

Thermogenesis elicited by skin cooling in anaesthetized rats: lack of contribution of the cerebral cortex

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Non-noxious cooling stimuli were delivered to the shaved back of urethane-chloralose-anaesthetized, artificially ventilated rats using a plastic bag containing water at 24–40°C. Cooling of the skin by 2–6°C increased the rate of whole body oxygen consumption (\dot{V}_{O_2}) and triggered electromyographic (EMG) activity recorded from the neck or femoral muscles. The cooling-induced \dot{V}_{O_2} responses did not depend on core (colonic) temperature and followed skin temperature in a graded manner. Pretreatment with the β -blocker propranolol (10 mg kg⁻¹, i.v.) greatly attenuated the \dot{V}_{O_2} response but did not affect the EMG response. On the other hand, pretreatment with the muscle relaxant pancuronium bromide (2 mg kg⁻¹, i.v.) affected the \dot{V}_{O_2} response very slightly but completely abolished the EMG activity. Accordingly, the cooling stimulus activated mainly non-shivering thermogenesis. Next, the contribution of the cerebral cortex to the cooling-induced thermogenesis was examined. Power spectral analysis of the electroencephalogram (EEG) showed that the cooling stimulus largely inhibited delta (0.5–3 Hz) waves, enhanced theta (3–8 Hz) waves, and slightly increased frequencies higher than 8 Hz. Pinching the hindpaw elicited changes in EEG similar to those elicited by skin cooling but did not increase the \dot{V}_{O_2} . Therefore, there was no relationship between changes in the EEG and the magnitude of thermogenesis. Finally, skin cooling increased the \dot{V}_{O_2} of decorticated rats but did not increase that of decerebrated rats. The results suggest that the subcortical forebrain structure, but not cortical activation, is indispensable for non-shivering thermogenesis elicited by cooling stimulation of the skin.

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Thermoafferent signals originating from cutaneous cold or warm receptors are not only involved in conscious temperature sensations but also play an important role in autonomic and behavioural responses of the organism to its thermal environment. Thermal information from the skin of the trunk is conveyed to the dorsal horn of the spinal cord. The thermoafferent pathway then projects to the lower brainstem structures and diverges further to sensory thalamic nuclei and regulatory hypothalamic areas (Hensel, 1981; Pierau, 1996). The hypothalamus, in particular the preoptic area (POA), contains neurones sensitive to temperatures of both local brain and skin, suggesting convergence and integration of peripheral and central thermal information (Boulant & Hardy, 1974). Because the POA exerts a diversity of thermoregulatory functions, including control of cutaneous blood flow for heat loss, muscle tone for shivering thermogenesis, and thermoregulatory behaviours, it is considered the primary

controlling structure in thermoregulation (Boulant, 1980; Hensel, 1981; Simon *et al.* 1986; Kanosue *et al.* 1998).

However, changes in firing rate of POA neurones in response to thermal stimulation of the scrotum (Kanosue *et al.* 1984, 1985) or the trunk skin (Berner & Heller, 1998) were always found to be associated with desynchronizations of the electroencephalogram (EEG). These studies suggest that the apparent responses of POA neurones to thermal stimulation of the skin were not specific but reflected EEG activation caused by the thermal stimulation. Berner & Heller (1998) therefore raised a question as to whether or not peripheral temperature information is integrated in the POA and claimed the possibility that thermal information of the skin is integrated lower in the neuroaxis. Indeed, Blatteis & Banet (1986) showed that microknife separation of the POA from the rest of the brain had no significant effect on thermoregulatory functions in warm or cold environments

and concluded that the POA is not essential for the integration of autonomic thermoregulatory responses.

A decrease in skin temperature elicits metabolic heat production, reflected by an increased oxygen consumption rate (\dot{V}_{O_2}). Although unequivocal evidence exists on the hypothalamic control of thermogenesis (Boulant, 1980; Kanosue *et al.* 1998), it is still an open question as to whether thermoafferent signals that reach the POA play an essential role in cold-induced thermogenesis. Heat production can also be elicited by non-thermal stimulation such as systemic administration of capsaicin (Kobayashi *et al.* 1998) or an intraduodenal infusion of hypertonic solutions (Osaka *et al.* 2001). Interestingly, these thermogenic responses do not require the forebrain, because decerebrated rats exhibited clear heat production responses to capsaicin (Osaka *et al.* 2000) and duodenal osmotic stimulation (Osaka *et al.* 2002). Similarly, Kenney *et al.* (2000) demonstrated that whole-body heating in chloralose-anaesthetized, midbrain-transected rats increased renal and splenic nerve activity and concluded that forebrain neural structures were not required for mediating these responses.

The aim of the present study was to examine the involvement of the forebrain and activation of the cerebral cortex in cold-induced thermogenesis. For this purpose, anaesthetized rats were used because their baseline metabolic rate is stable and various surgical or pharmacological manipulations are possible. Although anaesthetized animals retain only limited ability to maintain body temperature, in particular, by metabolic heat production, acute body cooling increases activity of the sympathetic nerves innervating the interscapular brown adipose tissue (Talan *et al.* 1996; Morrison, 1999) and elicits non-shivering thermogenesis (Nour *et al.* 1984; Talan *et al.* 1996). Moreover, anaesthetized animals can exhibit shivering in response to lowering body temperature (Asami *et al.* 1988; Zhang *et al.* 1995; Tanaka *et al.* 2001). In this study, I examined the mechanism of thermogenic responses of urethane–chloralose-anaesthetized, artificially ventilated rats to mild cooling of the skin. However, because no systematic study has ever been reported on skin cooling-induced thermogenesis in anaesthetized animals, I evaluated firstly whether this response is an adequate model for the study of cold-induced thermogenesis.

Methods

Male Wistar rats, weighing 350–500 g, were maintained at an ambient temperature of $24 \pm 1^\circ\text{C}$ with lighting between 07.00 h and 19.00 h for at least 1 week before

the experiments. They had free access to water and laboratory food. The care of animals and all surgical procedures followed National Institute of Health and Nutrition guidelines.

After induction of anaesthesia with 2–3% isoflurane in air and cannulation of a femoral vein and the trachea, the rats were kept anaesthetized intravenously with urethane (600 mg kg^{-1}) and α -chloralose (60 mg kg^{-1}). An electromyogram (EMG) was recorded with a pair of Teflon-coated flexible stainless-steel wires inserted into the femoral muscles, filtered at 150 Hz–3 kHz, and monitored on an oscilloscope. In some experiments, the EMG was also recorded from the dorsal neck muscles. Occasionally, the signals were digitized at 4 kHz and stored on a hard disk. A mixture of urethane ($70\text{--}80 \text{ mg kg}^{-1} \text{ h}^{-1}$) and chloralose ($7\text{--}8 \text{ mg kg}^{-1} \text{ h}^{-1}$) was continuously administered with the aid of a syringe pump (KDS100, KD Scientific, MA, USA), started 90–120 min after the initial anaesthesia. This anaesthetic regimen was determined and slightly adjusted in each rat so that paw pinching evoked EMG activity recorded from the same limb but did not elicit withdrawal responses. Pinching the contralateral paw did not evoke EMG activity from the limb measured for the activity. Animals were killed by an overdose of anaesthetic at the end of experiment.

