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Effects of arginine, L-alanyl-L-glutamine or taurine on neutrophil (PMN) free amino acid profiles and immune functions *in vitro*

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Summary. The objective of this study was to determine the effects of arginine, L-alanyl-L-glutamine (Ala-Gln) or taurine on polymorphonuclear leucocyte (PMN) free amino acid profiles, superoxide anion (O_2^-) generation, hydrogen peroxide (H_2O_2) formation and released myeloperoxidase activity (MPO). Arginine led to significant increases in PMN arginine, ornithine, citrulline, aspartate, glutamate and alanine concentrations as well as increased H_2O_2-generation and MPO activity while O_2^--formation was decreased. Ala-Gln caused significant increases in PMN free glutamine, alanine, asparagine, aspartate, glutamate, ornithine, arginine, serine and glycine concentrations and increased PMN immune functions. Taurine significantly increased PMN free taurine profiles, reduced PMN neutral amino acid content and decreased H_2O_2- and O_2^--formation while MPO was increased. Altogether, the pharmacological regimens which enhance the supply of arginine, Ala-Gln or taurine in whole blood significantly affect PMN "susceptible free amino acid pool". This may be one of the determinants in PMN nutrition considerably influencing PMN immune functions.

Keywords: Amino acids – Arginine – L-Alanyl-L-glutamine – Taurine – Neutrophil – Immune function

Introduction

Polymorphonuclear leucocytes (PMN) ensure an important part of non-specific cell-mediated immunity and play a crucial role in the host defense

mechanisms which protect the human organism from pathogenic microorganisms. This is because derangement of any PMN function may allow bacterial infection to develop and may contribute to a patient's morbidity and mortality (Krumholz et al., 1995 and 1993). Increasing evidence suggests that free intracellular amino acid turnover is important to the metabolic and physiological state of PMN as well as to the special inflammatory response functions of these cells (Bender, 1985). Various findings suggest that some "classic non-essential" amino acids like arginine, glutamine or taurine may have beneficial pharmacological value in modulating cellular metabolism as well as the immune response in rapidly proliferating cells such as PMN (Newsholme et al., 1999; Beaumier et al., 1996; Stapleton et al., 1998). Regarding arginine, important findings suggest that this amino acid has a strong potential as an immunomodulator and may alleviate post-traumatic immune depression or catabolic conditions as characterized by reduced leucocyte function (Evoy et al., 1998; Catz et al., 1995). Similarly, glutamine-dipeptides (i.e. L-alanyl-L-glutamine) have been shown to play important roles in cell-mediated immune functions and received interest in promotion of antimicrobial as well as antitumoral effector systems. L-Alanyl-L-glutamine, especially, seems to be an important substrate for immunocompetent and rapidly proliferating cells such as PMN (Roth et al., 1996). Taurine appears to play a pivotal role in important physiological leucocyte immune functions as well. The list of biological activities associated with taurine is impressive and includes antioxidation, membrane stabilization and detoxification (Stapleton et al., 1996 and 1998).

However, at present state of knowledge only poor information is available which describe and compare the precise effects of arginine, glutamine or taurine supplementation on "susceptible free intracellular amino acid pool" in PMN as well as on important PMN immune functions, although major pathophysiological aspects underlying remain still unclear.

The goal of this study was therefore:

1) To document the effects of arginine, L-alanyl-L-glutamine or taurine on free PMN amino acid concentrations. With regard to clinical relevance we have chosen an experimental design with whole blood, followed by rapid and highly selective enrichment of PMN, thereby preserving high cellular viability and integrity.
2) In a parallel study the effects of a arginine, L-alanyl-L-glutamine or taurine incubation of whole blood on released myeloperoxidase activity and both superoxide anion and hydrogen peroxide formation in isolated PMN were also investigated.

