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Transgenic superoxide dismutase mice differ in opioid-induced analgesia

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Abstract

Autoradiographic data from transgenic mice carrying the human Cu/Zn-superoxide dismutase gene demonstrate an increase in μ-opioid receptor concentration in dopaminergic-related areas and the central grey area. The relative potencies of μ-, δ- and κ-opioid receptor agonists to induce antinociception in heterozygous and homozygous superoxide dismutase transgenic mice as well as four inbred strains were assessed to determine the functional significance of the increased receptor concentration. Increased superoxide dismutase activity results in an increased sensitivity to μ-agonists in a gene dosage-dependent manner. SOD/Tg/hom mice were less sensitive to the δ-agonist than were SOD/Tg/het mice. The superoxide dismutase transgene did not affect κ-opioid receptor agonist sensitivity. These data suggest that δ-opioid receptors are not regulated in the same manner as μ-opioid receptors and that κ-opioid receptors are unaffected by superoxide dismutase activity.

Keywords: Transgenic mouse; Superoxide dismutase; Opiate receptor; Antinociception; Genetics

1. Introduction

Insertion of cloned DNA into the genome of a mouse provides a degree of experimental manipulation previously unavailable for studying complex systems. One example of transgenic technology use in experimental design is the insertion of the gene encoding the free radical metabolizing enzyme, human Cu/Zn superoxide dismutase (CuZnSOD), into the mouse genome (Epstein et al., 1987). The human CuZnSOD gene is located on the triplicate chromosome found in Down's syndrome (chromosome 21). Over-production of the superoxide dismutase gene in the transgenic mouse mimics the phenotypic consequence of increased gene dosage seen in Down's syndrome. The result of inserting this gene into the mouse genome is a several-fold increase in brain CuZnSOD-activity (Przedborski et al., 1992a). Heterozygous superoxide dismutase mice exhibiting intermediate increases in superoxide dismutase activity (2.6-fold) provide a valuable complement to the homozygous superoxide dismutase mice (5.7-fold mean increase) for assessing the gene dosage hypothesis. These transgenic mice, however, are more than a model for gene dosage observed in Down's syndrome. Mice that demonstrate a specific increase in superoxide dismutase activity offer a unique opportunity to explore numerous hypotheses related to the oxidative state of neuronal tissue and the importance of the free-radical metabolizing enzyme, superoxide dismutase. For example, superoxide dismutase transgenic mice have been used to demonstrate the importance of oxygen-based radicals in methamphetamine-, *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-, methylenedioxymethamphetamine (MDMA)-and methylenedioxyamphetamine (MDA)-induced neurotoxicity and lethality (Przedborski et al., 1992b; Cadet et al., 1994a,b). The neuroprotective effects of superoxide dismutase are hypothesized to occur via attenuation of excess superoxide radical pro-

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duction during increased dopaminergic activity (Rosenberg, 1988; Spina and Cohen, 1989).

Alterations in a key free-radical metabolizing enzyme such as superoxide dismutase may influence neuronal systems sensitive to oxidative stress and/or closely associated with free-radical producing systems such as the dopaminergic system. Neuronal opiate receptors fit both of these categories; neuronal opiate receptors are sensitive to oxidative stress (Marzullo and Hine, 1980; Hanissian and Tejwani, 1988) and are intimately associated with the dopaminergic system (Edley and Herkenham, 1984; Dilts and Kalivas, 1989; Unterwald et al., 1989; Spanagel et al., 1990). For this reason, changes in neuronal opiate-receptor concentration have recently been investigated using autoradiographic techniques (Kujirai et al., 1994). The results of this study demonstrated a significant region-specific increase in central nervous system μ-opiate receptor concentration in SOD/Tg mice. Increased μ-opiate binding was observed in the shell of the nucleus accumbens, substantia nigra pars compacta, the ventral tegmental area and the ventral part of the central grey area. Three of these four regions are significantly associated with the dopaminergic system. Thus, these mice not only provide a unique genotype to explore the importance of oxidative stress and superoxide dismutase activity in neuronal function, but they also provide a means to explore the behavioral contribution of specific regional changes in opiate receptor concentration.

