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Brain regional μ-opioid receptor function in rat lines selected for differences in alcohol preference

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Abstract

It has been suggested that opioid peptides play a role in the reinforcing effects of alcohol. The present study was designed to examine the function of the μ-opioid receptor system in rat lines selectively bred for alcohol preference (AA [Alko, Alcohol] rat line) and alcohol avoidance (ANA [Alko, Non-Alcohol] rat line). The functional coupling of μ-opioid receptors to G proteins was determined autoradiographically using Tyr-D-Ala-Gly-N(Me)Phe-Gly-ol-enkephalin-stimulated [^{35}S]GTPγS binding in brain cryostat sections. The binding was significantly increased in the striatal patches and substantia nigra reticulata of the AA rats in comparison with that of the ANA rats. Within the AA rat line, there was a significant positive correlation between 3 mg/kg morphine-induced locomotor activity and activation of G-proteins in the substantia nigra compacta and nucleus accumbens core. These results of the selective breeding experiment suggest that brain region-specific differences in μ-opioid receptor function may correlate with innate differences in alcohol preference.

Keywords: Alcohol preference; Opioid receptor; Morphine; Locomotor activity; (Selected rat line)

1. Introduction

The brain opioidergic system is involved in the positive reinforcing actions of alcohol and excessive alcohol consumption (Herz, 1997). Alcohol induces release of opioid peptides in the rodent brain (Gianoulakis et al., 1981; Seizinger et al., 1983) and also modifies the expression and function of opioid receptors (Charness et al., 1983, 1986, 1993). Alcohol consumption of rodents is effectively decreased by nonselective opioid receptor antagonists, such as naloxone and naltrexone (Altshuler et al., 1980; Froehlich et al., 1990; Hyytiä and Sinclair, 1993a; Sinclair, 1990; Wegelius et al., 1994; Weiss et al., 1990) and by subtype-selective μ-opioid receptor antagonists CTOP (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2) and naloxonazine (Froehlich et al., 1991; Honkanen et al., 1996; Krishnan-Sarin et al., 1995, 1998; Le et al., 1993). In agreement, the μ-opioid receptor knockout mice do not self-administer alcohol (Roberts et al., 2000).

Electrophysiological studies on brain slices and acutely dissociated ventral tegmental area neurons have also shown that behaviorally relevant alcohol doses (20–200 mM) increase the firing of dopaminergic ventral tegmental area neurons in a concentration-dependent manner (Brodie et al., 1990, 1999). Alcohol also increases the firing rate of dopaminergic neurons in the substantia nigra compacta and inhibits non-dopaminergic neurons in the substantia nigra reticulata probably by regulating the release of GABA (Gessa et al., 1985; Mereu and Gessa, 1985). In fact, the inhibitory effect of alcohol on substantia nigra reticulata neurons is thought to activate the dopaminergic neurons in substantia nigra compacta and increase their dopamine release in a similar way as μ-opioid receptor agonists cause disinhibition in the ventral tegmental area. These data clearly support the involvement of μ-opioid receptors in mediating the reinforcing effects of alcohol.

Genetic background plays an important role in the susceptibility to drug and alcohol addiction. Selected rat lines have been widely used as a tool for studying the behavioral, neurobiological and genetic factors in addiction. It is commonly concluded that all differences between the lines should somehow be linked to the behaviors that were originally used to select the lines in various experiments. An example of such an experiment is the alcohol-preferring AA (Alko, Alcohol) and alcohol-avoiding ANA (Alko, Non-

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Alcohol) rat lines that were selectively outbred for differences in voluntary alcohol drinking (Eriksson, 1968). There are many biobehavioral differences between these lines (Sinclair et al., 1989). The most interesting differences are related to the functions of the μ-opioid receptor system. Oral consumption of aqueous solution of etonitazene, a potent opioid receptor agonist, is higher in AA than ANA rats (Hyytiä and Sinclair, 1993b). μ-Opioid-induced locomotor activation is greater in the AA than ANA rats. The AA rats develop a large locomotor stimulation by acute morphine (3.0 mg/kg) while in the ANA rats or the nonselected Wistar rats the locomotor stimulant effect is marginal. The AA rats are behaviorally sensitized more readily than the ANA rats during repeated morphine treatment (Honkanen et al., 1999b). The μ-, δ- and κ-opioid receptor densities are greater in some brain regions such as the substantia nigra and striatal patches of the AA than ANA rats (Soini et al., 1998, 1999). The concentrations of opioid propeptide mRNA, opioid peptide mRNA and peptide contents are also different between the lines (Gianoulakis et al., 1992; Marinelli et al., 2000; Nylander et al., 1994).

