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Cold-induced thermogenesis mediated by GABA in the preoptic area of anesthetized rats

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Running title: Preoptic GABA-mediated thermogenesis

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Abstract

Bilateral microinjections of GABA (300 mM, 100 nl) or the $GABA_A$ receptor agonist muscimol (100 μM, 100 nl) into the preoptic area (POA) of the hypothalamus increased both the rate of whole-body oxygen consumption ($\dot{V}_{O_2}$) and the body core (colonic) temperature of urethane-chloralose-anesthetized, artificially ventilated rats. The most sensitive site was the dorsomedial POA at the level of the anterior commissure. The GABA-induced thermogenesis was accompanied by a tachycardic response and electromyographic (EMG) activity recorded from the femoral or neck muscles. Pretreatment with muscle relaxants (1 mg kg^{-1} pancuronium bromide plus 4 mg kg^{-1} vecuronium bromide, I.V.) prevented the GABA-induced EMG activity but had no significant effect on the GABA-induced thermogenesis. However, pretreatment with the β-adrenoceptor propranolol (5 mg kg^{-1}, I.V.) greatly attenuated the GABA-induced increase in $\dot{V}_{O_2}$ and tachycardic responses. Accordingly, the GABA-induced increase in $\dot{V}_{O_2}$ reflected mainly nonshivering thermogenesis. On the other hand, cooling of the shaved back of the rat by contact with a plastic bag containing 28 °C water also elicited thermogenic, tachycardic, and EMG responses. Bilateral microinjections of the $GABA_A$ receptor antagonist bicuculline (500 μM, 100 nl), but not those of vehicle saline, into the POA blocked these skin cooling-induced responses. These results suggest that GABA and $GABA_A$ receptors in the POA mediate cold information arising from the skin for eliciting cold-induced thermogenesis.

Key words: thermoafferent, neurotransmitter, nonshivering thermogenesis

Introduction

The preoptic area (POA) of the hypothalamus is considered to be the primary locus for body temperature regulation, because it integrates thermoafferent signals from the skin and other parts of the body and exerts control over the thermoefferent mechanisms (3, 8, 15, 25). However, the neurotransmitter that mediates thermoafferent signals to the POA is largely unknown, even though there is a great deal of electrophysiological and pharmacological evidence implicating a role for a variety of neurotransmitters, peptides, and cytokines (2, 6, 10, 32).

The POA contains GABAergic neurons (27), spontaneously releases GABA to the extracellular space (9, 29), and expresses $GABA_A$ receptors (7). Perfusion of the POA with the $GABA_A$ agonist muscimol induces hyperthermia, which is not affected by antipyretics and is thus independent of fever, in freely behaving rats (19). Both warm-sensitive and thermally insensitive neurons are inhibited by the GABAergic mechanism in the POA (28). Furthermore, it was recently reported that the extracellular GABA level in the POA was increased by acute cold exposure and decreased by heat exposure (14). Therefore, it is possible that GABA is involved in the mechanism of thermoregulation in the POA.

Body temperature is regulated by the balance between heat production and heat dissipation. Therefore, the muscimol-induced hyperthermia (19) can be caused either by an increase in heat production or by a decrease in heat dissipation. The former can be monitored by the whole-body O_2 consumption rate ($\dot{V}_{O_2}$); and the latter, by the temperature of the tail skin (T_{tail}). In the present study, the effects of microinjection of GABA and muscimol into the POA on the $\dot{V}_{O_2}$, T_{tail}, trunk skin temperature (T_s), and

core temperature (T_c) were investigated in anesthetized rats. Because administration of these agents increased the $\dot{V}_{O_2}$ without causing significant changes in T_{tail}, heat production was activated by a GABA-receptive mechanism in the POA. There are two forms of heat production: shivering and nonshivering thermogenesis. The relative contribution of these forms to the GABA-induced thermogenesis was examined by muscle relaxants to block shivering, and an adrenergic β-antagonist to block nonshivering, thermogenesis. Finally, the effects of the $GABA_A$ antagonist bicuculline on skin cooling-induced thermogenesis (17) were examined to elucidate the possible involvement of the GABAergic system in the neurotransmission of thermoafferent signals arising from the skin to the POA.

METHODS

Male Wistar rats, weighing 350–480 g, were maintained at an ambient temperature of 24 ± 1 °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 our institutional guidelines.

After induction of anesthesia with 2–3% isoflurane in air and cannulation of a femoral vein and the trachea, the rats were kept anesthetized 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 that had been inserted into the dorsal neck or femoral muscles, filtered at 150–3 kHz, and monitored on an oscilloscope. Occasionally, the signal was digitized at 4 kHz and stored on a

hard disk. A mixture of urethane (70–80 mg kg^{-1} h^{-1}) and chloralose (7–8 mg kg^{-1} h^{-1}) was continuously administered with the aid of a syringe pump (KDS100, KD Scientific, MA, USA) started 90–120 min after the initial anesthesia. Depth of anesthesia was checked by paw pinching, which 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 anesthetic at the end of experiment.

The rats were placed in a stereotaxic apparatus with their head fixed according to the coordinate system of Paxinos and Watson (20), and body temperature was maintained at 37–38 °C with a heating pad. The back of each rat was shaved between the caudal end of the forelimbs and the rostral end of the hindlimbs. Three thermocouples were glued to different sites on the shaved skin. The mean of these three readings was used as the measure of T_s. T_c was measured with a fourth thermocouple inserted ~50 mm into the anus. Occasionally, T_{tail} was measured with another thermocouple taped to the dorsal surface of the tail. The trunk and proximal part of 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 analyzer (LC-700E, Toray, Japan). Values were corrected for metabolic body size ($kg^{0.75}$). In some experiments, an electrocardiogram was recorded with needle electrodes subcutaneously inserted into the limbs of the rats and monitored on an oscilloscope. A counter (AT-601G, NihonKohden, Japan) was used to detect R-waves

and to calculate the heart rate. These signals were fed into a computer and recorded at 3- or 5-s intervals through a PowerLab system (ADInstrument, Australia) for on-line data display, storage, and off-line analysis. After the experiments, data were averaged over 30-s intervals.

