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Developmental and Hormonal Regulation of Thermosensitive Neuron Potential Activity in Rat Brain

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ABSTRACT

To understand the involvement of thyroid hormone on the postnatal development of hypothalamic thermosensitive neurons, we focused on the analysis of thermosensitive neuronal activity in the preoptic and anterior hypothalamic (PO/AH) regions of developing rats with and without hypothyroidism. In euthyroid rats, the distribution of thermosensitive neurons in PO/AH showed that in 3-week-old rats (46 neurons tested), 19.5% were warm-sensitive and 80.5% were nonsensitive. In 5- to 12-week-old euthyroid rats (122 neurons), 33.6% were warm-sensitive and 66.4% were nonsensitive. In 5- to 12-week-old hypothyroid rats (108 neurons), however, 18.5% were warm-sensitive and 81.5% were nonsensitive. Temperature thresholds of warm-sensitive neurons were lower in 12-week-old euthyroid rats (36.4 ± 0.2°C, $n = 15$, $p < 0.01$,) than in 3-week-old and in 5-week-old euthyroid rats (38.5 ± 0.5°C, $n = 9$ and 38.0 ± 0.3°C, $n = 15$, respectively). The temperature thresholds of warm-sensitive neurons in 12-week-old hypothyroid rats (39.5 ± 0.3°C, $n = 8$) were similar to that of warm-sensitive neurons of 3-week-old raats (euthyroid and hypothyroid). In contrast, there was no difference in the thresholds of warm-sensitive neurons between hypothyroid and euthyroid rats at the age of 3–5 weeks. In conclusion, monitoring the thermosensitive neuronal tissue activity demonstrated the evidence that thyroid hormone regulates the maturation of warm-sensitive hypothalamic neurons in developing rat brain by electrophysiological analysis.

INTRODUCTION

IT IS WELL KNOWN that hypothyroidism in rats causes decreased thermoregulation (1). The central nervous system (CNS), especially during its development, is under the control of thyroid hormone (2,3). The preoptic and anterior hypothalamic area (PO/AH) is a main thermosensitive region of the brain (4–8). Recent evidence indicates that thermoregulatory behavior did not reverse the propylthiouracil (PTU)-induced hypothermia, suggesting that PTU-induced hypothyroidism leads to a regulated reduction in body temperature (9). However, precise involvement of thyroid hormone toward the maturation of theromoresponsive hypothalamic neurons remains to be further clarified.

To understand the role of thyroid hormone during development of the brain, we focused on the analysis of hypothalamic neuronal thermosensitivity using developmental hypothyroid animal models. To apply the electrophysiological analysis of hypothalamic neuron maturation, we monitored the thermosensitive neural tissue potential activities in PO/AH regions of developing rats before and after exogenous heat loading. The monitoring of maturation of PO/AH thermosensitive neurons may be a useful method for understanding brain development and hormonal regulation.

MATERIALS AND METHODS

Euthyroid and hypothyroid male Wistar rats from different age groups were used: 3 weeks of age (5 euthyroid and 6 hypothyroid rats); 5 weeks of age (5 euthyroid and 6 hypothyroid rats); and 12 weeks of age (7 euthyroid and

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6 hypothyroid rats). The rats were housed in cages in a room maintained at approximately 21°C and 50% relative humidity. Lighting was timed for a 12-hour photocycle.

To cause the hypothyroid condition in rats, the mother of the litter received 0.02% PTU (Sigma, St. Louis, MO) to drink for 3 weeks (10,11).

Circulating thyroid hormone level (triiodothyronine [T_3] and thyroxine [T_4]) determined by radioimmunoassay (RIA) (Eiken ICL, JAPAN) revealed that both hormones in all hypothyroid rats were undetectable (normal range; T_3: 1.3–1.7 nmol/L, T_4: 48.9–56.6 nmol/L).

The animals in the experiment were anesthetized with urethane (1.0–1.2 g/kg, intraperitoneal) and placed in a stereotaxic apparatus. The animals' body temperatures were monitored during the experiment with thermodetectors and device MGAIII-219 (Nihonkohden, Tokyo, Japan) at different sites: in the rectum, in the brain (contralateral to the PO/AH recording site, cuadal from bregma, 4.0–5.0 mm, 3.0–4.0 mm aside from midline and 4.0–5.0 mm below the surface of the skull) and superficially on the back and abdominal skin.

