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Thermoregulatory Responses Following Injection of 5-Hydroxytryptamine Into the Septohippocampal Complex in Rats

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CUI, Y., T. F. LEE AND L. C. H. WANG. *Thermoregulatory responses following injection of 5-hydroxytryptamine into the septohippocampal complex in rats.* PHARMACOL BIOCHEM BEHAV 45(4) 935-939, 1993.—The present study investigated the change in thermoregulatory responses following microinjection of 5-hydroxytryptamine (5-HT) into the lateral septum and the hippocampus of unanesthetized, unrestrained rats. Intraseptal injection of 5-HT (5 to 20 μg) caused a dose-related fall in core temperature (T_b), which was associated with a decrease in heat production (HP). As the decrease in HP can not completely account for the magnitude of the decrease in T_b, increase in heat loss may also be involved in the 5-HT-induced hypothermia. In contrast to observed changes following intraseptal injection, no significant change in either T_b or HP was observed after microinjection of the same doses of 5-HT into the hippocampal areas, indicating that the hypothermic response to intraseptal injection of 5-HT is site specific. Further, the hypothermic response to intraseptal injection of 5-HT was only attenuated by systemic pretreatment with cyproheptadine, but not by naloxone or scopolamine, indicating that the hypothermic response is mediated by 5-HT receptor, but not by endogenous opioid and cholinergic systems.

5-Hydroxytryptamine Thermoregulation Lateral septum Hippocampus Hypothermia Heat production

SINCE the monoamine theory was proposed by Feldberg and Myers (7), 5-hydroxytryptamine (5-HT) has been implicated as one of the principle neurotransmitters in regulating body temperature (T_b). However, the role of this amine in thermoregulation still evokes controversy. When injected intracerebroventricularly, 5-HT elicits hyperthermia in cats, dogs, and monkeys, but hypothermia in goats, oxen, mice, and pigeons, and both hyper- and hypothermia in sheep, rabbits, and rats [for references see (15,27)]. Apart from differences in species and ambient temperature used, the divergent thermoregulatory responses of the animal to 5-HT could be attributed to anatomical specificity in site of application. This is evidenced by the fact that hyper- and hypothermia are observed following microinjection of 5-HT into the rostral and the preoptic anterior hypothalamic regions, respectively, of the cat and the rat [for references see (15)].

As compared with other monoamines, few studies have been carried out to examine the local sensitivity to 5-HT in sites other than the hypothalamus [for reference see (15)]. Circumstantial evidence indicates that the septohippocampal complex may play a role in thermoregulation. Electrical stimulation of the hippocampus in rats has been shown to elicit a rise and a fall in T_b in the warm and cold environment, respectively (18). Furthermore, thermosensitive neurons have also been found in the septum (10,16), and neuroanatomical circuits have been described between the hypothalamus and the septohippocampal complex (3,12,24). Using either autoradiographic or immunocytochemical technique, high densities of 5-HT fibers and terminals have been observed in the hippocampal and lateral septal areas in the rat brain (19,25). These raise the possibility that activation of the serotonergic system within the septal-hippocampal complex may invoke thermoregulatory responses. In order to test this hypothesis and to provide direct evidence, 5-HT was microinjected into the septum and the hippocampus of conscious rats and the ensuing thermoregulatory responses were documented.

METHOD

All experimental protocols used in the present study have received prior approval of the University of Alberta Animal Care Committee following the guidelines of the Canadian Council on Animal Care. Adult male Sprague-Dawley rats weighing approximately 400 g were used. They were housed individually at 20°C in a walk-in environmental chamber under 12L : 12D photoperiod. Food was rationed daily to maintain body weight around 400 g throughout the experimental period. Water was available at all times.

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Stereotaxic Procedure

Under halothane anesthesia, guide cannulae (23-ga stainless steel tubing) were implanted unilaterally into the lateral septum and the hippocampus of each rat utilizing the following stereotaxic coordinates: AP = 8.2 mm, L = 0.7 mm, H = 5.0 mm; and AP = 3.6 mm, L = 2.0 mm, H = 3.0 mm below the dura matter for septum and hippocampus, respectively (21). The tip of each guide tube was beveled and positioned 1.0 mm above the injection site in order to minimize damage to the actual injection site. After completion of the experiments, the precise anatomical location of the injection site was subsequently verified histologically.

