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Hibernation impact on the catalytic activities of the mitochondrial D-3-hydroxybutyrate dehydrogenase in liver and brain tissues of jerboa (*Jaculus orientalis*)

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Abstract

Background: Jerboa (*Jaculus orientalis*) is a deep hibernating rodent native to subdesert highlands. During hibernation, a high level of ketone bodies i.e. acetoacetate (AcAc) and D-3-hydroxybutyrate (BOH) are produced in liver, which are used in brain as energetic fuel. These compounds are bioconverted by mitochondrial D-3-hydroxybutyrate dehydrogenase (BDH) E.C. 1.1.1.30. Here we report, the function and the expression of BDH in terms of catalytic activities, kinetic parameters, levels of protein and mRNA in both tissues i.e brain and liver, in relation to the hibernating process.

Results: We found that: 1/ In euthemic jerboa the specific activity in liver is 2.4- and 6.4- fold higher than in brain, respectively for AcAc reduction and for BOH oxidation. The same differences were found in the hibernation state. 2/ In euthermic jerboa, the Michaelis constants, K_M BOH and K_M NAD^+ are different in liver and in brain while K_M AcAc, K_M NADH and the dissociation constants, K_D $NAD^+$and K_D NADH are similar. 3/ During prehibernating state, as compared to euthermic state, the liver BDH activity is reduced by half, while kinetic constants are strongly increased except K_D NAD^+. 4/ During hibernating state, BDH activity is significantly enhanced, moreover, kinetic constants (K_M and K_D) are strongly modified as compared to the euthermic state; i.e. K_D NAD^+ in liver and K_M AcAc in brain decrease 5 and 3 times respectively, while K_D NADH in brain strongly increases up to 5.6 fold. 5/ Both protein content and mRNA level of BDH remain unchanged during the cold adaptation process.

Conclusions: These results cumulatively explained and are consistent with the existence of two BDH enzymatic forms in the liver and the brain. The apoenzyme would be subjected to differential conformational folding depending on the hibernation state. This regulation could be a result of either post-translational modifications and/or a modification of the mitochondrial membrane state, taking into account that BDH activity is phospholipid-dependent.

Background

During winter period several mammals can survive inhospitable conditions by a series of metabolic adaptations which lead to hibernation. Jerboa (*Jaculus orientalis*), a model for deep hibernation, is a small rodent herbivore native to subdesert highlands in Morocco [1,2]. During cold period, jerboa accumulates lipid reserves as white adipose mass developing a seasonal obesity. During hibernation, the body temperature of jerboa decreases to around 9.8 ± 0.7°C and the heart frequency drops to 9.3 ± 1.5 beats/min in comparison with the active euthermic animal which exhibits a 37°C body temperature with a cardiac rhythm around 300 beats per minute [2]. Hibernation is characterized by a slow metabolic rate [3] and the survival of the hibernating animal depends on the accumulated lipids during the prehibernating state.

In a previous work [4], we have reported that during hibernation phases: 1/ SDS-PAGE of the mitochondrial protein pattern profile is modified; 2/ the yield of isolated mitochondria is increasead; 3/ the plasmatic parameters were subject to variations i.e. a decrease in the level of circulating glucose and triglycerides while the levels of D-3-hydroxybutyrate (BOH) (ketoneamia) and of urea increase; 4/ the activities of peroxisomal palmitoyl-CoA oxidase and urate oxidase and mitochondrial palmitoyl-CoA dehydrogenase are enhanced.

Other reports have shown that during hibernation, there is specific involvement of glycolytic enzymes: phosphofructokinase, hexokinase, pyruvate kinase [5], and glyceraldehyde-3-phosphate dehydrogenase [6,7], and of a lipogenic enzyme: the glycerol-3-phosphate dehydrogenase [8].

Lehninger et al. [9] reported that, the ketosis resulting from lipolysis increased release of fatty acids in liver and led to the production of ketone bodies as the consequence of a massive β-oxidation rate. Consequently proved by the increase in BOH level and the decrease in triglyceride level in the plasma [4]. Ketone bodies are produced by the liver in order to serve as energetic substrates compensating the lack of carbohydrates in tissues [10]. Changes of carbohydrate composition were previously observed during hibernation [11]. Indeed, during hibernation, carbohydrates are in short supply and lipid stocks become the main energetic source for the cells.

Consequently, the concept of balance in the levels of ketone bodies in the cell in different tissues appears evident during hibernation. In eukaryotic cells the ketone bodies are represented mainly by BOH and acetoacetate (AcAc). Those compounds are interconverted in mitochondria by D-3-hydroxybutyrate dehydrogenase (BDH) (E.C.1.1.1.30) which was previosuly described by Wakeman and Dakin [12] in dog liver.

In eukaryotic cells, BDH is a mitochondrial inner membrane enzyme slightly associated with the electron respiratory chain showing its active site oriented toward matricial side [13–17].

