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SEQUESTRATION OF ADENOSINE IN CRUDE EXTRACT FROM MOUSE LIVER AND OTHER TISSUES

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Summary

Adenosine (1 μM) was incubated in the presence of dialyzed crude tissue extract from mouse liver and its degradation determined. At high concentration of tissue extract, a fraction of adenosine was not metabolized. This phenomenon, termed sequestration of adenosine, was shown to be affected in the same way by the same factors (pH, salt, reducing agent and adenine) as those affecting the protection of adenosine against deamination in the presence of the purified cyclic AMP-adenosine binding protein/*S*-adenosylhomocysteinase from mouse liver (Sæbø, J. and Ueland, P.M. (1979) Biochim. Biophys. Acta 587, 333—340). These data point to a role of this protein in the sequestration of adenosine in crude extract.

The sequestration potency in crude extract could be determined by diluting the extract in the presence of a constant amount of adenosine deaminase added to the tissue extract. Under these conditions there was linearity of adenosine not available for degradation versus the concentration of tissue extract, and a total recovery of the sequestration potency of purified binding protein added to the crude extract was observed.

The tissue level of the cyclic AMP-adenosine binding protein/*S*-adenosylhomocysteinase in mouse liver was determined by two independent procedures based on the sequestration of adenosine and the hydrolysis of *S*-adenosylhomocysteine, respectively. The intracellular concentration was calculated to be 10 μM.

The sequestration of adenosine in crude extract from mouse, rat, rabbit and

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Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Mes, 2-(*N*-morpholino)-ethanesulfonic acid.

bovine tissues was determined and showed requirements similar to those of the sequestration in mouse liver extract.

The ability to sequester adenosine was high in liver and decreased in the following order: liver, kidney, adrenal cortex, brain, uterus, cardiac and skeletal muscle.

Introduction

Adenosine is protected against deamination to inosine by the enzyme adenosine deaminase in the presence of the homogenous cyclic AMP-adenosine binding protein/*S*-adenosylhomocysteinase from mouse liver [1—3]. This phenomenon, termed sequestration of adenosine, was observed in the absence of homocysteine showing that it is not dependent on enzymic formation or hydrolysis of *S*-adenosylhomocysteine. The sequestration process was promoted by alkaline pH, KCl, mercaptoethanol and inhibited by adenine [3]. In the light of these results the sequestration of adenosine in crude extract from mouse liver and other tissues was investigated. This study was undertaken to see if the cellular level of adenosine deaminase and adenosine binding protein(s) allows the protection of adenosine against deamination.

Materials and Methods

Chemicals. Sources of the reagents used have been given previously [1—3]. Inosine, hypoxanthine and allantoin were obtained from Sigma Chemical Co., St. Louis, U.S.A., and uric acid from Merck, Darmstadt.

Preparation of tissue extracts. Mouse, rat and rabbit were killed by cervical dislocation and the tissues rapidly removed and put in liquid nitrogen until use. Bovine tissues were obtained at a local slaughter house immediately after the animal was killed and the organs placed in liquid nitrogen. Liver and kidney from mouse, rat and rabbit, mouse and rat brain, bovine liver and bovine adrenal cortex were processed as follows. The tissues were thawed in 50 mM Tris-HCl, pH 7.6, containing 0.25 M sucrose and 10 mM EDTA (homogenization buffer) and homogenized in the same buffer (1 : 1, w/v) by six strokes in a Teflon glass homogenizer at 465 rev./min. The temperature was 0° C. Cardiac and skeletal muscle and bovine kidney were finely minced with scissors and homogenized (1 : 3, w/v) for 1 min with a Ultra-Turax homogenizer. The same buffer was used and the temperature was 2°C. The homogenates were centrifuged for 45 min at 100 000 $\times g_{av}$ and the supernatant so obtained dialyzed against 5 mM Hepes (pH 7.0) for 12 h at 2°C.

Purification of the cyclic AMP-adenosine binding protein/S-adenosylhomocysteinase. This was performed according to a procedure published previously [4,5].

