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The 5-HT$_{1A/1B}$-receptor agonist eltoprazine increases both catecholamine release in the prefrontal cortex and dopamine release in the nucleus accumbens and decreases motivation for reward and "waiting" impulsivity, but increases "stopping" impulsivity

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ABSTRACT

The 5-HT$_{1A/1B}$-receptor agonist eltoprazine has a behavioral drug signature that resembles that of a variety of psychostimulant drugs, despite the differences in receptor binding profile. These psychostimulants are effective in treating impulsivity disorders, most likely because they increase norepinephrine (NE) and dopamine (DA) levels in the prefrontal cortex. Both amphetamine and methylphenidate, however, also increase dopamine levels in the nucleus accumbens (NAc), which has a significant role in motivation, pleasure, and reward.

How eltoprazine affects monoamine release in the medial prefrontal cortex (mPFC), the orbitofrontal cortex (OFC), and the NAc is unknown. It is also unknown whether eltoprazine affects different forms of impulsivity and brain reward mechanisms.

Therefore, in the present study, we investigate the effects of eltoprazine in rats in the following sequence: 1) the activity of the monoaminergic systems using in vivo microdialysis, 2) motivation for reward measured using the intracranial self-stimulation (ICSS) procedure, and finally, 3) "waiting" impulsivity in the delay-aversion task, and the "stopping" impulsivity in the stop-signal task.

The microdialysis studies clearly showed that eltoprazine increased DA and NE release in both the mPFC and OFC, but only increased DA concentration in the NAc. In contrast, eltoprazine decreased 5-HT release in the mPFC and NAc (undetectable in the OFC). Remarkably, eltoprazine decreased impulsive choice, but increased impulsive action. Furthermore, brain stimulation was less rewarding following eltoprazine treatment. These results further support the long-standing hypothesis that "waiting" and "stopping" impulsivity are regulated by distinct neural circuits, because 5-HT$_{1A/1B}$-receptor activation decreases impulsive choice, but increases impulsive action.

1. Introduction

The 5-HT$_{1A/1B}$-receptor agonist eltoprazine is a relatively "old" drug that was originally developed as a serenic drug (Sybesma et al., 1991a, 1991b; De Boer, Koolhaas, 2005). Recently, PsychoGenics Inc. has used their SmartCube®, a high-throughput behavioral platform for detecting therapeutic efficacy, for comparing the behavioral profile of eltoprazine with those from their proprietary reference drug database (Alexandrov et al., 2015). Interestingly, it was shown that eltoprazine has a drug signature that resembles that of a variety of psychostimulant drugs (amphetamine, methylphenidate, and modafinil) and the norepinephrine (NE) reuptake inhibitor atomoxetine (Alexandrov et al., 2015). What all of these drugs have in common, despite the different working mechanisms, is that they increase NE and/or dopamine (DA) in the prefrontal cortex (Solanto, 1998), and enhance cognition and reduce impulsivity (Arnsten and Pliszka, 2011). In addition, ampheta-

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mine and methylphenidate also increase DA levels in the nucleus accumbens (NAc) and therefore are frequently abused for recreational purposes (i.e., to get high) (Stoops et al., 2003; Pierce and Kalivas, 1997). There is a growing body of literature that recognizes the importance of impulsivity as both a psychological construct and an endophenotype underlying ADHD and drug abuse (Urcelay and Dalley, 2012).

Different categories of impulsivity exist. 1) Impulsive action or"-stopping" impulsivity is the inability of individuals to stop a response that has already been initiated. This type of impulsivity can be measured with the stop-signal task (Dalley et al., 2011; Evenden, 1999). 2) Impulsive choice or"waiting" impulsivity as the inability to wait for a large reward over an immediate small reward. This tendency can be measured with delay-aversion/delay-discounting paradigms (Bari and Robbins, 2013; Sonuga-Barke et al., 1992). These different types of impulsivity probably have discrete underlying neural circuits, in which the medial prefrontal cortex (mPFC), the orbitofrontal cortex (OFC), and the nucleus accumbens (NAc) play an important role (Dalley et al., 2011).

Recent years have seen a renewed interest in eltoprazine, because this specific 5-$HT_{1A/1B}$-receptor agonist counteracts l-DOPA-induced dyskinesias in Parkinson's (Svenningsson et al., 2015). This suggests that eltoprazine also affects the dopaminergic system. It is widely accepted that the serotonergic and dopaminergic system are closely interconnected and exert regulatory control over each other (for review see: Assié et al., 2005; Diergaarde et al., 2008; Fink and Göthert, 2007a, 2007b). Thus, investigating the role of 5-$HT_{1A/1B}$-receptors and monoamine release on impulsivity is of special interest. The 5-$HT_{1A/1B}$-receptors may alter dopamine function and other neurotransmitters in complex ways, because they function both pre- and postsynaptically.

The objective of this paper is to investigate the effects of eltoprazine on the release-profile of 5-HT, NE and DA and their metabolites in the mPFC, OFC and NAc in rats. Both DA and 5-HT are involved in reward-related processes related to impulsivity (Kranz et al., 2010). We therefore also assessed the motivation for reward using an intracranial self-stimulation (ICSS) procedure. In addition, we examined the effects of eltoprazine on impulsive choice and impulsive action, as measured by the delay-aversion task and the stop-signal task, respectively.

