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MAGNETIC FIELD ELICITS HYPOTENSION MEDIATED BY PLATELET ACTIVATING FACTOR IN RATS INJECTED WITH IRON BEADS

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SUMMARY: Rats injected intravenously with iron beads (avg. diameter 3.4 μm; 1 g/kg body weight) were exposed to static or time-varying magnetic fields (400 gauss) for 5 min, which elicited a marked and rapid decrease in the mean arterial blood pressure (52 ± 7 mmHg, mean ± SE), lasting for 1-2 h. Hypotension was prevented or reverted by the platelet activating factor (PAF) antagonist SRI 63-675. The release of PAF from iron-loaded phagocytes may be due to magneto-orientational effects on membranes. This novel magnetic bioeffect can also be used for the study of PAF-mediated circulatory shock.

The search for biological effects of static magnetic fields has yielded largely negative results. However, a small group of organisms, including the magnetotactic bacteria, have been demonstrated to be sensitive to the extremely weak geomagnetic field (for a review see ref. 1). In an attempt to perturb the function of the macrophage system by non-immunological means the phenomenon of magnetically elicited hypotension was observed in rats injected with iron beads. While the presence of iron in this model sets it apart from experiments dealing with intact organisms, the sensitizing effect of the iron beads may lead to the recognition of a more general mode of interaction between biological entities and magnetic fields.

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Abbreviation used: PAF, platelet activating factor.

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MATERIALS AND METHODS

Male Sprague-Dawley rats (250-300 g) were equipped with permanent arterial (carotid) and venous (jugular) catheters using aseptic procedures the day preceding an experiment. Carbonyl iron powder (99 % Fe, non-magnetic; Sigma, St. Louis, Mo) was examined by scanning electron microscopy, and the particles were found to be spherical with an average diameter of 3.4 μm. The iron beads (0.1 g/ml) were suspended in saline containing 2 M sucrose and injected slowly through the venous catheter (2). SRI 63-675, a water-soluble receptor antagonist of PAF (3) was dissolved in saline (1 mg/ml) and injected IV. Before the injection of iron, the rats were placed into a plexiglass tube (5.5 X 23 cm) the ends of which were closed with aluminium grids. The plexiglass tube was inserted into the electromagnet (see below). Arterial blood pressure was monitored continuously during the experiment. After a few minutes in the tube the rats became quiet and their blood pressure was stable.

Magnetic field was generated by using the stator of a three-phase motor from which the rotor was removed. A regulated power supply was used to feed AC (60 Hz) or DC (ripple <1mV) to one set of field coils of the stator. The plexiglass tube containing the rat was inserted horizontally into the circular opening of the stator and both were cooled by a fan, thus the tube was kept at room temperature. The magnetic field in the empty tube was mapped by using the axial and transversal Hall-effect probes of a gaussmeter (Model 4048, F. W. Bell, Orlando, Fl) in AC or DC mode. The lines of force were vertical, and the variation of flux density over the cross section of the tube (at the middle vertical plane in the stator) was less than 15%. Along the axis of the tube, the flux density was 100% at the center, decreasing to 50, 25 and 10% at 2.8, 5.0 and 7 cm from the center, respectively. The flux densities given for various experiments were measured at the center.

Statistical significance was calculated by Student's t test.

RESULTS AND DISCUSSION

The hypotensive effect of iron-magnet treatment. The changes in mean arterial blood pressure in response to an intravenous injection of iron powder suspension and the exposure of the midsection of the rat's body to a static magnetic field are shown in Fig. 1. The magnetic field (flux density 400 gauss, for 5 min) by itself had no effect on the blood pressure (Fig. 1, top panel). The injection of iron powder suspension (1 g iron/kg bw) caused a transient drop in blood pressure of about 20 mmHg, which was normalized in 15 min (Fig. 1, middle); a similarly small decrease was seen when the magnetic treatment preceded the injection of iron beads (not shown). However, when the iron administration (at 0 min) was followed by magnetic exposure (starting 20 min after the iron) the blood pressure of the rats decreased rapidly to about 70 mmHg (Fig. 1, bottom). The hypotension lasted for more than an hour, then the blood pressure slowly returned to near-normal values. The animals were resting quietly in the tube dur-

