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Short communication

Anxiolytic-like effects of PHCCC, an allosteric modulator of $mGlu_4$ receptors, in rats

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Abstract

We examined the potential anxiolytic-like activity of (−)-*N*-phenyl-7-(hydroxyimino) cyclopropa[*b*]chromen-1*a*-carboxamide (PHCCC), an allosteric modulator of metabotropic glutamate$_4$ receptors ($mGlu_4$), after administration into the basolateral amygdala, using the conflict drinking Vogel test in rats as a model. The results indicate that PHCCC, but not 7-(hydroxyimino)cyclopropa[*b*]chromen-1*a*-carboxylate ethyl ester (CPCCOEt), the selective antagonist of group $mGlu_1$ receptors, showed significant, dose-dependent anticonflict effects without affecting the threshold current or water intake. The results indicate that positive allosteric modulation of $mGlu_4$ receptors may be a useful therapeutic approach to anxiety.

Keywords: Allosteric $mGlu_4$ receptor modulator; $mGlu_1$ receptor antagonist; Anxiety; Amygdala

1. Introduction

Several lines of evidence indicate that glutamate is involved in the pathophysiology of anxiety (for recent review see Chojnacka-Wojcik et al., 2001). The discovery of metabotropic glutamate receptors (mGlu receptors) and the subsequent identification of selective mGlu receptor ligands, which modulate the function of the glutamatergic system, triggered an intense search for novel and potentially safer pharmacotherapy for the treatment of nervous system disorders, including anxiety. MGlu receptors are members of a relatively new class of glutamate receptors linked to G-proteins. Eight different subtypes of mGlu receptors have been cloned so far (mGlu 1–8). On the basis of their sequence homology, coupling to effectors and pharmacology, mGlu receptors have been subdivided into three groups (groups I, II and III). Group I mGlu receptors (mGlu1 and mGlu5) are positively coupled to phospholipase C, while group II mGlu receptors (mGlu2 and mGluR3) and group III mGlu receptors (mGlu4, mGlu6, mGlu7 and mGlu8) are both negatively coupled to adenylate cyclase (Conn and Pin, 1997).

Data are accumulating that both agonists of group II metabotropic glutamate receptors (mGluR) and agonists of group III mGlu receptors exert anxiolytic-like effects in animals (Helton et al., 1998; Klodzinska et al., 1999; Palucha et al., 2004) and may become a new class of anxiolytic drugs. This notion is supported by clinical data suggesting that (+)-2-aminobicyclo-[3,1]hexane-2,6-dicarboxylic acid (LY354740), a group II mGlu receptor agonist, has an anxiolytic profile in humans without being sedative (Grillon et al., 2003). Group III mGlu receptors are G-protein-coupled receptors and undergo allosteric modulation (Conigrave and Franks, 2003). Therefore we decided to investigate whether PHCCC, a positive allosteric modulator of mGlu4 receptors (Maj et al., 2003; Marino et al., 2003), which belong to group III of the

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mGlu receptor family, exerts anxiolytic-like effects in rats. As the amygdala is a key structure in the regulation of anxiety (Davis, 1992), we decided to investigate possible anxiolytic-like effects of PHCCC after injections into the basolateral amygdala.

2. Materials and methods

2.1. Animals and housing

Male Wistar rats, weighing 250±20 g, were used in the study. The animals were caged individually (40×27×15 cm), on a natural day–night cycle and at a room temperature of 19–21 °C, with free access to food and tap water before the experiment. All the experiments were performed in the light phase of the natural light/dark cycle (from November till May) between 9 a.m. and 2 p.m. All experimental procedures were approved by the Animal Care and Use Committee at the Institute of Pharmacology, Polish Academy of Sciences in Krakow.