The purpose of the current experiments was to investigate the functional consequences of the observed region-specific increases in μ-opioid receptor concentration. The antinociceptive effects of two μ-opioid receptor agonists and a δ-and κ-opioid receptor agonist were determined in the transgenic superoxide dismutase mice (homozygous and heterozygous) and the host strain, CD1. In addition, the antinociceptive effects of these opioids were determined in two commonly available inbred mouse strains (BALB/ByJ, C57BL/6J) and two recombinant inbred mouse strains (CXBH/ByJ and CXBK/ByJ). These inbred strains were included in order to provide a framework for assessing the magnitude and relative change in antinociception produced by the transgene insertion. Regarding the potency of opioid-induced analgesia, these strains provide some of the most (CXBH/ByJ, BALB/ByJ) and least sensitive strains (C57BL/6J, CXBK/ByJ) available for comparison (Elmer et al., 1995a,b). In addition, the CXBK/ByJ mice are deficient in opiate receptor concentration in a region-specific manner that nearly mirrors the increases seen in the transgenic superoxide dismutase mice (Moskowitz and Goodman, 1985; Kujirai et al., 1994). The experiments described herein addressed two questions: (1) does superoxide dismutase gene dosage significantly influence the in vivo consequences of superoxide dismutase transgene insertion and (2) to what degree does the superoxide dismutase transgene influence the antinociceptive effects of opioids as compared to other commonly available genotypes?

2. Methods and materials

2.1. Animals

Adult male SOD/Tg/homozygous (SOD/Tg/hom), SOD/Tg/heterozygous (SOD/Tg/het), CD1 (CD), BALB/ByJ (BALB), C57BL/6J (C57) and male and female CXBK/ByJarc (BK) and CXBH/ByJarc (BH) mice (Jackson Laboratories and Addiction Research Center), 60–120 days old weighing approximately 23–30 g at the start of the experiment were used. Transgenic mice of strain Tg 218/3 were produced as previously described (Epstein et al., 1987; Cadet et al., 1994b). All animals were experimentally naive, housed in groups of 3–5 in a temperature-controlled room (21°C) with a 12-h light-dark cycle (07:00–19:00 h lights on), and given free access to Purina Laboratory Chow and tap water during the entire experimental procedure. The animals used in this study were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC) and the studies were conducted in accordance with the Guide for Care and Use of Laboratory Animals provided by the NIH and adopted by NIDA.

2.2. Determination of relative antinociceptive sensitivity

Morphine-, [D-Ala^2,*N*-Me-Phe^4,Gly-ol^5]enkephalin-(DAMGO), [D-Pen^{2,5}]enkephalin (DPDPE)-and *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1(pyrrolidinyl)-cyclohexyl]-benzeneacetamide methanesulfonate (U50488)-induced analgesia was measured by the hot-plate test (51°C). Morphine-induced antinociception was determined via a cumulative dosing procedure in which each strain received 1/2 log increments as a cumulative morphine dose (s.c.) every 20 min. Mice were tested on the hot-plate just prior to the next morphine dose. No more than four doses were administered to a subject. The dose range for each strain was determined in pilot studies. Morphine-treated mice at each dosing interval were compared to saline-treated mice tested with the same procedure. A cut-off time of 4 times each strain's saline control values was used to avoid tissue damage. Each dose-response curve for each strain involved 5–7 animals.

DAMGO-, DPDPE-and U50488-induced antinociception was determined 10 min post i.c.v. injection of a single dose of agonist. Injections (3 μl injection vol-

ume) were given as described by Haley and McCormick (1957) under light isoflurane anesthesia. U50488-induced antinociception was determined only in the transgenic and CD1 mice. A cut-off time of 4 times saline control values was used to avoid tissue damage. Each dose of DAMGO, DPDPE or U50488 for each strain involved 5–9 animals.

2.3. Data analysis

Analgesia data are presented as the maximum percent effect (%MPE) determined with the following formula: $100 \times [(\text{latency to paw-lick}) - (\text{saline baseline latency})] \div [(4 \times \text{baseline latency}) - (\text{baseline latency})]$. The ED_{50} values were derived from the regression analysis of the linear portion of each dose-response curve. A two-way analysis of variance (Genotype × Dose) was used to determine genetic differences in drug-induced antinociception. A linear correlation was used to assess genetic correlations within μ-agonists (morphine vs. DAMGO) and across opioid agonists (μ vs. δ). The ED_{50} was used as a measure of agonist potency for each inbred strain. The degree of genetic covariance (genetic correlation) between two phenotypes is an indication of the degree to which the actions of each agonist are mediated by the same mechanism (Crabbe et al., 1990).