The back of each rat was shaved between the caudal end of the forelimbs and the rostral end of the hindlimbs, and the rats were positioned prone on an operating table heated to $32\text{--}33^\circ\text{C}$. Three thermocouples were glued to different sites on the shaved skin. The mean of these three readings was used as the measure of skin temperature (T_s). Colonic temperature (T_c) was measured with a fourth thermocouple inserted $\sim 50 \text{ mm}$ into the anus. Tail skin temperature was occasionally measured with another thermocouple taped to the dorsal surface of the tail. The trunk and proximal part of the limbs were covered with a quilt to reduce heat dissipation. Respiration was maintained with an artificial respirator (Harvard pump 683). The intermittent expiratory gas from the respirator was introduced into a 30-ml reservoir, which was continuously ventilated with ambient air at a constant rate of 1 l min^{-1} . The difference in O_2 concentrations between reservoir and ambient air was measured with an O_2 analyser (LC-700E, Toray, Japan). Values were corrected for metabolic body size ($\text{kg}^{0.75}$). These signals were fed into a computer and recorded at 6-s intervals through a PowerLab system (ADInstruments, Australia) for on-line data display, storage, and off-line analysis. After the experiments, the data were averaged over 30-s intervals.

A pair of silver electrodes was placed on the somatosensory cortex for the bipolar recording of the EEG.

A stainless-steel screw was placed in the skull over the cerebellum and used as an earthed reference. Electrodes were secured in place by covering with a dental acrylic resin. The signal was filtered (0.5–30 Hz), digitized at 100 Hz, and stored on a hard disk. The power spectrum was computed from a 1-min EEG record by using the PowerLab's spectrum software, which divided the EEG record into 50% overlapping successive 46–48 segments, each of 256 data points, applied the fast Fourier transformation (FFT) with a cosine-bell window, and calculated the average spectrum from these FFTs.

Thermal stimulation of the skin was delivered by placing a plastic bag containing 24–40°C water on to the shaved back for 2 min instead of the quilt that covered the rat.

The bag was agitated every 30 s for 4–5 s to mix the water uniformly. Preliminary experiments showed that skin cooling for longer than 2 min or use of colder water resulted in a significant decrease in T_c ; and therefore I did not analyse the effects of such stimulation. The quilt was replaced in its original position soon after the 2-min thermal stimulation. This procedure kept T_s within the non-noxious range of 28–40°C (Defrin *et al.* 2002). The water bag was made of a polyethylene sheet 70- μm thick and had a bottom surface area of 110 mm \times 180 mm, and contained 340–360 ml of water. The bag covered not only the shaved back but also parts of the unshaved skin and the operating table on which the rat had been placed. The scrotum, tail, head and distal part of the limbs

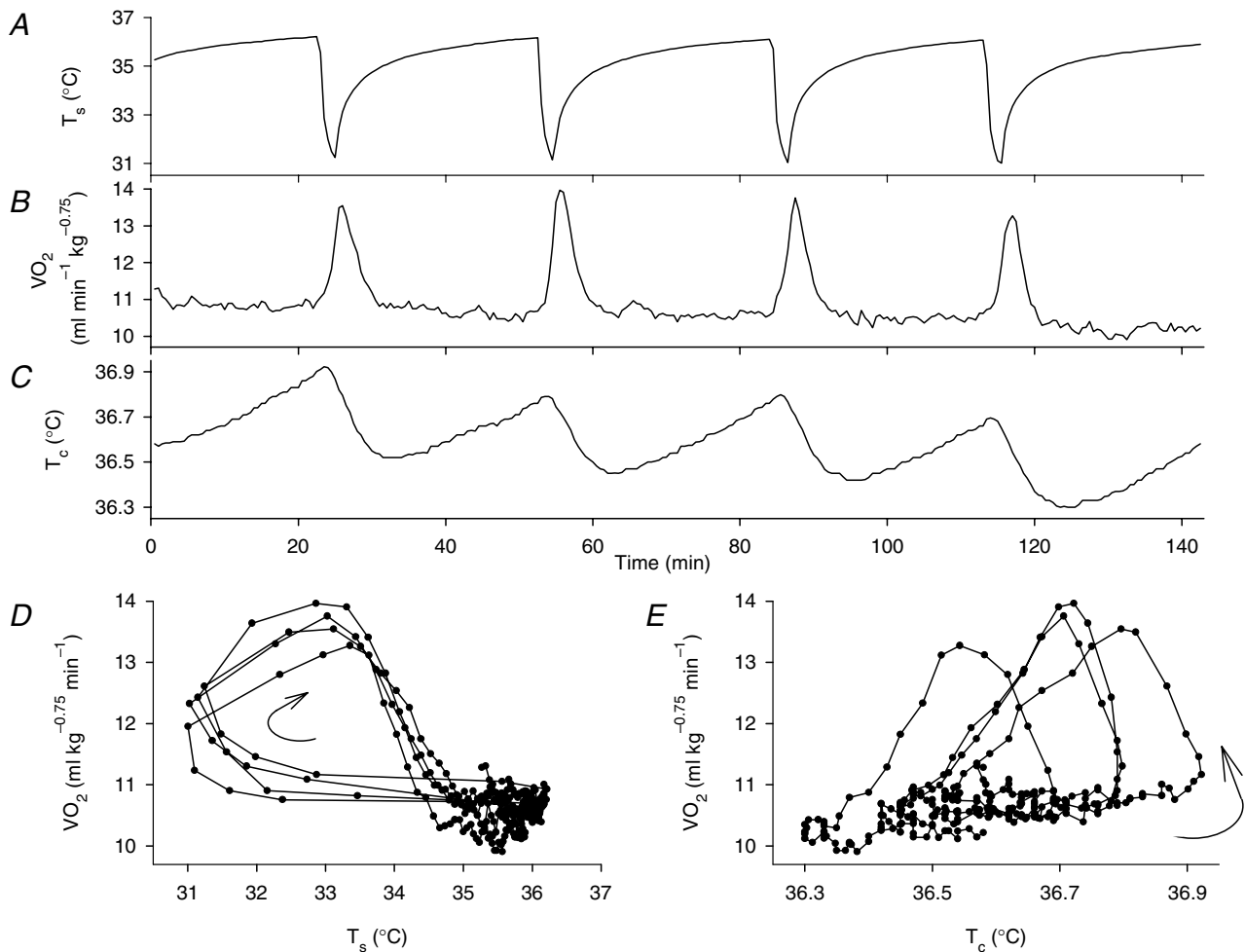


Figure 1. Thermogenic responses to skin but not core (colonic) temperature

A representative record showing T_s (A), \dot{V}_{O_2} (B) and T_c (C) in response to repeated skin cooling episodes with a plastic bag containing water at 28°C. D and E, sequential plots of \dot{V}_{O_2} versus T_s and T_c , respectively. Sequential points are joined by lines, and the direction of changes is shown by curved arrows. Although the \dot{V}_{O_2} response to skin cooling lagged behind T_s , resulting in hysteresis (D), the relationship between \dot{V}_{O_2} and T_s was constant in repeated cooling episodes. However, the relationship between \dot{V}_{O_2} and T_c varied during the experiment (E). These plots thus suggest that an input from the skin, but not from the core, determined the thermogenic response.

