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EFFECT OF POLYAMINES ON GLUTAMATE DEHYDROGENASE WITHIN PERMEABILIZED KIDNEY-CORTEX MITOCHONDRIA AND ISOLATED RENAL TUBULES OF RABBIT

ROBERT JARZYNA, TADEUSZ LIETZ and JADWIGA BRYŁA*
Institute of Biochemistry, Warsaw University, Al. Zwirki i Wigury 93, 02-089 Warsaw, Poland

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Abstract—The effect of polyamines on glutamate dehydrogenase {L-glutamate: NAD(P) oxidoreductase (deaminating) [EC 1.4.1.3]} activity has been studied in both permeabilized kidney-cortex mitochondria and isolated renal tubules of rabbit. Spermidine was the most potent inhibitor of glutamate synthesis in permeabilized mitochondria resulting in about 80% decrease of the enzyme activity at 5 mM concentration. Putrescine, α-monofluoromethylputrescine (MFMP) and (*R*,*R*)-δ-methyl-α-acetylenic-putrescine (MAP) were more efficient than spermine. The inhibitory action of polyamines was potentiated by an elevated NADH content in the reaction mixture. Increasing concentrations of either NH_4Cl, KCl or NaCl in the incubation medium resulted in a decrease of polyamine-induced inhibition of the enzyme activity, indicating that monovalent cations can compete with polyamines for the binding site at glutamate dehydrogenase. The inhibitory action of spermidine on glutamate synthesis was abolished by 2 mM ADP or 10 mM L-leucine, allosteric activators of the enzyme, as well as on the addition of either oxalate or sulphate at 20 mM concentrations. Spermidine did not affect glutamate formation when NADH was substituted by NADPH, suggesting an importance of the NADH binding to the inhibitory site of the enzyme for a decrease of reductive amination of 2-oxoglutarate by polyamine. Although spermidine did not influence glutamate deamination in the presence of NAD^+, it stimulated this process by about 70% when NAD^+ was substituted by $NADP^+$. In the presence of ADP the stimulatory effect of polyamine was not significant. The data indicate that in permeabilized rabbit kidney-cortex mitochondria the effect of polyamines on both glutamate formation and glutamate deamination via the reaction catalysed by glutamate dehydrogenase is dependent upon the coenzyme utilized by the enzyme. In the presence of NADH their inhibitory effect on the glutamate formation may be alleviated by allosteric activators of the enzyme, and concentrations of potassium, sodium, sulphate and oxalate. In isolated rabbit renal tubules incubated with 5 mM methionine sulfoximine and aminooxyacetate, in order to inhibit glutamine synthetase and aminotransferases, respectively, 5 mM spermidine decreased glutamate formation by about 30%, while putrescine and spermine did not significantly diminish the enzyme activity. In the presence of octanoate glutamate formation was reduced by about 30% by naturally occurring polyamines as well as MFMP and MAP, indicating that under these conditions NADH rather than NADPH is utilized as the coenzyme. In view of these data it is possible to suggest that polyamines may be of importance to control glutamate dehydrogenase activity under physiological conditions.

Key words: polyamines; glutamate dehydrogenase; rabbit kidney

Polyamines, naturally occurring polycationic molecules, are ubiquitous among eukaryotic cells and are known to reach intracellular concentrations approaching the millimolar range. They have mainly been associated with rapid cell growth and differentiation, but are now considered to be involved in a wide variety of cellular functions (for review see Refs 1–4). Many cells are capable of transporting polyamines [3, 5], so *de novo* biosynthesis can in principle be substituted by polyamine uptake from the environment. Moreover, putrescine, spermine and spermidine are all transported across the inner membrane and into the matrix of liver mitochondria [6, 7], where they affect several functions. Polyamines have been reported to activate ATP synthesis in rat liver mitochondria [8–10], pyruvate dehydrogenase phosphatase in rat adipocyte [11,12] and bovine kidney mitochondria [13]. Spermine is known to elevate calcium uptake in rat heart, liver and kidney mitochondria [14–16] and to regulate Ca^{2+}-sensitive intramitochondrial dehydrogenases, namely the pyruvate, NAD^+-isocitrate and 2-oxoglutarate dehydrogenases [15].

