(caudate putamen) was dissected. Total RNA was isolated using a Trizol reagent (Invitrogen Life Technology Japan, Tokyo, Japan). RNA amplification was conducted using the Illumina TotalPrep RNA amplification Kit (Applied Biosystems, Tokyo, Japan). The MouseRef-8 beadchip Kit (Illumina, Tokyo, Japan) was used for hybridization according to the manufacturer's instructions. Data were analyzed using the BeadStudio 3 Gene Expression Module (Illumina, Tokyo, Japan).

Of the 24,000 genes tested, 45 were downregulated and 37 were upregulated in ICER I-overexpressing mice compared with their wildtype littermates. Among the downregulated mRNAs, cocaine- and amphetamine-regulated transcript (CART) and prodynorphin (Pdyn) mRNA expression levels were reduced by approximately 50% in ICER I-overexpressing mice. The products of CART and Pdyn mRNAs are neurotransmitters expressed in brain regions associated with drug reward, including the nucleus accumbens and ventral tegmental area. CART knockout mice exhibited attenuated rewarding effects of drugs (Couceyro et al, 2005), and Pdyn knockout mice showed decreased cocaine-induced locomotor activity (Chefer and Shippenberg, 2006). ICER I-overexpressing mice, with decreased CART and Pdyn expression levels, consistently displayed attenuated METH-induced locomotor sensitization in the present study. Our results reveal the modulatory effects of the ICER/CART (Pdyn) pathway on METH-induced locomotor sensitization and provide an incentive for exploring the therapeutic potential of stimulating the ICER/CART (Pdyn) pathway in the treatment of drug abuse.

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ARTICLE

CINP

Active behaviours produced by antidepressants and opioids in the mouse tail suspension test

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Abstract

Most classical preclinical tests to predict antidepressant activity were initially developed to detect compounds that influenced noradrenergic and/or serotonergic activity, in accordance with the monoaminergic hypothesis of depression. However, central opioid systems are also known to influence the pathophysiology of depression. While the tail suspension test (TST) is very sensitive to several types of antidepressant, the traditional form of scoring the TST does not distinguish between different modes of action. The present study was designed to compare the behavioural effects of classical noradrenergic and/or serotonergic antidepressants in the TST with those of opioids. We developed a sampling technique to differentiate between behaviours in the TST, namely, curling, swinging and immobility. Antidepressants that inhibit noradrenaline and/or serotonin re-uptake (imipramine, venlafaxine, duloxetine, desipramine and citalopram) decreased the immobility of mice, increasing their swinging but with no effect on their curling behaviour. No differences were observed between antidepressants that act on noradrenergic or serotoninergic transmission. While opioid compounds also decreased the immobility of the mice [morphine, codeine, levorphanol, (−)-methadone, (±)-tramadol and (+)-tramadol], they selectively increased curling behaviour. Blocking opioid receptors with naloxone prevented the antidepressant-like effect of codeine, and μ-opioid receptor knockout decreased normal curling behaviour and blocked (±)-tramadol-induced curling, further demonstrating the reliability and validity of this approach. These results show that at least two behaviourally distinct processes occur in the TST, highlighting the antidepressant-like effects of opioids evident in this test. Furthermore, our data suggest that swinging and curling behaviours are mediated by enhanced monoamine and opioid neurotransmission, respectively.

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Key words: Antidepressants, behavioural despair, μ-opioid knockout, opioids, tail suspension test.

Introduction

There is substantial evidence implicating the opioid system in depression (Hegadoren *et al.* 2009), suggesting that compounds that enhance opioid neurotransmission may exert genuine antidepressant effects (Berrocoso & Mico, 2009*a*; Jutkiewicz, 2006; Tejedor-Real *et al.* 1998). Since the description of the 'opium cure' (Kraepelin, 1901), clinical reports have described the effectiveness of μ-opioid receptor (MOR) agonists in patients suffering depression, such as oxycodone, oxymorphone, tramadol and buprenorphine, especially in cases of refractory depression (Bodkin *et al.* 1995; Fanelli & Montgomery, 1998; Shapira *et al.* 2001; Spencer, 2000; Stoll & Rueter, 1999). We have studied the antidepressant-like effects of opioids and,

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in accordance with other studies (reviewed in (Berrocoso *et al.* 2009), we demonstrate the antidepressant-like activity of several opioids in animal paradigms of depression (Rojas-Corrales *et al.* 1998, 2004; Tejedor-Real *et al.* 1998). Indeed, we previously described a functional interaction between opioids and selective serotonin reuptake inhibitors (SSRIs) or noradrenaline (NA) reuptake inhibitors in the tail suspension test (TST: Berrocoso & Mico, 2009*a*), whereby both opioids and the more classic antidepressants decrease the time that the mice spend immobile (consistent with an antidepressant-like effect).

The mouse TST is a predictive behavioural test of antidepressant activity (Chermat *et al.* 1986; Steru *et al.* 1985, 1987) that is being increasingly used with the advent of transgenic mice. When mice are suspended by the tail they are subjected to short-term inescapable stress and they adopt an immobile posture. However, if antidepressant treatments are administered prior to the test, the mice will actively pursue escape-directed behaviours over longer periods of time. The increase in such activity (i.e. the decrease in immobility) in the TST is strongly correlated with antidepressant effects in humans (Cryan *et al.* 2005*a*). This clinical predictive value, together with the high degree of reliability in different laboratories, has led to the inclusion of the TST in almost all batteries used to screen new antidepressant drugs (Bravo *et al.* 2009), even though the TST in its traditional form cannot reliably detect specific modes of drug action. In our laboratory, previous studies with the TST suggested that while opioids decrease the time mice spend immobile they induce a pattern of activity that differs from that seen with classical monoaminergic antidepressants. To further investigate this effect, we have systematically analysed the behaviour of mice in the TST to compare the active behaviours induced by classical antidepressants and opioids.

Materials and method

Animals

Experiments were performed using male albino CD1 mice, male and female wild-type mice or heterozygous and homozygous MOR knockout (MOR-KO) littermates obtained by crossing heterozygous/heterozygous MOR-KO mice on a C57BL/6J genetic background (Sora *et al.* 2001). Animals were maintained under standard conditions: 12-h light/dark cycle (lights on 08:00 hours), *ad libitum* access to food and water and a constant temperature (21 ± 1 °C). Animals were housed in groups of 10 in standard polypropylene cages (1000 cm^2) and male and female mice shared the same room. All animal handling and procedures were performed in accordance with the European Communities directive 86/609-EEC and Spanish Law (RD 1201/2005) regulating animal research. The experimental protocols were approved by the Committee for Animal Experimentation of the University of Cádiz. All mice were experimentally naive, they weighed 25–30 g at the time of testing and they were only used once.

Drugs

The following drugs were used in this study: imipramine, desipramine, codeine and naloxone (Sigma-Aldrich-Quimica, UK); venlafaxine (Wyeth, USA); duloxetine (Eli Lilly, USA); citalopram, (±)-tramadol and (+)-tramadol (Grünenthal, Germany and Spain); morphine (Agencia Española de Medicamentos y Productos Sanitarios, Spain); (−)-methadone and levorphanol (RBI, USA). The selectivity of these compounds for opioid receptors (μ, δ and κ) and monoamine transporters (5-HT and NA) is summarized in Table 1.

All the drug solutions were prepared immediately before each trial and they were injected i.p., with the exception of naloxone, which was administered subcutaneously. All drugs were dissolved in physiological saline (NaCl 0.9%), with the exception of duloxetine, which was dissolved in distilled water, and the control animals received saline alone (NaCl 0.9%). All the solutions were injected in a volume of 10 ml/kg body weight 30 min prior to testing and the treatments were administered under blind conditions.

Tail suspension test

We used a modified form of the TST that was previously validated for NMRI mice (Steru *et al.* 1985). Accordingly, 30 min after injection the mice were individually suspended by the tail from an aluminium hook raised 20 cm above the floor using adhesive tape placed 2 cm from the tip of tail. The mice were positioned such that the base of their tail was aligned with the horizontal plane. Typically, mice demonstrated escape-oriented behaviour interspersed with successively longer bouts of immobility. Test sessions lasted for 6 min and they were videotaped and subsequently scored by a trained observer.

