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BRIEF COMMUNICATION

# Opioid Receptor Subtypes Mediating the Noise-Induced Decreases in High-Affinity Choline Uptake in the Rat Brain

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LAI, H. AND M. A. CARINO. *Opioid receptor subtypes mediating the noise-induced decreases in high-affinity choline uptake in the rat brain.* PHARMACOL BIOCHEM BEHAV 42(3) 553–558, 1992.—Acute (20 min) exposure to 100-dB white noise elicits a naltrexone-sensitive decrease in sodium-dependent high-affinity choline uptake in the frontal cortex and hippocampus of the rat. In the present study, the subtypes of opioid receptors involved were investigated by pretreating rats with microinjection of specific opioid-receptor antagonists into the lateral cerebroventricle before noise exposure. We found that the noise-induced decrease in high-affinity choline uptake in the hippocampus was blocked by pretreatment with either $\mu$-, $\delta$-, or $\kappa$-opioid-receptor antagonists, whereas the effect of noise on frontal cortical high-affinity choline uptake was blocked by a $\mu$- and $\delta$- but not by a $\kappa$-antagonist. These data further confirm the role of endogenous opioids in mediating the effects of noise on central cholinergic activity and indicate that different neural mechanisms are involved in the effects of noise on the frontal cortical and hippocampal cholinergic systems.

Noise  High-affinity choline uptake  Opioid receptor subtypes  Frontal cortex  Hippocampus

NOISE is a common form of "stressor" in the environment. In our previous research (9,11), we found that noise exposure affects the central cholinergic systems in the rat. Decreases in sodium-dependent high-affinity choline uptake (HACU) were observed in the frontal cortex and hippocampus of the rat after 20 min of acute exposure to 100-dB white noise. Repeated exposure to the noise leads to increases in the concentration of muscarinic cholinergic receptors in the brain. Further studies showed that these noise-induced neurochemical effects are mediated by endogenous opioids since they can be blocked by pretreatment with the narcotic antagonist naltrexone before noise exposure.

Different subtypes of opioid receptors exist in the brain and mediate the effects of endogenous opioids (2,5). In the present study, to further understand the neurological effects of noise, involvement of the three major subtypes of opioid receptors, namely, $\mu$, $\delta$, and $\kappa$, in the effects of noise on HACU in the brain of the rat were investigated. Specific antagonists of the different subtypes of opioid receptors were microinjected into the lateral cerebroventricle before noise exposure to block its effects.

## METHOD

### *Animals*

Male Sprague-Dawley rats (250–300 g) purchased from Tyler Laboratory (Bellevue, WA) were used in this study. They were housed three to a cage in a vivarium. The experimental environment was maintained on a 12 L:12 D cycle (light on 0700–1900 h) and at an ambient temperature of 23°C and relative humidity of 65%. Rats were provided with food and water ad lib.

### *Experimental Procedures*

At least 5 days before an experiment, a 20-ga stainless steel guide cannula was implanted in the rat for microinjection of drug unilaterally into the lateral ventricle of the brain. During cannula implantation, rats were anesthetized with pentobarbital sodium (50 mg base/kg, IP), and also injected with atropine methyl bromide (1 mg base/kg, IP) to prevent respiratory congestion. The rat was then positioned on a stereotaxic instrument (Kopf Instruments, Tujunga, CA) using a pair of blunt-tip ear-bars to prevent puncturing of the ear drums. The

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tip of the guide cannula was placed at 1.5 mm above the intended injection site, which, according to the rat brain stereotaxic atlas of Paxinos and Watson (16), corresponds to the coordinates of AP −1.0 mm, L ±1.6 mm, and DV −3.7 mm, with the bregma as the zero reference point. Rats were returned to the vivarium after surgery.

At least 2 days before an experiment, rats were moved to a room adjacent to the noise-exposure room. Animals were pretreated before noise-exposure with drugs to block the different subtypes of opioid receptors in the brain. Drug dosages and treatment procedure were according to those described in the literature (1,3,7,18,20).