Material and methods

The study was approved by the local ethics committee of the Justus Liebig University, Giessen. Twenty men between 23 and 37 years (30 ± 3.9) with an average height of 179.5 cm (range 171–189) and weight of 78.8 kg (range 74–93) were selected: those men with metabolic (diabetes etc.), cardiopulmonary, neurological or allergic diseases or men taking drugs were excluded. Whole blood samples (lithium-heparinate plastic tubes) were

withdrawn between 08:30 and 09:00 (after 10 hours of fasting) considering circadian variations.

The following amino acids were tested:

1) ***Arginine*** (Sigma, Deisenhofen, Germany): 0.02; 0.05; 0.1; 0.2; 0.5 and 1 mM.
2) ***L-Alanyl-L-glutamine*** (Dipetamin®, Fresenius, Bad Homburg, Germany [20 g L-alanyl-L-glutamine/100 ml; = 8.2 g L-Alanyl, 13,45 g L-glutamine; = 0,92 mol/L L-alanine and 0,92 mol/L L-glutamine]): 0.1; 0.25; 0.5; 1; 2.5 and 5 mM.
3) ***Taurine*** (Sigma, Deisenhofen, Germany): 0.02; 0.05; 0.1; 0.2; 0.5 and 1 mM.

All samples (inclusive control groups) were incubated for 60 min. The selected arginine, L-alanyl-L-glutamine and taurine concentrations corresponded to 0.2-, 0.5-, 1-, 2-, 5- and 10-fold of physiological plasma concentrations (see Bender et al., 1985 for physiological values). Originally glutamine was ommited from incubation because of both low water solubility as well as concerning its rapid breakdown to pyroglutamate and ammonium during sterilisation procedures. Dipetamin® is not fraught with such galenic problems. Solutions of arginine, L-alanyl-L-glutamine and taurine were prepared and diluted in Hank's balanced salt solution (HBSS; Sigma, Deisenhofen, Germany) and the pH in the test solution was adjusted to 7.4. One mL of whole blood was incubated with 25 μl of test solution (the final concentrations of arginine, L-alanyl-L-glutamine and taurine were as described above) for one hour at 37°C (vibrating water bath). The corresponding volumes of HBSS were added to the control tubes.

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

Precise details of our PMN-separation technique have been described previously (Mühling et al., 1999 and 2000). This method is a further development of the methods described by Eggleton et al. (1989) and Krumholz et al. (1993b and 1995) 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 acid analysis

Amino acids in PMN were quantified using previously described methods which fulfill the strict criteria required for ultrasensitive, comprehensive amino acid analysis, especially developed and precisely validated in our institute for this purpose (Mühling et al., 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 pMol per PMN-cell.

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

Superoxide anion production was measured by reduction of cytochrome C. 100 mg of cytochrome C (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 × g (10 min) opsonized zymosan was resuspended 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 × g, 20°C) as well as careful lysis of a few erythrocytes contaminating the pellet, the PMN-cells were re-suspended by adding diluted PBS® (Gibco, Karlsruhe, Germany) stock buffer. After administration of 7 mL PBS® stock buffer, the tubes were centrifuged at 350 × 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, Darm-stadt, Germany). After PMN isolation, 500 μL zymosan, 150 μL pool serum, 250 μL cytochrom 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 × 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 have been prepared, incubated and measured in the same way. *Hydrogen peroxide production* was also determined photometrically. The method based on horseradish peroxidase catalysed 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-destilled 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 have been 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 adding 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 extinction was measured (405 nm).

Statistical analysis

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

All tests were performed in duplicate. Thus, our PMN amino acid results are the means of two estimations. After the results were demonstrated to be normally distributed (Pearson Stephens test), statistical methods were performed including Bartlett 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 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 Mühling et al., 1999 and Krumholz et al., 1995).

Arginine

Effects on free amino acid pool in PMN

Concentrations of free intracellular amino acids were uneffected by 0.02; 0.05 and 0.1 mmol/L arginine, respectively. Following ≥0.2 mM arginine, significant increases in PMN glutamate, aspartate, ornithine arginine, citrulline and alanine profiles have been observed (Tables 1 and 2; Fig. 1). PMN free asparagine, glutamine, lysine, isoleucine, leucine, valine, methionine, serine, glycine, threonine, a-aminobutyrate, tyrosine, tryptophane, phenylalanine, histidine and taurine profiles remained unaffected.