The catalytic activity of BDH is lecithin-dependent [18,19]. Phospholipids induce a slight structural change of the active site permitting the enzymatic reaction [20]. As it has been reported [21], liver BDH catalyses the transformation of AcAc into BOH in the presence of NADH + H^+. Then, BOH is transported via the blood fluid to peripheral organs, especially brain, heart and kidney. Once in the extra-hepatic tissues, in the presence of NAD^+, BOH is converted into AcAc, which is used after its conversion to acetoacetyl-CoA, either as energetic fuel to produce ATP via the respiratory chain, or in the synthesis of fatty acids.

The catalytic mechanism of inter-conversion in liver and in peripheral tissues has been previously reported by our group [22]. Furthermore, it has been shown that acetoacetate is the main energetic donor in the new born animals [23] and during starvation [10]. On the other hand, it has been pointed out that the increasing BOH level in the plasma is the main source of energetic fuel for the brain metabolism and prevents brain damages during neonatal hypoxia/ischaemia [24]. Thus, there is a close relationship between ketone bodies, energy and lipid metabolism. Since obesity is characterized by the strong increase in the body lipid stock, the ketone body production could be enhanced in this metabolic situation.

We present here a study on the effect of cold exposure and hibernation of jerboa on the mitochondrial BDH function and expression level in liver, a ketone bodies producing tissue, and in brain, the main organ/tissue of ketone bodies as energetic source. The differences in BDH activities and kinetic parameters are coherent with the existence of two BDH enzymatic forms expressed in liver and in brain.

Results

Catalytic expression of BDH during hibernation process

BDH activity was measured at 37°C in different physiological states in liver, a ketone bodies producing tissue, and in brain, a main user tissue of ketone bodies as energetic fuel.

From the results presented in Table 1 it appears that : 1/ the enzymatic activities of BDH at 37°C (forward and reverse directions) are higher in liver than in brain tissue; 2/in liver or brain, the cold acclimatization

Table 1: Variation of BDH activity in both directions (oxidation of BDH and reduction of AcAc) in brain and liver measured at 37°C or 9°C under different physiological conditions. Values are given in nmol/min/mg mitochondrial protein in mean ± standard deviation of at least two experiments with 4 different animals for each condition at *P < 0.05 as compared to the active (A) or at **P < 0.05 as compared to the prehibernator (PH) calculated by ANOVA test.

Tissue	Reaction	Temperature °C	Euthermic (active)	Prehibernating	Prehibernating + ciprofibrate	Hibernating
Liver	AcAc → BOH	37°C	30.65 ± 1.77	14.86 ± 0.8 (× 0.48)*	19.1 ± 2.4 (× 0.62)* (× 1.28)**	42.87 ± 5.9 (× 1.4)* (× 2.88)**
	BOH → AcAc	37°C	33.11 ± 4.02	18.8 ± 1.4 (× 0.57)*	27.33 ± 2.7 (× 0.82)* (× 1.45)**	44.1 ± 2 (× 1.33)* (× 2.33)**
	BOH → AcAc	9°C	2.08 ± 0.24	-	-	6.84 ± 1.05 (× 3.29)*
Brain	AcAc → BOH	37°C	12.64 ± 1.9	10.33 ± 1.22 (× 0.82)*	8.19 ± 1.76 (× 0.65)* (× 0.79) **	16.11 ± 0.72 (× 1.27)* (× 1.56) **
	BOH → AcAc	37°C	5.14 ± 0.16	4.35 ± 0.48 (× 0.85)*	3.05 ± 0.32 (× 0.59)* (× 0.70) **	6.11 ± 0.64 (× 1.19)* (× 1.4) **
	BOH → AcAc	9°C	2.54 ± 0.59	-	-	3.33 ± 0.06 (× 1.31)*
			ratio	ratio	ratio	ratio
Liver / Brain	BOH → AcAc	37°C	6.4	4.3	8.9	7.2
	BOH → AcAc	9°C	0.8	-	-	2.0

Table 2: Variation of BDH activity ratios of both directions of reaction in liver and brain under different physiological conditionn (active, prehibernating, ciprofibrate – treated prehibernating and hibernating jerboas).

Ratio	Tissue	Euthermic (active)	Prehibernating	Prehibernating + ciprofibrate	Hibernating
Activity AcAc → BOH at 37°C	Liver	0.92	0.79	0.70	0.97
Activity BOH → AcAc at 37°C	Brain	2.45	2.37	2.68	2.63
Activity BOH → AcAc at 37°C	Liver	15.92	-	-	6.44
Activity BOH → AcAc at 9°C	Brain	2.02	-	-	1.83

(prehibernating) leads to a decrease of BDH-specific activity in both directions, whereas BDH activity is stimulated by combination of cold and food deprivation (hibernating) in both liver and brain, although with a higher effect measured in liver; 3/different kinetic properties of BDH from liver or brain revealed by the ratio of tissue-dependent specific activities strongly decreases from 37°C to 9°C in euthermic as well as in hibernating conditions. By comparing the activity ratio measured at 37°C and at 9°C for BOH oxidation, we note an 8 and 3 fold decrease respectively in euthermic and hibernating animals. In Table 2, the ratio indicates that the BDH specific activity in the liver is higher in the reverse direction (production of BOH) than the forward reaction (oxidation of BOH), while it is the opposite in the brain. On the other hand, in term of temperature dependency the BDH activity follows the Arrhenuis' law in liver of euthermic animals (doubling of activity every 10°C increase) while it does not, at all in brain and just partially in liver of hibernating jerboa (Table 2).