A three-barrel glass micropipette was used to apply drugs to the POA or neighbouring regions. Each barrel of the pipette contained 300 mM GABA (pH 7.2), 100 μM muscimol hydrobromide (pH 4.5), 500 μM (–)bicuculline methiodide (pH 5.8), 2% pontamine sky blue, or physiological saline solution. GABA was dissolved in distilled water, and other drugs were dissolved in physiological saline solution. The concentration of the GABA solution used was chosen because it is approximately equiosmotic (300 mosmol kg^{-1}) to the normal body fluids. The total tip diameter of the pipette was 30–40 μm. The solutions were ejected from the pipette with pressurized nitrogen by the aid of a pressure ejection system (Picosprizer, General Valve, NJ, USA) and an eight-way valved connector (No. 1103, Omnifit, UK). Injections were made bilaterally. The volume of injected solution was 100 nl on each side. In the case of the injections made into the midline, the volume of solution was also 100 nl. For ejection of the correct amount of a given solution, the displacement of the meniscus between the solution and the nitrogen gas in the pipette was observed through a dissecting microscope with an ocular micrometer. The ejection pressure was adjusted to deliver the solution for 20–30 s on each side.

The β-adrenoceptor blocker DL-propranolol hydrochloride was dissolved in physiological saline solution and I.V. administered at a dose of 5 mg kg^{-1}. A mixture of pancuronium bromide (1 mg kg^{-1}) and vecuronium bromide (4 mg kg^{-1}) was I.V. infused to paralyze the skeletal muscles. Effects of GABA were examined before and

7–20 min after administration of these drugs.

The method of thermal stimulation of the trunk skin was described previously (17). Briefly, a plastic bag containing 28 °C water was placed onto the shaved back for 2 min instead of the quilt that covered the rat. The water bag was made by a polyethylene sheet of 70-μm thickness, had the bottom surface area of 110 x 180 mm, and contained 340–360 ml water. The scrotum, tail, head, and distal part of limbs did not receive thermal stimulation. The bag was agitated every 30 s for 4–5 s to mix the water uniformly. The quilt was replaced in its original position soon after the 2-min thermal stimulation.

A volume of 20–30 nl pontamine sky blue solution was ejected from a barrel of the pipette for histological verification of injection sites at the end of experiment. The brains were perfused with 10% formalin through the carotid arteries. Coronal sections were cut at a 40-μm thickness on a freezing microtome, mounted on glass slides, and counterstained with 0.1% neutral red. The sites of injection were identified according to the rat brain atlases of Paxinos and Watson (20) and Swanson (26).

Data were presented as the mean ± S.E.M. Paired *t*-test was used to examine the difference in magnitude of GABA- or skin cooling-induced thermogenesis before and after administration of drugs. Statistical significance was defined as $P < 0.05$.

RESULTS

Figure 1 shows a representative example of the effects of skin cooling and microinjection of GABA, muscimol, or physiological saline into the medial POA of a

rat. Skin cooling increased the $\dot{V}_{O_2}$ and heart rate and temporally decreased T_c, which effects were similar to those observed previously (17). The microinjection of GABA increased the $\dot{V}_{O_2}$ during or within 30 s after the injection by 3.62 ± 0.56 ml $kg^{-0.75}$ min^{-1} (n = 15) at 5.0 ± 0.5 min, and then the $\dot{V}_{O_2}$ returned to the baseline level within 20 min. The GABA injection also increased the heart rate by 56 ± 13 beats min^{-1} at 4.2 ± 0.5 min (n = 13) and T_c by 0.27 ± 0.04 °C (n = 15) at 8–22 min after the injection. T_s was 36–38 °C and increased by 0.25 ± 0.04 °C after the GABA injection with a time course similar to that of T_c. On the other hand, injection of muscimol increased the $\dot{V}_{O_2}$ by 4.39 ± 0.34 ml $kg^{-0.75}$ min^{-1} (n = 6) for a period longer than 40 min (Fig. 1*B* and 2*C*). The muscimol injection increased T_c by 0.71 ± 0.14 °C and T_s by 0.85 ± 0.23 °C at 20–50 min post injection. T_{tail} was 29–32 °C and increased by 0.3–1.5 °C after the muscimol injection (n = 3, Fig. 2*F*), although it did not change significantly after the GABA injection (n = 6, Fig. 5*E*). Injection of physiological saline had no effect on the $\dot{V}_{O_2}$, heart rate, T_c, or T_s (n = 8).

The thermogenic effect of GABA and muscimol was restricted to an area in and around the dorsomedial POA. Figure 2*A* and *B* show specific sensitivity of the dorsomedial POA to GABA and the lack of a thermogenic response by the ventromedial, dorsolateral, or ventrolateral POA. Similarly, microinjection of muscimol into the dorsomedial POA, but not into the anterior commissure or ventromedial POA, elicited a thermogenic response (Fig. 2*C* and *D*). Figure 3 shows the site of GABA or muscimol microinjection and the $\dot{V}_{O_2}$ responses. The most effective injection sites were clustered in the dorsomedial POA at the level of the anterior commissure, although small responses were observed when microinjection was made into the dorsolateral POA and

vertical limb of the diagonal band of Broca. Injection of GABA into the median, periventricular, or ventral preoptic area had no effect on the $\dot{V}_{O_2}$.

Microinjection of GABA or muscimol usually elicited EMG activity recorded from the femoral or neck muscles. The GABA-induced EMG activity started simultaneously with or up to 3 min after the rise in $\dot{V}_{O_2}$ and lasted 8.0 ± 1.2 min (n = 11). The EMG consisted of several different-sized action potentials (Fig. 4*C*), suggesting concurrent activation of a limited number of muscle fibers. The discharge pattern was continuous and did not exhibit the rhythmic burst that is typically observed during shivering. In spite of the EMG activity, however, the rat did not exhibit any visible body movement. Because the result could suggest a contribution of muscle contractions to the GABA-induced thermogenesis, the effects of the muscle relaxants were examined. Administration of the muscle relaxants alone had various transient (< 5 min) and small (< 1 ml $kg^{-0.75}$ min^{-1}) effects on the $\dot{V}_{O_2}$: it was decreased (n = 5), increased (n = 3), or without effect (n = 1). However, the GABA-induced EMG activity was blocked effectively (cf., Fig. 4*C* and *D*), whereas the thermogenic response to GABA largely persisted (Fig. 4*A* and *B*). Fig. 4*E* summarises the increase in $\dot{V}_{O_2}$ of individual rats before and after administration of the muscle relaxants (n = 9). Although the response was slightly decreased from 3.76 ± 0.49 ml $kg^{-0.75}$ min^{-1} to 3.50 ± 0.47 ml $kg^{-0.75}$ min^{-1}, the difference was not statistically significant.

