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Selective agonists of metabotropic glutamate receptors elicit significant EEG effects when infused in the nucleus accumbens of rats

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Abstract

The effect of intra-accumbens infusion of selective group I ((*S*)-3,5-dihydroxyphenylglycine, DHPG), group II ((2*S*,3*S*,4*S*)-CCG/(2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)glycine, L-CCG-I) and group III ((L-(+)-2-amino-4-phosphonobutyric acid, L-AP4) metabotropic glutamate (mGlu) receptor agonists was studied in male Wistar rats. A computerised electroencephalographic (EEG) power spectral analysis was performed. While DHPG (400 nmoles) induced EEG and behavioural limbic seizures, L-CCG-I (400 nmoles) and L-AP4 (800 nmoles) induced a 'depressant' EEG with an increase in relative power in the slow-frequency bands and a decrease in relative power in the high-frequency bands) and behavioural effects. These results show for the first time that the stimulation of groups I, II and III mGlu receptors located in the nucleus accumbens significantly influences the EEG tracing in rats.

Keywords: Metabotropic glutamate receptor; Nucleus accumbens; EEG (electroencephalography); (Rat)

1. Introduction

The nucleus accumbens, which is considered a functional interface between the limbic and motor systems (Mogenson et al., 1980), receives glutamatergic afferents from limbic structures such as the amygdala and the hippocampus, as well as from the prefrontal cortex (Christie et al., 1987; Meredith et al., 1993). While in the past, research on glutamatergic pathways focused on ionotropic receptors, the role of metabotropic glutamate (mGlu) receptors is now under intensive investigation. On the basis of their amino acid sequence homology, effector systems and pharmacological profile, mGlu receptors have been classified in three groups (for review, see Pin and Duvoisin, 1995): group I (which stimulates phosphoinositide hydrolysis), group II and group III (which inhibit adenylyl cyclase).

The expression of mGlu receptors in the nucleus accumbens has been revealed by immunohistochemical (Baude et al., 1993; Shigemoto et al., 1993) and in situ hybridization studies (Ohishi et al., 1993; Testa et al., 1994). In in vivo experiments, the infusion of the mGlu receptor agonist, 1*S*,3*R*-ACPD ((1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid), in the rat nucleus accumbens increases dopamine release (Onho and Watanabe, 1995) and induces dopamine-dependent locomotor activation (Attarian and Amalric, 1997; Kim and Vezina, 1997). The notion that mGlu receptors play a functional role in limbic structures is also supported by the finding that some mGlu receptor agonists induce limbic seizures after intracerebroventricular (i.c.v.) administration in mice (Tizzano et al., 1995a,b), while amygdala kindling alters the expression of hippocampal mGlu receptors (Akbar et al., 1996) and induces changes in mGlu receptor-mediated responses in amygdala slices (Holmes et al., 1996; Neugebauer et al., 1997).

Since ligands with a high selectivity for the distinct mGlu receptor groups have been developed only recently, the specific role played by groups I, II and III mGlu receptors in the nucleus accumbens is not as yet fully understood. In vivo investigations are likely to provide insight into the ultimate function of mGlu receptors in the

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nucleus accumbens as an integrated network. The electroencephalographic (EEG) recording is a suitable model to study the effect of centrally acting drugs, because 'depressant' and 'activating' effects are generally distinguishable in laboratory animals (Ongini and Caporali, 1987; Popoli et al., 1995, 1996a,b, 1997). The fact that the nucleus accumbens projects to several subcortical structures, such as mesencephalic, pedunculopontine and thalamic areas (Pennartz et al., 1994), which are involved in the modulation of EEG activity (Rainnie et al., 1994; Steriade et al., 1993) further supports the suitability of such an experimental approach.

The aim of the present paper was to study the EEG effects induced by intra-accumbens (i.a.) injection of selective group I ((*S*)-3,5-dihydroxyphenylglycine, DHPG, Schoepp et al., 1994), group II ((2*S*,3*S*,4*S*)-CCG/(2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)glycine), L-CCG-I, Pin and Duvoisin, 1995) and group III ((L-(+)-2-amino-4-phosphonobutyric acid, L-AP4, Pin and Duvoisin, 1995) mGlu receptor agonists in rats.

2. Materials and Methods

2.1. Animals

Adult male Wistar rats (250–280 g) were used. The animals were kept under standardised temperature, humidity and lighting conditions, with free access to water and food. Animal care and use followed the directives of the Council of the European Communities (1986).

