Effects of Thermal Stimulation of Spinal Cord on Oxygen Consumption in Intact and Decerebrated Rabbits*

Mitsuo KOSAKA, Mariko FUJIWARA,
Nobu OHWATARI, Jun IWAMOTO and Yu-Jen FAN

Department of Environmental Physiology, Institute for Tropical Medicine, Nagasaki University, Nagasaki 852, JAPAN

Shyozo TAKABA and Yoshiaki ISOBE

Department of Physiology, Nagoya City University, School of Medicine, Nagoya 467, JAPAN

Abstract: 1. The effects of thermal stimuli applied to spinal cord on oxygen consumption have been compared in intact and decerebrated rabbits. In both groups, changes of oxygen consumption induced by cooling and heating of spinal cord were consistent. 2. Mean value of oxygen consumption was 8.8 ml/kg · min in intact rabbits. It was 9.8 in decerebrated rabbits. 3. In decerebrated rabbits, mean increase in percentage of oxygen consumption was 17.3% when spinal cord was cooled from normal state. It was 19.0% if a heated spinal cord was cooled suddenly. 4. When spinal cord was heated from the normal condition, the oxygen consumption was increased by 8.8% but using a cooled spinal cord brought −12.0% increase in oxygen consumption. 5. The data obtained from normal rabbits in similar experiment were almost the same. 6. From the present results, following tentative conclusion can be drawn: a) thermal stimuli applied to spinal cord have an important effect on pontine, medullary and spinal thermoregulatory centres. b) these centres are effective without the aid of hypothalamus. c) body temperature is hierarchically regulated in the process of evolution.

Key words: Temperature regulation, Decerebrated rabbits, Spinal thermal stimuli, Oxygen consumption

INTRODUCTION

Current interest is attracted to the problem of thermosensitive property of the spinal cord, since the property is similar to that of the hypothalamic structure as well as of the

thermoreceptor in the skin. In dogs, the spinal cord has been found to be a structure sensitive to selective thermal stimulation by Simon *et al.*, in 1963. Their finding based on the previous observation that the chronically spinalized rabbits at cervical level could maintain their body temperature constant in environmental temperature down to $20-18°C$, confirmed a hypothesis as follows; the central thermosensitivity is not restricted only in the hypothalamic area, but that thermosensitivity is a property of the entire central nervous axis.

Thus, since 1963, many experimental evidences for this concept have been reported in various kinds of mammals and birds. In rabbits, thermoregulatory responses elicited by cooling and heating the spinal cord closely resembled those following the same thermal stimulation of the hypothalamus (Kosaka *et al.*, 1975a, b).

The present investigation was performed to observe the effects of the spinal thermal stimulation in both intact and decerebrated rabbits, and further, to discuss the probability of extrahypothalamic areas concerning temperature regulation.

METHODS

A total of twenty-nine albino rabbits weighing 2.5-3.8 kg were used for this experiment. Under light anaesthesia (Sodium Pentobarbital 1.5-2.0 mg/kg), a trachea of the rabbit was opened to insert a canula of which the other end was connected with a respirometer. During experimentation, room temperature was kept constant at $24\pm1°C$.

All animals fixed on a stereotaxic apparatus in prone position, were submitted to the following surgical procedure in the conscious state without anesthesia.

In 17 rabbits successful midbrain transections (the midpontine pre-trigeminal transection: broken line in Fig. 1) were carried out without impairing spontaneous breathing.

A knife, specially designed not to impair the basilar artery at the pontine region was used for transection. The upper brain tissue of transection was removed by suction and the cut wound was covered with gelatine sponge containing blood coagulant which had neither effect on cardio-vascular nor motor functions. The peridural space of the
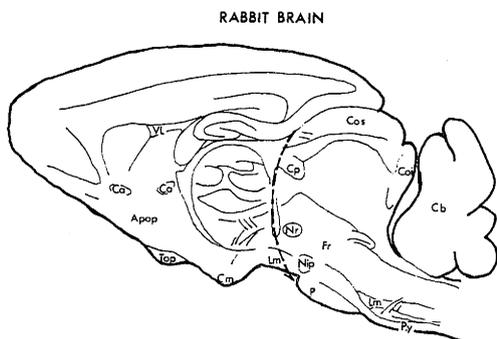
RABBIT BRAIN

Fig. 1 Schematic drawing of a midsagittal section of the rabbit brain showing the level of transection (broken line) Abbreviations: Ca, Commissura anterior; Cb, Cerebellum; Cm, Corpus mamillare mediale; Coi, Colliculus inferior; Cos, Colliculus superior; Cp, Commissura posterior; Apop, Area praeoptica; VL, Ventriculus lateralis; Top, Tractus opticus; Nip, Nucleus interpeduncularis; Nr, Nucleus ruber; Fr, Formatio reticularis; P, Pons; Py, Pyramid;

animal was carefully opened at the lumbosacral vertebra. A double barreled thermode of polyethylene tubing for thermal stimulation of the spinal cord was inserted into the peridural space and was carefully pushed upward to the lower thoracic region (Th 6-8).