Behavioural scoring

The procedure used to analyse the test sessions was similar to that described previously for the forced

Table 1. Summary of the inhibitory effects of antidepressants and opioids on monoamine uptake and opioid receptor binding

Drugs	μ	δ	κ	NA	5-HT	Reference
Antidepressants						
Imipramine	3700	12 700	1800	6.6	21	Raffa *et al.* (1992)
Venlafaxine	–	–	–	1260	74	Beique *et al.* (1998)
Duloxetine	–	–	–	3	1.8	Beique *et al.* (1998)
Desipramine	–	–	–	0.31	129	Owens *et al.* (1997)
Citalopram	–	–	–	3042	0.75	Owens *et al.* (1997)
Opioids						
With no reuptake-inhibiting activity						
Morphine	0.34	92	570	IA	IA	Raffa *et al.* (1992)
Codeine	160	5130	5970	IA	IA	Raffa *et al.* (1992)
With reuptake-inhibiting activity						
Levorphanol	0.42	3.61	4.2	1220	86.3	Codd *et al.* (1995)
(−)-Methadone	0.945	371	1860	702	14.1	Codd *et al.* (1995)
(±)-Tramadol	2100	57 600	42 700	709	990	Raffa *et al.* (1992)
(+)-Tramadol	1300	62 400	54 000	2510	530	Raffa *et al.* (1993)

NA, Noradrenaline; IA, inactive at 10 μM.
The assays were performed on rat brain samples and the data represent K_i values ± S.E.M. (nM).

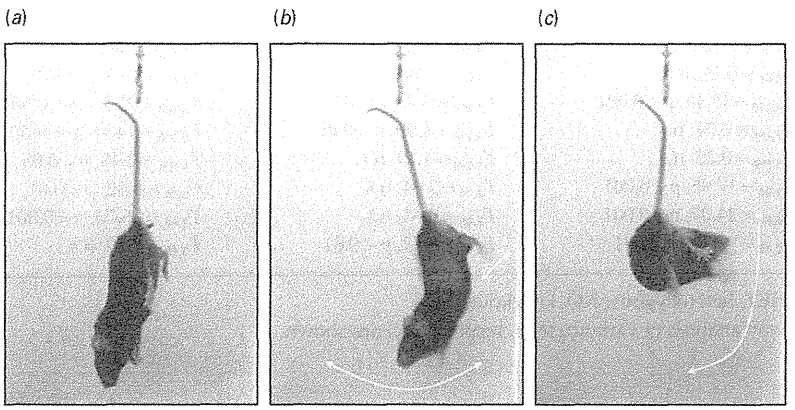

Fig. 1. Photographs illustrating the behaviours scored in the tail suspension test. (*a*) Immobility – the mouse hangs without engaging in any activity; (*b*) swinging – keeping its body straight, the mouse continuously moves its paws in a vertical position and/or moves its body from side to side; (*c*) curling – the mouse engages in active twisting movements.

swimming test (FST; Detke *et al.* 1995). A time-sampling technique was employed, whereby the predominant behaviour in each 5-s period of the 360 s test was recorded. The behaviours rated were: (1) immobility – a mouse was judged to be immobile when it hung by its tail without engaging in any active behaviour; (2) swinging – a mouse was judged to be swinging when it continuously moved its paws in the vertical position while keeping its body straight and/or it moved its body from side to side; (3) curling – a mouse was judged to be curling when it engaged in active twisting movements of the entire body (Fig. 1). The behavioural scoring was performed by a single experienced observer who was blind to the treatments. Several test sessions ($n = 30$ subjects) were then scored a second time by the observer to determine the test–retest reliability (i.e. two consecutive ratings by the same observer were compared). These sessions were then scored by a second blind observer to determine the inter-rater reliability.

Statistical analysis

The data were expressed as the mean + S.E.M. of the parameter measured and they were analysed by one-way analysis of variance (ANOVA) followed by the

Table 2. Effects of antidepressants and opioids on immobility and active behaviours in the tail suspension test

Drugs	Immobility	Swinging	Curling
Antidepressants			
(*a*) Imipramine	$F_{4,44}=3.34$, $p<0.05$	$F_{4,44}=3.55$, $p<0.05$	$F_{4,44}=0.69$, n.s.
(*b*) Venlafaxine	$F_{4,42}=7.82$, $p<0.001$	$F_{4,42}=3.30$, $p<0.05$	$F_{4,42}=0.13$, n.s.
(*c*) Duloxetine	$F_{6,68}=3.77$, $p<0.01$	$F_{6,63}=2.33$, $p<0.05$	$F_{6,63}=0.71$, n.s.
(*d*) Desipramine	$F_{3,32}=6.43$, $p<0.01$	$F_{3,32}=4.13$, $p<0.05$	$F_{3,32}=2.79$, n.s.
(*e*) Citalopram	$F_{5,51}=4.25$, $p<0.01$	$F_{5,51}=2.70$, $p<0.05$	$F_{5,51}=1.64$, n.s.
Opioids			
(*f*) Morphine	$F_{3,36}=5.36$, $p<0.01$	$F_{3,36}=12.02$, $p<0.001$	$F_{3,36}=26.78$, $p<0.001$
(*g*) Codeine	$F_{3,35}=3.13$, $p<0.05$	$F_{3,35}=3.70$, $p<0.05$	$F_{3,35}=9.35$, $p<0.001$
(*h*) Levorphanol	$F_{5,51}=3.14$, $p<0.05$	$F_{5,51}=7.15$, $p<0.001$	$F_{5,51}=13.52$, $p<0.001$
(*i*) (−)-Methadone	$F_{3,36}=3.44$, $p<0.05$	$F_{3,36}=3.61$, $p<0.05$	$F_{3,36}=8.61$, $p<0.001$
(*j*) (±)-Tramadol	$F_{3,36}=5.01$, $p<0.01$	$F_{3,36}=0.72$, n.s.	$F_{3,36}=5.85$, $p<0.01$
(*k*) (+)-Tramadol	$F_{3,33}=6.60$, $p<0.01$	$F_{3,33}=4.01$, $p<0.05$	$F_{3,33}=12.38$, $p<0.001$
Pharmacological opioid receptor blockade			
(*l*) Codeine	$F_{1,54}=7.93$, $p<0.01$	$F_{1,54}=2.19$, n.s.	$F_{1,54}=3.20$, n.s.
(*m*) Naloxone	$F_{2,54}=0.70$, n.s.	$F_{2,54}=12.23$, $p<0.001$	$F_{2,54}=16.26$, $p<0.001$
(*n*) Codeine × naloxone	$F_{2,54}=3.25$, $p<0.05$	$F_{2,54}=4.09$, $p<0.05$	$F_{2,54}=6.11$, $p<0.01$
MOR knockout			
(*o*) Gender	$F_{1,57}=0.32$, n.s.	$F_{1,57}=1.23$, n.s.	$F_{1,57}=0.02$, n.s.
(*p*) Genotype	$F_{2,57}=0.28$, n.s.	$F_{2,57}=1.23$, n.s.	$F_{2,57}=3.50$, $p<0.05$
(*q*) Gender × genotype	$F_{2,57}=0.73$, n.s.	$F_{2,57}=0.86$, n.s.	$F_{2,57}=0.12$, n.s.
(*r*) Genotype	$F_{2,60}=0.14$, n.s.	$F_{2,60}=1.50$, n.s.	$F_{2,60}=3.75$, $p<0.05$
(*s*) (±)-Tramadol	$F_{2,180}=45.44$, $p<0.001$	$F_{2,180}=8.78$, $p<0.001$	$F_{2,180}=21.31$, $p<0.001$
(*t*) Genotype	$F_{2,180}=0.54$, n.s.	$F_{2,180}=4.93$, $p<0.01$	$F_{2,180}=14.18$, $p<0.001$
(*u*) (±)-tramadol × genotype	$F_{4,180}=0.25$, n.s.	$F_{4,180}=1.19$, n.s.	$F_{4,180}=2.48$, $p<0.05$
(*v*) (±)-tramadol-WT	$F_{2,50}=17.95$, $p<0.001$	$F_{2,50}=2.84$, n.s.	$F_{2,50}=6.32$, $p<0.01$
(*w*) (±)-tramadol-HET	$F_{2,68}=24.07$, $p<0.001$	$F_{2,68}=0.61$, n.s.	$F_{2,68}=16.70$, $p<0.001$
(*x*) (±)-tramadol-KO	$F_{2,62}=9.77$, $p<0.001$	$F_{2,62}=6.12$, $p<0.01$	$F_{2,62}=1.59$, n.s.

MOR, μ-opioid receptor; WT, wild-type; HET, heterozygous; KO, knockout.
p values and F values from one- and two-way analysis of variance tests, respectively, are shown.

Dunnett's (for dose–response studies) or Tukey's test. For the mechanistic studies, the data were analysed using a two-way ANOVA followed by the Bonferrroni *post-hoc* test. The factors evaluated (between subjects) were codeine/(±)-tramadol treatment and naloxone treatment. A Pearson's correlation test was used to determine test–retest and inter-rater reliability and $p<0.05$ was considered statistically significant.

Results

In the TST employed here, the reliability of the scoring for each of the three behaviours contemplated was very high. Moreover, the test–retest reliabilities were: $r=0.89$ for immobility; $r=0.93$ for swinging; $r=0.88$ for curling. The concordance between raters was: $r=0.92$ for immobility; $r=0.86$ for swinging; $r=0.81$ for curling ($p<0.0001$).

