Seasonal Dynamics of Serotonin in the Duodenum of Hibernating Animals

L. V. Shestopalova, M. S. Vinogradova, and E. V. Dubinin

UDC 616.342-008.94:577.175.823]-07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 118, № 11, pp. 511-513, November, 1994
Original article submitted June 27, 1994

A correlation is shown between the duodenal content of serotonin in the red-cheeked souslik and the period of hibernation. The serotonin concentration in the tissue is maximal at the beginning of the hypothermia period and minimal at the end of this period. During spontaneous awakening (winter normothermia) the necessary amount of amine is produced.

Key Words: *serotonin; duodenum; hibernation; hypothermia*

Serotonin, a biogenic amine characterized by different physiological effects in the animal organism, appears to be the most interesting of the endogenous regulators of hibernation. Serotonin is a product of secretion of cellular elements of brain structures and of enterochromaffin (EC) cells of the peripheral organs. Previous studies have revealed different effects of "central" and "peripheral" serotonin in hibernating animals. The former is conducive to a smooth transition from a somnolent state to hibernation [4,9], while the latter is involved in thermoregulation and is needed for the development and maintenance of hypothermia [2,3,10]. Hence, there are good grounds for stating that serotonin helps set the hibernation mechanisms in motion.

Ninety percent of total serotonin is produced by elements of the enterochromaffin system of the gastrointestinal tract - EC cells [12]. Previously serotonin concentrations were measured in various portions of the stomach in the periods of deep torpor, summer activity, and spring awakening of red-cheeked sousliks [3,5]. In the present study we investigated the time course of serotonin and its principal metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the duodenum under various seasonal conditions, including the hibernation cycle.

Physiology Department, Novosibirsk State University; Department of Ecological Aspects in Morphology, Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk. (Presented by Yu. P. Borodin, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Red-cheeked sousliks (*Citellus erythrogenys* Brandt) caught in the steppes of the Novosibirsk Region were used in this work. These obligate hibernators hibernate 7 to 8 months a year, and their hibernation consists of cycles including periods of torpor (12-14 days) and short-term spontaneous awakenings (20 to 28 h). The animals were examined while in the state of summer activity, during winter hibernation, and after spring awakening. The hibernation cycle was studied in detail, and material for investigation was collected on days 1, 5-6, and 12 of hypothermia and during spontaneous awakening (winter normothermia). In spring the animals were examined on days 5-6 of hypothermia (March or beginning of April) and on days 1, 4, and 14 after spring awakening from winter hibernation (end of April - May). The concentration of serotonin and its metabolite 5-HIAA was

0007-4888/94/0011-1208$12.50

measured by a previously described technique [3] based on methods presented elsewhere [8,11,14]. The measurements were carried out using a Hitachi spectrofluorometer.

A total of 175 tissue samples from 46 animals were examined. Digital data were statistically processed using the Mann-Whitney nonparametric U test.

RESULTS

The highest serotonin concentrations are observed in the duodenum of "summer" animals (Table 1). The level of serotonin is reliably lowered during the period of deep torpor (days 5-6 of hibernation). The content of the amine changes in the course of the hibernation cycle. Its maximal value was observed at the beginning of the cycle on the first day of torpor after spontaneous awakening. The serotonin level drops as hypothermia progresses, and by day 12 of torpor it is reliably lower than on days 5-6 of hibernation. Spontaneous awakening is marked by an increase of the amine level, and when a new hibernation cycle begins, the serotonin concentration in the tissue is reliably higher than at the end of the period of hypothermia.

A very different picture is observed in spring. A reliable increase of amine concentration in the tissue was observed on days 5-6 of hibernation in April, compared to a similar period in the winter months. The serotonin level continues to rise after the sousliks awaken in spring, reaching 17.78 μg/g by day 14 of activity, which is close to its summer values.

The changes in the level of the main serotonin metabolite, 5-HIAA, are of a different type. Its maximal concentration is observed in the middle of torpor, and it drops perceptibly during spontaneous awakening. By the beginning of a new cycle of torpor the metabolite content is reduced in comparison with all periods of winter hypothermia.

The content of 5-HIAA is reduced in sleeping "April" animals, reliably differing from that in deep winter torpor. A certain tendency for this metabolite level to rise is observed on day 4 of activity in animals awakening from hibernation, but by day 14 its level drops. In "summer" active animals the levels of 5-HIAA are minimal.

