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Expression of three carnitine palmitoyltransferase-I isoforms in 10 regions of the rat brain during feeding, fasting, and diabetes

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Abstract

Inhibition of carnitine palmitoyltransferase-I (CPT-I) activity in the brain has been shown to decrease food intake in rats. We examined the expression of mRNA encoding all three known CPT-I isoforms (α, β, and γ) in 10 different major regions of the rat brain in normal, chow-fed rats, in fasting rats, and in insulin-dependent diabetic rats. Compared with the effects of fasting and diabetes on CPT-I mRNA in the liver and heart, there was either less effect or no effect depending on the particular brain region examined. These results suggest that the regulation of CPT-I mRNA levels is different in the brain than in other tissues. A surprising result of this study was the discovery of very high, unique expression of CPT-Iβ (the muscle isoform) in the cerebellum.

Mitochondrial fatty acid oxidation is known to be a significant source of energy for most cells and carnitine palmitoyltransferase-I (CPT-I), on the outer mitochondrial membrane, is the key regulatory enzyme for fatty acid β-oxidation in several tissues [1]. CPT-I catalyzes the transfer of fatty acyl groups from long-chain acyl-CoA to carnitine, producing acylcarnitine for transport into the mitochondrial matrix. This reaction is essential for the control of fatty acid oxidation, but also determines the availability of long-chain acyl-CoA for the synthesis of complex lipids. Although carnitine palmitoyltransferases are most often associated with the control of fatty acid oxidation, they are important in other processes such as fatty acid modulation of glucose-stimulated insulin release from pancreatic beta cells [2], in acyl group transport in the endoplasmic reticulum [3], and in mechanisms of appetite control in the brain [4–6].

Three isoforms of CPT-I have been cloned [7–9]. The most widely expressed and most extensively studied isoform is the so-called liver isoform, designated L-CPT-I or CPT-Iα [1,7]. The second isoform to be discovered was the skeletal muscle and adipose tissue specific isoform designated M-CPT-I or CPT-1β [1,8]. The most recently discovered isoform is the so-called brain-specific isoform, designated CPT-IC or CPT-Iγ [9]. The regulation of the first two isoforms is considerably different in terms of enzyme activity and gene expression. CPT-Iα is found in all cells except skeletal muscle cells and white and brown adipocytes and is primarily hormonally regulated. CPT-Iβ is highly expressed in skeletal muscle, brown and white adipocytes, and heart and is primarily fuel-regulated [1]. Nothing is known about the enzymatic action or the regulation of CPT-1γ, but it does bind malonyl-CoA tightly [9].

Malonyl-CoA, by virtue of its high-affinity binding to all CPT-I isoforms, is a potent physiological inhibitor of mitochondrial β-oxidation [1,9,10]. Since it is also a precursor for fatty acid synthesis as a substrate for fatty acid synthase, it is potentially involved in regulating both synthesis and degradation of fatty acids. In the brain, malonyl-CoA has recently been implicated in appetite control [4–6]. Experiments in which inhibitors of fatty acid synthase were injected intraperitoneally into mice resulted in cessation of feeding within 20 min [4,5]. These data suggested the hypothesis that a rise in brain malonyl-CoA reduces appetite. The malonyl-CoA level in brain was recently found to be low in fasted mice and rapidly increased on refeeding [6]. Furthermore,

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modulation of measured malonyl-CoA levels using inhibitors of fatty acid synthase (to increase malonyl-CoA) and acetyl-CoA carboxylase (decreased malonyl-CoA) increased and decreased food intake, respectively [6].

A potential problem for the malonyl-CoA hypothesis in appetite regulation is that in the best understood CPT system in which CPT-Iα predominates, the one found in the liver and kidney, the regulation of CPT-Iα mRNA and enzyme activity is inversely coordinated with the enzyme's sensitivity to inhibition by malonyl-CoA [11]. Thus, in these tissues, fasting is associated not only with decreased malonyl-CoA concentrations but also with a pronounced decrease in sensitivity of CPT-Iα to inhibition by malonyl-CoA as well as increased CPT-Iα mRNA and CPT activity [11]. Quantitatively, the greatest effect of fasting is on malonyl-CoA sensitivity, not malonyl-CoA concentration [12,13]. Furthermore, the change in sensitivity of hepatic CPT-Iα malonyl-CoA inhibition by fasting is prolonged over several hours, while both the changes in hepatic malonyl-CoA concentration and serum NEFA occurred quickly, preceding the increase in fatty acid oxidation by several hours [14]. In these experiments, the change in fatty acid oxidation rate was closely correlated with the change in malonyl-CoA sensitivity, not the change in malonyl-CoA concentration [14]. The same is not true for the muscle isoform, CPT-Iβ, which does not exhibit altered malonyl-CoA sensitivity in any state so far studied [1,12]. CPT-Iβ, which is currently thought not to be present in the brain, responds to altered malonyl-CoA concentration produced by changes in fuel supply (glucose, fatty acid, lactate/pyruvate, and ketone bodies), suggesting that CPT-Iβ is fuel regulated whereas CPT-Iα is hormonally regulated [1,10]. It has been reported that fasting and diabetes stimulate the expression of both CPT-Iα and CPT-Iβ isoforms in cardiomyocytes [1,15]. It is critical, therefore, to know distribution of all CPT-I isoforms in the brain and to know how expression of these isoforms changes in states of known increases in appetite such as fasting and insulin-dependent diabetes.

