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Vascular reactions during electrical stimulation

Vital microscopy of the hamster cheek pouch and the rabbit tibia

Vital microscopy during electrical stimulation was performed in the hamster cheek pouch and the rabbit tibia. Stimulation with DC of 5,20 or 50 μA or AC of 20 μA was demonstrated to cause macromolecular capillary leakage as evidenced by FITC-dextran fluorescence and histological demonstration of extravasated white blood cells. It was further demonstrated that the vascular leakage was blocked by administration of indomethacin. Pulsed electromagnetical fields did not visibly affect the vascular permeability within 3 h. As the same DC electrical stimulator has been demonstrated to increase the osteogenic capacity in similar titanium implants in the rabbit tibia, it is suggested that the observation of macromolecular leakage may predict a positive osteogenic response.

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Electrical and electromagnetical stimulation have been demonstrated to reinforce bone growth (Heckman et al. 1981, Zichner 1981), nerve repair (Ito & Bassett 1983) and skin healing (Kornikoff 1976, Bigelow et al. 1979). In spite of the frequently demonstrated positive effects of electrical stimuli on the healing response, no general consensus has been established as to the preferred type and quality of electrical stimulation in various experimental or clinical situations. For instance, beneficial effects on bone repair have been reported with the use of continuous direct currents (Bora et al. 1981), pulsing direct currents (Jörgensen 1977, Zichner 1981), alternating currents (Kraus & Lechner 1972) or pulsed electro-magnetical fields (Bassett 1982). Furthermore, invasive techniques may use different material and area of the electrodes and different current magnitudes, to mention only a few of the parameters which determine the quality of the electrical signal. Becker (1979) concluded that the presently used techniques in electrical stimulation differ so greatly among themselves that a common mechanism of action seems highly unlikely.

We have studied the influence of direct current on the vascular permeability in the hamster cheek pouch and rabbit metaphysary bone. In addition, a brief survey of the influence of alternating current and pulsed electromagnetical fields in the same animal models is presented.

Materials and methods

Animal groups

Twenty-one golden hamsters of both sexes, each weighing between 90 and 135 g, were used in the experiments. They were initially anaesthetized with 6 mg/100 g body weight pentobarbital and 2.5 mg/100 g body weight diazepam intraperitoneally, and additional pentobarbital was given in the cannulated femoral vein. The animal's temperature during the experiment was checked with a rectal thermistor. The animal was placed on a plexiglass plate, and the cheek pouch was everted and pinned out over a translucent plate. To get a 50–75 μm thin preparation, the upper epithelial layer was removed with microsurgical technique.

During the experiment the cheek pouch was superfused with a temperature-controlled (+36°C), modified Ringer solution, bubbled with N_2-CO_2 gas mixture (95% N_2 and 5% CO_2) (Romanus 1977).

The electrodes were placed in contact with but did not penetrate the cheek pouch. The distance between the electrodes was 22 mm (Figure 1). In the cases with electromagnetic stimulation, no electrodes were used, but instead coils were mounted on each side of the cheek pouch.

The 21 hamsters were divided into seven groups, each consisting of three animals. The stimulation levels were 5 μA DC, 20 μA DC, 50 μA DC, 20 μA AC, 5 μA DC followed by indomethacin 2 mg per 100 g

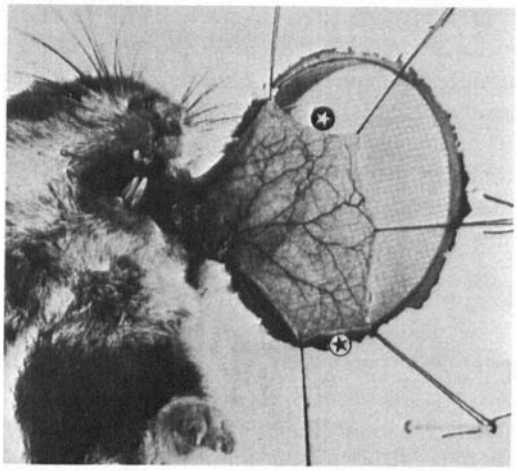

Figure 1. Hamster cheek pouch taken out for vital microscopy. The positioning of the electrodes is indicated by the two stars.

bw, pulsed electromagnetic fields of 15, 36 or 72 Hz and non-stimulated controls, the latter with the electrodes in contact with the pouch but the current switched off.

