Pflügers Arch. 342, 137–144 (1973)

Cold Sensitivity in the Spinal Cord of Sheep

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Received March 2, 1973

Summary. 1. Five clipped sheep with spinal thermodes, exposed to an air temperature of 20°C, had a resting oxygen consumption ($\dot{V}O_2$) of 5.9 ml/kg body weight · minute and a respiratory quotient (RQ) of 0.87. Cooling the spinal cord for 15 min from a mean temperature of 38.6°C down to 34.8°C caused $\dot{V}O_2$ to rise to 7.6 ml/kg · min ($P < 0.02$) and RQ to fall to 0.83 ($P < 0.001$). More intense cooling of the spinal cord to 32.6°C caused $\dot{V}O_2$ to increase to 9.6 ml/kg · min ($P < 0.01$) while RQ fell to 0.80 ($P < 0.01$). During spinal cooling the animals shivered. During the more intense spinal cooling, rectal temperature increased from 38.8°C to 39.2°C ($P < 0.02$).

2. Cooling the spinal cord to 32.6°C caused plasma free fatty acid (FFA) concentration to increase from a control value of 16.2 mg/100 ml to 22.7 mg/100 ml ($P < 0.01$). This increase in plasma FFA was completely abolished by prior administration of a sympathetic blocking drug.

3. These results show that the spinal cord of sheep is cold sensitive and that there is an increase in plasma FFA during spinal cooling, which is mediated via the sympathetic nervous system.

Key words: Temperature Regulation — Spinal Cord Temperature — Plasma FFA.

The hypothalamus has, for a long time, been known to be a sensor of central body temperature. More recently, the spinal cord (Simon, Rautenberg, Thauer, and Iriki, 1964) has also been shown to be temperature sensitive. In dogs, there is some evidence that the hypothalamus and spinal cord act as approximately equivalent sensors of temperature and both areas give thermoregulatory responses to warm and cool stimuli (Jessen and Mayer, 1971). In cattle the situation appears to be different. The hypothalamus is sensitive to both warming and cooling (Findlay and Ingram, 1961; Calvert, Clough, Findlay, and Thompson, 1972) and the spinal cord is sensitive to warming, but cold sensitivity appears to be almost completely absent (McLean, Hales, Jessen, and Calvert, 1970; Jessen, McLean, Calvert, and Findlay, 1972). This was the first ruminant animal to be studied. All other non-ruminant species investigated, and for which results are published, show spinal cold sensitivity. The effect of spinal cord cooling in sheep was therefore in-

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vestigated to see whether spinal cold sensitivity was present in this ruminant animal.

Hypothalamic and peripheral cooling of oxen both increase the plasma free fatty acid (FFA) concentration (Calvert *et al.*, 1972; Thompson and Clough, 1972) and peripheral cold in sheep also increases plasma FFA (Bost and Dorleac, 1965). The effect of spinal cooling on plasma FFA was therefore studied, and the mechanism controlling any change in FFA during spinal cooling was investigated.

Methods

Five Finnish Landrace × Dorset Horn sheep weighing between 38 and 54 kg were used. Their fleeces were shorn regularly so that the insulation of the animals did not vary. Spinal thermodes, consisting of U-shaped polyethylene tubes (outside diameter 1.6 mm; inside diameter 1.1 mm), were implanted under local anaesthesia as described by Jessen, Meurer and Simon (1967). In 3 animals, 3 U-shaped tubes were implanted, and in the other 2 animals, 2 U-shaped tubes were implanted. The thermodes were located in the peridural space and extended from the lumbar to the cervical spinal cord. A blind-ending polyethelyne tube was also inserted into the peridural space for measuring the spinal temperature. Since the relationship between the thermocouple, the thermodes, and the spinal detectors of temperature is not known this temperature provides only a vague indication of the temperature of the actual senser. The animals were allowed at least 2 weeks to recover from the operation.

