Analysis of abnormalities in purine metabolism leading to gout and to neurological dysfunctions in man

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A modelling approach is used to analyse diseases associated with purine metabolism in man. The specific focus is on deficiencies in two enzymes, hypoxanthine:guanine phosphoribosyltransferase and adenylosuccinate lyase. These deficiencies can lead to a number of symptoms, including neurological dysfunctions and mental retardation. Although the biochemical mechanisms of dysfunctions associated with adenylosuccinate lyase deficiency are not completely understood, there is at least general agreement in the literature about possible causes. Simulations with our model confirm that accumulation of the two substrates of the enzyme can lead to significant biochemical imbalance. In hypoxanthine:guanine phosphoribosyltransferase deficiency the biochemical mechanisms associated with neurological dysfunctions are less clear. Model analyses support some old hypotheses but also suggest new indicators for possible causes of neurological dysfunctions associated with this deficiency. Hypoxanthine:guanine phosphoribosyltransferase deficiency is known to cause hyperuricaemia and gout. We compare the relative importance of this deficiency with other known causes of gout in humans. The analysis suggests that defects in the excretion of uric acid are more consequential than defects in uric acid synthesis such as hypoxanthine:guanine phosphoribosyltransferase deficiency.

INTRODUCTION

Purine metabolism is central to the production of nucleotides. Permanent imbalances of metabolites associated with this pathway can lead to numerous diseases that include hyperuricaemia, gout, immune disorders, neurological abnormalities and severe depression. The pathway is also the target of therapeutic intervention in cancer and some viral diseases.

In a previous paper we developed a model of purine metabolism to elucidate the functioning and malfunctioning of the purine pathway [1]. The metabolic scheme for this model is depicted in Scheme 1 (all abbreviations are defined in Tables 1 and 2). Here we use this model to analyse how neurological dysfunctions and gout might be related to specific biochemical abnormalities in purine metabolism.

Two enzyme deficiencies in purine metabolism are known to lead to neurological dysfunctions in man. The two affected enzymes are HGPRT [2] and ASLI [3]. Although the association between neurological dysfunction on one hand and biochemical deficiencies in these two enzymes on the other hand have been documented unambiguously, the mechanisms connecting cause and effect are unclear.

Neurological dysfunctions associated with HGPRT deficiency form a complex of symptoms, also referred to as Lesch–Nyhan syndrome (LNS), that typically include spasticity, choreoathetosis, mental retardation and self-mutilation. Several hypotheses have been proposed for the mechanisms that relate HGPRT deficiency with these neurological dysfunctions; they will be discussed in the text. There are also alternative hypotheses about neurological dysfunctions due to ASLI deficiency, including the accumulation of S-AMP and of succinylaminoimidazolecarboxamide ribotide (S-AICAR) and its dephosphorylation products succinyladenosine and succinylaminoimidazolecarboxamide riboside [3–5]. Both enzymes whose deficiencies produce neurological dysfunctions (HGPRT and ASLI) catalyse two reactions of the purine pathway. HGPRT catalyses the salvage of hypoxanthine (v_{hprt}) and guanine (v_{gprt}), and ASLI catalyses the second step in the conversion of IMP to AMP and the eighth step in the conversion of PRPP to IMP.

One of the goals of this analysis is to scrutinize some hypotheses about the biochemical mechanisms that might result in neurological dysfunction. The approach is a biomathematical analysis that offers the unique opportunity of simulating experiments that otherwise would be impossible or unethical in animals or humans. For instance, we show here that it is useful to simulate an organism that is deficient in the flux v_{gprt} but shows normal activity in the associated flux v_{hprt}.

The specific strategy is to set the model parameters in such a way that they correspond to physiological or disease states. By evaluating the model settings and the resulting symptoms, one can gain insight into the importance and sensitivity of a model component of interest. Because each of these components corresponds uniquely to a metabolite or to some kinetic property of the pathway, the mathematical results can be translated directly into biochemical and clinical terms. Of particular importance are parameters that relate to HGPRT and ASLI deficiencies. For instance, we test whether simultaneous deficiencies in the two reactions catalysed by HGPRT lead to synergistic effects or whether these effects are essentially additive.

It is known that hyperuricaemia and gout, which are associated with HGPRT deficiency, can also be caused by other abnormalities in purine metabolism [6]. These abnormalities can be grouped into those associated with a decreased excretion of UA and those involving an increased synthesis of UA. In the latter case, one can further classify symptoms in those caused by increased activity of the PRPPS, insensitivity of AMPD to the inhibition of guanylates and phosphate, or to deficiencies in HGPRT. Finally, superactivity of PRPPS can stem from two different causes, such as an insensitivity of the enzyme to purine nucleotide inhibition or an enhanced catalytic activity of the

Abbreviations used: LNS, Lesch–Nyhan syndrome; S-AICAR, succinylaminoimidazolecarboxamide ribotide. Abbreviations of metabolites and enzymes are listed in Tables 1 and 2.

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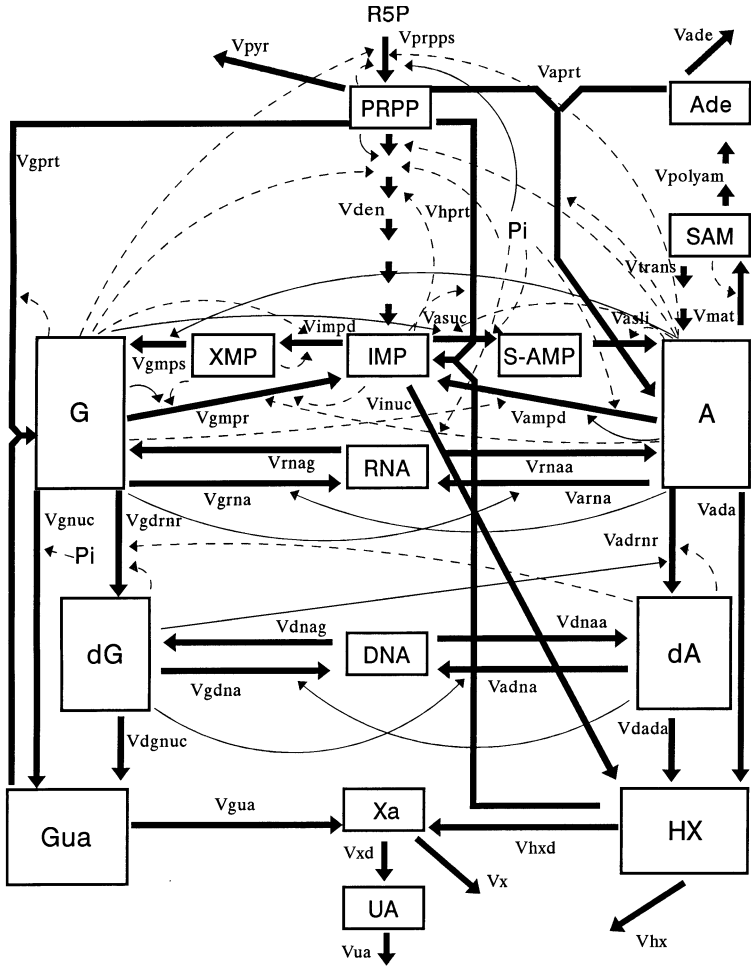