Administration of propranolol (5 mg kg^{-1}) alone decreased the $\dot{V}_{O_2}$ by 0.7–2 ml $kg^{-0.75}$ min^{-1} for 2–3 min (n = 5), and thereafter the $\dot{V}_{O_2}$ was 0.1–1 ml $kg^{-0.75}$ min^{-1} lower than the baseline level for 1–2 h. Pretreatment with propranolol greatly attenuated the GABA-induced thermogenesis (Fig. 5). All rats examined showed a decrease in their

thermogenic response to 11 ± 5 % (n = 5) of the control response at 9–17 min after propranolol administration (Fig. 5*F*). The magnitude of GABA-induced thermogenesis recovered to 30 ± 8 % (n = 5) of the control at 37–53 min and to 40 ± 1 % (n = 3) at 68–73 min. The heart rate was decreased by 50–140 beats min^{-1} following administration of propranolol, and thereafter it slowly recovered to the baseline level. The tachycardic response to the GABA injection was also greatly attenuated by the pretreatment with propranolol (Fig. 5*B*): it was decreased to 10 ± 5 % (n = 4) of the control response at 9–17 min after the administration of propranolol, was still suppressed to 7 ± 5 % (n = 4) at 37–53 min, and recovered to 24 ± 9 % (n = 3) at 70–100 min.

Effects of the microinjection of the $GABA_A$ receptor antagonist bicuculline into the POA on skin cooling-induced thermogenic, tachycardic, and EMG responses were investigated next (Fig. 6). Administration of bicuculline alone had variable effects on the $\dot{V}_{O_2}$: no effect (n = 2), a gradual increase by 1.4 ml $kg^{-0.75}$ min^{-1} at 10 min, a transient decrease followed by a slow increase by 0.5 ml $kg^{-0.75}$ min^{-1} (Fig. 6*B*, 1st administration), or a long-lasting decrease by 1.4 ml $kg^{-0.75}$ min^{-1} (Fig. 6*B*, 2nd administration); although the heart rate was always decreased by 51 ± 2 beats min^{-1} (n = 5) at 6.8 ± 0.5 min after administration of bicuculline (Fig. 6*C*). The range of basal T_c and T_s was 37.2–37.9 °C and 35.6–37.4 °C, respectively; and the change in $\dot{V}_{O_2}$ after administration of bicuculline had no correlation with these basal temperatures. Regardless of the changes in the baseline $\dot{V}_{O_2}$, however, skin cooling-induced thermogenesis was completely abolished by pretreatment with bicuculline. Moreover, skin cooling rather decreased the $\dot{V}_{O_2}$ on two occasions recorded from one rat after

administration of bicuculline (Fig. 6*B*). The average skin cooling-induced thermogenic response was significantly decreased from 4.0 ± 0.4 ml $kg^{-0.75}$ min^{-1} to –0.5 ± 0.4 ml $kg^{-0.75}$ min^{-1} (n = 5) at 7–16 min after the bicuculline administration (Fig. 6*K*). In parallel with the thermogenic response, the tachycardic response to skin cooling was also decreased from 91 ± 12 beats min^{-1} to 4 ± 13 beats min^{-1} (n = 5) after the bicuculline administration (Fig. 6*C*). The thermogenic and tachycardic responses to skin cooling recovered to 4.2 ± 0.4 ml $kg^{-0.75}$ min^{-1} and 84 ± 21 beats min^{-1}, respectively, at 32–46 min (n = 5). The skin cooling-induced EMG activity (Fig. 6*E* and *H*) was also blocked by pretreatment with bicuculline (Fig. 6*F* and *I*) and recovered at the subsequent cooling episode (Fig. 6*G* and *J*). On the other hand, microinjection of physiological saline into the POA had no effect on the skin cooling-induced thermogenic and tachycardic responses (Fig. 6*B*, *C* and *K*, n = 4).

DISCUSSION

Microinjection of GABA or muscimol into the POA increased the $\dot{V}_{O_2}$ and T_c, indicating a thermogenic response mediated by a GABA-receptive mechanism in the POA. The hyperthermic response agrees well with the results of a previous study on conscious rats (19). Because GABA is an inhibitory neurotransmitter, these results suggest that the GABA-receptive mechanism in the POA tonically suppresses thermogenesis and that the GABA-induced inhibition of the POA neurons activated thermogenesis by disinhibition of this system. Consistent with this notion, Chen *et al*. (5) demonstrated an inhibitory influence of preoptic neurons on nonshivering

thermogenesis: microinjection of the excitatory amino acid D,L-homocysteic acid into the POA attenuated nonshivering thermogenesis elicited by electrical stimulation of the ventromedial hypothalamus. Similarly, perfusion of the POA with tetrodotoxin induced hyperthermia and tachycardia in conscious rats, suggesting an inhibitory function of the POA with respect to heat production (13). On the other hand, administration of GABA in the present study had no significant effect on T_{tail}, which was within the baseline level of 29–32 °C. These results on T_{tail} suggest that the tail skin was in a steady vasoconstrictive state and did not exhibit a further vasomotor response to GABA.

The GABA-induced thermogenesis was greatly attenuated by pretreatment with the β-blocker propranolol but not significantly by that with the muscle relaxants. These results indicate that mainly nonshivering thermogenesis was elicited in the present study. However, they do not exclude the possibility of an involvement of preoptic GABA-receptive neurons in the mechanism of shivering thermogenesis. Microinjection of GABA or muscimol did evoke EMG activity, and pretreatment with bicuculline prevented the skin cooling-induced EMG activity in the present study. The POA reportedly regulates both shivering and nonshivering thermogenesis (3, 5, 33). Accordingly, it is possible that GABA is also involved in the increase in skeletal muscle tone during shivering thermogenesis, although the relative contribution of shivering to heat production was small under the present experimental conditions.

