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Reflex control of rat tail sympathetic nerve activity by abdominal temperature

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Abbreviations: CNS, central nervous system; Hex, hexamethonium chloride; SNA, sympathetic nerve activity; TRPV1 (channel), transient receptor potential vanilloid type 1 (channel)

The thermoregulatory reflex effects of warming and cooling in the abdomen were investigated in 4 urethane-anesthetized Sprague-Dawley rats. Animals were shaved and surrounded by a water-perfused silastic jacket. Skin temperature under the jacket was recorded by thermocouples at 3 sites and brain temperature was monitored by a thermocouple inserted lateral to the hypothalamus. A heat exchanger made from an array of silicon tubes in parallel loops was placed through a ventral incision into the abdomen; it rested against the intestinal serosa and the temperature of this interface was monitored by a thermocouple. Few- or multi-unit postganglionic activity was recorded from sympathetic nerves supplying tail vessels (tail SNA). Intra-abdominal temperature was briefly lowered or raised between 35–41 °C by perfusing the heat exchanger with cold or warm water. Warming the abdomen inhibited tail SNA while cooling it excited tail SNA in all 4 animals. We also confirmed that cooling the trunk skin activated tail SNA. Multivariate analysis of tail SNA with respect to abdominal, brain and trunk skin temperatures revealed that all had highly significant independent inhibitory actions on tail SNA, but in these experiments abdominal temperature had the weakest and brain temperature the strongest effect. We conclude that abdominal temperature has a significant thermoregulatory action in the rat, but its influence on cutaneous vasomotor control appears to be weaker than that of skin or brain temperatures.

Introduction

Body temperature control in mammals depends on behavioral and multiple reflex mechanisms.[1,2] All of these depend upon temperature signals which may be in the brain, spinal cord, skin and deep body stuctures.[3,4] The relative importance of each of these temperature signals differs, however, depending on which effector mechanism is being controlled.[5] For example, the skin temperature signal exerts dominant control over fusimotor neuron activation (a component of the shivering response), but is relatively less important for cutaneous vasomotor control.[5-7]

Mercer and Jessen[8] performed experiments on conscious goats in which hypothalamic and spinal cord temperatures were clamped at a normothermic level whist the rest of the body was warmed or cooled by an intravascular heat exchanger.[8] From observations on the metabolic (shivering) response to cooling and (in one animal) the panting response to heating, they concluded that the temperature signal from deep body structures had a substantial thermoregulatory effect independently of any contribution from spinal, hypothalamic or skin temperatures. In those experiments the independent influence of deep body temperature was comparable to that of spinal or hypothalamic temperatures.[8]

The question for us here is: where might those deep body temperature signals originate? A suggestion by Bligh[9] that temperature sensors in the heart or great veins might have a thermoregulatory influence was not supported by Cranston et al.,[10] who found no difference in the effect of cold saline injected in to the jugular vein or left atrium of conscious rabbits. A more promising avenue in the search for deep body thermosensors is in the abdomen. In conscious sheep, Rawson and Quick demonstrated that intra-abdominal warming by an implanted heating pad suppressed shivering and augmented panting.[11,12] Soon afterwards, Riedel and colleagues also obtained thermoregulatory responses from selectively warming and cooling an intra-abdominal thermode in rabbits.[13]

A convincing case has thus been made for the thermoregulatory importance of deep body structures outside the CNS, but the calculations are all based on evidence from large animals. Most modern thermoregulatory studies are done on rodents, for which there is no corresponding information. To our knowledge nobody has directly examined thermoregulatory reflex responses

