PREGANGLIONIC SYMPATHETIC ACTIVITY AND BARORECEPTOR RESPONSES DURING HYPOTHERMIA

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SUMMARY
In rabbits lightly anaesthetized with pentobarbitone and ventilated with 100% oxygen, hypothermia to 20–21°C was induced by bloodstream cooling, using an external coil primed with blood from another animal. Baroreceptor reflex activity was assessed from the reductions in multifibre preganglionic cervical sympathetic discharge and arterial pressure evoked by electrical stimulation of the aortic depressor nerve. Sympathetic activity increased down to levels of 26–28°C, with little change in arterial pressure or in the baroreceptor reflex. Below 25°C sympathetic discharge, heart rate, and arterial pressure fell progressively, and by 20–22°C the baroreceptor reflex was either abolished or greatly reduced; at these low temperatures many preganglionic neurones were still actively discharging.

Although its use in surgical procedures has diminished greatly in recent years, hypothermia retains its clinical importance as a deviation from normal physiology which is regarded as beneficial or harmful depending on circumstances. The dangers, and reasons for death, on exposure to low temperatures, have not been fully defined. In addition to these physiological aspects, it is usually considered that hypothermia reduces the requirement of general anaesthetic agents, and this has been demonstrated by measurement of MAC (Regan and Eger, 1967). Interactions of this kind may help to define the mechanism of action of anaesthetic agents.

The present study was intended to answer two questions: first, at what temperature does blockade of the neuronal pathways of the baroreceptor reflex occur?; second, what are the effects of hypothermia on directly recorded preganglionic sympathetic discharge?

METHODS
Complete experiments were carried out on six rabbits, weighing 3.0–3.5 kg, which were anaesthetized initially with sodium pentobarbitone injected slowly into an ear vein. The first dose was approximately 40 mg/kg, and was followed by 6–12 mg given through a femoral vein catheter at intervals of approximately 45 min, on the basis of previous experience (Biscoe and Millar, 1966). Gallamine triethiodide was injected intravenously in doses of 4 mg at similar intervals. Drugs were not administered at temperatures below 26°C.

Immediately after induction, the trachea was cannulated low in the neck and mechanical ventilation was started, using firstly air and subsequently 100% oxygen.

In addition to one femoral vein, both femoral arteries were cannulated; from one of these arterial pressure was displayed by means of a Statham P23A transducer and Devices amplifier and recorder (type M2). A “blood warming coil” (BR66, Baxter Laboratories Ltd) was filled with fresh blood (48 ml) which was withdrawn immediately prior to use from the carotid artery of another rabbit which had been lightly anaesthetized with intravenously-injected pentobarbitone (40 mg/kg), and given heparin 1000 units. Heparin was also given to the recipient animal, and the external circuit was then incorporated as a femoral arteriovenous shunt. Blood flow was maintained adequately through the coil by the (recipient) animal’s circulation, but was assisted in three experiments by a mechanical non-occlusive roller pump.

The animal’s temperature was measured by means of a thermometer inserted into the internal nares (in three experiments) or into the right external jugular vein. Hypothermia was gradually induced to approximately 20°C by immersion of the external coil in a water bath to which crushed ice was added.


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The left aortic (depressor) and preganglionic cervical sympathetic nerves were exposed by reflecting the larynx and pharynx in the mid-line. The aortic nerve was dissected free for stimulation through bipolar silver electrodes with constant-voltage wave shocks, using a Devices isolated stimulator (type 2533) and gated pulse generator (type 2521). Stimuli were applied for at least 20 sec, at a frequency of 100 impulses/sec, pulse width 100 /usec. The right aortic nerve was cut in two animals and in the other four experiments both sinus nerves were also divided. The left cervical sympathetic nerve was dissected free and used for recording. The nerves were covered with liquid paraffin, recordings being made from slips of sympathetic nerve, using bipolar platinum wire electrodes connected to a Tektronix type 122 preamplifier. The action potentials were displayed on an oscilloscope (Tektronix type 565) and could be photographed on 35 mm film. The signal from one vertical oscilloscope amplifier was fed to a pulse height selector; this converted the selected action potentials into standard pulses which were counted on a rate meter with variable time constant (Panax, RTM-4), whence a continuous record of the integrated discharge frequency was displayed on the Devices recorder.

