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Effect of GABAergic ligands on the anxiolytic-like activity of DOI (a 5-$HT_{2A/2C}$ agonist) in the four-plate test in mice

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Abstract 5-HTergic and GABAergic systems are involved in neurobiology of anxiety. Precedent studies have demonstrated that SSRIs possessed an anxiolytic-like effect in the four-plate test (FPT) at doses that did not modify spontaneous locomotor activity. This effect seems to be mediated through the activation of 5-HT_{2A} postsynaptic receptors. The purpose of the present study was to examine the implication of GABA system in the anxiolytic-like activity of DOI in the FPT. To achieve this, the co-administration of DOI (5-$HT_{2A/2C}$ receptor agonists) with $GABA_A$ and $GABA_B$ receptor ligands was evaluated in the FPT. Alprazolam, diazepam and muscimol (for higher dose) potentiated the anxiolytic-like effect of DOI. Bicuculline, picrotoxin and baclofen inhibited the anxiolytic-like effect of DOI. Flumazenil and CGP 35348 had no effect on the anxiolytic-like activity of DOI. These results suggest that the GABA system seems to be strongly implicated in the anxiolytic-like activity of DOI in the FPT.

1. Introduction

Although the physiopathology of anxiety disorders has not been elucidated yet, a lot of evidence support the implication of the serotonin (5-HT) and GABA systems. For a long time, benzodiazepines (BZDs) have been the first drugs for the treatment of anxiety disorders but these compounds involve several side effects including withdrawal symptoms, physiological dependence and sedation. More recently specific serotonin reuptake inhibitors (SSRIs) have proved to be efficient in the treatment of anxiety disorders in humans. Today, it seems essential to consider possible interactions between different systems because the study of a single neurotransmitter system is not sufficient to understand anxious disorders mechanism. The purpose of the present study is to examine the implication of GABA system in the anxiolytic-like effect of DOI in the FPT.

Animal's models of anxiety have been developed to be sensitive to the anxiolytic properties of BZDs (Jones et al., 1994; Kulkarni and Sharma, 1993; Stefanski et al., 1992). The four-plate test (FPT) in mice used in the present study (Aron et al., 1971) is based on exploration of novel surrounding

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suppressed by the delivery of mild electric foot shock contingent to quadrant crossing. This model has been demonstrated to be sensitive to BZDs. BZDs administration increases the number of punished passages accepted by mice (Bourin et al., 1992; Griebel et al., 2002; Jones et al., 1994; Klodzinska et al., 2004). To date, the study of the GABA system in the FPT has been limited to the utilisation of BZDs. However, the GABA system has been more widely studied in others behavioural models of anxiety by the utilisation of $GABA_A$ and $GABA_B$ ligands and other pharmacological agents (Cryan and Kaupmann, 2005; Dalvi and Rodgers, 1996). Sasaki et al. (2002) report an anxiolytic-like activity of the $GABA_A$ receptor agonist (muscimol) in the mouse EPM (Sasaki et al., 2002). On the other hand, GS39783 a new allosteric positive modulator at $GABA_B$ receptors possesses anxiolytic-like activity in the elevated zero maze in mice (Cryan et al., 2004). An intra-dorsal periaqueductal grey treatment with a $GABA_B$ receptor agonist, baclofen impaired escape in the elevated T maze in rats (Bueno et al., 2005). Several studies have demonstrated that mice deficient in glutamic acid decarboxylase gene, a key enzyme of GABA synthesis, exhibit increased anxiety-like responses in the auditory-cued fear conditioning paradigm and the elevated zero maze (Kash et al., 1999; Stork et al., 2003). On the other hand, the GABA transaminase inhibitor valproic acid has been found to reduce anxiety in the elevated plus maze (EPM) in mice (Dalvi and Rodgers, 1996). Moreover, different studies using $GABA_B$ knock out mice have demonstrated interesting results. Mombereau et al. (2004a,b; 2005) report that, in the light/dark box and the staircase test, $GABA_{B(1)}-/-$ and $GABA_{B(2)}-/-$ mice are more anxious than their wild-type.