Scheme 1 Metabolic scheme of purine metabolism in man

The system consists of 16 dependent variables, 2 independent variables and 37 reaction steps. One can distinguish several major pathways within purine metabolism: (1) the synthesis of nucleotides *de novo*, which includes v_{prpps}, the ten reactions that constitute the linear pathway from PRPP to IMP (v_{den}), and the branch point at IMP where the formation of adenylates and guanylates is regulated; (2) the catabolism of nucleotides to the degradation products HX, Gua, Xa and UA, including their excretion; (3) the salvage pathway for purine rings, which includes v_{aprt}, v_{gprt} and v_{hprt} (these processes recover purine rings in the form of adenine, guanine and hypoxanthine respectively before they are excreted); (4) the synthesis and degradation of nucleic acids. Each dependent variable is represented by a box; independent variables, such as R5P and P_i, are represented without boxes. Fluxes are represented by heavy arrows and coded with mnemonic abbreviations. Regulatory signals are represented by thin arrows, with inhibitions as broken lines and activations as solid lines. Abbreviations of metabolites and enzymes are listed in Tables 1 and 2.

Table 1 Abbreviations for metabolites of purine metabolism (Scheme 1)

Abbreviation	Metabolite or pool
A	Pool including adenosine, adenosine mono-, di- and tri-phosphate
Ade	Adenine
dA	Pool including deoxyadenosine, deoxyadenosine mono-, di- and tri-phosphate
dG	Pool including deoxyguanosine, deoxyguanosine mono-, di- and tri-phosphate
DNA	Deoxyribonucleic acid
G	Pool including guanosine mono-, di- and tri-phosphate
Gua	Pool including guanine, guanosine and deoxyguanosine
HX	Pool including hypoxanthine, inosine and deoxyinosine
IMP	Inosine monophosphate
P_i	Phosphate
PRPP	Phosphoribosylpyrophosphate
R5P	Ribose 5-phosphate
RNA	Ribonucleic acid
S-AMP	Adenylosuccinate
SAM	*S*-Adenosyl L-methionine
UA	Uric acid

mutant enzyme [7]. In this paper we quantify the effect that each one of these causes has on the concentration of UA.

METHODS

All results in this paper are based on mathematical analyses and computer simulations of the model depicted in Scheme 1. This model was previously formulated [1] as a power-law model in the form of a generalized mass action system within the framework of biochemical systems theory [8–12].

Model characteristics used to assess the relative importance of different causes of gout were parameter sensitivities and logarithmic gains. These are defined as percentage increases or decreases in the concentration of a variable after introducing a 1% increase in one of the parameters of the kinetic rate law, in an enzyme or in the concentration of an external effector. Sensitivities were formulated in the context of biochemical systems theory [13]. Numerical values of logarithmic gains were computed with the program ESSYNS [14], and parameter

Table 2 Abbreviations for enzymes of purine metabolism (Scheme 1)

Abbreviated flux	Enzyme abbreviation	Enzyme or reaction	EC number
V_{ada}	ADA	Adenosine deaminase	3.5.4.4
V_{ade}		'Adenine oxidation' (xanthine oxidase)	1.2.3.2 or 1.2.1.37
V_{adna}	DNAP	DNA polymerase (from deoxyATP)	2.7.7.7
V_{adrnr}	DRNR	Diribonucleotide reductase	1.17.4.1
V_{ampd}	AMPD	AMP deaminase	3.5.4.6
V_{aprt}	APRT	Adenine phosphoribosyltransferase	2.4.2.7
V_{arna}	RNAP	RNA polymerase (from ATP)	2.7.7.6
V_{asuc}	ASUC	Adenylosuccinate synthetase	6.3.4.4
V_{asli}	ASLI	Adenylosuccinate lyase	4.3.2.2
V_{dada}	ADA	Adenosine deaminase	3.5.4.4
V_{den}	ATASE	'*De novo* synthesis' (amidophosphoribosyltransferase)	2.4.2.14
V_{dgnuc}	3NUC	5′(3′) Nucleotidase	3.1.3.31
V_{dnaa}	DNAN	DNases (to dA)	Several enzymes
V_{dnag}	DNAN	DNases (to dG)	Several enzymes
V_{gdna}	DNAP	DNA polymerase (from deoxyGTP)	2.7.7.7
V_{gdrnr}	DRNR	Diribonucleotide reductase	1.17.4.1
V_{gmpr}	GMPR	GMP reductase	1.6.6.8
V_{gmps}	GMPS	GMP synthetase	6.3.4.1
V_{gnuc}	5NUC	5′-Nucleotidase	3.1.3.5
V_{gprt}	HGPRT	Hypoxanthine:guanine phosphoribosyltransferase	2.4.2.8
V_{grna}	RNAP	RNA polymerase (from GTP)	2.7.7.6
V_{gua}	GUA	Guanine deaminase	3.5.4.3
V_{hprt}	HGPRT	Hypoxanthine-guanine phosphoribosyltransferase	2.4.2.8
V_{hx}		'Hypoxanthine excretion'	Non-enzymic step
V_{hxd}	XD	Xanthine oxidase or xanthine dehydrogenase	1.2.3.2 or 1.2.1.37
V_{impd}	IMPD	IMP dehydrogenase	1.1.1.205
V_{inuc}	5NUC	5′-Nucleotidase	3.1.3.5
V_{mat}	MAT	Methionine adenosyltransferase	2.5.1.6
V_{polyam}	SAMD	'Polyamine pathway' (*S*-adenosylmethionine decarboxylase)	4.1.1.50
V_{prpps}	PRPPS	Phosphoribosylpyrophosphate synthetase	2.7.6.1
V_{pyr}		'Pyrimidine synthesis'	Several enzymes
V_{rnaa}	RNAN	RNases (to AMP)	Several enzymes
V_{rnag}	RNAN	RNases (to GMP)	Several enzymes
V_{trans}	MT	'Transmethylation pathway' (Protein O-methyltransferase)	2.1.1.24
V_{ua}		'Uric acid excretion'	Non-enzymic step
V_{x}		'Xanthine excretion'	Non-enzymic step
V_{xd}	XD	Xanthine oxidase or xanthine dehydrogenase	1.2.3.2 or 1.2.1.37

sensitivities with MATHEMATICA ([15], p. 961). The differential equations were solved numerically with the program MIST [16].

RESULTS

Relative importance of HGPRT deficiency as a cause of gout

Gout can have a number of causes. Many of them are found in purine metabolism but it is noted that there are others such as the Von Gierke syndrome and increases in glutathione reductase activity [6]. Five types of gout have been traced back to disorders of purine metabolism: (1) increased catalytic activity of PRPPS, (2) insensitivity of PRPPS to inhibition by purine nucleotides, (3) HGPRT deficiency, (4) insensitivity of AMPD to inhibition by guanylates and phosphate, and (5) dysfunctional renal excretion of UA, which is not a biochemical phenomenon but is none the less one of the major causes of gout in humans.

Each of these disorders can be characterized numerically in the model with a parameter sensitivity or logarithmic gain that quantifies by how much the steady-state concentration of UA is affected.

1. The effect of the activity of PRPPS on the steady-state concentration of UA is given by the logarithmic gain or control coefficient of UA with respect to PRPPS. This value is 0.403, which implies that a 1% increase in the PRPPS activity will increase the UA concentration by only 0.403%.

