Evidence that glutamine modulates respiratory burst in stressed rat polymorphonuclear cells through its metabolism into arginine

Christophe Moinard[1,2]*, Florence Caldefie-Chezet[1], Stephane Walrand[1,2], Marie-Paule Vasson[1] and Luc Cynober[2,3]

[1]*Laboratoire de Biochimie, Biologie Moléculaire et Nutrition EA 2416 and Centre de Recherche en Nutrition Humaine, Faculté de Pharmacie, Clermont-Ferrand, France*
[2]*Laboratoire de Biologie de la Nutrition, Faculté de Pharmacie, 4 avenue de l'Observatoire, 75270 Paris Cedex 06, France*
[3]*Laboratoire de Biochimie, Hotel-Dieu, AP-HP and Inserm U341, France*

Glutamine (GLN) and arginine (ARG) are recognized for their ability to modulate immune cell function. However, the metabolic pathways involved in their action remain unclear. It was recently shown that GLN- or ARG-enriched diets increased radical oxygen species (ROS) production by neutrophils from stressed rats. Since these two amino acids have a tied metabolism, we hypothesized that conversion between GLN and ARG (and its active metabolites NO$^\bullet$ and polyamines) might be involved. To test this hypothesis male Sprague–Dawley rats (n 117) were randomized into thirteen groups: rats in eleven groups were rendered catabolic by dexamethasone injection (1·5 mg/kg per d for 5 d) and 6·8 mmol either GLN, ARG or non-essential amino acids (NEAA; glycine, alanine and histidine)/kg per d were given by the enteral route; one group was pair-fed to the treated groups. The regimens of all the groups were rendered isonitrogenous by the addition of NEAA. The last group was not treated and was fed *ad libitum*. For each supplementation three subgroups were formed, each of which received a specific inhibitor: methionine sulfoximine (inhibitor of GLN synthase; 100 mg/kg per d), S-methylthiourea (inhibitor of inducible NO$^\bullet$ synthase (iNOS); 50 mg/kg per d) and difluoromethylornithine (inhibitor of ornithine decarboxylase (ODC); 50 mg/kg per d). Oxidative metabolism, intracellular H_2O_2, and extracellular $O_2^{\bullet-}$ production were measured in unstimulated and phorbol myristate acetate-stimulated polymorphonuclear neutrophils. GLN- and ARG-enriched diets increased respiratory burst by neutrophils (oxidative metabolism of 152 (SEM 24) and 138 (SEM 45) *v.* 57 (SEM 18) mV for GLN-, ARG- and NEAA-enriched diets respectively, $P<0.05$). *In vivo* inhibition of iNOS or ODC decreased ROS production induced by GLN and ARG. *In vivo* inhibition of GLN synthase did not modify the effect of ARG on ROS production. In conclusion, GLN and ARG modulate ROS production in neutrophils from stressed rats by the same pathway involving polyamine and NO$^\bullet$ synthesis.

Glutamine: Arginine: Polymorphonuclear cells: Polyamines: Nitric oxide: Respiratory burst

Response to injury is characterized by a hypermetabolic state and an alteration of protein metabolism (Jeevanandam, 1995). In particular, injury is also associated with marked changes in the concentrations of free amino acids, especially glutamine (GLN) and arginine (ARG), both in plasma and muscles (Cynober, 1989; Ardawi & Jamal, 1990; Ardawi & Majzoub, 1991). One current view is that the decrease in GLN and ARG availability is partly responsible for the dysimmunity often observed in stress (Newsholme *et al.* 1988; Brittenden *et al.* 1994; Pastores *et al.* 1994). Under these conditions, GLN- and ARG-enriched diets may have pharmacological effects on immunity. Many studies (for a review, see De Bandt & Cynober, 1998) have shown the ability of these two

