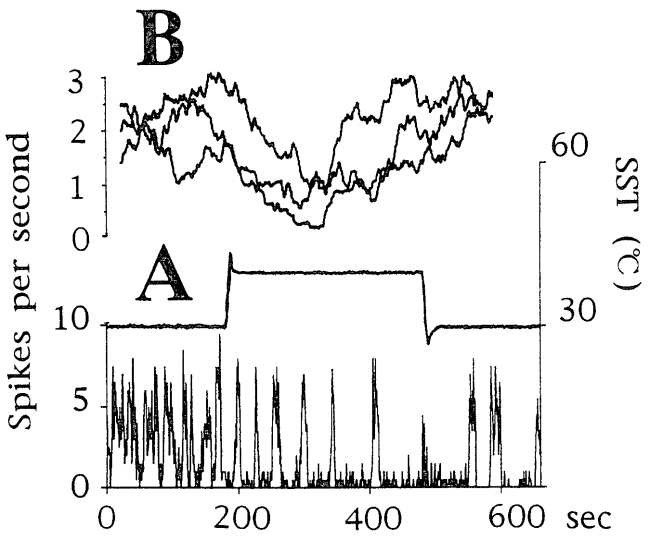

Fig. 1 A representative rate meter recording of the response of a supraoptic p-AVP neuron to warming (43℃) stimuli of the dorsal scrotal skins (**A**). The upper smoothed superimposed three responses were obtained from an another p-AVP neuron during repetitive warming stimuli (**B**).

tube (GDO-1.5, Narishige) filled with a 2% (wt/vol) solution of Pontamine sky blue dye in a 0.5 M sodium acetate buffer. The direct current resistance of the recording electrode was 6-11 MΩ. The SON was systematically explored while isolated rectangular pulses of 0.5 ms duration, at a 100-1500 μA intensity, were applied at 0.81 Hz through the stimulation electrode inserted into the neurohypophysis. Penetrations of recording electrodes were confined to an area 1.5-2.0 mm lateral to the midline, 7.3-8.0 mm rostral from the lamda, and 0.2-0.7 mm dorsal from the ventral hypothalamic surface according to the atlas by Albe-Fessard *et al*[3]. Conventional procedures were followed for amplification and display of the extracellular action potentials according to our previous study[1]. The antidromic identification of discharge activities of the p-AVP producing cells was followed by criteria proposed by Poulain and Wakerley[12].

For thermal stimulation of the scrotal skin, the dorsal region of the scrotal skin was clipped and unfolded. An attached surface (about 10.0 mm^2) of the thermode was placed on the midline of the dorsal portion so that it adhered to the skin of the scrotum. Skin temperature was measured by a thermocouple implanted inside the thermode that was in contact with the skin (BTC-201, Unique Medical).

The scrotal skin temperature (SST) was kept at 30℃ before and after stimulation, and SST was set and maintained at 43 or 15℃ for 5 min during the examination of responses of a particular SON neuron. Warming stimuli were always tested first, with cooling ones following the warming ones. In some responsive neurons, stimuli were repeatedly applied. The control and reaired mean discharge rate of a particular neuron was determined from 3 min before until 3 min after the stimulus.

All measurements, rectal temperature, SST, BP, heart rate (HR), and single neuronal activity were introduced to the MacLab system (MacLab/400, Bioresearch Center) via a regular or conventional high gain amplifier for microelectrode recording (MEZ-8201, Nihon Kohden Kogyo), and were analyzed.

ANOVA and Duncan's method were applied for analysis of the effect of stimulus on the SON neuronal activity. For determination of the mean response of the single unit activity, a simple smoothing method (normalizing points: 101) was applied.

All aspects of the present experiments were conducted in conformity with the guiding principles for the care and use of animals approved by the council of the Japan Physiological Society (2001).

RESULTS

The extracellular unit activity of a total of 51 neurons was recorded from the SON in the male rat (N = 23). Among them, 30 neurons exhibited a phasic pattern of the spontaneous discharge. An important