Antidepressants

All the antidepressants administered here induced similar behavioural changes in the TST, reducing the immobility in conjunction with an increase in swinging, exerting no effect on curling behaviour [see Table 2(*a–e*) for one-way ANOVA data]. For example, the tricyclic antidepressant imipramine (2.5–20.0 mg/kg) induced a dose-dependent decrease in immobility while increasing swinging behaviour at both 10 and 20 mg/kg ($p<0.05$ in all cases), without affecting curling behaviour (Fig. 2*a*). The 5-HT and NA reuptake inhibitors venlafaxine (2.5–20.0 mg/kg) and duloxetine (1.25–40.0 mg/kg) induced similar behavioural changes, significantly decreasing immobility and increasing swinging behaviour in a dose–dependent manner. Moreover, like imipramine, neither venlafaxine nor duloxetine altered curling

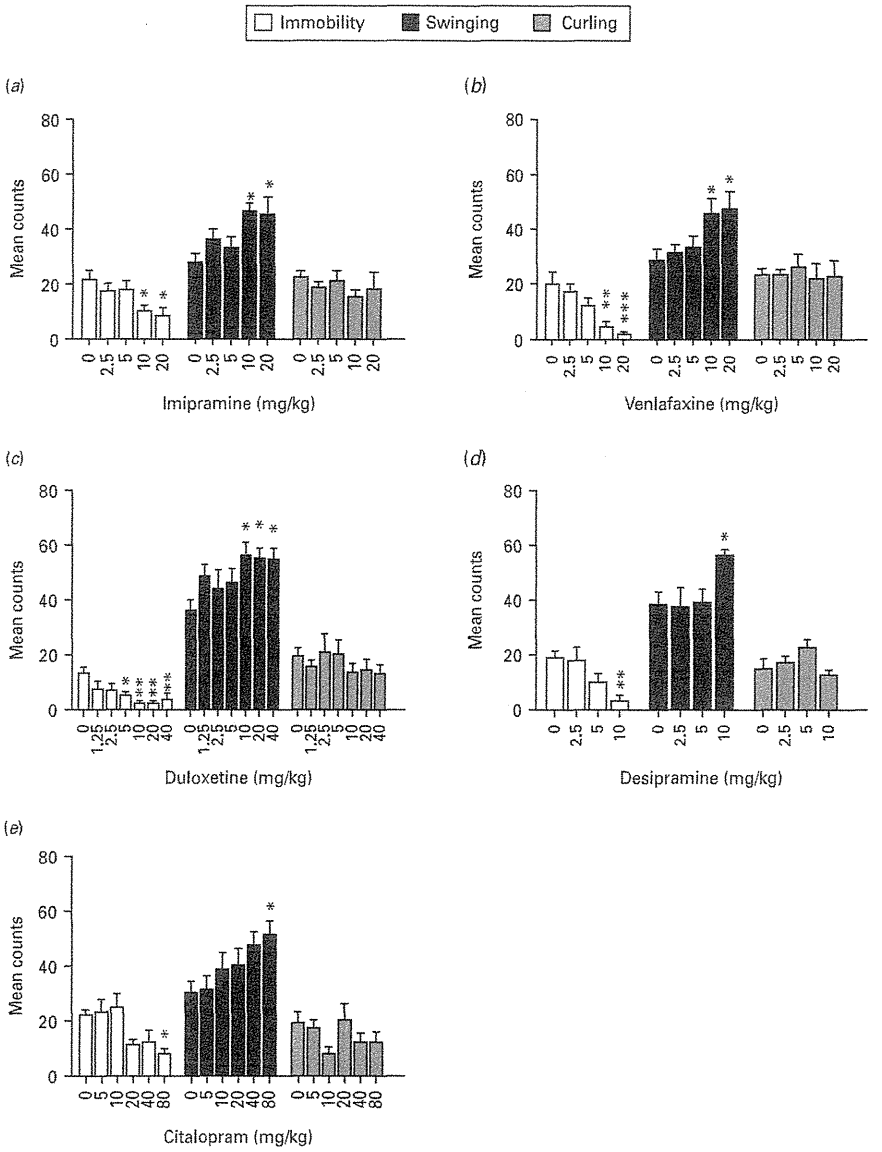

Fig. 2. Effects of (*a*) imipramine (2.5–20 mg/kg i.p.), (*b*) venlafaxine (2.5–20 mg/kg i.p.), (*c*) duloxetine (1.25–40 mg/kg i.p.), (*d*) desipramine (2.5–10 mg/kg i.p.) and (*e*) citalopram (5–80 mg/kg i.p.) on immobility and active behaviours in the tail suspension test. Drugs were administered 30 min before testing and the data represent the mean counts + S.E.M. from 8–11 animals per group. There were significant differences when compared to saline-treated mice (Dunnett's *post-hoc* test: * $p<0.05$; ** $p<0.01$; *** $p<0.001$).

behaviour (Fig. 2*b*,*c*). In a dose–response study to assess the antidepressant-like effects of the NA uptake inhibitor desipramine (2.5–10.0 mg/kg), 10 mg/kg desipramine reduced the time spent immobile ($p<0.01$) while increasing swinging ($p<0.05$), producing no effect on curling behaviour (Fig. 2*d*). Finally, the selective 5-HT uptake inhibitor citalopram (5–80 mg/kg) induced a behavioural pattern similar to that of the other antidepressants tested, decreasing immobility at 80 mg/kg ($p<0.05$) while increasing swinging behaviour ($p<0.05$), with no effect on curling behaviour (Fig. 2*e*).

Opioids

All the opioids tested induced a similar behavioural pattern in the TST, decreasing immobility while increasing curling behaviour [see Table 2(*f*–*k*) for

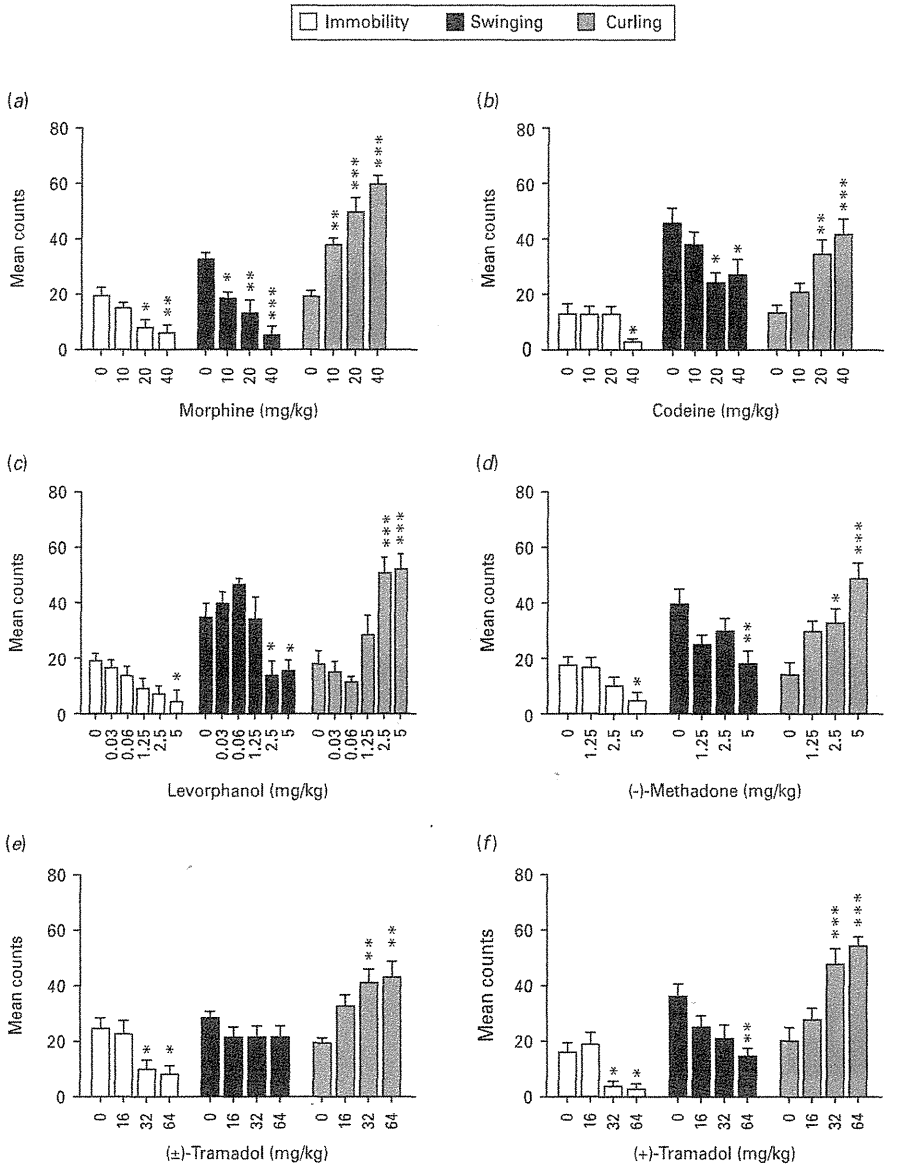

Fig. 3. Effects of (*a*) morphine (10–40 mg/kg i.p.), (*b*) codeine (10–40 mg/kg i.p.), (*c*) levorphanol (0.03–10 mg/kg i.p.), (*d*) (−)-methadone (1.25–5 mg/kg i.p.), (*e*) (±)-tramadol (16–64 mg/kg i.p.) and (*f*) (+)-tramadol (16–64 mg/kg i.p.) on immobility and active behaviour in the tail suspension test. Drugs were administered 30 min before testing and the data represent the mean counts + S.E.M. from 8–10 animals per group. There were significant differences when compared to saline-treated mice (one-way analysis of variance followed by Dunnett's *post-hoc* test: * $p<0.05$; ** $p<0.01$; *** $p<0.001$).

