Shivering in Intact and Spinal Rabbits During Spinal Cord Cooling

The initiation of shivering in anaesthetized[1] and unanaesthetized[2-4] animals by selective cooling of the spinal cord has proved the existence of central thermosensitive structures in the extracerebral section of the central nervous system[5]. In further experiments, indications have been found that effector mechanisms of cold shivering also exist at the spinal segmental level[6]. The present investigation was undertaken to compare the properties of the spinal shivering mechanism with those of the intact central nervous system. For this purpose the motor reactions on spinal cord cooling were studied in anaesthetized intact and spinal rabbits.

Method. 30 rabbits (2.3–3.5 kg) were anaesthetized by i.v. injection of Pernocton (70 mg/kg). In 15 animals the spinal cord was exposed by removing the arc of the 2nd cervical vertebra, and the spinal cord was transected at this level. The spinal animals were maintained by artificial respiration. In all animals a U-shaped thermode of polyethylene tubing was inserted into the peridural space extending from the 6th or 7th lumbar vertebra to the lower cervical region. Cooling of the spinal cord was performed, in the state of diminishing anaesthesia, in 2 min periods by perfusing the thermode with water of 20–25 °C. The experiments were carried out at constant ambient air temperatures of 32–33 °C to avoid shivering by external cold stimuli. Electromyograms were taken preferably from the lower thoracic and the lumbar sections of the dorsal trunk muscles. Pairs of needle electrodes were used as mass electrodes to determine the onset and the end of muscular activity during spinal cord cooling. Additionally, electromyograms were picked up by concentric needle electrodes to investigate the tremor patterns. The potentials were amplified by a condenser-coupled multistage amplifier, and were recorded by UV-direct-writing galvanometers. The temperatures of the vertebral canal and of the rectum were measured by thermocouples.

Results. In Figure 1 the electromyographical response to spinal cord cooling of a 3.0 kg normal rabbit (NR) and a 2.3 kg spinal rabbit (SR) is demonstrated. After the start of cooling, motor activity appeared within 10 sec in the normal and 15 sec in the spinal rabbit, when the temperature of the vertebral canal had fallen to 37.0 °C (NR) and 36.0 °C (SR) from the initial values of 39.2 °C (NR) and 38.6 °C (SR). In the intact animal the electromotor activity was accompanied by visible shivering, while slight palpable shivering was detected in the spinal animal. After the end of cooling, shivering diminished within 20 sec in the normal and 19 sec in the spinal rabbit at vertebral canal temperatures of 35.7 °C (NR) and 35.5 °C (SR). The rectal temperatures of both animals remained constant during the cooling periods at 39.6 °C (NR) and 39.1 °C (SR).

The experimental results obtained from 15 normal and 15 spinal rabbits are summarized in the Table. During spinal cord cooling visible, together with palpable, shivering appeared in 11 normal but only in 3 spinal animals. Additionally, palpable but not visible shivering was detected in 3 normal and 5 spinal rabbits. Thus, definite shivering tremor elicited by spinal cord cooling was observed in 14 normal and in 8 spinal animals. In these cases the electromyogram showed the discharges of multiple motor units. In 4 more spinal rabbits, showing neither visible nor palpable shivering during spinal cord

