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Down Regulating Mu Receptors in the Basolateral Complex of Amygdala Prevents Antinociception in the Rat

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Data from our laboratory indicate that application of the *mu* agonist DAMGO into the basolateral complex of amygdala (BLA) suppresses tail flick reflexes in anesthetized rats. This DAMGO-induced antinociception can be blocked by pretreatment of the BLA with the nonselective opioid antagonist naltrexone, the *mu* opioid antagonist β-FNA, or the G protein inhibitor pertussis toxin, suggesting that DAMGO's interaction with G protein-coupled *mu* receptors in the BLA leads to production of antinociception. The present study employing the gene control strategy was conducted to further investigate the direct action of DAMGO on the *mu* receptors in the BLA. Intra-BLA application of antisense oligodeoxynucleotides (ODN) against *mu* receptors blocked antinociception following intra-BLA injection of DAMGO. The amount of [^{3}H]-DAMGO binding to *mu* receptors in the amygdala was also reduced in the antisense ODN-pretreated rats. These data confirm the idea that antinociception induced by DAMGO in the BLA results from a direct interaction of DAMGO with *mu* receptors in the amygdala.

Key words : Mu receptor; Amygdala; Tail flick; Gene control; Antisense oligodeoxynucleotides (ODN); {^{3}H}-DAMGO binding

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Brainstem regions including the periaqueductal gray matter (PAG) and the rostral ventromedial medulla (RVM) are critical for the descending pain control. Cells in the PAG innervate neurons in the RVM that in turn send their fibers to the dorsal horn of the spinal cord, and activation of these brainstem cells inhibits activity of ascending nociceptive projection neurons in the dorsal horn (Basbaum and Fields, 1978, 1984). For example, electrical, opioid, or excitatory chemical stimulation of cells in the PAG or the RVM abolishes responsiveness to peripheral noxious stimulation in mammals (Bennet and Mayer, 1979; Carstens & Douglass, 1995; Mayer & Liebeskind, 1974; Pan, Tershner & Fields, 1997; Terashvili, Wu, Leitermann, Sun, Clithero, & Tseng, 2005; Young and Chambi, 1987), and this antinociception can often be blocked by application of opioid antagonists such as naloxone or naltrexone (Morgan, Gold, Liebeskind, & Stein, 1991; Swajkoski, Mayer, & Johnson, 1981; Terashvili et al, 2005).

The amygdala provides important forebrain inputs to the brainstem to activate the descending antinociceptive system. A considerable amount of evidence suggests the amygdala's strong neural and functional connections to the brainstem. For example, the amygdala sends dense neural projections to the PAG (Hopkins & Holstege, 1978; Rizvi, Ennis, Behbehani & Shipley, 1991). Electrical stimulation of amygdala cells or intra-amygdala application of opioid agonists or excitatory chemicals inhibits tail flick (TF) reflexes or paw-licking responses in rats as measured in the respective TF or formalin test (Helmstetter, Bellgowan & Tershner, 1993; Helmstetter, Tershner, Poore & Bellgowan, 1998; Kalivas, Gau, Nemeroff & Prange, 1982; McGaraughty, Farr & Heinricher, 2004; Oliveira & Prado, 2001; Shin, 2005; Shin & Helmstetter, 2005). This antinociceptive effect can be prevented by electrolytic lesions of the PAG or RVM, or by functional inactivation of cells in these regions (Helmstetter et al., 1998; McGaraughty et al., 2004; Oliveira & Prado, 2001).

Converging lines of existing data suggest that *mu* opioid synapse in the basolateral complex of the amygdala (BLA) including the basolateral and lateral nuclei (De Olmos, Alheid & Beltramino, 1985) may critically subserve opioid antinociceptive actions of the amygdala. Autoradiographic studies have shown that *mu* opioid receptors are mostly located in the BLA ratherthan in other amygdalar nuclei and are more densely localized than any other opioid receptor subtype in the BLA (Atweh & Kuhar, 1977; Mansour, Khachaturian, Lewis, Akil & Watson, 1987). Several pharmacological and behavioral approaches have demonstrated that the *mu*-preferential agonist morphine or the

mu-selective agonist [D-Ala^2, N-MePhe^4, Gly-ol^5]-enkephalin (DAMGO) is more effective in enhancing pain threshold in rats, when infused into the BLA rather than into any other amygdalar region (Helmstetter et al., 1993; McGaraughty et al., 2004; Nandigama & Borszcz, 2003).