Superficial rat head tissue was removed and the holes (1-mm diameter) in the cranial bone were prepared for stereotaxic insertion of the microelectrodes. Stainless-steel insulated microelectrodes (type: UJ 3002B, Nichonkohden), with 5-μm tip diameters, were used to record the extracellular neuronal electric activity (spikes) from PO/AH. The stereotaxic coordinates for the PO/AH were set as follows: (1) for 3-week-old rats: 0–0.5 mm rostral to bregma; 0–1.0 mm from the midline and 7.0–9.0 mm below the surface of the skull (12); (2) for 5-week-old rats: 0–0.5 mm caudal to bregma, 0–1.0 mm from the midline and 8.0–9.0 mm below the surface of the skull (13); (3) for 12-week-old rats: 0–1.0 mm caudal to bregma, 0–1.0 mm from the midline, and 8.0–10.0 mm below the surface of the skull (14). The experimental protocol consisted of extracellular recording of the neuronal spikes and testing their firing rates (imp/s) in response to the thermal stimulation resulting from rat head warming by lamp (40 W) or cooling down to 33°C by switching it off. The lamp was situated 10–20 cm from the animal and the animal's rectal temperature (37°C) was maintained by a blanket con-

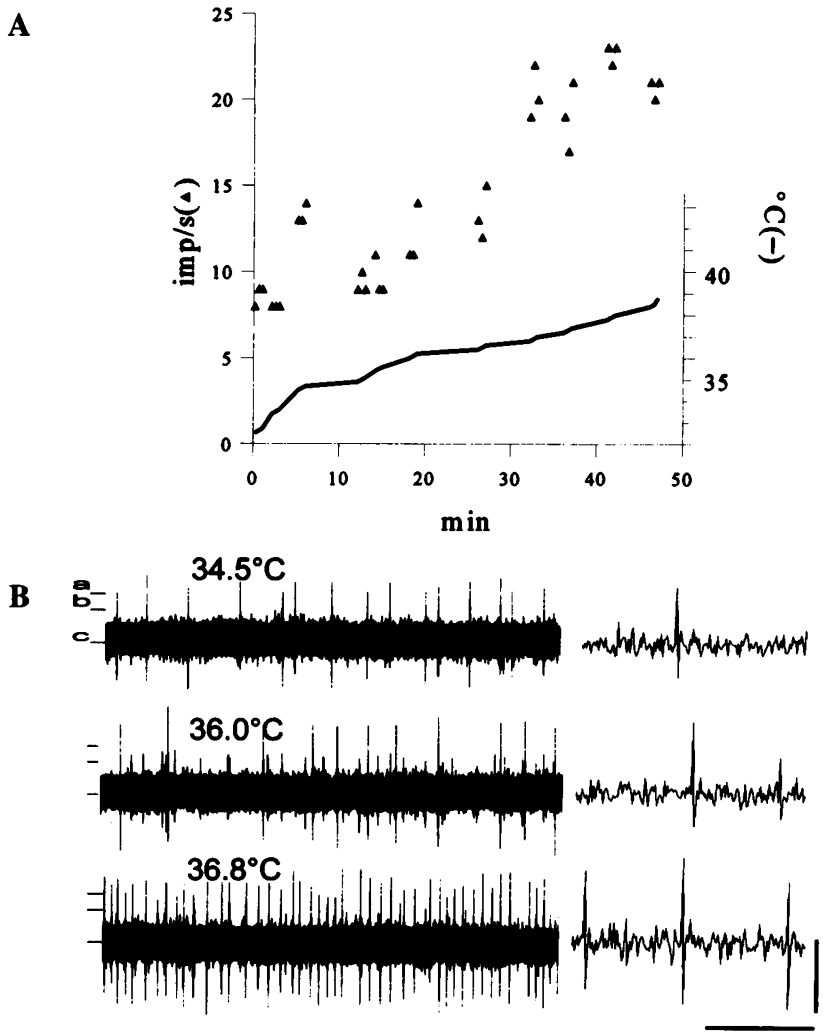

FIG. 1. An example of a warm-sensitive neuron recorded from PO/AH of euthyroid 12-week-old rats. **A:** A plot of firing rate (imp/s) in response of cerebral temperature (°C) changes. Every value of firing rate is result of 10-second record. **B:** Slow and fast records of neuronal activity at different cerebral temperatures. *a*,-level of discrimination of spike activity for the warm-sensitive neuron. *b*,-level of background noise. *c*,-zero level. Horizontal bar: −0.5 seconds and ×0.05 seconds for slow and fast records respectively; vertical bar: −0.1 mV.

troller, (ATB-1100, Nihonkohden). Spike discharges were amplified by a Biophysical Amplifier (AVB-11A, Nihonkohden) with a band pass of 200–3000 Hz, displayed during the experiments on an oscilloscope (VC-11, Nihonkohden) and stored to digital tape with a digital tape recorder (TEAC, Tokyo, Japan, RD-120TE DAT DATA RECORDER).

Averager/Histogram Analyzer (QC-111J, Nihonkohden) was used for window discrimination of spikes of different amplitudes, firing rate counting, and separation of background noise. Figure 1 demonstrates an example of firing rate changes and spikes of a warm-sensitive PO/AH neuron in response to cerebral temperature changes. A Thermal Array Recorder (Nihonkohden) and an AF-550 Waveform Analyzer (Ono Sokki, Japan) were used for the preparation of these records.

