Undernutrition during the preweaning period changes calcium ATPase and ADPase activities of synaptosomal fractions of weanling rats

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The presence of activities that hydrolyse externally added ATP to adenosine in synaptosomal preparations from various sources is well demonstrated. The hydrolysis of ATP to AMP can be mediated either by the concerted action of enzymes or by an ATP-diphosphohydrolase (*EC* 3.6.1.5; apyrase). Undernutrition during the preweaning period can delay the development of several enzymes involved in the metabolism of neurotransmitters or neuronal function. In young rats, the presence of an apyrase in synaptosomal preparations from cerebral cortex was investigated. The results suggested that the hydrolysis of externally added ATP and ADP can be mediated by a single enzyme. The effects of preweaning undernutrition on the hydrolysis of ATP and ADP were also investigated. In weanling rats, previous undernutrition caused a decrease of about 20% in the hydrolysis of both substrates in synaptosomal fractions.

ATP-diphosphohydrolase: Preweaning undernutrition: Synaptosomal ATPase and ADPase: Rat

During the postnatal period several anatomical, biochemical and physiological modifications occur in the central nervous system of altricial mammals. Some of the most prominent events that occur during this period of rapid brain growth are the formation of functional synapses and the increase in the activity of various enzymes involved in neurotransmitter metabolism and neuronal function (Abdel-Latif *et al.* 1970; Vernadakis & Arnold, 1980; Fiedler *et al.* 1987). Perinatal undernutrition impairs the generation of glial cells and myelin formation more than neuronal generation (Robain & Ponsot, 1978; Wiggins, 1982), impairing synaptogenesis and consequently the development of neuronal contacts (Cragg, 1972; Salas *et al.* 1974; Dyson & Jones, 1976). Perinatal undernutrition can also cause a marked reduction in the activity of various enzymes involved in neurotransmitter and energy metabolism, changing neurotransmitter and neuromodulator systems (Sereni *et al.* 1966; Adlard & Dobbing, 1971, 1972; Kissane & Hawrylewicz, 1975; Miller *et al.* 1977; Vanella *et al.* 1983; Vitiello & Gombos, 1987; Chanez *et al.* 1988; Vendite *et al.* 1988).

Recently, a low-affinity, Ca^{2+}-requiring, Mg^{2+}-independent ATPase has been described as associated with purified synaptic plasma membrane preparations from rat brain (Sorensen & Mahler, 1981). Additional studies of this calcium ATPase have demonstrated that the active site for hydrolysis of ATP is localized at the external surface of nerve-ending particles isolated from chicken forebrain, electric organ of *Torpedo marmorata*, and from various mammalian brain tissues (Sorensen & Mahler, 1982; Keller & Zimmerman, 1983; Nagy *et al.* 1983, 1986; Grondal & Zimmerman, 1986). The external localization and the insensitivity of this ecto-ATPase to inhibitors of ion-transporting ATPase (oligomycin, ouabain, sodium azide, sodium vanadate, and others) suggest that this activity is different from that of classical ATPases. Other studies have demonstrated that both ADPase and 5′-

nucleotidase (*EC* 3.1.3.5) activities are associated with isolated nerve-ending particles (Dowdal, 1978; Grondal & Zimmerman, 1986) and with synaptic membranes (Nagata *et al.* 1984). It has also been demonstrated that ATP and ADP can be degraded to AMP by the action of an ATP-diphosphohydrolase (*EC* 3.6.1.5; apyrase) in synaptosomal fractions from the hypothalamus of adult rats (Sarkis *et al.* 1986*b*: Schadeck *et al.* 1989). All these findings suggest that synaptosomal preparations contain enzymes that can be involved in the metabolism of ATP released in the synaptic cleft and that may be components of an enzyme chain that hydrolyses ATP to adenosine.

Since the maturation of various enzymes in the central nervous system seems to be delayed by perinatal undernutrition, we examined whether undernutrition during suckling changes the activity of hydrolysis of Ca-ATP and Ca-ADP in various subcellular fractions of cerebral cortex of 20-d-old rats. Since it has been proposed that the hydrolysis of externally added ATP and ADP to AMP in synaptosomal preparations obtained from the hypothalamus of adult rats may be mediated by an apyrase (Sarkis *et al.* 1986*b*; Schadeck *et al.* 1989), we also examined whether an apyrase is present in synaptosomal preparations from the cerebral cortex of young rats.

