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Effects of ornithine on neutrophil (PMN) free amino acid and α-keto acid profiles and immune functions *in vitro*

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Summary. The objective of this study was to determine the effects of ornithine on polymorphonuclear leucocyte (PMN) free amino- and α-keto acid profiles, superoxide anion (O_2^-) generation, hydrogen peroxide (H_2O_2) formation and released myeloperoxidase acitivity (MPO). Exogenous ornithine significantly increased PMN asparagine, glutamine, asparatate, glutamate, arginine, citrulline, alanine, α-ketoglutarate and pyruvate as intracellular ornithine increased. Concerning PMN immune function markers ornithine increased H_2O_2-generation and MPO acitivity while O_2^--formation was decreased. We believe therefore that ornithine is important for affecting PMN "susceptible free amino- and α-keto acid pool" although the mechanisms are not yet clear. This may be one of the determinants in PMN nutrition considerably influencing and modulating PMN host defense capability.

Keywords: Ornithine – Neutrophil – Amino acids – α-Keto acids – Immune function

Introduction

The neutrophil (polymorphonuclear leukocyte, PMN) ensures an important part of cell-mediated immunity and is the primary effector cell in the host defense mechanisms which protect the human organism from pathogenic microorganisms (Gabrilovich, 1999). The importance of PMNs is particularly apparent when their numbers are reduced or their functions become impaired (Witko-Sarsat et al., 2000; Mühling et al., 2001, 2000a, b). As a possible consequent of PMN malfunction, bacterial infection may develop and contribute to patient morbidity and mortality. (Bihari, 2002; Takrouri, 2002; Waitzberg et al., 2003). Ideally, the provision of optimal preconditions for PMN cells exists as an undisturbed function of major immunocompetent PMN biochemical pathways. Increasing evidence suggests that free intracellular amino acid and α-keto acid turnover is especially important for the metabolic and physiological state of PMN as well as to the special functions in the inflammatory response performed by these cells. (Moinard et al., 2002a, b; Metcoff et al., 1987; Metcoff, 1986; Mühling et al., 1999).

Various investigators suggest that ornithine may have particular beneficial pharmacological value in modulating cellular metabolism as well as in the immune response in rapidly proliferating cells such as PMN and therefore may alleviate post-traumatic immune depression or catabolic conditions as characterized by reduced leukocyte function (Moinard et al., 1999; Rodenas et al., 1998; Walters et al., 1995; Walters et al., 1998). Currently, the list of biological activities attributed to ornithine is quite extensive because ornithine participates in the urea and tricarboxylic acid cycle (TCA) (Evoy et al., 1998; Grimble and Grimble, 1998; Moinard et al., 2000; Witte and Barbul, 2003). Although its mode of action is not fully understood, ornithine is known to be involved in the secretion of anabolic hormones (e.g. growth hormone, insulin, etc.) and the synthesis of key metabolites such as glutamine, arginine, polyamines, nitric oxide and proline (Fauth et al., 1993; Moinard et al., 2002a; Vlaho and Sieberth, 1981; Walters et al., 1995; Walters et al., 1998; Wolfe and Gatfield, 1975).

However, the major biochemical pathways by which ornithine modulates major cellular functions, PMN dynamic free amino as well as the α-keto acid pool, and their important bactericidal functions have not yet been defined. Moreover, our current understanding of the rate of ornithine utilization and major pathways of metabolism raises some intriguing questions concerning possible therapeutic manipulation and whether important PMN antibacterial host defense mechanisms can be beneficially altered. Another important biological function of ornithine is its possible role as a tissue-specific immunonutrient.

The goals of this study are therefore:

1) To document the effects of ornithine (regarding its role in PMN immunonutrition) on PMN free intracellular amino- and α-keto acid concentrations.
2) To investigate the effects of ornithine on the activity of released myeloperoxidase, and on superoxide anion and hydrogen peroxide formation (as markers of PMN function) in order to show possible parallels with changes in PMN amino- and α-keto acid concentrations.
3) To examine whether a critical duration of exposure necessary to produce any significant effects.