2.2. Intramygdalar injections

The rats were operated on under equithesin anesthesia. Two stainless steel guide cannulae were implanted stereotaxically into the basolateral amygdala. The coordinates for injection were A+5.2; L+5.1; H+2.0, measured from bregma. The cannuale were fixed to the skull with stainless steel screws and dental acrylic cement. Seven days later, the rats were subjected to behavioral testing. Intrahippocampal injections of drugs were made using Hamilton microsyringes connected, via polyethylene tubing, to two stainless steel needles (0.3 mm o.d.). The injection needles were lowered 2 mm below the tip of the guide cannula, i.e. at the level of the basolateral amygdala. Solutions were administered bilaterally over 60 s. The injection needle remained in place for an additional 30–60 s before it was removed and replaced with a stylet. All compounds were dissolved in sterile saline with the addition of a minimal amount of 0.1 M NaOH (pH=7.2) and injected into the amygdala in a volume of 0.5 μl/site 10 min before the test. Control rats received vehicle.

2.3. Conflict drinking test (Vogel test)

A modification of the method of Vogel et al. (1971) was used. On the first day of the experiment, the rats were adapted to the test chamber for 10 min. After the adaptation period, the animals were deprived of water for 24 h and were then placed in the test chamber for 10 min with free access to the drinking bottle. Afterwards, they were allowed a 30-min free-drinking session in their home cage. After another 24-h water deprivation, the rats were again placed in the test chamber and were allowed to drink for 30 s. Immediately afterwards, drinking attempts were punished with an electric shock (0.5 mA). The impulses were delivered every 2 s (timed from the moment when a preceding shock was delivered) between the grid floor and the spout of the drinking bottle. Each shock lasted 1 s and if the rat was drinking when an impulse was released, it received a shock. The number of shocks accepted throughout a 5-min experimental session was recorded by an experimenter who observed a behavioral reaction (e.g., body jerks) of rats to the electric shock.

2.4. Shock threshold and free-drinking tests

To check for the possibility of drug-induced changes in the perception of the stimulus or in the thirst drive, which might have contributed to activity in the conflict-drinking test, the stimulus threshold was measured and a free-drinking experiment was carried out. In both cases the rats were treated in a manner similar to that described in the conflict drinking test. In the shock threshold test, the rats were placed individually in the box, and electric shocks were delivered through the grid floor. The shock threshold was determined stepwise by increasing manually the current (0.1, 0.2, 0.3, 0.4, 0.5 mA) delivered through the grid-floor until a rat showed an avoidance reaction (jump, jerk, or the like—recorded by an observer blind to the treatment) to an electric stimulus. There was a 15-s shock-free interval between steps.

In the free-drinking test, each animal was allowed to drink from the water spout. Licking was not punished. The total amount of water (ml) consumed in 5 min was recorded for each rat. In these tests and in the Vogel test the animals were used only once.

2.5. Histological analysis

On completion of each experiment the location of the infusion was verified visually. All the animals were killed on the final testing day; their brains were removed and

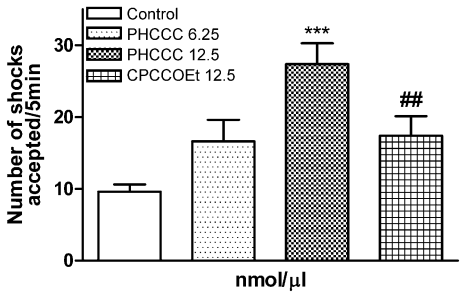

Fig. 1. Effects of PHCCC and CPCCOEt in the conflict drinking test after administration into the basolateral amygdala. Drugs were injected 10 min before the test. Values (expressed as means±S.E.M. (n=7–9 rats per group) indicate the number of shocks accepted during the 5-min experimental session. ANOVA as follows ($F(3,31)=8.325$; $P<0.001$). Symbols indicate significance of differences in Newman–Keuls multiple comparison test ***$P<0.001$ vs. control, ##$P<0.01$ vs. PHCCC 12.5 nmol.