To assess possible changes in the signal-to-noise ratio of the multifibre sympathetic preparation, the discharge was monitored audibly, and visually on the oscilloscope together with the output of the pulse height selector.

Pulmonary ventilation was adjusted, by changes in tidal volume and rate, to maintain a constant end-tidal CO₂ concentration of 3.5–4.0% throughout each experiment. In several experiments, arterial PO₂ was measured, and in an additional animal the effects of cooling and rewarming on the acid-base state were assessed specifically by frequent measurement of PaO₂, pH and base excess. The data were corrected for time and to temperature 37°C, according to the nomogram of Kelman and Nunn (1966).

### RESULTS

No technical difficulty was encountered during the cooling and warming procedure, nor was any transfusion reaction detected in any of the recipient animals. In two experiments, samples of blood were taken from the animal used for study (recipient) to assess possible haemolysis, but none was found. It appears, therefore, that under these experimental conditions blood can be safely transfused from one rabbit to another. Connection of the freshly filled external circuit to the animal's circulation caused only small and transient changes in arterial pressure.

#### Baroreceptor reflex responses.

Table I shows the percentage reduction in sympathetic discharge and mean arterial pressure induced by depressor nerve stimulation before the start of active cooling and at the temperature when the reflex was maximally reduced during hypothermia. The sympathetic response was totally inhibited in three of five experiments, at temperatures of 21.7–22.6°C (in one animal the sympathetic component was poorly reproduced). The pressure response was blocked at 21.4 and 22.6°C in two of the six animals. In the other experiments it was still possible to detect a weak baroreceptor reflex (sympathetic or pressure, or both) at temperatures as low as 20.0°C.

As temperature fell, the reduction in arterial pressure on baroreceptor nerve stimulation became progressively more delayed, while the time of onset of sympathetic inhibition was less affected.

Figure 1, from two experiments, shows typical recorded responses of sympathetic activity and arterial pressure to depressor nerve stimulation at

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<th>Percentage fall in arterial pressure</th>
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<tr>
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three temperatures. The lowest record, from one experiment, shows the baroreceptor reflex as previously described during light pentobarbitone anaesthesia (Biscoe and Millar, 1966); sympathetic activity falls quickly at the onset of stimulation, with a brief delay in the pressure response. The middle record, from another experiment, illustrates an active reflex at 27.6°C; here, sympathetic discharge had more than doubled from the level at 36°C. At a lower temperature of 20.6°C, as shown in the upper record, a greatly reduced baroreceptor reflex was still detectable with a delayed pressure response.

From the same experiment as that in the upper two records of figure 1, figure 2 shows a markedly inhibited baroreceptor reflex at 22.0°C nasal (upper record), and just after the start of rewarming at 23.0°C (lower record). Although the nasal temperature lagged behind blood temperature on rewarming, a small increase from this low level had a striking effect on the baroreceptor reflex (and also raised arterial pressure and sympathetic activity). It was confirmed also when recording temperature changes from the external jugular vein that the baroreceptor reflex reappeared promptly soon after the start of rewarming in all experiments.

Figure 3, from one experiment, shows the preganglionic discharge frequency (upper plot) during bloodstream cooling to 20.0°C (external jugular) followed by partial rewarming to 32.0°C, and also (lower plot) the levels during baroreceptor stimulation at various temperatures. The reflex was absent below a temperature of approximately 22.0°C.

A similar plot of arterial pressure, from another experiment, is shown in figure 4. The baroreceptor reflex response was greatly reduced, although still detectable, at 21.0°C (nasal).

From figures 2 and 3, it might be inferred that the magnitude of the reflex is enhanced over a certain temperature range. This conclusion cannot necessarily be drawn from the present studies, in view of possible interaction between hypothermia and variations in the background level of anaesthesia.
Fig. 3. Plot of preganglionic sympathetic activity (mean integrated discharge) during cooling from 35.0°C (external jugular temperature) to 20.0°C, followed by rewarming to 32.0°C. The lower plot (open circles) shows the levels during depressor nerve stimulation. The shaded area indicates the magnitude of the baroreceptor reflex.

