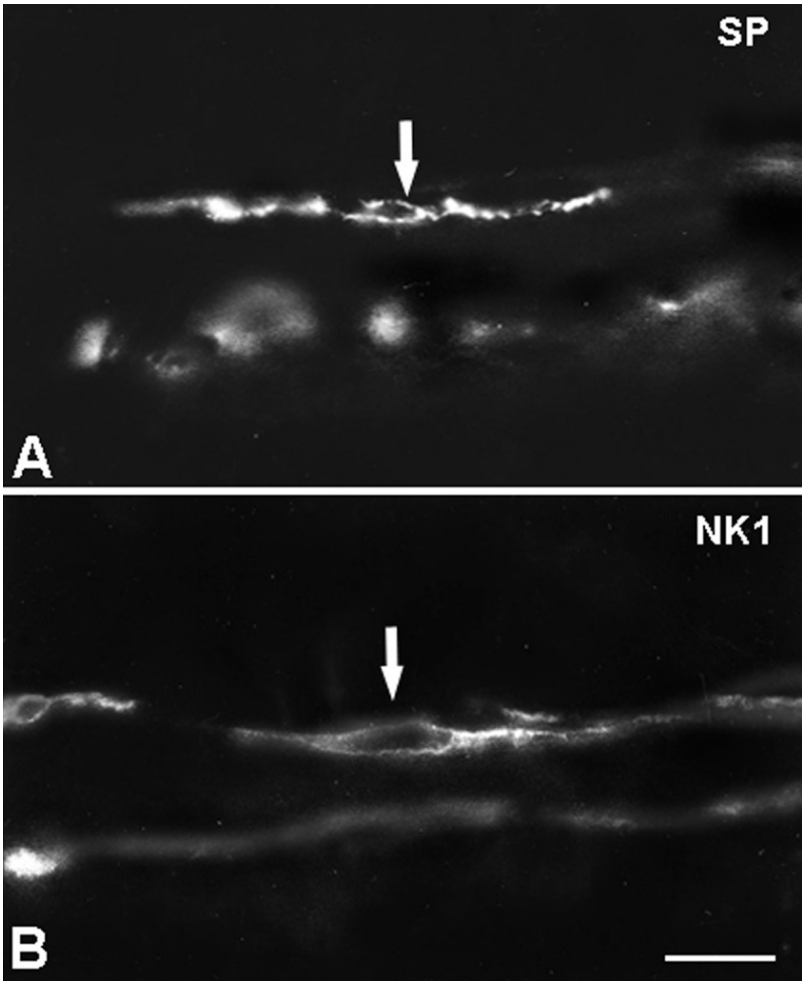

Fig. 1 Mouse ileum, transverse section of the gut wall. **A.** Substance P immunoreactivity (SP-IR). SP-IR is present within muscle and at the myenteric plexus level. At the deep muscular plexus (*arrow*) the SP-IR nerve endings are particularly numerous, have intense IR, and surround a SP-negative cell. **B.** Neurokinin 1 receptor-immunoreactivity (NK1r-IR). NK1r-IR is distributed in the myenteric plexus neurons and on spindle-shaped cells (*arrow*) located at the deep muscular plexus region. These cells appear as cells oriented parallel to the major axis of the circular muscle cells and having at the opposite poles two long, main processes. **A** and **B**: fluorescence microscope. Calibration bar = 10 μm. .

major axis of the circular muscle cells and frequently interconnected by obliquely oriented fibers; two or three varicose SP-IR axons often run together (Fig. 2A). Conversely, in the rat, these axons are significantly less numerous and always run as a single fiber (Fig. 2B).