It has been reported that gentamicin and some other aminoglycoside antibiotics behave like the naturally occurring polyamines spermine and spermidine in affecting certain reactions catalyzed by various cAMP- and Ca^{2+}-independent protein kinases from rat tissues [17], and phosphoinositide-specific phospholipase C [18]. Since in the previous communication from this laboratory we have

* Corresponding author. Tel. and FAX (4822) 232-046.

† Abbreviations: AOA, aminooxyacetate; DABS, 4-dimethylaminoazobenzene-4′-sulfonyl chloride; DFMO, α-difluoromethylornithine, eflornithine; MAP, (*R*,*R*)-δ-methyl-α-acetylenicputrescine; MFMP, α-monofluoromethylputrescine; MSO, methionine sulfoximine.

described the inhibitory effect of gentamicin and some other aminoglycosides on glutamate formation in both renal proximal tubules and kidney cortex mitochondria of rabbit [19], the aim of the present investigation was to study the effect of polyamines on the activity of glutamate dehydrogenase {L-glutamate: NAD(P) oxidoreductase (deaminating) [EC 1.4.1.3]} as a key enzyme of the nitrogen metabolism. Since putrescine analogues such as MFMP† and MAP have been recently discussed with respect to *in vivo* therapeutic effect of ornithine decarboxylase inhibition including cancer and autoimmune diseases [20] we have compared the action of these compounds on glutamate dehydrogenase activity with that exerted by naturally occurring polyamines.

MATERIALS AND METHODS

Animals and isolation of kidney-cortex tubules and mitochondria. Male white Termond rabbits were used throughout. Animals were maintained on the standard rabbit chow with free access to water and food. Rabbit kidney cortex tubules were isolated from the left kidney as described previously [21]. Mitochondria were isolated from kidney-cortex as reported by Harris *et al.* [22], but final wash was made with 0.3 M mannitol replacing sucrose. Mitochondria were permeabilized according to Matlib *et al.* [23] in order to abolish barrier for the transport of substrates and effectors of glutamate dehydrogenase.

Incubation of renal tubules. Isolated kidney cortex tubules were incubated at 37° in 25 mL-Erlenmeyer flasks sealed with rubber stoppers under atmosphere of O_2:CO_2 (95%:5%) in Krebs–Henseleit buffer, pH 7.4, in the presence of 2 mM 2-oxoglutarate and 10 mM NH_4Cl. The incubation mixture also contained 5 mM methionine sulfoximine and 2 mM aminooxyacetate in order to inhibit reaction catalysed by glutamine synthetase [24], and aspartate aminotransferase [25], respectively. For measurement of glutamate formation the reactions were stopped at the 60th min of incubation by the addition of 1 mL sample to 0.1 of 35% $HClO_4$.

Determination of glutamate. The amino acid produced via the reaction catalysed by glutamate dehydrogenase was determined by HPLC as its DABS derivative according to Chang *et al.* [26].

Assay of glutamate synthesis and glutamate deamination in permeabilized mitochondria. The glutamate dehydrogenase activity was measured spectrophotometrically at 340 nm [27] at 30°. In order to follow reductive amination the incubation medium contained 240 mM mannitol and 20 mM Tris–HCl, 0.15 mM NADH or NADPH, 2 mM EGTA and 0.2 mg of mitochondrial protein/mL. Concentrations of 2-oxoglutarate and NH_4Cl are indicated in legends and tables. The final pH was 7.4.

For enzyme assay in the deamination direction the reaction mixture consisted of 240 mM mannitol, 20 mM Tris–HCl, 1 mM NAD^+or $NADP^+$, 2 mM EGTA, 50 mM glutamate and a range of 0.2–0.3 mg of mitochondrial protein/mL. The final pH was 7.4. Reactions were started by additions of either 2-oxoglutarate (the reverse direction) or coenzyme (the forward direction).