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amino acids to modulate immune functions under physiological or pathological conditions. They are known to improve T-cell immmunity (Alexander, 1995; Robinson *et al.* 1999), to stimulate macrophage functions (Calder & Yaqoob, 1999; Wu *et al.* 2000) and polymorphonuclear neutrophil (PMN) bactericidal power (Moffat *et al.* 1996; Furukawa *et al.* 1997, 2000; Holm *et al.* 1999; Moinard *et al.* 1999). However, the mechanisms involved in these actions are still unclear. In a recent study (Moinard *et al.* 1999) we showed the ability of an enterally administered diet supplemented with GLN and ARG to stimulate H_2O_2 production by PMN. However, the mechanisms involved were not elucidated. We (Moinard *et al.* 2000) and others (Murphy & Newsholme, 1998) have shown that GLN may be an ARG precursor for $NO^{\bullet}$ synthesis in immune cells. These data suggest that these two amino acids have a tied metabolism and so their immunomodulatory effects may be related to a common pathway. We hypothesized that GLN may act via ARG or that ARG may act through GLN neosynthesis.

The aim of the present study was therefore to determine the metabolic pathways involved in GLN and ARG action on radical oxygen species (ROS) production by PMN from stressed rats. The catabolic state was obtained by daily intraperitoneal injection of glucocorticoids (i.e. dexamethasone; DEX). This model has been demonstrated to lead to a highly reproducible catabolic state in rats, including anorexia, protein breakdown and negative N balance (Odedra *et al.* 1983; Ardawi & Jamal, 1990; Parry-Billings *et al.* 1990; Minet-Quinard *et al.* 2000). In addition, glucocorticoids have immunosuppressive effects; they are responsible for changes in the distribution of leucocytes observed during stress (Shijo *et al.* 1998). These changes may have a significant impact on the functioning and effectiveness of the immune response (Shijo *et al.* 1998).

We evaluated the enhancing effect of GLN and ARG on respiratory burst using three different methods (chemiluminescence, flow cytometry and ferricytochrome reduction) in glucocorticoid-treated rats. We also used metabolic inhibitors in GLN- and ARG-supplemented stressed rats in order to block different metabolic pathways at the whole body level (Fig. 1): methionine sulfoximine (MSO), a specific inhibitor of GLN synthase (Heeneman & Deutz, 1993), to determine whether ARG acts through neosynthesis of GLN; S-methylthiourea (SMT), a specific inhibitor of inducible $NO^{\bullet}$ synthase (Ruetten & Thiemermann, 1996), to determine whether GLN and ARG act by a process dependent on $NO^{\bullet}$ synthesis (the active metabolite of ARG); α-difluoromethylornithine (DFMO), a specific inhibitor of ornithine decarboxylase (Nsi-Emvo *et al.* 1996) to determine whether GLN and ARG act via polyamine synthesis (deriving from ARG).

Materials and methods

Chemicals

All chemicals were purchased from Sigma (St-Quentin-Fallavier, France) except for 2′-7′-dichlorofluorescin diacetate (DCFH-DA; Acros, Noisy-le-Grand, France), Plasmagel® (Belon, Neuilly-sur-Seine, France), and DEX (Soludecadron®; Merck Sharp & Dohm, Riom, France).