Six adult (closed physes as evidenced by X-rays) Belgian hares, weighing between 4 and 6 kg, were used in the experiment. They were anaesthetized with 0.7 ml/kg bw Hypnorm, administered intramuscularly. Vital microscopy was made possible by an optical chamber (Albrektsson & Albrektsson 1978, Albrektsson 1980), (Figure 2).

The six rabbits were divided into two groups of three animals. One group was stimulated with a DC of 5 μA while the other group served as a control with the electrodes implanted but the current switched off.

Vital microscopy

Microscopic observations of hamsters were made in a Leitz Biomed intravital microscope, equipped with a Na 0.60 condensor, a green filter with maximum transmission at 5500 Å, ×4, ×10 long working distance objectives and ×10 oculars. A single reflex mirror camera and a television camera were connected to the vital microscope. For fluorescent light observations, the Leitz Ploem system was used while transillumination was utilized for ordinary light microscopy. During fluorescence microscopy the animals had been given FITC-dextran with a molecular weight of 150000 (hamsters) or 70000 (rabbits) (Svensjö et al. 1978)

In rabbit experiments intravital microscopic observations were performed in a Leitz intravital microscope with objectives ×2, ×5, ×6 and ×11 and oculars of ×8 to ×15 magnification. For the fluorescent light observations, the Leitz Ploem system was used while transillumination was utilized for studies in ordinary light. A single reflex mirror, a Vinten scientific and a Kotron television camera were connected to the microscope.

Histology

After each hamster experiment, the cheek pouch was carefully fixed to a plate and then quickly cut off. The pouch, still mounted on the plate, was then put in a formalaldehyde solution. This procedure was completed within 3 min. The pouch was then stained with Htx-eosin. The cheek pouch was examined with regard to extravasated white blood cells and oedema.

Electric stimulator

Stimulators designed to generate constant current within a range of 0–100 μA were used in the experiments. The current was set at a temperature of 38°C to compensate for a current change due to a higher temperature after implantation.

The current levels tested in the study were 5, 20 and 50 μA. Test measurements *in vivo* using titanium as a cathode and platinum-iridium as an anode showed that the supply voltage must be of at least 3.3 V to assure a proper function of the stimulator

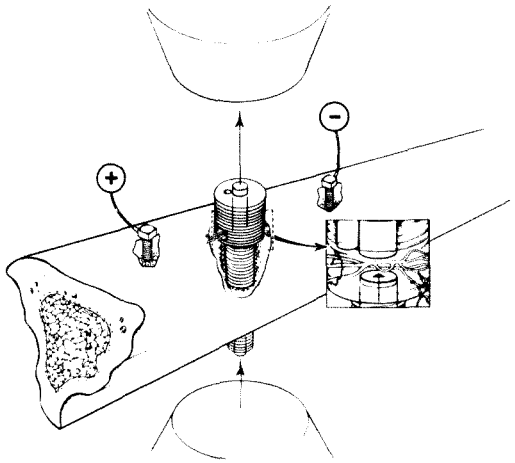

Figure 2. The vital microscopic bone chamber is inserted through the tibial bone of the rabbit. Bone and vessels will grow through the chamber during an incorporation time of the implant of about 6 weeks. It is then possible to transilluminate the ingrown tissue through an optical system inside the chamber. Electrical stimulation is not started before chamber integration has occurred, i.e. minimally 6 weeks after bone surgery.

for currents up to 50 μA with the present electrode areas.

