Warm and cold signals from the preoptic area: which contribute more to the control of shivering in rats?

Yi-Hong Zhang, Motoko Yanase-Fujiwara, Takayoshi Hosono and Kazuyuki Kanosue*

Department of Physiology, School of Allied Health Sciences, Osaka University Faculty of Medicine, Yamadaoka 2-2, Suita, Osaka 565, Japan

1. To find out whether the thermosensitive neurones in the preoptic area that control shivering are predominantly warm or cold sensitive, we tested the effects of injecting the excitatory amino acid L-glutamate at various sites in and adjacent to the preoptic area of anaesthetized rats shivering at ambient temperatures of 15–21 °C.

2. L-Glutamate injections (0·2 mM in 0·5–1·0 μl), as well as preoptic warming and electrical stimulation, suppressed shivering, whereas control saline injections had no effect. Effective sites were restricted to the anterior part of the preoptic area, and a tenfold lower concentration of L-glutamate did not influence shivering.

3. Injections of procaine (0·2 M) into the sites where L-glutamate suppressed shivering did not affect strong shivering activity, but facilitated shivering in three out of seven cases when shivering was weak or absent at higher ambient temperatures (25–30 °C).

4. L-Glutamate injections, as well as preoptic warming and electrical stimulation, also elicited vasodilatation of the paw skin and the tail. Procaine elicited vasoconstriction when it was applied during vasodilatation induced by local preoptic warming.

5. These results indicate that the contribution of the preoptic area to the control of shivering and vasomotion is influenced more by signals from warm-sensitive neurones than by signals from cold-sensitive neurones.

The preoptic area and anterior hypothalamus (POAH) plays an important role in body temperature regulation by responding to changes in local brain temperature and integrating information about other body temperatures. To explain signal processing in the POAH, Hammel (1968) proposed a model in which thermoregulatory effectors are controlled according to the balance between signals from high-Q_{10} and low-Q_{10} POAH neurones: heat loss is activated when signals from high-Q_{10} neurones predominate, and heat production is activated when signals from low-Q_{10} neurones predominate. Although the first study of single unit activity in the POAH (Nakayama, Eisenman & Hardy, 1961) had not found neurones with a negative temperature coefficient, cold-sensitive POAH neurones were found several years later (Eisenman & Jackson, 1967). Bligh, building on an idea of Wyndam & Atkins (1968), suggested that signals from hypothalamic and extrahypothalamic warm receptors are summed to form efferent signals driving heat loss, that signals from cold receptors are summed to form efferent signals driving heat production, and that there are inhibitory connections between these efferent paths (Bligh, 1973). Boulant, moreover, analysed the effect of peripheral thermal input on the firing rate and thermosensitivity of POAH neurones, and suggested that cold-sensitive neurones and high-frequency warm-sensitive neurones control heat production, while medium-frequency warm-sensitive neurones control cutaneous vasomotor tone and low-frequency warm-sensitive neurones control evaporative heat loss (Boulant, 1974).

All the neuronal models proposed so far coincide in postulating that heat loss is controlled by excitatory efferent signals that increase with increasing local brain temperature (warm signals), and that heat production is controlled by excitatory efferent signals that increase with decreasing local brain temperature (cold signals). Both *in vivo* and *in vitro* microelectrode surveys of the POAH, however, have found warm-sensitive neurones to be more plentiful than cold-sensitive neurones (Boulant, 1980; Nakayama, 1985; Hori, 1991). POAH control of heat production might therefore be due more to the inhibitory signals of warm-sensitive neurones than to the excitatory signals of cold-sensitive neurones (Benzinger,

* To whom correspondence should be addressed.

Pratt & Kitzinger, 1961; Anderson, Anderson & Gale, 1962). The present study tested this possibility by measuring shivering and, for comparison, vasomotor activity when the excitatory amino acid L-glutamate was injected into thermosensitive sites in the POAH of anaesthetized rats. Local POAH warming suppresses shivering and local cooling facilitates shivering (Hammel, Hardy & Fusco, 1960; Anderson *et al.* 1962; Kanosue *et al.* 1991). Therefore we reasoned that if L-glutamate suppressed shivering the main contribution to the control of shivering would be inhibitory signals from warm-sensitive neurones, and if L-glutamate enhanced shivering the main contribution would be excitatory signals from cold-sensitive neurones.

METHODS

Our experiments used fifty-five adult male Wistar rats (350–400 g) from a colony housed at 22 °C with free access to food and water. After anaesthesia was induced by 3% sevoflurane (Maruishi, Osaka, Japan) inhalation, ketamine hydrochloride (200 mg (kg body wt)$^{-1}$) was injected I.P. Ketamine was infused I.P. at 60 mg (kg body wt)$^{-1}$ h^{-1} throughout each experiment and additional doses of ketamine were given as needed to keep the depth of anaesthesia at or below the first plane of stage 3 (Lumb & Jones, 1984). This anaesthetic regime was approved by the Animal Care Committee of Osaka University Medical School.