Animals and study design

A total of 117 male Sprague–Dawley rats (3 months old) were used (Iffa Credo, L'Arbresle, France). After their arrival at our animal facility the rats were maintained on a standard chow diet (A04: 170 g proteins, 30 g fat, 590 g carbohydrates, and 210 g water/kg, fibre, vitamins and minerals; Usine d'Alimentation Rationnelle, Villemoisson-sur-Orge, France) and received water *ad libitum*. They were
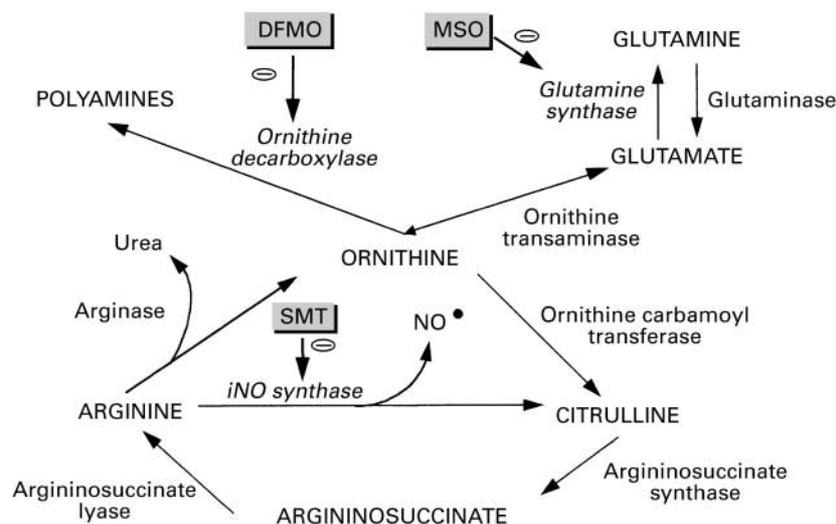

Fig. 1. Metabolic pathways involved in glutamine and arginine action on radical oxygen species produced by polymorphonuclear cells in stressed rats. Metabolic pathways were blocked (−) by metabolic inhibitors α-difluoromethylornithine (DFMO), methionine sulfoximine (MSO) and S-methylthiourea (SMT). iNO synthase = inducible $NO^{\bullet}$ synthase.

kept in individual cages in a controlled environment (constant temperature of 24°C) and light cycle (lights off 8.00–20.00 hours, lights on 20.00–8.00 hours). After this acclimatization period, the rats were randomized into thirteen groups (nine per group) as shown in Table 1.

For the next 5 d (day 0 to day 4) all the rats (except those that were pair-fed) had free access to the chow diet as described above. DEX-treated groups (i.e. groups 3 to 13, see Table 1) received a daily intraperitoneal injection of DEX (1·5 mg/kg per d) for 5 d (day 0 to day 4) as previously described (Minet-Quinard *et al.* 2000). The pair-fed group (PF) was pair-fed to DEX groups and received a daily isovolumic intraperitoneal injection of NaCl (9 g/l). The rationale for the study of a PF was the fact that DEX induces anorexia (Minet-Quinard *et al.* 2000); studying a PF thus isolates the effect of the treatment itself from the effects induced by marked anorexia. In addition to their oral chow diet the supplemented groups received enteral supplementation of GLN or ARG (6·8 mmol/kg per d) (day 0 to day 4) by oral administration as described previously (Lasnier *et al.* 1996). The dose of 6·8 mmol/kg per d corresponds to 1·00 g GLN/kg per d and 2·19 g ARG/kg per d. These doses are in the range of those used in the literature (Barbul, 1995; Fürst & Stehle, 1995; Farges *et al.* 1999; Moinard *et al.* 1999) and are an extrapolation of dosages used in human subjects allowing for the high metabolic rate of rats (Cynober, 1989). The enteral route was chosen to provide supplements, rather than mixing them with chow (Le Bricon *et al.* 1995), to be certain that rats suffering from anorexia received the targeted amount of pharmaconutrients. The enteral supplementation of all the groups was rendered isonitrogenous (up to 0·5 g N/kg per d) by addition of a mixture of non-essential amino acids (NEAA; glycine, alanine, histidine in equimolar amounts). DEX-NEAA and PF groups received only the NEAA mixture (0·5 g N/kg per d)

