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Coenzyme A and its thioester pools in fasted and fed rat tissues

Yuka Tokutake [a], Naoki Onizawa [b], Hiroki Katoh [c], Atsushi Toyoda [b,d], Shigeru Chohnan [a,c,*]

[a] Department of Applied Life Science, United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai, Fuchu, Tokyo 183-8509, Japan
[b] Department of Biological Production Science, United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai, Fuchu, Tokyo 183-8509, Japan
[c] Department of Bioresource Science, Ibaraki University College of Agriculture, 3-21-1 Chuo, Ami, Ibaraki 300-0393, Japan
[d] Department of Biological Production Science, Ibaraki University College of Agriculture, 3-21-1 Chuo, Ami, Ibaraki 300-0393, Japan

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ABSTRACT

Levels of three coenzyme A (CoA) molecular species, i.e., nonesterified CoA (CoASH), acetyl-CoA, and malonyl-CoA, in fasted and fed rat tissues were analyzed by the acyl-CoA cycling method which makes detection possible at the pmol level. Malonyl-CoA in brain tissues readily increased with feeding, and inversely, acetyl-CoA decreased. This phenomenon occurred in the cerebral cortex, hippocampus, cerebellum, and medulla oblongata, as well as in the hypothalamus which controls energy balance by monitoring malonyl-CoA. In the non-brain tissues, the sizes of the acetyl-CoA, malonyl-CoA, and CoASH pools depended on the tissues. The total CoA pools consisting of the above three CoA species in the liver, heart, and brown adipose tissue were larger and those of the perirenal, epididymal, and ovarian adipose tissues were much smaller, compared with those of other tissues including brain tissues. In addition, the response of each CoA pool to feeding was not uniform, suggesting that the tissue-specific metabolism individually functions in the non-brain tissues. Thus, a comprehensive analysis of thirteen types of rat tissue revealed that CoA pools have different sizes and showed a different response to fasting and feeding depending on the tissue.

1. Introduction

Coenzyme A (CoA) is an essential cofactor synthesized from pantothenate (vitamin B_5), cysteine, and ATP [1,2] and functions as an acyl group carrier in cells. The cofactor participates in numerous metabolic pathways, as it is estimated that about 4% of all enzymes utilize a nonesterified CoA (CoASH) or acyl-CoAs as a substrate [3]. The CoA biosynthetic pathway is composed of five steps, and is governed by pantothenate kinase (PanK) which catalyzes the initial step, ATP-dependent phosphorylation of pantothenate, since the enzyme activity is regulated through end products such as CoASH, acetyl-CoA, and malonyl-CoA [2]. Four PanK isoforms, i.e., PanK1α, PanK1β, PanK2, and PanK3, are catalytically active in mammals, and their differential expression depending on the tissue and *in vitro* feedback regulation by the CoA species were observed [2,4–10]. Meanwhile, overall *in vivo* behavior of the synthesized CoASH and its thioesters has not been sufficiently understood, although there is limited information on the contents of CoASH and acyl-CoA in mammalian tissues from the liver, heart, skeletal muscle, brain, and hypothalamus [10–26].

We conducted a comprehensive analysis of CoASH, acetyl-CoA, and malonyl-CoA in thirteen types of tissue from male and female rats under fed and fasted states, since it is known that the hypothalamic malonyl-CoA and the hepatic CoASH levels change with the transition from the fasted to the fed conditions [10,11,13,15,16,20]. The tissue CoA species were determined using a unique enzymatic method, the acyl-CoA cycling method, which can detect pmol levels of CoASH, acetyl-CoA, and malonyl-CoA separately [27–30]. Malonyl-CoA pools in the brain tissue clearly responded to feeding. The levels of malonyl-CoA not only in the hypothalamus, which recognizes malonyl-CoA as a mediator of feeding behavior [16,20], but also in the cerebral cortex, hippocampus, cerebellum, and medulla oblongata increased. On the other hand, the sizes and contents of the CoA pools from all the non-brain tissues tested were different and each CoA pool exhibited an intrinsic change with feeding.

2. Materials and methods

2.1. Handling of rats and extraction of CoA pools

Animal experiments were conducted in accordance with guidelines of the Ibaraki University Animal Research Committee. Male and female Wistar rats at 4-weeks of age were purchased from Kiwa Laboratory Animals Co. Ltd. (Wakayama, Japan) and were

* Corresponding author at: Department of Bioresource Science, Ibaraki University College of Agriculture, 3-21-1 Chuo, Ami, Ibaraki 300-0393, Japan. Fax: +81 29 888 8672.
E-mail address: chohnan@mx.ibaraki.ac.jp (S. Chohnan).

