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BIOPHYSICS OF COMPLEX SYSTEMS

The Effect of Insulin on the Heart Rate and Temperature of the Ground Squirrel *Spermofilus undulatus* during Arousal from Hibernation

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Abstract—The effect of insulin on the heart rate and body temperature, measured per rectum, of ground squirrels (*Spermophilus undulatus*) during triggered arousal from winter hibernation was studied. We found that the outcomes of insulin injection to hibernating ground squirrels varied in the course of arousal. During the first stage, while body temperatures were less than 10°C, the heart rates and rectal temperatures in both control and insulin-treated groups changed in the same manner. During the next stage of arousal, when the body temperature rose above 12°C, elevation of the heart rate and rectal temperature in the insulin-treated animals was significantly retarded and lasted 110 min compared to 80 min in the control group. Conversely, in the final stage of arousal at body temperatures above 20°C, the heart rate and body temperature increased more rapidly in the insulin-treated animals that reached normal body temperature within 40 min compared to 60 min in the control group. Suggested mechanisms of bidirectional effects of insulin on the heart rates and body temperatures in ground squirrels at the particular stages of arousal, with regard to the progression of endogenous insulin and glucose levels in the blood serum, are discussed.

Keywords: insulin, hibernation, ground squirrels, arousal
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INTRODUCTION

The possibility to control the level of metabolism in human and animal tissues to reduce the effects of ischemic injury in cells and organs has promise in clinical use, particularly in the treatment of stroke and heart attack [1, 2]. The particular attention of researchers is attracted by the mechanisms of natural hypobiosis, that is, mammalian hibernation, which allows a number of animal species to withstand the cold and lack of nutrition for many months due to stored subcutaneous fat and reduction in the metabolic rate to 1–5% of the initial level. Tissue cells of hibernators are adapted to low temperatures (0°C), retarded blood flow, and a sharp drop in oxygen and glucose [3–6]. Periods of hibernation itself (bouts) are periodically interrupted by short arousals that are vital to animals, in particular, for renewal and maintaining the conductivity of nerve cells [7, 8] and/or the restoration of energy substrates such as glucose and fatty acids, whose synthesis is not possible at low temperatures [9]. As an example, the Yakutian ground squirrel *S. undulatus* in the hibernation season awakes every 1–2 weeks of hibernation for a period of up to 12–14 h with an increase in the body temperature, as measured rectally (T_r), to ~36°C [10–16]. The duration of arousals for ground squirrels at the ambient temperature of approximately 1°C is 6 or 7 h. During experiments at room temperature (18–20°C) ground squirrels awake after 2.5–3 h (animals hibernate at 1–3°C in a dark room prior to the experiments). In the state of quiet wakefulness the heart rate (HR) of the ground squirrel is 130–160 bpm. During awakening from torpor HR increases from 3–4 bpm in the state of hibernation to 350–400 bpm when achieving the zone of increased thermogenesis. Thus, hibernating mammals have significant potential to prevent reperfusion injury when awakening from hibernation with no signs of functional disorders of brain or myocardium cells [7, 10, 13, 17, 18]. The results of the study of natural mechanisms that underlie the control of arousal processes in animals may be useful in the development of adequate methods that allow one to

Abbreviations: T_r, body temperature measured rectally; HR, heart rate; T_h, temperature in the heart area.

improve the level of brain- or heart-cell resistance of common animals (and humans) to ischemia/reperfusion under certain conditions.

Hibernating species are observed in all orders of mammals. It is believed that all mammals have a genetic predisposition to the torpor state [3, 19, 20]. Common physiological systems of the body, which function both in hibernating and nonhibernating warm-blooded animals, serve as the basis for the adaptation of hibernators to low temperatures [21]. It is also believed that winter hibernation is a manifestation of phenotypic adaptation: hibernating mammals do not differ from nonhibernating ones in their set of genes [22].