Body Temperature and Oxygen Consumption Measurements

One week postoperatively, each rat was transferred to a circular, Plexiglas water-jacketed metabolism chamber (20 cm × 20 cm, diameter × height) in which the ambient temperature could be controlled accurately at 20 ± 1°C. The T_bs of the rats were recorded continuously with a precalibrated temperature-sensitive radiotransmitter (Model T-M, Mini-Mitter Co.), which was implanted in the peritoneal cavity under halothane anesthesia at the same time as the guide cannula. Exhaust gas from the metabolism chamber was divided into two streams: one stream for O_2 measurement (Applied Electrochemistry O_2 analyzer, S-3A/II) after desiccation with Drierite and CO_2 removal with Ascarite, and the second stream for measurement of CO_2 after desiccation (Applied Electrochemistry CO_2 analyzer, CD-3A). Using an IBM personal computer interfaced with an ISAAC 91-I data acquisition system, oxygen consumption and CO_2 production were recorded and integrated simultaneously with transmitter signal. Heat production (HP) was calculated from oxygen consumption and respiratory quotient using Kleiber's equation (26), and the T_b was calculated using preestablished calibration curves for each individual transmitter.

Once placed in the test chamber, the animals were not handled throughout the experimental period. At least 1.5 h elapsed before drug injection in order to ensure that the T_b and HP had attained a stable baseline upon which the drug effects could be assessed accurately. Behavioral observations were also noted throughout the experimental period.

Drugs Used and Data Analysis

The following compounds were used in the present study: cyproheptadine hydrochloride (Sigma), 5-HT creatinine sulphate (Sigma), naloxone hydrochloride (Sigma), and scopolamine hydrobromide (RBI). The doses in the Results section are expressed in terms of the salt. Each drug was prepared immediately before an experiment in a pyrogen-free artificial cerebrospinal fluid (CSF) containing 0.01 mg/ml sodium metabisulfite. All test solutions were passed through a 0.22 μm Swinnex Millipore filter to ensure sterility. Either CSF or various doses of 5-HT was injected into either the septum or hippocampus in a dose volume of 1 μl. For antagonism studies, drugs were injected intraperitoneally (IP) in a dose volume of 1 ml/kg 15 min before central 5-HT injection.

Wilcoxon's signed ranks test was used for statistical comparisons on the effects of treatments in individuals of the same group. Significance was set at $p < 0.05$, unless otherwise stated.

RESULTS

Thermoregulatory Effect of Intraseptal Injection of 5-HT

Control rats exposed to 20°C had an initial mean T_b of 37.6 ± 0.28°C and resting HP of 1.47 ± 0.07 Kcal/rat/h (n = 8). As shown in Fig. 1, intraseptal injection of CSF caused a slight rise in T_b and HP, occurring within 10 to 20 min after injection, possibly due to general disturbance of the microinjection procedure. In contrast, injection of 5-HT (5 to 20 μg) induced a dose-related decrease in T_b. The HPs of rats receiving higher doses of 5-HT (10 and 20 μg) were significantly lower than that of the CSF controls within the first 20–30 min after injection (Fig. 1), suggesting that the hypothermic response is partly brought about by reducing the metabolic rate. In most cases, especially after receiving the highest dose of 5-HT, an increase in activity (exploring and digging) of the

