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Effects of Noradrenaline and 5-hydroxytryptamine on Thermoresponsive Neurons in Slice Preparations of Rat Brainstem

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Abstract Noradrenaline (NA) and 5-hydroxytryptamine (5-HT) have been thought to be involved in thermoregulation. In the present study, using slice preparations, we observe that NA inhibits the activities of most of the warm-responsive neurons but that 5-HT facilitates those of most of the warm-responsive neurons in the preoptic and anterior hypothalamic region. This conclusion supports the previous results which show that the serotonergic system activates heat-loss responses and that NA inhibits heat-loss responses during heat exposure. On the other hand, the result obtained in the dorsal raphe nucleus was opposite to that in the preoptic and anterior hypothalamic region when 5-HT was applied. This observation may be in part related to the autoregulation of 5-HT containing neurons in the dorsal raphe nucleus. In addition, the dose-response relations were determined using in vitro slice preparations. In both regions, NA and 5-HT started to effect the activities of the warm-responsive neurons at a minimum concentration of 5×10^{-6} mol, while the maximum responses were obtained at a concentration of 5×10^{-5} mol.

Key Words: Noradrenaline, 5-hydroxytryptamine, Thermoresponsive Neuron, Slice Preparation

Introduction

5-hydroxytryptamine (5-HT) and Noradrenaline (NA), which have been found in various regions of the central nervous system (CNS), are thought to be important neurotransmitters for brain functions. And since 5-HT and NA are found in high concentration especially in the hypothalamus, they have been considered to be closely related to the hypothalamic function involved in the accomplishment of autonomic responses. In 1963, Feldberg and Myers demonstrated that exogenous 5-HT and NA play important roles in the temperature regulation; Based on their own findings that microinjection of 5-HT into the cat's cerebral ventricle caused hyperthermia and NA hypothermia, they proposed a hypothesis that thermoregulatory responses depend on the relative concentration of these substances released in the hypothalamus[1]. Since then, several investigators have administered 5-HT and NA into the cerebral ventricle or the hypothalamic tissue of various species under test conditions. As a result, the general

belief that inter-species differences in change of body temperature is caused by exogenous amines prevails today[1-5]. The role of these amines in thermoregulation, however, has not yet been clarified.

On the other hand, since 1963, thermoresponsive neurons have been reported to be localized highly in the hypothalamus, thus playing important roles in thermoregulatory responses[6,7]. Hori and Nakayama investigated the effects of iontophoretically applied amines on the thermoresponsive neurons of the hypothalamus of rabbits, arriving at a conclusion consistent with that of the prior observation on the body temperature changes following microinjection of 5-HT and NA into the hypothalamus[8]. In rats, however, both 5-HT and NA were reported to suppress the warm-responsive neurons of the hypothalamus[9]. Murakami also applied amines to the thermoresponsive neurons in the hypothalamus in rat by the method of iontophoresis, but reported that there were a lot of thermoresponsive neurons without respond ing to amines[10]. This discrepancy may be due to that the dose of drugs ejected from the tip of micropipets can not be determined in the study using iontophoresis.

Besides the inter-species difference in the change of body temperature, many controversial results have been reported so far even in the same species of rat[11-14]. Day suggested that such inconsistent results obtained from rats are due to various dose of amines or distinct degree of restraint in rats in different laboratories and he verified his suggestion experimentally[15]. Experimental conditions, such as restraint, largely influence the results of in vivo experiments, single unit recordings inclusive.

Therefore, in order to exclude any possible influences by an unknown factor on the activity of neurons, as well as to determine the effective concentration of drugs around the neurons recorded, we have chosen the method of slice preparation of brain tissue. In the same manner, we have examined the effects of 5-HT and NA on the thermoresponsive neurons in several regions of the rat brainstem. The preoptic and anterior hypothalamic (PO/AH) region has been understood to contain the numerous thermoresponsive neurons and to play an important role in thermoregulation. Therefore, we made slice preparations of the PO/AH region as the hypothalamic slices. In addition, it is known that besides the PO/AH region, thermoresponsive neurons also localize at the raphe region in the mesencephalon[16-18]. There are some evidences indicating that the raphe system relay thermal information from the peripheral thermal detecter to the hypothalamus, playing an important role in thermoregulation[16,19]. For this reason, we made slice preparations at the level of the dorsal raphe (DR) nucleus in the mesencephalon.

