

20, 30 and 40 minutes after intra-dPAG drug treatment. Two-way ANOVA (factor 1: treatment; factor 2: time) followed by Duncan post hoc test revealed that intra-dPAG injection of CsA 1 nmol increased TFL in animals [treatment factor ($F_{3,15}=28.39$; $p < 0.05$); interaction ($F_{18,90}=3.23$; $p < 0.05$)]. In Experiment 2; animals were intra-dPAG injected with AM251 (10 pmol; a cannabinoid receptor antagonist, in order to prevent AEA binding to the cannabinoid system) or vehicle followed ten minutes later by local injection of CsA 0.1 nmol (an intrinsically inactive dose on nociception). Five minutes later, each animal was placed into the homecage of an aggressive conspecific mouse for a social defeat stress. The aggressive interaction was interrupted when the intruder mouse exhibited a submissive posture (i.e., defensive upright posture for a 3-sec period). Then, the intruder mouse was placed inside a transparent and perforated plastic bottle (7 cm x 7 cm x 15 cm) and kept inside the aggressor's cage for further five minutes (psychological stress). After that, further five TFL measures were recorded at 0, 5, 10, 20 and 30 minutes post-stress. Two-way ANOVA followed by Duncan post hoc test revealed that animals pre-treated with AM251 followed by CsA had potentiated the social defeat stress-induced analgesia [treatment factor ($F_{1,8} = 11.08$; $p < 0.05$); time factor ($F_{6,48} = 57.68$; $p < 0.05$); interaction ($F_{6,48} = 3.92$; $p < 0.05$)]. Intra-dPAG injection of CsA, at an intrinsically-inactive dose (0.1 nmol), potentiated and prolonged the social defeat stress-induced analgesia. Present results are suggestive that the role of vanilloid receptors located within the dPAG in the modulation of social defeat stress-induced analgesia in mice appears to be dependent on its phosphorylated state.

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P.1.g.069 Relationship between dose, plasma concentration and GABA-A receptor occupancy by partial GABA-A receptor modulators

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Background: Insufficient GABA neurotransmission in key neural circuits may lead to anxiety disorders. Enhancement of GABA channel function by positive modulators such as diazepam has been proven a successful anxiolytic therapy. Although diazepam and other benzodiazepines (BZs), agonists of GABA receptors are highly efficacious, significant adverse effects, such as sedation, amnesia, ataxia, and abuse liability limit their clinical usefulness in anxiety [1].

GABA receptors are ligand (GABA)-gated, heteropentameric chloride ion channels. About 16 different receptor subunits are arranged into pentameric assemblies. Spectrum of the clinical effects of BZs is mediated by the $\alpha 1$ -, $\alpha 2$ -, $\alpha 3$ - and $\alpha 5$ -containing GABA subtypes.

AZD7325 and AZD6280 are potent, selective GABA $\alpha 2,3$ receptor modulators. Both experimental drugs are functionally selective for the GABA $\alpha 2$ and GABA $\alpha 3$ receptor subtypes, with high affinity to $\alpha 2/3$ subunit ($K_i < 30$ nM) and low affinity to

$\alpha 5$ subunit. Preclinical pharmacological studies have indicated that AZD7325 and AZD6280 were fast-acting anxiolytics with reduced effects on cognition and without sedative side effects. Clinical neurophysiological studies using EEG suggest that AZD6280 and AZD7325 induced dose-dependent effects on saccadic peak velocity, marker of anxiolytic effect. Meanwhile both compounds in the Phase I clinical development program have demonstrated significantly smaller effect on body sway and cognitive performance compared to lorazepam [2,3].

Purpose: The aim of the present two PET studies was to determine receptor occupancy of AZD7325 and AZD6280 in humans using the GABA receptor radioligand [¹¹C]flumazenil.

Methods: Each of the PET studies consisted of two panels, with repeated weekly PET measurements, at baseline and after single dose administration. In total (both studies), twelve men, healthy volunteers, 21 to 34 years of age were examined. PET images were analysed using simplified reference tissue model, with pons as a reference region. Regional binding potentials (BPND) were obtained. The relationship between dose, plasma concentration of AZD7325, AZD6280 and GABA receptor occupancy was examined and apparent dissociation constant $K_{i,plasma}$ was estimated. Assessments of safety and tolerability of both tested drugs included recording of adverse events, vital signs, electrocardiogram, and laboratory tests.

Results: Dose-dependent, saturable binding of AZD6280 and AZD7325 was demonstrated. The pattern of regional drug binding to GABA receptors was dependent on the combination of the difference between non-selective binding of [¹¹C]flumazenil to the GABA receptors, subtype selectivity of tested drugs and regional GABA receptor subtype distribution. Maximum apparent receptor occupancy could be determined for both drugs using tolerated doses. The estimated $K_{i,plasma}$ for AZD7325 was 4 nmol/L, ID50 1.3 mg and for AZD6280 at approximately 90 nmol/L and 9 mg, respectively. There were no serious adverse events related to either of the tested drugs. In volunteers receiving AZD6280 doses of 20 mg and above CNS-related adverse events were observed.

Comments: The present PET studies of two novel $\alpha 2/3$ receptor subtype selective partial GABA receptor modulators confirmed their pharmacological activity as of partial agonists. High GABA receptor occupancy (>50%) by AZD7325 and AZD6280 could be reached without clear sedative effects.