The GABA-sensitive site for thermogenesis was localized in or near the dorsomedial POA at the level of the anterior commissure. The POA is composed of several histologically distinct subdivisions (24). Among these subdivisions, the ventromedial area has been proposed to participate in hyperthermia during fever (21,

22), and the ventrolateral area is particularly involved in the regulation of sleep (23). However, no report has hitherto identified any specific thermoregulatory function for the dorsomedial area; though thermosensitive neurons (11, 16) and GABA binding sites (1) were found to be distributed diffusely in and around the medial POA.

The $\dot{V}_{O_2}$ and heart rate changed roughly in a parallel fashion in response to the GABA injection and various other treatments employed in the present study, suggesting a common GABA-receptive mechanism for the thermogenic and tachycardic responses in the POA. However, although administration of the $GABA_A$ antagonist bicuculline alone always decreased the basal heart rate, it did not produce a consistent effect on the $\dot{V}_{O_2}$. These results suggest that the GABA-receptive mechanism for the control of thermogenesis is not totally identical to that governing the heart rate, and that the spontaneous release of GABA in the POA contributed to the basal heart rate but not significantly to that of the $\dot{V}_{O_2}$ under the present experimental conditions. On the other hand, β-adrenoceptors are critically involved in the peripheral mechanism of the GABA-induced thermogenic and tachycardic responses, because systemic administration of propranolol greatly attenuated both responses. Consistent with the present results, tachycardia induced by perfusion of the POA with muscimol was blocked by systemic administration of propranolol or adrenalectomy in halothane-anesthetized rats (18).

Local cooling of the POA reportedly increased the $\dot{V}_{O_2}$ and temperature of the brown adipose tissue in conscious rats, suggesting activation of nonshivering thermogenesis (12). The POA contains two types of thermosensitive neurons: cold-sensitive neurons are excited, and warm-sensitive neurons are inhibited, by local brain cooling.

Accordingly, it is likely that the preoptic cooling-induced thermogenesis was mediated either by excitation of the cold-sensitive neurons or by inhibition of the warm-sensitive ones. Because GABA inhibits spontaneous activity of warm-sensitive neurons in the POA (28), we may surmise that the GABA-induced thermogenesis was mediated by inhibition of warm-sensitive neurons rather than by excitation of cold-sensitive ones.

Thermogenic responses were elicited by skin cooling as well as by microinjection of GABA or muscimol into the POA. The skin cooling-induced thermogenesis, tachycardia, and EMG activity were blocked by microinjection of bicuculline into the POA. These results demonstrate that GABA and $GABA_A$ receptors in the POA exert a pivotal role in skin cooling-induced thermogenesis. Peripheral cold stimulation inhibits warm-sensitive neurons in the POA (4). Therefore, it may be inferred that cold information originating from the skin activates GABAergic fibers that terminate on warm-sensitive neurons in the POA, though further studies are necessary to verify this possibility. Alternatively, it is also possible that the GABA-receptive mechanism in the POA tonically inhibits some other key locus that receives the cold signal from the skin and has an excitatory effect on heat production. The dorsomedial hypothalamus has been suggested as such a locus (30).

Although the present study demonstrates the critical involvement of the GABA-receptive mechanism in the POA in cold-induced thermogenesis, the participation of neurotransmitters other than GABA cannot be excluded in the thermoafferent system. For example, serotonergic and noradrenergic systems have been proposed to mediate or modulate the afferent signals in the central thermoregulatory pathways (8, 31), though no definite evidence has been demonstrated. GABA and other transmitters can function in parallel or in series to mediate thermal

information, which affects various thermoregulatory functions, such as shivering and nonshivering thermogenesis, cutaneous vasoconstriction, and behavioral thermoregulation. Further studies are necessary to elucidate the relationships among neurotransmitters operating in the various thermoregulatory subsystems. Moreover, the location and properties of the GABAergic neurons that mediate thermogenesis remain to be clarified.

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Figure legends

Fig. 1. Thermogenesis elicited by skin cooling or microinjection of GABA or muscimol. A representative record shows T_s (*A*), $\dot{V}_{O_2}$ (*B*), heart rate (*C*) and T_c (*D*) in response to skin cooling and microinjection of GABA, muscimol, and physiological saline into the POA in a rat. The bars in *B* show the period of microinjection.

Fig. 2. Site-specific thermogenic effect of GABA and muscimol. Representative records show site-specific actions of GABA (*A* and *B*) and muscimol (*C* – *F*) on the $\dot{V}_{O_2}$ (*A* and *C)*, T_c (*B* and *D*), T_s (*E*), and T_{tail} (*F*). Note the different time scale in *A* and *B* vs. *C* – *F*. The bars show the period of microinjection. DMPO, dorsomedial preoptic area; VMPO, ventromedial preoptic area; DLPO, dorsolateral preoptic area; VLPO, ventrolateral preoptic area; AC, anterior commissure.

Fig. 3. Sites of GABA or muscimol injection shown on coronal sections of the rat brain. Circles and triangles show the injection sites of GABA and muscimol, respectively. Filled, dotted, and open symbols show where GABA or muscimol increased the $\dot{V}_{O_2}$ more than 3 ml $kg^{-0.75}$ min^{-1}, between 1 and 3 ml $kg^{-0.75}$ min^{-1}, and less than 1 ml $kg^{-0.75}$ min^{-1}, respectively. Numerals shown at the top of each section indicate the distance from bregma. Although the injections were made bilaterally, the sites in one hemisphere are shown for the sake of brevity. Data were obtained from 20 rats, each of which received 1–8 injections. Injection sites of these rats were marked with pontamine dye and histologically identified. Abbreviations: ACB, accumbens; BST, bed nucleus of the stria terminalis; HDB, horizontal limb of the diagonal band of

Broca; LPO, lateral preoptic area; MPO, medial preoptic area; MS, medial septum; OC, optic chiasma; ON, optic nerve; VDB, vertical limb of the diagonal band of Broca.

Fig. 4. Effects of muscle relaxants on the GABA-induced thermogenesis and EMG responses. Changes in $\dot{V}_{O_2}$ (*A*) and T_c (*B*) of a rat in response to repeated injections of GABA are shown before and after I.V. administration (arrow) of the muscle relaxants pancuronium (1 mg kg^{-1}) plus vecuronium (4 mg kg^{-1}). The thick horizontal bars in *A* show the period of GABA injection into the POA; and the thin bars indicated by "*c*" and "*d*" the period of the EMG record shown in *C* and *D*, respectively. Pretreatment with the muscle relaxants completely blocked the GABA-induced EMG activity. *E*, GABA-induced increase in $\dot{V}_{O_2}$ before and after administration of the muscle relaxants, showing lack of statistically significant change in the thermogenic response (n = 9). Each symbol and connecting line indicate an individual rat.

