Changes of characteristics of preoptic neurons and NA metabolism in hypothalamus of ground squirrel (*Citelleus Dautieus*) in different seasons and hibernating phases*

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Abstract The unit firing activities of neurons in the preoptic area (POA) of ground squirrel hypothalamic tissue slices were recorded and the metabolism of NA in hypothalamus was measured with high performance liquid chromatography (HPLC). Thermosensitivity, proportions, the critical temperature (Tc) and the lowest temperature (TL) of firing activity of the above-mentioned neurons, and NA metabolism in hypothalamus were compared in different seasons and hibernating phases. In comparison with that in summer euthermar, it was shown that (i) the percentage and thermosensitivity of the POA neurons varied respectively in the hibernating phases; (ii) TL and Tc of the POA neurons in winter, both euthermar and hibernation, were markedly decreased; (iii) the POA neurons in hibernation became much more sensitive to NA, and the response of cold-sensitive neurons to NA changed from inhibiting pattern in summer to exciting one in hibernation; (iv) the contents and metabolism of NA in hypothalamus decreased significantly in the entering phase and deep hibernation phase, while the metabolism of NA increased remarkably in the arousal phase. These changes might explain the regulatory mechanism how ground squirrel actively decreases body temperature (Tb) in entering into hibernation and quickly recovers body temperature in arousal phase.

Keywords: **hibernation, hypothalamus, thermosensitive neuron, noradrenaline, cold tolerance.**

From late fall or early winter on, the hibernator begins several cycles (hibernation bouts), each of which consists of hibernation entrance, deep hibernation and transient arousal until its waking in spring. The body temperature change of the hibernator during hibernation has two major features: in the deep hibernation state, its body temperature reaches 0℃ which is 1—2℃ higher than the environment temperature; while arousing, its body temperature can quickly come back to 33—35℃ in 2—3 h. Active body temperature regulation refers to these changes that are beyond the capability of the non-hibernator[1]. It was proved by many researches that the preoptic area (POA) of hypothalamus plays an important integrative role in the body temperature regulation in mammals, and that the hibernator has equal hypothalamic temperature sensitivity and capability of temperature regulation in the hibernation season and the non-hibernation season[1,2]. It is one of the most concerned core problems in the studies of hibernation if the POA neurons undertake some adaptive change in the unique body temperature regulation of the hibernator. Boulant and Wünenberg compared the temperature sensitivity and electric activity of the POA neurons of the hibernator with those of non-hibernator. Boulant reported that there was no significant

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difference between them when the temperature was above 30℃[2]; Wünenberg found that the lowest temperature of the spontaneous activity of the POA neurons in the hibernator was markedly different from that in the non-hibernator[3]. Since Boulant did not observe the situation below 30℃, and both of them made no systematic comparison among different hibernation phases and seasons, it remains unclear if the POA neurons change adaptively in different hibernation phases and seasons.

Maybe because of the obstacles in method and technology, there have been few reports on the property change of the POA neurons during hibernation. Since Hori et al. recorded the electric activity of thermosensitive neurons in the POA brain slice for the first time in 1980, great achievements have been made on the temperature sensitivity and electric activity of mammal POA neurons[5—7]. It has been proved by a mass of studies that brain slice can be made use of in investigating the electric activity and the cold tolerance of neurons[4,8]. These studies, combined with *in situ* studies, contributed to a better knowledge of the role of the hypothalamus thermosensitive neurons on thermal regulation.

Monoamine transmitters in the brain are involved in the temperature regulation. The contradictory roles of 5-HT and catecholamines (mainly norepinephrine) are major factors determining the activity of the POA in the thermal regulation, and thermal regulation roles of catecholamine in the brain vary with species and environment temperature[9,10]. It needs further proof if the content of NA in the hypothalamus and its metabolism are affected by hibernation phases and seasons, and furthermore if the temperature sensitivity of the POA neurons is affected by these changes of NA.

