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Mitochondrial Carnitine Palmitoyltransferase I Isoform Switching in the Developing Rat Heart*

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The expression pattern of mitochondrial carnitine palmitoyltransferase (CPT) enzymes was examined in the developing rat heart. Whereas the specific activity of CPT II increased ~3-fold during the first month of life, the profile for CPT I, which is composed of both liver (L) and muscle (M) isoforms, was more complex. Exposure of mitochondria to [^{3}H]etomoxir (a covalent ligand for CPT I), followed by fluorographic analysis of the membrane proteins, established that while in the adult heart L-CPT I represents a very minor constituent, its contribution is much greater in the newborn animal. Use of the related inhibitor, 2-[6-(2,4-dinitrophenoxy)hexyl]oxirane-2-carboxylic acid (specific for L-CPT I), allowed the activities of the two CPT I variants to be quantified separately. The results showed that in the neonatal heart, L-CPT I contributes ~25% to total CPT I activity (in V_{max} terms), the value falling during growth of the pups (with concomitant increasing expression of the M isoform) to its adult level of 2–3%.

Because the myocardial carnitine content is very low at birth and rises dramatically over the next several weeks, it can be estimated that L-CPT I (K_m for carnitine of only 30 μM compared with a value of 500 μM for M-CPT I) is responsible for some 60% of total cardiac fatty acid oxidation in the newborn rat; the value falls to ~4% in adult animals. Should these findings have a parallel in humans, they could have important implications for understanding the pathophysiological consequences of inherited L-CPT I deficiency syndromes.

The carnitine palmitoyltransferase (CPT)[1] enzyme system effects the entry of long chain fatty acids into the mitochondrial matrix for β-oxidation. CPT I, located on the outer membrane, catalyzes the transfer of acyl groups from coenzyme A to carnitine, the acylcarnitine so formed then traversing the inner membrane by means of a specific transporter. CPT II, on the matrix side of the inner membrane, reverses the transacylation reaction, regenerating acyl-CoA (1). Overall control of fatty acid transport, and thus of β-oxidation, is exerted at the level of CPT I by virtue of its unique inhibitability by malonyl-CoA, the product of the acetyl-CoA carboxylase reaction (1). Although first recognized in the context of hepatic ketogenesis and its regulation (2), the malonyl-CoA/CPT I interaction has since emerged as a key component of fuel "cross-talk" in a variety of non-hepatic tissues such as heart (3), skeletal muscle (4), and the pancreatic β-cell (5, 6). In addition, CPT I has attracted attention as a potential site of pharmacological intervention in poorly controlled diabetes mellitus where fatty acid oxidation is excessive and has a detrimental effect on glucose homeostasis (7, 8).

Efforts to dissect the mitochondrial CPT system[2] in terms of its structure/function/regulatory characteristics have been greatly enhanced by the recent isolation of cDNAs corresponding to the rat and human CPT II proteins (10–12) as well as those encoding rat and human liver CPT I (13, 14). Available evidence indicates that in both species CPT II (~71 kDa) is expressed as the same protein throughout the entire body (12, 15). By contrast, the regulated enzyme, CPT I, exists in at least two isoforms. These have been designated L-CPT I and M-CPT I, indicating their association with liver and skeletal muscle, respectively (16, 17). The two proteins differ not only in monomeric size (~88 and 82 kDa, respectively) but also in their profoundly different kinetic characteristics (K_m for carnitine, ~30 and 500 μM, respectively; I_{50}[3] for malonyl-CoA, ~2.7 and 0.03 μM, respectively (18)).

In examining the features of CPT I in adult rat heart mitochondria we made the unexpected observation that this tissue expresses both the M- and L-type enzymes (16, 17). The fact that the L variant contributed only ~2–3% to total CPT I activity was puzzling and difficult to rationalize teleologically. In the present study, therefore, we asked the following question: is the 98:2 ratio of M- to L-CPT I activity a fixed property of rat cardiac tissue, or is it possible that the L isoform (low K_m for carnitine) makes a more significant contribution in the newborn period when the carnitine content of the heart is known to be low (19)? As outlined below, it turns out that soon after birth L-CPT I is probably responsible for a major fraction of fatty acid oxidation in rat heart and that its decline during growth of the pups represents another striking example of developmental isoform switching in heart tissue. The findings might have pathophysiological significance in situations of inherited CPT I deficiency syndromes.

EXPERIMENTAL PROCEDURES

Animals—Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN) were maintained on regular Purina chow (4% fat) with

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[1] The abbreviations used are: CPT, carnitine palmitoyltransferase; L-CPT I, liver-type CPT I; M-CPT I, muscle-type CPT I; etomoxir, 2-[6-(4-chlorophenoxy)hexyl]oxirane-2-carboxylic acid; DNP-etomoxir, 2-[6-(2,4-dinitrophenoxy)hexyl]oxirane-2-carboxylic acid.

