



Helicobacter pylori growth stimulation by adrenaline detected by two methods[☆]

Lyudmila Boyanova^{*}, Romyana Markovska, Ivan Mitov

Department of Medical Microbiology, Medical University of Sofia, Zdrave street 2, 1431 Sofia, Bulgaria

ARTICLE INFO

Article history:

Received 31 May 2018

Received in revised form 3 August 2018

Accepted 12 August 2018

Available online 17 August 2018

Keywords:

Adrenaline

Helicobacter pylori

Stress

Hormone

Growth

ABSTRACT

Our aim was to assess growth effects of adrenaline on 32 *Helicobacter pylori* strains on 2.5% blood agar. By agar-well diffusion and disk diffusion methods, concentration-dependent growth stimulation occurred in $\leq 50.0\%$ and 36.8% of the strains, respectively. Growth inhibition was less frequent. Most strains were adrenaline-responsive and growth stimulation prevailed.

© 2018 Elsevier Inc. All rights reserved.

Fascinatingly, some aerobic/facultative and anaerobic bacteria can sense and respond to human stress hormones, thus influencing the infection course [Freestone, 2013]. Nonetheless, there is only very scanty information about catecholamine effects on *Helicobacter pylori* [Doherty et al., 2009].

The aim of this study was to assess adrenaline-induced growth effects on numerous *H. pylori* strains by 2 methods.

H. pylori strains from 32 untreated patients: 9 children and 23 adults with chronic gastritis (24 patients), acute gastritis (3), gastroesophageal reflux disease (1), duodenal ulcer (3) and Crohn disease (1 patient) were evaluated. Strain isolation and identification were performed as previously described [Boyanova et al., 2008]. The study was approved by the Ethical Committee of Medical University of Sofia.

Adrenaline dilutions were prepared from 1 mg/mL Adrenaline solution, Sopharma®, Bulgaria with sterile distilled water as a solvent as in another study [Roberts et al., 2002]. Activity of 1000, 100 and 10 pg/mL adrenaline against 32 *H. pylori* strains was tested by agar-well diffusion method (AWDM). Sterile distilled water was used as a control. Inocula (1 Mc Farland turbidity standard) were plated onto meat-peptone agar (National center of Infectious and Parasitic Diseases, NCIPD, Sofia, Bulgaria) plates containing 2.5% sheep blood (MPA). Wells (7-mm diameter) were made using a sterile stainless steel borer and were filled with 80 μ L of the solutions. The plates were incubated microaerophilically (Campy Pak envelopes, Oxoid) at 37 °C for

2–5 days. Zone diameters of growth changes were measured in millimeters. Some strains were plated onto both MPA and Mueller-Hinton agar (NCIPD) with 5% sheep blood (MHA).

Growth effects on 19 strains also was tested by disk-diffusion method (DDM) using the same adrenaline concentrations, media, *H. pylori* inocula (1 Mc Farland) and incubation as for AWDM.

Differences between groups were assessed with χ^2 test or Fisher's exact test, when appropriate.

Growth stimulation was observed as 1.5–2-fold larger colonies near adrenaline containing well/disk compared with the other colonies. By AWDM, 1000 pg/mL, 100 pg/mL and 10 pg/mL adrenaline produced growth stimulation in 50.0, 34.4 and 12.5% of the strains, and growth inhibition (lack of growth around the well) in 21.9, 15.6 and 6.2%, respectively (Table 1). By AWDM, the highest concentration produced growth stimulation significantly more often (in 50.0%, 16/32 strains) compared with growth inhibition (in 21.9%, 7/32, $P = 0.019$) and enhanced the growth of more strains (50.0%) compared with the lowest concentration (12.5%, $P = 0.002$). Within 10 strains plated onto both MPA and MHA, growth stimulation of three strains was observed only on the MPA.

In most studies on the catecholamine effects on bacteria, only single strain per species was used [Boyanova, 2017]; therefore, we evaluated numerous (32) *H. pylori* strains to detect strain-dependant differences in the response to adrenaline. The low blood concentration in the agar was used because the catecholamine effects have been better observed in nutrient-poor media [Boyanova, 2017]. Moreover, MPA, containing meat extract, peptone and agar, is a poorer medium compared with MHA, which additionally contains casein hydrolysate and starch.