We recently have investigated the selectivity of DAMGO for *mu* receptors by treating the BLA with various antagonists prior to DAMGO (Shin & Helmstetter, 2005). In this study, pretreatment of the BLA with the non-selective opioid antagonist naltrexone or the guanine nucleotide binding protein (G protein) inhibitor pertussis toxin can attenuate TF inhibition following injection of DAMGO into the BLA in rats as measured in the radiant heat-evoked TF test. Furthermore, pretreatment of the BLA with the *mu* antagonist *beta*-funaltrexamine (β-FNA) can also block the DAMGO-induced antinociception. These findings thus suggest that the opioid antinociception is attributable to a direct interaction of DAMGO with G protein-coupled *mu* receptors in the BLA.

The present study was conducted to further support the idea that DAMGO acts directly on *mu* receptors in the BLA to modulate nociception. We inhibited the synthesis of *mu* receptors in the BLA via intra-BLA application of antisense oligodeoxynucleotides (ODN) prior to the TF test with DAMGO injection into the same site. We further conducted *in vitro* [^{3}H]-DAMGO binding assays with same animals employed in this antinociception experiment to support the behavioral findings.

Materials and Methods

Experiment 1: TF testing Twenty-three Long Evans male rats (350-500 g before surgery) obtained from Harlan Sprague Dawley (Madison, WI) were used for TF testing. Animals were housed individually and allowed free access to rat chow and water. A 14:10 hr light/dark cycle was maintained in the vivarium. All experimental manipulations were conducted during the light portion of the cycle. The room temperature in the vivarium and the surgery/testing room was maintained at 22 ℃. All the present procedures on utilizing animals were approved by the Institutional Animal Care and Use Committee at the University of Wisconsin-Milwaukee.

The present preparations and procedures for Experiment 1 followed our previous standard methods (Helmstetter, Bellgowan & Poore, 1995; Helmstetter et al., 1998; Shin & Helmstetter, 2005; Tershner & Helmstetter, 2000) except the gene knock-down strategy. Prior to surgery each rat was injected intraperitoneally (i.p.) with both ketamine HCl (100 mg/ml/kg) and sodium pentobarbital (50 mg/ml/kg; 0.15 ml/rat) and

then mounted in a Kopf stereotaxic frame. Each animal was chronically implanted with bilateral 26 gauge stainless steel guide cannulae (Plastics One, C315G) above both the left and right BLA (AP, -3.0 mm; ML, ±5.2 mm; DV, -7.9 mm; relative to the bregma) and allowed seven days to recover.

The animals were then bilaterally given intra-BLA injections of either antisense ODN (2.50 μg/0.25 μl per side) targeting exon-1 (5'-CGC CCC AGC CTC TTC CTC T-3': 5'-untranslated region) or exon-4 (5'-GGG CAA TGG AGC AGT TTC TG-3': intracellular COOH terminus) of the *mu* receptor gene, mismatch ODN (2.50 μg/0.25μl per side; 5'-CGC CCC GAC CTC TTC CCT T-3'), or 0.9 % sterile isotonic saline vehicle (0.25 μl per side), via 33 gauge steel injection cannulae extending 1 mm beyond the guide cannula tips. The sequence of four bases from that of the antisense ODN (exon-1) was switched to produce the mismatch ODN. Intra-BLA injections of the ODNs and saline were conducted on days 1, 3 and 5. These sequences of ODNs, and the general procedures for inhibition of gene expression were based on those of Rossi and colleagues (Rossi, Leventhal, Pan, Cole, Su, Bodnar & Pasternak, 1997). Phosphodiester ODNs utilized in the current study were all synthesized by The Midland Certified Reagent Company (Midland, TX).

All the rats were TF-tested on day 6. Only animals that recovered 90% or more of their pre-operative body weight were used for TF testing. Before the TF testing session, each animal was anesthetized with an i.p. injection of sodium pentobarbital (50 mg/ml/kg). A polyethylene catheter (PE-20) was inserted 1.0 cm into the jugular vein, and its other tip was connected to a 10 cc syringe containing a solution of the anesthetic methohexital (10 mg/ml). As each rat was mounted in the stereotaxic frame, this anesthetic solution was continuously delivered throughout the TF testing session via an infusion pump (Razel Model A99) at a rate of 0.8 ml/hr, inducing a slight anesthesia.

