Effects of Intraventricular Neurotensin on Blood Pressure and Heat Balance in Rats

Osamu SHIDO and Tetsuo NAGASAKA

*Department of Physiology, School of Medicine, Kanazawa University,
Kanazawa, 920 Japan*

Abstract The effects of intraventricular neurotensin (NT) at doses of 0.1, 1.0, and 10.0 μg on blood pressure (BP), heart rate (HR), heat production (M), heat loss (H), and colonic temperature (T_{col}) were investigated in conscious rats in a direct calorimeter at 18 and 28°C. At 18°C, a 10.0 μg of NT significantly increased BP for several minutes after injection with prolonged bradycardia. The larger two doses (1.0 and 10.0 μg) significantly reduced M and T_{col} . In sinoaortic denervated rats, a 1.0 μg of NT elevated BP and decreased HR. The decrease in HR was significantly smaller than that in nerve intact rats, which indicates the occurrence of baroreflex with intraventricular NT. The changes in M and T_{col} in the denervated rats were, however, not statistically different from the intact rats. The baroreflexive suppression of metabolism seems to play a minimum role in the NT-induced hypothermia. H slightly increased for several minutes after central NT (1.0 and 10.0 μg) and significantly decreased thereafter. Thermal conductance significantly increased for a longer period of time after NT injection. At 28°C, 1.0 μg of NT increased H and M . It is concluded that central NT produced hypothermia by reducing M and enhancing H in the cool environment, but not at 28°C.

Key words: neurotensin, baroreflex, heat production, heat loss, hypothermia.

Centrally injected neurotensin (NT) has been known to have a profound effect on body temperature in various animals. In rodents and monkeys, NT injected into the cerebroventricle produces dose-dependent hypothermia especially in cold environment (BISSETTE *et al.*, 1976, 1982; BROWN and MILLER, 1982; LEE and MYERS, 1983; MARTIN *et al.*, 1980; NEMEROFF *et al.*, 1977, 1980), and metabolic reduction seems to play an important role in developing this hypothermia (CHANDRA *et al.*, 1981; MORA *et al.*, 1984). Intraventricular NT has also been known to affect the cardiovascular system of mammals, although the results

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remain controversial (RIOUX *et al.*, 1981; SUMNERS *et al.*, 1982). SUMNERS *et al.* (1982) observed that NT injected centrally caused an immediate and dose-dependent rise in blood pressure in conscious rats. Since several investigators observed that the arterial baroreflex following a rise in the systemic blood pressure suppressed shivering and nonshivering thermogenesis in conscious animals in cold environment (HOHTOLA *et al.*, 1980; NUNOMURA, 1983; SHIBATA, 1982; SHIDO *et al.*, 1984; WASSERSTRUM and HERD, 1977), the possibility exists that the baroreflex could contribute to hypothermia following intraventricular NT. Therefore, we attempted to examine the contribution of baroreceptor-mediated metabolic reduction to the NT-induced hypothermia in conscious rats at an ambient temperature of 18°C. The effect of intraventricular NT on heat loss mechanisms has not been established. Cutaneous vasodilation was suggested following central injection of NT in conscious monkeys (MORA *et al.*, 1984), but CHANDRA *et al.* (1981) reported vasoconstriction in conscious rats. We also investigated the heat loss response to central NT with direct calorimetry in conscious rats at 18 and 28°C.