EXPERIMENTAL

Chemicals

Nucleotides, ouabain, sodium azide, dithiothreitol (DTT), succinic acid, glucose-6-phosphate, *p*-nitrophenylphosphate and P^1P^5-di(adenosine-5′)-pentaphosphate (Ap5A) were obtained from Sigma Chemical Co. (St Louis, MO, USA). EDTA, calcium chloride, potassium chloride, pyrophosphate (PPi) and β-glycerophosphate were obtained from Merck (Rio de Janeiro, RJ, Brazil). Percoll was obtained from Pharmacia (Uppsala, AB, Sweden) and was routinely filtered through millipore AP15 pre-filters in order to remove aggregated, incompletely coated particles. All other reagents were of analytical grade.

General procedures

For assessing the homogeneity and condition of our synaptosomal preparations, Wistar rats (21 d of age) were used. They were maintained since birth on a 200 g protein/kg laboratory chow (Germani, Porto Alegre, RS, Brazil; for details see Dutra-Filho *et al.* 1989).

Rats were killed by decapitation and the brain was quickly removed. The cerebral cortex was dissected and placed in an ice-cold medium consisting of 0·32 M-sucrose, 1 mM-EDTA, and 0·25 mM-DTT, pH 7·4 (medium I). Rat cerebral cortex was homogenized in 10 vol. medium I with ten up-and-down strokes at approximately 1200 rev/min in a Teflon-glass homogenizer. The homogenate was centrifuged at 1000 ***g*** for 10 min to give the low-speed supernatant fraction S1. The S1 fraction was centrifuged for 20 min at 12000 ***g*** to give the P2 pellet. The P2 pellet was resuspended in a volume of medium I to give a protein concentration of approximately 3–4 mg/ml. Throughout the preparation the material was maintained at 0–4°.

Percoll gradient preparation

Discontinuous four-step Percoll gradients consisting of 160 (1·3 ml), 100 (1 ml), 50 (1 ml) and 20 (1 ml) ml Percoll/l medium I were made in 5 ml nitrocellulose tubes. The solutions of gradient were adjusted to pH 7·4 with hydrochloric acid, or sodium hydroxide, just before use.

Isolation of synaptosomes

The P2 fraction (0·7 ml) was layered gently over a freshly prepared gradient. The tubes were centrifuged in an ultracentrifuge swinging bucket rotor (Beckman, SW 50·1) at 22000 ***g*** for 20 min. The interfacial fractions and the pellet (fractions 1–5 with the numbering from top to bottom of the tube) were collected from the gradients by wide-tip disposable plastic transfer pipettes. As the fractions that banded between interfacial fractions contain little protein, only the interfacial fractions were analysed for lactate dehydrogenase (*EC* 1.1.1.27; LDH). The synaptosomal peak was determined by measuring occluded LDH (Morgan, 1976) in the presence of Triton X-100 (10 ml/l).

Enzyme assays

Samples were taken from the gradient and diluted when necessary to give an appropriate protein concentration. Since Percol was not removed, all enzyme assays were carried out in the presence of 0·5–2 ml Percoll/l. Control experiments demonstrated that this amount of Percoll did not interfere with any of the enzyme assays carried out in the present study. Nevertheless, the requisite amount of Percoll was added to the fractions that did not contain Percoll (homogenate, S1, P2 and microsomes). Unless otherwise stated, all enzyme assays were carried out in iso-osmotic mixtures.

Marker enzymes

Assay of LDH was carried out according to Whitaker (1969). Succinate dehydrogenase (*EC* 1.3.99.1; SDH) was assayed according to Sorensen & Mahler (1982). Na^+, K^+-ATPase (*EC* 3.1.6.4) was assayed according to Nagy & Delgado-Escueta (1984). Acetylcholinesterase (*EC* 3.1.1.7; AChE) was assayed according to Ellman *et al.* (1961), Glucose-6-phosphatase (*EC* 3.1.3.9; G6Pase) was determined by the method of Hubscher & West (1965).

