

The American Journal of Physiology
Original Contribution

Acute Exposure to a Moderate Strength Static Magnetic Field Reduces Edema Formation In Rats

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Running Title: Acute Static Magnetic Field Exposure Reduces Edema Formation In Rats

Funding: This work was supported by the National Institutes of Health – AT-00582

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ABSTRACT

External application of static magnetic fields (SMF), utilized specifically for the treatment of inflammatory conditions such as soft tissue injuries, has recently become popular as a complementary and/or alternative therapy with minimal investigation into efficacy or mechanism. Localized inflammation was induced via injection of inflammatory agents λ -carrageenan (CA) or histamine into rat hindpaws, alone or in conjunction with pharmacologic agents, resulting in a spatially and temporally defined inflammatory reaction. Application of a 10mT or 70mT, but not a 400mT, SMF for 15 or 30 minutes immediately following histamine-induced edema resulted in a significant, 20-50% reduction in edema formation. Additionally, a 2 hour, 70mT field application to CA-induced edema also resulted in significant (33-37%) edema reduction. Field application before injection or at the time of maximal edema did not influence edema formation or resolution, respectively. Together these results suggest the existence of a therapeutic threshold of SMF strength (below 400mT) and a temporal dependence of efficacy. Administration of pharmacologic agents directed at NO signaling and L-type Ca^{2+} channel dynamics in conjunction with SMF treatment and histamine-induced edema revealed that the potential mechanism of SMF action may be via modulation of vascular tone through effects on L-type Ca^{2+} channels in vascular smooth muscle cells.

Keywords: Static magnetic field, edema, microvascular tone

INTRODUCTION

External application of static magnetic fields, utilized specifically for the treatment of inflammatory conditions such as soft tissue injuries, has recently become popular as a complementary and/or alternative therapy with minimal investigation into efficacy or mechanism. While the literature supports a potential therapeutic benefit of pulsed electromagnetic field (PEMF) application to aid in the treatment of non-union bone fractures (2; 17) and osteoarthritis (5), the acceleration of wound healing (4;32;41) and the modulation of angiogenesis (14;42), direct evidence supporting the therapeutic use of static magnetic fields (SMF) is less established. Static magnetic fields are of particular interest as they are the primary field utilized in many of the over the counter products presently on the market, with approximately 5 billion dollars worldwide and 500 million dollars in the U.S. per annum being spent on magnetic field therapy (10).

Recent studies have demonstrated that localized SMF application can modulate microvascular tone in skeletal muscle (26) and cutaneous tissue (27;28) whereas global SMF application can modulate both blood pressure (29) and flow (21;46) both suggesting that SMF application may be effective in treating edematous tissue conditions. Clinical investigation of local, chronic SMF treatment on post-lipectomy patients revealed significant reduction of edema and pain when applied immediately after surgery (20). Additionally, application of a global, chronic SMF to pharmacologically induced synovitis demonstrated significant reduction in inflammatory infiltrate (44). Investigation of the specific mechanisms governing SMF action has been limited, however the evidence suggests that the SMF may act via alterations in Ca^{2+} flux or other enzymatic reactions (23;28;29;36;40). From these studies, the therapeutic application of magnetic fields for treatment of circulatory problems appears to be promising, but assessment of

acute application of static magnetic fields in an injured, compromised tissue has not been investigated.

Initiation of acute inflammation, manifested as redness, heat, pain and swelling, occurs in response to mechanical injury, ionizing radiation or invading pathogens. Each of these stimuli can independently activate the release/formation of inflammatory mediators such as histamine, bradykinin, platelet activating factor (PAF), TNF- α and prostaglandins from cells in the tissue. These mediators act on endothelial cells to increase vascular permeability and cause vasodilation and relaxation of smooth muscle cells via production of nitric oxide (NO), and act on Ca²⁺ channels through endothelial dependent and independent processes (13). As evidence suggests that SMF application can result in modulation of microvascular tone and flow, we hypothesize that acute application of SMF to an inflammatory injury may limit the formation of edema and therefore accelerate healing.