Few studies have examined the functional activity of opioid receptors in rat models of alcohol drinking behavior. In a recent study, the binding of guanosine 5′(γ)[^{35}S](thio)triphosphate ([^{35}S]GTPγS) to brain sections of the high-drinking AA rats after in vitro stimulation of μ-, δ- and κ-opioid receptors by the μ-opioid receptor-selective peptide DAMGO (Tyr-D-Ala-Gly-N(Me)Phe-Gly-ol-enkephalin), the δ-opioid receptor-selective peptide DPDPE (Tyr-D-Pen-Gly-Phe-D-Pen-enkephalin) and the non-peptide κ-opioid receptor-selective U-50488, respectively, was evaluated. The activation of G-proteins was greatest for μ-opioid receptors when compared to those of δ- and κ-opioid receptor subtypes (Hyytiä et al., 1999). The μ-opioid receptor densities were up-regulated during chronic naloxone treatment with osmotic minipumps (3.0 mg/kg/h for 7 days). The up-regulation was accompanied by an increase in receptor coupling to G protein activation and by a rebound elevation in alcohol intake after termination of naloxone treatment. These results suggested that high functional activity of μ-opioid receptors is associated with high alcohol drinking. Therefore, in the present study, we compared the functional activity of μ-opioid receptors between the alcohol-naïve AA and ANA lines of rats and tested the possible correlation between morphine-induced locomotor activity and G-protein activation within the AA rats.

2. Materials and methods

2.1. Animals

Adult (>3 months) AA and ANA rats were housed in groups of four to six in a room with controlled temperature (20 ± 2 °C) and humidity (55 ± 5%) on a 12-h light/dark cycle (lights on at 07:00 a.m.). Animals had free access to tap water and RM1 (E) pellet food (SDS, Witham, UK). All experimental procedures were approved by the Institutional Animal Care and Use Committees of the University of Turku and the National Public Health Institute, Helsinki.

2.2. Measurement of locomotor activity

Locomotor activity was measured in transparent Macrolon III cages (18 × 33 × 15 cm) that were placed inside a frame with seven photocells on each side of the frame (Cage Rack Activity System, San Diego Instruments, CA, USA). The photocells were located 5 cm above the surface of the cage bedding. The number of interruptions of successive photocells was used as the measure of forward horizontal locomotor activity and was recorded by a computer at 10-min intervals. During testing, rats were removed from their home cages and placed into measurement cages located in an adjacent experimental room. Rats (n = 9 for the AA line) were habituated to the test cages during the first three sessions. During the last 2 habituation sessions, the rats were first allowed to habituate to the cage for 15 min, after which they received a saline injection (1 ml/kg, s.c.). After the injection, locomotor activity was monitored for 90 min. On the morphine test session, the rats were injected with morphine (3 mg/kg, s.c.) after the 15-min habituation and then monitored for 180 min. All testing was conducted during the light phase of the light/dark cycle. For the brain receptor studies, these animals were sacrificed on the following day after measurement of locomotor activity.

2.3. [^{35}S]Guanosine 5′ -(γ-thio)triphosphate autoradiography

[^{35}S]GTPγS autoradiography was performed as described in detail by Hyytiä et al. (1999). Fourteen-micrometer-thick coronal brain sections were cut from the brains of naïve AA and ANA rats (n = 6 for the both lines) and from those of morphine-tested AA rats (n = 9) with a Leitz 1720 cryostat at levels 1.6, 1.2, −5.2, −5.6, and −5.8 mm from bregma (Paxinos and Watson, 1982). The sections were thaw-mounted on poly-L-lysine-coated slides, dried at room temperature and kept at −20 °C until used. The sections were thawed, preincubated at the room temperature using the liquid bubble method in 50 mM Tris–HCl (pH 7.4), 1 mM EDTA, 100 mM NaCl and 5 mM $MgCl_2$ for 20 min. After that the sections were incubated in the same buffer with 2 mM guanosine diphosphate (GDP, Sigma, St. Louis, MO, USA) for 60 min in the presence of 1 μM adenosine A_1 receptor blocker 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, Sigma) in order to eliminate a widespread adenosine A_1 receptor-dependent signal in basal conditions of [^{35}S]GTPγS autoradiography. The final incubation buffer with 225 pM [^{35}S]GTPγS (Du Pont De Nemours, New England Nuclear, Dreieich, Germany) was supplemented with 2 mM GDP, 1 mM dithiotreitol, 1 μM DPCPX and 0.1, 0.3, 1, 3 and 10 μM DAMGO (RBI,

