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SCIENTIFIC CORRESPONDENCE

Intrinsic inhibitory innervation of the stomach

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The intrinsic inhibitory innervation of the gastrointestinal tract is of great physiological and pharmacological significance for control of gut motor activity. The inhibitory response of the gastric musculature to electrical field stimulation (EFS) of enteric nerves in the presence of atropine and guanethidine can be pharmacologically inhibited by a combination of L-NAME, α-chymotrypsin and apamin. It is established that L-NAME blocks the NO-mediated response and α-chymotrypsin the VIP response. The neurotransmitter responsible for the inhibitory response that is sensitive to apamin, and hence likely acts on small-conductance potassium (K^+) channels, is the most controversial. Most evidence exists for ATP being this neurotransmitter. A complicating factor in the search for evidence for ATP as an inhibitory neurotransmitter has always been that ATP can act on several receptors and can cause contraction as well as relaxation (Huizinga & Den Hertog, 1979; Huizinga *et al*., 1981). In 1980 it was suggested that apamin prevents the opening of ATP and voltage-dependent potassium channels in guinea-pig *taenia caeci* (Maas *et al*., 1980). In the mouse gastric fundus, evidence was presented that ATP acts on P2 receptors to cause the apamin-sensitive fast inhibitory junction potential (Mashimo *et al*., 1996) or the associated relaxation (Mule & Serio, 2003). In the rat stomach, after blockade of NO and VIP responses, the remaining relaxation in response to EFS was blocked by apamin, the P2 receptor antagonist PPADS and ATP desensitization (Jenkinson & Reid, 2000). Another potential neurotransmitter, peptide histidine isoleucine, may be involved in relaxation not inhibited by L-NAME, α-chymotrypsin and apamin (Curro *et al*., 2002).

In a recent paper Curro *et al*. (2004) provide interesting data on the relative importance of inhibitory neurotransmitters to the rat stomach evoked by electrical stimulation of enteric nerves. However, their main conclusion is that the apamin-sensitive part of neural inhibition of the stomach is not mediated by ATP or carbon monoxide, but must involve another, so far unidentified transmitter. The experimental conditions employed by Curro and co-workers may not be the best to study the apamin-sensitive component of gastric relaxation in response to electrical stimulation of enteric nerves, since apamin did not reduce the amplitude of relaxation and only 'slightly' reduced the area under the curve of relaxation induced by field stimulation (15%), in a non-concentration-dependent manner. Interestingly, ATP caused a similar (19%) reduction of induced contraction and this was inhibited (88%) by apamin. The authors point out that apamin caused a marked inhibition of relaxation in the presence of L-NAME and anti-VIP-serum. However, since apamin did not produce a marked effect on its own, the anti-VIP-serum may have produced unwanted side effects.

The authors claim that the apamin-sensitive component of the relaxation was not affected by ATP desensitization. Desensitization of the preparation to ATP caused an 18% reduction in the EFS-evoked relaxation, consistent with ATP being involved in the apamin-sensitive component. The authors dismiss this argument by pointing out that the EFS-induced relaxation diminishes over time by a similar amount. With such strong, and undoubtedly variable, diminishing of the EFS response over time, and with the ATP response being relatively small, no firm conclusions should be made from these experiments. It would be interesting to see if an apamin-sensitive component was still present following ATP desensitization.

The authors conclude that the apamin-sensitive component was not sensitive to the P_2 receptor antagonist PPADS based on the observation that the relaxation in the presence of L-NAME and α-chymotrypsin was not significantly reduced by PPADS (7.1% reduction) However, L-NAME reduced the amplitude of relaxation by only 7.2%, which was significant. It seems likely that the apamin-sensitive component under the conditions of these experiments is not robust enough to be analysed in detail.

Another argument was that suramin did not block apamin-sensitive relaxation. However, suramin appeared to cause contraction of the tissue, which makes evaluation of its effect on the apamin-sensitive relaxation difficult.

In summary, although more evidence for a role of ATP in inhibitory innervation is certainly welcome, the study by Curro *et al*. (2004) does not conclusively rule out ATP as a neurotransmitter in the gastric fundus, and it may be premature to implicate another neurotransmitter.

We have appreciated discussions with Professor Adriaan Den Hertog.

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Reply to Huizinga *et al.*

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We will focus our discussion on the main arguments put forward by Huizinga *et al*. They state that

(1) 'Since apamin did not produce a marked effect on its own', but it did in the presence of L-NAME plus anti-VIP serum, 'the anti-VIP serum may have produced unwanted side effects'.
(2) The apamin-sensitive component of relaxation is 'very small' or 'minor', and so 'not robust enough to be studied'.
(3) 'Suramin caused marked contraction' and this 'did not allow an evaluation of its effect on the apamin-sensitive relaxation'.

Our comments are the following:

(1) When the effect induced by any of the inhibitory neurotransmitters is blocked, the reduction in relaxation amplitude is never marked. This indicates that the other neurotransmitters are able to compensate almost completely for the blocked component. The same concept applies to the relaxation AUC as long as the peptidergic component is preserved. That is why apamin did not produce a marked effect on its own. More definitive evidence comes from experiments in which non-nitrergic, nonpeptidergic conditions are used. With apamin, nonpeptidergic conditions must be achieved by the use of anti-VIP serum, since effective receptor antagonists are not available. We are not aware of the 'unwanted side effects' to which Huizinga *et al*. refer.
(2) The results of the experimental series with L-NAME plus anti-VIP serum, with and without apamin show that the apamin-sensitive component accounts for 40% of relaxation amplitude and 16% of relaxation AUC. This is undoubtedly a substantial component.
(3) We did not report that suramin caused contraction. Rather, it increased the AUC of EFS-induced relaxation. In any case, suramin markedly inhibited ATP-induced relaxation. Thus, it is an appropriate pharmacological tool to investigate the possible involvement of ATP in the NANC relaxation.

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