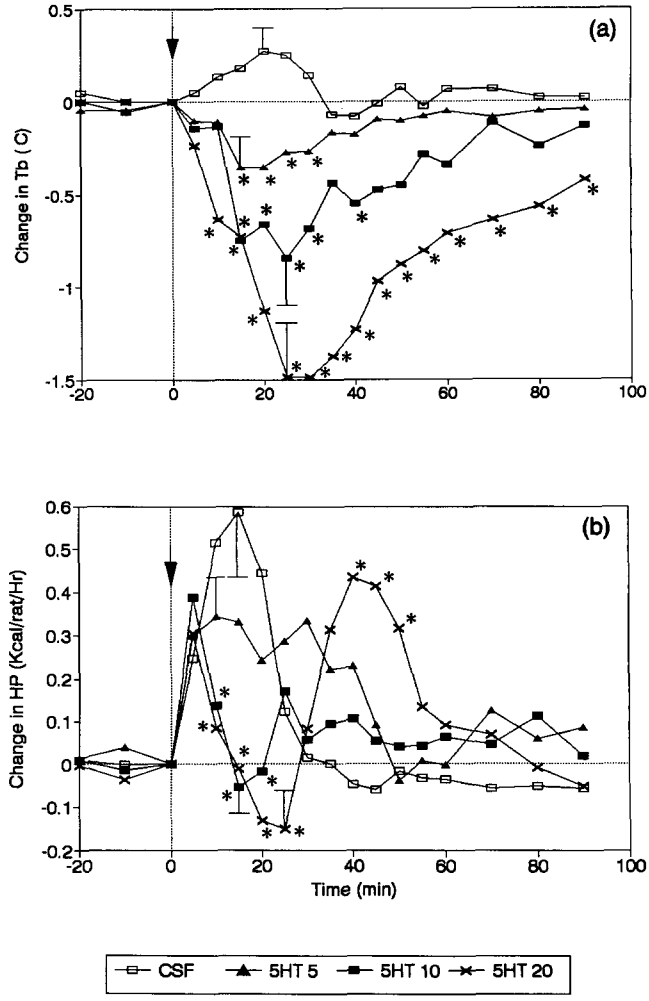

FIG. 1. Time course of changes in (a) core temperature and (b) heat production after intraseptal injection of CSF (□), 5-hydroxytryptamine 5 (▲), 10 (■), or 20 μg (X) in rats kept at 20°C. Each point represents the mean change from eight rats. The SE mean is represented by vertical bar and is shown only at the peak response value of each dose for clarity. Arrow indicates time of injection. *Significantly different from corresponding CSF control, $p < 0.05$ (Wilcoxon's signed ranks test).

animal was observed within the first 5 min immediately after injection. The rat then lay spread out on the floor of the metabolism chamber for the next 15–20 min. Thereafter, the animal returned to normal posture and displayed a slight increase in activity (grooming and licking).

A composite anatomical mapping of the microinjection sites into which 5-HT (20 μg) was injected is shown in Fig. 2. The most sensitive sites were found in the lateral septum. Injection of the same dose of 5-HT into either the medial septum or the caudate nucleus induced only a slight change in T_b and HP.

Thermoregulatory Effect of Intrahippocampal Injection of 5-HT

Figure 3 shows the time course of the metabolic and thermal responses to the microinjection of 5-HT into the hippocampus of rats kept at 20°C. In contrast to those observed following injection of 5-HT into the lateral septum, injection of 5-HT (either 5 or 20 μg) into the hippocampus only caused slight increases in both T_b and HP. None of these thermoregulatory changes were significantly different from that observed after CSF injection into the same site (Fig. 3). Furthermore, no notable change in the behavior of the rat was observed following intrahippocampal injection of either CSF or 5-HT.