[2] In this article the terms CPT I and CPT II refer exclusively to the mitochondrial enzymes, although it is recognized that proteins with CPT activity are also associated with peroxisomes and microsomes (9).

[3] The term I_{50} refers to the concentration of malonyl-CoA required to inhibit CPT I activity by 50% under defined assay conditions.

lighting from 1000 to 2200 h. Organ harvest was performed between 1100 and 1200 h.

Animals were bred in-house, with the day of birth designated as day 0 and weaning at day 21. The rats were killed by cervical dislocation, adult males (250–450 g) being first anesthetized by intraperitoneal injection of Nembutal. To obtain fetal tissue, timed pregnant females were purchased, and hearts were removed 1–2 days prepartum. The mothers were rendered unconscious by inhalation of Metofane prior to sacrifice.

Hearts from 1–3 litters of mixed sex were used for each analysis in animals between the fetal and 29-day-old stages of life. Individual males were used for adult time points.

Mitochondrial Preparation and Treatment—Hearts were rapidly removed, trimmed of visible blood vessels, and homogenized in ~10 ml/g, wet weight, of 250 mM sucrose, 5 mM Tris-HCl, pH 7.2 (Tissumizer; Tekmar Co., Cincinnati, OH; large probe, 10 s, setting 50). Mitochondria were prepared as described in method A of Ref. 18 and finally suspended in 150 mM KCl, 5 mM Tris-HCl, pH 7.2 (buffer A) at 4 ml/g of original tissue. For inhibitor treatment, the suspension was diluted with an equal volume of activation mixture (200 mM Tris-HCl, pH 7.2, 100 mM KCl, 12 mM $MgCl_2$, 12 mM ATP, 0.7 mM glutathione, 100 μM CoASH, and 1.2% bovine serum albumin) before addition of [^{3}H]etomoxir or unlabeled DNP-etomoxir to a concentration of 3 or 10 μM, respectively. Control samples lacked only the inhibitor. The tubes were then rocked gently for 1 h at room temperature (during this time the inhibitors become activated to their CoA derivatives, and it is in this form that they interact with CPT I, bonding covalently). Subsequently, the mitochondria were centrifuged at 12,000 × *g* for 15 min at 4 °C, resuspended in the same volume of 0.9% NaCl, 0.4% bovine serum albumin, and similarly resedimented. For samples treated with or without DNP-etomoxir, the final pellet was brought to 4 ml/g of original tissue in buffer A for assay of CPT I. The washed [^{3}H]etomoxir-labeled mitochondrial pellets were resuspended to 4 ml/g of original tissue in 5 mM potassium phosphate, pH 7.2, twice frozen and thawed in liquid N_2, and then centrifuged at 100,000 × *g* for 30 min at 4 °C. The labeled membranes were suspended to ~1 mg of protein/ml in SDS sample buffer and boiled for 3 min, and an aliquot of each sample containing ~5,000 cpm of protein-bound ^{3}H of the mixture was subjected to polyacrylamide gel electrophoresis followed by fluorography (20).

For measurement of CPT II activity mitochondria were pelleted by centrifugation and stored at −20 °C (studies not shown had established that CPT II is stable for months under these conditions). At the time of assay, the pellets were resuspended in buffer A (4 ml/g of original tissue), octyl glucoside was added to a concentration of 1% (w/v), and the mixtures were kept on ice for 30 min. Such treatment causes inactivation of CPT I and solubilization of CPT II in active form (21).

CPT activity was assayed at 30 °C in the direction of palmitoylcarnitine formation (18) in the presence of 50 μM palmitoyl-CoA, 1% bovine serum albumin (w/v) and the indicated concentrations of L-carnitine (containing L-[*methyl*-^{14}C]-carnitine). Reaction rates were linear over the time period studied, usually 4 min. With intact mitochondria, the assay monitors only CPT I, the activity of which is essentially completely inhibited by malonyl-CoA.

Protein was measured by the method of Lowry *et al.* (22).

Myocyte Isolation—Cardiac myocytes were isolated from 7-day-old litters or adult male rats by collagenase digestion of diced heart tissue and purification of released myocytes by centrifugation through 3% Ficoll as described (16, 23) except for the inclusion of 10 μg/ml DNase I during collagenase treatment. Preparations contained >90% myocytes as judged by microscopic examination. The cells, suspended in buffer A at a concentration of 0.4–1.0 mg of protein/ml, were broken using eight cycles of a tightly fitting glass homogenizer. The homogenate was then incubated in the absence or presence of DNP-etomoxir and assayed for CPT I as described above for heart mitochondria.

Measurement of Heart Carnitine Content—Hearts were rapidly excised, frozen in liquid N_2, and stored at −70 °C until the assay. At that time, samples of frozen tissue (250–500 mg) were pulverized under liquid N_2, and the powder was immediately homogenized in 3 ml of ice-cold 3% perchloric acid. After centrifugation, the supernatant was neutralized with KOH and used for the measurement of free and total carnitine (the difference representing short chain acylcarnitines, primarily acetylcarnitine) using an isotopic assay (24).