did not receive thermal stimulation. In most cases, the bag contained water at 28°C and lowered T_s by 4–5°C. The temperature of the operating table was increased by 1–2°C during repeated cooling episodes to compensate for the cooling-induced decrease in T_c . Preliminary experiments ($n = 5$) showed that the coefficient of variation of the decrease in T_s ranged between 1.2 and 3.8% when a rat received, at 30-min intervals, four consecutive applications of the bag containing water at the same temperature, thus indicating controlled and reproducible thermal stimulation in repeated trials.

The β -adrenoceptor blocker DL-propranolol hydrochloride was dissolved in physiological saline solution and i.v. administered at a dose of 10 mg kg^{-1} . The neuromuscular blocking agent pancuronium bromide (Mioblock, Sankyo, Japan) was infused i.v. at a dose of 2 mg kg^{-1} . A mixture of pancuronium (1 mg

$\text{kg}^{-1} \text{h}^{-1}$), urethane (70–80 mg $\text{kg}^{-1} \text{h}^{-1}$), and chloralose (7–8 mg $\text{kg}^{-1} \text{h}^{-1}$) was continuously infused to maintain the muscle relaxation and anaesthetic level. Skin cooling was applied before and 15–20 min after administration of the drugs. The effect of the drugs on the magnitude of cooling-induced thermogenesis was analysed using the paired t test.

Decerebration was achieved using a method previously described (Osaka *et al.* 1989). Briefly, the head was fixed in a stereotaxic apparatus, and a slit was made in the frontal plane of the skull at a position 4 mm rostral to the interaural line. An L-shaped small knife was bilaterally inserted into the brain through the slit with the aid of a micromanipulator until it made contact with the cranium floor. Care was taken not to damage the sagittal sinus. The level and completeness of the transection were later confirmed visually by examination of the cut on the ventral surface of the brain.

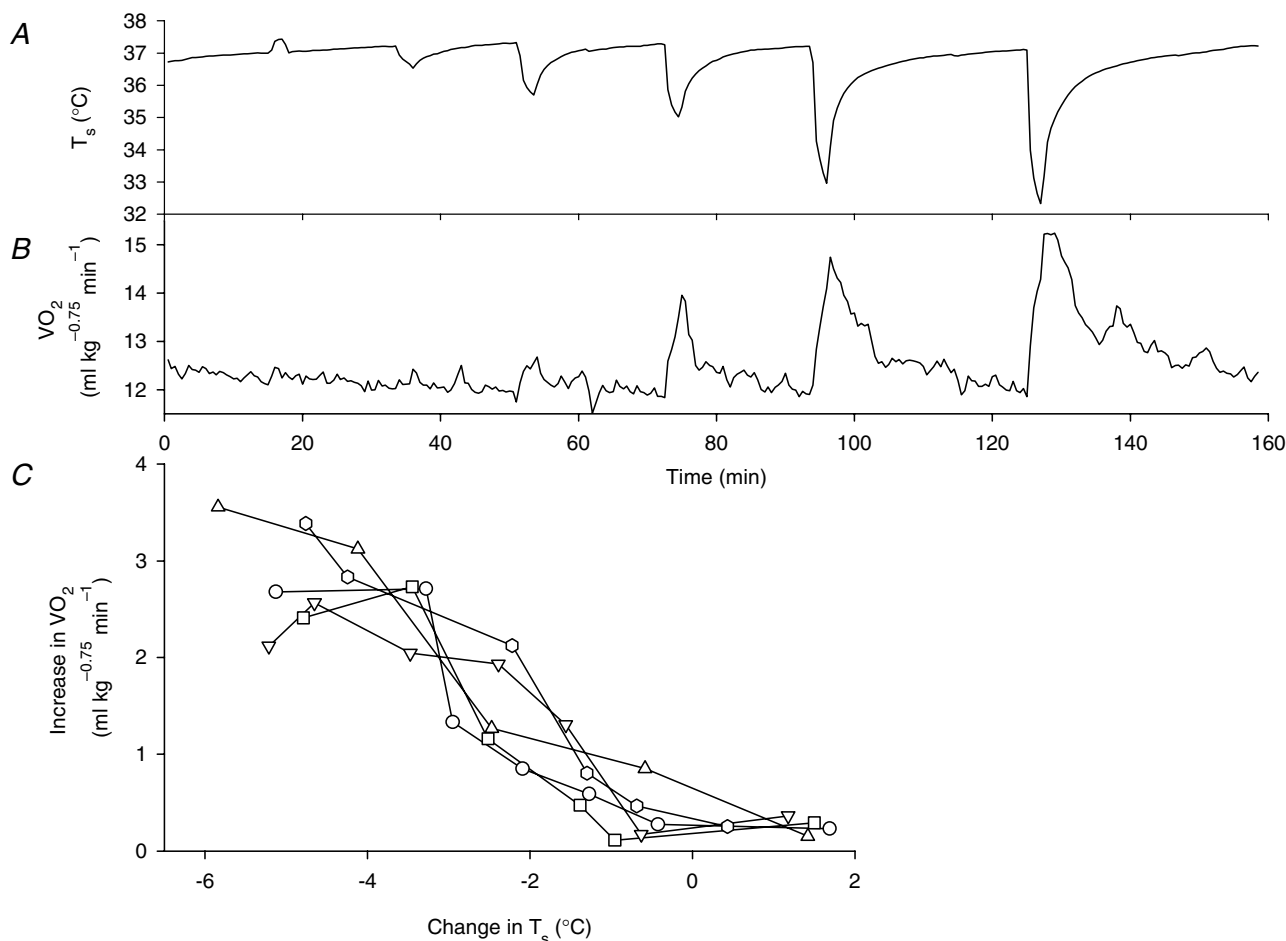


Figure 2. Cooling intensity-dependent thermogenic responses

Changes in T_s (A) and $\dot{V}O_2$ (B) of a rat in response to thermal stimulation of the skin delivered by a plastic bag containing water at 38, 36, 34, 32, 28, or 26°C. C, peak increase in $\dot{V}O_2$ plotted against change in T_s of five rats, showing that skin cooling of more than 1°C increased $\dot{V}O_2$ in an intensity-dependent manner. Each set of symbols and connecting lines shows data from the same rat.