3. Results

3.1. Relative antinociceptive sensitivity to μ-, δ- and κ-opioid receptor agonists

Fig. 1, panels A, B, C and D, shows opioid-induced antinociception for morphine, DAMGO, DPDPE and U50488, respectively. There was a significant effect of genotype on morphine, DAMGO and DPDPE antinociceptive dose-effect curves (Morphine: F(Genotype) df 6,189 = 4.9, $P < 0.0009$; DAMGO: F(Genotype) df 6,134 = 2.9, $P < 0.01$; DPDPE: F(Genotype) df 6,155 = 5.53, $P < 0.0001$). BH mice were the most sensitive to the analgesic effects of morphine and DAMGO. C57 mice were the least sensitive to the analgesic effects of morphine and DPDPE and equally insensitive to DAMGO as the BK and CD1 mice. ED_{50} values for each strain and each drug are shown in Table 1. The genetic correlations between sensitivity to morphine versus DAMGO and DAMGO versus DPDPE are shown in Fig. 2. There was a significant correlation across all genotypes between the μ-agonists, morphine and DAMGO ($R = 0.82$; $P < 0.05$). There was no genetic correlation between the μ-agonist DAMGO and the δ-agonist DPDPE.

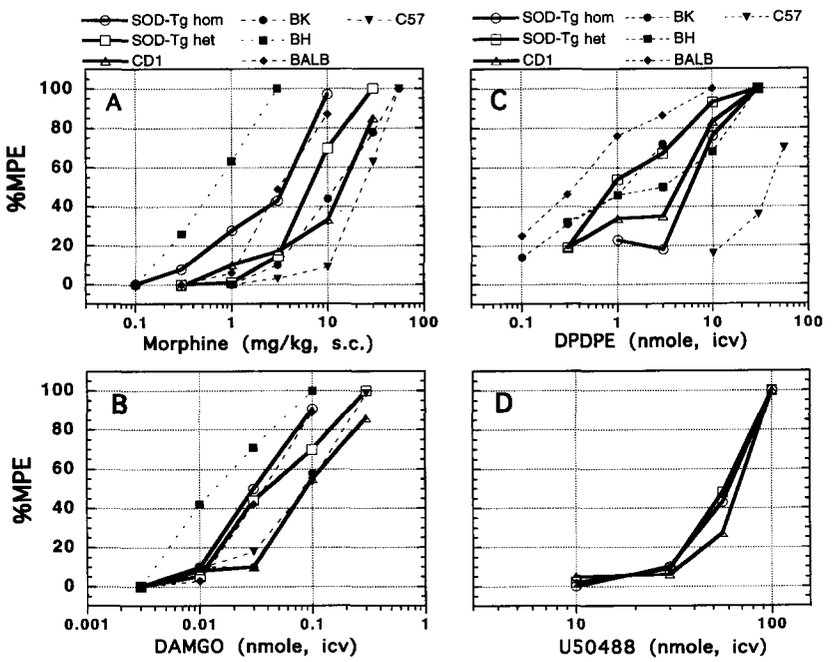

Fig. 1. Panels A, B, C and D show the antinociceptive effect of morphine, DAMGO, DPDPE and U50488, respectively, in two transgenic lines, the host 'strain' CD1, and four inbred mouse strains. Morphine dose-effect curves (panel A) were made via a cumulative dosing procedure (s.c.); each point represents the mean for 5–7 mice. DAMGO, DPDPE and U50488 dose-effect curves were made with single i.c.v. injections; each point represents the mean for 5–7 mice. Data for transgenic animals and CD1 dose-effect curves are represented by the solid lines.