Thin-layer chromatography. Chromatography was performed on polyethyleneimine-impregnated cellulose thin-layer sheets (0.25 mm) on glass plates (20 × 20 cm). Three solvent systems were used. The plates were developed in glacial acetic acid/water/*n*-butanol (1 : 1 : 2) as described by Fain and Shepherd [6] (system A). Adenosine, inosine, hypoxanthine, xanthine, uric

acid, AMP and ADP were separated in this system [6]. Adenine was only slightly separated from adenosine and allantoin showed the same mobility as uric acid. The second solvent was 2.5 M LiCl. The R_F values in this system were as follows: adenine 0.33, uric acid 0.35, adenosine 0.52, hypoxanthine 0.55, inosine 0.73, AMP 0.75 and allantoin 0.75. Finally, the plates were developed in isobutanol/ethanol/water (2 : 1 : 1) (system C). The R_F values for *S*-adenosyl-L-homocysteine, adenosine, adenine and inosine in this system have been given previously [2].

Preparation of S-[^{14}C]adenosylhomocysteine. [8-^{14}C]Adenosine (0.5 mM) and DL-homocysteine (3 mM) were incubated for 10 min at 37°C in the presence of purified *S*-adenosylhomocysteinase (100 μg/ml) from mouse liver. The incubation buffer was 5 mM Hepes buffer (pH 7.0) containing 2 mM 2-mercaptoethanol. The reaction was terminated by heating (100°C) for 5 min and the reaction products separated by thin-layer chromatography in system C. *S*-Adenosylhomocysteine was eluted from polyethyleneimine-cellulose powder into distilled water, lyophilized and redissolved to a final concentration of 0.7 mM. More than 98% of the radioactive material comigrated with *S*-adenosylhomocysteine in systems A–C.

Assay for S-adenosylhomocysteine hydrolase activity. This was performed as described elsewhere [7].

Assay for metabolism of adenosine in crude extract. Adenosine was incubated in the presence of tissue extract at 30°C under the conditions described in the legend to the separate figures or tables. Protein was denatured by mixing samples from the incubation mixture with perchloric acid (containing 1 mM of adenosine, adenine, inosine, hypoxanthine, allantoin, AMP and ADP) as described above. Neutralized samples [1] (25 μl) were chromatographed in systems A and B and the radioactivity residing in the separate spots was determined. The simultaneous determination of the metabolites by the two chromatographic systems differed by less than 7%.

Determination of protein. Protein was measured by the method of Klungsøyr using bovine serum albumin as standard [8].

Results

Metabolism of [^{14}C]adenosine in crude extract from mouse liver under various conditions.

Adenosine (1 μM) was incubated in the presence of crude extract from mouse liver. A fraction of adenosine was not deaminated to inosine and further metabolized to hypoxanthine, uric acid and allantoin. No xanthine could be detected. This fraction was increased in the presence of 2-mercaptoethanol (Fig. 1A), at alkaline pH (Fig. 1B), in the presence of KCl (Fig. 1C) and was decreased upon addition of adenine to the incubation mixture (Fig. 1D). Adenine could be detected at short time of incubation as shown in the presence of various concentrations of 2-mercaptoethanol in Fig. 1A.

Addition of high concentration of exogenous adenosine deaminase (50 units/ml) after 15 min of incubation and incubation for further 2 min did not decrease the fraction of adenosine not being metabolized (Fig. 1). This indicates the presence of sufficient high level of endogenous deaminase to convert

TABLE I

SEQUESTRATION OF [^{14}C]ADENOSINE BY TISSUE EXTRACTS UNDER VARIOUS CONDITIONS

[8-^{14}C]Adenosine (1 μM) was incubated for 0.5 and 15 min in the presence of extract from various tissues. Six incubation conditions (a—f) were used. a, 30 mM Mes buffer (pH 6.0) containing 150 mM KCl, 5 mM magnesium acetate and 20 mM 2-mercaptoethanol. b, 30 mM Hepes (pH 7.0) containing KCl, magnesium acetate and 2-mercaptoethanol. c, 30 mM Tris-HCl (pH 8.0) containing KCl, magnesium acetate and mercaptoethanol. d, as in b except that KCl was omitted. e, as in b but in the presence of 1 mM of adenine. f, as in b except that 2-mercaptoethanol was omitted. Ado, adenosine; aden, adenine; ino, inosine, and hx, hypoxanthine.