as a supplement to their regimen. In our experimental conditions, the NEAA mixture exerted neither pharmacological nor adverse effects on metabolic pathways (Minet-Quinard *et al.* 1999). MSO-, SMT- and DFMO-treated groups received MSO (100 mg/kg per d), SMT (50 mg/kg per d) and DFMO (50 mg/kg per d) respectively by the enteral route for 6 d (day −1 to day 4). The use of NEAA groups allowed us to determine whether the metabolic inhibitors are able to modify *per se* the parameters measured. We did not determine the effect of MSO in the GLN-supplemented group since GLN synthase is not involved in GLN catabolism. Dosages of inhibitors were selected on the basis of literature values (Nsi-Emvo *et al.* 1996; Ruetten & Thiemermann, 1996; Minet *et al.* 1997). Concerning the effect of inhibitors *per se*, GLN synthase activity in muscle (*extensor digitorum longus*) was determined using a colorimetric assay (Minet *et al.* 1997) and we previously demonstrated that GLN synthase activity was not detectable in muscles from MSO-treated rats (Moinard *et al.* 2002). In our experimental conditions, SMT abolished NO• secretion in stimulated macrophages (Moinard *et al.* 2000), which assess that SMT block NO synthase *in vivo*. In the case of DFMO, ornithine decarboxylase was not measured since this enzyme has a very short half-life and 24 h had elapsed since the last injection. However, it is well known that DFMO blocks ornithine decarboxylase activity in our experimental conditions (Messina *et al.* 1992; Morgan, 1994; Raul *et al.* 1995; Walters *et al.* 1998).

The *ad libitum* group (AL) received no treatment, and was fed *ad libitum*.

Animal care and experimentation complied with the rules of our institution, and two of us (L.C. and M.-P.V.) are authorized by the French Ministry of Agriculture and Forestry to use this type of experimental model.

After 24 h following the last injection, rats in the

Table 1. Treatment and nutritional supplementation of the thirteen groups studied

Groups	Injection	Amino acid Supplementation (6·8 mmol/kg per d and up to 0·5 g N/kg per d by addition of NEAA)			Metabolic inhibitors		
		NEAA	GLN	ARG	MSO (100 mg/kg per d)	SMT (50 mg/kg per d)	DFMO (50 mg/kg per d)
1. *Ad libitum* (AL)	None						
2. Pair-fed (PF)	NaCl	*					
3. DEX-NEAA	DEX	*					
4. DEX- GLN	DEX		*				
5. DEX-ARG	DEX			*			
6. DEX-NEAA-MSO	DEX	*			†		
7. DEX-NEAA-SMT	DEX	*				†	
8. DEX-NEAA-DFMO	DEX	*					†
9. DEX-GLN-SMT	DEX		*			†	
10. DEX-GLN-DFMO	DEX		*				†
11. DEX-ARG-MSO	DEX			*	†		
12. DEX-ARG-SMT	DEX			*		†	
13. DEX-ARG-DFMO	DEX			*			†

NEAA, non-essential amino acids; GLN, glutamine; ARG, arginine; MSO, methionine sulfoximine; SMT, S-methylthiourea; DFMO, α-difluoromethylornithine; NaCl, intraperitoneal injection of NaCl (9 g/l) during 5 d; DEX, intraperitoneal injection of dexamethasone (1·5 mg/kg per d) during 5 d.
* Amino acids were administered by the enteral route (from day 0 to day 4). All diets were made isonitrogenous (up to 0·5 g N/kg per d) by addition of a mixture of non-essential amino acids (glycine, histidine and alanine in equimolar amounts).
† Metabolic inhibitor was administered by the enteral route (day −1 to day 4).

post-absorptive state were anaesthetized and killed by decapitation.

Blood sampling

Blood was collected on calcium heparin to isolate PMN on a gradient of Ficoll-Hypaque (for determination of oxidative metabolism and superoxide anion generation) or by sedimentation (for H_2O_2 determination).

Procedures and analytical methods

Separation of polymorphonuclear cells for determination of superoxide anion generation. Blood was layered carefully on a double gradient of Ficoll-Hypaque (Histopaque 1083 and 1119; St Quentin Fallavier, France) with equal volumes. After centrifuging (700 *g*, 30 min, + 20°C), the Ficoll-Hypaque layers were removed and the PMN were transferred to a polypropylene tube (Falcon; Elvetec, Clermont Ferrand, France). The residual erythrocytes were destroyed by a short treatment with an ammonium chloride solution (ammonium chloride 16 mM, sodium hydrogen carbonate 1 mM, EDTA 0·01 mM).

