Am J Physiol Gastrointest Liver Physiol 298: G10–G13, 2010.
First published November 5, 2009; doi:10.1152/ajpgi.00426.2009.

Mounting evidence against the role of ICC in neurotransmission to smooth muscle in the gut

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Submitted 16 October 2009; accepted in final form 29 October 2009

Goyal RK, Chaudhury A. Mounting evidence against the role of ICC in neurotransmission to smooth muscle in the gut. *Am J Physiol Gastrointest Liver Physiol* 298: G10–G13, 2010. First published November 5, 2009; doi:10.1152/ajpgi.00426.2009.—How nerves transmit their signals to regulate activity of smooth muscle is of fundamental importance to autonomic and enteric physiology, clinical medicine, and therapeutics. A traditional view of neurotransmission to smooth muscles has been that motor nerve varicosities release neurotransmitters that act on receptors on smooth muscles to cause their contraction or relaxation via electromechanical and pharmacomechanical signaling pathways in the smooth muscle. In recent years, an old hypothesis that certain interstitial cells of Cajal (ICC) may transduce neural signals to smooth muscle cells has been resurrected. This later hypothesis is based on indirect evidence of closer proximity and presence of synapses between the nerve varicosities and ICC, gap junctions between ICC and smooth muscles, and presence of receptors and signaling pathways for the neurotransmitters and ICC. This indirect evidence is at best circumstantial. The direct evidence is based on the reports of loss of neurotransmission in mutant animals lacking ICC due to c-Kit receptor deficiency. However, a critical analysis of the recent data show that animals lacking ICC have normal cholinergic and purinergic neurotransmission and tachykinergic neurotransmission is actually increased. The status of nitrergic neurotransmission in c-Kit deficient animals has been controversial. However, reports suggest that *1*) nitrergic neurotransmission in the internal anal sphincter does not require ICC and *2*) the in vivo phenotype of ICC deficiency does not resemble nNOS deficiency. *3*) The most recent report, in this issue of the Journal, concludes that impaired nitrergic neurotransmission may be due to smooth muscle defects associated with c-Kit receptor deficiency.

bile gastritis; gastroparesis; intestinal phenotype of *Ws/Ws*; nitrergic neurotransmission

RARELY HAS THERE BEEN SO MUCH excitement in the field of gastrointestinal neuroscience and motility as has been with the proposed roles of interstitial cells of Cajal (ICC) in gastrointestinal function, diseases, and potential therapy (11, 15). One of the important functions proposed for a subset of ICC (intramuscular and deep muscular) has been to transduce neural signals to smooth muscle cells in the gut (15). In this issue of the Journal, Zhang and colleagues (27) report data on fundamentals of this hypothesis and conclude that ICC may not play a role in neurotransmission to the smooth muscles and propose that defects in the smooth muscles in c-Kit deficiency may be responsible for some of the reported abnormalities in neuromuscular transmission in ICC-deficient animal models (10, 27). This report tempers the "irrational exuberance" regarding the role of ICC in enteric neurotransmission and therefore invites for a critical evaluation of available evidence for the role of ICC in mediating neural signals to the smooth muscles.

The evidence for the role of ICC in neurotransmission includes indirect and direct components. A large body of indirect evidence has been presented to support the role of ICC in transducing neural signals to smooth muscle. Such indirect evidence include presence of signaling molecules for neurotransmitters in the ICC, proximity of nerve varicosity with ICC, synapselike contacts between nerve varicosities containing various neurotransmitters and ICC, presence of certain synapse-associated proteins in ICC, and presence of functional gap junctions between ICC and smooth muscle cells (15). However, most of the indirect evidence is questionable and, at best, circumstantial. For example: *1*) the evidence that ICC have receptors and intracellular signaling pathways for enteric neurotransmitters shows only that ICC can be targets to enteric neurotransmitters and does not prove that they transduce neural signals to smooth muscles (15); this is because similar receptors and intracellular signaling pathways for enteric neurotransmitters are well known to be present on the smooth muscles. *2*) It has been suggested that enteric nerve varicosities make $<$20-nm close contacts with ICC but not with smooth muscle cells (15). However, this conclusion is not supported by careful comparative studies. Mitsui and Komuro (14) made semiquantitative comparison of $<$20-nm close contact between the membranes of varicosities and ICC and smooth muscle cells and found similar close contacts of varicosities with ICC and smooth muscle. *3*) The evidence that nerve varicosities make electron microscopically recognizable synapse with ICC but not with smooth muscle cells is weak. Apart from anecdotal report, well-characterized synapse with presynaptic active zone and postsynaptic density has not been systematically documented. Because of the absence of convincing features of a synapse between nerve varicosities and ICC, these associations have often been called synapselike (8, 10, 14). In their studies, Mitsui and Komuro (14) found synapselike contacts of varicosities with both ICC and smooth muscle. *4*) Presence of SNAP25 and PSD95 in normal gut tissues and decrease in ICC-lacking W/W^v mice has been advanced as evidence for the presence of synapses between nerve varicosities and ICC (2). However, SNAP25 and PSD95 are not synapse-specific proteins and are widely distributed in synaptic as well as nonsynaptic regions (1, 4, 13). *5*) Synapse between nerve varicosity and ICC is proposed to involve neuropeptides such as VIP and neurokinin and nonvesicular transmitters such as nitric oxide. However, neurotransmission by neuropeptides and nonvesicular transmitters is not known to involve synapse (20, 22, 23). *6*) For argument's sake, even if it is accepted that there are synapses between nerve varicosities and ICC, that fact does not prove that these synapses with ICC are responsible for medi-