Tissue (concentration of protein)	Condition of incubation	Concentration of purine after 0.5 min of incubation (nmol/ml)				Concentration of purine after 15 min of incubation (nmol/ml)			
		Ado	Aden	AMP	Ino Hx Uric acid Allantoin	Ado	Aden	AMP	Ino Hx Uric acid Allantoin
Mouse liver (3 mg/ml)	a	0.20	0.05	0.34	0.41	0.22	<0.05	0.21	0.55
	b	0.27	0.24	0.12	0.37	0.49	<0.05	0.05	0.44
	c	0.32	0.30	0.07	0.31	0.49	<0.05	<0.05	0.47
	d	0.26	0.16	0.08	0.50	0.33	<0.05	<0.05	0.65
	e	0.39	0.08	0.12	0.40	0.06	0	0.06	0.88
	f	0.22	0.15	0.15	0.48	0.05	<0.05	0.13	0.82
Mouse kidney (3 mg/ml)	a	0.43	<0.05	<0.05	0.55	0.09	<0.05	<0.05	0.90
	b	0.51	0.06	<0.05	0.42	0.11	<0.05	<0.05	0.88
	c	0.62	0.07	0	0.30	0.13	<0.05	<0.05	0.86
	d	0.45	<0.05	0	0.52	0.09	<0.05	0	0.90
	e	0.56	<0.05	0	0.42	<0.05	0	0	0.97
	f	0.15	<0.05	<0.05	0.84	<0.05	<0.05	<0.05	0.98
Mouse heart (3 mg/ml)	b	0.73	<0.05	0.05	0.22	<0.05	<0.05	0.06	0.90
Mouse brain (4 mg/ml)	b	0.68	<0.05	0.08	0.23	0.06	<0.05	<0.05	0.91
Rat liver (3 mg/ml)	a	0.52	0.10	0.23	0.15	0.23	<0.05	0	0.76
	b	0.45	0.14	<0.05	0.39	0.34	<0.05	0	0.64
	c	0.51	0.18	<0.05	0.29	0.32	<0.05	<0.05	0.64
	d	0.38	0.13	<0.05	0.49	0.22	<0.05	<0.05	0.76
	e	0.53	0.08	<0.05	0.37	0.07	0	0	0.93
	f	0.39	0.12	<0.05	0.46	0.05	<0.05	<0.05	0.94
Rat kidney (3.0 mg/ml)	a	0.05	<0.05	<0.05	0.94	<0.05	0	<0.05	0.95
	b	0.09	<0.05	<0.05	0.89	0.05	<0.05	<0.05	0.94
	c	0.39	0.13	<0.05	0.47	0.09	<0.05	<0.05	0.90
	d	0.08	<0.05	<0.05	0.91	<0.05	<0.05	<0.05	0.96
	e	0.15	<0.05	<0.05	0.84	<0.05	0	0	0.99