ICC sub-types and NK1r-IR

ICC-DMP

In all animal species studied, the ICC-DMP express the SP-preferred receptor NK1r. NK1r-IR has a strong intensity, is distributed along the cell membrane at both cell body and processes and often has a punctuate aspect. Its distribution clearly defines the contour of these cells, allowing us to evaluate their shape and size and also to remark differences in the ICC-DMP morphology among animal species. For example, ICC labelled for NK1r appear in all animals species as cells oriented parallel to the major axis of the circular muscle cells and having at the opposite poles two long, main processes. Thin, short and ramified branches protrude laterally from the body and processes, by which these cells connect to each other and with the smooth muscle cells of the outermost portion of the circular muscle layer. The ICC body is elongated and spindle-shaped in the mouse and rat (Fig.1B, 2B, 3B) and oval or round in the guinea-pig (Fig.2A, 3A).

ICC-MP

In previous papers it was reported that the NK1r-IR was detectable on the ICC-MP contour in the guinea pig duodenum but not ileum [14]. Later [16], NK1r-IR was detected in the cytoplasm of the ileal ICC-MP, but only after receptor internalization was induced by the agonist SP. Therefore, it was suggested that the absence of immunoreactivity in rest conditions was due to a receptor conformation that masked the site for antibody inter-