2. The effect of PRPPS inhibition by nucleotides on the steady-state concentration of UA is quantified by the sensitivities of UA with respect to the parameter that quantifies the effect of the adenylate and guanylate pools in the PRPPS reaction. These values are −1.4 and −0.096 respectively, predicting that a 1% decrease in one of these kinetic parameters would increase the UA concentration by 1.4% or 0.096%.

3. The effect of HGPRT activity on the steady-state concentration of UA is quantified by the logarithmic gain or control coefficient of the UA concentration with respect to changes in HGPRT. This value is −0.177, indicating that a 1% decrease in HGPRT activity evokes an approx. 0.177% increase in UA concentration.

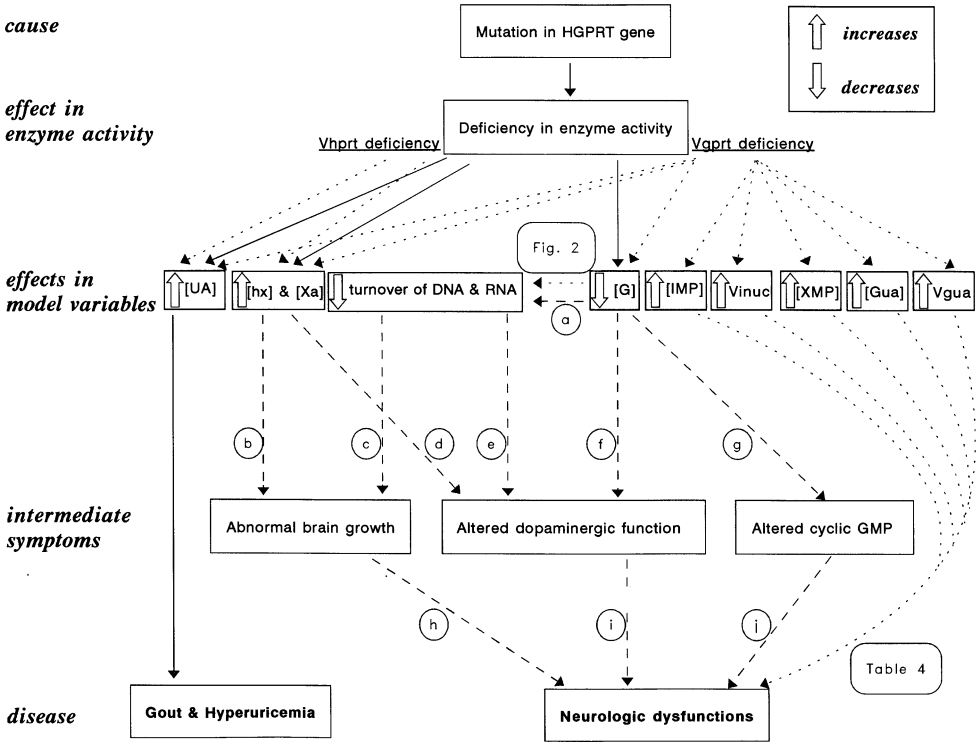
4. The effect of AMPD inhibition by guanylates and phosphate on the steady-state concentration of UA is quantified by the sensitivity of UA to the parameter that quantifies the effect of the guanylate pool and phosphate in the AMPD reaction. These values are −0.03 and −0.11 respectively, predicting that a 1% decrease in one of these kinetic parameters would increase the UA concentration by merely 0.03% or 0.11%.

5. The effect of reduced renal excretion is quantified by the sensitivity of UA to the parameter that quantifies the effect of UA on its own excretion (v_{ua}). This sensitivity in our model is

Table 3 Uric acid concentration after simulation of a 2-fold change in some parameters producing gout in humans

The normal UA concentration is 100 μM.

Parameter	Original value	Test value	Resultant UA concentration (μM)
PRPPS activity	0.899	1.798	131
Inhibition of PRPPS by adenylates and guanylates	−0.45 and 0.04	−0.225 and −0.02	195
HGPRT activity	12250	6125	112
Inhibition of AMPD by guanylates and phosphate	−0.03 and −0.1	−0.015 and −0.05	108
Effect of UA on its own excretion	2.21	1.105	10052



Scheme 2 Schematic representation of possible mechanisms responsible for diseases associated with HGPRT deficiency

Depicted are demonstrated relationships (solid arrows), hypothesized relationships (broken arrows) and relationships suggested in this work (dotted arrows). References to hypothesized relationships are: (a) [27–29]; (b) [20]; (c) [22]; (d) [20]; (e) [25]; (f) [17]; (g) [18]; (h) [20,21]; (i) [25]; (j) [18]. Relationships suggested by our model do not extend to secondary consequences of enzyme deficiencies because the corresponding secondary variables are not included in the model.

−4.6, which means that a 1 % decrease in the kinetic parameter of UA in v_{ua} leads to a 4.6 % increase in the concentration of UA.

These results suggest that a dysfunctional renal excretion of UA might be the most significant cause of gout in humans. A 1 % decrease in excretion leads to an almost 5-fold amplified increase in UA, whereas changes of the same magnitude in PRPPS activity, HGPRT activity, inhibition of adenylates over PRPPS, inhibition of guanylates over PRPPS, inhibition of guanylates over AMPD and inhibition of phosphate over AMPD seem to have considerably smaller effects on the concentration of UA.

Sensitivities and logarithmic gains (control coefficients) are, strictly speaking, defined for infinitesimally small changes from the physiological concentration (the so-called operating point). Although experience has shown that they are good predictors for changes of a few per cent, it is appropriate to analyse the effects of larger changes in each one of the above contributors to gout. The results of these analyses, presented in Table 3, demonstrate the reliability of the infinitesimal predictors. Even for larger changes, a dysfunctional renal excretion of UA has by far the strongest effect on the concentration of UA.

HGPRT deficiency as a cause of neurological dysfunctions

Numerous hypotheses have been proposed about the biochemical mechanisms that relate HGPRT deficiency to neurological dysfunctions as they are manifested in LNS. Generally accepted hypotheses include: GTP depletion [17–19], retarded cell division during brain development [20,21], altered levels of cGMP [18], increased concentrations of xanthine and hypoxanthine in the central nervous system [6,20,22], and altered levels of some neurotransmitters [23–26]. These hypotheses are not mutually exclusive but in some cases are consequences of each other.