ATPase and ADPase

Unless otherwise stated, the reaction medium for ATPase and ADPase assay contained 5 mM-KCl, 1·5 mM-$CaCl_2$, 0·1 mM-EDTA, 10 mM-glucose, 225 mM-sucrose, 45 mM-Tris–HCl buffer (pH 8·0) and 5 mM-sodium azide. Appropriate fractions (50 μl; 10–20 μg protein) were added to the reaction medium and the mixture was pre-incubated for 10 min at 37°. The enzyme reaction was started by the addition of ATP or ADP to a final concentration of 1 mM. Incubation periods were chosen in order to ensure the linearity of the reaction. The reaction was stopped by the addition of trichloroacetic acid (TCA) to give a final concentration of 50 ml/l. Controls with addition of fractions after TCA were run to correct for non-enzymic hydrolysis. All samples were run in duplicate or triplicate. Phosphate (Pi) was measured by the method of Fiske & Subbarow (1925). Enzyme activity was expressed as nmol Pi released/min per mg protein.

Assay of inorganic pyrophosphatase (EC *3.6.1.1*), *5′-nucleotidase and non-specific phosphatase activities*

The reaction medium and conditions were the same as those used in the assay of ATPase and ADPase, except that the reaction was initiated by the addition of one of these compounds (final concentration 1 mM): PPi, glucose-6-phosphate, *p*-nitrophenylphosphate, β-glycerophosphate or AMP in place of ATP or ADP.

Protein determination

Protein was determined by the method of Lowry *et al.* (1951) with crystalline bovine serum albumin as a standard.

Undernutrition

Rats were undernourished during the period while they were suckled by giving their dams a protein-deficient diet. The conditions were as previously reported (Mello *et al.* 1989), except that diets of 280 g (control) and 70 g (undernourished) casein/kg were used. On the day of birth (day 0), litters were culled to nine pups and dams were assigned at random to the 280 or 70 g casein/kg diets. The dams assigned to the 280 g/kg group received a 280 g casein/kg diet colour-coded with an inert green dye, and dams assigned to the 70 g/kg group received a 70 g casein/kg diet colour-coded with an inert red dye. Mothers that failed to rear at least eight pups until the day of the enzyme assays were not included in the experiments.

When the litters were 20 d old, two female rats from each litter were killed by decapitation. The brain was removed and the cerebellum and olfactory bulbs were discarded. The cerebrum was weighed and the cortex was dissected and processed as described previously.

Statistical analyses

Values are reported as means and standard deviations. Comparisons between groups were made by two-tailed paired or unpaired Student's *t* test, depending on the experiments.

RESULTS

Localization of synaptosomal fraction

The treatment of fractions with Triton X-100 resulted in a 2- (fraction 1) to 26-fold (fraction 4) increase in specific activity of LDH. The specific activity of LDH of the fraction that sedimented near the interphase of the 100-160 ml Percoll/l layers increased 26-fold with treatment with Triton X-100 (final concentration 10 ml/l), indicating a peak of intact synaptosome in this fraction (results not shown). This result is very similar to those reported by Nagy & Delgado-Escueta (1984) and Nagy *et al.* (1986). They have found that intact synaptosomes band at the 100–160 ml Percoll/l interphase and that the treatment of this fraction with Triton X-100 resulted in a 19- to 40-fold increase in the specific activity of LDH.

Marker enzymes

Table 1 shows the activity of marker enzymes in cerebral cortex homogenate and synaptosomal fraction. The amount of contamination of synaptosomal fraction by mitochondria, neural plasma membrane, microsome and soluble proteins was calculated in two ways: (*a*) taking the specific activity ratio of marker enzymes obtained in synaptosomes to that obtained in the homogenate (relative specific activity, RSA) and (*b*) by measuring the marker enzyme activity that could be recovered in the synaptosomal fraction (Table 1).

Considering the RSA of the various negative marker enzymes, it was found that the synaptosomal fraction presented an impoverishment of these markers (Table 1). This impoverishment relative to the homogenate was confirmed by the low levels of recovered activity of negative markers in synaptosomal fraction: approximately 5% contamination with SDH activity (mitochondrial marker), less than 2% contamination with Na^+, K^+-ATPase activity (plasma membrane marker), less than 1% with LDH (soluble protein marker), approximately 5% with AChE (plasma membrane and microsomal marker), and less than 3% with G6Pase (microsomal marker). The results obtained with mitochondrial and plasma membrane markers are very similar to those found by Nagy & Delgado-Escueta (1984) and Nagy *et al.* (1986) in their intact synaptosomes.