Table 1. Free acidic amino acid and acidic amide concentrations in PMN-cells following arginine incubation and free basic amino acid content in PMN-cells following L-alanyl-L-glutamine (Dipeptamin®) incubation of whole blood *in vitro*. PMN amino acid concentrations are given in 10^{-16} mol per PMN-cell (mean ± SD; n = 20). * = $p \leq 0.05$ versus control values; ** = $p \leq 0.01$ versus control values

Acidic amino acids, acidic amides				
Arginine	Control	0.2 mM	0.5 mM	1 mM
asparagine	0.40 ± 0.06	0.41 ± 0.06	0.42 ± 0.06	0.44 ± 0.06
glutamine	2.88 ± 0.60	3.02 ± 0.65	3.16 ± 0.68	3.31 ± 0.77
aspartate	2.79 ± 0.46	2.93 ± 0.48	3.34 ± 0.53*	3.54 ± 0.60**
glutamate	5.85 ± 0.87	6.55 ± 0.94	6.92 ± 1.04*	7.18 ± 1.08**
Basic amino acids				
Alanyl – glutamine	Control	1 mM	2.5 mM	5 mM
ornithine	0.45 ± 0.08	0.50 ± 0.09	0.53 ± 0.09	0.55 ± 0.10**
lysine	0.54 ± 0.09	0.58 ± 0.10	0.58 ± 0.10	0.61 ± 0.11
arginine	0.27 ± 0.05	0.30 ± 0.05	0.32 ± 0.06	0.34 ± 0.06**
citrulline	0.09 ± 0.02	0.09 ± 0.02	0.10 ± 0.02	0.11 ± 0.02

Table 2. Free neutral amino acid concentrations in PMN-cells following arginine, L-alanyl-L-glutamine (Ala-Gln; Dipeptamin®) or taurine incubation of whole blood *in vitro*. PMN amino acid concentrations are given in 10^{-16} mol per PMN-cell (mean ± SD; n = 20). *= $p \leq 0.05$ versus control values; **= $p \leq 0.01$ versus control values

Neutral amino acids				
Arginine:	Control	0.2 mM	0.5 mM	1 mM
serine	2.12 ± 0.44	2.19 ± 0.45	2.06 ± 0.43	2.01 ± 0.43
glycine	2.31 ± 0.47	2.21 ± 0.46	2.29 ± 0.46	2.25 ± 0.46
threonine	0.76 ± 0.11	0.77 ± 0.11	0.83 ± 0.13	0.79 ± 0.13
alanine	1.80 ± 0.33	1.98 ± 0.37	2.15 ± 0.45	2.28 ± 0.44**
Ala-Gln:	Control	1 mM	2.5 mM	5 mM
serine	2.02 ± 0.42	2.17 ± 0.43	2.46 ± 0.47	2.63 ± 0.55**
glycine	2.21 ± 0.44	2.41 ± 0.52	2.63 ± 0.56	2.82 ± 0.58**
threonine	0.75 ± 0.12	0.72 ± 0.11	0.78 ± 0.13	0.79 ± 0.14
alanine	1.82 ± 0.34	2.26 ± 0.43*	2.39 ± 0.47**	2.63 ± 0.51**
Taurine:	Control	0.2 mM	0.5 mM	1 mM
serine	2.20 ± 0.46	1.65 ± 0.35*	1.36 ± 0.29**	1.05 ± 0.22**
glycine	2.17 ± 0.41	1.73 ± 0.33*	1.28 ± 0.25**	0.98 ± 0.19**
threonine	0.69 ± 0.12	0.56 ± 0.10*	0.41 ± 0.07**	0.36 ± 0.06**
alanine	1.74 ± 0.33	1.54 ± 0.31	1.30 ± 0.27*	1.19 ± 0.24**