To test the hypothesis that locally applied acute static magnetic field exposure could significantly reduce edema formation and/or improve resolution, the hind-paw inflammation model was chosen. This model allowed for acute, localized application of static magnetic fields to an induced injury location and accurate quantification of the inflammatory response was facilitated by sequestration of inflammation to the paw itself. Two separate but related agents, λ -carrageenan (CA) and histamine, were chosen as severe and minor inflammatory stimuli, respectively, at the selected concentrations.

MATERIALS AND METHODS

All experiments were conducted in accordance with all rules and regulations set forth by the University of Virginia Animal Care and Use Committee.

Assessment of the efficacy of acute magnetic field exposure on edema formation and resolution was accomplished by utilizing a well established inflammatory model that has been used extensively for the detailed investigation of anti-inflammatory therapies. Inflammatory agents λ -carrageenan and histamine were injected locally in the paw of rats, alone or in conjunction with pharmacologic agents, resulting in a spatially and temporally defined inflammatory reaction. Magnetic field treatment was then applied locally to the inflamed paws at various strengths and durations in an attempt to elucidate the efficacy and/or mechanism(s) of static magnetic field action on induced edema.

The range of field strengths utilized in this study (7.5-400mT) fall into the range generally advertised for magnetic field therapy products (10) and are on the same order of magnitude as those in other investigations into the efficacy of SMF treatment. The duration of exposure for the acute application was chosen based upon the generally accepted time scale for application of cryotherapy following injury (15-30 minutes) (8) together with the mid-range of previous studies (1-40 minutes). Direct comparison of the field strengths utilized in these studies with the field strength of products on the market is difficult due to the fact that most manufacturers report the strength at the core of their magnets and do not know what the “active” strength is at the target site. This may explain the widely varying experiences regarding efficacy reported in the community at large; many devices are designed in such a way that the distance from the core of the magnet to the target site is too great for any effective field strength to remain at the target site. To address this deficiency, the magnets utilized in this study were carefully calibrated so that the “actual” field strength to which the tissue was exposed was known, rather than estimated based on a stated core strength.

Edema Induction. Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 150-210g were utilized and baseline paw volume measurements were taken by sequentially submerging right and left hindpaws up to the tibiotarsal joint in a pressure transducer driven plethysmometer (Kent Scientific, Torrington, CT), accurate to 0.01ml (stated by the manufacturer and calibrated by the user before each day of experiments); the changes in volume measured on the order of 0.1ml-1.0ml. Left hindpaws were injected sub-plantar under conscious conditions to maximize the inflammatory reaction with 0.1mL 1% or 0.5% λ -carrageenan (C-3889, Sigma Aldrich) (45) in sterile saline (0.9%), or 0.1mL 1mg/kg histamine (H-7125, Sigma Aldrich) (3) in de-ionized water to induce inflammation or 0.1mL of sterile saline or de-ionized water as vehicle controls, respectively. Left (treated with SMF or sham) and right (control) paw volume measurements were taken at regular intervals; every hour for 6 hours for λ -carrageenan (CA) treated paws and every 30 minutes for 3 h following histamine treatment. Right paws were either untreated, to ensure no systemic inflammation, or injected with vehicle control. Percent change in paw volumes from baseline measurements were calculated independently for both the left and right paws. Each animal was only injected in each paw one time; no animals were used for repeated measures and no data were excluded.

