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A Pharmacological Study of Veratrine-Induced Hyperthermia in the Rat: A Model of Neuroleptic Malignant Syndrome

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ABSTRACT

Stereotaxic microinjection of veratrine (50 μg in 1 μl of saline) into the preoptic anterior hypothalamus of rats which were intraperitoneally pretreated with haloperidol (1 mg/kg), significantly elevated body temperature (1.4 °C above normal body temperature) and produced abnormal behaviors. This microinjection also facilitated turnover of dopamine and serotonin in the regions of the thalamus and hypothalamus. Hyperthermia induced by haloperidol plus veratrine was significantly inhibited by systemic administration of serotonin antagonists (cyproheptadine 10 mg/kg, ritanserin 3 mg/kg). These findings suggest that hyperthermia in neuroleptic malignant syndrome is due to the dominant effect of serotonin in the thermoregulatory center either by blocking the dopamine receptor or by enhancing the serotonin secretion.

Key words: *Dopamine, Serotonin, Veratrine, Neuroleptic malignant syndrome*

Neuroleptic malignant syndrome (NMS) is a rare, but life-threatening complication of neuroleptic treatment. Its main symptoms are diffuse rigidity of the muscles often with extrapyramidal signs, an altered level of consciousness leading to either stupor or coma and dysfunction of the autonomic nervous system, such as hyperthermia, tachycardia and labile hypertension. A high incidence of NMS is recognized in the schizophrenic patients who have been given high potent dopamine (DA) receptor blockers such as haloperidol over a long period[14,17] and in the patients of Parkinson's disease from whom DA agonists (L-dopa[27,30], amantadine[28]) are suddenly withdrawn. The explanation[14] which emerged from clinical examinations of all these cases is that the content of DA becomes altered in hypothalamic thermoregulatory regions, leading to characteristic symptoms of hyperthermia.

It has been shown that injection of DA or its agonists decreases body temperature in a dose dependent manner[3,6]. On the contrary, serotonin (5-HT) and its analogue produce controversial changes in the body temperature in rats[20,22]; 5-HT_1 agonists produce hypothermic responses, while 5-HT_2 agonists provoke hyperthermia[11]. Since the receptor for 5-HT_2 is different from that for 5-HT_1[24], it is reasonable to assume that 5-HT_2 receptor is solely responsible for the rising action of body temperature.

These clinical as well as experimental findings indicate that body temperature may be regulated by the balance of the actions of DA-regulated and 5-HT-regulated systems in the hypothalamus. It has been speculated, therefore, that hyperthermic condition comes from either an increase in secretion of 5-HT by Na^+ channel modifier, veratrine, or from the blockade of dopamine receptor by its antagonists. An attempt was made to elucidate how DA and 5-HT are associated with the generation of hyperthermia in the rat, as a model of NMS, especially referring to the involvement of intracellular Ca^{2+} in the releasing mechanism of these transmitters in the central nervous system (CNS).

MATERIALS AND METHODS

Drugs

5-Hydroxy[G-^{3}H]tryptamine creatine sulphate was purchased from the Radiochemical Center (Amersham, specific activity 14 Ci/mmol U.S.A.). Veratrine hydrochloride, nicardipine hydrochloride, DL-propranolol hydrochloride, cyproheptadine hydrochloride, 5-hydroxytryptophan and carbidopa were purchased from Sigma (U.S.A.). Acetoxymethyl ester of fura-2 (fura-2/AM) was purchased from Dojindo laboratories. Verapamil hydrochloride, diltiazem hydrochloride, dantrolene sodium, ritanserin and fenfluramine hydrochloride were generously donated by Eizai Inc. (Japan), Tanabe Inc. (Japan), Yamanouchi Inc. (Japan), Janssen-Kyowa Inc. (Japan) and Fujisawa Inc. (Japan), respectively. All experiments with nicardipine were carried out in darkened conditions.

Experimental animals

Male wistar rats (290-310 g) were used in all experiments. They were housed five to a cage in a temperature controlled vivarium at 23°C on a 12 h light-dark cycle (light cycle between 7:00 and 19:00 h) and were provided with food and water *ad libitum*.

Cannula implantation

Stainless-steel cannula consisting of a guide tube (22 g, 0.71 mm o.d.) with a snug fitting trocar were used for the direct administration of the drug into the preoptic anterior hypothalamus (PO/AH)[18,19]. At the time of surgery, the rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). These cannula were implanted using the stereotaxic atlas and coordinates of Paxinos and Watson (1982)[23]. The coordinates followed were PO/AH : AP; 1.1 mm, L; 0.9 mm, DH; 8.5 mm below the surface of the scalp, using bregma as a reference. Drugs were injected through a inserted cannula connected to a 10 μl Hamilton microsyringe with Teflon tubing. The volume of injection was 1.0 μl at a rate of 1.0 μl/min. Sites of injection were verified at the end of the experiments using standard histological techniques in conjunction with the stereotaxic atlas of Paxinos and Watson[23].

Measurement of changes in body temperature

All experiments were performed between 2:00 and 5:00 p.m. at an ambient temperature of 23 ± 1°C at least 5 days after the cannula implantation. Rats were moved from the vivarium to the laboratory cases an hour before the experiments in order to habituate them to the experimental environment. Rectal temperature was monitored with a thermister probe (YSI-402) connected to a YSI-90TA digital thermometer (Yellow Springs Instrument CO, Yellow Springs, OH) installed in the rectum 8 cm away from anus.