Decortication was performed using method similar to that described by Huston (1983). After an extensive craniotomy, the neocortex, dorsal hippocampus, and most of the striatum were bilaterally removed by aspiration. The thalamus, caudal part of striatum, preoptic area and its adjacent septal nuclei, ventral hippocampus and amygdala remained largely intact. The surface of the exposed brain was covered with a saline-soaked gelatine powder (Gelfoam powder, Pharmacia & Upjohn, Sweden) to prevent gradual bleeding. The incision in the skin was closed with a cyanoacrylate adhesive. The effects of skin cooling were not examined until 1 h after surgery.

Results

Skin cooling with the bag containing water at 26–28°C for 2 min lowered the T_s by 4–5°C from the resting warm

temperature (35–38°C), and thereafter T_s recovered to its original level within 20–30 min (Fig. 1A). Baseline $\dot{V}O_2$ was $11.9 \pm 0.2 \text{ ml kg}^{-0.75} \text{ min}^{-1}$ (mean \pm s.e.m., $n = 31$). $\dot{V}O_2$ increased within 1 min after the onset of skin cooling and reached a peak increase by 2–3 $\text{ml kg}^{-0.75} \text{ min}^{-1}$ 1–2 min after the removal of the water bag (Fig. 1B). The sequential plot of $\dot{V}O_2$ against T_s (Fig. 1D) showed a 60–90 s delay of the $\dot{V}O_2$ response to the drop in T_s , and this plot also demonstrated that the relationship between $\dot{V}O_2$ and T_s was reproducible in repeated skin cooling episodes. The magnitude of the thermogenic responses to repeated stimuli of the same temperature varied slightly: the coefficient of variation of the increase in $\dot{V}O_2$ was $12.6 \pm 2.4\%$ (mean \pm s.e.m., $n = 5$) when a rat received four consecutive applications of cooling stimulation at the same temperature at 30-min

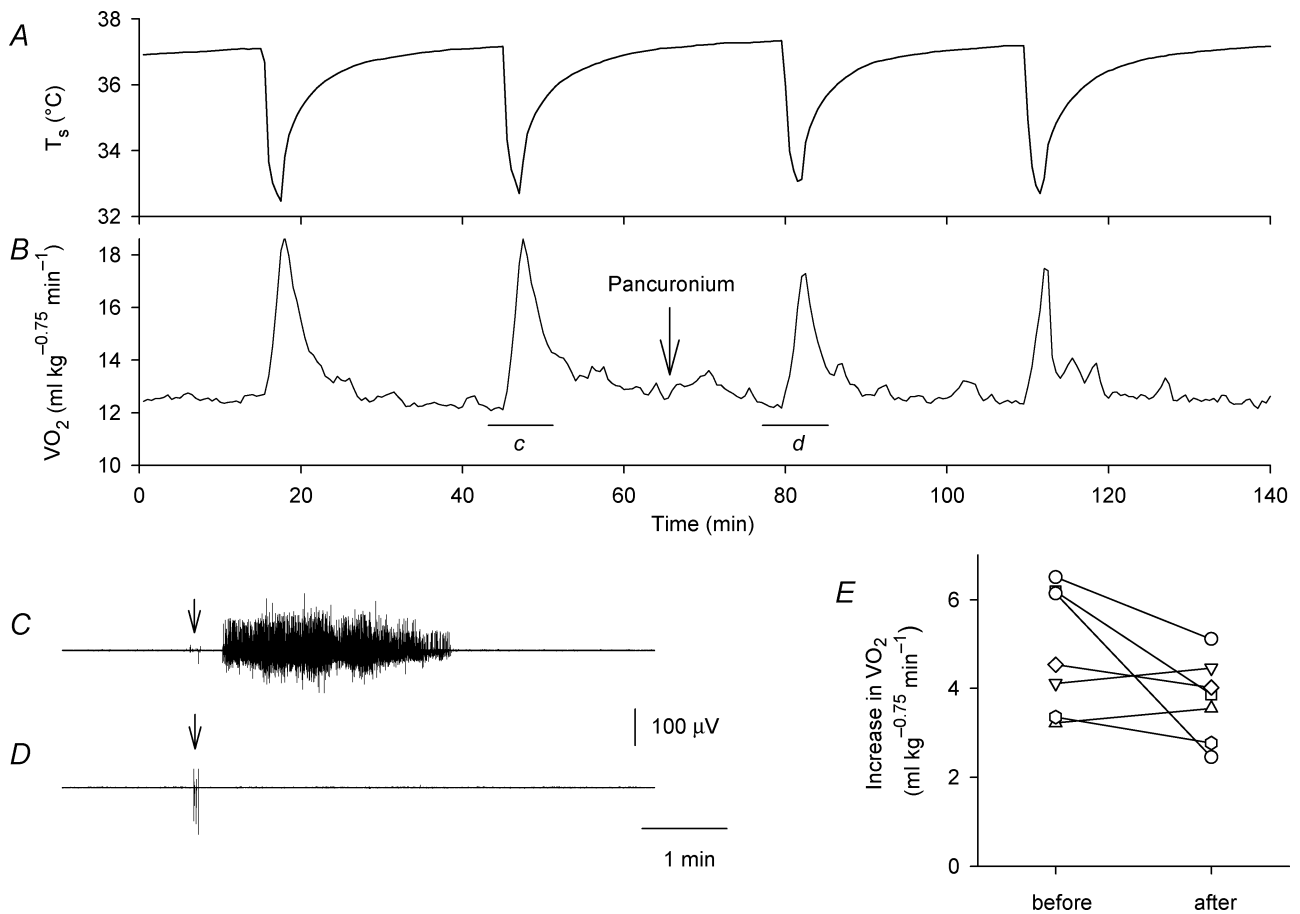


Figure 3. Effects of the muscle relaxant pancuronium bromide on skin cooling-induced thermogenesis and EMG responses

Changes in T_s (A) and $\dot{V}O_2$ (B) of a rat in response to skin cooling before and after administration of pancuronium (2 mg kg^{-1} , i.v., arrow). The horizontal bars 'c' and 'd' in B show the periods of the EMG records shown in C and D, respectively. The arrows in C and D show the onset of cooling stimulation. Administration of pancuronium completely blocked the cooling-induced EMG activity. E, cooling-induced increase in $\dot{V}O_2$ before and after administration of pancuronium in eight rats, showing the lack of a statistically significant change in the thermogenic response. Each set of symbols and connecting lines indicates an individual rat.

intervals. This condition lasted approximately 3 h, and the amplitude of responses decreased gradually thereafter. The duration of each thermogenic response varied by 5–30 min among preparations, but was approximately the same in the same rat. A single cooling episode gradually decreased T_c by 0.2–0.4°C 6–10 min after removal of the water bag (Fig. 1C). \dot{V}_{O_2} increased before the fall in T_c , reached its peak value during the falling phase of T_c , and declined to the baseline level when T_c reached its minimal level. Although the increase in \dot{V}_{O_2} was always associated with the initial drop in T_c , the sequential plot of \dot{V}_{O_2} against T_c (Fig. 1E) shows that there was no constant relationship between \dot{V}_{O_2} and T_c . On the other hand, the temperature of the tail skin was 28–32°C and decreased by 0.1–0.5°C in parallel with T_c after skin cooling, suggesting a steady vasoconstrictive state and a lack of further vasoconstriction in response to cooling stimulation.