Rat brain (3.2 mg/ml)	b	0.79	<0.05	0	0.20	<0.05	0	0	0.99
Rat uterus (4.5 mg/ml)	b	0.08	<0.05	0	0.91	<0.05	0	<0.05	0.95
Bovine adrenal cortex	a	0.59	<0.05	<0.05	0.34	0.15	<0.05	0.06	0.78
(3.0 mg/ml)	b	0.58	0.05	<0.05	0.34	0.15	<0.05	0.06	0.78
	c	0.44	0.10	<0.05	0.45	0.18	<0.05	0.09	0.72
	d	0.60	<0.05	0	0.37	0.12	<0.05	0.16	0.71
	e	0.72	<0.05	<0.05	0.23	<0.05	0	<0.05	0.95
	f	0.59	0.05	0	0.36	<0.05	0	0.23	0.75
Bovine liver	a	0.68	0.07	<0.05	0.23	0.39	<0.05	<0.05	0.55
(4.5 mg/ml)	b	0.56	0.11	0	0.33	0.33	<0.05	0	0.65
	c	0.60	0.15	0	0.25	0.30	<0.05	0	0.69
	d	0.27	0.09	0	0.64	0.25	<0.05	0	0.74
	e	0.59	<0.05	0	0.38	0.09	<0.05	0	0.90
	f	0.38	0.09	0	0.47	<0.05	<0.05	0	0.98
Bovine kidney	a	0.65	0.13	<0.05	0.20	0.40	<0.05	<0.05	0.52
(4.5 mg/ml)	b	0.60	0.20	<0.05	0.18	0.54	0.06	0	0.40
	c	0.44	0.40	<0.05	0.15	0.49	0.09	<0.05	0.41
	d	0.59	0.16	0	0.25	0.34	0.06	0	0.60
	e	0.75	<0.05	<0.05	0.20	0.15	<0.05	0	0.82
	f	0.52	0.18	<0.05	0.25	<0.05	<0.05	<0.05	0.95
Bovine heart (3.8 mg/ml)	b	0.05	<0.05	<0.05	0.93	<0.05	<0.05	<0.05	0.96
Rabbit liver	a	0.75	0.06	0	0.19	0.33	<0.05	0.06	0.59
(3.0 mg/ml)	b	0.75	0.12	<0.05	0.12	0.29	0.05	0.05	0.61
	c	0.80	0.15	0	0.05	0.26	<0.05	0.06	0.66
	d	0.68	0.09	<0.05	0.22	0.23	<0.05	<0.05	0.71
	e	0.82	<0.05	<0.05	0.14	0.10	<0.05	0.06	0.83
	f	0.74	0.10	<0.05	0.14	<0.05	<0.05	<0.05	0.93
Rabbit skeletal muscle	a	0.92	0	<0.05	0.07	<0.05	0	0.58	0.40
(4.5 mg/ml)	b	0.91	0	<0.05	0.08	0.06	0	0.43	0.51
	c	0.91	0	<0.05	0.08	0.10	0	0.12	0.78
	d	0.91	0	0	0.09	<0.05	0	<0.05	0.95
	e	0.92	0	<0.05	0.06	<0.05	0	0.25	0.74
	f	0.90	0	<0.05	0.09	0	0	0.45	0.55
Rabbit heart (4.5 mg/ml)	b	0.85	0	<0.05	0.11	<0.05	0	0.11	0.86