In this work, high performance liquid chromatography-electric detector was applied to the investigation of the content changes of NA and its main metabolite, 3-methoxy-4-hydroxyphenylglycel (MHPG), in the hypothalamus of the ground squirrel in different seasons and different hibernation phases; in the mean time, the changes of electric activity and temperature sensitivity of POA neurons and the effects of NA on them in different seasons and different hibernation phases were also explored with *in vitro* brain slice. This work will provide new information for the understanding on the unique thermal regulation mechanism of the hibernator and the roles of NA in hibernation.

1 Materials and methods

1.1 Animal grouping

Studies were carried out from June, 1991 to June, 1994. Adult ground squirrels were captured in the field. They were kept in cages individually, fed with standard fodder and fresh vegetable. In winter, they were moved into a cold-dark room (ambient temperature of (5±2)℃), a simulated natural hibernation environment, lighted upon 30 min with dim light only in the feed time and observation time. In the other seasons, they were kept under the condition of natural light and room temperature. They were divided into six groups without sex discrimination: (i) summer euthermar group, SE, the thorax skin temperature >33℃, number=55; (ii) winter euthermar group, WE, the thorax skin temperature >33℃, not in hibernation, number=9; (iii) entering hibernation group, EH , not in hibernation 24 h before sacrifice, but entering hibernation when tissue preparation, thorax skin temperature <20℃, number=8; (iv) deep hibernation group, DH , in deep hibernation 24 h before sacrifice, thorax skin temperature ≤9℃,

number = 36; (v) induced arousing group, IA, in deep hibernation and the thorax skin temperature ≤9℃, 1 h before sacrifice, arousing the animals with foreleg stimulation in the room temperature (15℃) and decapitating for tissue preparation when rectum temperature reached 20℃, number = 9; (vi) interbout group, IB, in deep hibernation and thorax skin temperature ≤9℃, 24 h before sacrifice, spontaneously arousing and thorax skin temperature ≥20℃ when tissue preparation, number = 11.

1.2 Electric activity recording in brain slice

As described by Dean and Boulant[5], 400 μm-thick frontal slices of the POA of the hypothalamus were prepared with a vibratome and positioned by referring the crossing anterior commissure and the optic chiasma. Slices were transferred to an incubation chamber and allowed to equilibrate for 90 min in artificial cortcospinal fluid (ACSF) at room temperature before entering a recording chamber. The chamber was constantly perfused (1—1.5 mL/min) with ACSF. After the slices were incubated for 60 min in ACSF, neuronal activity was recorded with extracellular glass microelectrodes filled with 3 mol/L NaCl. The composition of ACSF was (mmol/L): KCl 5, NaCl 124, $CaCl_2$ 2.4, $MgSO_4$ 1.3, KH_2PO_4 1.24, $NaHCO_3$ 26, glucose 10, oxygenated (95% O_2 + 5% CO_2) and maintained at pH 7.40. During perfusion this medium was always oxygenated. The temperature of the ACSF (also the temperature of the slices) was controlled by two thermostatic bathes and monitored with a thermocouple.

Single-unit spikes were identified and the integrated firing rate was monitored on a polygraph along with slice temperature. For each neuron, firing rate and temperature data were digitized and averaged and the curve of firing rate as a function of temperature was graphed on an X-Y plotter. Neuronal thermosensitivity or the thermal coefficient (m) of the firing rate was determined by the slope of the regression line over a range of no less than 3—4℃ in which the neuron was most thermosensitive[5]. Neurons were classified as warm sensitive (WS) if their thermal coefficients were no less than 0.8 imp/s·℃$^{-1}$, cold sensitive (CS) if their thermal coefficients were no higher than −0.6 imp/s·℃$^{-1}$. All other neurons were considered as temperature insensitive (TI). For each neuron, during the experiment, the critical temperature (Tc), at which firing activity of the warm or cold sensitive neurons was most sensitive to temperature[8], and the lowest temperature (TL) at which spontaneous firing activity disappeared, were examined. Only the data from the neurons which could restore to discharge when the incubation temperature rose back above TL and whose discharge ratio of signal to noise was greater than 3 were kept for statistical analysis.