Regarding preparations and procedures for TF testing, a custom-made radiant heat source was used that contained a 3 mm diameter aperture beneath which a 500 w projection bulb was mounted. The stainless steel apparatus was cooled with forced air. A thermocouple located inside the apparatus monitored the stimulus intensity on each trial. The surface of the heat source was maintained between 28 ℃ and 32 ℃. Deflecting the tail from the aperture resulted in the triggering of a photocell and a digital timing circuit that deactivated the heat source and subsequently recorded the TF latency. The heat source was set to produce baseline TF latencies of 4-6 sec. The same stimulus

temperature was employed throughout the test session. Unless a TF occurred within 20 sec, the trial was terminated to prevent tissue damage. When baseline TF latencies were stabilized at 4-6 sec, a standard testing protocol was initiated. TF latency was recorded every 2 min throughout the testing session. Immediately following the 5th baseline trial, DAMGO (Sigma) (0.25 μg/0.25 μl per side) was bilaterally infused into the BLA over 45 sec period.

After the TF testing, each animal was injected i.p. with sodium pentobarbital (100 mg/ml/kg) and its whole brain was extracted and then frozen until use for subsequent binding assays. In addition, brains from a set of three naive rats (350-500 g) that would be used to obtain a saturation curve were also removed and frozen.

Experiment 2: [^{3}H]-DAMGO binding assay

Brain tissue preparation

As illustrated in Figure 1, a range of brain tissue of naive or tested rats was obtained by coronal (AP, -0.80 and -4.80 mm relative to the bregma), horizontal (DV, -6.00 mm) and sagittal (ML, ±2.50 mm) sectioning. The procedure for brain tissue preparation is similar to that employed by Liu-Chen and Philips (1987). The tissue was homogenized with approximately 10 volumes of 0.32 M sucrose in 10 mM Tris-HCl buffer (pH 7.5) containing 10 mM glucose, 1 mM EGTA and 10 μM leupeptin, by using Teflon pestle-glass tissue grinder. The homogenate was centrifuged at 920 x g for 10 min and the supernatant was retained. The pellet was brought up with approximately 5 volumes of the sucrose solution and centrifuged again. The combined supernatant was then centrifuged at 40,000 x g for 20 min. The crude tissue fraction was brought up with approximately 5 volumes of 5 mM Tris-HCl buffer containing 1 mM EGTA and 10 μM leupeptin (pH 7.5), and was allowed to swell for 25 min at 0 ℃. The preparation was then homogenized to disrupt synaptosomes and centrifuged again at 40,000 x g for 20 min. The light brown pellet was removed and the top white loose pellet was swollen, disrupted and centrifuged again. The final pellet was brought up in 5 volumes of 50 mM Tris-HCl buffer containing 1 mM EGTA and 10 μM leupeptin (pH 7.5). The aliquots of tissue were stored at -70 ℃ until use.

[^{3}H]-DAMGO binding assays

The binding assay procedure was based upon previous methods (Liu-Chen, Yang, Li & Adams, 1995; Nishino, Su, Wong, Watkins & Chang, 1990; Wong, Su, Watkins & Chang, 1992). Tissue was incubated at room temperature for 30 min with 100 mM NaCl and 100 μM GDP in 50 mM Tris-HCl buffer (pH 7.4) containing

1 mM EGTA and 10 μM leupeptin (TEL buffer) and washed 3 times with 50 mM TEL buffer (pH 7.4) by resuspension and centrifugation. All binding assays were performed in the 50 mM TEL buffer containing 5 mM $MgCl_2$ for 2.5 to 3 hr at 25 ℃. Protein content was determined by the use of a bicinchoninic acid protein assay reagent.