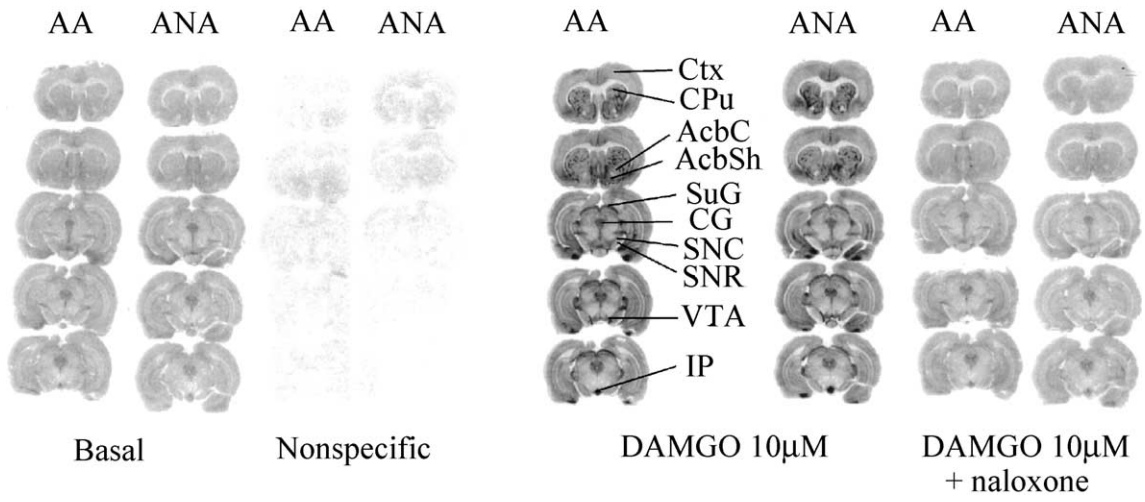

Fig. 1. Representative autoradiographic images of [^{35}S]GTPγS binding by μ-opioid receptor activation in the brain sections of alcohol-preferring AA and alcohol-avoiding ANA rats. The figure depicts the basal binding, the nonspecific binding in the presence of 10 μM GTPγS and the bindings stimulated by 10 μM DAMGO in the absence and presence of 10 μM naltrexone. Ctx, cortex; CPu, caudate-putamen; AcbC, nucleus accumbens core; AcbSh, nucleus accumbens shell; SuG, superior colliculus; Cg, central gray; SNC, substantia nigra compacta; SNR, substantia nigra reticulata; VTA, ventral tegmental area. IP, interpeduncular nucleus.

Natick, MA, USA) in the absence and presence of 10 μM naltrexone (RBI). Our preliminary studies revealed that 10 μM DAMGO gave maximal increase in [^{35}S]GTPγS binding in a naltrexone–sensitive manner. The sections were washed twice for 5 min in ice-cold 50 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$, dipped in ice-cold distilled water and dried under a stream of air in room temperature. Nonspecific binding was assessed in the presence of 10 μM unlabelled GTP-γ-S (Sigma). Autoradiograms were made by exposing Hyperfilm™-βmax (Amersham, Arlington Heights, IL, USA) to the labeled sections for 4 days in X-ray cassettes at 8–15 °C together with autoradiographic [^{14}C]-microscale standards (Amersham). The films were developed, the images scanned for pictures and binding densities deter-

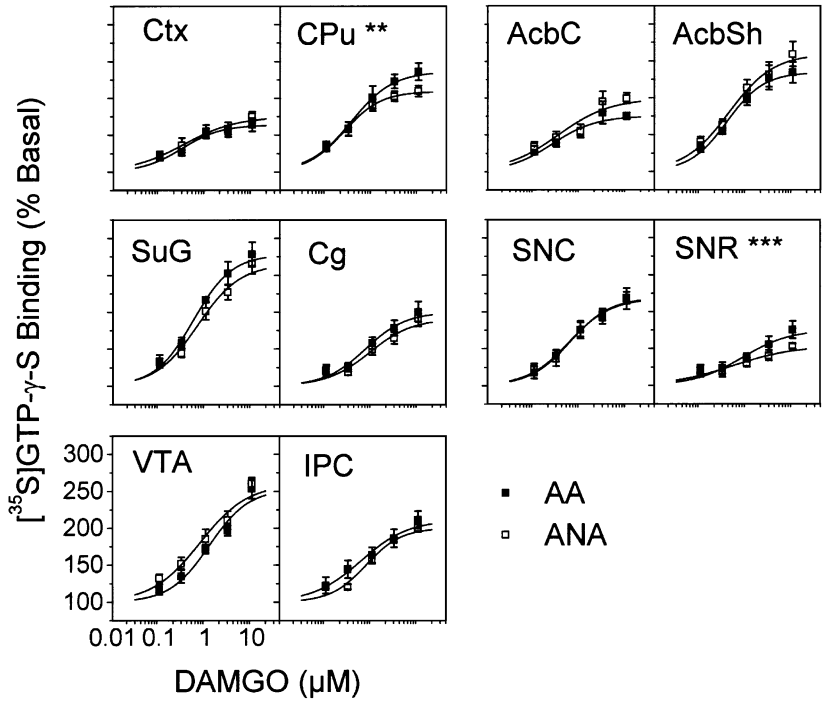

Fig. 2. Dose-dependent and saturable μ-opioid receptor-dependent G-protein activation in different brain regions revealed by [^{35}S]GTPγS autoradiography. The data are mean ± S.E.M. from six rats per line. Significant differences between the lines $^{**}P<0.01$, $^{***}P<0.001$.