Hibernating animals are a convenient model to determine how to cause the state of controlled hypobiosis based on natural physiological mechanisms [4, 17, 23–25]. A number of possible practical applications of hypothermia in clinical have been noted [26]. At the same time it has been noted that there are serious limitations on the use of pharmacological agents associated with a sharp increase in the risk of cardiac arrest during deep hypothermia for common warm-blooded animals and humans. The search for compounds, preferably of a natural origin, that can effectively influence the depth of the torpor state in hibernating animals may significantly accelerate the development of new methods for the clinical treatment for some diseases [2, 26].

The results of our previous studies [27, 28], as well as the literature data [29] suggest that insulin may play a significant role in the mechanisms of hibernator arousal from hibernation. The insulin content in the blood plasma of hibernating animals is significantly increased during the accumulation of subcutaneous fat by animals in the autumn prior to hibernation, and decreases in the hibernation season from December to January; at the same time the sensitivity of tissues to exogenous insulin decreases with significant suppression of its secretion [30]. An increased concentration of insulin in β-cells of the pancreas is also observed during hibernation, which contributes to a rapid increase in the content of this hormone in blood plasma during periodic arousals [31]. It has been shown that the insulin level in the blood of animals in winter during short arousals is much higher than its content in active animals in summer; the insulin sensitivity is also restored [28, 32–34]. The reversibility of this type of dysregulation is a sign of the presence of specific adaptive mechanisms in hibernating animals that control significant changes in the level of metabolic states, which may be based on the inherent ability of these animals to actively adjust the endocrine system and, consequently, lipid and glucose homeostasis [29, 35, 36].

In the present study we investigated the effect of insulin on the increase in body temperature, measured rectally, and HR of ground squirrels during arousal from hibernation. It was shown that insulin injection to ground squirrels to provoke arousal generally slows the awakening of animals from the state of torpor, while the nature and direction of the hormone effects depend on the stage of awakening and temperature of the animals.

MATERIALS AND METHODS

The experiments were carried out in Yakut isolates of long-tailed ground squirrel *Spermorhilus undulatus* of both sexes with weights of 500–800 g in accordance with the requirements of the European Convention for the Protection of Animals (1986, 86/609/EEC). Ground squirrels were caught in the summer in the neighborhoods of Yakutsk and kept under vivarium conditions in individual cages with natural light and free access to food and water. Before the hibernation season the cages with ground squirrels were transferred to a dark room at a temperature of 1–3°C. The study was carried out in the middle of the hibernation season from late December to early February.

Arousal was provoked on the fourth day of hibernation; prior to the experiment ground squirrels were transferred to a bright room with the temperature of 18–20°C. Previously, using telemetry we showed that awakening of ground squirrels from torpor via provocation of arousal occurs in a similar way to the natural arousal of animals, but slightly faster [13, 14, 37].

Removal of a ground squirrel from the nest and the experiment preparation took 5–7 min, after which the animals were injected intraperitoneally with insulin at the dose of 3 IU in 0.7 mL of saline solution. The same volume of saline solution was administered to control ground squirrels.

The body temperature of the ground squirrels was measured with an accuracy of ±0.1°C by a RST 1Q307 rectal temperature sensor (Switzerland), which was introduced into the colon to a depth of 6 cm. The cardiogram of the animals was recorded by an EEGP 4-02 electroencephalograph; recording electrodes were placed subcutaneously on the left front leg and above the shoulder bone of the animal. The measurement of HR and T_p was performed every 4 min after the beginning of the awakening. Some animals were decapitated at different stages of the awakening to compare the temperature in the heart area (T_h) with rectal T_r.