On the other hand, the BDH activity was measured at 37°C for BOH oxidation and for BOH formation in liver and in brain of ciprofibrate-treated jerboa. During the prehibernating state comparatively to the euthermic animals, we observed a decrease in this activity by 20 to 40 % depending on the tissues (Table 1). Ciprofibrate treatment of hibernating animal increases the ratio value (liver versus brain) of BDH activity by 40 % in the BOH oxidation direction comparatively to euthermics (Table 1).

Kinetic parameters of BDH during hibernation process

Kinetic parameters were determined in liver and in brain of jerboa at different states of cold adaptation (Table 3). In euthermic animals we find that the K_M BOH and the K_M NAD^+ are 3 and 5 respectively fold higher in the brain than in the liver, while there is no significant difference between both tissues for K_MAcAc, K_MNADH, $K_D$$NAD^+$ and K_DNADH. In the liver, during prehibernation, we observed an increase in the kinetic parameters except for $K_D$$NAD^+$ which decreased slightly, while in the brain no significant change was seen.

During hibernation, in the liver, a 5-fold decrease in $K_D$$NAD^+$ and a slight increase in K_MBOH were observed. Regarding other parameters, there is a weak decrease (from 18 % to 27%) except for K_MAcAc. In the brain, there is a decrease in K_M of 34 % for BOH and for NAD+ while the decrease is twice and 3 times greater for NADH and

Table 3: Variation of BDH kinetic parameters (K_M and K_D) for BOH, NAD^+, AcAc and NADH calculated from brain and liver enzymes activities under different physiological conditions. Values are given in mean ± standard deviation of at least two experiments with 4 different animals for each condition at *P < 0.05 as compared to the active (A) or at **P < 0.05 as compared to the prehibernating (PH) calculated by ANOVA test.

		BOH	NAD^+		AcAc	NADH, H^+	
Tissue	Physiological states	K_M (mM)	K_M (mM)	K_D (mM)	K_M (mM)	K_M (mM)	K_D (mM)
Liver	Euthermic(active)	0.633 ± 0.05	0.238 ± 0.015	2 ± 0.18	0.150 ± 0.01	0.074 ± 0.005	0.15 ± 0.01
	Prehibernating	1.06 ± 0.1 (× 1.67)*	0.55 ± 0.04 (× 2.3)*	1.33 ± 0.1 (× 6.8)*	1.026 ± 0.008 (× 6.8)*	0.28 ± 0.018 (× 3.8)*	0.4 ± 0.03 (× 2.66)*
	Hibernating	0.944 ± 0.07 (× 1.49)*	0.174 ± 0.014 (× 0.73)*	0.444 ± 0.035 (× 0.22)*	0.146 ± 0.01 (× 0.97)	0.058 ± 0.005 (× 0.78)*	0.12 ± 0.01 (× 0.8)*
Brain	Euthermic(active)	2 ± 0.15	1.187 ± 0.1	1.9 ± 0.1	0.216 ± 0.018	0.076 ± 0.005	0.16 ± 0.01
	Prehibernating	2.25 ± 0.2 (× 1.25)	1.35 ± 0.1 (× 1.13)	1.72 ± 0.1 (× 0.9)	0.287 ± 0.02 (× 1.33)*	0.066 ± 0.004 (× 0.87)*	0.2 ± 0.0175 (× 1.25)*
	Hibernating	1.33 ± 0.12 (× 0.66)*	0.787 ± 0.06 (× 0.66)*	3.11 ± 0.25 (× 1.64)*	0.067 ± 0.004 (× 0.30)*	0.035 ± 0.002 (× 0.46)*	0.9 ± 0.06 (× 5.62)*

AcAc respectively. Meanwhile, the K_D for NAD^+ and for NADH increase 1.6 and 5.6 fold respectively.

Western blotting

The BDH expression at protein level was determined by western blotting using an anti-BDH polyclonal antibody. Figure 1 showed no significant variation of BDH levels during prehibernation or hibernation states in both liver and brain. Treatment of jerboa with ciprofibrate during cold exposure does not modify the BDH protein levels either comparatively to euthermic or to prehibernating animals.

Northern blotting

The analysis of BDH mRNA level by northern blotting for different hibernation periods showed no significant variation in both brain and hepatic tissues (Figure 2). Treatment of jerboa with ciprofibrate during prehibernation phase does not change the level of BDH mRNA as compared to euthermic and prehibernating animals.

Discussion

In euthermic animals, the level of BDH activity is higher in liver, a ketone body producing tissue, than in brain, a ketone body consuming tissue, whatever the direction of the reaction (i.e. oxidation of BOH to AcAc or the formation of BOH from AcAc). In parallel, the difference in the activity levels is directly related to the kinetic constants (K_M) for all the substrates (i.e. BOH, AcAC, NAD^+ and NADH) between liver and brain. These results can be explained through the existence of two enzymatic forms of BDH in the two tissues. This is corroborated by a precedent work in which we proposed a molecular mechanism of BDH catalysis in the liver and in the peripheral tissues based on BDH conformational change [22]. Such a hypothesis has also been reported earlier in rat model, for the liver and the brain BDH [35] as well as in the goldfish model, *Carassius auratus* for liver and kidney BDH [36].