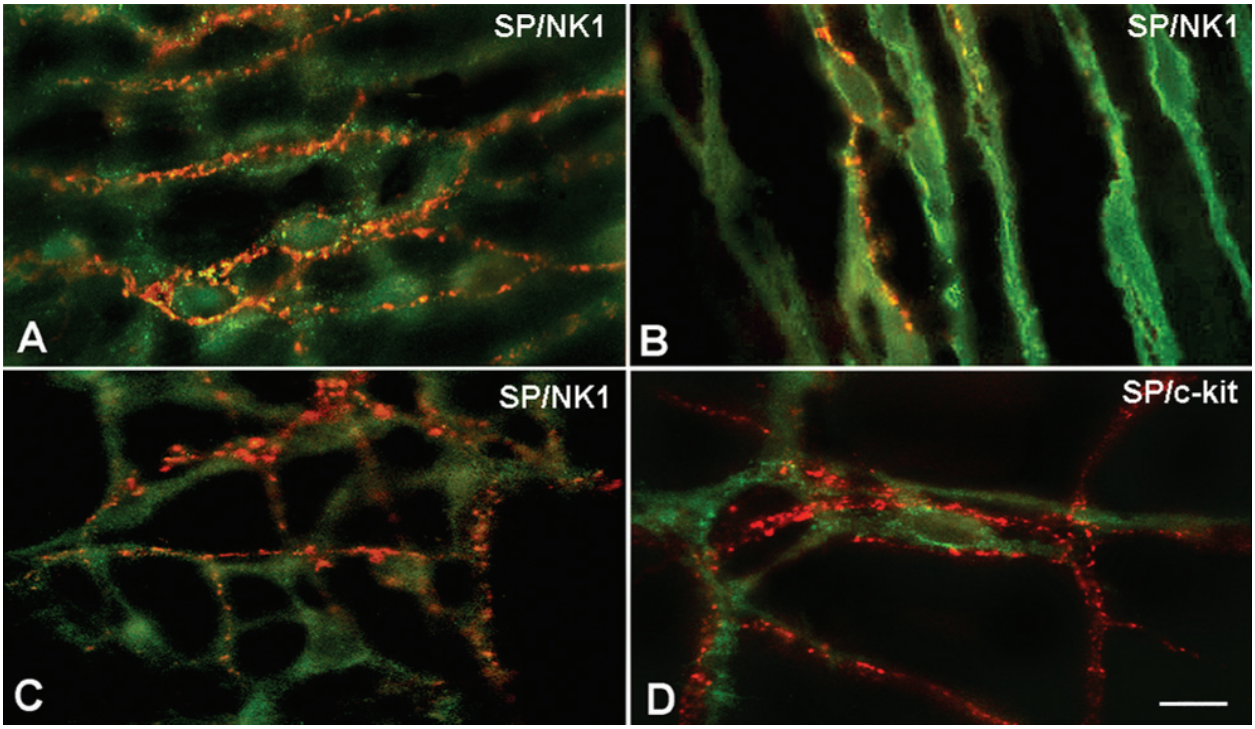

Fig. 2 **A-C**: Substance P- (SP-IR, in red) and Neurokinin 1 receptor-immunoreactivity (NK1r-IR, in green). **A.** Guinea pig ileum, whole mount. Deep muscular plexus. The SP-IR network has large and small meshes made of varicose fibers frequently interconnected by obliquely oriented fibers; two or three varicose axons often run together. Numerous SP-IR nerve endings are present around the body and along the processes of the NK1r-IR cells. All these cells are in contact with SP-IR nerve endings. **B.** Rat ileum, whole mount. Deep muscular plexus. The SP-IR network has large meshes and its varicose fibers run single and are apposed parallel to the main axis of the NK1r-IR cells; due to the scarce number of these fibers, many NK1r-IR cells are not contacting them. **C.** Guinea pig ileum, whole mount. Myenteric plexus. The SP-IR network has large meshes and varicose axons are few. The NK1r-IR cells are at the same level as the SP-IR nerve fibers, but forming separate networks. **D.** Substance P- (SP-IR, in red) and c-kit-immunoreactivity (c-kit-IR, in green). Many SP-IR nerve fibers run near one ICC-MP, but only few nerve endings abut on this cell. A-D: fluorescence microscope. Calibration bar = 10 µm.

action. One year later [17], it was demonstrated that also in rest conditions these cells have NK1r-IR that is distributed on the cell membrane at both cell body and processes (Fig. 2C). This NK1r-IR is, however, very faint (Fig. 3C). More recently [67], NK1r-IR was confirmed in these cells, but only after incubation with agonists, septide or SP and by using a commercial antibody.

ICC-IM

ICC-IM were reported to be never stained with non-commercial NK1r antibodies [62, 63], but the possibility that they did not recognize a slightly modified NK1r was considered. Indeed, more recently, intramuscular ICC of the human *antrum*, presumably those identifiable as ICC-SEP, were stained by using a monoclonal anti-NK1r antibody [29].

Colonic ICC

Up-to-now and at least in resting conditions, NK1r-IR was never observed in any colonic ICC population of the guinea pig [14], rat [62] and mouse (personal observations). Conversely, in the human colon, all colonic ICC were NK1r-labelled by using the non-commercial and widely used antibody [76].

Intestinal ICC in culture

NK1r and NK3r expression by RT-PCR was found in freshly dispersed ICC-MP obtained from the ileum and ICC-IM obtained from the gastric fundus [77]. Functional studies on cultured ICC from the mouse intestine showed that SP may modulate