An explanation of neurological dysfunctions in HGPRT

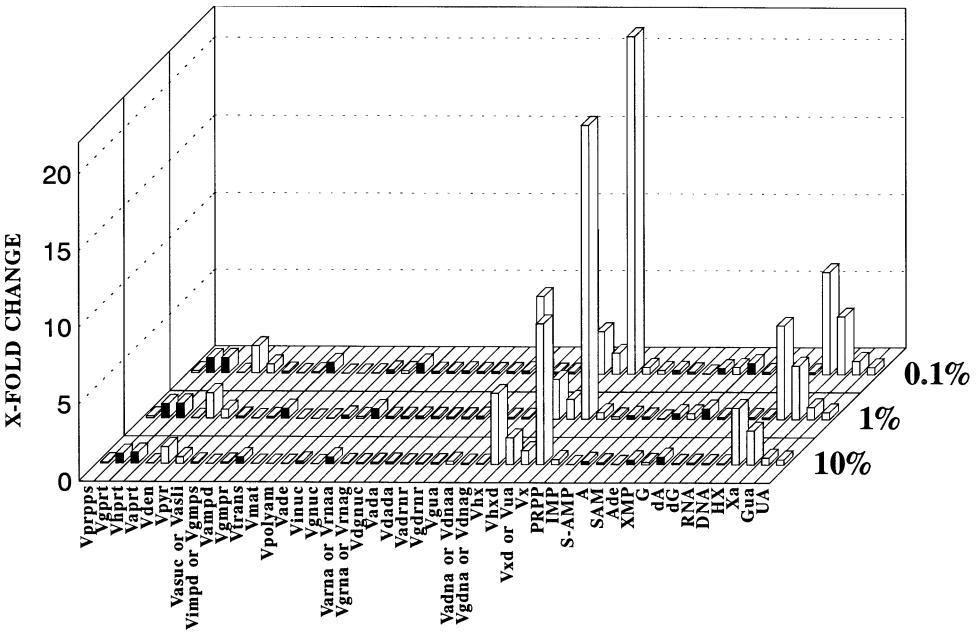


Figure 1 Multiplicative increases (light bars) and decreases (solid bars) for fluxes and concentrations within purine metabolism after simulations of 10%, 1% and 0.1% of normal activity in HGPRT

deficiency should ideally provide a complete chain of cause–effect relationships, beginning with the mutation of the enzyme and ending with a characterization of the neurological dysfunctions. Although such conclusive sequences of events are not fully established, Scheme 2 summarizes at least some primary mechanisms of diseases associated with HGPRT deficiency. Our biomathematical analysis provides a powerful tool for screening these mechanisms and for detecting patterns that relate biochemical abnormalities to physiological or neurological dysfunctions. The present model does not shed light on secondary consequences of the enzyme defect because these variables are not included in the model.

It is well known that partial deficiencies of HGPRT lead to hyperuricaemia and gout. If the enzyme catalyses less than 2% of normal activity, neurological problems, including mental retardation, appear. Moreover, if the enzyme activity is less than 0.2% of normal, patients show full-blown LNS, including self-mutilation [6].

Results of simulated 10%, 1%, and 0.1% activities of HGPRT are shown in Figure 1. Whereas it is evident that these marked deficiencies have an impact on most of the fluxes and metabolites of purine metabolism, some of the variables and fluxes are clearly more affected than others. Particularly noteworthy are: increases in fluxes of excretion of purines (v_{hx}, v_x and v_{ua}), increases in the two steps catalysed by xanthine dehydrogenase (v_{xd} and v_{hxd}), increases in the *de novo* synthesis of purines (v_{den}), increases in the concentrations of free purines (HX, Xa, Gua and UA), decreases in fluxes affecting degradation of the guanylate pool (v_{gmpr} and v_{gnuc}), and decreases in the concentration of guanylates (G). Some of these direct effects of HGPRT deficiency have been documented or hypothesized by others as responsible for neurological dysfunctions in HGPRT deficiency. In particular, the importance of GTP depletion has been noted [17–19], and increased concentrations of xanthine and hypoxanthine in the central nervous system have been suggested [6,20,22] as a cause of neurological dysfunction. Other suggestions have included a decreased synthesis of DNA and RNA, which would diminish cell division during brain development and the arborization of dendrites and dopamine terminals [19,20,25]. These suggestions are supported by our simulation results, which indicate decreases in concentrations and turnovers of nucleic acids (v_{arna}, v_{grna}, v_{rnaa}, v_{rang}, v_{adna}, v_{dnaa}, v_{gdnaa}, v_{dnag}, RNA and DNA).

It has been documented that the availability of guanylates is the rate-limiting step for the synthesis of nucleic acids [27–29]. Beyond this qualitative observation it is important to quantify the strength of guanylates in the control of the turnover of nucleic acids. If one can demonstrate that a decrease in nucleic acid turnover is only a consequence of guanylate pool depletion, the two main hypotheses shown in Scheme 2 are in fact two manifestations of the same root problem: the depletion of the guanylate pool.

To investigate this question, we performed simulations in which we subjected originally healthy subjects to an artificially decreased guanylate pool of 127 μM, which corresponds to the guanylate pool achieved in the simulation of 1% activity in the HGPRT deficiency (see [1]). This theoretical experiment cannot yet be implemented in animals or humans because it has so far proved impossible to maintain an artificially lowered or increased guanylate pool over an extended period of time. The results of this modelling experiment, as shown in Figures 2(a) and 2(b), strongly suggest that guanylate pool depletion is indeed responsible for essentially the entire decrease in DNA turnover and for most of the decrease in RNA turnover (96%). The effect of guanylate depletion is not the same for RNA and DNA turnover. For instance, there are large differences in the time span necessary for the variables to approach their steady-state values. In addition to these effects, the simulation suggests that guanylate pool depletion has some impact on HX accumulation, increases in v_{inuc}, decreases in v_{gmpr}, increases in IMP and XMP, and on some other variables that are less affected.

One could therefore expect that in LNS patients the turnover of DNA and RNA would return to normal if the guanylate pool

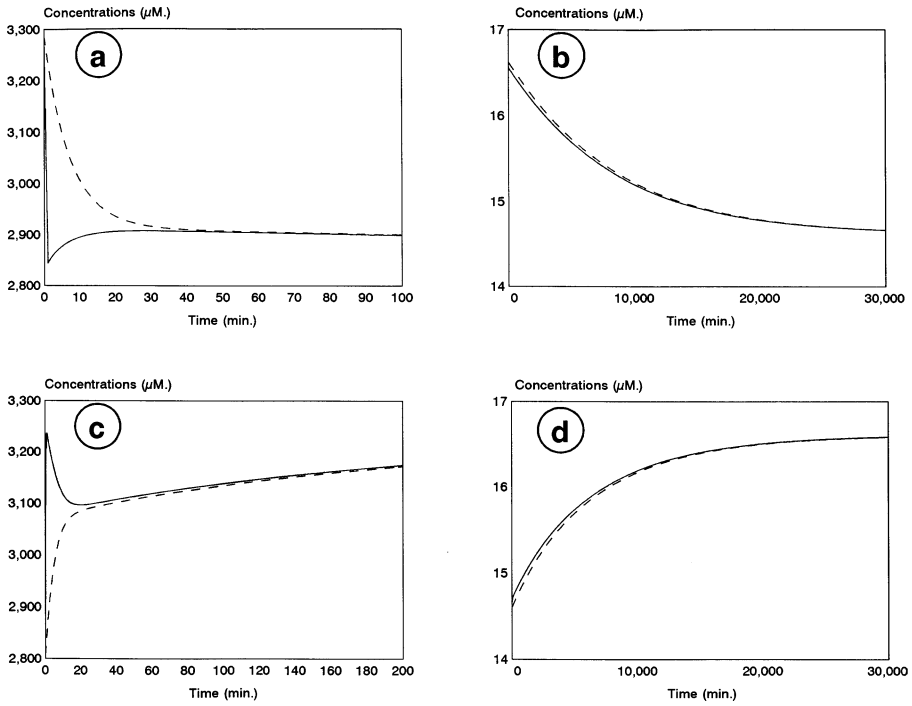


Figure 2 Dynamic behaviour of RNA and DNA after permanent changes in the guanylate pool

For modelling healthy subjects, the guanylate pool was permanently decreased to 127 μM. Responses of RNA and DNA pools are shown in (**a**) and (**b**) respectively, with solid lines representing synthesis of nucleic acids and broken lines representing their degradation. In a simulation of HGPRT deficiency, the guanylate pool was permanently increased to 400 μM. Responses of RNA and DNA pools are shown in (**c**) and (**d**) respectively. RNA and DNA turnover rates in healthy subjects are 3300 and 16.8 μmol/min per g body weight respectively, and in HGPRT-deficient patients they were 2819 and 12 μmol/min per g body weight respectively. See the text for further details.