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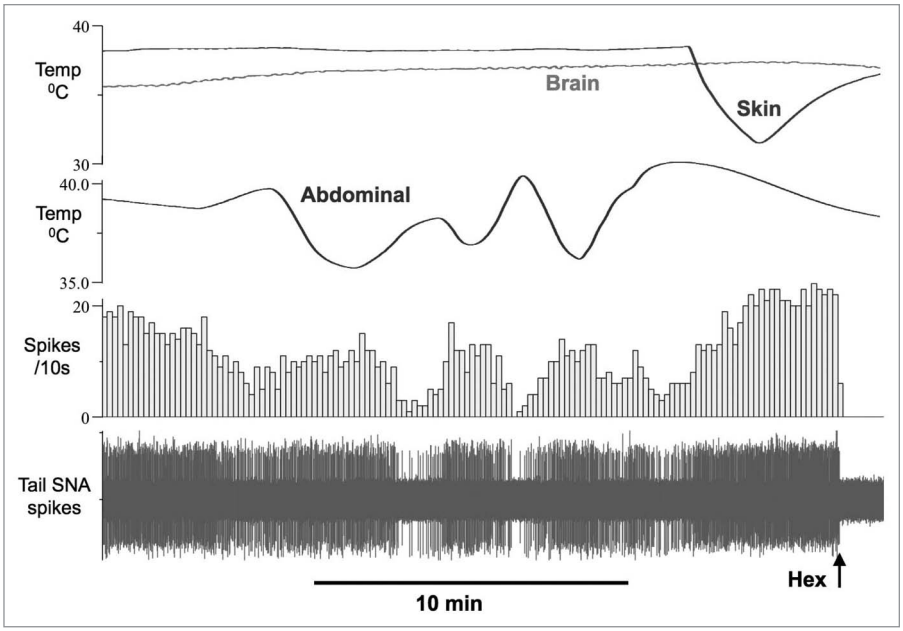

Figure 1. Chart record from one experiment showing brain, trunk skin and abdominal temperatures along with tail SNA as the raw spike record (bottom) and its 10 s spike count (above). The record spans several episodes of actively warming and cooling in the abdomen, which respectively inhibited and excited tail SNA. At the end of the record the trunk skin was cooled via the water jacket, and the resultant tail SNA was silenced by intravenous hexamethonium (Hex), proving that the activity was postganglionic sympathetic.

to extracranial deep body structures in the laboratory rat. We therefore set out to test whether manipulating intrabdominal temperature in rats would elicit thermoregulatory reflexes. Specifically we measured neural activity from the sympathetic vasomotor supply to the tail, its primary organ of heat exchange.[14]

Results

Confirming previous findings,[6,15,16] rat tail sympathetic nerve activity was strongly excited by cooling the skin (**Fig. 1**) and was inhibited by warming the skin (data not shown). Also in confirmation of previous findings,[6] changes in brain temperature were observed to have a strong influence over tail SNA. This may also be seen clearly in **Figure 1**.

Our present purpose, however, was to investigate the influence of experimental manipulation of intra-abdominal temperature on tail SNA. **Figure 1** shows examples of the effects of sequential warming and cooling of the intra-abdominal heat exchanger on tail SNA. The surface temperature of the heat exchanger was continuously monitored with a thermocouple, and temperatures were restricted to between approximately 35–41 °C. As may be seen from the chart record, warming the abdomen consistently inhibited tail SNA while cooling it increased tail SNA. This was demonstrated most clearly when brain and skin temperatures were held reasonably constant at levels that maintained a low tonic level of tail SNA firing. Clear examples of such responses were obtained from 4/4 rats.

Experimentally it proved very difficult to maintain skin and brain temperatures absolutely constant in the face of alterations in abdominal temperature. We therefore used multivariate analysis to extract the independent effects on tail SNA of abdominal temperature, independently of skin and brain temperatures. This analysis was performed on the sections of record where abdominal temperature was actively manipulated, while skin and brain temperatures were relatively constant. Also included were segments of data where the trunk skin was actively cooled, as at the end of the record shown in **Figure 1**.

In this analysis, the overall correlation between normalized tail SNA and the three temperatures under consideration (abdomen, brain and trunk skin) was highly significant ($f = 146$, $P < 0.0001$) and the adjusted R^2 value was 0.33. The independent correlations between of abdominal, skin and brain temperatures with tail SNA were all highly significant ($P < 0.001$), and their respective temperature coefficients were taken from their regression lines. The temperature coefficients for tail SNA responses were: - 0.73 ± 0.08 /°C for abdominal temperature, - 2.35 ± 014 /°C for brain temperature and - 1.5 ± 0.23 /°C for trunk skin temperature. These are shown plotted with their respective 95% confidence limits in **Figure 2**.

