Hypothermia and Gastric Lesions in Rats Exposed to Immobilization Stress

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Summary: Male Wistar rats were exposed to immobilization stress and changes in rectal temperature and incidence of gastric lesions were examined. Rectal temperature in immobilized rats significantly decreased within 2 hours of immobilization and the significant decrease continued up to 24 hours of immobilization. Hypothermia induced by stress rapidly recovered to control levels when the rats were released from 18-hours of immobilization. Based on macroscopical occurrence of gastric lesions after exposure to immobilization stress for 12 hours, rats were divided into two groups, i.e., affected or non-affected rats by gastric lesions. When examined retrospectively, the former showed significantly lower rectal temperatures than the latter. Pretreatment with a synthesis inhibitor of serotonin (5-hydroxytryptamine, 5-HT), p-chlorophenylalanine (PCPA) at 250 mg/kg completely abolished the stress-induced hypothermia. This suggests that stress-induced hypothermia may be mediated by the brain's 5-HT system.

Key words: immobilization stress — hypothermia — gastric lesion — rectal temperature — p-chlorophenylalanine — serotonin

Introduction

Gastric lesions induced by several types of stress are considered the primary animal model of the group of psychosomatic disorders known as "stress ulcer" (Brodie, 1971). Among these stresses, immobilization is a useful and common method for inducing stress in animals (Singh, 1971), in which psychological factors might be more involved.


We have reported that immobilization stress in the rat causes hypothermia and a significant increase in the content of 5-hydroxyindoleacetic acid (5-HIAA), a principal metabolite of serotonin (5-hydroxytryptamine, 5-HT) without alteration of 5-HT content in the whole brain (Tanaka et al. 1974). Rectal temperature is considered a useful, physiological index and a more conveniently measured parameter compared to other physiological indices, such as pulse rate and blood pressure. Moreover, we can collect data from
the animals during immobilization without sacrificing the animals.

In the present study, more detailed investigations were made to clarify changes in rectal temperature during immobilization stress and their relationship to the incidences of gastric lesions.

**Methods**

Male Wistar rats weighing 200-250 g were housed at constant room temperature (24 ± 1°C) and exposed to a 12:12 light-dark regimen (light on at 7 a.m. and off at 7 p.m.). Food and water were provided *ad libitum* except during the experiment.

Immobilization stress was employed by enclosing animals in a flexible wire mesh (3 × 3 mm) initially formed into a cone and then bent to conform to the size of the individual animals according to the method of Ader (1963). Rats were exposed to immobilization stress from 5:00 p.m. in all experiments. Control animals were deprived of food and water during the same period when the experimental rats were immobilized. Both immobilized and control animals were kept individually in the 20 × 30 × 25 cm compartments during the experiment in the room where the temperature was kept at 24 ± 1°C.

Rectal temperature was measured with an electronic thermister (Nihon Kohden) inserted 5 cm into the anus.

Four studies were undertaken independently. In the first study, twenty-four rats were divided into two groups, control and immobilization. Rectal temperature in both group was measured every 2 hours for 24 hours. In the second study, ten rats were immobilized for the first 18 hours and subsequently released from immobilization and left free in the compartment for the subsequent 2 hours, similarly with controls. Rectal temperature was measured during immobilization and the free period at times indicated in Figure 2.

In the third study, 46 rats were immobilized for 12 hours with rectal temperature measurements every 4 hours and then sacrificed by ether anesthesia. Stomachs were removed, opened along with the greater curvature, pinned on a cork board and fixed in 10 % formalin solution. Occurrence of gastric lesions was examined macroscopically. Then rats were divided into two groups depending on whether gastric lesions were observed or not, i.e., the affected group in which gastric lesions were developed and the non-affected group in which none developed. Their rectal temperatures which had been recorded prior to sacrifice were compared in a retrograde manner. In the fourth study, we examined the effect of the synthesis inhibitor of 5-HT, p-chlorophenylalanine (Koe and Weissman, 1966), on changes in rectal temperature induced by immobilization stress. Para-DL-chlorophenylalanine (PCPA, Sigma) was suspended in 0.3 % carboxymethylcellulose (CMC) solution. PCPA at 250 mg/kg or vehicle (0.3 % CMC solution) was injected intraperitoneally 16 hours before immobilization. Half of rats treated with PCPA or vehicle were immobilized for 12 hours and the remaining half served as respective controls. Rectal temperature was measured every 4 hours during 12 hours of immobilization period.