Table 1
Analgesic potency of opioids as a function of genotype

Strain	Morphine ED_{50} (s.c., mg/kg)	DAMGO ED_{50} (i.c.v., pmol)	DPDPE ED_{50} (i.c.v., nmol)	U50488 ED_{50} (i.c.v., nmol)
SOD/Tg/hom	1.5	27.5	7.0	71.2
SOD/Tg/het	8.1	45.8	1.1	68.1
CD1	10.3	95.6	3.1	80.0
BALB	2.8	35.8	0.3	
C57	22.1	70.2	29.0	
BH	0.8	15.4	2.1	
BK	3.7	81.8	1.0	

3.2. Specific effects of superoxide dismutase gene dosage on opioid-induced analgesia

Superoxide dismutase gene dosage significantly influenced the potency of morphine, DAMGO and DPDPE to produce analgesia (Morphine: F(SOD Genotype) df 2,111 = 4.9, $P < 0.009$; DAMGO: F(SOD Genotype) df 2,66 = 3.4, $P < 0.04$; DPDPE: F(SOD Genotype) df 2,84 = 2.9, $P < 0.05$). Within the transgenic and CD1 mice there was a rank order correlation between gene dosage and the potency of the μ-opioid receptor agonists, morphine and DAMGO, to produce analgesia. Although gene dosage significantly influenced DPDPE-induced analgesia, there was no clear relationship between gene dosage and the potency of DPDPE. There was no influence of genotype on the potency of U50488 to produce analgesia (U50488: F(SOD Genotype) df 2,58 = 0.04, ns).

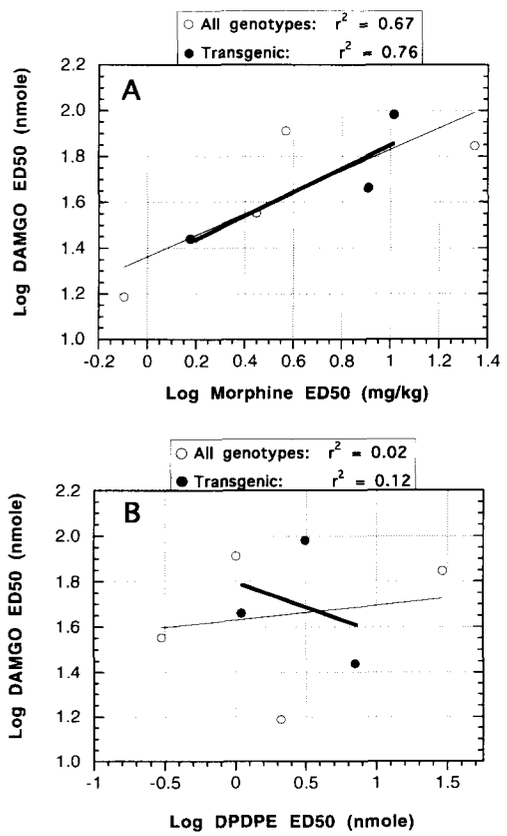

Fig. 2. Panels A and B represent the genetic correlation between sensitivity to the analgesic effects of morphine versus DAMGO and DAMGO versus DPDPE, respectively. Data for transgenic animals and CD1 points are represented by solid points. Each point represents the transgenic or inbred ED_{50} value for each opioid.

4. Discussion

The experiments described herein addressed two questions: (1) does superoxide dismutase gene dosage significantly influence the in vivo response to opioids and (2) to what degree does the superoxide dismutase transgene influence the antinociceptive effects of opioids as compared to other commonly available genotypes? One of the functional consequences of increased superoxide dismutase activity and μ-opioid receptor B_{max} in the SOD/Tg/hom mice was an increased sensitivity to the antinociceptive effects of the μ-opioid receptor agonists morphine and DAMGO; the SOD/Tg/het mice were intermediate in their sensitivity as compared to that of the host CD1 mice. Therefore, superoxide dismutase gene dosage appears to influence the relative sensitivity to μ-opioid receptor agonists in these transgenic mice. Interestingly, the SOD/Tg/hom mice are less sensitive to the δ-opioid receptor agonist, DPDPE, than are the SOD/Tg/het. There was no effect of the transgene on sensitivity to the κ-opioid receptor agonist U50488. In general, these data suggest that δ-opioid receptors are not regulated in a manner similar to μ-opioid receptors by superoxide dismutase activity and that κ-opioid receptors are unaffected by superoxide dismutase activity. These data are supported by the demonstrated independent inheritance of sensitivity to μ- versus δ-opioid receptor agonists (see Fig. 2).