Table 1. *Marker enzyme activities in cerebral cortex homogenates (H) and synaptosomal fractions (SYN) from 21-d-old rats*

(Mean values and standard deviations. SDH activity is expressed as nmol 2,6-dichloroindophenol reduced/min per mg protein. Na^+, K^+-ATPase and G6Pase activities are expressed as nmol phosphate/min per mg protein. LDH activity is expressed as nmol lactate consumed/min per mg protein. AChE activity is expressed as nmol acetylthiocholine consumed/min per mg protein)

	H			SYN				
Marker enzyme	Mean	SD	*n*	Mean	SD	*n*	RSA	Percentage recovered
SDH	12·7	3·1	5	7·9	2·4	5	0·62	4·5
Na^+,K^+-ATPase	83·1	20·7	5	18·9	6·5	5	0·23	1·3
LDH	353·3	35·1	3	27·0	3·1	3	0·08	0·7
G6Pase	17·2	1·8	3	6·1	1·9	3	0·35	2·1
AChE	63·2	14·9	4	39·7	5·9	4	0·63	5·0

RSA, specific activity of SYN: that of H; SDH, succinate dehydrogenase (*EC* 1.3.99.1); Na^+, K^+-ATPase, Na^+, K^+-dependent ATPase (*EC* 3.1.6.4); LDH, lactate dehydrogenase (*EC* 1.1.1.27); G6Pase, glucose-6-phosphatase (*EC* 3.1.3.9); AChE, acetylcholinesterase (*EC* 3.1.1.7).

Table 2. *Effects of disruption of synaptosomes and inhibitors on the hydrolysis of ATP and ADP by synaptosomal and P2‡ fractions from 21-d-old rats*

(Mean values and standard deviations. ATPase and ADPase activities are expressed as nmol phosphate/min per mg protein. The incubation media were used as described on p. 275, except that sodium azide was not included in the media unless its effect was examined. Synaptosomes were disrupted by hypo-osmotic shock in 6 mM-Tris–hydrochloric acid buffer, pH 8·0 for 1 h)

		Synaptosomal fraction			P2		
Preparation	Substrate	Mean	SD	*n*	Mean	SD	*n*
Intact	ATP	102·0	8·5	4	160·9	5·2	5
	ADP	52·1	6·2	4	73·3	4·3	4
Disrupted	ATP	112·6	5·2	4	—		
	ADP	56·3	4·7	3	—		
+Sodium azide (5 mM)	ATP	93·2	7·7	4	124·3**	7·2	5
	ADP	48·0	6·5	4	64·8	7·6	3
+Ouabain (1 mM)	ATP	98·7	10·0	5	—		
	ADP	51·7	6·2	3	—		
+Lanthanum (0·1 mM)	ATP	118·5	16·2	2	—		
	ADP	54·7	3·7	2	—		
+Potassium fluoride (30 mM)	ATP	9·2†††	3·5	4	—		
	ADP	2·9†††	1·1	4	—		

** Mean value was significantly different from intact P2 ($P < 0·01$).
††† Mean value was significantly different from intact synaptosome ($P < 0·001$).
‡ For details, see p. 274.

Effect of disruption and inhibitors on ATP and ADP hydrolysis

Table 2 shows the effects of disruption and inhibitors on Ca-ATP and Ca-ADP hydrolysis activity of the synaptosomal fraction. Disruption of the synaptosomal fraction caused an increase of about 10% in the hydrolysis of both ATP and ADP; however, this increase was not statistically significant. Nagy *et al.* (1986) have described a 7% increase in the Ca^{2+}-