The PMN were then washed twice with PBS, resuspended and adjusted to 10^6 cells/ml with Hank's balanced salt solution. Cell viability was determined by trypan blue exclusion and was more than 95 %. May-Grunwald-Giemsa staining of leucocytes was carried out to determine the efficiency of our separation, and gave more than 80 % PMN in each preparation.

Determination of oxidative metabolism by chemiluminescence assay. Oxidative metabolism was measured by chemiluminescence. Luminol-dependent chemiluminescence was measured using a luminometer (Model 1250; LKB Pharmacia, Trappes, France) with disposable polypropylene tubes at 37°C. PMN (5×10^5 cells) were pre-incubated for 5 min at 37°C. Luminol was then added to the medium at a final concentration of 10^{-9} M. Time was recorded from the addition of the stimulating agent (phorbol myristate acetate, 10^{-6} M). All the results are expressed as the maximum peak values (mV) corresponding to the maximal activation state.

Determination of extracellular superoxide generation by ferricytochrome C reduction. Extracellular $O_2^{\bullet-}$ generation was measured by reduction of ferricytochrome C (horse-heart type III) as previously described (Vasson *et al.* 1994). Briefly, PMN (5×10^5 cells) pre-incubated for 5 min were added to a ferricytochrome C (0·4 nM) solution and stimulated with phorbol myristate acetate (10^{-6} M) for 10 min at 37°C. The final volume of the reaction mixture was adjusted to 0·5 ml with PBS. Incubation was stopped by placing the tubes in an ice bath for 10 min. After centrifuging (400 *g*, 10 min, 4°C), the absorbance of the supernatant fractions was measured at 545 nm (DU540 spectrophotometer; Beckman-Coulter, Gagny, France). The amounts of extracellular $O_2^{\bullet-}$ produced were calculated from the absorbance using an extinction coefficient of 20 nM/cm. The results are expressed in picomoles of released $O_2^{\bullet-}$/min per 10^6 cells.

Measurement of intracellular hydrogen peroxide production by polymorphonuclear cells. Leucocytes were

isolated and intracellular H_2O_2 was measured as previously described (Moinard *et al.* 1999). In brief, leucocytes were isolated after gravity sedimentation. During leucocyte oxidative burst non-fluorescent intracellular dichlorofluorescin is oxidized by H_2O_2 to highly fluorescent 2'-7'-dichlorofluorescein. PMN were discerned among leucocyte populations and 2'-7'-dichlorofluorescein fluorescence of PMN was measured using a flow cytometer (Epics, Coulter, USA). PMN were selected on the basis of their size and granularity by the combination of low-angle forward scattered and right-angle scattered laser light. For each determination, 2500 PMN were counted.

Results are expressed as the ratio of fluorescence produced by phorbol myristate acetate-stimulated leucocytes to fluorescence produced by non-stimulated cells.

Statistical analysis

Data are expressed as mean values and SEM. Comparisons between sets of data were made using the Kruskal–Wallis H test followed by the Newman–Keuls test when appropriate as indicated in Table and Fig. legends. Programme Conversationnel de Statistiques pour les sciences et le Marketing (PCSM) software was used (Deltasoft, Grenoble, France). Values of $P < 0.05$ were considered significant.

Results

Effect of arginine and glutamine on oxidative metabolism in stimulated polymorphonuclear cells

Oxidative metabolism evaluated by chemiluminescence was increased under GLN and ARG supplementation

Table 2. Oxidative metabolism, extracellular $O_2^{\bullet-}$ and intracellular H_2O_2 production by phorbol myristate acetate-stimulated polymorphonuclear cells (PMN) from rats fed *ad libitum* (AL), pair-fed (PF) or treated with dexamethasone (DEX) (1·5 mg/kg per d during 5 d) and supplemented with non-essential amino acids (NEAA; DEX-NEAA), glutamine (GLN; DEX-GLN) or arginine (ARG; DEX-ARG)§