Materials—Sources have been given in Refs. 16–18 and 24.

RESULTS

Fig. 1 depicts the profile of CPT I and CPT II activities in heart mitochondria from rats at various stages of development.

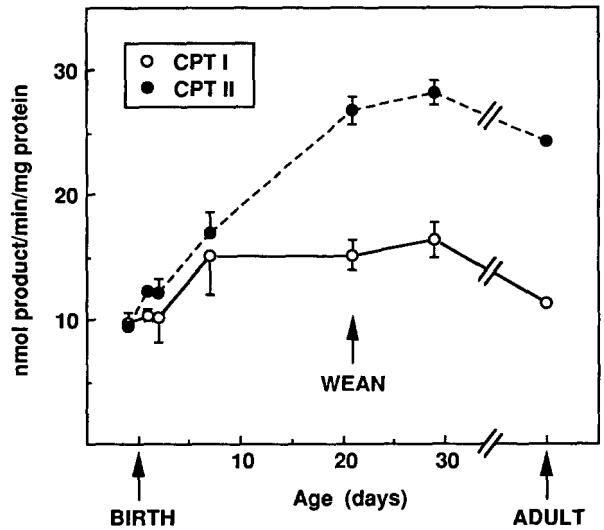

FIG. 1. **Activities of mitochondrial CPT I and CPT II in the developing rat heart.** Mitochondria were prepared and assayed for CPT I and CPT II in the presence of 200 μM carnitine as described under "Experimental Procedures." Values are means ± S.E. for three or four independent experiments at each time point. Where *error bars* are not shown they lie within the *symbol*.

These assays were conducted using our standard procedure in which carnitine was present at a concentration of 200 μM. It is seen that the activity of CPT I (measured in intact mitochondria) remained at its prepartum level until 2 days after birth. Over the next 5 days the value rose by ~50% and remained constant out to the 29-day time point. By contrast, CPT II activity, measured in octyl glucoside-solubilized mitochondria, increased steadily throughout the course of development, reaching a value at day 29 that was almost 3 times that seen at day −1. The levels of both enzymes declined somewhat between day 29 and adulthood.[4] The CPT II component is not considered further here.

The data for CPT I shown in Fig. 1 do not discriminate between the two isoforms of the enzyme, M and L, previously shown to be expressed in adult rat heart (16, 17). To obtain qualitative insight into whether the ratio of these isozymes might vary during development we made use of the irreversible inhibitor, [^{3}H]etomoxir, which, in its CoA ester form, interacts covalently with both proteins (causing complete inactivation) and thus serves as a radiolabeled tagging agent (16, 17). Accordingly, heart mitochondria from juvenile and adult rats were exposed to [^{3}H]etomoxir in the presence of ATP and CoASH, after which the membranes were subjected to SDS-polyacrylamide gel electrophoresis followed by fluorography. As seen from Fig. 2, both L- and M-CPT I were present at all stages of postnatal development. This was also true in late fetal tissue (results not shown). The important point is that the relative labeling intensity of the higher M_r L-CPT I compared with the faster migrating M-CPT I can clearly be seen to diminish with increasing age of the litters.

In order to quantify changes in the actual activities of the two CPT I isoforms, mitochondria were preincubated in the absence or presence of DNP-etomoxir (together with ATP and CoASH), a maneuver that completely inhibits L-CPT I while leaving the M variant unaffected (17). Because the K_m of L-CPT I for carnitine is substantially lower than that of M-CPT I (30 *versus* 500 μM (18)) the presence of the former is more readily

[4] The activities of CPT I and CPT II shown here cannot be compared in absolute terms, since the latter was measured in the presence of octyl glucoside, which might alter its kinetic properties.

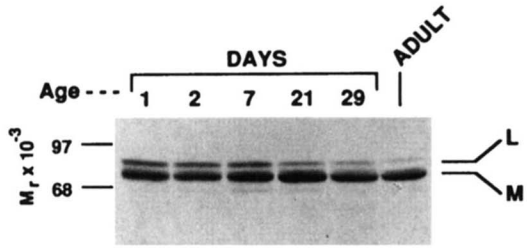

FIG. 2. **SDS-polyacrylamide gel electrophoresis analysis of [^{3}H]etomoxir-labeled rat heart mitochondrial membranes.** Preparation, labeling, and analysis of mitochondrial samples are detailed under "Experimental Procedures." *L* and *M* indicate the migration positions of L- and M-CPT I, respectively. Typical results are shown.

detected in assays conducted at low levels of this substrate (17). Thus, a concentration of 20 μM was chosen for the studies illustrated in Fig. 3. As seen in *panel A*, while DNP-etomoxir caused ~23% inhibition of CPT I activity in adult hearts, this value was in the region of 70–75% in late fetal and newborn animals and fell progressively with postnatal development. The actual activity of each CPT I isoform under these assay conditions is seen in *panel B*, which reveals their distinct developmental profiles. Over the first month postpartum the level of M-CPT I rises rapidly and changes little thereafter. L-CPT I displays the converse pattern. All of the assays in the experiment of Fig. 3 were repeated using 10 μM carnitine. Qualitatively similar results were obtained, although, as expected, the percentage of inhibition of CPT I activity by DNP-etomoxir at each time point was even more pronounced (data not shown).