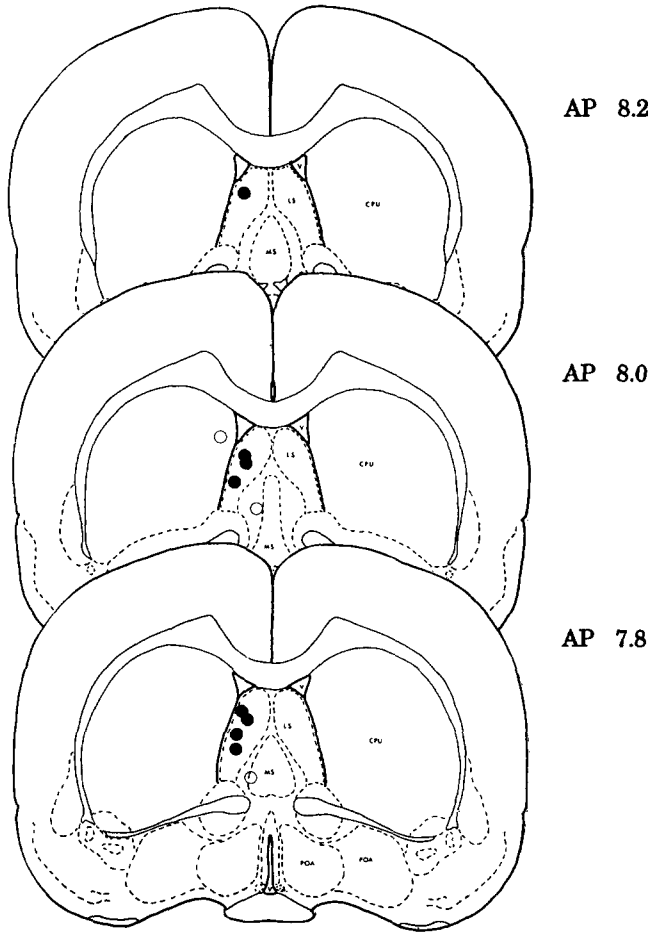

FIG. 2. Anatomical loci in the rat at which 20 μg 5-HT was microinjected to elicit either a drop (>0.5°C) (●) or no change (○) in core temperature. Abbreviations used: CPU = striatum, LS = lateral septum, MS = medial septum, POA = preoptic anterior hypothalamus, V = lateral ventricle.

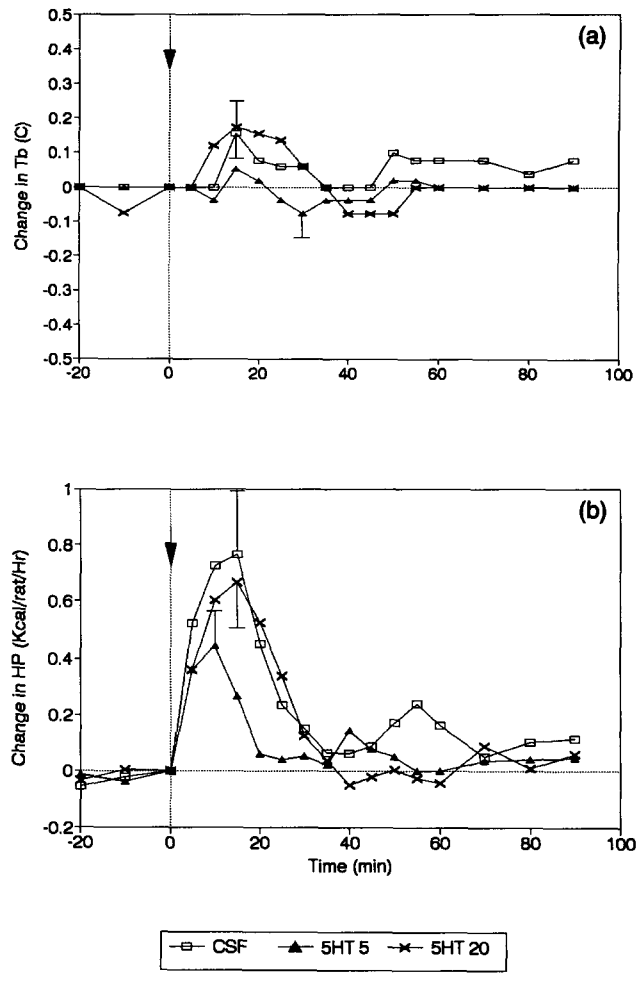

FIG. 3. Time course of changes in (a) core temperature and (b) heat production after intrahippocampal injection of CSF (□), 5-hydroxytryptamine 5 (▲) or 20 μg (X) in rats kept at 20°C. Each point represents the mean change from eight rats. The SE mean is represented by vertical bar and is shown only at the peak response value of each dose for clarity. Arrow indicates time of injection.

Effect of Various Antagonists on Intraseptal 5-HT-Induced Hypothermia

To investigate the specificity of the intraseptal 5-HT-elicited hypothermia, the effects of various receptor antagonists on the thermoregulatory response induced by the optimal dose of 5-HT (20 μg) were examined and the results are summarized in Table 1. Intraperitoneal pretreatment with cyproheptadine (2.5 mg/kg), a 5-HT receptor antagonist, significantly attenuated the thermoregulatory responses to intraseptal injection of 20 μg 5-HT (Table 1). After cyproheptadine pretreatment, the changes in behavioral activities typically observed after 5-HT injection were not seen. In contrast, the 5-HT induced hypothermic response was not affected by pretreating the animal with either scopolamine (1 mg/kg) or naloxone (5 mg/kg) at doses which have been shown previously