The experiments were performed at a temperature of 20° C in a climatic chamber to which the animals were previously accustomed. In the morning of an experiment food was not given. Each experiment consisted of a control period of at least 1 hour during which a manifold, close to the thermodes, was perfused. The thermodes were then perfused for 15 min, with water from a thermostatically controlled bath ($\pm$ 0.1°C) at a rate of 70 $\pm$ 10 ml/min for each tube, and then this was followed by another control period. The rate of perfusion through the thermodes was measured with a rotameter. The temperature of water entering and leaving the thermodes was also measured. The minimum water temperature used for perfusion was 25° C and this cooled the spine to 30.1° C. A minimum of 30 min was allowed between periods of spinal cooling. Temperatures were measured with copper-constantan thermocouples which were accurate to $\pm$0.1° C. The animals' oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were measured continuously using an open circuit with a ventilated head cage. A rotameter was used to measure the rate at which air passed through the cage, and a para-magnetic oxygen analyser (Servomex type OA 137) and an infra-red carbon dioxide analyser (Hilger and Watts model RM) were used to measure the difference in oxygen and carbon dioxide concentration between cage inlet and outlet air. The Brouwer equation (Brouwer, 1965) was used to calculate heat production from $\dot{V}O_2$ and $\dot{V}CO_2$.

To study the effect of spinal cooling on plasma FFA concentration, the experiments were repeated and $\dot{V}O_2$ was not measured. A polyethylene cannula filled with 3.8% trisodium citrate was placed aseptically in the external jugular vein a least 1 hour before the experiment began. Three blood samples were taken at 10 min intervals before and after 15 min of spinal cooling. During the cooling period blood samples were taken at 5 min intervals. The total and individual plasma FFA's were measured using gas-liquid chromatography as described previously (Thompson and Clough, 1970). This experiment was repeated following sympathetic nerve

blockade obtained by injecting bethanidine (BW 467 C60) (Boura, Copp, Green, Hodson, Ruffell, Sim, Walton and Grivsky, 1961) into the jugular vein. The dose used was 0.4 mg/kg body wt.

The paired t-test was used to compare the results obtained during different experimental conditions.

Results

The Effect of Spinal Cooling on Oxygen Consumption and Respiratory Quotient

The experiment shown in Fig. 1 demonstrates the response obtained from two different intensities of spinal cord cooling in the most sensitive animal. The resting $\dot{V}O_2$ during this experiment was 5.2 ml/kg · min and the respiratory quotient (RQ) was 0.88. Cooling the spinal cord from 38.7°C to 35.3°C caused the $\dot{V}O_2$ to increase to 7.4 ml/kg · min while RQ fell to 0.80. Cooling the spinal cord to 33.5°C caused $\dot{V}O_2$ to rise to 9.2 ml/kg · min while RQ fell to 0.76. As shown in Fig. 1 rectal temperature increased during the spinal cooling. By measuring the inlet and outlet temperature of the water and the flow through the thermodes, the total heat lost by the animal to the water

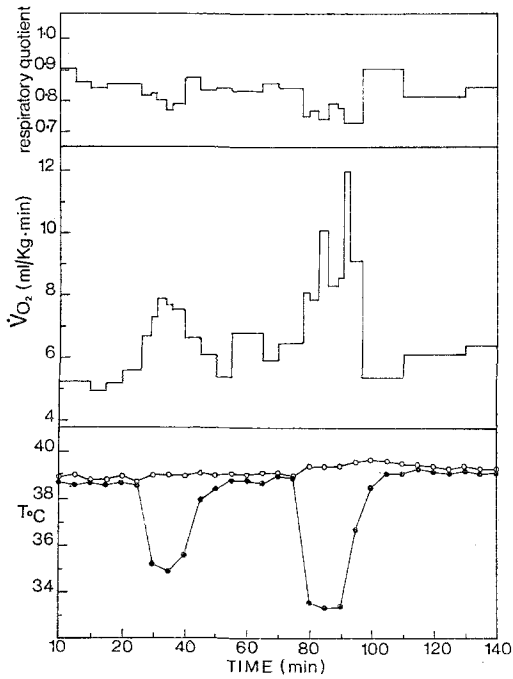

Fig. 1. The effect of spinal cooling on spinal temperature (•——•), rectal temperature (o——o), oxygen consumption and respiratory quotient of a sheep

Table 1. The effect of two intensities of spinal cooling on the oxygen consumption, respiratory quotient and rectal temperature of 5 sheep (Mean ± S.E.M.) (One experiment on each animal)