[☆] ORCID ID: 0000-0001-9622-0873

^{*} Corresponding author. Tel.: +359 2 91 72 730.

E-mail address: lboyanova@hotmail.com (L. Boyanova).

Table 1Growth effect of adrenaline on *H. pylori* clinical strains assessed by agar well diffusion and disk diffusion methods.

Data / Method	Details	Agar-well diffusion ^a				Modified disk diffusion ^a			
Agent Concentrations	Adrenaline pg/mL	1000	100	10	0 (control)	1000	100	10	0 (control)
Amount	pg/well or pg/disk	80	8	0.8	0.0	5	0.5	0.05	0.0
Study on MPA ^d	No. of strains tested	32	32	32	32	19	19	19	19
Growth stimulation	No. of strains	16	11	4	0	7	4	2	0
	% of strains	50.0 ^{e,f}	34.4	12.5 ^e	0	36.8	21.0	10.5	0
	Mean diameter (mm)	17.2	15.4	10.0	7.0	17.3	14.5	10.0	6.0
	Range (mm)	9.0–26.0	10.0–20.0	9.0–12.0	7.0–7.0	10.0–30.0	12.0–20.0	10.0–10.0	6.0–6.0
Growth inhibition	No. of strains	7	5	2	0	2	1	1	0
	% of strains	21.9 ^f	15.6	6.2	0	10.5	5.3	5.3	0
	Mean diameter (mm)	13.0	11.4	10.0	7.0	10.5	NA ^d	NA	6.0
	Range (mm)	11.0–16.0	10.0–13.0	10.0–10.0	7.0–7.0	10.0–11.0	9.0	9.0	6.0–6.0

^a Diameter of the well, 7 mm, diameter of the disk, 6 mm; ^bcontrol well/disk, ^ccontrol well/disk,^c MPA- meat-peptone agar with 2.5% sheep blood.^d NA-non-appropriate.^e Statistically significant differences ($P = 0.002$).^f Statistically significant differences ($P = 0.019$).

Indeed, in some strains in the present study, the growth enhancement was found on MPA but was lacking on MHA.

Although dense inocula (McFarland 2–4 opacity standard) have been used for *H. pylori* susceptibility testing [Mégraud and Lehours, 2007], we used less dense inocula since in the study of O'Donnell et al. [2006], low inoculum density was important to detect catecholamine induced effects on some bacterial species.

Common human plasma concentrations of adrenaline are 23–85 pg/mL, the levels increasing up to 20-fold under stress conditions [Carron et al., 2012, Jentsch et al., 2013]. However, gastrointestinal catecholamine levels are difficult to be determined due to their enzymatic changes and amounts in foods [Boyanova, 2017]. In the present work, adrenaline concentrations attainable under conditions of stress were evaluated. Growth increase of some anaerobes such as *Prevotella* and *Fusobacterium* spp. has also been observed by ≤ 1000 pg/mL catecholamines [Roberts et al., 2002, Jentsch et al., 2013].

Stress hormones can increase bacterial growth by facilitating bacterial iron acquisition and use [Boyanova, 2017]. For instance, in some studies on anaerobic bacteria, noradrenaline and adrenaline at relatively low concentrations (< 1000 pg/mL) enhanced the bacterial growth [Boyanova, 2017]. Cell-surface receptors and iron-binding siderophore secretion are important for the bacterial iron acquisition [Doherty et al., 2009]. An assumption can be made that the lack of growth effect or the inhibition in some strains in our study may be associated with lack of siderophore production or cell-surface receptor mutations reported by Velayudhan et al. [2000].

Doherty et al. [2009] found *H. pylori* growth increase in a dose-dependent and strain-independent pattern. Similarly, in the present study, growth stimulation by 1000 pg/mL adrenaline was found 4-fold more often compared with 10 pg/mL concentration by AWDM and 3.5-fold more often than by DDM. There were two growth effects: stimulation and, less frequently, inhibition. Differences can be due to the different adrenaline concentrations, media and reading techniques in the two studies [Doherty et al., 2009]. Like in the present study, strain-specific effects in the same species (*Cutibacterium acnes*) were found in catecholamine studies on anaerobes [Roberts et al., 2002].