By comparing our results obtained through the method of slice preparation with those obtained in in vivo iontophoresis unit studies which has been carried out so far, we studied how 5-HT and NA in various levels of the brainstem take part in thermoregulatory responses.

Methods

Hypothalamic and mesencephalic single units were recorded in vitro tissue slices prepared from 200-250 g male rats of Wistar strain. The procedures for tissue slice preparation are similar to those described by Yamamoto and McIlwain[20]. Rats were decapitated and the brains were quickly removed. Each brain was sectioned and several slices of the hypothalamus or the mesencephalon were obtained using a guillotine type razor-blade slicer (Y.H Slicer, Hotta Rika, model YH-10S). Slices were incubated for about one hour before the experiment at 37°C in preincubation chamber filled with the Krebs-Ringer solution. The Krebs-Ringer solution had the following composition in mM at PH 7.4: NaCl 124; KCl 5; KH_2PO_4 1.24; $MgSO_4$ 1.3; $CaCl_2$ 2.6; $NaHCO_3$ 26; and glucose 10, equilibrated with gas mixture (95% O_2 and 5% CO_2). After one hour preincubation, the slice

was transferred to the recording chamber which was perfused with the Krebs-Ringer solution. The temperature of tissue slice was changed by controlling the temperature of the Krebs-Ringer solution and was measured by a thermocouple placed near the slice. Single unit activity was extracellulary recorded with glass microelectrodes filled with Pontamine sky blue dissolved in 0.5M sodium acetate (10–40 MΩ in resistance). Action potentials were amplified, displayed on an oscilloscope and monitored with a loud speaker. These firing rates were counted every 10 second, converted to analog voltages and displayed on a pen recorder together with the slice temperature.

The drugs, NA and 5-HT, were injected into the perfusate through the side route, into which a microsyringe pump pushed forward a syringe containing either NA or 5-HT to add amines (the concentration of amines was 100 μg/ml each in the syringe.). The concentration of NA or 5-HT in the recording chamber (Cre) was determined by three factors; The concentration of the substances in the syringe (Csy) (usually it was 100μg/ml for each drug), the flow rate of perfusate (F) (0.8–2.5ml/min), and the volume of the solution of monoamines added to the perfusate per a given time period (V) (0.02 ml–0.2 ml/min).

The equation is as follows:

$Cre = Csy \times V/(F+V)$

To test the accuracy of this equation, we added the solution of glucose in the same way as we conducted the amine administration. For each minute within a four minute interval, the fluid in the recording chamber was drawn out and the concentration of the glucose was measured. Next the concentration of glucose measured was compared with the calculated concentration. As a result, the measured concentration reached a maximum at four minutes and the calculated concentration was almost identical to the substantial value measured at this time.