Table 1
EC_{50} values for DAMGO for DAMGO stimulation of [^{35}S]GTPγS binding in various regions of brains in AA and ANA rats

Brain region	AA	ANA
Cortex	0.30 ± 0.06	0.33 ± 0.07
Caudate-putamen	0.34 ± 0.04	0.24 ± 0.01
Nucleus accumbens core	0.25 ± 0.08	0.34 ± 0.13
Nucleus accumbens shell	0.37 ± 0.04	0.41 ± 0.05
Superior colliculus	0.54 ± 0.05	0.67 ± 0.12
Central gray	0.72 ± 0.13	0.89 ± 0.24
Substantia nigra compacta	0.58 ± 0.07	0.61 ± 0.08
Substantia nigra reticulata	0.80 ± 0.22	0.50 ± 0.19
Ventral tegmental area	1.21 ± 0.27	0.76 ± 0.18
Interpeduncular nucleus	0.58 ± 0.07	0.75 ± 0.15

Values are mean ± S.E.M.of six rats per line. Two-way ANOVA between the rat lines did not reach significance in any of the brain regions.

mined by using MCID AIS programs (Imaging Research, St. Catharines, Canada) as described (Korpi et al., 1995), scaling the densities with the aid of the microscale standards. The brain areas analyzed were identified using an atlas of the rat brain (Paxinos and Watson, 1982) and adjacent thionin-stained sections.

2.4. Statistical analysis

Statistical analysis was performed either by using one-way ANOVA (basal and nonspecific binding differences between the strains), two-way ANOVA (regional differences between the strains) or three-way ANOVA (rat line and brain region as factors and DAMGO concentrations as repeated measures) and followed by Tukey's test (SPSS v. 9.0, Chicago, IL, USA). EC_{50} values were fitted with sigmoidal logistic function using Microcal Origin 5.0 program (OriginLab, MA, USA). Stimulation achieved with 10 μM DAMGO was considered as maximum. Pearson correlation coefficients were calculated between 10 μM DAMGO-stimulated [^{35}S]GTPγS binding values and morphine-induced locomotor activity scores in individual AA rats ($n = 9$) using Prism program (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Brain regional opioid receptor agonist-stimulated [^{35}S]GTPγS binding in alcohol-naïve AA and ANA rats

Basal and nonspecific binding levels are shown in representative sections in Fig. 1. The basal [^{35}S]GTPγS binding was somewhat unevenly distributed while the nonspecific binding was low and evenly distributed throughout the brain. There was no significant rat line difference in the basal binding [AA 190.1 ± 8.1 nCi/g vs. ANA 179.9 ± 9.4

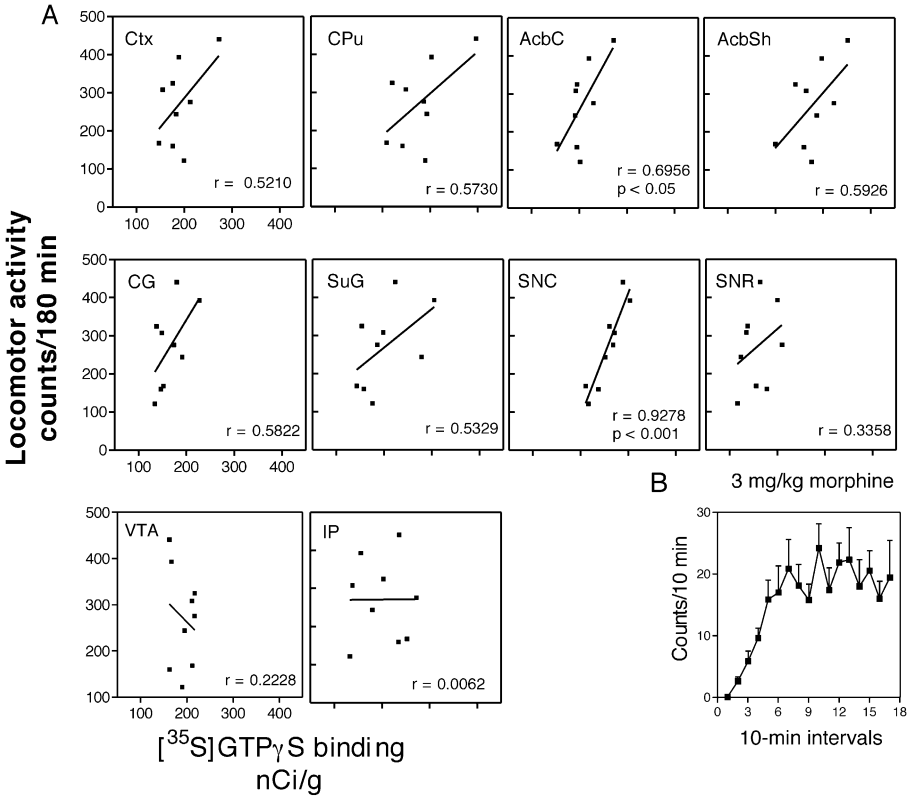

Fig. 3. (A) Correlation between morphine-induced locomotor activity and 10 μM DAMGO-stimulated [^{35}S]GTPγS binding in brain areas of nine AA rats. Locomotor activity values were summed up from values measured for 180 min in 1-min intervals following the acute morphine treatment (3 mg/kg). (B) Effects of acute treatment with morphine (3 mg/kg) on the locomotor activity values for 180 min. Mean ± S.E.M. for the nine rats used in panel A.

