Extracorporeal Shock Waves Stimulate Frog Sciatic Nerves Indirectly via a Cavitation-Mediated Mechanism

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ABSTRACT Shock waves (SWs) are single pressure pulses with amplitudes up to over 100 MPa, a rise time of only a few nanoseconds, and a short duration of approximately 2 μs. Their clinical application for stone destruction causes pain, indicating nerve stimulation by SWs. To examine this phenomenon, sciatic nerves of frogs were exposed to SWs in an organ bath. The SWs were generated with an experimental Dornier lithotripter model XL1 at an operating voltage of 15 kV. The nerves were mounted in a chamber which allowed electrical nerve stimulation and the registration of electrically and SW-induced compound action potentials (SWCAPs). The chamber was filled with frog Ringer's solution. In a standardized protocol, 100 SWs were administered at a rate of 1/min to each nerve preparation and a total of four experiments were performed. The first experiment established that 95.0 ± 4.7% of administered SWs induced action potentials which were lower in amplitude (1.45 ± 1.14 versus 1.95 ± 0.95 mV, $p = 0.004$) but similar in shape to electrically induced compound action potentials. In a second experiment, it was shown that the site of origin of the SWCAPs could be correctly determined by simultaneous recording of action potentials at both ends of the nerve. The mechanism of shock wave stimulation was examined by experiments 3 and 4. In experiment 3, in contrast to the previous experiments, SW exposure of the nerves was performed 6 cm outside the shock wave focus. This resulted in a mean probability of inducing a SWCAP of only 4%. After gas bubble administration, this probability increased to 86% for the first SW released immediately after bubble application and declined to 56% for the second, 21% for the third, to 0 for the 10th SW after fluid injection. This indicates that cavitation, the interaction between shock waves and gas bubbles in fluid or tissues, was involved in SWCAP generation. In experiment 4, nerves were again exposed in the focus, however, the Ringer's solution surrounding the nerve was replaced by polyvinyl alcohol (PVA). PVA is a solution with low cavitation activity. In PVA, the excitability was markedly diminished to 11.0 ± 5.1% compared with 96.0 ± 4.4% in control nerves exposed in Ringer's solution. In conclusion, bioeffects of SWs on nervous tissue appear to result from cavitation. It is suggested that cavitation is also the underlying mechanism of SW-related pain during extracorporeal SW lithotripsy in clinical medicine.

INTRODUCTION

Excitation of nerve fibers by short, rapid mechanical stimuli is a well-known phenomenon that was first described in the eighteenth century (Tigerstedt, 1880) and is familiar to anyone who has struck an elbow nerve. More detailed experimental work on mechanical nerve stimulation dates back to the 1930s and 1970s (Goldman, 1974; Gray, 1954; Julian and Goldman, 1962). Using single lobster axons it was established that stimuli had to be rapid to set off action potentials, while slow mechanical compression had no effect. Short stimuli of 0.5 ms duration were as effective as longer ones. A transient increase in ion permeabilities induced by compression or stretching of the axon membrane resulting in depolarization was suggested as the basic mechanism of mechanical nerve stimulation (Julian and Goldman, 1962). There was little experimental interest in these phenomena for the last 20 to 30 years.

Over the last decade, extracorporeal shock waves (SWs) have gained wide acceptance in clinical medicine. Shock wave lithotripsy is the standard therapy for kidney stones and a treatment option for a subset of gallstones (Sackmann et al., 1990). Shock waves are single pressure pulses with high amplitudes of several tens to over 100 MPa, a rise time of only few nanoseconds, and a short pulse duration of a few microseconds (Coleman and Saunders, 1989). SW treatment is performed under analgesic medication or general anesthesia as every single pulse is accompanied by a short lasting pain sensation. This indicates that the extremely rapid shock wave action is able to stimulate nervous tissue in the SW path (Schelling et al., 1989).

To examine the excitation of nerves by shock waves in more detail, sciatic nerves of frogs were exposed to SWs in an organ bath and the induced compound action potentials were registered. Evidence is presented that SWs do not directly stimulate nerves in spite of their high pressures and short rise times. Their effect is instead mediated by acoustic cavitation, which is the interaction of SWs with small gas bodies or bubbles occurring in either fluids or tissues (Crum, 1982).