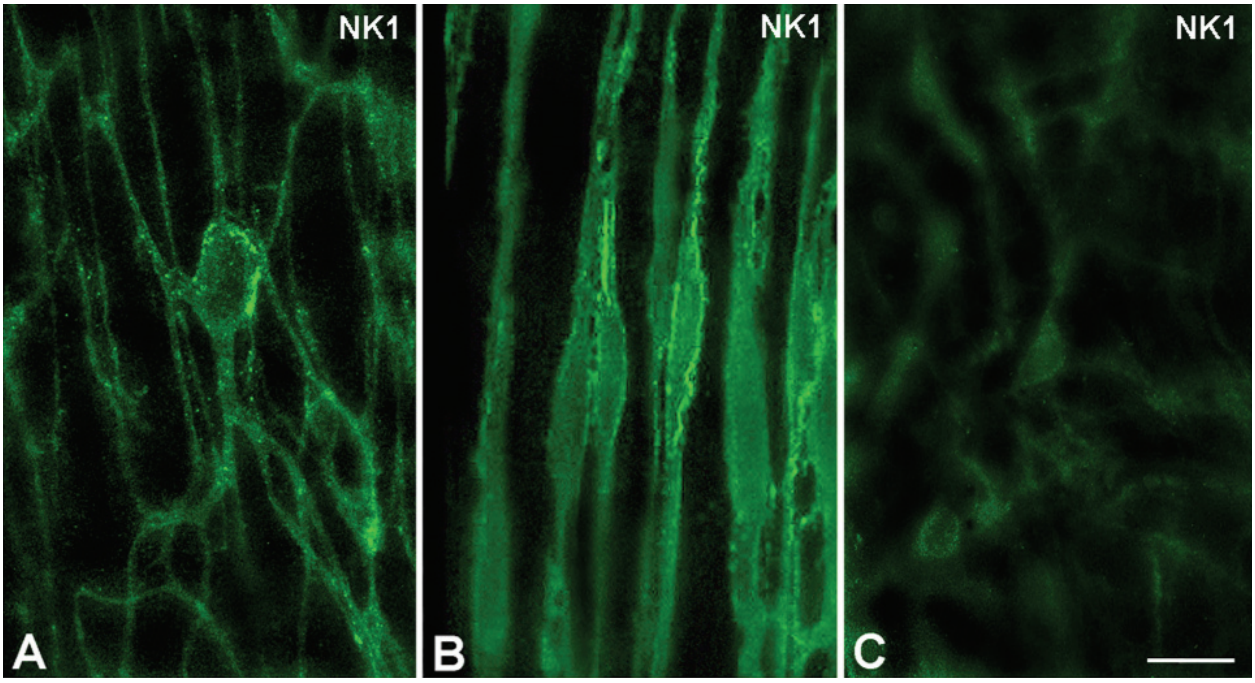

Fig. 3 Neurokinin 1 receptor-immunoreactivity (NK1-IR). **A.** Guinea pig ileum. Whole mount, deep muscular plexus. NK1r-IR is intense, distributed along the cell membrane at both cell body and processes and has a punctuate aspect. The cell body is oval or round with thin, short and ramified branches by which these cells connect to each other. **B.** Rat ileum. Whole mount, deep muscular plexus. The body of the NK1r-IR cells is elongated and spindle-shaped and have short lateral processes and a strong IR. **C.** Guinea pig ileum. Whole mount, myenteric plexus region. NK1r-IR is faint and distributed along the cell membrane at both cell body and processes. The cell body is round or triangular and two-three branches protrude laterally by which these cells connect to each other. **A-C**: fluorescence microscope. Calibration bar = 10 μm.

intestinal motility by acting on ICC *via* the release of intracellular Ca^{+2} induced by NK1r stimulation [78]. The authors, however, did not specify which types of ICC were cultured.

ICC-like cells outside of the gut

NK1r-IR cells were observed in the arterial wall [79] and were considered to be ICC for their ultrastructural features. These cells, however, are c-kit-negative. NK1r-IR cells were also found in the stroma of the human exocrine pancreas [80].

Myoid cells

In the mouse gut only [17], NK1r-IR is distributed along the contour of a non-neuronal cell type that, contrary to ICC, is c-kit-negative and α-smooth muscle actin-positive (Fig. 4A). Similar to the ICC, these NK1r-labelled cells appear as having an elongated shape and short, thin branches (Fig. 4B). These cells are located in the villous stroma, especially at the tips of the villi, very close to the basal surface of the lining epithelium. Under the electron microscope, these cells can be identified as spindle-shaped cells sharing myoid features, oriented with their major axis parallel to the major axis of the villous and interconnected by short, thin branches. Varicose nerve fibers rich in synaptic vesicles are constantly seen near them. These cells represent a well-known cell type frequently encountered within the villi of mammalian small intestine and called *myoid cells* [81]. Numerous SP-IR varicose fibers are present in the villous and it has been proposed they might regulate the excitability of these NK1r-IR cells keeping them able to respond to the mechanical or locally generated chemical stimuli [82]. Since this cell type, as well as a rich SP inner-