nCi/g, mean ± S.E., $n=6$; $F(1,106)=0.622$, $P=0.432$] or in the nonspecific binding [AA 69.8 ± 1.6 nCi/g vs. ANA 74.2 ± 1.6 nCi/g; $F(1,106)=3.930$ $P=0.005$]. A three-way ANOVA on the DAMGO-stimulated [^{35}S]GTPγS binding revealed statistically significant interactions between brain region and dose [F (40,540) = 8.90, $P<0.001$] and region and rat line [$F(8,540)=2.025$, $P=0.042$]. In addition, significant main effects of region ($P<0.001$), dose ($P<0.001$) and rat line ($P=0.035$) on [^{35}S]GTPγS binding values were found. Neuroanatomical distribution of DAMGO-stimulated [^{35}S]GTPγS binding was similar to that in our previous study on AA rats (Hyytiä et al., 1999). The magnitude of DAMGO-stimulated [^{35}S]GTPγS binding varied between the different brain areas from 1.8 to 2.8 fold over the basal binding (Fig. 2). The regions with high DAMGO-stimulated [^{35}S]GTPγS binding included the ventral tegmental area, superior colliculus, interpeduncular nucleus, substantia nigra compacta, nucleus accumbens shell and caudate-putamen. Within the caudate-putamen, the stimulated binding occurred in patches. The brain areas that differed between the lines were the caudate-putamen [$F(1,35)=8.711$, $P=0.0045$] and substantia nigra reticulata [$F(1,35)=5.603$, $P=0.0203$]. The regional EC_{50} values did not differ between the lines (see Table 1).

3.2. Correlation of opioid receptor agonist-stimulated [^{35}S]GTPγS binding and morphine-induced locomotor activity in AA rats

The AA rats display high locomotor stimulation in response to low morphine doses (Honkanen et al., 1999b). We wanted to ascertain whether this response within a population of AA rats is correlated with the amount of DAMGO-stimulated G protein activation in various brain regions determined 24 h after the behavioral testing. There was rather large variation both in locomotor activity scores and binding values (Fig. 3). Morphine-induced locomotor activity was significantly positively correlated with the overall maximal DAMGO-stimulated [^{35}S]GTPγS binding in the substantia nigra compacta and nucleus accumbens core (Fig. 3). There were no significant correlations between the EC_{50} values of DAMGO and the locomotor activity values (data not shown).

4. Discussion

Experiments with selected lines of rodents are a powerful means of establishing genetic correlations between the selected features and other behaviors or parameters. Although there are many neurochemical differences between the alcohol-preferring AA and the alcohol-avoiding ANA rats, the morphine-induced hyperlocomotion in the AA rats (Honkanen et al., 1999b) is the only clear genetic behavioral correlate that is unrelated directly to alcohol effects and that can simply form the basis of pathophysiological hypothesis of the abnormally high alcohol preference of the AA rats. Since alcohol releases opioid peptides (De Waele and Gianoulakis, 1993; De Waele et al., 1994; Gianoulakis et al., 1981; Seizinger et al., 1983), the high reinforcement from alcohol drinking in the AA rats can be due to the released endogenous opioid peptides. This would also correlate with locomotor stimulant actions of alcohol drinking (Päivärinta and Korpi, 1993). In the present study, we extend this finding firstly by showing that μ-opioid receptor function in situ is increased in several relevant brain regions of the AA rats in comparison with the ANA rats, and secondly by revealing that within the AA rat line there exists a positive correlation between μ-opioid receptor activation and morphine-induced hyperlocomotion. Therefore, our results together with previous data indicate the correlation of altered μ-opioid receptor function and alcohol drinking behavior in our model system.

The activation of G-proteins by DAMGO was dose-dependent and saturable, and the percentual stimulation by DAMGO ranged from 180% to 280%, suggesting regional variation in receptor coupling efficiency and/or receptor density. In our previous study, a positive correlation was observed between the ligand binding site densities and G-protein activation in brain sections for μ-opioid receptors under conditions of induced up-regulation of opioid receptors (Hyytiä et al., 1999). This indicates that the maximal stimulations in the present study strongly depend on the number of active μ-opioid receptors. Earlier we have demonstrated higher μ-opioid receptor ligand binding in the substantia nigra and caudate-putamen patches of naïve AA than ANA rats assessed with both antagonist and agonist binding assays (Soini et al., 1998, 1999). We now found a higher G-protein activation in the AA than ANA rats in the same brain regions. Unexpectedly, we also found intense activation of G-proteins in the ventral tegmental area, the area previously undetectable by autoradiographic assays in several studies because of very sparse μ-opioid receptor content (Soini et al., 1998, 1999; Mansour et al., 1994). Yet, there was no significant difference between the AA and ANA rat lines in the activation of G-proteins in the ventral tegmental area. This is the area of dopaminergic cell bodies of the mesolimbic dopaminergic pathway which is thought to be involved in the reinforcing and locomotor activity-stimulating properties of drugs of abuse (Wise and Bozarth, 1987). The distribution of μ-opioid binding sites generally corresponded well with the agonist-stimulated G-protein activation (Hyytiä et al., 1999). Unfortunately, the ventral tegmental area was not among the analyzed brain areas in that study. However, some differences have been observed between the density of receptors and agonist-stimulated G-proteins, due to variation in coupling efficiency. In a recent study (Maher et al., 2000), variation in the relationship between μ-opioid receptor binding and DAMGO-stimulated [^{35}S]GTPγS binding was demonstrated in the rat brain. The amplification factor (the ratio between the B_{max} of receptor binding and the B_{max} of agonist-activated G-proteins) for

DAMGO was different between brain regions, ranging from 8 to 38. That kind of differences in coupling could account for the different physiological actions of opioids.