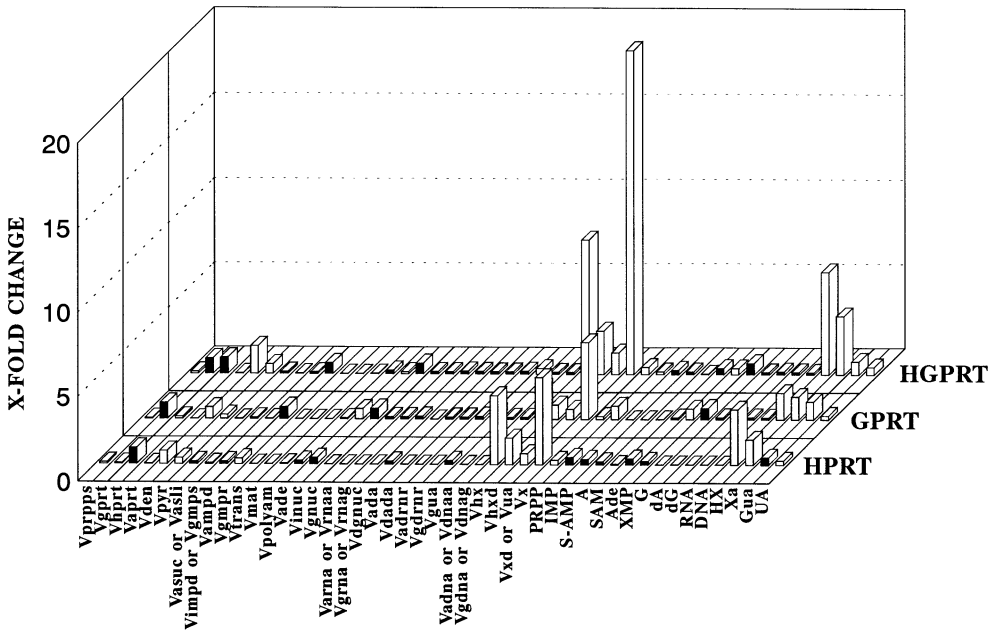


Figure 3 Multiplicative increases (light bars) and decreases (solid bars) for fluxes and concentrations within purine metabolism after simulation of 1% activity in v_{hprt} with 100% activity in v_{gprt} (HPRT), 1% activity in v_{gprt} with 100% activity in v_{hprt} (GPRT), and 1% activity in both v_{hprt} and v_{gprt} (HGPRT)

could be re-established at its normal concentration. To test this we performed a second model experiment. Beginning with a (simulated) HGPRT-deficient individual, we reset the concentration of the guanylate pool to a constant, normal concentration of 400 μM, letting all other variables follow the dynamics given by the metabolic system. The model responses pertaining to

Table 4 Summary of results of the model analyses

1. Metabolites and fluxes affected by v_{gprt} deficiency but not affected by v_{hprt} deficiency
   - Decreases in the concentrations of deoxynucleotides and nucleic acids and the corresponding turnover rates (dA, dG, RNA, DNA, V_{adrnr}, V_{gdrnr}, V_{dada}, V_{dgnuc}, V_{arna}, V_{grna}, V_{rnaa}, V_{rang}, V_{adna}, V_{dnaa}, V_{gdnaa} and V_{dnag})
   - Decreases in guanylate concentrations and their dephosphorylation flux (G and V_{gnuc})
2. Metabolites and fluxes affected by v_{hprt} deficiency but not affected by V_{gprt} deficiency
   - Decreases in adenylosuccinate and adenylate concentrations and in adenylate catabolism (S-AMP, A, V_{ada}, V_{asuc} and V_{asli})
3. Metabolites and fluxes oppositely affected by V_{gprt} deficiency and V_{hprt} deficiency
   - Increases in Gua, IMP and XMP concentrations
   - Increases in V_{gua} and V_{inuc}
   - Decrease in V_{gmpr}
4. All other metabolites and fluxes are either unaffected by HGPRT deficiency or affected by both reactions in a similar fashion

RNA and DNA are shown in Figures 2(c) and 2(d). They show that the forced re-establishment of normal concentrations of guanylates in patients with LNS results in the re-establishment of normal turnover of nucleic acids. The time scales for RNA and DNA again are quite different. These results suggest that guanylate pool depletion and decreased turnover of nucleic acids are different manifestations of the same mechanism leading to neurological dysfunctions.

Two reactions are catalysed by HGPRT: v_{hprt} and v_{gprt}. Figure 1 indicates that a deficiency in either reaction affects almost all metabolites and fluxes of purine metabolism. A third reaction that recycles purine free bases to nucleotides is catalysed by adenine phosphoribosyltransferase (v_{aprt}), which recycles adenine to AMP. Simulation of a drastically decreased activity to 1 % in this enzyme results in almost no changes in purine metabolism, and only the concentration and excretion of adenine are affected by this deficiency [1]. These findings are consistent with clinical observations on patients whose only symptom is an increased concentration of the adenine degradation products 8-hydroxyadenine and 2,8-dihydroxyadenine [30,31].

One could surmise that only one of the two reactions v_{gprt} and v_{hprt} might be responsible for symptoms associated with HGPRT deficiency. To test this hypothesis experimentally or clinically seems to be very difficult because patients with HGPRT deficiency always seem to be similarly affected in both reactions at the same time. In contrast, such a test is readily executed in our biomathematical model, because we can 'create' a hypothetical patient deficient in v_{hprt} but normal with respect to v_{gprt} or vice versa.

We performed model analyses with a 1 % activity only in v_{gprt} and with a 1 % activity only in v_{hprt}, retaining the other reaction catalysed by HGPRT at the normal level. The results in Figure 3 suggest that not just one reaction is responsible for all the observed changes in metabolites and fluxes; in general, both reactions, v_{gprt} and v_{hprt}, have similarly strong effects on purine metabolism. However, a closer look qualifies this overall pattern, and we can find several metabolites and fluxes that are affected by only one of the two reactions. A summary of results is given in Table 4.

Two current hypotheses allege that GTP depletion and decreased turnover of nucleic acids are the predominant causes of neurological dysfunctions associated with purine metabolism. Our findings imply that these two causes might be the consequence solely of a low activity in v_{gprt}. In fact, the modelling results suggest that v_{gprt} deficiency leads primarily to guanylate pool depletion and secondarily to a decrease in turnover of nucleic acids. The third hypothesis postulates increased concentrations of xanthine and hypoxanthine in the central nervous system. Figure 3 suggests that increases in these metabolites are due to an additive effect of the two reactions catalysed by HGPRT.