(Mean values and standard errors of the mean for nine rats per group)

	Oxidative metabolism (mV/5·10⁵ cells)		Extracellular $O_2^{\bullet-}$ (pmol/10^6 PMN per min)		Intracellular H_2O_2 (arbitrary units)	
	Mean	SEM	Mean	SEM	Mean	SEM
AL	51	14	0·86	0·16	3·8	0·6
PF	6	2	0·39	0·08	1·6*	0·2
DEX-NEAA	57	18	0·56	0·11	7·0*†	0·7
DEX-GLN	152*‡	24	1·41*†‡	0·20	11·2*†‡	0·6
DEX-ARG	138*†‡	45	0·83	0·35	9·9*†‡	1·3

* Mean value within a column was significantly different from that of the AL group: $P < 0.05$ (by ANOVA and Newman–Keuls test, or Kruskal–Wallis test for extracellular $O_2^{\bullet-}$).

† Mean value within a column was significantly different from that of the PF group: $P < 0.05$ (by ANOVA and Newman–Keuls test, or Kruskal–Wallis test for extracellular $O_2^{\bullet-}$).

‡ Mean value within a column was significantly different from that of the DEX-NEAA group: $P < 0.05$ (by ANOVA and Newman–Keuls test or Kruskal–Wallis test for extracellular $O_2^{\bullet-}$).

§ For details of study groups and procedures, see Table 1 and p. 690.

Table 3. Oxidative metabolism, extracellular $O_2^{\bullet-}$ and intracellular H_2O_2 production by stimulated polymorphonuclear cells (PMN) from dexamethasone (DEX)-treated rats (1·5 mg/kg per d during 5 d) and supplemented with either non-essential amino acids (NEAA; DEX-NEAA), NEAA + methionine sulfoximine (MSO) (DEX-NEAA-MSO), NEAA + S-methylthiourea (SMT) (DEX-NEAA-SMT) or NEAA + α-difluoromethylornithine (DFMO) (DEX-NEAA-DFMO)*

(Mean values and standard errors of the mean for nine rats per group)

	Oxidative metabolism (mV/5·10^5 cells)		Extracellular $O_2^{\bullet-}$ (pmol/10^6 PMN per min)		Intracellular H_2O_2 (arbitrary units)	
	Mean	SEM	Mean	SEM	Mean	SEM
DEX-NEAA	57	18	0·56	0·11	7·0	0·7
DEX-NEAA-MSO	69	23	0·25	0·06	5·0	0·7
DEX-NEAA-SMT	42	6	0·34	0·09	6·0	0·8
DEX-NEAA-DFMO	31	20	0·54	0·10	5·0	0·4

* For details of study groups and procedures, see Table 1 and p. 690.

(DEX-GLN, DEX-ARG v. AL, PF and DEX-NEAA; $P<0.05$) (Table 2). $O_2^{\bullet-}$ release was increased in rats supplemented with GLN (DEX-GLN v. DEX-NEAA; $P<0.05$). H_2O_2 production by PMN from DEX-treated rats was increased (DEX-NEAA v. AL; $P<0.05$). This effect was specifically due to DEX because H_2O_2 production by PMN from PF was decreased (AL v. PF; $P<0.05$). Also, in another series of experiments, the same result was obtained in DEX rats in the absence of NEAA supplementation (data not shown). Supplementation with GLN and ARG further raised H_2O_2 production by stimulated PMN (DEX-GLN, DEX-ARG v. DEX-NEAA; $P<0.05$).