In the liver, the BDH activity is similar in both directions of the reaction. This can be related to the perfect reversibility of the reaction and allows this tissue to maintain both BOH and AcAc availability at a steady-state level necessary for homeostasis.

In the brain of jerboa, our results show that the direction of BDH activity reaction is favoured in the reduction of AcAc. This is unexpected because in the rat brain the direction of the reaction is forwarded in the direction of BOH oxidation. Such BOH from liver is providing AcAc and NADH as energetic sources in the brain tissue [37]. Taking into account the body temperature of hibernating jerboa drops to around 9°C [4], the measured BDH activity at lowered temperature in the direction of BOH oxidation is similar in both tissues. Thus showing that the response of BDH activity to low temperatures is different in liver and brain. Indeed, the comparison of activities measured at 37°C or at 9°C show a stronger decrease in BDH activity in liver (16 fold) than in brain (2 fold). All these results support our hypothesis about the existence of two BDH enzymatic forms at least one in the brain and another one in the liver.

During prehibernating period, in which jerboa accumulates fatty acids in adipose tissue leading to a decrease in circulating triglycerides and in ketone body rates [4], the slowdown of BDH activity in liver and in brain seems to be due to the decrease in the production of its substrate (i.e. BOH or AcAc). Furthermore, cold exposure seems to favour the liver enzymatic reaction in the direction of BOH oxidation resulting in AcAc production. Such increased AcAc level could allow both, i,e supply brain with this energetic compound and further constitution of an AcAc pool for lipogenesis. This is corroborated by the

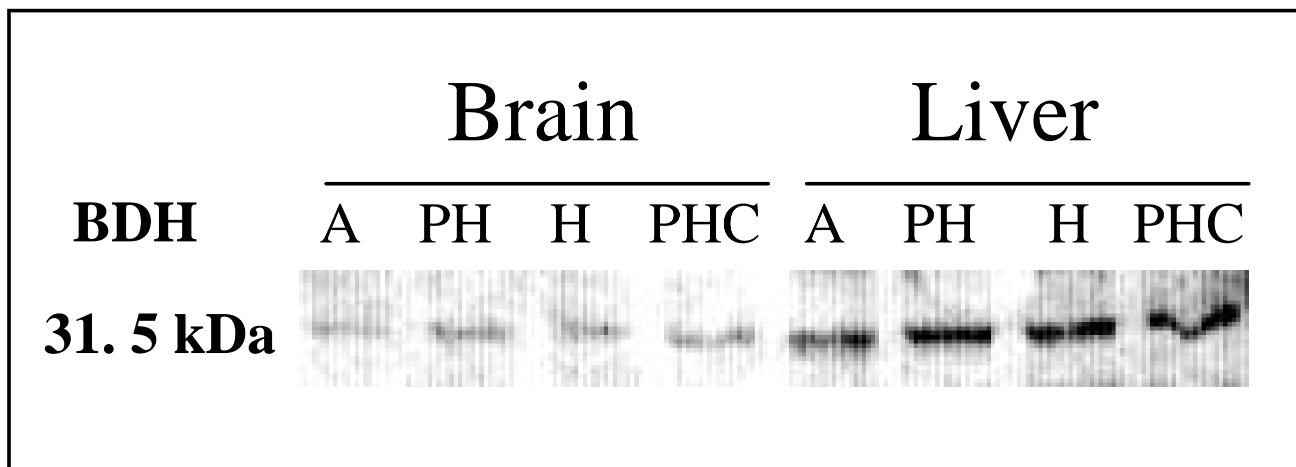

Figure 1
western blotting analysis of BDH in brain and liver of euthermic active (A), prehibernating (PH), prehibernating ciprofibrate-treated (PHC) or hibernating jerboa (H). The solubilised proteins were subjected to 12.5 % SDS-polyacylamide gel electrophoresis and transferred on nitrocellulose. BDH was detected with anti-rat liver BDH polyclonal antibody.

change in the affinity of the enzyme which is increased for NAD^+ and decreased for NADH. Meanwhile, in the brain, cold exposure had no effect on the reaction direction as indicated by the measured affinity of the enzyme for NAD^+ and NADH. On the other hand, cold adaptation during prehibernation had no effect on the synthesis rate of BDH protein and mRNA as analyzed by western and northern blotting respectively. These results seem to indicate that cold exposure induces regulation of BDH at the catalytic level in the liver but not in the brain. During hibernation, jerboa uses fatty acid stock to survive leading to an important production of ketone bodies [4]. Thus the increase in BDH activity compared to euthermic animals, particularly in the liver at 9°C, seems to provide the ketone bodies during this phase.

