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A Novel Anxiogenic Role for the Delta Opioid Receptor Expressed in GABAergic Forebrain Neurons

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ABSTRACT

BACKGROUND: The delta opioid receptor (DOR) is broadly expressed throughout the nervous system; it regulates chronic pain, emotional responses, motivation, and memory. Neural circuits underlying DOR activities have been poorly explored by genetic approaches. We used conditional mouse mutagenesis to elucidate receptor function in GABAergic neurons of the forebrain.

METHODS: We characterized DOR distribution in the brain of Dlx5/6-CreX*Oprd1*$^{fl/fl}$ (Dlx-DOR) mice and tested main central DOR functions through behavioral testing.

RESULTS: The DOR proteins were strongly deleted in olfactory bulb and striatum and remained intact in cortex and basolateral amygdala. Olfactory perception, circadian activity, and despair-like behaviors were unchanged. In contrast, locomotor stimulant effects of SNC80 (DOR agonist) and SKF81297 (D_1 agonist) were abolished and increased, respectively. The Dlx-DOR mice showed lower levels of anxiety in the elevated plus maze, opposing the known high anxiety in constitutive DOR knockout animals. Also, Dlx-DOR mice reached the food more rapidly in a novelty suppressed feeding task, despite their lower motivation for food reward observed in an operant paradigm. Finally, c-fos protein staining after novelty suppressed feeding was strongly reduced in amygdala, concordant with the low anxiety phenotype of Dlx-DOR mice.

CONCLUSIONS: We demonstrate that DORs expressed in the forebrain mediate the described locomotor effect of SNC80 and inhibit D_1-stimulated hyperactivity. Our data also reveal an unanticipated anxiogenic role for this particular DOR subpopulation, with a potential novel adaptive role. In emotional responses, DORs exert dual anxiolytic and anxiogenic roles, both of which may have implications in the area of anxiety disorders.

Keywords: Conditional gene knockout, Delta opioid receptor, Emotion, GABAergic forebrain neurons, Locomotion, Motivation

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Mu, delta, and kappa opioid receptors are distributed throughout the nervous system and play a central role in pain control, hedonic homeostasis, and emotions (1,2). In the last decade, the delta opioid receptor (DOR) has emerged as an attractive target to reduce chronic pain (3,4). The DOR receptor is also a key player in several brain processes (5), including the regulation of emotional responses (6), impulsivity (7), and learning and memory (8), and has raised interest in areas of both neurologic and psychiatric disorders. Emotional responses represent an important aspect of DOR function. Preclinical studies established a general beneficial role for DOR in reducing levels of anxiety and depressive-like behavior, and delta agonists are in clinical trial for the treatment of mood disorders (3,5).

The DORs are broadly expressed in the central and peripheral nervous systems. In mice, quantitative autoradiographic binding (9–11) shows particularly abundant protein levels in the olfactory bulb (OB), cortex, striatum, and amygdala. Moderate DOR levels are also found in interpeduncular and pontine nuclei, hippocampus, spinal cord (SC), and dorsal root ganglia, and low levels are found in hypothalamus, thalamus, mesencephalon, and brainstem (12). A knock-in mouse line expressing functional fluorescent DORs (13) has allowed anatomic studies of DOR expression with cellular and subcellular details in dorsal root ganglia (14), enteric neurons (14–17), and hippocampus (15,16). Refined mapping of DOR expression in mice is now possible (18) and provides a basis for understanding DOR activities in the brain and periphery. Analyses of DOR distribution in the human brain show expression concordant with rodent studies in cortical regions and limbic structures such as hippocampus and amygdala as well as basal ganglia and hypothalamus (19–22).

At the present time, neuron populations and brain circuits where DORs operate in the nervous system have been poorly

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explored. In pain research, local pharmacology at the level of dorsal root ganglia and SC has indicated a role for peripheral DORs in pain control (23), and a conditional genetic approach has demonstrated that DORs expressed in small primary nociceptive neurons are essential to reduce persistent pain and mediate delta opioid analgesia (24). In the brain, local pharmacology has provided evidence for an anxiolytic role of DORs at the level of cingulate cortex (25), hippocampus (26), and amygdala (27,28). However, neural populations engaged in DOR-mediated mood control have not been examined by genetic approaches, and DOR-mediated mechanisms underlying motivational and emotional responses or learning and memory remain unexplored.

