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Thermal analgesia induced by 30-min exposure to 1 μT burst-firing magnetic fields is strongly enhanced in a dose-dependent manner by the α_2 agonist clonidine in rats

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Abstract

Most of the research concerning analgesia following brief exposures to physiologically patterned weak magnetic fields has focused upon their morphine-related properties. However, the α-adrenergic system interacts with morphine-induced analgesia. In the present study we found that prazosin, phenylephrine, and yohimbine did not augment the robust analgesia to thermal stimuli in rats evoked by whole-body exposures to a 1 μT, burst-firing magnetic field presented once every 4 s for 30 min. However, the α_2 agonist clonidine enhanced the field-induced analgesia in a dose-dependent manner that reflected a receptor-saturation response. Potentiation between the field and clonidine was evident at 0.2 mg/kg and approached asymptote at 1 mg/kg. The combination of the effects from exposure to the magnetic field and the clonidine explained more than 75% of the variance in the change in nociceptive thresholds from baseline levels. The possibility that properly patterned weak magnetic fields could be a powerful adjunct to pharmacological treatments of pain is considered.

Keywords: α_2-Adrenergic receptor; Clonidine; Burst-firing; Thermal stimuli; Yohimbine; Prazosin; Phenylephrine

For the past decade most researchers studying the potential analgesic effects from physiologically patterned weak magnetic fields have focused upon the opioid system [2,6–8]. Extremely low frequency patterns of magnetic fields have been shown to induce levels of analgesia that were similar to specific dosages of morphine [2,7,8]. The analgesic effects have been blocked totally or partially by some antagonists of the μ receptor such as naloxone [2].

Activations of opioid and α_2 receptors inhibit transmission of nociceptive responses at spinal and supraspinal levels [4]. There is evidence that stimulation of the α_2- and α_1-adrenergic receptor may augment opioid-induced analgesia [12,14]. In the present experiments, we examined the possibility that another receptor system known to affect analgesia, the α_2 receptors of the adrenergic family of receptors, could be responsible for the analgesia produced by specific physiologically patterned magnetic fields.

One hundred and four, 8–12-month-old Wistar albino male rats that had been obtained from Charles River (Quebec) served as subjects in two major blocks of experiments. All rats were treated in accordance with the guidelines for the Canadian Council of Animal Care. The rats were housed three per cage in standard wire metal cages within standard rooms whose temperatures were maintained at 20 ± 1 °C. The light–dark cycle was 12 h:12 h with the onset of light at 07:30 h local time. Food and water were available ad libitum. All testing occurred during the early mid-light phase, between 08:00 and 12:00 h.

Each rat was tested on an Omnitech thermal plate whose temperature was maintained at 55 °C. The apparatus (26 cm × 26 cm) was enclosed within a Plexiglas chamber (18 cm high) so the rat could not escape. The rat was removed from the chamber immediately after two consecutive licks of either hind foot or a maximum of 60 s had elapsed (in order to minimize tissue damage). Each rat was tested three times each day for two consecutive days. The first trial, defined as the baseline trial, was followed 30 min (after 30 min of treatment) later by a second trial. A third trial was given 30 min later or 30 min after the cessation of the magnetic

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field treatment. To employ each rat as its own control, the latencies in seconds to respond for the second and third trials were subtracted from the latency of the first trial. The procedure was repeated for the second day.

Exposures to the chamber within which the magnetic fields were generated were conducted in a room that was not associated with testing or housing. An IBM XT computer was employed to operate software (complex-2© S.A. Koren) that converted a column of 230 numbers between 0 and 255 by a custom-constructed digital-to-analogue converter to voltages between −5 and +5 V. Neutral polarity was represented as the value 127. The graphical representation of the field, with the values between −5 and +5 plotted along the vertical axis and the order of the values down the column plotted along the horizontal axis, showed a wave form that imitated the burst-firing characteristics of limbic neurons [13]. The duration of each number or point was 3 ms and the duration of the entire pattern was 690 ms. It was presented once every 4 s for 30 min. The graphic pattern has been shown elsewhere [13]. The specifics of the field strengths within the exposure volume are also presented elsewhere [10]. The median value in the central 85% of the volume within which the rat ambulated freely was between 500 nT and 1 μT.