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fed standard laboratory chow (MF from Oriental Yeast Co. Ltd., Tokyo, Japan) and water *ad libitum* for 6 weeks (until 10-weeks old) under a 12-h light (6:00 am to 6:00 pm)/12-h dark (6:00 pm to 6:00 am) cycle at 22 ± 1 °C in an individual cage. The male and female rats were divided into two groups each which were composed of three individuals. One group was starved for 16 h (fasted rats), and another was fed the chow for 2 h after 16-h of starvation (fed rats). The body weights of rats used in this work were as follows: the fasted male rats, 358.5 ± 27.7 g (means ± SEM [n = 3]); the fed male rats, 313.8 ± 16.9 g; the fasted female rats, 204.8 ± 8.4 g; the fed female rats, 208.0 ± 7.4 g. Thirteen types of tissue, i.e., cerebral cortex, hippocampus, hypothalamus, cerebellum, medulla oblongata, liver, spleen, kidney, heart, skeletal muscle from soleus muscle, perirenal adipose tissue, brown adipose tissue, epididymal adipose tissue (male), and ovarian adipose tissue (female), were removed from both the starved and fed rats and the CoA pools were extracted by homogenization of the tissues in 400 μl of 0.6 M sulfuric acid per each 100 mg of tissue. For complete extraction of the intracellular CoASH and its thioesters and inactivation of endogenous enzymes, the homogenized tissues were kept at 4 °C overnight. After centrifugation at 9000*g* at 4 °C for 10 min, 1 M Tris (0.05 vol. of the supernatant) was added to the supernatant and the resulting solution was carefully adjusted to around pH 6.5 with NaOH on ice. The neutralized solution was kept at −80 °C overnight and the clear extract was recovered by centrifugation after thawing. CoASH, acetyl-CoA, and malonyl-CoA in the extract were stable for at least a week at 4 °C.

2.2. Measurement of CoASH, acetyl-CoA, and malonyl-CoA in the tissue extract

CoASH, acetyl-CoA, and malonyl-CoA in the extracts were measured by the acyl-CoA cycling method using malonate decarboxylase [27–30]. The enzyme was purified from *Pseudomonas putida* JCM 20089 (formerly IAM 1177) as previously described [31]. The reaction mixture of the acyl-CoA cycling method contained 50 mM Tris–HCl (pH 7.2), 1 mM 2-mercaptoethanol, 10 mM $MgSO_4$, 50 mM malonate, 10 mM ATP, 1 U of malonate decarboxylase, and the tissue extracts containing acetyl-CoA and/or malonyl-CoA (2.5–80 pmol) in 400 μl. The cycling reaction was initiated by the addition of malonate decarboxylase, and the mixture was incubated at 30 °C for 20 min, followed by the addition of 1 U of acetate kinase from *Escherichia coli* ([EC 2.7.2.1], Roche Diagnostics GmbH, Mannheim, Germany). After 20 min of incubation, 0.2 ml of 2.5 M neutralized hydroxylamine was added, and the incubation was continued for an additional 20 min at 30 °C. The reaction was terminated by adding 0.6 ml of 10 mM ferric chloride dissolved in 25 mM trichloroacetic acid-1 M HCl. The A_{540} of the acetohydroxamate formed was measured. Every assay was performed in duplicate. Separate determinations of malonyl-CoA and CoASH in the tissue extracts were accomplished by eliminating acetyl-CoA with citrate synthase (EC 4.1.3.7) and by converting CoASH to acetyl-CoA with phosphate acetyltransferase (EC 2.3.1.8), respectively, before the measurements using the acyl-CoA cycling method. The reaction mixture for the citrate synthase treatment contained 50 mM Tris–HCl (pH 7.2), 10 mM $MgSO_4$, 2 mM oxaloacetate, 1 U of citrate synthase from porcine heart (Roche Diagnostics GmbH), and the tissue extracts in 1 ml. The reaction was carried out at 25 °C for 20 min and terminated by placing the reaction tube on ice slush. The reaction mixture for CoASH measurement contained 50 mM Tris–HCl (pH 7.2), 1 mM 2-mercaptoethanol, 10 mM $MgSO_4$, 10 mM ammonium sulfate, 0.1 mM acetylphosphate, 5 U of phosphate acetyltransferase from *Bacillus stearothermophilus* (Sigma–Aldrich Inc., St. Louis, MO, USA), and the tissue extracts in 1 ml. After incubation at 25 °C for 20 min, the reaction was terminated by filtration through a Millipore Ultracel YM-30 ultrafilter to eliminate the phosphate acetyltransferase.