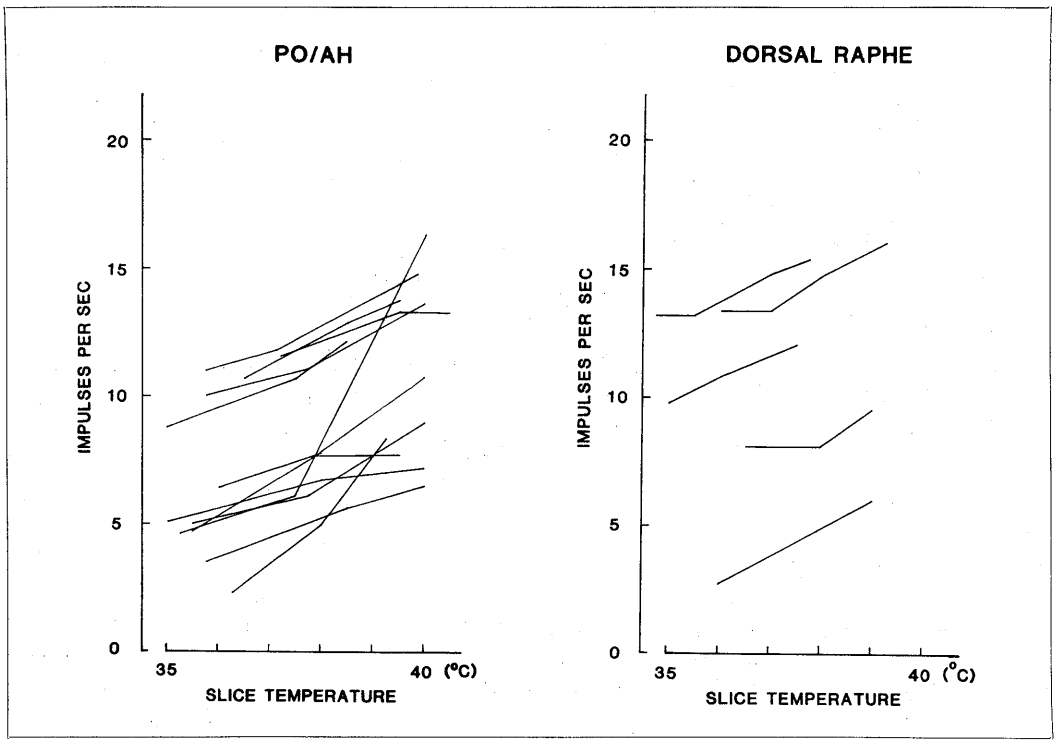

Fig. 1 Thermal response curve of the warm-responsive neurons in the PO/AH region and the DR nucleus.