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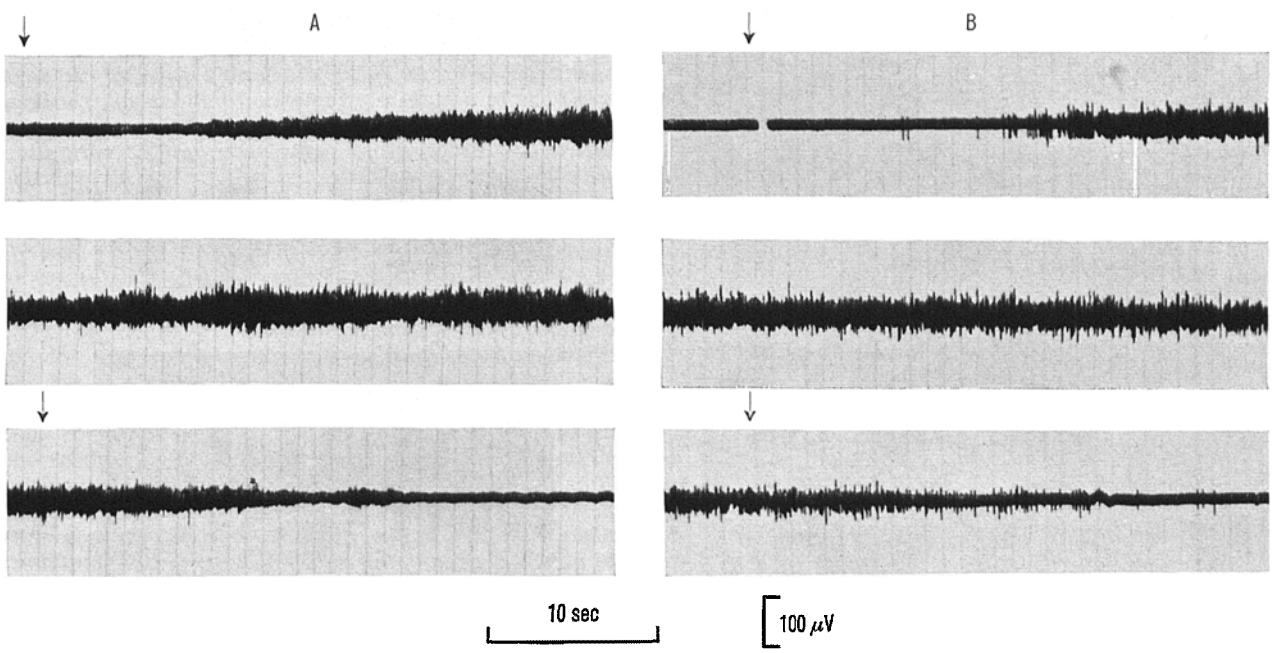

Fig. 1. Electromotor activity during spinal cord cooling in anaesthetized normal (A) and spinal (B) rabbits. Upper records: start of cooling (indicated by arrows) and start of motor activity. Middle records: marked activity during cooling. Lower records: end of cooling (indicated by arrows) and end of motor activity.

cooling, an increase of motor activity was observed in the electromyogram; it consisted, however, of the discharge of only one single motor unit. No motor activity was found in 1 normal and in 3 spinal rabbits.

The mean threshold values of the vertebral canal temperature at the start and at the end of electromotor activity are given in the lower column of the Table. In spinal rabbits motor activity appeared, on the average, at lower vertebral canal temperatures than in intact animals. The threshold difference could, however, not be proved by statistic analysis. This is explained by the fact that in spinal animals the threshold temperatures were distributed over a wide range between 30.4 °C and 37.7 °C. Especially, low thresholds ranging between 30.4 °C and 35.3 °C were found in the animals in which only an increase in electromotor activity had been observed. If only those spinal animals were regarded which showed visible or palpable shivering (8 cases, the data are given in the Table in parenthesis) the mean threshold temperature for the start of motor activity was found to be identical with that of normal animals. The mean threshold temperature for the end of motor activity after spinal cord cooling was, however, significantly lower in spinal animals, even if the animals without definite shivering were excluded.

The electromyograms recorded in 14 normal and 12 spinal rabbits were compared with respect to the tremor pattern. As it is demonstrated in Figure 2, periodic changes of tremor intensity – shivering bursts – could be observed in spinal rabbits as well as in normal animals. Only 2 out of 14 normal rabbits did not show this tremor pattern. In the spinal animals, however, this pattern was observed only in 3 cases. In 2 of them the pattern was apparently induced by the artificial respiration (Figure 2C), while in 1 case (Figure 2B) the shivering bursts seemed to be independent of the artificial respiratory rhythm. In the remaining cases of the spinal animals, a continuously increased motor activity was observed during spinal cord cooling (Figure 1B).

Discussion. The experimental results have confirmed the previous observations[6] that a cold-induced tremor may originate in the isolated spinal cord. The ability for cold shivering is, however, reduced in the spinal rabbits. The rate of success in evoking definite shivering by spinal cord cooling was smaller in spinal animals. Additionally, the threshold temperature of the beginning and the end of motor activity was, in several cases, considerably lower. This impairment may partly be due to the surgical interference, which is inevitable for spinal transection. Those spinal rabbits showing visible or palpable shivering had, with regard to the onset of tremor, identical thresholds with the normal rabbits, in which shivering had been observed regularly. The fact, however, that, even in the definitely shivering spinal rabbits, a lower threshold temperature for the end of motor activity was found, demonstrates the injury to the tremor-producing system caused by spinal transection. The typical electromyographic pattern of shivering which was found in most normal rabbits, was observed only in 3 out of 12 spinal animals. This also indicates that the mechanism of shivering is altered by the exclusion of supraspinal influences.

