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Pathways involved in alanyl-glutamine-induced changes in neutrophil amino- and α-keto acid homeostasis or immunocompetence

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Summary. We examined the effects of DON [glutamine-analogue and inhibitor of glutamine-requiring enzymes], alanyl-glutamine (regarding its role in neutrophil immunonutrition) and alanyl-glutamine combined with L-NAME, SNAP, DON, β-alanine and DFMO on neutrophil amino and α-keto acid concentrations or important neutrophil immune functions in order to establish whether an inhibitor of •NO-synthase [L-NAME], an •NO donor [SNAP], an analogue of taurine and a taurine transport antagonist [β-alanine], an inhibitor of ornithine-decarboxylase [DFMO] as well as DON could influence any of the alanyl-glutamine-induced effects. In summary, irrespective of which pharmacological, metabolism-inhibiting or receptor-mediated mechanisms were involved, our results showed that impairment of granulocytic glutamine uptake, modulation of intracellular glutamine metabolisation and/or de novo synthesis as well as a blockade of important glutamine-dependent metabolic processes may led to significant modifications of physiological and immunological functions of the affected cells.

Keywords: Alanyl-glutamine – DON – L-NAME – SNAP – β-Alanine – DFMO neutrophil – Amino acids – α-Keto acids – Immune function

Introduction

Upon closer consideration of individual amino acids that appear to play important roles in the metabolic demands and immunological functions of neutrophils, the acid amide glutamine is worthy of special mention (Alpers, 2006; Andrews and Griffiths, 2002; Castell, 2003; Newsholme, 2001; Wischmeyer, 2003). Already in the year 1949, Ehrensvard et al. found that glutamine represents an important factor supporting the in vitro cultivation of various cell types. Continuing on from these initial observations it was also shown over the last years that cells of the immune system feature a particularly high glutamine utilisation. T- and B-lymphocytes, lymphokine activated killer cells, macrophages and particularly neutrophil show high degrees of glutamine dependence regarding their metabolic and immunological functions (Ardawi, 1988; Ardawi and Newshome, 1986; Brand et al., 1989; Curi et al., 1986, 1997, 1999; Furukawa et al., 2000a; Mühling et al., 2002, 2005; Ziegler et al., 1998). The most recent research has shown that glutamine deficiency both in vivo and in vitro leads to a significant reduction of bactericidal activity, phagocytosis, cytokine formation as well as a clear decrease in oxygen radical production (reactive oxygen species; ROS) (Andrews and Griffiths, 2002; Knight, 2000; Moinard et al., 1999, 2002a; Mühling et al., 2002, 2005; Newsholme, 1996, 2001; Newsholme and Calder, 1997). However, new findings have shown that only at most a third of the intragranulocytically enriched glutamine is metabolized and thereby completely oxidized by the first enzyme that utilises glutamine, namely glutaminase, despite its high enzyme activity (Castell et al., 2004; Curthoys and Watford, 1995; Curi et al., 1997, 1999). By far the greatest proportion of intracellular glutamine appears to be metabolized to glutamate, aspartate (e.g. via the citric acid cycle) as well as alanine and thereby stored in the form of anabolic substrates and nitrogen carriers (Curi et al., 1999; Engel et al., 2003; Leighton et al., 1987; Metcoff, 1986; Newsholme and Calder, 1997; Newsholme, 2001). It is presumed that this compartmentalization of

energy-rich and intracellularly highly-active substrates principally serves the immunological tasks of neutrophils (Rose and Jalan, 2006; Furukawa et al., 1997a, b). Neutrophils must at any time be able to react to a pathogenic stimulus using their own specific defence mechanisms (phagocytosis, degranulation processes, secretion of reactive oxygen metabolites, etc.) (Lee et al., 2005). This requires a direct and rapid activation of intracellular substrates for energy generation, DNA, RNA and lipid synthesis, and also of course the production of immunologically active proteins, enzymes and cytokines (Metcoff et al., 1973; Metcoff, 1986; Furukawa et al., 2000a, b; Newsholme et al., 1987a). An especially interesting fact is that neutrophil migrating into inflammatory areas induce a local glutamine depletion in the extracellular matrix surrounding them due to a massive intracellular glutamine consumption. As a result of this there is a risk that enduring inflammatory processes may in fact induce pathophysiologically significant functional losses in all cell types participating in an inflammatory event (Noble, 2005; Wischmeyer, 2003). Based on these findings, some have even suggested that glutamine, which in itself is not essential, is converted to a ''conditional'' essential amino acid when the cellular requirement reflected in the uptake from the extracellular space exceeds the cells-own synthetic rate (Lacey and Wilmore, 1990). This might happen when there is increased metabolic demand or when catabolic conditions prevail (e.g. with operations, trauma, infectious and tumour disease). Leading on from this hypothesis, an ever more accepted opinion is that an additional, so-called ''immunonutritive'', supplementation of glutamine might exert a positive influence on the above-mentioned disease process and patient outcome (Alpers, 2006; Andrews and Griffiths, 2002; Boelens et al., 2001; Castell, 2003; Engel et al., 2003; Morlion et al., 1998; Scheid et al., 2004). Of special importance regarding the potential therapeutic use of glutamine in an ''immunonutritive'' therapy are the considerable chemical and galenic problems associated with singular glutamine application: i.e. temperature, pH and anion-concentration-dependent chemical instability, thermal instability upon sterilization, very poor water solubility (e.g. *Ala-Gln*: 568 g/l H_2O; *Gly-Gln*: 154 g/l H_2O; *Gln*: 36 g/l H_2O), and its rapid breakdown to toxic substances such as pyroglutamate or ammonia (Dechelotte et al., 2006; Fuentes-Orozo et al., 2004; Jacobi et al., 1998, 1999; Kircher et al., 2002; Mühling et al., 2002, 2005; Rogero, 2006; Scheid et al., 2004; Wischmeyer, 2003). These incalculable side effects of singular glutamine therapy are unacceptable both from a scientific and therapeutic viewpoint, and do indeed demand the development of chemically and galenically more stable glutamine-containing dipeptides (e.g. L-alanyl-L-glutamine). These are applied in aqueous form intravenously, and because of their rapid hydrolysis in human plasma (broken down both by plasma [≈50 nMol/ml/min] and membrane-bound hydrolases) they are designed to ensure a glutamine-analogous biological activity; the half-life with bolus application of an L-alanyl-L-glutamine dipeptide in healthy individuals is approx. 3–4 minutes (Albers et al., 1988, 1989; Fürst et al., 1997; Hubl et al., 1989). According to earlier results and our own preliminary findings, the plasma and intragranulocytic glutamine and alanine concentrations can increase by 2–3 fold upon application of L-alanyl-L-glutamine dipeptides. Because of the high alanine metabolism of many cells and tissues, application of dipeptides based on alanine-glutamine is more recommendable than for example dipeptide combinations with tyrosine or glycine (Coster et al., 2004; Fürst et al., 1997; Fürst, 2001).

However, signal transmission as well as major intracellular pathways through which glutamine-dipeptides modulate major cellular functions, neutrophil dynamic free amino and α-keto acid pools and important bactericidal functions remain to be defined (Bracco, 2005; Cynober, 2001; Dechelotte et al., 2006; Dhaliwal and Heyland, 2005; Kircher et al., 2002; Wischmeyer, 2003). Moreover, our current understanding based on the use of glutamine-dipeptides in ''immunonutrition'' raises some intriguing questions about potential therapeutic manipulation and whether important neutrophil antibacterial host defence mechanisms can be altered to the benefit of the individual.

The goals of this study were therefore:

1. To document the effects of *6-diazo-5-oxo-L-norleucine* [*DON*] on neutrophil free intracellular amino acid and α-keto acid concentrations as well as on the activities of released myeloperoxidase (MPO) and the formation of both superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2) (as markers of neutrophil function, and in order to show possible parallels with changes in neutrophil amino acid concentrations).
2. To explore the effects of *alanyl-glutamine* (regarding its role in neutrophil immunonutrition) and *alanyl-glutamine* combined with *N_ω-nitro-L-arginine-methylester-hydrochloride* [*L-NAME*], *S-nitroso-N-acetyl-penicillamine* [*SNAP*], *6-diazo-5-oxo-L-norleucine* [*DON*], *β-alanine* [*β-Ala*] and *α-difluoro-methyl-ornithine* [*DFMO*] on neutrophil amino acid and α-keto acid concentrations or important neutrophil immune functions [released myeloperoxidase (MPO), the formation

of superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2)], in order to establish whether an inhibitor of •NO-synthase [*L-NAME*], an •NO donor [*SNAP*], an analogue of glutamine and inhibitor of glutamine-requiring enzymes [*DON*], an analogue of taurine and taurine transport antagonist [*β-Ala*] as well as an inhibitor of ornithine-decarboxylase [*DFMO*] can influence any of the *alanyl-glutamine*-induced effects.

Materials and methods

The study was approved by the local ethics committee of the University Hospital Giessen and Marburg. Ten men (29 ± 6 years) with an average height of 179.0 cm (±9 cm) and weight of 73.1 kg (±11 kg) were selected: males 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 08:00 and 09:00 (after 10 hours of fasting) taking circadian variation into account.

6-Diazo-5-oxo-L-norleucine (DON)

The following concentrations were tested in addition to a control for each substance:

1. *DON* (100 μM)
   [DON: 6-diazo-5-oxo-L-norleucine; analogue of glutamine and inhibitor of glutamine-requiring enzymes; Sigma, Deisenhofen, Germany]
2. *Alanyl-glutamine combined with L-NAME, SNAP, DON, β-Ala or DFMO*

   2.1 *Alanyl-glutamine* (5 mM; Ala-Gln)
   2.2 *Ala-Gln* (5 mM) + *L-NAME* (1 mM)
   2.3 *Ala-Gln* (5 mM) + *SNAP* (100 μM)
   2.4 *Ala-Gln* (5 mM) + *DON* (100 μM)
   2.5 *Ala-Gln* (5 mM) + *β-Ala* (10 mM)
   2.6 *Ala-Gln* (5 mM) + *DFMO* (1 mM)

[*Ala-Gln*: L-alanyl-L-glutamine, Dipeptamin®, Fresenius, Bad Homburg, Germany; *L-NAME*: N_ω-nitro-L-arginine-methylester-hydrochloride; inhibitor of nitric oxide (•NO) synthase; Calbiochem, Bad Schwalbach, Germany; *SNAP*: S-nitroso-N-acetyl-penicillamine; exogenous nitric oxide donor (•NO-release: 5.6 μM/min); Sigma, Deisenhofen, Germany; *β-Ala*: β-alanine; analogue of taurine and taurine transport antagonist; Sigma, Deisenhofen, Germany; *DFMO*: α-difluoro-methyl-ornithine; irreversible inhibitor of ornithine decarboxylase; Sigma, Deisenhofen, Germany]

Neutrophils were incubated with DON for 10, 60 or 120 min to examine if there was a critical duration of exposure necessary to produce any significant effects or for 120 min with Ala-Gln or Ala-Gln combined with L-NAME, SNAP, DON, β-Ala or DFMO. The final concentrations selected corresponded, consistently with previous work, to those used in our own preliminary studies (not published). Solutions were prepared and diluted in Hank's balanced salt solution (HBSS; Sigma, Deisenhofen, Germany), and the pH in the test solution was confirmed as 7.4. 1 ml of whole blood was incubated with 25 μl of test solution (final ornithine concentrations were as described above) at 37 °C using a shaking 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 sulphonyl 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 Mühling et al., 1999, 2003).