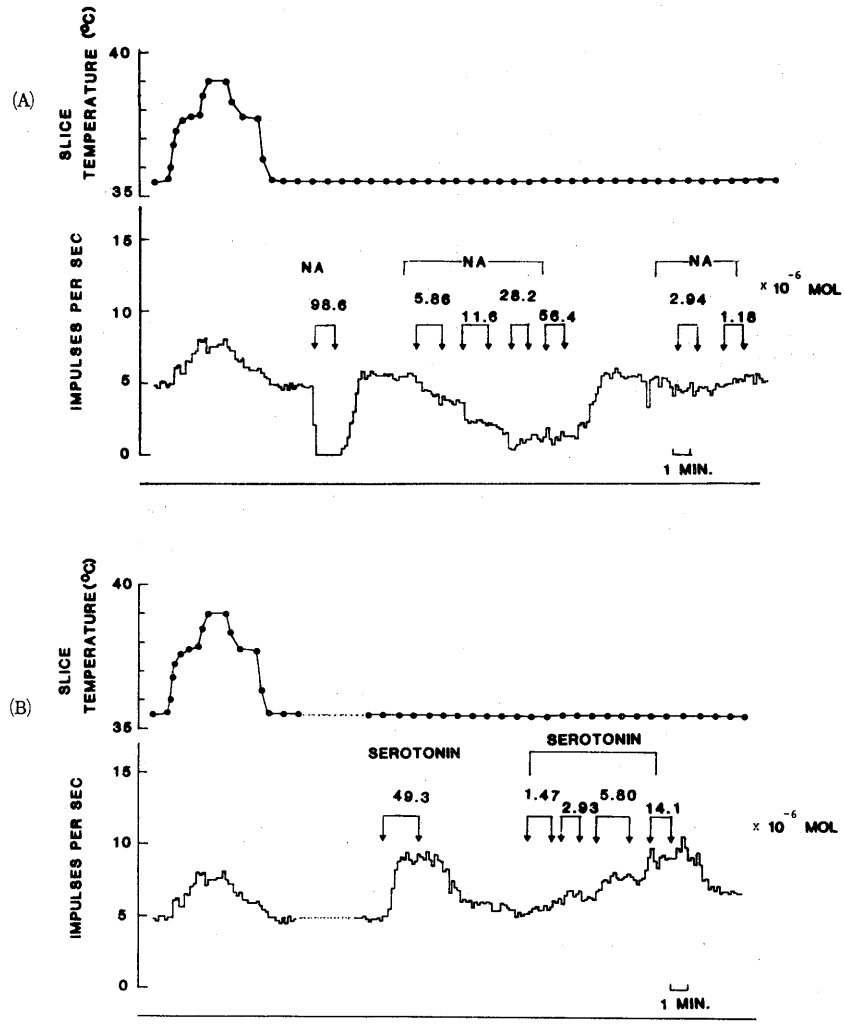

Fig. 2 Typical case of two warm-responsive neurons in the PO/AH region with application of amines. A: A warm-responsive neuron whose activity was inhibited by NA. B: A warm-responsive neuron which increased its firing rates by 5-HT. The concentration of amines is expressed as mol concentration.

Results

Forty-three units in the PO/AH region and twenty-eight units in the DR nucleus were examined for their responsiveness to local temperature change and to the drugs. Of forty-three units in the PO/AH region, seventeen units incresed their firing rates with the rise in slice temperature (i.e. warm -responsive unit). One unit decreased its firing rate with the rise in slice temperature (i.e. cold-responsive unit). Twenty-five units were thermally insensitive units which did not respond to the temperature change. Among the units identified in the DR nucleus, sixteen units were warm-responsive, three were cold-responsive, and nine were insensitive units. Clearly, the proportion of the units classified based on thermosensitivity varied between regions of the brainstem, indicating regional specificity.

Fig. 1 shows the thermal response curve of the warm-responsive neurons obtained in the PO/AH and the DR nucleus. In the PO /AH region and the DR nucleus, the firing rates range between 2-11 imp/sec at the