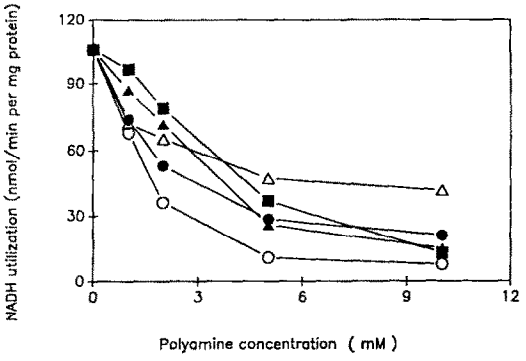

Fig. 1. Effect of various concentrations of polyamines on glutamate formation in permeabilized kidney cortex mitochondria. Open triangle, spermine; filled circle, putrescine; filled square, MFMP; filled triangle, MAP; open circle, spermidine: Concentrations of 2-oxoglutarate and NH_4Cl were 1 mM and 25 mM, respectively. SEM values were less than symbol sizes.

Chemicals. Putrescine, spermidine, spermine, AOA, MSO, DABS and EGTA were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). DFMO, MFMP and MAP were generous gifts of Marion Merrell Dow Research Institute (Strasbourg, France). NADH, $NADP^+$ and NAD^+ were from Reanal (Budapest, Hungary). NADPH was from Merck (Darmstadt, Germany), while Tris was from Serva (Heidelberg, Germany). Other chemicals were of analytical grade from Polish Chemicals (Gliwice, Poland).

Expression of results. Data shown are means ± SEM for three separate experiments. The statistical significance of differences was calculated by Student's *t*-test, $df = 2$.

RESULTS

Effect of polyamines on glutamate dehydrogenase activity in isolated mitochondria

Figure 1 presents the activities of glutamate dehydrogenase in the presence of 0.15 mM NADH and 25 mM NH_4Cl, in the direction of glutamate synthesis as a function of concentration of several polyamines. It is evident that the inhibitory action of particular polyamine increased proportionally to its concentration in the incubation mixture. At each concentration spermidine was the most efficient inhibitor of glutamate synthesis. Analogues of putrescine such as MFMP and MAP, being potent inhibitors of ornithine decarboxylase (*cf.* [20] for review) exhibited a similar effect on glutamate formation to that of putrescine, while cadaverine did not practically affect the enzyme activity (not shown). Spermine was less inhibitory than putrescine, while arginine, ornithine and eflornithine did not affect the enzyme reaction. Since 5 mM spermidine inhibited efficiently glutamate formation and higher concentration of this polyamine did not further

Table 1. Effect of spermidine on both glutamate formation and glutamate deamination in permeabilized rabbit kidney-cortex mitochondria

Spermidine	Glutamate formation NADH	Glutamate formation NADPH	Glutamate deamination NAD^+	Glutamate deamination $NADP^+$
–	67.1 ± 2.2	33.6 ± 2.1	13.3 ± 1.7	4.2 ± 0.1
+	12.5 ± 3.4*	34.5 ± 2.7	14.7 ± 0.9	7.0 ± 0.5*

Glutamate dehydrogenase activity in the direction of glutamate formation was determined in the presence of 1 mM 2-oxoglutarate, 25 mM NH_4Cl and 5 mM spermidine where indicated. Other conditions are described in Materials and Methods. Both glutamate formation and glutamate deamination are expressed in nmol/min per mg protein.
* $P < 0.05$ versus corresponding control without spermidine.

Table 2. Effect of spermidine on glutamate dehydrogenase activity in the presence of various concentrations of NADH

NADH (μM)	Glutamate formation −Spermidine	Glutamate formation +Spermidine
100	83.7 ± 3.0	27.3 ± 3.8*
150	74.7 ± 2.3	16.0 ± 1.6†
200	66.3 ± 2.1	13.3 ± 1.6†
250	50.7 ± 3.1‡	1.0 ± 0.8†

Glutamate dehydrogenase activity in the direction of glutamate formation was determined in mannitol medium in the presence of 1 mM 2-oxoglutarate and 25 mM NH_4Cl. Enzyme activity is expressed in nmol/min per mg protein.
* $P < 0.01$, † $P < 0.02$ versus corresponding control without spermidine, ‡ $P < 0.05$ versus control in the presence of 100 μM NADH.