First, saturation binding was conducted with naive rats' amygdalar tissue using a range of eight [^{3}H]-DAMGO (NIDA) (51.00 Ci/mmol) concentrations (0.1 nM to 10 nM). Total binding was carried out in triplicate for each [^{3}H]-DAMGO concentration, in a final volume of 1 ml with 0.7 mg protein/tube. Based on this assay, 7.5 nM [^{3}H]-DAMGO was used for binding assays on the amygdalar tissue from rats that had been pretreated with ODNs. Total

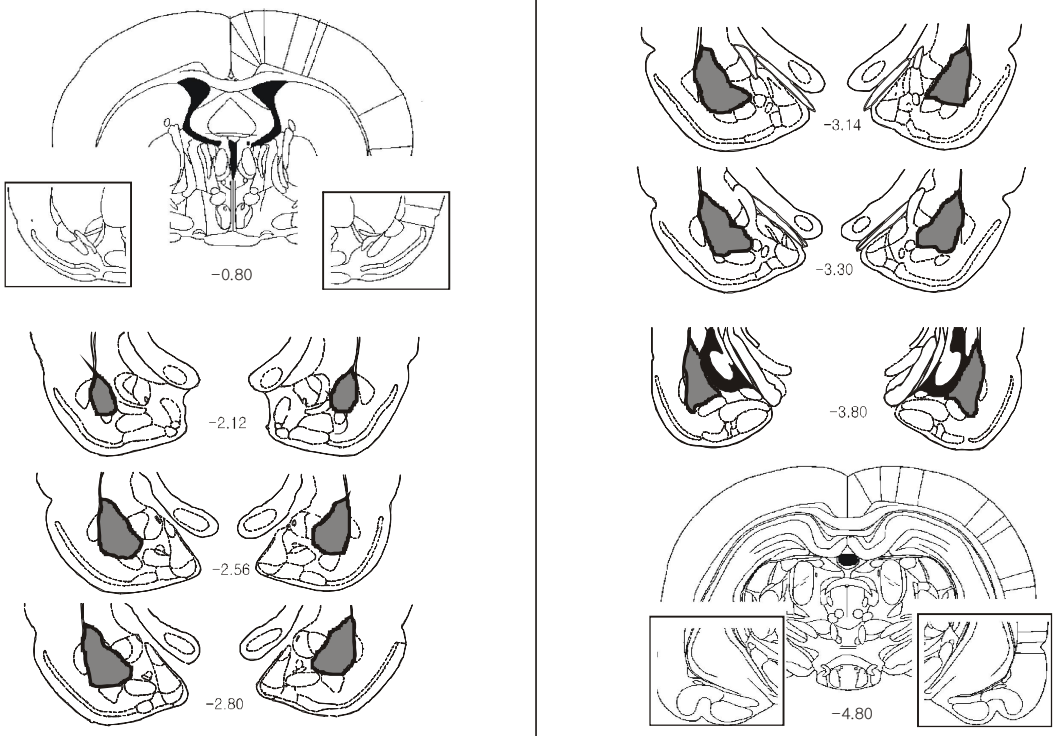

Fig. 1. Serial illustrations of the bilateral BLA and its adjacent regions of rats employed for (^{3}H)-DAMGO binding assays. A range of brain tissue (from -0.80 mm through -4.80 mm relative to the bregma) dissected out by coronal (AP, -0.80 mm and -4.80 mm), horizontal (DV, -6.00 mm) and sagittal (ML, ±2.50 mm) sectioning was utilized for the binding assays. Shaded regions (grey) represent BLA injection targets. The numbers in the middle of the diagrams indicate distances (mm) posterior to the bregma. Diagrams at -0.80 mm and -4.80 mm display how the brain tissue was obtained.

binding was performed in triplicate with 7.5 nM of [^{3}H]-DAMGO for each experimental group. For non-specific binding, naltrexone (1 mM) was applied along with 7.5 nM [^{3}H]-DAMGO to one set of tissue for each group.

Following incubation, separation of bound from free radioligand was conducted by rapid filtration with a Brandel 24 sample cell harvester over GF/B filters under vacuum. Radioactivity trapped in the filters was determined by liquid scintillation counting.

Results

TF testing Twenty rats were accepted for statistical analyses. Three animals were excluded due to their reduced body weight (less than 90 % relative to their pre-surgery weight) on the testing day. Figure 1 depicts bilateral injection targets of the BLA. Reconstruction of the brain region was made with aid of a rat brain atlas (Paxinos & Watson, 1998).