Measurement of monoamines in the regions of thalamus and hypothalamus

Monoamines in thalamus and hypothalamus were measured by the method of Yokota (1989)[31]. The rats were quickly killed by microwave application (5 KW 0.9 sec) one hour after stereotaxic microinjection. 5-HT, 5-hydroxyindol acetic acid (5-HIAA), DA, 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured by high-performance liquid chromatography. Isoprotenelol was used as an internal standard. An analysis column of Hiber Richlosorb RP-1 was used for separation.

Measurement of [^{3}H]-5HT

Release of [^{3}H]-5HT from rat hypothalamic slices were monitored with the slightly modified Gothert's (1980) method[9]. Slices of hypothalamus (at 0.4 mm in thickness) were incubated in physiological salt solution containing 0.1 μM [^{3}H]-5HT creatine sulphate for 30 min at 37°C under gentle bubbling with 95% O_2 - 5% CO_2 gas. At the end of incubation, the slices were transferred to superfusion chambers in which physiological salt solution flowed at a rate of 0.5 ml/min. The composition of the physiological salt solution was (in mM): NaCl, 118; KCl, 4.8; $CaCl_2$, 1.3; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; ascorbic acid, 0.06; EDTA, 0.03; glucose, 10 nialamide, 0.01; pH7.4 gassed with O_2/CO_2 (95/5%). In the experiments using high K^+ as depolarizing stimulus, the slices were stimulated twice with 30 mM KCl for 4 min each at 60 min (S_1) and 90 min (S_2) after the start of superfusion. In veratrine stimulation, the sliced preparation were soaked in Ca^{2+}-free solution containing 1.0 mM EGTA and were subjected to Ca^{2+}-free solution with 10 μg/ml (about 15 μM) veratrine for 4 min twice each at 60 min (S_1) and 110 min (S_2). Each superfusate was collected at 5 min intervals. At the end of these release experiments, the radioactivity of both the slices and superfusate was determined by liquid scintillation spectrometry.

Assay of intrasynaptosomal [Ca^{2+}]i

Synaptosomes were obtained by the method of Gray and Whittaker (1962)[10]. Cerebrum except the cerebellum and ponsmedulla was dissected out from the rat brain. The synaptosomes was suspended in Hepes buffer to give the tissue concentration 0.15 g-equivalent/ml (about 2.6-3.1 mg/ml of protein). The composition of Hepes buffer was (mM): NaCl, 125; KCl, 5.0; $CaCl_2$, 1.0; NaH_2PO_4, 1.2; $MgCl_2$, 1.2 $NaHCO_3$, 5; glucose, 6; HEPES, 25; pH 7.4. [Ca^{2+}]i was measured by the modified method of Komulainen and Bondy (1987)[15,16].

Statistics

The statistical significance was determined by Student's two-tailed t-test in comparing values and by Mann-Whitney U-test in comparing curves.

RESULTS

Changes in body temperature induced by microinjection of veratrine into PO/AH in rats pretreated with haloperidol

1 μl of sterile saline of containing 0 μg, 5 μg, 50 μg, 100 μg veratrine were microinjected into PO/AH in rats to which haloperidol (1 mg/kg) had been intraperitoneally given 30 min before injection of veratrine. Rectal temperature was monitored successively in control, in haloperidol and each 15 min after veratrine injection from 0 to 60 min. Injection of more than 50 μg veratrine into PO/AH significantly increased rectal temperature [Fig. 1(A)]. Change in body temperature by administration of haloperidol plus veratrine (50 μg) and that by administration of saline plus veratrine showed a statistical significance of $p<0.001$ [Fig.1(B)]. This suggests that hyperthermia is mediated by the release of monoamines by the application of veratrine in PO/AH.

Changes in the contents of metabolites of DA and 5-HT in thalamus and hypothalamus [Table 1(A), (B)]

A stereotaxic microinjection of saline (1μl) or ver-

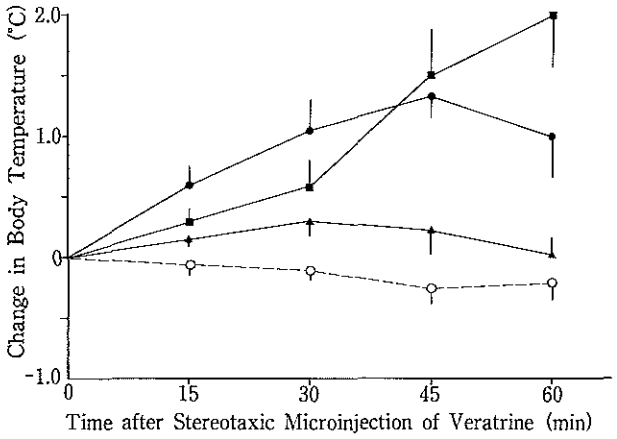

Fig. 1(A). Body temperature responses to different doses of veratrine into PO/AH in rats pretreated with haloperidol. Pretreatment with haloperidol did not significantly affect base-line body temperature (38.22 ± 0.05, n = 32). The results were means ± S.E.M. of eight determinations presented as degree of temperature change from base-line temperature. ○: Haloperidol (1 mg/kg) + Saline (1 μl). ▲: Haloperidol (1 mg/kg) + Veratrine (5 μg in 1 μl of saline). ●: Haloperidol (1 mg/kg) + Veratrine (50 μg in 1 μl of saline). ■ : Haloperidol (1 mg/kg) + Veratrine (100 μg in 1 μl of saline).