There was a significant positive correlation between morphine-induced locomotor activity and DAMGO-stimulated [^{35}S]GTPγS binding in the AA rats in the nucleus accumbens and substantia nigra. The nucleus accumbens (ventral striatum) is a projection area of the mesolimbic dopaminergic pathway originating in the ventral tegmental area. The neurons in the nigrostriatal dopaminergic pathway project from the substantia nigra compacta mainly to the caudate-putamen (dorsal striatum) (Björklund and Lindvall, 1984). Morphine increases the firing of dopamine neurons in substantia nigra compacta/ventral tegmental area by disinhibiting the GABAergic interneurons, which subsequently decreases the tonic inhibition of these neurons (Di Chiara and North, 1992; Johnson and North, 1992). Basal dopamine release is lower in the caudate-putamen and nucleus accumbens in the AA rats than ANA rats (Honkanen et al., 1999a; Mikkola et al., 2000), but the dopamine metabolism-stimulating and dopamine-releasing acute actions of morphine are greater in the caudate-putamen but not in the nucleus accumbens of the AA than ANA rats (Honkanen et al., 1999a; Mikkola et al., 2000). Therefore, the importance of the nigrostriatal rather than the mesolimbic pathway in our AA/ANA rat model is emphasized in dopaminergic activation, although it should be noted that some cross-projections exist from the ventral tegmental area to the caudate-putamen and from the substantia nigra compacta to the nucleus accumbens (Fallon and Loughlin, 1995). While the activation of nigrostriatal pathway is typically related to stereotypic behavior rather than to horizontal locomotion, receptors in the substantia nigra may also mediate the locomotor effects of μ-opioid receptor agonists (Morelli et al., 1989). Local infusion of μ-opioids into the nucleus accumbens increases locomotor activity and this behavioral effect has been shown to be independent of the mesolimbic DA system, because it could not be blocked by the neuroleptic injection into the nucleus accumbens nor by the destruction of mesolimbic DA system with 6-hydroxydopamine (Kalivas et al., 1983). For these reasons, the higher μ-opioid sensitivity of the AA rats may be due to enhanced μ-opioid receptor activation in the nigrostriatal dopaminergic pathway. It should be added that the increased opioid sensitivity of the AA rats is not detectable in spinal function since morphine-induced antinociception is identical in the AA and ANA rats in the tail-flick analgesia test (Honkanen et al., 1995). Provided that there are no differences in μ-opioid receptor subtypes or posttranslational modifications between the spinal cord and forebrain, we can speculate that the genetic correlation deals with altered receptor number, G-protein coupling and/or opioid effects rather than altered receptor structure.

The μ-opioid-induced locomotor activity has been demonstrated in numerous studies, but it has been difficult to show enhanced activity with alcohol. Namely, alcohol-induced locomotor activity is scarcely seen when alcohol is administered intraperitoneally (Cunningham et al., 1993; Frye and Breese, 1981). The same is also true for the AA and ANA rats, as alcohol administered intraperitoneally either acutely or repeatedly has no effect on locomotor activity in the AA, ANA and Wistar rat lines (Honkanen et al., 1999b). However, locomotor activation is measurable in AA rats when alcohol is consumed by voluntary drinking (Päivärinta and Korpi, 1993). Therefore, the observed alterations in the opioid receptor system of the AA rats and their correlations with the regional opioid-induced hyperactivity in AA rats may contribute to increased alcohol preference of these rats.

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References

Altshuler, H.L., Phillips, P.E., Feinhandler, D.A., 1980. Alteration of ethanol self-administration by naltrexone. Life Sci. 26, 679–688.

Björklund, A., Lindvall, O., 1984. Dopamine-containing system in the CNS. In: Björklund, A., Hökfelt, O. (Eds.), Classical Transmitters in the CNS, Part I. Elsevier, Amsterdam, pp. 55–122.

Brodie, M.S., Shefner, S.A., Dunwiddie, T.V., 1990. Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area in vitro. Brain Res. 508, 65–69.

Brodie, M.S., Pesold, C., Appel, S.B., 1999. Ethanol directly excites dopaminergic ventral tegmental area reward neurons. Alcohol., clin. exp. res. 23, 1848–1852.

Charness, M.E., Gordon, A.S., Diamond, I., 1983. Ethanol modulation of opiate receptors in cultured neural cells. Science 222, 1246–1248.

Charness, M.E., Querimit, L.A., Diamond, I., 1986. Ethanol increases the expression of functional delta-opioid receptors in neuroblastoma × glioglioma NG108-15 hybrid cells. J. Biol. Chem. 261, 3164–3169.