The mean firing rates of discriminated single neurons were counted in 10-second intervals every 0.2°C–0.5°C of cerebral temperature change.

Firing rates of neurons were tested with cerebral heating at a rate not faster than 0.5°C/min, through the range 33°C–41°C. The records of some neuronal spike activities were repeated to confirm the constant position of the microelectrode tip. To find new neuronal spike activity the microelectrode was moved deeper until the next spike was recorded and cerebral warming continued. The thermal coefficient was used to characterize the temperature response of the neurons (7). Neurons with firing rates changed at a rate less than 0.8 imp/s°C were classified as nonthermosensitive neurons (nonsensitive). Neurons that displayed an increase in firing rates with rising cerebral temperature at a rate of 0.8 imp/s or higher were warm-sensitive neurons. For nonlinear warm-sensitive neurons, stepwise regression analysis was applied to determine the thermal thresholds, ie, the cerebral temperature which the thermal coefficient changes beyond 0.8 imp/s°C.

After the above experiments, the animals were cannulated through the heart and blood samples were collected for T_4 and T_3 estimation, and perfused later through the heart with 10% formalin/4% paraformaldehyde. Samples of the animals' brains were taken for histological confirmation of microelectrode tip position. Statistical analysis comparable parameters are described as mean values standard errors of the mean (SE). Differences between samples were tested for statistical significance by means of a distribution-free test (Wilcoxon) with a significance limit of $p < 0.05$. Distributions of types of thermosensitive neurons (units) were analyzed by chi-square test.

RESULTS

The results of neuronal spike activity recorded from PO/AH regions of euthyroid and hypothyroid 3-, 5- and 12-week-old rats are summarized in Table 1. The relative quantity of warm-sensitive and nonsensitive neurons tends to be different in euthyroid rats of 3 and 5 weeks of age ($p = 0.08$), and 3 and 12 weeks of age ($p = 0.13$). The increase of warm-sensitive neurons was observed during the development of euthyroid rats: from 19.5% (3 weeks) to 34.3% and 32.7% (5 and 12 weeks, respectively).

The analyses showed the decrease in the relative quantity of warm-sensitive PO/AH neurons from 33.6% (euthyroidism) to 18.5% (hypothyroidism) in rats 5–12 weeks of age.