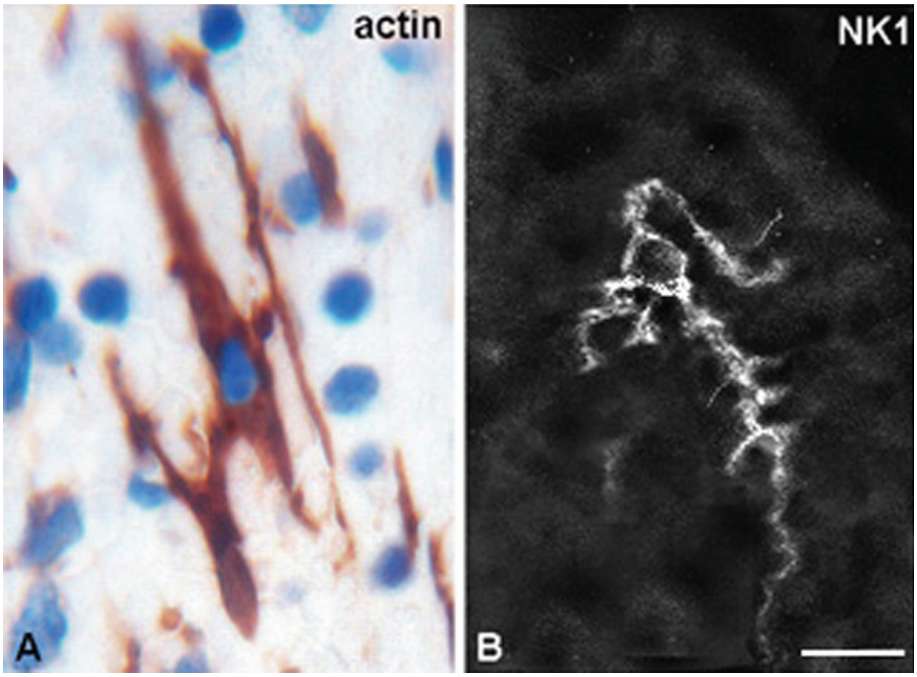

Fig. 4 *Myoid cells* in the villi of the mouse ileum. Transverse section. **A.** A ramified *myoid cell* labelled with an α-smooth muscle actin antibody, light microscope. **B.** A ramified *myoid cell* labelled with a NK1r antibody, fluorescence microscope. **A** and **B**: bar = 10 μm.

vation, is also present in other animal species, such as guinea pig and rat, the lack of NK1r-IR on their *myoid cells* might be due to the presence of an NK1r subtype not recognized by the antibody used.

ICC and SP-IR nerve fibers

Double-labelling confirmed that ICC receive SP-IR terminals but also showed that the neurokinin receptors do not form clusters apposed to these terminals [14, 15, 64, 66]. The relationships between ICC and SP-IR nerve fibers show similarities as well as differences among animal species.

Several studies have shown that the ICC-DMP expressing NK1r are very close to SP-containing nerves [15, 16, 64] and have quantified their relationships, demonstrating that up to 90% of the nerve endings are closely apposed to these cells. This suggests an extensive excitatory control of ICC, likely influencing circular muscle contraction [14, 15, 27]. In three animal species (rat, guinea pig and mouse), a more intense staining of the DMP by an NKA antibody as compared to an SP antibody and an unexpected NK2r-and NK3r-IR at the inner portion of the circular muscle layer were observed [17]. These data suggest that DMP varicosities contain a large amount of NKA and allow suggesting that ICC-DMP and inner circular muscle cells are under a complex tachykinin control through all the three types of NK receptors.

The network formed by SP-IR nerve fibers and that formed by ICC-DMP are closely associated in all animals species [27]. In the guinea-pig, the SP-IR nerve fibers surround the ICC body and processes and at least two of these fibers are in contact with one ICC (Fig. 2A). In contrast, in the rat, the SP-IR nerve fibers run single and apposed parallel to the ICC main axis. Notably, due to the scarce number of these fibers, many ICC are not contacting them (Fig. 2B). Statistical evaluation of the number of SP-IR nerve fibers per ICC revealed that these nerves are significantly more numerous in the guinea-pig than in the rat, while the SP-IR varicosities abutting to the ICC-DMP body and processes are similarly numerous in these two species. Interspecies differences do not correlate with differences in the NK1r expression by the ICC-DMP. However, it is well known that SP released by the nerve endings diffuses easily in the intercellular space and, therefore, can reach also the ICC not in contact with the SP-IR nerve fibers. These interspecies differences do implicate a different tachykinergic control of the ICC-DMP. In the guinea-pig, this control can act simultaneously and with the same efficacy on almost all the