Material and methods

The study was approved by the local ethics committee of the Justus Liebig University, Giessen. Ten men between 24 and 38 years (31 ± 4.1) with an average height of 179.0 cm (range 171–188) and weight of 79.3 kg (range 73–92) were selected. Those men with metabolic (e.g. diabetes, etc.), cardiopulmonary, neurological or allergic diseases or men taking drugs were excluded. Whole blood samples (lithium-heparinate plastic tubes) were withdrawn between 08:00 and 09:00 (after 10 hours of fasting) with consideration of circadian variations.

Ornithine

1) To document any dose-dependent effects PMN were incubated with different ornithine concentrations (0.025, 0.05, 0.1, 0.25 and 0.5 mM) for 120 min. The selected ornithine concentration corresponded to 10-fold the clinically achieved plasma concentrations (see Bender et al. (1985) for physiological values).
2) To examine if there is a critical duration of exposure necessary to produce any significant effects PMN were incubated with ornithine (0.5 mM) for 10, 30, 60 or 120 min.

Solutions of ornithine were prepared and diluted in Hank's balanced salt solution (HBSS; Sigma, Deisenhofen, Germany), and the pH in the test solution was confirmed to be 7.4. One milliliter of whole blood was incubated with 25 μl of test solution (final ornithine concentrations were as described above) at 37°C using a vibrating water bath. Corresponding volumes of HBSS were added to the control tubes. Before further processing all fractions were immediately cooled in an ice water bath at 4°C and 100 μg/ml phenyl methyl sulfonyl fluoride (PMSF), 10 μg/ml leupeptin, 10 μg/ml pepstatin, as well as 10 μg/ml antipain (all acquired from Sigma, USA) were added to each plastic heparin tube before the blood samples; these additions served to inhibit proteases.

Highly selective separation of polymorphonuclear leucocytes (PMN) from whole blood

Precise details of our PMN-separation technique have been described previously (Mühling et al., 2000a, 1999). This method is a further development of the methods described by Eggleton et al. (1989) and Krumholz et al. (1995, 1993) which allows a very rapid and selective enrichment of PMN preserving high cellular viability and integrity from very small quantities of whole blood.

Chromatographic amino and α-keto acid analysis

Amino- and α-keto acids in PMN were quantified using previously described methods which fulfill the strict criteria required for ultrasensitive, comprehensive amino- and α-keto acid analysis, especially developed and precisely validated in our institute for this purpose (for details see Mühling et al. (2003, 1999). The coefficients of variations for both method reproducibility and reproducibilities of the retention times were within normal range. PMN amino acid concentrations are given in 10^{-16} Mol per PMN-cell, PMN α-keto acid concentrations are given in 10^{-17} Mol per PMN-cell.

Superoxide anion (O_2^-), hydrogen peroxide production (H_2O_2) and activity of released myeloperoxidase (MPO) were determined photometrically using modifications of known methods validated in our institute for this purpose (Krumholz et al., 1995, 1993).

Superoxide anion production was measured by reduction of cytochrome C. Cytochrome C (100 mg, type IV, Sigma, Deisenhofen, Germany) was dissolved in 30 ml PBS® glucose buffer (Gibco, Karlsruhe, Germany). The solution was portioned and stored at −20°C. Opsonized zymosan (Sigma, Deisenhofen, Germany) was used to stimulate PMN. It was evoked by incubating 100 mg zymosan with 6 ml pool serum for 30 min at 37°C. After washing with saline and centrifuging at $350 \times g$ (10 min) opsonized zymosan was re-suspended in 10 ml PBS® glucose buffer, portioned and stored at −20°C. Whole blood was incubated either with arginine, L-alanyl-L-glutamine or taurine. These preparations were then incubated for 60 min at 37°C (vibrating water bath). The PMN were thereafter isolated using a modified PMN separation technique as mentioned above. After stepwise (15 min and 5 min) centrifugation procedures ($350 \times g$, 20°C) as well as careful lysis of a few erythrocytes contaminating the pellet, the PMN cells were resuspended by adding diluted PBS® (Gibco, Karlsruhe, Germany) stock buffer. After administration of 7 ml PBS® stock buffer, the tubes were centrifuged at $350 \times g$ for 5 min (20°C). The supernatant was decanted. Samples with a PMN purity <96% and those with more than 4% dead cells were discarded. The PMN concentration (0.8×10^6/ml) was adjusted by adding PBS® containing 9.99 g/l glucose (Merck, Darmstadt, Germany). After PMN isolation, 500 μl zymosan, 150 μl pool serum, 250 μl cytochrome C and 500 μl isolated PMN suspension (0.8×10^6/ml) and again arginine, L-alanyl-L-glutamine or taurine were poured into a test tube. A preparation containing 500 μl buffer instead of zymosan was used for zero adjustment. After incubation for 15 min at 37°C, the reaction was stopped by putting the test tube into ice water. After centrifugation ($350 \times g$; 3 min, 4°C) extinction of the supernatant was measured photometrically (546 nm; Digitalphotometer 6114S®; Eppendorf, Germany). The amount of superoxide anions measured resulted from the extinction coefficient of cytochrome C (Rick, 1977). All control probes were prepared, incubated and measured in the same way.