Charness, M.E., Hu, G., Edwards, R.H., Querimit, L.A., 1993. Ethanol increases delta-opioid receptor gene expression in neuronal cell lines. Mol. Pharmacol. 44, 1119–1127.

Cunningham, C.L., Niehus, J.S., Noble, D., 1993. Species difference in sensitivity to ethanol's hedonic effects. Alcohol 10, 97–102.

De Waele, J.P., Gianoulakis, C., 1993. Effects of single and repeated exposures to ethanol on hypothalamic beta-endorphin and CRH release by the C57BL/6 and DBA/2 strains of mice. Neuroendocrinology 57, 700–709.

De Waele, J.P., Kiianmaa, K., Gianoulakis, C., 1994. Spontaneous and ethanol-stimulated in vitro release of beta-endorphin by the hypothalamus of AA and ANA rats. Alcohol., clin. exp. res. 18, 1468–1473.

Di Chiara, G., North, R.A., 1992. Neurobiology of opiate abuse. Trends Pharmacol. Sci. 13, 185–193.

Eriksson, K., 1968. Genetic selection for voluntary alcohol consumption in the albino rat. Science 159, 739–741.

Fallon, J.H., Loughlin, S.E., 1995. In: Paxinos, G. (Ed.), Substantia Nigra, in The Rat Nervous System. Academic Press, San Diego.

Froehlich, J.C., Harts, J., Lumeng, L., Li, T.K., 1990. Naloxone attenuates voluntary ethanol intake in rats selectively bred for high ethanol preference. Pharmacol. Biochem. Behav. 35, 385–390.