Materials and methods

Animals. Male Sprague–Dawley rats, 200–250 g, were fed Purina Rodent chow (Ralston Purina, Richmond, IN, USA) or were starved for 48 h before brains were removed for analysis. Ketotic diabetic rats were produced by intraperitoneal injection with streptozotocin (150 mg/kg) and were used 2 days after injection when ketone bodies in the urine exceeded 80 mg/dl as measured using Multistix 10SG (Bayer, IN, USA). To investigate expression within different regions of the central nervous system, whole brains were sectioned following anatomical lines.

RNA extraction and cDNA synthesis. Brain sections were homogenated in 4 ml RNA-Stat 60 (Tel-Test "B," TX, USA). RNA isolation was carried out according to the protocol supplied by the manufacturer, except that we repeated the extraction to decrease the possibility of contamination by genomic DNA. Extracted RNA was treated with DNase-I (Ambion, TX, USA). For reverse transcription we used 2 μg total RNA. Synthesis of single-stranded cDNA was done using Superscript reverse transcriptase II-RNase H(−) (Invitrogen, USA). Total cDNA was diluted with DEPC-water 1:100 times and analyzed by real-time quantitative polymerase chain reaction (RTQ-PCR) on an iCycler (Bio-Rad, USA).

RTQ-PCR quantification of cDNA. Primers were designed to have a size of about 23 bp and approximately 50% G/C content (Table 1). The target fragment sizes were <100 bp. RTQ-PCR was performed in a total volume of 25 μl consisting of 12.5 μl of 2× SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK), 2.5μl of each primer (100 nM), 2.5μl DEPC–water, and 5μl cDNA diluted 1:100 (∼50 pmol). For RTQ-PCR the following protocol was used: 10 min at 95 °C for denaturation, 40 cycles for amplification and temperature annealing at 62 °C, and extension at 72 °C. A DNA melt curve was run on all samples to insure that all amplicons were consistent with the intended targets and to avoid secondary products. Single-stranded cDNA sequences approx. 100 bp were used as standards. Dilution series from 10^{-4} to 10^{-10} pmol were established for each standard curve. The correlation coefficient and amplification efficiencies were calculated by iCycler software. The correlation coefficient was always greater than 0.99 and PCR efficiency was between 94% and 100% in all experiments. For normalization of PCR results we used 18S RNA as an endogenous control.

Results

Effects of fasting and diabetes on expression of CPT-Iα in rat liver, heart, and brain

Fasting for 48 h produced an increase in hepatic CPT-Iα mRNA of 7 ± 1-fold and diabetes caused an increase

Table 1
Primers for RTQ-PCR and GenBank Accession Nos. were used

Genes	Forward primer	Reverse primer	Sequence
Rat CPT-1α	291F	366R	
	cgg ttc aag aat ggc atc atc	tca cac cca cca cca cga t	L07736
Rat CPT-1β	547F	620R	
	caa aca tca ctg ccc aag ctt	ggc cgc aca gaa tcc aag t	NM_013200
Rat CPT-1γ	971F	1037R	
	tgc ata ccc tgc tcc tgt atc	ccc atc agc aag gtc ggt	XM_218625
Rat 18S RNA	452F	524R	
	cgg cta cca cat cca agg aa	ttt cgg tca cta cct ccc cg	X01117

of 14 ± 2-fold (Fig. 1). In the heart fasting resulted in an increase of 1.4 ± 0.2-fold while diabetes caused a 4 ± 1-fold increase in CPT-Iα mRNA. Clearly, the effects of fasting and diabetes were similar in the two tissues, but the effects were much reduced in the heart. We were not able to see any effects of fasting or diabetes on CPT-Iα mRNA in whole brain (data not shown) but there was a very small effect in the medial basal hypothalamus and the motor cortex (Fig. 1). These data indicate that the large changes caused by fasting and diabetes in the liver were diminished in the heart and almost non-existent in the brain.