Furthermore, the comparison of BDH activities measured at 37°C and at 9°C in both brain and liver tissues indicates that there is a change in the liver BDH conformation when the animal enters hibernation. Indeed, the Q_{10} (defined as the ratio of enzymatic activity rate at the given temperature [t] to a 10°C higher temperature [t+10]) of BDH in euthermic animals is largely higher than 2, while in hibernating jerboa the Q_{10} is near 2. This conformational changing process is strengthened by the increase in the affinity of the enzyme and for its coenzymes during hibernation. In the brain, hibernation has no effect on the activity variations between 37°C and 9°C compared to euthermia. However, the BDH affinity for its coenzymes strongly decrease at the lower temperatures. This latter effect is also in agreement with the conformational modifications of BDH between the two physiological states (active euthermic and hibernating). Moreover, analysis of both mRNA and protein levels by northern and western blotting respectively does not reveal any variations.

Ciprofibrate is a hypolipemic agent, which to a large extent increases the degradation of lipids in both mitochondria and in peroxisomes [38]. Previously we reported, that BDH activity is inhibited in euthermic jerboa liver after treatment with ciprofibrate while this activity is still unchanged in peripheral organs[39].

In the present work we have studied BDH expression in jerboa treated with ciprofibrate during prehibernating state where animal accumulates lipid reserves. By contrast

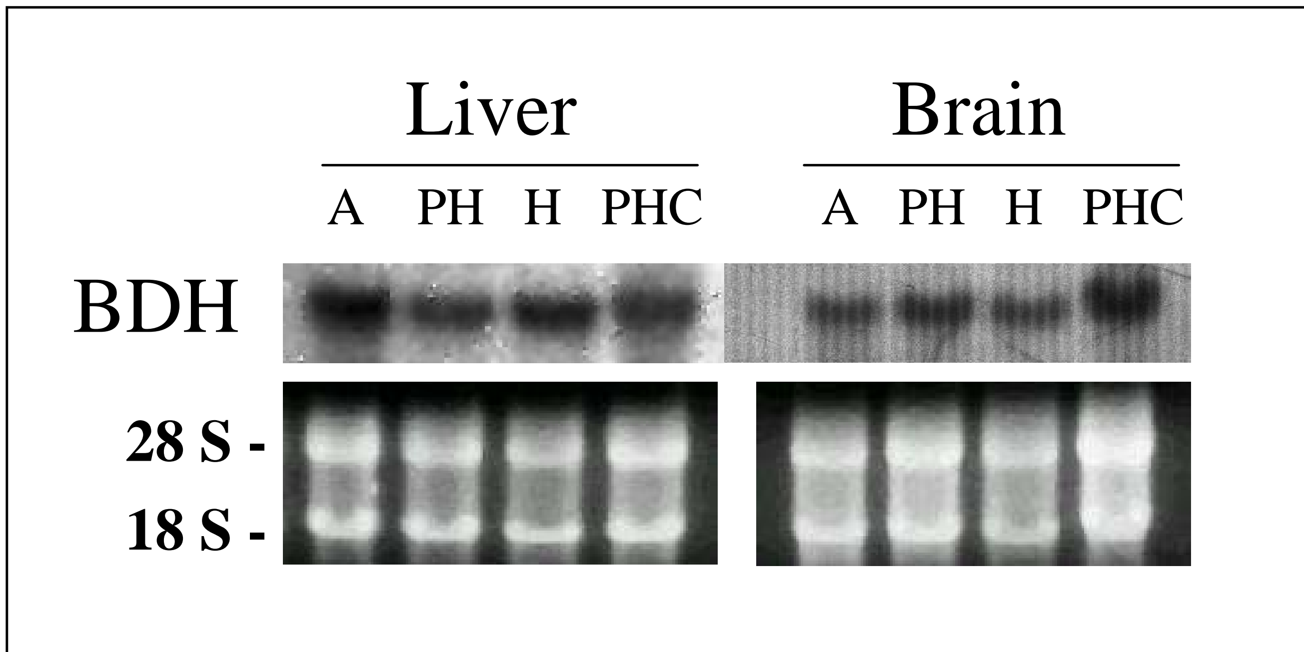

Figure 2
northern blotting analysis of BDH mRNAs. Total RNA (10 μg) was isolated from brain and liver tissues of euthermic active (A), prehibernating (PH), prehibernating ciprofibrate-treated (PHC) or hibernating jerboa (H) and subjected to northern blotting analysis using rat liver BDH cDNA probe. Standardization was made with 28 S and 18 S ribosomal RNAs.

to our previous work about euthermic jerboa [39], treatment with ciprofibrate during prehibernation significantly stimulates BDH activity in liver while it slightly inhibits this activity in brain. Furthermore, the treatment with ciprofibrate has no effect on both mRNA and protein BDH level.

We showed that ciprofibrate treatment of prehibernating jerboa decreases significantly the level of circulating triglycerides. Such a decrease is lower than the one recorded during hibernation [4]. One could suggest, in the light of our finding, that the fatty acid degradation in liver during hibernation and during the ciprofibrate treatment of prehibernating jerboa provokes a change in mitochondrial membrane lipid composition. These modifications would stimulate the BDH activity in both cases, knowing that the plasmatic BOH level strongly increases during hibernation but not after ciprofibrate treatment of prehibernating jerboa [4]. This leads us to propose that the treatment with ciprofibrate of prehibernating jerboa inhibits the release of ketone bodies by the liver into the blood. So, by such a transport inhibition of ketone body efflux from the liver, the brain is deprived of those energetic compounds leading to lower BDH activity. Elsewhere, Kabine et al [40] observe that the starvation-induced hibernation provokes the rapid death of all animals treated during prehibernation with ciprofibrate.

