Effect of midbrain stimulations on thermoregulatory vasomotor responses in rats

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1. Efferent projections eliciting vasodilatation when the preoptic area is warmed were investigated by monitoring tail vasomotor responses of ketamine-anaesthetized rats when brain areas were stimulated electrically (0·2 mA, 200 μs, 30 Hz) or with the excitatory amino acid D,L-homocysteic acid (1 mM, 0·3 μl).

2. Both stimulations elicited vasodilatation when applied within a region extending from the most caudal part of the lateral hypothalamus to the ventrolateral periaqueductal grey matter (PAG) and the reticular formation ventrolateral to the PAG.

3. Vasodilatation elicited by preoptic warming was suppressed when either stimulation was applied within the rostral part of the ventral tegmental area (VTA).

4. Sustained vasodilatation was elicited by knife cuts caudal to the VTA, and vasodilatation elicited by preoptic warming was suppressed by cuts either rostral to the VTA or in the region including the PAG and the reticular formation ventrolateral to it.

5. These results, together with the results of earlier physiological and histological studies, suggest that warm-sensitive neurones in the preoptic area send excitatory signals to vasodilatative neurones in the caudal part of the lateral hypothalamus, ventrolateral PAG and reticular formation, and send inhibitory signals to vasoconstrictive neurones in the rostral part of the VTA.

The preoptic area plays a key role in body temperature regulation by integrating information about local brain temperature and other body temperatures, and by sending efferent signals to various effector organs. This area is known to contain thermosensitive (warm- and cold-sensitive) neurones transducing local brain temperature into nervous signals, but the efferent connections of these neurones remain to be elucidated (Nakayama, 1985; Kanosue, Yanase-Fujiwara, Hosono & Zhang, 1994*b*; Boulant, 1996). For example, nothing is known about the axonal projections of the preoptic neurones contributing to thermoregulation, or the contribution of these neurones to a specific effector response. Skin vasodilatation occurring in response to local warming of the preoptic area of the rat has been analysed in an attempt to explore these pathways (Ishikawa, Nakayama, Kanosue & Matsumura, 1984). It was recently found that this vasodilatation is elicited mainly by the activation of warm-sensitive neurones, since glutamate injected into the preoptic area elicits vasodilatation and procaine injected there elicits vasoconstriction (Zhang, Yanase-Fujiwara, Hosono & Kanosue, 1995*b*). Efferent pathways for this response descend from the preoptic area through the medial forebrain bundle (Kanosue, Hosono & Yanase-Fujiwara, 1994*a*).

The purpose of the present study was to find out where the preoptic neurones carrying these efferent signals terminate. Brain areas ranging from the caudal portion of the hypothalamus to the midbrain were explored by observing the tail vasomotor responses elicited by electrical stimulations and by injections of the excitatory amino acid D,L-homocysteic acid (DLH). This was followed by investigating the effects of knife cuts on the vasomotor response elicited by preoptic warming.

Preliminary reports of this work have been published in abstract form (Zhang, Yamada, Yanasa-Fujiwara, Hosono, Chen & Kanosue, 1995*a*).

METHODS

Ninety-one adult male Wistar rats (350–450 g) were used in this work. They were housed at 22 °C with free access to food and water. After anaesthesia was induced by 3% sevoflurane inhalation (Maruishi, Osaka, Japan), ketamine hydrochloride (200 mg kg^{-1})

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was injected intraperitoneally and a needle was placed in the peritoneal cavity so that ketamine could be continuously infused at 60 mg kg^{-1} h^{-1} throughout each experiment with an infusion pump (CMA/100; Carnegie, Stockholm, Sweden). Additional doses of ketamine were given as needed to keep the depth of anaesthesia at or below the first plane of stage three (surgical anaesthesia; Lumb & Jones, 1984). This anaesthetic regime had been 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 an electrode–thermocouple assembly (Kanosue *et al.* 1991) was implanted with its tip in the right preoptic area 1·0–1·5 mm from the mid-line, between 0·5 mm anterior and 0·5 mm posterior to the bregma, and 9·0–9·5 mm below the skull surface. This assembly consisted of an insulated stainless steel tube (0·4 mm i.d., 0·6 mm o.d.) with a bared sharp tip used for thermal stimulation and a copper–cobalt thermocouple glued inside for monitoring local brain temperature (T_{hy}). Previous work has shown that tissue around the electrode is not damaged as long as T_{hy} is not raised above 42 °C (Kanosue, Zhang, Yanase-Fujiwara & Hosono, 1994*c*). This assembly was fixed to the skull with dental cement. A small hole was opened in the right skull surface, 0·5–2·5 mm lateral from the mid-line and 3·0–7·0 mm posterior to the bregma for inserting a stimulation electrode/cannula or microknife. A polyethylene catheter (Imamura, Tokyo, Japan; PE-50 filled with heparin saline, 50 U ml^{-1}) was implanted in the left femoral artery to monitor arterial blood pressure and heart rate.

Experimental procedures

Still anaesthetized as described above, each rat was put into a climatic chamber (30 cm × 40 cm × 80 cm) and a thermocouple for measuring rectal temperature (T_{re}) during the experiment was inserted 6 cm past the anal sphincter and fixed with surgical tape. Another thermocouple for measuring tail temperature (T_{tail}) was fixed to the lateral surface of the tail 10 cm from the base.