Based on the measured activity of the two CPT I isoforms at 10 and 20 μM carnitine (Fig. 3) and on the known K_m value of each adult enzyme for this substrate (see above), it is possible, using the Michaelis-Menten equation, to transform the data into a V_{max} format if it is assumed (not unreasonably) that throughout development the K_m of each enzyme remains unchanged. The picture obtained is shown in Fig. 4. It is seen that the combined maximal capacity of L-CPT I plus M-CPT I rises from ~20 to 45 nmol · min^{-1} · mg of protein^{-1} during the suckling period and falls back to ~30 nmol · min^{-1} · mg of protein^{-1} in adult animals. This is accompanied by a marked change in the contribution of L-CPT I to overall enzyme activity, the value being ~20–25% in the perinatal stage and declining to ~2–3% in the adult heart.

Since changes in the cellular structure of cardiac muscle are also occurring in the neonatal period (25), the possibility that the high level of L-CPT I detected during this time (Figs. 1–4) derived from non-myocyte cell types had to be considered. We therefore prepared myocytes that were >90% in purity from 7-day-old and adult hearts. The cells were then broken (under conditions that maintained mitochondrial integrity); incubated with DNP-etomoxir, ATP, and CoASH; and subsequently assayed for CPT I at 20 μM carnitine. In two separate experiments the percentage of inhibition of enzyme activity by the etomoxir derivative was 44.6 and 50.2 in 7-day-old cells and 19.4 and 24.2 in adult cells. These values are very similar to those obtained with mitochondria isolated from whole heart tissue (Fig. 3*A*), lending confidence that the changing ratio of L- to M-CPT I observed in the experiments of Figs. 1–4 did indeed reflect events in the cardiac myocyte itself.

In light of the fact that the L- and M-isoforms of CPT I have such different affinities for carnitine (but similar K_m values for palmitoyl-CoA (26)), it follows that the contribution of each enzyme to the process of fatty acid oxidation in the developing heart will be a function not only of its relative abundance but also of the tissue carnitine concentration at any given time. To

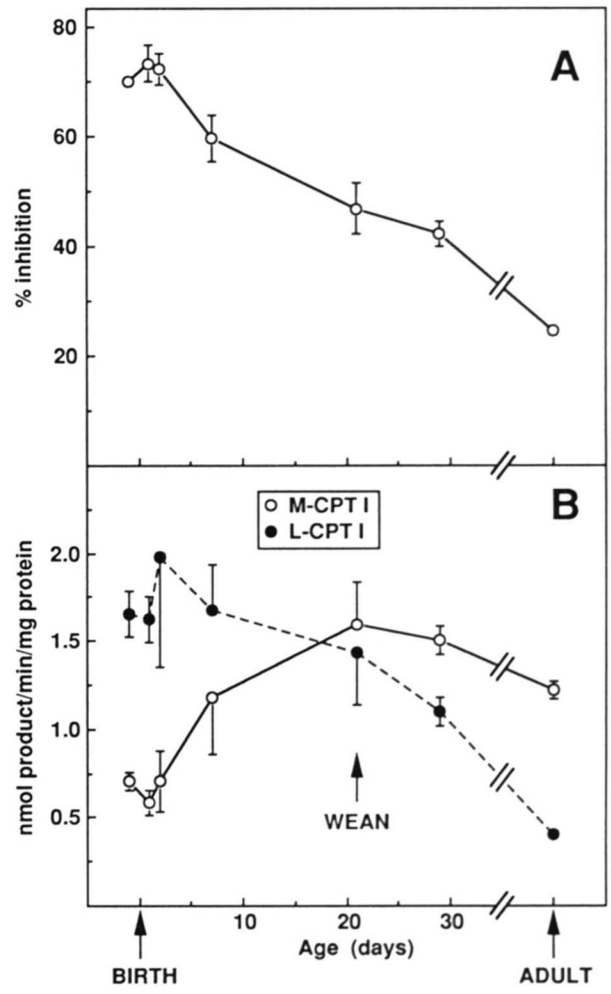

FIG. 3. **Use of DNP-etomoxir to discriminate between L- and M-CPT I in heart mitochondria.** Mitochondria were preincubated with ATP and CoASH in the absence or presence of DNP-etomoxir, washed, and then assayed for CPT I activity using a carnitine concentration of 20 μM. The percentage of suppression of overall activity by the inhibitor (due to selective loss of L-CPT I) is shown in *panel A*. *Panel B* depicts the actual activities of the two CPT I isoforms. The contribution of L-CPT I represents the difference in activity between untreated samples (L-CPT I + M-CPT I) and those treated with DNP-etomoxir (M-CPT I). Values are means ± S.E. for three to five independent experiments at each time point.