Discussion

Temperature signals from deep body structures have long been known to contribute to thermoregulatory reflexes, but what structures give rise to those signals is incompletely known.[8] Extracranial sensors evidently contribute significantly to this signal: it has been estimated that less than half is due to temperature sensors in the brain.[3,17] The contribution of abdominal temperature sensors to this signal is our present concern.

In their classic experiments on abdominal thermosensitivity in conscious sheep, Rawson and Quick used a heating pad that was attached to the dorsal abdominal wall and which also contacted the rumen and intestine. These workers showed that intra abdominal heating on either side depressed the animal's metabolic heat production in a cold environment and augmented its panting and evaporative heat loss in a warm environment.[11,12] Critically, they showed that the effects of unilateral warming were abolished by section of the splanchnic nerves on that side.[12] They concluded that intra abdominal heating activated warm-sensitive receptors in the abdominal wall and/or abdominal viscera, whose afferent fibers ran in the splanchnic nerves and whose activity influenced thermoregulatory responses in an appropriate manner.[11,12]

In the rabbit, Riedel and colleagues[13] used a small perfused heat exchanger that was stitched to the dorsal abdominal wall

between the vena cava and renal vessels. In most cases it did not contact the stomach. When this was selectively warmed in the conscious animal, it increased respiratory rate and vasodilated the skin.[13] Cooling the exchanger to 36 °C reduced respiratory rate slightly, but further cooling had no extra effect. These authors concluded that intra-abdominal warm receptors, located in or near the dorsal abdominal wall, were responsible for these thermoregulatory responses; the ineffectiveness of further cooling below 36 °C suggested that cold receptors were either absent or did not contribute to this response.[13] In a later paper, Riedel showed that this intra-abdominal thermal stimulus activated warm-sensitive afferent fibers in the splanchnic nerve; he found no cold-sensitive afferents there.[18] Similar warm-sensitive afferent fibers (slowly adapting) were later recorded from the rat splanchnic nerve in vitro.[19] The latter study also noted that most thermosensitive afferent activity appeared to originate from dorsal, paravertebral regions of the abdomen.[19] Neither of the latter studies reported finding cold-responsive splanchnic afferents, but cold-sensitive abdominal afferents were identified in the splanchnic nerve of the cat by Gupta and coworkers.[20]

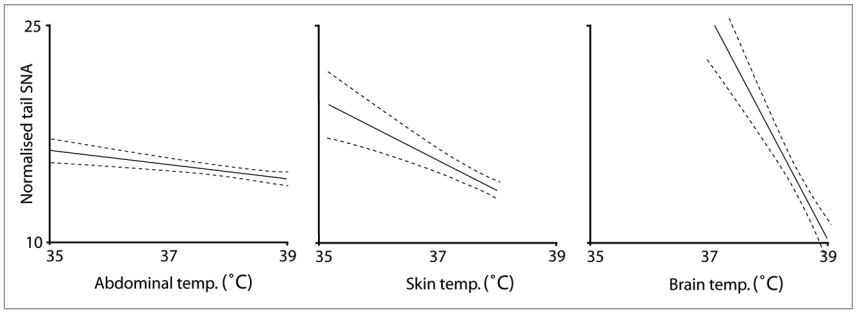

Figure 2. Normalized tail SNA responses to (left to right) abdominal, trunk skin and brain temperatures, calculated by multiple regression analysis of data from the 4 experiments. The respective regression lines are shown with their 95% confidence limits (dashed). All slopes were significantly different from zero (see text).