The rat was put on a heating pad (10 cm × 10 cm) and covered with a blanket. A thermocouple between the abdomen and the heating pad measured abdominal skin (pad) temperature. Change in tail blood flow could be clearly inferred from the change in T_{tail} whenever the difference between deep body and ambient temperatures was at least 5 °C, so the ambient temperature was kept at 26–28 °C throughout each experiment. Because the tail of a ketamine-anaesthetized rat with a T_{re} of about 37 °C was usually vasoconstricted even during preoptic warming to 42 °C, each rat was first warmed by keeping the pad temperature at 40 °C, gradually raising T_{re}, while preoptic warming to 42 °C was carried out repeatedly. For preoptic warming, radio frequency current (500 kHz; Lesion Generator RF-4; Radionics, Burlington, MA, USA) was passed between the tip of the electrode–thermocouple assembly and a subcutaneous needle electrode in the back. When T_{re} surpassed some threshold value between 38·5 and 40·5 °C, preoptic warming elicited vasodilatation of the tail. Then T_{re} was maintained at this level and recordings were started.

Stimulation study

Seventy-nine rats were used in this series of experiments. A stainless steel tube (0·5 mm o.d.) for drug injection and electrical stimulation was lowered into the brain until its tip reached 4·0–5·0 mm below the skull surface. This 'electrode/cannula' was insulated except for a 0·5 mm distance at the tip, which was used as the cathode for electrical stimulation. A polyethylene tube connected the electrode/cannula to a 5 μl microsyringe (Hamilton, Reno, NV, USA). The preoptic area was first warmed at 0·1–0·5 °C min^{-1} by increasing the current intensity until tail vasodilatation occurred. The current was then turned off. The temperature of the vasodilated tail always decreased after this preoptic warming was stopped. The preoptic area was then warmed to keep T_{hy} 1·0–1·5 °C below the threshold for tail vasodilatation, and an electrical stimulation (0·2 mA; 30 Hz; 0·2 ms) was applied for 2 min. If T_{tail} rose in response to this electrical stimulation, D,L-homocysteic acid (DLH; 1 mM; pH 7·4; osmolality, 290 mosmol kg^{-1}; 0·3 μl in physiological saline) was injected through the same electrode/cannula. The solution was made just before the experiment, and pH and osmolality were adjusted by adding sodium hydoxide and sodium cloride. The preoptic area was then warmed to produce tail vasodilatation and, while T_{hy} was maintained high enough to sustain the vasodilatation, electrical or chemical stimulation was applied to test for vasoconstrictive effects. The electrode/cannula was then advanced 0·5–1·0 mm deeper and all these procedures were repeated. Finally, DC current (1 mA, 0·5 s) was passed through the electrode/cannula to mark the position of the tip.

The effect of vehicle control injection was tested in a separate series of experiments with six other rats. After a site where DLH injection produced a vasodilatative or vasoconstrictive response had been located using the procedures described above, the electrode/cannula was withdrawn, rinsed with physiological saline (0·9% NaCl; pH 7·4, 290 mosmol kg^{-1}), and connected to a microsyringe containing physiological saline. It was then lowered into the brain until its tip was at the stereotaxic co-ordinates of the site where DLH injection had just been found to be effective. Saline (0·3 μl) was then injected under the same protocol followed during the DLH injection.

In a later series of experiments evaluating the effect of blood pressure changes on tail blood, the pencil-type probe of a laser doppler flow meter (ALF 21; Advance, Tokyo, Japan) was set perpendicular to and about 5 mm from the lateral surface of the tail. The position of the probe tip was optimized by warming the preoptic area to induce tail vasodilatation and adjusting the probe position to produce the highest flow meter output. The flow meter output was fed into a computer together with all the temperature, blood pressure, and heart rate data. Tail conductance was calculated as follows: (1) blood pressure was normalized by the value in a period when no stimulus was applied; (2) tail blood flow was normalized by the maximum value during preoptic warming, because the thermoregulatory vasomotion of the rat tail occurs in an on–off fashion (Young & Dawson, 1982); and (3) tail conductance was calculated as normalized blood flow/normalized blood pressure.

Knife-cut study

Twelve rats were used in this series of experiments. After preoptic warming was confirmed, as in the stimulation study, to elicit tail vasodilatation, a microknife consisting of a thin stainless steel tube containing a wire blade (Blatteis, Haar, Banet & Hensel, 1982) was used to make a transection in a coronal plane 4·5–7·0 mm posterior to the bregma. The transection was made by first lowering the knife into the brain with the wire blade sheathed, extending the blade, and then lowering and raising the knife three times. After the knife cut, the vasomotor response to preoptic warming was observed for 2 h.

An increase in T_{tail} in response to electrical or chemical stimulation was defined as a post-stimulation T_{tail} more than 1 °C above the baseline T_{tail} before the stimulation. And a decrease in T_{tail} was defined by the presence of a clear notch in rising phase of the T_{tail} during preoptic warming – that is, when the difference between the two lines extrapolated from the rises in T_{tail} before and after the stimulation (see Fig. 2) was more than 1 °C. Increases and decreases

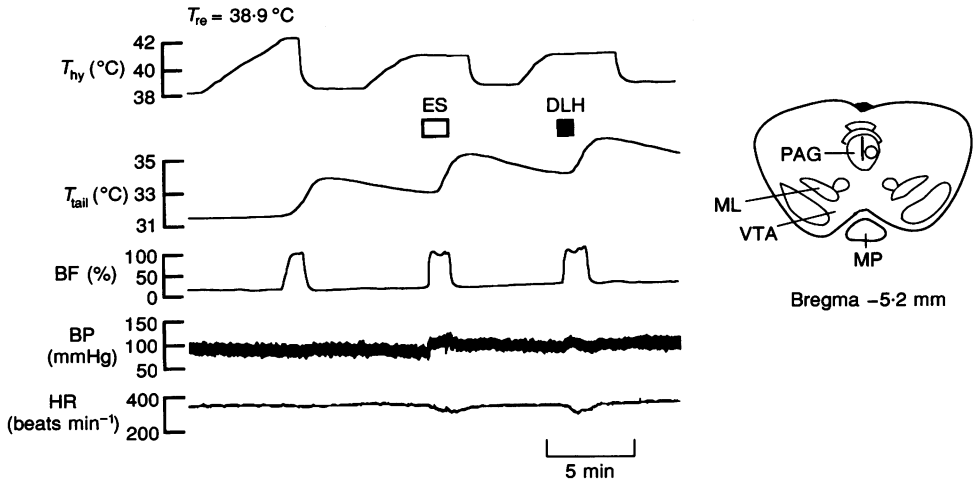

Figure 1. Vasomotor activity changes elicited by preoptic warming, electrical stimulation and the application of D,L-homocysteic acid (DLH) to the periaqueductal grey matter