Highly selective separation of neutrophils from whole blood

Precise details of our neutrophil-separation technique have been described previously (Mühling et al., 1999, 2003). This method is a further development of the methods described by Eggleton et al. (1989) and Krumholz et al. (1993, 1995a, b) that allows a very rapid and selective enrichment of neutrophils while preserving high cellular viability and integrity from very small quantities of whole blood.

Chromatographic amino and α-keto acid analysis

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

Superoxide anion (O_2^-), hydrogen peroxide production (H_2O_2) and the activity of released myeloperoxidase (MPO)

This was determined photometrically using modifications of known methods validated in our institute for this purpose (for further details see Krumholz et al., 1993, 1995a, b; Mühling et al., 2002 as well as Rick, 1977).

Statistical analysis

Statistical analysis and interpretation of the results were performed in close co-operation with colleagues from the Department of Medical Statistics, Justus Liebig University Giessen. All tests were performed in duplicate. Thus our neutrophil amino acid results represent 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 as significant. The data are given as arithmetic means ± standard deviations (mean ± SD).

Results

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

Effects of DON on neutrophil amino and α-keto acid pools or immune functions

DON induced significant incubation time-dependent (≥60 min) reductions in intracellular pools of the following amino acids: asparagines (asn), glutamine (gln), aspartate (asp), glutamate (glu), ornithine (orn), arginine (arg), citrulline (cit), hypotaurine (h-tau), serine (ser), glycine (gly), threonine (thr) and alanine (ala) (≈ −34–55%)

Table 1. Effects of *6-Diazo-5-oxo-L-norleucine* [DON (100 µM)] incubated with whole blood for 10, 60 and 120 min on free intracellular *acidic amino acid*, *acidic amide* and *basic amino acid* concentrations in neutrophils [PMN] (10^{-16} mol per PMN-cell; mean ± SD; $n = 10$)

Acidic amino acids, acidic amides, basic amino acids					
	control	DON		control	DON
10 min					
asn	0.37 ± 0.06	0.36 ± 0.06	Orn	0.45 ± 0.07	0.48 ± 0.08
gln	2.80 ± 0.58	2.48 ± 0.53	Lys	0.61 ± 0.11	0.60 ± 0.11
asp	2.83 ± 0.45	2.89 ± 0.50	Arg	0.32 ± 0.06	0.30 ± 0.05
glu	5.90 ± 0.86	5.28 ± 0.83	Cit	0.12 ± 0.02	0.12 ± 0.03
60 min					
asn	0.38 ± 0.06	0.30 ± 0.05*	Orn	0.46 ± 0.08	0.38 ± 0.07*,#
gln	2.88 ± 0.60	1.93 ± 0.45*,#	Lys	0.67 ± 0.12	0.60 ± 0.11
asp	2.94 ± 0.48	1.91 ± 0.46*,#	Arg	0.33 ± 0.07	0.26 ± 0.06*,#
glu	6.01 ± 0.88	3.78 ± 0.81*,#	Cit	0.12 ± 0.03	0.09 ± 0.02*
120 min					
asn	0.36 ± 0.05	0.20 ± 0.04*,#	Orn	0.47 ± 0.09	0.29 ± 0.06*,#
gln	2.70 ± 0.56	1.29 ± 0.27*,#	Lys	0.64 ± 0.13	0.57 ± 0.12
asp	2.72 ± 0.43	1.38 ± 0.29*,#	Arg	0.32 ± 0.06	0.17 ± 0.04*,#
glu	5.79 ± 0.85	2.63 ± 0.54*,#	Cit	0.11 ± 0.02	0.07 ± 0.02*,#

* $p \leq 0.05$ versus control values; # $p \leq 0.05$ versus 10 min

Table 2. Effects of *6-Diazo-5-oxo-L-norleucine* [DON (100 µM)] incubated with whole blood for 10, 60 and 120 min on free intracellular *branch-chain amino acid* (*BCAA*), *taurine*, *hypotaurine* and *neutral amino acid* concentrations in neutrophils [PMN] (10^{-16} moles per neutrophil-cell; mean ± SD; $n = 10$)

Branch-chain amino acids (BCAA), taurine, hypotaurine neutral amino acids					
	control	DON		control	DON
10 min					
ile	0.40 ± 0.07	0.41 ± 0.07	Ser	2.29 ± 0.45	2.29 ± 0.47
leu	0.69 ± 0.16	0.72 ± 0.17	Gly	2.39 ± 0.50	2.36 ± 0.49
val	0.58 ± 0.12	0.55 ± 0.13	Thr	0.73 ± 0.12	0.70 ± 0.12
met	0.15 ± 0.03	0.17 ± 0.03	Ala	1.80 ± 0.36	1.73 ± 0.35
tau	41.0 ± 7.8	38.9 ± 6.7	Aba	0.28 ± 0.05	0.28 ± 0.05
h-tau	1.54 ± 0.34	1.76 ± 0.39			
60 min					
ile	0.39 ± 0.07	0.50 ± 0.09*	Ser	2.19 ± 0.43	1.72 ± 0.38*,#
leu	0.70 ± 0.15	0.89 ± 0.17*	Gly	2.30 ± 0.49	1.70 ± 0.37*,#
val	0.55 ± 0.11	0.67 ± 0.12*	Thr	0.69 ± 0.11	0.59 ± 0.10
met	0.16 ± 0.03	0.15 ± 0.03	Ala	1.79 ± 0.32	1.36 ± 0.28*,#
tau	40.3 ± 7.4	34.1 ± 6.2	Aba	0.26 ± 0.04	0.24 ± 0.04
h-tau	1.30 ± 0.32	0.90 ± 0.26*			
120 min					
ile	0.38 ± 0.07	0.66 ± 0.13*,#	Ser	2.08 ± 0.41	1.13 ± 0.25*,#
leu	0.67 ± 0.13	1.21 ± 0.26*,#	Gly	2.32 ± 0.47	1.14 ± 0.27*,#
val	0.59 ± 0.11	1.13 ± 0.26*,#	Thr	0.66 ± 0.10	0.37 ± 0.08*,#
met	0.17 ± 0.04	0.17 ± 0.03	Ala	1.98 ± 0.32	1.04 ± 0.23*,#
tau	39.8 ± 7.2	32.1 ± 6.1*,#	Aba	0.26 ± 0.05	0.17 ± 0.04*,#
h-tau	1.39 ± 0.21	0.67 ± 0.17*,#			

* $p \leq 0.05$ versus control values; # $p \leq 0.05$ versus 10 min

(Tables 1 and 2). The contents of the following amino acids on the other hand were raised significantly: isoleucine (ile), leucine (leu) and valine (val) (≈ +73–91%) (Tables 1 and 2). Furthermore DON induced additional modifications in the intragranulocytic α-aminobutyrate (aba) level (−34%) and particularly the taurine (tau) level

Table 3. Effects of *6-Diazo-5-oxo-L-norleucine* [DON (100 μM)] incubated with whole blood for 10 and 120 min on free intracellular *α-ketoglutarate* [KG], *pyruvate* [PYR], *α-ketobutyrate* [KB], *α-ketoisovalerianate* [KIV], *α-ketoisocapronate* [KIC], *p-hydroxy-phenylpyruvate* [PPY] and *α-keto-β-methylvalerianate* [KMV] concentrations in neutrophils [PMN] (10^{-17} mol per PMN-cell; mean ± SD; $n = 10$)

α-Keto acids	10 min		120 min	
	control	DON	control	DON
KG	1.37 ± 0.27	1.14 ± 0.25	1.19 ± 0.24	$0.56 \pm 0.14^{*,\#}$
PYR	5.96 ± 1.19	5.24 ± 1.11	5.76 ± 1.03	$2.92 \pm 0.76^{*,\#}$
KB	4.20 ± 0.89	3.65 ± 0.77	4.39 ± 0.86	$2.46 \pm 0.61^{*,\#}$
KIV	2.20 ± 0.49	1.89 ± 0.42	2.31 ± 0.38	$1.43 \pm 0.34^{*,\#}$
KIC	0.38 ± 0.08	0.32 ± 0.07	0.36 ± 0.07	$0.20 \pm 0.05^{*,\#}$
PhePYR	0.31 ± 0.07	0.26 ± 0.06	0.32 ± 0.07	$0.19 \pm 0.04^{*,\#}$
KMV	0.80 ± 0.16	0.65 ± 0.13	0.75 ± 0.13	$0.43 \pm 0.11^{*,\#}$

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

(−19%) (Tables 2 and 3). In addition, DON induced significant reductions in all α-keto acids examined in this paper (≈ −38–55%) as well as a very clear reduction in all the studied granulocytic immunofunctional markers (Tables 3 and 4).