2.2. Surgery

Under Equitesin anaesthesia (3 ml/kg), animals were placed in a Kopf stereotaxic apparatus and stainless steel guide cannulae (22 G, Plastics ONE) were bilaterally inserted 3 mm above the upper boundary of the nucleus accumbens ($A = +2.4$, $L = +3.3$; $V = -4.5$ mm from bregma, sagittal suture and dura, respectively, according to the Atlas by Pellegrino et al., 1979). Guide cannulae were fixed with dental acrylic to the skull surface. Stainless steel stylets were inserted into the cannulae to prevent occlusion. Screw cortical electrodes were implanted at the level of the frontal cortex and fixed with dental acrylic to the skull surface. A recovery period of 5–6 days was allowed before testing. Correct cannula placement was ascertained by postmortem histological investigation. Only data from animals showing the appropriate injection sites on both sides were included in the analysis.

2.3. Experimental procedure

Rats were randomly assigned to the following treatments: vehicle (distilled water or phosphate-buffered saline), DHPG (200 and 400 nmoles), L-CCG-I (200 and 400 nmoles), L-AP4 (400 and 800 nmoles). Each group, except for the DHPG 400-nmole group (in which only four rats were included owing to the appearance of long-lasting seizures), was composed of six animals. Injection needles (28 G) extending 3 mm below the guide were inserted into the cannulae and drugs were bilaterally infused into the nucleus accumbens at a rate of 0.2 μl/min by means of a microdrive pump (injection volume: 1 μl). Half of the total dose was infused in each side. At the end of drug infusion, each animal was put in a cylindrical Plexiglas container and connected to an Ote (model 10b) polygraph. The EEG was then continuously recorded over 60 min. The methods used for EEG recording and analysis have been described elsewhere (Popoli et al., 1997). Briefly, sequential power spectra of 20-s EEG epochs (1 epoch every min) were analysed by fast Fourier transformation with a frequency resolution of 0.35 Hz (software by Enrico Staderini, Italy).

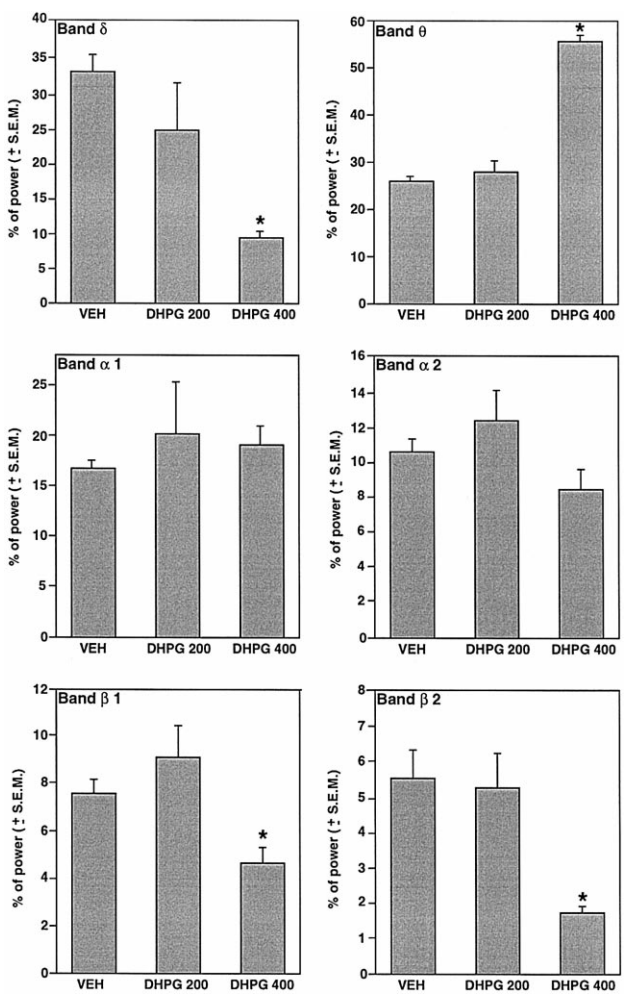

Fig. 1. EEG effects (relative power distribution) induced by intra-accumbens infusion of the selective group I mGlu receptor agonist DHPG in rats. Bars represent the means ± S.E.M. from four (DHPG 400) or six (vehicle and DHPG 200) experiments. Doses are expressed as nmoles. Half of the total dose was infused in each side. * $P < 0.05$ vs. vehicle (distilled water), according to one-way ANOVA followed by Dunnett's test.