MATERIALS AND METHODS

Experimental set-up

Sciatic nerves from mature *Rana esculenta* (body weight approximately 50 g) were obtained from the Institute of Physiology, University of Munich. The animals were used for various purposes at this institution and not specifically sacrificed for these experiments. The nerves were mounted in a Perspex chamber 7.5 × 12.5 × 8 cm (length × width × height) in size (Fig. 1). They were pulled through two lateral orifices 5 mm above the bottom,

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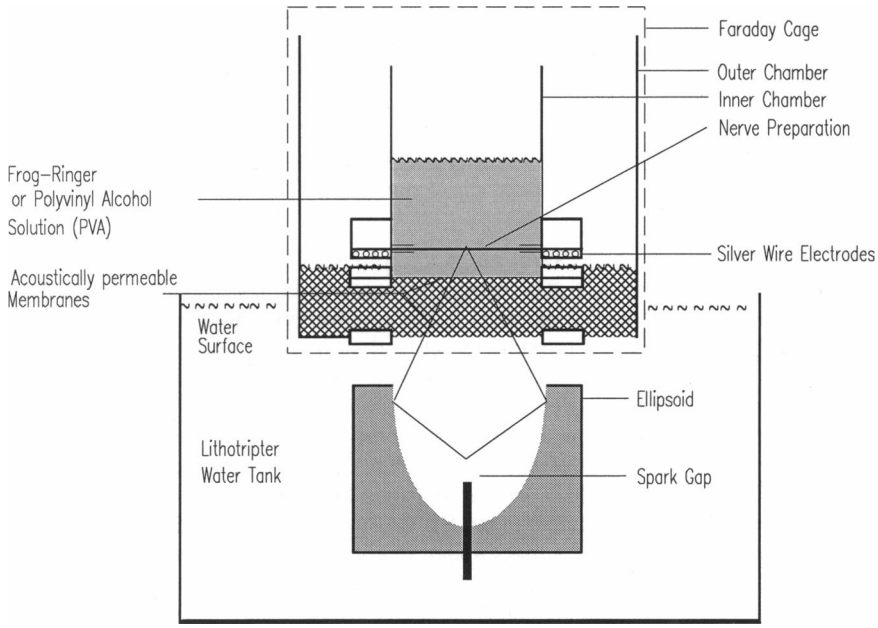

FIGURE 1 Schematic diagram of the experimental setup. The sciatic nerve was pulled through both lateral openings of the inner chamber and placed on four silver electrodes on each side. Two outer electrodes on one side were used for bipolar electrical stimulation and the two pairs of inner electrodes for simultaneous recording of SWCAPs on both nerve endings. The outer chamber was mounted on a precisely adjustable gantry and partially submerged into the lithotripter water tank to allow acoustic coupling to the SW source (gantry not shown).

which were sealed afterward with 20% gelatin in Ringer's solution. Thus, the fluid level in the chamber could be raised to 3 cm above the level of the nerve.

The chamber was filled with frog Ringer's solution containing NaCl, 98 mM; KCl, 3.2 mM; $CaCl_2$, 2 mM; glucose, 10 mM; and HEPES, 10 mM, with the pH adjusted to 7.6. In group 1 of experiment 4, a polyvinyl alcohol (PVA) solution was used to fill the chamber. It was prepared by dissolving 100 g of PVA powder (Merck, Darmstadt, Germany) in 900 ml of frog Ringer's solution and the pH was adjusted to 7.6. PVA is a solution of high viscosity with low cavitation activity when used as a bathing environment during SW exposure (Delius and Gambihler, 1992a). It was chosen for the experiment because its acoustic impedance is nearly identical to the impedance of water and its SW attenuation is relatively low, albeit higher than the attenuation of water (Hayakawa, 1989). All experiments were performed at room temperature, 19 to 21°C.

The chamber was equipped with silver electrodes for bipolar external electrical stimulation and recording of electrically induced compound action potentials (ELCAPs) or SW-induced compound action potentials (SWCAPs). It was surrounded by a much larger outer chamber made of the same material and covered with a grounded metal wire net to serve as a Faraday cage (Fig. 1). Both chambers were floored with an acoustically transparent polyvinyl chloride film, 0.05 mm thick, to allow SW entry through the bottoms of both compartments. The distance between both membranes was 10 mm. The outer chamber was filled with frog Ringer's solution except in group 2 of experiment 4, where PVA was used; the fluid level was kept below the electrode level, but it allowed bubble-free acoustic coupling to the inner chamber.

Shock waves

The SW generator was an experimental Dornier XL 1 lithotripter (Dornier Medizintechnik GmbH, Germering, Germany). The principles of electrohydraulic SW generation have been described earlier (Forssmann et al., 1977). A lithotripter SW is composed of a large positive pressure peak with a duration of approximately 2 μs followed by a much smaller tensile wave that lasts up to 10 μs (Coleman et al., 1987). The discharge voltage of the lithotripter was 15 kV at a capacitance of 80 nF. According to recently published measurements using polyvinylidenedifluoride needle hydrophones (Müller, 1990), the peak positive pressure in the geometric focus of the shock wave field is approximately 50 MPa at this voltage. The focal region, defined as the isobar representing 50% of maximum pressure, has the shape of a cigar and extends 22 mm in direction of the longitudinal axis of the shock wave field and 5.0 mm perpendicular to it (Müller, 1990). The focus was marked by the intersection of two laser beams.