Each rat was mounted in a stereotaxic apparatus according to the co-ordinate system of Paxinos & Watson (1986), and in forty-one rats an electrode–thermocouple assembly (Kanosue *et al.* 1991; Kanosue, Hosono & Yanase-Fujiwara, 1994*a*; Kanosue, Zhang, Yanase-Fujiwara & Hosono, 1994*c*) was implanted with its tip in the left preoptic area 0·5–1·5 mm from the mid-line, between 0·5 mm anterior and 1·0 mm posterior to bregma, and 9·0–9·5 mm below the skull surface. This assembly was an insulated stainless-steel tube (0·4 mm i.d., 0·6 mm o.d.) with a bared sharp tip used for thermal and electrical stimulations and with a copper–cobalt thermocouple for monitoring local hypothalamic temperature (T_{hy}) glued inside. In a preliminary experiment we measured the temperature gradient around the electrode in a rat in which two thin thermocouples (0·35 mm o.d.) were implanted 1·0 and 3·5 mm posterior to the electrode and with their tips at the same depth as the electrode tip. When the temperature measured with the thermocouple in the electrode was increased 5 °C by radio-frequency warming current, the increase in brain temperature 1·0 and 3·5 mm from the electrode was 2·5 and 0·7 °C, respectively. Tissue around the electrode was not damaged as long as T_{hy} was not raised above 42 °C (Kanosue *et al.* 1994*c*). A stainless-steel guide tube (0·5 mm o.d.) for drug microinjection was implanted into the right preoptic area 0·5–2·5 mm from the mid-line, between 1·0 mm anterior and 1·5 mm posterior to bregma, and the tip of this tube was 4·5–9·5 mm below the skull surface. The electrode–thermocouple assembly and guide tube were fixed to the skull with dental cement.

In the other fourteen rats a guide tube rather than an electrode–thermocouple assembly was implanted, also into the left preoptic area.

Experimental procedures

Shivering. Each rat, still anaesthetized as described above, was put into a climatic chamber (30×40×80 cm), and the rectal temperature (T_{re}) was measured by a thermocouple inserted into the rectum to 6 cm past the anal sphincter while an electromyogram (EMG) was recorded bilaterally from needle electrodes in the m. quadriceps femoris. The signal was amplified (gain 2000 from 10 Hz to 1 kHz), and an integrated EMG was obtained by rectifying the EMG signal and filtering it through a low-pass filter (5 Hz). An injection cannula (0·3 mm o.d.) for drug application was inserted into the guide tube so that its tip protruded 0·5 mm beyond the tip of the guide tube. A polyethylene tube connected the injection cannula to a 5 μl Hamilton microsyringe.

When the operation was finished, the T_{re} of the rat was close to 37 °C and its EMG did not exhibit shivering activity. When the rat was then exposed to an ambient temperature (T_a) of 18 °C, shivering began as T_{re} gradually decreased (Fig. 1). Experiments started when the EMG signal became continuous and stable. If the shivering was too weak or too vigorous, T_a was adjusted to within the range 15–21 °C. Each rat was then given, in random order, three stimuli: preoptic warming, electrical stimulation, or drug application. For preoptic warming, radio-frequency warming current (500 kHz; Lesion Generator RF-4; Radionics, Burlington, MA, USA) was passed between the tip of the implanted electrode–thermocouple assembly and a subcutaneous needle electrode in the back. T_{hy} was increased at 0·1–0·5 °C min^{-1} by increasing the current intensity until there was a clear change in EMG activity or until T_{hy} reached 42 °C. The current was then turned off. The electrode–thermocouple assembly was also used to apply electrical stimulations (0·2–0·5 mA, 200 μs at 20 Hz for 1–2 min). Drug solutions were injected in a volume of 0·2–1·0 μl and at a rate of 0·3–0·5 μl min^{-1}. The solutions tested were 0·2 and 0·02 mM sodium L-glutamate, 0·2 M procaine hydrochloride, and control saline (0·9% NaCl, pH 7·4). The drugs were dissolved in sterile saline immediately before the experiment. In some experiments T_a was increased to 25–30 °C and the drug effects were also tested when EMG activity was weak or absent.

Vasomotion. The experimental procedure was the same as in the shivering experiment except that skin temperatures rather than EMG activity were monitored. The thermocouple for measuring tail temperature (T_{tail}) was fixed by surgical tape to the lateral surface of the tail 10 cm from the base. Thermocouples attached to both hind paws measured skin temperature (T_s). The rat was put on a heating pad (10×10 cm) and covered with a blanket. A thermocouple between the abdomen and the heating pad measured abdominal skin (pad) temperature. Vasomotor activity could be clearly inferred from the changes in T_{tail} and T_s when the difference between deep body and ambient temperatures was at least 5 °C. Ambient temperature was therefore kept at 26–28 °C throughout each experiment. The tail and skin of a ketamine-anaesthetized rat with a T_{re} of about 37 °C were usually vasoconstricted even during preoptic warming to 42 °C. The rat was therefore first warmed by keeping the pad temperature at 40–42 °C and gradually raising the T_{re}; during this rise in the T_{re}, preoptic warming to 42 °C with radio-frequency warming current was carried out repeatedly. When T_{re} was between 38·5 and 40·5 °C, preoptic warming elicited vasodilatation of the tail or skin. Then T_{re} was maintained at this level while preoptic warming, electrical stimulation or drug application was carried out as in the shivering experiment.