To achieve maximal receptor blocking effect, drug treatment procedures were as follow: For $\mu$-receptor blockade, rats were given ICV injection of $\beta$-funaltrexamine ($\beta$-FNA) (10 $\mu$g in 5 $\mu$l sterile pyrogen-free physiological saline) 24 h before noise exposure. Controls for $\mu$-antagonist treatment were given ICV injection of 5 $\mu$l physiological saline 24 h before exposure. For $\delta$-receptor blockade, naltrindole (NTD) (10 $\mu$g in 5 $\mu$l physiological saline) was injected into the lateral cerebroventricle immediately before noise exposure. Drug treatment controls were given similar injection of physiological saline. For blockade of $\kappa$-receptors, rats were given ICV injection of nor-binaltorphimine (BNI) (50 $\mu$g in 5 $\mu$l sterile water) at 30 min before exposure to noise. Drug treatment controls were similarly injected with 5 $\mu$l sterile water. All three opioid antagonists were purchased from Research Biochemical Inc. (Natick, MA).

During ICV injection, rats were lightly restrained. A stainless steel injection cannula (30 ga) was inserted into the guide cannula and injection started 30 s after insertion and lasted for 1 min. The injection cannula was withdrawn 30 s after injection. Depending upon the drug treatment, animals were either returned to their home cages (rats injected with the $\mu$- or $\kappa$-antagonist) and exposed to noise at later times or subjected to noise exposure immediately (rats treated with the $\delta$-antagonist). We have previously showed that the light restraint and ICV injection procedure do not significantly affect HACU in the brain of the rat.

For noise exposure, rats were placed in individual exposure cages and exposed to 100-dB noise for 20 min. The exposure cage was a Plexiglas cylinder (15 cm in diameter, 24 cm in length) with 11 rows of holes (1 cm in diameter, 6 holes per row) drilled equally spaced longitudinally on the wall. One end of the cage was sealed closed and the other end was a removable door. The cage was put on its side. A platform made of plastic rods was built longitudinally in the cylinder (3 cm from the lower side), through which waste could fall. The rat had sufficient room to move freely in the cage. A loudspeaker (Speakerlab, Seattle, WA; Model KR 4580) was mounted 30 cm above the cage and activated by a white-noise generator (Lehigh Valley Electronics, Boston, MA, Model 581-02) powered by an amplifier system (Hewlett-Packard 467A power amplifier and 6215A power supply). The frequency range of the noise generator was up to 40 kHz. Noise level was set a 100 dB and found to be uniform inside the cage as monitored by a sound meter (B & K, Inc., Seattle, WA). The ambient noise level inside the cage was 60 dB, which came mostly from the ventilation system in the room. Rats used as control for the noise exposure were sham exposed, that is, they were given similar drug or vehicle treatment and then placed in similar exposure cages for 20 min without the white noise turned on.

At the end of the exposure period, rats were sacrificed by decapitation at a workbench close by. The frontal cortex and hippocampus were dissected on ice for assay of HACU. The frontal cortex consisted of the cerebral cortex anterior to the optic chiasm with the olfactory tubercles and anterior portions of the striatum and septum removed. Sites of ICV injection were visually confirmed for each rat by tracing the guide cannula track in the brain during brain dissection.

From the individual brain tissue, HACU was determined using a method described previously by us (10). Brain tissue was homogenized in 2 ml 0.32 M sucrose solution with a glass homogenizer. The homogenate was centrifuged at 100 × $g$ for 10 min and the supernatant was then recentrifuged at 17,000 × $g$ for 15 min. The pellet was reconstituted in 2 ml 0.27 M sucrose. Of this synaptosomal preparation, 0.1 ml was added to each of a set of tubes containing 0.9 ml of a buffer (containing 4% dextrose, 126 mM NaCl, 1.28 mM $Na_2HPO_4$, 4.75 mM KCl, 1.27 mM $CaCl_2$, and 1.42 mM $MgCl_2$, pH 7.2), 0.5 $\mu$M choline chloride, and 0.4 $\mu$Ci [$^3$H]choline (80 Ci/mmol, New England Nuclear, Newton, MA). Non-sodium-dependent choline uptake was determined by addition of 2 $\mu$M hemicholinium-3 (Sigma Chemical Co., St. Louis, MO) to a similar set of tubes. The samples were transferred from

