Changes in cold- and heat-defence following electrolytic lesions of raphe nuclei in the guinea-pig

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Abstract. In conscious guinea-pigs the effects of electrolytic lesions of the nucleus raphe magnus (NRM) and of adjacent areas in the lower brainstem on cold- and heat-defence were studied. Changes in core temperature, heat production and heat loss effectors as well as their threshold temperatures were compared in the same animals before and after lesioning. As an additional index of heat loss, ear skin temperature and a derived parameter - vasomotor index - were also measured. Three days after NRM lesion the fall in core temperature evoked by an exposure to 14 - 15°C was smaller than before lesion, furthermore the body temperature threshold for shivering increased. Cold-induced heat production was also higher following NRM lesions. Lesions outside the NRM or sham-operation did not influence cold-defence. After NRM lesion heat-defence was also improved; the rise in core temperature elicited by an exposure to 36 - 37°C was smaller and the body temperature threshold for ear skin vasoconstriction during recovery from hyperthermia decreased. No change in respiratory evaporative heat loss could be observed after NRM lesion. Lesions outside the NRM or sham-operation did not influence heat-defence. An attempt has been made to explain the observed improvements in cold- and heat-defence by discussing relevant data on mechanisms in central temperature control.

Key words: Temperature regulation -- Cold-defence -- Heat-defence -- Nucleus raphe magnus lesion -- Body temperature thresholds

Introduction

Recently interest has been increased on the role of the lower brainstem in various aspects of central body temperature control [see 4, 5]. One of the sites of importance at that level of the brain has proved to be the raphe system, traditionally looked upon as an anatomical and functional entity with similar neurochemical architecture in different mammalian species [14, 16]. Although new lines of evidence from a large number of studies utilizing a sophisticated neurochemical repertoire have demonstrated considerable heterogeneity in the "chemical wiring" within the different raphe nuclei, some convergence in our understanding of the function of this part of the brain has occurred during the last decade.

One major area of research has been involved in studies on the role of some raphe areas in modulation of nociceptive spinal mechanisms [1, 8]. However, non-noxious information from peripheral receptors, including thermal information within the physiological range also reaches the central controlling sites through the lower brainstem. It has been suggested that the nucleus raphe magnus (NRM) may act as a relay station of warm-receptive information ascending from the skin [3, 7, 19]. The same raphe nucleus has been shown to be the origin of pathways projecting to the hypothalamus [2], the activation of which could specifically influence warm- and cold-responsive hypothalamic units in the guinea-pig [3]. Also in the guinea-pig, electrical stimulation of NRM inhibited cold-induced thermogenesis, a response not influenced by the interruption of descending pathways known to inhibit cutaneous sensory inputs probably at the level of the dorsal horn in the spinal cord [10].

In the present study guinea-pigs were exposed to a standard cold environment before and after lesioning of the NRM to see if cold-defence would change in the manner expected from the results of the electrical stimulation experiments mentioned above. Furthermore, standard heat exposure was applied in the same animals to test the idea of the role of ponto-medullary raphe also in influencing heat-loss reactions, since electrical stimulation of the NRM could increase the activity of hypothalamic warm-responsive units [3].

Preliminary results of the present study have been reported elsewhere [17].

Methods

Twenty-one guinea-pigs of either sex aged 5 - 7 weeks, with an initial body weight of 310 - 380 g, were used. The animals were held at an ambient temperature of 21 - 24°C before the first experiment and also during the ensuing week which elapsed between the first and the second thermophysiological experiment.

On the morning of the experiment thermocouples were introduced into the colon (8 cm from the anus), under the back skin and on the inner surface of one of the ear-lobes using halothane anaesthesia. Electrodes for measuring integrated electrical activity were inserted into the thigh and masseter muscles on the right side and sutured in place. After the effect of the anaesthetic had worn off, the guinea-pig was fixed to a plexiglass plate with strings bound gently around the lower end of the four extremities as well as with a plaster tape around the back.

The course of the experiments was as follows:

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A. Thermophysiological experiment 1

1. The animal was placed into a metabolic chamber with wall and air temperature held at 28.5°C (range 28.0–29.0°C) for 1.5–2 h, during which time body temperatures and metabolic rate became stabilized at their normal resting levels.

2. A cold exposure of 14–15°C range was then applied for 1 h, followed by a 2-h period of exposure to 28–29°C.

3. A heat-exposure of 36–37°C was then initiated, which lasted until all signs of heat-defence (i.e. polypnea, wall and air temperature held at 28.5°C) had developed (1–1.5 h).

4. An additional exposure to 28–29°C concluded the experiment leading to recovery from the previous heat-induced hyperthermia.

5. Under halothane anaesthesia all electrodes and thermocouples were removed and the animal placed back into its cage.

B. Operation

Three days later an i.p. injection of nembutal (40 mg per kg body weight) was given and the animal’s head placed in a stereotaxic apparatus according to the co-ordinate system of Rössner [15]. After drilling holes in the midline of the skull and incising the dura a stainless steel lesioning electrode (0.1 mm in diameter with a 0.1 mm free end) was lowered aiming at the NRM and adjacent brainstem sites.