SMF Application. Following injection, each animal was anesthetized with inhalational isoflurane (Iso-Thesia, Vetus Animal Health, Burns Veterinary Supply, Rockville Center, NY). Anesthesia was induced at a concentration of 2.5% isoflurane delivered in pure oxygen at a flow rate of 1.0 L/min via a VetEquip vaporizer (#911103, VetEquip Inc., Pleasanton, CA) and maintained for the duration of magnetic field exposure at a concentration of 1.5% and a flow rate of 1.0 L/min. During anesthesia, the animals were placed prone on a heating pad to maintain physiologic temperature of 37°C and the hindlimbs were loosely taped to a plexiglass stage to

ensure reproducible orientation of the paw for treatment. The magnet treatment was applied within 30 seconds of injection via a lexan positioning device that placed the magnet directly over the injected paw, 2mm from the surface. Sham treated animals were injected with a vehicle control (saline or D.I. water) and subjected to identical immobilization and anesthesia treatment, the only difference being that no magnet was placed over the paw. At the conclusion of exposure, animals were removed from the isoflurane, awoke almost immediately, and were free to roam around their cage for the duration of the experiment.

Pharmacological Intervention. The pharmacological agents utilized for determination of the mechanism of static magnetic field action; L-arginine (L-Arg, Sigma, 15 μ M) was co-administered with the 0.1mL of histamine locally in the paw to minimize systemic action and (-)BAYK8644 (Sigma, 3mg/kg) was administered intraperitoneally (i.p.), concurrently with the histamine sub-plantar, in 0.1ml dimethylsulfoxide (DMSO, Sigma) due to its insolubility in saline or de-ionized water.

Magnetic Field Distribution Calibration. A magnetic calibration system developed in our laboratory to generate three-dimensional maps of the magnetic flux density (26) was utilized to assess the strength and uniformity of the magnets used in these experiments (Figure 1). The magnets utilized in these experiments (Magnotherapy Inc., Rivera Beach, FL and Engineered Concepts, Birmingham, AL) each measuring ~3.5cm in diameter were scanned with a 2mm resolution over a 5cm² area, 2mm from the surface of the magnet. This separation distance corresponds to the distance between the surface of the magnet and the surface of the paw. During exposure, each magnet was positioned above the plantar surface of the left (treated) paw so that uniform portion of the field encompassed the entirety of paw. Magnetic fields penetrate tissue and bone without distortion, however the strength decreases as $\sim 1/d^2$ where d is the

distance from the core of the magnet (6). The thickness of a rat paw was measured to be 0.5cm, therefore the distribution of the magnetic field through the thickness of the paw was a gradient, each magnet varying in magnitude by approximately $x \cdot 0.25$ mT from the plantar to the dorsal surface of the paw, where x represents the strength at the plantar surface. Graphical representation of the two-dimensional projections (a, c) and three-dimensional surfaces (b, d) of the flux distribution on the plantar (a, b) and dorsal (c, d) surfaces of the paw for each of the magnets utilized are presented in Figure 1. Figure 1A depicts the 70mT field utilized in all but the dose response experiments and Figure 1B and 1C depict the magnets used for dose response determination.

Statistical Analysis. All statistical significance was determined by utilizing a 2-Way Analysis Of Variance (ANOVA) and post hoc comparisons with Tukey's test assuming a p-value < 0.05 to be statistically significant (Sigma-Stat Software, SPSS; Chicago, IL).

RESULTS

15 and 30 minute SMF application reduces histamine but not carrageenan induced edema

Figure 2 shows the average temporal inflammatory response as represented by percent volume increase from the measured pre-injection volume for CA and histamine treated paws exposed for 15 or 30 minutes to a 70mT SMF immediately after injection. These results demonstrate that carrageenan (CA) injection results in a more complex edema response, with a 70% maximum volume change, fully manifested 4 hours after injection, whereas histamine-induced inflammation results in a 30% maximal volume change that peaks 30 minutes post-injection. This deviation in volume and duration is due to the differing inflammatory cascades that are stimulated by CA vs. histamine.

Magnet application for 15 or 30 minutes did not consistently reduce the edema formation resulting from CA injection (Figure 2A, B respectively) whereas histamine-induced edema formation was significantly reduced by the 15 and the 30-minute magnet application but to varying degrees (Figure 2C, D respectively). Fifteen-minute magnetic field exposure significantly reduced histamine-induced edema formation by 40-65% at all but the final timepoint (Figure 2C). Thirty-minute exposure also reduced histamine-induced edema formation significantly (20-25%, Figure 2D) at all but the last two timepoints, but to a lesser extent than 15-minute exposure. Injection of CA vehicle control, saline, or histamine vehicle control, de-ionized water, resulted in no measurable change in paw volume (closed diamonds, Figure 2A-D).