Preoptic warming was made when T_{tail} and T_s were stable and more than 4 °C below T_{re}. The temperature of the vasodilatated skin always decreased after preoptic warming stopped, and the next stimulus was applied when the change in skin temperature became less than 0·5 °C min^{-1}. In some experiments, drugs were applied during vasodilatation elicited by maintaining T_{hy} about 0·5 °C above the threshold for vasodilatation.

At the end of the experiment, 0·5 μl of Pontamine Sky Blue in sodium acetate was injected through the cannula to mark the position of the cannula tip. The anaesthetized rat was then perfused with saline followed by 10% formalin. Electrode and cannula positions were verified in frozen 50 μm brain sections stained with Toluidine Blue.

Data are given in the text as mean values ± S.D.

RESULTS

Shivering

Shivering activity was investigated in sixteen rats with an electrode–thermocouple assembly and a guide tube and in fourteen rats with two guide tubes. At forty-one sites in or near the preoptic area the effects of injecting 0·2 mM L-glutamate were tested. The mean T_{re} during the test was 34·5 ± 0·9 °C and the T_a was 18·4 ± 2·0 °C. In the rats with an electrode–thermocouple assembly, T_{hy} during L-glutamate application was 34·2 ± 1·3 °C.

L-Glutamate applications at twenty-three out of forty-one sites suppressed shivering: shivering usually became weak and disappeared 10–30 s after the start of infusion and reappeared less than 1 min after infusion stopped (Fig. 2). The short-lasting effect of L-glutamate is seen in Fig. 2*B*, where 0·2 μl of 0·2 mM L-glutamate solution was intermittently infused five times at 30 s intervals. Integrated EMG activity decreased briefly with each infusion. L-Glutamate never facilitated shivering and control saline never had an effect when it was infused at sites where L-glutamate infusion had suppressed shivering (7 sites in 7 rats). The lower concentration of L-glutamate (0·02 mM) also had no effect on shivering when it was infused into the same sites at which 0·2 mM L-glutamate had suppressed shivering (7 sites in 7 rats). The sites where L-glutamate suppressed shivering were in the anterior part of the preoptic area at the level of the anterior commissure, and the caudal portion of the diagonal band (Fig. 3). Shivering was not affected by L-glutamate injected into the posterior part of the preoptic area (0·8 mm posterior to bregma).

Local warming of the preoptic area suppressed shivering in all sixteen rats (Figs 1 and 2). Although we did not systematically study the distribution of sites where warming suppressed shivering, they were more broadly distributed than the sites where L-glutamate infusion suppressed shivering (Fig. 3). Electrical stimulations at an intensity less than 0·5 mA also suppressed shivering at nine of sixteen sites (in 16 rats) where warming suppressed shivering (Figs 1 and 2).

The infusion of procaine at seven of the sites (in 7 rats) where L-glutamate infusion suppressed shivering (see for example Fig. 4) had no effect when shivering was stable at a T_a of 19·4 ± 0·9 °C (T_{re} = 34·3 ± 0·9 °C). Procaine did not influence shivering as clearly as L-glutamate: procaine facilitated or elicited shivering (Fig. 4) in three of seven cases when shivering was weak or absent at a T_a of 26·5 ± 2·3 °C and a T_{re} of 35·2 ± 1·1 °C. This effect lasted 5–10 min.