Table 1
Effects of PHCCC on the shock threshold and amount of water drunk by water-deprived rats

Compounds	Dose (nmol/μl)	*N*	Shock threshold (mA)	Water consumption (ml)
Vehicle	–	7	0.5±0.03	10.9±1.3
PHCC	12.5	7	0.40±0.03 ns	7.7±0.6 ns
CPCCOEt	15	7	0.40±0.02	7.8±0.4

All compounds were administered into the basolateral amygdala 10 min before the test. *N*=number of rats per group. The values shown are the means±S.E.M.

stored in a 10% formalin solution. To identify the position of the cannula tracts, the frozen brains were cut in the coronal plane in a Cryo-cut. Only the data from rats in which the cannulae were located bilaterally in the intended structure were included in the analyses.

2.6. Analysis of the data

All the data are expressed as the means±S.E.M. The results were analyzed with a one-way analysis of variance (ANOVA). Specific comparisons were carried out with the Newman–Keuls multiple comparison test using GrapPad Prism software.

2.7. Drugs

(−)-*N*-Phenyl-7-(hydroxyimino) cyclopropa[*b*]chromen-1*a*-carboxamide (PHCCC) was a gift from Dr Fabrizio Gasparini, Novartis, Basel, Switzerland. 7-(Hydroxyimino) cyclopropa[*b*]chromen-1*a*-carboxylate ethyl ester (CPCCOEt) was purchased from Tocris.

3. Results

PHCCC, an allosteric modulator of mGlu4 receptors, administered into the basolateral amygdala at doses of 6.25 and 12.5 nmol/μl rat exerted an anxiolytic-like effect, significantly ($P<0.001$) increasing (by 285% after the higher dose) the number of shocks accepted during the experimental session in the conflict drinking test (Fig. 1), ($F(3,31)=8.325$; $P<0.001$). The possibility that the efficacy of the tested compounds was due to a reduced perception of the stimulus or to an increased thirst drive was excluded, since these compounds tested at doses effective in the conflict drinking test did not change the threshold current or water intake compared to vehicle treatment (Table 1). As PHCCC is also an antagonist of $mGlu_1$ receptors, we used its close analogue, CPCCOEt, a selective antagonist of group $mGlu_1$ receptors, as a control; however, it was without any anxiolytic-like effect after injection into the basolateral amygdala at a dose of 12.5 nmol/μl rat.

4. Discussion

Previous studies have shown that intrahippocampal injections of group III mGlu receptor agonists (Tatarczynska et al., 2001, 2002; Palucha et al., 2004) induce anxiolytic-like effects in rats, indicating that group III mGlu receptors may emerge as promising target for the treatment of anxiety. The observation that PHCCC, a positive allosteric modulator of $mGlu_4$ receptors, produced an anxiolytic effect by itself indicates that this approach is valid. PHCCC is a close structural analogue of CPCCOEt. Both compounds are noncompetitive antagonists of $mGlu_1$ receptors (Maj et al., 2003, Marino et al., 2003) with similar affinity, but PHCCC has about 30% the efficacy of CPCCOEt. CPCCOEt, which has no positive allosteric modulatory effects on $mGlu_4$ receptors, was inactive when injected into the basolateral amygdala. This strongly indicates that the positive allosteric modulation of $mGlu_4$ receptors is responsible for the anxiolytic-like effects of PHCCC, perhaps due to interactions with an endogenous transmitter, glutamate. Benzodiazepines, allosteric modulators of $GABA_A$ receptor function, serve as an example that such substances can be used as drugs. Positive allosteric modulators may become a more efficient drug than receptor agonists because receptor desensitization or down-regulation is not expected to occur. The in vivo anxiolytic action of PHCCC supports the hypothesis that activation of $mGlu_4$ receptors, belonging to the III group of mGlu receptors, represents a possible approach to the treatment of anxiety. Stimulation of group III mGlu receptors (which are localized presynaptically) leads to inhibition of glutamate release (Schoepp, 2001). Decreased glutamatergic transmission, which leads to overall inhibitory effects in the central nervous system, may have consequences similar to the effect of increased GABA-ergic transmission exerted by benzodiazepines, an important class of anxiolytic drugs. Further experiments to investigate interactions between PHCCC and group III mGlu receptor agonists/antagonists are under way.

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