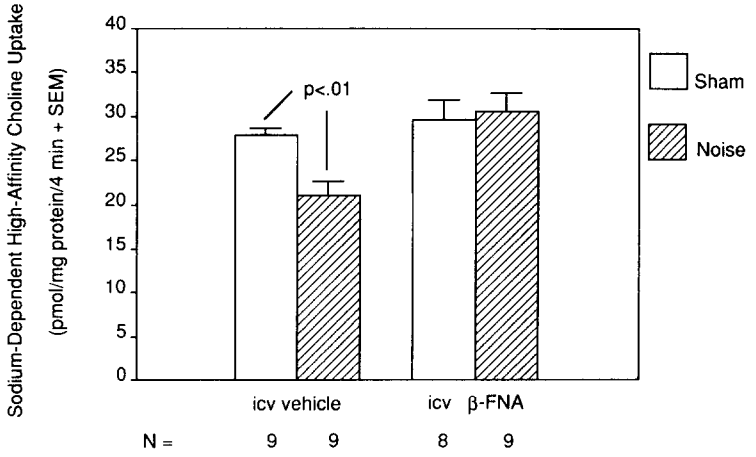


FIG. 1. Effect of pretreatment with the $\mu$-antagonist $\beta$-FNA on noise-induced decrease in HACU in the frontal cortex of the rat.

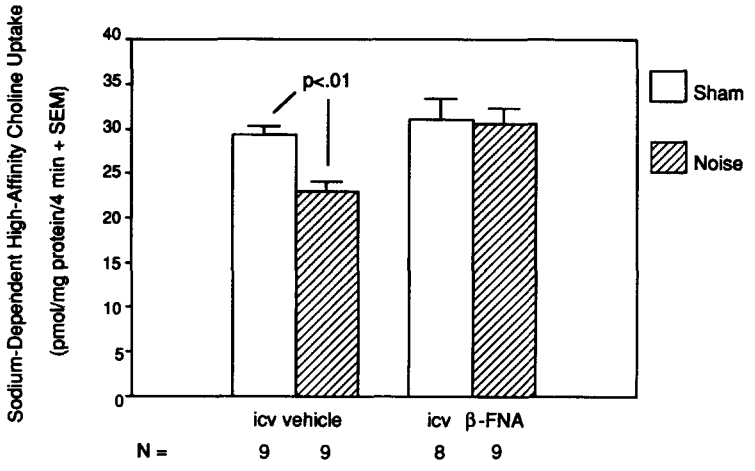


FIG. 2. Effect of pretreatment with β-FNA on noise-induced decrease in HACU in the hippocampus of the rat.

an ice-water bath to a water bath at 37°C for incubation for 4 min. Uptake was terminated by returning the samples to the ice-water bath. Synaptosomes were collected by centrifugation at 7000 × *g* for 25 min. The supernatant was discarded and the pellet was surface washed with 1 ml ice-cold 0.9% saline. The saline was removed and the pellet was dissolved overnight with 0.7 ml hyamine hydroxide (ICN Biochemicals, Inc., Costa Mesa, CA). Ten milliliters of Cytoscint (ICN Biochemicals, Inc.) were then added. Radioactivity was determined by liquid scintillation technique at 50% counting efficiency. HACU was determined as the difference in uptakes in the absence and presence of hemicholinium-3 and expressed in pmol/mg protein/4 min. Protein concentration of the synaptosomal preparation was determined by the method of Lowry et al. (14) using bovine serum albumin as external standards.

*Data Analysis*

Data of HACU from the frontal cortex and hippocampus were analyzed by two-way analysis of variance (ANOVA) for significance of treatment (noise and drug treatment) and treatment interaction (noise × drug treatment) effects. Differences between the two treatment groups were compared by the Newman-Keuls test. A difference at $p < 0.05$ was considered statistically significant.