Effects of Ala-Gln and Ala-Gln combined with L-NAME, SNAP, DON, β-Ala or DFMO on the free amino acid pools in neutrophils

The combined incubation of Ala-Gln with the above described ''metabolism modulators'' led, unlike singular application, to significant and decisive findings (Figs. 1–3). Combined administration of Ala-Gln and L-NAME induced no effect that exceeded singular application of Ala-Gln. Although SNAP + Ala-Gln in neutrohils, compared with Ala-Gln singular application, induced significantly lower intracellular asparagine, glutamine, aspartate, glutamate and alanine levels, the results were nevertheless still significantly above the control findings (e.g. alanine: Ala-Gln: +106%; Ala-Gln + SNAP: +43%) (Figs. 1, 2). For serine, glycine and threonine as well, SNAP induced a concentration decrease compared to Ala-Gln application alone (Fig. 2). Nevertheless this reduction remained more or less within the range of control values (e.g. serine: Ala-Gln: +44%; Ala-Gln + SNAP versus Ala-Gln: −25%). DON produced a very clear reduction in important primary amino acids (Figs. 1–3). Although the contents of cellular asparagine, glutamine, ornithine, arginine, citrulline, serine, glycine and alanine were decreased significantly compared to Ala-Gln singular application, they were still within control ranges. Only regarding the granulocytic aspartate (Ala-Gln: +63%; Ala-Gln + DON: −18%), glutamate (Ala-Gln: +68%; Ala-Gln + DON: −16%), hypotaurine (Ala-Gln + DON versus Ala-Gln: −41%), threonine (Ala-Gln: +15%; Ala-Gln + DON: −26%) and branch-chain amino acid levels (for example isoleucine: Ala-Gln + DON versus Ala-Gln: +23%) did significant intracellular concentration decreases or changes occur. β-Ala in combination with Ala-Gln induced significant reductions in granulocytic hypotaurine (Ala-Gln + β-Ala versus Ala-Gln: −24%) and taurine (Ala-Gln + β-Ala versus Ala-Gln: −21%) (Fig. 3). In addition, β-Ala also exerted additive effects on the content of neutral amino acids (for example glycine: Ala-Gln: +45%; Ala-Gln + β-Ala: +88% and alanine: Ala-Gln: +106%; Ala-Gln + β-alanine: +154%) (Fig. 2). DFMO added to Ala-Gln (similar to L-NAME) induced an increase in intragranulocytic ornithine (Ala-Gln + DFMO versus Ala-Gln: +61%), arginine (Ala-Gln + DFMO versus Ala-Gln: +32%) and citrulline (Ala-Gln + DFMO versus Ala-Gln: +21%) that exceeded the effects of singular Ala-Gln application (Fig. 1).

Effects of Ala-Gln and Ala-Gln combined with L-NAME, SNAP, DON, β-Ala or DFMO on the free α-keto acid pools in neutrophils

Additional application of SNAP, DON, β-alanine or DFMO to Ala-Gln also induced important Ala-Gln-inde-

Table 4. Effects of *6-Diazo-5-oxo-L-norleucine* [DON (100 μM)] incubated with whole blood for 10, 60 and 120 min on neutrophil (PMN) superoxide anion generation [fmol O_2^-/(PMN × min)], hydrogen peroxide production [fmol H_2O_2/(PMN × min)] and on PMN myeloperoxidase activity ([units/l supernatant]; mean ± SD; $n = 10$)

O_2^-, H_2O_2, MPO	10 min		60 min		120 min	
	control	DON	control	DON	control	DON
O_2^-	3.536 ± 0.551	3.047 ± 0.509	3.461 ± 0.538	$1.994 \pm 0.466^{*,\#}$	3.317 ± 0.528	$1.465 \pm 0.310^{*,\#}$
H_2O_2	1.324 ± 0.225	1.135 ± 0.206	1.239 ± 0.219	$0.678 \pm 0.151^{*,\#}$	1.154 ± 0.195	$0.472 \pm 0.111^{*,\#}$
MPO	0.685 ± 0.175	0.542 ± 0.153	0.646 ± 0.161	$0.371 \pm 0.108^{*,\#}$	0.605 ± 0.153	$0.219 \pm 0.064^{*,\#}$

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

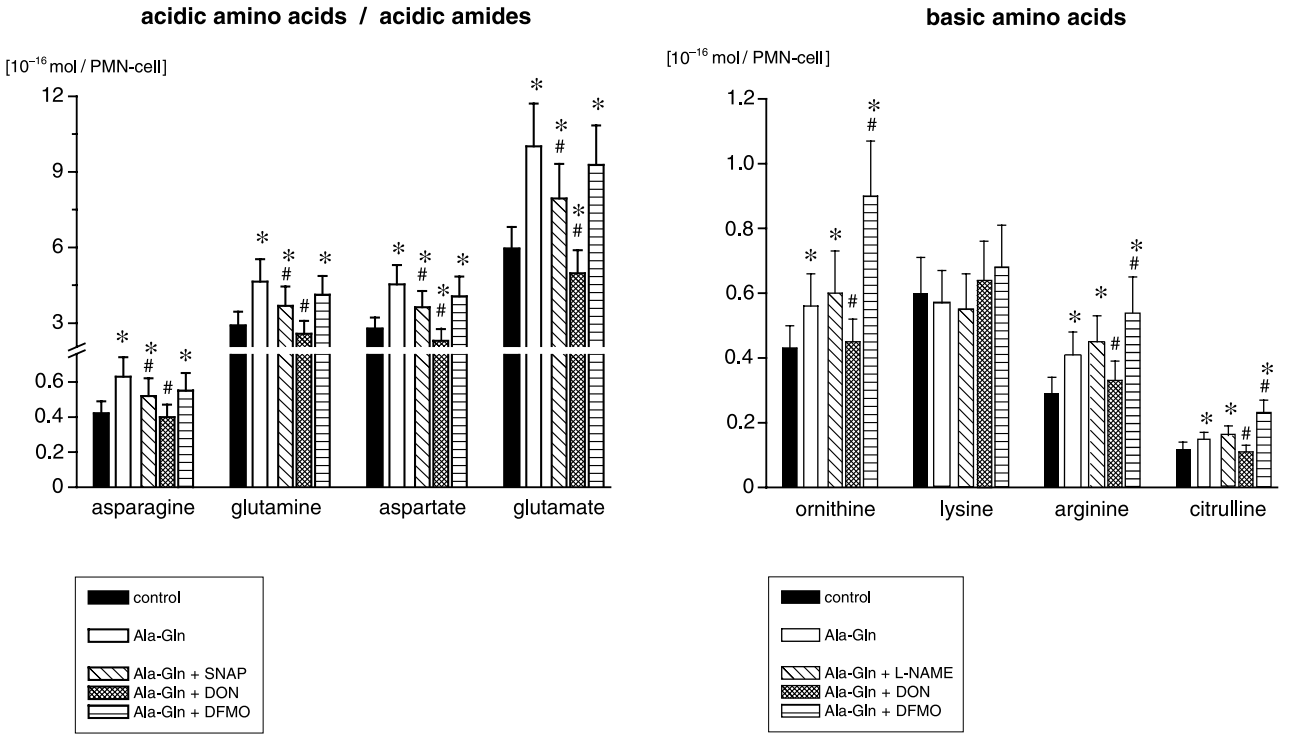

Fig. 1. Effects of *alanyl-glutamine* (Ala-Gln; 5 mM) incubated with whole blood for 120 min combined either with N_{ω}-*nitro-L-arginine-methylester-hydrochloride* [L-NAME (1 mM)], *S-nitroso-N-acetyl-penicillamine* [SNAP (100 µM)], *6-Diazo-5-oxo-L-norleucine* [DON (100 µM)] or α-*difluoro-methyl-ornithine* [DFMO, (1 mM)] on free intracellular *acidic amino acid*, *acidic amide* and *basic amino acid* concentrations in neutrophils [PMN] (10^{-16} moles per PMN-cell; mean ± SD; $n = 10$). $^{*}p \leq 0.05$ versus control values; $^{\#}p \leq 0.05$ versus alanyl-glutamine

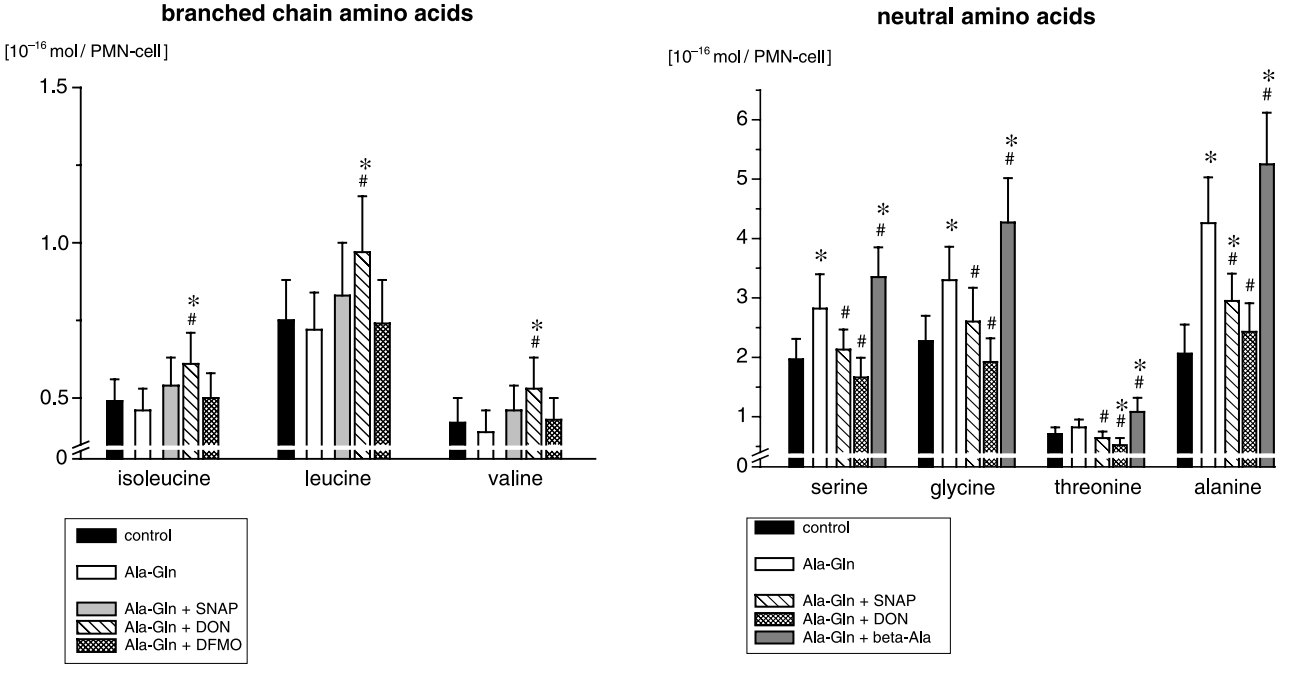

Fig. 2. Effects of *alanyl-glutamine* (A–G; 5 mM) incubated with whole blood for 120 min combined either with *S-nitroso-N-acetyl-penicillamine* [SNAP (100 µM)], *6-Diazo-5-oxo-L-norleucine* [DON (100 µM)], β-*alanine* [β-Ala (10 mM)] or α-*difluoro-methyl-ornithine* [DFMO, (1 mM)] on free intracellular *branch-chain amino acid* (*BCAA*) and *neutral amino acid* concentrations in neutrophils [PMN] (10^{-16} moles per PMN-cell; mean ± SD; $n = 10$). $^{*}p \leq 0.05$ versus control values; $^{\#}p \leq 0.05$ versus alanyl-glutamine