Regarding thermal stimulation of the spinal cord, selective heating and cooling was carried out by perfusing the thermode with hot (45-52°C), co'd (10-20°C) and neutral (38-39°C) water. Due to limited heat exchange through the tubing walls, the temperature measured in the peridural space was about 42°C on the average during heating and about 32-34°C during cooling and 38-39°C during neutral state (Kosaka *et al.*, 1969). Animals fixed on a stereotaxic apparatus in prone position were submitted to following experiment of 3-5 hours.

For estimation of oxygen consumption, the trachea cannula was connected with a Benedict Roth's respirometer (13.5 liters, Bell Factor 41.4 ml/mm). Gaseous temperature in the respirometer was attentively kept constant by faint cooling the gases in the closed circuit (Kosaka *et al.*, 1975b). Oxygen consumption estimated in V-atps in every 15 minutes period was converted into V-stpd after referring to the coefficient table of Peters. Peters' coefficient was caculated from the fact that soda-lime absorbs 20% water vapor in the closed circuit system, therefore, V-stpd was reduced from the ambient temperature and pressure and 80% water vapor saturated in the present investigation.

The other experimental indicators such as temperatures of the vertebral canal, rec- tum, various parts of the skin as well as gaseous temperature in the respirometer were measured by thermocouples connected either with an electric thermometer (Ellab. Copen- hagen) or DC-recorder. Respiratory frequency was derived from the spirometer excur- sions. Electromyograms were recorded with pairs of needle electrodes from the lumbar dorsal trunk muscles.

After experiment all animals were sacrificed and the rest brain tissues were fixed with Muller's solution for serving macroscopic observation.

RESULTS

Figure 2a shows a simultaneous recording of various theromoregulatory responses of an intact rabbit during cooling and heating the spinal cord. Temperature changes in the spinal cord were detected as a change in the vertebral canal temperature (Tv). During heating the spinal cord, rectal temperature (Tr) seemed to fall slightly in consequence of of appearing heat loss responses such as thermal polypnea and vasodilation of the skin. During spinal cooling, temperature of rectum tended to rise in accordance with appearance of cold shivering, slight decrease of respiratory frequency (RR) and vasoconstriction of the skin. Thus, coherent heat production and heat loss responses could be induced by spinal cord cooling and heating, respectively.

The change in oxygen consumption of the same intact rabbit was continuously re- corded by means of Benedict Roth's Respirometer. Figure 2 b demonstrates a small por-
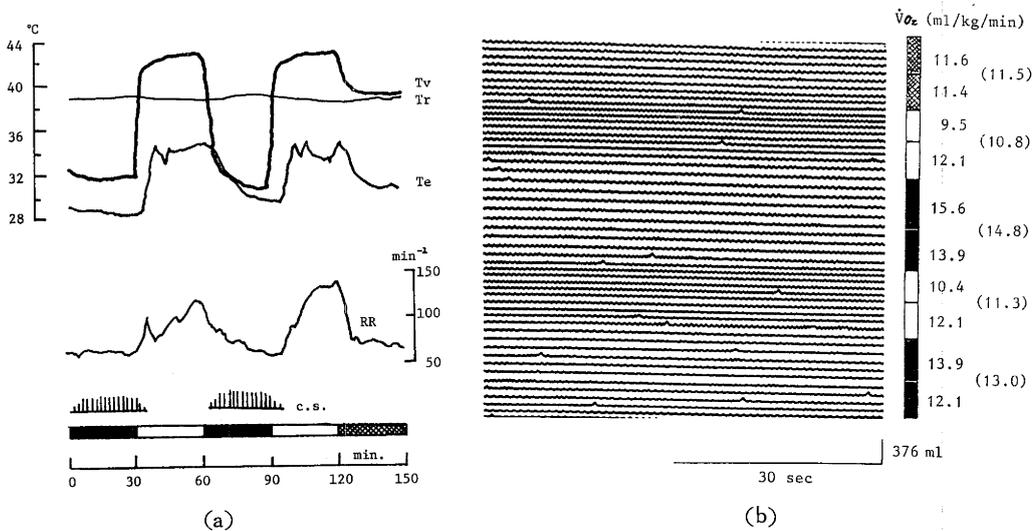
Fig. 2 Thermoregulatory responses (a) and oxygen consumption (b) to cooling and heating the spinal cord in normal rabbit.