one-way ANOVA data]. Opioids that do not inhibit monoamine reuptake, such as morphine (10–40 mg/kg), attenuated immobility and swinging behaviour in a dose-dependent manner, while robustly increasing curling behaviour (Fig. 3*a*). Similar effects were observed for codeine (10–40 mg/kg), which as reported elsewhere (Berrocoso & Mico, 2009*a*), reduced immobility at 40 mg/kg ($p<0.05$), decreased swinging ($p<0.05$) and increased curling behaviour when administered at 20 or 40 mg/kg ($p<0.01$ and $p<0.001$, respectively; Fig. 3*b*). Opioids with monoamine uptake-inhibiting activity such as levorphanol (0.03–10.00 mg/kg) and (−)-methadone (1.25–5.00 mg/kg) induced a behavioural pattern similar to that seen for morphine and codeine, significantly reducing immobility ($p<0.05$ in both cases) and swinging behaviour and increasing curling behaviour (Fig. 3*c*,*d*). The behaviour elicited in response to (±)-tramadol

(16–64 mg/kg) differed slightly from that provoked by the other opioids (Fig. 3*e*) and, specifically, a significant reduction in immobility ($p<0.05$) and an increase in curling behaviour ($p<0.01$) at 32 and 64 mg/kg was not coupled with any effect on swinging behaviour. By contrast, the dextro enantiomer (+)-tramadol, which binds to the MOR and inhibits 5-HT reuptake more strongly than (±)-tramadol (Raffa *et al.* 1993), produced similar effects to the other opioids studied (Fig. 3*f*), decreasing immobility and increasing curling behaviour at 32 and 64 mg/kg and decreased swinging at 64 mg/kg ($p<0.01$; Fig. 3*f*).

Role of opioid receptors in antidepressant-like effects

Pharmacological blockage

To confirm the role of the opioid system in curling behaviour, an effective dose of codeine (40 mg/kg) was co-administered with the opioid receptor antagonist, naloxone [0.5–2.0 mg/kg; Fig. 4, Table 2(*l–n*)]. Two-way ANOVA revealed a significant effect of codeine on immobility ($p<0.01$), while swinging and curling behaviour remained unchanged. Two-way ANOVA also revealed significant effects of naloxone on swinging ($p<0.0001$) and curling behaviours ($p<0.001$). Furthermore, a significant interaction between factors was observed for all three behaviours: immobility ($p<0.05$); swinging ($p<0.05$); curling ($p<0.01$). Indeed, a Bonferroni's analysis revealed that naloxone significantly blocked the effects of codeine on immobility, swinging and curling ($p<0.01$ in all cases; Fig. 4).

MOR-KO study

To determine whether curling behaviour involves the activation of MORs, we evaluated the behaviour of C57BL/6J MOR-KO mice in the TST, both males and females. Initially, we investigated the effects of gender and genotype [Table 2(*o–q*)] and while a two-way ANOVA revealed no significant effect of gender and no gender × genotype interaction, a significant effect of genotype was evident [$p<0.05$, Table 2(*p*)]. As expected, a subsequent unpaired Student's *t* test revealed no significant difference in behaviour between male and female knockout mice (data not shown). Thus, the data from the male and female mice were pooled for the subsequent studies. While we were unable to detect any differences in the behaviour of heterozygous MOR-KO mice from their wild-type littermates, the homozygous knockout mice displayed significantly less curling behaviour compared to their wild-type littermates [$p<0.05$, Tukey's test; Fig. 5, Table 2(*r*)]. This result supports the hypothesis that curling behaviour involves MOR activation.

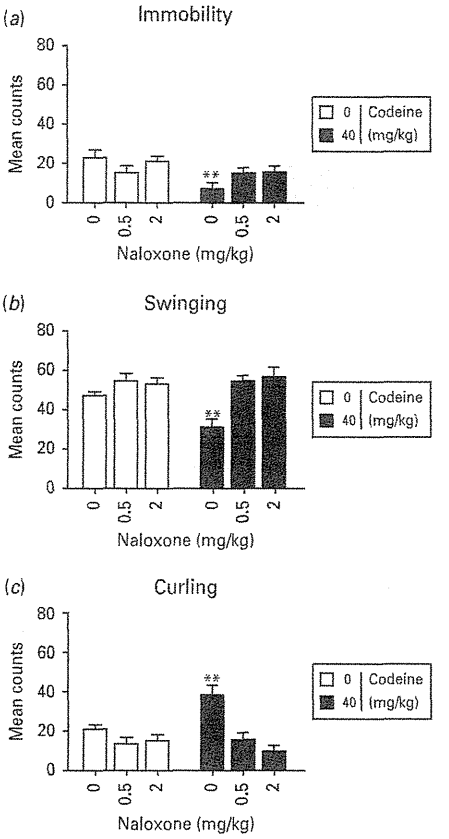

Fig. 4. Opioid receptor involvement in the effects of codeine on immobility, curling and swinging behaviour in the tail suspension test in mice. Codeine (40 mg/kg i.p.) and the opioid receptor antagonist naloxone (0.5–2 mg/kg s.c.) were administered 30 min before testing and the data represent the mean counts +S.E.M. from 10 animals per group. There were significant differences when compared to saline-treated mice (two-way analysis of variance followed by Bonferroni *post-hoc* test: $^{**}p<0.01$).

The opioid (±)-tramadol acts through both MOR and 5HT/NA transporters and, hence, we explored its effects on C57BL/6J MOR-KO mice over a range of active doses (32–64 mg/kg). Two-way ANOVA revealed a significant effect of (±)-tramadol on all the behaviour studied [Table 2(*s–u*); $p<0.001$] and a significant effect of genotype on swinging ($p<0.01$) and curling ($p<0.001$). Interestingly, the interaction of both factors ((±)-tramadol × genotype) was only significant for curling ($p<0.05$). To study the effect of (±)-tramadol on different behaviours, we performed a one-way ANOVA, followed by the Dunnett's test [Table 2(*v–x*)]. Results showed that (±)-tramadol

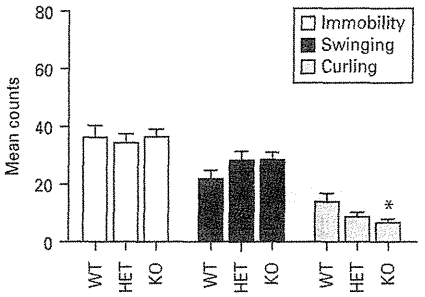

Fig. 5. Immobility, curling and swinging behaviour in wild-type (WT), heterozygous (HET) and homozygous μ-opioid receptor knockout (KO) mice in the tail suspension test. Data represent the mean counts +S.E.M. from 16–26 animals per group. There were significant differences when compared to saline-treated mice (one-way analysis of variance followed by Tukey's *post-hoc* test: * $p<0.05$). Data from male and female mice were pooled.

significantly decreased immobility to a similar degree in all three genotypes (Fig. 6), although the effect of (±)-tramadol on active behaviour varied according to the genotype. Thus, at both doses (±)-tramadol significantly increased swinging behaviour in knockout mice alone ($p<0.05$ and $p<0.01$, respectively), while curling behaviour was significantly increased at 64 mg/kg in wild-type and heterozygous mice ($p<0.01$ and $p<0.001$, respectively; Fig. 6). Note that the effect of (±)-tramadol was similar in CD1 and C57BL/6J wild type mice (Figs. 3*e*, 6).