**Results**

Rectal temperature of immobilized rats was significantly reduced within 2 hours of immobilization stress compared to temperatures of control animals, as shown in Figure 1. These significant decreases continued up to 24 hours of immobilization. Control animals showed virtually constant temperature except for slight fluctuations due to diurnal variation. The mean difference in temperature between the two groups ranged from 1.1°C to 2.5°C but averaged approximately 2°C from 8 hours...
to 24 hours. Variations of temperature in the immobilized rats were larger than those in controls and tended to increase depending upon the duration of the immobilization period.

In the second study (Figure 2), the rectal temperature in immobilized rats was significantly lower than that in controls, as similarly observed in the first study; however, the lowered temperature in the immobilized rats rapidly recovered to the levels of controls after they were released from immobilization. Statistical significance was still obtained 15 min after release but not at 60 min and there was no difference at all between the two groups 120 min later.

In the third study, thirty rats were affected by gastric lesions and 16 rats not affected. Affected rats showed significantly lower temperature at every time of immobilization than non-affected animals (Figure 3).

PCPA by itself failed to affect rectal temperature; however, pretreatment with the drug completely abolished the stress-induced hypothermia observed in animals pretreated with vehicle (Figure 4).

**Figure 1.** Changes in rectal temperature during immobilization stress for 24 hours.

Each value indicates the mean±S.E.M. of 12 rats. Statistically significant as compared to controls: *P<0.05, **P<0.01, ***P<0.001

**Figure 2.** Changes in rectal temperature during immobilization for 18 hours and subsequent 2 hours free from the prior immobilization.

Each value indicates the mean±S.E.M. of 10 rats. Statistically significant as compared to controls: **P<0.01, ***P<0.001 (∆P<0.10)

**Figure 3.** Changes in rectal temperature of rats affected or non-affected by gastric lesions.

The values in affected and non-affected groups indicates the mean±S.E.M. of 30 and 16 rats, respectively. Statistical significances between the two groups are: *P<0.05, **P<0.01, ***P<0.001

See text for details.
Figure 4. Effect of pretreatment with p-chlorophenylalanine (PCPA) on stress-induced changes in rectal temperature in rats. Rats in the top group were injected intraperitoneally with 0.3% carboxymethylcellulose (CMC) solution and those in the bottom group with PCPA at 250 mg/kg 16 hours before immobilization.

Each value indicates the mean ± S. E. M. of 10 rats. Statistically significant as compared to respective controls: *P<0.05, **P<0.01, ***P<0.001

See text for details.

Discussion

Although there have been various reports concerned with thermoregulation in rats, few studies have been made in changes in body temperature in immobilized rats. Grant (1950) reported that restraint stress caused hypothermia in rabbits which varied depending on the environmental temperature and suggested a relationship between hypothermia and stress-induced emotion.

In rats, we have already reported that hypothermia occurs during immobilization stress (Tanaka et al. 1974). The present study shows that rectal temperature in the rat is significantly decreased within 2 hours of immobilization and that hypothermia induced by immobilization continues up to at least 24 hours of stress. This finding is consistent with our previous finding (Tanaka et al. 1974) and the recent work by Amar and Sanyal (1981). Variations of rectal temperature in immobilized rats increased depending on the course of immobilization. This may be due to increases in individual differences in response to continuous stress. Control animals showed slight variation in rectal temperature, higher in the dark period and lower in the light period, which appears to result from diurnal rhythm of body temperature (Friedman and Walker, 1968).