In an effort to address the apparent discrepancy in efficacy between the 15 and 30 minute magnet treatment on histamine-induced edema, a protocol was adopted that allowed for investigation of the confounding effects of additional immobilization as well as additional anesthesia on the efficacy of magnet application. The animals were subjected to an additional 15-minutes of anesthesia immediately following the 15-minute magnet treatment, allowing for the comparison of the resulting edema with the 30-minute (anesthetized) sham and 30-minute magnet treated paws. Interestingly, the additional 15 minutes of anesthesia reduced the magnitude of edema reduction (15-30%, Figure 2E) to that of the 30 minute treatment. These results demonstrate that the additional anesthesia and/or immobilization reduces the originally observed additional efficacy of the 15 minute treatment over the 30 minute treatment.

A 2 hour SMF application reduces carrageenan induced-edema

To address the differing efficacy of magnet application in preventing edema formation in CA vs. histamine-induced edema, the CA dose was reduced by half (0.1ml of 0.5% CA as opposed to 0.1ml of 1%) inducing an increase in volume proportional to that of histamine. Application of the 70mT magnet for 15-minutes to this reduced magnitude CA-induced edema was not successful in significantly reducing the edema formation (Figure 2F). A shortened timeline with more frequent volume measurements was then adopted for an additional set of experiments to assess, with greater temporal resolution, the effect of magnet treatment on the edema formation with this reduced CA dose (Figure 2G). Again, no significant edema reduction was observed between sham and magnet treated paws. Finally, based on the previously presented data showing that application of the SMF for half the time to histamine-induced maximal edema (15 of the 30 minutes to max. edema formation) resulted in significant edema reduction, the SMF was applied for a 2-hour duration (2 of the 4 hours to max. CA-induced edema formation) to this reduced dose CA-induced edema. Interestingly, this 2 hour application resulted in a significant (33-37%) reduction in edema formation (Figure 2H).

SMF application is most effective when applied at the time of injury

Based on the data presented that a 15-minute magnet application is the most effective in reducing histamine-induced edema formation, investigation of the influence of magnetic field exposure on edema resolution was completed. Histamine treated animals were injected as previously described and the 70mT SMF was applied for 15 minutes at the time of maximal edema (30 minutes post-injection), resulting in no significant enhancement of edema resolution (Figure 3A). Furthermore, a 15-minute magnetic field pre-treatment applied just prior to

histamine injection also resulted in no significant reduction in edema formation or resolution (Figure 3B).

SMF efficacy is dose dependent

To address whether the reduction in histamine-induced edema formation was dose dependent, a dose-response experiment was completed via application of a 400mT and 10mT in addition to the 70mT field utilized for all previous experiments. Surprisingly, the 400mT field (open squares, Figure 4) did not reduce edema formation to any degree whereas the 10mT field (open triangles, Figure 4) significantly reduced the edema by 25-55% at all but the last timepoint, similarly to the 70mT field (40-65%, open circles, Figure 4). This data supports published assertions that there exists a “physiologic window” of effective magnetic field strengths (21;46). In light of this data, the remaining experiments were conducted utilizing the 70mT magnet.

SMF application may act via L-type Ca²⁺ channels

After establishing that the magnetic field application can significantly reduce histamine-induced edema formation, investigation of the mechanism of action was attempted via administration of pharmacologic agents in conjunction with histamine stimulation and magnetic field exposure. Utilization of histamine as the injected inflammatory mediator, as opposed to CA, allows for very specific investigation of the early phase of the acute inflammatory response as the cascade of events is better defined and can be manipulated pharmacologically.

