

## BRIEF COMMUNICATION

# Quaternary Naltrexone Reveals the Central Mediation of Conditional Opioid Analgesia<sup>1</sup>

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CALCAGNETTI, D J, F J HELMSTETTER AND M S FANSELOW *Quaternary naltrexone reveals the central mediation of conditional opioid analgesia* PHARMACOL BIOCHEM BEHAV 27(3) 529-531, 1987 — Earlier research has demonstrated that when rats are placed in a context associated with mild electric shock (1 mA/0.75 sec), environmental cues alone can produce conditional analgesia that suppresses pain sensitivity on the formalin test. This analgesia appears to be mediated by endogenous opioids since it is reversed by the centrally active opioid antagonists naloxone and naltrexone. Two experiments attempted to determine if peripheral or central opioids mediate this analgesia by employing quaternary naltrexone (QNTX), an antagonist which does not readily penetrate the blood-brain barrier at moderate doses. Intracerebroventricularly administered QNTX (10 µg) significantly reversed conditional analgesia, whereas intraperitoneally injected QNTX did not affect formalin-induced behavior. These results suggest that the conditional analgesia produced by our procedures is mediated by central, not peripheral, opioid mechanism(s).

Intracerebroventricular Quaternary naltrexone	Conditional analgesia Endogenous opioids	Stress-induced analgesia Pavlovian conditioning	Formalin test
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A central prediction of the Perceptual-Defensive-Recuperative (PDR) model, proposed by Bolles and Fanselow (1980), is that originally neutral stimuli that have become associated with a painful event through Pavlovian conditioning will acquire the ability to engage a defensive behavior system referred to as fear [2]. Activation of this system produces species specific defensive responses (such as freezing) and a reduction of sensitivity to nociceptive stimuli (analgesia). In more operational terms, it has been demonstrated that if a rat is placed in a context that was associated with mild electric footshock the situational cues alone can produce a naloxone reversible conditional analgesia capable of suppressing pain related behaviors on the formalin test [6,8]. Earlier research has demonstrated that this analgesia is mediated, at least partially, by endogenous opioids since intraperitoneal (IP) injections of the opioid antagonists, naloxone (NX, 10 mg/kg) [6] and naltrexone (NTX, 7 mg/kg) [8], eliminate the suppression of formalin-induced recuperative behavior thus reversing conditional analgesia. However, it is unknown if peripheral or central opioid mechanisms are primarily responsible for conditional analgesia.

The present experiments attempted to address this issue

by using naltrexone methobromide (QNTX), a quaternary form of naltrexone that does not readily penetrate the blood-brain barrier when administered in moderate doses [3]. If blockade of peripheral opioid receptors, using this quaternary antagonist, fails to reverse conditional analgesia, then a central opioid mechanism is suggested. In Experiment 1, QNTX was administered IP to block peripheral opioid receptors. To provide simultaneous indices of pain sensitivity and fear responses we employed the formalin test [4,9]. The doses were selected based on prior research using this drug in aversive learning situations [12].

## EXPERIMENT 1

## METHOD

*Subjects*

The subjects were 22 female rats of the Long Evans strain raised in the Dartmouth Psychology Department colony. All subjects were 180 days old at the time of testing. The rats were maintained in a colony room (14/10 hr light/dark cycle, with dark onset at 7:00 p.m.), individually housed in hanging stainless steel cages, and provided with ad lib food (Prolab

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3000) and tap water. They were trained and tested in an isolated room maintained at 23°C during the last two hours of their light cycle.

### Procedure and Apparatus

After five days of adaptation to transportation and handling, conditioning took place in one of four observation chambers (23.5 × 29 × 19.5). The chambers were illuminated with a 7.5 W white light bulb which allowed the experimenter to observe the subjects' behavior through a 30 × 30 cm clear plastic window in the front wall of the sound attenuating chest. Ventilation fans were adjusted to provide background noise at 73 dB (C scale). The chambers were cleaned after each rat with a 5% ammonium hydroxide solution. Two hr prior to dark onset on the training day each rat was placed into its assigned chamber. Four min after placement in the chamber each rat received a total of three 1 mA/0.75 sec footshocks spaced 20 sec apart. The rats were removed 20 sec after the last shock and returned to their home cage for about 24 hr.