The present study further indicates that stress-induced hypothermia very rapidly recovers to control levels when the rats are released from immobilization. This suggests that hypothermia is caused by immobilization itself. Recently, Athey and Iams (1981) reported that both spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto rats (WKY) show hypothermia, when exposed to cold-restraint stress, and that the former has a significantly lower core temperature. The decreased core temperature in their study was much lower than that in our study, as shown in mean values of 25.4°C (SHR) and 29.1°C (WKY). This seems to be due to the difference in environmental temperature, as pointed out by Grant (1950) and Avery (1972).

The stress-induced hypothermia was completely abolished by pretreatment with a synthesis inhibitor of 5-HT, PCPA (Koe and Weissman, 1966). In rats, Morgan et al. (1975) demonstrated the increases in 5-HT turnover by immobilization stress in the cerebral cortex but not in the diencephalon. We have noted, by measuring simultaneously 5-HT and 5-HIAA, that
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5-HT turnover in areas such as the diencephalon and amygdala is increased by the same immobilization stress as used in the present study (Tanaka et al. 1975). Together with these findings, it is suggested that immobilization-induced hypothermia may result from increased turnover of 5-HT during stress.

However, numerous reports, including ours, have also demonstrated that NA turnover is increased by immobilization stress not only in the whole brain but also in various brain regions in rats (Corrodi et al. 1968, 1971; Fuxe et al. 1970; Palkovits et al. 1975; Kvetnanský et al. 1977; Tanaka et al. 1979, 1980, 1981). We cannot exclude the possibility that noradrenergic system might be involved in the stress-induced hypothermia.

Since Feldberg and Meyers (1963) suggested a new concept of temperature regulation by amines in the hypothalamus, numerous conflicting reports have been made on the involvement of brain monoamines and acetylcholine in thermoregulation in some species (Cooper et al. 1965; Meyers and Yaksh, 1968; Meyers, 1974; Lomax et al. 1969; Beckman, 1970; Avery, 1971, 1972; Grawshaw, 1972). Cooper et al. (1965) pointed out the possibility of species-differences in response to locally applied monoamines, and Meyers (1974) emphasized the important distinction between intraventricular and intrahypothalamic route of administration of a monoamine. To date, it is difficult to draw a general view of thermoregulation mechanisms where many neurochemical systems are probably involved.

The suggestion that hypothermia may be closely related to the brain's 5-HT system, such a conclusion is not conclusive, since PCPA was administered only systematically in the present study and thermoregulation mechanisms are probably more complicated. However, the idea is consistent with the suggestion made by Amar and Sanyal (1981) that the late phase of hypothermia between 45-120 min of immobilization is mediated by the 5-HT system.

The present study reveals that rats affected by gastric lesions show greater decreases in rectal temperature compared to non-affected animals. This indicates that a relationship may exist between hypothermia induced by stress and development of gastric lesions and that hypothermia may be a useful index in predicting the occurrence of gastric lesions. A similar relationship between body temperature and ulcer formation was observed in the activity-stress ulcer paradigm developed by Paré (1977). Athey and Iams (1981) showed that SHR with a lower core temperature than indicated a less positive ulcer index than WKY when exposed to cold-restraint. This is inconsistent with our data, which seems to be due to the strain-difference, such a specific strain as SHR or due to different experimental conditions, since they employed cold-restraint stress and we used only restraint stress.

The present study demonstrates that immobilization stress causes hypothermia in rats which is reversed by systemic administration of PCPA. This suggests that stress-induced hypothermia is closely related to brain 5-HT and that rectal temperatures are a useful physiological index in the assessment of bodily changes caused by stressful stimuli, since it can be measured simply and continuously without sacrificing animals.

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References

ADER, R. (1963). Gastric erosions in the rat: Effects of immobilization at different point


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