2. Material and methods

2.1. Compounds

Eltoprazine (1-[2,3-dihydro-1,4-benzodioxin-5-yl]-piperazine hydrochloride, synthesized by Psychogenics Inc, USA), has high affinitiy for the 5-HT_{1A} and 5-HT_{1B} receptor subtypes (K_i =40 and 52, respectively) (Schipper et al., 1990). Eltoprazine was dissolved in 0.9% NaCl and administered intraperitoneally (i.p.) in a volume of 2 ml/kg. Doses were 0, 1, and 3 mg/kg eltoprazine in both the microdialysis- and ICSS experiments and 0, 0.25, 0.5, and 1 mg/kg eltoprazine in the impulsivity tests. In all experiments, the drugs or vehicle (NaCl) were administered 30 min before testing.

2.2. Animals

Male Wistar rats (in total 116) obtained from Harlan (The Netherlands), weighing 125–175 g on arrival. Seventy-two animals were used for the microdialysis experiment (mPFC: n=24; OFC: n=24; NAc: n=24); 16 for intracranial self-stimulation; 12 for delay-aversion and finally, and 16 for the stop-signal task. The subjects were randomly divided over the different experimental groups. Animals weighed between 250 and 360 g at the time of microdialysis experiments, when they were ca. 10–12 weeks of age. During impulsivity testing, the rats weighed between 350–500 g and were ca. 4–6 months of age. The rats were socially housed, four per cage. For the microdialysis experiments, animals were housed singly directly after surgery until the experiment the next day. All animals were kept on a 12 h light/dark cycle with lights on between 6:00 A.M. and 6:00 P.M., and rooms were temperature (21 ± 2 °C) and humidity (50 ± 10%) controlled. Food and water were available ad libitum except during ICSS training and the delay-aversion task, during which they received ca. 75% of their ad libitum intake. All experiments were approved by the Ethical Committee for Animal Research of Utrecht University, The Netherlands. During the experiments every effort was made to minimize animal pain, distress and discomfort.

2.3. Surgery

Rats were anesthetized by inhalation of isoflurane gas (2–3%), mixed with nitrous oxide and oxygen and animals were placed in a stereotaxic instrument (Kopf, David Kopf Instruments). Lidocaine hydrochloride (2%) was applied in the incision as a local anesthetic. All animals received Rimadyl (5 mg/kg, subcutaneously) for pain relief.

In the microdialysis experiment Cuprophan microdialysis probes were implanted in the mPFC (MAB 4.7., 3 mm CU), the OFC (MAB 4.6., 2 mm CU), and the NAc (MAB 4.7., 2 mm CU) of rats as part of three separate cohorts. For the mPFC, the incisor bar was lowered to the coordinates at −3.3 mm, AP: +3.2 mm, ML: +0.8 mm, DV: −4.0 mm from bregma and skull. The incisor bar was lowered to coordinates of the OFC at −3.3 mm, AP: +3.2 mm, ML: +2.5 mm, DV: −6.2 mm from bregma and skull. For the NAc, the incisor bar was lowered to the coordinates −3.3 mm, AP: +1.5 mm, ML: +1.8 mm, DV: −8.4 mm from bregma and skull (Paxinos, 2007). Probes were anchored with three screws and dental cement on the skull. After microdialysis probe implantation, animals were housed individually until the end of the experiment. For the intracranial self-stimulation (ICSS) experiments, bipolar ICSS electrodes (Plastics One, cut to 11 mm in length) were implanted into the lateral hypothalamus (LH). Coordinates were AP: −0.5 mm from bregma; ML: ± 1.7 mm; DV: −8.3 mm from dura. The incisor bar was adjusted to 5 mm above the interaural line (Pellegrino et al., 1979). Electrodes were anchored with four screws and dental acrylic on the skull.

2.4. Microdialysis experiment

One day after surgery, microdialysis experiments were carried out in awake, freely moving animals. Although 1 d after surgery the animals may not be fully recovered from the operation, most neuroscientists (including our group) perform microdialysis experiments within the optimal window of 24–48 h after probe insertion (Westerink et al., 1987). Microdialysis probes produce gliosis extending 200–300 μm from the track by 3–7 days after implantation, which is not observed 1 d after probe implantation (Hascup et al., 2009; Benveniste and Diemer, 1987). In line with this observation, it has also been shown that astrocytes around the guide cannula and microdialysis probe increase over time and this may clog the microdialysis membrane (Georgieva et al., 1993). Conducting microdialysis experiments immediately after probe insertion, however, are not recommended, because probe insertion is well known to cause localized tissue damage that compromises the blood-brain barrier to small molecules, but is re-established after 24 h (Benveniste et al., 1987; Morgan et al., 1996; Hascup et al., 2009; Benveniste and Hüttemeier, 1990).

The tubing was pre-rinsed with Ringer solution (147 mM NaCl, 2.3 mM KCL, 2.3 mM $CaCl_2$, and 1 mM $MgCl_2$) with use of a KdScientific Pump 220 series (USA) at constant flow rate of 20 μl/h for approximately 14 h. before every experiment. At the beginning of the test day animals were connected to a dual channel swivel (type 375/D/22QM), which allowed them to move freely. During microdialysis, the pump rate was set at 1.5 μl/min. Two h after connection, ten 30-min samples were manually collected in vials containing 15 μl of 0.1 M acetic acid and frozen at −20 °C. At the end of the test day samples were transferred to −80 °C until analysis with HPLC. After two h of