In conclusion, our results reveal 1/ the presence of two distincts enzymatic forms of BDH in liver and brain tissues. This has been also reported for the hepatic glutamate dehydrogenase in Richardson's ground squirrel [41], and 2/ that BDH from liver and from brain is subject to differential regulation depending on the hibernation state. This regulation could be a result of post-translational modifications and/or a modifications of mitochondrial membrane state, knowing that the BDH activity is phospholipid-dependent. The local environment of the protein may result in an important kinetic change. A change of physical properties of the mitochondrial membrane related to the hibernation process has been also reported in ground squirrel [42]. Furthermore, post-trans-

lational regulation during hibernation was reported for glyceraldehyde-3-phosphate dehydrogenase in jerboa (*Jaculus orientalis*) [6,7]. A further cloning of BDH gene(s) will put forward the properties and the role of this enzyme. Indeed, the cloning experiment would allow to establish if there are two genes, two mRNA splice variants and/or self specific post-translational modifications.

Methods

Animals

Male young adult jerboa (*Jaculus orientalis*) 4 to 6 months old (110–140 g body weight) were captured in the sub-desert of eastern Morocco. They were acclimatized in the laboratory for 3 weeks at 22 ± 2°C with food (rabbit diet biscuits; Aliments UAR-Villemoisson, Orge, France) and salad. The circadian rhythm was 10 h light and 14 h dark. For pre-hibernating and hibernating states, a group of animals (4 per cage) was kept with food in a cold room (6°C) for 3 weeks. This group was called the pre-hibernator group (PH). A second group was kept under the same conditions as PH, except that the food contained ciprofibrate (3 mg/kg body weight/day), and was called the ciprofibrate-treated pre-hibernator group (PHC). The third group was housed as PH, and at the end of 3 weeks of the prehibernation period, the food was removed leading to hibernation after 24 to 36 hours, so called group (H). These animals were sacrificed on the sixth day of hibernation. The reference group corresponds to euthermic active animals (A). Animals were used in agreemant with laws in both Morocco and France and animal experiments were approved by the moroccan veterinary office of laboratory animal welfare.

Liver and brain mitochondria isolation

The jerboas were decapitated and liver and brain were rapidly removed for mitochondrial preparation according to Fleischer et al [25]. Protein content was estimated with Bio-Rad assay according to Bradford [26] using bovine serum albumin as standard.

Enzymatic activity measurement

BDH activity was measured as described in [27] at 37°C by following NADH production at 340 nm ($\varepsilon = 6.22 \times 10^3$ $M^{-1}.cm^{-1}$) using a cold disrupted mitochondrial preparation (0.2 mg of protein/assay) in a medium containing 10 mM potassium phosphate, 0.5 mM EDTA, 1.27 % redistilled ethanol, 0.3 mM dithiothreitol at pH 7.35, in the presence of 2 mM NAD^+ and 2.5 μg rotenone (final addition to prevent NADH re-oxidation by the respiratory chain). The assay was started by the addition of D,L-3-hydroxybutyrate to 10 mM final concentration. Kinetic parameters of BDH were determined by measuring the initial rate at 37°C in a standard medium as above described for the oxidation of D-3-hydroxybutyrate using the following coenzyme/substrate concentrations: [NAD^+] = 0.2, 0.4, 0.8 or 2 mM ; [D,L-3-hydroxybutyrate] = 1.25, 2.5, 4.5 or 10 mM or in the same medium without NAD^+ and rotenone and in the presence of varying acetoacetate concentration (0.2, 0.4, 0.6 or 0.8 mM) and NADH concentrations (0.2, 0.4, 0.6 or 0.8 mM). In all cases, the NaCl concentration of the medium was adjusted in order to keep constant salt concentration [27]. Graphical determination of parameters was made from mathematical analysis according to the method of Cleland [28]. For all additional experimental conditions, see legend of figures and tables.

Western blotting analysis

The solubilised proteins (50 μg) were subjected to 12.5 % SDS-polyacylamide slab gel electrophoresis using the method of Laemmli [29], then transferred on nitrocellulose filter according to Towbin et al. [30] BDH was dectected by an anti-rat liver BDH polyclonal antibody [31].

Northern blotting analysis

Total RNAs were obtained from brain and liver tissues previously frozen in liquid nitrogen and stored at -80°C using the LiCl method as described by Auffray and Rougeon [32]. Northern blots were performed as described by Cherkaoui-Malki et al [33]. Hybridization was done at 42°C overnight in 50% formamide, 5 × Denhardt's, 5 × SSC (Sodium Saline Citrate) and 0.1% SDS. The filters were washed twice at 42°C for 30 min in 2 × SSC, 0.5% SDS and once at 65°c for 30 min in 1 × SSC, 0.1% SDS. Rat BDH cDNA probe was described earlier [34]. Northern blots were normalized with the 18 S and 28 S ribosomal RNAs.