Figure 2A and B shows the thermogenic responses of a rat to various intensities of thermal stimulation of the skin, and Fig. 2C summarizes the responses of five rats. Skin cooling by more than 2°C elicited thermogenesis, the magnitude of which increased with the intensity of cooling. However, skin warming by 1–2°C had no effect on \dot{V}_{O_2} , and skin cooling by 1°C also did not elicit a significant increase in \dot{V}_{O_2} above the level of baseline fluctuations.

The rats usually had no significant background EMG activity, indicating a lack of spontaneous activity of the skeletal muscles. Skin cooling always elicited EMG activity recorded from the femoral or neck muscles (Fig. 3C; Fig. 4C and D). The EMG consisted of a series of continuous action potentials, and did not exhibit the rhythmic burst pattern that is typically observed during shivering. The rats also exhibited momentary

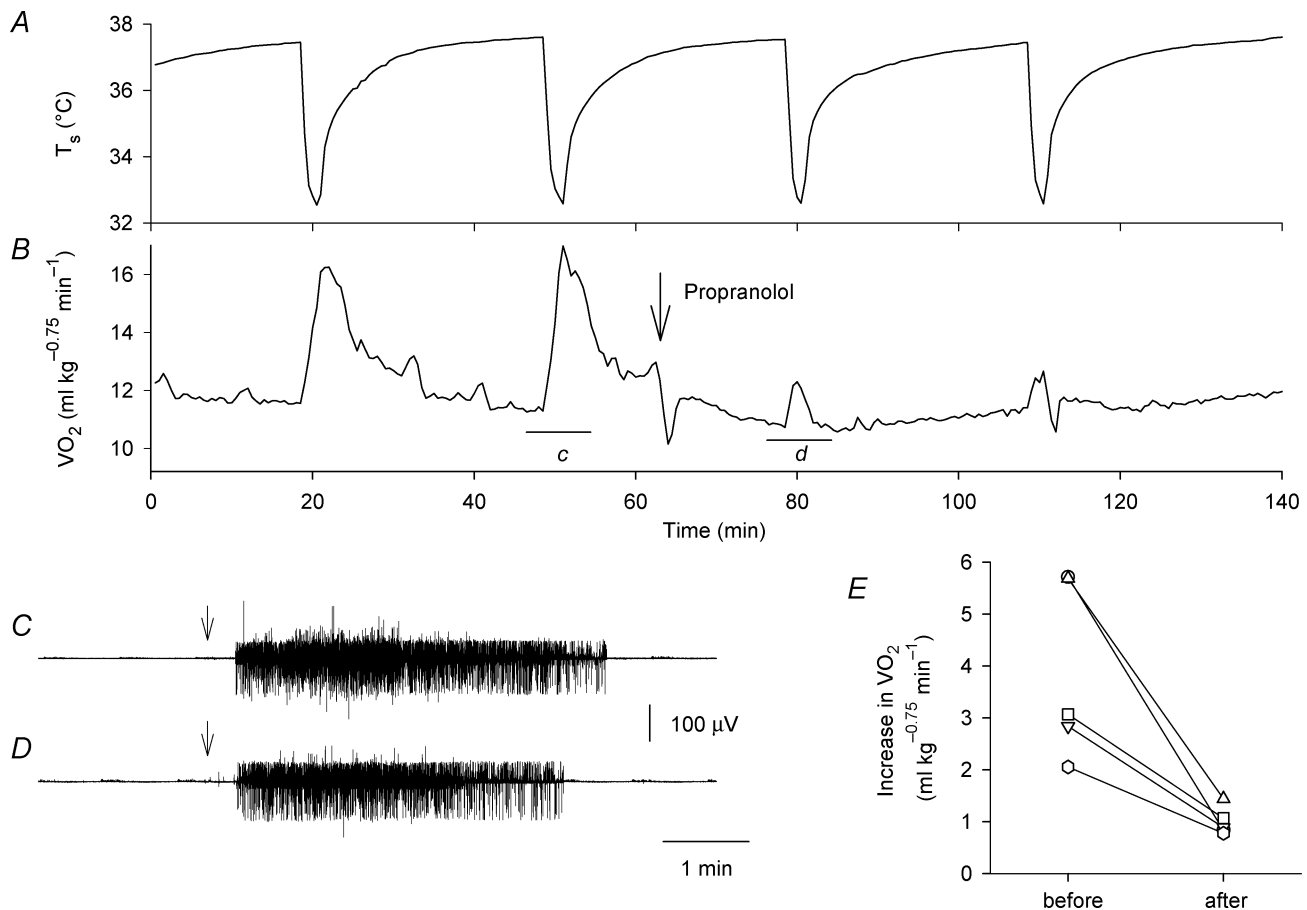


Figure 4. Contribution of β -adrenoceptors to skin cooling-induced thermogenesis

Effects of propranolol (10 mg kg⁻¹, i.v., arrow in B) on skin cooling-induced changes in T_s (A) and \dot{V}_{O_2} (B). The horizontal bars 'c' and 'd' in B show the periods of the EMG records shown in C and D, respectively. The arrows in C and D show the onset of cooling stimulation. Administration of propranolol had no effect on the cooling-induced EMG activity. E, cooling-induced increase in \dot{V}_{O_2} before and after administration of propranolol in five rats, showing significant attenuation of thermogenesis. Each set of symbols and connecting lines indicates an individual rat.

shivering-like reactions around the neck 1–5 times during the 2-min cooling period, though they did not usually move the lower portions of their bodies. However, the skin cooling-induced EMG activity occurred continuously, and the activity lasted for 1–10 min even when the rats did not move their bodies, as judged by palpation and visual observation. On the other hand, skin warming did not elicit any body movement or EMG activity. These observations could suggest that shivering made a contribution to skin cooling-induced thermogenesis, though there was no definite relationship between the increase in \dot{V}_{O_2} and the occurrence of body movements. Therefore, the effects of the muscle relaxant pancuronium bromide were examined. Administration of pancuronium did not affect baseline \dot{V}_{O_2} but effectively blocked cooling-induced body movements and EMG activity (Figs 3C and D).