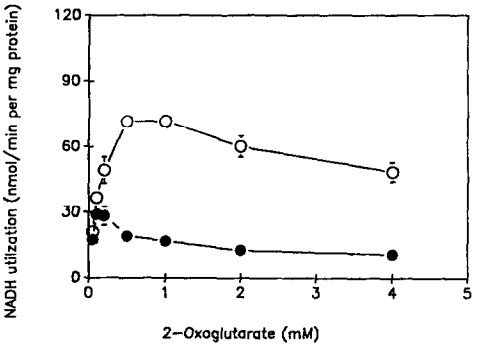

Fig. 2. Effect of various 2-oxoglutarate concentrations on the inhibition of glutamate formation by spermidine in permeabilized kidney cortex mitochondria. Open circle, no spermidine; filled circle, with 5 mM spermidine. Concentration of NH_4Cl was 25 mM. SEM values were less than symbol sizes when not indicated.

decrease the enzyme activity, spermidine was applied at 5 mM concentration in all subsequent experiments.

It is necessary to point out that at a low, 2.5 mM, NH_4Cl level in the incubation mixture 1 mM spermidine was also an efficient inhibitor of glutamate formation, resulting in about 50% decrease of the enzyme activity (16.7 ± 0.7 and 9.3 ± 0.4 nmol/min per mg protein without and with spermidine, respectively).

As shown in Table 1, in the presence of NADH glutamate dehydrogenase activity was inhibited by 5 mM spermidine by about 80%, when assayed in the direction of glutamate synthesis. In contrast, this polyamine did not affect this reaction when NADH was substituted by NADPH in the incubation mixture. This may suggest, that NADH binding to the inhibitory site of the enzyme is of importance for the action of polyamines on the reductive amination of 2-oxoglutarate. The spermidine did not affect glutamate deamination in the presence of NAD^+ as coenzyme. Moreover, it stimulated this process for about 70% when NAD^+ was substituted by $NADP^+$.

The data presented in Table 2 show that in the presence of elevated NADH concentrations the inhibitory effect of spermidine on the glutamate formation was increased suggesting an enhancement by polyamine of association of reduced coenzyme with the inhibitory site of the enzyme [28]. Since the binding of reduced coenzyme to the inhibitory site of glutamate dehydrogenase enhances binding of 2-oxoglutarate to the enzyme–coenzyme complex, resulting in substrate inhibition by 2-oxoglutarate [29], we have studied the effect of spermidine on glutamate formation in the presence of increasing 2-oxoglutarate concentrations. As shown in Fig. 2, the polyamine markedly decreased the enzyme activity practically at all 2-oxoglutarate concentrations.

When NH_4^+ ions were added at increasing concentrations to the reaction mixture a progressive release of the inhibitory action of the polyamine was observed (Table 3). However, in the presence of 100 mM concentration of NH_4Cl spermidine still decreased the glutamate formation by about 30%. Similar effects were observed on the addition of increasing K^+ concentration in the reaction medium. Among the monovalent cations studied sodium ions were most effective preventing at 50 mM concentration the inhibition of enzyme activity by spermidine. In view of these observations it is possible to conclude that spermidine and monovalent cations bind at the same site of the enzyme.

The addition of 10 mM L-leucine or 2 mM ADP, allosteric activators of glutamate dehydrogenase [30, 31], abolished completely the inhibitory effect of spermidine (Table 4) and other polyamines on glutamate formation (not shown). The stimulatory

Table 3. Effect of spermidine on glutamate formation in the presence of various concentrations of NH_4Cl, KCl and NaCl