Compound action potentials

The recording electrodes of the inner chamber were connected to two preamplifiers (×100 gain) and the signals registered and displayed using a two-channel digital oscilloscope (Fluke Philips PM3335). The oscilloscope was triggered by the optically detected (remote photo diode) spark discharge flash of the lithotripter. As a positive control, all nerves could be electrically stimulated by two additional electrodes at one end of the nerve (Fig. 2) with ELCAPs registered on the opposite end. Square waves of 0.2 ms duration at a voltage of 8 times the threshold for stimulation were used (Grass SD 5 Stimulator, Grass Medical Instruments, Waltham, MA). ELCAPs or SWCAPs were digitized and stored on an interfaced personal computer. Signal filtration and analysis was performed off line using standard signal processing software (Turbolab, Stemmer, Puchheim, Germany). Latencies of ELCAPs were measured from the beginning of the electrical artifact to the upstroke of the action potential on the far end of the nerve. SWCAP latencies were measured from the first downstroke of the electrical artifact produced by the SW generator discharge to the first upstroke of the SWCAP (Fig. 3). There was a delay of 19 ± 0.0 μs (mean from 50 measurements) between the downstroke of the electrical artifact and the spark discharge flash of the lithotripter which triggered the registration of SWCAPs. SW propagation

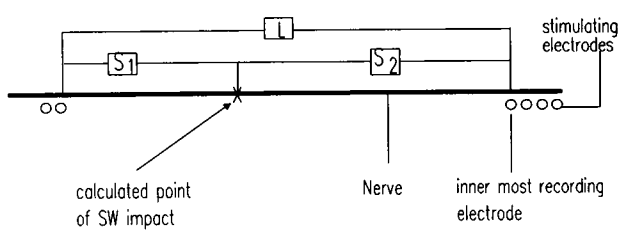

FIGURE 2 Schematic drawing of the nerve and position of recording electrodes. For description of variables see text and Eqs. 1 and 2. The distance L between the two innermost recording electrodes measured 52 mm in the experimental setup. S_1 is the distance between the point of SW impact and the innermost recording electrode on one side and S_2 the corresponding value on the opposite side.

