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Relationships Between Serum T_4, T_3, Cortisol and the Metabolism of Chemical Energy Sources in the Cobra During Pre-hibernation, Hibernation and Post-hibernation

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Abstract. -This study analyzed the variation of the serum thyroxine (T_4), triiodothyronine (T_3), and cortisol in the cobra (*Naja naja* Linnaeus) in pre-hibernation, hibernation, and post-hibernation. The variations were compared with the changes of indexes of cobra metabolism in those three periods, such as oxygen consumption, serum glucose, serum triglyceride, glycogen content of liver, and triglyceride content of fat bodies. From pre-hibernation to hibernation, the change of metabolic indexes of the cobra indicated that the metabolic rate tends to decline because of the very low levels of the three serum hormones in pre-hibernation and falling temperature. Low temperature during hibernation limited the activities of the three serum hormones which were rising at higher levels during hibernation. From hibernation to post hibernation, the rising temperature allowed the three serum hormones to increase markedly, stimulating metabolism gradually. Therefore the metabolic rate of the cobra during post-hibernation tended to rise again. The metabolic indexes of the cobra in post-hibernation showed a significant increase in metabolic rate, which had close relation with the high levels of the three serum hormones. The indexes of metabolism of the cobra indicate that it is hepatic glycogen and not fat that hibernating cobras use as their main energy source.

Key Words: Reptilia, Serpentes, Elapidae, *Naja naja*, cobra, thyroxine, triiodothyronine, cortisol, metabolism, energy metabolites, chemical energy sources.

Introduction

Hibernation is an adaptive strategy of ectotherms for keeping out of the cold during winter. The metabolic rate of the hibernating reptiles is apparently different from that of active ones, as a result of physiological adaptation. The endocrine system and its close relation to metabolism may play an important role in physiological adaptation. Maher (1965) pointed out that the maximum metabolic response in lizards to thyroxine occurred at 3°C; Wilhoft (1966) found that injections of thyroxine in lizards increased their metabolism. Musucchia (1984) suggested that the injection of cortisol into hamsters during hibernation or hypothermia could achieve the same result as the injection of glucose, which might prolong the survival of hamsters under the same conditions. We consider that the metabolism of energy metabolites in hibernating snakes must have a close relation to the control of serum thyroxine (T_4), triiodothyronine (T_3), and cortisol. This study may provide a way to find the mechanism of snake hibernation and guidance for maintaining snakes.

Methods

This study was carried out from September 1987 to May 1988. Cobras *Naja naja* (Linnaeus) used as experimental animals were captured at Changlou, Fujian Province, China. We studied serum T_4, T_3, and cortisol, and related indexes of metabolism in cobras during pre-hibernation, hibernation, and post-hibernation.

Cobra groups: 12 adult cobras (6 males, 6 females) were captured in May 1987, weighing 176-273 g. These cobras had been fed in our college snake garden for 4 months before the experiment. On September 9, 1987, 6 cobras were taken out of the snake garden and tested as a pre-hibernation group (Pre G). On October 2, 1987, the remaining 6 cobras were transferred from the snake garden into a dark cement pool with a perforated metal window above. The temperature in the pool was similar to the atmosphere. On

February 10, 1988, 3 cobras were taken out from the pool and tested as a hibernation group (H G), and on May 7, the last three cobras were tested as a post-hibernation group (Post G).

Determination of oxygen consumption: The oxygen consumption of each group was determined using the method of Dong et al. (1986) corrected by the authors.

Heart beats and respiratory state: Heart rate and respiratory state of each group were measured by routine methods.

Analysis of three hormones: Blood was drawn from the posterior vena cava. Serum T_4, T_3, and cortisol of each group were analyzed by radioimmunoassay by means of kits produced by the Institute of Shanghai Biologicals. Each sample was analyzed twice.

Analysis of serum energy metabolites: Serum glucose and serum triglycerides of each group of snakes were analyzed by clinical chemistry methods, and each sample was analyzed twice.