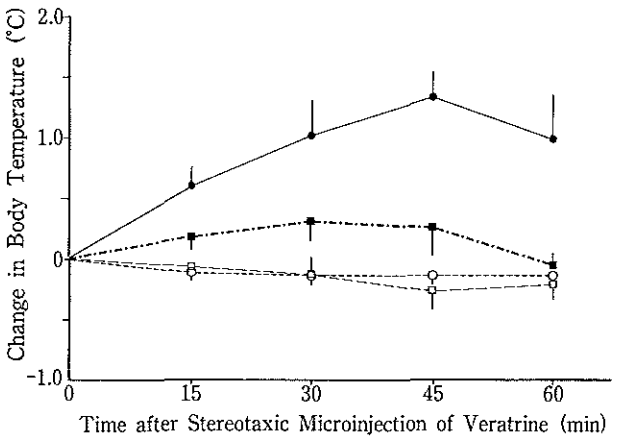

Fig. 1(B). Effect of pretreatment of haloperidol on change in body temperature induced by microinjection of veratrine into PO/AH. Pretreatment with haloperidol or saline did not significantly affect base-line body temperature (38.20 ± 0.10, n = 16, 38.24 ± 0.12, n = 16, respectively). The results were means ± S.E.M. of eight determination presented as degree of temperature change from base-line temperature. ○: Saline (1 ml/kg) + Saline (1 μl). □: Haloperidol (1 mg/kg) + Saline (1 μl). ●: Haloperidol (1 mg/kg) + Veratrine (50 μg in 1 μl of saline). ■ : Saline (1 ml/kg) + Veratrine (50 μg in 1 μl of saline).

Table 1(A). Changes in content of metabolites of DA and 5-HT in the region of thalamus and hypothalamus after stereotaxic microinjection of veratrine (VRT)

		DOPAC	HVA	DA	5-HIAA	5-HT	(nmol/g)
SAL + SAL	(6)	0.74 ± 0.06	0.21 ± 0.05	3.30 ± 0.44	3.35 ± 0.40	6.89 ± 0.13	
HPD + SAL	(6)	1.22 ± 0.12[a]	0.66 ± 0.13[a]	2.83 ± 0.12	3.96 ± 0.26	7.20 ± 0.15	
HPD + VRT	(6)	1.95 ± 0.26[b]	1.22 ± 0.29[b]	3.19 ± 0.26	5.47 ± 0.40[b]	6.97 ± 0.20	

[a]$p<0.01$, significantly higher compared to corresponding values of rats intraperitoneally pretreated with saline (SAL). [b]$p<0.05$, significantly higher compared to corresponding values of microinjection of SAL into PO/AH in rats pretreated with haloperidol (HPD). All values were means ± S.E.M. of six determinations.

Table 1(B). Turnover of DA and 5-HT in the thalamus and hypothalamus after microinjection of veratrine (VRT)

		Ratio of metabolic rate of monoamines	
		(DOPAC + HVA)/DA	5-HIAA/5-HT
SAL + SAL	(6)	0.31 ± 0.07	0.49 ± 0.05
HPD + SAL	(6)	0.67 ± 0.09[a]	0.54 ± 0.02
HPD + VRT	(6)	0.97 ± 0.09[b]	0.78 ± 0.04[b]

[a]$p<0.01$, significantly higher compared to corresponding values of rats intraperitoneally pretreated with saline (SAL). [b]$p<0.05$, significantly higher compared to corresponding values of microinjection of SAL into PO/AH in rats pretreated with haloperidol (HPD). All values were means ± S.E.M. of six determinations.

atrine (50 μg in 1 μl of saline) into PO/AH in cannula implanted rat was performed 30 min after the pretreatment with intraperitoneal injection of saline (1 ml/kg) or haloperidol (1 mg/kg). The intraperitoneal application of haloperidol (1 mg/kg) significantly increased the content of DOPAC ($p<0.01$) and HVA ($p<0.01$), two major metabolites of DA, but produced no changes in 5-HIAA, a metabolite of 5-HT. With microinjection of veratrine (50 μg) into PO/AH in rats pretreated with haloperidol, the levels of DOPAC and 5-HIAA were significantly higher than those in haloperidol alone ($p<0.05$). This alkaloid also facilitated turnover of both DA; (DOPAC+HVA)/DA ($p<0.05$) and 5-HT; 5-HIAA/5-HT ($p<0.05$).