Oxidative metabolism in stimulated polymorphonuclear cells from rats receiving inhibitors

In the control groups (DEX-NEAA) the inhibitors used had

Table 4. Oxidative metabolism, extracellular $O_2^{\bullet-}$ and intracellular H_2O_2 production by stimulated polymorphonuclear cells (PMN) from dexamethasone (DEX)-treated rats (1·5 mg/kg per d during 5 d) and supplemented with either arginine (ARG; DEX-ARG), ARG + methionine sulfoximine (MSO) (DEX-ARG-MSO), ARG + S-methylthiourea (SMT) (DEX-ARG-SMT) or ARG + α-difluoromethylornithine (DFMO) (DEX-ARG-DFMO)†

(Mean values and standard errors of the mean for nine rats per group)

	Oxidative metabolism (mV/5·10^5 cells)		Extracellular $O_2^{\bullet-}$ (pmol/10^6 PMN per min)		Intracellular H_2O_2 (arbitrary units)	
	Mean	SEM	Mean	SEM	Mean	SEM
DEX-ARG	139	44	0·83	0·35	9·9	1·3
DEX-ARG-MSO	101	17	0·30	0·10	7·9	1·2
DEX-ARG-SMT	32*	8	0·21	0·04	3·7*	0·5
DEX-ARG-DFMO	38*	6	0·71	0·19	6·7*	1·1

* Mean value within a column was significantly different from that of the DEX-ARG group: $P<0.05$ (by ANOVA and Newman–Keuls test).
† For details of study groups and procedures, see Table 1 and p. 690.

Table 5. Oxidative metabolism, extracellular $O_2^{\bullet-}$ and intracellular H_2O_2 production by stimulated polymorphonuclear cells (PMN) from dexamethasone (DEX)-treated rats (1·5 mg/kg per d during 5 d) and supplemented with either glutamine (GLN; DEX-GLN), GLN + S-methylthiourea (SMT) (DEX-GLN-SMT) or GLN + α-difluoromethyl-ornithine (DFMO) (DEX-GLN-DFMO)†

(Mean values and standard errors of the mean for nine rats per group)

	Oxidative metabolism (mV/5·10^5 cells)		Extracellular $O_2^{\bullet-}$ (pmol/10^6 PMN per min)		Intracellular H_2O_2 (arbitrary units)	
	Mean	SEM	Mean	SEM	Mean	SEM
DEX-GLN	152	24	1·41	0·20	11·2	0·6
DEX-GLN-SMT	40*	11	0·33*	0·11	3·3*	0·4
DEX-GLN-DFMO	40*	9	0·29*	0·10	4·8*	0·6

* Mean value within a column was significantly different from that of the DEX-GLN group: $P<0.05$ (by ANOVA and Newman–Keuls test, or Kruskal–Wallis test for extracellular $O_2^{\bullet-}$).
† For details of study groups and procedures, see Table 1 and p. 690.

no effect on oxidative metabolism, extracellular $O_2^{\bullet-}$ or H_2O_2 production by stimulated PMN (Table 3).

In the ARG-supplemented groups, oxidative metabolism was decreased in rats treated with SMT and DFMO (DEX-ARG-SMT, DEX-ARG-DFMO v. DEX-ARG; $P<0.05$). However, the three inhibitors did not significantly modify extracellular $O_2^{\bullet-}$ generation. Concerning intracellular H_2O_2 production by PMN, only SMT and DFMO abolished the enhancing effect of ARG (DEX-ARG-SMT, DEX-ARG-DFMO v. DEX-ARG; $P<0.05$) (Table 4).

In GLN-supplemented groups the use of SMT and DFMO abolished the GLN-induced increase in oxidative metabolism, extracellular $O_2^{\bullet-}$ generation and intracellular H_2O_2 production by PMN (DEX-GLN-SMT, DEX-GLN-DFMO v. DEX-GLN; $P<0.05$) (Table 5).