The electrode cables were attached to the stimulator by means of specially designed pin connectors incorporated in the stimulator body. The electrodes consisted of a bone screw of titanium if used as the cathode and of platinum-iridium (90 per cent Pt-10 per cent Ir) if used as the anode. The electrodes were shaped as screws with a diameter of 2 mm, a slope of 0.4 mm and a length of 4 mm.

The electromagnetic coils delivered a repetitive single pulse, 380 MS (positive going), quasirectangular waveform, repeating at 15 Hz (one animal), 36 Hz (one animal) or 72 Hz (one animal).

Results

In the vital microscopic models, the tissues were observed with regard to macromolecular leakages and changes in blood flow velocities.

Macromolecular leakage as observed by vital microscopy

All cheek pouches stimulated with DC (5–50 μA) or AC (20 μA) showed a large amount of macromolecular leakages after varying time (Table 1). The leakage sites were localized on the capillary level. The leakages were small and seemed to be concentrated in the area between the anode and the cathode without any noticeable preference for any of the electrodes. Nor was there any systemic pattern in the localization of the leakages which appeared first. No changes in blood flow velocity were noticeable to the naked eye. Localized blood flow standstill was not observed. Regardless of whether the stimulation was performed with 5, 20 or 50 μA DC or with 20 μA AC, there were no differences in the type of fluorescence observed.

Table 1. Observed time (min) for electrical stimulation before first appearance of macromolecular leakage in the hamster cheek pouch model. Three animals were examined in each group

5 μA	20 μA	50 μA
30	100	75
50	105	150
80	125	160

In three animals, indomethacin was given i.v. when macromolecular leakages were first observed in fluorescent light. It was demonstrated that such infusions of indomethacin were capable of counteracting an already established leakage. The macromolecular leakage was found to be decreased after a restitution period of 15 min and completely disappeared after 30 min, in spite of a continuous stimulation with 5 μA DC.

In the hamsters where electromagnetical stimulation was used or in the control group, no macromolecular leakages were observed during a follow-up period of 3 h. No visible individual differences in blood flow were noted when these two latter groups were compared.

The bone tissue of rabbits subjected to DC of 5 μA showed a large number of macromolecular leakages. The times for appearance of the leakages were 50, 55 and 60 min, respectively, after onset of the electrical stimulation. The leakages were all on the capillary level and, after a short time (5–10 min) they showed a tendency to conglomeration similar to that observed in the hamster cheek pouch model. The leakages occurred over the whole area under observation and were not apparently related to either the region close to the cathode or that close to the anode. There were no apparent changes in blood flow velocity. The control rabbits did not show any macromolecular leakages during the observation period of 3 h. There were no visible changes in blood flow velocity in the control group.

Histological observations of white cell extravasation and oedema

Routine histological examinations showed a large number of extravasated white blood cells in the electrically stimulated cheek pouches. There were no differences between the animals which were stimulated with DC of 5, 20 or 50 μA or AC of 20 μA with respect to the number of extravasated white blood cells (WBCs). In the group where the leakage was "washed away" with indomethacin, WBC nevertheless appeared in the tissue in an amount not separable from what was seen in the DC/AC stimulated animals. Practically no WBC extravasation was noted in electromagnetically stimu-

