Trop. Med., 29 (4), 241−249, December, 1987

Autonomic Nervous Tones in Chronic Spinal Rats

Katsuhiko TSUCHIYA, Peter George RIWA* and Mitsuo KOSAKA

Department of Environmental Physiology, Institute of Tropical Medicine, Nagasaki University, 12−4 Sakamoto-machi, Nagasaki 852, Japan

Abstract: Sympathetic and parasympathetic nervous tones and ability of temperature regulation were compared in spinal-intact control rats and in chronic spinal rats. The lower cervical cord of male Wistar rats was transected under pentobarbital anesthesia, and the rats were reared in a room of 30℃. For the experiment, each rat was placed in wire-meshed small cage in the climatic chamber (30℃, 60%, r.h.). Ninety minutes after the beginning of recording, atropine sulfate (1mg/kg, i.p.) or propranolol hydrochloride (8mg/kg, i.p.) was injected. Cardiac parasympathetic and sympathetic tones were evaluated by heart rate (HR) change after the medication. Rectal temperature (Tre), tail skin temperature (Ttail) and HR were evaluated for 30 min before the medication. Mean Tre (M. ± S.E.) in control was 38.0±0.1℃, and those in the 1st, 2nd and 3rd week after spinalization were 36.1±0.2℃, 37.0±0.3℃ and 37.3±0.3℃, respectively. Resting HR (HR(norm.)) in control was 369±6 beats/min, and those in the 1st, 2nd and 3rd week after spinalization were 330±19 beats/min, 344±10 beats/min and 341±9 beats/min, respectively. The excess temperatures (dT) of the tail skin over the environmental temperature in the 1st to 3rd week were significantly higher ($p<0.05$) than in the control. In chronic spinal rats, there was a negative correlation between the increase in heart rate by atropine (HR(p)) and HR(norm.), and a positive correlation between the decrease in heart rate by propranolol (HR(s)) and HR(norm.). In control rats, parasympathetic tone (PT) and sympathetic tone (ST) were calculated as 22.5% and 24.4%, while in chronic spinal rats (from 1st to 6th week after spinalization), PT and ST were 16.6% and 15.7%, respectively. From these results it is presumed that the change in parasympathetic tone also may be a contributing factor in the recovery of the autonomic functions in chronic spinal animals.

Key words: Spinal rat, Sympathetic parasympathetic tone, Temperature regulation, Heart rate, Atropine, Propranolol

*JICA trainee from Occupational Health Unit, Ministry of Labour and Manpower, Dar es Salaam, Tanzania

Received for Publication, October 27, 1987.
Contribution No. 1993 from the Institute of Tropical Medicine, Nagasaki University

INTRODUCTION

It is well known that the depressed autonomic function observed in spinalized animals, gradually recovers in the spinal level day by day. There are many reports relating to recoveries in temperature regulation (Thauer, 1935; Simon, 1974), cold shivering (Kosaka and Simon, 1968), efferent activity of spinal sympathetic nerve concerning with systemic pressure control (Arddel *et al.*, 1982), differential control of the peripheral and visceral blood flow by spinal sympathetic nerves (Walther *et al.*, 1971), and micturition, defecation reflexes and *etc.* But the detail mechanism of such a recovery process in these functions and an establishment of a new balance of the sympathetic and parasympathetic nervous tones have not been fully explained. Therefore, the aim of this study was to clarify the recovery process especially in the relationship between regulatory mechanisms of body temperature and systemic circulation in chronic spinal animals.