Table 5 Activities of V_{gprt} and V_{hprt} in different families with HGPRT deficiency and degree of neurological dysfunction

Values in columns V_{gprt} and V_{hprt} are the means for the family, expressed as percentages of normal activity, in erythrocyte haemolysates. The individuals within each family have similar values of V_{gprt}, V_{hprt} and the ratio of V_{gprt} to V_{hprt}.

Family	V_{gprt} (%)	V_{hprt} (%)	V_{gprt}/V_{hprt}	Neurological dysfunctions	Reference
I family	0.09	0.06	1.5	No	[41]
D family	17.8	12.6	1.41	No	[41]
M family	3.8	2.8	1.37	No	[41]
T family	0.075	0.07	1.03	No	[41]
S family	9.8	10.2	0.96	Borderline	[41]
G family	8.4	9.6	0.87	No	[41]
N family	0.31	0.36	0.87	No	[41]
R family	0.5	0.9	0.55	Borderline	[41]
J family	0.76	1.6	0.49	Borderline	[41]
S_2 family	0.009	0.03	0.3	Yes	[41]
L family	0.51	10.6	0.05	Yes	[41]
LNS	< 0.004	< 0.01	'0.4'	Yes	[41]
5-6	0.87	1.75	0.5	No	[42]
E. L.	4	9	0.45	Yes	[43]
II	1.85	9.6	0.19	Yes	[44]
P	12.3	97.3	0.13	Yes	[45]

At present no definite conclusions can be drawn from the literature about the relative significance of v_{gprt} and v_{hprt}; however, clinical data provide some circumstantial evidence. HGPRT deficiency can be the result of a large number of different mutations [19,32,33], and it is to be expected that these mutations might differ in their effects on v_{hprt} and v_{gprt}. Thus one should expect to find HGPRT-deficient patients with different degrees of deficiency in either v_{gprt} or v_{hprt}, and potentially with correlated degrees of neurological dysfunction. Indeed, there are a few clinical studies that provide these types of data. In Table 5 we summarize studies executed with erythrocyte haemolysates. Other investigations have used intact fibroblasts [34] but the authors mention methodological problems with measuring v_{gprt}; this caused us not to include these data in Table 5.

Most clinical data pertaining to v_{gprt}, v_{hprt} and the degree of documented neurological dysfunctioning in HGPRT-deficient patients consist of HGPRT measurements in erythrocyte haemolysates [35]. Several attempts have been made to correlate the enzyme activities with neurological dysfunctions but no convincing results have been obtained [34,35]. Some patients with

gout as their only clinical symptom showed lower enzyme activities than patients with neurological dysfunctions. Examples are patient E. L. and patient II, who showed relatively high enzyme activities and marked neurological dysfunctions, and the I family and the T family, who exhibited very low enzyme activities without neurological dysfunctions.

Better results are obtained if one considers the ratio of v_{gprt} to v_{hprt} instead of one of the two activities alone. It seems that the lower this ratio is in erythrocyte lysates, the higher is the risk of developing neurological dysfunctions (see Table 5). Furthermore it seems that v_{gprt} has an important role in the development of neurological dysfunctions in HGPRT-deficient patients but that v_{hprt} deficiency has a counteracting effect. It is noted that Page et al. [34], also pointed out the importance of considering both v_{gprt} and v_{hprt}, as well as their ratio, even though their results and ours are different. The differences might be due to the fact that Page et al. used intact fibroblasts but also to their self-acknowledged methodological problems in the measurements of v_{gprt}. Their results with respect to the ratio v_{gprt}/v_{hprt} are therefore not quite comparable with those obtained with erythrocyte lysates and have not been taken into account for our conclusions. Obviously further research in this area is needed for definite conclusions.

The model suggests that six characteristics of purine metabolism are affected by the ratio of v_{gprt} to v_{hprt} (Figure 3). None of these has previously been postulated as a responsible contributor to neurological dysfunctions in LNS. One of these changes, the decrease in the flux of GMP reductase, is not very likely to be of importance because the flux of v_{gmpr} is not significant in the central nervous system. The remaining changes are related to increases in the concentrations of IMP, XMP and Gua, and to increases in the fluxes v_{gua} and v_{inuc}. Thus we can formulate as a new hypothesis that some or all of these metabolites and fluxes might be contributors to neurological dysfunctions. Scheme 2 shows how this hypothesis fits into the overall picture of LNS.

To test whether only the ratio of v_{gprt} and v_{hprt} is significant or whether the absolute values of these fluxes are important, we performed simulations with the same ratio of v_{gprt} to v_{hprt} but changing enzyme activities. These simulations suggest that the variables mainly affected by the ratio are IMP, XMP, Gua, v_{inuc} and v_{gua}, and that the variables not affected by the ratio of fluxes are HX and Xa (results not shown). This implies that HX and Xa are not correlated with neurological dysfunctions in a readily recognizable pattern.

It should be noted that in some of these simulations IMP, XMP, Gua, v_{inuc} and v_{gua} showed decreases instead of increases in comparison with the normal concentrations. No patient with LNS has been reported with one or some of these variables decreased instead of increased, and this reinforces our hypothesis that the biochemical mechanisms leading to neurologic dysfunctions are related to increases in IMP, XMP, Gua, v_{inuc} or v_{gua}. To analyse this aspect further, we performed simulations that corresponded to the percentage activities of v_{gprt} and v_{hprt} in each of the families or patients in Table 5. The five metabolites or fluxes proposed above as possible contributors to neurological dysfunctions were increased in all simulations (results not shown).

Clinical findings suggest that neurological dysfunctions are associated only with severe enzyme deficiencies. As a quantitative hypothesis, one could deduce that variables responsible for neurological dysfunctions might fulfil two requisites: (1) these variables should be almost unaffected as long as the enzyme activities are larger than 10 % of normal, and (2) they would be increasingly affected if enzyme activities fell below 10 % of normal. In contrast, gout also accompanies smaller enzyme deficiencies, which would suggest that the metabolites and fluxes associated with gout and hyperuricaemia (HX, Xa and UA) would change more gradually over the possible range of enzyme activities.

It seems that significant clinical symptoms are observed if enzyme activities fall below approx. 10 % of their normal activities. To test which variables of the model would lead to such extreme decreases in activity, we simulated nine degrees of HGPRT deficiency in both reactions and plotted the value of each metabolite or flux against the deficient enzyme activity. The results are presented in Figure 4. The figure suggests that UA does not increase significantly even if the enzyme activity falls below 10 % of normal. Furthermore Xa and HX change gradually when the enzyme activity is decreased from 100 % to 0 %. In contrast, other metabolites and fluxes fulfil both criteria previously mentioned. They change only slowly or remain essentially constant if HGPRT deficiency is not too severe, but change quite markedly in the low activity range (from 10 % to 0 %) of HGPRT. This effect pattern is in agreement with manifestations of neurological dysfunctions in patients within this range of HGPRT deficiency. This fact is also consistent with recent work on potential contributors to neurological dysfunctions, which focuses less and less on the accumulation of Xa and HX.