Analysis of hepatic glycogen: Fresh snake liver of each group was weighed and cut into pieces. After having been in boiling water for 5 minutes, the liver pieces were homogenized. The homogenate was in boiling water for another twenty minutes and filtered immediately. The filtered homogenate was mixed with 95% alcohol – A. R. (mean analysis reagent) to twice the volume and kept at room temperature for ten minutes before it was centrifuged for twenty minutes at 3000 rpm. The supernate was drawn out, the sediment (glycogen) soluble in hot water was estimated by the following calculation:

Hepatic glycogen (g/Kg(BW)) = total glycogen content in liver/body weight (BW)

Analysis of fat body triglycerides: Fresh snake fat bodies of each group was weighed and a small weighed part was homogenized in a fixed volume of n-heptane (C_7H_{16}). The fat tissue was extracted in this way three times. Triglycerides of fat bodies extracted in n-heptane were analyzed by clinical chemical methods.

Triglycerides of fat body(g/Kg(BW)) = triglyceride content in fat body/body weight (BW)

Results

1. Variations of the three serum hormones during Pre G, HG and Post G

The levels of serum T_4, T_3, and cortisol of Pre G were the lowest in the three cobra groups. The level of serum cortisol of HG was markedly lower than that of post G. The levels of T_4 and T_3 of HG were not distinctly different from those of Post G (Table 1).

2. Variations of the oxygen consumption and energy metabolites during Pre G, HG and Post G

The oxygen consumption of the HG group was significantly the lowest of the three cobra groups. The oxygen consumption of Pre G was not significantly different from that of Post G.

The contents of serum glucose of both

TABLE 1. Experimental results of the three serum hormones and t tests.

Groups	N or N'	Serum T_3 (ng/ml)	Serum T_4 (ng/ml)	Serum cortisol (ng/ml)
Pre-hibernation (A, 22.9°C)	6	0.2303	0*	369.0
Hibernation (B, 13°C)	3	0.3607	0.700	1509
Post-hibernation (C, 24.9°C)	3	0.5467	1.233	6953
t_{AB} value	7	3.967**	4.399**	12.35**
t_{BC} value	4	1.767	1.153	2.808**
t_{AC} value	7	4.703**	4.792**	5.168**

* Too small to measure. **Significant at 0.05.

TABLE 2. Experimental results of oxygen consumption by energy substances, and t tests.

Groups	N or N'	Oxygen consumption (ml O_2/hr • kg(BW*))	Serum glucose (mg%)	Serum triglyceride (mg%)	Hepatic glycogen (g/kg(BW))	Fat body triglycerides (g/kg(BW))
Pre hibernation (A, 22.9°C)	6	127.6	193.9	102.9	47.99	10.29
Hibernation (B, 13°C)	3	54.9	50.40	18.73	0.3825	16.04
Post-hibernation (C, 24.9°C)	3	788.6	143.5	24.4	0.03084	17.61
t_{AB}	7	9.731**	7.742**	6.542**	2.680**	1.108
t_{BC}	4	2.791**	5.473**	0.679	2.985**	0.1615
t_{AC}	7	2.316	2.220	2.165	2.699**	1.736

* BW = Body Weight. **$P < 0.05$

Pre G and Post G were apparently higher than that of HG. But the content of serum glucose of Pre G was not significantly different from that of Post G.

The hepatic glycogen content of the cobras appeared to decline during hibernation. The hepatic glycogen content of HG was markedly less than that of Pre G, and the hepatic glycogen of Post G was less than that of HG.

Similar to the variation of serum glucose, the content of serum triglycerides of Pre G was higher than that of HG, but there were not significant differences between the serum triglycerides of post G and that of HG, and between that of Pre G and that of Post G. The triglyceride contents of fat bodies in the three cobra groups did not have any statistical differences from one another (Table 2).

Discussion

1. Variations of the oxygen consumption and energy metabolites in the cobras during the three periods

The contents of serum glucose and triglycerides of Pre G were relatively high due to the cobras active intake before entering hibernation, which was advantageous for the storage of energy metabolites for overwintering. The oxygen consumption was large during this period, but the contents of glucose and triglycerides of HG were apparently at low level. Oxygen consumption decreased markedly in this period. The hepatic glycogen content during hibernation became 99% less than during pre-hibernation. The triglyceride content of fat bodies of the HG group seemed to rise a little, perhaps due to some triglyceride synthesis at the beginning of hibernation.

These results indicate that the metabolic rate of cobras during hibernation remains at a low level and oxygen consumption, serum glucose, and serum triglyceride content fell markedly, and that hepatic glycogen is used as the main energy source instead of fat body triglycerides. In post-hibernation, the serum glucose content rose significantly and the content of hepatic glycogen decreased by 92 percent less than that of HG, showing that cobras had used hepatic glycogen to the greatest degree for evoking their activities. This variation provided a good situation for cobras to come out from hibernation, and at the same time, oxygen consumption increased greatly. The rising serum triglycerides of post G also meant that the cobras' fatty metabolism began to become active.