Effects of ritanserin, cyproheptadine, and DL-propranolol on the hyperthermia induced by microinjection of veratrine (50 μg in 1 μl of saline) into PO/AH in rats pretreated with haloperidol (1 mg/kg, i.p.) 30 min before veratrine injection

In order to elucidate the role of serotonergic or beta-adrenergic system in the hyperthermia induced by haloperidol plus veratrine, ritanserin, a 5-HT_2 antagonist, cyproheptadine, a classical 5-HT antagonist and DL-propranolol, a beta-adrenergic an-

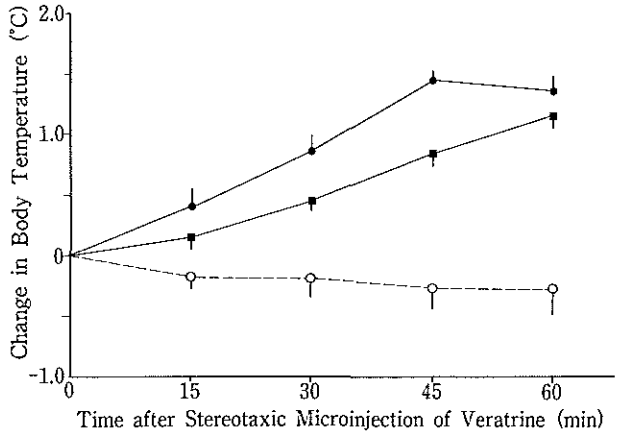

Fig. 2. Effect of ritanserin on hyperthermia induced by microinjection of veratrine into PO/AH in rats pretreated with haloperidol. Pretreatment with haloperidol and ritanserin did not significantly affect base-line body temperature (38.24 ± 0.10, n = 16). The results were means ± S.E.M. of eight determinations presented as degree of temperature change from base-line temperature. ■ : Haloperidol (1 mg/kg) + Ritanserin (3 mg/kg) + Saline (1 μl). ●: Haloperidol (1 mg/kg) + Saline (1 ml/kg) + Veratrine (50 μg in 1 μl of saline). ○: Haloperidol (1 mg/kg) + Ritanserin (3 mg/kg) + Veratrine (50 μg in 1 μl of saline).

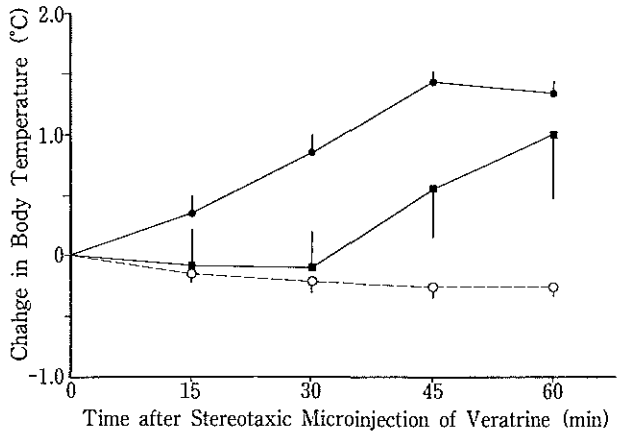

Fig. 3. Effect of cyproheptadine on hyperthermia induced by microinjection of veratrine into PO/AH in rats pretreated with haloperidol. Pretreatment with haloperidol and cyproheptadine did not significantly affect base-line body temperature (38.21 ± 0.08, n = 16). The results were means ± S.E.M. of eight determinations presented as degree of temperature change from base-line temperature. ■ : Haloperidol (1 mg/kg) + Cyproheptadine (10 mg/kg) + Saline (1 μl). ●: Haloperidol (1 mg/kg) + Saline (1 ml/kg) + Veratrine (50 μg in 1 μl of saline). ○: Haloperidol (1 mg/kg) + Cyproheptadine (10 mg/kg) + Veratrine (50 μg in 1 μl of saline).

tagonist were administered intraperitoneally 15 min before the cerebral injection of veratrine. 3 mg/kg ritanserin, 10 mg/kg cyproheptadine and 40 mg/kg DL-propranolol significantly inhibited this hyperthermia ($p<0.05$, $p<0.05$, $p<0.001$, respectively)

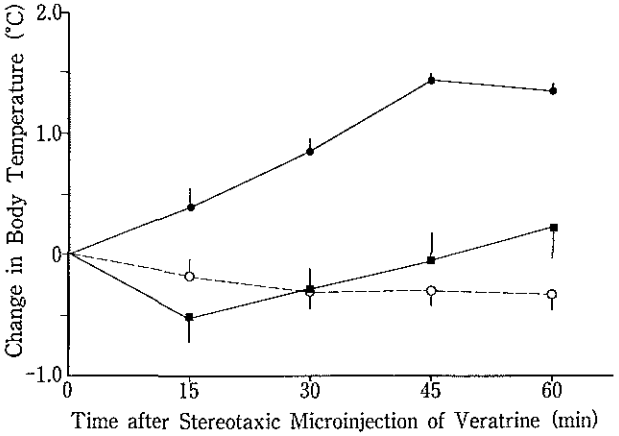

Fig. 4. Effect of DL-propranolol on hyperthermia induced by microinjection of veratrine into PO/AH in rats pretreated with haloperidol. Pretreatment with haloperidol and DL-propranolol did not significanly affect base-line body temperature (38.16 ± 0.09, n = 16). The results were means ± S.E.M. of eight determinations presented as degree of temperature change from base-line temperature. ○: Haloperidol (1 mg/kg) + DL-propranolol (40 mg/kg) + Saline (1 μl). ●: Haloperidol (1 mg/kg) + Saline (1 ml/kg) + Veratrine (50 μg in 1 μl of saline). ■ : Haloperidol (1 mg/kg) + DL-propranolol (40 mg/kg) + Veratrine (50 μg in 1 μl of saline).