Four-way analyses of variance with two within subject levels (repeated measures for two trials and two days) and two between subject levels (drug and field conditions) were completed for all experiments. Post hoc tests included Tukey's ($P < 0.05$) and correlated *t*-tests where appropriate. All analyses employed SPSS software for a VAX 4000 computer. Omega-squared estimates, which reflect the amounts of variance in the changes in response latencies explained by treatments, were calculated to represent effect size.

In experiment 1, the potential interactions between drugs that affect the α_1- and α_2-adrenergic receptors and the magnetic field-induced analgesia were measured. Immediately following the baseline measurements, rats (four to six rats/treatment) were injected subcutaneously with either 5 mg/kg of the α_1 agonist phenylephrine, 0.5 mg/kg of the α_1 antagonist prazosin, 5 mg/kg of the α_2 agonist clonidine, 2 mg/kg of α_2 the antagonist yohimbine, or physiological saline (0.9%, 1 ml/kg). Dosages were selected on the bases of our pilot studies and the results of published research [12,14]. All rats were randomly assigned to both magnetic field and drug conditions.

The results are shown in Fig. 1. Analysis of variance showed a statistically significant difference in the change in response latencies as a function of the type of drug that was injected [$F(4, 30) = 40.49$, $P < 0.001$; $\omega^2 = 74\%$] and exposure or non-exposure to the magnetic field [$F(1, 30)=14.7$, $P < 0.001$; $\omega^2 = 7\%$]. A statistically significant two-way interaction was found between drug treatment and magnetic field treatment [$F(4, 30) = 3.27$, $P < 0.05$; $\omega^2 = 6\%$].

Post hoc analyses indicated: (1) the rats that received the clonidine and were exposed to a sham field condition exhibited significantly greater analgesia than the rats exposed to the sham field and injected with either saline, prasozin, yohimbine, or phenylephrine, and (2) all of the groups exposed to the magnetic field conditions exhibited stronger analgesia than the rats exposed to the sham field and saline conditions. The rats exposed to the burst-firing field *and* the clonidine exhibited twice the latency to respond than those that received the clonidine and the sham field. The rats receiving the yohimbine exhibited a mild but statistically significant analgesia compared to controls but only after the field had been removed for 30 min.

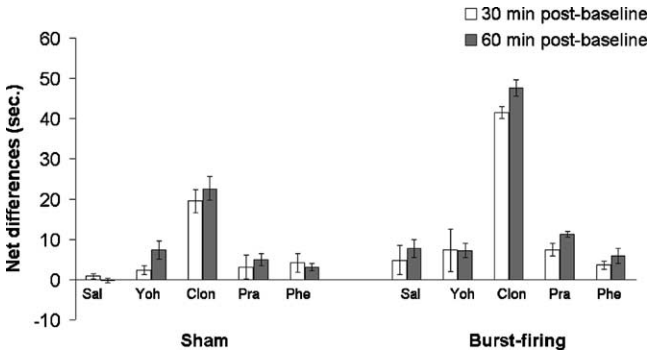

Fig. 1. Means and standard errors of the mean (vertical bars) for the net differences in response latency between baseline and after treatment for rats receiving one of the α_1- or α_2-adrenergic agonists or antagonists and exposed either to sham field or to the magnetic field (burst presented every 4 s). $N = 4$–6/group.

Based upon the results of the first block of experiments, the dose-dependent curve for the interaction between the magnetic field-induced analgesia and clonidine was established in experiment 2. After baseline measurements were taken rats were injected subcutaneously with one of the following dosages of clonidine: 0.1, 0.2, 0.5, 0.9, 1.0, or 5.0 mg/kg. Another group received saline. All rats were randomly assigned to both magnetic field and drug conditions. There were four to six rats per treatment condition. Figs. 2 and 3 show the response latencies 30 min after the injection of the drug and exposure to the magnetic field and 60 min after the injection of the drug and 30 min after the termination of the field. To facilitate presentation of the effect scaling of the horizontal axis (dosages) was not adjusted in order to enhance the inflection in the response curve.