The water content of the cerebral cortex, cerebellum, and liver were determined by drying at 105 °C.

3. Results

3.1. CoA pools in the brain tissues

Changes in the size and composition of CoASH and short chain acyl-CoAs in the thirteen types of tissue in response to fasting and feeding were examined (Table 1). First, we shall describe the behavior of the CoA pools in the brain tissues, i.e., cerebral cortex, hippocampus, hypothalamus, cerebellum, and medulla oblongata. It is well known that the hypothalamic malonyl-CoA level increases depending on feeding and this regulates food intake in mice [16,20]. The increase of the hypothalamic malonyl-CoA level according to the transition from the fasted to the fed state was also observed in rats, and resulted in a 4.0-fold increase from 0.234 to 0.934 nmol/g of tissue in males and 3.1-fold from 0.264 to 0.826 nmol/g in females. Surprisingly, the dramatic rise of the malonyl-CoA level by feeding occurred not only in the hypothalamus but also in the cerebral cortex (a 13.0-fold increase in males and 16.1-fold in females), hippocampus (4.3- and 9.2-fold), cerebellum (4.0- and 4.2-fold), and medulla olongata (2.4- and 3.0-fold), although the metabolite was a minor molecular species in all brain tissues during starvation. It is noteworthy that the malonyl-CoA level in the cerebral cortex changes within a wider range than that in the hypothalamus. The malonyl-CoA level in the fasted rat brain was suppressed, even though available acetyl-CoA exists there. On the other hand, the acetyl-CoA levels decreased inversely in proportion to the increase in the malonyl-CoA levels. These findings indicate that malony-CoA levels in the brain are strictly controlled by acetyl-CoA carboxylase activity which is sensitive to a cellular energy state [18,20,24]. CoASH (nonesterified CoA) levels in the brain tissues showed an individual differential response to the change of energy state which was attributed to fasting and feeding: little change in the hypothalamus, an increase in the cerebral cortex and cerebellum, and a decrease in the hippocampus and medulla oblongata. Total CoA pools are expressed here as the sum of acetyl-CoA, malonyl-CoA, and CoASH which are detectable by the acyl-CoA cycling method. The sizes of the total CoA pools seem to be kept within a narrow range in the cerebral cortex, hypothalamus, and cerebellum, although the cerebral cortex and cerebellum formed slightly larger pools by feeding, corresponding to the rise of the CoASH pools. Meanwhile, the sizes in the hippocampus and medulla oblongata became almost half with the transition from the fasted to the fed state. This phenomenon was mainly due to the decrease of CoASH pool, and it is not known whether the CoASH pools were really suppressed by the enzymatic decomposition of CoASH or apparently decreased by a conversion of CoASH into long chain acyl-CoAs which cannot be measured by the unique enzymatic assay method.

A significant difference between males and females was not observed in the three CoA pools from the brain tissues, although the difference was detected only in the CoASH pool from the hippocampus. The sizes of the acetyl-CoA, malonyl-CoA, and CoASH pools in the brain tissues seem to change in response to the supply of energy source regardless of whether male or female.

3.2. CoA pools in the non-brain tissues

Next, the CoA pools from the liver, spleen, kidney, heart, skeletal muscle, perirenal adipose tissue, brown adipose tissue, epididymal adipose tissue (male), and ovarian adipose tissue (female) and

Table 1
CoA pools in fasted and fed rat tissues.