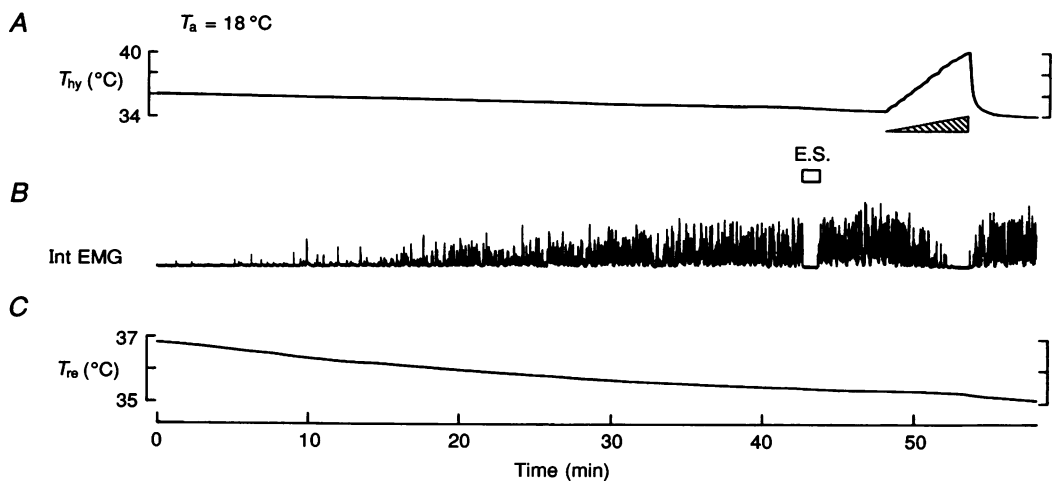

Figure 1. Induction of shivering in a ketamine-anaesthetized rat exposed to cold

A, local hypothalamic temperature (T_{hy}) record. The hatched bar roughly indicates the change in radio-frequency warming current. *B*, integrated electromyogram (Int EMG) of thigh muscle. The open bar indicates electrical stimulation (E.S., 0·4 mA, 200 μs, 20 Hz) applied with the same electrode as that used for heating. *C*, rectal temperature (T_{re}) record over the same time period. The ketamine anaesthetic was applied at 60 mg (kg body wt)$^{-1}$ h^{-1} and the ambient temperature (T_a) was 18 °C.

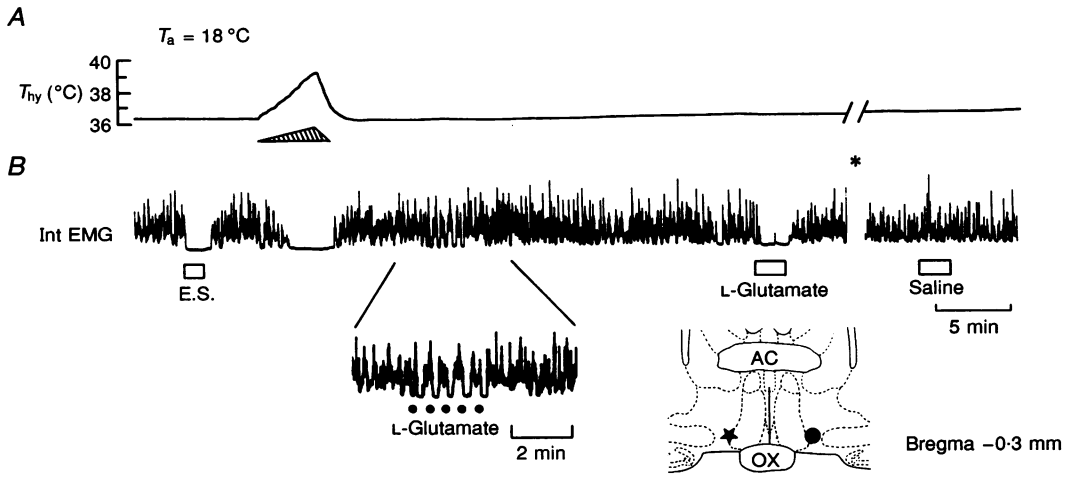

Figure 2. Changes in thigh muscle shivering in response to unilateral preoptic warming, electrical stimulation and injection of L-glutamate or control saline

A, local hypothalamic temperature (T_{hy}) record. The hatched bar roughly indicates the change in radio-frequency warming current. The ambient temperature (T_a) was 18 °C. *B*, integrated EMG (Int EMG) of the thigh muscle. The filled circles under the time-expanded record mark injections of 0·2 μl of 0·2 mM L-glutamate in 1·0 ml saline. The cannula for saline injection was set during the period marked with the asterisk. Open bars indicate electrical stimulation (E.S., 0·4 mA, 200 μs, 20 Hz), L-glutamate and control saline injection. The inset shows the location of the electrode–thermocouple tip (★) and the tip of the injection cannula (●). AC, anterior commisure; OX, optic chiasm.

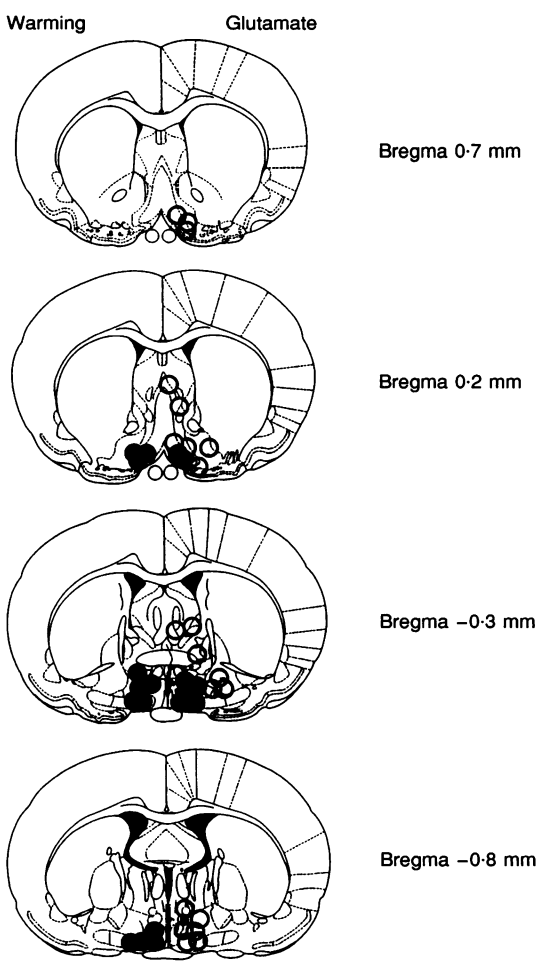

Figure 3. Representative locations of the tips of the injection cannula for L-glutamate injection (right-hand side) and the tips of the warming electrodes (left-hand side) in shivering experiments

Filled circles show where L-glutamate injection or warming suppressed cold-induced shivering and open circles show where L-glutamate had no effect on shivering. At all sites, warming suppressed shivering.