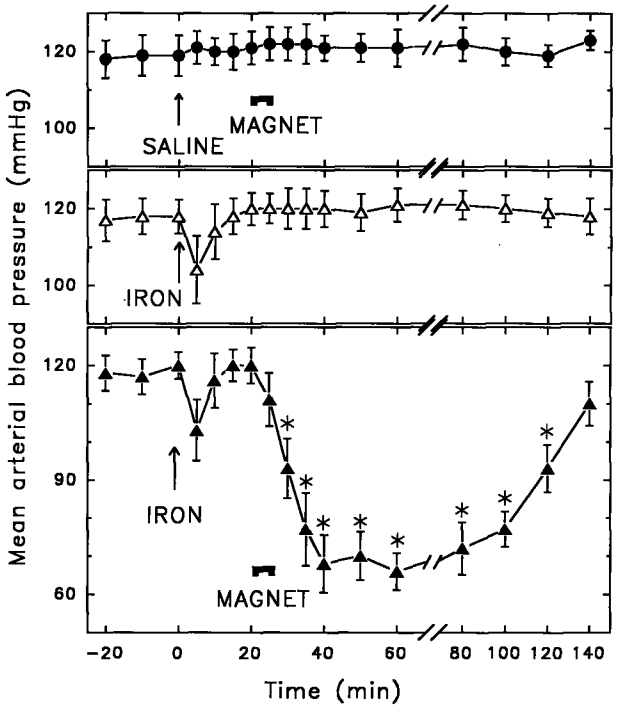

Fig. 1. Effect of iron-magnet treatment on the blood pressure of rats. Iron beads (1 g/kg; triangle symbols) or the suspending medium (circles) were injected intravenously at 0 min. Twenty min later the rats were exposed to a static magnetic field of 400 gauss centered at the midsection of the body for 5 min (filled symbols). Symbols show means $\pm$ SE, n=5; * indicates significant difference ($p<0.05$) as compared to time-matched values in rats not exposed to the magnetic field (middle panel).

ing the magnetic exposure. Rats removed from the tube during the hypotensive period were conscious but subdued and had difficulty walking. As the blood pressure returned to normal the rats became alert, too, and survived for a week at least. The same effect of iron-magnet treatment on behaviour was also observed in mice. Histological examination was performed in rats sacrificed 1 and 24 hours after iron-magnet treatment or iron administration only. Most iron beads were found in the macrophages of the spleen and liver (Kupffer cells), and many in the lung. A few beads were seen in kidney glomeruli. Beside occassional intestinal hemorrhage, light microscopy did not reveal any changes due to magnetic exposure.

No hypotension was caused when the magnetic field was centered at the upper (head and shoulders) or lower third of the rats body. Latex beads (1 μm diameter) did not sensitize the rats to the hypotensive effect of magnetic exposure (not shown). Thus,

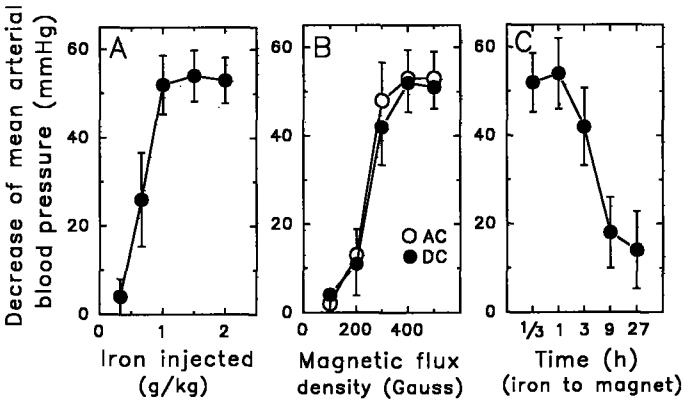

Fig. 2. Decrease of blood pressure in response to iron-magnet treatment. The experimental conditions described in Fig. 1 were varied with respect to the dose of iron (A), or the flux density of static (DC) or oscillating (sinusoidal, 60 Hz AC) magnetic field (B). The time elapsed between the injection of iron beads and the onset of the magnetic exposure was varied in panel C. Symbols indicate means ± SE, n=5.

hypotension resulted when the magnetic field acted on iron beads ingested by macrophages in the liver and spleen.