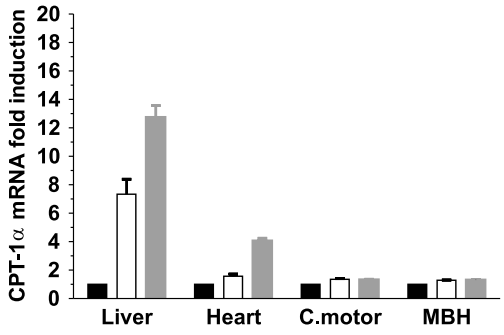

Fig. 1. Fold induction of CPT-1α mRNA expression by fasting and streptozotocin-induced diabetes in the different tissues.

Expression of CPT-Iα, CPT-Iβ, and CPT-Iγ in different regions of the rat brain

We found the expression of CPT-Iα mRNA to be much greater (12 ± 2-fold) than CPT-Iβ throughout the brain with the sole exception of the cerebellum, where there was 8.4 ± 0.8 times greater CPT-Iβ mRNA expression compared with all other regions of the brain. CPT-Iα mRNA was twice the level of CPT-Iβ mRNA in the cerebellum (Fig. 2). When CPT-Iα mRNA was compared with CPT-Iγ mRNA, CPT-Iα was significantly greater (1.7 ± 0.2-fold) in all regions of the brain. The expression of CPT-Iα mRNA in the hypothalamus