TABLE 1

EFFECTS OF INTRAPERITONEAL PRETREATMENT WITH CYPROHEPTADINE (2.5 mg/kg), NALOXONE (5 mg/kg) OR SCOPOLAMINE (1 mg/kg) ON MAXIMAL CHANGES IN CORE TEMPERATURE AND HEAT PRODUCTION INDUCED BY INTRASEPTAL INJECTION OF 5-HT (20 μg)

Treatment (n = 7)	Max. Change in T_b (°C)	Max. Change in HP (kcal/h/rat)
CSF + saline	+0.24 ± 0.15	+0.67 ± 0.14
CSF + cyproheptadine	+0.23 ± 0.11	+0.62 ± 0.26
CSF + naloxone	−0.08 ± 0.17	+0.25 ± 0.22
CSF + scopolamine	+0.31 ± 0.17	+0.70 ± 0.23
5-HT + saline	−1.54 ± 0.17*	−0.32 ± 0.06*
5-HT + cyproheptadine	+0.02 ± 0.21†	+0.08 ± 0.11†
5-HT + naloxone	−1.43 ± 0.16*	−0.37 ± 0.13*
5-HT + scopolamine	−1.26 ± 0.22*	−0.28 ± 0.15*

*Significantly different from CSF + saline control, $p < 0.05$.
†Significatly different from 5-HT + saline control, $p < 0.05$.

to attenuate the thermoregulatory responses induced by cholinergic and opioid receptor agonists, respectively (5,13) (Table 1). As shown in Table 1, neither of these antagonists by themselves, at the dose used in the present study, affected the thermoregulatory responses of the rat.

DISCUSSION

Most of the literature indicates that the hypothalamus is the primary central serotonergic site that participates in thermoregulation (15). However, as reported with many other neurotransmitters, extrahypothalamic areas may also be involved in temperature regulation. Previously, an increase in hippocampal 5-HT was observed in rats as the environmental temperature was elevated (23), implying that the hippocampal serotonergic system may be involved in regulating heat loss. To examine whether the septohippocampal serotonergic is involved in the heat loss processes, thermoregulatory responses to exogenously applied 5-HT to these areas were investigated in rats kept at 20°C when the tone in the heat loss pathways was presumably minimal. Intrahippocampal injection of 5-HT in doses used in the present study did not cause any significant changes in thermoregulatory responses of rats kept at 20°C. This result does not agree with previous observation that the hippocampal 5-HT may be involved in the heat loss pathways. Furthermore, as an increase in 5-HT turnover was also observed in other brain areas in the previous study, the serotonergic pathway within the hippocampus may possibly be activated as a secondary response to the heat stress (23). Taken together, it appears that the serotonergic system within the hippocampus may not be involved, at least, in regulating the heat loss processes.

In contrast to the response observed following the injection of 5-HT into the hippocampus, injection of 5-HT into the lateral septum caused a dose-dependent decline in T_b. The thermoregulatory change appears to be very site specific, because only a small change in T_b was observed when injections were made slightly outside of the lateral septal region. Because this decline in T_b was associated with a dose-related decrease in HP, the hypothermia observed after intraseptal injection of 5-HT may involve suppression of metabolic rate. Even though it was not measured in the present study, an increase in heat loss may also be responsible for the 5-HT-induced hypothermia. This is evident on three counts: a) the T_b started to decline without a decrease in HP immediately after injection; b) the decrease in HP could not completely account for the magnitude of the decrease in T_b (an increase, rather than a decrease, in HP was observed in the slight hypothermia induced by the lowest dose of 5-HT); and c) the behavioral changes (increase activity, prone body extension, and grooming) observed after intraseptal 5-HT injection were similar to the heat dissipation responses elicited during exposure to warm environments (22). Our suggestion that an increase in heat loss is also involved in intraseptal 5-HT-induced hypothermia is indirectly supported by a previous study involving injection of 5-HT into the lateral ventricle (20), which is anatomically adjacent to the lateral septum. In that study, a decline in T_b after 5-HT injection was accompanied with a rise in the tail skin temperature (20), suggesting an increase in heat dissipation. Based on these observations, it may be concluded that a concomitant decrease in metabolic rate and an increase in heat loss are associated with the hypothermia evoked by intraseptal 5-HT injection.

