

CROSSTALK

CrossTalk opposing view: Interstitial cells are not involved and physiologically important in neuromuscular transmission in the gut

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In the gut, smooth muscle bundles are separated by intramuscular spaces containing nerve fibres and interstitial cells including intramuscular interstitial cells of Cajal (ICC-IM), a subset of a family of ICC (Komuro 2006). Traditionally, smooth muscle cells (SMCs) are believed to transduce the action of neurotransmitters that cause muscle contraction or relaxation by a process called direct neuromuscular transmission (NMT). Neuro-ICC-IM and neuro-smooth muscle (SM) transmissions regulate functions of ICC and SMCs, respectively. Initially proposed by Imaizumi & Hanna (1969), Sanders and colleagues championed the idea that the smooth muscle responses to nerve stimulation required mandatory transduction by the ICC-IM (indirect NMT) (Burns *et al.* 1996; Ward *et al.* 2000). The role of the smooth muscles was considered simply to mount mechanical contraction or relaxation in response to electrical signals transduced in ICC-IM. It has also been suggested that both ICC-IM and SMCs transduced neural signals to the smooth muscle. According to one view, ICC-IM is involved only in certain situations (direct *or* indirect NMT) (Bhetwal *et al.* 2013; Klein *et al.* 2013). According to another, ICC-IM is involved in parallel with smooth muscles (direct *and* indirect NMT) (Groneberg *et al.* 2013). However, most of the available data continue to support the traditional view of

direct NMT, not requiring ICC-IM (Goyal & Chaudhry 2010) (see Table 1).

The traditional model: direct NMT

The direct NMT is supported by the following: (1) close synapse-like contacts of motor nerve varicosities with smooth muscles (Komuro 2012), (2) the presence of nitrergic and cholinergic signalling molecules in the smooth muscles (Bhetwal *et al.* 2013; Cobine *et al.* 2014; Lies *et al.* 2014a), (3) the presence of nitrergic and cholinergic signalling in the smooth muscles (Wang *et al.* 1996; Zhang *et al.* 1998), (4) expected effects of agonists of neurotransmitters in isolated smooth muscle (Wang *et al.* 1996; Zhang *et al.* 1998), and (5) demonstration of active generation of nitrergic inhibitory junction potential in the SMC (He & Goyal 2012).

Mandatory role of ICC-IM: indirect NMT

An assumed lack of direct NMT led to the conclusion of a mandatory role of ICC-IM in nitrergic NMT. This conclusion was supported by the following: (1) an assumed lack of direct innervation of smooth muscle and presence of exclusive close synaptic contacts of nerve varicosities with ICC-IM (Sanders *et al.* 2014a), (2) the abundance of the nitric oxide sensitive-guanylate cyclase (NO-GC) in ICC-IM but not in smooth muscle (Lies *et al.* 2014a), and (3) the reported loss of nitrergic NMT in ICC-IM-deficient W/W^v mice (Burns *et al.* 1996). The mandatory role of cholinergic transmission was based on the reported loss of cholinergic NMT in ICC-IM deficiency (Ward *et al.* 2000; Klein *et al.* 2013).

However, as summarized below, there is substantial evidence against the mandatory role of the ICC in nitrergic NMT.

First, close synapse-like junctions between the nerves and ICC are also present

between the nerves and the smooth muscles (Mitsui & Komuro 2002). However, true synapses with a synaptic cavity are present at neither neuro-ICC nor neuro-smooth muscle junctions (Komuro 2012).

Second, a key assumption in the model of the role of ICC-IM in NMT is that the gap junctions conduct electrical potentials from ICC-IM to smooth muscles (Sanders *et al.* 2014a). Although gap junctions have been identified, their functional efficiency in conducting electrical signals has been shown to be poor (Sibave *et al.* 2006; Daniel *et al.* 2007).

Third, NO-GC is present in the smooth muscle, ICC and PDGFR α ⁺ fibroblast-like cells and abundance of NO-GC does not correlate with greater function. PDGFR α ⁺ fibroblast-like cells have the highest concentration of NO-GC, yet they do not participate in NMT (Cobine *et al.* 2014; Lies *et al.* 2014a).

Forth, several studies have reported that nitrergic inhibitory junction potential (IJP) and smooth muscle relaxation are preserved in ICC-IM-deficient W/W^v mice and W^s/W^s rats (Sivarao *et al.*, 2001, 2008; Huizinga *et al.* 2008; Zhang *et al.* 2010). Most recently, Sanders and colleagues (2014b) suggested that the discrepant results may be due to an incomplete loss of ICC in the interrogated tissues. They reported that complete absence of ICC-IM was required for a loss of nitrergic relaxation. However, in the absence of ICC-IM, there is no barrier between neurally released neurotransmitter and SMCs, leaving direct NMT intact (Bhetwal *et al.* 2013; Klein *et al.* 2013). Therefore, the report of loss of nitrergic neurotransmission in the absence of ICC in W/W^v mice (Sanders 2014b) is difficult to reconcile.