At the beginning of our study it was important to choose of the insulin dose correctly. The standard dose used in common animals is 0.3–0.8 IU per kg body weight at the temperature of approximately 37°C. The ground squirrel in a state of deep hibernation decreases T_p to 2–3°C, while the respiratory rhythm, heart rate, and metabolic level decreases by hundreds of times. In the pilot experiments, we used doses at the concentration range from 0.12 to 12 IU. The dose of 3 IU per animal with a weight of 500–600 g (5 IU per 1 kg of body weight) showed the most

significant result and was chosen as the main dose for the study. The concentration of 3 IU is higher than the concentration accepted as the standard for common animals by an order of magnitude, but one should consider that the physiological and metabolic processes in the body of a hibernating ground squirrel may be two orders of magnitude lower compared with those of common laboratory animals. When we used insulin doses from 0.12 to 12 units in ground squirrels we never observed seizures, which would indicate an overdose. The dissolving of crystalline insulin was carried out in an acidified medium (0.003 M HCl, pH 2.7–3.0) until its complete dissolution.

The samples of the blood serum were collected from ground squirrels in the hibernating state ($T_h = 3–5$°C) during awakening at a T_h of approximately 20–22°C and 28–32°C, which corresponds to T_r of 10–12°C and 18–20°C, respectively [14], and in the active state between bouts of hibernation. The whole blood was centrifuged at 3500 *g* for 10 min. The resulting blood serum was stored at –20°C. The insulin content in the blood serum of the ground squirrel was determined by immunoradiometric method with an Insulin(E)Irma test kit (Immunotesh Insulin kit, catalog no. 3210, Immunotesh, Czech Republic) using two kinds of mouse monoclonal antibodies against various epitopes of its molecule.

The measurement of the glucose level in the blood of the ground squirrel was performed using a Accu-Chek Go glucometer (Roshe, Germany). The experiments on the insulin and glucose levels in the blood serum of the animals were performed in the fasting state.

The Origin 5.0 software package (Microcal Origin, United States) was used for the statistical analysis of the data.

RESULTS

The removal of ground squirrels from the nest and administration of insulin or saline solution initiated the arousal of the animals. Figure 1 shows the curves of the increase in the heart rate of the ground squirrels when awakening from the hibernating state according to the groups (injected with insulin and control animals).

At the beginning of the awakening the HR of ground squirrels was at a minimum and equal to 5–6 bpm; 40 min after the beginning of the awakening HR increased to 30–40 bpm and almost did not differ between the groups of animals. Subsequently, the growth of heartbeats in the groups began to differ significantly: by the 80th min of the awakening at T_r = 10–12°C the values of HS were on average 130 bpm in the control animals, while in the ground squirrels with the insulin injection similar values were achieved only by the 110th min of the awakening. At the 120th min of the awakening the HR increased to 350 bpm in the control ground squirrels, whereas the animals with the insulin injection achieved this level only by the 150 min from the beginning of the awakening.

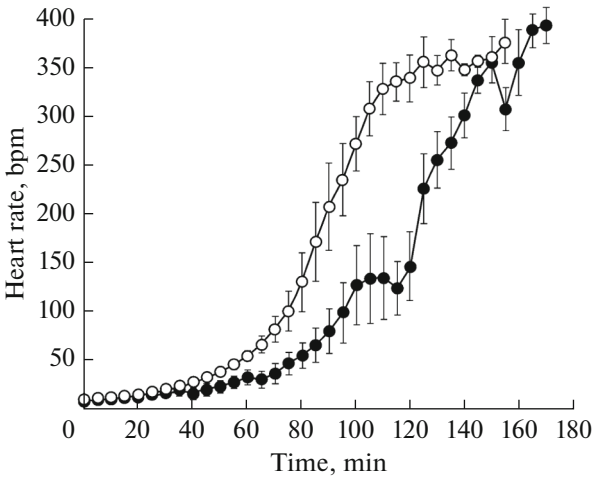

Fig. 1. The effect of insulin on the HR of ground squirrels during awakening from the state of hibernation. Bright circles, control animals; dark circles, animals injected with insulin. Curves are averaged in groups of five ground squirrels; confidence intervals for the 5% significance level are shown.

Figure 2 shows the changes in the rectal temperature during the awakening from hibernation.