With respect to the cerebral temperature, the firing rates of PO/AH warm-sensitive neurons detected in our study were classified as linear or nonlinear (7,15). The firing rates of the majority of warm-sensitive neurons showed nonlinear dependence on cerebral temperature with a thermal coefficient higher than 0.8 imp/s°C in the range of 36°C–40°C (Figs. 2 and 3). The warm-sensitive responses of these neurons were observed after the cerebral temperature reached a specific temperature called "threshold." Before reaching the threshold, the thermal coefficients were lower than 0.8 imp/s°C. We identified with linear temperature dependence in 7 of 22 warm-sensitive PO/AH neurons in 5-week-old euthyroid rats, 4 of 19 in 12-week-old euthyroid rats and only 1 of 12 in 5-week-old hypothyroid rats. All PO/AH warm-sensitive neurons in 3-week-old rate (euthyroid and hypothyroid) and 12-week-old hypothyroid rats had nonlinear dependence on cerebral temperature.

Comparison of the nonlinear warm-sensitive neurons with respect to their thermal coefficients around the thresholds did not reveal significant differences between 3-, 5-, and 12-week-old euthyroid rats, and between euthyroid and hypothyroid rats (Table 2). Also, at the estimated points of the thresholds of nonlinear warm-sensitive neurons among the different age groups, there were not differences of firing rates between hypothyroid and euthyroid rats. On the other hand, with respect to cerebral temperature values of the thresholds of nonlinear warm-sensitive neurons, there were differences depending on age and thyroid function of developing rats (Fig. 3, Table 3). Thus, 3- and 5-week-old euthyroid rats had PO/AH warm-sensitive neurons with higher thermal thresholds than in 12-week-old euthyroid rats by 2.1°C ($p = 0.005$) and 1.6°C ($p = 0.001$), respectively. Nonlinear PO/AH warm-sensitive

TABLE 1. SUMMARY OF PO/AH UNITS IN EUTHYROID AND HYPOTHYROID RATS OF 3, 5, AND 12 WEEKS OF POSTNATAL AGE

Age	*3 weeks*	*5 weeks*	*12 weeks*	*5 weeks + 12 weeks*
Euthyroid rats				
All units	46	64	58	122
Nonsensitive	37 (80.5%)	42 (65.7%)	39 (67.3%)	81 (66.4%)
Warm-sensitive	9 (19.5%)	22 (34.3%)	19 (32.7%)	41 (33.6%)
Hypothyroid rats				
All units	41	51	47	108
Nonsensitive	37 (90.3%)	49 (80.4%)	39 (83.0%)	88 (81.5%)
Warm-sensitive	4 (9.7%)	12 (19.6%)	8 (17.0%0	20 (18.5%)

Statistical evaluation by chi-square tests of 3 vs. 5 weeks, 3 vs. 12 weeks, and 5 vs. 12 weeks were $p = 0.08$, 0.13 and 0.85, respectively. PO/AH, preoptic and anterior hypothalamic regions.