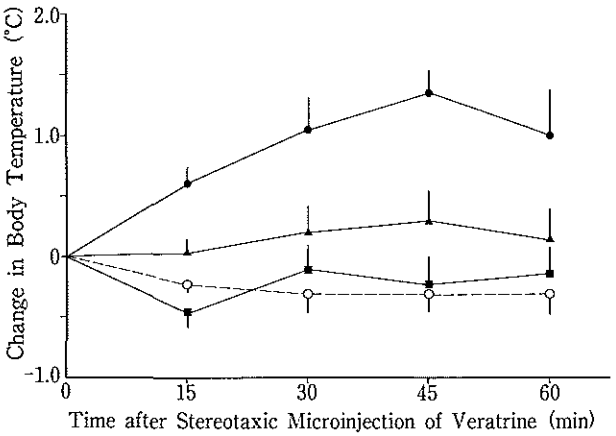

Fig. 5. Effect of dantrolene on hyperthermia induced by microinjection of veratrine into PO/AH in rats pretreated with haloperidol. Pretreatment with haloperidol and dantrolene did not significantly affect base-line body temperature (38.22 ± 0.09, n = 16). The results were means ± S.E.M. of eight determinations presented as degree of temperature change from base-line temperature. ○: Haloperidol (1 mg/kg) + Dantrolene (40 mg/kg) + Saline (1 μl). ●: Haloperidol (1 mg/kg) + Saline (1 ml/kg) + Veratrine (50 μg in 1 μl of saline). ▲: Haloperidol (1 mg/kg) + Dantrolene (20 mg/kg) + Veratrine (50 μg in 1 μl of saline). ■ : Haloperidol (1 mg/kg) + Dantroline (40 mg/kg) + Veratrine (50 μg in 1 μl of saline).

[Fig. 2, 3, 4]. These results suggest that this hyperthermia is mediated by the serotonergic and bateadrenergic system.

Table 2(A). Dose response effect of intraperitoneal administration of fenfluramine (FEN) on change in body temperature

			Change in body temperature (△°C)				
			Time after administration of SAL or FEN				
			0	15	30	45	60 (min)
SAL		(8)	0	−0.09 ± 0.01	−0.10 ± 0.02	−0.13 ± 0.01	−0.13 ± 0.02
FEN	3.0 mg/kg[a]	(8)	0	0.22 ± 0.20	0.48 ± 0.23	0.75 ± 0.28	1.08 ± 0.26
	7.5 mg/kg[b]	(8)	0	0.35 ± 0.07	0.58 ± 0.10	1.13 ± 0.12	1.39 ± 0.14
	15.0 mg/kg[b]	(8)	0	0.50 ± 0.07	0.87 ± 0.09	1.24 ± 0.18	1.89 ± 0.12

[a]$p<0.01$ or [b]$p<0.001$, significantly higher compared to the saline (SAL) curve. The results were means ± S.E.M. of eight determinations presented as degree of temperature change from base-line temperature.

Table 2(B). Effect of dantrolene (DAN) on hyperthermia induced by intraperitoneal administration of fenfluramine (FEN) or 5-hydroxytryptophan (5-HTP)

		Change in body temperature (°C)				
		Time after administration of SAL, FEN or 5-HTP				
		0	15	30	45	60 (min)
SAL + SAL	(8)	0	−0.09 ± 0.01	−0.10 ± 0.02	−0.13 ± 0.01	−0.13 ± 0.02
SAL + DAN 40 mg/kg	(8)	0	−0.11 ± 0.05	−0.22 ± 0.07	−0.23 ± 0.07	−0.25 ± 0.03
FEN + SAL	(8)	0	0.35 ± 0.07	0.58 ± 0.10	1.13 ± 0.12	1.39 ± 0.14
FEN + DAN 40 mg/kg	(8)	0	0.11 ± 0.12	0.67 ± 0.16	1.36 ± 0.21	1.45 ± 0.22
5-HTP + SAL	(8)	0	0.46 ± 0.10	0.82 ± 0.20	1.87 ± 0.18	2.14 ± 0.26
5-HTP + DAN 40 mg/kg	(8)	0	0.37 ± 0.14	0.92 ± 0.17	1.56 ± 0.15	1.94 ± 0.15

The results were means ± S.E.M. of eight determinations presented as degree of temperature change from base-line temperature. SAL: saline.

Table 3. Effect of dantrolene on an increase in $[Ca^{2+}]i$ in synaptosomes induced by high K^+ or by veratrine

		Increase in $[Ca^{2+}]i$ (nM)			
Addition		High K^+		Veratrine	
Dantrolene	0 μM	298 ± 16	(5)	249 ± 13	(5)
	1 μM	307 ± 16	(6)	241 ± 10	(5)
	10 μM	202 ± 3[a]	(6)	188 ± 7[a]	(5)

[a]$p<0.05$, significantly lower compared to corresponding values with stimulation of high K^+ or veratrine in the absence of dantrolene. All values were means ± S.E.M. of five to six determinations.