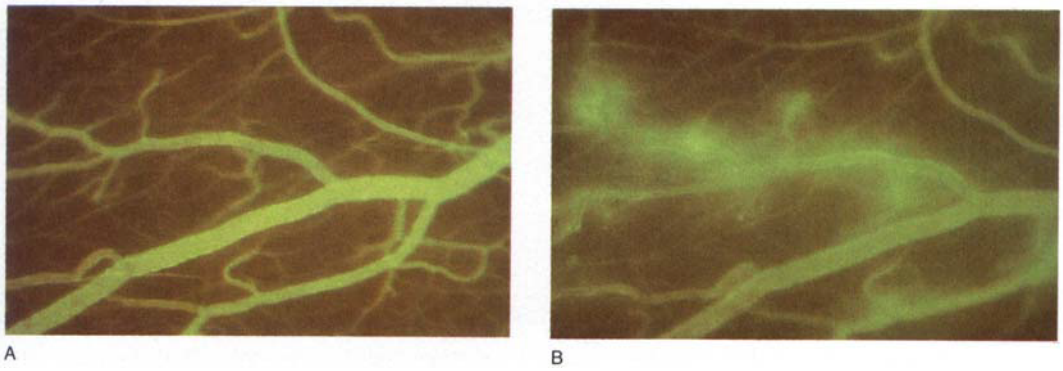

Figure 3. Electrical stimulation with DC of 5, 20 or 50 μA or AC of 20 μA caused a typical capillary leakage in the hamster cheek pouch as well as in the rabbit tibial bone. A is a control cheek pouch before electrical stimulation while B demonstrates macromolecular leakage in the same vascular bed after DC-stimulation with 20 μA.

lated animals or in the controls. Oedema was not observed in any animal.

Discussion

Vascular reactions to direct currents in the hamster cheek pouch

Vital microscopy in ordinary light did not reveal any changes in the vasculature of the cheek pouch during or immediately after the applied DC:s. No blood flow alterations were recorded. We could thus not verify the blood flow standstill that has previously been described during DC-stimulation of mesenteric capillaries at a current of 0.015–20 μA (Lübbers et al. 1976, Weigelt & Schwarzmann 1981), presumably because of a greater distance between the electrodes and the closest capillaries in our experiment.

In the present experiment, FITC-dextran was used to indicate possible vascular leakages in fluorescent light. Continuous observations during a 3-h period, when electrodes had been implanted but no current turned on, demonstrated that spontaneous vascular leakage did not occur. The present study clearly demonstrates that a continuous DC of 5–20 μA magnitude will increase the vascular permeability in the microcirculation of the hamster cheek pouch (Figure 3). This inflammatory response was verified by histological sectioning of the cheek pouches. Treatment with indomethacin, an anti-inflammatory drug, was demonstrated to block the capillary leakage elicited by electrical stimulation. Pulsed electromagnetical fields did not, over the short follow-up period of 3 h, provoke any vascular leakage in the observed animals.

Each electrically stimulated hamster was thoroughly investigated to find out whether leakages occurred predominantly in proximity to either the cathode or the anode. However, the macromolecular leakages were demonstrated to be evenly spread in the field, without any noticeable preference to any of the electrodes. Furthermore, stimulation with AC of 20 μA produced a similar leakage pattern to that observed after DC, which indicates that the observed vascular reaction is a general one rather than specifically related to the electrodes.

Reference electrode measurements indicated cathodic tissue potentials of 0.60 V at 5 μA to 1.28 V at 50 μA. These figures from the hamster cheek pouch model are similar to measurements in bone tissue where the same electrical stimulator was tested (Buch et al. 1984).

Observations in bone tissue

Previous control experiments with the bone chamber have revealed no tendency to spontaneous vascular leakage after administration of FITC-dextran over a follow-up period of 3 h. In contrast, electrical stimulation with 5 μA produced macromolecular leakage of a pattern similar to that observed in the hamster cheek pouch. This finding is of particular interest since Buch et al. (1984), using a similar experimental model in the rabbit tibia, were able to demonstrate a significantly increased bone for-

mation after electrical stimulation at the same level as tested in the present experiment. Hence, the vascular leakage elicited by this type of electrical stimulation does not interfere with bone formation. Instead, the vascular reaction may bear a causal relationship to the osteogenic process.

The observations made in the present study show that electrical stimulation may give rise to an early inflammatory reaction in bone as well as in soft tissues and that this reaction may be hampered by the administration of indomethacin. The same electrical stimulation has also been shown to elicit osteogenesis in earlier experiments (Buch et al. 1984). This osteogenic response may hypothetically be triggered by the preceding vascular exchange. This assumption does not, however, rule out the possibility that the same type of vascular reaction but of a more extensive degree may actually hamper bone formation. We believe our results may motivate further experiments to investigate whether vascular leakage may be used as an indication of a bone-stimulating effect of the applied currents.

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