From top to bottom: hypothalamic temperature (T_{hy}), tail temperature (T_{tail}), tail blood flow (BF), blood pressure (BP), and heart rate (HR). The open bar indicates electrical stimulation (ES; 0·2 mA, 0·2 ms, 30 Hz) and the filled bar indicates application of DLH (1 mM in 0·3 μl saline). T_{re}, averaged rectal temperature. In the inset, ○ indicates the position of the electrode/cannula tip. ML, medial lemniscus; MP, mammillary nucleus; PAG, periaqueductal grey; and VTA, ventral tegmental area.

in blood pressure were defined as changes of more than 10% from the pressure before the stimulation.

At the end of each experiment, the anaesthetized rat was killed by first perfusing with saline and then with 10% formalin. The position of the electrode/cannula and the extent of knife cut were verified in 40 μm brain sections stained with Toluidine Blue.

Data are expressed in the text as means ± S.D., and the significance of differences was evaluated with Student's t test.

RESULTS

Electrical and chemical stimulations

When tail vasomotor activity was monitored during electrical stimulations at 144 sites ranging from the caudal part of the medial forebrain bundle to the midbrain, T_{tail} rose or fell depending on the site of stimulation. Figure 1 shows a typical example of the rise in T_{tail}. Preoptic warming elicited an increase in T_{tail} at a threshold T_{hy} of 42·3 °C.

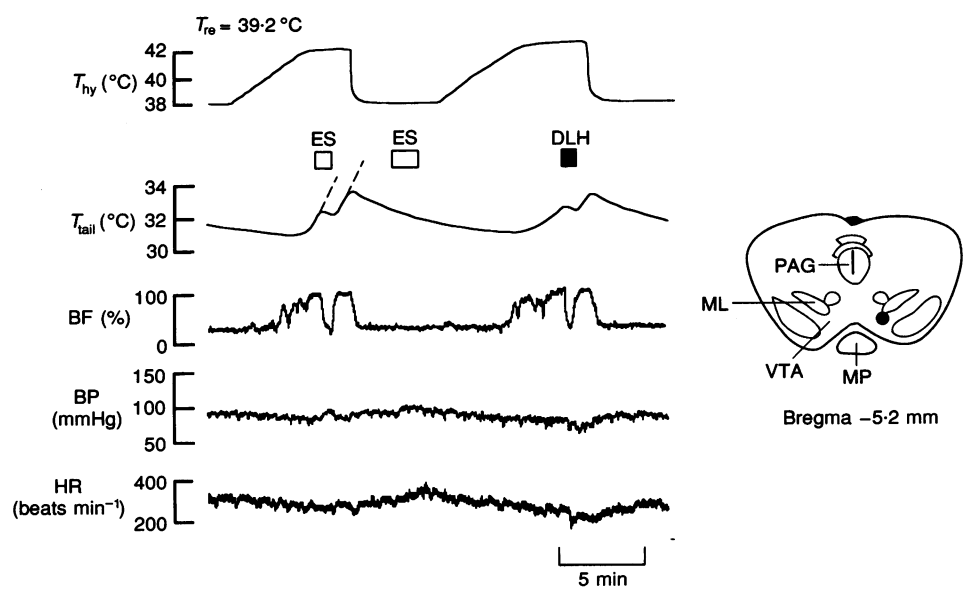

Figure 2. Vasomotor activity changes elicited by preoptic warming, electrical stimulation, and the application of DLH to the ventral tegmental area

The open bars indicate electrical stimulation (ES; 0·2 mA, 0·2 ms, 30 Hz) and the filled bar indicates application of DLH (1 mM in 0·3 μl saline). Dashed extrapolated lines in the T_{tail} record are for evaluating the decrease in T_{tail}. In the inset, ● indicates the position of the electrode/cannula tip. Abbreviations as in Fig. 1.

The rise in T_{tail} was preceded by an increase in tail blood flow. When an electrical or DLH stimulation was applied to the lateral periaqueductal grey matter (PAG) while T_{hy} was maintained at 41 °C, both stimulations elicited an increase in tail blood flow and T_{tail}. Although the size and duration of the blood flow increase elicited by DLH application were almost identical to those of the response elicited by electrical stimulation, blood pressure increased markedly in the latter but only slightly in response to the former. The region in which electrical stimulation produced rises in T_{tail} extends rostro-caudally from the hypothalamus to the midbrain (Fig. 3*A*). It starts from the caudal portion of the lateral hypothalamus, curves dorsally to the aqueducts, and extends to the PAG and the reticular formation ventrolateral to the PAG. Effects of DLH application were tested at those sites where electrical stimulation elicited an increase in T_{tail}. The sites at which a rise in T_{tail} was also elicited by DLH injection are predominantly in a region extending from the most caudal part of the lateral hypothalamus to the rostral PAG and its adjacent reticular formation (Fig. 3*B*).