However, cooling-induced thermogenesis largely persisted after administration of pancuronium (Fig. 3A and B). Figure 3E summarizes the increase in \dot{V}_{O_2} of individual rats before and after administration of pancuronium. Although the average magnitude of cooling-induced thermogenesis decreased from $5.07 \pm 0.50 \text{ ml kg}^{-0.75} \text{ min}^{-1}$ to $3.96 \pm 0.37 \text{ ml kg}^{-0.75} \text{ min}^{-1}$ (mean \pm s.e.m., $n = 8$) after administration of pancuronium, the difference was not statistically significant.

Administration of propranolol alone (10 mg kg^{-1}) decreased \dot{V}_{O_2} by $2\text{--}3 \text{ ml kg}^{-0.75} \text{ min}^{-1}$ for 3–5 min ($n = 5$), and thereafter the baseline level of \dot{V}_{O_2} was $0.5\text{--}1.5 \text{ ml kg}^{-0.75} \text{ min}^{-1}$ lower than the control level for 1–2 h. Figure 4A and B show that pretreatment with propranolol largely, but not completely, attenuated cooling-induced thermogenesis. All five rats examined showed a great decrease in their thermogenic response to

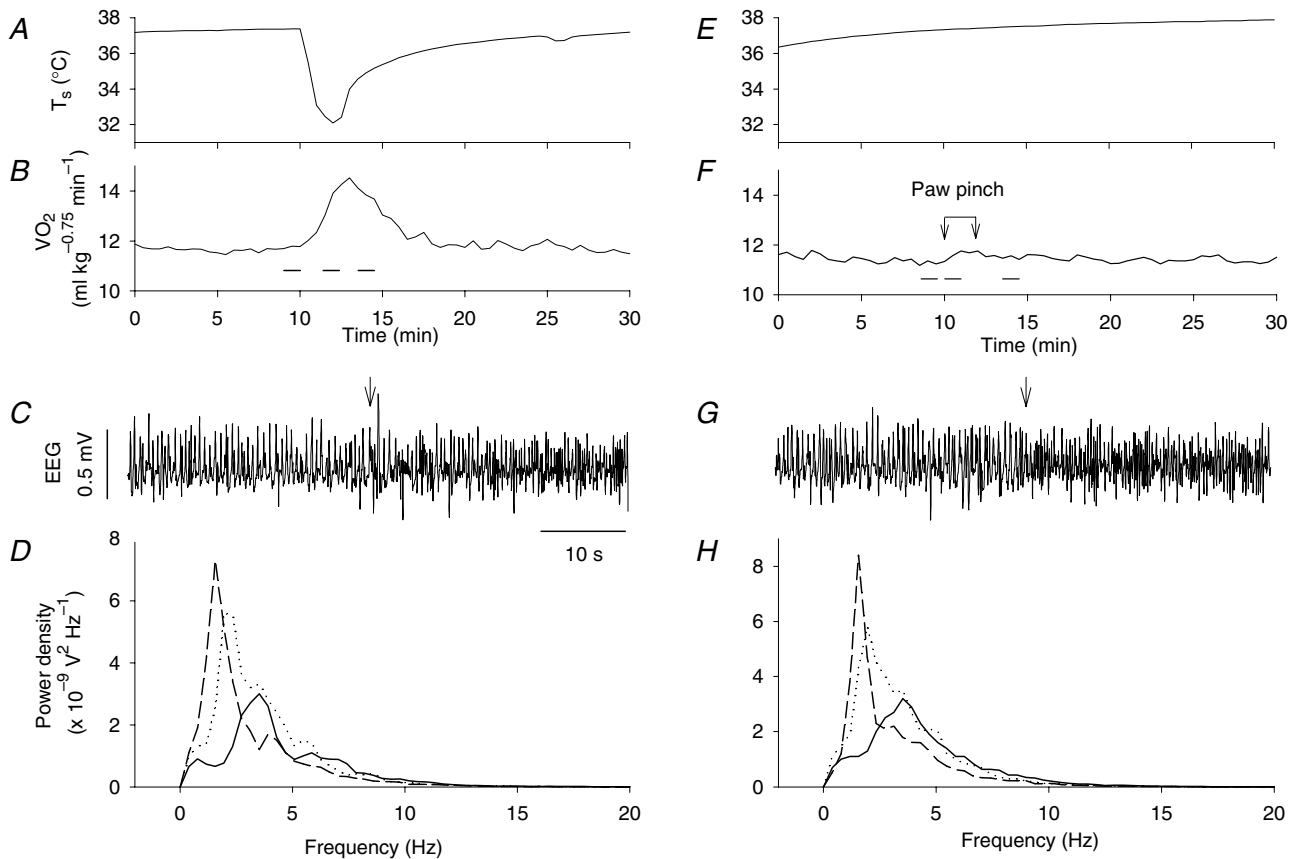


Figure 5. Changes in EEG elicited by skin cooling and paw pinch

Effects of skin cooling and paw pinching on T_s (A and E), \dot{V}_{O_2} (B and F), EEG (C and G), and the power spectrum of the EEG (D and H) recorded from a rat. The horizontal bars in B and F show the periods used to calculate power spectra of the EEG records shown in D and H, respectively. The arrows connected by a bar in F show the period of paw pinching. Dashed, continuous, and dotted lines in D and H show the respective power spectra obtained before, during, and after skin cooling (D) or paw pinching (H). The arrows in C and G show the onset of stimulation. Although the EEG was similarly affected by skin cooling and paw pinching, the thermogenic response was specific to skin cooling.

skin cooling after propranolol administration (Fig. 4E). On the other hand, cooling-induced EMG activity was not affected by administration of propranolol (Fig. 4C and D).