ASLI deficiency as a cause of neurological dysfunctions

ASLI deficiency and HGPRT deficiency are similar in many ways. Like HGPRT, ASLI catalyses two reactions of the purine pathway, namely the conversion of S-AICAR into aminoimidazolecarboxamide ribotide, which constitutes the eighth of the ten reactions of the synthesis of purines *de novo* (v_{den}), and that of S-AMP into AMP (v_{asli} in Scheme 1). Deficiencies in ASLI also can lead to neurological abnormalities, such as severe mental retardation and autism. The symptomatology of ASLI deficiency is not the same as that of HGPRT, but it is quite similar. This suggests that the mechanisms resulting in mental retardation might be closely related in the two types of deficiency. As with HGPRT deficiency, there are two degrees of deficiency that, in this case, are accompanied by different degrees of mental retardation. The degree of neurological dysfunction has been demonstrated to be closely related to the ratio of v_{den} to v_{asli} [36,37].

Two subtypes of the deficiency have previously been distinguished. Type I entails severe mental retardation; the ratio of succinyladenosine to aminoimidazolecarboxamide riboside in this case is approx. 1, and the activities of v_{den} and v_{asli} are both approx. 30 % of the normal level in fibroblasts. Type II is characterized by slightly decreased mental acuteness; the ratio of succinyladenosine to aminoimidazolecarboxamide riboside is approx. 4, the activity of v_{den} is approx. 30 %, and the activity of v_{asli} is only approx. 3 % of the physiological level in fibroblasts. These clinical observations suggest that in ASLI deficiency the activity of v_{den} is mainly responsible for mental retardation. It has furthermore been hypothesized that v_{asli} deficiency is protective against the consequences of v_{den} deficiency [4].

We modelled 1 % activity in v_{asli}, leaving the activity in v_{den} at 100 %. The new steady state achieved under these conditions turned out to be exactly the same as that for healthy subjects, with only one exception, namely that the concentration of S-AMP had increased to 21 μM from 0.2 μM for normal subjects (results not shown).

We did not expand the model to account for the ten reaction steps in the flux v_{den} (see Scheme 1) and to simulate the deficiency of ASLI for the substrate S-AICAR, because theoretical considerations tell us what the results would be. In order for one variable inside the *de novo* pathway to affect some other variables of the purine metabolism pathway, the logarithmic gain of this

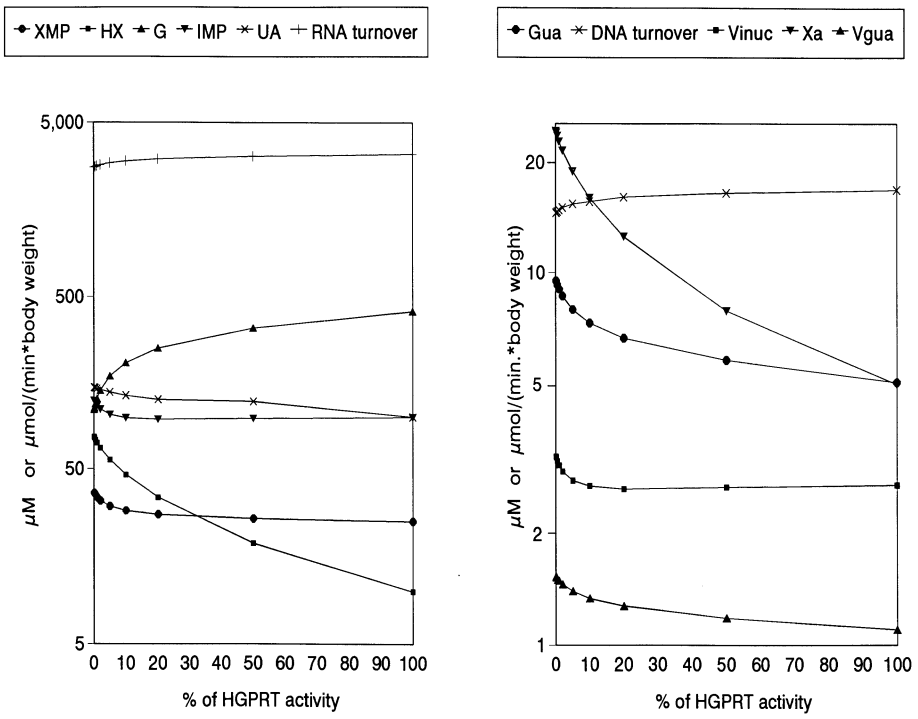


Figure 4 Changes in variables proposed as primary causes of neurological dysfunctions in HGPRT deficiency

The plots were obtained for 100%, 50%, 20%, 10%, 5%, 2%, 1%, 0.5%, 0.2% and 0.1% HGPRT activity in both reactions catalysed by the enzyme. Units on the *y*-axis are μM for metabolites and μmol/min per g body weight for fluxes. See [1] for unit details.

variable with respect to the flux (v_{den}) must be different from 0. However, in a linear pathway in which no dependent variable modifies the first step, the logarithmic flux gains of the pathway correspond exactly to the kinetic orders of the first reaction [1]. Thus no enzyme inside the *de novo* pathway can change the overall flux of v_{den} except the first enzyme of this pathway (amidophosphoribosyltransferase), and consequently none of these enzymes can affect any of the variables of purine metabolism. The only variable that could be affected by a deficiency of ASLI in catalysing S-AICAR is the concentration of substrate (S-AICAR), which would be increased. This is indeed observed in the simulation of v_{asli} deficiency because the pathway leading from IMP to A is strictly linear and no variable of the linear S-AMP pathway (S-AMP) affects the first reaction of this pathway (see Scheme 1).

These considerations lead to the following conclusion: if no metabolite inside the *de novo* pathway affects the enzyme amidophosphoribosyltransferase, and if S-AMP does not affect the flux v_{asuc}, the only variables in purine metabolism affected by ASLI deficiency are its own substrates, S-AMP and S-AICAR. This theoretical result is consistent with three pieces of evidence from the literature.

1. Clinical measurements were performed in patients with ASLI deficiency, and the only documented metabolic abnormalities were found to be increased concentrations of S-AMP and S-AICAR and its dephosphorylated products (succinyladenosine and succinylaminoimidazolecarboxamide riboside) [3,5,38]. One can conclude from our model results, combined with these findings, that the mechanisms leading to neurological dysfunctions in patients with either HGPRT or ASLI deficiency are not the same and not even closely related, because HGPRT deficiency results in a decreased rather than an increased concentration of S-AMP.

2. Our results support the hypothesis that accumulation of S-AMP and S-AICAR and/or of their dephosphorylated derivatives, rather than changes in other variables of purine metabolism, leads to neurological dysfunctions in ASLI deficiency. Thus the mechanisms responsible for neurological dysfunctions in ASLI deficiency are likely to be associated with those metabolites.

3. Finally, the model approach supports the finding that neither of the two reactions catalysed by ASLI is the rate-limiting step in the synthesis of purines [4].

DISCUSSION

The results of our analyses yield a tentative ranking of the relative importance of the primary metabolic dysfunctions that lead to hyperuricaemia and gout. These dysfunctions, listed in decreasing order of importance, are:

(1) changes in the effect of UA on its own excretion;

(2) changes in the inhibition of PRPPS by adenylates and guanylates;

(3) changes in PRPPS activity;

(4) changes in HGPRT activity; and

(5) changes in the inhibition of AMPD by guanylates and phosphate.

