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# THE BEHAVIOURAL DEPRESSION OF HIPPOCAMPAL KINDLED RATS IS ATTENUATED BY SUBCUTANEOUS AND INTRACEREBROVENTRICULAR NALTREXONE

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## Abstract

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1. Two questions were asked: Does naltrexone attenuate the behavioural depression (BD) in other models of limbic epilepsy besides amygdala kindling? Does intracerebroventricular (ICV) administration produce the same effects as subcutaneous injection, i.e., attenuation of the BD.
2. Male wistar rats with bipolar electrodes implanted bilaterally in the dorsal hippocampus and a metal cannula in the lateral ventricle were kindled through 1 electrode and EEG recorded through the contralateral electrode.
3. Subcutaneous (sc) and ICV naltrexone administration attenuated the BD of hippocampal kindled rats.
4. These results further implicate the brain opioid system in the postictal phase of kindling and possibly epilepsy.

Keywords: amygdala kindling, behavioural depression, epilepsy, hippocampal kindling, naltrexone, opiate receptors

Abbreviations: after-discharge (AD), behavioural depression (BD), electroencephalographic (EEG), intracerebroventricular (ICV), subcutaneous (sc)

## Introduction

Kindling is an experimental model for limbic epilepsy. It entails the administration of one low amperage electrical stimulation per day, to a limbic structure. It is characterized by the gradual lengthening of the evoked after-discharge (AD) with the accompanying appearance of behavioural components such as immobility, head nodding and chewing, and finally a tonic-clonic convulsion. This is the ictal phase. There is also a postictal phase following the convulsion in which the animal is temporarily torpid or

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immobile. During this phase, which has been termed postictal depression or behavioural depression, the animal displays drastically reduced responsiveness to environmental stimuli. Frenk et al. (1979) were able to eliminate this phase or reduce its duration by pretreating the animals with the opiate receptor antagonist naloxone. Specifically, rats injected with 10 mg/kg naloxone before the kindling session got up and started walking around immediately or soon after the convulsion terminated. Thus, Frenk et al. proposed that there is an opioid component to this postictal phase. This result has now been replicated several times in amygdala-kindled rats pretreated with naloxone or naltrexone (Stone et al., 1982; Jarvis & Freeman, 1983).

We were interested in whether this result was generalizable to other limbic epilepsy models and in particular to one with a more pronounced and well-defined BD. We chose to study hippocampal kindling as it has a prominent BD of 3-10 min. Secondly we asked whether central, as well as peripheral, administration would be effective. Naltrexone was chosen as the opiate receptor antagonist because it has a longer half life than naloxone, which means that it is active 1 hour post injection. We felt that a 1 hr injection-experiment interval was better for the ICV experiment as it is very hard to obtain stable convulsion parameters from hippocampal kindled rats injected ICV at 30 min or less (Cottrell, 1983 personal observation).

## Methods

Animals and surgery. Male wistar rats bred in the Rudolf Magnus Institute were used in these experiments. The rats were anesthetized with Hypnorm and implanted with bipolar electrodes made of twisted, nichrome-coated stainless-steel wire (0.15 mm). There were 3 types of implants: one with bilateral amygdala electrodes (incisor bar at 0, AP -1.5 from bregma, L ±4.4, V -8.3 mm), one with bilateral dorsal hippocampal electrodes (AP -2.4, L ±2.0, V -3.7 mm) and one with bilateral dorsal hippocampal electrodes (see above) and a lateral ventricle cannula on the side of the stimulating electrode (AP +0.5, L +1.5, V -4.8 mm, tilted 10° anteriorally). The rats were housed individually in a room with a 14:10 LD cycle and allowed a minimum of one week for recovery.

Kindling procedure. The rats were stimulated for one second, once per day through the left electrode. The stimulation (60 Hz, 1 msec bipolar square wave pulses) was provided by a 2-channel Grass S88 stimulator through 2 stimulus isolation units. The stimulus intensity was set at near-threshold for AD-elicitation for each rat. EEG was recorded through the contralateral electrode in order to determine the duration of the electric after-discharge. Behavioural measures were simultaneously noted. Animals were considered kindled after exhibiting convulsions on 4 consecutive days. The threshold stimulus intensity which would evoke reliable seizures was then determined and used in subsequent test sessions. For the three groups the stimulus intensity ranged from 2.25-8.25 ($\bar{x}$=3.9), 1.25-5.00 ($\bar{x}$=2.3) and 1.75-8.25 ($\bar{x}$=3.4) volts, respectively.