Tissues	CoA species	Male		Female		ANOVA		
		Fasting	Feeding	Fasting	Feeding	Feed	Sex	Sex × Feed
Cerebral cortex	A	1.867 ± 0.128	0.677 ± 0.261	2.297 ± 0.080	0.603 ± 0.287	**	ns	ns
	M	0.093 ± 0.036	1.213 ± 0.072	0.077 ± 0.009	1.239 ± 0.112	**	ns	ns
	CoA	1.457 ± 0.073	2.431 ± 0.191	1.373 ± 0.150	3.437 ± 0.850	**	ns	ns
	Total	3.417 ± 0.185	4.321 ± 0.246	3.747 ± 0.118	5.278 ± 0.775	*	ns	ns
Hippocampus	A	1.535 ± 0.123	1.118 ± 0.334	2.042 ± 0.250	0.696 ± 0.102	**	ns	ns
	M	0.170 ± 0.029	0.724 ± 0.049	0.087 ± 0.025	0.803 ± 0.053	**	ns	ns
	CoA	3.050 ± 0.426	0.419 ± 0.098	5.195 ± 0.352	0.727 ± 0.092	**	**	*
	Total	4.755 ± 0.467	2.260 ± 0.352	7.324 ± 0.507	2.226 ± 0.050	**	*	**
Hypothalamus	A	1.188 ± 0.055	0.816 ± 0.107	1.609 ± 0.135	0.639 ± 0.019	**	ns	*
	M	0.234 ± 0.012	0.934 ± 0.072	0.264 ± 0.017	0.826 ± 0.122	**	ns	ns
	CoA	4.049 ± 0.756	3.064 ± 1.278	4.113 ± 1.121	4.893 ± 0.856	ns	ns	ns
	Total	5.472 ± 0.800	4.815 ± 1.204	5.986 ± 1.211	6.358 ± 0.845	ns	ns	ns
Cerebellum	A	3.382 ± 0.086	1.891 ± 0.356	3.785 ± 0.237	1.285 ± 0.130	**	ns	ns
	M	0.354 ± 0.056	1.418 ± 0.120	0.375 ± 0.038	1.586 ± 0.051	**	ns	ns
	CoA	1.400 ± 0.884	2.339 ± 0.802	0.792 ± 0.172	3.564 ± 0.161	*	ns	ns
	Total	5.135 ± 0.882	5.648 ± 0.500	4.952 ± 0.244	6.435 ± 0.248	ns	ns	ns
Medulla oblongata	A	1.588 ± 0.015	0.569 ± 0.180	1.660 ± 0.175	0.598 ± 0.031	**	ns	ns
	M	0.296 ± 0.032	0.711 ± 0.114	0.255 ± 0.044	0.758 ± 0.027	**	ns	ns
	CoA	7.373 ± 0.439	4.002 ± 0.495	7.056 ± 0.944	3.598 ± 0.313	**	ns	ns
	Total	9.257 ± 0.461	5.283 ± 0.755	8.972 ± 1.098	4.954 ± 0.265	**	ns	ns
Liver	A	7.215 ± 1.129	9.857 ± 0.933	6.608 ± 0.572	9.099 ± 1.424	*	ns	ns
	M	0.818 ± 0.104	1.003 ± 0.032	0.773 ± 0.124	0.830 ± 0.076	ns	ns	ns
	CoA	22.253 ± 7.015	4.194 ± 1.932	15.721 ± 4.575	0.173 ± 0.148	**	ns	ns
	Total	30.287 ± 7.683	15.054 ± 1.754	23.103 ± 4.615	10.102 ± 1.438	*	ns	ns
Spleen	A	2.376 ± 0.311	2.123 ± 0.071	1.471 ± 0.143	2.013 ± 0.088	ns	*	ns
	M	0.504 ± 0.102	0.448 ± 0.056	0.891 ± 0.023	0.479 ± 0.072	**	*	*
	CoA	4.510 ± 1.571	5.400 ± 0.437	2.722 ± 0.629	3.608 ± 0.358	ns	ns	ns
	Total	7.391 ± 1.288	7.971 ± 0.412	5.085 ± 0.495	6.100 ± 0.264	ns	*	ns
Kidney	A	5.132 ± 0.738	4.246 ± 0.180	4.735 ± 0.264	3.648 ± 0.452	ns	ns	ns
	M	0.434 ± 0.070	0.230 ± 0.039	0.708 ± 0.055	0.309 ± 0.052	**	*	ns
	CoA	1.949 ± 0.140	nd[a]	0.687 ± 0.253	nd	–	–	–
	Total	7.516 ± 0.609	4.476 ± 0.185	6.130 ± 0.074	3.956 ± 0.411	**	*	ns
Heart	A	5.604 ± 0.660	7.748 ± 0.224	2.620 ± 0.446	6.409 ± 0.359	**	**	ns
	M	1.748 ± 0.151	1.452 ± 0.063	2.908 ± 0.259	2.015 ± 0.340	*	**	ns
	CoA	11.047 ± 2.412	5.094 ± 0.709	7.720 ± 0.934	nd	–	–	–
	Total	18.399 ± 3.085	14.295 ± 0.869	13.248 ± 0.820	8.424 ± 0.095	*	*	ns
Skeletal muscle	A	1.541 ± 0.263	0.674 ± 0.069	1.368 ± 0.264	0.974 ± 0.117	*	ns	ns
	M	0.448 ± 0.107	0.604 ± 0.052	0.748 ± 0.143	0.525 ± 0.020	ns	ns	ns
	CoA	1.572 ± 0.572	1.042 ± 0.073	2.544 ± 0.376	0.939 ± 0.182	*	ns	ns
	Total	3.561 ± 0.915	2.320 ± 0.103	4.659 ± 0.548	2.438 ± 0.272	*	ns	ns
Perirenal adipose tissue	A	0.186 ± 0.048	0.274 ± 0.006	0.163 ± 0.023	0.091 ± 0.039	ns	*	*
	M	0.182 ± 0.020	0.140 ± 0.032	0.144 ± 0.041	0.171 ± 0.005	ns	ns	ns
	CoA	0.214 ± 0.028	0.269 ± 0.101	0.261 ± 0.041	0.141 ± 0.023	ns	ns	ns
	Total	0.582 ± 0.085	0.683 ± 0.078	0.567 ± 0.062	0.403 ± 0.049	ns	ns	ns
Brown adipose tissue	A	5.902 ± 2.823	11.324 ± 1.510	7.014 ± 0.721	10.239 ± 1.807	*	ns	ns
	M	2.712 ± 0.243	3.107 ± 0.568	3.346 ± 1.779	3.514 ± 0.709	ns	ns	ns
	CoA	7.865 ± 2.510	5.888 ± 3.182	19.694 ± 6.810	11.445 ± 2.159	ns	ns	ns
	Total	16.479 ± 5.527	20.319 ± 4.966	30.054 ± 7.304	25.197 ± 3.342	ns	ns	ns