METHODS

Adult male rats of Wistar strain were used for this experiment. Under pentobarbital anesthesia (50mg/kg, i.p.), laminectomy was performed to expose the lower cervical cord and the lowest end of the cervical cord was transected by aspiration. After the operation, antibiotic (Sulbenicillin, Takeda) was injected intramuscularly. To avoid hypothermia due to spinalization, in the room of 30℃ (50%, r.h.) spinalized rats were reared with plenty of wood shaves in an individual box with dimensions 22cm×32cm×7cm, of which one side was meshed. Seven intact rats, heat acclimated to 30℃ for more than one month, were used as the control rats. In chronic spinal rats, 21 out of 34 experiments were performed in heat-acclimated rats, but the other 13 experiments were in non heat-acclimated rats. Under ether anesthesia, needle electrodes were attached to the both axially areas for detecting electrocardiogram (ECG). A thermistor probe was inserted into the rectum more than 5cm beyond the anus. Another thermistor probe was attached on the ventral surface in the middle part of the tail and the tip of probe was covered by a layer of an adhensive tape. During the experiment, rats were individually placed in the loosely fitting wire-meshed cage under conscious condition, and the cage containing a rat was suspended in the air of the climatic chamber of 422 liters of which air temperature was kept constant at 30℃ (60%, r.h.) (Tsuchiya, 1986). ECG, rectal temperature (Tre) and tail skin temperature were recorded continuously. The experiments were performed between 9 o'clock and 18 o'clock. Heart rate (HR) curve was drawn by counting R-R intervals of ECG by a counter (Nihon Koden, AT 600G). All the parameters were recorded simultaneously on the UV oscillograph (Type 5L, Sanei). Ninety minutes after the beginning of the recording atropine sulfate 1mg/kg (Tanabe) or propranolol hydrochloride 8mg/kg (Sumitomo) was injected intraperitoneally (Tipton and Taylor, 1965; Barnes and Eltherington, 1973). Parasympathetic tone (PT), sympathetic tone (ST) and intrinsic heart rate (HR(o)) were calculated after Lin and Horvath (1972)

as follows: HR(norm.)=HR(o)+HR(s)−HR(p)···(1), HR(atrop.)=HR(o)+HR(s)···(2), HR(prop.)=HR(o)−HR(p)···(3). From (1), (2) and (3), HR(p)=HR(atrop.)−HR(norm.), HR(s)=HR(norm.)−HR(prop.)*. HR(norm.) is the mean heart rate for 30 min before injection of drugs. HR(atrop.) and HR(prop.) are heart rate evaluated after 10 min for atropine and 20 min after propranolol injection, respectively. HR(p) and HR(s) respesent changes in heart rate by parasympathetic and sympathetic activities, respectively. Tones of the cardiac parasympathetic and sympathetic nervous system were calculated as a ratio to the intrinsic heart rate, as follows; PT=HR(p)/HR(o)×100, ST=HR(s)/HR(o)×100.

The statistical significance of change in parameters was determined by Mann-Whitney U-test.

Results

Both heart rate and rectal temperature in chronic spinal rats placed in the wire-meshed cage gradually decreased. Mean values of Tre, HR and the excess temperature (dT) of the tail skin over the environmental temperature were calculated from the values piked up at 7 points in every 5 min for 30 min (from 60th min of the beginning of the recording to 90th min). These results are summarised in Table 1 and illustrated in Fig. 1. The mean value of heart rate (HR(norm.)) in control animals was 369±6 beats/min and the mean values in the 1st, 2nd, 3rd and 4-6th week after the spinalization were 330±19 beats/min, 344±10 beats/min, 341±9 beats/min and 377±12 beats/min, respectively. The value in the 3rd week was significantly ($p<0.05$) low compared to that in the control. The mean Tre in the control rats was 38.0±0.1℃, and mean Tre in the 1st, 2nd and 3rd week after the spinalization were 36.1±0.2℃, 37.0±0.3℃ and 37.3±0.3℃, respectively. These values were significantly low ($p<0.05$) compared to the

Table 1. Mean values (M ± S. E.) of rectal temperature (Tre), excess temperature (dT) and heart rate (HR) in control (spinal-intact) rats and chronic spinal rats in the 1st, 2nd, 3rd and 4−6th week after spinalization. These mean values were calculated from values observed at 7 time-points every 5 min for 30 min before of injection of drugs (from 60th min to 90th min after the beginning of recording). Asterisks (*) indicate the statistical significance ($p<0.05$) compared to the values in control by testing with Mann-Whiteny U-test