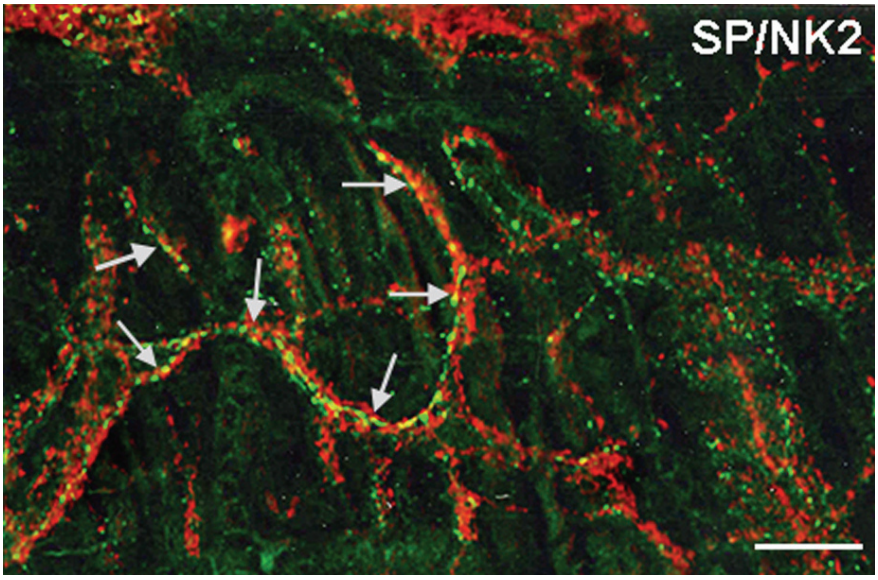

Fig. 5 Substance P- (in red) and NK2r- (in green)-immunoreactivity (SP/NK2r-IR) in the rat ileum. Whole mount. Numerous SP- and NK2r-IR varicose fibers at the myenteric plexus area. Many but not all of the NK2r-IR varicosities overlap the SP-IR ones (*arrows*). In the background, the spindle-shaped and green-labelled cells are the smooth muscle cells of the longitudinal muscle layer that are NK2-IR. Confocal microscope. Calibration bar = 20 μm.

ICC-DMP. In the rat, up to half of the ICC-DMP may be under immediate control of tachykinergic innervation; the other half of ICC may become a target of SP action under conditions of increased release of the tachykinin. Alternatively, differences between animal species could be related to the NK1r affinity to the agonist: a low NK1r affinity in the presence of a high concentration of the agonist in the guinea-pig, a high NK1r affinity in the presence of a low concentration of the agonist in the rat. At the MP, the ICC were seen at the same level as the SP-IR nerve fibers, but forming separate networks (Fig. 2C), and the SP-IR nerve endings closely apposed to the ICC-MP (Fig. 2D) were less numerous than those apposed to the ICC-DMP [27]. A reduced number of NK1r on ICC-MP fits well with the scarcity of closely apposed SP-IR nerve endings.

Briefly, SP-innervation shows differences between DMP and MP, which imply a different tachykinergic control of the ICC-DMP compared to the ICC-MP. Still from a functional point of view, the different richness in the SP-innervation of ICC-DMP and ICC-MP together with the very intense NK1r-IR of the former and very faint NK1r-IR of the latter lead us to conclude that the ICC-DMP may play a major role and the ICC-MP a minor role in the excitatory neurotransmission. However, it cannot be excluded that some of the SP-IR nerve endings abutting on ICC-DMP are sensory. This hypothesis is not in contrast with data recently obtained in a functional study which demonstrates that activation of NK1r on the ileal ICC results in an increase in slow wave frequency and distension-induced peristaltic activity [83]. In many cases, NK1 receptors may mediate action on ICC and no indirect innervation of smooth muscle cells.