Stress and *H. pylori* combination can be deleterious to patients. Guo et al. [2009] found higher gastric *H. pylori* colonization and mucosal injury in stressed mice compared with controls. Stress has been associated with peptic ulceration, either alone or in association with *H. pylori* [Fink, 2011].

In our study, at concentrations achievable under conditions of stress, the catecholamine stimulated the growth of many *H. pylori* strains with sometimes striking diameters (≥ 26.0 mm) of the growth increase found by both AWDM and DDM.

Although some bacteria have exhibited preference for noradrenaline/dopamine over adrenaline [Freestone, 2013], most (71.9%, 23/32) *H. pylori* strains in this study were adrenaline responsive. Notably, growth stimulation prevailed over growth inhibition, which may be of clinical importance. Moreover, the catecholamines also can induce increased virulence gene expression and biofilm behavior changes leading to worsening of the infection [Boyanova, 2017]. The present study emphasizes the need for further extensive evaluation of stress hormone effects on *H. pylori*.

In conclusion, most *H. pylori* strains were responsive to adrenaline concentrations achievable under stress conditions. The prevalent adrenaline-associated growth change was the growth increase, which can explain, at least partly, the synergistic stress-related mucosal damages and the worsening of *H. pylori*-associated diseases under conditions of stress.

Acknowledgments

This research was funded by Grant/Contract B02/17 (12.12.2014) from the National Science Fund at the Ministry of Education and Science of Bulgaria entitled “Complex study of *Helicobacter pylori* virulence and resistance factors and epidemiology of the infection”.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Boyanova L. Stress hormone epinephrine (adrenaline) and norepinephrine (noradrenaline) effects on the anaerobic bacteria. *Anaerobe* 2017;44:13–9.
- Boyanova L, Gergova G, Nikolov R, Davidkov L, Kamburov V, Jeleu C, et al. Prevalence and evolution of *Helicobacter pylori* resistance to 6 antibacterial agents over 12 years and correlation between susceptibility testing methods. *Diagn Microbiol Infect Dis* 2008; 60:409–15.
- Carron M, Veronese S, Gomiero W, Foletto M, Nitti D, Ori C, et al. Hemodynamic and hormonal stress responses to endotracheal tube and ProSeal laryngeal mask airway™ for laparoscopic gastric banding. *Anesthesiology* 2012;117:309–20.
- Doherty NC, Tobias A, Watson S, Atherton JC. The effect of the human gut-signalling hormone, norepinephrine, on the growth of the gastric pathogen *Helicobacter pylori*. *Helicobacter* 2009;14:223–30.
- Fink G. Stress controversies: post-traumatic stress disorder, hippocampal volume, gastroduodenal ulceration. *J Neuroendocrinol* 2011;23:107–17.
- Freestone P. Communication between bacteria and their hosts. *Scientifica (Cairo)* 2013; 2013:361073.
- Guo G, Jia KR, Shi Y, Liu XF, Liu KY, Qi W, et al. Psychological stress enhances the colonization of the stomach by *Helicobacter pylori* in the BALB/c mouse. *Stress* 2009;12: 478–85.

- Jentsch HF, März D, Krüger M. The effects of stress hormones on growth of selected periodontitis related bacteria. *Anaerobe* 2013;24:49–54.
- Mégraud F, Lehours P. *Helicobacter pylori* detection and antimicrobial susceptibility testing. *Clin Microbiol Rev* 2007;20:280–322.
- O'Donnell PM, Aviles H, Lyte M, Sonnenfeld G. Enhancement of in vitro growth of pathogenic bacteria by norepinephrine: importance of inoculum density and role of transferrin. *Appl Environ Microbiol* 2006;72(7):5097–9.
- Roberts A, Matthews JB, Socransky SS, Freestone PP, Williams PH, Chapple IL. Stress and the periodontal diseases: effects of catecholamines on the growth of periodontal bacteria in vitro. *Oral Microbiol Immunol* 2002;17:296–303.
- Velayudhan J, Hughes NJ, McColm AA, Bagshaw J, Clayton CL, Andrews SC, et al. Iron acquisition and virulence in *Helicobacter pylori*: a major role for FeoB, a high-affinity ferrous iron transporter. *Mol Microbiol* 2000;37:274–86.