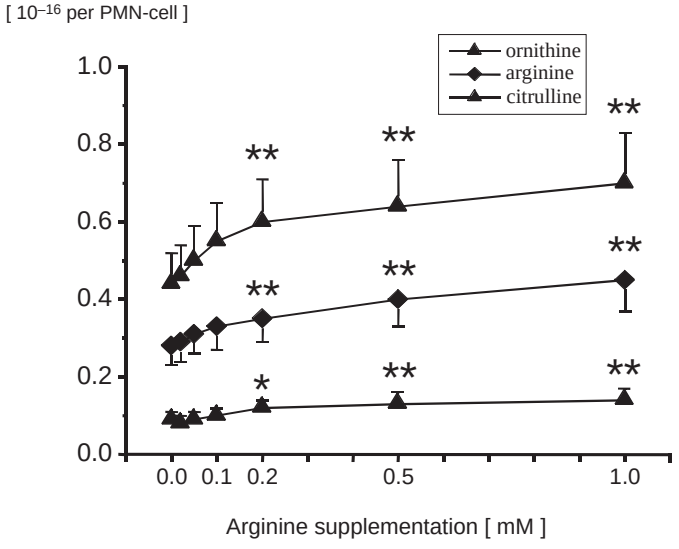

Fig. 1. Free basic amino acid content in PMN-cells following arginine incubation of whole blood *in vitro*. PMN amino acid concentrations are given in 10^{-16} mol per PMN-cell (mean ± SD; n = 20). *= $p \leq 0.05$ versus control values; **= $p \leq 0.01$ versus control values

Effects on oxidative response and myeloperoxidase activity

All PMN immune functions tested were uneffected up to 0.1 mM arginine. In the presence of higher arginine supplementation (0.2, 0.5 and 1 mM), superoxide anion generation decreased significantly. Moreover, arginine (0.5 and 1 mM) caused significant increases in hydrogen peroxide formation and myeloperoxidase activity (Fig. 4).

L-Alanyl-L-glutamine

Effects on free amino acid pool in PMN

Concentrations of free intracellular amino acids were uneffected by 0.1; 0.25 and 0.5 mM L-alanyl-L-glutamine, respectively. Incubation with ≥1 mM L-alanyl-L-glutamine caused significant increases in PMN free intracellular glutamine, asparagine, aspartate, glutamate, ornithine, serine, glycine and alanine concentrations (Tables 1 and 2; Fig. 2). All other PMN free amino acids tested remained uneffected from L-alanyl-L-glutamine supplementation.

Effects on oxidative response and myeloperoxidase activity

All PMN immune functions tested were uneffected up to 0.5 mM L-alanyl-L-glutamine. Incubation with ≥1 mM L-alanyl-L-glutamine led to significant increases in superoxide anion and hydrogen peroxide production as well as myeloperoxidase activity (Fig. 5).