METHODS

Animals and preparations. Male Wistar rats, initially weighing 280–320 g, were used. They were housed individually in rat cages in an animal room at $24 \pm 1^\circ\text{C}$ with a 12:12 LD cycle and were provided laboratory rat chow and tap water ad libitum. In all rats, a 22-gauge stainless steel cannula was stereotaxically implanted into the left lateral ventricle under pentobarbital sodium ($50 \text{ mg} \cdot \text{kg}^{-1}$, i.p.) anesthesia. The location of the cannula was confirmed by the spontaneous flow of cerebrospinal fluid from the cannula. Thereafter, a steel stylet was inserted into the cannula to prevent obstruction. From 2 days after surgery, the rats were loosely restrained in a cylindrical wire mesh cage for 4 hr almost every day for over 8 days to get them accustomed to the experimental situations. After the acclimation period, the rats underwent a second operation under pentobarbital anesthesia. A polyethylene catheter (o.d. 0.6 mm, i.d. 0.3 mm) filled with heparinized saline for measuring the mean arterial blood pressure (BP) was inserted into the abdominal aorta via the right femoral artery, tunnelled subcutaneously and brought out through the back skin of the neck thereafter. Additionally in 4 rats, sinoaortic deafferentation (KRIEGER, 1964) was performed with a longitudinal cervical incision. One day after the second surgery, the rats were used in the experiment. After all experiments, the rats were sacrificed with a large dose of anesthetics for gross examination of the location of the ventricular cannula.

Measurements. On the day of the experiment, rats were loosely restrained in the cylindrical wire mesh cage. A thermister probe was inserted 6 cm into the colon and fixed with vinyl tape, and 4 copper wire electrododes were fixed on the chest of rats. A stainless steel injection needle connected by a polyethylene tube

containing NT solution was inserted 0.5 mm beyond the tip of the ventricular cannula. To avoid the diffusion of the drug from the needle into the ventricle, a very fine air bubble had been sucked inside the needle. A vinyl chloride pan filled with vegetable oil was set underneath the rat cage to collect their urine and feces to avoid the evaporations from the wastes. After these manipulations were over, rats in cages were transferred into a direct calorimeter sized $12 \times 23 \times 12$ cm (NAGASAKA *et al.*, 1979). The ambient temperature (T_a) had been chosen to be either 18 or $28 \pm 0.02^\circ\text{C}$, and the temperature controlled dry air ($2 \text{ l} \cdot \text{min}^{-1}$) was introduced into the calorimeter.

BP was monitored with a pressure transducer (MPU-0.5, Toyo Baldwin, Tokyo) via the arterial catheter, and heart rate (HR) was counted every 2 min from the ECG recordings. Oxygen consumption was measured with a Beckman O_2 analyzer (Model 755, USA) and the rate of heat production (M : $\text{W} \cdot \text{m}^{-2}$) was calculated as $\text{RQ}=0.83$. Colonic temperature (T_{col}) was measured with the thermistor. Dry heat loss ($R+C$) was measured with the direct calorimeter and evaporative heat loss (E) was monitored with a humidity sensor (HMI-11, Vaisala, Helsinki), and both were calculated as $\text{W} \cdot \text{m}^{-2}$. All variables except HR were continuously recorded on potentiometers (MC 6735, Watanabe Sokki, Tokyo; SP-H6P, Riken Denshi, Tokyo). After the records had been stabilized for 90 min, 0.1, 1.0, or 10 μg of NT (Peptide Institute Protein Research Foundation, Osaka) dissolved in 3 μl saline was injected into the left lateral ventricle of rats. The measurements continued for the following 60 min. The rats received only one dose of NT and were not repeatedly used in experiments.

Statistics. The results are presented as the mean $\pm$ S.E. Statistical evaluations of the data was performed by a one-way analysis of variance, and the statistical significance of difference between mean values was assessed by paired or unpaired Student's *t*-test. The level of significance was set at $p < 0.05$.

RESULTS

Tables 1 and 2 summarize the mean resting values and the maximal responses of BP, HR, T_{col} , M , total heat loss (H), ($R+C$), and E to the intraventricular NT at 0.1, 1.0, and 10.0 μg at 18°C . Intraventricular saline (3 μl) produced no significant change in any variables and the results were abbreviated. Figure 1 shows the changes of BP and HR at 18°C . The 1.0 and 10.0 μg of NT produced an immediate rise in BP, but the rise was significant only for the period from 6 to 12 min after injection of the 10.0 μg of NT. The 0.1 μg of NT did not affect BP. Both the 1.0 and 10.0 μg of NT produced a significant and long-lasting bradycardia. The 0.1 μg of NT did not again affect HR. Figure 2 shows the changes in T_{col} , M , and H following NT injection at 18°C . The 10.0 μg of NT produced a significant hypothermia with a marked reduction of M . The fall in T_{col} was over 2.0°C , and the period of hypothermia clearly continued for over