A study carried out in our laboratory has demonstrated the strong anxiolytic-like activity of SSRIs, serotonin and noradrenaline reuptake inhibitors (SNRIs) in the FPT (Bourin et al., 1992; Hascoet et al., 2000). In addition, 5-HT_{2A} receptors have been found to be implicated in the anxiolytic-like mechanism of a SSRI paroxetine and a SNRI venlafaxine in the FPT (Nic Dhonnchadha et al., 2005). 5-HT_2 receptor agonists (BW 723C86, DOI and RO 60-0175) have been shown to induce anxiolytic-like effect in two mouse models of anxiety the EPM and the FPT, 5-HT_2 receptor antagonists being without effect in these tests (Nic Dhonnchadha et al., 2003a). However, some authors (Nastiti et al., 1991) have found anxiogenic-like effect following after an acute administration of DOI in ultrasonic "distress" vocalization model in mice. In the same way, 5-HT_2 receptor antagonists, such as ritanserin and ketanserin have demonstrated an anxiolytic-like effect in animal models of anxiety and clinical results (for references, see Nic Dhonnchadha et al., 2003a; Griebel, 1996). In our model, the anxiolytic-like effect of DOI was antagonized by SR 46349B (a specific 5-HT_{2A} receptor antagonist) in the FPT and the EPM (Nic Dhonnchadha et al., 2003a,b).

Recently, the study of the noradrenergic system implication in the anxiolytic-like activity of DOI (5-$HT_{2A/2C}$ agonist), two SSRIs (paroxetine and citalopram) and two SNRIs (venlafaxine and milnacipran) in the FPT, has only demonstrated a regulation by α_2-noradrenergic heteroreceptors (Masse et al., 2005, 2006). The lesion of noradrenergic and dopaminergic pathways and the depletion of serotonergic system did not modify the anxiolytic-like effect of DOI (Masse et al., 2006) (unpublished data for lesion of dopaminergic system). These results suggest the implication of another neurotransmitter system in the mechanism of action of DOI in the FPT.

Many electrophysiological and pharmacological studies have observed links between the GABAergic and the 5-HTergic systems but their inter-relations in anxiety are not clearly explained yet (Abi-Saab et al., 1999; Forchetti and Meek, 1981; Tao et al., 1996).

In the present study, DOI (5-$HT_{2A/2C}$ receptor agonists) was co-administered with benzodiazepine receptor agonists (alprazolam and diazepam) and benzodiazepine receptor antagonist (flumazenil), $GABA_A$ receptor agonist (muscimol), $GABA_A$ receptor antagonists (bicuculline and picrotoxin), $GABA_B$ receptor agonist (baclofen) and $GABA_B$ receptor antagonist (CGP35348) in the FPT.

2. Experimental procedures

2.1. Animals

Male Swiss mice (Janvier, France), weighing on average 20 ± 2 g, on the day of the study, were used. These animals were housed in groups of 18 for a minimum of 1 week prior to experiments, at a constant temperature (20 °C) and a standard light cycle (lights on between 07:00 and 19:00 h). There was free access to food and water. Naive mice were used for each experiment. Mice were allocated randomly to the treatment groups (n = 10 or 12). All experiments were conducted in accordance with the ethical rules of the French Ministry of Agriculture for experiments with laboratory animals (No. 87.848).

2.2. Drugs

- 5-$HT_{2A/2C}$ receptor agonist: DOI hydrochloride (Sigma, France)
- benzodiazepine ligands: alprazolam (Sigma, France), diazepam (Sigma, France), flumazenil (Tocris, France)
- $GABA_A$ receptor ligands: muscimol (Sigma, France), picrotoxin (Sigma, France) and bicuculline (Sigma, France)
- $GABA_B$ receptor ligands: baclofen (Sigma, France), CGP 35348 (Sigma, France)

DOI, muscimol, picrotoxin, bicuculline, baclofen and CGP 35348 were dissolved in distilled water. Alprazolam and diazepam were suspended in a solution of distilled water with 5% of Tween 80 (Merck, Germany).

2.3. Experimental design

Testing was performed between 09:00 and 13:00 h. All tests were performed in a quiet room. The mice were kept in this room at least 1 h before the test. After injection (vehicle or treatment), the mice were placed in their holding cage in order to reduce any neophobic response to the test-room environment. The acute administration of GABA ligands was performed 45 min before the test and 30 min before the test for the acute administration of DOI via the intraperitoneal (i.p.) route, in a volume of 0.5 ml/20 g of bodyweight. All doses of drugs chosen in the present work were based on previous studies. Each mouse received two administrations of drugs or vehicle in accordance with the protocol.

2.4. Psychopharmacological tests

2.4.1. Actimeter test (Boissier and Simon, 1965)

The spontaneous activity of naive animals was recorded using a photoelectric actimeter (Orga System, Changé, France). This