In man, both in the gastric *corpus* and *antrum*, SP-IR nerve fibers run apposed to the cell bodies and processes of the intramuscular ICC and most of them are in contact with these cells [75]. In addition, these ICC express NK1r [29].

Co-localization of SP- and NK2r-IR

The relationship between NK2r-IR nerve varicosities and SP-IR perikarya and nerve varicosities have been evaluated in the enteric plexuses of the guinea-pig and rat ileum [84]. Many but not all of the NK2r-IR varicosities detected at the MP level overlapped the SP-IR ones (Fig. 5), while none of those at the submucous plexus were co-localized. The majority of the nerve varicosities at the MP that showed the double labeling surrounded SP-negative neurons and only a few of them were seen close to SP-IR neurons. Similarly, NK2r- and SP-IR co-localized in many of the varicosities present within the longitudinal muscle layer, whereas only a few of the SP-IR varicose fibers located within the circular muscle layer and the DMP were also NK2r-IR. These

data, together with those showing that DMP varicosities contain a larger amount of NKA, further indicate that ICC-DMP are under a complex tachykinin control.

NK1r-IR ICC-DMP and SP-IR nerves during gut development

A study of the time course of appearance of NK1r on ileal ICC during rat development has shown that NK1r is very early expressed by the ICC-DMP [15]. Indeed, at birth, ICC-DMP already possessed NK1r-IR, although irregularly distributed onto their plasma membrane. At the end of the first week, the labelling reached maximum intensity and was distributed uniformly over the entire plasma membrane, with no further changes in the adult life. Similarly, SP-IR in nerve varicosities of the DMP appeared very early after birth. These data indicate that, very early in postnatal life, the DMP contains SP-IR nerve endings whose final targets seem to be the ICC. Interestingly, the intensity of the labelling for the NK1r was at its maximum at 7 days of postnatal life, while that for SP reached the highest intensity in the adult age. TEM studies [85] have shown that ICC-DMP acquire the adult features during the first week of post-natal life and that their differentiative steps are temporally related to those of the nerve plexuses. The difference between the earliest ages and adulthood in the NK1r distribution on the ICC-DMP probably depends on a progressive membrane re-organization of the receptors in a cell that increases in size and changes in shape. Moreover, the high expression of NK1r with a peculiar distribution, different from that observed in the adult animal and not exactly correlating with richness in SP-IR nerve fibers, could imply a trophic role of these receptors during a fundamental period of ICC differentiation.

Pathological conditions

Intestinal inflammation

Studies on neurokinin receptor expression in pathological conditions are extremely scarce while changes in SP in the intestinal wall during experimental inflammation in animals or inflammatory bowel diseases in humans have been widely documented [70], but data remain controversial. For example in humans, an increase [86] and a decrease [87] in SP-IR fibers have been described in ulcerative colitis. In animals a large majority of data indicates a decrease in intestinal SP content in different inflammatory models [70]. A role of SP, and also NKA, in the development of intestinal inflammation has been proposed since antagonists of NK1r and NK2r have been found to attenuate the severity of a colitis induced by trinitrobenzene sulfonic acid in rats [88, 89]. Moreover, an increased expression of NK1r has been found in lymphoid aggregates and blood vessels of colonic samples from patients with Crohn's disease [90]. However, most of the data on the role of SP and NK receptors in intestinal inflammation concern the acute phase of the inflammatory process.