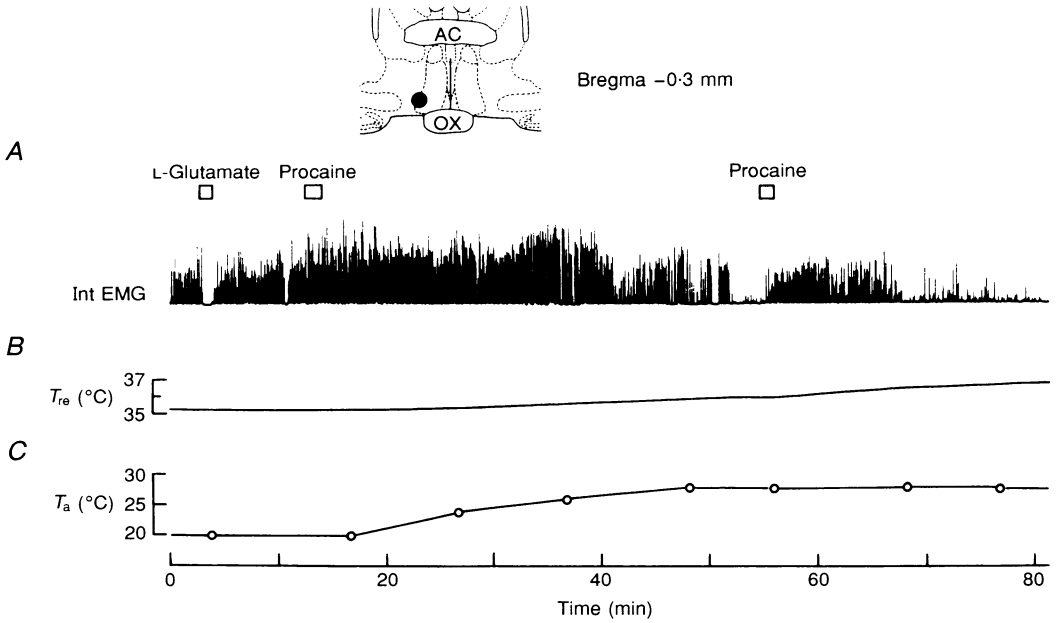

Figure 4. Shivering activity changes elicited by preoptic application of L-glutamate or procaine

A, integrated EMG (Int EMG) record of the thigh muscle. The open bars indicate applications of L-glutamate (0·2 mM in 0·6 μl saline) and procaine (0·2 M in 1·0 μl saline). *B* and *C*, rectal temperature (T_{re}; *B*) and ambient temperature (T_a; *C*) over the same time period. In the inset, ● indicates the position of the tip of the injection cannula.

Vasomotion

Twenty-five rats not used for shivering experiments were implanted with an electrode–thermocouple assembly and a guide tube, and vasomotion experiments were carried out at a T_{re} of 39·5 ± 0·6 °C and a heating pad temperature of 39·9 ± 1·2 °C. When the preoptic area was not warmed, T_{hy} was 38·9 ± 0·7 °C. Figure 5 shows one example of vasomotor responses to L-glutamate injection. Local

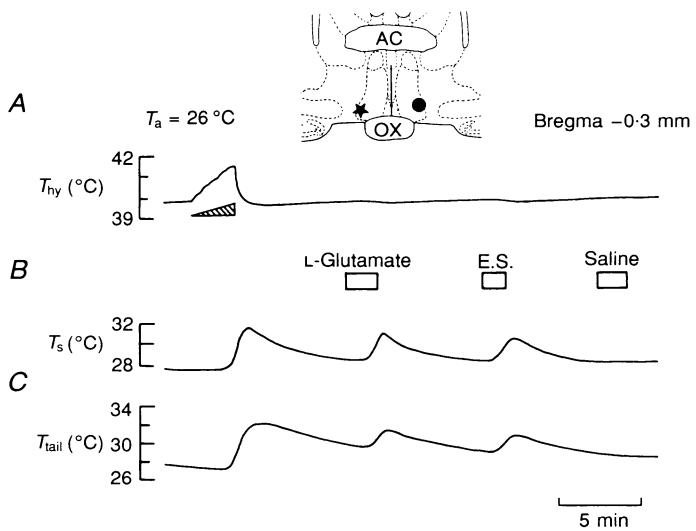

Figure 5. Vasomotor activity changes elicited by preoptic warming, electrical stimulation and application of L-glutamate