NH_4Cl (mM)	KCl (mM)	NaCl (mM)	Glutamate formation −Spermidine	+Spermidine
12			51.3 ± 5.1	5.4 ± 0.8*
25			66.3 ± 8.5	10.9 ± 2.1†
50			79.4 ± 2.5	23.0 ± 4.6†
100			112.0 ± 4.5	73.0 ± 0.5†
25	12		89.0 ± 2.3	19.3 ± 0.7‡
25	25		92.3 ± 3.1	28.3 ± 1.7‡
25	50		97.3 ± 5.2	41.3 ± 3.3†
25	100		104.0 ± 1.2	70.1 ± 2.6*
25		12	92.3 ± 3.1	24.7 ± 0.5‡
25		25	108.8 ± 5.0	48.3 ± 1.9*
25		50	113.3 ± 5.0	89.3 ± 3.8
25		100	117.3 ± 4.8	102.7 ± 4.8

Glutamate dehydrogenase activity in the reverse direction was determined in the presence of 1 mM 2-oxoglutarate without or with 5 mM spermidine. Mannitol concentration was 240 mM unless KCl or NaCl was added. In samples containing either KCl, NaCl or increased NH_4Cl mannitol was decreased to achieve the same ionic strength. Other conditions are described in Materials and Methods. Glutamate formation is expressed in nmol/min per mg protein.

* $P < 0.02$, † $P < 0.05$, ‡ $P < 0.01$ versus corresponding control without spermidine.

Table 5. Reversal by oxalate and sulphate of the inhibitory action of spermidine on glutamate formation in rabbit kidney-cortex mitochondria

Oxalate	Sulphate	Glutamate formation −Spermidine	+Spermidine
0		70.0 ± 3.3	9.3 ± 2.6*
5		80.3 ± 4.7	31.7 ± 2.9†
10		82.3 ± 4.7	43.7 ± 3.7†
20		96.7 ± 2.7	85.7 ± 2.2
	0	74.2 ± 4.5	9.9 ± 1.3*
	5	70.5 ± 3.5	19.6 ± 2.1*
	10	70.7 ± 4.5	34.1 ± 4.1†
	20	70.7 ± 5.0	66.7 ± 4.0

Glutamate formation was measured in the presence of 1 mM 2-oxoglutarate. Incubation mixture contained ammonium sulphate or ammonium oxalate, where indicated, supplemented with either Tris-sulphate, Tris-oxalate or NH_4Cl in order to achieve 25 mM concentration of ammonium ions. In control samples 25 mM NH_4Cl was added as a source of ammonia. Concentrations of oxalate or sulphate are given as mM, while enzyme activity is expressed in nmol/min per mg protein.

* $P < 0.01$, † $P < 0.05$ versus corresponding control without spermidine.

Table 4. Reversal by ADP and L-leucine of the spermidine action on glutamate formation and glutamate deamination in rabbit kidney-cortex mitochondria

Additions	Glutamate formation −Spermidine	+Spermidine	Glutamate deamination −Spermidine	+Spermidine
None	70.0 ± 2.0	12.7 ± 0.7*	4.2 ± 0.1	7.0 ± 0.5†
2 mM ADP	146.3 ± 5.7‡	145.0 ± 4.1	15.2 ± 0.1‡	16.2 ± 0.8
10 mM Leu	165.7 ± 7.5‡	172.3 ± 6.2	ND	ND

Glutamate dehydrogenase activity in the reverse direction was determined in the presence of 0.15 mM NADH, 1 mM 2-oxoglutarate and 25 mM NH_4Cl, while the glutamate deamination was measured with 1 mM $NADP^+$. Other conditions are described in Materials and Methods. Results are expressed in nmol/min per mg protein.

* $P < 0.01$, † $P < 0.05$ versus the corresponding control with no spermidine. ‡ $P < 0.01$ versus the corresponding control with no additions.

effect of spermidine on glutamate deamination observed in the presence of $NADP^+$, was not significant on the addition of ADP into the reaction mixture.

As shown in Table 5, increasing concentrations of both oxalate and sulphate resulted in a progressive decrease of the inhibitory action of spermidine on glutamate formation in the presence of NADH as coenzyme. On the addition of these anions at 20 mM concentrations the polyamine did not affect the enzyme activity. Among other anions studied, both malonate and phosphate did not release the inhibitory action of spermidine, while sulphite, malate, citrate or maleate decreased markedly glutamate synthesis also in the absence of the polyamine (not shown). Thus, the protective action of both oxalate and sulphate against the inhibitory action of spermidine, is likely to be specific for these particular anions.