Treatment	Spinal cord temperature (°C)	Oxygen consumption (ml/kg · min)	RQ	Change in rectal temperature (°C)
Control	38.6 ± 0.03	5.9 ± 0.24	0.87 ± 0.019	—
Spinal cooling 1	34.8 ± 0.42	7.6 ± 0.64	0.83 ± 0.016	+0.2 ± 0.07
Spinal cooling 2	32.6 ± 0.50	9.6 ± 0.73	0.80 ± 0.017	+0.4 ± 0.08

may be calculated. This may then be compared with the total extra heat produced by the animal. In the example shown in Fig. 1 this ratio of heat produced to heat lost was approximately 5:1. Averaged over a total of 40 cooling periods in 5 animals, the ratio of heat produced to heat lost was 3.3:1.

Table 1 shows the effect of two levels of spinal cooling on $\dot{V}O_2$, RQ and rectal temperature of the 5 sheep. Cooling the spinal cord from a mean of 38.6°C down to 34.8°C, significantly increased $\dot{V}O_2$ by 1.7 ± 0.41 (Mean ± S.E.M.) ml/kg · min ($P < 0.02$) from a control value of 5.9 ml/kg · min. Cooling the spine to 32.6°C increased $\dot{V}O_2$ by 3.7 ± 0.59 ml/kg · min ($P < 0.01$). The difference in $\dot{V}O_2$ between the two cooling periods is also significant ($P < 0.01$). During both cooling periods the animals were observed to be shivering. The resting RQ of 0.87 was significantly reduced during both the less intense ($P < 0.001$) and the more intense ($P < 0.01$) cooling periods. The resting rectal temperature was 38.8°C and this increased by 0.4 ± 0.08°C ($P < 0.02$) during the more intense cooling period.

The Effect of Spinal Cooling on Plasma Free Fatty Acids

Fig. 2 shows the effect of spinal cooling on the FFA of 5 sheep. The total FFA was mostly made up of palmitic, stearic and oleic acids, with small amounts of palmitoleic, linoleic and linolenic acids. The total FFA level during the control periods before and after spinal cooling was an average of 16.2 ± 1.10 mg/100 ml plasma. During the last 5 min of spinal cooling this had increased significantly by 6.5 ± 0.93 ($P < 0.01$) to 22.7 mg/100 ml. This increase resulted from increases in all of the major FFA but, as can be seen from Fig. 2, the oleic acid increased more than the other major fatty acids, and its percentage of the total FFA increased from 35.7% to 39.5% ($P < 0.01$). The spinal cord temperature during these experiments was 32.6°C which is the same as the more intense cooling period shown in Table 1. In control

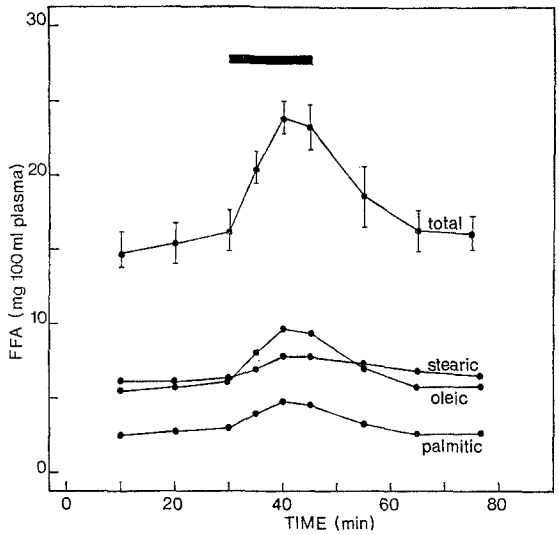

Fig. 2. The effect of spinal cooling (temperature of spinal cord 32.6 ± 0.44°C) on the plasma concentration of free fatty acids in 5 sheep (Mean ± S.E.M.) (One experiment on each animal). The period of spinal cooling is represented by a black bar

experiments the spinal thermodes of 2 sheep were perfused with water at body temperature and this had no detectable effect on the plasma FFA.