Hydrogen peroxide production was also determined photometrically using a method based on horseradish peroxidase catalyzed oxidation of phenol red by hydrogen peroxide. Phenol red (Sigma, Deisenhofen, Germany) and horseradish peroxidase (Type II, Sigma, Deisenhofen,

Germany) were added to PMN which had been stimulated by opsonized zymosan. Phenol red was dissolved in double-distilled water (10 g/l). Horseradish peroxidase was dissolved in PBS® glucose buffer (5 g/l). After incubation of whole blood with either arginine, L-alanyl-L-glutamine or taurine for 60 min at 37°C, PMN were isolated as described above. Isolated PMN were stimulated by opsonized zymosan. The final preparation consisted of 500 μl zymosan, 125 μl pool serum, 12.5 μl horseradish peroxidase, 12.5 μl phenol red, 12.5 μl sodium azide (200 mmol/l; Merck, Darmstadt, Germany), 500 μl PMN suspension (2×10^6 PMN cells/mL) and again arginine, L-alanyl-L-glutamine or taurine (15 min, 37°C). After adding of 25 μl 1N sodium hydroxide solution (Merck, Darmstadt, Germany), the extinction was measured photometrically at 623 nm. All control probes were prepared, incubated and measured in the same way.

Activity of released myeloperoxidase: 1 mmol/l 2,2′-azino-di-(3-ethyl-benzthiazoline) sulphonic acid (ABTS, Sigma, Deisenhofen, Germany) was dissolved in 0.1 mol citrate buffer (Behring, Marburg, Germany; pH 7.4). The preparation of whole blood was incubated either with arginine, L-alanyl-L-glutamine or taurine for 60 min at 37°C. After this incubation, 100 μl isolated PMN suspension (2×10^6/ml) was once more incubated with 0.5 μg cytochalasin B (Sigma, Deisenhofen, Germany) and again with arginine, L-alanyl-L-glutamine or taurine for 5 min (37°C). After addition of 100 μl opsonized zymosan and supplementation of arginine, L-alanyl-L-glutamine or taurine in order to keep the concentration constant, the preparation was incubated again for 10 min (37°C). Then 1 ml ABTS solution was added. After centrifugation (700 × g, 5 min, 20°C), 1 ml supernatant was removed and mixed with 1 μl hydroxide peroxide solution (30%; Merck, Darmstadt, Germany) and the extinction coefficient was measured at 405 nm.

Statistical analysis

Statistical analysis and interpretation of the results were performed in close cooperation with colleagues from the Department of Medical Statistics, Justus Liebig University Giessen.

All tests were performed in duplicate. Thus our PMN amino acid results are the mean of two estimations. After the results were demonstrated to be normally distributed (Pearson-Stephens test), statistical methods were performed including Bartlett's test to check homogeneity of variance ($p \leq 0.1$). If the requirements were met, ANOVA analysis was conducted. If the requirements were not fulfilled, the Friedmann test was performed. Probability levels of $p \leq 0.05$ versus control were considered significant. The data are given as arithmetic means ± standard deviations (mean ± SD).

Results

The free intracellular amino and α-keto acid concentrations, superoxide anion formation, hydrogen peroxide generation as well as activity of released myeloperoxidase obtained in the control cells were within normal physiological ranges (see (Krumholz et al., 1995, 1993; Mühling et al., 2003, 1999).