	Control	1st week	2nd Week	3rd Week	4th-6th Week
Number of experiments (n)					
Drug treatment Atropine	7	7	5	4	3
Drug treatment Propranolol	7	2	7	2	4
Rectal temperature (Tre), (℃) (dT=Ttail-Ta)	38.0 ± 0.1	36.1 ± 0.2*	37.0 ± 0.3*	37.3 ± 0.3*	38.1 ± 0.5
Excess temperature of the tail skin over the air temperature (dT), (℃)	0.5 ± 0.2	1.5 ± 0.4*	1.5 ± 0.4*	2.0 ± 0.2*	1.2 ± 0.4
Heart rate (HR), (beats/min)	369 ± 6	330 ± 19	344 ± 10	341 ± 9*	377 ± 12

* $HR(p) = HR(atrop.) - HR(norm.)_{atrop.}$
$HR(s) = HR(norm.)_{prop.} - HR(prop.)$
$HR(norm.) = (HR(norm.)_{atrop.} + HR(norm.)_{prop.}) / 2$
$HR(norm.)_{atrop.}$: HR(norm.) before atropine treatment
$HR(norm.)_{prop.}$: HR(norm.) before propranolol treatment

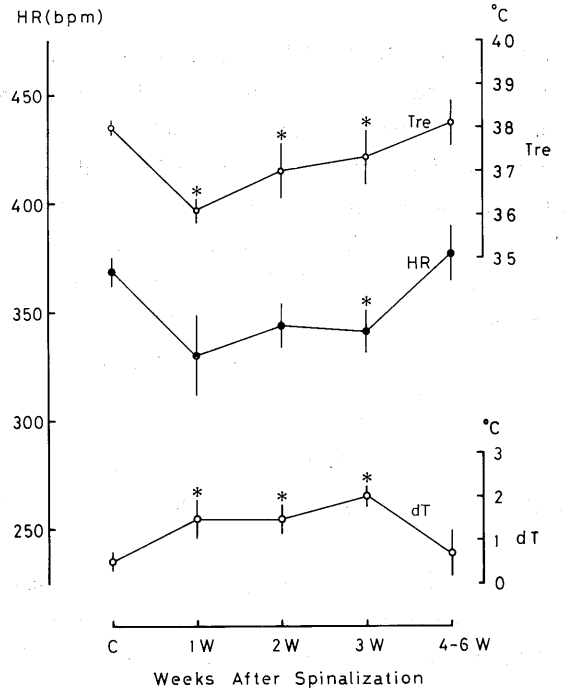

Fig. 1. Rectal temperature (Tre), heart rate (HR) and excess temperature (dT) of the tail skin over the air temperature (dT=Ttail-Ta) in spinal-intact rats in the control (C) and in chronic spinal rats of the 1st (1W), 2nd (2W), 3rd (3W) and from 4th to 6th (4-6W) week after spinalization. Solid and open circles with vertical bars represent mean and standard errors of values measured at every 5 min for 30 min (from 60th to 90th min after beginning of recording) before injection of the drugs. Asterisks (*) indicate the statistical significance ($p<0.05$) compared to the values in control. Air temperature was kept at 30℃ (60%, r.h.).

value in the control. The mean Tre in the 4-5th week was 38.1±0.5℃. The excess temperatures (dT) of the tail skin in the 1st, 2nd and 3rd week after the spinalization were significantly high ($p<0.05$) compared to that in the control.

In order to evaluate the autonomic nervous tones, atropine or propranolol was injected intraperitoneally at the 90th min after the beginning of the recording. Mean HRs in 7 control rats after administration of each drug are demonstrated in Fig. 2-A. Fig. 2-B shows mean HR of 19 experiments of atropine treatment as well as of 15 experiments of propranolol treatment in chronic spinal rats at various weeks after the spinalization. Details about each week of experiments after spinalization are shown in Table 1. In control rats the heart rate after injection of atropine sulfate 1mg/kg increased and attained the maximal value at 5 to 10 min after injection, and then reduced toward the initial level. The mean value of HR was 451±10 beats/min at 10 min, 444±7 beats/min at 20 min and 438±8 betats/min at 30 min after injection. The differences among these three values were not statistically significant. On the other hand, in chronic spinal rats, the mean value of heart rate was 405±5 beats/min at 10 min, 388±5 beats/min at 20 min and 376±6 beats/min at 30 min after atropine injection. The latter two values were significantly low ($p<0.05$) compared to that at 10 min. By injection of propranolol 8 mg/kg, HR constinuously decreased. At 20 min after injection, mean HR in control and