At the initial stage of the awakening the growth rate for T_r was similar in the groups of animals: approximately 0.1°C/min; by the 80th min T_r reached the value of 8–9°C in both groups. As in the case of HR, further growth dynamics for the rectal temperature began to differ significantly between the groups of animals. The control ground squirrels increased the rate of the T_r growth to 0.3°C/min and by the 140th min the temperature of the animals rose to 28–30°C; further the T_r growth rate slightly slowed and by the moment of the wakefulness of the animals at the 160th min their temperature reached approximately 34°C. In ground squirrels with the insulin injection after 80 min of awakening the T_r growth rate did not increase and by the 140th min it was only 14°C on average. However, the T_r growth rate then rapidly increased to 0.4°C/min (higher than in the control) and as a result it reached ~34°C by the 180th min.

The inset (Fig. 2) shows the excess of the temperature of the ground squirrels in the heart area (T_h) over the rectal temperature (T_r) during the process of the awakening from hibernation. It can be seen that the growth of T_h is faster and at the phase of increased thermogenesis it exceeds the values of T_r by 10–12°C. The differences of the temperatures are gradually leveled by the moment of the wakefulness of the animals.

The table shows the results of the measurement of insulin and glucose levels in the blood serum of the

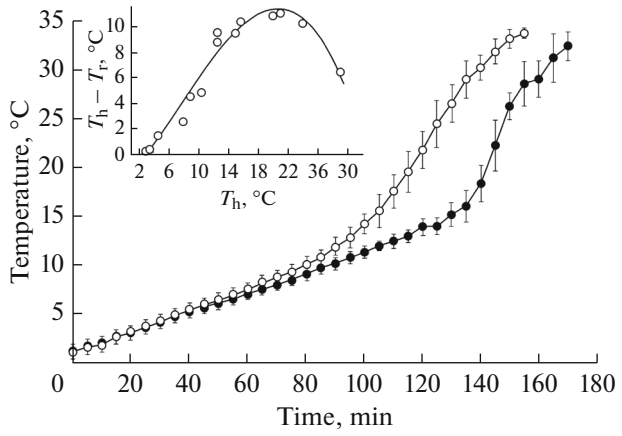

Fig. 2. The effect of insulin on the temperature of ground squirrels during awakening from the state of hibernation. Bright circles, controls; dark circles, ground squirrels injected with insulin. The curves are averaged in groups of five ground squirrels, confidence intervals for 5% significance level are shown. Changes in the ratio of the values of the rectal temperature and the temperature in the heart area during awakening of ground squirrels are given in the inset.

ground squirrels in the following physiological states: (a) in deep hibernation at a body temperature of 3–5°C; (b) during awakening at T_r = 10–12°C (which corresponds to T_h = 18–20°C); (c) at T_r > 13–15°C (which corresponds to T_h = 29–34°C) (d) in the winter active state between the bouts of hibernation; (e) in the summer active period for comparison.

It can be seen from the data shown in the table that the content of glucose and insulin in the blood serum of ground squirrels varied depending on the physiological state of the animals. The lowest concentrations of insulin and glucose were noted in the hibernating ground squirrels: 11.68 ± 1.77 and 5.6 ± 0.7 mmol/L, respectively. During the process of the awakening when the heart temperature of ground squirrels reached approximately 20°C (T_r = 10–12°C) the concentration of endogenous insulin was approximately two times higher than that in the hibernating animals; at the same time, as can be seen from the table, the glucose content did not differ from that in the state of hibernation. At the phase of increased thermogenesis, when T_h reached 29–34°C (T_r = 13–15°C), the level of endogenous insulin was increased almost three times compared with its level during hibernation (34.47 ± 3.72/11.68 ± 1.77); at the same time the glucose content was also increased by almost two times compared with the level typical for hibernating, as well as active summer, animals. After the ground squirrels awakened a slightly elevated level of insulin in the blood serum was noted, whereas the glucose content was reduced to a level comparable to that in the state of hibernation.

DISCUSSION

As our studies showed, insulin is capable of influencing the character of the awakening of ground squirrels from the hibernation state and has both inhibitory and activating properties depending on the stage of the awakening and body temperature of an animals.