Effects of ornithine on free amino acid pool in PMN

Concentrations of free intracellular amino acids were uneffected by 0.025 and 0.05 mM ornithine (PMN incubation for 120 min), respectively. Following ≥0.1 mM ornithine (PMN incubation for 120 min), significant increases in PMN ornithine, arginine and citrulline (Fig. 1) as well as in PMN glutamate, glutamine, asparagine, aspartate, and alanine profiles have been observed (Fig. 2). Moreover, following 0.5 mM ornithine (PMN incubation for ≥30 min) significantly increased PMN ornithine, arginine, citrulline, glutamate, glutamine, asparagine, aspartate, and alanine (Table 1). PMN free lysine, isoleucine, leucine, valine, methionine, taurine, hypotaurine, serine, glycine, threonine, a-aminobutyrate, tyrosine, tryptophane, phenylalanine and histidine profiles remained uneffected.

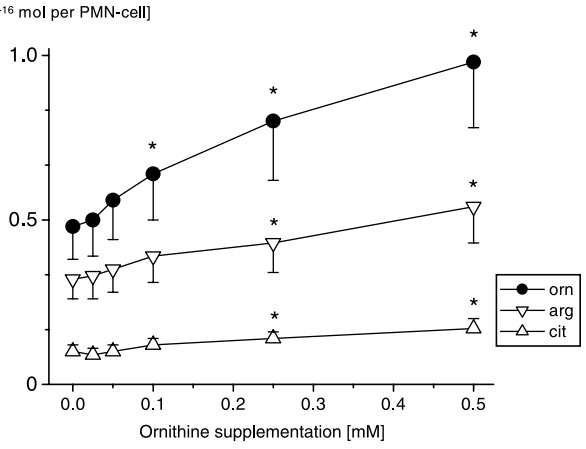

Fig. 1. Free intracellular ornithine, arginine and citrulline concentrations in PMN-cells following *ornithine* incubation (0, 0.25, 0.05, 0.1, 0.25 and 0.5 mM; 120 min) of whole blood *in vitro*. PMN amino acid concentrations are given in 10^{-16} Mol per PMN-cell (mean ± SD; n = 10). *$p \leq 0.05$ versus control values

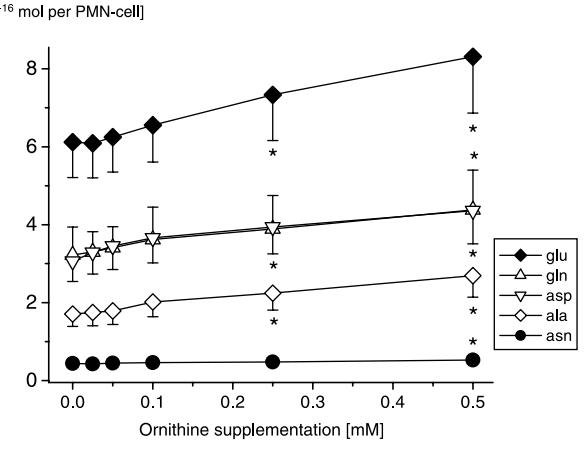

Fig. 2. Free intracellular glutamine, glutamate, asparagine, aspartate and alanine concentrations in PMN-cells following *ornithine* incubation (0, 0.25, 0.05, 0.1, 0.25 and 0.5 mM; 120 min) of whole blood *in vitro*. PMN amino acid concentrations are given in 10^{-16} Mol per PMN-cell (mean ± SD; n = 10). *$p \leq 0.05$ versus control values

Table 1. Effects of *ornithine* (0.5 mM) incubated with whole blood for 10, 30, 60 and 120 min on free intracellular acidic amino acid, acidic amide, basic amino acid and alanine concentrations in PMN (10^{-16} Mol per PMN-cell; mean ± SD; n = 10)