On the next day (test day) pain sensitivity was measured with the formalin test [5]. Each rat was injected subcutaneously (0.05 ml) under the dorsal surface of the right hind paw with 15% formalin in saline (SA). Five min later the rats were randomly assigned to one of four drug groups and injected IP with 0, 3, 10 and 30 mg/kg of QNTX (a gift of Dr. H. Merz, Boehringer-Ingelheim) and returned to the home cage. Fifteen min after drug injection (thus 20 min after formalin) the rats were placed in the chamber in which they received shock the day before and their behavior scored for 8 min with a time sampling procedure [8]. Every 8 sec each rat's behavior was scored as belonging to one of the four following categories: (1) *Freezing*—defined as complete body immobility except for those required by respiration, (2) *Paw Lifting*—in which the animal raises and holds the formalin treated paw elevated and close to its body, (3) *Paw Licking*—defined as any licking or contact of the treated paw with the animal's mouth, (4) *Activity*—all other behaviors, such as locomotion and grooming, were scored as general activity [6]. Statistical analyses were performed by overall one way ANOVA followed, when appropriate, by linear trend analyses. No shock was administered on this test day.

### RESULTS

The percentage of total samples was determined for the categories of recuperation (defined as the sum of paw lick and paw lift scores) and freezing. These data were subjected to separate one way ANOVA's. No significant differences were found for recuperation,  $F(3,18)=1.2, p=0.32$ , or freezing,  $F(3,18)=1.83, p=0.18$ . The percent recuperative means for peripherally administered doses of QNTX (0, 3, 10 and 30 mg/kg) were 3.4 (SD 3.29,  $n=5$ ), 0 (SD 0,  $n=5$ ), 10 (SD 17.37,  $n=6$ ) and 1.2 (SD 2.04,  $n=6$ ) respectively. Since none of the animals in the 3 mg/kg group showed any recuperative behavior the assumption of homogeneity of variances for the ANOVA was violated. Therefore, we performed a supplemental analysis using the Kruskal-Wallis test. The results with this nonparametric test confirmed the results with the ANOVA in that there was no reliable between groups difference ( $H=5.02, p=0.17$ ). Thus peripherally administered QNTX did not significantly affect conditional analgesia at the doses tested using these procedures.

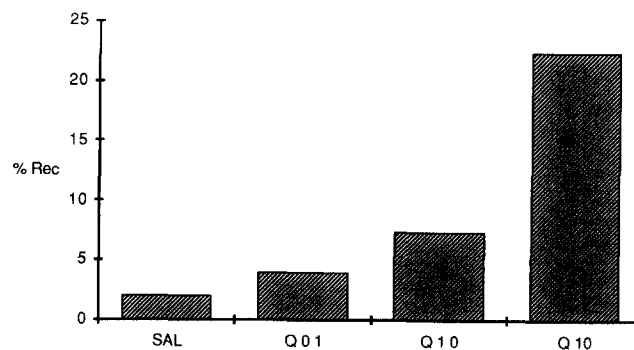


FIG 1 Shows the mean percent recuperation scores for four ICV doses of QNTX (0, 0.1, 1 and 10 µg/rat)

### EXPERIMENT 2

Experiment 1 demonstrated that IP QNTX failed to suppress conditional analgesia. Since the centrally and peripherally active tertiary form of naltrexone does attenuate conditional analgesia, this result suggests that peripheral opioid receptors are not involved in conditional analgesia. However, the possibility exists that QNTX did not work because its unique molecular structure resulted in a compound with less receptor affinity than tertiary naltrexone. To further test the hypothesis that the effects of naloxone and naltrexone upon recuperation are indeed centrally mediated, we administered QNTX intracerebroventricularly (ICV). The doses were selected based upon research using the drug in other aversive learning situations [1].

### METHOD

#### Subjects

The subjects were 20 naive female rats of the Long Evans strain similar to those used in Experiment 1. All subjects were about 180 days old at the time of surgery. The rats were housed, maintained, trained and tested as specified in Experiment 1.

