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Short Communication

Control of APN/CD13 and NEP/CD10 on sperm motility

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Abstract

Aminopeptidase N (APN/CD13) and neutral endopeptidase (NEP/CD10) are enzymes present in human sperm cells and involved in regulation of sperm motility of noncapacitated spermatozoa. We investigated the involvement of APN/CD13 and NEP/CD10 in motility and in kinematic parameters of human capacitated spermatozoa. Sperm cells isolated by a discontinuous Percoll gradient (40%–80%) followed up by swim-up techniques were incubated with the APN/CD13-specific inhibitor, leuhistin (100 μmol L^{-1}), and the NEP/CD10-specific inhibitor, thiorphan (1 μmol L^{-1}). The complete inhibition of both APN/CD13 and NEP/CD10 improved sperm motility. Spermatozoa incubated with the APN/CD13-specific inhibitor leuhistin showed asymmetrical trajectories, whereas sperm trajectories were more regular after treatment with the NEP/CD10-specific inhibitor thiorphan. In conclusion, APN/CD13 and NEP/CD10 modulate the motility of capacitated spermatozoa, although each of the enzymes seems to participate in the control of different aspects of sperm motility. Therefore, both inhibitors may be useful for sperm activation at different functional stages of spermatozoa.

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Keywords: APN/CD13, human, hyperactivation, kinematic, motility, NEP/CD10, sperm

1 Introduction

Sperm motility appears to be essential for natural reproduction and is an important feature and currently the most reliable predictor of male factor infertility [1, 2]. Activation of sperm motility occurs upon release from the male and is modified during the transport of spermatozoa through the female reproductive tract. When deposited inside female reproductive tract, sperm cells develop a progressive motility which must develop to hyperactive motility when they arrive to the oviduct [2, 3].

Aminopeptidase N (APN/CD13) and neutral endopeptidase 24:11 (NEP/CD10) are surface membrane multifunctional enzymes and both are present in human sperm cells [4, 5]. Therefore, the aim of this study was to investigate the involvement of APN/CD13 and NEP/CD10 in motility of human capacitated spermatozoa.

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2 Materials and methods

2. 1 Reagents

Leuhistin was obtained from Calbiochem (La Jolla, CA, USA) and thiorphan was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2. 2 Methods

Human semen was obtained from healthy donors by masturbation after 2–3 days of abstinence; the donors were normozoospermic according to World Health Organization standards [6]. Ethical approval was obtained from the Ethics Committee of the University of

the Basque Country and from the Cruces Hospital Ethics Committee. Informed consent was obtained from all donors. Samples were ejaculated into sterile containers and allowed to liquefy at 37 ºC for 30 min before processing.

Spermatozoa were isolated using a discontinuous Percoll gradient (40%–80%) followed by a swim-up procedure and they were capacitated for 5 h at 37 ºC in 5% CO_2 in G-IVF (Vitrolife, Göteborg, Sweden). Isolated sperm cells were resuspended to ~50 × 10^6 cells per mL and incubated with the APN-specific inhibitor leuhistin (100 μmol L^{-1}), the NEP-specific inhibitor thiorphan (1 μmol L^{-1}) or the corresponding solvent (control aliquots). At these concentrations, both enzymes were completely inhibited [4]. The percentage of motile sperm and kinematic parameters were measured using an SCA (Sperm Class Analyzer) system following the WHO recommendations [6]: grade A sperm (rapidly progressive), grade B (slow/sluggish progressive), grade C (nonprogressive motility) and grade D (immobile), progressive motility (grade A+B sperm), curvilinear velocity (VCL), straight-linear velocity (VSL), average velocity (VAP), linearity index (LIN = VSL/VCL), straightness index (STR = VSL/VAP), oscillation index (WOB = VAP/VCL), amplitude of lateral head displacement (ALH) and beat-cross frequency (BCF). The Mann–Whitney nonparametric test was used to compare normalized data ([treatment−control]/control × 100) between different time periods of incubation.