Effects of dantrolene on the hyperthermia induced by microinjection of veratrine (50 μg in 1μl of saline) into PO/AH in rats pretreated with haloperidol (1 mg/kg, i.p.) 30 min before veratrine injection

Dantrolene is a therapeutic drug for NMS[5]. In order to elucidate whether dantrolene could attenuate the hyperthermia induced by haloperidol plus veratrine, dantrolene (20 mg/kg, 40 mg/kg) was administered i.p. 15 min before the cerebral injection of veratrine. Dantrolene (20 mg/kg, 40 mg/kg) significantly inhibited this hyperthermia ($p<0.01$, $p<0.001$, respectively) in a dose dependent manner (Fig. 5).

Effects of dantrolene on hyperthermia induced by fenfluramine or 5-hydorxytryptophan

Intraperitoneal injection of fenfluramine, a 5-HT releasing agent, elicited a dose related increase in rectal temperature [Table 2(A)]. 5-hydroxytrptophan (150 mg/kg, i.p.), a 5-HT precursor, plus carbidopa (25 mg/kg, i.p.), a peripheral decarboxylase inhibitor, also produced hyperthermia. Dantrolene (40 mg/kg, i.p.) could not keep the body temperature from increasing under the action of either fenfluramine (7.5 mg/kg, i.p.) or by 5-hydroxytryptophan (150 mg/kg, i.p.) [Table 2(B)].

Effects of dantrolene on elevation of free intrasynaptosomal $[Ca^{2+}]i$ induced by high K^+ or veratrine (Table 3)

The resting level of $[Ca^{2+}]$; was 485 ± 25 nM (n = 32) when 1 mM Ca^{2+} was present in the incubation medium. The mean increases of intrasynaptosomal $[Ca^{2+}]i$, stimulated by 50 mM KCl and by veratrine (67 μg/ml about 100 μM) were 298 ± 16 nM (n = 5) and 249 ± 13 nM (n = 5), respectively. Dantrolene (10 μM) significantly attenuated both high K^+- and veratrine-induced $[Ca^{2+}]i$ increase, by 32.2% ($p<0.05$) and by 24.4% ($p<0.05$), respectively.

Effects of Ca^{2+} channel blockers on release of [^{3}H]5-HT induced by high K^+ or veratrine from slices of hypothalamus

Release of [^{3}H]5-HT from slices of hypothalamus was examined in response to each stimulation with 30 mM KCl and with 10 μg/ml veratrine (Fig. 6). The release of [^{3}H]5-HT induced by high K^+ from slices of hypothalamus was completely suppressed

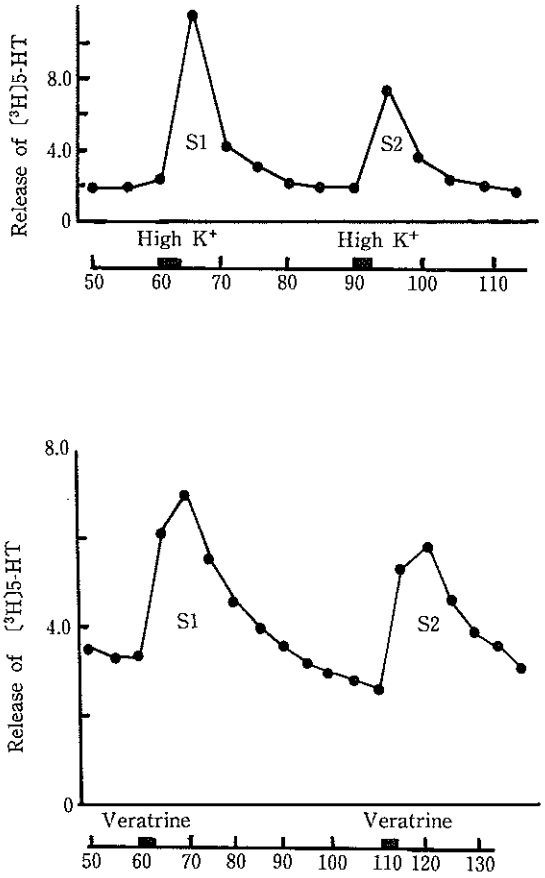

Fig. 6. The release of [^{3}H]5-HT stimulated by high K^+ and veratrine.

in the Ca^{2+}-free medium containing 1 mM EGTA. However, the release of tritium by veratrine (10 μg/ml, about 15 μM) was recognized even in this Ca^{2+}-free medium. The first stimulation period (S_1, was used as control and Ca^{2+} channel blockers were added to the medium 20 min before the second stimulation period (S_2) and remained present throughout the rest of the superfusion. Drug-induced changes of release of [^{3}H]5-HT were compared with the quotient S_2/S_1 which is the ratio of tritium released by the first (S_1) and second stimulation (S_2). The ratio (S_2/S_1) by high K^+ and by veratrine were found to be fairly constant at 0.71 ± 0.03 (n = 18) and 0.78 ± 0.03 (n = 18), respectively.