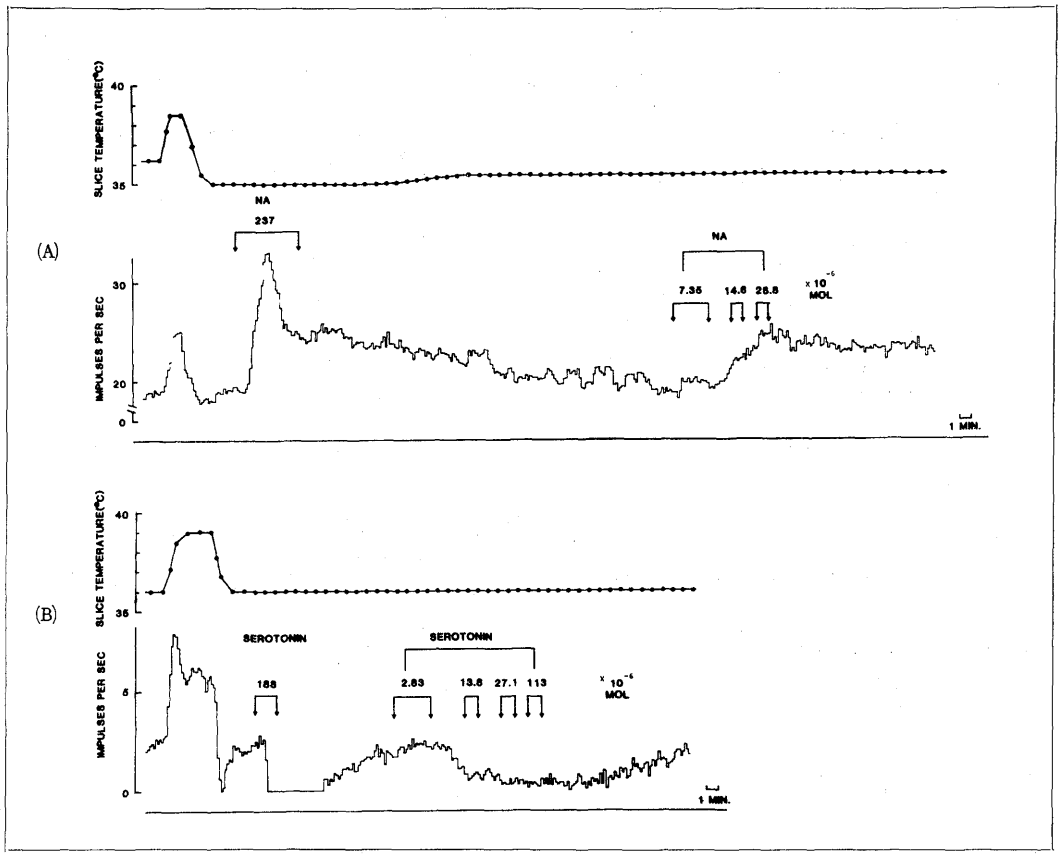

Fig. 3 Two examples of warm-responsive neurons in the DR nucleus. A: A warm-responsive neuron examined its responsiveness to NA. B: A warm-responsive neuron that was tested for responsiveness to 5-HT. The concentration of amines is expressed as mol concentration.

slice temperature of 36°C. The mean thermal coefficient of the warm-responsive neurons is 1.06 imp/sec°C in the DR nucleus and 1.2 imp/sec°C in the PO/AH region.

THE RESPONSIVENESS TO AMINES

After the thermosensitivity was examined for each unit, amines were added to the perfusate with keeping the slice temperature constant. Injection of amines terminated after the change of the firing rates had reached a constant level. At first, a high concentration of amines were injected to test the responsiveness of the neuron to amines. After the firing rates retured to the preinjection level following cessation of the administration, various concentrations of amines, from low to high concentration, were in turn applied to the neuron.

In Fig. 2, the lower half shows a representative example of a warm-responsive neuron in the PO/AH region whose activity was increased by administration of 5-HT. High concentration (49.3×10^{-6} M) of 5-HT caused the maximum response and as the concentration of 5-HT increased from 1.47×10^{-6} mol to 14.1×10^{-6} mol in a step by step manner, the firing rate increased gradually and eventually reached stationary discharge. The upper half depicts another example of a warm-responsive neuron in the same region

Table Responsiveness of the thermoresponsive neurons to amines

Hypothalamic Neuron		Warm-responsive Neuron	Temperature Insensitive Neuron	Cold-responsive Neuron
Noradrenaline	↑	0	1	0
	→	2	3	0
	↓	11	10	1
Total		13	14	1
5-hydroxy-tryptamine	↑	10	7	0
	→	0	2	1
	↓	1	9	0
Total		11	18	1

Mesencephalic Neuron		Warm-responsive Neuron	Temperature Insensitive Neuron	Cold-responsive Neuron
Noradrenaline	↑	6	2	1
	→	2	3	0
	↓	3	1	1
Total		11	6	2
5-hydroxy-tryptamine	↑	4	0	0
	→	1	2	0
	↓	8	5	3
Total		13	7	3

↑ : Facilitatory response ↓ : Inhibitory response
→ : No response

which decreased its firing rates with an administration of NA. The stepwise increase in the concentration of NA, ranging from 5.86×10^{-6} mol to 56.4×10^{-6} mol, caused the inhibitory effect on the firing rates of the warm-responsive neuron.

Fig. 3 shows examples of two warm-responsive neurons in the DR nucleus. A warm-responsive neuron in the lower half decreased its activity with the application of 5-HT and a stepwise increase in the concentration of 5-HT caused a gradual inhibition on the warm-responsive neuron. A warm-responsive neuron depicted in the upper half increased its firing rates in response to NA and as the concentration of NA increased, the response became more effective.

Table summarizes the responses of the neurons to various doses of 5-HT and NA in the PO/AH region and the DR nucleus. As noted in the table, in the PO/AH region, most of the warm-responsive neurons responded to 5-HT with increasing their firing rates, while they responded to NA with decreasing their firing rates. As for the thermally insensitive neurons, NA decreased