A, local hypothalamic temperature (T_{hy}). The hatched bar roughly indicates the change in the radio-frequency warming current. *B* and *C* show hindpaw (T_s; *B*) and tail skin temperature (T_{tail}; *C*). Open bars indicate the application of L-glutamate (0·2 mM in 1·0 μl saline), electrical stimulation (E.S., 0·35 mA) and control saline. Ambient temperature (T_a) was 26 °C. The inset shows the location of the electrode–thermocouple tip (★) and the tip of the injection cannula (●).

warming or electrical stimulation of the preoptic area elicited increases in paw and tail skin temperatures. L-Glutamate injection into the contralateral preoptic area (roughly opposite the site of the warming) and electrical stimulation elicited similar increases in skin temperature, whilst saline injection did not affect skin temperature. L-Glutamate injection (0·2 mM) produced vasodilatation at eleven of twenty-five sites, and these eleven sites were all within the preoptic area (Fig. 6). Saline injected into the sites where L-glutamate produced vasodilatation (6 sites in 6 rats) did not affect vasomotor activity.

Local warming of the POAH elicited vasodilatation in all twenty-five rats. Electrical stimulation at an intensity less than 0·5 mA also elicited vasodilatation at seven out of ten sites where warming produced vasodilatation (Fig. 5).

We tested the effects of procaine injection by using six rats in which L-glutamate had been confirmed to elicit vasodilatation. In all cases procaine produced constriction of dilated blood vessels but had no effect when it was injected while blood vessels were constricted. In the sample record shown in Fig. 7, preoptic warming elicited tail vasodilatation at a threshold T_{hy} of 41·2 °C. After T_{tail} returned close to the baseline level, T_{hy} was increased and maintained at 40·7 °C, a level which alone did not produce vasodilatation but at which additional L-glutamate injection did. Then T_{hy} was maintained slightly above the threshold and procaine was injected when T_{tail} became high as a result of vasodilatation. T_{tail} soon began to decrease and in 10 min increased again. Subsequent procaine injection, when T_{tail} was returning to the baseline level, had no effect on the vasomotor activity.

DISCUSSION

Local warming of the POAH suppresses cold-induced shivering. Is this response due to excitation of warm-sensitive neurones or to inhibition of cold-sensitive neurones? Electrical stimulation of the same sites, using the same electrodes, also suppressed shivering, which suggests that the excitation of POAH neurones is necessary for the suppression of shivering. Because electrical stimulation excites not only cell bodies but also passing fibres, this suppression might be due to excitation of passing fibres. The suppression of shivering by local application of L-glutamate, however, indicates that the suppression of shivering is due to the excitation of cell bodies. Although for L-glutamate we have to consider the possibility of depolarization block (that is, inhibition rather than excitation of cell bodies), we never observed the opposite response – facilitation of shivering – when

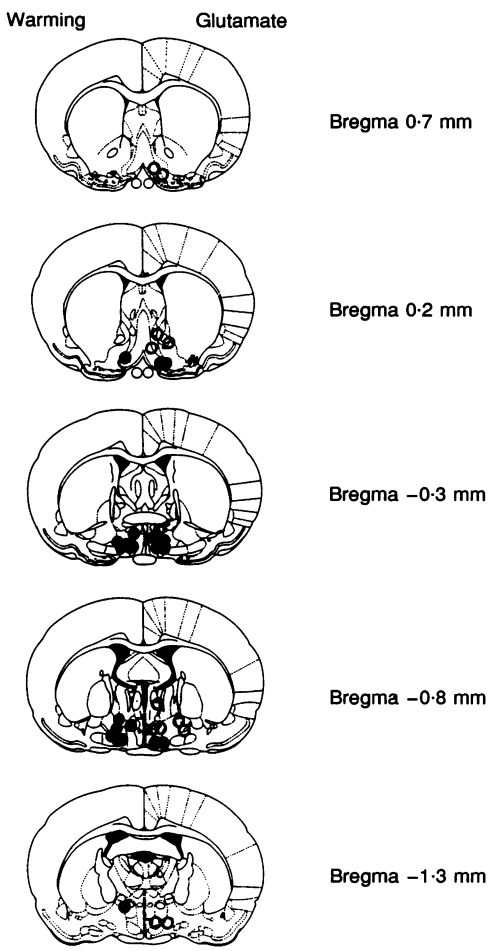

Figure 6. Representative locations of the tips of the injection cannula for L-glutamate injection (right-hand side) and the tips of the warming electrodes (left-hand side) in vasomotor experiments

Filled circles show where L-glutamate injection or warming produced skin vasodilatation and open circles show where L-glutamate had no effect on vasomotor activity. At all sites, warming produced vasodilatation.

L-glutamate was given at a lower concentration. Furthermore, the local anaesthetic procaine tended to facilitate shivering, although this might have been due to an effect on passing fibres. It is therefore very likely that the main contribution of the POAH to the control of shivering consists of inhibitory signals from warm-sensitive neurones.