Nicardipine (10 μM) significantly reduced high K^+-induced release of [^{3}H]5-HT by 25% ($p<0.05$) and release by veratrine by 71.2% ($p<0.001$). Diltiazem (100 μM) significantly reduced the high K^+-induced [^{3}H]5-HT release by 47.1% ($p<0.01$). Furthermore, diltiazem (1 μM, 10 μM, 100 μM) significantly reduced the veratrine-induced release in a dose dependent manner by 48.6%, 77.1% and 87.1% ($p<0.01$, $p<0.001$, $p<0.001$, respectively). Verapamil (100 μM) significantly reduced the high K^+-evoked [^{3}H]5-HT release by 47.9% ($p<0.01$) and the veratrine-induced release by 87.7% ($p<0.001$) [Fig. 7(A), 7(B)].

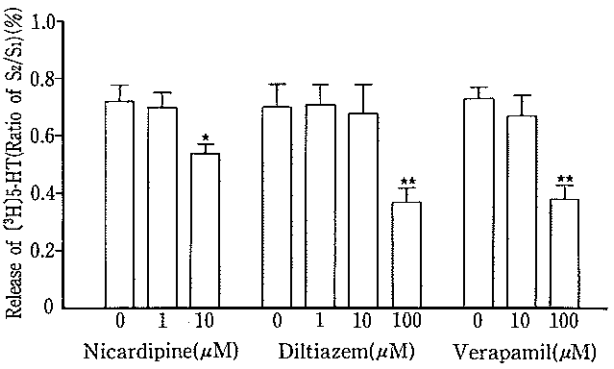

Fig. 7(A). Effect of Ca^{2+} channel blockers on release of [^{3}H]-5HT induced by high K^+ from slices of hypothalamus of rat brain. *$p<0.05$ or **$p<0.01$, significantly lower compared to corresponding values with stimulation of high K^+ in the absence of Ca^{2+} channel blockers. All values were means ± S.E.M. of four to six determinations.

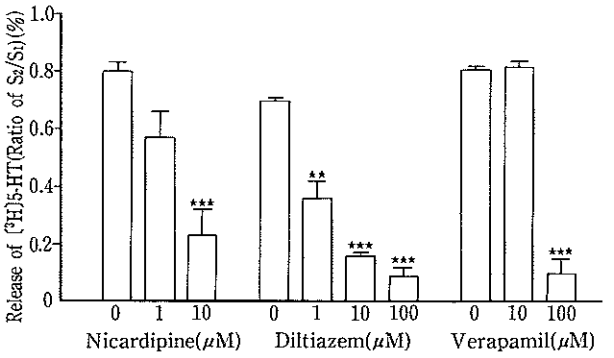

Fig. 7(B). Effect of Ca^{2+} channel blockers on release of [^{3}H]-5HT induced by veratrine from slices of hypothalamus of rat brain. **$p<0.01$ or ***$p<0.001$, significantly lower compared to corresponding values with stimulation of veratrine in the absence of Ca^{2+} channel blockers. All values were means ± S.E.M. of five to six determinations.

Effects of Ca^{2+} channel blockers on the hyperthermia induced by microinjection of veratrine (50 μg in 1μl of saline) into PO/AH in rats pretreated with haloperidol (1 mg/kg, i.p.) 30 min before veratrine injection [Table 4]

The systemic application (15 min before veratrine injection) of Ca^{2+} channel blockers such as nicardipine, diltiazem and verapamil could not inhibit the hyperthermia induced by haloperidol plus veratrine. This unexpected result could be explained by either nonpermeability of Ca^{2+} channel blockers through the blood brain barrier or poor sensitivity to these durgs in the Ca^{2+} channel of CNS.

DISCUSSION

This experiment has shown that hyperthermia can be induced by the injection of veratrine into PO/AH when the dopaminergic system is pharmacologically blocked. Since veratrine increases not only membrane permeability to Na^+[1)] but also mobiliza-

Table 4. Effect of Ca^{2+} channel blockers on hyperthermia induced by microinjection of veratrine (VRT) into PO/AH in rats pretreated with haloperidol (HPD)

		Change in body temperature (°C)				
		Time after microinjection of SAL or VRT				
		0	15	30	45	60 (min)
HPD + SAL + SAL	(8)	0	−0.06 ± 0.08	−0.09 ± 0.12	−0.25 ± 0.16	−0.21 ± 0.13
HPD + SAL + NDP 10 mg/kg	(8)	0	−0.13 ± 0.07	−0.18 ± 0.07	−0.15 ± 0.11	−0.21 ± 0.12
HPD + SAL + DZM 40 mg/kg	(8)	0	−0.23 ± 0.08	−0.25 ± 0.04	−0.31 ± 0.03	−0.33 ± 0.03
HPD + SAL + VML 40 mg/kg	(8)	0	−0.13 ± 0.06	−0.15 ± 0.08	−0.22 ± 0.09	−0.22 ± 0.09
HPD + VRT + SAL	(8)	0	0.39 ± 0.15	0.84 ± 0.13	1.43 ± 0.09	1.35 ± 0.10
HPD + VRT + NDP 10 mg/kg	(8)	0	0.31 ± 0.08	0.68 ± 0.08	0.95 ± 0.11	1.19 ± 0.12
HPD + VRT + DZM 40 mg/kg	(8)	0	0.58 ± 0.14	1.01 ± 0.21	1.11 ± 0.26	1.40 ± 0.14
HPD + VRT + VML 40 mg/kg	(8)	0	0.35 ± 0.08	0.89 ± 0.21	1.21 ± 0.22	1.59 ± 0.36

The results were means ± S.E.M. of eight determinations presented as degree of temperature change from base-line temperature. SAL: saline, NDP: nicardipine, DZM: diltiazem, VML: verapamil.

tion of the intracellular Ca^{2+}[26], availability of free Ca^{2+} is increased in the presynaptic terminals, leading to the enhanced neurotransmitter release. Moreover, the microinjection of veratrine into the PO/AH significantly increases the content of 5-HT metabolites, 5-HIAA and that of DA metabolites, HVA and DOPAC. Thus, it is reasonable to assume that this hyperthermia is due to the increase in neurotransmitter release in PO/AH.