3 Results and discussion

The inhibition of APN/CD13 and NEP/CD10 by

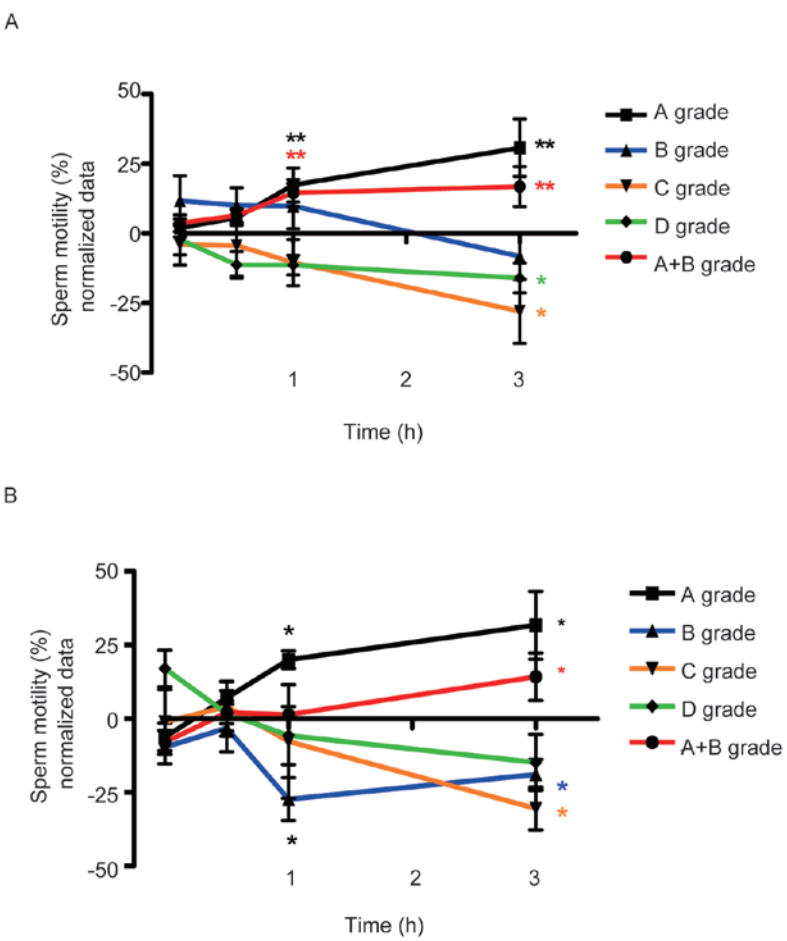

Figure 1. Effect of APN/CD13 inhibition (A) and NEP/CD10 inhibition (B) on sperm motility at 0 (2 min), 0.5, 1 and 3 hours of incubation. Percentage of A, B, C, D and progressive (A+B) grade sperm after the addition of leuhistin (100 μmol L^{-1}). Normalized data are expressed as mean + SEM. ($^{*}P < 0.05$, $^{**}P < 0.01$, compared with control as 0%; $n = 7$).

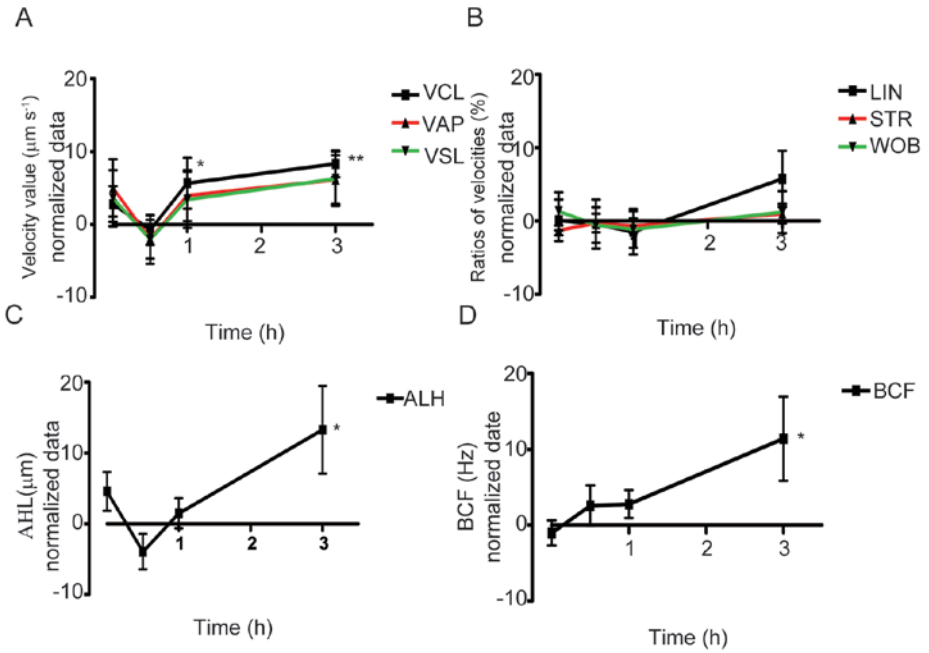

Figure 2. Effect of APN/CD13 inhibition on kinematic parameters at 0 (2 min), 0.5, 1 and 3 h of incubation. (A) Velocity values: VCL, VSL and VAP (μm/s); (B) ratios of the velocities: LIN, STR and WOB (%); (C) amplitude of lateral head of displacement: AHL(μm); (D) BCF (Hz) during the time period on leuhistin treatment. Normalized data are expressed as mean + SEM ($^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* control as 0%; $n = 7$). Abbreviations: VCL, curvilinear velocity; VSL, straight-linear velocity; VAP, average velocity; LIN = VSL/VCL, linearity index; STR = VSL/VAP, straightness index; WOB = VAP/VCL, oscillation index; ALH, amplitude of lateral head displacement and BCF: beat-cross frequency.