Vasoconstrictive effects elicited by electrical stimulation and DLH injection are shown in Fig. 2. Tail temperature increased when the preoptic area was warmed to 42 °C, and electrical stimulation of the ventral tegmental area (VTA) produced immediate decreases in T_{tail} and blood flow. These decreases terminated when the stimulation ended. Electrical

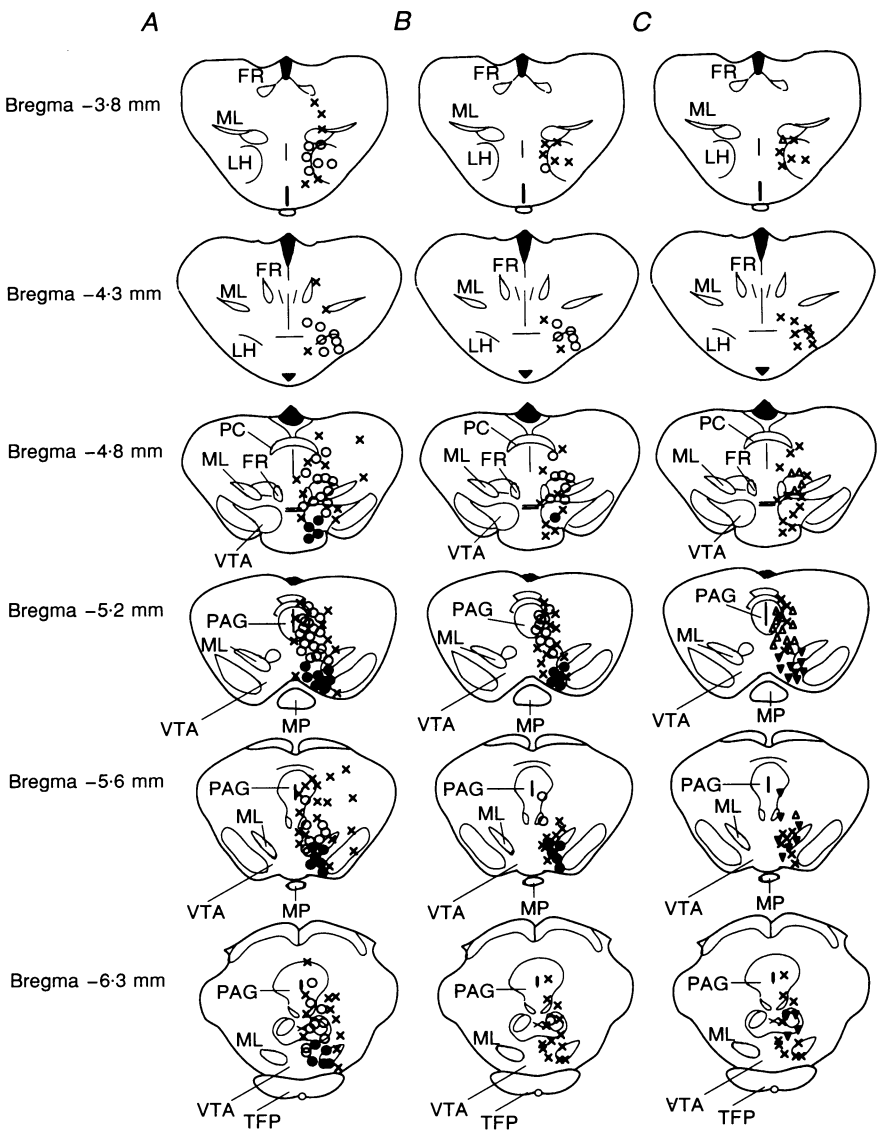

Figure 3. Locations of electrode/cannula tips for electrical stimulation (*A*) and application of DLH (*B* and *C*)

A and *B*, ○ and ● show where electrical stimulation (*A*) or DLH injection (*B*) elicited increases and decreases, respectively, in tail temperature. × shows where electrical stimulations or DLH injections were ineffective. *C*, △ and ▲, respectively, show where DLH injection increased and decreased blood pressure by more than 10%. FR, fasciculus retroflexus; LH, lateral hypothalamus; PC, posterior comissure; and TFP, transverse fibres pons. Other abbreviations as in Fig. 1.

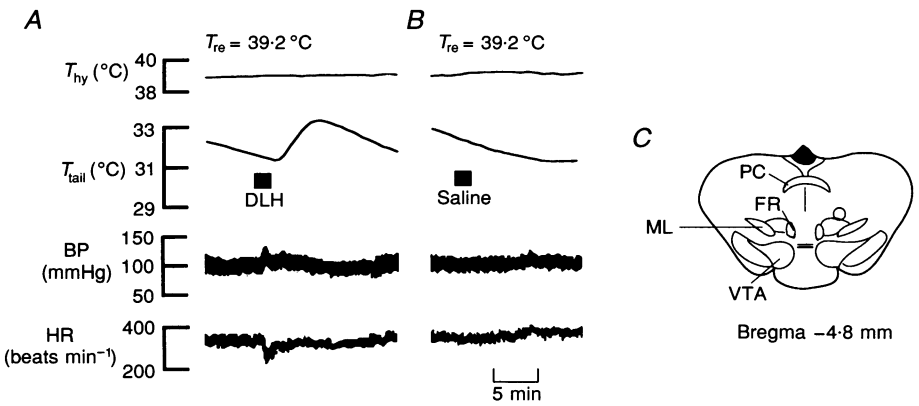

Figure 4. Tail vasomotor changes elicited by the application of DLH (*A*) and control vehicle (*B*) to the reticular formation

The closed bars indicate application of DLH (1 mM in 0·3 μl saline) or control vehicle (0·3 μl saline). In the inset, ○ indicates the position of the electrode/cannula tip. Abbreviations as in previous figures.

stimulations applied when the preoptic area was not warmed affected neither T_{tail} nor blood flow. Decreases in T_{tail} and tail blood flow were also elicited when DLH was injected into the same site during the vasodilatation elicited by preoptic warming. Blood pressure was not notably influenced by electrical stimulation, but it decreased slightly after DLH stimulation. The region containing sites where electrical stimulation produced decreases in T_{tail} starts at the rostral part of the VTA and continues caudally along the ventromedial portion of the midbrain (Fig. 3*A*). Chemical stimulation, however, produced decreases in T_{tail} only when the DLH was injected into the rostral part of the VTA (Fig. 3*B*).