    (a) A simultaneous recording of various thermoreguatory responses induced by cooling and heating the spinal cord. Coherent heat production and heat loss responses were induced by spinal cord cooling and heating, respectively.

    Brush like figures on the black blocks indicate visible cold shivering induced by cooling the spinal cord. Tv: vertebral canal temperature, Tr: rectal temperature, Te: ear skin temperature, RR: respiratory rate, c. s.: cold-shivering. Black block: cooling the spinal cord, White black: heating the spinal cord, and Hatched block: neutral temperature of the spinal cord.

    (b) A continuous recording of oxygen consumption during cooling and heating the spinal cord in the rabbit.

    A small portion of a continuous recording of oxygen consumption by mean of Benedict Roth's Respirometer is demonstrated. Each interval of two lines of respiratory curve indicates an amount of oxygen consumed in 3 minutes.

    Calibration of gaseous volume: the volume of oxygen was converted from V-atps into V-stpd after referring to the coefficient table of Peters.

tion of a continuous recording of oxygen consumption corresponded to those of Figure 2 a on the respirometer. Each line showing respiratory curve allows to observe a gross tidal volume and respiratory rate. Each interval of two lines of respiratory curve indicates an amount of oxygen consumed in 3 minutes in the state of ATPS, i. e., ambient temperature and pressure, with water vapor saturated. Wide interval, therefore, indicates a larger oxygen consumption compared with narrow one.

    Before thermal stimulation of the spinal cord, this animal has consumed 8.8 ml/kg·min oxygen calculated in V-stpd.

    Subsequent to this period, the spinal cord was cooled for 30 minutes, where the value of oxygen consumption was calculated 13.0 ml/kg·min in V-stpd. After the cooling period, the spinal cord was heated for 30 minutes, where oxygen consumption was decreased and the value of 11.3 ml/kg·min corresponded to 13% lower than the value of 13.0 ml/kg·min during cooling period. Similar application of thermal stimuli on the spi-

nal cord was repetitively performed as shown in this figure. The effect of stimuli oxygen consumption tend to be similar to that mentioned above. After thermal stimulation of the spinal cord, (the hatched portion, the upper part of the figure), the value of oxygen consumption was recovered as 11.5 ml/kg·min.

Figure 3a demonstrates the response of a decerebrated unanesthetized rabbit applied to variations of spinal cord temperature. When decerebrated, rabbits could also respond to spinal cord cooling and heating with adequated changes of activity of the autonomic thermoregulatory effectors. Under the conditions of a thermo-neutral of 26°C, the animal maintained a core temperature close to normal. Lowering spinal canal temperature resuted in an immediate drop of ear skin temperature (Te) indicating cutaneous vasoconstriction, in a decrease of respiratory rate, and an increase of electromyographically indentified shivering, although responses were less distinct than those in intact animals. Heating of the spinal cord induced opposite changes of all recorded parameters.
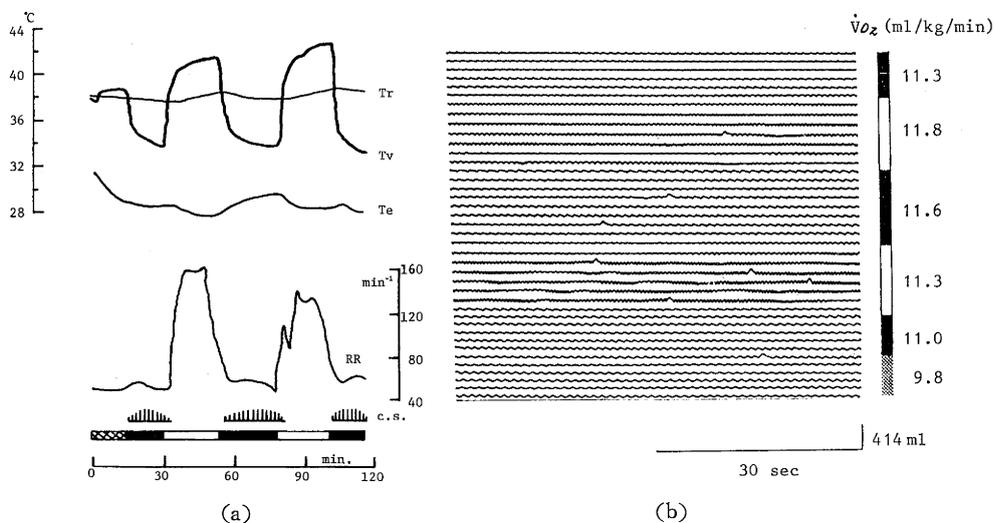

Fig. 3 Thermoregulatory responses (a) and oxygen consumption (b) to cooling and heating the spinal cord in the decerebrated rabbit. Abbreviations are same as in Fig. 2.