60 min. The period of reduction in M continued for 40 min with the maximal metabolic suppression of $30 \text{ W} \cdot \text{m}^{-2}$ at approximately 30 min after the NT injection. H slightly increased for the first 20 min and significantly decreased there-

Fig. 1. Changes in mean blood pressure (ΔBP) and heart rate (ΔHR) from resting levels following various doses of NT injection into lateral ventricle. Values are means and vertical bars are S.E. Time 0 indicates the time of NT injection. Triangles, at dose of $0.1 \mu\text{g}$ of NT; circles, at dose of $1.0 \mu\text{g}$; squares, at dose of $10.0 \mu\text{g}$. Black symbols, significantly different from resting values.

Table 1. Resting values of mean blood pressure (BP), heart rate (HR), colonic temperature (T_{col}), metabolic rate (M), total heat loss (H), dry heat loss ($R+C$), and evaporative heat loss (E) at 18°C .

Dose (μg)	BP (mmHg)	HR (bpm)	T_{col} ($^\circ\text{C}$)	M ($\text{W} \cdot \text{m}^{-2}$)	H ($\text{W} \cdot \text{m}^{-2}$)	$R+C$ ($\text{W} \cdot \text{m}^{-2}$)	E ($\text{W} \cdot \text{m}^{-2}$)
0.1	125.1	485.3	37.20	95.2	97.1	85.0	12.1
($n=6$)	± 0.8	± 6.8	± 0.14	± 2.9	± 2.6	± 1.7	± 1.3
1.0	123.7	458.1	37.21	91.9	94.5	84.7	9.7
($n=7$)	± 2.4	± 12.7	± 0.27	± 3.8	± 1.3	± 1.3	± 0.4
10.0	123.7	482.6	37.26	94.4	94.4	85.7	8.6
($n=7$)	± 2.8	± 7.0	± 0.17	± 2.8	± 2.5	± 2.2	± 0.4

Values (mean $\pm$ S.E.) were obtained as the means of three data sets before NT injection. The numbers in parentheses indicate the numbers of rats.

Table 2. Maximal changes of mean blood pressure (ΔBP), heart rate (ΔHR), colonic temperature (ΔT_{col}), metabolic rate (ΔM), total heat loss (ΔH), dry heat loss ($\Delta(R+C)$), and evaporative heat loss (ΔE) following various doses of NT injection into lateral ventricle at 18°C.

Dose (μg)	ΔBP (mmHg)	ΔHR (bpm)	ΔT_{col} ($^{\circ}C$)	ΔM ($W \cdot m^{-2}$)	ΔH ($W \cdot m^{-2}$)	$\Delta(R+C)$ ($W \cdot m^{-2}$)	ΔE ($W \cdot m^{-2}$)
0.1 ($n=6$)	ns	ns	ns	ns	ns	ns	ns
1.0 ($n=7$)	ns	-125.9 ± 17.1	-1.48 ± 0.25	-31.5 ± 4.5	-10.8 ± 1.1	-9.6 ± 1.1	ns
10.0 ($n=7$)	+15.4 ± 5.3	-179.0* ± 7.0	-2.54* ± 0.29	-40.0 ± 4.2	-11.4 ± 2.6	-12.2 ± 2.3	ns

Values (mean $\pm$ S.E.) are the maximum changes within 60 min after NT injection.

* Significantly different from 1.0 μg of NT injected group. ns, no significant change.

Fig. 2. Changes in colonic temperature (ΔT_{col}), metabolic rate (ΔM), and total heat loss (ΔH) from resting levels following various doses of NT injection into lateral ventricle. Values are means and vertical bars are S.E. Time 0 indicates the time of NT injection. Symbols are the same in Fig. 1.