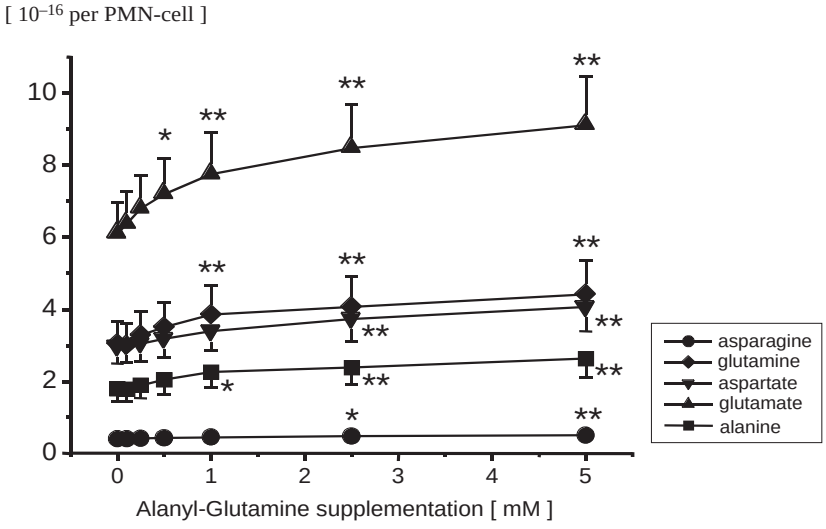

Fig. 2. Free acidic amino acid, acidic amide and alanine content in PMN-cells following L-alanyl-L-glutamine incubation of whole blood *in vitro*. PMN amino acid concentrations are given in 10^{-16} mol per PMN-cell (mean ± SD; n = 20). * = $p \leq 0.05$ versus control values; ** = $p \leq 0.01$ versus control values

Taurine

Effects on free amino acid pool in PMN

Concentrations of free intracellular amino acids were uneffected by 0.02; 0.05 and 0.1 mM taurine, respectively. Incubation with 1 mM taurine caused an significant increase in PMN taurine profiles itself (Fig. 3). Moreover, significant decreases in PMN intracellular neutral amino acid profiles have closely been connected with taurine (≥0.2 mM) incubation (Table 2). All other PMN free amino acids tested remained uneffected from taurine incubation.

Effects on oxidative response and myeloperoxidase activity

All PMN immune functions tested were uneffected up to 0.2 mM taurine. Treatment with higher taurine concentrations (≥0.5 mM) significantly decreased superoxide anion and hydrogen peroxide formation. Myeloperoxidase activity was increased in the presence of 1 mM taurine only (Fig. 6).

Discussion

Arginine

The incubation of whole blood with arginine concentrations ≥0.2 mM, led, as expected, to significant increases in PMN free arginine concentrations. Surprisingly, arginine incubation also significantly increased PMN free ornithine

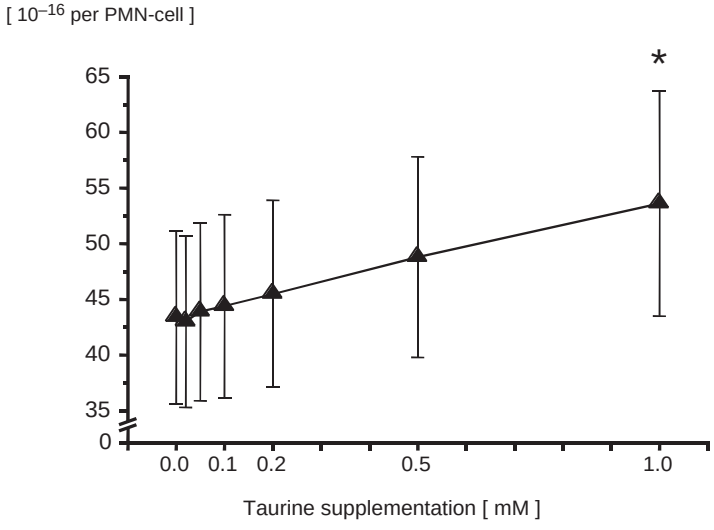

Fig. 3. Free taurine content in PMN-cells following taurine incubation (n = 20) of whole blood *in vitro*. PMN taurine concentration is given in 10^{-16} mol per PMN-cell (mean ± SD; n = 20). *= $p \leq 0.05$ versus control values