explore this matter further we measured both the total[5] and free carnitine content of the heart throughout the course of development (Fig. 5). The data for total carnitine are very similar to those reported by us earlier (19) and reaffirm the fact that this is low in the fetal heart and rises rapidly in the postnatal period. A similar profile was found with regard to free (unesterified) carnitine, although at all time points between days −1 and +29 it represented less than half of the total pool. Both free and total carnitine increased further between the 29-day-old and adult stage of life. By dividing the values for free carnitine in nmol/g, wet weight, of tissue by 0.7 (to obtain an approximation of the cytosolic carnitine concentration in μM) and combining these with the data of Fig. 4 (showing the percentage of contribution of each CPT I isoform to total enzyme activity) it is possible, again using the Michaelis-Menten equation, to estimate the relative *functional* contri-

[5] The term "total carnitine" does not include long chain acylcarnitines. These are removed during perchloric acid treatment of the tissue and, in any event, ordinarily make up only a small fraction of the total carnitine pool.

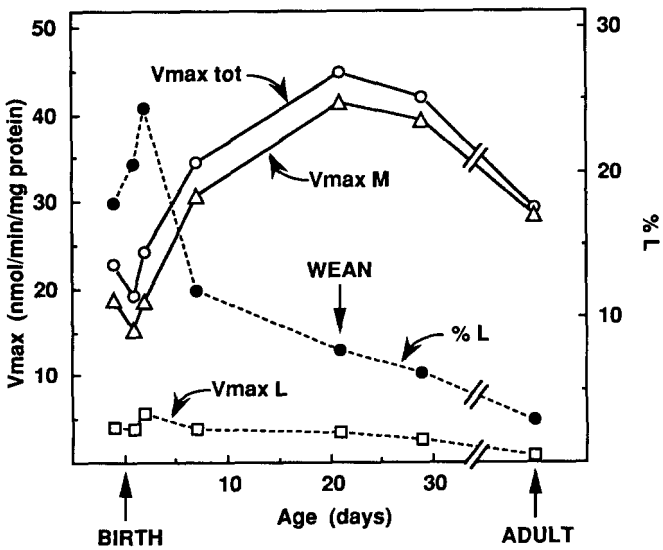

FIG. 4. **Calculated V_{max} activities of L- and M-CPT I in heart mitochondria.** V_{max} values for liver (*L*) and muscle (*M*) CPT I isoforms are derived from the data of Fig. 3 as explained under "Results." *% L* represents the contribution of the liver isoform.

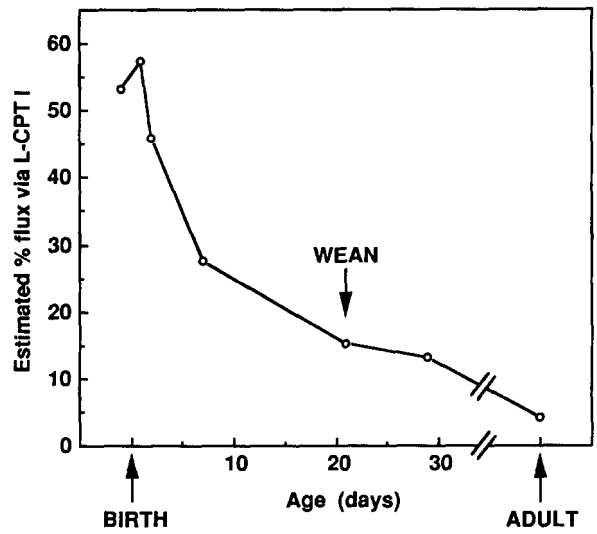

FIG. 6. **Theoretical contribution of L-CPT I to overall CPT I flux in the developing rat heart.** Results are expressed as the percentage of CPT I flux estimated to occur via L-CPT I. Derivation of data is detailed under "Results."

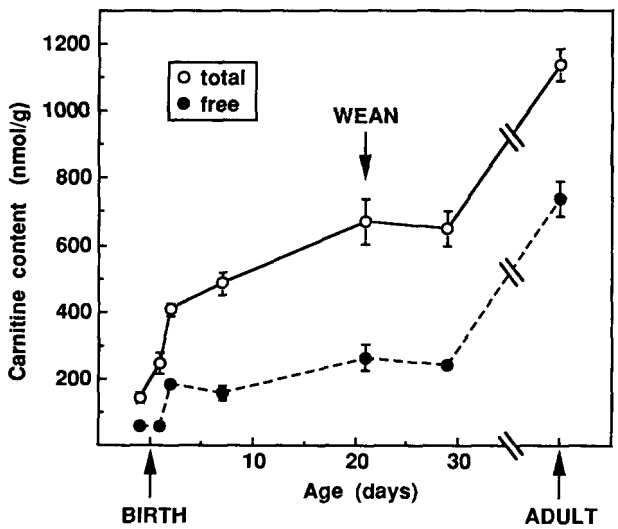

FIG. 5. **Carnitine content of the developing rat heart.** Free and total (free plus esterified) carnitine was assayed in neutralized perchloric acid extracts of heart tissue. Values are means ± S.E. for three or four independent measurements at each time point.