Injection procedure. Physiological saline was injected 1 hr before the kindling session, sc or ICV for 2-3 days until the rat exhibited stable AD and BD durations. Naltrexone (ENDO, New York) was then injected. The sc injections were administered in a volume of 0.5 ml, the ICV injections in 1 µl saline. Saline was injected for 2 days following the naltrexone administration. All kindling sessions were done at approximately the same time each day.

Histological analysis. At the end of the experiment standard histological techniques were used to verify that the electrode sites were within the amygdaloid complex or dorsal hippocampus and that the cannula was within the lateral ventricle.

Statistical analysis. Data were analyzed for mean duration of AD and BD (BD was defined as the number of minutes taken by the rat to resume walking about the test cage after termination of the convulsion). Saline baseline scores were calculated and each animals' naltrexone score was compared against its own baseline score: Naltrexone AD or BD - Saline AD or BD = Δ Min Score. Statistical analyis was performed by paired t-tests.

## Results

The results of the first experiment - the effect of sc naltrexone on amygdaloid kindled seizures - are presented in Table 1. In this experiment, we replicated the finding that naltrexone significantly ($t = -2.557$, $p<0.05$) attenuates the duration of the BD and at a lower concentration (0.48 mg/kg) than previously used (10 mg/kg). There was no effect on the duration of the AD.

Table 1

Effect of sc Naltrexone on AD and BD Duration in Amygdala Kindled Rats

| Naltrexone[b] | n[c] | Δ Min Score[a] AD | BD |
|---|---|---|---|
| 0.48 | 11 | -0.176 ± 0.173 | -0.742 ± 0.290* |
| 0.24 | 10 | -0.103 ± 0.099 | -0.443 ± 0.631 |

[a]Difference score is presented in minutes ± S.E.
[b]dose in mg/kg was injected sc 1 hr before the kindling session
[c]n = number of rats; *p<0.05 (paired t-test)

In the second experiment, the effects of sc naltrexone on hippocampal kindled seizures was tested. As can be seen from Table 2, the BD of hippocampal kindled rats was quite sensitive to naltrexone. The duration was significantly attenuated down to a dose of 0.06 mg/kg. There was no effect on the duration of the AD.

Table 2

Effect of sc Naltrexone on Duration of AD and BD in Hippocampal Kindled Rats

| Naltrexone[b] | n[c] | Δ Min Score[a] AD | BD |
|---|---|---|---|
| 0.48 | 10 | -0.113 ± 0.136 | -2.747 ± 1.020** |
| 0.24 | 9 | -0.184 ± 0.145 | -1.473 ± 0.317* |
| 0.12 | 9 | -0.036 ± 0.045 | -1.601 ± 0.658** |
| 0.06 | 13 | -0.162 ± 0.086 | -1.364 ± 0.319 |
| 0.002 | 14 | -0.086 ± 0.108 | -0.261 ± 0.489 |

[a]Difference score is presented in minutes ± S.E.
[b]dose in mg/kg was injected sc 1 hr prior to kindling session
[c]n = number of rats; *p<0.05, **p<0.01

The results of the third experiment - the effect of ICV naltrexone on hippocampal kindled seizures - are presented in Table 3. ICV naltrexone had no effect on AD duration, however at a dose of 1 µg the BD was significantly attenuated ($t = -3.478$, $p<0.05$).

Table 3

Effect of ICV Naltrexone on Duration of AD and BD in Hippocampal Kindled Rats

| Naltrexone[b] | n[c] | Δ Min Score[a] AD | BD |
|---|---|---|---|
| 1.0 | 7 | -0.017 ± 0.067 | -1.600 ± 0.460* |
| 0.1 | 10 | -0.020 ± 0.055 | -0.705 ± 0.625 |

[a]Difference score is presented in minutes ± S.E/.
[b]dose in µg/kg was injected ICV 1 hr prior to the kindling session
[c]n = number of rats; *p<0.05

## Conclusions

The opiate receptor antagonist naltrexone attenuates the BD following amygdala kindled seizures and hippocampal kindled seizures. This suggests that it is not the site of action that is important but rather the phenomenon of BD and that the endogenous opioid system is involved in this postictal stage of seizures. Quite low doses were effective: 0.48 mg/kg and 0.06 mg/kg for amygdala and hippocampal seizures, respectively. The BD of a hippocampal kindled seizure was more sensitive than the BD of a amygdala kindled seizure; i.e., a lower dose was effective. Whether this sensitivity result is related to the differences between the two types of BD's or to the site of the foci is unknown at this time. ICV administration of naltrexone was also effective: 1 µg significantly attenuated the BD. This implicates - perhaps more directly - a role for the brain opioid system in the postictal stage of kindled seizures and thus perhaps in limbic epilepsy.

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