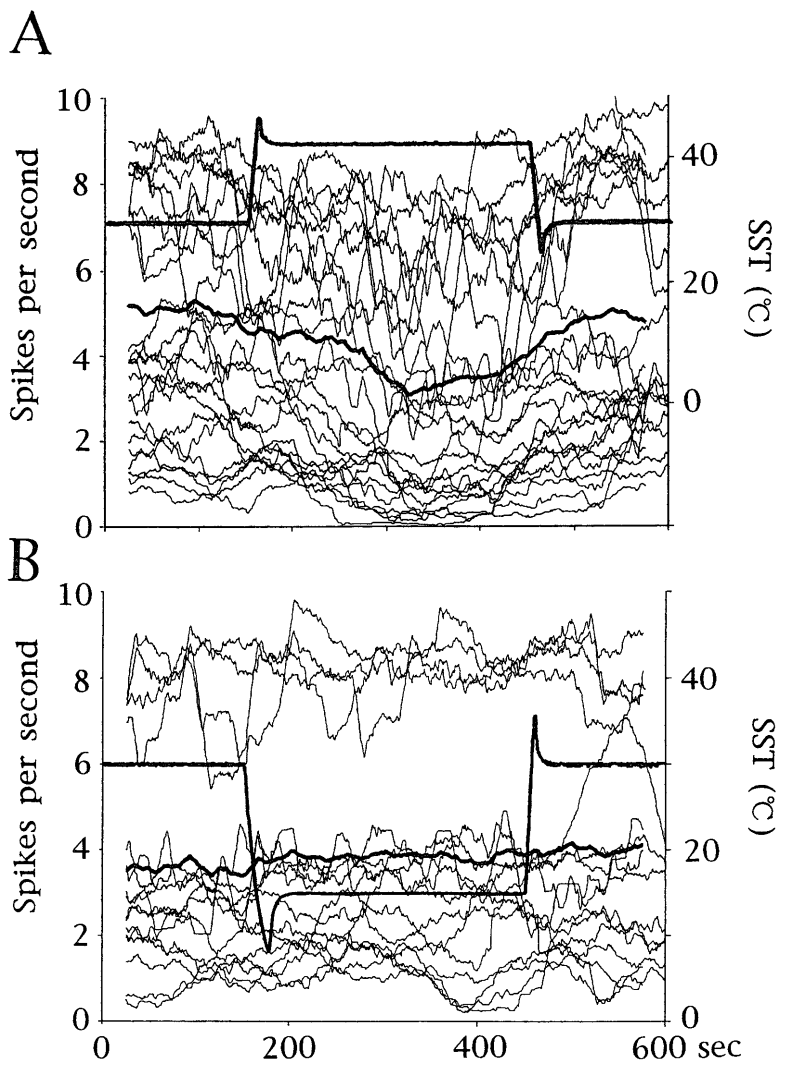

Fig. 2 Each neuronal response (thinner lines) and mean response (thicker curve line) to warming (**A**) and cooling stimuli (**B**) of the scrotal skin in all p-AVP neurons examined. Using a moving average method, each rate meter recording (thinner lines) are smoothed and superimposed. Thicker straight line shows the scrotal skin temperature (SST, ℃).

Table 1. Effects of cooling and warming of the scrotal skin on the burst activity of the supraoptic p-AVP neuron

Parameter	Cooling (15℃) N=16		Warming (43℃) N=28	
	Control	Stimulus	Control	Stimulus
Number of burst (Burst/min)	4.9±0.8*	4.7±1.0	5.5±0.7	3.3±0.5**
Interval of burst (sec)	18.5±3.4	17.7±3.0	14.5±2.6	19.8±2.6
Firiny rate in burst (spikes/sec)	7.5±0.6	7.4±0.5	7.4±0.4	7.5±0.5

*; mean±SEM, **; $P<0.05$.

Table 2. The mean blood pressure (MBP) and heart rate (HR) before (3 min), during (5 min) and after (3 min) the warming or cooling stimulus on the scrotal skin in 4 rats

Stimulus		Before	During	After
Warming	MBP*	69.3±3.7***	69.5±3.7	68.9±3.9
(N****=16)	HR**	310.8±7.1	310.0±7.5	312.1±7.8
Cooling	MBP	70.8±3.7	70.6±3.6	70.1±3.7
(N=16)	HR	305.1±7.7	304.8±7.8	305.0±7.6

*, mmHg; **, beats/min; ***, mean±SEM; ****, trial number.

criterion for the classification as a phasic type was that the firing discharge activity was interrupted by distinct periods of complete silence, which began and terminated abruptly during observations. Other SON 12 neurons had a firing pattern with a constant discharge rate, and were designated as a continuous type in this study. Thus, they satisfied the necessary conditions proposed by Poulain and Wakerley[12]. According their study, the former type of neurons were presumed "vasopressin (p-AVP) neurons", the latter "oxytocin (p-OXT) neurons". These two types of unit activities were recorded well within the nuclear boundary of the SON. Nine SON cells presented such difficultly in determining their obvious firing pattern because of their lower discharge rate that they were excluded from further examination.