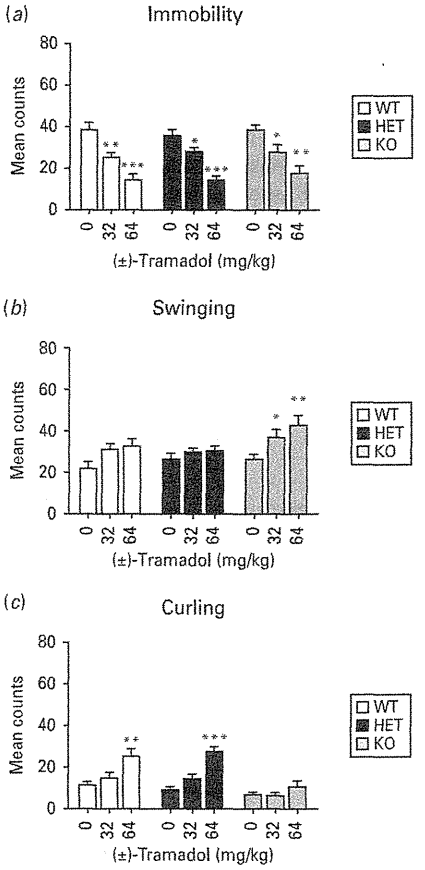

Fig. 6. Effects of (±)-tramadol (32–64 mg/kg i.p.) on immobility, curling and swinging behaviour in wild-type (WT), heterozygous (HET) and homozygous μ-opioid receptor knockout (KO) mice in the tail suspension test. (±)-Tramadol was administered 30 min before testing and the data represent the mean counts +S.E.M. from 15–24 animals per group. There were significant differences when compared to the corresponding saline-treated controls (one-way analysis of variance followed by Dunnett *post-hoc* test: * $p<0.05$; ** $p<0.01$; *** $p<0.001$). Data from male and female mice were pooled.

Discussion

The current study describes a modified means of scoring the TST, which differentiates between two active behaviours, swinging and curling. Using this approach, it was evident that antidepressants and opioids induce distinct active behaviours in the TST. While antidepressants increased swinging behaviour but had no effect on curling, opioids increased curling behaviour. Importantly, both antidepressants and opioids diminish the immobility of the mice, the traditional measure of antidepressant-like activity in the TST.

A modified form of the rat FST previously described a behavioural sampling technique that could distinguish between antidepressants with noradrenergic and serotonergic modes of action (Cryan *et al.* 2002). Hence, we employed a similar sampling technique to quantify and distinguish active behaviours induced by antidepressant treatment in the TST. While the traditional method of scoring the TST only measures the duration of immobility, we scored the frequency of the distinct behaviours in 5-s intervals throughout the test session. The results obtained using this approach do not differ from those obtained when each of the behaviours of interest are timed (Berrocoso & Mico, 2009*a*), yet they provided important information on the active behaviours that may be adopted when immobility is reduced. Significantly, our data are consistent with the strain differences previously observed in the TST (Cryan *et al.* 2005*a*; Liu & Gershenfeld, 2001) as CD1 mice were less immobile than C57BL/6J mice. Along similar lines, the immobility we observed in the dose–response experiments performed was in accordance

with the effects of antidepressants observed in other such screening tests. For example, the decrease in immobility following 10 and 20 mg/kg venlafaxine administration here is similar to its effect on mice in the FST (Berrocoso & Mico, 2009*b*). These findings validate the behavioural sampling technique and confirm that immobility is not to be confused with active behaviours.

The present study demonstrates that antidepressant compounds that differentially block the reuptake of NA and/or 5-HT induce a stereotypic behavioural profile that involves decreased immobility and increased swinging, with no effect on curling behaviour. These results implicate enhanced noradrenergic and/or serotenergic neurotransmission in swinging behaviour. Interestingly, and in agreement with previous data regarding the poor effect of SSRIs in animal models of depression (Berrocoso & Mico, 2009*a*; Lucki *et al.* 2001; Petit-Demouliere *et al.* 2005), a much higher dose of citalopram than that of other classes of antidepressants (noradenergic or dual) is required to significantly decrease immobility and increase swinging in CD1 mice. However, this behavioural sampling technique was unable to differentiate between antidepressants that selectively increase 5-HT or noradrenergic neurotransmission, unlike the modified version of the rat FST (Detke *et al.* 1995). It should be noted that, to date, all behavioural patterns in mouse tests of depression (including the FST) have been attributed to either serotoninergic or catecholaminergic signalling. The reasons for this restriction have not yet been elucidated but it suggests that differences in the coping strategies employed by mice and rats may be reflected in distinct active behaviours.

One of the main findings in this study is the clear demonstration of the antidepressant-like effect exerted by opioids, as witnessed by a decrease in immobility. These findings are consistent with previous studies using the TST or other behavioural tests to screen for depression or antidepressant effects using similar doses of these opioids (Fichna *et al.* 2007; Rojas-Corrales *et al.* 2002; Tejedor-Real *et al.* 1993, 1998). In the learned helplessness paradigm, morphine exerts an antidepressant-like effect and is antagonized by naloxone, indicating that it is indeed mediated by the opioids (Besson *et al.* 1996; Tejedor-Real *et al.* 1995). Antidepressant effects of other MOR agonists, such as (−)-methadone and levorphanol, have also been reported in the rat learned helplessness test (Rojas-Corrales *et al.* 2002). Thus, our findings validate the use of the TST as a test to screen for antidepressant-like activity of compounds that modulate opioid neurotransmission. Furthermore, the characteristic reduction in immobility indicative of antidepressant-like activity was accompanied by a consistent increase in curling behaviour with all the opioids evaluated. The pharmacological blockade of this behaviour by naloxone suggests that the antidepressant-like effect of opioid drugs is mediated by the opioid system and not other neurotransmission systems. Furthermore, the increase in curling behaviour seems to be accompanied by a decrease in swinging. However, it is important to note that the observed dose-dependent reduction in swinging may be a consequence of the increase in curling, i.e. if the animals spend a substantial amount of the time curling then the time left for other behaviours is reduced. While opioids caused a 3- to 6-fold decrease in immobility at the highest doses, swinging was only reduced ~2-fold (with the exception of morphine), indicating that many opioids induce a shift towards relatively more swinging in the 'non-curling' periods. This would suggest a contribution (although less relevant) of the monoaminergic system in the antidepressant-like effect of opioids.

It could be argued that curling behaviour reflects an opioid-induced increase in spontaneous motor activity or, alternatively, the induction of Straub tail, an S-shaped dorsiflexion of the mouse tail, produced by contraction of the sacro-coccygeal dorsalis muscles. Such effects would suggest that curling behaviour is not an escape-oriented behaviour but, rather, that it can be considered as a false positive score in the TST. However, we previously showed that codeine (40 mg/kg) did not modify spontaneous motor activity or coordination (Berrocoso & Mico, 2009*a*). In addition, spontaneous locomotion is not modified in the genetic knockout of the MOR (Ide *et al.* 2010), ruling out any possible increase in spontaneous motor activity. While Straub tail may be produced by high doses of opiates (Bilbey *et al.* 1960; Narita *et al.* 1993), genetic blockade of MORs significantly and specifically decreases curling behaviour without modifying immobility, indicating that Straub tail and curling are modulated by different mechanisms. Therefore, it seems unlikely that these events are confounded in the analysis of active behaviours in the TST.

While many studies have described antidepressant-like effects of δ (Tejedor-Real *et al.* 1998; Torregrossa *et al.* 2005, 2006) and κ (Mague *et al.* 2003; Pliakas *et al.* 2001; Shirayama *et al.* 2004) receptor antagonists, the specific roles of these opioid receptors on opioid-induced behaviour was not evaluated here. Hence, further studies with opioids that specifically act through these receptors will be necessary to determine their individual contributions. Indeed, since δ-opioid receptors are thought to be critical for the analgesic

activity of tricyclic antidepressants (Benbouzid *et al.* 2008), it would be interesting to evaluate their possible contribution to the effect observed in the TST. Finally, in the light of experiences with the FST (Cryan *et al.* 2005*b*), it would also be very interesting to test other effective or potential antidepressants that do not directly target monoamines (such as ketamine, AMPA potentiators or CRF1 antagonists) in order to determine how they affect the active behaviours described above.

We evaluated our modified scoring method using (±)-tramadol, a compound that has both opioid and monoaminergic effects. This compound is a weak agonist of the MOR and, like many antidepressant drugs, it inhibits the reuptake of 5-HT and NA. When compared to its parental compound, (+)-tramadol binds more potently to MOR and it inhibits the reuptake of 5-HT (Raffa *et al.* 1993; see Table 1). Indeed, (±)-tramadol decreases the immobility of CD1 mice and is suggestive of an antidepressant-like action (Berrocoso *et al.* 2006; Rojas-Corrales *et al.* 2002, 2004; Yalcin *et al.* 2007), further validating our sampling method. While (±)-tramadol significantly increased curling behaviour (consistent with an increase in MOR activity) it had no effect on swinging behaviour, despite its monoaminergic mode of action. Interestingly, (+)-tramadol, which possesses greater affinity for MOR, acted like a typical opioid, decreasing immobility and swinging behaviour and increasing curling. These data are consistent with the stronger affinity of (±)-tramadol and (+)-tramadol for MOR than for 5-HT/NA transporters (Table 1).