#### Surgery, Drugs and Injection

Rats were anesthetized with 100 mg/ml/kg Ketamine Hydrochloride. A local anesthetic (2% lidocaine) was injected under the scalp. A 22 gauge stainless steel outer cannula was implanted into the right lateral ventricle (coordinates used were 0.4 mm posterior to bregma, 1.4 mm lateral to midline, and 3.1 mm ventral to the surface of the cortex) with the skull leveled between lambda and bregma. QNTX was dissolved in 0.9% filtered SA (Millex-GV 0.22 µm, Millipore Corp., Bedford, MA). Filtered 0.9% SA also served as the control injection. The ICV injections were performed by backloading the drug up a 28 gauge internal cannula (Plastic Products, Roanoke, VA) into PE-50 tubing (Intramedic No. 7411). An injection volume of 4 µl was delivered by a 5 µl syringe (Hamilton No. 7002) at a rate of 4 µl/40 sec.

#### Procedure

The subjects were given a minimum of six days recovery prior to the beginning of conditioning. All subjects were handled and had their cannula caps removed daily for five days prior to training to familiarize them with the procedure.

The rats were run with the same procedure and apparatus as specified in Experiment 1 except that five min after the formalin injection the rats received ICV injections of QNTX (0, 0.1, 1 and 10  $\mu\text{g}/\text{rat}$ ) instead of IP injections. Fifteen min after drug injection (thus 20 min after formalin) the rats were placed in their assigned chambers and scored as described in Experiment 1.

At the conclusion of the experiment, all subjects were overdosed with Nembutal and injected ICV with 4  $\mu\text{l}$  of India ink (Hunt/Speedball No 3338). Approximately 5–15 min after injection of the ink tracer they were perfused transcardially with SA followed by 10% formalin. The brains were removed and coronal sections were made along the cannula tract. Positive cannula placement was verified for each rat by the presence of ink lining the epithelial cells of the lateral, third and fourth ventricles. Eighteen of the 20 rats showed positive cannula placements. Due to procedural error two rats missed ink injections, however, visual inspection showed the tip of the cannula within the ventricle. Therefore, all 20 rats were included in the analysis. When the data analysis was performed without those two rats it did not alter the statistical results.

#### RESULTS

An overall one way ANOVA of recuperative scores revealed reliable group differences,  $F(3,16)=6.58$ ,  $p<0.004$ . Linear trend analysis revealed a significant linear component for dose,  $F(1,16)=16.04$ ,  $p<0.001$ . Figure 1 depicts the mean percent recuperative scores in rats given the four doses of QNTX ICV. These data indicate that intracerebroventricularly administered QNTX produced a significant dose-related reversal of conditional analgesia. The mean recuperative score for the rats which received 10  $\mu\text{g}$  QNTX was nine times higher than the SA group. One way ANOVA for ICV freezing scores revealed no significant differences,

$F(3,16)=1.8$ ,  $p=0.18$ , nor were any expected given these procedures [7–9]. We did not observe hyperexcitability or seizure-like activity after ICV administration of any dose of QNTX tested.

#### DISCUSSION

QNTX (10  $\mu\text{g}$ ) given ICV on the test day significantly ( $p<0.001$ ) reversed conditional analgesia in that drug treated subjects recuperated on the average of nine times more than controls using the formalin test. On the other hand, peripherally administered QNTX, at doses as high as 30 mg/kg, failed to significantly reverse conditional analgesia. These findings are consistent with the hypothesis that the effects of naloxone and naltrexone on conditional analgesia measured by the formalin test are centrally mediated. Peripheral opioid receptors do not appear to be involved in this form of conditional analgesia.

Freezing was not significantly affected by QNTX. These results argue against suppression of locomotor mechanisms by QNTX. Since peripherally administered naloxone (10 mg/kg) [10], has no effect on baseline pain sensitivity using the formalin test, but does reverse conditional analgesia [6,8], it seems reasonable to assume that the primary effect of ICV QNTX is indeed a reversal of analgesia. And since conditional analgesia is not affected by hypophysectomy [6] nor IP QNTX, it seems that this analgesia is mediated by a neural-opioid mechanism [11].

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