The superoxide dismutase transgene does not increase or decrease the potency of morphine or DPDPE to a level outside the range of values found in commonly available inbred mouse strains. ED_{50} values for the transgenic mice were within the range of values found in four inbred mouse strains chosen for divergent response to opioids. Insertion of the transgene into a relatively insensitive outbred strain (CD1) significantly improved the possibility of demonstrating enhancement of μ-opioid receptor agonist antinociception by increased superoxide dismutase activity. These data demonstrate the importance of the background genotype on the phenotypic expression of genetic manipulations (Coleman and Hummel, 1975). In general, these data agree with previous reports describing a significant influence of genotype on sensitivity to opi-

oids and divergent response to μ-agonists in the opiate receptor-rich BH versus μ-opioid receptor deficient BK mice (Marek et al., 1990; Elmer et al., 1995b; Moskowitz and Goodman, 1985). The lack of a genetic correlation between μ-and δ-opioid receptor agonist sensitivity and differential modulation of μ- versus δ-opioid receptors by superoxide dismutase confirm independent inheritance and regulation of these two opiate receptors.

Insertion of the human CuZnSOD gene into the mouse genome may have increased μ-opioid receptor concentration by direct alteration of superoxide free radical concentration and consequent effects on μ-binding. Conversely, random insertion of the transgene may have disrupted genome function related to μ-opioid receptor regulation. With regard to the latter point, the superoxide dismutase transgene is located on chromosome 3 whereas the μ-opioid receptor has been mapped to chromosome 10 (Kozak et al., 1994; Shi et al., 1994). Distinct chromosomal locations do not rule out the potential insertion of the transgene in other areas related to the regulation of μ-opioid receptor number. However, there are several lines of evidence to support the direct involvement of superoxide dismutase activity in the regulation of μ-receptor number and function. First, superoxide dismutase activity plays a critical role in regulating the redox state of the cell (Cadet, 1988), which in turn significantly influences in vitro μ- and δ-opioid binding (Marzullo and Hine, 1980; Bowen and Pert, 1982) and in vivo antinociception (Marzullo and Hine, 1980). Transition cations such as Cu^{2+} inhibit opiate receptor binding, an effect that is reversed with thiol reducing reagents (Marzullo and Hine, 1980). The ability of thiol reducing agents to reverse Cu^{2+} inhibition of opiate binding is in rank order correlation with their negative redox potential. Addition of low concentrations of H_2O_2 to striatal slices enhances μ-binding while the thiol reducer, dithiothreitol, reduces μ-binding and increases δ-binding (Bowen and Pert, 1982). Thus, transgenic alteration of superoxide dismutase activity may alter opiate receptor binding by altering the status of redox buffers in the cell. A second more indirect effect of superoxide dismutase activity may stem from the regulation of intracellular H_2O_2 and its subsequent effects on membrane viscosity (Cadet, 1988). H_2O_2-induced lipid peroxidation of phospholipids results in decreased membrane viscosity. Decreases in membrane viscosity significantly inhibit opioid binding (Lazar and Medzihradsky, 1992). Increased superoxide dismutase may increase intracellular H_2O_2 and may ultimately increase lipid peroxidation. This would result in decreased viscocity and subsequent decreases in δ-opioid receptor binding. This might help to explain the effects of delta ligands in the SOD/Tg mice since δ-opioid receptor binding appears to be more sensitive to these effects than μ-opioid receptor binding (Lazar and Medzihradsky, 1992).

Regardless of the mechanism by which the superoxide dismutase transgene altered μ-opioid receptor concentration, the result is a genotype with specific changes in μ-opioid receptor concentration in regions intimately linked to dopaminergic systems and thought to be important in the analgesic, stimulant and reinforcing effects of opioids. The empirical consequence seen thus far is an increase in the antinociceptive potency of the μ-agonists morphine and DAMGO. Future studies using the superoxide dismutase transgenic mice are planned to assess the consequences of region specific differences in μ-opioid receptors in the stimulant and reinforcing effects of opioids. Since there is evidence to suggest differential involvement of the shell versus the core of the nucleus accumbens in drug-reinforced behavior (Robledo and Koob, 1993) and since the increases in μ-opioid receptors appear to be functionally relevant, these transgenic mice may provide a unique behavioral genetic approach to the investigation of drug-reinforced behavior.

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