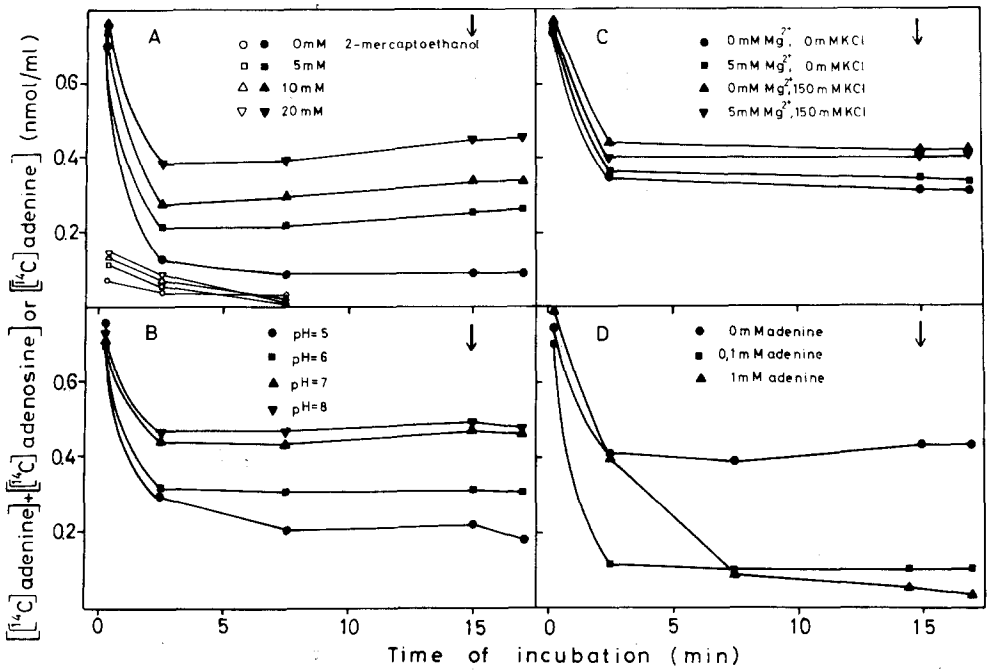

Fig. 1. Effect of 2-mercaptoethanol, pH, potassium chloride, magnesium and adenine on the sequestration of adenosine in crude extract from mouse liver, (A) [8-^{14}C]Adenosine (1 μM) was incubated in the presence of mouse liver cytosol (1 mg of protein/ml) for the time indicated. The incubation buffer was 30 mM Hepes (pH 7.0) containing 5 mM magnesium acetate, 150 mM KCl and various concentrations of 2-mercaptoethanol (0—20 mM). After 15 min of incubation, the incubation mixture was supplemented with 50 units/ml of adenosine deaminase (arrow) and incubated for further 2 min. The sum of the concentration of adenosine and adenine (filled symbols) is plotted against time of incubation. Open symbols represent the concentration of adenine. (B) The experimental design was as in (A) except that the incubation buffers were 30 mM Mes, pH 5.0 or pH 6.0, or 30 mM Hepes buffer (pH 7.0) or 30 mM Tris-HCl, pH 8.0. All buffers contained 5 mM magnesium acetate, 150 mM KCl and 20 mM 2-mercaptoethanol. (C) The experiment was performed as above except that the incubation was conducted in the absence or presence of KCl or magnesium acetate or both as shown on the figure. The incubation buffer was 30 mM Hepes (pH 7.0) containing 20 mM 2-mercaptoethanol. (D) The incubation was performed in the presence of increasing concentrations of adenine (0—1 mM). The incubation buffer was 30 mM Hepes (pH 7.0) containing 5 mM magnesium acetate, 150 mM KCl and 20 mM 2-mercaptoethanol.

adenosine available for deamination to inosine.

A fraction (up to about 30%) of adenosine was converted to a compound which was mainly identified as AMP. This phenomenon was nearly totally dependent on Mg^{2+} and KCl and was more pronounced at low pH (Table I). Formation of AMP was also observed after the tissue extract was subjected to prolonged dialysis and gel filtration on Sephadex G-25 to remove substances of low molecular weight from the proteins. This could be explained by phosphorylation of adenosine by adenosine kinase using protein-bound ATP as phosphoryl donor. Phosphorylation of adenosine in crude extract in the absence of added ATP has been reported by others [9]. The slight increase in the amount of adenosine observed after the initial fall (Fig. 1) is probably effected by conversion of AMP to adenosine catalyzed by 5′-nucleotidase known to be present in the soluble fraction of cell homogenate [10].

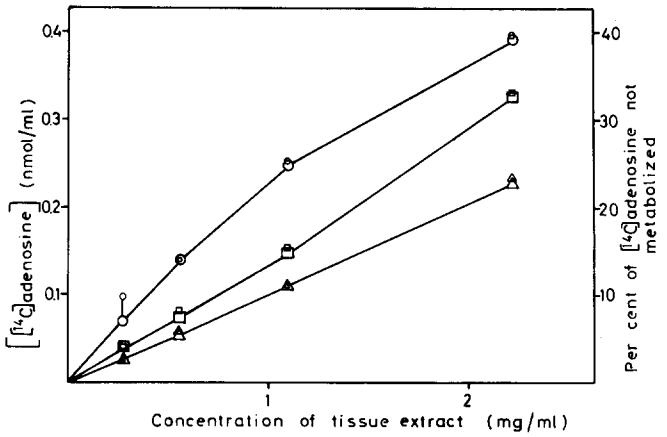

Fig. 2. Sequestration of adenosine as a function of the concentration of mouse liver extract in the presence of a constant amount of adenosine deaminase. [8-^{14}C]Adenosine (1 μM) was incubated in the presence of mouse liver extract which was supplemented with adenosine deaminase at a final concentration of 0.50 unit/ml (△), 0.25 unit/ml (□) or 0.125 unit/ml (○). After 15 min of incubation (small symbols) the incubation was made 50 units/ml in adenosine deaminase and incubated for further 2 min (big symbols). The incubation buffer was 30 mM Tris-HCl (pH 8.0) containing 150 mM KCl and 20 mM 2-mercaptoethanol. The concentration of adenosine is plotted versus concentration of protein.