To test the specificity as well as the possible involvement of other neurotransmitters in intraseptal 5-HT induced hypothermia, various receptor antagonists were used. Intraperitoneal pretreatment with cyproheptadine, a 5-HT receptor antagonist, completely abolished the 5-HT induced hypothermia, suggesting that the thermoregulatory effects of 5-HT were elicited by activation of central 5-HT receptors. The endogenous opioid systems have been shown to interact with the serotonergic system in numerous physiological and pathophysiological responses (2,17). In addition, we had previously shown that intraseptal injection of metenkephalinamide caused hypothermia by suppressing metabolic HP (13). These observations raise the possibility that the endogenous opioid system may be involved in the intraseptal 5-HT-induced hypothermia. However, this does not appear to be the case, as intraperitoneal pretreatment with naloxone, an opioid receptor antagonist, failed to attenuate the 5-HT-induced thermoregulatory changes. The other neurotransmitter which may possibly be involved in intraseptal 5-HT induced hypothermia is acetylcholine, as a) a significant fall in T_b has been reported after implantation of crystalline carbachol, a cholinergic agonist, in the lateral septum of the rat (11) and b) the major projection from the septum to the hippocampal formation has been demonstrated to be cholinergic (14). To test this possibility, scopolamine, a cholinergic receptor antagonist, was used. Similar to the observations with naloxone, pretreating the animal with scopolamine had no effect on thermoregulatory responses consequent to intraseptal injection of 5-HT, suggesting that the cholinergic system is not involved in 5-HT-induced hypothermia. As the doses of naloxone and scopolamine used in the present study had previously been shown to attenuate the thermoregulatory responses induced by their respective agonists (5,13), the failure of these antagonists to reverse the 5-HT-induced hypothermia should not be due to insufficient dosages. Thus, the present results not only demonstrate that the observed hypothermia is mediated specifically by 5-HT receptors, but also suggest that both endogenous opioid and cholinergic systems are not involved in this response.

Although the present pharmacological study implicates a thermoregulatory role for the serotonergic system in the lateral septum of the rat, its functional significance in temperature regulation is unclear. Ample evidence suggests that the central serotonergic system is involved in the heat loss pathway. Hyperthermia and an increase in mortality have been

observed in rats pretreated with p-chlorophenylalanine, a 5-HT synthesis inhibitor, when exposed to a warm ambient temperature (6,8). Similarly, systemic pretreatment with cyproheptadine reduced the ability of the rat to cope with heat, but not cold (4). As drugs were administered systemically in these studies, the involvement of the septal serotonergic system in heat dissipation is a possibility. Moreover, the central serotonergic system has been implicated in sleep (1), during which heat loss increases and T_b declines, and the septohippocampal complex has been recognized as a key area in regulating the sleep process (9). It is, therefore, equally plausible that the septal serotonergic system, via its involvement in sleep, could also affect temperature regulation unique to this physiological state. The demonstration of the functional significance of the septal 5-HT involvement in thermoregulation clearly represents the next important challenge.