The administration of (±)-tramadol to MOR-KO mice provided further evidence of its specific mode of action. Thus, (±)-tramadol showed similar antidepressant-like effects in all three genotypes (wild-type, heterozygous and knockout), as indicated by the decrease in immobility. However, the increase in curling behaviour in wild-type mice suggests that (±)-tramadol acts through the opioid system, although the increase in swinging induced by (±)-tramadol in MOR-KO mice suggests that it enhances monoaminergic neurotransmission. Heterozygous mice displayed a profile similar to that of wild-type mice, indicative of a predominant opioid-mediated effect. It is also noteworthy that (±)-tramadol does not apparently affect swinging in wild-type mice, despite its well-known influence on monoamine transporters. The decrease in immobility but not swinging indicates a shift towards more swinging in the 'non-curling' periods. However, (±)-tramadol-induced swinging is more pronounced in MOR-KO mice, indicating that curling induced by MOR activation might mask the effects on swinging. These findings demonstrate the utility of this approach to explore the mode of action of monoaminergic/opioidergic compounds. Furthermore, we show that (±)-tramadol has both a central monoaminergic and opioidergic activity and that it can elicit antidepressant-like effects, even when one of these signalling mechanisms is blocked. This finding may be particularly relevant in pathological conditions in which both receptor profiles are modified.

Depression displays remarkable inter-individual variation in terms of symptoms and drug response. For example, opioid therapy has been successful in treating some refractory cases of depression such as when using the MOR agonists, oxycodone and oxymorphone, the partial agonist, buprenorphine, and the atypical opioid (±)-tramadol (Bodkin *et al.* 1995; Fanelli & Montgomery, 1998; Shapira *et al.* 2001; Spencer, 2000; Stoll & Rueter, 1999). Accordingly, alterations to the opioid system may underlie the neuroendocrine abnormalities observed in some groups of patients with this illness (Kennedy *et al.* 2006). Indeed, significant alterations in MOR activation have been observed in several brain areas (e.g. rostral region of the anterior cingulate) in patients with a major depressive disorder who did not respond to SSRI treatment. Interestingly, these alterations were correlated with corticotropin and cortisol plasma levels. Thus, although it remains to be confirmed, it is possible that the behaviour's affect in the TST could serve to predict different symptom clusters or therapeutic effects.

In summary, using a novel approach to scoring the TST, two specific active behaviours can be characterized that enable serotonergic/noradrenergic antidepressants to be distinguished from opioid compounds: swinging and curling. While traditional antidepressants that inhibit serotonin and/or NA reuptake decrease immobility and increase swinging behaviour, opioids, having decreased immobility, increase curling behaviour. Analysing these active behaviours may be useful to evaluate the mode of action of opioids and of opioids that also display monoaminergic properties, providing an important means of analysing the antidepressant effects of opioid compounds.

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Statement of Interest

Dr Berrocoso has served as a consultant for Grünenthal GmbH. Dr Ikeda has received research grants from Esai and Fujifilm. Dr Uhl has a patent regarding MOR gene (oprm1). Dr Mico has received research grants from, or served as a consultant for, Grünenthal GmbH, Eli Lilly and Company, Pfizer Inc, Takeda, Lundbeck, Pierre Fabre and Boehringer Ingelheim.

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シンポジウム

精神医学研究の到達点と展望*

依存性薬物作用の解明が拓く新しい精神医学**

池田和隆[1)]

Key words

Substances of abuse, NMDA receptor, Opioid, Analgesia, AD/HD

依存性薬物と精神疾患

依存性薬物は，物質使用障害のみならず，広く精神疾患と関連している。覚せい剤であるメタンフェタミンや幻覚剤であるフェンサイクリジン(PCP)の摂取が統合失調症様の症状を引き起こすことはよく知られている。また，アルコールや各種の依存性薬物の摂取は，うつ病の重大なリスクファクターである。このように依存性薬物が精神疾患を誘発する一方で，依存性薬物は精神疾患の治療薬としても広く用いられている。メチルフェニデートは注意欠如多動性障害(AD/HD)やナルコレプシーの治療薬であり，ベンゾジアゼピン系の薬物は睡眠薬，抗不安薬，アルコール依存離脱期の治療薬であり，モルヒネなどのオピオイドはがん性疼痛治療に欠かせない精神腫瘍学における主要な薬剤である。このように依存性薬物は，人類にとって諸刃の剣であり，さまざまな精神疾患と密接に関わっている。本稿では，筆者らの最近の研究成果を交えながら，依存性薬物の作用機序の解明が新たな精神医学の展開につながる可能性を論じたい。

物質使用障害の治療薬の探索

アルコール依存や薬物依存などの物質使用障害は，患者本人にとっても社会にとってもきわめて深刻な問題である。物質使用障害の治療は難しく，特効薬はないが，依存性物質の作用や代謝のメカニズムの解明から，治療法や治療薬が開発されている。たとえば抗酒剤は，アルコール代謝物のアセトアルデヒドの代謝を抑えることで，アルコール摂取によって不快感が生じるようにする薬剤である。また，ベンゾジアゼピン系の薬物は，アルコールが作動させるガンマアミノ酪酸(GABA)受容体チャネルをアルコールに代わって作動させることで，アルコール離脱期の症状を緩和する。

筆者らは，依存性物質の作用機序を解明するために，G蛋白質活性型内向き整流性カリウム(GIRK)チャネルに注目している。GIRKチャネルは，オピオイド受容体やD_2ドーパミン受容体などのGi/o型G蛋白質と共役する受容体の活性

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** Update of Psychiatry via Uunderstanding Effects of Substances of Abuse

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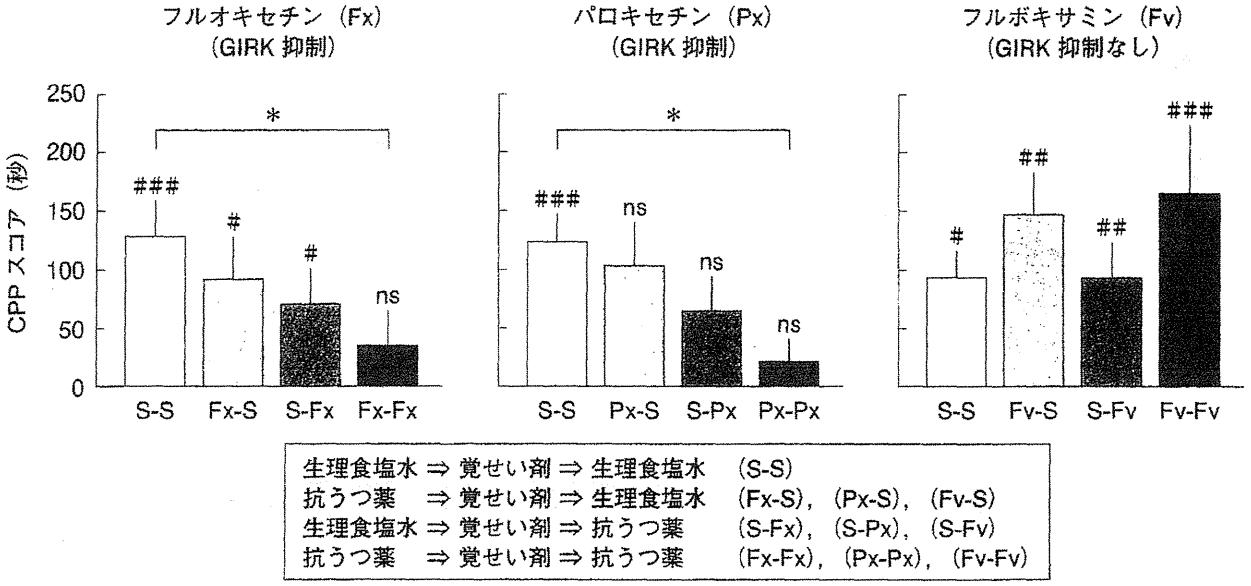

図1　GIRK 抑制作用のある抗うつ薬による覚せい剤嗜好性の減弱
覚せい剤に対する嗜好性を調べる薬物条件付け場所嗜好性試験において，条件付け前および嗜好性試験の前に生理食塩水または抗うつ薬(フルオキセチン，パロキセチン，フルボキサミン)を投与した。生理食塩水または抗うつ薬の投与順によるマウス群を図の下部に示す。