In the present study, we used a Dlx5/6 driver Cre line to achieve genetic inactivation of the DOR gene in forebrain GABAergic neurons. We obtained a conditional knockout mouse line that lacks receptors in two main DOR expression sites (i.e., OB and striatum), including caudate putamen (CPu) and nucleus accumbens (NAc). These mice retain full receptor density in the basolateral amygdala (BLA), which represents a third main site with the densest DOR protein levels. We then examined these mice in behaviors known to engage these brain structures and possibly to recruit DOR-mediated controls. Our data reveal an unexpected anxiogenic role for this particular DOR population, which contrasts with the known overall anxiolytic role of the receptor.

METHODS AND MATERIALS

Animals

The DOR-floxed (*Oprd1*$^{fl/fl}$ or control mice) mouse line was described previously (24). Mice were crossed with CMV-Cre mice or Dlx5/6-Cre mice to produce constitutive knockout (CMV-Cre*XOprd1*$^{fl/fl}$ or CMV-DOR) and conditional knockout (Dlx5/6-*Oprd1*$^{fl/fl}$ or Dlx-DOR) mouse lines (see details in Supplement 1). For all behavioral experiments, the Dlx-DOR mice were compared with their control littermates mice. The CMV-DOR mice were also tested in the anxiety-related tests (Supplement 1). Experiments were performed on animals 6–18 weeks old, housed two to four per cage under standard laboratory conditions (12-hour dark/light cycle light on at 7 AM). Food and water were available ad libitum. In the chocolate pellet self-administration experiment, we used male mice only. For other experiments, both male and female mice were used, and data were pooled because statistical analysis showed no significant gender effect. All experimental procedures were carried out in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) and were approved by the local ethical committee (Comité d'Éthique pour l'Expérimentation Animale, Institut de Génétique et de Biologie Moléculaire et Cellulaire-Mouse Clinical Institute).

Quantitative Reverse Transcriptase Polymerase Chain Reaction

Sampling of brain regions, RNA extraction, and quantification were performed according to a previous study (29,30) and are briefly described in Supplement 1.

Autoradiographic Binding Assay

Sections were cut from control, Dlx-DOR, and CMV-DOR brains ($n = 3$) for determination of total DOR binding using [^{3}H] deltorphin-1 as the radiolabeled ligand. On the day of the experiment, sections were thawed and processed according to established protocols (31,10), with minor modifications. Film exposure, development, and analysis were performed as previously described by Kitchen *et al.* (10). Further details are described in Supplement 1.

Agonist-Stimulated [^{35}S]GTPγS Binding Assays

Membrane preparations and [^{35}S]GTPγS binding assays were performed on brain regions from control, Dlx-DOR, and CMV-DOR mice as described (Supplement 1) (32).

Behavioral Assays

Locomotion, depressive-like behaviors (forced swim and tail suspension tests), anxiety-related behaviors (light/dark box, elevated plus maze, and open field tests), novelty suppressed feeding (NSF) tests, and food self-administration experiments were performed as described in Supplement 1.

Drugs

The nonpeptidic DOR agonist SNC80 and the dopamine D_1 receptor agonist SKF81297 were used at doses according to previous studies (33,34). See preparation in Supplement 1.

c-Fos Protein Immunoreactivity

Measures of c-fos protein expression were performed as reported (35). Further details about sections processing are provided in Supplement 1.

Statistical Analysis

Statistical differences were determined by analysis of variance (ANOVA) (StatView 5; SAS Institute Inc, Cary, North Carolina) followed by Bonferroni/Dunn post hoc analysis. The *F* values and experimental degrees of freedom are included in the Results section. For experiments with two groups, a Student *t* test was used. The level of statistical significance was set at $p < .05$. For the behavioral tests during which data were obtained at several periods during the same session (locomotor tests, the open field test, and despair-like behavior paradigms), ANOVA repeated measures was used.

RESULTS

Dlx-DOR Mice Show DOR Deletion Mainly in OB and Striatum

We used the Cre-*LoxP* strategy to inactivate the DOR gene (*Oprdm1*) in forebrain areas. Because DORs are mainly expressed in GABAergic neurons (16,36,37), we mated floxed-DOR (*Oprd1*$^{fl/fl}$) mice (24) with Dlx-Cre5/6 mice that express Cre recombinase in the forebrain GABAergic neurons (38) to produce conditional (Dlx5/6-Cre*XOprd1*$^{fl/fl}$ or Dlx-DOR) mutant mice. We first analyzed DOR transcripts throughout the nervous system using quantitative reverse transcriptase polymerase chain reaction analysis (Figure 1A). In mutant mice, DOR messenger RNA expression was undetectable in OB and striatum, including CPu and NAc; was partially reduced in frontal cortex and amygdala; and showed normal