Oxygen consumption of the same decerebrated rabbit presented in Fig. 3a was shown in Fig. 3b. Sbusequent recording to the transsection, this rabbit has shown 9.8 ml/gk·min oxygen calculated in V-stpd in the thermo-neutral condition (hatched line), and this value is closely resemble to the normal rabbit. Spinal cord cooling increased oxygen consumption of about 12%.

The results of change in oxygen consumption induced by cooling and heating the spinal cord in twenty normal (a) and decerebrated (b) rabbits are summarized in Fig. 4. n indicates the temperature of the spinal cord which kept constant at neutral (rectal) temperature, that is, 39°C. h and c mean the spinal temperature at 42°C and 34°C, respectively. The mode of spinal thermal stimulation was divided into four groups as follows;

NORMAL   **DECEREBRATE**
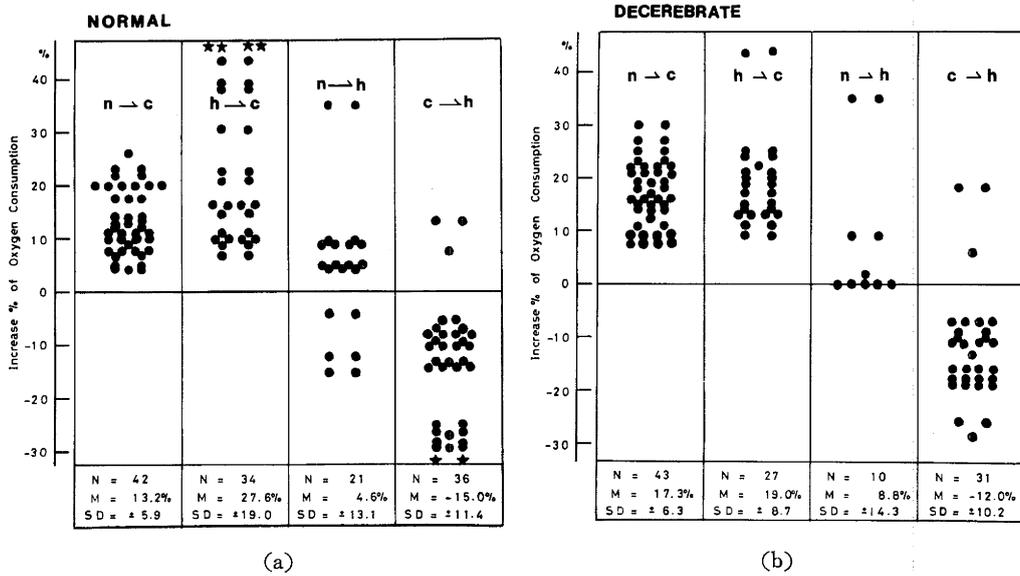
(a)   (b)

Fig. 4 Comparison of changes in oxygen consumption induced by cooling and heating the spinal cord of normal (a) and decerebrate (b) rabbits

        Changes in oxygen consumption of twenty rabbits elicited by spinal thermal stimuli are summarized. Mode of stimulation: neutral-cool indicates that temperature of the spinal cord before cooling is kept constant at neutral (rectal) temperature (details see text). Data significanty different from the values before thermal stimualtion within 1% level. Difference of the values of increase percent of oxygen consumption between n-c and h-c is statistically highly significant with p value 0.01 calculated from the Student's t test. But there are not statistically significant between normal (a) and decerebrate (b) group.

n→c : before cooling spinal cord temperature was kept constant at rectal temperature.

h→c : before cooling, spinal temperature at 42°C or so,

n→h : before heating, spinal cord temperature was kept at rectal temperature, and

c→h : before heating, spinal temperature at 34°C or so.

     Both 42°C and 34°C of spinal cord temperature at the beginning of the thermal stimulation described in h→c and c→h column in both figures indicated that the spinal cord was exposed in the state of heating and cooling, respectively. In normal subjects, it was obvious that oxygen consumption in both cases of n→c and h→c was significantly increased, and difference of the increased value of oxygen consumption between n→c and h→c was also statistically significant (p<0.01). While slight increase of oxygen consumption was observed during heating as demontrated in n→h column, however, the change of value was not statistically significant i.e., within 1% level.