Fig. 4. Plot of mean arterial pressure during cooling from 37.0°C (nasal) to 21.0°C, followed by rewarming to 34.0°C. The lower plot (open circles) shows the levels during depressor nerve stimulation. The shaded area indicates the magnitude of the depressor reflex.
Preganglionic sympathetic activity during hypothermia

Preganglionic sympathetic discharge.

A gradual increase in signal-to-noise ratio could have occurred when recording from multifibre nerve preparations over the relatively long time period of the present experiments, so that preganglionic sympathetic action potentials which were originally below the gating threshold may have become included subsequently in the total count. This would have tended to exaggerate a true increase, and minimize a decrease, occurring some time after the initial control measurements. However, from continuous audible and visual monitoring of the discharge, and from previous experience of these nerve preparations, it is considered unlikely that this would have changed the findings to be described, although a quantitative error in the absolute level of sympathetic activity may have been introduced subsequent to control measurements; this would not have influenced the accurate assessment of the baroreceptor reflex.

Preganglionic discharge gradually increased as temperature was lowered from the control levels of 35–38°C; in the six experiments, sympathetic activity reached a peak level within the temperature range 25.0–31.6°C, the maximum increase averaging 136%. A reduction in the discharge to less than the initial level did not occur except below 25.0°C. The maximum fall, by 34%, on average, was seen within the narrow temperature range 20.0–22.2°C, lower levels of temperature not being attained in these experiments.

When rewarming began, sympathetic activity showed rapid increases; in the three experiments in which temperature was recorded from the external jugular vein, a mean temperature rise from 20.2°C to 24.0°C was accompanied by a greater than 100% increase in the discharge frequency. The rise in nasal temperature on rewarming was slower than that in the external jugular vein, and since it lagged behind the physiological changes at that time, it was probably a poorer indicator of brain temperature.

Figure 5 shows filmed oscilloscope records from one experiment. Sympathetic discharge is still very active at 24.0°C (external jugular temperature, fig. 5b) compared to 33.5°C (fig. 5a), although heart rate is much slower. At 20.5°C (fig. 5c), however, the discharge rate is markedly reduced and there is pronounced bradycardia.

Arterial pressure and heart rate.

Mean arterial pressure, which averaged 111 mm Hg before active cooling was started, was little changed (119 mm Hg) down to the temperature range 28–30°C, and was slightly reduced, to 104 mm Hg, at 24–26°C. As with the other measurements, the most notable effect occurred below about 25°C. Thus, mean arterial pressure was reduced to an average of 57 mm Hg (five of the six measurements being in the range 17–66 mm Hg) at temperatures of 20–23°C. Rewarming caused a gradual rise in pressure.

Progressive slowing of heart rate, from the high control levels of about 400 beats/min (normal for the anaesthetized rabbit), was a characteristic feature of hypothermia. For example, when temperatures of 20.5°C had been reached in three animals, heart rates had fallen to 48, 55 and 64 beats/min. A progressive increase in heart rate accompanied rewarming.

Acid-base state.

In one experiment, in which no nerve recordings were made (and therefore with minimal surgical preparation), acid-base measurements were made regularly during bloodstream cooling down to 21.0°C, followed by rewarming to 35.4°C (table II). The PaO2 measurements, which were corrected to 37.0°C (Kelman and Nunn, 1966), suggested that maintenance of an almost constant end-tidal concentration was successful in regulating the arterial carbon dioxide tension in these experiments. Also, there was no indication that the technique of cooling or rewarming caused an important degree of meta-
Zimmer and Martin (1959) demonstrated a reduced pressor response in dogs in the range 25-28 °C. Similar findings were obtained in man (Blair et al., 1963). The lowest temperatures studied by these workers were 21°C, and raised static pressure in the carotid sinus. The temperature at which conduction was blocked in 15 cervical sympathetic fibres averaged 6.1°C, and Paintal (1965a, fig. 3) shows an apparently normal action potential recording from a pulmonary stretch fibre at 21°C. However, he also showed that at about 20°C, the conduction velocity in vagus and saphenous nerves was reduced by the order of 60% (Paintal, 1965b). The resulting temporal dispersion of incoming baroreceptor volleys could mean that the threshold for excitation of central synapses was not reached. Added to this is the likelihood of direct depressant effects of cooling on central synaptic transmission.

