Author's response to reviews

Title: Nitric oxide—an endogenous inhibitor of gastric acid secretion in isolated human gastric glands

Authors:

Mrs Anna Berg (annbe@ibk.liu.se)
Stefan MD Redeén (stefan.redeen@lio.se)
Dr Ann-Charlott ASS.PROF. Ericson (loter@ibk.liu.se)
Sven Erik PROF. Sjostrand (svesj@imv.liu.se)

Version: 2 Date: 10 Jun 2004

Dr Christopher Gadd
Assistant Editor
BMC Journals

Dear Chris,

Thank you for the extended deadline on our re-submission. We have made changes in the manuscript according to reviewer's comments.

Sincerely
Lotta (Ann-Charlott) Ericson

Reviewer's report
Nitric oxide—an endogenous inhibitor of gastric acid secretion in isolated human gastric glands
Title: 18 February 2004 Version: Date:
Lars Fandriks Reviewer:
Reviewer's report:
General
The authors report that NO acts inhibitory on acid secretion in isolated human gastric glands. The method used is well-established and experimental procedures are skillfully performed. However, the number of experiments are generally low and no statistical analysis is presented. It follows that I am very concerned about the statistical robustness of the reported data in relation to the conclusions drawn.

------------------------------------------------------------------------------------------------------------------------------------------------------------------------

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Statistical significance of stated effects are not analysed. For example, in Results L-arg is stated to decrease the AP ratio but when consulting Fig 2a the effect is not obvious, at least not in comparison to SNP. Furthermore, fig 2b shows convincingly that administration of the NOS-inhibitor L-NAME was associated with increased AP ratio indicating an inhibitory action by NO. No effect was obtained in presence of D-NAME. However, only two such D-NAME experiments were performed. At least a doubling of the number of these experiments has to be done in order to convince me that the isomer was without effect. If this is the case, such a stereospecificity will strengthen strongly the assumption that NOS mediated NO formation inhibits the acid secretion in this preparation. Also the db-cAMP experiments are very few (n=2 or 3) thus being a weak basis for the conclusions. Although the paper is mainly confirmative some more experiments are needed together with a proper statistical analysis before it could be considered for publication.
The number of experiments has been increased and statistical analyses have been made. Statistical significances are stated.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. Background: The references to NO mediated influence on duodenal and gastric bicarbonate secretions (ref 9, 10, 11) are not entirely correct. Please revise.

2. All secretory data are given as % of maximal secretion in response to histamine or db-cAMP as calculated separately for each individual. Please state in text if this calculation was based on single analysis or duplicate, triplicates?

3. Immunohistochemistry: Please state in how many of the individuals this analysis was performed and that preparation in absence of the primary antibody was unstained. Other isoforms of NOS were not analyzed; Please include in Discussion the reasons.

O Revisions have been made according to reviewer's wishes.

Reviewer's report
Nitric oxide-an endogenous inhibitor of gastric acid secretion in isolated human gastric glands Title: 15 March 2004 Version: Date:
koji Takeuchi Reviewer:
Reviewer's report:
General
This paper describes the effect of nitric oxide (NO), both occurred endogenously and given exogenously, on histamine- and cAMP-stimulated gastric acid secretion in the isolated human gastric gland preparations in vitro, using [14C]-aminopyrine. The results seem to be interesting and consistent, yet some additional data should be required to support the author?s conclusion.

O The number of experiments has been increased.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Lots of data have so far reported concerning the influence of NO on acid secretion, and the results remained controversial. The present study supports the inhibitory effect of NO on acid secretion at the parietal cells, consistent with the finding by Brown et al. This is the first study using human isolated gastric glands, yet to raise the originality the following points should be addressed.

1. To further support the inhibitory effect of endogenous NO on acid secretion, the amount of NO should be measured in the present isolated gland preparation before and after addition of L-arginine or the NO synthase inhibitors such as L-NAME or L-NNA.

O We have chosen not to measure the amount of NO, but instead focus on the effect of the NOS-inhibitors. Since L-NAME, but not D-NAME, causes such an obvious increase in acid output we draw the conclusion that endogenous NO-production is involved.
2. The authors discussed the possible involvement of cGMP in the inhibitory action of NO. This point should be verified in the present study. It would not be difficult to confirm the idea whether cGMP plays a mediator role in the negative action of NO in acid secretion. The authors should examine the effect of dbcGMP on histamine- and cAMP-stimulated [14C]-aminopyrine uptake or the effect of methylene blue (guanylate cyclase inhibitor) on the action of NO donors.

O A study in our laboratory concerning the possible involvement of cGMP in the regulation of gastric acid secretion is going on. However, preliminary data indicate that, to gain sufficient information on that matter, isolated parietal cells should be used. Since this question is not satisfactory answered with this particular method, we will present these results in a separate article in the near future.

3. Isolated gastric gland preparations may include enterochromaffin-like (ECL) cells, the most important endocrine cells for acid secretion. This histamine containing cells are capable of releasing histamine in response to cAMP. Several studies showed the inhibitory action of NO on histamine release from ECL cells. It is possible that NO suppresses cAMP-stimulated acid secretion by inhibition at both parietal cells and ECL cells. The authors should make extensive discussion on this point.

O This matter is dealt with in the discussion.

Minor comments
page 4, line 12: Takeuchi and coworkers.
page 4, line 3-4 from the bottom: the experiments reported in Refs. 13 and 14 were in vivo but not in vitro.
Figure 3: The legend did not well correspond to this figure. The authors should replace (a) and (b) in the legend or the figure.

O Revisions have been made according to reviewer's wishes.