Altered cannabinoid CB₁ receptor mRNA expression in mesencephalon from mice exposed to repeated methamphetamine and methanandamide treatments

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Abstract

OBJECTIVES: Since among others also our previous studies suggested an interaction between the endocannabinoid system and methamphetamine brain mechanisms we focused on possible changes in relative expression of cannabinoid CB₁ receptor mRNA in mesencephalon from mice sensitized by repeated treatments to methamphetamine stimulatory effects and cross-sensitized by cannabinoid CB₁ receptor agonist methanandamide pre-treatment.

METHODS: The Open Field Test was used to measure changes in terms of behavioural sensitization or cross-sensitization to drug effects on locomotion in male mice treated repeatedly with either methamphetamine or methamphetamine after pre-treatment with methanandamide. After each measurement one third of animals were sacrificed and the brain was stored. RNA was isolated from the midbrain and used for reverse transcription and subsequent real-time PCR.

RESULTS AND CONCLUSION: The evaluation of behavioural drug effects showed both development of sensitization to methamphetamine stimulatory effects after repeated treatment and cross-sensitization to them by pre-treatment with cannabinoid receptor CB₁ agonist methanandamide. Real-time PCR analyses revealed an increase in CB₁ receptor mRNA expression after the first dose of methanandamide followed by decrease after the combined treatment with methamphetamine challenge dose. Our findings suggest that particularly repeated pre-treatment with CB₁ agonist methanandamide can elicit increase in the mRNA expression level at least in the mouse mesencephalon neurons associated with cross-sensitization to methamphetamine stimulatory effects.
INTRODUCTION

Repeated administration of various psychotropic drugs can elicit behavioural sensitization – a phenomenon characterised by gradually increasing response to the drug (Robinson & Berridge 1993). This phenomenon has been well described for majority of addictive substances including amphetamines (Kameda et al. 2011) and cannabinoids (Rubino et al. 2003). An increased response to the tested drug may be also elicited by previous repeated administration of a drug different from the drug tested, which is termed as cross-sensitization. Cross-sensitization was observed, for example, after repeated treatment with tetrahydrocannabinol to heroin (Singh et al. 2005).

It has been identified, that the crucial neuronal circuits essential for the development of sensitization involve namely dopaminergic, glutamatergic, GABAergic and serotonergic projections between VTA, nucleus accumbens, prefrontal cortex, hippocampus and amygdala (Ago et al. 2008). Particularly, the mesolimbic dopaminergic projection from the VTA to nucleus accumbens is considered as the most important for effects associated with reward properties of abused drugs (Kalivas et al. 1993). Stimulation of cannabinoid CB₁ receptors present on GABAergic and glutamatergic nerve terminals negatively regulates the release of GABA and glutamate and that way influence the mesolimbic DA functions (Chiang & Chen 2007). The endocannabinoid system consists of cannabinoid receptors (CB₁, CB₂), their endogenous ligands (endo-cannabinoids), and enzymes for their biosynthesis and degradation. It is known, that CB₁ receptors located in VTA on presynaptic glutamatergic and GABAergic neurons act as retrograde inhibiting modulators and influence their input to VTA dopaminergic neurons which is believed to activate the reward pathway of addictive substances (Maldonado et al. 2006).

The first results from our laboratory suggesting an interaction between the endocannabinoid system and methamphetamine brain mechanisms were obtained in the rat I.V. drug self-administration model (Vinklerova et al. 2002). Later we have created an original experimental paradigm showing development of behavioural sensitization to psychostimulant methamphetamine effects and also cross-sensitization elicited by cannabinoid CB₁ receptor agonist methanandamide pretreatment (Landa et al. 2006a;b) confirming that there exists some relationship between the endocannabinoid system and methamphetamine effect processing.

The present study was designed with respect to results obtained in our previous behavioural studies as well as in the preliminary pilot studies focusing on CB₁ receptor expression (Landa & Jurajda 2007a;b) and density (Sulcova et al. 2007) in rodent mesencephalon, and to data confirming that structures responsible for the development of behavioural sensitization to psycho-stimulants (including methamphetamine) are parts of mesencephalon (namely VTA) with high CB₁ receptor density (Ago et al. 2008). The attention was focused on possible changes revealed by quantitative polymerase chain reaction (qPCR) in relative expression of CB₁ receptor mRNA in mouse mesencephalon during a) sensitization to methamphetamine and b) cross-sensitization to methamphetamine induced by repeated pretreatment with CB₁ receptor agonist methanandamide.

MATERIAL AND METHODS

Animals

Male mice (strain ICR, TOP-VELAZ s. r. o., Prague, Czech Republic) with an initial weight of 18–21 g were used. They were randomly allocated into two treatment groups. Experimental sessions in the behavioural part of the experiment were always performed in the same light period between 1:00 p.m. and 3:00 p.m. in order to minimise possible variability due to circadian rhythms.