Epididymal adipose tissue	A	0.592 ± 0.191	0.537 ± 0.060	–	–	ns	–	–	
	M	0.136 ± 0.019	0.062 ± 0.004	–	–	ns	–	–	
	CoA	0.149 ± 0.050	0.281 ± 0.035	–	–	ns	–	–	
	Total	0.877 ± 0.251	0.881 ± 0.045	–	–	ns	–	–	
Ovarian adipose tissue	A	–	–	0.147 ± 0.011	0.141 ± 0.012	ns	–	–	
	M	–	–	0.144 ± 0.029	0.108 ± 0.062	ns	–	–	
	CoA	–	–	0.044 ± 0.009	nd	–	–	–	
	Total	–	–	0.335 ± 0.036	0.249 ± 0.055	ns	–	–	

Male and female rats were fed the standard laboratory chow *ad libitum*, and then 13 types of tissue listed in the table were removed from the fasted rats that were starved for 16 h and from the fed rats that were refed for 2 h after 16-h starvation. Acetyl-CoA, malonyl-CoA, and CoASH pools in the tissues were determined using the acyl-CoA cycling method. "A", "M", "CoA", and "Total" indicate acetyl-CoA, malonyl-CoA, CoASH, and the total CoA which is defined here as the sum of the three CoA pools, respectively. All data are expressed as nmol/g of tissue and means ± SEM ($n = 3$). Statistical significance was assessed by the Two-way ANOVA: ns, not significant; $^{*}P < 0.05$; $^{**}P < 0.01$. In the epididymal adipose tissue (male) and ovarian adipose tissue (female), the Student's *t*-test was employed.

[a] Not detected.

were estimated. The larger total CoA pools were formed in the liver, heart, and brown adipose tissue compared with those of the other tissues including the brain tissues. On the other hand, the sizes of the total CoA pools from the perirenal, epididymal, and ovarian adipose tissues were markedly smaller, showing less than 0.9 nmol/g of tissue. In perirenal adipose tissues, each CoA pool changed little through the fasted and the fed state and there was no significant difference between males and females. The size of the total CoA pools in the epididymal adipose tissue (male), however, was substantially larger than that in the ovarian adipose tissue (female), and both pools hardly responded to feeding.