Froehlich, J.C., Zweifel, M., Harts, J., Lumeng, L., Li, T.K., 1991. Impor-

tance of delta opioid receptors in maintaining high alcohol drinking. Psychopharmacology 103, 467–472.
Frye, G.D., Breese, G.R., 1981. An evaluation of the locomotor stimulating action of ethanol in rats and mice. Psychopharmacology 75, 372–379.
Gessa, G.L., Muntoni, F., Collu, M., Vargiu, L., Mereu, G., 1985. Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. Brain Res. 348, 201–203.
Gianoulakis, C., Woo, N., Drouin, J.N., Seidah, N.G., Kalant, H., Chretien, M., 1981. Biosynthesis of beta-endorphin by the neurointermediate lobes from rats treated with morphine or alcohol. Life Sci. 29, 1973–1982.
Gianoulakis, C., de-Waele, J.P., Kiianmaa, K., 1992. Differences in the brain and pituitary beta-endorphin system between the alcohol-preferring AA and alcohol-avoiding ANA rats. Alcohol., clin. exp. res. 16, 453–459.
Herz, A., 1997. Endogenous opioid system and alcohol addiction. Psychopharmacology 129, 99–111.
Honkanen, A., Ovaska, T., Korpi, E.R., 1995. Defective habituation to nociceptive stimulation in alcohol-avoiding ANA rats. Psychopharmacology 120, 21–27.
Honkanen, A., Vilamo, L., Wegelius, K., Sarviharju, M., Hyytiä, P., Korpi, E.R., 1996. Alcohol drinking is reduced by a mu1- but not by a delta-opioid receptor antagonist in alcohol-preferring rats. Eur. J. Pharmacol. 304, 7–13.
Honkanen, A., Hyytiä, P., Korpi, E.R., Ahtee, L., 1999a. Effects of morphine on metabolism of dopamine and serotonin in brains of alcohol-preferring AA and alcohol-avoiding ANA rats. Alcohol 18, 3–10.
Honkanen, A., Mikkola, J., Korpi, E.R., Hyytiä, P., Seppälä, T., Ahtee, L., 1999b. Enhanced morphine- and cocaine-induced behavioral sensitization in alcohol-preferring AA rats. Psychopharmacology 142, 244–252.
Hyytiä, P., Sinclair, J.D., 1993a. Responding for oral ethanol after naloxone treatment by alcohol-preferring AA rats. Alcohol., clin. exp. res. 17, 631–636.
Hyytiä, P., Sinclair, J.D., 1993b. Oral etonitazene and cocaine consumption by AA, ANA and Wistar rats. Psychopharmacology 111, 409–414.
Hyytiä, P., Ingman, K., Soini, S.L., Laitinen, J.T., Korpi, E.R., 1999. Effects of continuous opioid receptor blockade on alcohol intake and up-regulation of opioid receptor subtype signalling in a genetic model of high alcohol drinking. Naunyn-Schmiedeberg's Arch. Pharmacol. 360, 391–401.
Johnson, S.W., North, R.A., 1992. Opioids excite dopamine neurons by hyperpolarization of local interneurons. J. Neurosci. 12, 483–488.
Kalivas, P.W., Widerlov, E., Stanley, D., Breese, G., Prange Jr., A.J., 1983. Enkephalin action on the mesolimbic system: a dopamine-dependent and a dopamine-independent increase in locomotor activity. J. Pharmacol. Exp. Ther. 227, 229–237.
Korpi, E.R., Wong, G., Lüddens, H., 1995. Subtype specificity of γ-aminobutyric acid type A receptor antagonism by clozapine. Naunyn-Schmiedeberg's Arch. Pharmacol. 352, 365–373.
Krishnan-Sarin, S., Jing, S.L., Kurtz, D.L., Zweifel, M., Portoghese, P.S., Li, T.K., Froehlich, J.C., 1995. The delta opioid receptor antagonist naltrindole attenuates both alcohol and saccharin intake in rats selectively bred for alcohol preference. Psychopharmacology 120, 177–185.
Krishnan-Sarin, S., Wand, G.S., Li, X.W., Portoghese, P.S., Froehlich, J.C., 1998. Effect of mu opioid receptor blockade on alcohol intake in rats bred for high alcohol drinking. Pharmacol. Biochem. Behav. 59, 627–635.
Le, A.D., Poulos, C.X., Quan, B., Chow, S., 1993. The effects of selective blockade of delta and mu opiate receptors on ethanol consumption by C57BL/6 mice in a restricted access paradigm. Brain Res. 630, 330–332.
Maher, C.E., Selley, D.E., Childers, S.R., 2000. Relationship of mu opioid receptor binding to activation of G-proteins in specific rat brain regions. Biochem. Pharmacol. 59, 1395–1401.
Mansour, A., Fox, C.A., Thompson, R.C., Akil, H., Watson, S.J., 1994. mu-Opioid receptor mRNA expression in the rat CNS: comparison to mu-receptor binding. Brain Res. 643, 245–265.
Marinelli, P.W., Kiianmaa, K., Gianoulakis, C., 2000. Opioid propeptide mRNA content and receptor density in the brains of AA and ANA rats. Life Sci. 66, 1915–1927.
Mereu, G., Gessa, G.L., 1985. Low doses of ethanol inhibit the firing of neurons in the substantia nigra, pars reticulata: a GABAergic effect? Brain Res. 360, 325–330.
Mikkola, J.A., Honkanen, A., Piepponen, T.P., Kiianmaa, K., Ahtee, L., 2000. Effects of repeated morphine on cerebral dopamine release and metabolism in AA and ANA rats. Pharmacol. Biochem. Behav. 67, 783–791.
Morelli, M., Fenu, S., Di Chiara, G., 1989. Substantia nigra as a site of origin of dopamine-dependent motor syndromes induced by stimulation of mu and delta opioid receptors. Brain Res. 487, 120–130.
Nylander, I., Hyytiä, P., Forsander, O., Terenius, L., 1994. Differences between alcohol-preferring (AA) and alcohol-avoiding (ANA) rats in the prodynorphin and proenkephalin systems. Alcohol., clin. exp. res. 18 (5), 1272–1279.
Päivärinta, P., Korpi, E.R., 1993. Voluntary ethanol drinking increases locomotor activity in alcohol-preferring AA rats. Pharmacol. Biochem. Behav. 44, 127–132.
Paxinos, G., Watson, C., 1982. The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.
Roberts, A.J., McDonald, J.S., Heyser, C.J., Kieffer, B.L., Matthes, H.W., Koob, G.F., Gold, L.H., 2000. mu-Opioid receptor knockout mice do not self-administer alcohol. J. Pharmacol. Exp. Ther. 293, 1002–1008.
Seizinger, B.R., Bovermann, K., Maysinger, D., Hollt, V., Herz, A., 1983. Differential effects of acute and chronic ethanol treatment on particular opioid peptide systems in discrete regions of rat brain and pituitary. Pharmacol. Biochem. Behav. 18 (Suppl. 1), 361–369.
Sinclair, J.D., 1990. Drugs to decrease alcohol drinking. Ann. Med. 22, 357–362.
Sinclair, J.D., Le, A.D., Kiianmaa, K., 1989. The AA and ANA rat lines, selected for differences in voluntary alcohol consumption. Experientia 45, 798–805.
Soini, S.L., Ovaska, T., Honkanen, A., Hyytiä, P., Korpi, E.R., 1998. Brain opioid receptor binding of [^{3}H]CTOP and [^{3}H]naltrindole in alcohol-preferring AA and alcohol-avoiding ANA rats. Alcohol 15, 227–232.
Soini, S.L., Honkanen, A., Hyytiä, P., Korpi, E.R., 1999. [^{3}H]Ethylketocyclazocine binding to brain opioid receptor subtypes in alcohol-preferring AA and alcohol-avoiding ANA rats. Alcohol 18, 27–34.
Wegelius, K., Honkanen, A., Korpi, E.R., 1994. Benzodiazepine receptor ligands modulate ethanol drinking in alcohol-preferring rats. Eur. J. Pharmacol. 263, 141–147.
Weiss, F., Mitchiner, M., Bloom, F.E., Koob, G.F., 1990. Free-choice responding for ethanol versus water in alcohol preferring (P) and unselected Wistar rats is differently modified by naloxone, bromocriptine, and methysergide. Psychopharmacology 101, 178–186.
Wise, R.A., Bozarth, M.A., 1987. A psychomotor stimulant theory of addiction. Psychol. Rev. 94, 469–492.