1.3 Recording the effects of NA and its receptor blockers on electric activity of the POA neurons

During the recording of the electric activity of POA neurons, the effects of NA on firing activity of the POA neurons were examined in 25 SE animals and 20 DH animals at 37℃. The drug application method of Scott's was followed[6]. Five stainless iron pipettes with 0.1 mm inner diameter were filled with NA, Prazosin (α1 blocker, 1% solution prepared with ACSF and 20% N, N-dimethyl-acetylamine), Yohimbine (α2 blocker, 1% solution prepared with ACSF) or ACSF. Under a microscope, the pipettes were propelled where they were 0.5 mm distant from the tip of microelectrode. The doses of the drugs were 1 μL and drug administration time was 5 s.

The effects of NA were judged by the ratio of the greatest firing rate (FR_1) after drug application to the average firing rate (FR_0) before the drug application: they were facilitating action if

$FR_1/FR_0 > 1.3$; inhibiting action if $FR_1/FR_0 < 0.7$; and the others were classified as no marked action (the variation range of firing rate before drug application was lower than ± 0.2).

1.4 Measuring the content changes of NA and MHPG in hypothalamus in different seasons and hibernation phases

With HPLC, the content changes of NA and MHPG in hypothalamus were investigated (14 SE animals, 10 WE animal, 10 EH animals, 10 DH animals and 9 IB animals). Chromatography condition: brain tissues were homogenated with pre-cooled 400 μL 0.2 mol/L perchloric acid (containing 0.1% antiscorbutic acid) in ice bath, the homogenate was centrifuged at 45 000 *g* for 15 min, twice; an amount of 80 μL supernatant with internal standard 3,4-dihydroxybenzylamine, DHBA was injected into HPLC system. The work condition of liquid chromatograph (L-ECD-6A) and electro-chemical detector (LC-6A): mobile phase contained 0.15 mol/L citric acid, 0.18 mol/L NaOH, 1.0 sodium octyl-sulphate and 7% methanol (pH = 4.0); the work potential of detection cell was 0.72 V; standards including NA, MHPG and DHBA were products of Sigma's.

All data were given as $\pm$ SD, and the Student's $\bar{X}$ test was used for statistic analysis.

2 Results

2.1 Changes of temperature sensitivity and firing activity of POA neurons in different seasons and hibernation phases

The proportions of the neurons, temperature sensitivity, and Tc and TL of firing activity varied with seasons and hibernation phases (table 1).

Table 1 Comparison of characteristics of thermosensitive neurons in hypothalamic tissue slices from ground squirrels in different seasons and hibernating phases

		SE group	WE group	EH group	DH group	IA group	IB group
	n	35	12	16	19	7	3
	%	27.3	29.3	51.6	15.5	18.5	7.5
WS	m/Hz·℃$^{-1}$	2.0±1.3	2.0±0.6	2.5±1.1[a)]	1.5±0.7[a)]	2.2±2.2	1.1±0.2[a)]
	Tc/℃	37.0±2.3	34.0±2.4[b)]	37.3±1.3	32.6±2.7[b)]	35.0±2.7	32.0±0.6[b)]
	TL/℃	32.2±3.5	23.1±6.1[b)]	21.1±7.2[b)]	18.2±9.6[b)]	17.6±8.4[b)]	17.3±3.4[b)]
	n	12	6	4	15	5	11
	%	9.4	14.6	12.9	12.2	12.5	27.5
CS	m/Hz·℃$^{-1}$	−1.3±0.6	−0.8±0.1[a)]	−1.4±0.4	−0.8±0.2[a)]	−0.9±0.3[a)]	1.0±0.5[a)]
	Tc/℃	36.8±1.7	30.8±1.5[b)]	34.5±3.7	31.3±3.2[b)]	35.5±2.1	31.6±3.6[b)]
	TL/℃	30.6±3.8	16.7±6.9[b)]	4.3±8.3[b)]	21.1±4.7[b)]	18.0±7.9[b)]	11.9[c)]±6.1[b)]
	n	81	23	11	50	28	26
TI	%	63.3	56.1	35.5	72.3	69.0	65.0
	TL/℃	29.3±4.2	14.4±6.4[b)]	22.1±5.6[b)]	14.6±6.3[b)]	14.4±6.4[b)]	8.2[c)]±2.8[b)]

