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Short communication

Class I mGlu receptor antagonist 1-aminoindan-1,5-dicarboxylic acid blocks contextual but not cue conditioning in rats

Karin Sandager Nielsen, Euan M. Macphail, Gernot Riedel *

Department of Psychology, University of York, Heslington, York YO1 5DD, UK

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Abstract

It is widely believed that metabotropic glutamate (mGlu) receptors play a potential role in memory formation. However, the particular function of different classes of mGluRs, or even subtypes, remains elusive. We show here that intraperitoneal injection of the class I selective antagonist 1-aminoindan-1,5-dicarboxylic acid (AIDA) in concentrations of 0.18 or 1.8 mg/kg 25 min prior to acquisition training blocks hippocampus-dependent contextual, but not hippocampus-independent cue, conditioning in rats. These data provide the first evidence for a specific role of mGlu receptors, class I in particular, in hippocampus-dependent learning tasks.

Keywords: Metabotropic glutamate (mGlu) receptor; Contextual conditioning; AIDA (1-aminoindan-1,5-dicarboxylic acid)

1. Introduction

Metabotropic glutamate (mGlu) receptors are a family of currently 8 members ($mGlu_{1-8}$) of G-protein-coupled receptors, which have been grouped based on their amino acid sequence similarity, transduction mechanism and pharmacological profile against selective agonists (for review, see Pin and Duvoisin, 1995). Currently, three classes of mGlu receptors are distinguished; class I ($mGlu_1$ and $mGlu_5$) is coupled via phospholipase C to inositide hydrolysis, class II ($mGlu_2$ and $mGlu_3$) and class III ($mGlu_{4,6-8}$) are negatively linked to the formation of cyclic AMP.

It has been shown by means of the broad antagonist MCPG (*R,S*-α-methyl-4-carboxyphenylglycine) that activation of mGlu receptors is a prerequisite for the expression of late phases of long-term potentiation (for review, see Riedel and Reymann, 1996). More recently, behavioural investigations have supported the notion that mGlu receptors also play important roles in some forms of learning (for review, see Riedel, 1996; Riedel et al., 1996). Pharmacological application of various antagonists and agonists induces memory deficits in spatial, but not in non-spatial, paradigms (Riedel et al., 1995) suggesting a potential role in hippocampus-dependent learning processes. However, compounds which have been tested previously have been neither class nor subtype-specific and it still remains to be investigated whether learning and memory deficits are due to the selective blockade of one particular class/subtype of mGlu receptors, or due to a combined block of several classes/subtypes.

This question concerning a differential involvement of the different mGlu receptor classes has been addressed by means of administration of AIDA, 1-aminoindan-1,5-dicarboxylic acid, a compound recently synthesised by Pellicciari et al. (1995). It has been found to be a selective antagonist for $mGlu_1$ expressed in baby hamster kidney cells with an IC_{50} value of 214 μM (Costantino and Pellicciari, 1996), but is devoid of any action on class II and III mGluRs. AIDA, therefore, is much more potent than MCPG which has an IC_{50} value of 700 μM (Annoura et al., 1996).

We therefore designed behavioural experiments that compare contextual conditioning, which is disrupted by hippocampal lesions (Jarrard, 1995), and cue conditioning to a tone, which is not.

2. Materials and methods

Experimentally naive male hooded Lister rats (320–350 g), purchased from a local dealer, were housed in pairs with free access to water and food in temperature-con-

* Corresponding author. Present address: Centre for Neuroscience, University of Edinburgh, Crichton Street, Edinburgh EH8 9LE, UK. Tel.: (44-131) 650-4569; Fax: (44-131) 650-4579.

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trolled holding rooms ($21 \pm 2°C$) under a 12/12 h light/dark regime.

Training for aversive classical conditioning to context and tone was performed in a temperature-controlled testing room in a conditioning chamber (Campden Instruments; 25 cm L × 21 cm D × 19 cm H) which was placed in a sound-attenuated chamber, but the front door remained open to allow video observation during the session. The box was equipped with a grid floor (stainless steel) through which shocks were passed at a fixed intensity (0.25 mA). A house light (3 W) in the ceiling illuminated the box during the session and an overhead speaker was used to deliver the conditioned stimulus (CS, tone = 10 kHz, 80 dB). A fan provided a continuous background noise of 65 dB.