It is generally accepted that PO/AH is deeply involved in thermoregulation, which is regulated with the projection of monoaminergic neuron[2,4,12]. It has been found that the dopaminergic system in PO/AH plays a significant role in reducing body temperature[7,18,25], but the serotonergic system causes inconsistent thermoregulatory responses[20,22]. Activation of 5-HT_2 receptor in CNS in rats produces hyperthermia, whereas that of 5-HT_1 receptor produces hypothermia[11].

This hyperthermia induced by haloperidol plus veratrine may be caused by a relative predominance of actions of 5-HT in PO/AH. In other words, hyperthermic response mediated by 5-HT_2 receptor may overcome hypothermic response by the DA receptor when DA receptor is blocked by haloperidol. This assumption has been experimentally verified, because 5-HT antagonists, ritanserin and cyproheptadine could attenuate this hyperthermia.

In line with the observation that beta-adrenergic system also controls the temperature regulation[13], it has been found that the direct injection of noradrenaline (NA) into the PO/AH in rats causes hyperthermia[19]. Feibei and Schiffer[8] reported that the severity of NMS is associated with the high urinary levels of NA metabolites. In these experiments, beta-blocker, DL-propranolol, effectively inhibited this hyperthermia. Therefore, hyperactivities of the noradrenergic pathways may also be responsible for hyperthermia of NMS, accompanying tachycardia and hypertension.

In taking account of all these experimental findings, it is suggested that a key of factors involved in this hyperthermia is mobilization of the intracellular Ca^{2+} in the nerve endings. Dantrolene, which suppresses Ca^{2+} release from the sarcoplasmic reticulum in skeletal muscle[21], inhibited an increase in $[Ca^{2+}]i$ in synaptosomes induced by veratrine. It is suggested here that dantrolene may exert an inhibitory action through reducing $[Ca^{2+}]i$ in CNS, besides the ordinary pharmacological effect on skeletal muscle. Intraperitoneal application of dantrolene also inhibited the hyperthermia induced by haloperidol plus veratrine in a dose dependent manner. On the contrary, dantrolene failed to reduce hyperthermia induced by fenfluramine and 5-hydroxytryptophan. The mechanism in which an increase in 5-HT release caused by fenfluramine or 5-hydroxytryptophan is that these drugs drastically accumulate in serotonergic nerve terminals, thereby kicking out 5-HT from the nerve endings[29]. Thus, the release of 5-HT evoked by fenfluramine and 5-hydroxytryptophan is independent of Ca^{2+} mobilization. From these findings, dantrolene is considered to inhibit the release of monoamines by interfering with the intracellular Ca^{2+} mobilization in the presynaptic nerve endings.

In conclusion, it is suggested that the hyperthermia in NMS is caused by the relative predominance of actions of the serotonergic system in the thermoregulatory center of the hypothalamus either by blockade of DA receptor by neuroleptics or by enhancement of secretion of 5-HT by antidepressants (5-HT uptake inhibitor) (Fig. 8).

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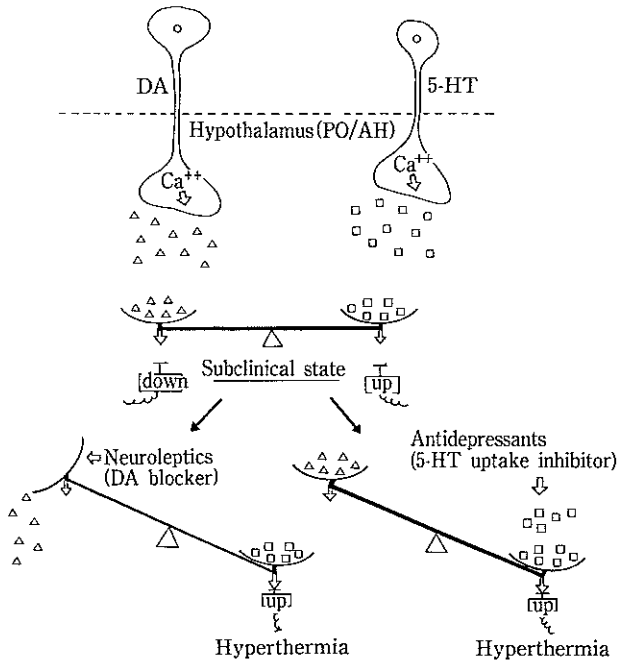

Fig. 8. Relative predominance of actions of serotonergic system on the onset of hyperthermia in MNS.

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