Measurement of sequestration potency for adenosine in crude tissue extract

The amount of adenosine (1 μM) not available for deamination at various concentrations (0.5—7 mg/ml) of liver extract was determined at pH 8.0 and in the absence of Mg^{2+}. Incubation was run for 15 min and thereafter for 2 min in the presence of exogenous adenosine deaminase (50 units/ml). The amount of adenosine sequestered was non-linear with respect to the concentration of tissue extract and increased per mg of protein upon dilution of the extract (data not shown) probably because of increased time of interaction of the nucleoside with adenosine binding protein(s). When the cytosol was diluted in the presence of a constant amount of adenosine deaminase, linearity of the amount of adenosine sequestered versus the concentration of tissue extract was obtained (Fig. 2). The slope of the graph increased by decreasing the concentration of exogenous adenosine deaminase (Fig. 2). From the linear part of the curve the sequestration potency (pmol of adenosine/mg of protein) could be determined.

Determination of the tissue level of cyclic AMP-adenosine binding protein/S-adenosylhomocysteinase in mouse liver

The sequestration potency of the purified cyclic AMP-adenosine binding protein/*S*-adenosylhomocysteinase and of crude extract from mouse liver were determined under the same conditions (Fig. 3). A total recovery of the sequestration potency of the purified cyclic AMP-adenosine binding protein/*S*-adenosylhomocysteinase (8 pmol of adenosine/μg of protein) was observed when the protein was added to a ten-fold concentration range of crude extract (Fig. 3B). From these data (Fig. 3) it could be calculated that cyclic AMP-adenosine binding protein/*S*-adenosylhomocysteinase accounts for 1.8% of the soluble proteins in mouse liver.

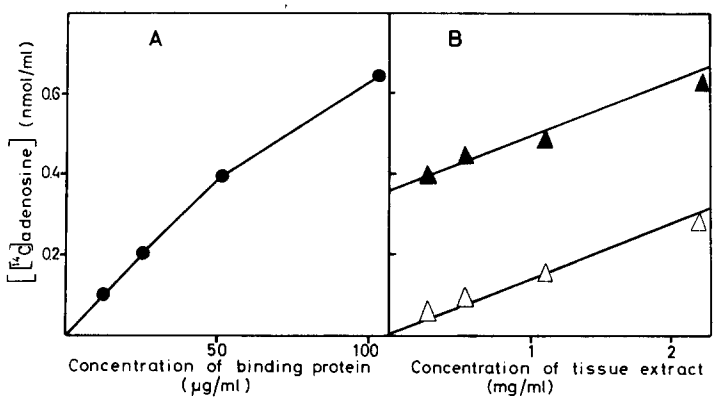

Fig. 3. Sequestration of adenosine by the purified binding protein and the recovery of its sequestration potency in crude liver extract. (A) The sequestration of adenosine by the purified protein was determined as described in legend to Fig. 2. The concentration of adenosine deaminase was 0.25 unit/ml and the incubation time 15 + 2 min. (B) The sequestration of adenosine at various concentrations of tissue extract from mouse liver (△) and in tissue extracts supplemented with 50 μg/ml of purified binding protein (▲) was determined as above.

S-Adenosylhomocysteine was hydrolyzed at a rate of 0.54 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ and 0.0092 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ in the presence of purified cyclic AMP-adenosine binding protein/*S*-adenosylhomocysteinase and liver cytosol, respectively. The enzyme activity of the purified protein was totally recovered when added to the crude cytosol (data not shown). These data are consistent with cyclic AMP-adenosine binding protein/*S*-adenosylhomocysteinase accounting for 1.7% of the proteins in mouse liver cytosol.

About 84 g of soluble proteins could be extracted from 1 kg of mouse liver [11] which corresponds to 1.5 g of cyclic AMP-adenosine binding protein/*S*-adenosylhomocysteinase. Assuming that intracellular water comprises 70% of the tissue wet weight, the cellular concentration of this protein is about 10 μM.