At the initial low temperature stage of the awakening, when the temperature of the ground squirrels did not exceed 8–9°C, there was no significant effect of the insulin injection compared with the control group of animals. At the next stage of the awakening, which progressed in the zone of increased thermogenesis with a rapid growth of HR and T_r for the control ground squirrels, in the group of animals injected with insulin the growth of these parameters was retarded for approximately 80 min compared with the control (from the 40th to 120th min; Figs. 1 and 2). However, at the final stage of the awakening a rapid increase in HR and T_r that exceeded the growth of these parameters in the control ground squirrels in the zone of increased thermogenesis was observed in the animals injected with insulin.

It is known that in the beginning of the awakening of ground squirrels from hibernation the major thermogenic organ is brown adipose tissue located in close proximity to vital organs: heart, lungs, spinal cord, and blood vessels to the brain. The influx of warm blood to the tissues of the periphery is limited by vasoconstriction in this period [16, 38, 39]. With the warming of the animals the general blood supply to the cold periphery tissues and warmer organs, especially the heart, is restored, which temporarily reduces the increase of the temperature and HR [15]. This relatively short but characteristic period insignificantly affects the statistical curve of the HR and T_r values in the control group; however, it is clearly manifested in the ground squirrels injected with insulin; the increase in the heart rate stops and even falls during the time interval from the 100th to the 120th min of the awakening (Fig. 1), which on average slows down the duration of the initial awakening period for 80 min (from the 40th to the 120th min). These animals undergo the following stage of the awakening, the increased thermogenesis zone, even more actively compared with the control.

Thus, the effect of insulin on the arousal of ground squirrels has a pronounced phase character. The inhibitory effect begins to occur significantly at T_r higher than 10–12°C; the most pronounced effect of insulin is noted at the HR values above 130–140 bpm, when the increase in the heart rate stops. The activating effect of insulin is observed when T_r exceeds 18–20°C (130–160 min).

The character of insulin effects, depending on the stage of the awakening and temperature of the animal, is apparently determined by the adaptation of the body of the hibernator to optimal arousal from the hiberna-

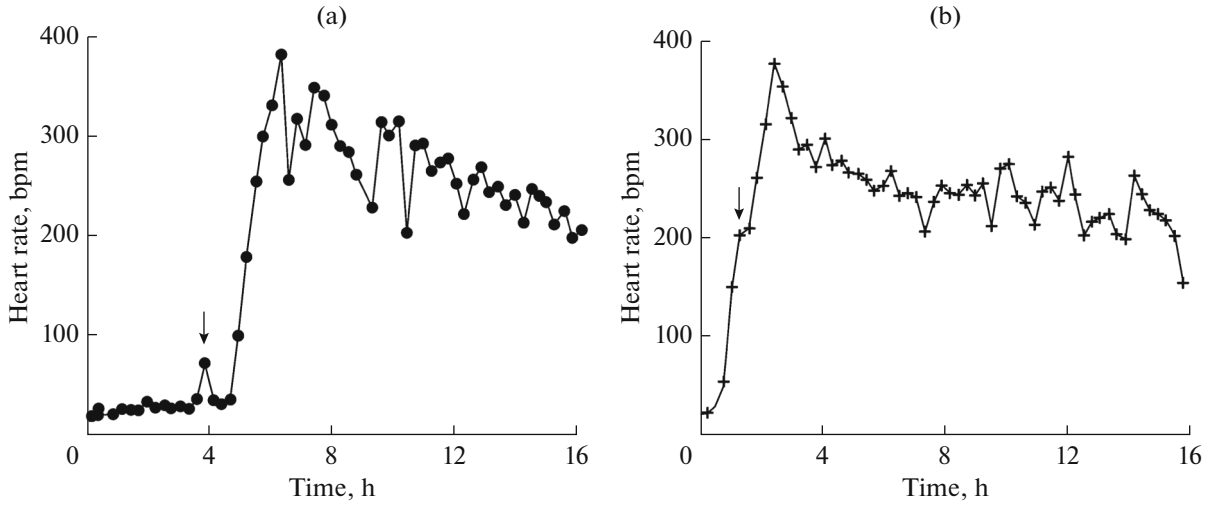

Fig. 3. Examples of changes in the heart rate of the ground squirrel obtained by telemetry during natural (a) and triggered (b) arousal from the state of hibernation. Time (h) is along the abscissa; HR (bpm) is along the ordinate. The characteristic temporary slowdown of HR growth is indicated by the arrow.