List of Abbreviations

AcAc: acetoacetate, BDH: D-3-hydroxybutyrate dehydrogenase, BOH : D-3-hydroxybutyrate.

Autors' contributions

MK and MCM conducted animals treatment and northern blotting. MK and MSE conducted the biochemical and molecular experiments. AH realised the western blotting. MSE, MCM and NL supervised the work of MK and AH, wrote the first draft of the manuscript and oversaw subsequent drafting and editing of the manuscript.

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References

1. Hooper ET and El Hilali M: **Temperature regulation and habits in two species of Jerboa, genus *Jaculus*.** *J Mammal* 1972, **53:**574-593.

2. El Hilali M and Veillat JP: ***Jaculus orientalis* : A True Hibernator.** *Mammalia* 1975, **39:**401-404.
3. Nedergaard J and Cannon B: **Mammalian hibernation.** *Philos Trans R Soc Lond B Biol Sci* 1990, **326:**669-686.
4. Kabine M, Cherkaoui-Malki M, Clémencet M-C, El Kebbaj MS and Latruffe N: **Peroxisomal Changes During Hibernation of Jerboa (*Jaculus orientalis*).** *J Am Oil Chem Soc* 1998, **75:**275-280.
5. El Hachimi Z, Tijane M, Boissonnet G, Benjouad A, Desmadril M and Yon JM: **Comparison of muscle phosphofructokinase from euthermic and hibernating Jaculus orientalis. Purification and determination of the quaternary structure.** *Comp Biochem Physiol* 1992, **B.102:**507-513.
6. Soukri A, Valverde F, Hafid N, El Kebbaj MS and Serrano A: **Characterization of muscle glyceraldehyde-3-phosphate dehydrogenase isoforms from euthermic and induced hibernating *Jaculus orientalis*.** *Biochim Biophys Acta* 1995, **1243:**161-168.
7. Soukri A, Hafid N, Valverde F, El Kebbaj MS and Serrano A: **Evidence for a posttranslational covalent modification of liver glyceraldehyde-3-phosphate dehydrogenase in hibernating jerboa (*Jaculus orientalis*).** *Biochim Biophys Acta* 1996, **1292:**177-187.
8. Berrada W, Naya A, Iddar A and Bourhim N: **Purification and characterization of cytosolic glycerol-3-phosphate dehydrogenase from skeletal muscle of jerboa (*Jaculus orientalis*).** *Mol Cell Biochem* 2002, **231:**117-127.
9. Lehninger AL, Sudduth HC and Wise JB: **D-β-hydroxybutyric dehydrogenase of mitochondria.** *J Biol Chem* 1960, **235:**2450-2455.
10. Kante A, Malki MC, Coquard C and Latruffe N: **Metabolic control of the expression of mitochondrial D-β-hydroxybutyrate dehydrogenase, a ketone body converting enzyme.** *Biochim Biophys Acta* 1990, **1033:**291-297.
11. Zimny ML, Packman H and Haydel R: **Carbohydrate metabolism in ground squirrels during the summer season.** *Comp Biochem Physiol* 1969, **31:**211-216.
12. Wakeman AJ and Dakin HD: **On the decomposition of β-oxybutyric and acetoacetic acid by enzyme of the liver.** *J Biol Chem* 1909, **6:**373-389.
13. Wise JB and Lehninger AL: **The stability of mitochondrial D-β-hydroxybutyrate dehydrogenase and its relationship to respiratory chain.** *J Biol Chem* 1962, **237:**1363-1370.
14. Nielsen NC, Zahler WL and Fleischer S: **Mitochondrial D-β-hydroxybutyrate dehydrogenase. IV. Kinetic analysis of reaction mechanism.** *J Biol Chem* 1973, **248:**2556-2562.
15. Latruffe N and Gaudemer Y: **Properties and kinetic mechanism of D-β-hydroxybutyrate dehydrogenase from rat liver submitochondrial particles. Comparative study of the effects of differents thiols.** *Biochimie* 1974, **56:**435-444.
16. Gaudemer Y and Latruffe N: **Evidence for penetrant and nonpenetrant thiol reagents and their use in the location of rat liver mitochondrial D(-)-β-hydroxybutyrate dehydrogenase.** *FEBS Lett* 1975, **54:**30-34.
17. McIntyre JO, Bock HG and Fleischer S: **The orientation of D-beta-hydroxybutyrate dehydrogenase in the mitochondrial inner membrane.** *Biochim Biophys Acta* 1978, **513:**255-267.
18. Sekuzu I, Jurtshuk P and Green DE: **On the isolation and properties of D-β-hydroxybutyrate dehydrogenase of beef heart mitochondria.** *Biochem Biophys Res Commun* 1961, **6:**71-75.
19. Gazzotti P, Bock H and Fleischer S: **Interaction of D-β-hydroxybutyrate apodehydrogenase with phospholipids.** *J Biol Chem* 1975, **250:**5782-5790.
20. Latruffe N, El Kebbaj MS, Morpain C and Bernengo JC: **Fluorescence study of the phospholipid dependent cnoformational changes of D-β-hydroxybutyrate dehydrogenase Membrane proteins.** *BioRad laboratories, San Diego* 1987:95-107.