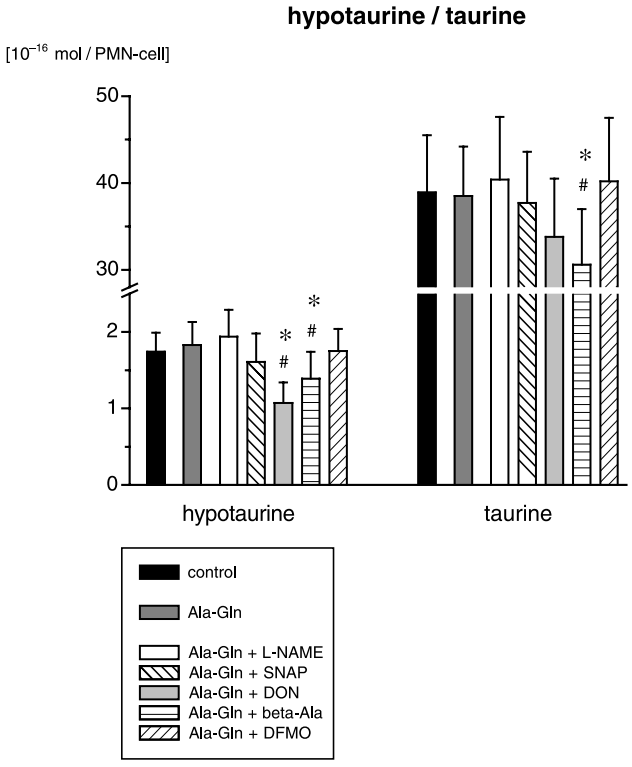

Fig. 3. Effects of *alanyl-glutamine* (A–G; 5 mM) incubated with whole blood for 120 min combined either with N_ω*-nitro-L-arginine-methylester-hydrochloride* [L-NAME (1 mM)], *S-nitroso-N-acetyl-penicillamine* [SNAP (100 μM)], *6-Diazo-5-oxo-L-norleucine* [DON (100 μM)], *β-alanine* [β-Ala (10 mM)] or *α-difluoro-methyl-ornithine* [DFMO, (1 mM)] on free intracellular *hypotaurine* and *taurine* concentrations in neutrophils [PMN] (10^{-16} mol per PMN-cell; mean ± SD; $n = 10$). $^{*}p \leq 0.05$ versus control values; $^{\#}p \leq 0.05$ versus alanyl-glutamine

pendent effects (Table 5). While L-NAME induced no effect exceeding that of singular Ala-Gln, significant reductions in α-ketoglutarate (Ala-Gln: +182%; Ala-Gln + SNAP: +81%) and pyruvate (Ala-Gln: +133%; Ala-Gln + SNAP: +63%) that were both clearly raised by singular Ala-Gln occurred after SNAP application, although these values remained significantly above the respective control values. Upon DON application, however, there were significant decreases in all measured α-keto acids that were below the control findings (for example α-ketoglutarate: Ala-Gln: +182%; Ala-Gln + DON: −14% and DON −53%; pyruvate: Ala-Gln: +133%; Ala-Gln + DON: −21% and DON: −49%; and α-ketoisovalerianate: Ala-Gln + DON versus Ala-Gln: −34%, and DON: −39%). Only with the cellular α-ketobutyrate content did β-ala induce additive effects that exceeded singular alanyl-glutamine application (Ala-Gln + β-Ala versus Ala-Gln: +58%).

Effects of Ala-Gln and Ala-Gln combined with L-NAME, SNAP, DON, β-Ala or DFMO on neutrophil oxidative response and myeloperoxidase activity

Alanyl-glutamine led to an increased formation of superoxide anions and hydrogen peroxide and combined with L-NAME additional significant increases were observed (e.g. O_2^-: Ala-Gln + L-NAME versus Ala-Gln: +44%) (Fig. 4). The activity of myeloperoxidase released by neutrophils on the other hand was not influenced by L-NAME application. In combination with alanyl-glutamine, SNAP and DON (Fig. 4) induced decreases in superoxide anion and hydrogen peroxide generation that were raised by alanyl-glutamine on its own (for example H_2O_2: Ala-Gln: +131%; Ala-Gln + SNAP: +14%; Ala-Gln + DON: −19%). DON alone, however, led to a reduction below control values. The "nutritively"-induced increase in myeloperoxidase activity was also significantly inhibited

Table 5. Effects of *alanyl-glutamine* (A–G; 5 mM) incubated with whole blood for 120 min combined either with N_ω*-nitro-L-arginine-methylester-hydrochloride* [L-NAME (1 mM)], *S-nitroso-N-acetyl-penicillamine* [SNAP (100 μM)], *6-Diazo-5-oxo-L-norleucine* [DON (100 μM)], *β-alanine* [β-Ala (10 mM)] or *α-difluoro-methyl-ornithine* [DFMO, (1 mM)] on free intracellular *α-ketoglutarate* [KG], *pyruvate* [PYR], *α-ketobutyrate* [KB], *α-ketoisovalerianate* [KIV], *α-ketoisocapronate* [KIC], *p-hydroxy-phenylpyruvate* [PPY] and *α-keto-β-methylvalerianate* [KMV] concentrations in neutrophils [PMN] (10^{-17} mol per PMN-cell; mean ± SD; $n = 10$)

α-Keto acids	control	A–G	A–G + L-NAME	A–G + SNAP	A–G + DON	A–G + β-Ala	A–G + DFMO
KG	1.01 ± 0.16	2.85 ± 0.51*	2.93 ± 0.59*	1.82 ± 0.45*,#	0.87 ± 0.18*,#	2.66 ± 0.60*	2.47 ± 0.62*
PYR	5.38 ± 1.05	12.54 ± 2.41*	11.70 ± 2.84*	8.79 ± 1.89*,#	4.27 ± 1.08*,#	13.72 ± 2.97*	9.95 ± 2.23*,#
KB	3.66 ± 0.80	4.23 ± 1.01	3.93 ± 1.04	3.37 ± 0.66	2.69 ± 0.63*,#	6.68 ± 1.84*,#	3.96 ± 0.95
KIV	1.83 ± 0.35	1.90 ± 0.37	1.71 ± 0.42	1.64 ± 0.39	1.25 ± 0.31*,#	2.11 ± 0.52	2.24 ± 0.49
KIC	0.33 ± 0.06	0.35 ± 0.08	0.30 ± 0.06	0.29 ± 0.06	0.25 ± 0.05*,#	0.39 ± 0.11	0.37 ± 0.09
PPY	0.28 ± 0.05	0.33 ± 0.07	0.30 ± 0.06	0.27 ± 0.06	0.23 ± 0.04*,#	0.35 ± 0.08	0.30 ± 0.07
KMV	0.65 ± 0.10	0.61 ± 0.16	0.64 ± 0.12	0.59 ± 0.13	0.50 ± 0.13*,#	0.84 ± 0.23	0.71 ± 0.17

$^{*}p \leq 0.05$ versus control values; $^{\#}p \leq 0.05$ versus alanyl-glutamine

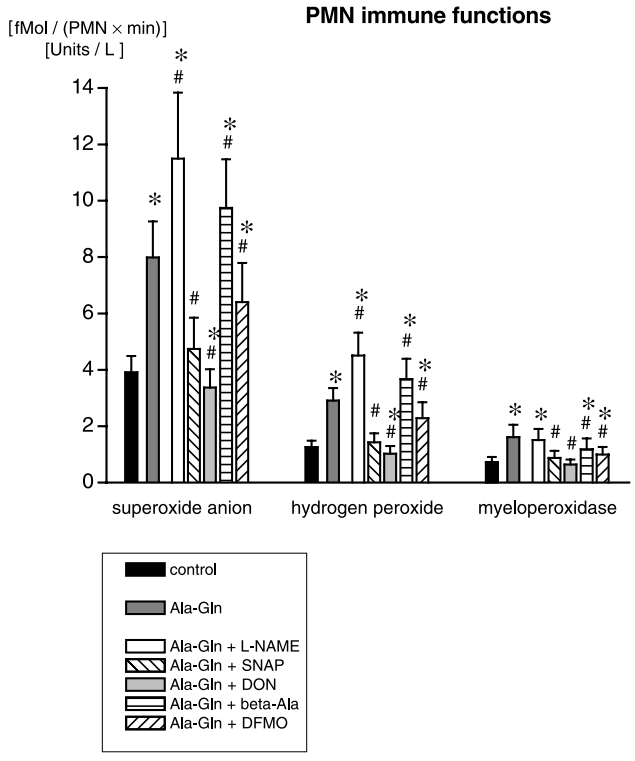

Fig. 4. Effects of *alanyl-glutamine* (A–G; 5 mM) incubated with whole blood for 120 min combined either with N_{ω}*-nitro-L-arginine-methylester-hydrochloride* [L-NAME (1 mM)], *S-nitroso-N-acetyl-penicillamine* [SNAP (100 μM)], *6-Diazo-5-oxo-L-norleucine* [DON (100 μM)], *β-alanine* [β-Ala (10 mM)] or *α-difluoro-methyl-ornithine* [DFMO, (1 mM)] on neutrophil (PMN) superoxide anion generation [fmol O_2^-/(PMN × min)], hydrogen peroxide production [fmol H_2O_2/(PMN × min)] and on PMN myeloperoxidase activity ([units/l supernatant]; mean ± SD; $n = 10$). $^{*}p \leq 0.05$ versus control values; $^{\#}p \leq 0.05$ versus alanyl-glutamine

by SNAP and DON (Ala-Gln + SNAP versus Ala-Gln: −46%; Ala-Gln + DON versus Ala-Gln: −61%). The results for alanyl-glutamine combined with SNAP or DON revealed no differences from control findings. β-alanine in combination with alanyl-glutamine led to a significant increase in granulocytic superoxide anion production (Fig. 4). With alanyl-glutamine there were additive effects exceeding effects of singular incubation (Ala-Gln + β-Ala versus Ala-Gln: +22%). Similar effects were seen with hydrogen peroxide formation, where there were also further significant increases in the measured values (Ala-Gln + β-Ala versus Ala-Gln: +26%). Regarding the myeloperoxidase activity the combination of β-alanine and alanyl-glutamine also led to significant effects. Here the results observed were clearly above the control values (Ala-Gln + β-Ala versus control: +64%). DFMO in a qualitatively similar manner to SNAP produced an inhibition of all the tested immune functions (Fig. 4). The findings obtained here for alanyl-glutamine (e.g. O_2^-: Ala-Gln + DFMO versus Ala-Gln: −20% or versus control: +64%) were completely above the respective control values.