Local warming, electrical stimulation and L-glutamate application all had the same effect on vasomotion: vasodilatation. Procaine produced the opposite response: it elicited vasoconstriction when applied to an already vasodilated rat. Considerations similar to those in the case of shivering therefore suggest that excitation of POAH neurones by local warming is responsible for eliciting vasodilatation. That is, signals from warm-sensitive neurones would predominate over signals from cold-sensitive neurones in the control of vasomotor activity as well as in the control of shivering. Whether the contributions of POAH thermosensitivity to the control of other thermoregulatory responses – especially non-shivering thermogenesis and behavioural cold defence – can also be attributed mainly to the signals from warm-sensitive neurones is, however, still an open question.

The inhibitory action of warm-sensitive (high-Q_{10}) neurones on heat production has already been incorporated in the neuronal models proposed so far (Hammel, 1968; Bligh, 1973; Boulant, 1974), and this inhibition has been assumed to occur inside the POAH. The POAH seems to work only for generating efferent signals of warm-sensitive neurones and the inhibition seems to occur somewhere along the efferent pathway, the exact site of which is not yet known.

If skin vasomotion and shivering are both controlled by signals from warm-sensitive POAH neurones, do these signals come from the same neurones? We recently found that the left and right sides of the POAH exchange information for vasomotor control (Kanosue *et al.* 1994*a*) but that the two sides contribute independently to the control of shivering (Kanosue *et al.* 1994*c*). Shivering and vasomotion are thus controlled by different POAH networks, even though the two networks are located close to, or intermingled with, each other (Kanosue, Yanase-Fujiwara, Hosono & Zhang, 1994*b*).

There has long been debate over whether POAH cold-sensitive neurones are inherently thermosensitive or simply interneurones inhibited by warm-sensitive neurones (Hori, Nakashima, Kiyohara, Shibata & Hori, 1980; Kelso & Boulant, 1982; Boulant, Curras & Dean, 1989). The present study shows that if inherently cold-sensitive neurones exist, they do not play an essential role in the control of shivering and vasomotion. The existence of inherently warm-sensitive POAH neurones, on the

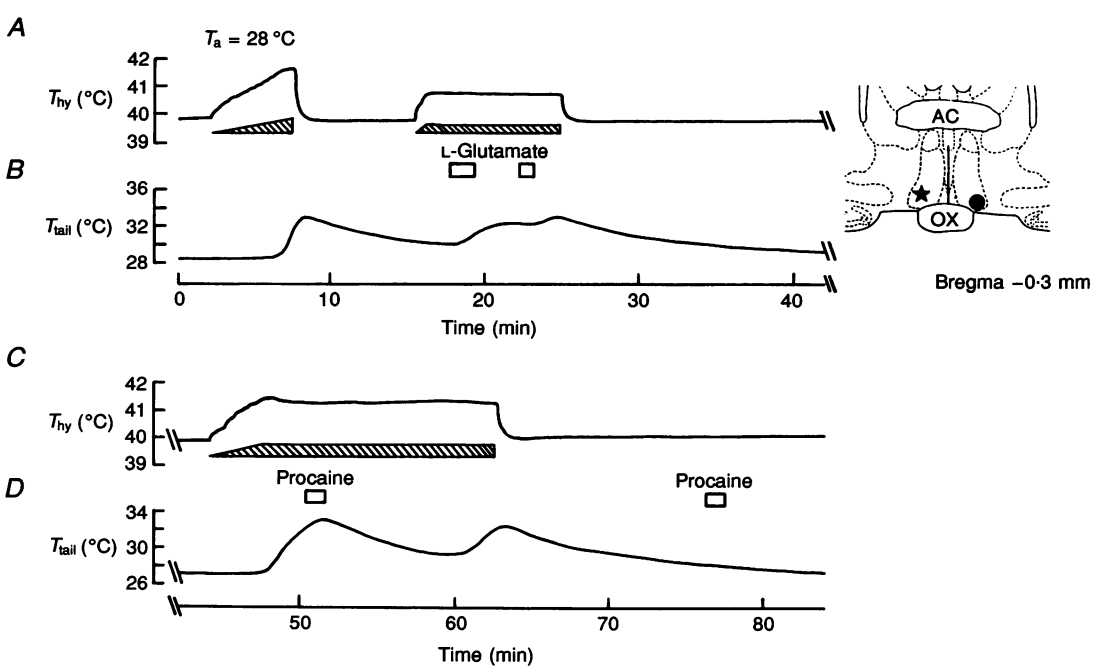

Figure 7. Vasomotor activity changes elicited by preoptic warming and application of L-glutamate or procaine

A and *B*, local hypothalamic (T_{hy}; *A*) and tail skin temperatures (T_{tail}; *B*) after preoptic warming and L-glutamate (0·2 mM) applications, indicated by the open bars. *C* and *D*, T_{hy} (*C*) and T_{tail} (*D*) after preoptic warming and procaine (0·2 M) applications (open bars). The hatched bars roughly indicate the changes in radio-frequency warming current. Ambient temperature (T_a) was 28 °C. The inset shows the location of the electrode–thermocouple tip (★) and the tip of the injection cannula (●).

other hand, is well established (Hori *et al.* 1980; Kelso *et al.* 1982; Kiyohara, Hirata, Hori & Akaike, 1990). Because the excitatory effect of L-glutamate is non-specific, the present data do not indicate whether the neurones sending efferent signals to the lower brain for vasomotor or shivering control are inherently warm sensitive, or whether they are thermally insensitive and driven by synaptic inputs from inherently warm-sensitive neurones.