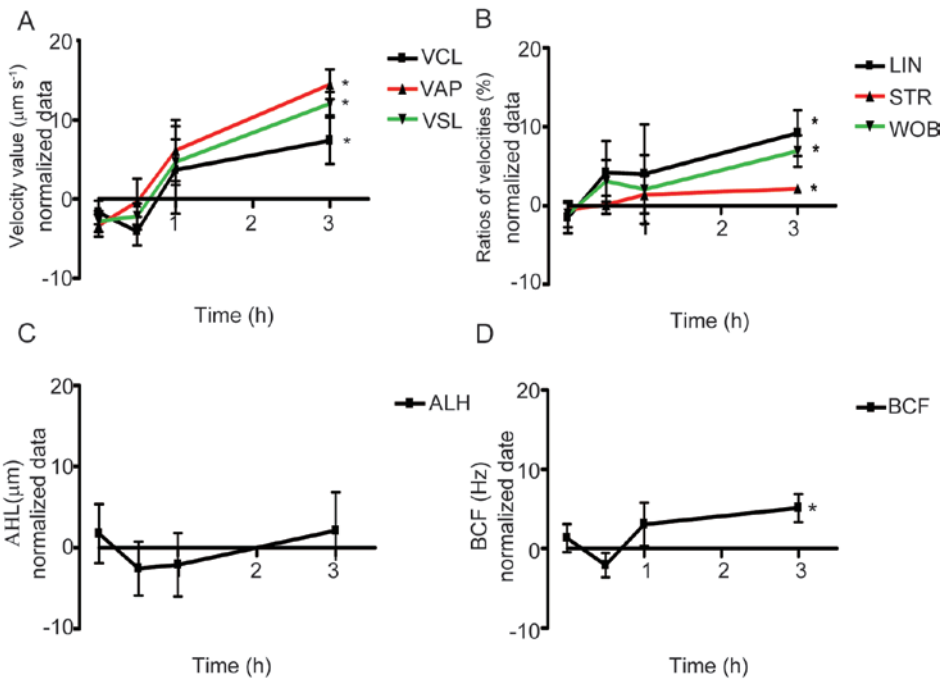

Figure 3. Effect of NEP/CD10 inhibition on kinematic parameters at 0 (2 min), 0.5, 1 and 3 h of incubation. (A) Velocity values: VCL, VSL and VAP (μm s^{-1}); (B) ratios of the velocities: LIN, STR and WOB (%); (C) AHL (μm); (D) BCF (Hz) during the time period on thiorphan treatment. Normalized data are expressed as mean + SEM ($^{*}P < 0.05$, compared with control as 0%; $n = 5$). Abbreviations: VCL, curvilinear velocity; VSL, straight-linear velocity; VAP, average velocity; LIN = VSL/VCL, linearity index; STR = VSL/VAP, straightness index; WOB = VAP/VCL, oscillation index; ALH, amplitude of lateral head displacement and BCF: beat-cross frequency.

leuhistin and tiorphan, respectively, improved sperm motility (Figures 1A and B), but spermatozoa showed distinctly different trajectories after leuhistin and thiorphan incubation. Kinematic parameters define sperm trajectories; they should be useful to establish the difference between progressive and hyperactive motility [7–9]. After leuhistin treatment, sperm cells showed more asymmetric trajectories characteristic of hyperactivation [8, 10], because the inhibitor increased VCL, ALH and BFC after incubation for 3 h (Figures 2A, C and D). High values of these parameters are important, because only hyperactivated sperm can leave the fallopian tube isthmus and penetrate the oocyte zona pellucida [11]. On the other hand, after thiorphan incubation, sperm cells showed more regular trajectories than those in the control aliquots characteristic of progressive motility, because thiorphan increased all velocity values (Figure 3A), linearity indices (Figure 3B) and the BCF (Figure 3D) of sperm cells, whereas AHL remained unaltered (Figure 3C). Only spermatozoa with regular and good progressive motility are able to swim through the female reproductive tract [7]. Our results suggest that APN/CD13 and NEP/CD10 control different aspects of sperm motility and that both inhibitors may be useful for sperm activation at different functional stages of spermatozoa.

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