21. Williamson DH, Bates MW, Page MA and Krebs HA: **Activities of enzymes involved in acetoacetate utilization in adult mammalian tissues.** *Biochem J* 1971, **121:**41-47.
22. El Kebbaj MS and Latruffe N: **Alkylation at the active site of the D-3-hydroxybutyrate dehydrogenase (BDH), a membrane phospholipid-dependent enzyme, by 3-chloroacetyl pyridine adenine dinucleotide (3-CAPAD).** *Biochimie* 1997, **79:**37-42.
23. Latruffe N, Feuvrier MN, Bichet N and Gaudemer Y: **Variations of D(-)-β-hydroxybutyrate dehydrogenase activity of rat liver mitochondria during post-natal development.** *Biochimie* 1978, **60:**71-76.
24. Dardzinski BJ, Smith SL, Towfighi J, Williams GD, Vannucci RC and Smith MB: **Increased plasma beta-hydroxybutyrate, preserved cerebral energy metabolism, and amelioration of brain damage during neonatal hypoxia ischemia with dexamethasone pretreatment.** *Pediatr Res* 2000, **48:**248-255.
25. Fleischer S, McIntyre JO and Vidal JC: **Large-scale preparation of rat liver mitochondria in high yield.** *Methods Enzymol* 1979, **55:**32-39.
26. Bradford MM: **A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding.** *Anal Biochem* 1976, **72:**248-254.
27. El Kebbaj MS, Latruffe N and Gaudemer Y: **Presence of an essential arginyl residue in D-beta-hydroxybutyrate dehydrogenase from mitochondrial inner membrane.** *Biochem Biophys Res Commun* 1980, **96:**1569-1578.
28. Cleland WW: **Prediction of initial velocity and inhibition patterns by inspection.** *Biochim Biophys Acta* 1963, **67:**104-107.
29. Laemmli UK: **Cleavage of structural proteins during the assembly of the head of bacteriophage T4.** *Nature* 1970, **227:**680-685.
30. Towbin H, Staehelin T and Gordon J: **Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications.** *Proc Natl Acad Sci USA* 1979, **76:**4350-4354.
31. Coquard C, Adami P, Cherkaoui-Malki M, Fellmann D and Latruffe N: **Immunological study of the tissue expression of D-β-hydroxybutyrate dehydrogenase, a ketone body-converting enzyme.** *Biol Cell* 1987, **59:**137-143.
32. Auffray C and Rougeon F: **Nucleotide sequence of a cloned cDNA corresponding to secreted mu chain of mouse immunoglobulin.** *Gene* 1980, **12:**77-86.
33. Cherkaoui-Malki M and Caira F: **Northern blot analysis of rat liver mRNA encoding for peroxisomal Acyl-CoA oxidase.** *in Peroxisomes* Edited by: *Latruffe N, Bugaut M. Springer Verlag, Berlin*; 1993:61-73.
34. Bailly A, Lone YC and Latruffe N: **Post-transcriptional analysis of rat mitochondrial D-3-hydroxybutyrate dehydrogenase control through development and physiological stages.** *Biol Cell* 1991, **73:**121-129.
35. Dombrowski GJ, Cheung GP and Swiatek KR: **Evidence for the existence of enzymatic variants of beta-hydroxybutyrate dehydrogenase from rat liver and brain mitochondria.** *Life Sci* 1977, **21:**1821-1829.
36. Leblanc PJ and Ballantyne JS: **Novel aspects of the activities and subcellular distribution of enzymes of ketone body metabolism in the liver and kidney of the goldfish, Carassius auratus.** *J Exp Zool* 2000, **286:**434-439.
37. Latruffe N and Gaudemer Y: **Inhibition of beta-hydroxybutyrate oxidation in rat liver mitochondria or submitochondrial particles by monocarboxylates transport inhibitors.** *Biochimie* 1978, **60:**677-680.
38. Lazo O, Contreras M and Singh I: **Effect of ciprofibrate on the activation and oxidation of very long chain fatty acids.** *Mol Cell Biochem* 1991, **100:**159-167.
39. El Kebbaj MS, Malki MC and Latruffe N: **Properties of peroxisomes from jerboa (*Jaculus orientalis*).** *Eur J Cell Biol* 1996, **70:**150-156.
40. Kabine M, Clémencet M-C, Bride J, El Kebbaj MS, Latruffe N and Cherkaoui-Malki M: **Changes of peroxisomal fatty acid metabolism during cold acclimatization in hibernating jerboa (*Jaculus orientalis*).** *Biochimie* 2003.
41. Thatcher BJ and Storey KB: **Glutamate dehydrogenase from liver of euthermic and hibernating Richardson's ground squirrels: evidence for two distinct enzyme forms.** *Biochem Cell Biol* 2001, **79:**11-19.
42. Raison JK and Lyons JM: **Hibernation: alteration of mitochondrial membranes as a requisite for metabolism at low temperature.** *Proc Natl Acad Sci USA* 1971, **68:**2092-2094.