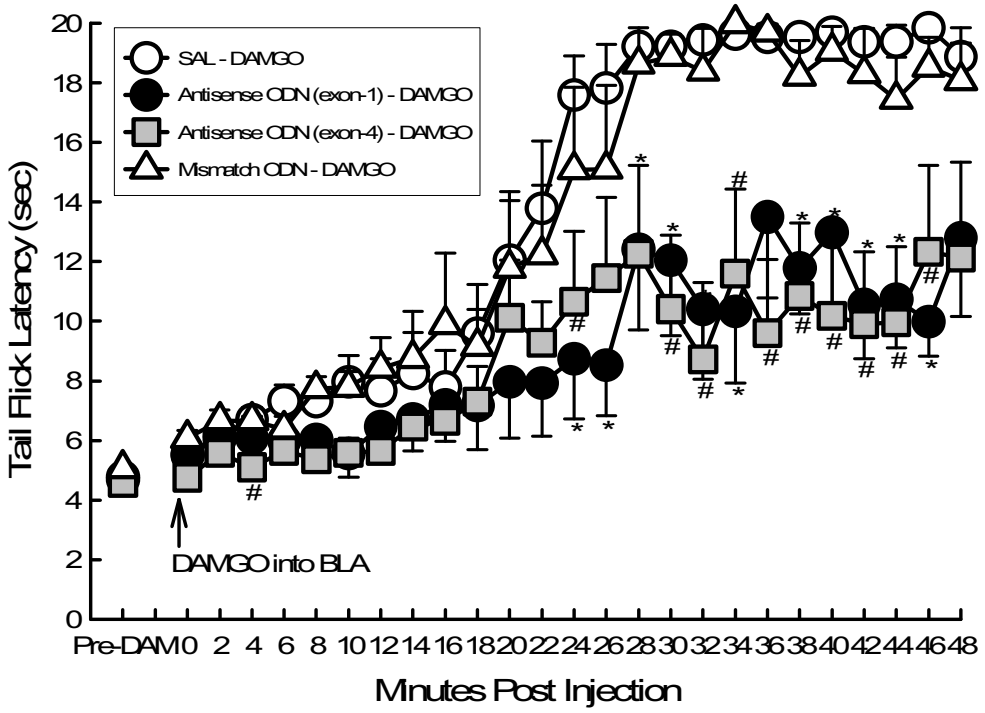

Fig. 2. Time-dependent curves representing mean TF latencies. * and # indicate statistical significance from Tukey HSD comparisons of mean TF latencies for antisense ODN (exon-1 and exon-4, respectively) pretreated rats with those for saline pretreated animals. Antisense ODN (exon-1 or exon-4); antisense ODN targeting exon-1 or exon-4, respectively. Mismatch ODN; mismatch ODN targeting exon-1. SAL; saline. BLA; the basolateral complex of amygdala. Pre-DAM; baseline time period prior to injection of DAMGO

Figure 2 illustrates mean TF latencies for pre- and post-DAMGO injection trials. TF latency data were analyzed with a repeated measures analysis of variance (ANOVA) that was followed by post hoc comparisons employing Tukey HSD test ($\alpha = 0.05$). Mean TF latencies for five pre-DAMGO baseline trials were averaged and used for a baseline reference, since the TF latencies did not differ between groups and change over time.

Application of DAMGO into the BLA elevated TF latencies over time. An omnibus F test on TF latency data for post-DAMGO injection period produced a significant main effect for time ($F_{24, 384} = 35.12$, $p < 0.001$).

Pretreatment of the BLA with antisense ODN targeting exon-1 or exon-4 of the *mu* receptor gene blocked the DAMGO-induced antinociception. The overall test showed a significant main effect for group ($F_{3, 16} = 5.44$, $p < 0.01$). Subsequent post hoc comparisons on the group further revealed that during the post-DAMGO injection period, mean TF latencies for rats pretreated with antisense ODN targeting exon-1 or exon-4 were lower than those for animals given saline or mismatch ODN control