The parameters of the iron-magnet treatment. For an analysis of the experimental conditions leading to hypotension the parameters of the iron-magnet treatment (described in Fig. 1) were varied as indicated in Fig. 2., and the magnitude of the decrease in blood pressure was determined. Fig. 2A shows the hypotensive response with respect to the dose of iron. While about 0.6 g/kg iron was needed to sensitize the rats to the hypotension-inducing action of the magnetic field, pretreatment with 1 g/kg iron powder per kg body weight achieved the maximum effect.

The magnetic flux density necessary to induce a marked hypotension in iron-treated rats was 300 gauss (Fig. 2B); further increase of field strength enhanced the effect only slightly. Importantly, the effects of static (DC) and oscillating (60 Hz AC) magnetic fields were almost identical (Fig. 2B). It was also observed that the hypotensive effect of the static magnetic field was not diminished when the magnetic flux was increased gradually (from 0 to 400 gauss in 2 min, then 400 gauss for 5 min) as compared to the case when the 400 gauss magnetic field was switched on instantly. Therefore, the induction of electric currents is not likely to play a role. Further, the significance of magneto-mechanical movements of the beads appears to be negligible, too,

since no translational movement ("dragging") occurs in the sinusoidally ocillating field, and the spherical shape of the beads excludes rotation (or "prodding"; ref. 4,5).

The time elapsed between the administration of iron and the magnetic exposure ("iron to magnet" time, Fig. 2C) was critical with respect to the occurrence of hypotension. To allow for the clearance of the iron beads from the bloodstream, the earliest time point of magnetic exposure was 20 min after the administration of iron. A hypotensive response of similar magnitude was obtained 1 hr after iron injection. However, the hypotension was less marked when the magnetic exposure was delayed further, and it was totally absent in some rats on the next day (27 h in Fig. 2C). Further, the rats were refractory to a second magnetic exposure following a hypotensive iron-magnet treatment. When hypotension was elicited as shown in Fig.1 (bottom panel), a second magnetic exposure during the hypotensive period did not produce a further decrease of blood pressure. Moreover, no hypotension was provoked by a second magnetic exposure even after the normalization of blood pressure (not shown). The time window for the magnetic treatment to be effective can be explained by the dependence of the magnet-induced hypotension on a one-time, transient post-phagocytic event.

The role of platelet activating factor (PAF). Macrophages are known to increase the synthesis of a number of products following phagocytosis (6), from among which prostaglandins and platelet-activating factor (PAF) are capable of inducing systemic hypotension (7,8). PAF also induces intestinal necrosis (9). No palliative effect was achieved by the administration of indomethacin (5 mg/kg) 5, 30 or 60 min prior to magnetic exposure (not shown). However, the hypotension induced by the iron-magnet treatment was prevented and reverted by SRI 63-675, a specific receptor antagonist of PAF (3). Intravenous administration of SRI 63-675 five minutes before the magnetic exposure of the rats completely prevented the development of hypotension (Fig. 3, open squares). The drug was equally effective when administered before iron injection (not shown). Even after the development of hypotension due to iron-magnet treatment, the blood pressure increased rapidly when SRI 63-675 was injected, and returned to near-normal values in 10 minutes (Fig. 3, filled squares). Therefore, it was concluded that the hypotensive effect of the iron-magnet treatment is mediated by PAF.