in spinal rats were 279±8 beats/min and 291±10 beats/min, respectively. These values were not significantly different compared to those at 10 min and at 30 min after injection. From the reason above mentioned, HR(atrop.) and HR(prop.) were evaluated at 10 min for atropine cases and at 20 min for propranolol after injection, respectively.

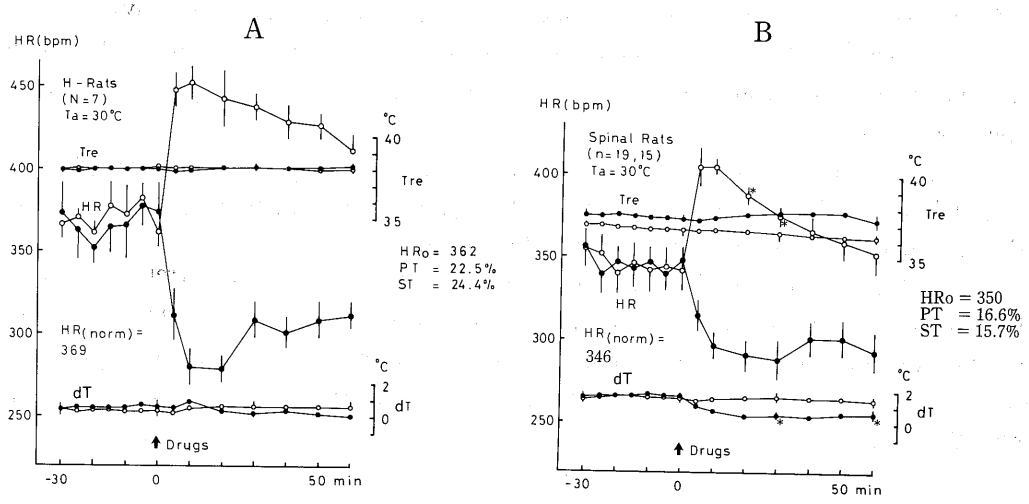

Fig. 2. Changes in rectal temperature (Tre), heart rate (HR) and excess temperature (dT) of the tail skin over the air temperature in spinal-intact rats as the control (A) and in chronic spinal rats (B). Air temperature was kept constant at 30℃ (60%, r.h.). Solid arrows indicate the injection of atropine sulfate (1mg/kg, i.p.) or propranolol hydrochloride (8mg/kg, i.p.). Open and solid circles with vertical bars are mean values (M. ± S.E.) when atropine or propranolol was injected, respectively. Asterisks (*) on lines of dT and HR indicate the statistical significance ($p<0.05$) compared to those at just before (at 0 min) and at 10 min after injection of drugs, respectively.