n, Number of neurons recorded in the POA slices; CS, cold sensitive neuron; DH, deep hibernation group, $n = 36$; EH, entering hibernation group, $n = 8$; IA, induced arousing group, $n = 9$; IB, interbout group, $n = 11$; m, thermal coefficient of the firing rate; SE, summer euthermar group, $n = 55$; Tc, the critical temperature at which the firing activity of warm sensitive or cold sensitive neuron became most sensitive along with the temperature increase; TL, the lowest temperature at which the firing activity disappeared; TI, temperature insensitive neuron; WE, winter euthermar group, $n = 9$; WS, warm sensitive neuron. a) $P < 0.05$, b) $P < 0.01$, WE (EH, DH, IA, IB) vs. SE; c) $P < 0.01$, IB vs. WE.

2.1.1 Relative quantity (table 1: %). The relative quantity of warm sensitive (WS) neurons was slightly influenced by seasonal change, and greatly influenced by hibernation phase switch: the result of the proportion of WS neurons in WE group was similar to that of previous studies which were performed on summer ground squirrel and rat[2,11]; the WS neuron proportion of the SE group was 51.6%, almost twice of the proportion of the EH group which was 27.3%; the relative numbers of WS neuron in DH group and IB group reduced to 15.5% and 7.5% respectively. The proportion of the CS neuron increased in winter and with the progress of hibernation. In IB group, it was about three times as much as that in SE group. Compared with the proportion of thermal insensitive neurons in SE group, that in DH group increased slightly, while that in EH group decreased markedly.

2.1.2 Temperature sensitivity (table 1: m). Compared with that in SE group, the temperature sensitivity (m) of the CS neurons increased in winter and in hibernation except that in EH group. The values of m of the WS neurons in DH and IB group decreased, while that in EH group increased.

2.1.3 Lowest temperature of firing (table 1: TL). The firing activity of the neuron in summer ground squirrel terminated at 25℃; the TLs of the neuron in winter and hibernation were obviously reduced. The TLs of WS neurons decreased in the order of WE group, EH group, DH group, IA group, IB group, and the TLs of CS neurons were lowered in the order of EH group, DH group, IA group, WE group, IB group. The cold tolerance of the POA neurons was the strongest.

2.1.4 Critical temperature (table 1: Tc). Compared with that in SE group, the Tc of WS and CS neurons in WE group, DH group and IB group were markedly lowered, and the Tc in EH group and IA group had no significant change.

2.2 Effects of NA on spontaneous firing of POA neurons

The effects of NA on the spontaneous firing of neurons in POA brain slices were recorded. The brain slices were prepared with summer animals and deep hibernation animals respectively. The results are shown in table 2 and figure 1.

Table 2 Effects of NA on the spontaneous firing activity of POA neurons in summer euthermic and deep hibernating ground squirrels

Group	Type of neuron	n	Effect of NA		
			+ (C: ng/mL)	− (C: ng/mL)	nc (C: ng/mL))
SE	WS	21	19 (130 ± 47)	0	2(270 ± 0)
(n = 25)	CS	3	0	3 (135 ± 56)	0
	TI	32	15 (135 ± 62)	9 (236 ± 161)	8 (270 ± 0)
DH	WS	15	9 (65 ± 15[a)])	0	6 (270 ± 0)
(n = 20)	CS	4	4(45 ± 15[a)])	0	
	TI	34	11 (72 ± 35[a)])	19 (56 ± 22[a)])	4 (270 ± 0)

C, Threshold concentration of NA to affect the firing rate of thermosensitive neurons; +, exciting response; −, inhibiting response; nc, no change. a) $P < 0.01$, DH vs. SE. The other abbreviations are the same as those in table 1.

The following conclusion could be drawn from table 2 and figure 1.

The firing activities of most of WS neurons and CS neurons in SE group were facilitated and inhibited by NA respectively, and the effects of NA increased with the quantity of NA applied. It showed that the main effects of NA on summer animals were facilitation of heat loss and inhibition of heat production.

All four CS neurons in DH group were excited by NA and the effect increased with the quantity of NA applied. The proportion of WS neurons responsive to NA application was markedly reduced, while that of non-responsive neurons increased. It was suggested that the major influences of NA to POA thermosensitive neurons in DH animals were facilitation of heat production and inhibition of heat loss.