Tail vasomotor activity was not affected by physiological saline injected into five sites where DHL injections elicited increases in T_{tail} (Fig. 4) and into six sites where DLH injections elicited decrease in T_{tail} (Fig. 5).

Mean blood pressure just before DLH injection was 83 ± 19 mmHg ($n = 83$), and the injections sometimes produced an increase or decrease in blood pressure, depending on where it was injected. Increases in blood pressure were observed when DLH was injected into the ventrolateral PAG, mainly at the level 5·2 mm posterior to the bregma, and its adjacent reticular formation (Fig. 3*C*). On the other hand, injections into more ventral portions of the midbrain, including the VTA, elicited decreases in blood pressure. The 'pressor area' includes the region where DLH injection elicited increases in T_{tail}, and the 'depressor area' includes the region where DLH injection elicited decreases in T_{tail}. Eleven of the twenty-six T_{tail} increases elicited by DLH were accompanied by increases in blood pressure, and seven of the eleven T_{tail} decreases were accompanied by decreases in blood pressure (Table 1). To test whether the T_{tail} increase accompanying a pressor response is due to vasodilatation or is a secondary effect of the rise in blood pressure, tail

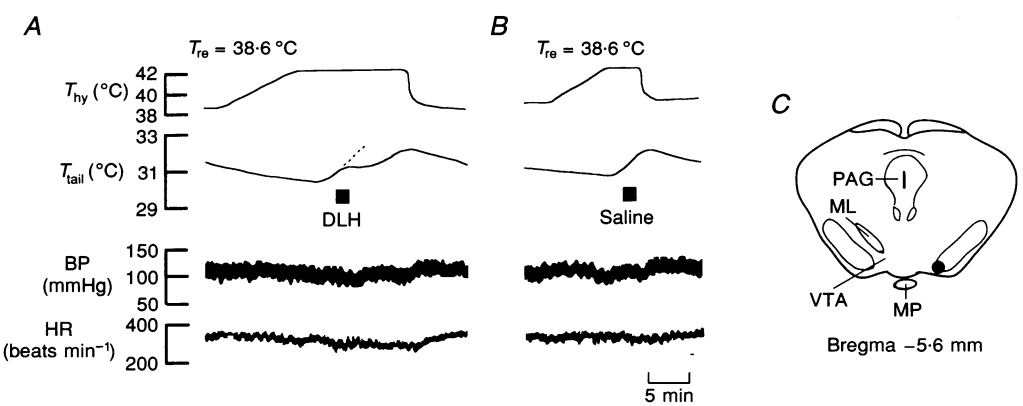

Figure 5. Tail vasomotor changes elicited by the application of DLH (*A*) and control vehicle (*B*) to the ventral tegmental area

The closed bars indicate application of DLH (1 mM in 0·3 μl saline) or control vehicle (0·3 μl saline). Note that T_{tail} increase started before DLH or saline injection, which indicates that this vasodilatation was the response to preoptic warming. Extrapolated rise in T_{tail} is indicated by the dashed line. In the inset, ● indicates the position of the electrode/cannula tip. Abbreviations as in previous figures.

Table 1. Changes in blood pressure and tail skin temperature elicited by DLH injections

	Blood pressure		
T_{tail}	Increase	Decrease	No change
Increase	11	3	12
Decrease	0	7	4
No change	7	10	29

conductance was calculated in the eight cases in which both tail blood flow and blood pressure increased in response to DLH injection into the ventrolateral PAG. Similarly, to test whether the T_{tail} decrease accompanying a depressor response is due to vasoconstriction or is secondary to the drop in blood pressure, tail conductance was calculated in the five cases in which both tail blood flow and blood pressure decreased in response to DLH injection into the rostral VTA.

Figure 6 shows a tail conductance change occurring in response to DLH injection into the 'pressor area'. Preoptic warming produced increases in T_{tail} and blood flow. Because the increased tail blood flow was not accompanied by an increase in blood pressure, it was paralleled by an increase in tail conductance. DLH injection into the PAG elicited increases in T_{tail} and blood flow as clear as those elicited by preoptic warming. In this case blood pressure increased by 50% and conductance also increased by 20%. The latency of the peak conductance increase after DLH injection was 74 s, more than twice the 28 s latency of the peak blood pressure increase. Note that electrical stimulation of the site of the DLH injection elicited a similar or even greater increase in blood pressure, but that the increase in tail blood flow was smaller and there was no increase in conductance. In all eight cases, tail conductance increased when DLH was injected into the pressor area in the ventrolateral PAG. The mean conductance increase was $37 \pm 15\%$, and the mean blood pressure increase was $45 \pm 27\%$. Both values are significantly different from zero ($P < 0{\cdot}01$). The latency of the conductance peak was 53 ± 18 s, which was significantly longer than the latency of blood pressure peak, 27 ± 11 s ($P < 0{\cdot}05$).

A conductance change occurring in response to a DLH injection into the 'depressor area' is shown in Fig. 7. In this case DLH was injected into the border of the VTA and the medial lemniscus while tail blood flow was high during preoptic warming. The injection elicited a decrease in tail blood flow lasting 1 min and a corresponding decrease in T_{tail}. Blood pressure decreased by 40%. Tail conductance decreased by 50% and this decrease was much sharper than the fall in blood pressure. Conductance decreased in all five cases in which DLH was injected into the depressor area in the rostral VTA. The mean conductance decrease was $52 \pm 21\%$ and the mean blood pressure decrease was $23 \pm 15\%$. Both values are significantly different from zero ($P < 0{\cdot}05$). The latency of the conductance decrease was 27 ± 5 s, which was significantly shorter than that of the blood pressure decrease, 70 ± 15 s ($P < 0{\cdot}01$).