Amino acids	Ornithine			
	10 min	30 min	60 min	120 min
Acidic amino acids, acidic amides, alanine				
Control				
Asparagin	0.41 ± 0.06	0.40 ± 0.06	0.37 ± 0.05	0.42 ± 0.07
Glutamin	2.92 ± 0.61	2.90 ± 0.60	2.86 ± 0.59	2.98 ± 0.67
Aspartat	3.02 ± 0.53	3.12 ± 0.56	3.07 ± 0.55	3.04 ± 0.55
Glutamat	6.26 ± 0.96	6.13 ± 0.92	6.18 ± 0.94	6.39 ± 0.99
Alanin	1.86 ± 0.38	1.80 ± 0.36	1.79 ± 0.32	1.90 ± 0.40
Orn (0.5 mM)				
Asparagin	0.40 ± 0.07	0.41 ± 0.07	0.42 ± 0.07	0.50 ± 0.09$^{*,\#}$
Glutamin	2.99 ± 0.64	3.39 ± 0.73	3.81 ± 0.86$^{*,\#}$	4.11 ± 0.93$^{*,\#}$
Aspartat	3.31 ± 0.66	3.69 ± 0.74	4.18 ± 0.80$^{*,\#}$	4.44 ± 0.98$^{*,\#}$
Glutamat	6.79 ± 1.16	7.43 ± 1.35	8.21 ± 1.47$^{*,\#}$	8.87 ± 2.04$^{*,\#}$
Alanin	2.04 ± 0.43	2.29 ± 0.48	2.62 ± 0.53$^{*,\#}$	2.90 ± 0.60$^{*,\#}$
Basic amino acids				
Control				
Ornithin	0.45 ± 0.08	0.46 ± 0.08	0.47 ± 0.09	0.46 ± 0.08
Lysin	0.64 ± 0.11	0.61 ± 0.11	0.67 ± 0.12	0.63 ± 0.11
Arginin	0.34 ± 0.07	0.33 ± 0.07	0.32 ± 0.06	0.33 ± 0.07
Citrullin	0.12 ± 0.03	0.11 ± 0.03	0.12 ± 0.03	0.12 ± 0.03
Orn (0.5 mM)				
Ornithin	0.56 ± 0.11	0.71 ± 0.16$^{*,\#}$	0.80 ± 0.18$^{*,\#}$	1.05 ± 0.26$^{*,\#}$
Lysin	0.62 ± 0.12	0.67 ± 0.13	0.66 ± 0.13	0.62 ± 0.13
Arginin	0.40 ± 0.08	0.43 ± 0.09*	0.52 ± 0.10$^{*,\#}$	0.65 ± 0.13$^{*,\#}$
Citrullin	0.12 ± 0.03	0.14 ± 0.03	0.17 ± 0.04$^{*,\#}$	0.20 ± 0.05$^{*,\#}$

$^{*}p \leq 0.05$ versus control values; $^{\#}p \leq 0.05$ 120 min versus 10 min

Effects of ornithine on free α-keto acid pool in PMN

Following low ornithine doses (0.05 mM) concentrations of free intracellular α-keto acids remained uneffected. In the presence of ≥0.25 mM ornithine, significant increases in PMN α-ketoglutarate and pyruvate were observed (PMN incubation for 120 min; Table 2). PMN free α-ketobutyrate, α-ketoisovaleriate, α-ketoisocapronate, p-hydroxy-phenylpyruvate and α-keto-β-methylvaleriate profiles remained uneffected.

Table 2. Effects of *ornithine* (0.05, 0.25 and 0.5 mM) incubated with whole blood for 10 and 120 min on free intracellular α-ketoglutarate and pyruvate concentrations in PMN (10^{-17} Mol per PMN-cell; mean ± SD; n = 10)

α-Keto acids	Ornithine			
	Control	0.05 mM	0.25 mM	0.5 mM
Orn (10 min)				
α-KG	1.15 ± 0.30	1.21 ± 0.28	1.26 ± 0.36	1.43 ± 0.44
PYR	6.13 ± 1.27	6.09 ± 1.23	6.24 ± 1.36	6.85 ± 1.43
Orn (120 min)				
α-KG	1.44 ± 0.32	1.51 ± 0.34	1.88 ± 0.40$^{*,\#}$	2.14 ± 0.47$^{*,\#}$
PYR	6.21 ± 1.13	6.91 ± 1.25	7.76 ± 1.52$^{*,\#}$	8.56 ± 2.11$^{*,\#}$

$^{*}p \leq 0.05$ versus control values; $^{\#}p \leq 0.05$ 120 min versus 10 min

Effects of ornithine on oxidative response and myeloperoxidase activity

All PMN immune functions tested were uneffected up to 0.025 mM ornithine. In the presence of higher ornithine supplementation ($\geq$0.05 mM), superoxide anion generation decreased significantly. Moreover, ornithine ($\geq$0.1 mM) caused significant increases in hydrogen peroxide formation and MPO activity (Fig. 3). In the presence of 0.5 mM ornithine, hydrogen peroxide formation and released MPO acitivity significantly increased. A statistically significant reduction of hydrogen peroxide formation was also effected. Relevant changes in PMN immune functions tested mainly occurred with PMN incubation for 30 min or longer.