bution of L-CPT I to overall cardiac fatty acid oxidation in the developing rat. The composite data are shown in Fig. 6. The key finding is that in the immediate newborn period the calculated fraction of total fatty acid flux through the β-oxidation pathway of heart mitochondria that is supported by L-CPT I approaches 60%. This value falls sharply during suckling as the relative expression of L-CPT I diminishes and the tissue carnitine content rises, the majority of theoretical flux shifting to the high K_m M-CPT I, which itself is increasing in expression over this time frame. In the adult rat, ~96% of CPT I catalysis would be predicted to occur via the muscle isoform.

DISCUSSION

The present study was prompted by our recent observation that the adult rat heart expresses both liver and muscle isoforms of mitochondrial CPT I (16, 17). Here we have explored the development of the cardiac CPT enzymes with emphasis on the distribution of the two CPT I variants.

Early reports had described a major increase in CPT activity in rat heart during the postnatal period (27, 28). However, at the time of those studies the complexities of the CPT system were not as well understood than they are now, and the assay procedures employed did not adequately discriminate between the CPT I and CPT II components. By carrying out the CPT measurements in intact and octyl glucoside-solubilized mitochondria it is now possible to make this distinction (21). Using this methodology it is evident that while the specific activity of both CPT I and CPT II increases between the time of birth and weaning there is a greater -fold change for CPT II (~3) compared with CPT I (~1.5). It should be noted, however, that the mitochondrial content of the heart cells is also increasing during this period (25), which would exaggerate the changes in CPT I and CPT II levels when data are expressed on a per gram of tissue basis. The most striking aspect of the experiment depicted in Fig. 1 is the marked change in the ratio of CPT II to CPT I when assays are performed at our standard carnitine concentration of 200 μM.

The data of Fig. 1, however, do not describe the situation fully, since the activity noted for CPT I represents the sum of two kinetically different components, the L and M isoforms of the enzyme. We took two approaches to examining the apparent CPT I profile more closely by analyzing the contribution of each variant to total CPT I activity. Using [^{3}H]etomoxir to label both proteins, fluorographic analysis revealed that they do not follow identical paths of developmental expression. The results with adult heart confirmed our previous finding (16, 17) that both L- and M-CPT I are present but that the former is a very minor constituent. In contrast, the relative intensity of the L-CPT I signal was clearly seen to be higher in the perinatal period. Quantification of the catalytic activity of each isoform was achieved by the use of DNP-etomoxir, which selectively inhibits the liver enzyme (17). Three important points emerged. First, it could now been seen that the sum of the V_{max} for total CPT I increased only ~2-fold between days 1 and 29 after birth, returning to an intermediate value in the adult rat. Second, the V_{max} capacity of L-CPT I remained fairly consistent from the time of birth to weaning and then fell by a factor of ~3 as the animals reached maturity, in keeping with the fluorographic data alluded to above. Importantly, however, the contribution of L-CPT I to total enzyme activity in V_{max} terms was as high as 25% in the perinatal period, falling precipitously during suckling and to its low point of ~2–3% in the adult rat.

Third, this change in the ratio of L- to M-CPT I in the developing heart was shown to be a characteristic of the cardiac myocytes themselves and not of non-myocyte cell type(s).

To gain some appreciation of what these findings might mean as regards the quantitative contribution of L-CPT I to cardiac fatty acid oxidation in the developing rat, the data must be viewed in the context of the carnitine content of the heart at any given stage of life. We had previously shown that total (free plus esterified) carnitine levels in rat heart are low at birth and increase rapidly during the suckling period (19). Here we confirm this finding and extend it to the status of the free carnitine pool, which is also seen to be very low at birth, rising some 5-fold during suckling and by a further 3-fold in adult animals. When the changes in carnitine concentration are considered together with the shifting ratio of L- to M-CPT I, it can be estimated that the functional contribution of L-CPT I to overall cardiac CPT flux, while only ~4% in the adult rat, is almost 60% in 1-day-old pups. Whereas in the adult animal carnitine is largely synthesized in liver (both *de novo* and from butyrobetaine supplied from skeletal muscle), the major source of this material for the newborn rat is the mother's milk (19). This may provide a teleological explanation for the high expression of L-CPT I in the neonatal heart. After birth, the animal's diet is rich in fat (derived from maternal milk), and fatty acids, presumably together with ketone bodies, represent important energy sources for heart function (19). However, the M isoform of CPT I would be unable to operate efficiently at this time, since sufficient carnitine has not yet accumulated in the tissue. A transient high expression of the low K_m L-CPT I would provide a mechanism for fatty acylcarnitine synthesis despite the low tissue content of carnitine. As suckling continues and the heart carnitine pool expands, the L form of CPT I gradually disappears, with concomitant increased expression of the high K_m M variant, which then becomes the near exclusive vehicle for initiating the fatty acid oxidation process. In Fig. 6 we have provided a theoretical estimate of the contribution of L-CPT I to cardiac fatty acid oxidation as the rat pup matures. It must be emphasized that nonideal kinetic behavior of the CPT I isozymes with regard to substrates, as well as subcellular compartmentation of the latter, would influence the actual values, but the trend is solidly apparent.