Compared with that of pre G, the oxygen consumption of post G was relatively large, but the serum glucose and serum triglyceride content of post G were relatively low. The reasons may be: 1) that the pre G cobras were not fasting so that their contents of serum glucose and triglycerides were relatively high, 2) that the post G cobras had so little hepatic glycogen as to be unable to raise their serum glucose and triglyceride contents as high as those of the Pre G group, and 3) that the rising temperature and the high serum hormone level after hibernation accelerated the organs and tissues to take in glucose and triglycerides from the blood. Among the three reasons the latter was the most important because of the apparent rise in oxygen consumption. This amount indicated that the metabolism in the organs and tissues had been enhanced markedly.

2. Variation of the three serum hormones

Serum T_3 of HG was 56.6% higher than that of Pre G, and serum T_3 of Post G 51.7% higher than that of HG. Similar to serum T_3, serum T_4 of Pre G was too low to be tested, but T_3 of HG rose significantly, and T_3 of Post G continued to rise 43.2% more than that of HG. As a result, serum T_3 and T_4 were increasing steadily during hibernation. This pattern of serum T_4 was similar to the results reported by Nauleau, et al. (1987).

A comparison between the variations of serum T_3 and serum T_4 can provide some important information about the secretive state of the thyroid gland. Before hibernation, serum T_4 was very low, and serum T_3 was higher, being in a dominant position, but in hibernation, serum T_4 was twice as great as serum T_3, though both of them had increased. These variations were caused by the increased activity of the thyroid gland during hibernation. Turner and Bagnara (1976) suggested that the activity of the thyroid gland of ectotherms was low both in summer and during pre-hibernation. It was enhanced during hibernation, reaching a peak during post-hibernation. The thyroid gland mainly secretes T_4, which is the precursor of T_3. The secreted T_4 is converted into T_3 in the blood or in the tissues and organs. The function of stimulating metabolism by T_3 is at least three times stronger than that by T_4. The activity of the cobra thyroid gland was at a low level in pre-hibernation. The synthesized and secreted T_4 was very low and a portion of T_4 was converted into T_3 thus the concentration of serum T_4 was at a low level and the level of T_3 was relatively high. After the cobra entered hibernation, the secretive function of the thyroid gland began to become active and produce more and more T_4, therefore serum T_4 dominated at a higher level though both T_4 and T_3 increased in the blood. The variation of serum T_4 and T_3 in post G was the same as in HG. The very high levels of serum T_4 and T_3 in post G were of great advantage to the cobra enhancing its metabolic rate for arising from hibernation. Besides these, the results in Table 1 and Table 2 also indicate that the levels of serum T_4, and T_3, of HG were higher than that of Pre G, but the amount of oxygen consumption of HG was less than that of Pre G. This seemed to be self-contradictory because both T_4, and T_3, were able to stimulate metabolism. These phenomena resulted in the falling temperature, inhibiting the function of hormones. Wilhoft (1966) pointed out that the function of T_4 stimulating metabolism appeared to be inactive in many reptiles at low body temperature, and to be active only at higher temperature. Stimulation of metabolism was carried out in such a way that T_4 and T_3 were capable of inducing the synthesis of aerobic metabolic enzymes. The low levels of serum T_4 and T_3 in pre-hibernation indicated that the metabolic rate of cobras in this period tends to fall.

The metabolic enzymes which had been synthesized were still in an active state because of the higher temperature, thus the pre G cobras had a high oxygen consumption. The metabolic rate of HG, though serum T_4 and T_3 levels had risen, was at a low level due to the low temperature during hibernation which inhibited the function of T_3 (T_4) and the activity of metabolic enzymes synthesized before. The cobras of HG had a small oxygen consumption. The very high levels of serum T_4 and T_3 gradually performed the function of stimulating metabolism in the phase at the end of hibernation as the temperature was rising.