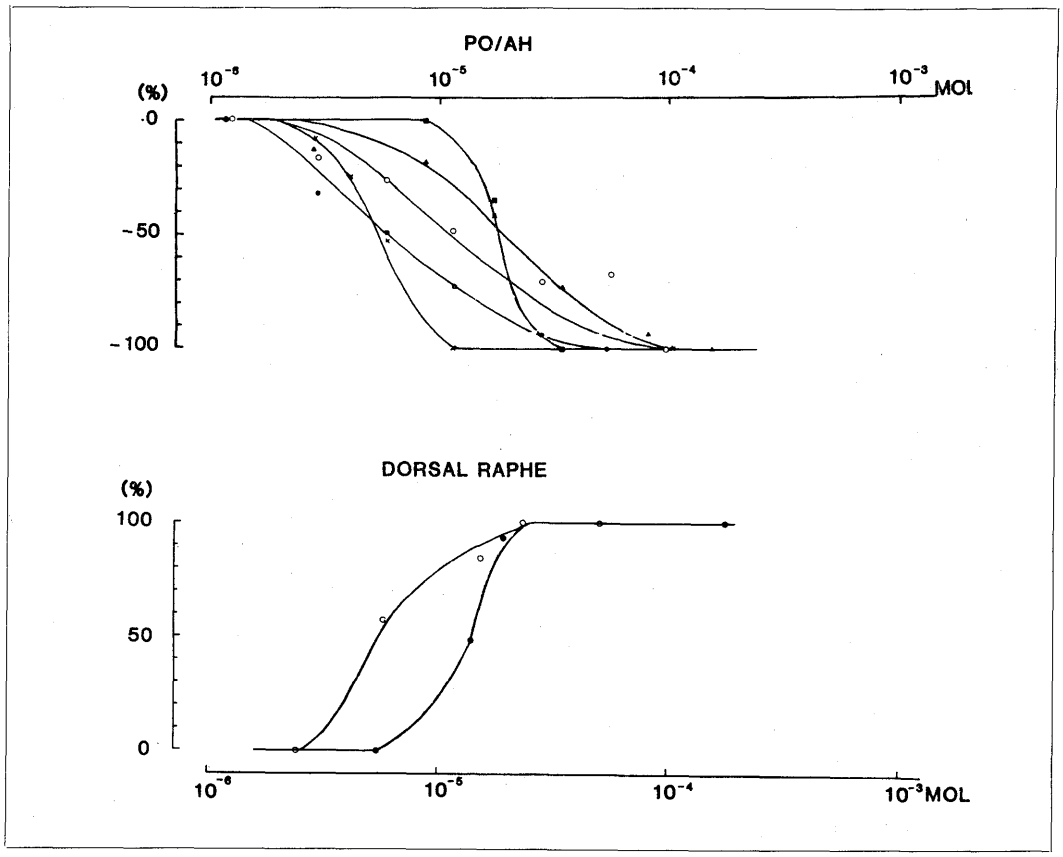

Fig. 4 The dose-response relationship when the warm-responsive neurons in the PO/AH region and the DR nucleus changed their activities upon application of NA. Response was expressed as the percentage change. The maximum change in the firing rates from the basal value was considered as the 100% response.

the firing rates in most of them, similar to the case of the warm-responsive neurons. However, 5-HT increased or decreased the activities of insensitive neurons in equal proportion, which is a different trend from those of warm-responsive neurons.

In the DR nucleus, however, more than 50% of the warm-responsive neurons decreased their firing rates with an application of 5-HT. With NA application, the warm-responsive neurons increased or decreased their firing rates. On the other hand, 5-HT decreased the activities of all of cold-responsive neurons and five of seven insensitive neurons. We can see the essential differences between the effects of amines on neurons in the PO/AH region and those in the DR nucleus.

THE DOSE-RESPONSE RELATION

In the in vivo single unit experiment, it is impossible to know the actual concentration of the drugs near the neurons injected. Using the in vitro tissue slices, however, the relationship between the amine's concentration and the response of the amine-treated neurons could be explored by bathing them with perfusate containing variable doses of the amines.; In particular, we could assess