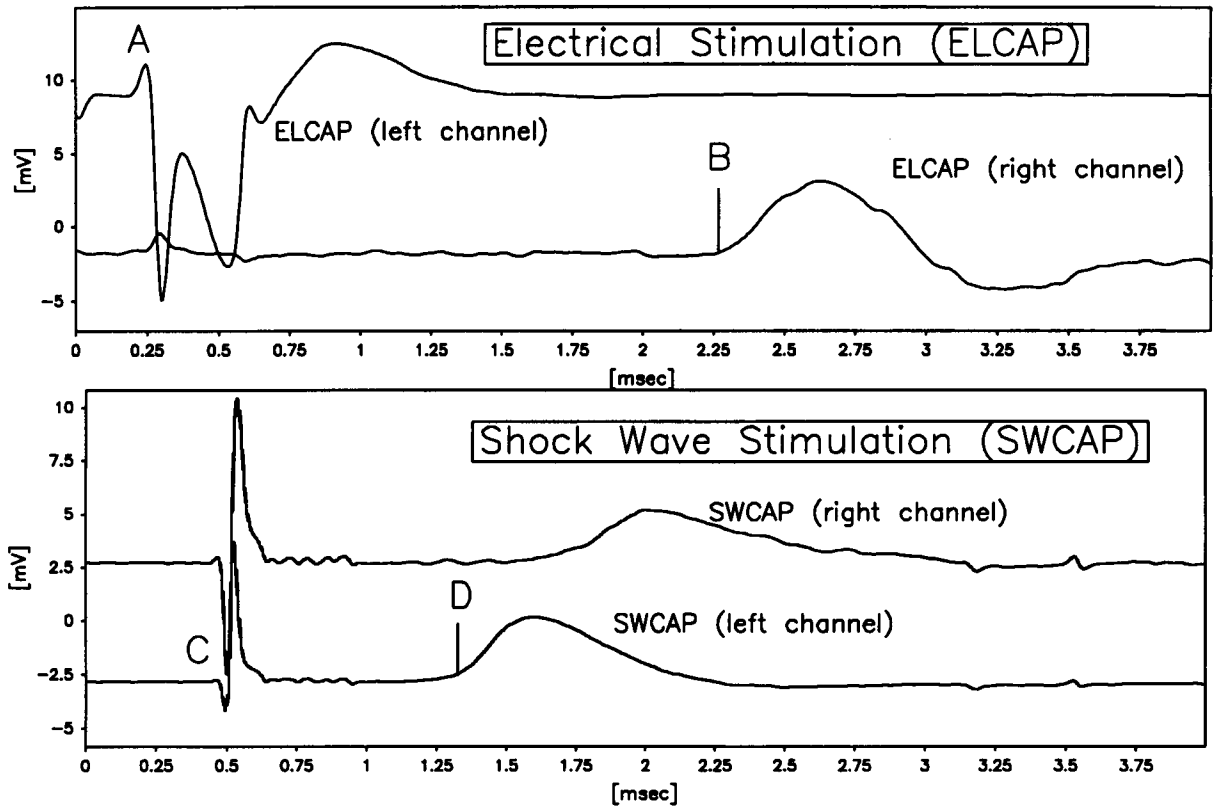

FIGURE 3 ELCAP (*top*) and SWCAP (*bottom*) with signals registered simultaneously on both ends of the nerve. Latencies of ELCAPs were measured from the beginning of the downstroke of the electrical stimulus (*A*) to the beginning of the upstroke of the compound action potential (*B*). The broad stimulus artifact at point (*A*) is due to the very short distance between stimulus and recording electrodes on the left side of the nerve which results in a fusion complex of stimulus and ELCAP. Latencies of SWCAPs were measured from the beginning of the downstroke of the electrical SW artifact (*C*) to the beginning of the upstroke of the SWCAP (*D*) for both channels. The calculated origin of the initial depolarization of the SWCAP in this experiment was 2.46 cm from the left recording electrode.

from the spark gap to the SW focus took 150 μs (experiments 1, 2, 4) and to 6 cm above the focal area 190 μs (experiment 3). These values were subtracted from latency measurements. Amplitudes of ELCAPs and SWCAPs were determined by baseline to peak measurements.

Experimental protocols

Control experiments without SW application

Six nerves in frog Ringer's solution and four nerves in PVA solution were mounted into the Perspex chamber and electrically stimulated every 10 min but not exposed to SWs. These sham experiments lasted 100 min and these preparations served as controls to delineate the effects of the bathing solution, the manipulating of the nerves, and drying of the nerve endings on the electrodes.

Experiment 1: SWCAP generation

In this and the following experiments, 100 SWs were administered at a rate of 1/min to all preparations to provide a long recovery time and to allow storage of signals. SW-exposed nerves were electrically stimulated after every 10th administered shock wave to ensure their excitability.

During experiment 1, the size and shape of SWCAPs was registered and compared with ELCAPs. Four nerves in frog Ringer's solution were positioned directly into the focus. In addition, possible indirect nerve stimulation by electromagnetic interference was ruled out. This was done by shielding the nerves intermittently from mechanical SW impact by interposing 20-mm strong styrofoam pads (20 × 20 cm in size) between the SW generator and the outer chamber. Styrofoam completely prevents the passage of acoustic waves but has little influence on the electromagnetic field.

Experiment 2: SWCAP localization

The site of origin of SW induced compound action potentials was related to the site calculated from simultaneous recordings on both sides of the nerve. Assuming a single point of origin of the SWCAP and a constant propagation velocity across the nerve, the site of origin of SWCAPs can be calculated according to the following equations and should correspond to the areas exposed to SWs during the experiments:

$$S_1 = L/(T_2/T_1 + 1) \quad (1)$$

and

$$S_2 = L - S_1 \quad (2)$$

where L is the distance between the innermost recording electrodes, T_1 the latency between shock wave release and the first vertical upstroke of the SWCAP on one side, T_2 the corresponding latency of the SWCAP on the other side, and S_1 the distance between the point of SW impact and the innermost recording electrode and S_2 the corresponding value on the opposite side (Figs. 2 and 3).

To verify the calculated points of origin, four nerves were shielded by a styrofoam pad (4 × 3 cm in size and 3 mm thick) positioned 3 mm below the nerve in the inner chamber. A small opening (10 × 10 mm) was created in the pad with its center 15 mm from the left and 37 mm from the right electrodes. The opening was positioned on the longitudinal axis of the shock

wave field. Localization of the opening determined the site of acoustic nerve stimulation as only the part of the nerve immediately opposite to the opening was exposed to SWs. Twenty SWs were initially administered and then the styrofoam pad turned by 180°, shielding the opposite part of the nerve. Then the next 20 SWs were applied. This procedure resulted in SW exposure of both ends of the nerve, essentially sparing its middle part.

Experiment 3: air bubble injection

The experiment was performed to delineate the influence of cavitation on the incidence of SWCAPs. Twelve nerves were assigned to two groups of six and positioned on the longitudinal SW axis 6 cm beyond the geometric shock wave focus. One hundred shock waves were applied to the nerves of group 1 and SWCAPs registered. In group 2, in addition to this protocol, 50 ml of Ringer's solution were aspirated from the inner chamber after every 10th administered SW and forcefully reinjected through a 10-gauge needle. This generated local turbulence and seeded small gas bubbles into the solution. Care was taken that the flow did not hit the nerve. SWs were administered within 15 s after fluid injection.

Experiment 4: PVA as a bathing medium

The role of cavitation was further investigated with 10 nerves assigned to two groups of five. They were positioned directly in the focus, 100 shock waves were applied, and SWCAPs registered. In group 1, five nerves were exposed with the inner chamber filled with PVA and the outer chamber with frog Ringer's solution. In group 2, five nerves were exposed in frog Ringer's solution with the outer chamber filled with PVA. Thus, SWs in group 2 traveled 10 mm in PVA as compared with 5 mm in group 1 until they reached the nerve.