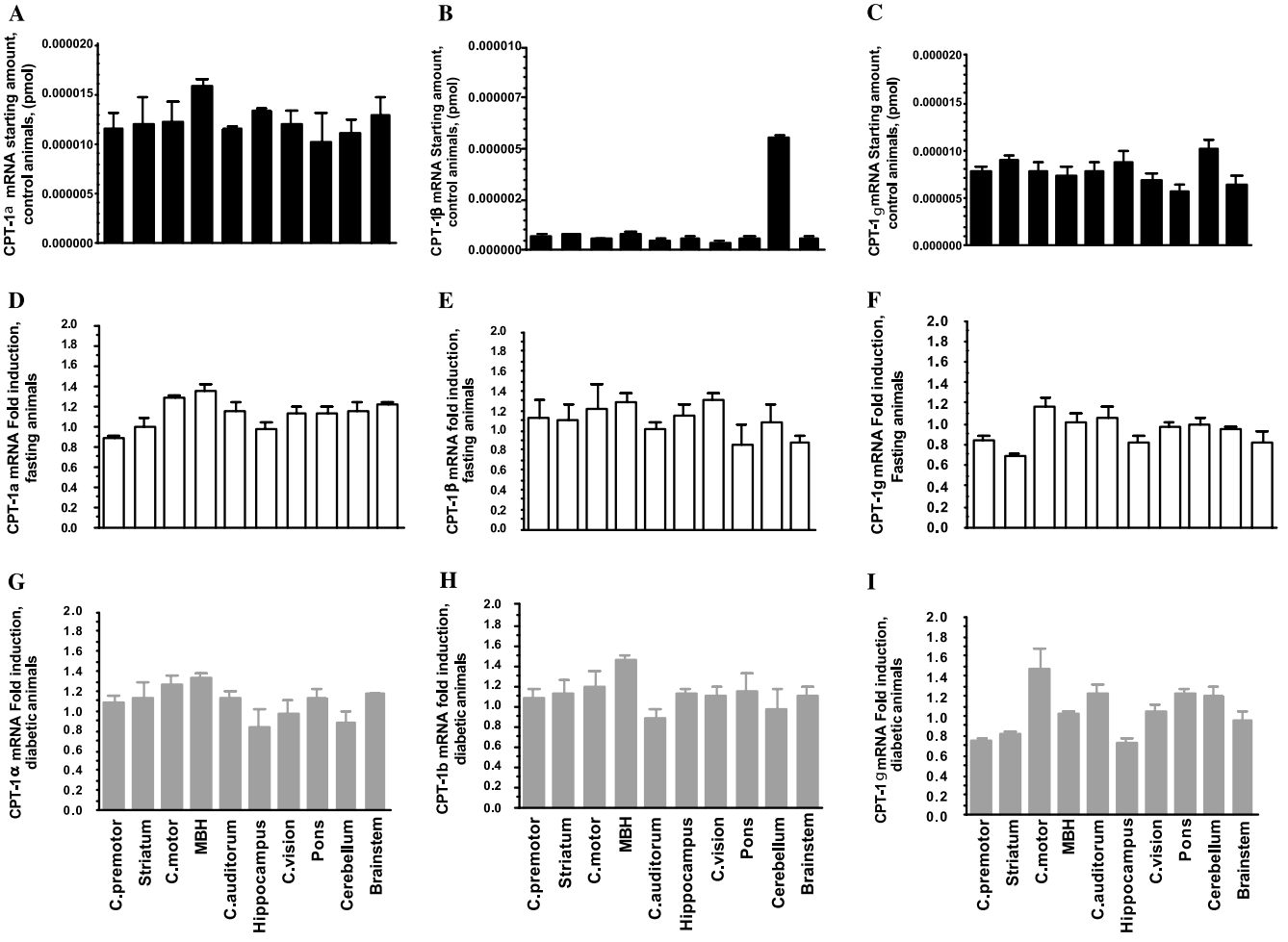

Fig. 2. Expression of CPT-1 isoforms mRNA within different rat brain regions by control, fasted, and diabetes animals. (A,B,C) Quantification of CPT-1α (A), CPT-1β (B), CPT-1γ (C) mRNA by RTQ-PCR in the brain regions of control animals. Data have been normalized to 18S RNA. (D,E,F) CPT-1α (D), CPT-1β (E), and CPT-1γ (F) mRNA fold induction by fasting. (G,H,I) CPT-1α (G), CPT-1β (H), and CPT-1γ (I) mRNA fold induction by streptozotocin-induced diabetes within 10 rat brain regions.

was 1.3 ± 0.2 times higher compared with average expression in other regions of the brain. There was also some difference in expression of CPT-Iγ in cerebellum (1.3 ± 0.08 times the average expression). Neither CPT-Iα nor CPT-Iγ differed greatly in other brain regions.

Expression of rat CPT-Iα, CPT-Iβ, and CPT-Iγ genes during fasting and in diabetes

Neither CPT-1α, CPT-1β nor CPT-1γ expression in the brain was patently affected by starvation or streptozotocin-induced diabetes rats (Fig. 2). Our data indicate that CPT-1α expression was significantly increased in medium basal hypothalamus 1.33 ± 0.04 times in diabetes and 1.35 ± 0.08 times in fasting animals. Both CPT-Iα and CPT-Iβ in the motor cortex were increased 1.3 ± 0.2-fold and 1.4 ± 0.2 times, respectively. For the rest of the samples we could not find significant changes. Expression of the CPT-1γ mRNA was decreased in striatum by fasting (0.69 ± 0.08) and diabetes (0.82 ± 0.02 times). Thus, only minor changes in expression of mRNAs encoding the CPT-I isoforms were found to be associated with fasting and diabetes in the brain.

Discussion

This is the first study in which the expression of CPT-Iα, CPT-Iβ, and CPT-Iγ has been studied in the brain simultaneously. CPT-Iβ mRNA was originally planned as a negative control, as there has been no previous report of its expression in brain. The results presented here clearly demonstrate that expression of the members of CPT-I family is not greatly different in various regions of the brain except for CPT-Iβ expression in the cerebellum. The discovery of high levels of CPT-Iβ mRNA in cerebellum was surprising because previous reports suggested that CPT-Iβ expression was limited to muscle and adipose cells [1,8,10]. It will be important in the future to examine the possible reasons for this high expression of CPT-Iβ in the cerebellum. This may relate to the higher sensitivity of CPT-Iβ to inhibition by malonyl-CoA or to its higher K_m for carnitine. One of the most important functions of the cerebellum is the control of contraction of skeletal muscles and it may be necessary for both cerebellum and muscle to have a similar regulation of fatty acid oxidation. It may be that the cerebellum needs to be more responsive to changes in fuel supply just as the skeletal muscle does.

Although mRNA for the CPT-Iα isoform is predominant throughout the brain, future studies should examine the role of CPT-Iγ, since it is present throughout the brain and is second to CPT-Iα in mRNA expression only by a factor of 2. Furthermore, CPT-Iγ has a very high affinity for binding malonyl-CoA and could presumably be as likely to be involved in mechanisms of appetite regulation as CPT-Iα, especially since its expression in brain is much greater than in other tissues [9].

Our studies do not refute the malonyl-CoA hypothesis for appetite regulation in brain, but seem to support it since we do not see the strong regulation of CPT-Iα in brain that is seen in liver. This might explain how a change in malonyl-CoA in the brain can have a rapid effect [6] while a change in malonyl-CoA in the liver is followed by changes in fatty acid oxidation several hours later when the sensitivity of CPT-Iα to inhibition by malonyl-CoA changes [14]. Our results would suggest that malonyl-CoA sensitivity of CPT-I isoforms is not regulated in brain as it is in other tissues since fasting and diabetes do not have the same effects on these isoforms that are seen in liver and heart. Furthermore, previous measurements of malonyl-CoA sensitivity in whole brain indicated that sensitivity is not changed [16]. It appears that the most important change in the CPT system in brain is the change in malonyl-CoA concentration [6].

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