Histamine stimulation results in a transient increase in microvascular permeability and leakage accompanied by vasodilation. The increase in permeability is thought to result from gap

formation in intracellular junction complexes (12;18;33;43;1) initiated by intracellular Ca^{2+} influx (7;38), which acts via action of phospholipase C and generation of inositol triphosphate. This flux can also initiate Ca^{2+} influx from the extracellular space (38) as well as liberate nitric oxide (NO), resulting in the endothelial mediated vasodilation characteristic to histamine stimulation (47), further exacerbating fluid exudation. Generation of NO and its action on guanylate cyclase to increase cGMP concentrations (22) and administration of L-arginine (19) have also been shown to contribute to histamine-induced increased permeability (15). While the histamine response is only one of the early phase mediators and therefore only one element of the acute inflammatory response per se, the isolation of this pathway minimizes the number of variables thus facilitating a more direct investigation of the mechanism(s) involved in magnet treatment.

Studies suggest that EMF exposure may activate NO production via activation of NOS resulting in modification of the vascular tone through cGMP pathways and influencing the downstream Ca^{2+} flux (23;24). Other work has suggested that SMF application may act via influence on Ca^{2+} dynamics and NOS to regulate blood pressure and local microvascular tone (28;29). Studies have also recorded an effect of SMF on Ca^{2+} dynamics in other, non-vascular cell types and systems (36;37;30;31;39), suggesting that application of a SMF may impact edema formation via influencing the Ca^{2+} signal directly or indirectly through the NO signaling cascade, resulting in modulated dilation and permeability.

Based on these findings, agonists of both nitric oxide synthesis and L-type calcium channel signaling were utilized in an effort to elucidate the mechanism by which SMF exposure significantly reduces induced edema. L-arginine, the substrate for nitric oxide synthesis, was co-administered sub-plantar with histamine (0.1ml) to potentiate edema formation as has been

shown previously (9). The 70mT magnetic field or sham was applied for 15 minutes and volumes were measured every 30 minutes for 3 hours (Figure 5A). Edema formation was significantly potentiated, 36-87%, by the co-administration of L-arginine + histamine + sham (closed squares, Figure 5A) vs. histamine + sham treatment alone (closed circles, Figure 5A). This potentiation was suppressed 25-35% by application of the magnetic field (open squares, Figure 5A), but the total volume level was not reduced to the level of histamine + magnet alone (open circles, Figure 5A). Injection of L-arginine in de-ionized water did result in a measurable increase in paw volume and this can possibly be attributed to the potentiation of the small histamine release in response to the injection itself.

Concurrent administration of Ca²⁺ channel agonist BAY K8644 (34) i.p. and histamine sub-plantar (Figure 5B) resulted in no significant change in the histamine swelling characteristics between the histamine + sham (closed circles, Figure 5B) and the histamine + BAY + sham (closed squares, Figure 5B). Interestingly, application of the magnetic field to the BAY treated animals (open squares, Figure 5B) did not elicit a significant decrease in edema similar to that previously observed in the histamine + magnet (open circles, Figure 5B) experiments.

DISCUSSION

This study demonstrates that SMF exposure significantly reduces induced edema in a time and dose dependent manner. Histamine-induced edema was significantly reduced by both a 15 and 30 minute SMF, whereas CA-induced edema required a 2 hour application to elicit a similar reduction. The effective dose duration appears to be related to the time to maximal edema formation. In the case of histamine-induced edema the maximally effective tested dose duration was 15 minutes, and in the CA-induced case it was 2 hours, both corresponding to 50%

of the time to maximal edema formation; suggesting that the SMF must be applied for a sufficient fraction of the edema-formation period to have a significant effect. This finding is supported by two existing studies evaluating effects of a PEMF to CA-induced edema which reported a significant decrease in paw volume with an exposure time of 3-4 hours (25;48). Additionally, we found that application of the field is required at the time of injury, as exposure before or after maximal edema formation yields no significant edema reduction.