Discussion

Intracellular glutamine levels amounted on average to 3×10^{-16} moles per neutrophil (PMN) cell (≈ 700 μmol/l l PMN cell volume [NCV]; plasma: 536 ± 81 μmol/l). The intracellular glutamate content at almost 6×10^{-16} mol per PMN cell (≈ 1400 μmol/l NCV; plasma: 31 ± 14 μmol/l) was approximately twice as high. The intragranulocytic α-keto acids, α-ketoglutarate and pyruvate associated with glutamine and glutamate metabolism could also be exactly quantified. Their concentrations at 1.0×10^{-17} mol per PMN cell (≈ 22 μmol/l NCV; plasma: 7 ± 2 μmol/l) and 5.5×10^{-17} mol per PMN cell (≈ 130 μmol/l NCV; plasma: 110 ± 35 μmol/l), respectively, were on average about an order of magnitude lower. Further interesting findings were also obtained for asparagine, aspartate and alanine. Their intracellular contents were on average 0.45×10^{-16} mol (≈ 100 μmol/l NCV; plasma: 58 ± 11 μmol/l), 2.75×10^{-16} mol (≈ 600 μmol/l NCV; plasma: 12 ± 3 μmol/l) and 2×10^{-16} mol (≈ 450 μmol/l NCV; plasma: 357 ± 83 μmol/l) per PMN cell, respectively. However, when comparing the intra versus the extracellular concentration gradient [i:e], the results painted a very different picture: glutamine (i:e: 1.3), alanine (i:e: 1.3) and pyruvate (i:e: 1.2) were almost plasmaisotonic and as such featured high intracellular concentrations, while glutamate (i:e: 45.2), aspartate (i:e: 50), α-ketoglutarate (i:e: 3.1) and asparagine (i:e: 1.7) with low plasma concentrations apparently accumulated within the neutrophils. The composition of these free amino acid and α-keto acid pools does not appear to be arbitrary in any way (Mühling et al., 2002, 2005; Newsholme and Calder, 1997; Newsholme, 1996). The maintenance of high intragranulocytic glutamate, aspartate, α-ketoglutarate and also glutamine levels may represent a metabolic correlate for the underlying (patho) physiological functions and requirements of neutrophils (Curi et al., 1988, 1989a, b, 1999; Nussler, 1999). As lymphocyte studies have shown, the large intragranulocytic glutamate, aspartate and asparagine levels presumably arise not mainly from a purely transmembraneous amino acid influx (e.g. through the transport system X^-_{AG}), but much rather from the very high intracellular synthesis of glutamine (Ardawi and Newsholme, 1986; Ardawi, 1988; Frei et al., 1975, 1980; Pithon-Curi et al., 2004). Glutamine in particular seems to fulfil the substantial metabolic

demands for a potent intracellular metabolic precursor in these cells (Borghetti et al., 1979; Brand et al., 1989; Curi et al., 1986, 1988, 1989a, b, 1997, 1999).

From these findings it is absolutely necessary to know whether significant metabolic and immunofunctional changes can also be induced in neutrophils by directly or indirectly influencing intragranulocytic glutamine levels (Lagranha et al., 2005). Studies in the last years have indeed shown that artificial reductions in extracellular glutamine concentration are associated with significant impairments of granulocyte function, such as phagocytosis, bactericidal activity or oxygen radical production (Andrews and Griffiths, 2002; Furukawa et al., 1997a, b, 2002; Moinard et al., 1999, 2002a, b; Mühling et al., 2002, 2005; Newsholme, 2001). According to the results presented here the glutamine dependence of cellular function in neutrophils seems to be comparable with that of other leukocytic cells (e.g. T- and B-lymphocytes, lymphokine activated killer cells, macrophages and monocytes) (Kircher et al., 2002; Pithon-Curi et al., 2004). As in neutrophils, an experimentally induced glutamine reduction in these cells leads to a significant reduction in immunological function and impairments in metabolic activities (e.g. reductions in cellular proliferation rate, phagocytosis, secretion and degranulation of immunologically active enzymes and cytokines). A modulation of immunofunctional and metabolic-physiological activities, however, can not be induced by merely reducing leukocyte glutamine uptake (Novogrodsky et al., 1977).

As current studies in macrophages and monocytes have shown, intracellular inhibition of directly glutamine-dependent metabolic processes and enzymes, e.g. induced by application of specific glutamine analogues [e.g. 6-diazo-5-oxo-L-norleucine (DON)], leads in the same way to a significant reduction of important cellular immune reactions (e.g. production of TNF-α and interleukin-8) and metabolic functions (Murphy and Newsholme, 1997, 1998, 1999; Nascimento et al., 2005). Our own results here have confirmed such effects for the first time also for the free intragranulocytic amino acid and α-keto acid pools as well as for important granulocytic immunofunctional parameters. DON not only induced, presumably via additional competitive inhibition of the glutamine transport system (i.e. ASC, L, N, etc.), a clear reduction in intragranulocytic glutamine content, but also led to an almost ubiquitous influencing of all examined amino acid as well as α-keto acid pools (Pithon-Curi et al., 2002a, b; Piva et al., 1992). Here one has to view the modifications of glutamate, aspartate and basic amino acids as well as the reduction in intracellular taurine levels not balanced by a compensatory rise in neutral amino acids as particularly grave. Also, the further cellular metabolism to their metabolically-physiologically active α-keto acids seems to be severely influenced by an inhibition of glutamine metabolism. Because of these findings it is not surprising that significant changes in the immunofunctional parameters studied here could be observed in a manner consistent with findings obtained in macrophages, lymphocytes or monocytes (Pithon-Curi et al., 2004; Piva et al., 1992). Indeed our results lead us to postulate that glutamine alongside glucose is an important metabolic precursor also for neutrophils. The enzymatic constellation required for a direct or indirect glutamine metabolisation and glutamine synthesis is certainly present in neutrophils as it is in other cell types (Curthoys and Watford, 1995; Kobayashi et al., 2005; Witko-Sarsat et al., 2000). The primary intracellular metabolisation of glutamine to glutamate is of special metabolic significance here. The enzyme complex required for that, a phosphate-dependent glutaminase, is especially active in neutrophils ($\approx 56\,\mathrm{nmol/min/mg}$ protein) (Ardawi et al., 1991; Castell et al., 2004; Curi et al., 1997). The enzymatic activity in neutrophils is comparable here with that present in kidney cells and far exceeds the enzyme kinetics found in lymphocytes or macrophages (Curthoys and Watford, 1995; Newsholme, 2001). The activity of this enzyme can be significantly influenced by extra- and intracellular stimuli, the highest conversion rates being found after stimulation by proinflammatory mediators and catecholamines (Curi et al., 1999). As a result, the glutaminase reaction ensures the demand-driven conversion of the intragranulocytic carbon and nitrogen store glutamine to its intracellularly important metabolic product glutamate during neutrophil activation. The glutamate arising from the glutaminase occupies a key position as a so-called ''intracellular turntable for amino nitrogen metabolisation'', since it can be very rapidly converted into other essential cell substrates (Haberle et al., 2005). Amongst the first reactions to be mentioned here are reversible transaminations involving the release of NH_3 (Korpela et al., 1981). Alanine aminotransferase for example transfers the amino group of glutamate to pyruvate resulting in the production of alanine and α-ketoglutarate (Kobayashi et al., 2005; Witko-Sarsat et al., 2000; Venizelos and Hagenfeldt, 1985). Aspartate aminotransferase on the other hand produces aspartate and α-ketoglutarate where the amino group is bound to oxalacetate. An oxidative deamination of glutamate can also lead to α-ketoglutarate formation in neutrophils according to Washizu et al. (1998). In the form of α-ketoglutarate, the carbon backbone arising from glutamine oxidation can