化によって開口するとともに，エタノールによっても開口するチャネルであり，さまざまな依存性物質の情報伝達に関わると考えられる[14]。

実際，このチャネルに異常を持つウィーバーマウスでは，オピオイドやエタノールの作用が減弱することを見出している[7,11]。さらに最近，GIRK チャネルを阻害するフルオキセチンやパロキセチンがマウスにおけるメタンフェタミン嗜好性を減弱させること，類似の抗うつ薬に分類されるフルボキサミンでは GIRK チャネル阻害能が弱くメタンフェタミン嗜好性を減弱させる効果が認められないことなどを見出した[12,13,15,26,27]（図1）。また，鈴木らにより薬物嗜好性を減弱させることが示されているイフェンプロジル[24]も，GIRK チャネルを阻害することを見出した[16]。つまり，薬物嗜好性を減弱させる効果があるフルオキセチン，パロキセチン，イフェンプロジルは GIRK チャネル阻害能を持ち，薬物嗜好性を減弱させる効果がないフルボキサミンには GIRK チャネル阻害能がなかった。このほか，GIRK チャネルの GIRK2，GIRK3 サブユニットを欠損したマウスでは，コカインの自己投与が減弱することが報告されている[19]。また，最近の学会などで，イフェンプロジルが鎮咳薬依存患者において著効したことや，アルコール依存患者での奏効例が報告されている。以上より，GIRK チャネルは依存性物質による報酬効果と密接に関連していると考えられ，GIRK チャネルの阻害剤には薬物依存治療薬としての可能性が期待される。

フェンサイクリジンと依存，統合失調症

フェンサイクリジン(PCP)は麻酔薬として開発されたが，麻酔からの回復期に幻覚などの精神病様症状が現われることから開発が中止された薬物である。PCP は，幻覚剤として乱用されており依存性物質である。また，乱用により，統合失調症と類似した症状が誘発されることから，動物に PCP を投与した統合失調症モデル動物が作製されて研究に広く用いられている。PCP の作用点は NMDA 受容体チャネルであり，異なるサブユニットで構成される NMDA 受容体チャネルのいずれにおいても PCP によって同様に阻害されることが示されている[29]。PCP の作用機序を調べ

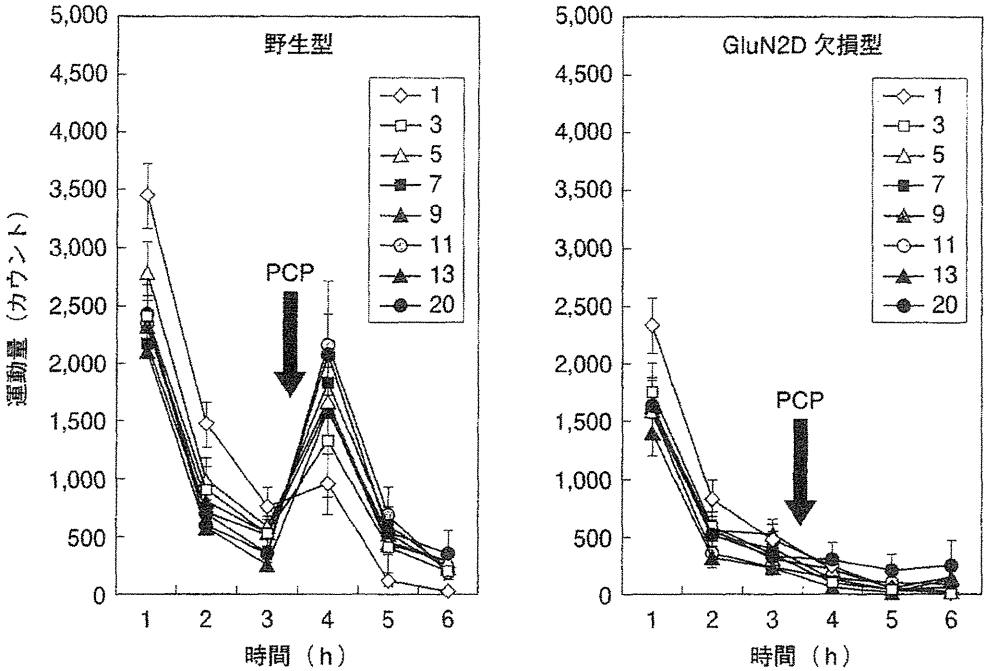

図2　GluN2D 欠損マウスにおける PCP による運動量亢進効果の消失
野生型マウスおよび GluN2D 欠損マウスに対して，1，3，5，7，9，11，13，20 日目に PCP を投与し，移所運動量試験を行った。

るため，NMDA 受容体チャネルサブユニットの GluN2A と GluN2D の遺伝子欠損マウスにおいて，移所運動量試験とマイクロダイアリシス分析による細胞外ドーパミン量の測定を行った[2]。野生型マウスでは，PCP 投与後に活動量の亢進および細胞外ドーパミン量の上昇がみられるのに対して，GluN2D 欠損マウスでは，このような PCP の効果が全くみられなかった。さらに，PCP を連続投与すると，感受性亢進が起こり，連続投与後の運動量の亢進は初回投与後の運動量の更新よりも有意に大きなものとなるが，この感受性亢進も GluN2D 欠損マウスでは全く観察されなかった（図 2）。GluN2D サブユニットは，PCP の効果発現において必須の分子であるといえる。さらに，GluN2D 遺伝子多型が統合失調症と関連することが示されていることから[17]，GluN2D の研究は，薬物依存だけでなく統合失調症の病態メカニズムの解明にもつながる可能性が考えられる。

GluN2D サブユニットは 15 年以上前に筆者が cDNA クローニングや遺伝子欠損マウスの作製・解析を担当したサブユニットである[5,6]。GluN2D サブユニットは，マウスの胎生期から生後 2 週齢において強い発現を脳で示すが，成獣においては発現量が低下し，限定的な発現となる[28]。GluN2D 遺伝子欠損マウスでは，移所運動量の低下などが認められるが，顕著な行動異常は報告されておらず，光学顕微鏡レベルでの脳組織も正常である[6,18]。GluN2D 欠損マウスにおいて PCP の効果が消失している原因としては，成獣において限局的に発現している GluN2D が PCP の標的である可能性や発達期に GluN2D が欠損しているために何らかの脳の機能変化が起こる可能性などが考えられる。今後さらに PCP 効果における GluN2D の役割およびそのメカニズムを研究することで，PCP 依存や PCP 誘発性精神病の病態メカニズムが解明されると期待できる。

オピオイドと精神腫瘍学

2007 年にがん対策基本法が施行され，早期からの適切な緩和医療が求められるようになった[21]。緩和医療チームには精神科医の参加が必須

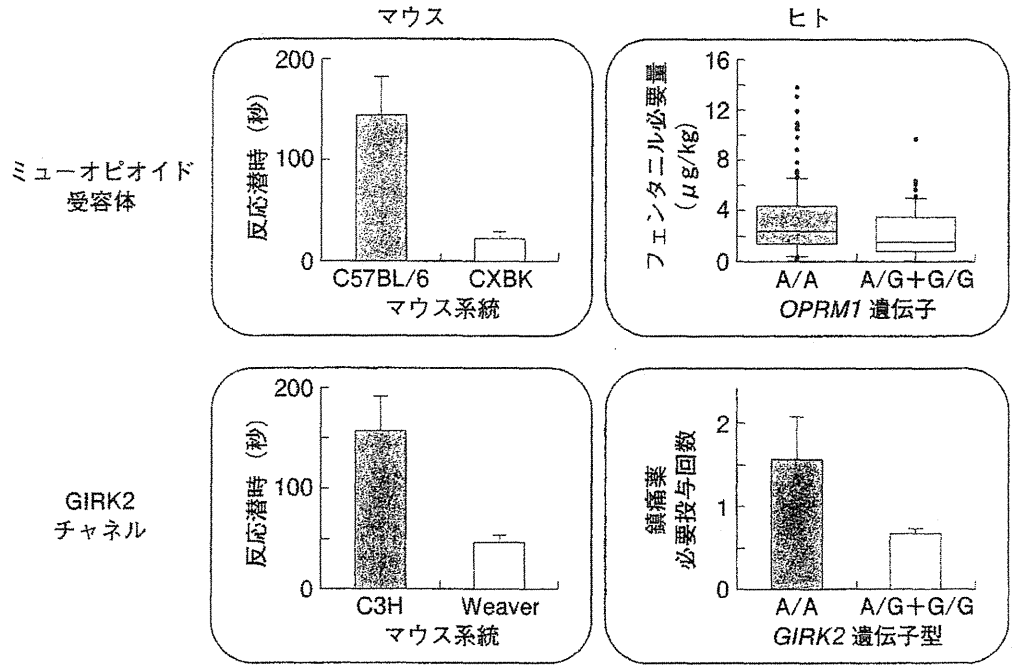

図3　マウスおよびヒトにおけるミューオピオイド受容体およびGIRKチャネルの遺伝子配列の違いと麻薬性鎮痛薬感受性の違いとの関連

ミューオピオイド受容体遺伝子の塩基配列に差異があるCXBKマウスでは，野生型マウス(C57BL/6)と比べて麻薬性鎮痛薬の効果が減弱している。ヒトにおいても，ミューオピオイド受容体遺伝子(*OPRM1*)の多型と術後鎮痛に必要なフェンタニル量が関連する。また，GIRKチャネルのGIRK2サブユニットの遺伝子配列に変異があるウィーバーマウスでは，野生型マウス(C3H)と比べて麻薬性鎮痛薬の効果が減弱している。ヒトにおいても，*GIRK2*遺伝子の多型と術後鎮痛に必要な鎮痛薬投与回数が関連する。

