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The Role of the NMDA Receptor in *Helicobacter pylori*–Induced Gastric Damage

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Infection by *Helicobacter pylori* affects 50% of the world's population. It is well accepted that infection results in gastritis in all subjects, peptic ulcer disease in 20% of the probands, and greatly increases the risk of gastric cancer, the second most frequent cause of cancer-related death worldwide.^{1,2} More controversial is the hypothesis that eradication of the organism increases gastroesophageal reflux disease.³ Even though the importance of this gastric pathogen has been recognized for more than a quarter of a century, it is still not clear as to how the organism generates its pathologic consequences.

Based on the work presented in this paper,⁴ there is an ammonia-dependent intracellular pH elevation with activation of *N*-methyl-D-aspartate (NMDA) channels, and thus calcium entry, with the possible consequence of gastritis and its sequelae. The ammonia is generated from gastric juice urea by the very high levels of bacterial urease.

Urease is vital for gastric infection; the NH₃ and H₂CO₃ generated are able to maintain the periplasmic pH at about 6.1, thus enabling colonization of the human stomach and that of animal models, a unique property of this organism.⁵ The critical properties of this urease system are the acid activated urea channel, UreI, enabling urea access to intracytoplasmic bacterial urease, essential for gastric infection⁶ and cytoplasmic and membrane-bound periplasmic carbonic anhydrases, also important for infection.^{5,7} Trafficking of urease to the cytoplasmic membrane in acid is dependent on two 2-component systems, HP0244 and HP0165/0166, and the presence of UreI in the membrane. UreI also conducts NH₃, CO₂ and even NH₄⁺, resulting in a key consequence of this complex set of interactions, namely, the

rapid generation of NH_3 on the surface of the bacteria that are adhering to gastric surface cells, readily able to penetrate the epithelial cells.

It is, therefore, entirely appropriate that this paper investigates the effect of either ammonium chloride addition directly or urea on 2 gastric cell lines in the presence of *H pylori*. As a result of this study, a novel finding appears, namely that the toxicity of the $\text{NH}_3/\text{NH}_4^+$ generated by bacterial urease depends on the expression of NMDA channels in gastric epithelial cells, channels previously well described in nervous tissue. There is evidence for its presence in, for example, the renal tubule and in human colon cancer.^{8,9} These channels are activated by glutamate and glycine and are responsible for Ca^{2+} entry into cells. Normal human gastric juice contains up to 3 mmol/L urea, perhaps more in patients with hepatic failure. It has been shown that ammonia is toxic to gastric cells.^{10,11} Ammonia is a highly membrane-permeable gas and thus enters the cells, whereupon it is protonated, forming the cation NH_4^+ , and alkalizing the cell cytoplasm.¹² In contrast to acidification, recovery from alkalization is very slow because the cation NH_4^+ is membrane impermeant in the absence of a NH_4^+ transporter. Elevated pH compromises mitochondrial function.¹³ Furthermore, increase of pH results in Ca^{2+} entry, further adding to mitochondrial distress with generation of reactive oxygen species. In neurons, $\text{NH}_3/\text{NH}_4^+$ at high concentration results in cell death by increasing calcium entry via NMDA channels.¹⁴ Furthermore, it has been observed that this toxicity is less likely in gastric cancer cells owing to transcriptional down-regulation of NMDA channel subunits in many gastric cancer cell lines.¹⁵ These considerations set the scene for the work performed by these authors.

Two cell lines were used: RGM1, derived from normal rat gastric mucosa, and MKN28 cells that are tumor derived. It is known that cancer cells do not express all the necessary subunits of the NMDA receptor;¹⁵ hence, these latter cells were transfected with a NMDAR2B plasmid to provide full NMDA receptor expression.

Two initial types of experiments were carried out, either the addition of $\text{NH}_3/\text{NH}_4^+$, or the addition of urea to cells co-cultured with either urease positive or urease negative *H pylori*. Monitoring of intracellular calcium showed initially a transient increase (<10 minutes) and after longer incubation an enhanced vesicular quantity of this ion. It was further shown that cyclic adenosine monophosphate plays an essential role in this calcium response. A similar response was elicited by the addition of urea to MKN28 cells co-cultured with urease positive, but not urease negative *H pylori*. These changes were blocked by MK801, a potent inhibitor of NDMA-mediated calcium influx. The authors then showed that cell viability was decreased in correspondence to the changes in cell calcium either with $\text{NH}_3/\text{NH}_4^+$ addition or urea to wild-type *H pylori*. This was likely owing to apoptosis, as evidenced by enhanced expression of pro-apoptotic markers such as BAK and BAX. These effects were likely mediated by an action of increased cell calcium on mitochondria, as shown by a reduction in mitochondrial potential and adenosine triphosphate and increase in cytochrome oxidase that was prevented by the addition of the intracellular calcium buffer, BAPTA-AM. To identify the major source of the increased intracellular calcium, inhibition of ER calcium efflux by 2-APB resulted in restoration of cell viability. The effect was also attenuated by blockade of proteolysis by cathepsin or calpain by E64D. Finally, staining of intact gastric

mucosa by NR2B antibody showed localization to parietal cells, as well as to gastric surface cells and chief cells with differential effects of the presence of *H pylori* with urea.

There are several questions raised by this intriguing paper. First, what is the role of the NMDA receptor in nonexcitable cells? Are there activating ligands present in gastric juice such as glutamate or glycine at high enough concentrations for long enough? Second, how does $\text{NH}_3/\text{NH}_4^+$ result in activation of the NMDA channel? Most likely, elevation of intracellular pH that is long lasting compared with acidification as the causal effect. Administration of CO_2 or acetate would then blunt the toxic effect. However, changes of pH would have little effect on plasma membrane potential, because epithelial cells express high chloride conductance, essentially clamping membrane potential. High intracellular pH results in an increase of the pH gradient across the mitochondrial membrane with a compensatory decrease in membrane potential, as observed. It would have been of interest to measure cell pH as a function of time after $\text{NH}_3/\text{NH}_4^+$ addition or urea to *H pylori* co-cultured cells. Finally, because memantine is a safe NMDA channel antagonist approved for human use, it would be of real interest to evaluate treatment with memantine in infected animal models with respect to the induced gastritis and to see if these data could be extrapolated to humans.

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