A representative example of reversible changes in the neuronal activity of p-AVP cells when the SST was changed from 30℃ to 43℃ is shown in Fig. 1. The control firing discharge rates of p-AVP cells before warming stimuli were 5.0±0.7 (N=28) spikes/sec. The warming temperature in SST significantly inhibited the firing discharge rate (3.6±0.6 Hz, $P<0.001$) of these examined cells. The control firing discharge rates of p-AVP cells before cooling stimuli were 3.6± 0.5 Hz spikes/sec (N=16). The cooling in SST, on the other hand, had induced insignificant changes in the discharge rate in p-AVP cells (3.9±0.7 spikes/sec, *N. S.*) during cooling. The discharge rates after stimuli were 4.8±0.8 (warming) and 4.0±0.7 Hz (cooling), respectively. Each response in all p-AVP neurons examined is shown in Fig 2.

The burst activities for all p-AVP cells examined were further analyzed before and during application of the stimulus. The mean number, interval and firing rate of the burst are shown in Table 1. The warming stimulus significantly decreased the number of bursts generated per minute ($P<0.05$). The two other parameters in warming and cooling were not affected.

In the p-OXT cells examined, no significant change in their discharge rate could be detected during either warming (1.2±0.2 Hz, N=11) or cooling (1.5±0.3 Hz, N=11) stimuli when compared with that during the control period (1.3±0.2 and 1.5±0.2 Hz, respectively).

Mean blood pressure (MBP) and heart rate (HR) were measured continually from 4 rats and the effects of thermal stimuli on the scrotal skin were tested. Neither MBP nor HR, showed significant changes during either warming or cooling stimuli as shown in Table 2.

DISCUSSION

Thermoreceptor afferent inputs derived from the scrotal skin to the hypothalamus have been reported in a number of past studies[6,8,14,15,17]. However, to date there has been no direct evidence showing that the magnocellular neuroendocrine system receives the thermal influences from the peripheral organs, including the skin. This study is the first to show that the supraoptic p-AVP neurons were inhibited by the warming stimuli applied to the scrotal skin without variation of the body temperature.

Local hypothalamic cooling causes a diuresis in the goat[16] and monkey[5], and warming causes antidiuresis in the dog[9]. These reports suggest that the hypothalamic temperature, in other words, the body core temperature, affects AVP release. In male rats, similar results were reported by Matsumura *et al*[10]., namely, that the paraventricular neurosecretory neurons with a pbasic firing pattern, p-AVP cells, were accelerated by warming of the preoptic and anterior hypothalamus.

Considering these past studies collectively suggests that an increase or decrease in body core temperature respectively induces an acceleration or inhibition of the AVP release for thermoregulatory function. As is generally known in thermoregulation for rela-

tively small mammals such as the rat and mouse, the role of the peripheral skin thermoreceptors should play a more important role in the regulation of the body temperature than the central hypothalamic and visceral thermoreceptors. The different heat capacity of the body and ratio of the body surface/weight may cause the body to install a different thermoregulation mechanism. Indeed, AVP is well known as a very potent vasoconstrictor in the peripheral vessel, followed by endotheline *in vitro*[13]. Therefore, in the rat, an increased or decreased release of AVP may respectively induce a vasoconstriction or vasodilatation of the skin, including the scrotum, as an initial stage of the thermoregulation. In fact, at the level of the hypothalamus, AVP was suggested to work as an antipyretic in the case of a fever[11]. Further studies are required to clarify the relationship between the altered plasma AVP concentration and the initial stage of the thermoregulation.

Another possibility for explanation of the paradox is that the applied thermal stimuli were noxious. However, this is unlikely because the temperature stimuli applied in this study were 15 and 43℃. These temperatures have not been considered to be noxious[4], and are within the range used in previous reports[7,10]. Furthermore, in our previous study[2], we revealed that the paraventricular p-OXT cell responds to a pinching stimulus applied to the skin. These were always accompanied with a transient increase in MBP and HR. In this study, however, p-OXT cells were not at all responsive to the thermal stimuli, and neither BP nor HR were affected during stimuli. This may directly indicate that the applied thermal stimulus was not noxious.

Another proposal that the changed cardiovascular factors may indirectly exert influence on the firing activity of the p-AVP cells examined is also unlikely because the MBP and HR during the thermal stimuli did not significantly vary.

The time course that could be detected in the mean response of the p-AVP cell was not entirely stimulus dependent. It is worth noting that the delay of the peak response was about 2-3 min. This would depend upon characteristics of the thermal receptors and/or the expanse of the stimulated area on the scrotal skin rather than the central mechanism. In fact, it is generally well known that the discharge activity of the thermal afferent of the skin is a so-call "rapidly adapting" one. The combined total of the activated afferent signals may make a final characteristic response in the p-AVP cell of the hypothalamus. Nevertheless, the possibility that it partially depends upon the central neuronal mechanism can not be neglected in this study.

In conclusion, the present study is first to demonstrate that the SST (scrotal skin temperature) affects the firing or releasing activity of the p-AVP but not that of the p-OXT cell in the SON. This result strongly suggests the AVP may take part in the initial stage of the thermoregulatory mechanism in the male rat.

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