be fed directly into the tricarboxylic acid cycle and in so doing becomes indirectly available as a substrate for the enzymes of the urea cycle (e.g. in the form of aspartate via oxalacetate) (Curi et al., 1997, 1999; Huynh, 2005; Newsholme et al., 1987a, b). In this way the regulation of glutamine metabolisation can also modulate the formation of arginine and ornithine and with that the synthesis of •NO required for neutrophil activation (Coleman, 2001; Moinard et al., 2002a, b; O'Dowd and Newsholme, 1997). Other reaction pathway involving intracellular glutamine transformation to arginine and ornithine might also be catalyzed by the enzymes pyrroline-5-carboxylate synthase, ornithine aminotransferase, ornithine carbamyltransferase and carbamylphosphate synthase (Igarashi and Kashiwagi, 2000; Murphy and Newsholme, 1998). The conversion of malate to oxalacetate by the $NADP^+$-dependent malate dehydrogenase is especially important for the metabolisation of α-ketoglutarate by the enzyme chain of the TCA-cycle (Newsholme, 1996, 2001; Newsholme and Calder, 1997). Glutamine can then ultimately be converted to pyruvate, from which the final products of glutamine catabolism, namely lactate, acetyl-CoA and CO_2, can then be formed (Venizelos and Hagenfeldt, 1985; Curi et al., 1988). Pyruvate is also regarded as a precursor for the de novo synthesis of glucose from α-ketoglutarate (Junghans, 2005). The key enzyme here is fructose-1,6-bisphosphatase which is also found in leukocytes (Schrijver and Hommes, 1975). According to Newsholme (1996), Newsholme and Calder (1997), Curi et al. (1997, 1999) as well as Castell et al. (2004), Castell (2003), only a small proportion of the carbon arising from glutamine is oxidized (approx. 10–30%) completely to carbon dioxide, while the majority is converted to glutamate, aspartate and alanine before it is metabolized further. Alanine in particular (like glutamine) serves here as a high-energy store of carbon and nitrogen for the cell, since it can be converted rapidly into glutamate and pyruvate under the influence of alanine aminotransferase (Curi et al., 1986, 1988). In cells with a high metabolic activity per se (e.g. kidney cells) or also in those that must abruptly increase their substrate turnover in response to a stimulus (e.g. neutrophils), this might explain why in the presence of anabolic metabolic situations or phases of raised substrate supply there is not only a raised glutamine uptake, but also a significant amount of de novo intracellular glutamine synthesis (Haberle et al., 2005; Rose and Jalan, 2006). The de novo synthesis of glutamine as a metabolic precursor is mainly carried through the enzymatic transformation (glutamine synthase) of glutamate. Glutamine accumulated or synthesized in such a way is not just an important cellular energy supplier or precursor especially for amino acid metabolites. It can also be an important nitrogen or carbon donor for the synthesis of other key intracellular substances. Amongst other things glutamine acts as a metabolic precursor in different cells and organs for purine nucleotides, or in the form of aspartate for pyrimidine nucleotides (Curi et al., 1999). Because of the high intragranulocytic glutamine as well as alanine, glutamate and aspartate levels, the cell can rapidly resort to (particularly with raised demand under pathological conditions) the metabolic precursors required for the synthesis of RNA and DNA as well as the formation of immune-functional enzymes, proteins and amino sugars (Curi et al., 1999; Metcoff et al., 1973; Metcoff, 1986). Further possible precursors for intragranulocytic serine and glycine synthesis include glutamine in the form of glutamate or pyruvate. Serine and glycine can be very easily interconverted (serine- and glycine-hydroxymethyltransferase). Conversely, e.g. during neutrophil activation, both amino acids can be fed in the form of pyruvate into the TCA cycle (Newsholme et al., 1987b; Venizelos and Hagenfeldt, 1985). Glycine in neutrophils fulfills other immune-functional tasks in the same way as glutamine. Especially worthy of mention here is the condensation to the tripeptide glutathione (γ-Glu-Cys-Gly) (Sahoo et al., 1998). This reaction pathway is of high importance especially for leukocytic cells such as neutrophils, but an essential task of a intracellularly reduced glutathione is to protect the cell against attack by reactive oxygen species such as the highly membrane permeable H_2O_2 (Carr and Winterbourn, 1997). Disorders in intragranulocytic glutathione metabolism, as in certain forms of inborn or acquired reductions in NADPH-dependent glutathione-reductase activity, are not of an insignificant pathophysiological or clinical importance. The metabolisation of glutamine by the TCA cycle does not just supply intracellular carbon and nitrogen precursors for the above mentioned metabolic pathways, but also NADPH (Board et al., 1990; Frei et al., 1975; Junghans et al., 2006; Newsholme et al., 1987a, b). NADPH arises from the reactions of isocitrate-, α-ketoglutarate and malate dehydrogenase as well as from the transformation of pyruvate by pyruvate dehydrogenase. Immunofunctionally, NADPH has an essential role for neutrophils, because of its further metabolisation by membranous NADPH oxidase. The activation of this enzyme complex supplies superoxide anions and in this way forms an essential component of granulocytic immune defence (Ikeda et al., 2003; Knight, 2000). An increase in glutamine metabolisation during an inflammatory event therefore leads not only to essential metabolic pre-

cursors, but also increases the relative activity of immediately available intragranulocytic redox systems (Jacobi et al., 1998, 1999).

The rapid release of glutamine and alanine into blood after an additive L-alanyl-L-glutamine application also led to dose-dependent and incubation time-dependent increases in intragranulocytic glutamine and alanine supplies, whereby alanine, like glutamine, is taken up unto the cells via specific amino acid transporters (e.g. systems A, L or ASC) (Jacobi et al., 1998, 1999; Mühling et al., 2002, 2005). In parallel, significant concentration and incubation time-dependent increases in other important intragranulocytic amino acids and α-keto acids (glutamate, aspartate, α-ketoglutarate, pyruvate, ornithine, arginine, serine, glycine) were also shown. Although this is not surprising, nevertheless, as already explained in detail, the metabolic and enzymatic preconditions for the further intragranulocytic metabolisation of both amino acids and for an accumulation of metabolically active glutamine and alanine-dependent metabolic products are certainly present also in neutrophils. Consistent with the immunofunctional findings from other research groups, the increase in intragranulocytic glutamine and alanine levels as well as the influencing of glutamine and alanine-dependent amino acid and α-keto acid substrate levels in response to L-alanyl-L-glutamine application led to parallel and very marked increases in extragranulocytically secreted superoxide anion and hydrogen peroxide concentrations as well as to a clear increase in extracellular myeloperoxidase activity (Furukawa et al., 1997a, b, 2000a, b; Pithon-Curi et al., 2002, 2003). Analogously with the already quoted results from other groups, the present findings showed that an additive application of L-alanyl-L-glutamine can lead to a stimulus-dependent maintenance or even enhancement of intragranulocytic amino acid and α-keto acid pools as well as essential granulocytic immune functions, and not just with unimpaired metabolic function. Here the observed effects were not just restricted to a sole influencing of direct glutamine- and alanine-dependent metabolic pathways (consistent with the results of singular L-alanyl-L-glutamine application), but there were also significant modulations of other important intragranulocytic amino acid and α-keto acid pools (arginine, ornithine, taurine, α-ketoglutarate, pyruvate). The importance of the additional influencing of individual intragranulocytic metabolic pathways for the observed amino acid and α-keto acid changes and the reported immune effects was revealed by the results of combining L-alanyl-L-glutamine with ''metabolism modulators''. For example, although parallel application of a glutamine analogue (DON) led as expected to a wide ranging inhibition of all L-alanyl-L-glutamine dependent singular effects, the quantitative changes compared with singular DON application were far less distinct. L-alanyl-L-glutamine could however significantly modulate and in all substantially weaken the influence of an artificially-induced extracellular •NO increase (e.g. using SNAP) on the intragranulocytic amino acid and α-keto acid pools as well as the immune functional parameters observed in this study (Huynh and Chin-Dusting, 2006; Moinard et al., 2002b; O'Dowd and Newsholme, 1997). A nutritively-mediated maintenance of the intracellular substrate homoeostasis of important intracellular, direct or indirect amino acid precursors (such as glutamate, aspartate, ornithine, α-ketoglutarate, glutathione) induced by L-alanyl-L-glutamine therefore seems to be an important precondition for protecting neutrophils against attack by noxious pharmacological and catabolic influences (Oehler and Roth, 2003, Oehler et al., 2002). Additional findings by us confirm these results: according to the results presented here L-alanyl-L-glutamine can also significantly compensate the metabolic and immunofunctional effects induced by inhibition of intragranulocytic ornithine metabolism. As a result of the glutamine-specific maintenance of intracellular ornithine decarboxylase activity, noxious metabolic influences, induced by inhibitors of the said enzyme complex, can be significantly weakened or even annulled (Newsholme, 2001). Similar effects were also found with lymphocytes and natural killer cells.

Despite the promising findings presented here, there is an urgent need for further research on the potential immunonutritive application of glutamine-dipeptides, since the maintenance or even promotion of granulocytic metabolism and immune functions in critically ill patients does not necessarily lead to a clinical improvement of the disease event itself (Alpers, 2006; Saito et al., 1999; Scheid et al., 2004; Schroder et al., 1990). This statement is based on a pathophysiological problem that can accompany the migration of immunonutritively metabolically and functionally improved neutrophils into an inflammatorily damaged and potentially glutamine depleted extravascular matrix (Dechelotte et al., 2006; Bracco, 2005; Cynober, 2001). Here there is a major risk of an additional granulocyte-mediated injury to all cells and tissue structures present there (Yeh et al., 2006; Nussler et al., 1999; Parry-Billings et al., 1990). These pathophysiological processes are of particular clinical and therapeutic relevance especially when the granulocytic secretory and degranulation processes can not be balanced by a similarly nutritionally promoted system of cellular- and matrix-dependent pro-

tective functions, e.g. because the systemic supply of the inflammation region itself (oedema formation, raised substrate consumption, etc.) is significantly altered or impaired pathophysiologically (Brandon, 2004; Buchmann, 2003; Castell, 2003). The glutamine-mediated functional increase in the granulocytic O_2^-–H_2O_2–MPO system might paradoxically induce injury to the body's own tissue and organs and in so doing aggravate the course of the disease inadvertently (Dhaliwal and Heyland, 2005; O'Dowd and Newsholme, 1997).

References

Albers S, Wernerman J, Stehle P, Vinnars E, Fürst P (1988) Availability of amino acids supplied intravenously in healthy man as synthetic dipeptides: kinetic evaluation of L-alanyl-L-glutamine and glycyl-L-tyrosine. Clin Sci (Lond) 75: 463–468

Albers S, Wernerman J, Stehle P, Vinnars E, Fürst P (1989) Availability of amino acids supplied by constant intravenous infusion of synthetic dipeptides in healthy man. Clin Sci (Lond) 76: 643–648

Alpers DH (2006) Glutamine: Do the data support the cause for glutamine supplementation in humans? Gastroenterology 130: S106–S116

Andrews FJ, Griffiths RD (2002) Glutamine: essential for immune nutrition in the critically ill. Br J Nutr 87 [Suppl 1]: S3–S8

Ardawi MS (1988) Glutamine and glucose metabolism in human peripheral lymphocytes. Metabolism 37: 99–103

Ardawi MS, Newsholme EA (1986) The transport of glutamine into rat mesenteric lymphocytes. Biochim Biophys Acta 856: 413–420

Ardawi MS, Majzoub MF, Kateilah SM, Newsholme EA (1991) Maximal activity of phosphate-dependent glutaminase and glutamine metabolism in septic rats. J Lab Clin Med 118: 26–32

Bender DA (1985) Amino acid metabolism, 2nd ed. Chichester, New York, Brisbane, Toronto, Singapore

Board M, Humm S, Newsholme EA (1990) Maximum activities of key enzymes of glycolysis, glutaminolysis, pentose phosphate pathway and tricarboxylic acid cycle in normal, neoplastic and suppressed cells. Biochem J 265: 503–509

Boelens PG, Nijveldt RJ, Houdijk AP, Meijer S, van Leeuwen PA (2001) Glutamine alimentation in catabolic state. J Nutr 131: 2569S–2577S

Borghetti AF, Kay JE, Wheeler KP (1979) Enhanced transport of natural amino acids after activation of pig lymphocytes. Biochem J 182: 27–32

Bracco D (2005) Glutamine: a double edge sword in the intensive care unit? Crit Care Med 33: 2692–2694

Brand K, Fekl W, von Hintzenstern J, Langer K, Luppa P, Schoerner C (1989) Metabolism of glutamine in lymphocytes. Metabolism 38: 29–33