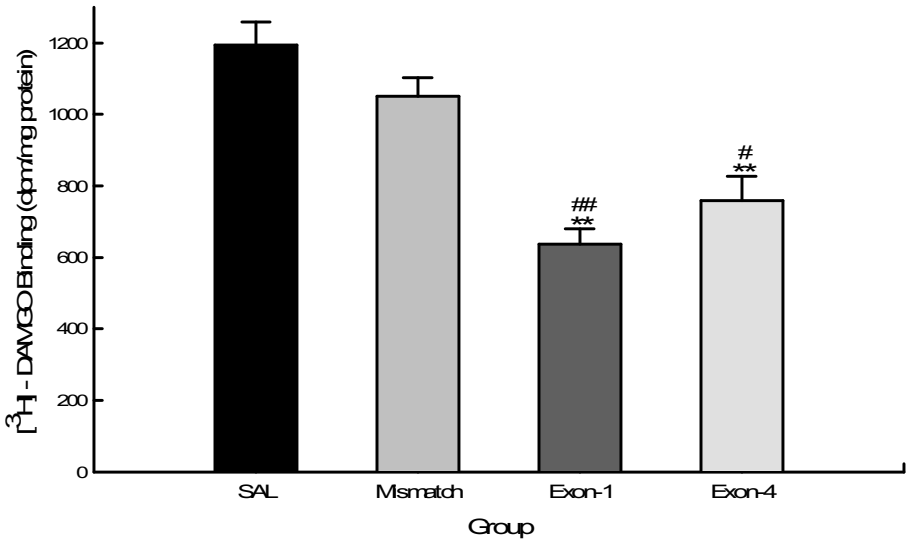

Fig. 3. Mean concentration of radioactivity representing amount of specific binding of [^{3}H]-DAMGO. ** ($p < 0.01$) indicate a statistical significance relative to saline-pretreated subjects, resulting from Tukey HSD comparisons. # ($p < 0.05$) or ## ($p < 0.01$) indicates a statistical significance relative to animals pretreated with mismatch ODN. dpm/mg protein; disintegration per minute, per milligram of protein. Exon-1 or Exon-4; antisense ODN targeting exon-1 or exon-4, respectively. Mismatch; subjects pretreated with mismatch ODN (targeting exon-1). SAL; subjects pretreated with saline.

injections.

Moreover, the ability of down-regulation of the *mu* receptors to block the DAMGO effect became more pronounced over time, as implicated by a significant group by time interaction ($F_{72, 384} = 2.15$, $p < 0.001$).

Binding assay The portion of specific binding of [^{3}H]- DAMGO was obtained by subtracting the amount of non-specific binding from that of total binding (Emmerson, Liu, Woods & Medzihradsky, 1994). Figure 3 illustrates the amount of the specific binding of 7.5 nM [^{3}H]-DAMGO. Data analysis for the binding was conducted via one-way ANOVA followed by Tukey HSD post hoc comparisons ($\alpha = 0.05$).

Pretreatment with antisense ODNs inhibited [^{3}H]-DAMGO binding. A significant effect for group was seen in the overall F test ($F_{3, 8} = 13.30$, $p < 0.01$). Subsequent post-hoc comparisons found that animals given intra-BLA injections with antisense ODN targeting exon-1 or exon-4 reduced the [^{3}H]-DAMGO binding. The comparisons resulted in significant differences in the amount of [^{3}H]-DAMGO binding between saline or mismatch and antisense ODN (exon-1 or exon-4)-pretreated groups, while no significant difference was observed between saline and mismatch ODN-pretreated control groups.

Discussion

The gene knock-down strategy is a useful tool and often employed to control expression of specific genes. A perfect complementary match of nucleotide sequences between mRNA and antisense ODN interferes with protein synthesis via blocking transcription or translation of the mRNA (Schlingensiepen, Brysch & Schlingensiepen, 1997; Wahlestedt, 1994). In contrast, even a slight mismatch[1] of the sequences between the two is incapable of preventing the protein synthesis (Schlingensiepen et al., 1997; Wahlestedt, 1994). Therefore, in blocking specific receptor proteins, this gene regulation strategy can be preferred to applying antagonistic compounds that are believed to occasionally produce a non-selective or less effective antagonism depending on some factors including their concentrations or injection sites (Shin & Helmstetter, 2005; Tam & Liu-Chen, 1986).

In the present study, administration of DAMGO into the BLA inhibited TF reflexes in anesthetized rats, replicating many previous observations from our laboratory (Helmstetter et al., 1995; Helmstetter et al., 1998; Shin & Helmstetter, 2000; Shin & Helmstetter, 2005;

1) Mismatch ODN control is used to check whether injection of antisense ODN itself may induce effects other than a gene-specific treatment.

Tershner & Helmstetter, 2000). This analgesic effect was blocked by inhibiting the synthesis of *mu* receptors via repeated pretreatment of the BLA with antisense ODN targeting exon-1 or exon-4 for the *mu* receptor. These findings are consistent with existing data that intracerebroventricular (i.c.v.) or intra-PAG injections of antisense ODN directed against exon-1 or exon-4 for the *mu* receptor can prevent antinociception induced by the *mu* agonist morphine or β-casomorphine (Rossi et al., 1997; Sancez-Blazquez, DeAntonio & Rodriguez-Diaz, 1999).