tion state. Brown adipose tissue located in close proximity to vital organs is a type of "spark plug" for a number of energy substrates in hibernating animals at the beginning of awakening [38, 39]. It is known that insulin, in addition to its basic functions, is capable of performing the role of an "energy regulator" of brown adipose tissue, significantly saving its reserves for the optimal use during the awakening of an animal [40, 41].

Considering the importance of this issue for our further analysis, we determined the features of changes in the heart rate of the ground squirrel during natural (spontaneous) and triggered arousal from hibernation. These data were obtained previously by telemetry. In the natural habitat arousal is provoked, for example, when rats or mice, which are very dangerous to sleeping animals, penetrate into the nest of the ground squirrel. In experimental practice it is sometimes necessary to provoke the awakening of the ground squirrel during a certain period of the bout of hibernation.

Figure 3a shows the curve of the HR changes in one of the ground squirrels during natural arousal from hibernation.

At the initial stage of the awakening the characteristic temporary slowdown of the HR growth associated with the moment of the influx to the organs of the cooled peripheral blood (indicated by an arrow) is clearly seen. At the following stage of the awakening (in the phase of increased thermogenesis) the heart rate increases rapidly (up to 400 bpm). After the final awakening of the ground squirrel the HR is gradually reduced to the level of quiet wakefulness.

A similar curve was obtained in the same ground squirrel when arousal was provoked during the next bout of hibernation (Fig. 3b). The main difference lies in the initial stage of the awakening, which is faster during the provocation of arousal and has a less pronounced short-term slowdown that occurs when the HR is approximately 200 bpm.

In our experiments (Figs. 1, 2) the procedure of the removal of the ground squirrel from the nest and the injection of insulin or saline solution almost provoked the awakening of the animals. In the control group the awakening from hibernation took place in a similar manner to that observed during the provocation of arousal by telemetry (Figs. 1, 3b). In ground squirrels injected with insulin the increase of the HR and the awakening from the state of hibernation were slowed for tens of minutes. Comparing this result (Fig. 1) with the data on natural arousal of the ground squirrel (Fig. 3a), whose tissues also contain a certain amount of endogenous insulin, one can note the following: a) there is a certain similarity in the character of the HR slowdown at the low temperature stage of awakening in the natural and triggered arousal of the ground squirrel from the state of hibernation; b) moderate additional injection of exogenous insulin to the ground squirrels significantly prolongs the awakening of the animals from the hibernation state, while the procedure of the injection itself provoked acceleration of the awakening; c) the effect of insulin on the heart rate of the ground squirrels has a pronounced phase character, depending on the temperature of the animals: the initial low temperature stage of the awakening is characterized by a temporary slowdown of the HR growth; the values of HR grow rapidly with increasing temperature of the ground squirrels over 18–20°C, exceeding the HR of the control animals in the phase of increased thermogenesis.

A temporary slowdown of the HR and T_r growth of the ground squirrels at the beginning of the awakening

is directly associated with the following factors that were mentioned above: first, is the ability of insulin to suppress a rapid increase in temperature by brown adipose tissue [40, 41]; secondly, the factor is directly linked to this moment: the influx of cold peripheral blood to the organs, which is limited by vasoconstriction prior to this period [38, 39]. The association of these factors has an adaptive character, which provides the optimum arousal of animals from the hibernation state with the lowest energy consumption.