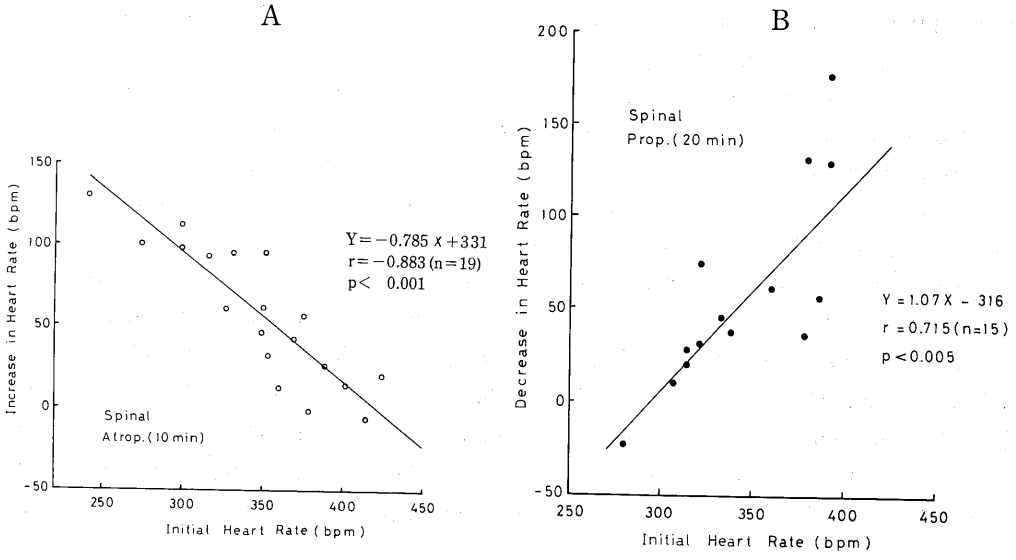

Fig. 3. (A): Relationship between the initial heart rate (HR(norm.)) and the increase in heart rate (HR(p)) at 10 min after injection of atropine sulfate (1mg/kg, i.p.) in spinal rats. (B): Relationship between HR (norm.) and the decrease in heart rate (HR(s)) at 20 min after injection of propranolol hydrochroride (8mg/kg, i.p.) in spinal rats. Regression lines were determined by the least square method.

In control heat acclimated rats (N=7), initial HR (HR(norm.)), intrinsic heart rate HR(o), parasympathetic tone (PT) and sympathetic tone (ST) were 369 beats/min, 362 beats/min, 22.5% and 24.4%, respectively. On the other hand, in 34 experiments (19 cases of atropine treatment, 15 of propranolol) of chronic spinal rats, HR (norm.), HR(o), PT and ST were 346 beats/min, 350 beats/min, 16.6% and 15.7%, respectively.

As shown in Fig. 2-B, a continuous decrease in the excess temperature (dT) of the tail skin was observed after propranolol injection but not in the atropine case in chronic spinal rats.

The increase in heart rate by atropine treatment (HR(p)) evaluated at 10th min after injection and the decrease in heart rate by propranolol (HR(s)) at 20 min after injection in spinal rats were plotted against the initial resting heart rate (HR(norm.)). As shown in Fig. 3-A and B, there was a significant ($p<0.001$) negative correlation between them in atropine case. The equation of the regression line obtained by the least square method was $Y=-0.785X+331$. In the case of propranolol treatment, threre was a significant ($p<0.005$) positive correlation between them. The equation of the regression line was $Y=1.07X-316$.

Discussion

In this experiment, heart rate, rectal temperature, excess temperature (dT) of the tail over the environmental air and effects of injection of atropine or propranolol were compared in the spinal-intact control and the chronic spinal rats at 30℃ of room temperature. Autonomic nervous tones concerned with the cardiac regulation were calculated by the method of Lin and Horvath (1972) (Walsh, 1969; Cameron, 1979). Both parasymapthetic and sympathetic tones in chronic spinal rats were lower than those in heat-acclimated control rats.

Spinal rats were reared in a warm room of 30℃. During the experiment, Tre decreased gradually in a wire-meshed cage suspended in the air of 30℃. It is presumably due to following reasons: peripheral vasodilation especially in the tail, and increase of heat loss under poor heat insulative condition of the wire-meshed cage suspended in the air. On the other hand, control rats under the same condition, vasodilation was not observed in the tail. In control rats heart rate incresed during the first 20 min and then gradually decreased to the initial level. Rectal temperature increased slightly.

Excess temperature of the tail (dT) is an index to know the change in the tail blood flow (Rand *et al.*, 1965). Mean dT of chronic spinal rats in the 1st week to the 3rd week were about 2℃ at air temperature of 30℃, which indicated the sustained tail vasodilation. Heart rate and rectal temperaturre were low in the 1st week after spinalization, but these parameters gradually recovered day by day after spinalization (Tsuchiya, 1986).