Knife cuts

Each knife cut was made through the PAG and its adjacent reticular formation or through the VTA, where electrical and DLH stimulation produced changes in vasomotor activity.

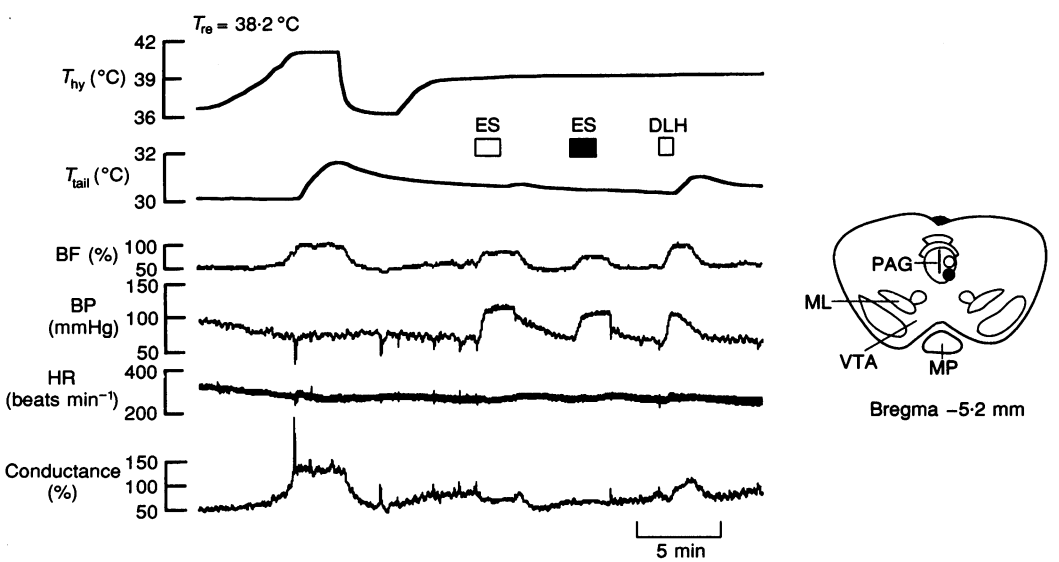

Figure 6. Tail blood flow and conductance changes elicited by preoptic warming, electrical stimulation, and the application of DLH to the PAG

The first electrical stimulation (ES; 0·2 mA, 0·2 ms, 30 Hz) and injection of DLH (1 mM in 0·3 μl saline) were applied to the point shown by the ○ in the inset, and the second electrical stimulation was applied to the point shown by the ●. Abbreviations as in previous figures.

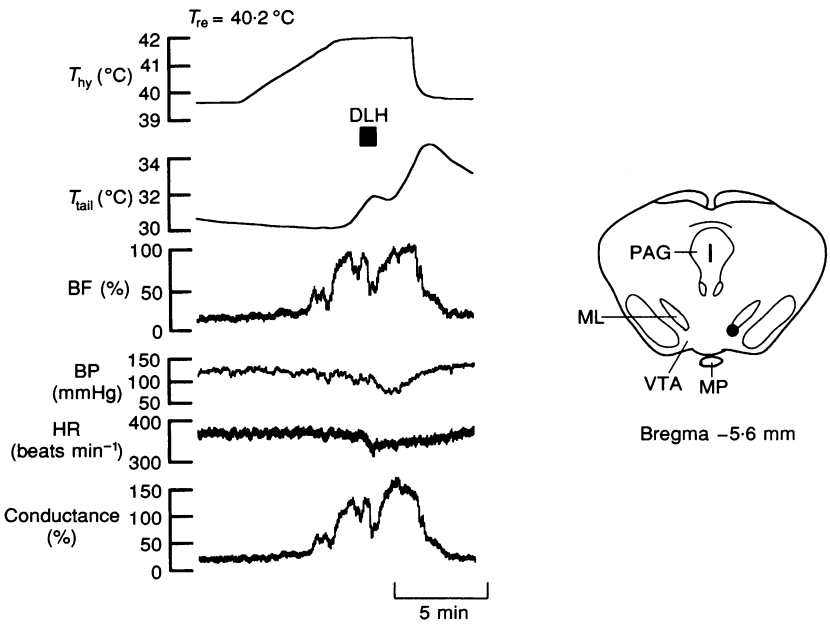

Figure 7. Tail blood flow and conductance changes elicited by preoptic warming and the application of DLH to the VTA

The injections of DLH (1 mM in 0·3 μl saline) were applied to the point shown by the ● in the inset. Abbreviations as in previous figures.

Two kinds of effects were obtained. One was that the tail vasodilatation occurring in response to preoptic warming did not occur after the knife cut. An example is shown in Fig. 8. Before the cut, preoptic warming elicited an increase in T_{tail} and a slight bradycardia. After a knife cut in the PAG and its ventrolateral area, vasodilatation did not appear in response to preoptic warming, but the bradycardia during warming was as clear as that before the knife cut. This type of effect was obtained when the knife cut extended through the PAG and the reticular formation ventral to the PAG in the plane rostral to the pontine nucleus, or rostrally through a more ventral portion of the midbrain including the rostral border of the VTA (Fig. 10).