ATPase activity in synaptosomes after disruption of the fraction. Ouabain, a classical Na^+,K^+-ATPase inhibitor, had practically no effect on ATP and ADP hydrolysis. This result is in accordance with the findings of Nagy *et al.* (1986) and Grondal & Zimmerman (1986) who reported a similar effect of ouabain on Ca^{2+}-ATPase. The effect of sodium azide, a mitochondrial inhibitor, was assessed in the synaptosomal and P2 fraction. Azide caused an inhibition of 9% in the hydrolysis of ATP and ADP of the synaptosomal fraction. This inhibition was not statistically significant. Nagy *et al.* (1986) reported that 1 mM-sodium azide causes a 14% inhibition of Ca^{2+}-ATPase activity of the synaptosomal fraction; however, these authors did not report whether this inhibition was significant. On the other hand, sodium azide caused a significant 23% inhibition of Ca^{2+}-ATPase activity of the P2 fraction ($P < 0.01$). This effect may be related to an inhibition of an ATPase associated with mitochondria. Lanthanum, a $Ca^{2+} + Mg^{2+}$-ATPase inhibitor, did not alter the hydrolysis of either ATP or ADP. Potassium fluoride, which inhibits apyrase from pig pancreas (LeBel *et al.* 1980), caused a significant and parallel reduction in the hydrolysis of both ATP and ADP ($P < 0.001$).

Inorganic pyrophosphatase, non-specific phosphatases and 5′-nucleotidase

The presence of inorganic pyrophosphatase in the synaptosomal preparations was excluded because no Pi was released when 1·0 mM-PPi was added to the incubation medium in place of ATP and ADP (n 6). Neither was there hydrolysis of the other phosphate esters tested: glucose-6-P (n 5), p-nitrophenylphosphate (n 4), and β-glycerophosphate (n 4). The presence of a 5′-nucleotidase in our assay system was excluded because AMP was not hydrolysed (n 4).

The presence of contaminant adenylate kinase (*EC* 2.7.4.3) was excluded because the potent and selective adenylate kinase inhibitor, Ap5A (Lienhard & Secemski, 1973) at a concentration of 10 μM did not affect either ADP or ATP hydrolysis by the synaptosomal preparation. Mean values for ATP and ADP hydrolysis in the presence of Ap5A were 91·3 (SD 5·8) and 44·7 (SD 3·9) respectively, and in the absence of Ap5A the values for ATP and ADP were 94·2 (SD 4·1) and 42·0 (SD 3·3) respectively (n 3).

Mixed-substrate assay

To exclude the possibility that the hydrolysis of ATP and ADP was due to concerted activity of an ATPase with an ADPase, ATP and ADP were added to the incubation medium at saturating concentrations at the same time. In this condition the rate of Pi release was intermediate between the rates obtained when each one was added alone: thus with 1 mM-ATP the mean was 90·3 (SD 3·4) nmol Pi/min per mg protein, with 1 mM-ADP the mean was 46·3 (SD 2·8) nmol Pi/min per mg protein, and with 1 mM-ATP + 1 mM-ADP a mean of 70·3 (SD 3·2) was obtained (n 5). In the presence of a single enzyme, rates of Pi production in the mixed-substrate reaction should be close to the average value found for each individual substrate, whereas if two enzymes existed the rate for the mixed reaction should be the sum of the values found for the individual substrates. Results from these experiments support the existence of one enzyme with two substrates (Dixon & Webb, 1979) in synaptosomal preparations from young rats. This procedure is a classical treatment for characterizing apyrases (Harper *et al.* 1978; LeBel *et al.* 1980; Sarkis *et al.* 1986*a*).

Effect of undernutrition on body and brain weights of 20-d-old rats

Table 3 shows the body-weights and brain weights of 20-d-old rats. Undernutrition reduced the body-weight of deprived rats to 33% of that of well-nourished animals ($P < 0.001$). The brain weight of undernourished rats was decreased to 82% of that of well-nourished animals ($P < 0.001$).