Serum cortisol was increasing continuously during hibernation. Serum cortisol of HG was 2.8 times higher than that of pre G, and that of post G which continued to rise by 3.6 times more than that of HG. Musacchia (1984) reported that the survival of animals during hibernation or hypothermia had a close relation to the level of serum cortisol in their bodies. An injection of cortisol into hamsters during hibernation or hypothermia could yield the same result as an injection of glucose, and might increase the survival of hamsters under the same conditions. He also reported that glucocorticosteroids played an important role in animals arising from hibernation or hypothermia. Some clues perhaps could be found for reptiles from the experimental results on hamsters, although they were very different in other respects.

The results in Table 2 indicated that the hepatic glycogen content of HG was very low. It was difficult for cobras to continue hibernating while only using so little an amount of hepatic glycogen as a energy source. They must receive energy metabolites in other ways. Gluconeogenesis may be an important process in which cortisol has a close relation. The surprarenal cortex of cobras during hibernation became active gradually and the level of serum cortisol became higher and higher, which was related to accelerating gluconeogenesis and to the cobra's arising from hibernation. The variation of cortisol showed that fat metabolism became active after cobras were aroused out of hibernation.

3. Relationship between the three serum hormones and energy metabolites

In pre-hibernation, the function of T_4 and T_3 in inducing synthesis of aerobic metabolic enzymes was weak because of the low levels of serum T_4 and T_3. The metabolism of cobras in this period was under the control of the enzymes which had been synthesized before. The metabolic rate of cobras tended to decrease from pre-hibernation to hibernation as the temperature was falling and as the amount of enzyme reduced (synthesizing less and degrading). During hibernation, when the temperature was low, cobras had a low metabolism under the control of the low-temperature isozymes, though the levels of serum hormones were at relatively higher. The low serum glucose and triglyceride contents implied that the decreased metabolism of cobras in hibernation, and the reduction of glycogen in the liver indicated the metabolic rate of cobras at a certain level. In post-hibernation, high temperature provided a favorable factor for hormones to perform their functions, and T_3 (T_4) induced synthesis of enzymes and cortisol accelerated the gluconeogenesis and fat catabolism. Serum glucose was increasing markedly though the hepatic glycogen was low, and quite a lot of glucose was produced from gluconeogenesis. Serum T_4 and T_3 play an important role in accelerating somatic cells to take in energy metabolites and in raising metabolism.

In short, during hibernation the cobra nearly used up all the hepatic glycogen but consumed little fat body triglyceride. Body weight tended to be lost significantly during the period after the snake was aroused out of hibernation. Perhaps fat bodies were used up because there was little hepatic glycogen. The high level of cortisol indicated the tendency for fat catabolism.

4. One putative pattern

From the above results, we consider that perhaps the cobra has formed a seasonal regulation of endocrine and that hibernation is controlled by this mechanism. When the season for hibernation comes, the contents of serum T_4, T_3, and cortisol decrease so that the metabolic system of all organs and tissues in cobras is controlled effectively. The metabolic rate of cobras tends to go down. With temperature falling and the three serum hormones reducing, the remaining metabolic enzymes in cobras become inactive gradually (content less and activity low) and cobras go into hibernation keeping their metabolic rate at a fairly low level under the control of the low temperature isozymes, and after physical regulation. This suggests that the external factor for cobra hibernation is the fall of temperature, while the internal factor is the drop of the three serum hormone levels. In hibernation, the contents of serum T_4, T_3, and cortisol increases gradually, but the levels of the three serum hormones are not high enough to accelerate metabolism of the organs and tissues, and the low temperature inhibits the activities of the three serum hormones. When the levels of serum T_4, T_3, and cortisol are high enough, the metabolism of the organs and tissues in cobras tends to go up. With the temperature rising, the three serum hormones induce the synthesis of aerobic metabolic enzymes in the organs and tissues and accelerate the metabolism of energy metabolites. The metabolic rate rises and the cobra recovers from hibernation. This suggests that the external factor for the cobra to come out of hibernation is the rise of temperature, while the internal factor is the rise of the three serum hormone levels.

5. Conclusion

Serum T_4, T_3, and cortisol at low levels were involved in the regulation of depressing the metabolism of cobras before hibernation. The low temperature during hibernation inhibited activity of the three serum hormones, and the metabolism was at a low level. With the rising temperature, the levels of serum T_4, T_3, and cortisol at very high levels accelerated the metabolic rate of cobras which resulted in arousal from hibernation. Cobras in hibernation used hepatic glycogen as their main energy source instead of fat body triglycerides.

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