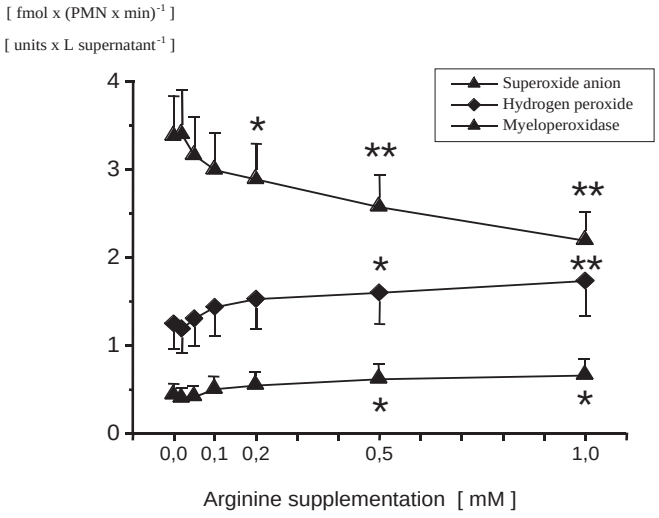

Fig. 4. Superoxide anion production [fMol × (PMN × min)$^{-1}$], hydrogen peroxide production [fMol × (PMN × min)$^{-1}$] and myeloperoxidase activity [units × L supernatant^{-1}], (mean ± SD; n = 20), of PMN-cells following arginine incubation *in vitro*. *= $p \leq 0.05$ versus control values; **= $p \leq 0.01$ versus control values

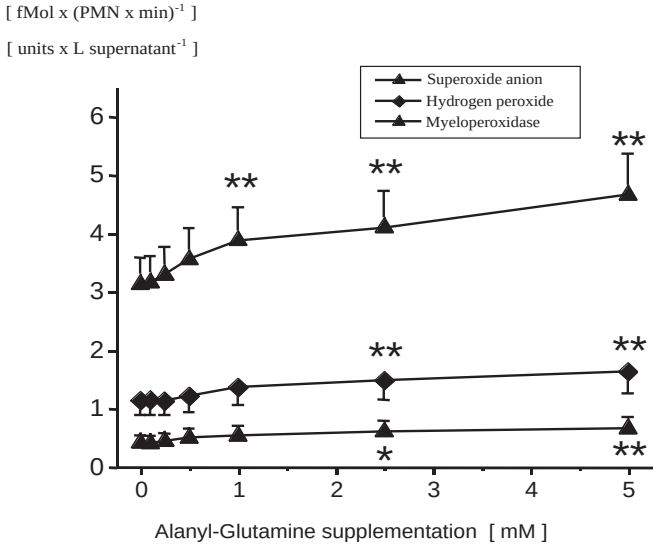

Fig. 5. Superoxide anion production [fMol × (PMN × min)$^{-1}$], hydrogen peroxide production [fMol × (PMN × min)$^{-1}$] and myeloperoxidase activity [units × L supernatant^{-1}], (mean ± SD; n = 20), of PMN-cells following L-alanyl-L-glutamine (Ala-Gln) incubation *in vitro*. *= $p \leq 0.05$ versus control values; **= $p \leq 0.01$ versus control values

and citrulline as well as glutamate, aspartate and alanine concentrations as well. This observation has not been described before in PMN-cells. Although our study does not allow to decide whether the effects were direct (as the result of direct PMN intracellular arginine conversion into amino acid metabolites) or secondary to metabolic changes induced by arginine incuba-

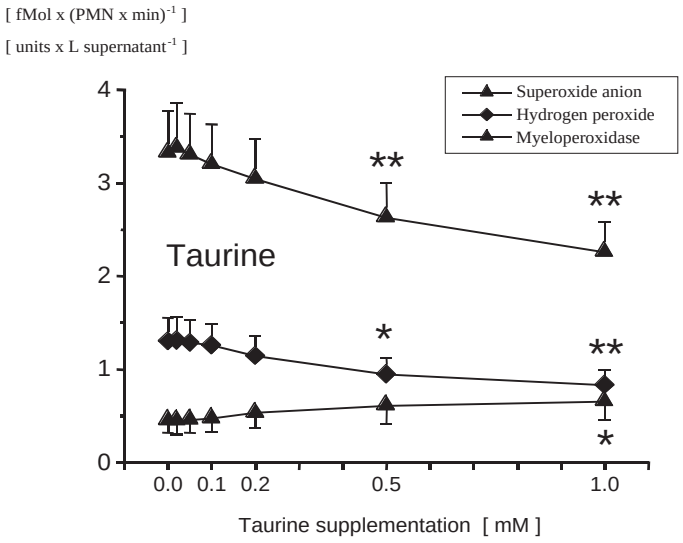

Fig. 6. Superoxide anion production [fMol × (PMN × min)$^{-1}$], hydrogen peroxide production [fMol × (PMN × min)$^{-1}$] and myeloperoxidase activity [units × L supernatant^{-1}], (mean ± SD; n = 20), of PMN-cells following taurine incubation *in vitro*. *= $p \leq 0.05$ versus control values; **= $p \leq 0.01$ versus control values