In DH group, the thresnolds of WS and CS neurons to NA stimulation were markedly lower than that in SE group, and so were the thresholds of thermal insensitive neurons. The proportion of thermal insensitive neurons which were responsive to NA increased in DH group , especially that of thermal insensitive neurons inhibited by NA. The results indicated that the sensitivity of POA neurons to NA increased significantly in deep hibernation animals.

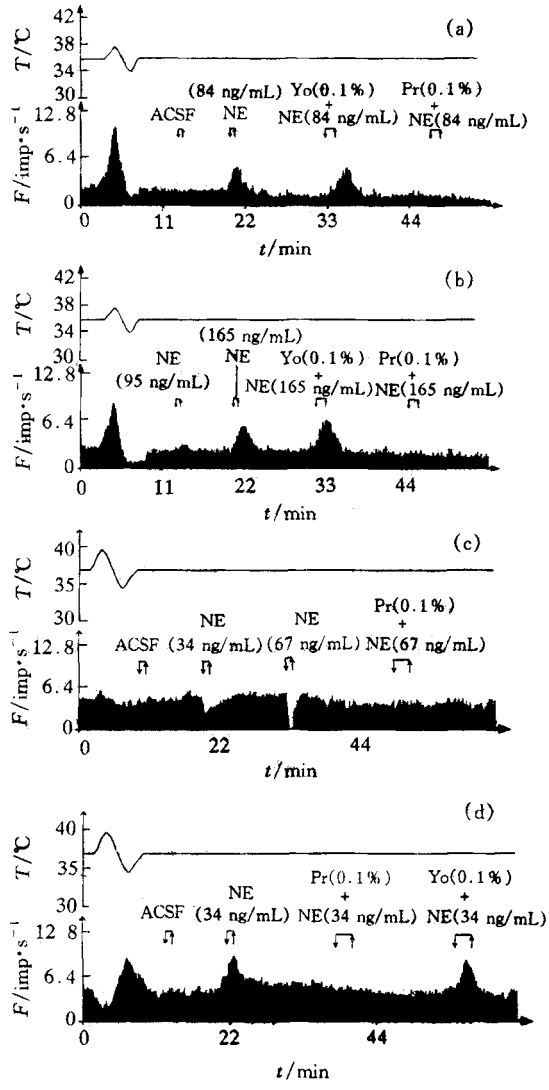

Fig. 1. Effects of NA, prazosin and yohimbine on the spontaneous firing activity of POA neurons in summer euthermic and deep hibernating ground squirrels. (a) Warm sensitive neuron in deep hibernating ground squirrels; (b) warm sensitive neuron in summer euthermic ground squirrels; (c) temperature insensitive neuron in deep hibernating ground squirrels; (d) cold sensitive neuron in deep hibernating ground squireels; ACSE, artificial cortcospinal fluid; Pr, prazosin; Yo, yohimbine.

2.3 Effects of NA blockers on NA action on POA neuron firing

On the POA neurons of DH animals and SE animals whose firing was influenced by NA, the effects of prazosin (NA α1 blocker) and Yohimbine (NA α2 blocker) on NA action were investigated (table 3 and figure 1).

The following conclusion could be drawn from table 3.

The facilitation and inhibition of NA on thermosensitive neurons could be abrogated by prazosin, not by Yohimbine, indicating that the action of NA on thermosensitive neurons is mediated not by α2 receptor but by α1 receptor. This situation was not influenced by season and hibernation phase switch, while the action of NA on thermal insensitive neurons was related to both α1 and α2

receptors.

Table 3 Effects of NA-receptor antagonists on the effects of NA on spontaneous firing activity of POA neurons in summer euthermic and deep hibernating ground squirrels

Group	Type of neuron	n	Prazosin		Yohimbine	
			block	no	block	no
SE	WS(+)	8	7	1	0	8
	CS(−)	3	3	0	0	3
	TI(+)	11	6	5	3	8
	TI(−)	7	3	4	2	5
DH	WS(+)	4	4	0	0	4
	CS(+)	3	3	0	0	3
	TI(+)	10	5	5	3	7
	TI(−)	12	7	5	2	10

Block, the blocking effect of NA-receptor antagonists; no: no effect of NA-receptor antagonists; (+), exciting response of neuron to NA; (−), inhibiting response of neuron to NA. The other abbreviations are the same as those in table 1.