Brandon WJ (2004) Clinical controversies: the role of glutamine counterpoint. Nutr Clin Pract 19: 312–313

Buchman A (2003) The role of glutamine: counterpoint. Nutr Clin Pract 18: 391–396

Carr AC, Winterbourn CC (1997) Oxidation of neutrophil glutathione and protein thiols by myeloperoxidase-derived hypochlorous acid. Biochem J 327: 275–281

Castell L (2003) Glutamine supplementation in vitro and in vivo, in exercise and in immunodepression. Sports Med 33: 323–345

Castell L, Vance C, Abbott R, Marquez J, Eggleton P (2004) Granule localization of glutaminase in human neutrophils and the consequence of glutamine utilization for neutrophil activity. J Biol Chem 279: 13305–13310

Coleman JW (2001) Nitric oxide in immunity and inflammation. Int Immunopharmacol 1: 1397–1406

Coster J, McCauley R, Hall J (2004) Glutamine: metabolism and application in nutrition support. Asia Pac J Clin Nutr 13: 25–31

Curi R, Newsholme P, Newsholme EA (1986) Intracellular distribution of some enzymes of the glutamine utilisation pathway in rat lymphocytes. Biochem Biophys Res Commun 138: 318–322

Curi R, Newsholme P, Newsholme EA (1988) Metabolism of pyruvate by isolated rat mesenteric lymphocytes, lymphocyte mitochondria and isolated mouse macrophages. Biochem J 250: 383–388

Curi R, Williams JF, Newsholme EA (1989a) Formation of ketone bodies by resting lymphocytes. Int J Biochem 21: 1133–1136

Curi R, Williams JF, Newsholme EA (1989b) Pyruvate metabolism by lymphocytes: evidence for an additional ketogenic tissue. Biochem Int 19: 755–767

Curi R, Newsholme P, Pithon-Curi TC, Pires-de-Melo M, Garcia C, Homem-de-Bittencourt Junior PI, Guimaraes AR (1999) Metabolic fate of glutamine in lymphocytes, macrophages and neutrophils. Braz J Med Biol Res 32: 15–21

Curi TC, De Melo MP, De Azevedo RB, Zorn TM, Curi R (1997) Glutamine utilization by rat neutrophils: presence of phosphate-dependent glutaminase. Am J Physiol 273: C1124–C1129

Curthoys NP, Watford M (1995) Regulation of glutaminase activity and glutamine metabolism. Annu Rev Nutr 15: 133–159

Cynober LA (2001) Do we have unrealistic expectations of the potential of immuno-nutrition? Can J Appl Physiol [Suppl 26]: S36–S44

Dechelotte P, Hasselmann M, Cynober L, Allaouchiche B, Coeffier M, Hecketsweiler B, Merle V, Mazerolles M, Samba D, Guillou YM, Petit J, Mansoor O, Colas G, Cohendy R, Barnoud D, Czernichow P, Bleichner G (2006) L-alanyl-L-glutamine dipeptide-supplemented total parenteral nutrition reduces infectious complications and glucose intolerance in critically ill patients: the French controlled, randomized, double-blind, multicenter study. Crit Care Med 34: 598–604

Dhaliwal R, Heyland DK (2005) Nutrition and infection in the intensive care unit: what does the evidence show? Curr Opin Crit Care 11: 461–467

Eggleton P, Gargan R, Fisher D (1989) Rapid method for the isolation of neutrophils in high yield without the use of dextran or density gradient polymers. J Immunol Methods 121: 105–113

Ehrensvard G, Fischer A, Stjernholm R (1949) Protein metabolism of tissue cells in vitro. The chemical nature of some obligate factors of tissue cell nutrition. Acta Physiol Scand 18: 218–227

Engel JM, Mühling J, Weiss S, Lu L, Simonis Y, Menges T, Hempelmann G (2003) Low plasma glutamine after multiple trauma: relationship with intracellular glutamine in polymorphonuclear neutrophils during prolonged ICU stay. Acta Anaesthesiol Scand 47: 707–713

Frei J, Aellig A, Nessi P (1975) Enzyme system and coenzymes involved in the energy metabolism of leukocytes. Function and metabolism of polymorphonuclear neutrophils. Ann Biol Clin (Paris) 33: 459–464

Frei J, Maillard M, Markert M, Aellig M (1980) Energetic metabolism of leukocytes. XI. Presence and function of creatine kinase in leukocytes. Enzyme 25: 258–264

Fuentes-Orozco C, Anaya-Prado R, Gonzalez-Ojeda A, Arenas-Marquez H, Cabrera-Pivaral C, Cervantes-Guevara G, Barrera-Zepeda LM (2004) L-alanyl-L-glutamine-supplemented parenteral nutrition improves infectious morbidity in secondary peritonitis. Clin Nutr 23: 13–21

Fürst P (2001) New developments in glutamine delivery. J Nutr 131: 2562S–2568S

Fürst P, Pogan K, Hummel M, Herzog B, Stehle P (1997) Design of parenteral synthetic dipeptides for clinical nutrition: in vitro and in vivo utilization. Ann Nutr Metab 41: 10–21

Furukawa S, Saito H, Fukatsu K, Hashiguchi Y, Inaba T, Lin MT, Inoue T, Han I, Matsuda T, Muto T (1997a) Glutamine-enhanced bacterial killing by neutrophils from postoperative patients. Nutrition 13: 863–869

Furukawa S, Saito H, Inaba T, Lin MT, Inoue T, Naka S, Fukatsu K, Hashiguchi Y, Han I, Matsuda T, Ikeda S, Muto T (1997b) Glutamine-enriched enteral diet enhances bacterial clearance in protected bacterial

peritonitis, regardless of glutamine form. J Parenter Enteral Nutr 21: 208–214

Furukawa S, Saito H, Inoue T, Matsuda T, Fukatsu K, Han I, Ikeda S, Hidemura A (2000a) Supplemental glutamine augments phagocytosis and reactive oxygen intermediate production by neutrophils and monocytes from postoperative patients in vitro. Nutrition 16: 323–329

Furukawa S, Saito H, Matsuda T, Inoue T, Fukatsu K, Han I, Ikeda S, Hidemura A, Muto T (2000b) Relative effects of glucose and glutamine on reactive oxygen intermediate production by neutrophils. Shock 13: 274–278

Haberle J, Gorg B, Rutsch F, Schmidt E, Toutain A, Benoist JF, Gelot A, Suc AL, Hohne W, Schliess F, Haussinger D, Koch HG (2005) Congenital glutamine deficiency with glutamine synthetase mutations. N Engl J Med 353: 1926–1933

Hubl W, Druml W, Langer K, Lochs H (1989) Influence of molecular structure and plasma hydrolysis on the metabolism of glutamine-containing dipeptides in humans. Metabolism 38: 59–62

Huynh NN, Chin-Dusting J (2006) Amino acids, arginase and nitric oxide in vascular health. Clin Exp Pharmacol Physiol 33: 1–8

Igarashi K, Kashiwagi K (2000) Polyamines: mysterious modulators of cellular functions. Biochem Biophys Res Commun 271: 559–564

Ikeda S, Kudsk KA, Le T, Zarzaur BL, Johnson CD (2003) Glutamine improves impaired cellular exudation and polymorphonuclear neutrophil phagocytosis induced by total parenteral nutrition after glycogen-induced murine peritonitis. Shock 19: 50–54

Jacobi CA, Ordemann J, Zuckermann H, Docke W, Volk HD, Muller JM (1998) Effect of alanyl-glutamine in postoperative total parenteral nutrition on postoperative immunosuppression and morbidity. Preliminary results of a prospective randomized study. Langenbecks Arch Chir Suppl Kongressbd 115: 605–611

Jacobi CA, Ordemann J, Zuckermann H, Docke W, Volk HD, Muller JM (1999) The influence of alanyl-glutamine on immunologic functions and morbidity in postoperative total parenteral nutrition. Preliminary results of a prospective randomized trial. Zentralbl Chir 124: 199–205

Junghans P, Derno M, Pierzynowski S, Hennig U, Eberhard RP, Souffrant WB (2006) Intraduodenal infusion of alpha-ketoglutarate decreases whole body energy expenditure in growing pigs. Clin Nutr 25: 489–496

Kircher B, Eibl G, Enrich B, Latzer K, Herold M, Niederwieser D (2002) The role of L-alanyl-L-glutamine in the immune response in vitro. Wien Klin Wochenschr 114: 702–708

Knight JA (2000) Review: free radicals, antioxidants, and the immune system. Ann Clin Lab Sci 30: 145–158

Kobayashi SD, Voyich JM, Burlak C, De Leo FR (2005) Neutrophils in the innate immune response. Arch Immunol Ther Exp (Warsz) 53: 505–517

Korpela H, Holtta E, Hovi T, Janne J (1981) Response of enzymes involved in the metabolism of polyamines to phytohaemagglutinin-induced activation of human lymphocytes. Biochem J 196: 733–738

Krumholz W, Demel C, Jung S, Meuthen G, Knecht J, Hempelmann G (1995a) The effects of thiopentone, etomidate, ketamine and midazolam on several bactericidal functions of polymorphonuclear leukocytes in vitro. Eur J Anaesthesiol 12: 141–146

Krumholz W, Endrass J, Knecht J, Hempelmann G (1995b) The effects of midazolam, droperidol, fentanyl, and alfentanil on phagocytosis and killing of bacteria by polymorphonuclear leukocytes in vitro. Acta Anaesthesiol Scand 39: 624–627

Krumholz W, Demel C, Jung S, Meuthen G, Hempelmann G (1993) The influence of intravenous anaesthetics on polymorphonuclear leucocyte function. Can J Anaesthesiol 40: 770–774

Lacey JM, Wilmore DW (1990) Is glutamine a conditionally essential amino acid? Nutr Rev 48: 297–309

Lagranha CJ, de Lima TM, Senna SM, Doi SQ, Curi R, Pithon-Curi TC (2005) The effect of glutamine supplementation on the function of neutrophils from exercised rats. Cell Biochem Funct 23: 101–107

Lee CH, Chiu WC, Chen SC, Wu CH, Yeh SL (2005) Effects of glutamine-containing total parenteral nutrition on phagocytic activity and anabolic hormone response in rats undergoing gastrectomy. World J Gastroenterol 11: 817–822

Leighton B, Curi R, Hussein A, Newsholme EA (1987) Maximum activities of some key enzymes of glycolysis, glutaminolysis, Krebs cycle and fatty acid utilization in bovine pulmonary endothelial cells. FEBS Lett 225: 93–96