tion of whole blood (i.e. uptake of arginine and amino acids by PMN produced from other blood cells), we have demonstrated that arginine supplemented to whole blood significantly increased PMN free amino acids *in vitro* which are important metabolic precursors or substrates for various PMN metabolic pathways. The pattern of PMN amino acid changes following arginine incubation favour the hypothesis that increases in PMN free arginine concentrations are followed by arginine conversion into amino acid derivatives (i.e. ornithine, glutamate, aspartate). Especially increases in PMN ornithine and citrulline concentrations, which have also been directly connected with increases in PMN free arginine levels suggest significant intracellular conversion within PMN. For example, arginase has significantly been isolated in PMN to a relatively high amount (Reyero and Dorner, 1975; Sahoo et al., 1998). Moreover, among all amino acids tested, the levels of aspartate, glutamate and alanine were also significantly increased. Regarding these findings we suggest an anabolic role of arginine supplemented to whole blood *in vitro* by increasing PMN amino acids which are important in the supply of nitrogen, carbon and energy. Indeed, there is a body of evidence suggesting that supplemented arginine increases PMN immune function and especially ornithine but also glutamate may share the immunostimulatory as well as the secretagogue effects of arginine (Evoy et al., 1998; Kriegbaum et al., 1987). This would partly explain significant arginine dependent changes in PMN immune functions. Arginine incubation of PMN distinctly increased both hydrogen peroxide generation and myeloperoxidase activity. Indeed, various authors suggest that arginine may enhance immune function and improves the host's capacity to resist infection (Angele et al., 1999; Wyatt et al., 1993; Seth et al., 1994). 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 hypothetized that increases in PMN free intracellular arginine especially inhibits free superoxide anion generation as evidenced by an inverse correlation between PMN arginine derived •NO formation and superoxide anion formation generation (Kausalya and Nath, 1998; Mulligan et al., 1991; Roy et al., 1996; Fierro et al., 1996). With respect to our findings the authors suggest that dietary arginine supplementation should receive new attention because of its role in modulation of PMN "susceptible free amino acid pool" as well as essential PMN immune functions.

L-Alanyl-L-glutamine

L-Alanyl-L-glutamine supplementation to whole blood ($\geq$1 mM) significantly increased free intracellular glutamine as well as alanine profiles in PMN indicating increased uptake of L-alanyl-L-glutamine by PMN from whole blood (Roth et al., 1996). Connected with these findings, we observed significant increases in further important PMN free intracellular amino acids (i.e. glutamate, aspartate, asparagine, ornithine, arginine, citrulline, serine and glycine) as well. This may indicate both increased uptake of L-alanyl-L-glutamine metabolites from whole blood and/or high glutamine utilization by PMN. These findings would favour the latter hypothesis because high rates of glutamine uptake and utilisation, especially by rapidly dividing cells such as PMN, have previously been described (Newsholme et al., 1999), Curi et al., 1999). Increasing evidence suggest that not only glucose but also glutamine in PMN provides and maintains both nitrogen and carbon for the synthesis of macromolecules and acts as an oxidative fuel for energy production (Castell et al., 1994). Not only glutamine but glutamate as well may have been a substrate for further metabolical processes producing aspartate, asparagine as well as neutral amino acids such as serine and glycine. (Curi et al., 1999). Changes in PMN aspartate levels especially may also have essential consequences for PMN respiratory fuel sources because aspartate, just as glutamate, enters the tricarboxylic acid cycle which complete enzymatic equipement has previously been described in PMN (Fauth et al., 1990). Interestingly, exogenous L-alanyl-L-glutamine supplementation also increased PMN arginine and ornithine profiles. Regarding the pattern of PMN amino acid changes, we hypothetize that PMN, similar to macrophages or monocytes, may contain sufficient activity of the enzyme sytems required to convert glutamine to arginine and ornithine (Murphy and Newsholme, 1998). Moreover, L-alanyl-L-glutamine supplementation significantly increased antimicrobicidal functions of human PMN *in vitro*, suggesting an anabolic role of L-alanyl-L-glutamine for PMN. Until now, little information is available concerning the fuel that PMN-cells use for important bactericidal functions, but various