Evidence against a mandatory role of the ICC cholinergic NMT is the failure to reproduce the reported loss of cholinergic NMT in W/W^v mice (Ward *et al.* 2000;

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Table 1. Proposed models of involvement of intramuscular interstitial cells of Cajal (ICC-IM) in NMT in the gut and experimental results supporting them

	Involvement of ICC-IM in NMT			
	None	Mandatory	Optional	Complementary
Type of NMT	Direct	Indirect	Direct or indirect (situational)	Direct and indirect (dual)
Proposers	Traditional	Burn <i>et al.</i> 1996; Ward <i>et al.</i> 2000	Klein <i>et al.</i> 2013; Bhetwal <i>et al.</i> 2013	Groneberg <i>et al.</i> 2013
Neurotransmitters involved	All transmitters	Nitrgergic and cholinergic	Nitrgergic and cholinergic	Nitrgergic only
Lack of evidence for true synaptic innervation of the ICC-IM*	Consistent	Against	Against	Consistent
Evidence for direct innervation of SM*	Consistent	Against	Against	Consistent
Evidence for the presence of functional signalling molecules in SM*	Consistent	Against	Consistent	Consistent
Lack of evidence for effective conduction from ICC-IM to SM via gap junctions*	Consistent	Against	Against	Against
Origin of nitrgergic inhibitory junction potential in SM*	Consistent	Against	Against	Consistent
Preserved nitrgergic NMT in ICC deficiency*	Consistent	Against	Consistent	Consistent
Loss of nitrgergic inhibition with presumed deletion of NO-GC from ICC*	Against?	Consistent	Against	Consistent
Preserved cholinergic NMT in ICC deficiency*	Consistent	Against	Against	Consistent

*See text for discussion of the evidence.

Zhang *et al.* 2011; Bhetwal 2013; Goyal 2013).

Optional role of ICC-IM: direct or indirect NMT

Klein *et al.* (2013) reported that 'ICC-specific' deletion of protein kinase G1 (*Prkg1*) abolished nitrgergic NMT, but deletion of ICC left nitrgergic neurotransmission intact. To explain these paradoxical findings the authors made an intriguing proposal, that when present ICC-IM are involved in NMT, but when ICC-IM is absent, direct NMT takes place. This model was based on the presence of assumed true neuro-ICC-IM synapses that can restrict the accessibility of the released nitric oxide (NO) to the smooth muscle (Beckett *et al.* 2005). However, no true synapses have been identified between ICC and smooth muscles (Komuro 2012; Goyal

& Chaudhury 2013). Moreover, NO is a highly diffusible gas (diffusion constant $3300 \mu^2 s^{-1}$) (Lancaster 1997). Therefore, ICC-IM cannot restrict it to having effects on adjacent smooth muscles. A similar unsubstantiated model was used to explain the persistence of cholinergic responses in W/W^v mice (Bhetwal *et al.* 2013; Goyal 2013).

Complementary role of ICC-IM: direct and indirect NMT

For nitrgergic NMT, Groneberg and colleagues (2013) reported that cell-specific knockdown of NO-GC in SMCs or ICC did not affect nitrgergic relaxation. However, double knockdown of NO-GC in both SMCs and ICC abolished nitrgergic NMT (Groneberg *et al.* 2013). Other studies have reported that incomplete deletion of NO-GC in ICC results in a dominant loss

of nitrgergic relaxation (Lies *et al.* 2014b; Groneberg *et al.* 2015). Moreover, Klein *et al.* 2013 has reported that partial deletion of *Prkg1* abolishes nitrgergic NMT, but partial deletion of ICC does not (Klein *et al.* 2013). It is not clear why incomplete deletion of NO-GC has a more profound effect on nitrgergic NMT than incomplete deletion of ICC. It is possible that *cKIT-CreER*^{T2} mutants may also affect NO-GC in smooth muscles. Validation studies using immunohistochemistry are not sensitive enough to reveal changes in NO-GC in smooth muscles (Groneberg *et al.* 2015). Finally, a complementary role of ICC in NMT is difficult to comprehend in the absence of a functional gap junction that can transmit signals from the ICC to SMC (Sibave *et al.* 2006; Daniel *et al.* 2007). A complementary role of cholinergic NMT is also speculated but without any experimental data to support it (Groneberg *et al.* 2015).

Conclusion

Overall, available evidence is most consistent with the traditional model of direct NMT without the involvement of ICC-IM. Parallel neuro-ICC and neuro-SM transmissions may independently regulate the functions of the ICC-IM and SMCs, respectively. There is little evidence for an obligatory or optional involvement of ICC-IM in NMT. A complementary role and physiological importance of ICC-IM in nitrergic NMT remain speculative. Moreover, it is unlikely that ICC-IM would only be involved in nitrergic NMT while a variety of other neurotransmitters will use direct NMT.

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Additional information

Competing interests

None declared.