2.4 Variation of NA content in hypothalamus of ground squirrel

From table 4, the contents of NA and MHPG in hypothalamus of winter euthermar were higher than those of summer euthermar, but the ratios of the two substances, which reflect the metabolic rate, were similar in the two groups. The contents of NA and MHPG, the ratios of the two substances decreased in EH and DH animals compared with those of SE animals. The contents of NA were higher and the contents of MHPG were lower in IB animals than those in SE animals, therefore, the ratios of the two substances increased.

Table 4 Variation of NA and MHPG content in the hypothalamus of ground squirrel (*Citelleus Dauricus*) during seasons and hibernating bouts (ng/g wet brain)

Group	SE	WE	EH	DH	IB
NA	587.9±19.7	816.9 ±40.5[a)]	442.5±42.8[a)]	516.5±47.0[a)]	360.3±36.1[a)]
MHPG	274.5±26.7	421.5±78.3[a)]	88.6±24.5[a)]	51.9±25.2[a)]	298.1±47.7[a)]
MHPG/NA	0.46±0.05	0.52±0.09	0.20±0.07[a)]	0.10±0.08[a)]	0.84±0.12[a)b)c)]

a) $P<0.01$, WE (EH, DH, IB) vs. SE; b) $P<0.01$, IB vs. EH (DH); c) $P<0.01$, IB vs. WE (SE). The other abbreviations are the same as those in table 1.

3 Discussions

In this work, the properties of the POA thermosensitive neurons in different seasons and different hibernation phases, and effects of NA on them were investigated. Miller et al. reported that the POA neurons of hibernating ground squirrel stopped their firing activities *in situ*. That result made their another finding, i.e. the body temperature of animals in deep hibernation still fluctuated rhythmically, unexplainable[12]. Our results showed that the TL of WS, CS and thermal insensitive neurons were lowered markedly in hibernation, even to the extent of 5—6℃, indicating from the point of cytological physiology that the thermal regulation mechanism is not ineffective in hibernation. However, the total number of thermosensitive neurons decreased and the proportion of thermal insensitive neurons increased in deep hibernation animals, suggesting that the function of thermal regulation be depressed. The lowering of TL of POA neurons in ground

squirrel brain slice was similar to Wünenberg's result that the TL of *in situ* POA neurons of gold hamster decreased in hibernation[3]. The lowering of TL may reflect improvement of cold tolerance, which may contribute to what makes the hibernator keep its thermal regulation ability with body temperature varying in a range of more than 30℃. The marked lowering of Tc of WS and CS neurons means that the thresholds of heat loss and production decrease to render great cold tolerance to cold adaptive animals. The lowering of Tc may be the reflection of down regulation of T_{set} in the property of neuron, then may provide some evidence for the hypothesis that T_{set} would be lowered in hibernation[1]. Moreover, the TL of thermal insensitive neurons was lowered more markedly, but its role in hibernation is inconclusive. It is suggested that the firing of thermal insensitive neurons might act as a reference signal in the process of the production of "set point" of thermal regulation. Besides their involvement in the activities of autonomic nervous system such as drinking and eating, the POA thermal insensitive neurons were involved in thermal regulation as interneurons[6]. In conclusion, the neural mechanism of cold tolerance in hibernation has not been well understood.

The changes of proportions of POA thermosensitive neurons and thermosensitivity of neurons (m) match with the season and hibernation phase switch. On the one hand, the relative quantity of WS neurons and the value of m increased at ground squirrel's entering hibernation, suggesting that when entering hibernation the hibernator does not react to cold stimulus with heat production as in non-hibernation seasons but actively lowered its body temperature to adapt itself to the environment. The above phenomena might be determined by annual rhythm of biological clock, not or not completely by environment. On the other hand, at waking from hibernation, the proportion and m value of WS neurons markedly increased, while those of CS neurons decreased. Then these changes reduced heat loss and increased heat production. Therefore, these changes might contribute to the ability that the hibernator can raise its body temperature quickly from extremely low point at waking. Miller et al.[13] investigated the effects of hibernation and low Tb on the firing activity and thermosensitivity of suprachiasmatic nuclei (SCN) with the same methods as ours, and proved that the quantity of CS neurons in SCN remarkably increased. This result was in consistence with ours.