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ating neural signals to smooth muscles. *7*) If ICC mediates neural signals to smooth muscles, the nature of the signals, electrical or biochemical, and mode of the mediation remains unexplained. Low-resistance "gap junctions" between ICC and smooth muscle cells have been suggested to be the mode of transmission of signals from ICC to smooth muscle (15). However, evidence for functional transmission through the gap junctions is lacking (7, 17).

Direct evidence for the role of ICC in neurotransmission is based on studies showing that loss of ICC is associated with loss of neuromuscular neurotransmission. There are two well-established models of ICC deficiency, namely *W/W^v* mice and *Ws/Ws* rats, both of which are due to c-Kit deficiency. These models have been used to investigate the role of ICC in neurotransmission including cholinergic and tachykinergic excitatory and nitrergic and purinergic inhibitory signals.

Cholinergic excitatory neurotransmission has long been known to be responsible for atropine-sensitive fast excitatory junction potential (EJP) in the smooth muscles and muscle contraction in response to electrical field stimulation (EFS) of the muscle strips. Ward and colleagues (24) were first to report that cholinergic neurotransmission is impaired in *W/W^v* mice. However, these studies did not account for the direct well-known pharmacomechanical actions of cholinergic stimulation on smooth muscles. Moreover, Zhang et al. (27) report that cholinergic neurotransmission to smooth muscles is unaffected in *W/W^v* mutants. These studies do not support the role of ICC in cholinergic neurotransmission.

Tachykinergic excitatory neurotransmission is responsible for slow EJP. Huizinga and colleagues (10) investigated the status of tachykinergic neurotransmission in *Ws/Ws* rats and found no evidence of impaired tachykinergic transmission; instead they found that tachykinergic responses were markedly increased in the mutant rats. The increase in tachykinin responses was associated with increase in tachykinergic nerves. The mechanism of increased tachykinergic nerves in c-Kit deficiency is not known. However, enhanced tachykinergic neural responses may mask the EFS-induced inhibitory responses under nonadrenergic, noncholinergic (NANC) conditions unless the tachykinergic responses are also suppressed (10). In any case, there is no direct evidence that tachykinergic neurotransmission is mediated by ICC.

Purinergic inhibitory neurotransmission is responsible for fast inhibitory junction potential (IJP) and the fast component of the relaxation. All investigators, including the champions of the hypothesis that ICC transduce neural signal to smooth muscles, agree that purinergic inhibitory responses are not affected by lack of ICC in *W/W^v* mouse and *Ws/Ws* rat animal models in both electrophysiological and mechanical studies (10, 21). Thus there is no evidence that ICC mediate purinergic inhibitory neurotransmission.

Nitrergic inhibitory neurotransmission is responsible for slow IJP and slower component of the relaxation. Burns and colleagues (3) found that *W/W^v* mice have loss of nitrergic IJP and muscle relaxation, which led them to propose that ICC may transduce neural signals from nitrergic nerves to smooth muscle cells. Since then, large number of reports in *W/W^v* mice and *Ws/Ws* rats have produced contradictory evidence regarding the role of ICC in nitrergic neurotransmission (25, 27). These conflicting results may be due to differences in experimental conditions and technical reasons (10). Huizinga and colleagues (10) reported that responses to EFS in electrical and mechanical studies in ICC-deficient animals may be affected by associated abnormalities of reduced muscle tone, increased phasic contractions, and increased tachykinergic responses in these mutants. The increased overlapping excitatory tachykinergic response may mask the inhibitory responses; when the tachykinergic responses are blocked, ICC-deficient rats have normal nitrergic inhibitory responses. In a well-designed study using a large sample size, Zhang and colleagues (27) report that EFS under NANC conditions produces a nitrergic IJP in the lower esophageal sphincter (LES) that has a bimodal distribution. In 52% of the animals, the IJP was normal in amplitude and duration but was markedly reduced in the remaining 48% of the mutant mice. The authors carefully investigated the reason for this confusing finding and found that this variation in *W/W^v* mutants was not due to possible differences in loss of ICC in the two subgroups. They concluded that ICC is not involved in nitrergic neurotransmission.

For clinical and therapeutic purposes, it is important to examine the intact phenotype of ICC-deficient animal models. If ICC play a critical role in nitrergic inhibitory neurotransmission, ICC-lacking mice should have phenotype resembling nNOS$-/-$ mice. Sivarao and colleagues (19) compared LES and pyloric sphincter (PS) phenotypes in intact *W/W^v* and nNOS$-/-$ mice. They found that the LES was hypotensive with normal nitrergic relaxation, resembling incompetent LES in *W/W^v* mutant mice. In nNOS$-/-$ mutant, the LES was normotensive with impaired nitrergic relaxation to swallowing and vagal stimulation, resembling achalasia. These studies revealed completely different phenotypes of LES in *W/W^v* and nNOS$-/-$ mutant mice and suggested that nitrergic neurotransmission was not seriously affected in *W/W^v* mutants.