Statistics

The probability of SWCAPS after SW exposure was calculated by dividing the number of positive recordings by the number of experiments performed in each group. Student's *t*-test was used to compare mean latencies and amplitudes between ELCAPs and SWCAPs. A p value below 0.05 was considered significant.

RESULTS

In the six nerves without SW stimulation (sham experiments), ELCAPs decreased from 2.62 mV to 0.17 mV and latencies increased from 1.90 to 3.12 ms. These effects were probably caused by drying of the free ends of the nerve on the electrodes. Similar effects were observed in all of the following experiments.

Experiment 1: SWCAP generation

During this and the following experiments, approximately 10% of all SWs resulted in signals that could not be easily distinguished from electrical artifacts on the oscilloscope screen. They were usually found toward the end of the experiment where the voltage of control electrically evoked compound action potentials had decreased. These recordings were regarded as equivocal and excluded from further analyses.

The overall appearance of SWCAPs was similar to ELCAPs (Fig. 3), and 95.0 ± 4.7% of all SWs that could be analyzed during this experiment resulted in compound action potentials. Intermittent shielding of the nerves from mechanical SW impact resulted without exception in complete disappearance of the SWCAPs with prompt return of the recordings when the styrofoam pads were removed. This excluded triggering of compound action potentials by electromagnetic interference.

SWCAPs showed lower mean amplitudes (1.45 ± 1.14 versus 1.95 ± 0.95 mV, $p = 0.004$) and shorter latencies (0.95 ± 0.183 versus 1.81 ± 0.24 ms, $p < 0.001$) when compared with electrically evoked potentials. The calculated points of origin of the SW evoked action potentials were narrowly distributed along the middle of the nerve with a maximum number of SW impacts at 2.5 cm from the innermost recording electrodes (Fig. 4). These points were closer to the recording electrodes than the electrical stimulation electrodes on the opposite end of the nerve. This explains the shorter latencies of SWCAPs.

All nerves remained electrically excitable throughout this and the following experiments. The decrease in amplitudes of both SWCAPs and ELCAPs over time from a maximal observed value of 6.02 mV to a minimum of 0.02 mV for SWCAPs and from 4.03 mV to 0.48 mV for ELCAPs corresponded to the expected decrease from sham experiments.

Experiment 2: verification of SWCAP localization

When styrofoam pads with openings were used to shield the nerve, the calculated origins of all analyzed SWCAPS were found within the areas exposed to SW impact in all experiments. When the styrofoam pad was turned by 180°, the opposite end of the nerve was subject to SW impact and the

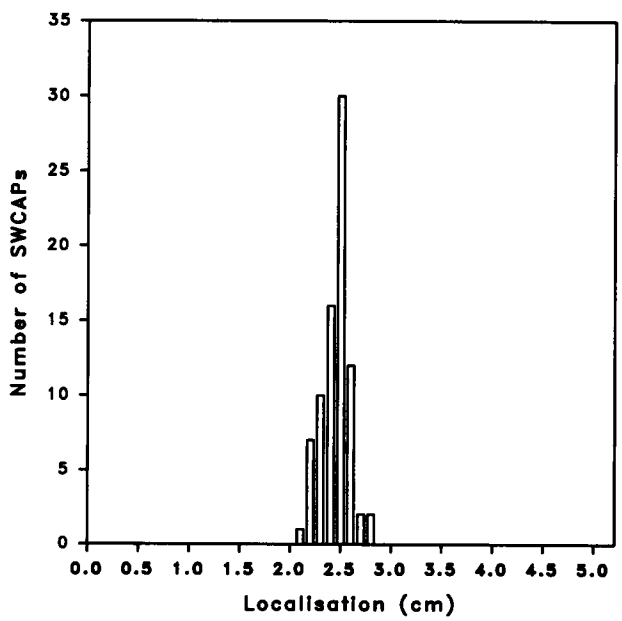

FIGURE 4 Calculated distribution of origins of SWCAPs after SW exposition of a nerve in frog Ringer's solution. During this experiment, the nerve was placed into the SW focus (experiment 1). For graphical representation, the length of the nerve was divided into 1-mm segments and plotted on the horizontal axis. The number of SWCAPs resulting from each segment are represented by vertical bars.

calculated origins of depolarization were closer to the opposite electrode (Fig. 5). No calculated point of SW impact lay in an area covered by styrofoam.

Experiment 3: air bubble injection

Six centimeters behind the focus, the probability of eliciting a SWCAP in both groups was highest for the first seven administered SWs immediately after beginning of the experiments and declined rapidly from 100% for the first, second, and third SW to 33% for the fourth and to 8.3% for the seventh SW. It was essentially zero for the following 8 to 100 SWs of group 1. Rapid fluid injection in group 2 after every 10th administered SW led to a reappearance of excitation. Mean SWCAP probability reached a maximal value of 0.86 for the first SW after bubble injection in this group and declined steadily to 0.56 for the second, 0.21 for the third, 0.09 for the fourth, to 0 for the 10th SW after bubble application (Fig. 6). SWCAP amplitudes declined from 1.15 ± 1.30 mV for the first SW to 0.97 ± 0.84 mV for the fourth, to 0.11 ± 0.10 mV for the ninth SW after gas bubbles. The electrical excitability of the nerve preparations remained unchanged in both groups and all nerves could still be electrically stimulated at the end of the experiments.