In the guinea pig, Romanovsky and Blatteis demonstrated that cutaneous dilatation followed intra-abdominal heating, but their experiments did not allow them to distinguish reflex responses to local heating from those due to whole body heating.[21] In the pig, Ingram and Legge[22] could produce neither any panting in response to selective abdominal warming nor any inhibition of panting by abdominal cooling. This was despite the existence of strong thermoregulatory reflexes from hypothalamic and spinal cord temperatures in that species.[22] It therefore seems likely that differences exist between species in their thermoregulatory responses to abdominal temperature. The exact positioning of the thermode may also be an important factor.[13]

A further consideration is that abdominal TRPV1 channels, presumably signaling via afferent neurons, tonically suppress autonomic cold defenses in rodents.[23] Those channels, however, are tonically stimulated under normal conditions not by temperature signals but by protons (low pH) and possibly endovanilloids.[24] This mechanism should not have had any involvement in responses to abdominal cooling: whether the TRPV1-bearing afferent fibers would have responded to the mild warming stimuli used here is unknown, though we think it unlikely.

To the best of our knowledge, no previous study has systematically examined thermoregulatory reflexes from the abdomen of the rat. We were able to show clear cutaneous vasoconstrictor and vasodilator responses to cooling and warming the abdomen within the physiological range of temperatures. Directly recording sympathetic neural activity allowed us to obtain rapid reflex responses to brief temperature changes, minimising heat exchange to the rest of the animal. In contrast to previous workers,[11-13] we placed the thermode not at the dorsal abdominal wall but against the intestinal serosa, with some contact to the ventral abdominal wall. Nevertheless, this stimulus produced robust thermoregulatory reflex responses to small temperature changes within the physiological range. We did not cool the abdomen below 35 °C so we cannot be sure whether the rat, unlike the rabbit,[13] shows responses attributable to intra-abdominal cold receptors.

Our experiments found that the effect on tail SNA of warming and cooling intra-abdominally was relatively weak—weaker than the effect of trunk skin (which was directly manipulated by means of a water-perfused jacket, see **Fig. 1**)—and weaker than the action of brain temperature. It is important to note two points, however. First, brain temperature was not independently manipulated in these experiments, so its signal would also reflect temperature changes experienced by other internal organs, including the spinal cord. The effect on tail SNA of selectively manipulating brain temperature is documented elsewhere.[6] Second, the skin contact area of the blanket was 3–4 times greater than the contact area of the abdominal heat exchanger. The difference between their thermoregulatory sensitivities could thus have been overestimated. It is also possible that stronger effects of abdominal temperature on tail SNA might have been found if the heat exchanger had been larger or if it had been placed more dorsally, where Riedel and colleagues found the most sensitive region in rabbits,[13,18] and Adelson and colleagues found the commonest source of thermosensitive splanchnic afferents in rats.[19]

The afferent pathway conveying abdominal thermosensitivity in rats is presently unknown, though the evidence noted above suggests that the splanchnic nerves are most likely responsible. The vagus, however, may contain warm-and cold-sensitive afferents supplying abdominal viscera. Thermosensitive afferents have been recorded in the vagus of the cat,[25] and neurons expressing thermosensitive ion channels have been shown to supply abdominal viscera in the mouse.[26] The influence of vagal afferents on thermoregulation is unproven, however.

In summary, we have shown directly for the first time that warming and cooling abdominal viscera within the physiological temperature range can drive thermoregulatory reflexes to the rat tail vasomotor nerve supply. The influence of abdominal temperature was independent from those of skin and brain

temperatures, but under the conditions of the experiment it was weaker than either. It seems inadequate to account for the large missing component of deep body temperature influencing thermoregulation, if indeed such a component exists in small animals like rodents.[17] Its physiological role needs to be determined.

Materials and Methods

Experiments on four adult male Sprague Dawley rats (340–70 g) are reported here. All experiments were performed in accordance with guidelines of the National Health and Medical Research Council of Australia and were approved by the Animal Experimentation Ethics Committee of the Florey Institute.

Surgical preparation

Animals were anesthetized initially with pentobarbital sodium (60 mg/kg, i.p.), and the hair over the trunk and neck was shaved. The trachea was cannulated, and then animals were artificially ventilated with 2% isoflurane (Forthane; Abbott Australia Pty Ltd.) in pure oxygen for the duration of the surgical preparation. Respiratory pressure and expired CO_2 concentration were continually monitored by a pressure transducer and CO_2 analyzer (ADC), respectively. Ventilation was adjusted to keep expired CO_2 between 3 and 4.5%. The right femoral artery and vein were cannulated to monitor blood pressure and administer drugs, respectively. A jacket made of Silastic tubing[6] placed around the animal's shaved trunk was perfused with water at predetermined temperatures to warm or cool the skin. The surface area in contact with the animal's trunk skin was ~120 cm^2.