Sivarao and colleagues (18) also investigated gastric phenotype in *W/W^v* mutant mice and found that in the *W/W^v* mutants the pyloric sphincter was hypotensive with normal nitrergic relaxation. These mutants also showed evidence of bile reflux. Consistent with this finding, frequent bile reflux, gastritis, and gastric ulcers have also been reported in ICC-lacking *Ws/Ws* rats (12, 26). On the other hand, pyloric sphincter was normotensive with impaired nitrergic relaxation in mice lacking nNOS. Moreover, the stomach in nNOS$-/-$ mice revealed gastric stasis and gastric bezoars (18). Thus findings in ICC-lacking *W/W^v* mice and *Ws/Ws* rats mimic gastric phenotype of bile gastritis. On the other hand, findings in nNOS-lacking nNOS$-/-$ mice resemble diabetic gastroparesis. These observations further fail to support the view that nitrergic neurotransmission is lost in ICC deficiency and that ICC play a critical role in nitrergic neurotransmission.

Overall, the available data show that ICC is clearly not involved in purinergic or tachykinergic neurotransmission. The role of ICC in cholinergic neurotransmission is questionable and is at best considered to be controversial. Even if it is assumed that ICC may participate in cholinergic and nitrergic neurotransmission, the proposed involvement of ICC in selective neurotransmissions will be difficult to explain. Moreover, a recent study showing that neuromuscular transmission in the internal anal sphincter does not require ICC further suggests that ICC are not necessary involved in neurotransmission (5).

It has recently been proposed that some of available data may be consistent with a partial role of ICC in neurotransmission, so that there may be direct neurotransmission to smooth muscle and indirect neurotransmission through ICC

(11, 14). The reported partial loss of inhibitory responses was due to selective loss of nitrergic neurotransmission with preservation of the purinergic transmission (3). Our review did not discover any experimental evidence for partial inhibition of inhibitory response in ICC-deficient animals.

If ICC is not essential for neurotransmission, what is responsible for the controversial reports of impaired nitrergic neurotransmission in mouse models lacking ICC due to c-Kit deficiency? c-Kit (CD117) is a receptor tyrosine kinase that is involved in survival, proliferation, and differentiation of stem cells. It is possible that c-Kit deficiency leads to several cellular abnormalities in addition to the loss of ICC. Therefore, some of the observed abnormalities in the models of c-Kit deficiency are not to be attributed to the loss of ICC but to effects of c-Kit deficiency on enteric nerves and smooth muscle cells. It has been reported that c-Kit deficient models have hypotensive muscle with increased phasic contractions and increased tachykinergic innervation (10, 19). Zhang et al. (27) also show that *W/W^v* mutant mice have hyperpolarized muscle with reduced unitary potentials that may explain reduced resting tone in the smooth muscles and reduced relaxation in some mutant mice.

The resting depolarized state and tone of smooth muscles has been reported to due to resting Cl^- conductance mediated by Ca^{2+}-activated Cl^- channels in smooth muscle cells whose suppression by nitric oxide has been show to be responsible for nitrergic IJP (6, 9). Cl^- channels that are suppressed by cGMP and nitric oxide have been identified in isolated smooth muscles (28). Zhang and colleagues (27) present preliminary data to speculate that c-Kit deficiency may be associated with abnormal responses to pharmacological modulation of intracellular Ca^{2+} stores. However, these findings do not explain why tissues from only half the mutant mice show abnormalities in inhibitory junction potentials whereas the responses in other half of the animals are normal. To explain these findings, Zhang and colleagues (27) speculate that c-Kit deficiency may be associated with marked cellular variability in Ca^{2+}- store and Ca^{2+}-activated Cl^- channel signaling pathway. Further studies are needed to test these hypotheses.

In summary, two important points regarding the role of ICC in neurotransmission in the gut are now becoming increasingly clear. One, the foundation for the hypothesis that ICC is responsible for transmitting neural signals to smooth muscles in the gut appears to be based on a slippery slope and is now unraveling (16). Therefore, pending further reproducible and convincing evidence to the contrary, utilizing this hypothesis in defining pathophysiology and treatments of clinical disorders may be premature and unwise. Two, c-Kit deficiency does not equal ICC deficiency, since ICC deficiency is only one of the manifestations of c-Kit deficiency. c-Kit receptor pathway deficiency may cause smooth muscle dysfunction independent of the lack of the ICC. Effect of c-Kit deficiency on the smooth muscles function requires careful study.

GRANTS

This review was supported in part by a National Institute of Diabetes and Digestive and Kidney Diseases Grant to R. K. Goyal (DK 062867).

DISCLOSURES

There are no potential conflicts (financial, professional, or personal).

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