The other kind of effect was a sudden vasodilatation after the knife cut. In the example shown in Fig. 9, after the vasodilatation was elicited by preoptic warming, the knife cut was made when the tail was vasoconstricted, indicated by the decrease in T_{tail} after the preoptic warming was terminated. T_{tail} increased abruptly after the knife cut, and the vasodilatation was so strong that a decrease in T_{hy} accompanied the rise in T_{tail}. This type of vasodilatation without preoptic warming was produced when the knife cut

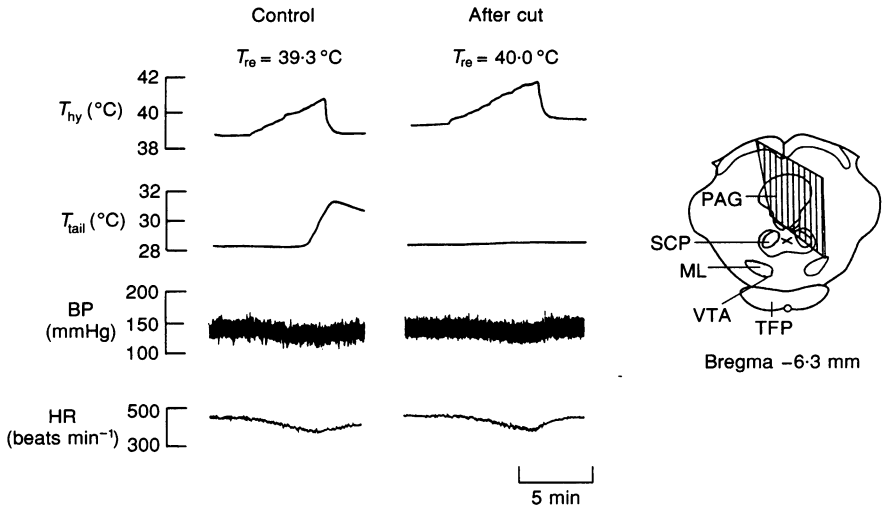

Figure 8. Tail vasomotor response to preoptic warming before and after a knife cut in the dorsomedial part of the midbrain

In the inset, the hatched area indicates the extent of the knife cut. Abbreviations as in previous figures.

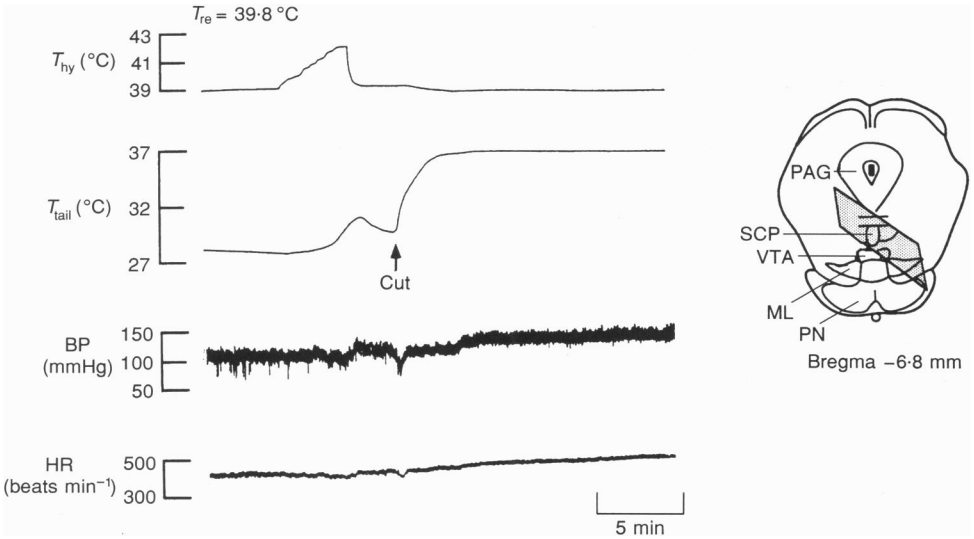

Figure 9. Tail vasomotor response to preoptic warming and to a knife cut in the pons
In the inset, the shaded area indicates the extent of the knife cut. PN, pontine nucleus. Other abbreviations as in previous figures.

was in the ventral part of the midbrain. These ventral cuts are more caudal than those that produced the loss of vasodilatation (Fig. 10).

DISCUSSION

Microinjection of DLH produces changes in T_{tail} – that is, changes in tail blood flow – only when the DLH is injected into certain sites, which in turn indicates that the cell bodies of neurones mediating the responses are located in those sites. The sites where DLH injections elicited increases in T_{tail} ranged from the caudal most edge of the lateral hypothalamus to the border of the ventrolateral PAG and the reticular formation. The sites where DLH injections elicited decreases in T_{tail} were in the rostral part of the VTA. Increases in T_{tail} occurring without changes in blood pressure can be attributed to tail vasodilatation, and decreases in T_{tail} occurring without changes in blood pressure can be attributed to tail vasoconstriction. But in some cases, the T_{tail} increases elicited by DLH injection into the rostral PAG were accompanied by increases in blood pressure, and the T_{tail} decreases elicited by DLH injection into the VTA were accompanied by decreases in blood pressure (Fig. 3*C* and Table 1).

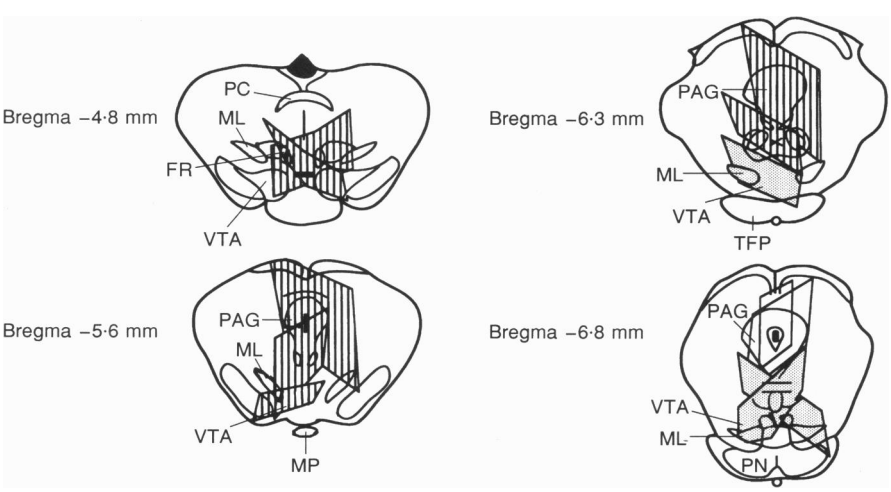

Figure 10. Extent of knife cuts
The hatched areas show the extent of knife cuts after which the vasodilatative response to preoptic warming disappeared. The shaded areas show the extent of knife cuts that without preoptic warming elicited vasodilatation. Open areas show the extent of knife cuts that did not affect the vasomotor response to preoptic warming. Abbreviations as in previous figures.