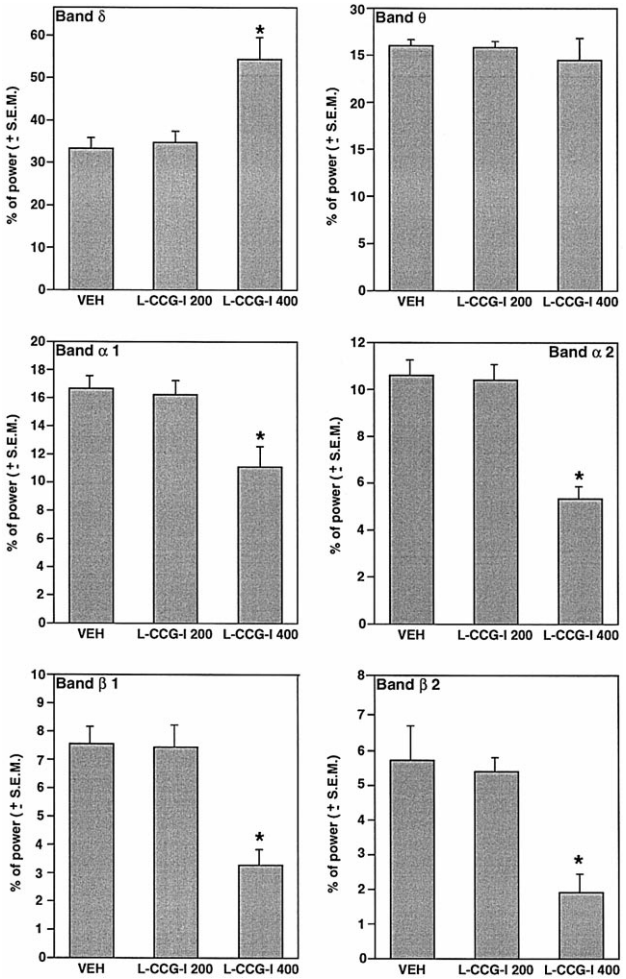

Fig. 2. EEG effects (relative power distribution) induced by intra-accumbens infusion of the selective group II mGlu receptor agonist L-CCG-I in rats. Bars represent the means ± S.E.M. from six experiments. Doses are expressed as nmoles. Half of the total dose was infused in each side. * $P < 0.05$ vs. vehicle (distilled water), according to one-way ANOVA followed by Dunnett's test.

All the power spectra relevant to an EEG tracing were recorded on a optical disk (940 MB, RPS) and then analysed to calculate the relative power in each frequency band. The following bands were considered (Kropf and Kuschinsky, 1993; Popoli et al., 1997): 1.2–4 Hz (δ), 4.35–7 Hz (θ), 7.35–9.5 Hz (α_1), 9.85–12.5 Hz (α_2), 12.85–16 Hz (β_1), 16.35–30 Hz (β_2).

Gross behaviour was continuously observed during EEG recording.

One-way analysis of variance (ANOVA) followed by Dunnett's test were used for the statistical analysis of the results.

2.4. Drugs

All compounds were obtained from Tocris Cookson (Bristol, UK). DHPG and L-CCG-I were dissolved in distilled water. L-AP4 was dissolved in phosphate-buffered saline. Each solution was freshly prepared immediately before infusion.

3. Results

3.1. Effects of the group I mGlu receptor agonist DHPG

Nine to twenty minutes (mean 14 ± 3) after the i.a. injection of 400 nmoles DHPG, the EEG tracing showed a predominant rhythm of about 7 Hz, as revealed by the spectral analysis that showed a significant peak (> 50% of total power) within the θ band (Fig. 1). A reduction of power was observed in the remaining bands, except for α_1 (which includes most of the 7–8 Hz range). Behaviourally, these EEG effects were paralleled by the appearance of limbic seizures very similar to those reported by Tizzano et al. (1995a,b) in mice (clonic forelimb contractions,