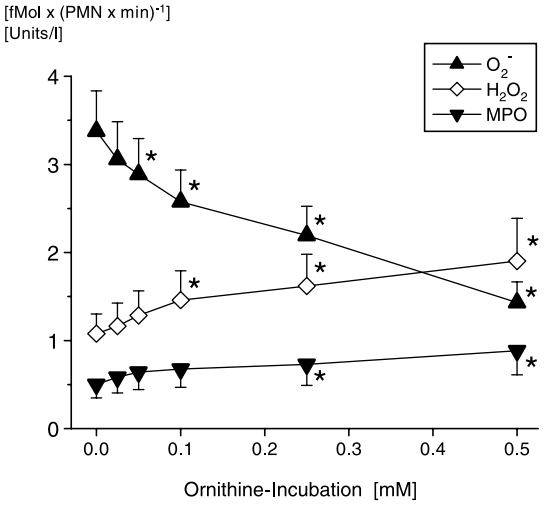

Fig. 3. Effects of different *ornithine* concentrations (0, 0.25, 0.05, 0.1, 0.25 and 0.5 mM) incubated with whole blood for 120 min on PMN superoxide anion production [fmol/(PMN • min)$^{-1}$], hydrogen peroxide formation [fmol/(PMN • min)$^{-1}$] and myeloperoxidase activity [units/l supernatant]; (mean $\pm$ SD; n = 10). *p $\leq$ 0.05 versus control values

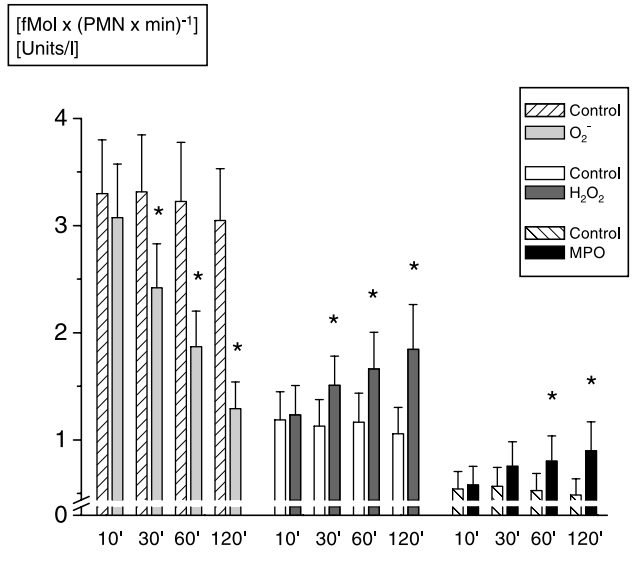

Fig. 4. Effects of *ornithine* (0.5 mM) incubated with whole blood for 10, 30, 60 and 120 min on PMN superoxide anion production [fmol/(PMN• min)$^{-1}$], hydrogen peroxide formation [fmol/(PMN • min)$^{-1}$] and myeloperoxidase activity [units/l supernatant]; (mean $\pm$ SD; n = 10). *p $\leq$ 0.05 versus control values; #p $\leq$ 0.05 120 min versus 10 min