The Effect of Bethanidine on the Increase in Plasma Free Fatty Acids during Spinal Cooling

30 min before the spinal cooling, bethanidine (0.4 mg/kg) was administered as a single intravenous injection. This caused the control level of total FFA to fall and for the 10 min preceding and the 30 min following spinal cooling, the average concentration of the total FFA's was 9.3 mg/100 ml plasma. During spinal cooling there was a slight but insignificant increase in plasma FFA level which averaged 0.4 ± 0.76 mg/100 ml ($P > 0.1$).

Discussion

The experiments described above show that the spinal cord of sheep is sensitive to cold. These experiments do not show any quantitive relationship between the level of cooling and the increase in $\dot{V}O_2$, but they do show that at more severe levels of cooling the increase in $\dot{V}O_2$ is larger. Cooling the spinal cord to 32.6°C caused $\dot{V}O_2$ to increase by 63%. Jessen and Mayer (1971) found an increase of 100% in the

heat production of dogs with a similar spinal temperature. The sheep is thus less sensitive than the dog to spinal cooling, however the increase in rectal temperature during spinal cooling and the ratio of 3.3:1 for heat produced against heat lost during the cooling both confirm that there is a metabolic response to cooling the spinal cord of sheep. The small response to spinal cooling in oxen has been attributed to their large body size (Jessen *et al.*, 1972), but the steers used by McLean *et al.* (1970) were young and had a similar body weight to the sheep used in this experiment, suggesting that variation in body size does not by itself explain these variations in spinal cold sensitivity. The reason why spinal cooling of oxen did not produce an increase in rectal temperature is not clear, but observations made during pilot experiments with sheep may be important in this respect. It was found that fully fleeced sheep were much less sensitive to spinal cooling than were shorn sheep. The warm skin of a heavily fleeced animal could inhibit the cold signals generated in the spinal cord. The coat of oxen, although not as thick as that of a sheep, provides excellent insulation and it may be that if their coats were clipped, spinal cooling would be more effective.

The fall in RQ during spinal cooling and the increase in plasma FFA are also important. The increased $\dot{V}O_2$ during cold exposure of sheep is accompanied by a fall in RQ (Graham, Wainman, Blaxter, and Armstrong, 1959) and an increase in plasma FFA (Bost and Dorleac, 1965). From their RQ measurements Graham *et al.* (1959) calculated that the extra heat produced during cold exposure of sheep is due entirely to oxidation of fat. The finding that spinal cooling also lowers RQ and increases plasma FFA thus shows that in this respect cold exposure and spinal cooling represent equivalent stimuli to the animal.

The increase in the percentage of oleic acid in the plasma, during spinal cooling, suggests that the increase in plasma FFA represents an increase in mobilisation and not a decrease in utilisation. The adipose tissue triglycerides of sheep are rich in oleic acid (Duncan and Garton, 1967) and hormonal stimulation of rat adipose tissue *in vitro* has been found to cause a preferential release of unsaturated acids (Hollenberg and Angel, 1963). For either of these reasons plasma oleic acid concentration would increase more than palmitic or stearic acid concentration during fat mobilisation. An intravenous infusion of noradrenaline increases plasma FFA and also the percentage of oleic acid in the plasma of sheep (Noble, Thompson, and Moore, 1969).

The increase in plasma FFA during cold exposure of sheep is probably mediated via the sympathetic nervous system because their urinary excretion of noradrenaline and adrenaline increases during cold exposure (Webster, Heitman, Hays, and Olynyk, 1969), and the increase in FFA in the cold is not prevented by denervating the

adrenal medullae (Bost and Dorleac, 1965). The increase in plasma FFA during cold exposure of oxen can be completely abolished by sympathetic blockade (Thompson and Clough, 1972) and the present results show that the increase in plasma FFA during spinal cooling is also mediated via the sympathetic nervous system since it too can be abolished by sympathetic neurone blockade. Changes in sympathetic activity have been previously shown to occur during both spinal cooling and heating (Walther, Iriki, and Simon, 1970).

These experiments thus show that the spinal cord of sheep is sensitive to cold, and that the increase in plasma FFA during spinal cooling is mediated via the sympathetic nervous system.

The authors would like to thank the Wellcome Foundation Limited for supplying bethanidine sulphate.

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