Fig. 5. Effects of the β-adrenoceptor blocker propranolol (5 mg kg^{-1}, I.V., arrow in *A*) on GABA-induced changes in $\dot{V}_{O_2}$ (*A*), heart rate (*B*), T_c (*C*), T_s (*D*), and T_{tail} (*E*). The bars in *A* show the period of GABA injection into the POA. *F*, GABA-induced increase in $\dot{V}_{O_2}$ before and after administration of propranolol in five rats, showing significant attenuation of thermogenesis. Each symbol and connecting line indicate an individual rat.

Fig. 6. Blockade of skin cooling-induced thermogenesis, tachycardia, and EMG activity by microinjection of bicuculline into the POA. Representative recordings

show T_s (*A*), $\dot{V}_{O_2}$ (*B*), heart rate (*C*), and T_c (*D*) in response to repeated skin cooling episodes. Bilateral microinjections of bicuculline methiodide (500 μM, 100 nl), but not those of the same amount of physiological saline, into the POA suppressed the thermogenic and tachycardic responses to skin cooling. The arrows in *B* indicate the time of administration of bicuculline or physiological saline. *E-J*, EMG recorded from femoral muscles, each corresponding to the cooling episode (*e-j*) in *A*. The arrows in *E-J* indicate the onset of cooling stimulation. Pretreatment with bicuculline reproducibly prevented the skin cooling-induced EMG activity. *K*, skin cooling-induced increase in $\dot{V}_{O_2}$ before and after administration of bicuculline (open symbols, n = 5) and physiological saline (closed symbols, n = 4). Each symbol and connecting line indicate an individual rat.

Figure 1

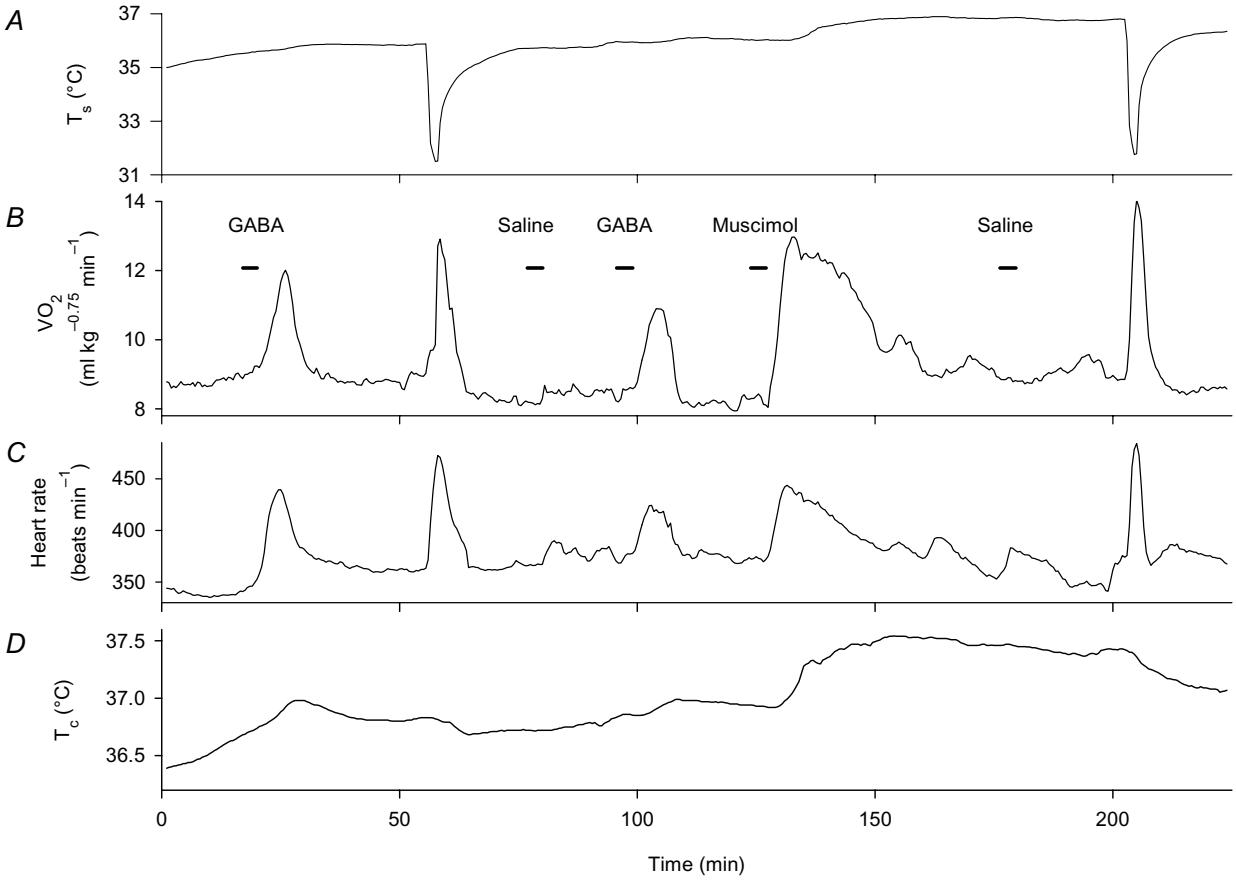

A
37
35
33
31
T_s (°C)
B
14
12
10
8
VO_2 (ml kg$^{-0.75}$ min^{-1})
GABA
Saline
GABA
Muscimol
Saline
C
Heart rate (beats min^{-1})
450
400
350
D
37.5
37.0
36.5
T_c (°C)
0
50
100
150
200
Time (min)