Contextual versus cue conditioning was performed using a paradigm based on that described by Phillips and Ledoux (1992). Briefly, rats were transferred to the conditioning box on day 1 and habituated for 20 min. They were then removed and returned to their home cages. Since pilot studies suggested that the first presentation of the tone leads to considerable amounts of freezing, we performed a session to reduce unconditioned suppression on day 2. Rats were placed in the conditioning box and allowed to move freely for 140 s. Then, a tone was presented for 20 s without any consequence. Rats remained in the test box for a further 30 s and then returned to their home cages. Training on days 3–5 consisted of 2 trials per day (inter-trial interval 60 s). After 2 min of adaptation, a period of 20 s prior to the CS (pre-CS = context) was video-recorded, followed by a 20 s CS (tone) and a 500 ms foot shock (US). After the second trial, rats remained in the conditioning chamber for further 30 s. Freezing (complete absence of movements except respiratory activity) was continuously scored for both pre-CS and CS periods. The behaviour recorded by video camera was scored for freezing by two experimenters independently; scores were averaged when the difference was smaller than 2 s; if the difference was greater, the amount of freezing was rescored by both experimenters until data were in line with this criterion. Only freezing during trial 1 was taken into account since freezing during trial 2 is confounded by the lingering effects of the US presented moments earlier during trial 1.

Previous work suggests that changes in activity due to drug injection might interfere with aversive conditioning in that an increase in overall activity could easily explain reduced amounts of freezing during pre-CS and CS periods (Good and Honey, 1997). Therefore, we also monitored the activity of the animals for 2 min on day 3 prior to onset of trial 1. Lateral and vertical movements were recorded as the rat's snout crossing lines bisecting upper and lower, and right and left halves of the front opening of the conditioning chamber, respectively.

AIDA (Tocris Cookson) was dissolved in equimolar NaOH and further diluted with saline (0.9%). Two different concentrations adjusted to pH 7 were used: 1.8 mg/kg ($n = 5$), 0.18 mg/kg ($n = 5$); saline injection served as control ($n = 5$). AIDA/saline was injected intraperitoneally (i.p., 0.5 ml per 100 g) 20–25 min prior to conditioning on days 3–5. If 100% of the AIDA entered and was uniformly distributed in the brain (presumed brain volume of a 300 g rat 2 ml), a dose of 0.18 mg/kg would result in a final concentration of 100–150 μM which is below the IC_{50} of 214 μM.

3. Results

The results of contextual versus cue conditioning are summarised in Fig. 1. Animals treated with AIDA showed no gross motor abnormalities and normal righting reflexes. Whereas controls increased the amounts of freezing during trial 1 of the context periods (20 s pre-CS) over days 4 and 5 (Fig. 1A), AIDA, 0.18 mg/kg and 1.8 mg/kg, treated rats did not. Analysis of variance (ANOVA) with repeated measures confirmed a significant main effect of group ($F(2,12) = 7.1$, $P < 0.01$) and a group by day interaction ($F(4,24) = 3.35$, $P < 0.05$). Performance in cue conditioning (Fig. 1B), however, was similar in all groups. Al-