Effect of polyamines on glutamate synthesis in isolated renal tubules

As shown in Table 6, in isolated renal tubules 5 mM spermidine decreased glutamate formation by about 30% while both putrescine and spermine insignificantly diminish the enzyme activity. Octanoate, entering mitochondria independently of carnitine and activated inside mitochondria [32], increased the rate of glutamate formation by about 30%, probably due to an elevation of mitochondrial $NADH/NAD^+$ ratio [21]. In the presence of octanoate naturally occurring polyamines as well as putrescine analogues (MFMP and MAP) decreased glutamate formation by about 20–30%, indicating that under these conditions polyamines may be of importance for regulation of glutamate dehydrogenase activity in renal tubules.

Table 6. Effect of various polyamines on glutamate formation in isolated rabbit renal tubules

Polyamines	−Octanoate	+Octanoate
None	72.1 ± 5.6	107.3 ± 3.1
Putrescine	64.3 ± 3.8	73.6 ± 4.0*
MAP	ND	87.0 ± 0.5*
MFMP	ND	77.7 ± 0.6†
Spermidine	50.0 ± 1.4*	76.3 ± 1.3†
Spermine	54.9 ± 4.2	77.0 ± 2.1*

Isolated rabbit renal tubules were incubated as described in Materials and Methods with or without 0.5 mM octanoate. Polyamines were added at 5 mM concentrations, where indicated.

* $P < 0.05$ and † $P < 0.02$ versus the corresponding values with no polyamines.

DISCUSSION

The results presented in this paper show that in permeabilized kidney-cortex mitochondria the effect of polyamines on glutamate dehydrogenase activity is dependent upon the direction of reaction catalysed by the enzyme as well as on the coenzyme. The inhibitory effect of polyamines on the reductive amination of 2-oxoglutarate in the presence of NADH as coenzyme in addition to the lack of their inhibitory action on the enzyme operating in the direction of glutamate catabolism, observed in the presence of NAD^+ as coenzyme, (*cf.* Table 1) is similar to that reported for aminoglycosides [19]. There are, however, some differences in the action of aminoglycosides and polyamines on glutamate dehydrogenase activity. (a) In contrast to aminoglycosides the inhibitory action of spermidine is abolished by both ADP and L-leucine (*cf.* Table 4), allosteric activators of glutamate dehydrogenase [30, 31]. (b) Gentamicin-induced decrease of glutamate formation occurred at 15 mM sulphate concentration in reaction medium [19] while the inhibitory effect of spermidine on this process was released by both sulphate and oxalate (*cf.* Table 5). (c) In agreement with reports concerning inhibition of phosphatidylinositol-specific phospholipase C [33, 34], ornithine decarboxylase [35] and pyruvate carboxylase [36], the inhibitory potential of aminoglycosides seems to correlate with the number of amino groups in the antibiotic [19], whereas among the polyamines the triamine spermidine is more efficient than the diamine putrescine and the tetramine spermine in diminishing of glutamate formation (*cf.* Fig.1). (d) In contrast to spermidine, gentamicin inhibits glutamate formation in the presence of NADPH (unpublished results). The above discrepancies between aminoglycoside antibiotics and natural polyamines suggest that the mechanism of action of these compounds on glutamate synthesis via the reaction catalysed by glutamate dehydrogenase may differ. Some differences between aminoglycoside neomycin and spermidine have also been reported with respect to their effect on phosphatidylinositol kinase activity [37].

Various salts are known to affect dissociation of the coenzyme from the glutamate dehydrogenase–coenzyme complex [38]. According to Srinivasan [39] specific anions can bind to the γ-carboxylate site by anionic interaction altering the kinetic and thermodynamic parameters of the enzyme–coenzyme complex. This could explain a release of the inhibitory action of spermidine on glutamate formation in the presence of both sulphate and oxalate (*cf.* Table 5). In addition, the effect of sulphate and oxalate on the inhibition by spermidine can also be related to a binding and neutralization of these particular anionic compounds to the cation spermidine. Our findings do not provide, however, sufficient information to allow us to speculate on the precise mechanism by which these specific anions abolish the inhibition of glutamate dehydrogenase activity by polyamines.