## RESULTS

Data on the effect of pretreatment with the μ-opioid receptor antagonist β-FNA on noise-induced decreases in HACU in the frontal cortex and hippocampus are presented in Figs. 1 and 2, respectively. Two-way ANOVA of the data of the frontal cortex showed nonsignificant noise effect, $F(1, 31) = 3.1$, nonsignificant, but significant β-FNA, $F(1, 31) = 10.89$, $p < 0.005$, and noise × β-FNA interaction, $F(1, 31) = 5.15$, $p < 0.05$, effects. Data showed that ICV pretreatment with β-FNA blocked the effect of noise on frontal cortical HACU (Fig. 1).

Similar analysis of the data of the hippocampus shows

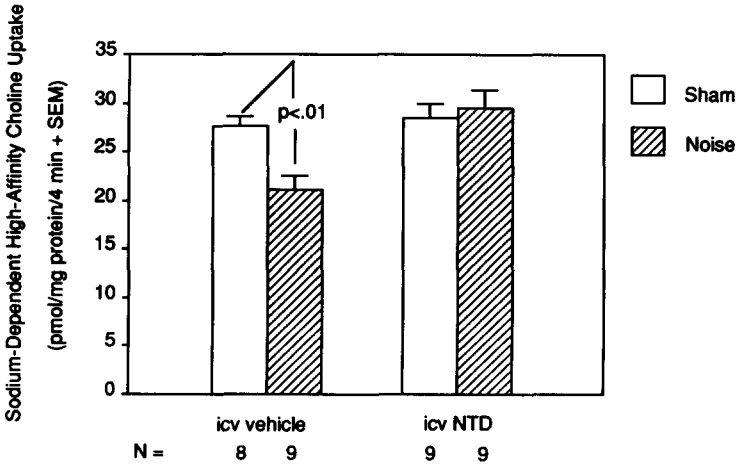


FIG. 3. Effect of pretreatment with the δ-antagonist NTD on noise-induced decrease in HACU in the frontal cortex of the rat.

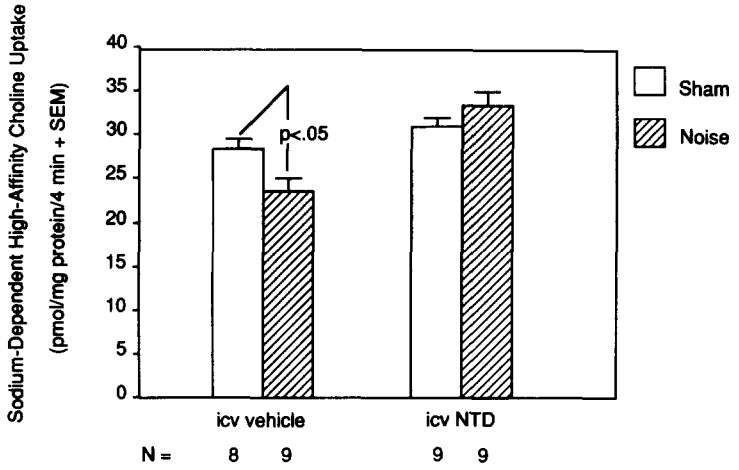


FIG. 4. Effect of pretreatment with NTD on noise-induced decrease in HACU in the hippocampus of the rat.

significant noise, $F(1, 31) = 5.02$, $p < 0.05$, and β-FNA, $F(1, 31) = 9.43$, $p < 0.005$, effects, but no significant noise × β-FNA interaction effect, $F(1, 31) = 3.74$, nonsignificant. The noise-induced decrease in HACU in the hippocampus was blocked by the drug pretreatment (Fig. 2).

Data on the effect of pretreatment with the δ-opioid receptor antagonist NTD on noise-induced decreases in HACU in the frontal cortex and hippocampus are presented in Figs. 3 and 4, respectively. Two-way ANOVA showed nonsignificant noise effect, $F(1, 31) = 3.23$, nonsignificant, but significant NTD, $F(1, 31) = 8.79$, $p < 0.01$, and noise × NTD interaction, $F(1, 31) = 5.76$, $p < 0.025$, effects on frontal cortical HACU. Pretreatment with the δ-antagonist blocked the noise-induced decrease in HACU in the frontal cortex (Fig. 3).