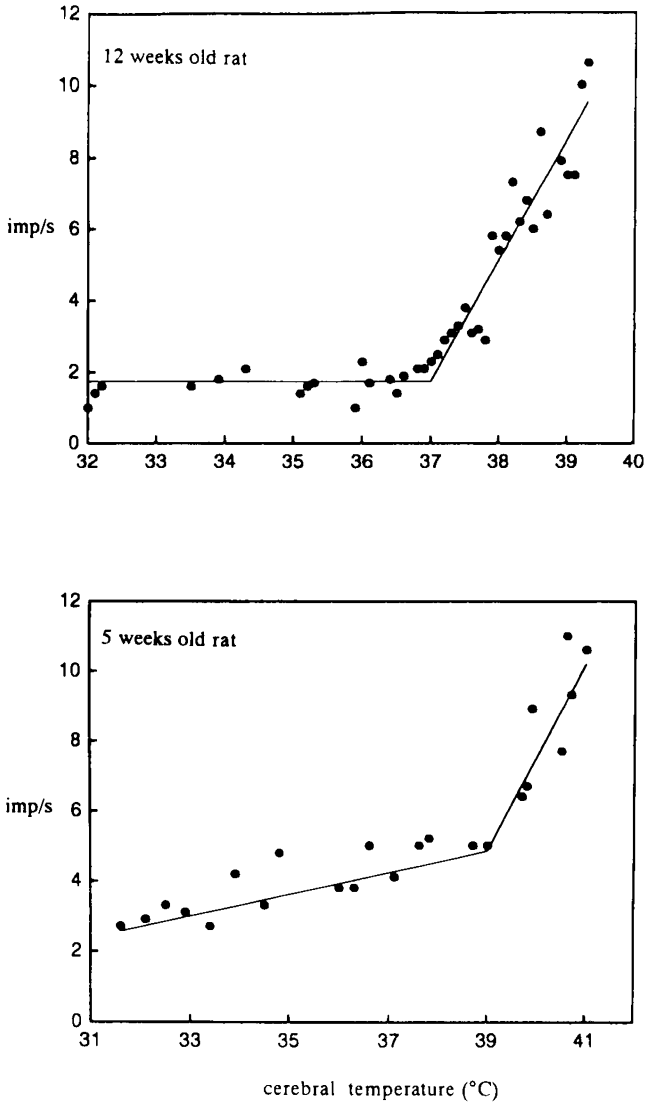

FIG. 2. Representative scatter-plot figure of relationship between firing rates (imp/s) and cerebral temperature (°C), recorded in preoptic and anterior hypothalamic regions (PO/AH) of 5- and 12-week-old euthyroid rats. The thermal threshold of 5-week-old rats is higher than that of 12-week-old rats.

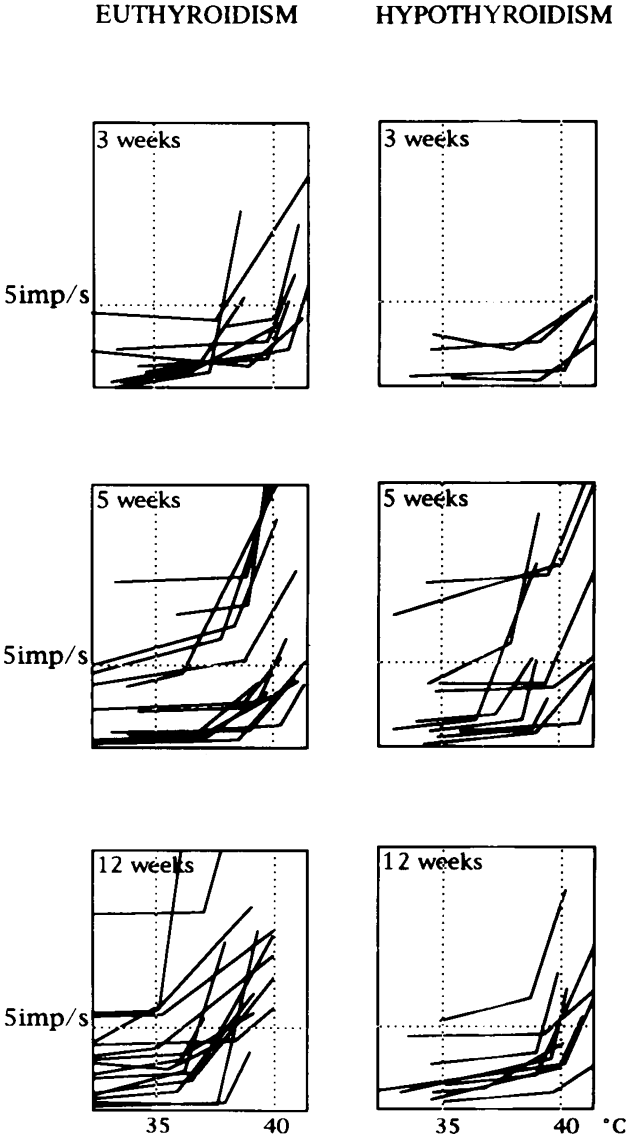

FIG. 3. Nonlinear warm-sensitive neurons recorded in preoptic and anterior hypothalamic regions (PO/AH) of 3-, 5-, and 12-week-old euthyroid and hypothyroid rats (-cerebral temperature, imp/s-firing rate). The thermal threshold of nonlinear warm sensitive neurons is shifted (maturated) age-dependently in euthyroid rats but not in hypothyroid rats.

neurons of hypothyroid 12-week-old rats had significantly higher thermal thresholds (39.5 ± 0.3°C) than those in euthyroid 12-week-old rats (36.4 ± 0.2°C, $p = 0.0004$).