The tail was placed in a bath whose edges were sealed with 4% agar. A window of skin was removed to expose the nerves running parallel to the lateral vein. Selected nerves were then dissected and placed over a black Perspex dissecting plate ready for splitting and recording.

On completion of surgery, the isoflurane anesthesia was discontinued and replaced with urethane (1.0 –1.2 g/kg, i.v.) over a period of 20–30 min. The effectiveness of anesthesia was assessed at intervals throughout the experiment by testing the corneal and withdrawal reflexes. Additional doses of urethane (25–50 mg, i.v.) were given if necessary to abolish those reflexes.

Tail sympathetic nerve fiber recording

Postganglionic sympathetic nerve activity (SNA) was recorded from tail sympathetic nerves as described previously.[16] Few- or multifiber spike activity was recorded differentially with respect to a nearby strand of connective tissue, amplified (10,000 fold), and filtered (15 – 600 Hz with a 50Hz notch). The activity was continuously monitored using an oscilloscope, and recorded using a CED Power 1401 interface and Spike2 software (Cambridge Electronic Design, Cambridge, UK) at a sampling rate of 5kHz. Spikes that passed across a selected threshold voltage were discriminated and counted in 5 or 10 s bins. Before starting the experimental protocol, tail SNA recordings were identified functionally by their excitatory response to cooling the skin for 30 – 60 s. At the end of experiment, hexamethonium chloride (50 mg/kg in saline; Sigma) was given intravenously to confirm that recorded activity was from sympathetic postganglionic fibers.

Temperature measurements

Cutaneous, colorectal, abdominal, and brain temperatures were continuously measured using copper-constantan thermocouples (Physitemp Instrument Inc. Clifton, NJ, USA). Brain temperature was measured by a thermocouple stereotactically placed through a 1mm diameter burr hole, 8 mm deep to the dorsal skull surface, lateral to the hypothalamus. Colorectal temperature was measured from a thermocouple inserted 5–7 cm into the rectum. Trunk skin temperature was measured as the mean of three thermocouples secured by sutures to the trunk skin at three points beneath the silastic jacket. Abdominal temperature was measured by small thermocouple fixed to the surface of the abdominal heat exchanger.

Abdominal temperature manipulation

The abdominal heat exchanger was made from six parallel 15 cm loops of 2 mm diameter silastic tubing, bonded in a single plane (exchange area 18 cm^2 on each side).The array and thermocouple were placed on the serosa of the gut through a small midline incision in the abdominal wall. The thermocouple wire and the two ends of the array tubes were exteriorised in the midline, and the incision firmly closed with 4.0 silk sutures. Care was taken to avoid contact between the tubes and skin. To warm or cool the abdomen, 50–200 ml of hot or cold water was injected rapidly through these tubes, using a series of 50 ml syringes. This altered abdominal temperature by ~3–5 °C.

Between periods of heating or cooling, colorectal temperature was maintained between 37 and 38 °C by adjusting water flow through the Silastic jacket. Flow through the jacket was maintained between 150 and 180 ml/min and the water temperature between 39 and 42 °C.

Data analysis

Five second tail SNA spike counts, together with corresponding brain, trunk skin and abdominal temperatures, were extracted from selected sections of the chart record in each experiment. A total of 75 min of data were analyzed, with approximately equal contributions from each of the 4 rats. The numbers of active units counted, few- or multiunit, differed between animals. To give equal weighting to data from each animal, the 5s spike counts were normalized to a maximum of 25 in each rat (the approximate average from 4 data sets). Data from the 4 rats were then combined in a multiple regression analysis (Microsoft Excel) to analyze the influence of abdominal, brain and trunk skin temperatures on tail SNA.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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