The present antinociception is believed to arise from DAMGO's action on the mu_1 receptor in the BLA. It has been documented that the MOR-1 (i.e., the first cloned *mu* receptor) gene contains four exons (i.e., exon-1, 2, 3, and 4) and this receptor has its several splice variants (Chen, Mestek, Liu, Hurly & Yu, 1993; Pan, Xu., Bolan, Chang, Mahurter, Rossi & Pasternak, 2000; Pasternak, Pan. Xu, Yu, Xu & Pasternak, 2004; Thompson, Mansour, Akil. & Watson, 1993). Pharmacological data indicate that the mu_1 receptor as a splice variant of the MOR-1 is encoded by exon-1 and exon-4 and is critical for supraspinal *mu* opioid antinociception (Pasternak & Standifer, 1995; Rossi et al., 1995). For example, repetitive intracerebroventricular (i.c.v.) or intra-PAG injections of antisense ODN directed against exon-1 or exon-4 of the MOR-1 gene can prevent antinociception observed after application of the *mu* agonist morphine into the same site in the rodent as measured in the TF test (Rossi et al., 1995, 1997). In contrast, in these studies, antisense probes targeting exon-2 or exon-3 of the MOR-1 gene fail to block this opioid antinociception. Notably, DAMGO serves as mu_1 agonist in supraspinal regions as with morphine (Heyman, Williams, Burks, Mosberg & Porreca, 1988; Paul, Bodnar, Gistrak & Pasternak, 1989; Sanchez-Blazquez et al, 1999), and the present antinociception induced by intra-BLA injection of DAMGO can be effectively prevented by antisense ODN against exon-1 or exon-4 of the MOR-1 gene just as the morphine-produced pain inhibition can be. Accordingly, these viewpoints suggest that the present DAMGO-induced antinociception is attributable to DAMGO working on the BLA mu_1 receptor.

Our current study utilized the same gene inhibition as employed in a previous study (Rossi et al., 1997) in which intra-PAG applications (on days 1, 3 and 5) of antisense ODN (10 μg/μl at concentration) against exon-1 or exon-4 of the MOR-1 gene can block elevation in TF latencies in rats following application of the *mu* agonist morphine into the same site. However, this same antisense probe is unable to affect antinociception that is induced by the *delta* agonist deltorphin injected into the PAG. Thus,

these data represent the *mu* antisense ODN's specificity for the *mu* receptor. Furthermore, in this study, the *mu* antisense ODN's blockade of the *mu* antinociception seen after i.c.v. infusion of morphine becomes entirely abolished, when the same animals are TF-tested again on several days after the last injection of the *mu* antisense ODN. This observation indicates functional reversibility of the target *mu* receptor protein, suggesting a non-toxic effect of the pretreatment with the ODN. Therefore, in light of all these accounts, the present *mu* antisense ODN's blockade of DAMGO's action can be attributed to the selective down-regulation of *mu* receptors in the BLA.

The present binding study found that the amount of [^{3}H]-DAMGO binding to *mu* receptors is significantly reduced in rats pretreated with antisense ODN directed against exon-1 or exon-4, whereas the [^{3}H]-DAMGO binding is not affected by DNA control (mismatch ODN) or saline control treatment. These results are in agreement with previous pharmacological observations. That is, down-regulation of *mu* receptors via i.c.v. pretreatment over 5 days with antisense ODN, but not with cerebrospinal fluid or mismatch ODN controls, has been observed to reduce the amount of [^{3}H]-DAMGO binding to the *mu* receptors (Adams, Chen, DeRriel, Yin, Adler & Liu-Chen, 1996). Thus, our current observations from the binding study firmly support the present behavioral findings that BLA treatment with antisense ODN against exon-1 or exon-4 of the MOR-1 gene can effectively prevent DAMGO-produced elevation in TF latencies.