Figure 2

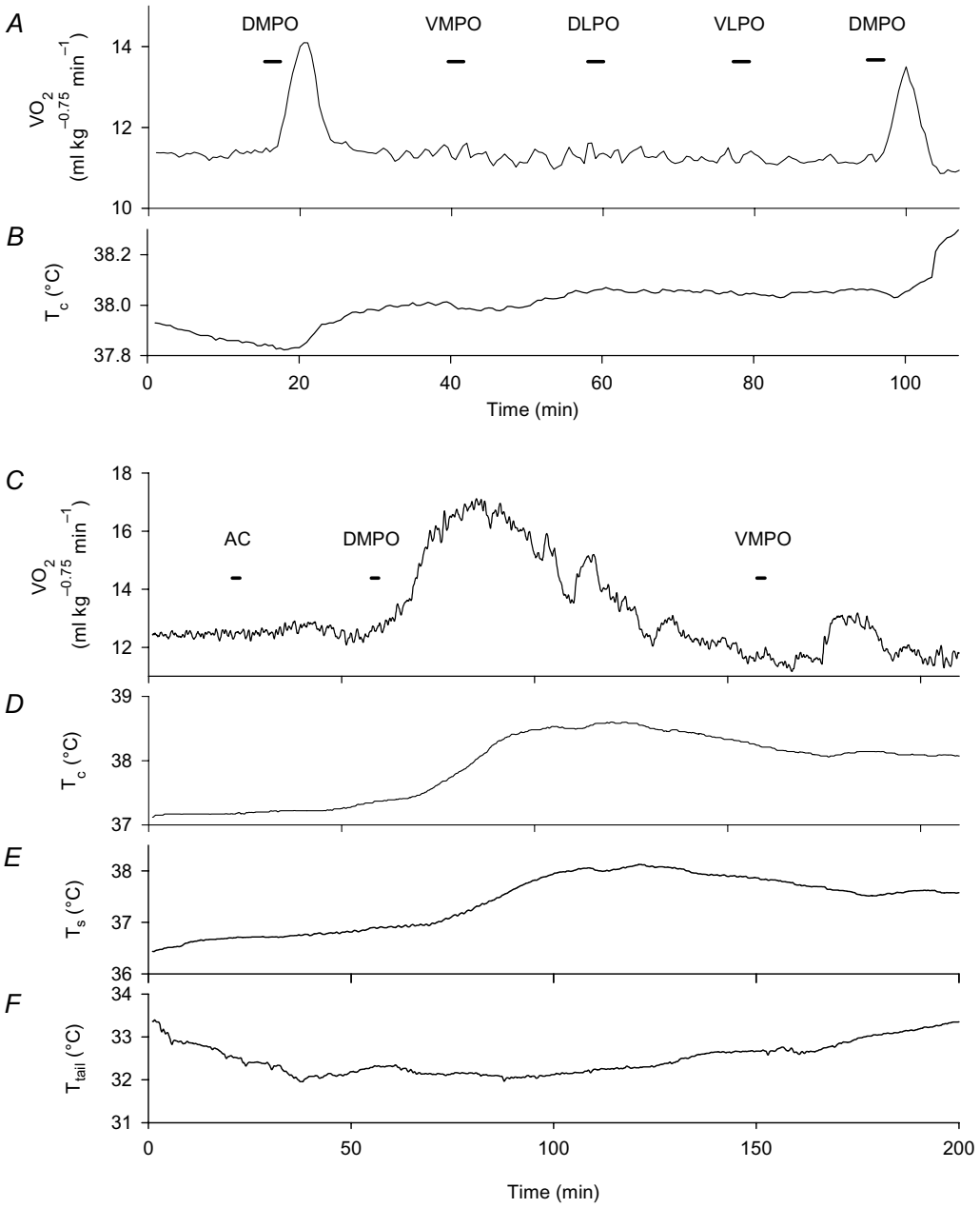

A
DMPO
VMPO
DLPO
VLPO
DMPO
VO_2 (ml $kg^{-0.75}$ min^{-1})
14
12
10
B
T_c (°C)
38.2
38.0
37.8
0
20
40
60
80
100
Time (min)
C
AC
DMPO
VMPO
VO_2 (ml $kg^{-0.75}$ min^{-1})
18
16
14
12
D
T_c (°C)
39
38
37
E
T_s (°C)
38
37
36
F
T_{tail} (°C)
34
33
32
31
0
50
100
150
200
Time (min)

Figure 3

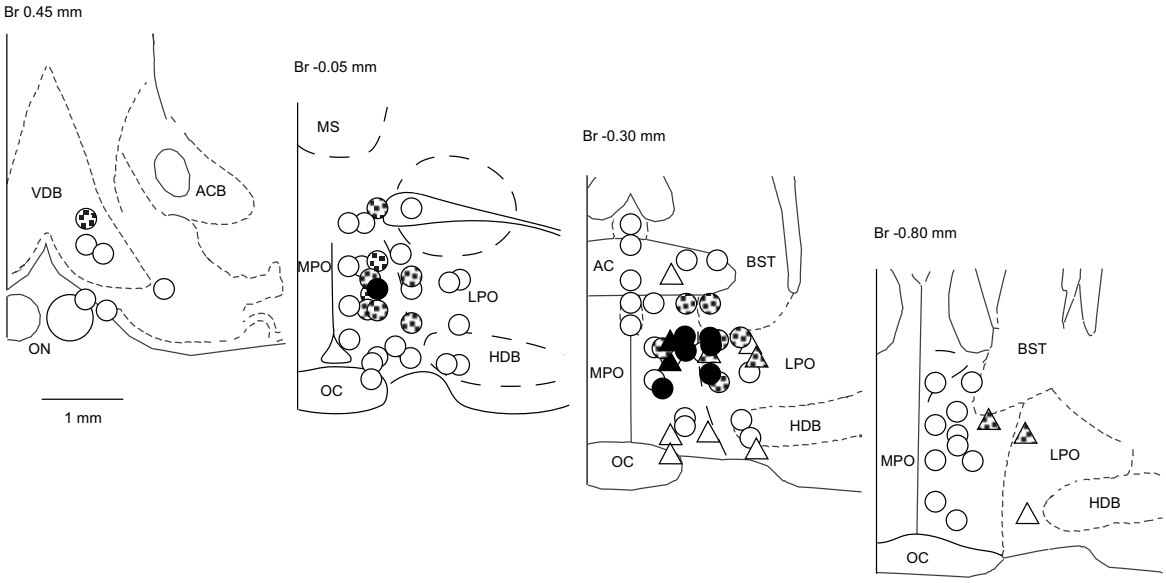

Br 0.45 mm
VDB
ACB
ON
1 mm
Br -0.05 mm
MS
MPO
LPO
HDB
OC
Br -0.30 mm
AC
BST
MPO
LPO
HDB
OC
Br -0.80 mm
BST
MPO
LPO
HDB
OC