A study of serotonin metabolism in the small intestine of hibernating animals over a year revealed the following regularity: the content of serotonin and its metabolite changes in accordance with the function of serotonin-producing cells (EC). Previously we presented the data of an electron-microscopic study of duodenal EC cells in hibernating animals and of morphometric analysis of their organelles in different seasons [7]. In the "summer" cells the synthetic processes and secretion accumulation in the form of basally disposed electron-dense polymorphic secretory granules predominate. In summer the "utilization" index (ratio of 5-HIAA to serotonin levels) of the duodenum is only 0.21 (Fig. 1). This may be because serotonin is being stored up in order to ensure its participation in the hypothermic reactions of the organism in the period of lowered body temperature. In "winter" animals in a state of torpor, active disintegration of secretory granules is observed, and the amine is not produced by the cells. This iindicates that it is mobilized from the cells. Red-cheeked sousliks do not eat during the entire winter season. Hence, the amine cannot be involved in regulating digestion in winter, because the gastrointestinal tract is completely shut down. The withdrawal of EC cell secretion against the background of a slowed course of all physiological processes indicates the contribution of serotonin to maintenance of the normal course of hibernation.

A study of the time course of serotonin concentrations in winter helps trace a clear-cut relationship between its levels in the intestine and the functional status of the animal. Comparison of morphological and biochemical data showed that spontaneously awakening animals produce serotonin

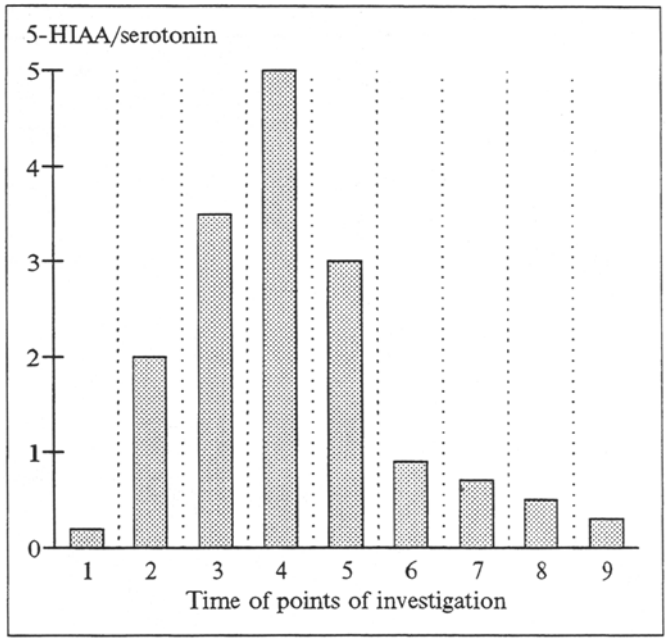

Fig. 1. Seasonal changes in the utilization index (5-HIAA/serotonin) in the duodenum of red-cheeked sousliks. *1*) summer activity; *2*) day 1 of torpor; *3*) days 5-6 of torpor; *4*) day 12 of torpor; *5*) spontaneous awakening; *6*) days 5-6 of spring torpor; *7*) day 1 after spring awakening; *8*) day 4 after spring awakening; *9*) day 14 after spring awakening.

TABLE 1. Changes in the Content of Serotonin and Its Metabolite in the Duodenal Tissue

Status of animals	Concentration, μg/g	
	serotonin	5-HIAA
Summer activity, 37°C	22.17/19.21–24.06/(3)	4.68/3.10–6.64/(3)
Day 1 of winter hibernation, 3–5°C	6.10/4.73–10.62/(5)	12.86/7.97–17.83/(5)
Days 5–6 of hibernation, 3–5°C	5.58/3.9–7.35/(5)	21.25/13.97–26.65/(5)
Day 12 of hibernation, 3–5°C	3.08/2.26–4.04/(5)	15.86/7.30–32.74/(5)
Spontaneous awakening, 35–36°C	4.14/2.80–5.66/(5)	13.02/5.85–22.83/(5)
Spring hibernation, days 5–6, 5–6°C	9.27/7.01–10.96/(5)	8.42/7.46–10.96/(5)
Spring awakening: day 1, 37°C	9.19/6.58–12.28/(5)	8.21/6.93–10.63/(4)
Day 4, 37°C	13.54/9.46–16.27/(5)	10.08/9.63–10.46/(5)
Day 14, 37°C	17.78/12.93–21.98/(8)	7.76/5.29–9.05/(7)