Our findings that sympathetic discharge increased to a peak within the temperature range 28–32°C, but always declined below approximately 25°C, are supported by information recently identified in the Italian literature (Passatore, Innocenti and Cardona, 1969). The latter workers studied intact cats, which showed similar changes to those described here; in decerebrate animals, however, preganglionic cervical discharge and arterial pressure fell away progressively as temperature was lowered from normal levels. The inference from their studies, therefore, is that the sympathetic responses are mainly mediated by regions above the mid-collicular region (probably hypothalamus).

It may be of clinical relevance that the peak increase of sympathetic discharge in these experiments occurred roughly within the temperature range when cardiac dysrhythmias, or ventricular fibrillation, are anticipated in man.

The relative ease with which rabbits could be cooled and would withstand the procedure was surprising in view of their often-acknowledged lack of experimental robustness. Although arterial pressure was low and bradycardia was pronounced, with occasional dropped beats when temperatures approached 20°C, no animal developed ventricular fibrillation.

### REFERENCES

Expressions anévrillies ont accusé une diminution progressive de décharges sympathiques, la fréquence cardiaque et la barorecepteur. Au-dessous d'une température de 25°C, les en ce qui concerne la pression artérielle ou le réflexe sympathique s'est accrue lorsque la température corporelle et intéressant la pression artérielle, engendrées par stimulation électrique du nerf dépresseur aortique. L'activité sympathique s'est abaissée jusqu'à 26 à 28°C, avec peu de changements à une température de 20 à 22°C, le réflexe barorécepteur était, soit absent, soit considérablement affaibli. A ces températures basses, de nombreux neurones pré-ganglionnaires présentaient encore une nette activité à type de décharges.

**ACTIVITÉ DES FIBRES SYMPATHIQUES PREGANGLIONNAIRES ET REPONSES DES BARORECEPTEURS AU COURS D’UNE HYPOTHERMIE**

Chez des lapins légèrement anestésiés au pentobarbital et soumis à une ventilation comportant 100% d’oxygène, une hypothermie de l’ordre de 20 à 21°C a été induite par refroidissement du flux sanguin en recourant à un rouleau externe alimenté par le sang provenant d’un autre animal. L’activité réflexe des barorécepteurs a été déterminée à partir des réductions affectant les décharges au niveau des multiples fibres pré-ganglionnaires du sympathique cervical et intéressant la pression artérielle, engendrées par stimulation électrique du nerf dépresseur aortique. L’activité sympathique s’est accrue lorsque la température corporelle s’est abaissée jusqu’à 26 à 28°C, avec peu de changements en ce qui concerne la pression artérielle ou le réflexe barorécepteur. Au-dessus d’une température de 25°C, les décharges sympathiques, la fréquence cardiaque et la pression artérielle ont accusé une diminution progressive et à une température de 20 à 22°C, le réflexe barorécepteur était, soit absent, soit considérablement affaibli. A ces températures basses, de nombreux neurones pré-ganglionnaires présentaient encore une nette activité à type de décharges.

**ZUSAMMENFASSUNG**


**RESUMEN**

En conejos ligeramente anestesiados con pentobarbitona y ventilados con 100% de oxígeno, fue inducida una hypotermia a 20–21°C por una corriente sanguínea refrigerada, usando un serpentín externo cebado con sangre de otro animal. La actividad del reflejo barorreceptor fue valorada partiendo de las reducciones de la descarga multifibrilar preganglionar del simpático cervical y la presión arterial producida por estimulación eléctrica del nervio depressor aórtico. La actividad simpática aumentaba por debajo de los límites de 26–28°C; la descarga simpática, frecuencia cardiaca y presión arterial descendían progresivamente, y a 20–22°C se abolía o reducía considerablemente el reflejo barorreceptor; a estas temperaturas bajas muchas neuronas preganglionares se descargaban todavía activamente.