One of the main functions of insulin is glucose transport from the blood into the cells where it is converted into energy. The results of our research on the administration of exogenous insulin during provocation of arousal in ground squirrels are comparable with those obtained during the measurement of endogenous insulin and glucose content in the blood of the ground squirrels in the period of the awakening from hibernation. As can be seen from the table, when the temperature in the area of the heart reached approximately 20°C the concentration of endogenous insulin was approximately two times higher than its level in hibernating animals; at the same time the glucose content still practically did not differ from that in the hibernation state. The glucose content increased rapidly only in the zone of increased thermogenesis and at the heart temperature of 29–34°C it was two times higher compared with its concentration in hibernating animals. As it can be seen from table, the most intense growth of insulin level occurs at the final stage of the awakening: when the heart temperature reaches 29–34°C the hormone content is almost three times higher than its level in the state of hibernation. A few hours after awakening, when the ground squirrels again fall into the sleepy state while preparing for the next bout of hibernation, the content of insulin and glucose levels in the blood of animals began to decrease and was comparable to that observed in the state of deep hibernation (see table), although the temperature of the animals was still at a quite high level (~34°C).

According to the literature the predominant source of energy for the brain and heart of awakening animals is not glucose, but ketone bodies, which are fatty-acid derivatives [5]. The stores of glucose that accumulated due to catabolism of ketones are intensively consumed at the final stage of the wakening, when insulin also begins to have a pronounced activating effect on myocardial contractile function.

In animals that are in a state of hibernation, blood glucose is not regulated by exogenously administered insulin, while glucagon causes a significant increase in its concentration [36]. According to our data the serum glucose level of hibernating ground squirrels is at a minimum and corresponds to the state of starvation. At the same time at the final stages of the awakening when the heart temperature rises to 29–34°C it increases by almost two times, indicating the determining role of carbohydrate substrates during the warming of animals.

Our data are consistent with the results of studies carried out in other hibernator species: hedgehog and dormouse [31–33]. In particular, it was shown that the level of insulin in the blood of hibernating hedgehogs is very low, but increases significantly during spontaneous awakening. [32] The plasma insulin level of the garden dormouse began to grow at the body temperature of animals above 17°C and reached the maximum value at 26°C [36].

The respiratory quotient of the awakening ground squirrels at the initial stage of warming is approximately 0.78, indicating a mixed lipid-carbohydrate type of energy substrates [42]. The respiratory quotient at the final stage of the awakening is equal to 1; thus, carbohydrates are the predominant energy substrate during this period.

The oxidative activity of brown fat is approximately 2.5 times higher than that of the myocardium and 5.5 times higher than that of skeletal muscle [38], while at the following stage of warming a greater role is played by skeletal muscles as the system with the largest level of heat production in the body. According to assessments made in [38], in common warm-blooded animals with a weight of approximately 0.5 kg muscles account for approximately 60% of the consumed oxygen. In hibernators this assessment is probably higher due to specific adaptive mechanisms that accelerate the warming of the animals. The awakening of ground squirrels in this period is accompanied by severe muscle tremors; at the same time reversible changes occur in the composition of contractile proteins of ground squirrel muscles, leading to contractions with a lower efficiency, but increased heat release [43].

According to our data, the administration of insulin slows the awakening of ground squirrels, which is associated with the stabilization of the glucose content at a reduced level. It can be assumed that insulin induced hypoglycemia until a certain moment limits the availability of glucose to the tissues, which is apparently associated with the special role of glucose in the awakening processes [5].

At the final stages of the awakening additionally administrated insulin may contribute to glucose utilization, which is indicated by an increase in the growth rate of the heart rate at a heart temperature of more than 25°C, which corresponds to the maximum value of the respiratory quotient. The data obtained previously on the isolated papillary muscle of the ground squirrel heart also support this idea. When the papillary muscle was isolated at various stages of the awakening, insulin had virtually no effect on the contractile activity of the muscle during the awakening from the state of hibernation and it began to cause a significant inotropic effect only at the final stage of the awakening in the heart temperature range of 29–35°C [28].