Table 3. *Effect of preweaning undernutrition (dams given 70 g casein/kg diet; well-nourished group 280 g casein/kg diet) on body and cerebral weight of 20-d-old rats*

(Mean values and standard deviations. Lactate dehydrogenase (*EC* 1.1.1.27, LDH) activity expressed in nmol/min per mg protein. Triton-treated synaptosomes were pre-incubated for 5 min in the presence of 10 ml Triton X-100/l)

Dietary group...	280 g casein/kg			70 g casein/kg		
	Mean	SD	*n*	Mean	SD	*n*
Body-wt (g)	48·6	2·4	9	16·0***	2·1	9
Brain wt (mg)	967·5	27·1	9	769·9***	40·4	9
LDH						
Intact synaptosomes	30·0	11·7	6	31·1	7·3	6
Triton-treated synaptosomes	530·5	127·8	6	532·7	66·7	6

*** Mean value significantly different from that for well-nourished animals ($P < 0·005$).

Table 4. *Effects of preweaning undernutrition (dams given 70 g casein/kg diet; well-nourished group 280 g casein/kg diet) on Ca^{2+}-ATPase and Ca^{2+}-ADPase activity of various subfractions of cerebral cortex† of 20-d-old rats*

(Mean values and standard deviations. ATPase and ADPase activities are expressed in nmol phosphate/min per mg protein)

Dietary group...		280 g casein/kg			70 g casein/kg		
Cerebral fraction	Substrate	Mean	SD	*n*	Mean	SD	*n*
H	ATP	148·1	5·0	4	146·3	2·4	4
	ADP	66·7	2·4	4	68·8	1·7	4
S1	ATP	128·0	0·7	4	130·4	7·7	4
	ADP	61·5	2·9	4	65·2	7·4	4
P2	ATP	136·2	8·5	9	115·1***	6·2	9
	ADP	66·7	7·4	8	55·2*	6·1	8
SYN	ATP	96·1	6·6	7	78·0**	5·8	7
	ADP	47·2	4·5	6	37·8*	3·9	6

Mean values were significantly different from those for well-nourished animals: * $P < 0·05$, ** $P < 0·01$ or *** $P < 0·005$.

† Fractions obtained by differential centrifugation: H, homogenate; S1, low-speed supernatant fraction; P2, postmitochondrial pellet; SYN, synaptosomal fraction. For details see pp. 274–275.

Effects of undernutrition on ATPase and ADPase activity

Table 4 shows the effects of undernutrition on the ATPase and ADPase activities of various fractions of the cerebral cortex of 20-d-old rats. Undernutrition had no significant effects on either ATPase or ADPase activity of either the homogenate or S1 fraction. On the other hand, undernutrition caused significant reductions of 15% in Ca^{2+}-ATPase ($P < 0·005$) and of 17% in Ca^{2+}-ADPase ($P < 0·05$) activities of P2, and a significant reduction of 19% in both ATPase ($P < 0·01$) and ADPase ($P < 0·05$) activities of the synaptosomal fraction.

DISCUSSION

The results obtained from young rats suggest that the hydrolysis of both ATP and ADP in the synaptosomal preparations is due to an ATP diphosphohydrolase. The following evidence excluded the possibility of enzyme combinations that could mimic an apyrase activity in our assay: (1) no adenylate kinase was detected, excluding a combination of this enzyme with an ATPase on the hydrolysis of ADP, (2) no inorganic pyrophosphatase activity was detected, excluding the possibility of a concerted action of an ATP pyrophosphatase (*EC* 3.6.1.8) and inorganic pyrophosphatase, and (3) the rate of hydrolysis of both nucleotides (ATP and ADP), when they were added together, was equal to the arithmetic mean of the activities found for each one separately, suggesting the presence of a single enzyme with two substrates. Regarding the mixed-substrate experiments, there remains the possibility of cross-inhibition between two substrates at the level of two different enzymes. However, the effects of ATPase inhibitors (Table 2) suggest that well-known ATPases are not involved in the hydrolysis of ATP and give further support to the presence of one enzyme that hydrolyses ATP and ADP.

The undernutrition imposed in the present work resulted in a deficit in body-weight greater than previously reported in rats undernourished when they are being suckled. At 20 d of age, undernourished rats had a deficit in body-weight of 67% compared with well-nourished animals (Table 3). The deficit achieved in the present work is comparable to those obtained with combined gestational and lactational undernutrition (Adlard & Dobbing, 1971, 1972).