Table 3. Resting values and the maximal changes following 1.0 μg of intraventricular NT injection in mean blood pressure (BP), heart rate (HR), colonic temperature (T_{col}), metabolic rate (M), total heat loss (H), dry heat loss ($R+C$), and evaporative heat loss (E) in 4 sinoaortic denervated rats at 18°C.

	BP (mmHg)	HR (bpm)	T_{col} (°C)	M ($\text{W}\cdot\text{m}^{-2}$)	H ($\text{W}\cdot\text{m}^{-2}$)	$R+C$ ($\text{W}\cdot\text{m}^{-2}$)	E ($\text{W}\cdot\text{m}^{-2}$)
Resting values	147.7* ± 2.4	493.4 ± 10.5	36.71 ± 0.16	95.6 ± 2.6	93.4 ± 3.8	81.6 ± 3.2	11.8 ± 1.0
Maximal changes	+47.5* ± 4.1	-63.4* ± 15.3	-1.36 ± 0.19	-29.9 ± 5.7	-9.9 ± 3.1	-9.3 ± 2.6	ns

Values are mean $\pm$ S.E. * Significantly different from 1.0 μg of NT injected group in Tables 1 and 2. ns, no significant change.

after. There was no significant change in E . The 1.0 μg of NT produced similar changes in T_{col} , M , and H , although the magnitudes of the changes in T_{col} and M were evidently less. The maximum reduction of M was $20 \text{ W}\cdot\text{m}^{-2}$ occurring approximately 25 min after the injection of NT. The 0.1 μg of NT did not affect T_{col} , M , and H .

Table 3 summarizes the effects of sinoaortic denervation in the responses to an intraventricular injection of NT (1.0 μg) at T_{a} of 18°C. In the sinoaortic denervated rats ($n=4$), the resting BP was significantly higher than that observed in the nerve intact control rats. In contrast to the insignificant increase in BP in the control rats, the vasopressor action of NT was significantly greater in the denervated rats. However, the reduction of HR was greatly suppressed, and the magnitude of decrease in HR was statistically significant. In the denervated rats, the 1.0 μg NT significantly decreased T_{col} , M , and H . The maximum decreases in these parameters were approximately the same as those in the control rats.

An additional experiment was done at 28°C in another 7 rats. The 1.0 μg of intraventricular NT produced a significant elevation of BP by 17.5 mmHg but did not affect HR. The changes of T_{col} after NT injection were various (hyperthermia was observed in 2 rats and hypothermia in 3 rats) and not statistically significant. M , H , ($R+C$), and E increased significantly following intraventricular administration of NT by 13.9, 10.5, 6.4, and $4.1 \text{ W}\cdot\text{m}^{-2}$, respectively.

DISCUSSION

The intraventricularly injected NT at doses ranging from 1.0 μg to 10.0 μg elevated BP in conscious rats at 18 and 28°C. The results supported the report of SUMNERS *et al.* (1982) who observed the dose-dependent rise in BP following NT injection. At 18°C, the 1.0 μg of central NT produced a significant decrease in HR by 125.9 beats per min (bpm) in the control rats. In the sinoaortic de-

nervated rats, however, the same dose of NT decreased HR by only 63.4 bpm. About a half of the bradycardia following NT injections may be attributed to the sinoaortic baroreflex reduction of HR.

It has been shown that the baroreflex suppresses shivering and nonshivering thermogenesis of conscious animals in cool environments (HOHTOLA *et al.*, 1980; NUNOMURA, 1983; SHIBATA, 1982; SHIDO *et al.*, 1984; WASSERSTRUM and HERD, 1977). Recently, we observed that an intraventricular injection of angiotensin II produced a decrease in metabolism and a fall in body temperature with a concomitant rise in BP and bradycardia (SHIDO *et al.*, 1985). Since hypothermia and metabolic suppression were markedly diminished in the sinoaortic denervated rats, the conclusion was that the hypothermic response to the central angiotensin II was attributed to the sinoaortic baroreflexive suppression of metabolism. Thus, if the central NT raises BP, it is possible that the NT-induced hypothermia is attributed to the baroreflex-mediated metabolic reduction. As shown in Tables 2 and 3, however, the maximum decreases in M and T_{col} after the $1.0 \mu\text{g}$ of NT were the same in the control and the sinoaortic denervated rats, and the baroreflex seemed to play a minimum role in the NT-induced hy-