Under conscious condition, positive correlations have been reported between heart rate and symapthetic activity in the rat (Riksten *et al.*, 1984) and also between heart rate and oxygen consumption in rodents (Morhardt and Morhardt, 1971). Therefore heart rate

is an important factor relating to the recovery of the spinal sympathetic activity and the heat production. In this study the increase in heart rate by atropine (HR(p)) and the decrease in heart rate by propranolol (HR(s)) were polotted against the initial heart rate (HR(norm.)). There were a negative correlation between HR(p) and HR(norm.) and a positive one between HR(s) and HR(norm.). The increased HR after atropine injection (HR(atrop.)) in chronic spinal rats reduced more quickly than in the spinal-intact group.

It was reported that autonomic nervous tones were changed by thermal acclimation in rats, (LeBlanc and Côté, 1967). It was also reported that parasympathetic tone in hot environment was higher than that in cold environment in rats (Tsuchiya, 1987). In this study, to neglect the effects of environmental temperature and thermal acclimation on the autonomic tones, heat-acclimated rats were used as the control. In 21 out of 34 experiments of chronic spinal rats, heat-acclimated rats, were used. But in the other 13 experiements, non-heat-acclimated ones which were expected to show an increase in parasympathetic tone in hot environment were used.

Ardell *et al.* (1982) observed in chronic spinal cats a recovery of the sympathetic nerve activity and gradual increase in reducing effect of blood pressure by administrations of ganglion blocker (hexamethonium bromide). The mean blood pressure in chronic spinal group was about 66% of that in control at 1 month after the spinalization in the same anesthetic condition. In this study, both parasympathetic and sympathetic tones in chronic spinal rats were lower than those in the control. An increase in sympathetic activity and a decrease in parasympathetic one in chronic spinal rats may have an advantageous effect for the recovery from the depressed condition due to the spinalization.

As shown in Fig. 2, a decrease of dT indicating the vasoconstriction in the tail was observed after administration of propranolol but not after the atropine in spinal rats. Because the systemic blood pressure (BP) was not measured during this experiment, there are not enough informations about the effects of the change in BP after medications on blood circulation in the tail. Propranolol is a selective beta-adrenergic blocker. Arterio-venous anastomoses, AVA, are aboundant in the rat tail. These AVA are innervated by the sympathetic vasoconstrictors (Kondo, 1972; Gemmel & Hales, 1977). Nikerson and Collier (1975) described that beta-adrenergic blocking agents have minimal direct effect on the peripheral vasculacture and peripheral resistance is increased as a result of compensatory sympathetic reflex (Nies *et al.*, 1973). From these facts, it is also suggested that the spinal symapthetic nervous system participats in the tail vasoconstriction after the propranolol administration.

ACKNOWLEGEMENT

Authors would like to thank colleagues in Department of Environmental Physiology, Institute of Tropical Medicine, Nagasaki University for helpful discussions and preparing the manuscript.

References

1) Ardell, J. L., Barman, S. M. & Gebber, G. L. (1982): Sympathetic nerve discharge in chronic spinal cat. Am. J. Physiol., 243, H463−H470.

2) Barnes, C. D. & Eltherington, L. G. (1973): Drug dosage in laboratory animals, a handbook. 2nd Ed. University of California Press, Berkeley, Los Angeles, London.

3) Cameron, J. S. (1979): Autonomic nervous tone and regulation of heart rate in the goldfish, *Carassius auratus*. Comp. Biochem. Physiol., Vol. 63C, 341−349.

4) Gemmell, R. T. & Hales, J. R. S. (1977): Cutaneous arteriovenous anastomoses present in the tail but absent from the ear of the rat. J. Anat., 124, 355−358.

5) Kondo, H. (1972): An electron microscopic study on the caudal glomerulus of the rat. J. Anat., 113, 341−358.

6) Kosaka, M. & Simon, E. (1968): Der zentralnevöse spinale Mechanismus des Kältezitterns. Pflügers Arch., 302, 357−373.

7) LeBlanc, J. & Côté, J. (1967): Increased vagal activity in cold-adapted animals. Canad. J Physiol. Pharmacol., 45, 745−748.

8) Lin, Y. C. & Horvath, S. M. (1972): Autonomic nervous control of cardiac frequency in the exercise-trained rat. J. Appl. Physiol., 33, 796−799.