It is unclear why the proportions, thermosensitivity and sensitivity to NA of POA neurons change with the switches of season and hibernation phase. It can be hypothesized that the CS neuron supplements origin from thermal insensitive neurons or potential CS neurons which show their function in cold environment. Unlike WS neurons, the POA CS neurons mostly belong to secondary neuron and their thermal properties are modulated by two kinds of synaptic input. One consists of excitatory postsynaptic potential (EPSP) from another CS neuron and inhibitive postsynaptic potential (IPSP) from thermal insensitive neuron; the other consists of EPSP from thermal insensitive neurons and IPSP from WS neurons[7]. Therefore, the excitement of CS neurons and inhibition of WS neurons at low temperature can disinhibit potential CS neurons. In this work, it is proved that the contents and metabolism of NA in hypothalamus in ground squirrel had drastic changes during season and hibernation phase switches: in deep hibernation, the contents of NA and MHPG decreased consistently with low metabolic rate and inhibition of thermoregulation in this phase. In interbout phase, metabolic rate of NA was increased for large heat production and then quick recovery of Tb in later waking. Continuous low level of NA and its metabolic rate might promote the sensitivity of POA thermosensitive neurons to NA. It was reported that in cold

environment, the firing rate of noradrenergic neurons increased, so did the release and catabolic rate of NA in these neurons, causing the contents of NA to decrease drastically in the brain area into which they project. The contents of monoamine changed in the brain of rats with a long term of artificial low Tb, therefore the thermosensitivity and electric excitability of POA neurons were modified[14]. It could be inferred that probably similar to the supersensitivity of denervated tissues, the promotion of the thermosensitivity of POA neurons result from increase of receptor affinity with NA caused by decrease of NA content in the brain area. With regard to the possibility that increase of membrane receptor density results in the promotion of thermosensitivity of POA neurons, there is no conclusion.

Not only thermosensitivity but also reaction patterns to NA of POA neurons in hibernating animals changed. The reaction of CS neurons to NA was inhibition in summer, but excitement in deep hibernation. In addition, the relative quantity of WS neurons with excitatory reaction to NA was reduced in deep hibernation. The action of NA on thermoregulation depended on Ta[7], and effects of 5-HT on thermoregulation of the hibernator varied with season switch[10]. The alteration of the reaction pattern of thermosensitive neurons might relate to modification of signal transduction system of NA, and studies on its mechanism will be greatly conducive to the knowledge of mechanism of hibernation and cold tolerance.

The following phenomena were worth being paid attention to. The Tb of both IA and IB groups were in recovery state, their thorax skin temperature was approximately 20℃, but the variation of proportions of thermosensitive neurons in the two groups was different. Compared with DH group, the proportion of WS neurons decreased and that of CS neurons increased in IB group. In IA group, there was few change. From point of Tb recovery, the variation of proportion of thermosensitive neurons reflected the intimate relationship between restoration of Tb in IB phase and functional state of POA. However, the activity of POA neurons in animals in IA group fell behind with the physiological activities, that is , the thermoregulation centre in POA did not carry out its regulatory activities of enhanced heat production when the animals were artificially aroused, suggesting that the rise of Tb not be due to regulation of POA. If so, there might be another heat production centre besides POA. Haak et al. considered that at least parts of the brain areas controlling spontaneous arousing belonged to POA[9]. Beckman[15] proposed the following hypothesis: sensitivity of hypothalamus to temperature variation increased with the progression of hibernation, the heat production inhibition initiated at the beginning of hibernation was gradually weakened in the course of hibernation. Only in POA did the "progressive irritability" occur. Our work indicated that the progressive irritability in POA might relate with the changes of neural proportions, thermosensitivity, sensitivity to NA, and metabolism of NA. The evolution of the hibernator is the cause of the progressive irritability and related adaptivity.

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