Sequestration of adenosine at increasing concentrations of tissue extract

The amount of adenosine not available for deamination was determined at increasing concentrations of tissue extract and at various concentrations (1, 10 and 100 μM) of adenosine (Fig. 4). By increasing the concentration of extract beyond 10 mg of protein/ml, only a slight increase or decrease in the amount of adenosine sequestered, was observed. A sequestration capacity of at least 800 pmol/mg of protein could be calculated from the data presented in Fig. 4.

Degradation of [^{14}C]adenosine in crude extract from various tissues

The effect of pH, KCl, 2-mercaptoethanol and adenine on the degradation of adenosine (1 μM) in crude extracts from various tissues from mouse, rat, rabbit and cow was investigated (Table I). The concentrations of metabolites were determined at short time of incubation (30 s) and at long time of incubation (15 min) in order to detect a possible formation of adenine and to estimate the fraction of adenosine not available for deamination, respectively. No significant decrease in the amount of adenosine sequestered was observed after

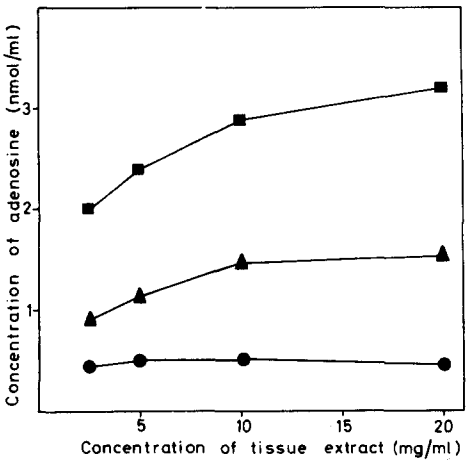

Fig. 4. Sequestration of adenosine at increasing concentrations of tissue extract. [8-^{14}C]Adenosine at concentrations of 1 μM (●), 10 μM (▲) or 100 μM (■) was incubated in the presence of various concentrations of tissue extracts. The incubation buffer was 30 mM Hepes buffer (pH 7.0) containing 150 mM KCl, 5 mM magnesium acetate and 20 mM 2-mercaptoethanol. After 15 min of incubation, the incubation mixture was supplemented with 50 units/ml of adenosine deaminase and incubated for further 2 min.

TABLE II

COMPARISON OF THE SEQUESTRATION POTENCY FOR ADENOSINE OF EXTRACTS FROM VARIOUS TISSUES

[8-^{14}C]Adenosine (1 μM) was incubated in the presence of various concentrations of tissue extracts prepared from the tissues listed in the left row of the table. The incubation buffer was 30 mM Tris-HCl (pH 8) containing 150 mM KCl and 20 mM 2-mercaptoethanol. The concentration of adenosine deaminase was 0.25 units/ml. After 15 min of incubation the incubation mixture was made 50 units/ml in adenosine deminase and incubated for further 2 min and the amount of adenosine present after 17 min of incubation which differed by less than 5% from the values obtained after 15 min of incubation, was plotted against the concentration of tissue extract. From the linear part of the curve, the amount of adenosine sequestered (pmol/mg of protein) was determined.

Tissue	Relative sequestration potency	
	pmol of adenosine/mg of protein	Percent
Mouse		
Liver	177	100
Kidney	39	22
Heart	3	1.7
Brain	7	4.0
Rat		
Liver	100	56
Kidney	25	14
Heart	2	1.1
Brain	8	4.5
Uterus	4	2.3
Rabbit		
Liver	51	29.0
Heart	2	1.1
Skeletal muscle	1	0.6
Bovine		
Liver	114	64
Kidney	67	38
Heart	2	1.1
Adrenal cortex	27	15

further 1 min of incubation in the presence of exogenous adenosine deaminase (50 units/ml) (data not shown).