DISCUSSION

Our data demonstrated a developmental change of the composition of thermosensitive neuronal activities of PO/AH regions and the maturation of warm-sensitive neurons in rat brain with age, which is in concordance with previous results (12,16). Because the consequence of thyroid hormone insufficiency on ontogenetic development of thermosensitive neurons in PO/AH regions had not been investigated using hypothyroid rats of different ages, we showed the first evidence of the decrease in the relative quantity of warm-sensitive neurons in PO/AH regions in comparison with that of euthyroid rats. Thus, the relative quantitative distribution of temperature-sensitive and nonsensitive neurons in PO/AH showed the immaturity of development of warm-sensitive neurons at comparably earlier stages and in a hypothyroid state.

Increasing spontaneous firing rates of PO/AH neurons were observed during development in newborn rats (12), which suggests an immaturity of firing rates of hypothalamic neurons even by 21 days of age. On the other hand, subsequent neuronal differentiation in PO/AH regions could be observed from 3 weeks of age to puberty in rats (17), despite cessation of synaptogenesis in the preoptic area of the hypothalamus by 24 days of age (18,19). However, our data demonstrated that maturation of PO/AH

TABLE 2. AVERAGED FIRING RATES (imp/s) OF PO/AH UNITS (ALL, NON-SENSITIVE, WARM- AND COLD-SENSITIVE) AT CEREBRAL TEMPERATURE 38°C IN 3-, 5-, AND 12-WEEK-OLD EUTHYROID (E) AND HYPOTHYROID (H) RATS

Postnatal age (weeks)	*3*	*5*	*12*
E imp/s	2.4 ± 0.2 (n = 46)	4.4 ± 0.5 (n = 64)	6.1 ± 0.6 (n = 58)
H imp/s	1.7 ± 0.2 (n = 41)	2.4 ± 0.3 (n = 61)	2.5 ± 0.3 (n = 47)
p = 0.0001			
Nonsensitive	ntc		
E imp/s	2.4 ± 0.8 (n = 37)	3.4 ± 0.7 (n = 42)	4.8 ± 1.0 (n = 39)
H imp/s	1.8 ± 0.7 (n = 37)	1.9 ± 0.8 (n = 49)	2.9 ± 0.6 (n = 39)
p = 0.0001			
Warm-sensitive			
E imp/s	2.9 ± 0.4 (n = 9)	6.3 ± 1.0 (n = 22)	8.9 ± 1.4 (n = 19)
H imp/s	1.4 ± 0.4 (n = 4)	4.6 ± 0.8 (n = 12)	3.1 ± 0.6 (n = 8)
p = 0.008			

PO/AH, preoptic and anterior hypothalamic regions.

neurons, particularly warm-sensitive ones, continued after 21 postnatal days in rats and, although it was delayed by hypothyroidism. Even though we tried to minimize the interference form the peripheral signal, hypothyroidism itself might modify the activity of hypothalamic neurons involved in temperature regulation in response to heat reduction such as activation of heat-saving mechanisms (vasoconstriction, piloerection) as well as shivering. However, the pattern of the neuronal activities observed suggest comparable alteration in the developmental response as well as the effect of hypothyroidism.

We confirmed the maturation of warm-sensitive neurons in PO/AH in the late stages of postnatal ontogenesis by examining the neuronal thermal thresholds, ie, temperatures at which the thermal coefficient became equal to or higher than 0.8 imp/s in nonlinear warm sensitive neurons. We found that the thresholds of warm-sensitive neurons in 12 week old rats were decreased by 2.1°C and 1.6°C in comparison with those at 3 and 5 weeks of age, respectively.