The presence of L-CPT I in rat heart might also have relevance to the still puzzling question of how fatty acid oxidation can ever proceed in cardiac tissue given the fact that its content of malonyl-CoA has been found to fluctuate between ~1 and 4 nmol/g, wet weight, under various conditions (18, 29, 30) and that M-CPT I displays such a low I_{50} value for the inhibitor (~0.03 μM). Presumably, most of the cellular malonyl-CoA is in a bound state and unavailable for interaction with CPT I. The additional possibility is now raised that expression of some L-CPT I, whose I_{50} for malonyl-CoA is 100-fold higher than that of M-CPT I (18), may permit fatty acid oxidation to occur at a basal level even if the free concentration of malonyl-CoA were to rise into an inhibitory range for the muscle enzyme. In the rabbit, the heart content of malonyl-CoA has been reported to decrease after birth in association with a lowered activity of acetyl-CoA carboxylase and acceleration of fatty acid oxidation (30). A greater expression of L-CPT I in the perinatal period (if indeed this applies to rabbit myocardium) would presumably offset the higher malonyl-CoA level and allow fatty acid oxidation to occur at a measurable rate.[6]

Although the present work adds CPT I to the growing list of proteins known to undergo isoform switching during cardiac development (25), we do not yet understand which signals are responsible for the phenomenon. Some regulatory factors must exist that are specific to cardiac tissue. While the L-CPT I level in the heart changes little during the first 48 h after birth (Fig. 4), expression of the enzyme in liver rises 4-fold during this time (31). Conversely, whereas hepatic expression of CPT II remains constant in the neonatal period (31), the present study shows a substantial up-regulation in the heart. The differential response of the two tissues likely involves dietary components and changes in circulating hormone levels. The intriguing possibility exists that carnitine itself plays a role, in light of the fact that its concentration in liver and heart changes reciprocally in the neonatal rat (Ref. 19 and Fig. 5). It remains to be established whether skeletal muscle also expresses some L-CPT I at an early age, since carnitine levels in this tissue would also be predicted to be low at birth.

Apart from liver, heart, and, as recently documented, fibroblasts (14), the range of tissues expressing L-CPT I is not known. However, we suspect that kidney and the pancreatic β-cell fall into this category.[7] Nor has it been established at this juncture that the changing pattern of cardiac CPT I isoforms noted here in the developing rat has a parallel in humans (although we have detected both L and M forms of CPT I in adult human heart mitochondria labeled with [^{3}H]etomoxir). If it does, it could have important implications for individuals with the homozygous form of the so-called "hepatic" CPT deficiency syndrome where CPT I has been found to be deficient both in liver and fibroblasts (32). The classic symptoms emphasized to date have been severe hypoketotic hypoglycemia during episodes of starvation in infancy, sometimes resulting in death (33). Is it possible that disturbances of heart and kidney function, both of which have been reported in such patients (34), have resulted, at least in part, from diminished CPT I activity in these tissues? Finally, the fact that CPT I is now known to exist in at least two different forms (and possibly more) will need to be considered in the design of CPT I inhibitors should such agents be employed for therapeutic purposes.