Two-way ANOVA also showed no significant noise effect, $F(1, 31) = 1.24$, nonsignificant, but significant NTD, $F(1, 31) = 21.31$, $p < 0.005$, and noise × NTD interaction, $F(1, 31) = 7.5$, $p < 0.025$, effects on hippocampal HACU. The effect of noise on hippocampal HACU was blocked by pretreatment with the δ-antagonist (Fig. 4).

Effects of pretreatment with the κ-opioid receptor antagonist BNI on noise-induced decreases in frontal cortical and hippocampal HACU are presented in Figs. 5 and 6, respectively. Two-way ANOVA showed significant noise effect, $F(1, 31) = 24.64$, $p < 0.005$, but no significant BNI, $F(1, 31) = 2.18$, nonsignificant, and noise × BNI interaction, $F(1, 31) = 2.5$, nonsignificant, effects on HACU in the frontal cortex. Pretreatment with the κ-antagonist did not block the effect of noise. Frontal cortical HACU of the noise-exposed/BNI-treated animals was significantly smaller than that of the sham-exposed/BNI-treated rats (Fig. 5).

Two-way ANOVA of the data from the hippocampus showed significant noise effect, $F(1, 30) = 11.56$, $p < 0.005$,

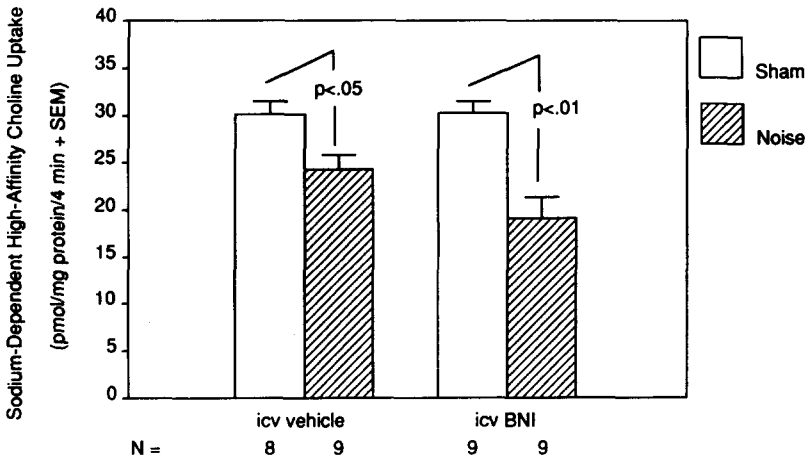


FIG. 5. Effect of pretreatment with the κ-antagonist BNI on noise-induced decrease in HACU in the frontal cortex of the rat.

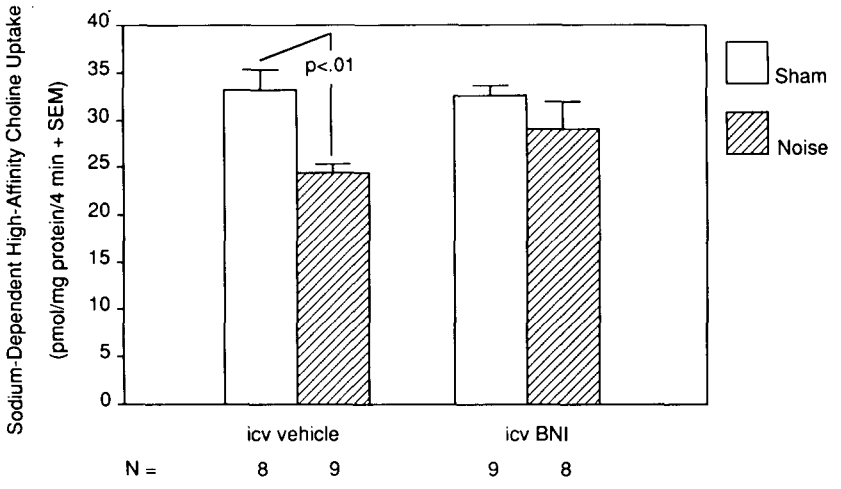


FIG. 6. Effect of pretreatment with BNI on noise-induced decrease in HACU in the hippocampus of the rat.

but no significant BNI, $F(1, 30) = 1.24$, nonsignificant, and noise × BNI interaction, $F(1, 30) = 2.23$, nonsignificant, effects. Pretreatment with BNI blocked the noise-induced decrease in HACU in the hippocampus (Fig. 6).