Discussion

Exogenous ornithine ($\geq$0.1 mM) incubated in whole blood siginificantly increased PMN intracellular ornithine content (requiring over 30 min exposure at the highest concentration). This is not surprising taking into consideration that ornithine can enter immune cells by various different active transport mechanisms (i.e. sodium-coupled) or by diffusion, a process which has been described for various cells (Evoy et al., 1998; Fürst, 2000). When intracellular ornithine increases, the concentrations of the basic amino acids arginine and citrulline as well as glutamine, glutamate, asparagine, aspartate and alanine were significantly elevated, drawing attention to ornithin's role as an important intracellular substrate of various amino acid pathways (Bansal and Ochoa, 2003; Calder, 2003; Grimble, 2001; Kirk and Heys, 2003; Montejo et al., 2003). Moreover, ornithine incubation also significantly increased PMN free α-ketoglutarate and pyruvate concentrations without influencing the other α-keto acids tested as well. Although our study does not allow one to infer whether the effects were direct (as the result of direct PMN intracellular ornithine conversion into amino and α-keto acid metabolites) or metabolic changes induced by ornithine incubation of whole blood (i.e. uptake of ornithine and amino and α-keto acids by PMN produced from other blood cells), we were able demonstrate reproducibly that ornithine supplemented to whole blood significantly increased PMN free amino acids *in vitro*. The pattern of PMN amino and α-keto acid changes following ornithine incubation favour the hypothesis that increases in PMN free ornithine concentrations are followed by ornithine conversion into amino acid derivatives (i.e. arginine, glutamate and α-ketoglutarate). Since an initial increase in arginine and citrulline were observed (amino acids next to ornithine in the urea cycle), some urea cycle activity is suggested (Fauth et al., 1993). The incubation of PMN at elevated free ornithine concentrations is of physiological importance since ornithine plasma and tissue concentrations are increased in many metabolic diseases like urea cycle disorders and in

post-surgery trauma (Vlaho and Sieberth, 1981; Wolfe and Gatfield, 1975). Since arginase has been isolated in PMN to a relatively high amount ornithine is a precursor for polyamine synthesis. (Guarnieri et al., 1987; Moinard et al., 2000; Thomas et al., 1983; Walters et al., 1992). Moreover, out of all of the amino acids tested, the levels of aspartic acid, glutamic acid, glutamine, asparagine, alanine and α-ketoglutarate and pyruvate, were also significantly increased. Regarding these findings we suggest an anabolic role of ornithine supplemented to whole blood *in vitro* by increasing PMN amino acids which are important in the supply of nitrogen and carbon for the synthesis of makromolekules and act as an oxidative fuel for energy production (Bansal and Ochoa, 2003; Bihari, 2002; Fürst, 2000; Moinard et al., 2002b; Singh et al., 2002). This has been showed primarily by increases in PMN α-ketoglutarate and pyruvate concentrations. We therefore suggest that ornithine may enter the tricarboxylic acid cycle (whose complete enzymativ equipement has been described previously) via aspartate and glutamate which were also elevated following ornithine supplementation (Evoy et al., 1998; Fürst and Stehle, 1995).

This would partly explain significant ornithine dependent changes in PMN immune functions. Ornithine incubation of PMN distinctly increased both hydrogen peroxide generation and myeloperoxidase activity. Indeed, various authors suggest that ornithine may enhance leukocyte immune function and improves the host's capacity to resist infection (Evoy et al., 1998; Grimble and Grimble, 1998). Indeed, Moinard et al. (1999), Roch-Arveiller et al. (1996) and Walters et al. (1998, 1995) described that essential antimicrobial functions of human leukocytes particularly depend on intracellular ornithine concentrations. Moreover, Metcoff et al. (1989, 1988, 1987 and 1986) mentioned that a combination of PMN free aspartate, ornithine, arginine, glutamate and glutamine profiles in particular were highly predictive regarding the levels of PMN energy charge ands protein synthesis. Consequently, we suggest that nutritional or pharmacological regimens which enhance the supply of ornithine to PMN may have considerable value in modulating essential PMN functions. In regards to both hydrogen peroxide as well as superoxide anion formation our controversial results suggest that arginine supplementation not only augmented but simultaneously inhibited free radical generation in human PMN. Although single time point measurements of PMN immune functions may be misleading, these findings confirm previous investigations which postulated that increases in PMN free intracellular arginine metabolized from ornithine, for example, inhibits free superoxide anion generation as evidenced by an inverse correlation between PMN arginine derived •NO formation and superoxide anion formation generation (Catz et al., 1995; Kausalya and Nath, 1998; Moinard et al., 2000; Rodenas et al., 1998, 1996; Wheeler et al., 1997).

From our results, it is clear that ornithine fulfills the criteria for a potent molecule in the regulation of the dynamic amino and α-keto acid pools as well as in modulation of PMN host defense mechanisms and immunoregulation. However, further research is necessary to clarify ornithine's role in vivo therapeutic immunoregulatory or immunomodulatory properties.

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