To find out whether these changes in T_{tail} were simply secondary to the changes in systemic pressure rather than due to changes in vasomotor tone, tail blood flow was measured and the tail conductance changes occurring in response to DLH injection into these 'pressor' and 'depressor' areas (Figs 6 and 7) were calculated. Whenever T_{tail} clearly increased in response to DLH injection into the pressor area, tail conductance increased. Likewise, whenever T_{tail} clearly decreased in response to DLH injection into the depressor area, tail conductance decreased. An increased T_{tail} under the present experimental conditions must therefore have been at least partly due to vasodilatation of the tail, even when it was accompanied by an increase in blood pressure, and a decreased T_{tail} must have been at least partly due to vasoconstriction of the tail, even when it was accompanied by a decrease in blood pressure.

The heart rate of our ketamine-anaesthetized rats ranged between 240 and 450 beats min^{-1} (309 ± 43, $n = 83$). This did not differ between groups in which DLH injections elicited vasodilatation (300 ± 34, $n = 26$), vasoconstriction (310 ± 45, $n = 11$) or no change in vasomotor state (307 ± 44, $n = 46$). Therefore, this large variation does not affect the conclusions of the present study.

The vasodilatation produced by DLH injection into the vasodilatative area found in the present study does not itself necessarily mean that synaptic connections there link efferent fibres from neurones in the preoptic area with neurones that drive the vasodilatation that occurs in response to preoptic warming. However, knife cuts transecting the vasodilatative area suppressed the vasodilatation elicited by preoptic warming. Earlier studies have shown that efferent fibres from the preoptic area descend through the medial forebrain bundle (Kanosue *et al.* 1994*a*). The vasodilatative area found in the present study is the continuation of this pathway. The present results confirmed that electrical stimulation of the medial forebrain bundle rostral to the vasodilatative area produces vasodilatation, and histological studies have also shown that there are projections from the preoptic area to the caudal part of the lateral hypothalamus, PAG and reticular formation ventrolateral PAG (Conrad & Pfaff, 1976; Fahrbach, Morrell & Pfaff, 1986; Simerly & Swanson, 1988). Therefore, efferent signals from the preoptic area are probably relayed in the vasodilatative area found in the present study. These signals are probably excitatory, because efferent signals for thermoregulatory vasomotion from the preoptic area are mainly generated by warm-sensitive neurones (Zhang *et al.* 1995*b*).

Likewise, histological studies have shown that there are efferent projections from the preoptic area to the VTA (Conrad & Pfaff, 1976; Fahrbach *et al.* 1986; Simerly & Swanson, 1988), and activity of VTA neurones is modulated by electrical stimulation of the preoptic area (Sakamoto, Suga & Sakuma, 1993). Therefore, preoptic neurones working for thermoregulatory vasomotor control probably project to the VTA. If so, warm-sensitive neurones in the preoptic area inhibit the vasoconstrictive neurones in the VTA. The region in which only electrical stimulations elicited vasoconstriction extends caudally from the area where DLH injection elicited vasoconstriction and lies along the ventromedial part of the midbrain. Vasoconstrictive neurones in the rostral part of the VTA probably send fibres through this region. Indeed, knife cuts transecting this region produced sudden vasodilatation even when the preoptic area was not warmed, and this suggests that neurones in the rostral VTA generate tonic signals.

Although thermoregulatory vasodilatation does not occur in anaesthetized rats at normal body temperatures (37–38 °C), even when the preoptic area is warmed, vasodilatation occurs when the body is warmed enough to surpass an elevated threshold temperature. And the same pattern of interaction between hypothalamic and extrahypothalamic temperatures evident in the unanaesthetized rat persists in the anaesthetized rat: the higher the extrahypothalamic temperature, the lower the threshold hypothalamic temperature for vasodilatation (Ishikawa *et al.* 1984). The basic neuronal circuits for thermoregulatory vasomotor control, would seem therefore to have been working even in anaesthetized rats used in the present study.

The rectal temperature at which hypothalamic warming elicited vasodilatation in the present study differed between animals and fell into a range of 38·5 to 40·5 °C. This large variation might have been due to small differences in the depth of anaesthesia as well as to the inevitable animal-to-animal differences in the position of the tip of the thermode. The tip was always placed within the preoptic area, but the location of the thermosensitive area most relevant to the control of vasodilatation is not known (Zhang *et al.* 1995*b*). A higher rectal temperature would, therefore, have been required when vasodilatation was elicited by hypothalamic warming when the thermode was further from the centre of the thermosensitive area.

The present study suggests that warm-sensitive neurones in the preoptic area send excitatory signals to the vasodilatative neurones in the caudal part of the lateral hypothalamus, the ventrolateral PAG, and the reticular formation adjacent to the PAG, and that they send inhibitory signals to vasoconstrictive neurones in the rostral part of the VTA. Further studies are required to elucidate the physiological function of each of the separate pathways for thermoregulatory vasomotor control, to find out whether the same or different neurones send axons to the vasodilatative and vasoconstrictive area, and to find out where in the preoptic area these thermosensitive neurones are located.

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