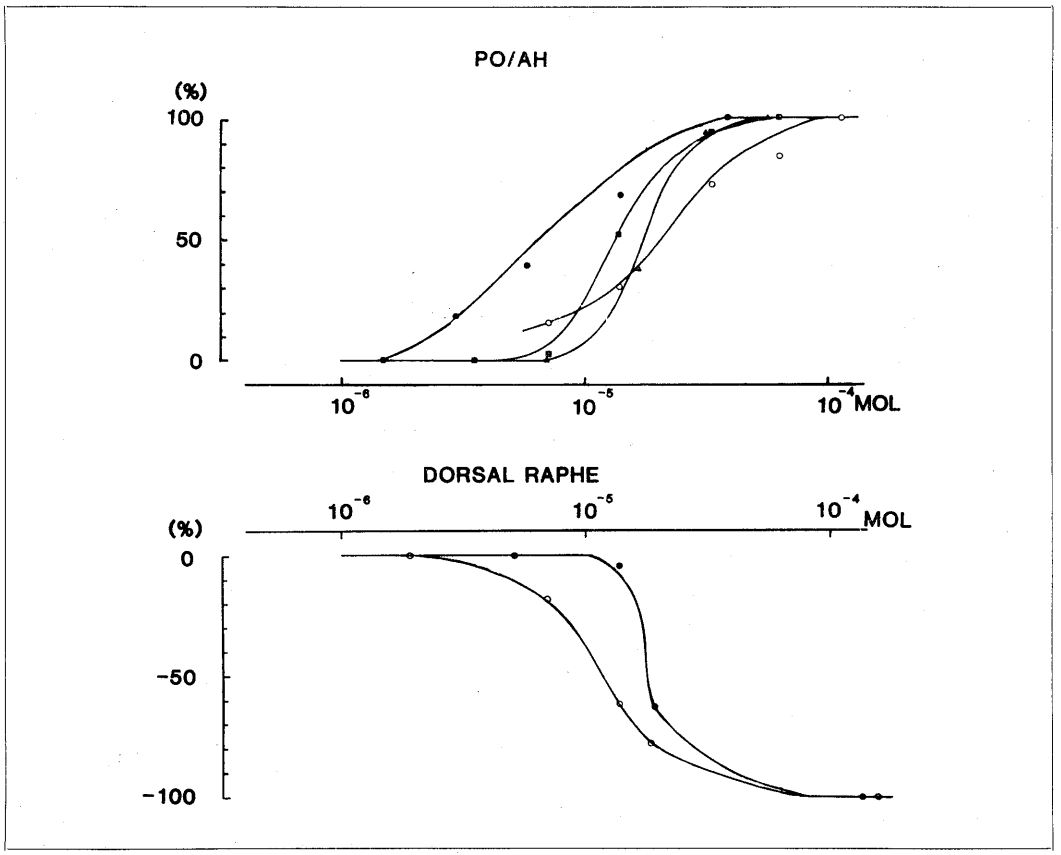

Fig. 5 The dose-response relationship when 5-HT changed the activities of the warm-responsive neurons in the PO/AH region and the DR nucleus. Response was expressed as the percentage change. The maximum change in the firing rates from the basal value was considered as the 100% response.

these relationships by examining the change in the firing rates of the warm-responsive neurons when perfusate containing various concentration of amines was applied. First, the maximum increase or decrease from the basal firing rates was determined and was considered to be a 100% change. Then, a value of some change by a given dose of amines from the base was divided by the 100% value in order to determine the percentage change in the firing rates of the neuron. These percentages were plotted against the logarithmic value of the concentration of amines. Fig. 4 shows the concentration-response relationships when NA decreased the firing rates of the warm-responsive neurons located in the PO/AH region and increased in the DR nucleus. In both cases, NA started to effect on the activities of the warm-responsive neurons at a minimum concentration of 5×10^{-6} mol. The maximum responses were obtained at a concentration of 5×10^{-5} mol. Fig. 5 shows the concentration-response relationships when 5-HT increased the activities of the warm-responsive neurons located in the PO/AH and decreased those in the DR nucleus. As shown in Fig. 5, 5-HT changed the activities of the warm-responsive neurons in the PO/AH region and the DR nucleus at a minimum concentration of 5×10^{-6} mol. This change reached a maximum at a concentration of 5×10^{-5} mol. Thus the warm-responsive neurons in both regions responded to NA and 5-HT within the same range of concentration.