In the mammalian kidney both sulphate and oxalate are filtered in the glomeruli and reabsorbed or secreted, respectively, by the proximal tubule [40, 41]. Moreover, oxalate has been found to be transported into mitochondria [42] while sulphate at least in liver might be either generated from cysteine intramitochondrially [43] or transported across the membrane via the dicarboxylate carrier [44]. Thus, both oxalate and sulphate may exert their effects on the mitochondrial metabolism. The opposite effect of negative charged compounds to that exhibited by polyamines has also been observed with respect to tyrosine protein kinase from human erythrocytes [45]. In addition, ADP, an allosteric activator of glutamate dehydrogenase, has also been considered as "superanion" by virtue of an adenine moiety in addition to the ribose pyrophosphate, that tightly binds to the enzyme making ADP more difficult to displace [46]. The effects of L-leucine, another allosteric activator of the enzyme, are the result of a direct effect on the enzyme together with a relief of high substrate inhibition [47].

The action of monovalent cations such as Na^+ and K^+ has been observed to influence the participation of 2-oxoglutarate in the reaction by glutamate dehydrogenase [48]. Since according to Pena-Diaz *et al.* [48] the combination between the enzyme-reduced coenzyme–NH_4^+ complex and 2-oxoglutarate takes more favourably in Na^+- than in K^+-media, a complete release of the inhibitory effect of spermidine on the enzyme activity in the presence of Na^+ ions in comparison with the partial one on the additions of K^+ ions to the reaction medium (*cf.* Table 3) is understood. Potassium has also been reported to protect mitochondria from the inhibitory action of spermine on state 4 respiration [9], suggesting a competition for the same biding site at the mitochondrial membrane.

Evaluation of intramitochondrial polyamine concentration is difficult. Both spermidine and spermine associated with the isolated mitochondria were found to be approximately 0.5 mM. However, it is unlikely that they reflect qualitatively or quantitatively the polyamine levels prior to cell homogenization, given to the rigors of the isolation procedures [9]. Since at low 2.5 mM NH_4^+ concentration present in the reaction medium the inhibitory effect of 1 mM spermidine on glutamate formation was higher than

that observed at extremely unphysiological 25 mM NH_4Cl concentration, it is likely, that mitochondrial levels of polyamines can efficiently influence the glutamate formation in the presence of physiological NH_4^+ concentration (0.4–3.3 mM in the lumen of rat proximal tubules *in vivo*) [49, 50]. It is possible, therefore, that mitochondrial glutamate dehydrogenase utilizing NADH in the direction of glutamate production may be affected by polyamines in the intact cell, under conditions of low ADP, oxalate and sulphate concentrations. The data with the use of isolated renal tubules (*cf.* Table 6) confirm this suggestion. In addition, it seems likely that ADP and L-leucine, allosteric activators of the glutamate dehydrogenase, as well as oxalate, sulphate and monovalent cations may alleviate the action of these polycations. In isolated mitochondria glutamate dehydrogenase reacts with both NAD(H) and NADP(H) [51]. In view of the inhibitory effect of polyamines on glutamate formation in renal tubules (*cf.* Table 6) it seems likely, that under *in vivo* conditions glutamate dehydrogenase interacts with NADH rather than NADPH.

Similarly to naturally occurring polyamines MFMP and MAP, inhibitors of ornithine decarboxylase [20], considered as potent antitumour agents appeared to be negative modulators of glutamate formation in both kidney cortex mitochondria (Fig. 1) and renal tubules (Table 6), while eflornithine did not affect glutamate dehydrogenase activity. In view of these observations, the therapeutic benefit of putrescine analogues remains to be determined. In addition, it should be pointed out that MAP has been reported to be more toxic than eflornithine [52].

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