The contents of insulin and glucose in the blood serum of the ground squirrel *S. undulatus* at different stages of awakening from hibernation, as well as in the active summer period

Ground squirrels	Insulin (μIU/L)	Glucose (mM/L)
Winter hibernating (January–February)	11.68 ± 1.77 (n = 8)	5.57 ± 0.66 (n = 10)
Awakening (T_h = 20°C; February–March)	21.41 ± 1.20 (n = 2)	5.42 ± 1.50 (n = 6)
Awakening (T_h = 29–34°C; February–March)	34.47 ± 3.72$^{\$}$ (n = 7)	10.47* ± 1.36 (n = 6)
Winter active (between bouts of hibernation, January–February)	16.03 ± 2.45* (n = 6)	6.07 ± 0.55 (n = 6)
Summer active (late June–July)	18.20 ± 2.27* (n = 8)	8.28 ± 0.35* (n = 12)

* $P < 0.05$; $^{\$}$ $P < 0.002$ (compared with hibernating ground squirrels).

Our studies show the high plasticity of the endocrine system of hibernators during the regulation of glucose homeostasis, which is part of the adaptive program of the body for optimum transition of animals to a new level of metabolism.

The sharp rise in the blood insulin level (by almost three times) seemingly could cause a decrease in the blood glucose level, but in reality its significant increase is observed (see table), which may be due to active immobilization of glucose from various depots. It has long been established that the glucose reserves are stored mainly in the liver and in other tissues of hibernators in the form of glycogen, which are preserved during hibernation and actively mobilized to the blood plasma of animals during arousal [44]. It is believed that at this time glucose is especially necessary for the functioning of the central nervous system. At the same time at the most energy intensive stage of the awakening a high level of glucose, which apparently is preserved for the final stage of the awakening, is noted. This can be explained by the existence of some other energy sources that operate in the zone of increased thermogenesis that do not require significant consumption of glucose. As was shown in [5], the main energy substrate for the brain and heart of hibernating animals during the arousal from hibernation is not glucose, but ketone bodies, which are fatty-acid derivatives. Preferential utilization of fat reserves in the hibernation period leads to an increase of ketone bodies in the blood plasma, which not only reduces glucose uptake in the muscle and heart, but also provides an energy supplement to various organs due to subsequent oxidation of ketone bodies [45]. All these changes may contribute to the preservation of glucose during hibernation.

Thus, the results of a study on intact ground squirrels given in this paper show that in addition to the previously noted activating property of insulin at the final stage of awakening, this hormone is capable of providing an inhibitory effect at the initial low temperature stage of the awakening.

The activating effect of insulin before the awakening of the ground squirrels is probably mediated by the mechanisms of its receptor action on glucose transport. The analysis shows that the inhibitory effect of insulin on the initial low temperature stage of the awakening is associated with the ability of this hormone to play the role of an energy regulator of brown adipose tissue, whose main consumption occurs at the final stage of the awakening, in addition to its other functions. The optimal use of brown fat is vital for hibernating animals, considering that arousals of ground squirrels have a multiple periodic nature during the hibernation season. The reserve of brown adipose tissue is also vital for the final spring awakening of ground squirrels, when food is not available along with a sharp increase in the metabolic level: increased hormonal activity is associated with the reproduction of animals at a negative daily average ambient temperature.

As our studies show, the final stage of the awakening occurs during a simultaneous increase in the level of endogenous insulin and glucose concentration in the blood; the administration of an additional moderate dose of exogenous insulin increases the rate of the awakening from the hibernation state in this period, which was retarded at the initial stage of the awakening. This emphasizes the important issue that the availability of glucose is a limiting factor for the process of the ground squirrel awakening.

The preliminary results of this study were presented at the International Symposium on Biological Motility (Pushchino, 2016) [46].

The paper is dedicated to the blessed memory of S.G. Kolaeva, the initiator of the studies on hibernation problems at the Institute of Cell Biophysics of the Russian Academy of Sciences.

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