Apart from the differences in the intensity and the pattern of the cold-induced muscular tremor between intact and spinal rabbits, the results give clear evidence that essential properties of a basic cold defence mecha-

Responses of motor activity to spinal cord cooling in anaesthetized normal and spinal rabbits, and temperatures corresponding with the start of cooling and with the onset and the end of motor activity. Temperatures in parenthesis: spinal rabbits with visible and/or palpable shivering

Responses to spinal cord cooling	Normal rabbits $n = 15$	Spinal rabbits $n = 15$
Non-response	1	3
Positive response:		
Visible and palpable shivering	11	3
Palpable shivering	3	5
Only electromotor activity	0	4
Temperatures in animals with positive response (mean values ± S.D.)		
	Normal rabbits $n = 14$ °C	Spinal rabbits $n = 12$ ($n = 8$) °C
Rectal temperature	38.7 ± 0.68	37.2 ± 1.33 (37.7 ± 0.72)
Vertebral canal temperatures:		
Start of cooling	38.5 ± 0.75	37.3 ± 1.18 (37.8 ± 0.83)
Start of motor activity	36.0 ± 0.85	35.2 ± 1.90 (36.2 ± 0.70)
End of motor activity	36.8 ± 1.18	35.3 ± 1.12 (35.7 ± 0.96)

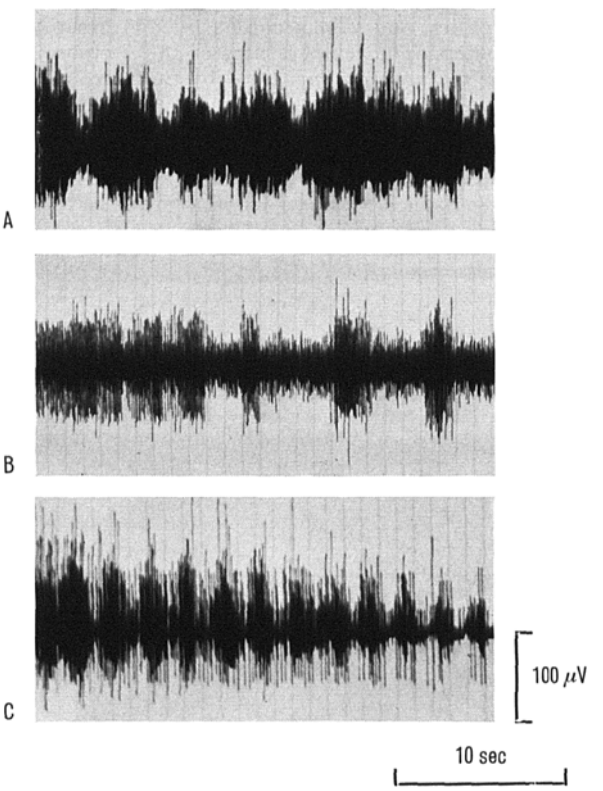

Fig. 2. Phasic pattern of electromotor activity during spinal cord cooling in anaesthetized normal (A) and spinal (B and C) rabbits. The pattern in C corresponds to artificial respiration.

nism do exist at the spinal level of the central nervous system. This corresponds with earlier findings[7] that a restoration of thermoregulatory mechanisms may take place, to some extent, in chronic spinal animals.

Zusammenfassung. Bei spinalen wie auch bei intakten, leicht narkotisierten Kaninchen konnte durch Kühlung des Rückenmarkes Muskelzittern ausgelöst werden. Im Vergleich zu den intakten Tieren war der Effekt der Rückenmark-Kühlung bei spinalen Tieren schwächer, und die Temperatur zu Beginn und bei Beendigung der elektromyographisch erfassten Aktivität war niedriger, bzw. streute in einem weiteren Temperaturbereich. Die während des Kältezitterns intakter Tiere häufig zu beobachtenden phasischen Aktivitätsschwankungen im Elektromyogramm waren bei spinalen Tieren nur selten nachweisbar.