The fraction of adenosine not metabolized was less than 5% (0.05 nmol/ml) in mouse, rat, rabbit and bovine heart, rat uterus and mouse and rat brain, and only the results obtained at pH 7 are given. Adenine formation could be detected in most of these tissues (Table I). The sequestration of adenosine in extract from mouse and rat kidney, rat liver, bovine adrenal cortex and rabbit skeletal muscle is stimulated by alkaline pH, 2-mercaptoethanol and KCl and inhibited by adenine (Table I), as shown for mouse liver extract (Fig. 1). The effect of these factors on adenine formation at short time of incubation paralleled their effect on the sequestration of adenosine (Table I) as previously shown for the purified cyclic AMP-adenosine binding protein/*S*-adenosylhomocysteinase from mouse liver [3]. The same results were obtained for bovine liver and kidney and rabbit liver except that the sequestration of adenosine did not show the highest value at alkaline pH (Table I).

Comparison of the sequestration potency for adenosine in various tissues

The data cited in the preceding paragraph do not provide information allowing the comparison of the sequestration potency in different tissues as the level of adenosine deaminase differs from one tissue to another [12]. This problem was circumvented by determining the sequestration of adenosine in the presence of constant amount of exogenous adenosine deaminase as described above (Fig. 2). The sequestration potency is high in liver, kidney and adrenal cortex, low in cardiac and skeletal muscle. Intermediate values were obtained in brain and uterus (Table II).

Discussion

The observation that the protection of adenosine against deamination in crude extract from mouse liver shows the same requirements as the sequestration of adenosine by the purified cyclic AMP-adenosine binding protein/*S*-adenosylhomocysteinase, points to a role of this protein in the sequestration of adenosine in crude extract. This suggestion is reinforced by the fact that the same tissue level for this protein was obtained by calculation based on sequestration of adenosine and hydrolysis of *S*-adenosylhomocysteine.

Meyskens and Williams reported that a small portion of adenosine seems unavailable for metabolism in extract from erythrocytes [13]. The reason for further attention not being paid to this phenomenon is obvious. Under the conditions of assay for adenosine deaminase [14], adenosine kinase [15] and *S*-adenosylhomocysteine synthase [16,17], the concentration of adenosine is higher than used in the experiments presented here, and the tissue extract is highly diluted to meet with the requirement of linearity of enzyme activity versus time and concentration of enzyme.

The results presented in Fig. 4 show that the amount of adenosine not available for deamination does not decrease when the concentration of soluble proteins approaches the level existing in the cell. This indicates that the sequestration of adenosine is not an artifact of the dilution process. Furthermore, a large fraction of adenosine is not available for deamination at 1 μM

(Figs. 1 and 4) and 10 μM (Fig. 4) of adenosine which is in the order of magnitude reported for the tissue level of this nucleoside [18—21]. These observations together with the fact that the cellular level of cyclic AMP-adenosine binding protein/*S*-adenosylhomocysteinase in mouse liver (10 μM) is in the upper range reported for intracellular proteins [22], suggest that a substantial fraction of adenosine may be sequestered in vivo.

At low concentration of adenosine, the intracellular nucleoside seems mainly to be phosphorylated to AMP [6,18,23]. Formation of *S*-adenosyl-L-homocysteine is not a major metabolic fate of adenosine [6]. The metabolic flow is probably in the direction of hydrolysis of *S*-adenosyl-L-homocysteine [24]. These data point to some objections to the experiments presented in this paper. The sequestration of adenosine has not been determined under the conditions optimal for the phosphorylation of adenosine to AMP (i.e. in the presence of ATP) and in the presence of homocysteine and *S*-adenosyl-L-homocysteine. However, under these conditions, studies on the sequestration would be obscured by inhibition of adenosine binding by AMP and ADP [5], and a continuous formation of adenosine from AMP and *S*-adenosyl-L-homocysteine catalyzed by the enzymes 5′-nucleotidase [10] and *S*-adenosylhomocysteinase, respectively.

The ability to sequester adenosine, which is high in liver, kidney and adrenal cortex, moderate in brain and low in cardiac and skeletal muscle (Table I), parallels the tissue level of *S*-adenosylhomocysteinase in these tissues [17,25,26]. Furthermore, the sequestration of adenosine shows similar requirements in the various tissues tested (Table I). This indicates that adenosine is sequestered through its interaction with *S*-adenosylhomocysteinase in mouse liver and other tissues.

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