The nonlinear characteristics of temperature versus firing rate response curves of warm-sensitive neurons are reported to be clearly related to their thermosensitive properties of tetrodoxin-sensitive, noninactivating sodium (Na^+) channels (7,20). But the mechanisms of the maturation of thermosensitive PO/AH neurons and the role of thyroid hormone in these phenomena have not been clarified. On the other hand, developmental changes in Na^+ current densities during postnatal maturation had been identified in different types of neurons (21,22). Recently, selective upregulation of Na^+ current density by T_3 in hippocampal neurons from postnatal rats (23) and different Na^+-K^+-adenosinc triphosphatase (ATPase) subunits in the developing rat cerebellum and cortical neurons had been reported (24–26). Therefore, the effects of thyroid hormone on thermosensitive neurons and their maturation may involve a change in the Na^+ channels in PO/AH and other regions of the brain. Alternatively, an indirect relationship between thyroid hormone and immature thermosensitive neurons may be caused by some additional peptide factors and/or neuromediators that modulate the maturation of warm-sensitive neurons. Thyrotropin-releasing hormone (TRH) especially is one of the candidate

TABLE 3. AVERAGE SENSITIVITY OF NONLINEAR WARM-SENSITIVE PO/AH UNITS ABOVE AND BELOW THE TEMPERATURE THRESHOLDS AND AVERAGE VALUES OF TEMPERATURE THRESHOLDS IN EUTHYROID (E) AND HYPOTHYROID RATS 3, 5, AND 12 WEEKS OF AGE

	Sensitivity below threshold (imp/s °C)	*Temperature at threshold (imp/s)*	*Frequency at threshold (imp/s °C)*	*Sensitivity above threshold (imp/s °C)*
3 weeks of age				
E mean ± SE (n = 9)	0.06 ± 0.05	38.5 ± 0.5	2.1 ± 0.3	2.9 ± 0.6
H mean ± SE (n = 4)	0.06 ± 0.1	39.0 ± 0.4	1.5 ± 0.4	1.3 ± 0.3
3 weeks of age				
E mean ± SE (n = 15)	0.20 ± 0.04	38.0 ± 0.3	4.5 ± 1.1	3.7 ± 0.9
H mean ± SE (n = 11)	0.18 ± 0.07	38.8 ± 0.3	3.7 ± 1.0	3.3 ± 0.4
12 weeks of age				
E mean ± SE (n = 15)	0.18 ± 0.04	36.4 ± 0.2	3.6 ± 0.7	3.5 ± 0.6
H mean ± SE (n = 4)	0.25 ± 0.06	39.5 ± 0.3	3.1 ± 0.6	2.6 ± 0.4
n.s.		P =0.0001	n.s.	n.s.

n.s., not significant.

factors, because TRH is also upregulated in hypothyroidism (29), and simultaneously TRH can suppress warm-sensitive neurons in the PO/AH regions (30,31). Another possible modulator for PO/AH neuronal development is nerve growth factor (NGF) which is upregulated by thyroid hormone age-dependently (32) and has been shown to be an important regulator of voltage-gated ion channels (33). There are, however, no data on ontogenetic interactions of thermosensitive neurons in PO/AH regions with TRH and/or NGF at hypothyroidism. At the same time, our study showed that, there were no differences in the nonlinear properties (thresholds) and the quantity of warm-sensitive PO/AH neurons of younger (3–5 weeks of age) hypothyroid rats in comparison with euthyroid ones of the same age. These facts suggest that the relationship between thyroid hormone and the properties of thermosensitive neurons in 3–5 week-old rats may be still weak and immature. Conversely, in 12-week-old hypothyroid rats thermal thresholds were almost the same as those in 3–5 week old euthyroid rats and substantially higher (by 3.1°C) than those of 12 weeks old euthyroid rats. This evidence indicates the possibility of a critical period (from 5 to 12 weeks postnatal life) in maturation of properties of PO/AH warm-sensitive neurons, which is disturbed in hypothyroid rats.

Furthermore, we must consider the role of extrahypothalamic influences in the developing PO/AH neuronal system, although there was no apparent reverse correlation in thermal thresholds of warm-sensitive neurons (cerebral temperature of the thresholds) versus peripheral, rectal, and skin temperatures of rats.

In summary, our data demonstrated the electrophysiological properties of warm-sensitive neurons, their relative quantitative change in PO/AH regions during ontogenesis and as a consequence of hypothyroidism in rats. These data indicate a crucial role of thyroid hormone on neuronal maturation in the hypothalamic thermoresponsive regions.

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