These changes and their implications are discussed below.

The model analysis yields the result that the kinetic parameter representing the effect of UA on its own excretion is the most influential factor, and that small defects in the excretion of UA can produce a large accumulation of this metabolite. Increases in

the synthesis of UA also lead to accumulation and are recognized as important causes of gout [7]. However, the model suggests that increased synthesis has smaller consequences than decreased excretion. This theoretical result is indeed supported by radioisotopic studies that have shown that only 10 % of gouty subjects overproduce UA. Statistically, one would expect that many more subjects would have defects in the synthesis of UA than in its excretion, because UA synthesis involves more individual processes than UA excretion. Consequently there is much greater chance of mutations affecting UA synthesis than UA excretion. However, most of the deficiencies in UA synthesis are inconsequential and probably remain undetected, and only the most severe dysfunctions are diagnosed when a subject presents with gout. In contrast, even small defects in excretion can produce a significant accumulation of UA and are thus detected.

These causes of gout listed in decreasing order let us predict which magnitude of the primary cause or defect will be necessary to deal with the symptoms of gout. For example, patients with PRPPS superactivity show symptoms of gout with no more than a 2-fold increase in the enzyme activity [39,40] (the logarithmic gain of UA with respect to PRPPS is 0.4). However, patients with HGPRT deficiency develop gout at an approx. 10-fold decrease in their enzyme activity [6], which corresponds to a lower logarithmic gain of UA with respect to HGPRT (-0.177).

Two different pathophysiologies are associated with HGPRT deficiency. The first includes direct metabolic abnormalities as they are manifested in gout, such us overproduction of purines, increased concentrations of UA, xanthine and hypoxanthine, and increased excretion of these metabolites. Figures 1 and 4 imply that these abnormalities are present even if the enzyme deficiency is minor, and that all relevant metabolites and fluxes (HX, Xa, UA, v_x, v_{hx}, v_{ua} and v_{den}) are altered even in cases of moderate HGPRT deficiency. This theoretical result agrees with the clinical observation that patients with enzyme activities between 2 % and 20 % show symptoms of gout and hyperuricaemia. Figure 3 shows that the metabolic abnormalities associated with HGPRT deficiency are due to deficiencies in both reactions catalysed by this enzyme, v_{gprt} and v_{hprt}.

The second pathophysiology associated with HGPRT deficiency is neurological dysfunction, which can show itself as LNS with mental retardation, spasticity, choreoathetosis and self-mutilation. The mechanistic relationships between these dysfunctions and HGPRT deficiency are still not fully understood. It is known, however, that severe enzyme deficiency is necessary to evoke these neurological dysfunctions. Figure 1 shows that most of the variables in the model are affected when the activity of the enzyme is approx. 0.1 % of normal. At this drastically decreased activity, essentially every component of the system is affected, which might explain why it is difficult to pinpoint which metabolite or flux is primarily responsible for neurological dysfunctions in LNS patients.

Although this remains a difficult question, the biomathematical analysis in this paper helps us in the search for metabolites and fluxes that are particularly important in the underlying biochemical mechanisms that lead to the neurological pathophysiology of LNS. The analyses led to the hypothesis that the ratio of v_{gprt} to v_{hprt} is significantly correlated with neurological dysfunctions, and in fact this is consistent with some measurements of v_{gprt} and v_{hprt} in patients. If this hypothesis is true, the processes leading to LNS must be strongly affected by v_{gprt} activity, and, at the same time, the activity of v_{hprt} must have an opposite effect. Figure 3 indicates that only a very few metabolites and fluxes exhibit these characteristics, but none of them have previously been proposed as being responsible for neurological dysfunctions in HGPRT-deficient patients. It was therefore appropriate to compare our findings with hypotheses suggested by other authors (see Scheme 2).

To confirm or reject the hypothesis that the ratio of v_{gprt} to v_{hprt} is correlated with neurological dysfunctions in HGPRT deficiency, we propose to (1) measure activity of HGPRT in erythrocyte lysates of HGPRT-deficient patients, by using both substrates, hypoxanthine and guanine; (2) report both activities as percentages of activities in healthy subjects, because otherwise different laboratory procedures would preclude direct comparisons; and (3) report the ratio of v_{gprt} to v_{hprt} together with the degree of neurological dysfunctions exhibited by each patient. These results could be evaluated statistically to quantify correlations between neurological dysfunctions and the two activities v_{gprt} and v_{hprt}, or the ratio of v_{gprt} to v_{hprt}. At present not enough data are available, but on the basis of the cases presented in Table 5, the threshold of the v_{gprt}-to-v_{hprt} ratio might be approx. 0.5, with lower values for patients with neurological dysfunctions, and higher values for hyperuricaemia without neurological dysfunctions. Such a threshold, if it could be established and confirmed, could be an interesting prenatal screening tool. The proposed clinical analysis would also reveal whether IMP, XMP, Gua, v_{inuc} or v_{gua} have prime roles in a biochemical association between HGPRT deficiency and neurological dysfunctions.

Other possible contributors to neurological dysfunction in HGPRT-deficient patients that were discussed in the paper (see Figure 1) are increases in hypoxanthine and xanthine, guanylate pool depletion, and decreases in nucleic acid turnover. These turned out not to be independent of each other. In particular, Figure 2 suggests that a decrease in nucleic acid turnover might only be a consequence of guanylate pool depletion. Model analyses similar to those in Figure 2 confirmed that maintaining decreased concentrations of HX, Xa and UA in HGPRT-deficient patients had almost no effect on other variables in the model (results not shown). This insensitivity with respect to changes in Xa and HX and the pattern of change of these two metabolites as a response in the simulation of different degrees of HGPRT deficiency (Figure 4) seem to indicate that increased levels of Xa and HX are merely symptoms of HGPRT deficiency rather than causes of neurological dysfunctions (Figure 4). Moreover, these variables are not affected at all by changes in the v_{gprt}-to-v_{hprt} ratio as we would expect for metabolites associated with neurological dysfunction (results not shown).

The conclusion of these analyses is that guanylate pool depletion is the most likely cause of neurological dysfunctions and that the re-establishment of the guanylate pool might be the most promising target for drug therapy in patients with LNS. However, if it can be confirmed that the ratio of v_{gprt} to v_{hprt} is strongly correlated with neurological dysfunctions in LNS patients, the metabolites and fluxes proposed in this paper as particularly influential should be considered in the exploration of mechanisms that yield neurological dysfunctions.

Deficiencies in ASLI are also known to lead to mental retardation, and because there is a certain parallelism between this enzyme deficiency and HGPRT deficiency we explored the possibility that the same biochemical mechanisms could be relevant in the two enzyme deficiencies. Our results suggest that this is not a realistic hypothesis, because the only consequences of ASLI deficiency turned out to be the accumulation of S-AMP and S-AICAR (results not shown), which is in agreement with clinical measurements performed in other labs. In contrast, HGPRT deficiency led to a decrease in S-AMP to one-half (Figure 1), which implies that HGPRT deficiency and ASLI deficiency have opposite effects on purine metabolism and that the mechanisms involved in neurological dysfunctions are different for the two enzyme deficiencies.

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