The sizes of acetyl-CoA pools tended to be larger during feeding in the liver, heart, and brown adipose tissue, all which possessed larger total CoA pools, as mentioned above. That in the spleen remained steady during feeding, and those in the kidney and skeletal muscle were suppressed. Malonyl-CoA was also a minor CoA species in the non-brain tissues. The malonyl-CoA pools did not change significantly, whereas in the spleen and kidney the pools decreased by almost half through the transition from the fasted to the fed state. It is reported that in mice, the malonyl-CoA pools in the skeletal muscles are little changed by fasting and feeding [17]. In rats, significant change in malonyl-CoA pools of the skeletal muscle was not observed either. The hepatic CoASH level markedly decreased with feeding and consequently this depression led to the lower total CoA pool than that during fasting, in agreement with previous results [10,11,13,15]. This is considered to be caused by a degradation of CoASH in accordance with the expression of mouse nudix hydrolase 7 (Nudt7), a liver-specific CoA-phosphodiesterase [32], with food intake [10,33,34]. This decrease during feeding also occurred in the CoASH pools of the kidney, heart, and skeletal muscle, although there is no information on the behavior of the CoA-phosphodiesterase in those tissues. Appreciable sexual dimorphism was detected only in the acetyl-CoA and malonyl-CoA pools from the heart.

4. Discussion

Changes in the size and composition of the pools of acetyl-CoA, malonyl-CoA, and nonesterified CoA (CoASH) in rat tissues were estimated (Table 1). The hypothalamic malonyl-CoA readily responded to the carbon source and dramatically increased. Acetyl-CoA which is a substrate of acetyl-CoA carboxylase inversely decreased. In the murine hypothalamus malonyl-CoA functions as a mediator of feeding behavior and its responsivity to feeding has been well studied together with acetyl-CoA carboxylase [16,18,20–22,24]. In addition, we found here that the brain tissues, except for the hypothalamus, also formed considerably larger malonyl-CoA pools with the acetyl-CoA pools decreasing according to feeding. The levels of both CoA molecular species seem to be governed by the same metabolic regulation involving acetyl-CoA carboxylase in the central nervous system, although the role of regulation in each tissue is still unknown. On the other hand, the acetyl-CoA, malonyl-CoA, and CoASH pools from the eight types of non-brain tissue each showed a different response to feeding, indicating that a tissue-specific metabolic pathway preferentially works in the eight types of non-brain tissue. The decrease of the CoASH pool in the liver was particularly noteworthy, and the degradation can be explained by the behavior of the liver-specific CoA-degradative enzyme [10,11,13–15,32–34].

The intracellular levels of CoASH and CoA thioesters are strictly controlled by four PanK isoforms in eukaryotic cells [2]. These enzymes are inhibited by CoASH and acyl-CoAs, and show different responses to CoA molecular species: for murine PanK1α and PanK3, acetyl-CoA > malonyl-CoA > CoASH [5,6]; murine Pank1β, acetyl-CoA > malonyl-CoA and CoASH functions as its activator [4–6]; murine and human PanK2 are very sensitive to CoA species

[7–9]. Furthermore, the expression of the PanK isoforms depends on the tissue. In the murine brain, PanK2 and PanK3 are the dominant species, whereas the expression level of PanK1 ($\alpha + \beta$) is much lower [4,9]. Our result showing that total CoA pools in the brain tissues were suppressed compared with those in the liver, which possesses PanK1, (which is less sensitive to acetyl-CoA and malonyl-CoA), in addition to PanK3 [4,6,9], agrees well with the tissue-specific expression of the PanK species mentioned above. Acetyl-CoA is the most effective CoA molecular species to inhibit PanK activities and the acetyl-CoA levels in the cerebral cortex, cerebellum, and liver, varied within the range of 0.603–2.297, 1.285–3.785, and 6.608–9.857 nmol/g of tissue, respectively. These values are estimated to be 0.815–3.104 μM, 1.765–5.199 μM, and 10.62–15.85 μM as an intracellular acetyl-CoA concentration based on the following water contents measured in this study; 74.0 ± 2.1% (mean ± SEM [$n = 3$]) in the cerebral cortex, 72.8 ± 1.8% in the cerebellum, and 62.2 ± 2.7% in the liver. These concentrations are in accordance with the IC_{50}s of PanK1α at 5 μM [5], PanK1β at 10–32 μM [4–6], and PanK3 at 1 μM [6], although PanK2, of which mutant proteins lead to neurodegeneration by iron accumulation in brain [35], shows a very low value at 62.5 nM [9]. Thus, the total CoA contents in tissue would be primarily controlled by the the tissue-specific distribution of the four PanK species in combination with the acetyl-CoA which is the most potent inhibitor of PanKs among CoA species. Further comprehensive analyses of rat and/or mouse tissues using the acyl-CoA cycling method would be necessary for understanding the overall *in vivo* CoA flux, especially acetyl-CoA and malonyl-CoA as a metabolic intermediate and a regulatory substance.

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