It was also noted that the slopes of the recovery portion of the volume curves do not differ between sham and SMF treated groups and therefore it can be argued that the passive recoil of the tissue, lymphatic uptake, and venous reabsorption are not affected by the SMF application, as this would be manifested in a change in the rate of recovery. Taken together these results suggest that SMF treatment is not beneficial for the resolution of induced edema, but is useful in this model solely for the prevention of edema formation.

Whereas other studies have demonstrated physiological effects in tissues exposed to very high field strengths, on the order of 7-8T (9;16;35), it was found that a 400mT field did not have any influence on edema formation, suggesting that there may exist an upper limit of magnetic field strength that results in suppression of edema. The remaining two field strengths investigated, the 70mT field and 10mT field, were successful in reducing edema volume, but to different extents. The 70mT field reduced the edema formation to a greater degree than the 10mT field, but it was not 7 times more effective, suggesting that the response is non-linear and there may exist a saturation point, perhaps related to the available substrate for SMF action. Past studies have suggested that a lower limit of 1mT exists for eliciting a physiological affect from SMF application (21;46), but this is the first data suggesting a possible upper limit as well. The lower level may simply reflect the existence of a threshold level of activated substrate necessary

to elicit a measurable response. The fact that the response is completely abolished above the yet to be determined upper threshold might also suggest that some other response is activated that masks the desired response. The mechanistic basis for the existence of such a “physiological window”, however, requires further investigation.

Determination of potential mechanisms involved in the observed physiological responses to magnetic field exposure is ongoing, but no definitive mechanism or pathway has previously been identified. While studies have reported that magnetic field applications have decreased voltage-sensitive channel activation in GH3 cells (36), increased second messenger levels in human skin fibroblasts (30) and FNC-B4 neuronal cells (31), and increased microvessel dilation mediated by NO signaling (23), the results are mostly confounding as they are from different cell types in vitro and tissues in vivo with varying magnetic field dosages, durations and applied both locally and globally, thus complicating the assessment of therapeutic value. By locally applying the SMF in conjunction with pharmacological alteration of endothelial-dependent production of NO, or the Ca²⁺-induced contractile state in smooth muscle cells, we can begin to assess the mechanism(s) of SMF action and determine the validity of our hypotheses that SMF application might modulate the permeability and/or dilation resulting from histamine-induced edema.

Looking first at the results attained by agonizing NO production in conjunction with local SMF exposure, we find that although the magnitude of edema is elevated by increasing NO production, the degree of the edema reduction (the area encompassed between the magnet treated and sham curves, Figure 5A) is not altered, suggesting that the magnet acts independently of NO production. Conversely, we find that by agonizing L-type Ca²⁺ channels, we eliminate the edema reduction evoked by application of the SMF. Taken together, these results suggest that the SMF may act to open/activate L-type Ca²⁺ channels in VSMCs, increasing the intracellular

Ca²⁺ concentration, inducing constriction and therefore limiting edema formation. This data does not, however, give any insight into the additional Ca²⁺ handling mechanisms such as Ca²⁺ reuptake into the intracellular stores such as the sarcoplasmic reticulum (SR), which also influences the intracellular Ca²⁺ concentration. Additional studies utilizing pharmacological interventions targeted at intracellular stores need to be completed to solidify the argument that the SMF acts to increase the intracellular Ca²⁺ concentration. As active dilation occurs in a transient fashion during edema formation, this conclusion can possibly explain the time-sensitive nature of magnet application. However, in conjunction with the active dilation, permeability is also transiently regulated during edema formation and therefore further investigation of the direct effect of SMF application on permeability is warranted.

Furthermore, these conclusions provide a possible explanation regarding the observed greater SMF effect when applied to histamine-induced edema for 15 minutes versus 30 minutes. We found that 15 minutes of additional anesthetic immediately following the 15 minute application resulted in the same magnitude of edema reduction as a 30 minute application, suggesting that the additional time under anesthesia may be influencing the efficacy of the magnet. Since both the 15 minute and 30 minute sham exposures resulted in the same volume curves we can conclude that the additional anesthetic by itself does not influence the edema formation and therefore it may be interacting with the action of the SMF. As isoflurane is a known vasodilator (11), it is possible to conceive that this dilation competes with, in the case of 30 minute application, or reverses, in the case of 15 minute application followed by 15 minutes of additional anesthesia, the constriction induced by the magnet resulting in a smaller magnitude of edema reduction. Ideally, it would be best to conduct the SMF application experiments under conscious conditions as this eliminates any confounding affect of anesthesia, but immobilization

in this case was required to ensure that the magnet application was unvarying for each experiment. Additional experiments could address this possibility utilizing other techniques for conscious magnet application.