The increasing dosages of clonidine were associated with a systematic increase in the thresholds to respond to the thermal stimuli [$F(6, 50) = 54.48$, $P < 0.001$; $\omega^2 = 64\%$]. Rats exposed to the magnetic field also displayed systematically higher thresholds compared to those exposed to the sham fields [$F(1, 50) = 92.02$, $P < 0.001$; $\omega^2 = 16\%$]. There was a strong and statistically significant interaction between the dosages of clonidine and the application of the magnetic field [$F(6, 50) = 7.03$, $P < 0.001$; $\omega^2 = 9\%$]. The profile of the analgesic responses for the different groups did not differ during the second day of testing except that all groups showed mild but statistically significant ($P < 0.05$) reductions in their response latencies.

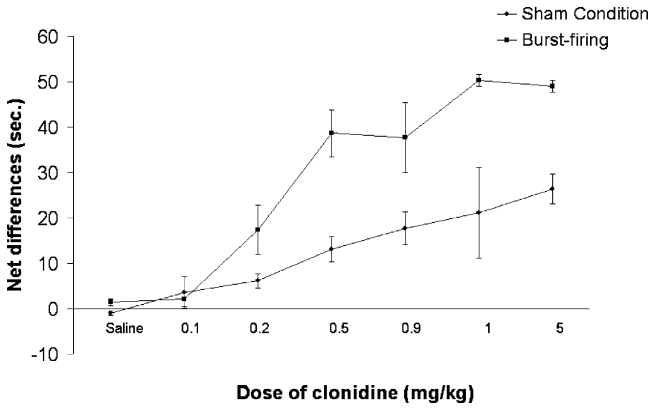

Fig. 2. Means and standard errors of the mean (vertical bars) for the net differences in response latency between baseline and 30 min after treatment for rats receiving either physiological saline or one of several dosages of clonidine and exposed either to the sham field or to the magnetic field. $N = 4$–6/group.

Post hoc results for the first day of treatment indicated that the source of the interaction was the increased slope in analgesia for rats that had been exposed to the magnetic field and had received any of the dosages (0.2–5.0 mg/kg) above 0.1 mg/kg. The greatest increase in the slope for the stronger analgesic effects from the combination of magnetic field and clonidine treatments occurred for dosages of 0.2 and 0.5 mg/kg. Higher dosages did not increase the elevated response latency whose levels approached the value required for removal of the rats from the testing according to humanitarian criteria. This effect was evident immediately after the removal from the field (Fig. 2) and 30 min after removal from the field (Fig. 3).

The results of the current study replicates previous studies demonstrating that 30-min exposure to a particular temporally patterned magnetic field whose intensity ranged between 500 nT and 1 μT can elevate the threshold of response to thermal stimuli [2,7,8]. The delayed response latency reflects analgesia rather than inhibited ambulation [3]. The initial concept of whole-body application of a burst-firing field once every 4 s to the organism was derived from the work of Liebeskind and coworkers in 1980 [9] who had found that foot shock delivered once every 4 s rather than continuously remarkably enhanced analgesic responding.

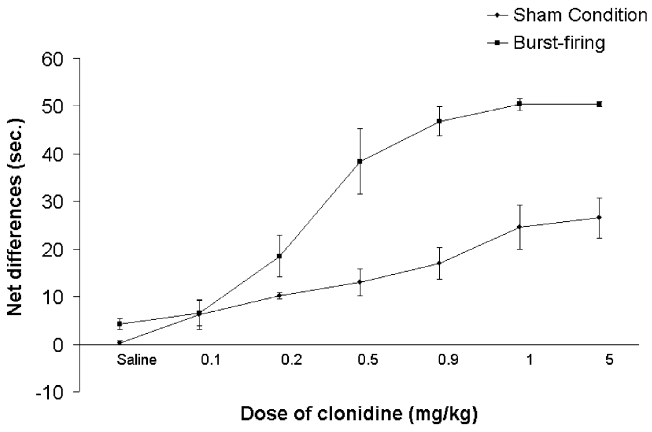

Fig. 3. Means and standard errors of the mean (vertical bars) for the net differences in response latency between baseline and 30 min after cessation of the 30-min treatment for rats receiving either physiological saline or one of several dosages of clonidine and exposed either to the sham field or to the magnetic field. $N = 4$–6/group.