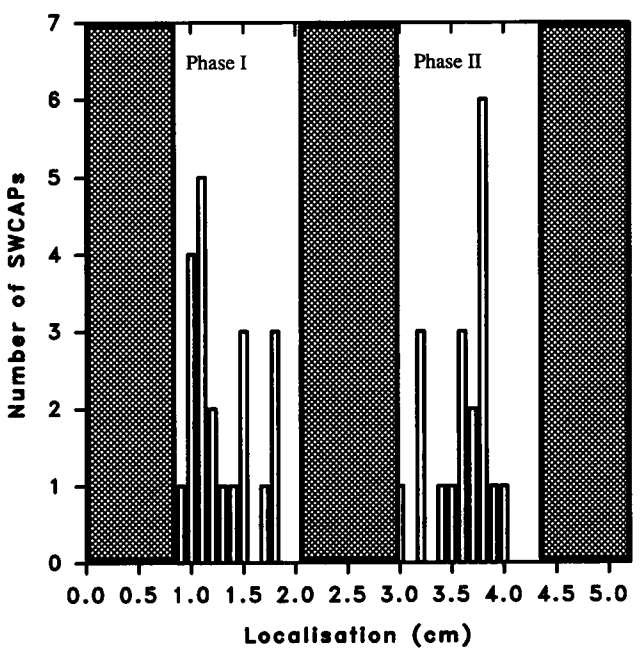

FIGURE 5 Calculated origins of the initial depolarization after SW impact from experiment 2 (verification of SWCAP localization). The total length of nerve is represented on the horizontal axis and was divided into segments of 1 mm. The number of SWCAPs resulting from each segment is represented by vertical bars. During phase I of the experiment, the nerve was shielded by a small styrofoam pad with an opening of 10 × 10 mm with its center located approximately 15 mm from the left recording electrode, and 20 SWs were applied. The styrofoam pad was then turned 180° (phase II), the left side of the nerve shielded, and the next 20 SWs administered. Shaded areas indicate areas covered by styrofoam (drawn to scale). This procedure resulted in an asymmetric distribution of SW origins which were opposite the openings in the pad in all cases.

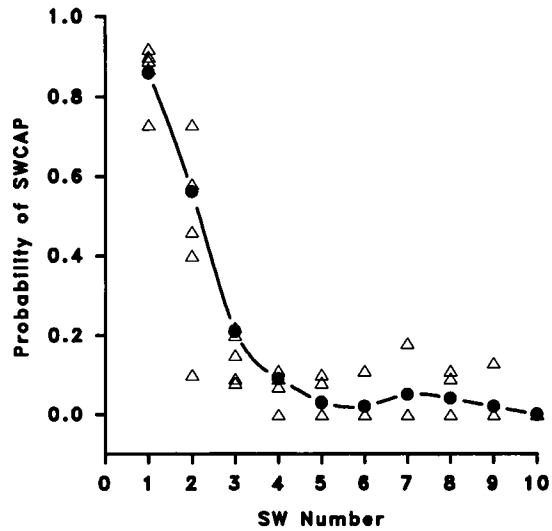

FIGURE 6 Probability of a SWCAP during 10 serially administered SWs after the administration of air bubbles (group 2). Air bubbles were brought into the solution by withdrawing 50 ml of frog Ringer's and forceful reinjection of that volume 30 s before release of the first SW. The following nine SWs were administered at a rate of 1/min. After the 10th SW, bubble application was repeated and the next 10 SW administered until 100 SWs were applied. Six experiments were performed, and each triangle indicates the mean of SW probabilities of 1 experiment, whereas circles show mean values over all experiments.

Four SWs administered at the beginning of the experiments (one from group 1 and three in group 2) resulted in SWCAPs of high amplitudes and shorter latencies than usually observed (2.84 ± 1.65 mV mean amplitude and a mean latency of 0.52 ± 0.13 ms). Small gas bubbles adherent to the nerves could be observed in three of these four cases. The bubbles rapidly disappeared with repetitive SW application. These recordings were excluded from further analyses.

Experiment 4: PVA as a bathing medium

Excitability by SWs of the nerves of group 1 which were bathed in PVA solution was markedly diminished compared to the nerves of group 2 which were bathed in frog Ringer's solution. In group 1, only 11.0 ± 5.1% (mean ± SD from five experiments) of all analyzed SWs resulted in a SWCAP (Fig. 7) versus 96.0 ± 4.4% in group 2. While the incidence of SWCAPS was comparable in both groups for the first 10 to 20 SWs, it declined rapidly thereafter in group 1, and reached 0 in four of five experiments after more than 40 SW had been administered. In two cases of group 1, after the full series of 100 SWs had been administered, the PVA solution bathing the nerve was removed and replaced by frog Ringer's solution. The excitability of the nerve preparation immediately returned, and five consecutively administered SWs resulted in SWCAPs.