ANDERSON, H. T., ANDERSON, B. & GALE, C. (1962). Central control of cold defence mechanisms and the release of 'endopyrogen' in the goat. *Acta Physiologica Scandinavica* **54**, 159–174.

BENZINGER, T. H., PRATT, A. W. & KITZINGER, C. (1961). The thermostatic control of human metabolic heat production. *Proceedings of the National Academy of Sciences of the USA* **47**, 730–739.

BLIGH, J. (1973). Neural models of temperature regulation. In *Temperature Regulation in Mammals and other Vertebrates*, pp. 174–191. North-Holland, Amsterdam.

BOULANT, J. A. (1974). The effect of firing rate on preoptic neuronal thermosensitivity. *Journal of Physiology* **240**, 661–669.

BOULANT, J. A. (1980). Hypothalamic control of thermoregulation. Neurophysiological basis. In *Handbook of the Hypothalamus*, ed. MORGANE, P. J. & PANKSEPP, J., pp. 1–82. Dekker, New York.

BOULANT, J. A., CURRAS, M. C. & DEAN, J. B. (1989). Neurophysiological aspects of thermoregulation. In *Advances in Comparative and Environmental Physiology*, ed. WANG, L. C. H., pp. 117–160. Springer, Berlin.

EISENMAN, J. S. & JACKSON, D. C. (1967). Thermal response patterns of septal and preoptic neurons in cats. *Experimental Neurology* **19**, 33–45.

HAMMEL, H. T. (1968). Regulation of internal body temperature. *Annual Review of Physiology* **30**, 641–710.

HAMMEL, H. T., HARDY, J. D. & FUSCO, M. M. (1960). Thermoregulatory responses to hypothalamic cooling in unanesthetized dogs. *American Journal of Physiology* **198**, 481–486.

HORI, T. (1991). An update on thermosensitive neurons in the brain: from cellular biology to thermal and non-thermal homeostatic functions. *Japanese Journal of Physiology* **41**, 1–22.

HORI, T., NAKASHIMA, T., KIYOHARA, T., SHIBATA, M. & HORI, N. (1980). Effect of calcium removal on thermosensitivity of preoptic neurons in hypothalamic slices. *Neuroscience Letters* **20**, 171–175.

KANOSUE, K., HOSONO, T. & YANASE-FUJIWARA, M. (1994*a*). Hypothalamic network for thermoregulatory vasomotor activity. *American Journal of Physiology* **267**, R283–388.

KANOSUE, K., NIWA, K., ANDREW, P. D., YASUDA, H., YANASE, M., TANAKA, H. & MATSUMURA, K. (1991). Lateral distribution of hypothalamic signals controlling thermoregulatory vasomotor activity and shivering in rats. *American Journal of Physiology* **260**, R485–493.

KANOSUE, K., YANASE-FUJIWARA, M., HOSONO, T. & ZHANG, Y.-H. (1994*b*). Hypothalamic network for thermoregulation: old but still unanswered question. In *Integrative and Cellular Aspects of Autonomic Functions: Temperature and Osmoregulation*, ed. PLESHKA, K. & GERSTBERGER, R., pp. 253–257. John Libbey Eurotext, Paris.

KANOSUE, K., ZHANG, Y.-H., YANASE-FUJIWARA, M. & HOSONO, T. (1994*c*). Hypothalamic network for thermoregulatory shivering. *American Journal of Physiology* **267**, R275–282.

KELSO, S. R. & BOULANT, J. A. (1982). Effect of synaptic blockade on thermosensitive neurons in hypothalamic tissue slices. *American Journal of Physiology* **243**, R480–490.

KIYOHARA, T., HIRATA, M., HORI, T. & AKAIKE, N. (1990). Hypothalamic warm-sensitive neurons possess a tetrodotoxin-sensitive sodium channel with a high Q_{10}. *Neuroscience Research* **8**, 48–53.

LUMB, W. V. & JONES, E. W. (1984). *Veterinary Anaesthesia*, pp. 199–211. Lea & Febiger, Philadelphia.

NAKAYAMA, T. (1985). Thermosensitive neurons in the brain. *Japanese Journal of Physiology* **35**, 375–389.

NAKAYAMA, T., EISENMAN, J. S. & HARDY, J. D. (1961). Single unit activity of anterior hypothalamus during local heating. *Science* **134**, 560–561.

PAXINOS, G. & WATSON, C. (1986). *The Rat Brain in Stereotaxic Coordinates*, 2nd edn. Academic Press, San Diego.

WYNDAM, C. H. & ATKINS, A. R. (1968). A physiological scheme and mathematical model of temperature regulation in man. *Pflügers Archiv* **303**, 14–30.

Acknowledgements

We thank Randy Kaul for his critical reading of and comments on this manuscript. This study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (Grants No. 02670066 and No. 04454143).

Received 6 July 1994; accepted 31 October 1994.