The spontaneous EEG consisted of mainly synchronized slow waves: power spectral analysis revealed that the proportions of delta (0.5–3 Hz), theta (3–8 Hz) and higher frequency (> 8 Hz) waves were 45–63%, 33–44% and 3–10% of the total power of the EEG, respectively ($n = 6$). Skin cooling abruptly changed the EEG to a theta-dominant state (Fig. 5A–D): the theta waves occupied 40–64% of the total power of the EEG during skin

cooling, although the power of higher frequency waves also increased, to 7–14%. The EEG recovered to the delta wave-dominant state within 2 min after cooling stimulation, when \dot{V}_{O_2} was still higher than the baseline level. Noxious pinching of the hindpaw also changed the EEG to the theta wave-dominant state (Fig. 5E–H). The spectral composition of the EEG during paw pinching (Fig. 5H) was very similar to that during skin cooling (Fig. 5D). However, in contrast to skin cooling-induced thermogenesis, paw pinching increased \dot{V}_{O_2} only very slightly (Fig. 5F). Although in two to three tests in each of six rats examined paw pinching could increase \dot{V}_{O_2} to 1 ml

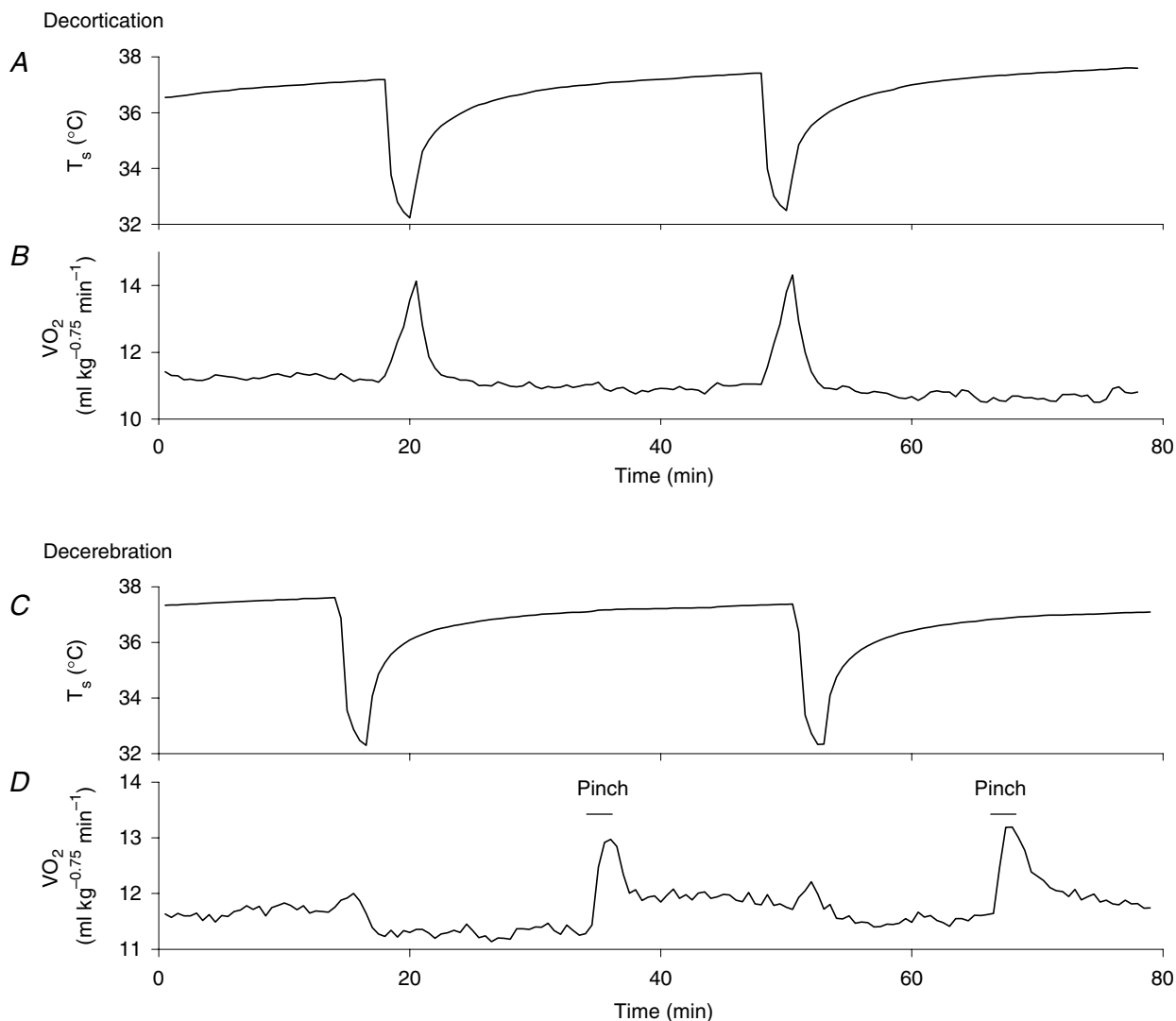


Figure 6. Involvement of the basal forebrain in skin cooling-induced thermogenesis

T_s (A and C) and \dot{V}_{O_2} (B and D) of decorticated (A and B) or decerebrated (C and D) rats. Thermogenic responses of a decorticated rat to skin cooling were similar to those of intact rats. However, a decerebrated rat examined ~2 h after stopping the infusion of urethane–chloralose solution exhibited a very small increase in \dot{V}_{O_2} followed by a period of inhibition, although paw pinching elicited significant thermogenic responses. These responses were reproducible in repeated tests. The bars in D show the period of paw pinching.

$\text{kg}^{-0.75} \text{ min}^{-1}$, the magnitude of thermogenesis induced by pinching was always smaller than that elicited by skin cooling in the same rat.

Two groups of four rats each were either decorticated or decerebrated. Their baseline \dot{V}_{O_2} value ($11\text{--}12 \text{ ml kg}^{-0.75} \text{ min}^{-1}$) and occasional shivering-like reactions during the cooling episode were similar to those observed in intact rats. Whereas skin cooling elicited normal thermogenic responses in the decorticated rats (Fig. 6A and B), it decreased the \dot{V}_{O_2} of decerebrated rats by $0.3\text{--}1 \text{ ml kg}^{-0.75} \text{ min}^{-1}$ for 5–20 min. To confirm the lack of a thermogenic response in the decerebrated rats, I examined the effect of skin cooling ~ 2 h after stopping the infusion of anaesthetics in two decerebrated rats. They exhibited a slight (at most $0.5 \text{ ml kg}^{-0.75} \text{ min}^{-1}$) increase in \dot{V}_{O_2} during the cooling episode, followed by a period of inhibition (Fig. 6C and D). However, pinching the hindpaw of these rats reproducibly increased \dot{V}_{O_2} by $1.2\text{--}1.7 \text{ ml kg}^{-0.75} \text{ min}^{-1}$ (Fig. 6D).

Discussion

A model of non-shivering thermogenesis: the skin cooling-induced \dot{V}_{O_2} response in anaesthetized rats

Mild non-noxious cooling of the skin elicited thermogenesis in anaesthetized rats. Deep anaesthesia generally blocks non-shivering thermogenesis (Albanese *et al.* 1994), whereas animals can exhibit thermogenic responses to cold under light anaesthesia (Nour *et al.* 1984). The anaesthetic regimen employed in the present study was based on the presence of EMG activity and the absence of withdrawal movements of the limb to paw pinching. There were, however, some variations in the magnitude of the \dot{V}_{O_2} response during repeated cooling episodes in a rat. The cause of this variation partly reflected the slight differences in baseline levels of T_c and \dot{V}_{O_2} in each cooling episode and might have been caused by minute changes in the depth of anaesthesia during the experiments. However, an average variation of 12.6% in the \dot{V}_{O_2} responses was considered sufficiently reproducible to allow an examination of the effects of the various treatments employed in the present study.