Figure 4

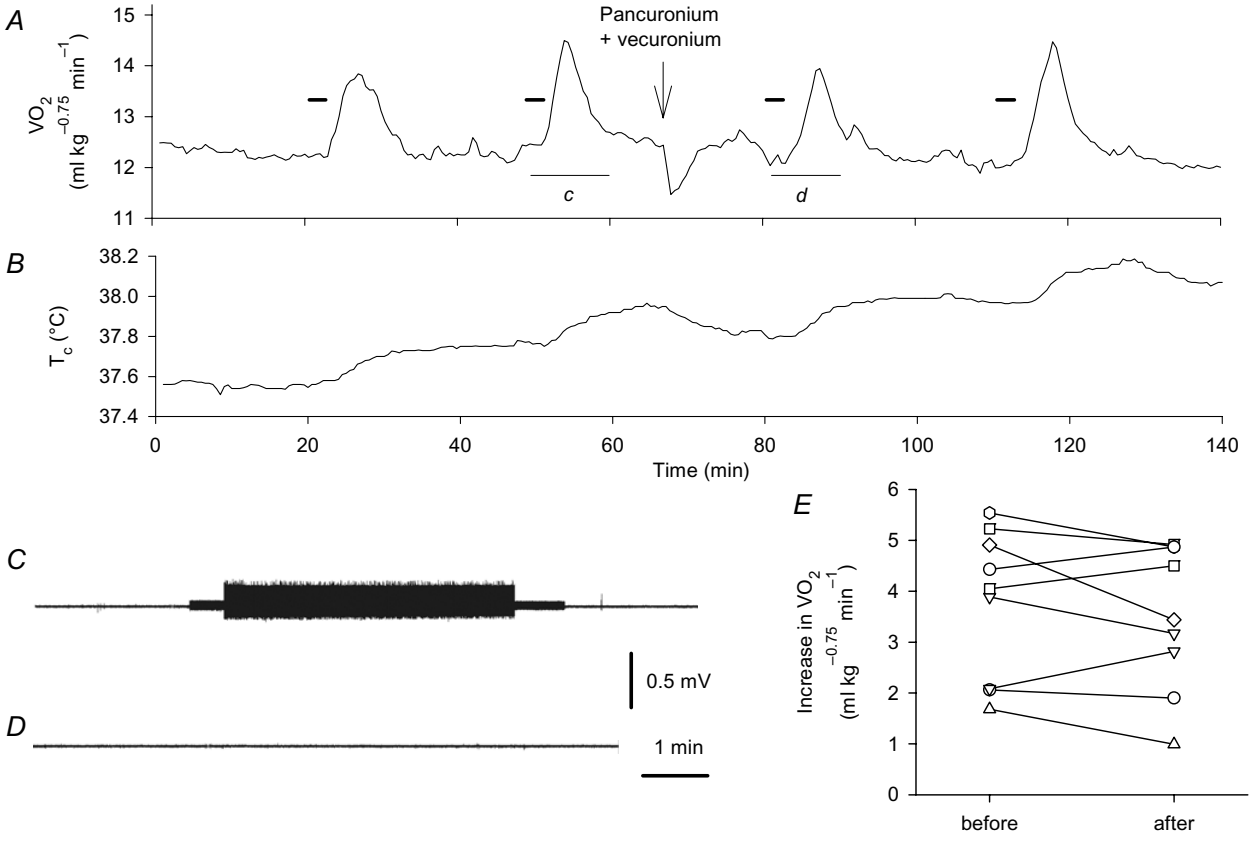

A
Pancuronium
+ vecuronium
VO_2 (ml kg$^{-0.75}$ min^{-1})
15
14
13
12
11
c
d
B
T_c (°C)
38.2
38.0
37.8
37.6
37.4
0
20
40
60
80
100
120
140
Time (min)
C
0.5 mV
D
1 min
E
Increase in VO_2 (ml kg$^{-0.75}$ min^{-1})
6
5
4
3
2
1
0
before
after