Discussion

The amino acids GLN and ARG are considered immunonutrients (De Bandt & Cynober, 1998; Calder & Yaqoob, 1999; Wu *et al.* 2000). This term was introduced to describe the effects of specific nutrients on immune functions. The present study, in agreement with the literature (Moffat *et al.* 1996, Moinard *et al.* 1999; Furukawa *et al.* 2000), shows the ability of GLN and ARG to increase respiratory burst of PMN from stressed rats. GLN is utilized by immune cells. In particular, Pithon Curi *et al.* (1997) have already characterized the glutaminase activity in PMN. This glutaminase activity was higher than that reported for lymphocytes and macrophages, which confirms the importance of GLN for the functioning of these cells. These authors also reported that less than 1 % GLN was totally oxidized, confirming that GLN is not only a fuel for these cells but may generate other metabolites (i.e. ARG or polyamines, which may play a key role in the cells). Concerning ARG, two pathways exist in PMN. First, the arginase pathway in which ARG is converted into urea and ornithine, which generates aliphatic polyamines

by the action of ornithine decarboxylase. Second, ARG is the substrate for NO$^\bullet$ synthesis; NO$^\bullet$ is synthesized by the action of NO$^\bullet$ synthase resulting in the formation of NO$^\bullet$ and citrulline (Cynober *et al.* 1995). In the present study we determined which of these metabolic pathways were involved at the whole body level in GLN- and ARG-mediated actions. For this purpose enzymic inhibitors were used. Their utilization did not modify respiratory burst in control groups (i.e. DEX-NEAA-treated groups). This result indicates that in basal conditions the metabolic inhibitors did not affect the parameters measured and that only the modifications induced by GLN or ARG were affected by the metabolic inhibitors.

The use of MSO showed that the action of ARG is not related to GLN synthesis. However, ARG stimulates respiratory burst through NO$^\bullet$ and polyamine pathways. Several studies have shown the ability of NO$^\bullet$ to control NADPH-dependent oxidase activity (the first enzyme involved in radical oxygen species production) (Seth *et al.* 1994; Catz *et al.* 1995; Fujii *et al.* 1997). However, this effect of NO$^\bullet$ on NADPH oxidase is controversial. These differences may be explained by the results of Pieper *et al.* (1994), who showed a biphasic effect of NO$^\bullet$ on NADPH-oxidase activity. For these authors this mediator has a stimulatory effect at low dose and an inhibitory effect at high dose. The effect of polyamines on ROS production was previously observed *in vitro* (Walters *et al.* 1998); treatment with DFMO produced dose-dependent inhibition of the respiratory burst in PMN. Our results confirm that *in vivo* ARG may be a polyamine precursor that in turn stimulates ROS production in stimulated PMN. Concerning GLN, we showed that it stimulates oxidative metabolism via NO$^\bullet$ and polyamines generation. But how does GLN generate NO$^\bullet$ and polyamines? Recently, several studies have investigated the relationship between GLN and NO$^\bullet$. O'Dowd & Newsholme (1997) have evidenced the involvement of GLN in NO$^\bullet$ production by stimulated PMN. They showed that the PMN possess the ability to produce ARG from GLN. However, these observations were made *in vitro*. For the first time our study has shown the ability of GLN supplementation *in vivo* to be an NO$^\bullet$ precursor in neutrophils.

To the best of our knowledge there are few available data on the relationship between GLN and polyamine pathways; Kandil *et al.* (1995) have established that GLN stimulates ornithine decarboxylase dose- and time-dependently in a porcine jejunal enterocyte cell line. These data suggest that GLN may be able to stimulate ornithine decarboxylase in neutrophils. Also, GLN could be a precursor of polyamine synthesis (see Fig. 1). This last point deserves further study.

In conclusion, we demonstrate that both ARG and GLN stimulate oxidative metabolism in stressed rats via NO$^\bullet$ and polyamine generation. However, the present study did not allow us to determine which one of these two pathways is predominant. Further study, using labelled molecules, is required to quantify the metabolic flux at the whole-body level and in PMN. It would be useful to determine whether these observations are specific to neutrophils or may be extended to other cells and tissues. GLN and ARG have many common actions on immunity

and protein metabolism. In practice, they are known to improve patient outcome, to enhance immunity and post-traumatic N metabolism. However, there are few data available on the mechanistic action of these nutrients in catabolic patients. Knowledge of their action would help optimize their use in clinical practice. Further studies are necessary to determine which of these two amino acids is more efficacious in catabolic patients.

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