REFERENCES

1. McGarry, J. D., Woeltje, K. F., Kuwajima, M., and Foster, D. W. (1989) *Diabetes Metab. Rev.* **5,** 271–284
2. McGarry, J. D., Mannaerts, G. P., and Foster, D. W. (1977) *J. Clin. Invest.* **60,** 265–270
3. Saddik, M., Gamble, J., Witters, L. A., and Lopaschuk, G. D. (1993) *J. Biol. Chem.* **268,** 25836–25845
4. Duan, C., and Winder, W. W. (1992) *J. Appl. Physiol.* **72,** 901–904
5. Prentki, M., Vischer, S., Glennon, M. C., Regazzi, R., Deeney, J. T., and Corkey, B. E. (1992) *J. Biol. Chem.* **267,** 5802–5810
6. Chen, S., Ogawa, A., Ohneda, M., Unger, R. H., Foster, D. W., and McGarry, J. D. (1994) *Diabetes* **43,** 878–883
7. Foley, J. E. (1992) *Diabetes Care* **15,** 773–784
8. McGarry, J. D. (1992) *Science* **258,** 766–770
9. Murthy, M. S. R., and Pande, S. V. (1994) *J. Biol. Chem.* **269,** 18283–18286
10. Woeltje, K. F., Esser, V., Weis, B. C., Sen, A., Cox, W. F., McPhaul, M. J., Slaughter, C. A., Foster, D. W., and McGarry, J. D. (1990) *J. Biol. Chem.* **265,** 10720–10725
11. Finocchiaro, G., Taroni, F., Rocchi, M., Martin, A. L., Colombo, I., Tarelli, G. T., and DiDonato, S. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 661–665
12. McGarry, J. D., Sen, A., Brown, N. F., Esser, V., Weis, B. C., and Foster, D. W. (1992) in *Current Concepts in Carnitine Research* (Carter, A. L., ed) pp. 137–151, CRC Press, Boca Raton, FL
13. Esser, V., Britton, C. H., Weis, B. C., Foster, D. W., and McGarry, J. D. (1993) *J. Biol. Chem.* **268,** 5817–5822
14. Britton, C. H., Schultz, R. A., Zhang, B., Esser, V., Foster, D. W., and McGarry, J. D. (1995) *Proc. Natl. Acad. Sci. U. S. A.,* **92,** 1984–1988
15. Woeltje, K. F., Esser, V., Weis, B. C., Cox, W. F., Schroeder, J. G., Liao, S.-T., Foster, D. W., and McGarry, J. D. (1990) *J. Biol. Chem.* **265,** 10714–10719
16. Weis, B. C., Esser, V., Foster, D. W., and McGarry, J. D. (1994) *J. Biol. Chem.* **269,** 18712–18715
17. Weis, B. C., Cowan, A. T., Brown, N., Foster, D. W., and McGarry, J. D. (1994) *J. Biol. Chem.* **269,** 26443–26448
18. McGarry, J. D., Mills, S. E., Long, C. S., and Foster, D. W. (1983) *Biochem. J.*

[6] If the carnitine content of rabbit myocardium also increases during postnatal development this would obviously make an additional contribution to the enhancement in fatty acid oxidation capacity observed by Lopaschuk *et al.* (30).

[7] N. F. Brown, B. C. Weis, J. E. Husti, D. W. Foster, and J. D. McGarry, unpublished observations.

214, 21–28
19. Robles-Valdes, C., McGarry, J. D., and Foster, D. W. (1976) *J. Biol. Chem.* **251,** 6007–6012
20. Declercq, P. E., Falck, J. R., Kuwajima, M., Tyminski, H., Foster, D. W., and McGarry, J. D. (1987) *J. Biol. Chem.* **262,** 9812–9821
21. Woeltje, K. F., Kuwajima, M., Foster, D. W., and McGarry, J. D. (1987) *J. Biol. Chem.* **262,** 9822–9827
22. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275
23. Glick, M. R., Burns, A. H., and Reddy, W. J. (1974) *Anal. Biochem.* **61,** 32–42
24. McGarry, J. D., and Foster, D. W. (1985) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed) pp. 474–481, Verlag Chemie, Weinheim, and Academic Press, New York
25. Jones, C. T., and Rolph, T. P. (1985) *Physiol. Rev.* **65,** 357–430
26. Mills, S. E., Foster, D. W., and McGarry, J. D. (1984) *Biochem. J.* **219,** 601–608
27. Wittels, B., and Bressler, R. (1965) *J. Clin. Invest.* **44,** 1639–1646
28. Warshaw, J. B. (1972) *Dev. Biol.* **28,** 537–544
29. Awan, M. M., and Saggerson, E. D. (1993) *Biochem. J.* **295,** 61–66
30. Lopaschuk, G. D., Witters, L. A., Itoi, T., Barr, R., and Barr, A. (1994) *J. Biol. Chem.* **269,** 25871–25878
31. Thumelin, S., Esser, V., Charvy, D., Kolodziej, M., Zammit, V. A., McGarry, D., Girard, J., and Pegorier, J.-P. (1994) *Biochem. J.* **300,** 583–587
32. Demaugre, F., Bonnefont, J.-P., Mitchell, G., Nguyen-Hoang, N., Pelet, A., Rimoldi, M., DiDonato, S., and Saudubray, J.-M. (1988) *Pediatr. Res.* **24,** 308–311
33. Saudubray, J.-M., Mitchell, G., Bonnefont J.-P., Schwartz, G., Nuttin, C., Munnich, A., Brivet, M., Vassault, A., Demaugre, F., Rabier, D., and Charpentier, C. (1992) in *New Developments in Fatty Acid Oxidation* (Coates, P. M., and Tanaka, K., eds) pp. 271–288, Wiley-Liss, Inc., New York
34. Bergman, A. J. I. W., Donckerwolcke, R. A. M. G., Duran, M., Smeitink, J. A. M., Mousson, B., Vianey-Saban, C., and Poll-The, B. T. (1994) *Pediatr. Res.* **36,** 582–588