Figure 5

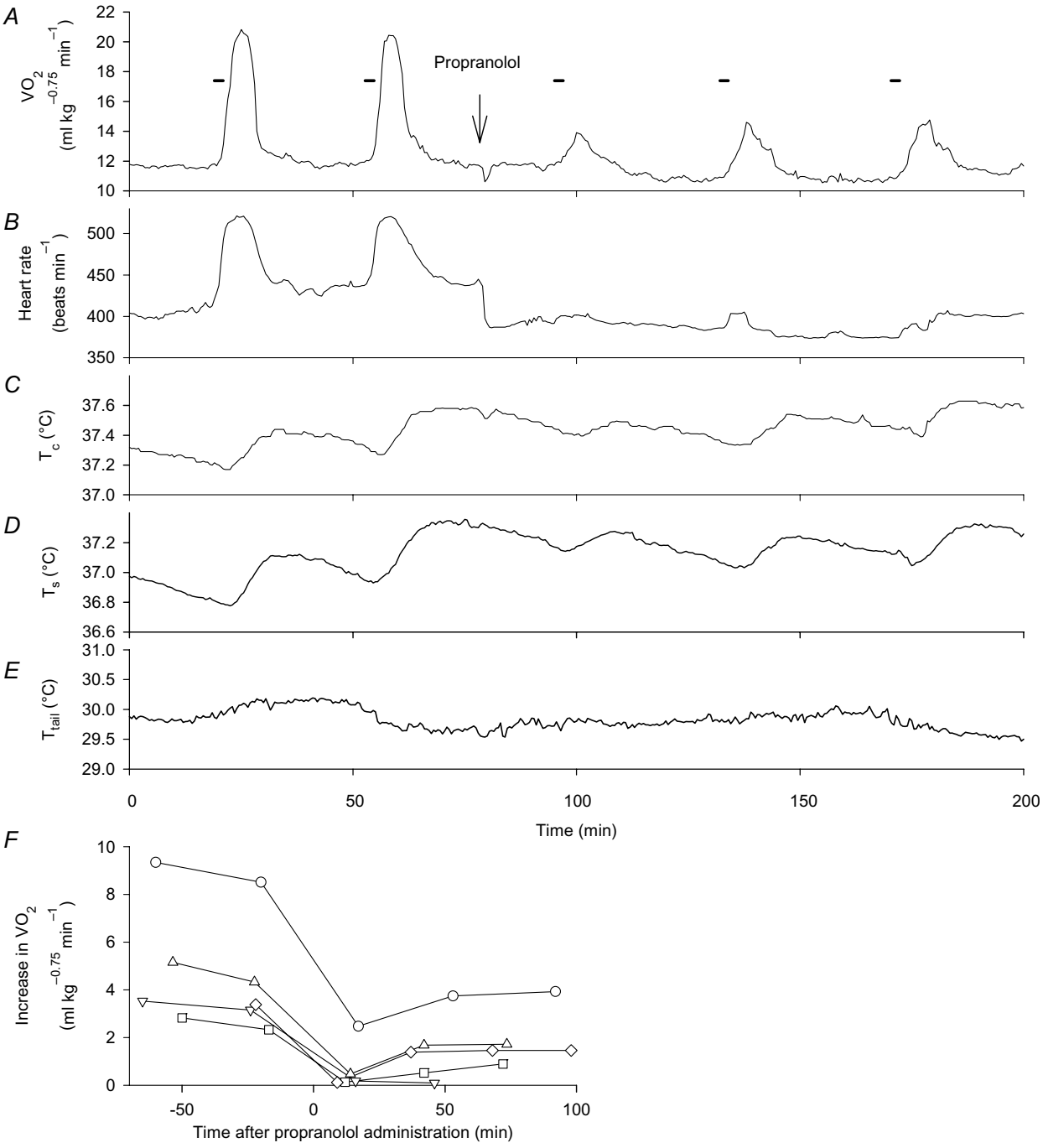
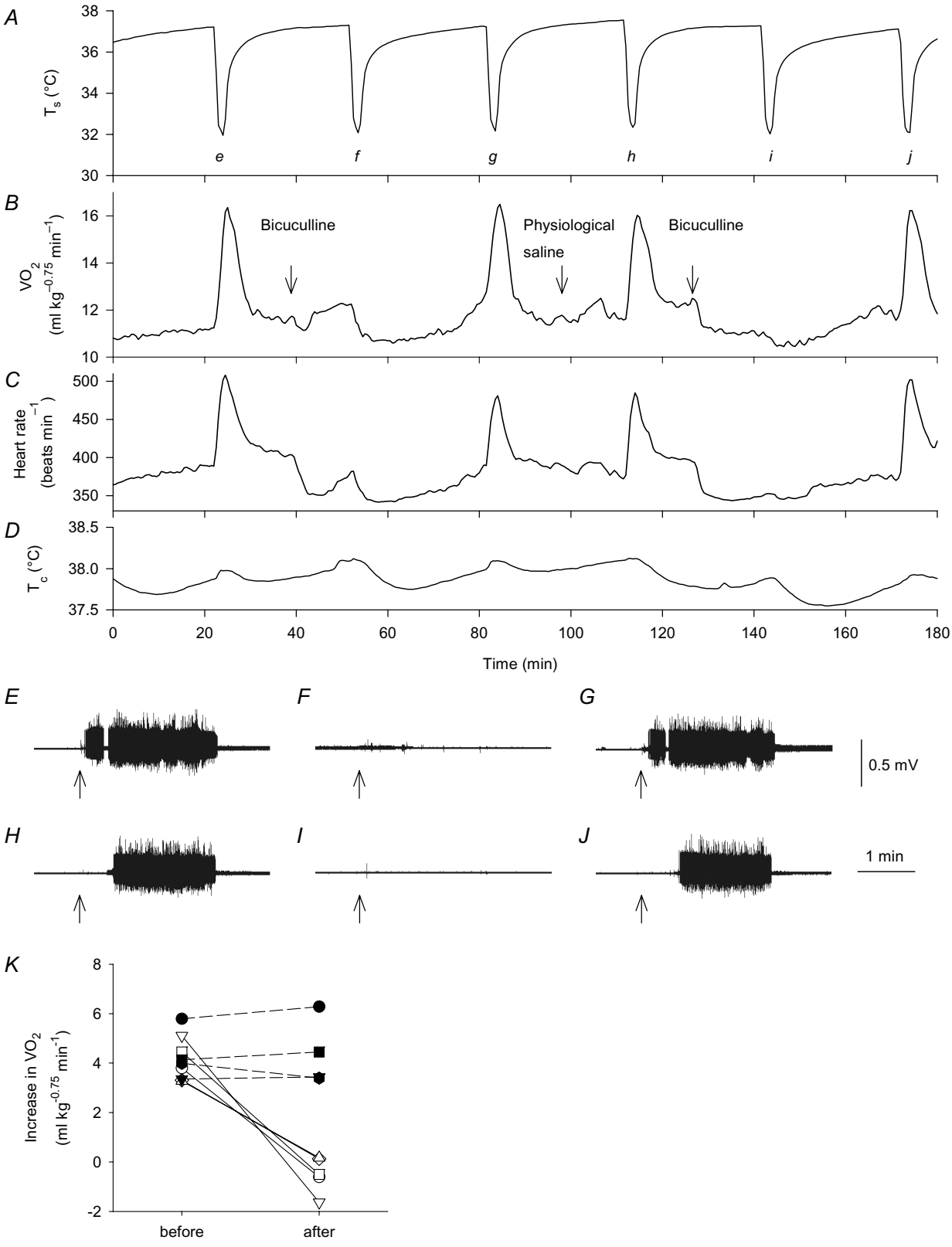

A
$\dot{V}O_2$ (ml kg$^{-0.75}$ min^{-1})
Propranolol
B
Heart rate (beats min^{-1})
C
T_c (°C)
D
T_s (°C)
E
T_{tail} (°C)
Time (min)
F
Increase in $\dot{V}O_2$ (ml kg$^{-0.75}$ min^{-1})
Time after propranolol administration (min)

Figure 6


A
B
C
D
E
F
G
H
I
J
K
Bicuculline
Physiological saline
Bicuculline
e
f
g
h
i
j
Time (min)
0.5 mV
1 min
before
after