investigators described that essential antimicrobial functions of human leucocytes strongly depend on intracellular glutamine concentrations (Chang et al., 1999; Amores-Sanchez and Medina, 1999; Ruggeberg et al., 1997). Moreover, Metcoff et al. (1983) mentioned that a combination of PMN free aspartate, glycine, ornithine, arginine, glutamate and glutamine profiles especially were highly predictive regarding the levels of PMN energy charge. Consequently, we suggest that nutritional or pharmacological regimens which enhance the supply of alanine and glutamine to PMN may have considerable value in modulating essential PMN functions (Ogle et al., 1998; Furukawa et al., 1997; Grimble and Grimble, 1998). Summing up, our results confirm the beneficial effects of L-alanyl-L-glutamine in at least important arms of PMN metabolism as well as in PMN immune function and may add further evidence for the possible advantage of including this amino acid in the advanced therapeutical standards (intensive care unit etc.).

Taurine

Incubation of whole blood with high taurine concentrations (1 mM) significantly increased intracellular taurine profiles. This effect has been described in various other cell types as well (Lin et al., 1988). Alterations in PMN intracellular neutral amino acid concentrations, which were also connected with taurine incubation, may draw attention to its role as an important intracellular osmoregulator (Chen and Kempson, 1995). As expected, increases in intracellular taurine levels decreased intracellular neutral amino acid profiles which may impart osmoprotection to PMN-cells (Horio et al., 1997; Fugelli et al., 1995). Our results suggest that neutral amino acids may interfere with intracellular taurine levels and may, therefore, represent a homeostatic reaction to changes in intracellular osmoregulation (Trachtmann et al., 1998; Law, 1998). Concerning PMN immune functions, following taurine incubation, decreases in both superoxide anion generation as well as in hydrogen peroxide formation have been observed. Moreover, taurine supplementation led to an increase in released myeloperoxidase activity. These results confirm several previous *in vitro* studies as well as clinical findings (Stapleton et al., 1996 and 1998; Schuller-Levis et al., 1990, 1994; Mc Loughlin et al., 1991; Raschke et al., 1995). These studies hypothetized that increased presence of abundant extracellular and intracellular taurine may act as an antioxydant by both preventing oxidative damage and protecting from the attack of chlorinated oxidants as well as inactivation of important enzymes including myeloperoxidase. Therefore, in regards to former findings (Coble et al., 1994; Masuda et al., 1986; Schuller-Levis et al., 1990) we assume that taurine supplementation may "strengthen immunological functions" of PMN, but further study is necessary to examine the precise biochemical effects of taurine supplementation on PMN immunocompetence.

Overall, for the first time the effects of arginine, L-alanyl-L-glutamine or taurine on PMN free intracellular amino acid concentrations and essential PMN immune functions have been investigated. Although this study was not able to confirm any relationship between PMN free amino acid profiles and

their immune functions, however, there is considerably significance to pharmacological regimens which enhance the supply of arginine, L-alanyl-L-glutamine or taurine to whole blood. These regimens influence PMN amino acid concentrations and the "susceptible intracellular amino acid pool" may be one of the determinants in cell nutrition. Moreover, changes in PMN-cell nutrition caused by enhanced supply of arginine, L-alanyl-L-glutamine or taurine in whole blood may have considerable influence on essential PMN immune functions.

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