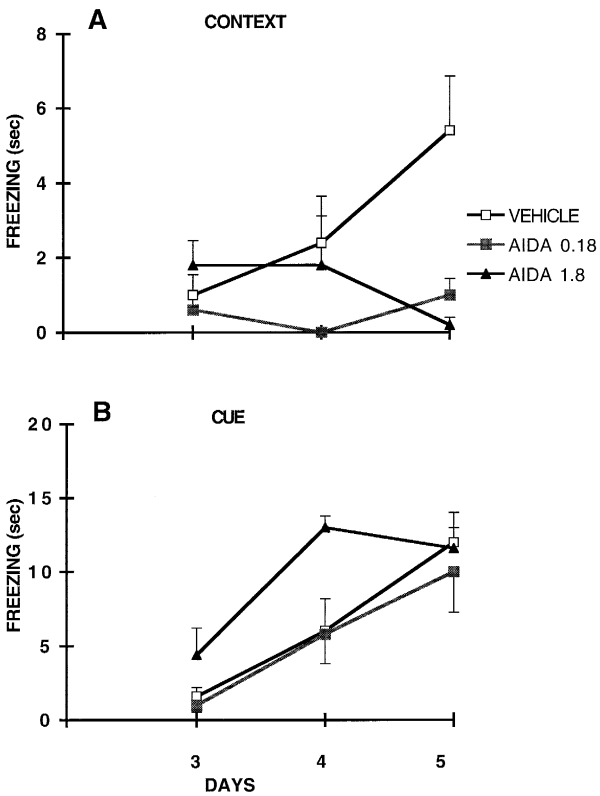

Fig. 1. AIDA blocks contextual, but not cue, conditioning. (A) Freezing (s) to context is recorded as a measure for memory on three consecutive days; only trial 1 is shown. AIDA (0.18 and 1.8 mg/kg) treated rats did not improve performance over days compared with vehicle-injected controls. Means ± S.E.M. (B) Freezing to tone (cue) was not different between groups. Means ± S.E.M.

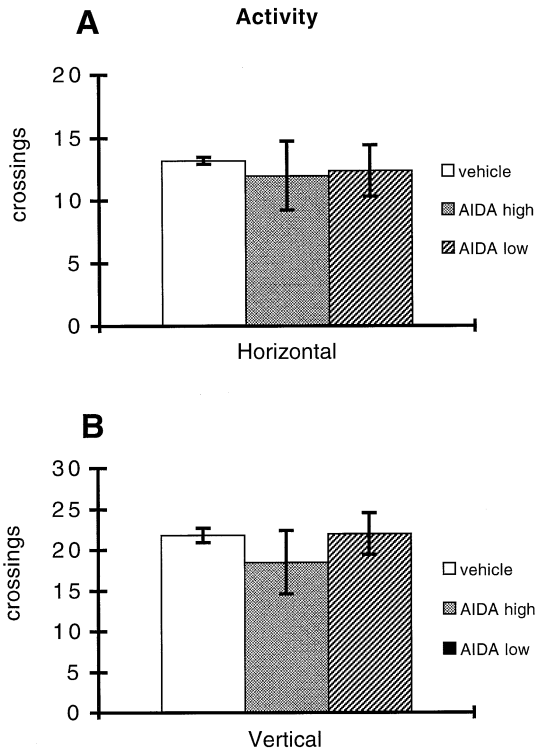

Fig. 2. Overall activity is not altered by administration of AIDA. Activity was monitored as snout crossings over lines bisecting the box into upper and lower (A, horizontal crossings) as well as right and left halves (B, vertical crossings). Two minutes on the first acquisition day prior to the first trial were recorded. Means ± S.E.M.

though animals of the AIDA 1.8 mg/kg group showed slightly higher amounts of freezing on days 3 and 4, ANOVA revealed no significant effect of group ($F(2,12) = 2.8$, $P < 0.05$) and no group by day interaction ($F(4,24) = 2.3$; $P < 0.05$).

Overall activity (horizontal and vertical snout crossings) on day 3 during 2 min prior to the first trial is shown in Fig. 2. The number of both horizontal (Fig. 2A) and vertical (Fig. 2B) crossings did not differ between groups ($F < 1$ for both horizontal and vertical crossings). Activity was not monitored on subsequent acquisition days because it would be confounded by learning due to previous trials.

4. Discussion

Most previous behavioural studies applying antagonists of mGlu receptors have used MCPG which acts on a wide range of different subtypes. The results have provided compelling evidence that mGlu receptor activation may be a prerequisite for some forms of learning, in particular spatial learning in the water maze (Richter-Levin et al., 1994) and spatial alternation in the Y-maze (Riedel et al., 1994, 1995). Brightness discrimination learning, in contrast, was found not to be affected by mGlu receptor antagonists (Riedel et al., 1995) suggesting that hippocampus-dependent learning is particularly sensitive to mGlu receptor blockade. We demonstrate here that contextual conditioning is abolished in the presence of AIDA, a novel mGlu receptor antagonist, but cue conditioning is not.