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P.1.g.070 Amygdala inactivation produces antinociception without changing threatening-induced pain inhibition in mice

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Exposure of animals to environmentally aversive situations [e.g., an open elevated plus maze (oEPM: 4 open arms) or to a predator]

elicits intense antinociceptive effects, defensive behaviors (e.g., head dipping, stretch attend posture and flatback) and changes in levels of corticosterone. The underlying systems and mechanisms involved in these effects are still poorly understood. Considering that the amygdaloid complex plays a role in the modulation of aversive stimuli and emotional responses related to fear as well as pain processing, the present study attempted to investigate the role of this forebrain structure in the modulation of nociceptive response in mice subjected to the formalin test and exposed to a glass cage (protected environment) or to the open elevated plus maze (threatening situation). Accordingly male Swiss mice ($n=3-6$ /group) were subjected to a stereotaxic surgery in order to implant guide cannulas bilaterally in the amygdala. Five to seven days (recovering time) after surgical procedure all animals received injection of 2.5% formalin (50 μ L) into the dorsal right hind paw (nociceptive test) followed 15 minutes later by a bilaterally injection of 0.1 μ L of saline or cobalt chloride (CoCl₂: non-selective synaptic inactivating) within the amygdaloid complex. Animals were then placed either in the glass cage (vehicle/glass cage or CoCl₂/glass cage) or the oEPM (vehicle/oEPM or CoCl₂/oEPM) and the time spent (in seconds) licking the formalin injected paw was recorded during a 10-min period. Two-way ANOVA revealed a decrease on the time spent licking the formalin-injected paw in animals treated with CoCl₂ or exposed to the oEPM (treatment factor $F_{1,15}=34.05$; environmental factor $F_{1,15}=179.11$; interaction $F_{1,15}=31.04$; $p < 0.05$). Duncan post hoc test confirmed that CoCl₂ injection decreased the time spent licking the formalin-injected paw in animals exposed to the glass cage compared to vehicle-treated animals also exposed to the glass cage (vehicle: 150.8 ± 9.3 ; CoCl₂: 65.3 ± 8.7). oEPM-exposed animals spent lower time spent licking the formalin injected paw than glass-cage exposed animals ($p < 0.05$), an effect that was not changed by CoCl₂ (vehicle: 8.7 ± 1.8 ; CoCl₂: 6.7 ± 3.9). These results suggest that the amygdala plays an important role in the modulation of nociceptive response induced by formalin test in a protected environment (glass cage). In this context, amygdala inactivation seems to have disinhibited the descending pain inhibitory pathway, which, in turn, led to a decrease in pain perception (less time licking the formalin-injected paw). Although further experiments are needed to clarify the role of the amygdala in the modulation of defensive behaviors of mice exposed to the oEPM, it seems that chemical inactivation of amygdala did not change the oEPM-induced antinociception, suggesting that this limbic structure does not modulate this type of environmentally induced pain inhibition.

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P.1.g.072 Genetic polymorphisms of OPRM1 gene and their impact on methadone maintenance treatment in opioid addicts: A systematic review of the literature

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Introduction: Methadone, a long-acting opioid receptors agonist, is effective in treating opioid addiction, however, an interindividual variability exists. Mu1 opioid receptor (MOR), which is encoded by the OPRM1 gene, is the preferential binding target

of methadone. Many single nucleotide polymorphisms (SNP) were identified within the OPRM1 gene. Some of these SNPs may influence methadone maintenance treatment (MMT) and thus explain this variability.

Objectives: Our purpose was to identify all relevant published studies having as objective the detection of correlations between OPRM1 SNPs and methadone required doses as well as the efficacy of MMT, then, to summarize results in order to evaluate the necessity of OPRM1 SNPs genotyping in opioid addicts prior to the onset of MMT.

Methods: A systematic literature search of PUBMED was conducted to identify studies published until March 2015 using the following keyword combination (methadone or MMT) AND (mu1 or oprm1 or MOR or SNP or polymorphism or gene). All abstracts were reviewed to assess the relevance of the studies. We excluded duplicate papers and samples.

Results: Our literature search yielded 333 articles. Eight of them were included in the review with a total of 1704 opioid addicts undergoing MMT. All of the studies are multicentric: Two are prospective, the other ones are retrospective. Subjects have different ethnic origins. The most focused on SNP was rs1799971(A118G). Results concerning its influence on doses and efficacy of methadone were conflicting. Five studies found no correlation between rs1799971 SNP and methadone required doses as well as the efficacy of MMT. One study concluded to that carriers of the G allele need higher methadone doses. The same result was reported by another study, however, this finding depended of the ABCB1 gene allele. Other OPRM1 SNPs might influence MMT. A study conducted in Taiwan suggested that subjects with the rs2075572 C allele require higher methadone doses. An association between poor response to methadone and the presence of GG-172 (rs6912029) or GG-1510 (rs12205732) genotypes was reported in individuals of Arab origins. Another study conducted among Jewish addicts found that carriers of the rs558025 G allele require lower methadone doses. Finally and according to a Spanish study, rs 1074287 doesn't seem to interfere with methadone pharmacodynamics.

Conclusion: There is evidence that OPRM1 polymorphisms have an impact on MMT. Findings concerning Rs1799971 SNP are contradictory. Other SNPs might influence either response to methadone or its required dose, a duplication of these findings is needed.

Furthermore, variations in other genes acting in conjunction with the OPRM1 gene might interfere with methadone pharmacodynamics which has to be taken into consideration during future studies. Based on literature published to date, we can not enunciate recommendations concerning the need of OPRM1 SNP genotyping prior to the initiation of MMT in opioid addicts.

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