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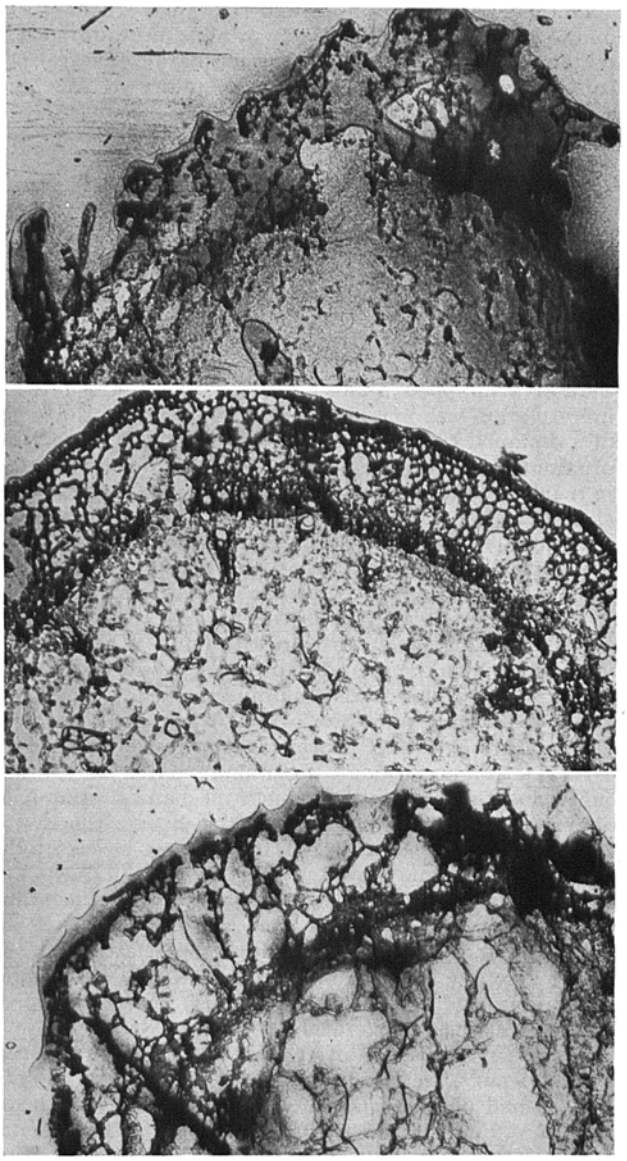
Autoradiographic Evidence of Tritiated Indolyl-3-Acetic Acid in Epicotyl Tissue of *Phaseolus coccineus*

One of the difficulties involved in obtaining autoradiographic evidence of the tissue in which indolyl-3-acetic acid (IAA) is translocated in a polar manner is the fact that the polar transport mechanism is saturated at very low concentrations of IAA[1]. Therefore, radioactively-labelled IAA with a high specific activity is required in order to obtain sections with radioactivity above background. Moreover, because IAA is soluble in water and alcohols, the usual procedures of fixing, embedding and autoradiography cannot be used.

Tritium-labelled IAA (generally labelled), with a specific activity of 112 mc/m mole was used in these experiments in an attempt to show the tissues involved in translocating this compound. Segments of epicotyl 6 mm in length were cut from 1 cm below the hook of 7-day-old dark-grown seedlings of *Phaseolus coccineus* (Scarlet Runner beans). All manipulations of seedlings were carried out using a safelight having peak transmittance at 525 nm and cut offs at 500 and 560 nm. Donor concentrations of $10^{-4}M$ IAA were applied in cylindrical agar blocks using a series of treatments. The treatments included normal and horizontal orientation of the tissue segments and different durations of diffusion.

In order to prevent leaching and to localize the radioactivity incorporated during the treatments, it was necessary to freeze the tissue and to section it without it coming in contact with any solvent, and without it being thawed. Before freezing, the tissue was marked with ink at positions for which sections were desired, ca. $^1/_4$, $^1/_2$ and $^3/_4$ of the way along the segments.

The segments were flash-frozen in isopentane cooled in dry ice. The frozen segments were transferred, packed in dry ice, to a cryostat where they were maintained overnight at $-20\,°C$. They were then sectioned with a microtome contained in the cryostat. Using a camel's hair brush, the sections (ca. 14μ thick) were transferred from the microtome knife to slides previously treated with Haupts solution. The slides were transferred to pre-cooled flasks connected to the freeze drying apparatus. The flasks containing the open slide boxes were immersed in an ice and salt bath below $-10\,°C$ to maintain the tissue



Tissue radioautographs of sections of *Phaseolus coccineus* epicotyl segments after treatment with IAA-H^3 from donor agar blocks (X 45). Top: tissue oriented normally, diffusion time 3 h. Middle: same as above, but the sections were fixed instead of being developed. Bottom: tissue laid horizontally, diffusion time 3 h.

[1] M. H. M. Goldsmith and K. V. Thimann, Pl. Physiol., *37*, 492 (1962).