Given the specificity of AIDA against class I mGlu receptors (Pellicciari et al., 1995; Costantino and Pellicciari, 1996), our results suggests a potential role of $mGlu_1$ and $mGlu_5$ receptors in hippocampus-dependent forms of learning. Similar deficits in contextual, but not cue, conditioning have been reported for hippocampal lesions (Phillips and Ledoux, 1992). We therefore suggest that the learning/memory deficits observed in this study may be due to selective blockade of class I mGlu receptors in the hippocampal formation.

Others have suggested that an impairment in the performance in contextual conditioning might occur because of an increase in the overall activity of hippocampal animals (Good and Honey, 1997). Several lines of evidence, however, suggest that AIDA-induced side effects could not account for the memory deficit. (1) We found no gross motor disturbances. (2) When monitored as horizontal and vertical crossings, the activity of drug-treated animals was found to be identical to that of saline-injected controls. (3) Although injected with AIDA, animals performed normally in conditioning to the tone (cue), suggesting that the drug neither acts as an anxiolytic nor an analgesic.

In conclusion, our data provide strong evidence for a role of class I mGlu receptors in hippocampus-dependent, but not hippocampus-independent, forms of learning.

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References

Annoura, H., Fukunaga, A., Uesugi, M., Tatsuoka, T., Horikawa, Y., 1996. A novel class of antagonists for metabotropic glutamate receptors, 7-(hydroxyimino)cyclopropa[*b*]chromen-1*a*-carboxylates. Bioorg. Med. Chem. Lett. 6, 763–766.

Costantino, G., Pellicciari, R., 1996. Homology modelling of metabotropic glutamate receptors (mGluRs): structural motifs affecting binding modes and pharmacological profile of mGluR1 agonist and competitive antagonists. J. Med. Chem. 39, 3998–4006.

Good, M., Honey, R.C., 1997. Dissociable behavioral effects of selective lesions to hippocampal sub-systems. Behav. Neurosci. (in press).

Jarrard, L.E., 1995. What does the hippocampus really do?. Behav. Brain Res. 71, 1–10.

Pellicciari, R., Luneia, R., Costantino, G., Marinozzi, M., Natalini, B., Jakobsen, P., Kanstrup, A., Lombardi, G., Moroni, F., Thomsen, C., 1995. 1-Aminoindan-1,5-dicarboxylic acid – a novel antagonist at phospholipase C-linked metabotropic glutamate receptors. J. Med. Chem. 38, 3717–3719.

Phillips, R.G., Ledoux, J.E., 1992. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav. Neurosci. 106, 274–285.

Pin, J.P., Duvoisin, R., 1995. The metabotropic glutamate receptors: structure and functions. Neuropharmacology 34, 1–26.

Richter-Levin, G., Errington, M.L., Maegawa, H., Bliss, T.V.P., 1994. Activation of metabotropic glutamate receptors is necessary for longterm potentiation in the dentate gyrus and for spatial learning. Neuropharmacology 33, 853–857.

Riedel, G., 1996. Function of metabotropic glutamate receptors (mGluRs) in learning and memory. Trends Neurosci. 19, 219–224.

Riedel, G., Reymann, K.G., 1996. Metabotropic glutamate receptors in long-term potentiation and learning and memory. Acta Physiol. Scand. 157, 1–19.

Riedel, G., Wetzel, W., Reymann, K.G., 1994. (*R*,*S*)-α-Methyl-4-carboxyphenylglycine (MCPG) blocks spatial learning in rats and LTP in the dentate gyrus in vivo. Neurosci. Lett. 167, 141–144.

Riedel, G., Wetzel, W., Reymann, K.G., 1995. Metabotropic glutamate receptors in spatial and nonspatial learning in rats studied by means of agonist and antagonist application. Learning Memory 2, 243–265.

Riedel, G., Wetzel, W., Reymann, K.G., 1996. Comparing the role of metabotropic glutamate receptors in long-term potentiation and in learning and memory. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 20, 761–789.