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REFERENCES

1. Borbely, A. A.; Tobler, I. Endogenous sleep-promoting substances and sleep regulation. Physiol. Rev. 69:605–670; 1989.
2. Brush, F. R.; Shain, C. N. Endogenous opioids and behavior. In: Brush, F. R.; Levine, S., eds. Psychoendocrinology. New York: Academic Press; 1989:379–435.
3. Conrad, L. C. A.; Pfaff, D. W. Axonal projections of medial preoptic and anterior hypothalamuic neurons. Science 190:1112–1114; 1975.
4. Cox, B.; Davis, A.; Juxon, V.; Lee, T. F.; Martin, D. A role for an indoleamine other than 5-hydroxytryptamine in the hypothalamic thermoregulatory pathways of the rat. J. Physiol. (Lond.) 337:441–450; 1983.
5. Cox, B.; Lee, T. F. Is acetylcholine involved in a dopamine receptor mediated hypothermia in mice and rats? Br. J. Pharmacol. 62:339–347; 1978.
6. Cronin, M. J. p-chlorophenylalanine hyperthermia in a warm environment: Reversal with 5-hydroxytryptophan. Brain Res. 112:194–199; 1976.
7. Feldberg, W.; Myers, R. D. A new concept of temperature regulation by amines in the hypothalamus. Nature 200:1325; 1963.
8. Giacchino, J. L.; Schertel, E. R.; Horowitz, J. M.; Horwitz, B. A. Effect of p-chlorophenylalanine on thermoregulation in unrestrained rats. Am. J. Physiol. 244:R299–302; 1983.
9. Heller, H. C. Hibernation: Neural aspects. Annu. Rev. Physiol. 41:305–321; 1979.
10. Hori, T.; Kiyohara, T.; Nakashima, T. Thermosensitive neurons in the brain—The role in homeostatic function. In: Mercer, J. B., ed. Thermal physiology. New York: Elsevier; 1989:3–12.
11. Hulst, S. G. T. Intracerebral implantation of carbachol in the rat: Its effect on water intake and body temperature. Physiol. Behav. 8:865–872; 1972.
12. Kiyohara, T.; Hori, T.; Shibata, M.; Nakashima, T.; Osaka, T. Neural inputs to preoptic thermosensitive neurons—Histological and electrophysiological mapping of central connections. J. Therm. Biol. 9:21–26; 1984.
13. Lee, T. F.; Nürnberger, F.; Jourdan, M. L.; Wang, L. C. H. Possible involvement of septum in seasonal changes in thermoregulatory responses to met-enkephalinamide in ground squirrels. In: Lomax, P.; Schönbaum, E., eds. Thermoregulation: Research and clinical applications. Basel: Karger; 1989:200–203.
14. Milner, T. A.; Loy, R.; Amaral, D. G. An anatomical study of the development of the septo-hippocampal projection in the rat. Dev. Brain Res. 8:343–371; 1983.
15. Myers, R. D.; Lee, T. F. Neurochemical aspects of thermoregulation. In: Wang, L. C. H., ed. Advances in environmental and comparative physiology, vol. 4. Berlin: Springer-Verlag; 1989: 161–203.
16. Nakashima, T.; Hori, T.; Kiyohara, T. Intracellular recording analysis of septal thermosensitive neurons in the rat. In: Mercer, J. B., ed. Thermal physiology. New York: Elsevier; 1989:69–72.
17. Oka, T. 5-HT and narcotic-induced hypothermia. Gen. Pharmacol. 9:151–154; 1978.
18. Osaka, T.; Hori, T.; Kiyohara, T.; Shibata, M.; Nakashima, T. Changes in body temperature and thermosensitivity of preoptic and septal neurons during hippocampal stimulation. Brain Res. Bull. 13:93–98; 1984.
19. Parent, A.; Descarries, L.; Beaudet, A. Organization of ascending serotonin systems in the adult rat brain. A radioautographic study after intraventricular administration of [^{3}H]5-HT. Neuroscience 6:115–138; 1981.
20. Popova, N. K.; Konusova, A. V. Brain and peripheral effects of serotonin on thermoregulation. Biogen. Amines 3:125–134; 1985.
21. Pellegrino, L. J.; Pellegrino, A. S.; Cushman, A. J. A stereotaxic atlas of the rat brain. New York: Plenum Press; 1979.
22. Roberts, W. W.; Mooney, R. D.; Martin, J. R. Thermoregulatory behaviors of laboratory rodents. J. Comp. Physiol. Psychol. 86:693–699; 1974.
23. Simmonds, M. A. Effect of environmental temperature on the turnover of 5-hydroxytryptamine in various areas of rat brain. J. Physiol. (Lond.) 211:93–108; 1970.
24. Staiger, J. F.; Nürnberger, F. The efferent connections of the lateral septal nucleus in the guinea pig: Projections to the diencephalon and brainstem. Cell Tissue Res. 264:391–413; 1991.
25. Steinbusch, H. W. M. Distribution of serotonin-immunoreactivity in central nervous system of the rat—cell bodies and terminals. Neuroscience 4:557–618; 1981.
26. Wang, L. C. H. Modulation of maxmimum thermogenesis by feeding in the white rat. J. Appl. Physiol. 49:975–978; 1980.
27. Wang, L. C. H.; Lee, T. F. Temperature regulation. In: Levine, S.; Brush, R., eds. Psychoendocrinology, vol. 2, Behaviors and regulation of species adaptation. New York: Academic Press; 1989:437–539.