In conclusion, these studies are the first to demonstrate that acute, localized static magnetic field exposure of moderate field strength (5-100mT), when applied immediately after an inflammatory injury, can result in significant reduction of edema formation. One proposed mechanism of SMF action is through modulation of vascular tone via L-type Ca^{2+} channels in vascular smooth muscle. These results support the further study of acute application of static magnetic fields for the therapeutic treatment of vascular pathologies related to dysregulation of microvascular tone.

GRANTS

This work was supported by a grant from the National Institutes of Health (NCCAM) AT-00582

Figure 1. Graphical representation of the two-dimensional projections (a, c), and three-dimensional surfaces (b, d) of the magnetic field densities utilized. A) 70mT static magnetic field at the plantar (a, b) and dorsal (50mT) (c, d) surface of treated paws. B) 400mT static magnetic field at the plantar (a, b) and dorsal (250mT) (c, d) surface of treated paws. C) 10mT static magnetic field at the plantar (a, b) and dorsal (7.5mT) (c, d) surface of treated paws. Units are in mT.

Figure 2. SMF application reduces CA and histamine-induced edema with differing exposure durations. A, B) 15 and 30 minute SMF application, respectively, to CA-induced edema does not reduce edema formation. C, D) 15 and 30 minute 70mT SMF application, respectively, to histamine-induced edema significantly reduces edema formation. E) An additional 15 minutes of isoflurane accounts for the reduced efficacy of 30 min SMF application to histamine-induced edema when compared to the 15 minute 70mT application. F) 15 minute 70mT SMF application to a reduced dose CA-induced edema does not result in edema reduction, and G) greater measurement resolution of the early stage of CA-induced edema formation does not reveal a significant edema reduction resulting from a 15 minute 70mT SMF application. H) 2 hour 70mT SMF application to the reduced dose CA-induced edema results in a significant reduction in edema formation suggesting that the 15-minute application duration is insufficient to elicit a positive response with CA stimulus. ** = $p < .005$, * = $p < .05$ sham vs. magnet, ++ = $p < .005$, + = $p < .05$ Sham vs. magnet + sham. Data are means +/-S.E.

Figure 3. SMF application is most effective when applied at the time of injury. Application of the 70mT SMF for 15 minutes before, A) or at the time of maximal histamine-induced edema B) results in no significant edema reduction. Data are means +/-S.E.

Figure 4. Edema reduction by SMF application is dose dependent in histamine stimulated paws. Application of a 10mT and 70mT SMF for 15-minutes significantly reduced edema formation but application of a 400mT SMF had no effect on histamine-induced edema. ** = $p < .005$, sham vs. 70mT magnet, ++ = $p < .005$, + = $p < .05$ sham vs. 10mT. Data are means +/-S.E.

Figure 5. SMF application may act via L-type Ca^{2+} channels and not via NO signaling in histamine stimulated paws. A) Co-administration of L-arginine with histamine followed by a 15-minute 70mT SMF exposure resulted in a significant reduction in the L-arginine potentiated edema but not to the level of SMF treated histamine alone. B) Concurrent administration of BAYK8644 (i.p.) with histamine followed by a 70mT SMF exposure resulted in abolition of the initial edema reduction observed with SMF exposure. Data are means +/-S.E. ++ = $p < 0.005$ L-arg sham vs. sham, ## = $p < 0.005$, # = $p < 0.05$ L-arg sham vs. L-arg magnet.

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Figure 1