Metcoff J (1986) Intracellular amino acid levels as predictors of protein synthesis. J Am Coll Nutr 5: 107–120

Metcoff J, Wikman-Coffelt J, Yoshida T, Bernal A, Rosado A, Yoshida P, Urrusti J, Frenk S, Madrazo R, Velasco L, Morales M (1973) Energy metabolism and protein synthesis in human leukocytes during pregnancy and in placenta related to fetal growth. Pediatrics 51: 866–877

Moinard C, Chauveau B, Walrand S, Felgines C, Chassagne J, Caldefie F, Cynober LA, Vasson MP (1999) Phagocyte functions in stressed rats: comparison of modulation by glutamine, arginine and ornithine 2-oxoglutarate. Clin Sci (Lond) 97: 59–65

Moinard C, Caldefie-Chezet F, Walrand S, Vasson MP, Cynober L (2002a) Evidence that glutamine modulates respiratory burst in stressed rat polymorphonuclear cells through its metabolism into arginine. Br J Nutr 88: 689–695

Moinard C, Caldefie F, Walrand S, Tridon A, Chassagne J, Vasson MP, Cynober L (2002b) Effects of ornithine 2-oxoglutarate on neutrophils in stressed rats: evidence for the involvement of nitric oxide and polyamines. Clin Sci (Lond) 102: 287–295

Morlion BJ, Siedhoff HP, Joosten U, Koller M, Konig W, Furst P, Puchstein C (1996) Immunomodulation after parenteral glutamine administration in colorectal surgery. Langenbecks Arch Chir Suppl Kongressbd 113: 342–344

Morlion BJ, Stehle P, Wachtler P, Siedhoff HP, Koller M, Konig W, Furst P, Puchstein C (1998) Total parenteral nutrition with glutamine dipeptide after major abdominal surgery: a randomized, double-blind, controlled study. Ann Surg 227: 302–308

Mühling J, Fuchs M, Dehne MG, Sablotzki A, Menges T, Weber B, Hempelmann G (1999) Quantitative determination of free intracellular amino acids in single human polymorphonuclear leukocytes. Recent developments in sample preparation and high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 728: 157–166

Mühling J, Fuchs M, Sablotzki A, Fleck C, Krüll M, Dehne MG, Gonter J, Weiss S, Engel J, Hempelmann G (2002) Effects of arginine, L-alanyl-L-glutamine or taurine on neutrophil (PMN) free amino acid profiles and immune functions in vitro. Amino Acids 22: 39–53

Mühling J, Fuchs M, Campos ME, Gonter J, Engel JM, Sablotzki A, Menges T, Weiss S, Dehne MG, Krüll M, Hempelmann G (2003) Quantitative determination of free intracellular α-keto acids in neutrophils. J Chromatogr B Analy Technol Biomed Life Sci 789: 383–392

Mühling J, Nickolaus KA, Halabi M, Fuchs M, Krüll M, Engel J, Wolff M, Matejec R, Langefeld TW, Welters ID, Menges T, Dehne MG, Sablotzki A, Hempelmann G (2005) Alterations in neutrophil (PMN) free intracellular alpha-keto acid profiles and immune functions induced by L-alanyl-L-glutamine, arginine or taurine Amino Acids 29: 289–300

Murphy C, Newsholme P (1997) Glutamine as a possible precursor of L-arginine and thus nitric oxide synthesis in murine macrophages. Biochem Soc Trans 25: 404S

Murphy C, Newsholme P (1998) Importance of glutamine metabolism in murine macrophages and human monocytes to L-arginine biosynthesis and rates of nitrite or urea production. Clin Sci (Lond) 95: 397–407

Murphy C, Newsholme P (1999) Macrophage-mediated lysis of a beta-cell line, tumour necrosis factor-alpha release from bacillus Calmette-Guerin (BCG)-activated murine macrophages and interleukin-8 release from human monocytes are dependent on extracellular glutamine concentration and glutamine metabolism. Clin Sci (Lond) 96: 89–97

Nascimento SB, Sousa RB, Martins MJ, Souza GA, Souza MH, Guerrant RL, Cunha FQ, Ribeiro RA, Brito GA (2005) Glutamine depletion

potentiates leucocyte-dependent inflammatory events induced by carrageenan or Clostridium difficile toxin A in rats. Immunology 116: 328–336

Newsholme EA (1996) The possible role of glutamine in some cells of the immune system and the possible consequence for the whole animal. Experientia 52: 455–459

Newsholme EA, Newsholme P, Curi R (1987a) The role of the citric acid cycle in cells of the immune system and its importance in sepsis, trauma and burns. Biochem Soc Symp 54: 145–162

Newsholme EA, Calder PC (1997) The proposed role of glutamine in some cells of the immune system and speculative consequences for the whole animal. Nutrition 13: 728–730

Newsholme P (2001) Why is L-glutamine metabolism important to cells of the immune system in health, postinjury, surgery or infection? J Nutr 131: 2515S–2522S

Newsholme P, Gordon S, Newsholme EA (1987b) Rates of utilization and fates of glucose, glutamine, pyruvate, fatty acids and ketone bodies by mouse macrophages. Biochem J 242: 631–636

Noble DW, Avenell A (2006) Glutamine-containing parenteral nutrition: another piece in the jigsaw? Crit Care Med 34: 893–894

Novogrodsky A, Tate SS, Meister A (1977) Uptake and utilization of L-glutamine by human lymphoid cells; relationship to gamma-glutamyl transpeptidase activity. Biochem Biophys Res Commun 78: 222–229

Nussler AK, Wittel UA, Nussler NC, Beger HG (1999) Leukocytes, the Janus cells in inflammatory disease. Langenbecks Arch Surg 384: 222–232

O'Dowd Y, Newsholme P (1997) Evidence for the involvement of glutamine in nitric oxide (NO) production by immunostimulated neutrophils. Biochem Soc Trans 25: 403S

Oehler R, Roth E (2003) Regulative capacity of glutamine. Curr Opin Clin Nutr Metab Care 6: 277–282

Oehler R, Pusch E, Dungel P, Zellner M, Eliasen MM, Brabec M, Roth E (2002) Glutamine depletion impairs cellular stress response in human leucocytes. Br J Nutr 87 [Suppl 1]: S17–S21

Parry-Billings M, Evans J, Calder PC, Newsholme EA (1990) Does glutamine contribute to immunosuppression after major burns? Lancet 336: 523–525

Pithon-Curi TC, Trezena AG, Tavares-Lima W, Curi R (2002a) Evidence that glutamine is involved in neutrophil function. Cell Biochem Funct 20: 81–86

Pithon-Curi TC, Levada AC, Lopes LR, Doi SQ, Curi R (2002b) Glutamine plays a role in superoxide production and the expression of p47phox, p22phox and gp91phox in rat neutrophils. Clin Sci (Lond) 103: 403–408

Pithon-Curi TC, Schumacher RI, Freitas JJ, Lagranha C, Newsholme P, Palanch AC, Doi SQ, Curi R (2003) Glutamine delays spontaneous apoptosis in neutrophils. Am J Physiol Cell Physiol 284: C1355–C1361

Pithon-Curi TC, De Melo MP, Curi R (2004) Glucose and glutamine utilization by rat lymphocytes, monocytes and neutrophils in culture: a comparative study. Cell Biochem Funct 22: 321–326

Piva TJ, McCabe MG, McEvoy-Bowe E (1992) The kinetics of glutamine transport in bovine lymphocytes. Biochem Int 26: 309–316

Rick W (1977) Klinische Chemie und Mikroskopie. Springer, Berlin Heidelberg New York

Rogero MM, Tirapegui J, Pedrosa RG, de Castro IA, de Oliveira Pires I (2006) Effect of alanyl-glutamine supplementation on plasma and tissue glutamine concentrations in rats submitted to exhaustive exercise. Nutrition 22: 564–571

Rose C, Jalan R (2006) Congenital glutamine deficiency with glutamine synthetase mutations. N Engl J Med 354: 1093–1094

Sahoo G, More T, Singh VK (1998) A comparative study on certain enzymes of the granulocyte from different ruminant species. Comp Immunol Microbiol Infect Dis 21: 319–325

Saito H, Furukawa S, Matsuda T (1999) Glutamine as an immunoenhancing nutrient. J Parenter Enteral Nutr 23: S59–S61

Scheid C, Hermann K, Kremer G, Holsing A, Heck G, Fuchs M, Waldschmidt D, Herrmann HJ, Sohngen D, Diehl V, Schwenk A (2004) Randomized, double-blind, controlled study of glycyl-glutamine-dipeptide in the parenteral nutrition of patients with acute leukemia undergoing intensive chemotherapy. Nutrition 20: 249–254

Schrijver J, Hommes FA (1975) Activity of fructose-1,6-diphosphatase in human leukocytes. N Engl J Med 292: 1298–1299

Schröder MT, Schäfer G, Schauder P (1990) Characterization of glutamine transport into resting and concanavalin A-stimulated peripheral human lymphocytes. J Cell Physiol 145: 155–161

Souba WW (1993) Glutamine and cancer. Ann Surg 218: 715–728

Venizelos N, Hagenfeldt L (1985) Pyruvate and acetate oxidation by leukocytes in vitro. Activation of the pyruvate dehydrogenase complex by uncoupling of oxidative phosphorylation. Scand J Clin Lab Invest 45: 335–340

Washizu T, Kuramoto E, Abe M, Sako T, Arai T (1998) A comparison of the activities of certain enzymes related to energy metabolism in leukocytes in dogs and cats. Vet Res Commun 22: 187–192

Wischmeyer PE (2003) Clinical applications of L-glutamine: past, present, and future. Nutr Clin Pract 18: 377–385

Witko-Sarsat V, Rieu P, Descamps-Latscha B, Lesavre P, Halbwachs-Mecarelli L (2000) Neutrophils: molecules, functions and pathophysiological aspects. Lab Invest 80: 617–653

Yeh CL, Hsu CS, Yeh SL, Lin MT, Chen WJ (2006) Dietary glutamine supplementation reduces cellular adhesion molecule expression and tissue myeloperoxidase activity in mice with gut-derived sepsis. Nutrition 22: 408–413

Ziegler TR, Bye RL, Persinger RL, Young LS, Antin JH, Wilmore DW (1998) Effects of glutamine supplementation on circulating lymphocytes after bone marrow transplantation: a pilot study. Am J Med Sci 315: 4–10

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