Discussion

In the present study, the effects of 5-HT and NA on the thermoresponsive neurons were examined by the method of tissue slice preparations in vitro. As a consequence, 5-HT facilitated most of the warm-responsive neurons in the hypothalamus, the excitation of which has been thought to induce heat-loss responses. The present result is in agreement with the previous reports that serotonergic system in the brain in rats is activated during heat-exposure[19]. The insensitive neurons responded to 5-HT by increasing or decreasing their firing rates. This can be well explained by the fact that the insensitive neurons are concerned with various autonomic functions in the hypothalamus. NA had inhibitory effect on the warm-responsive neurons, suggesting that it might inhibit the heat-loss responses. However, NA decreased the activities of most of the insensitive neurons, as well as those of the warm-responsive neurons, suggesting that NA acts as a general inhibitory neurotransmitter in the hypothalamus. In fact, a previous report by Bligh et. al. indicated that the change of the body temperature by administration of amines depends on the ambient temperature and that NA might lower the activity of the thermoregulatory nervous system in the hypothalamus[4]. In 1971, Bligh proposed the neuronal model of the role of amines in the thermoregulatory center. According to his model, NA has cross-inhibitory effects on the neuronal pathway of both heat-loss and heat-gain responses. The model also proposes that 5-HT acts as a neurotransmitter facilitating heat-loss pathway. The general inhibitory action of NA and the facilitatory action of 5-HT on the warm-responsive neurons in the present study agree with the role of amines in Bligh's model.

In the mesencephalon, 5-HT lowered the activities of the warm-responsive neurons, cold-responsive neurons and insensitive neurons. These are opposite results to those obtained in the hypothalamus. In 1973, Hori and Nakayama revealed that in rabbits, 5-HT facilitated the warm-responsive neurons and inhibited the cold-responsive neurons in the hypothalamus, but in the mesencephalon 5-HT inhibited the warm-responsive neurons and facilitated the cold-responsive neurons[8].

They describe that the amine-sensitivity may be a different factor from temperature sensitivity. The present study shows the results similar to those by Hori and Nakayama and the same interpretation may be possible. In the present study, however, 5-HT inhibited the activities of both of the warm-responsive and the cold-responsive neurons. The effects of various substances on the thermoresponsive neurons have been examined and it has been indicated that these substances acted on the warm-responsive neurons with the opposite manner to on the cold-responsive neurons. However, that is not always the case with amines. Serotonin-containing neurons, most of which are involved in the raphe system in the mesencephalon, are understood to send their axons to the upper or lower central nervous system and to also regulate themselves in a way that their axon branches recurrently innervate themselves. This can explain the fact that most of the neurons in the DR nucleus are inhibited by 5-HT.

In addition to the amine-sensitivity of the thermoresponsive neurons, dose-response relationships were obtained between the applied doses of amines and the responses to amines of the thermoresponsive neurons in the hypothalamus and the mesencephalon. The present results show that 5-HT and NA, the concentrations of which ranged from 10^{-6} mol to 5×10^{-5} mol, altered the activities of the warm-responsive neurons in the hypothalamus and the mesencephalon. The total volume of cerebrospinal fluid (CSF) of a rat whose body weight is 300g might be calculated to be about 0.7cc, since a 60 kg human has about 140cc CSF. The dosage level of 0.08μg to 4.25μg is necessary to make the concentration of amines from 10^{-6} mol to 5×10^{-5} mol in the cerebral ventricle in rats when these substances were ICV administered. Therefore, it is desirable that the dose of amines injected into the cerebral ventricle ranges from 0.1μg to 4.0μg. Although many controversial reports about the effects of amines injected into the CNS on the body temperature in rats have been accumulated so far, these discrepancies may be, in part, due to the large doses of amines.

Consequently by the method of tissue slice preparations in vitro, results were obtained that better explain the effects of amines on the thermoresponsive neurons in the CNS involved in thermoregulatory responses than the results from in vivo experiment. Moreover, such a conclusion suggests the importance of elimination of the influences by various autonomic functions throughout a living body in vivo.

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