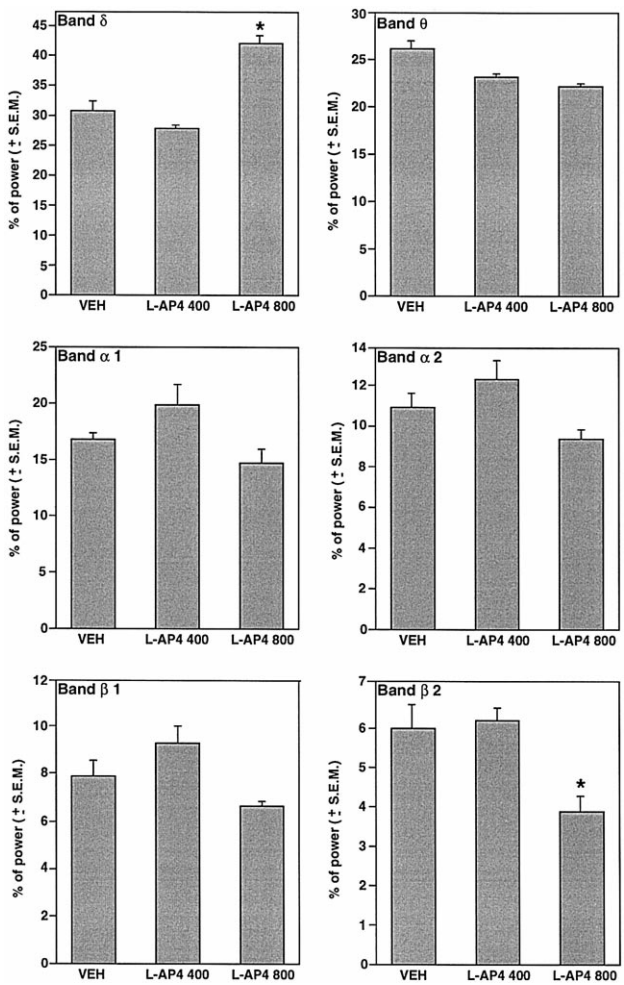

Fig. 3. EEG effects (relative power distribution) induced by intra-accumbens infusion of the selective group III mGlu receptor agonist L-AP4 in rats. Bars represent the means ± S.E.M. from six experiments. Doses are expressed as nmoles. Half of the total dose was infused in each side. * $P < 0.05$ vs. vehicle (phosphate buffered saline), according to one-way ANOVA followed by Dunnett's test.

praying stance, loss of balance). Both the EEG and behavioural effects lasted 2.5–3 h. In the observation period that followed the animals seemed to recover completely, but for ethical reasons only four rats, instead of the six originally planned, were treated with this dose of DHPG.

No remarkable EEG or behavioural effects were observed after the injection of DHPG 200 nmoles.

3.2. Effects of the group II mGlu receptor agonist L-CCG-I

The i.a. injection of 400 nmoles L-CCG-I induced the appearance of slow waves in the EEG tracing. As shown in Fig. 2, the spectral analysis revealed a significantly increased power in the slowest band (δ) and a significantly reduced power in higher frequency bands. Behaviourally, spontaneous motor activity was nearly abolished, while sporadic episodes of stereotyped grooming and chewing were observed.

The lower dose of L-CCG-I did not induce noticeable EEG or behavioural effects.

3.3. Effects of the group III mGlu receptor agonist L-AP4

After the injection of L-AP4 800 nmoles, slow waves appeared in the EEG tracing. This feature was sporadically replaced by brief episodes of EEG activation characterised by the appearance of low-voltage, high-frequency waves. Fig. 3 shows that power significantly increased in band δ and significantly decreased in band β_2. The animals' spontaneous motility was reduced with interposed episodes of grooming and behavioural arousal.

At the dose of 400 nmoles L-AP4, there were only brief periods of EEG arousal (occurrence of low-voltage, high-frequency waves), with no significant changes in the spectral analysis. The animals showed episodes of grooming, chewing, yawning, head-weaving, tremors and ear twitching.