In a study conducted during jejunal inflammation induced by the nematode *Nippostrongylus brasiliensis* it was immunohistochemically determined that both NK receptors and SP were altered in the muscle coat and that these alterations persisted when inflammation had spontaneously resolved 30 days post-infection [18]. This study was unique in that it considered NK1r-IR on ICC. In control animals, NK1r-IR cells, identified as ICC for their c-kit-IR, formed a continuous layer at the DMP. On the contrary, this layer was discontinuous at 14 days after infection, since there were large areas devoid in these cells. At 30 days after infection, neither NK1r-IR nor c-kit-IR cells were found at the DMP. On the contrary, NK1r-IR, that is usually undetectable on ICC-MP in the rat in resting conditions, could easily be detected on these cells 14 days after infection and the NK1r-IR was distributed on the plasma membrane and in the cytoplasm. At 30 days after infection, NK1r-IR was no longer identifiable on the ICC-MP. At day 14, when inflammmation peaks, there was a reduction in NK1r at myenteric neurons and in SP-IR nerve endings and NK2r loss at the circular muscle layer. The SP decrease persisted at day 30, whereas neurons and circular muscle cells re-expressed NK1r and NK2r, respectively. These changes, likely associated with impairment in tachykinergic control of jejunal functions, were interpreted as leading to the alterations of motility and sensitivity to distension described in these animals.

Muscular dystrophy (mdx mice)

In a mouse strain used to study muscular dystrophy, the *mdx* mice, ICC do not express dystrophin, a molecule usually present on their plasma membrane in normal animals [91], but still express c-kit [40]. Interestingly, a functional impairment and altered ultrastructural features were found for all gastric ICC populations [40]. ICC-DMP were seen to maintain an intense NK1r-IR (personal observation), but whether NK1r-IR is present or absent on the other ICC sub-types of these animals is not known. No information is also available on SP innervation. Further studies extended to all gut regions might give important information on this topic.

c-kit mutants

Several strains of c-kit mutant mice and rats have been studied. In particular, the strain of mice with a mutation in the W locus has been widely used to study the function of ICC, starting ten years ago [36, 92, 93]. None of these studies, however, has considered whether NK1r expression changes in the various ICC sub-types and whether variations in SP expression are present in the related nerve endings. In the ileum of We/+ mice, NK1–IR at the ICC-DMP is practically absent although c-kit-IR at these cells and SP-IR at enteric neurons and intramuscular nerves appear normal (Faussone-Pellegrini *et al.*, submitted). This is of high interest and deserves further studies since, contrarily to ICC-MP, the ICC-DMP are reported to be unaffected in these animals. This animal model, as well as other c-kit mutants and stem cell factor mutants, could be particularly appropriate to study the tachykininergic control on the ICC sub-types and might help in understanding the role these cells play in intestinal motility.

Conclusions

Immunohistochemical studies have shown that ICC, in particular the ICC-DMP, are closely apposed to SP-IR nerve endings and express its preferred receptor, the NK1r. It is noteworthy that neither NK2r nor NK3r antibodies label ICC. Combined studies of immunohistochemistry and pharmacology have demonstrated that the NK1r on ICC is a true receptor since in the presence of the agonist (SP) it aggregates on the plasma membrane and internalizes in the cytoplasm.

In the gut, NK1r- and c-kit-co-staining has always demonstrated that the NK1r-labelled cells are also c-kit-labelled and, therefore, it is generally assumed that these cells are ICC. Conversely, not all the c-kit-labelled cells (ICC) are NK1r-IR and several possibilities can be forwarded to explain why some of the ICC are NK1r-negative. This datum is also highly intriguing from a functional point of view. In fact, the different NK1r expression, together with the different SP-innervation among the ICC sub-types, supports the hypothesis that *i*) there are distinct ICC populations, *ii*) each of them is under a different tachykininergic control and, likely, *iii*) each ICC sub-type plays a different role.

On the basis of literature data, it seems reasonable to conclude that ICC may play different roles in the excitatory neurotransmission, but it has not to be excluded that some ICC might be implicated in both motor and sensitive SP innervation. Studies on pathological conditions are few and those on mutant strains are practically absent. This kind of study is recommended, since it might help in understanding the functional relationships between ICC and tachykininergic nerves.

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