There was no significant difference between the amplitudes of SW and electrically evoked compound action potentials, regardless of the medium surrounding the nerve (1.70 ± 0.90 versus 1.50 ± 1.14 mV in PVA and 2.33 ± 2.20 versus 2.00 ± 0.95 mV in frog Ringer's solution).

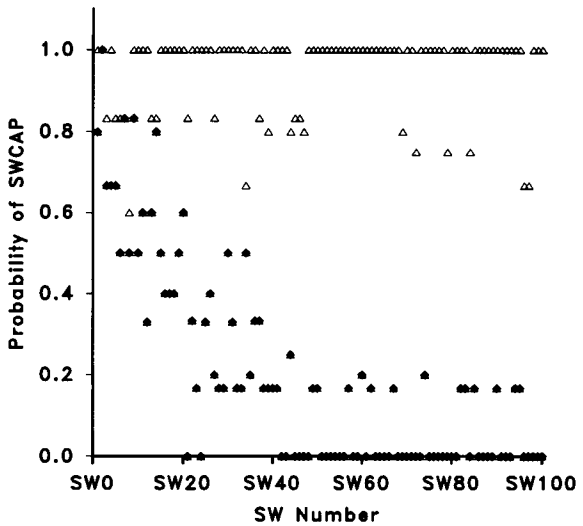

FIGURE 7 Effect of the bathing medium on the probability of eliciting a SWCAP. △, SW exposure in frog Ringer's solution (group 2); ▲, PVA solution as a bathing medium (group 1). SW probability was calculated for each individual SW by dividing the number of SWCAPs identifiable on the oscilloscope screen by the number of experiments (n = 5) performed. Because approximately 10% of all SWs could not be analyzed due to electrical artifacts, these probabilities are not always equal to 0, 20, 40, 60, 80, or 100%.

DISCUSSION

This in vitro study demonstrates that extracorporeal shock waves generate action potentials in frog nerves. They originated from a narrow area within the shock wave focus. SWCAPs looked similar to ELCAPs, indicating that identical types of nerve fibers were excited. A detailed analysis of a possible different sensitivity of different fiber types to SWs was not intended in this study and precluded by our set-up, due to both electromagnetic field and mechanic vibrational disturbances from the shock wave generator.

During the initial testing of the experimental arrangement (data not shown), SWCAPs were only seen during shock wave exposure of nerves in the focus; there was no consistent response a few centimeters beyond the focus. We therefore assumed that the high pressures in the focal area were directly responsible for the shock wave effect. This would have indicated that the steep pressure gradient of the shock pulse, which is in the nanosecond range, resulted directly in axonal depolarization. Direct depolarization was also suggested from a previous experiment in which frog sciatic nerves were exposed to shock waves generated by mechanical impact of a steel ball on a plate (Wehner and Sellier, 1981). A similar direct effect has been described with mechanical stimuli in the microsecond range. The response to this stimulation has been explained by mechanical compression leading to a distortion of the axonal contents, strain of the membrane, and a resulting increase in permeability with depolarization (Julian and Goldman, 1962; Yamada and Sakada, 1961). Under these conditions, an increased in vivo mechanosensibility of axons has been reported after partial demyelination (Calvin et al., 1982; Howe et al., 1977; Smith and McDonald, 1980).

It is, however, not self-evident that shock wave effects are directly caused by the pressure pulse, since it is currently debated which role cavitation plays in the generation of biological shock wave effects. Cavitation is the generation and movement of bubbles in a fluid or tissue (Crum, 1982). It is a very powerful process involved even in the destruction of hard materials. It has been known since the beginning of this century that cavitation is the mechanism that induces the surface erosion of ship propellers and turbine blades and finally leads to their failure. Detailed investigations on bubble movements during cavitation were started in the 1960s (Naudé and Ellis, 1961). Since it is a very fast process with crucial events occurring in the microsecond range, high speed photography has to be used for its observation (Lauterborn, 1974). The major finding in these studies was that a moving cavity generated near a solid surface, for example by optical breakthrough with a laser, collapses asymmetrically under formation of a water jet onto the surface. It does not collapse symmetrically because the surface impedes the flow of water in direction of the bubble center. A surface pit generated by the collapse has the same diameter as the water jet and is considered to be the primary damaging event (Tomita and Shima, 1986; Vogel et al., 1989). More recently, research focused on a second mechanism of water jet formation. A shock wave that hits a stable gas bubble in a fluid induces a strong jet in this bubble. There is evidence that this interaction between a shock wave and a pre-existing gas bubble is an even stronger mechanism of damage formation (Dear and Field, 1988; Dear et al., 1988). Using the same lithotripter as the one employed in our study, it has recently been demonstrated that the interaction of lithotripter-generated shock waves with air bubbles generates water jets with a speed of 400–700 m/sec, which is nearly as fast as a rifle bullet (Philipp et al., 1993).