The results of the ATPase and ADPase assays demonstrated that undernutrition while rats are being suckled decreases these activities in P2 and synaptosomal fractions (Table 4). The specific activities of ATPase and ADPase of both P2 and synaptosomal fractions were lowered by undernutrition to about 18% of well-nourished values. The other fractions studied (total homogenate and S1) were not affected by undernutrition, suggesting that the deficit is associated with fractions enriched in synaptosomes. It is interesting to note that undernutrition caused a parallel decrease in the hydrolysis of both nucleotides.

Various subcellular fractions of the brain possess ATPase activities. Ca^{2+}-ATPase activities have been reported to be associated with mitochondria, microsomes, and plasma membranes (Nagy *et al.* 1983). Thus, it became important to assess the contamination of these structures in our synaptosomal preparation. Mitochondrial contamination in the synaptosomal fraction did not exceed 5% (Table 1). Furthermore, a mitochondrial ATPase inhibitor was included in the reaction medium in the present study, excluding practically all mitochondrial ATPase contamination. This was shown by the fact that sodium azide caused a 23% inhibition in the Ca^{2+}-ATPase activity of P2 (a fraction enriched in mitochondria and synaptosomes), while in the synaptosomal fraction the inhibition of ATP hydrolysis was not statistically significant (9%, Table 2). The contamination with plasma membranes was low in our synaptosomal preparation as judged by Na^+, K^+-ATPase (less than 2%), suggesting that the contamination by disrupted synaptosomes and postsynaptic attachments was low (Table 1). In addition, the ratio of LDH measured in the intact synaptosomes to that in Triton-treated synaptosomes demonstrated that less than 10% of this activity is present in intact synaptosomes (Table 3), which also indicates that most of the synaptosomes in this fraction were intact. Contamination of the synaptosomal fraction with microsomes, as judged by G6Pase, was also low (less than 3%). Furthermore, we also measured Ca^{2+}-ATPase and ADPase in microsomal fractions obtained from the cerebral cortex of well-nourished and undernourished animals (results not shown). No significant differences were detected between nutritional groups in either nucleotidase activity, suggesting that the deficits found in these activities in synaptosomal and P2 preparations are not due to microsomal contamination.

Other reports in the literature have demonstrated that perinatal undernutrition can retard the development of several enzymes involved in neurotransmitter metabolism and neuronal function (Sereni *et al.* 1966; Adlard & Dobbing, 1971; Kissane & Hawrylewicz, 1975; Stern *et al.* 1975; Vitiello & Gombos, 1987; Chanez *et al.* 1988). The results of the present study demonstrated that undernutrition between birth and weaning can also decrease the activity of both ATP and ADP hydrolysis in fractions enriched in synaptosomes of 20-d-old rats.

Although no specific experiments were done to demonstrate that these activities are localized at the external surface of synaptosomal plasma membrane, the low contamination with plasma membranes, the low activity of LDH (indicating few broken synaptosomes in our preparation), the fact that disruption of synaptosomes did not increase the hydrolysis of either ATP or ADP, and considering that the adenine nucleotides added to the incubation medium cannot enter the synaptosomes (Krueger *et al.* 1977; Grondal & Zimmerman, 1986) all suggest that these activities are due to ecto-enzymes.

Recently it has been demonstrated that an apyrase is present at the external surface of synaptosomes of the hypothalamus of rats (Schadeck *et al.* 1989). In the present study, undernutrition was shown to cause a similar decrease in both ATPase and ADPase activities which may suggest that a single enzyme is hydrolysing both nucleotides. However, in the present work we have obtained only partial evidence that ATPase and ADPase activities resulted from the action of a single enzyme. Thus, it is also possible that two enzymes are being affected to the same extent by undernutrition.

The results of the present study demonstrate that undernutrition while rats are being suckled decreases ATPase–ADPase activities associated with synaptosomal preparations. Probably these activities are due to an ecto-enzyme and may be involved in ATP and ADP degradation in the synaptic cleft. It has been suggested that these activities, together with a 5′-nucleotidase, could be a component of an enzyme chain that produces adenosine in the synaptic cleft (Keller & Zimmerman, 1983; Nagy *et al.* 1986; Grondal & Zimmerman, 1986; Schadeck *et al.* 1989). Whether the observed decrease in both ATPase and ADPase of synaptosomal preparations of the cerebral cortex of 20-d-old rats would lead to deficits in the production of adenosine in the synaptic cleft remains to be investigated.

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