4. Discussion

The present results show for the first time that the i.a. injection of selective group I, II and III mGlu receptor agonists induces significant EEG effects in rats. The selective group I mGlu receptor agonist DHPG induced behavioural limbic seizures paralleled by a dramatic increase in EEG power in the θ band. Pure θ rhythm is typically recorded from limbic structures (Alonso and Llinás, 1989), and in the frontal cortex of the rat this frequency band only accounts for approximately 25% of the total power. The observation that DHPG markedly increases the percentage of power lying in the θ band, together with the appearance of limbic seizures, indicates that group I mGlu receptor stimulation in the nucleus accumbens strongly activates limbic structures. The finding that DHPG had convulsant effects agrees with the recent report by Ghauri et al. (1996), who showed that intracerebroventricular (i.c.v.) DHPG dose dependently induced seizures in DBA/2 mice. The proconvulsant action of group I mGlu receptors is also supported by the finding that the selective antagonist (*S*)-4-carboxyphenylglycine, (*S*)-4CPG, inhibits both audiogenic and chemically induced seizures in mice (Dalby and Thomsen, 1996).

The i.a. administration of the selective group II mGlu receptor agonist L-CCG-I (400 nmoles) induced behavioural depression. The appearance of slow waves in the EEG tracing and the significant modification of the relative frequency distribution (i.e., increased power in the δ band and reduced power in the β band) are also considered to reflect 'depressant' effects (Popoli et al., 1996a,b). These findings are apparently at odds with the report by Tizzano et al. (1995b), who showed that intrathalamic L-CCG-I induced limbic seizures similar to those elicited by 1*S*,3*R*-ACPD in mice. However, these convulsant effects of L-CCG-I were observed only at doses (micromoles) much higher than those used in the present investigation. Conversely, when administered i.c.v. at doses of 400–500 nmoles, L-CCG-I antagonizes both audiogenic and ACPD-induced seizures in mice (Tizzano et al., 1995b; Dalby and Thomsen, 1996). Moreover, our finding of depressant effects elicited by i.a. injection of a selective group II mGlu receptor agonist is in full agreement with recent in vitro studies showing that L-CCG-I depresses synaptic transmission in amygdala neurons (Holmes et al., 1996; Neugebauer et al., 1997) and inhibits excitatory synaptic responses in nucleus accumbens slices (Manzoni et al., 1997).

The observation that the stimulation of group II mGlu receptors elicited opposite effects with respect to group I mGlu receptor stimulation may account for the conflicting results obtained with the nonselective groups I–II mGlu receptor agonist 1*S*,3*R*-ACPD. Although convulsant effects have been reported after central (Tizzano et al., 1995a,b) or peripheral (McDonald et al., 1993) administration of 1*S*,3*R*-ACPD in mice, the drug shows significant protective effects against both sound-induced seizures in DBA/2 mice and β carboline-induced convulsions in non-epileptic mice (Dalby and Thomsen, 1996). In rats, 1*S*,3*R*-ACPD has been reported either to possess cataleptogenic effects after i.c.v. administration (Kronthaler and Schmidt, 1996) or to stimulate locomotor activity after i.a. injection (Attarian and Amalric, 1997; Kim and Vezina, 1997).

At the dose of 800 nmoles, the selective group III mGlu receptor agonist L-AP4 induced 'depressant' EEG and behavioural effects. This finding agrees with previous reports showing that L-AP4 depresses synaptic transmission in both control and kindled amygdala neurons (Neugebauer et al., 1997) as well as in nucleus accumbens slices (Manzoni et al., 1997). The observation that—with respect to L-CCG-I—higher doses of L-AP4 were needed

to induce EEG depression is also consistent with the rank order of agonist potency reported in the above studies. In in vivo investigations, centrally administered L-AP4 has been reported to protect mice against DHPG-induced seizures (Tizzano et al., 1995b) and to inhibit epileptogenesis in amygdala-kindled rats (Abdul-Ghani et al., 1997). It should also be noted, however, that the occurrence of convulsant effects has been reported after i.c.v. injection of group III mGlu receptor agonists both in normal and epilepsy-prone rats (Ghauri et al., 1996). Our finding of some subconvulsant effects (tremors, ear twitchings) after the injection of 400 nmoles L-AP4 further complicates the understanding of the role of group III mGlu receptors in epileptogenesis. In order to clarify the function of these receptors, further studies should be aimed at evaluating the effect of different doses of selective ligands in different brain areas.

In conclusion, pronounced EEG effects occurred after i.a. injection of selective mGlu receptor agonists. While group I mGlu receptor stimulation induced EEG and behavioural seizures, depressant EEG and behavioural effects were observed after the stimulation of group II, and—at certain doses—of group III mGlu receptors. These results provide in vivo evidence of the functional importance of mGlu receptors located in the nucleus accumbens.

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