Previous experiments have also suggested that cavitation is strongly involved in the generation of biological shock wave effects which have been examined in detail in several organs like the liver and kidney (Delius and Gambihler, 1992b; Recker et al., 1992). Tissue damage mainly consists of vessel wall defects, hemorrhages, and thrombus formation. Shock waves of lithotripters have been shown to generate by their tensile wave gas bubbles in tissues which are detectable by ultrasound (Delius and Gambihler, 1992b). There is evidence that the interaction between a shock wave and gas bubbles is a crucial event for the formation of tissue damage. Tissue damage was increased when the interaction was facilitated by a fast shock wave administration rate (Delius et al., 1990a,b) and also when small gas bubbles of micrometer size were injected into the hepatic artery during shock wave administration to the liver (Prat et al., 1992).

Based on this evidence, it seems reasonable to assume that excitation of nervous tissue by shock waves might not be generated by a direct shock wave effect, but cavitation and, more specifically, the interaction between shock waves and gas bubbles could be responsible for generation of action potentials.

This assumption was corroborated by an accidental observation during an initial experiment. The inner chamber holding the nerve became leaky, and when additional frog Ringer's solution was added from a syringe, the excitability of the preparation appeared to be increased and SWCAPs could be induced even many centimeters beyond the focal area. This observation led to the design of experiment 3 where the influence of gas bubbles was systematically examined. In retrospect, the setup corresponded to the one published by Prat et al. (1992) who found in an in vivo study a dramatic increase in tissue damage in rabbit livers when gas bubbles were injected during shock wave application.

Experiment 4 of this study was also designed to differentiate further between a direct shock wave and a cavitation effect. Suppression of cavitation effects in high viscosity fluids has previously been used for this purpose (Brümmer et al., 1989). Attenuation of the shock wave itself by PVA as an alternative explanation was excluded by putting a jacket of PVA between both membranes of the experimental setup in group 2. This should have prevented SWCAP induction in frog Ringer's solution. A third approach, suppression of cavitation by hyperbaric pressure, was not feasible in this case, as hyperbaric pressure affects nerve conduction as well.

Shock waves are single ultrasonic pulses of high pressure. Continuous wave ultrasound has been shown to induce different effects in nerve preparations than we saw with single SWs, namely reversible and irreversible nerve block (Young and Henneman, 1961a,b). In vivo, diagnostic levels of ultrasound can disrupt myelination (Ellisman et al., 1987), and high intensities have been shown to induce focal brain lesions (Fry et al., 1970). That ultrasound may induce cavitation in tissues has long been known (Hug and Pape, 1954), and high intensity lesions are believed to result from cavitation (Fry et al., 1970). An ultrasound-induced increase in temperature has been claimed to be important in the generation of lesions at low intensities (Fry et al., 1970). Reversible membrane depolarization by continuous wave ultrasound has been previously described using a rat muscle preparation (Zakharov et al., 1989). Its onset corresponded to the onset of cavitation. This corroborates our view of the role of cavitation as a mechanism of membrane stimulation. Thermal effects of lithotripter shock waves can be clearly excluded (Filipcynski and Piechocki, 1990), but electron microscopic studies are required to delineate the morphological changes induced by shock waves on nervous tissue.

The interaction of SWs with nervous tissue carries two other important clinical implications: SW-related pain and cardiac dysrhythmia. Pain during SW treatment can be severe and often requires analgesic medication (Schelling et al., 1992) or general anesthesia (Weber et al., 1988). It represents a multifactorially determined phenomenon which depends on the administered SW energy and the SW repetition rate (Schelling et al., 1992; Weber et al., 1988). SW treatment can cause cardiac dysrhythmia, which requires synchronization of SW release to the patient's electrocardiogram to achieve generation of the SWs in the absolute refractory period of the ventricle (Weber et al., 1988). The results of this study present evidence that both phenomena may indeed be caused by cavitation- mediated stimulation of cardiac tissue (Delius et al.,1994) or nerve fibers. Pain is mediated by small, slow conducting A-fibers and C-fibers, however, and based on the conduction latencies of our SWCAPs, they resulted from large diameter A-fibers. The stimulation of small, slow-conducting fibers by SWs could therefore not be proven directly. We assume, however, that the principal reaction of a nerve fiber, be it small or large, to a shock wave is similar, and that the same mechanism of stimulation should be in operation.

In summary, data from this in vitro study show that SW exposure of frog sciatic nerve results in compound action potentials similar to those seen after electrical stimulation. Bioeffects of SWs on nervous tissue appear to result from cavitation. It is suggested that cavitation is also the underlying mechanism of SW-related pain during lithotripsy.

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