9) Morhardt, J. E. & Morhardt, S. S. (1971): Correlations between heart rate and oxygen consumption in rodents. Am. J. Physiol., 221, 1580−1586.

10) Nies, A. S., Evance, G. H. & Shand, D. G. (1973): Regional hemodynamic effects of beta-adrenergic blokade with propranolol in the unanesthetized primate. Am. Heart J. 85, 97−102.

11) Nikerson, M. & Collier, B. (1975): Drugs inhibiting adrenergic nerves and structures innervated by them. pp533-564, *In* Goodman, L. S. and Gilman, A. Ed. The pharmacological basis of therapeutics. Macmillan Publishing, New York.

12) Rand, R. P., Burton, A. C. & Ing, T. (1965): The tail of the rat, in temperature regulation and acclimatization. Canad. J. Physiol. Pharmacol. 43, 257−267.

13) Ricksten, S. -E., Lundin, S. & Thoren, P. (1984): Spontaneous variations in arterial blood pressure, heart rate and sympathetic nerve activity in conscious normotensive and spontaneously hypertensive rats. Acta. Physiol. scand., 120, 595−600.

14) Simon, E. (1974): Temperature regulation: The spinal cord as a site of extrahypothalamic thermoregulatory functions. Rev. Physiol. Biochem. Pharmacol., 71, 1−76.

15) Thauer, R. (1935): Wärmeregulation und Fiberfähigkeit nach operativen Eingriffen am Nervensystem homoiothermer Säugetiere. Pflügers Arch. ges. Physiol., 236, 102−147.

16) Tipton, C. M. & Taylor, B. (1965): Influence of atropine on heart rates of rats. Am. J. Physiol., 208, 480−484.

17) Tsuchiya, K, (1986): Change in tail skin temperature during exposure to various ambient temperatures in chionic spinal rats. Trop. Med., 28, 129−142, 1986.

18) Tsuchiya, K, (1987): Autonomic nervous tone in heat acclimated rats. J. Physiol. Soc. Japan, 49, 576.

19) Walsh, R. R. (1969): Heart rate and its neural regulation with rising body temperature in anesthetized rats. Am. J. Physiol., 217, 1139−1143.

20) Walther, O. -E., Riedel, W., Iriki, M. & Simon, E. (1971): Differentiation of sympathetic activity at the spinal level in response of central cold stimulation. Pflügers Arch., 329, 220−230.

慢性脊髄ラットの自律神経 Tone

土屋勝彦，P. G. Riwa*，小坂光男（長崎大学熱帯医学研究所環境生理）
*JICA 研究コース研究生

慢性脊髄動物の自律神経の Tone を Lin and Horvath の方法で測定評価し，脊髄無傷動物の場合と比較検討した。Wistar 系雄ラットの頚髄下端を Pentobarbital 麻酔下で吸引除去し，室温30℃で飼育したものを慢性脊髄ラットとした．実験は室温30℃において，無麻酔，半拘束状態で約90分放置した後に，硫酸アトロピン 1mg/kg 又は塩酸プロプラノロール 8mg/kg を腹腔内に投与し，その後の心拍数の変化から交感及び副交感神経の活動による心拍の増加及び減少幅を計算し，それらの intrinsic な心拍数（HR(o)）(計算で求められた）に対する百分率を求め，各々副交感神経の Tone (PT) 及び交感神経の Tone (ST) とした．30℃に順化した脊髄無傷の対照群では，HR(o): 362 beats/min, PT: 22.5%, ST: 24.4%と計算された．一方脊髄切断後，1週から6週後の34例（アトロピンとプロプラノロールの合計例数）の慢性脊髄ラットでは，HR(o): 350 beats/min, PT: 16.6%, ST: 15.7%と計算された．慢性脊髄動物の交感・副交感神経の Tone は，脊髄無傷の対照群に比して低い値を示した．この事から，慢性脊髄動物の体温調節能等の自律機能の回復に，副交感神経の Tone の変化も含めた新しい自律神経バランスの形成の関与が示唆された．

熱帯医学　第29巻　第4号　241－249頁，1987年12月