The most novel result of this study is that small and clinically relevant dosages of the α_2 agonist clonidine, which has been shown to significantly increase morphine-induced analgesia [12], interacted with the application of the temporally patterned magnetic field to produce a powerful analgesic effect. The magnitude of the absolute effect, in terms of response latencies (in second), was greater than a single dosage of 8 mg/kg of morphine [1]. In fact the analgesic effect was so robust that most of the rats that received the magnetic field and dosages of clonidine above 0.5 mg/kg were removed from the hot plate to prevent the risk of tissue damage.

The α_2 antagonist yohimbine at the dosage employed in this study has been shown to decrease morphine-induced analgesia and elicit hyperalgesia in Lewis and Fischer 344 rats [5]. However, the administration of the α_2-adrenergic antagonist did not decrease the analgesic response and did not interact with the effects from the exposure to the magnetic field. The administration of yohimbine in attempts to block the clonidine-induced analgesia remains ambiguous. When yohimbine was administered to counteract the effects of clonidine, the variability in thermal latencies was so great that there were no statistically significant differences between saline-injected rats and those injected with yohimbine and clonidine. The means and standard deviations for the net thermal increases were 4.21 (10.64) for the saline injected rats and 17.51 (12.13) for the yohimbine and clonidine rats.

The fact that yohimbine did not block the clonidine-induced analgesia may arise from two possibilities: (1) the dosage of yohimbine was too weak to block the effects of clonidine fully or (2) the specificity of clonidine for the α_{2c} receptor was too great for a non-specific antagonist such as yohimbine to block the analgesic effects. The explanation is most likely a combination of these two processes. Interestingly enough, yohimbine did not block the analgesia induced by the burst-firing magnetic field on its own and this may indicate that a more complex phenomenon is occurring rather than a simple receptor-mediated process. The dosage of the α_1-adrenergic receptor antagonist prazosin, which has been shown to facilitate morphine-induced analgesia in a dose-dependent manner [12], also did not affect the magnetic field-induced analgesia. The α_1 agonist phenylephrine, which was not effective, was included for methodology symmetry and because analgesic properties had been variable [14,15].

The ogive or S-shape of the response curve for analgesia for the groups of rats that received the magnetic field plus the increasing dosages of clonidine could be argued to reflect the saturation of a specific receptor. This particular

dose-dependent shape for groups given both the drug and the magnetic field might also be considered typical of a high-affinity receptor rather than a non-specific or low-affinity receptor considering the range of concentrations of clonidine that produced the minimum to maximum change.

Close inspection of Figs. 2 and 3 reveals that the application of the magnetic field for rats receiving 0.2 mg/kg of clonidine was equivalent to rats receiving five times (1 mg/kg) the concentration of this compound without the field. For the rats that received the field plus dosages of 0.9 mg/kg or higher, the effect of the field would be equivalent to dosages above 10 mg/kg of clonidine assuming the trend shown for the sham-field group continued beyond the ones we tested. That the enhanced analgesia was an artifact of clonidine-induced immobility is not likely [3].

The mechanism by which this particular magnetic field and different dosages of clonidine interacted cannot be determined from these experiments. The intracellular effects of α_2 receptors appear to be primarily mediated by adenylyl cyclase. We have calculated that the electric field induced within the rat from the rate of change generated in our computer-generated, physiologically patterned magnetic fields would be sufficient to produce electric currents in the order of nA assuming the conductivity of extracelluar fluid is about 50 Ω cm [11]. Whether or not the particular temporal pattern of small current intensities induced by the field would be sufficient to alter the configuration of the protein comprising the specific α_2-adrenergic receptor, thus enhancing its binding capacity for clonidine, must still be investigated.

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