Fig. 3. Changes in mean blood pressure (ΔBP) and colonic temperature (ΔT_{col}) from resting values following $1.0 \mu\text{g}$ of NT injection into lateral ventricle in two examples. Solid lines and dotted lines show different examples. Time 0 indicates the time of NT injection.

pothemia. The NT caused a rapid rise of BP which lasted only for a short period of time (Fig. 1) and it sometimes even failed to raise BP. Figure 3 illustrates two different examples of the responses to central NT ($1.0 \mu\text{g}$) on BP and T_{co1} at 18°C . In one rat (shown as solid lines), a marked and sharp rise in BP and a rapid fall in T_{co1} were noticed. No such rapid changes in BP and T_{co1} were observed in the other rat (shown in dotted lines). The magnitudes of hypothermia of the two examples were, however, almost the same. Therefore, it may be said that baroreflexive suppression of metabolism can contribute to the initiation of the fall in T_{co1} .

At 18°C , the effect of central NT on heat loss was biphasic (Fig. 2). H increased for the initial 20 min after an injection of 1.0 or $10.0 \mu\text{g}$ of NT and significantly decreased thereafter. However, the decrease in H may not directly indicate the decrease in cutaneous circulation, because the concomitant fall in body temperature decreased the temperature gradient from the deep body to the chamber wall. As an index of cutaneous blood flow, thermal conductance (K) was calculated with an equation of $K=H/(T_{\text{co1}}-T_w)$ (T_w : temperature of chamber wall). The K significantly increased from the resting values of 4.919 ± 0.062 or $5.059 \pm 0.149 \text{ W}\cdot\text{m}^{-2}\cdot^\circ\text{C}^{-1}$ after an injection of $0.1 \mu\text{g}$ or $10.0 \mu\text{g}$ of NT (Fig. 4). No significant fall in K was observed within 60 min after the NT injection, which indicated rather a prolonged cutaneous vasodilation. At 28°C , the $1.0 \mu\text{g}$ of NT also produced a significant increase in $(R+C)$, E , H , and K . These results suggest that the central NT may facilitate heat loss mechanisms in rats.

It is uncertain whether NT plays a role in the body temperature control mechanisms. CHANDRA *et al.* (1981) and LEE and MYERS (1983) reported the poikilothermic effect of intraventricular NT in the rat. They observed NT-induced hypothermia below 22°C of ambient temperature and hyperthermia over

Fig. 4. Changes in conductance (ΔK) from resting values following various doses of NT injection into lateral ventricle. Values are means and vertical bars are S.E. Time 0 indicates the time of NT injection. Symbols are the same in Fig. 1.

30°C of T_a . In the present study, body temperature response to NT at T_a of 28°C was various, hyperthermia occurred in 2 rats and hypothermia in 3 rats, although hypothermia was consistently observed following central NT at 18°C. The ambient temperature of 28°C may not be high enough to produce NT-induced hyperthermia. Anyhow, this result seems to suggest the poikilothermic action of intraventricular NT rather than a thermolytic property. Furthermore, the fact that central NT increased both heat production and heat loss at 28°C seems to disagree with the idea that NT participates in the central mechanisms of body temperature regulation, and may support the conclusion of MORA *et al.* (1984) that NT does not play a role in the central mechanisms mediating the body temperature control.

In summary, the central NT produced pressor and hypothermic effects in conscious rats in a cool environment. However, the central pressor action of NT seemed to be less potent and the effect of arterial baroreflex may be the minimum in causing suppression of metabolism. The reductions of metabolism and cutaneous vasodilation were profound and persistent after central NT at 18°C. On the contrary, both heat production and heat loss increased following NT at 28°C. It seems doubtful that central NT acts in thermoregulatory centers in rats.

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