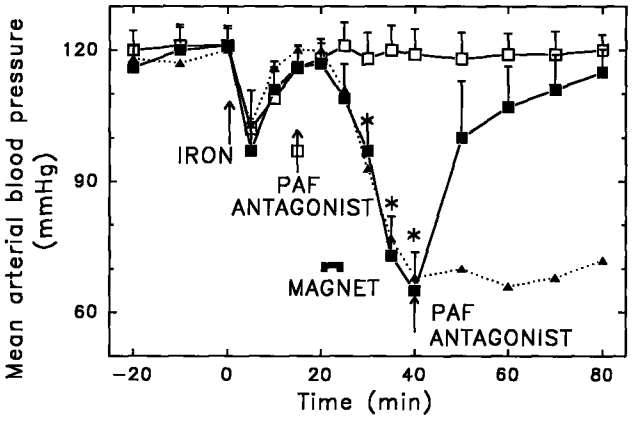

Fig. 3. Effect of the PAF antagonist SRI 63-675 on the hypotension elicited by iron-magnet treatment. Rats were treated as described in Fig. 1, and SRI 63-675 (3 mg/kg) was injected intravenously 5 min before (open squares) or 20 min after (filled squares) the onset of magnetic exposure. For comparison, the values from the bottom panel of Fig. 1 are also shown (triangles). Symbols show means ± SE, n=5; * indicates significant difference ($p<0.05$) as compared to time-matched values of rats not exposed to magnetic field shown in the middle panel of Fig. 1.

How can the magnetic field stimulate the release of PAF? It has been shown that the cells secrete only a fraction of the PAF that they produce (10,11,12). Leakage from the macrophages due to the motion of the beads, or an effect of induced currents were shown above to be unlikely under the conditions applied. A probable mechanism of PAF release might be connected with the the magneto-orientational effect of static magnetic fields. Anisotropic macromolecules (e.g. DNA) and ordered molecular assemblies, such as retinal rods and various biological membranes, orient themselves in strong magnetic fields (in the order of 10^4-10^5 gauss; ref. 13). There is a strongly suggestive relationship between the present observations and the findings of Liburdy, Tenforde and Magin (14), who elicited the rapid release of encapsulated cytosine arabinofuranoside (ARA-C) from large unilamellar phospholipid vesicles by exposing them to uniform static magnetic fields (>100 gauss) at temperatures approaching phase transition. The permeability effect showed a sigmoidal dependence on magnetic flux density; a similar sigmoid dependence of hypotension development on magnetic flux density was found in the present study (Fig. 2B). The rapid change in permeability has been attributed to the magnetic orientation of phospholipid domains, passing through an unstable state in which magnetic

forces produce boundary layer separations leading to the release of encapsulated solutes (1). A similar perturbation of membranes may elicit the release of PAF leading to hypotension, since most of the newly synthesized PAF remains bound to cellular lipid membranes (11,15,16). Alternatively, it may increase the availability of substrates for PAF synthesis or remodeling.

An important difference between earlier experiments using homogenous static magnetic fields (reviewed in 1) and the present model is the presence of iron beads in this system, which renders the animals susceptible to magnetic fields. Whereas tissues and cells do not distort the magnetic field considerably, the microscopic iron beads of high magnetic permeability concentrate the lines of force, creating high flux density and an extremely steep magnetic gradient in their vicinity (4), which may actually elicit the molecular events. In this respect there might be some similarity between the present model and the organisms containing magnetite crystals, notably the magnetotactic bacteria (17). While these inclusions aid the perception of weak magnetic fields, it is not clear how this information is processed by the cells (18).

Though the interactions between biomolecules and magnetic fields in the presence of intracellular iron beads are not clear yet, the involvement of the cellular membranes of macrophages appears likely in this novel model of magnetically induced hypotension. Finally, this phenomenon could be applied as a "remote-control" model for the study of PAF-mediated circulatory shock states, including anaphylactic, traumatic, hemorrhagic, endotoxic and septic shock (8).

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