であり，精神腫瘍学は精神医学においても一層重要な位置づけとなった。緩和医療において主要な薬剤は，オピオイド性鎮痛薬であり，典型的な依存性薬物である。一方，オピオイドの感受性には大きな個人差があり，効果的な疼痛治療を妨げている。そして，このような薬剤感受性個人差には，環境要因だけではなく，遺伝要因もあると考えられている[9]。

筆者らは，オピオイド感受性個人差の遺伝要因を，マウスおよびヒトにおいて探索している。モルヒネ感受性が減弱しているCXBKマウスは，ミューオピオイド受容体の遺伝子配列に差異を持つことを見出し[3,8]，ヒトにおいてもこの遺伝子領域の多型がオピオイド性鎮痛薬の感受性と関連することを見出した[1,4]（図3）。また，GIRKチャネルのGIRK2サブユニットに変異を持つウィーバーマウスでは，モルヒネ鎮痛効果やアルコールによる鎮痛効果が減弱していることを見出すとともに[7,11]，ヒトにおいても*GIRK2*遺伝子の多型がオピオイド感受性と関連することを見出した[20]（図3）。このような研究がさらに進むことで，個々人の鎮痛薬感受性や副作用出現リスクを遺伝子検査によって予測することが可能になり，テーラーメイドの疼痛治療に道が拓かれると期待できる。

中枢刺激薬とAD/HD

AD/HDは，小学生の4～7%が罹患するきわめて頻度の高い小児精神疾患であり，患者の自尊心の低下や学習の遅れを招くだけでなく，学級崩壊など周囲を巻き込んだ問題に発展することもある。治療薬として国内外で広く用いられているメチルフェニデートは，中枢刺激薬であり，その作用機序は覚せい剤と類似している。

筆者らは，メチルフェニデートの主要作用点と考えられているドーパミントランスポーター(DAT)に注目し，その遺伝子欠損マウスにおけるメチルフェニデートの効果を検討した。興味深いことに，DAT欠損マウスは多動や学習障害を示し，これらの障害はメチルフェニデート投与によって顕著に改善した。これらの結果から，DAT欠損マウスは，典型的なAD/HDモデル動物といえるが，DATが存在しない動物においてメチルフェニデートが奏効したことから，メチルフェニデートの主要標的はDATではないことが示唆された。さらに，マイクロダイアリシス分析により，DAT欠損マウスにおいてもメチルフェニデート投与後に，前頭前野で細胞外ドーパミン量が上昇することが明らかになり，メチルフェニデートによるAD/HDの治療メカニズムに前頭前野でのドーパミン上昇が関連する可能性が考えられた。

依存性薬物の作用機序解明とその医療応用

依存性薬物は，脳内報酬系など精神を担う根源的な脳機能に影響することで，一見関係が薄いと考えられる精神疾患とも関係している。依存性薬物の作用機序の解明は，さまざまな精神疾患の病態の解明や治療法の開発に寄与する可能性がある。

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野生由来近交系マウス系統における *Oprm1* 遺伝子多型とモルヒネ感受性の関連性*

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モルヒネやフェンタニルといった麻薬性鎮痛薬は，慢性痛の疼痛治療や外科手術における周術期の疼痛治療など様々な医療行為に使用されている．世界保健機関（WHO）により公表されたWHO方式がん性疼痛治療指針には，その基本5原則の1つに，“for the individual”（個々の患者の痛みに見合った鎮痛薬の量で治療すること）という項目があり，麻薬性鎮痛薬の最適量が各個人で著しく異なることは広く知られている．日本における麻薬性鎮痛薬の使用量はアメリカ・ドイツ・カナダの約1/20，フランス・イギリス・イタリアの約1/2～1/7で，他の先進諸国に比べて圧倒的に少ない．その理由の1つとして，麻薬性鎮痛薬は鎮痛作用のほかにも吐き気・嘔吐，便秘や呼吸抑制といった様々な副作用を引き起こし，こういった鎮痛薬の作用には上述のような著しい個人差が存在するため，各個人に最適な疼痛治療が困難であることが挙げられる．日本では副作用の面がクローズアップされてしまい，他国に比べて使用機会が少ないと考えられる．そのため，鎮痛薬感受性の個人差を引き起こす分子メカニズムを明らかにすることは，日本における各個人に最適な疼痛治療の推進につながると考えられる．

鎮痛薬感受性個人差には遺伝子要因，環境要因や生活習慣要因など様々な要因が関与すると考えられている．近年，ヒト遺伝子多型と鎮痛薬感受性（鎮痛効果や最適鎮痛薬量など）との関連解析が行われ，鎮痛薬感受性個人差に関連する遺伝子多型が複数同定されている．しかし，いくつかの遺伝子多型については再現性が得られないという報告もあり，より統制された解析が必要である．多要因が関与する疾患・病態における遺伝子要因について解析する場合，近交系マウス系統における系統間差異の解析が有効な解析モデルの1つである．近交系マウス系統において，系統内では遺伝子塩基配列はほぼ一致し，系統間では様々な遺伝子の塩基配列に違いが見られることから，特に鎮痛薬感受性個人差のような，健常人における遺伝子要因の解析モデルとして有効であると考えられる．

μオピオイド受容体（MOP）はモルヒネなど主要な麻薬性鎮痛薬の分子ターゲットである．MOP遺伝子（*Oprm1*）欠損マウスでは，モルヒネの鎮痛作用はほぼ失われ，行動量亢進，報酬作用，身体依存，耐性等の副作用についても減弱していることから，MOPはモルヒネの鎮痛作用や副作用において重要な役割を果たしていると考えられる．また，*Oprm1* ヘテロ遺伝子欠損マウスではモルヒネの鎮痛作用，行動量亢進や報酬作用が半減していることから（MOP-haploinsufficiency），モルヒネの鎮痛作用，副作用においてはMOP必要量の閾値は高く，野生型マウスにおけるMOP発現量の半分以上は必要であると考えられる．そのため，MOPが重要な働きを果たす鎮痛薬感受性において，*Oprm1* 遺伝子多型の影響は大きいと考えられる．

本研究では，野生由来の近交系マウス系統を用いて *Oprm1* 遺伝子多型を同定し，モルヒネ鎮痛作用に影響を及ぼす *Oprm1* 遺伝子多型の解析を行った．

方　法

本研究では，実験用マウス1系統（C57BL/6），野生由来近交系マウス9系統（BFM/2, BLG2, CHD, HMI, KJR, MSM, NJL, PGN2, SWN），愛玩用マウス1系統（JF1）の計11系統を用いた．BLG2（ブルガリア），CHD（中国），HMI（台湾），KJR（韓国），MSM（日本・三島市），NJL（デンマーク），PGN2（カナダ），SWN（韓国）の各マウス系統は，静岡県三島市にある国立遺伝学研究所が世界各地（括弧内に表示）で捕獲された野生マウスから樹立し・維持している近交系マウス系統である．BFM/2マウス系統は，フランスの研究者が有するオリジナルストックから国立遺伝学研究所が樹立した近交系マウス系統である．また，JF1マウス系統はデンマークで見つかった愛玩用マウスから国立遺伝学研究所が樹立した近交系マウス系統であるが，遺伝子解析の結果，日本の愛玩用マウス由来であることが明らかとなっている．このマウス系統はエンドセリン受容体B遺伝子に変異を有し難聴を呈することから，聴覚が正常な復帰突然変異体のJF1-s^{+}系統を解析に用いた．

これら“Mishima battery of inbred mouse strains”（三島近交系マウスバッテリー）を用いて，モルヒネ（10 mg/kg i.p. injection）の鎮痛作用をtail-flick試験およびhot-plate試験により検討した．鎮痛作用は% of maximal possible effect（%MPE）[{(latency with morphine injection)－(latency

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