We presently did not introduce antisense probes against exon-2 and exon-3 of the MOR-1 gene due to a lack of their encoding of the mu_1 receptor (Pasternak & Standifer, 1995; Rossi et al., 1997). For example, i.c.v. pretreatment with antisense ODN against exon-2 or exon-3 of the MOR-1 gene is incapable of preventing morphine-induced antinociception (Rossi et al., 1997). Rather, the exon-2 and exon-3 are known to encode another MOR-1 subtype that is responsible for supraspinal antinociception induced by an exon-1 and exon-4 insensitive *mu* agonist such as morphine-6β-glucuronide (M6G) (Pasternak & Standifer, 1995; Rossi et al., 1995, 1997). This evidence thus suggests that the exon-2 and exon-3 function independently of exon-1 and exon-4 encoding the mu_1 receptor that is, as has been stated above, related to supraspinal morphine or DAMGO-induced antinociception. Therefore, the present pretreatment with antisense ODN directed against only exon-1 and exon-4 of the MOR-1 gene is thought to be sufficient to probe DAMGO's action on the *mu* receptor in the amygdala.

The primary site of action of compounds

presently applied into the amygdala is believed to be the target BLA. Existing autoradiographic and *in situ* hybridization data state that the BLA has the highest density of *mu* opioid receptors and a large amount of *mu* receptor mRNAs, in the amygdala (Atweh & Kuhar, 1977; Bunzow, Zhang, Bouvier, Saez, Ronnekleiv, Kelly & Grandy 1995; Mansour et al., 1987; Mansour, Fox, Thompson, Akil & Watson, 1994). More notably, our recent autoradiographic work has demonstrated that [^{3}H]-DAMGO (0.125 μCi/0.25 μl per side) infused into the BLA of the rat has limited spread (less than 10% of the radioactive chemical) to structures outside the target region BLA (Shin & Helmstetter, 2005). Thereby, the current intra-BLA injected compounds are suggested to act directly on cells in the BLA.

As with the present study, the standardized animal model utilizing anesthetic protocols is frequently employed to provide a good neural basis for investigating antinociceptive neural circuitry in awake organisms encountering threats. A major reason is that anesthetized animals that can produce more stable baseline responses such as TF reflexes often share same antinociceptive circuitry in the brain with conscious animals (Bellgowan & Helmstetter, 1998; Foo & Helmstetter, 2000). Thus, our current *mu*-selective antagonistic effect in the anesthetized animals is likely to induce a similar effect in awake animals.

Taken together, administration of DAMGO into the BLA inhibits radiant heat TF reflexes in anesthetized rats. Repetitive pretreatment of the BLA with antisense ODN targeting exon-1 or exon-4 of the MOR-1 gene not only prevents production of the antinociception following DAMGO infusion into the BLA, but also reduces the amount of [^{3}H]-DAMGO binding to the *mu* receptor in the amygdala. These findings thus firmly support the idea that the robust antinociceptive effect observed after intra-BLA injection of DAMGO is related to DAMGO's interaction with *mu* opioid receptors in the amygdala.

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편도체 기저외측핵의 뮤 수용기 수의 하향조절은 항유해 효과를 방해한다

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우리의 선행 연구에 따르면 뮤 효능제인 DAMGO를 편도체 기저외측핵(BLA)에 적용할 때 쥐에게서 방사열자극으로부터의 꼬리회피반응이 억제된다. 이 DAMGO로 유발된 항유해 효과는 비선택적 아편 길항제인 날록손, 뮤 아편 길항제인 베타-FNA, 또는 G 단백질 억제제인 퍼터시스 독소에 의해 사전 차단될 수 있다. 이는 DAMGO가 BLA에 있는 G 단백질 결합성 뮤 수용기와 상호작용해서 항유해 효과를 유발함을 시사한다. 유전자 조절법을 활용하는 본 연구는 DAMGO가 BLA의 뮤 수용기에 직접 작용하는가를 심도있게 조사했다. 뮤 수용기를 표적으로 안티센스 올리고디옥시뉴클리오티드는(ODN)를 BLA에 적용할 때, 같은 뇌부위에 DAMGO 주입으로 인해 유발된 항유해 효과가 차단되었다. 편도체의 뮤 수용기에 결합하는 방사성 표지된 DAMGO의 양도 안티센스 ODN을 사전처치 받은 쥐에게서 감소했다. 이들 자료는 BLA에 DAMGO를 주입해서 유발된 항유해 효과가 편도체에서 DAMGO와 뮤 수용기의 직접적인 상호작용에 기인한다는 생각을 확증해준다.

주제어 : 뮤 수용기, 편도체, 꼬리회피반사, 유전자 조절, 안티센스 올리고디옥시뉴클리오티드, 방사성 DAMGO 결합