A plastic bag containing 340–360 g of water was used to deliver thermal stimulation. This procedure necessarily also involved mechanical stimulation due to the weight of the water. However, it is unlikely that thermogenic responses were elicited by mechanical stimulation, because skin warming by $1\text{--}2^\circ\text{C}$ had no effect on \dot{V}_{O_2} and because skin cooling increased \dot{V}_{O_2} in a temperature-dependent manner. A water-perfused jacket has been used to study the

effects of thermal stimulation of the trunk skin on single unit activity of medullary raphe neurones (Dickenson, 1977; Rathner *et al.* 2001) and on sympathetic fibres supplying the tail (Owens *et al.* 2002) in anaesthetized rats. However, the water bag method employed in the present study was simple and changes in T_s were of a similar magnitude and time course to those observed with the water jacket method, the main difference being that in the present study the stimulated area was limited to the dorsal skin of the trunk.

A decrease in either peripheral temperature or core temperature will produce an increase in metabolic heat production. Although skin cooling-induced thermogenesis was accompanied by a slight ($0.2\text{--}0.4^\circ\text{C}$) decrease in T_c , a larger ($1\text{--}7^\circ\text{C}$) decrease in T_c is generally employed to elicit body cooling-induced thermogenesis (Nour *et al.* 1984; Asami *et al.* 1988; Zhang *et al.* 1995; Talan *et al.* 1996; Tanaka *et al.* 2001). Moreover, in the present study, the increase in \dot{V}_{O_2} was well correlated with T_s but not with T_c . These results suggest that afferent signals originating from the skin, but not from the core, largely contributed to the thermogenesis observed. However, because the present experiments were not designed to measure the effect of core temperature in isolation, we cannot rule out a possible contribution from thermoreceptors in the spinal cord (Simon, 1974) or in the wall of the abdominal cavity (Riedel *et al.* 1973) to cold-induced thermogenesis.

There are two general types of heat production responses: shivering and non-shivering thermogenesis. The relative contributions of shivering *versus* non-shivering thermogenesis can be determined experimentally by giving muscle relaxants to block shivering or antagonists of the β -adrenoceptor to block non-shivering thermogenesis. Skin cooling occasionally evoked shivering-like body movements and always elicited EMG activity recorded from neck or femoral muscles. Since these observations suggested an involvement of shivering thermogenesis, the effects of the muscle relaxant pancuronium were examined. However, pretreatment with pancuronium did not significantly attenuate the skin cooling-induced thermogenesis, while it effectively blocked the body movements and EMG activity. Moreover, decerebrated rats did not exhibit thermogenesis but showed the muscular activity in response to skin cooling. Therefore, shivering contributed only a little amount, if at all, to the present thermogenic response. On the other hand, pretreatment with the β -blocker propranolol greatly attenuated the skin cooling-induced thermogenic response but did not affect the EMG response, indicating that non-shivering

thermogenesis was mainly activated in the present experimental conditions. However, I cannot strictly exclude some involvement of shivering thermogenesis because skin cooling did elicit muscular activity and pretreatment with propranolol did not completely abolish the thermogenic response. Also, the threshold temperature of shivering thermogenesis is generally lower than that of non-shivering thermogenesis (Gautier, 2000). It is possible that in the present experimental conditions skin cooling activated both non-shivering and shivering thermogenesis, but the relative contribution of shivering to heat production was negligible.

The \dot{V}_{O_2} response to skin cooling always lagged behind T_{sk} , resulting in hysteresis. The time lag probably reflected various processes of thermogenesis. These include, for example, (1) neural processing, such as the temporal summation of impulses in the afferent/efferent pathways, (2) the biochemical processes in the cells where thermogenesis occurs, and (3) the lag between the O_2 consumption in the cells and the decrease in pulmonary O_2 concentration. In addition, the delayed participation of the core thermoreceptors could also contribute to the time lag.

Involvement of the basal forebrain and lack of the contribution of the cerebral cortex in skin cooling-induced thermogenesis

Both skin cooling and paw pinching similarly attenuated the delta waves and enhanced the theta waves in the EEG, suggesting that the cerebral cortex was affected non-specifically by these stimuli. However, this non-specific influence could not account for the skin cooling-induced thermogenesis, because skin cooling elicited significantly larger thermogenic responses than did paw pinching. Although thermal stimulation of the scrotum or the trunk skin desynchronized the EEG in rats anaesthetized with 1–1.5 g kg⁻¹ urethane (Kanosue *et al.* 1984, 1985; Berner & Heller, 1998), the changes in the EEG that occurred in the present study were largely limited to the frequency range below 8 Hz, indicating the absence of arousal patterns. Similarly, Rathner *et al.* (2001) showed that skin cooling excited medullary raphé neurones without accompanying changes in the EEG of rats anaesthetized with isoflurane. Thus, cortical activation is not indispensable for the full expression of skin cooling-induced thermogenesis.

Decorticated rats still exhibited thermogenic responses to skin cooling, which result also demonstrated that any effect of cortical arousal could not account for the skin cooling-induced thermogenesis. However, decerebrated rats did not increase \dot{V}_{O_2} in response to thermal

stimulation. Because the cerebral transection was made between the hypothalamus and midbrain, the subcortical forebrain structure is responsible for this response. It is likely that the POA in the hypothalamus is critically involved in this response, because bilateral microinjections of bicuculline, a GABA_A receptor antagonist, into the POA blocked skin cooling-induced thermogenesis (author's unpublished observation). On the other hand, paw pinching elicited small but significant thermogenic responses in decerebrated rats when supplemental anaesthetics were not administered. Thus, the lower brainstem or spinal cord could mediate thermogenesis elicited by nociceptive stimulation.

The present study demonstrated that cutaneous thermoafferents reaching the basal forebrain, possibly the POA of the hypothalamus, are indispensable to non-shivering thermogenesis. Thermoregulation is achieved by several hierarchically arranged subsystems, including the spinal cord, lower brainstem, and hypothalamus (Satinoff, 1978). The lack of thermoregulatory deficits in chronic rats subjected to microknife separation of the POA from the rest of the brain (Blatteis & Banet, 1986) could be attributable to the compensatory function of the intact subsystems. Similarly, many classic studies have shown the recovery of thermoregulatory abilities in animals or patients after partial or complete transections of the spinal cord, although these responses were much reduced in comparison with those in intact animals or patients and were not sufficient to maintain body temperature. To understand the organization and compensatory function of the thermoregulatory system, it is critical to define neural pathways and transmitters among thermoregulatory subsystems. In particular, our knowledge on thermoafferent pathways is still quite fragmentary. The preparation employed in the present study will be useful for elucidating the organization of thermoafferents and the mechanisms underlying non-shivering thermogenesis.

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