     On the other hand, it is clear that marked decrease of oxygen consumption was induced by successional heating from the cooling period, since mean increase of 13.2% of oxygen consumption at the beginning of heating returned at least to the value of

pre-cooling level by successional heating the spinal cord in the case of the column c→h.

Nevertheless, the findings of excess increase of oxygen consumption in h→c compared with n→c as well as of marked decrease of oxygen consumption in c→h still suggest the suppressive effect on metabolic action concerning the secondary chemical regulation of body temperature.

In decerebrated rabbits (Fig. 4b), mean increase in percentage of oxygen consumption was 17.3% when spinal cord was cooled from normal state. It was 19.0% if a heated spinal cord was cooled suddenly. When spinal cord was heated from the normal condition, the oxygen consumption was increased by 8.8% but using a cooled spinal cord brought −12.0% increase in oxygen consumption. The date obtained from both normal and decerebrated rabbits were almost the same, and were not statistically significant.

DISCUSSION

Evidence has been accumulated that thermosensitive structures must exit in the spinal cord (Jessen et al., 1968; Kosaka et al., 1969), in addition to the well known cutaneous thermal receptors and the thermally sensitive neurons of the preoptic region (Hardy et al., 1964; Nakayama et al., 1963). In dogs, there is some evidence that the hypothalamus and spinal cord act as approximately equivalent sensors of temperature and both areas give thermoregulatory responses to warm and cool stimuli (Jessen and Mayer, 1971).

In the present investigation, decerebrated rabbits were submitted thermal stimulation of the spinal cord, and compared the thermoregulatory responses with those of the intact animals. These stimulations are reported not to elicit major changes of heart rate (Iriki et al., 1972). Comparison of these time courses with those typical for thermoregulatory responses to the same stimulus in intact rabbits was considered as a valuable means to identify autonomic thermoregulatory functions in decerbrated animals.

Its has shown that the time courses of the changes in oxygen consumption, skin temperatures, and respiratory rate induced by alterations of spinal cord temperature in decerebrated rabbits closely resemble those observed in intact animals. Furthermore, it has reported that thermoregulatory responses of rabbits impairing preoptic hypothalamus (PO/AH) by X-ray irradiation were similar to those of PO/AH intact animal although heat loss capability was slightly reduced (Ohwatari et al., 1983, Kosaka et al., 1984).

The lower brain stem is a site where temperature signals from the spinal cord can be transformed effectively into adequate drives controlling autonomic thermoregulatory effectors, especially the vasomotor tone of the skin vessels and the ventilation of the upper respiratory tract (Kosaka et al., 1975b). Thus, additional support is contributed to the concept that the temperature regulation system is composed of hierarchically organized control loops located in the spinal cord and in various sections of the brain stem with the

anterior hypothalamus and possibly also cortical structures as the highest level of integration (Walther *et al.*, 1971).

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正常および除脳ウサギの脊髄温度刺激による酸素消費量の変化

小坂光男・藤原真理子・范　育仁・大渡　伸・岩元　純（長崎大学熱帯医学研究所・疫学―環境生理）
高羽祥三・磯部芳明（名古屋市立大学医学部・生理学教室）

視床下部外中枢温度感受性の概念に立脚して，無麻酔・弱拘束条件下で，正常および除脳ウサ

ギに選択的脊髄温度刺激を加えて誘発される体温調節反応，特に熱産生反応に関する酸素消費量の変化を観察・記録し次の結果を得た.

(1) 平常環境で正常ウサギの平均酸素消費量は 8.8ml/kg・min，除脳ウサギのそれは 9.8ml/kg・min を示した.

(2) 除脳ウサギの選択的脊髄冷却で酸素消費量の平均増加率は17.3％，脊髄加温状態からの急峻な冷却では19.0％の増加率を示した.

(3) 除脳ウサギの脊髄加温での平均増加率は8.8％，脊髄冷却状態からの急峻な加温では，逆に12.0％の減少を示した.

(4) これらの結果と，同様な実験条件下で正常ウサギから得られた酸素消費量変化との間に統計的に有意な差異を示さなかった.

以上の研究結果から，脊髄温度刺激効果は視床下部を介することなく，中脳一橋，延髄，脊髄といった視床下部外中枢温度感受性組織における体温調節中枢機構に直接的に作用すること，これは進化過程からみて体温が中枢神経幹軸の階層状機構によって調節されていることを示唆する.