## DISCUSSION

Data from the present experiment confirm our previous finding that endogenous opioids mediate the noise-induced decreases in HACU in the frontal cortex and hippocampus of the rat. The data further identify that the effect on the frontal cortex involves $\mu$- and $\delta$-opioid receptors, whereas that on the hippocampus involves $\mu$-, $\delta$-, and $\kappa$-opioid receptors. Since the effect of noise can be blocked by direct injection of drugs into the lateral cerebroventricle, the endogenous opioid mechanisms involved are located centrally in the brain.

The three subtypes of opioid receptors are present in many regions of the CNS of the rat, including the areas containing the cell bodies and the axonal terminals of the neurons of the septohippocampal and basaliscortical cholinergic pathways (2, 15, 23). The brain loci at which endogenous opioids modulate the noise-induced decrease in central HACU are not known. This information will require local microinjection of opioid antagonists into various regions of the brain.

Even though it is well established that endogenous opioids in the brain modulate the transmission of central cholinergic systems, the mechanism and roles played by the different subtypes of opioid receptors remain unclear. Different opioid receptor subtypes are involved in the control of release of acetylcholine in different brain areas and species of animals (6). In vitro studies (12) using brain slices from the hippocampus and frontal cortex of the rat showed that various opioid agonists had no significant effect on spontaneous release of acetylcholine from the tissue. However, $K^+$-evoked release of acetylcholine from hippocampal slice was depressed by $\mu$-agonists, but not by $\delta$- or $\kappa$-agonists, whereas release from frontal cortical slices was depressed by $\kappa$-, but not by $\mu$- or $\delta$-, agonists. In an in vivo study, the turnover rate of acetylcholine from the hippocampus was decreased by administration of $\mu$- or $\delta$-agonists to the rat, whereas $\kappa$-agonist had no significant effect. For the frontal cortex, none of the opioid agonists studied had a significant effect on the turnover rate of acetylcholine (22). This picture is further complicated by the finding that opioid agonists can cause both decrease or increase in acetylcholine turnover rate in the brain, probably dependent upon the dosage and type of opioid agonist studied. For example, morphine has been shown to both increase and decrease the release of acetylcholine in the frontal cortex and hippocampus of the rat (4,19,21).

It is not surprising that different experimental conditions reveal different mechanisms of interaction between endogenous opioids and acetylcholine in the brain. Endogenous opioids and their receptors are present in almost all areas of the brain. Thus, conceivably the activity of central cholinergic systems can be modulated by different neural mechanisms or pathways involving endogenous opioids as a link in the pathway. The locus of action can be directly on the cell bodies or terminals of the cholinergic neurons or it can be remote from the cholinergic pathways affected. In the present study, the effect of noise on cholinergic systems involves all three subtypes of endogenous opioid receptors, suggesting a complex sequence of neural mechanisms involved.

It is interesting to note that the effect of noise on hippocampal and frontal cortical HACU can be attenuated by blockade of more than one subtype of the opioid receptors. A similar finding has also been reported in the stress-induced decrease in luteinizing hormone in the rat. The effect can equally be blocked by $\mu$- and $\kappa$-opioid antagonists (17). This would imply that the functions of the different subtypes of opioid receptors are linked in series or that they are functionally coupled so that activation of more than one type is needed to produce an effect on the cholinergic pathways. Another explanation for the finding is that the endogenous opioids released as a result of noise exposure can act on different receptor subtypes present in the postsynaptic membrane. For example, $\beta$-endorphin can act on both $\mu$- and $\delta$-receptors (8), whereas dynorphin can interact with $\mu$, $\delta$, and $\kappa$-opioid receptors (13). Thus, each subtype of receptors contributes to the postsynaptic effect of the endogenous opioids activated by noise. Blockade of any one subtype by a specific antagonist will attenuate the effect.

## ACKNOWLEDGEMENT

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