Note. The number of animals examined is given in parentheses.

in the required amounts, this raising its level in the intestinal tissue during winter normothermia and during the first day of the new hibernation cycle. Along with other endogenous factors, a reduction of the amine concentration by the end of torpor evidently alters the body temperature and activates many physiological processes, as a result of which the animal awakens. Metabolites are withdrawn during the periodic awakenings and the reserve of bioactive substances needed for the normal course of hibernation is replenished. The accumulation of 5-HIAA resulting from the depressed function of its withdrawal occurring in sousliks [6] probably contributes to the switch of the "torpor-activity" phases and stimulates awakening.

The serotonin "utilization" index is demonstrative for this period (Fig. 1). It is high in the tissues of a spontaneously awakened animal (3.14) and continues to rise in the course of the period of torpor, its value by day 12 of hypothermia being as high as 5.15. This is indicative of continuous and active catabolism of serotonin during torpor, thus proving that this biogenic amine performs an important function in winter by maintaining hypothermia.

The rise of the serotonin level in animals after spring awakening from winter hibernation may be due to regeneration of the epithelial lining and the resumption of digestion in the gastrointestinal tract. In parallel with stabilization of the duodenual function, a gradual deposition of an appreciable portion of the synthesized serotonin for the next hibernation season occurs in EC cells. Serotonin utilized in summer goes for the requirements of the intensively working gastrointestinal tract and for maintenance of the necessary basal level of the amine which influences cell proliferation and the development of behavioral, cardiovascular, and other physiological reactions in the summer period [1,4,13].

Hence, the data on the seasonal dynamics of serotonin levels indicate an important role of this duodenal amine in the development and maintenance of hypothermia in hibernating animals.

The authors are grateful to Dr. A. L. Markel' for giving us the opportunity to work with the Hitachi spectrofluorometer.

REFERENCES

1. E. A. Gromova, *Serotonin and Its Role in the Body* [in Russian], Moscow (1966).
2. D. A. Ignat'ev, G. S. Sukhova, and V. P. Sukhov, *Zh. Evol. Biokhim. Fiziol.*, **27**, № 2, 206-210 (1991).
3. N. N. Kudryavtseva, *On the Contribution of Serotonin to the Mechanisms of Hibernation* [in Russian], Author's synopsis of Thesis submitted for the degree of Candidate of Biological Sciences, Novosibirsk (1976).
4. N. K. Popova, E. V. Naumenko, and V. G. Kolpakov, *Serotonin and Behavior* [in Russian], Novosibirsk (1978).
5. K. V. Svechnikov, *The Peripheral Serotonin System in Some Physiological States and Stress* [in Russian], Author's Synopsis of Thesis submitted for the degree of Candidate of Medical Sciences, Novosibirsk (1988).
6. P. W. Hochachka and G. N. Somero, *Strategies of Biochemical Adaptation*, Saunders College Publishing (1973).
7. L. V. Shestopalova, M. S. Vinogradova, O. A. Ponomareva, and E. V. Dubinin, *Byull. Eksp. Biol. Med.*, **115**, № 2, 119-122 (1993).
8. G. Curson and A. R. Green, *Brit. J. Pharmacol.*, **39**, № 3, 653-655 (1970).
9. D. D. Feist and W. A. Galster, *Comp. Biochem. Physiol.*, № 48a, 623-662 (1974).
10. L. Jansky, *Physiol. Res.*, **40**, № 2, 155-159 (1991).
11. R. P. Maikel, R. H. Cox, J. Saillant, and F. P. Miller, *Int. J. Neuropharmacol.*, **7**, 275-281 (1968).
12. R. H. Resnick and S. J. Gray, *Gastroenterology*, **41**, 119-121 (1961).
13. T. Tutton, *Virchows Arch. [B]*, **16**, 79-87 (1974).
14. S. M. Schlumpf, W. Lichtensteiger, H. Langemann, *et al.*, *Biochem. Pharmacol.*, **23**, 2337-2446 (1974).