In 14 guinea-pigs electrolytic lesions were made by using 1.0–1.5 mA current for 10 s at 2.4–6.0 V. One leg of the animal serving as reference site. In all cases two lesions, 0.3–0.6 mm apart were placed sagittally from each other. In 7 guinea-pigs no current was passed through the electrode (sham-operation). The electrode was then withdrawn, the wound sutured and the animal placed back into its cage.

C. Thermophysiological experiment 2

Three days after surgery the guinea-pig was again supplied with the electrodes and thermocouples (this time the left ear-lobe and the left masseter and thigh muscles having been used) and procedure A was repeated. Since the duration of the heat-exposure varied in different guinea-pigs during experiment A, care was taken to expose each guinea-pig to heat for the same time as was the case in the first experiment.

After an overdose of Nembutal the animal’s brain was quickly removed and placed into a buffered formaldehyde solution. The localization of lesions were evaluated using standard histological methods.

The following physiological variables were measured during the whole course of experiments A and C:

1. Colonic temperature ($T_{colon}$).
2. Subcutaneous temperature on the back ($T_{back}$).
3. Surface temperature of the ear-lobe ($T_{ear}$).
4. Integrated electrical activity in the two muscle groups (EMA).
5. Respiratory evaporative heat loss (REHL), using temperature and humidity data at the inlet and near the outlet air flowing through the metabolic chamber.
6. Metabolic rate (MR) by measuring oxygen consumption (open system) using a paramagnetic oxygen analyser.

The variables listed were measured consecutively by a digital voltmeter and sampled every 30 s by a Hewlett-Packard 9603A measurement and control system and stored on a magnetic tape. All these data as well as mean body temperature ($T_b = 0.5 \times T_{colon} + 0.5 \times T_{back}$) and ear-lobe vasomotor index (VMI) were recorded on-line by a Hewlett-Packard printer.

Mean body temperature thresholds for shivering thermogenesis ($T$ - SH), for rise in metabolic rate ($T$ - MR) were used to characterize the heat production response during cold-exposure. Similarly, the mean body temperature threshold for REHL ($T$ - REHL) was utilized to judge the onset of rise in evaporative heat loss during heat-exposure. To get a deeper insight into the functioning of heat-loss effectors an index of the vasomotor tone, VMI was utilized (Szelényi and Székely, unpublished). There is some information in the literature that — similar to the rabbit’s ear temperature — ear temperature of the guinea-pig also shows signs of thermoregulatory vasomotor reactions [9]. To get information on changes in $T_{ear}$ independent of changes in core temperature ($T_{colon}$) and ambient temperature ($T_a$), the following equation was used:

$$VMI = \frac{T_{ear} - T_a}{T_{colon} - T_a}$$

Except for extremely rapid changes in the respective temperatures, VMI can be a useful indicator of vasomotor tone on ear-skin and theoretically its value can range from almost 0 to 1 (full vasoconstriction and vasodilatation, respectively). Preliminary experiments showed that VMI did not change reliably during heat-exposure to indicate vasodilatation-threshold. After transfer of the animal to a near-thermoneutral $T_a$, however, a slow recovery from the heat-induced hyperthermia (while $T_{colon}$ and $T_{ear}$ are declining practically in parallel) gives way to a steep fall in $T_{ear}$ indicating vasoconstriction. When calculated in the way given above, VMI does not change during the first phase of recovery from hyperthermia, then there is a very steep fall followed by a second steady-state of the VMI value (see inset in Fig. 1). The $T_b$ at which the start of this sudden decrease in VMI indicates onset of vasoconstriction thus may be taken as a threshold of vasomotor heat-defence in the guinea-pig (Fig. 1). This body temperature threshold ($T$ - VMI) represents another estimate of heat-defence; the lower the $T$ - VMI, the later the animal stops vasodilatation which contributes to recovery from hyperthermia.

For statistical analysis the one-sample and the multiple $t$-test were used. For the calculations of thresholds linear regression analysis was applied.

Results

Histological evaluation of the lesion sites revealed that the 14 guinea-pigs with brainstem lesions could be divided into two sub-groups according to the localization and extension of lesions (Fig. 2). In 7 guinea-pigs much of the NRM as well as — in 4 cases — the ventral part of the nucleus raphe pontis (NRP) had been destroyed (“NRM-lesion”). In the other 7 guinea-pigs the lesions either were localized more laterally — thus leaving the raphe system intact — or destroyed only the NRP or more distal and dorsal parts of the median lower brainstem. These animals were therefore grouped as “lesions outside the NRM”. These latter lesions, though heterogenous in terms of their localization, can be regarded as an homogeneous group from the point of view of their leaving the NRM and its immediate surroundings intact.