Apparatus

Locomotor activity was measured using an open-field equipped with Actitrack (Panlab, S.L., Spain). This device consists of two square-shaped frames that deliver beams of infrared rays into the space inside the square. A plastic box is placed in this square to act as an open-field arena (base 30 × 30 cm, height 20 cm), in which the animal can move freely. The apparatus software records locomotor activity of the animal by registering the beam interruptions caused by movements of the body. Using this equipment we have determined the Distance Travelled (trajectory in cm per 3 minutes).

Drugs

Vehicle and all drugs were always given in a volume adequate to drug solutions (10 ml/kg).

(+)Methamphetamine, (d-N,a-Dimethylphenyl-ethylamine; d-Desoxyephedrine), (Sigma Chemical Co.) dissolved in saline.

(R)-(+)Methanandamide, (R)-N-(2-hydroxy-1-methylethyl)-5Z, 8Z, 11Z-eicosotetraenamide) supplied pre-dissolved in anhydrous ethanol 5 mg/ml (Tocris Cookson Ltd., UK) was diluted in saline to the concentration giving the chosen dose to be administered to animals in a volume of 10 ml/kg; vehicle therefore contained an adequate part of ethanol (a final concen-
Methamphetamine and methanandamide treatments alter cannabinoid CB₁ receptor mRNA expression

Procedure

Mice were randomly divided into 2 groups (n₁=24, n₂=24) and all were given vehicle on Day 1 (10 ml/kg). There were no applications from Days 2 to 6. For the next seven days animals were daily treated intraperitoneally as follows: a) n₁: methamphetamine at the dose of 2.5 mg/kg/day, b) n₂: methanandamide at the dose of 0.5 mg/kg. On Day 14 all animals were given intraperitoneally methamphetamine at the dose of 2.5 mg/kg (challenge dose).

Changes in locomotion were measured for the period of 3 minutes in the open field on Days 1 (1st record), 7 (2nd record) and 14 (3rd record) 15 minutes after drug application to assess sensitizing phenomenon. After each measurement one third of both groups was decapitated (75 minutes after drug administration) and the brain was stored in RNAlater (Ambion). For RNA isolation we used excised mesencephalon only. The total RNA was isolated by means of RNAEasy Mini Kit (Qiagen) and the subsequent reverse transcription was performed with Omniscript RT Kit (Qiagen) and RNase OUT Ribonuclease Inhibitor (Invitrogen). Relative expression of CB₁ receptor (assay Mn00432621_s, Life Technologies) was compared to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA (assay Mn99999915_1g, Life Technologies) using real time cycler ABI SDS 7000 (AppliedBiosystems). All real time PCR reactions were performed using TaqMan Gene Expression Master Mix (Life Technologies).

Data analysis

As the data was not normally distributed (according to the Kolmogorov-Smirnov test of normality), non-parametric statistics were used: Mann-Whitney U test, two-tailed (statistical analysis package STATISTICA – StatSoft, Inc., Tulsa, USA).

RESULTS

In the behavioural part of the study (Figure 1), the treatments in the group n₁ caused significant increase (p<0.01) in locomotion after the 1st application of methamphetamine (M) compared to the application of vehicle (V₁) (see Figure 1; V₁ versus M). The challenge dose of M produced a significant increase in Distance Travelled (p<0.05) in animals pre-treated repeatedly with M when compared to the animals after the 1st application of M (see Figure 1; M versus M/M).

The 1st applications of methanandamide (CAN) compared to the application of vehicle (V₂) evoked in the group n₂ significant decrease (p<0.01) in locomotion (see Figure 1, V₂ versus CAN). The challenge dose of M produced a significant increase in Distance Travelled (p<0.01) in animals pre-treated repeatedly with甲amphetamine.
CAN when compared to the animals after the 1st application of CAN (see Figure 1; CAN versus CAN/M).

Real-time PCR results showed no significant changes after various treatments in the group n1 (see Figure 2; V1 and M versus M/M). The treatments in the group n2 caused significant increase ($p<0.01$) in relative expression of CB1 receptor mRNA after the 1st application of CAN compared to the application of vehicle (V2) (see Figure 2; V2 versus CAN). The challenge dose of M produced a significant decrease in relative expression of CB1 receptor mRNA ($p<0.05$) in animals pre-treated repeatedly with CAN when compared to the animals after the 1st application of CAN (see Figure 2; CAN versus CAN/M).

There was no significant change in relative expression of CB1 receptor mRNA between animals after the MET challenge dose (those were pre-treated with MET) and animals after the MET challenge dose (those were pre-treated with CAN) – see Figure 2; M/M versus CAN/M.

**DISCUSSION**

The behavioural part of this study confirmed both development of sensitization to methamphetamine stimulatory effects on mouse locomotor behaviour during its repeated administration and cross-sensitization to such effects caused by pre-treatment with cannabinoid CB1 receptor agonist methanandamide prior to methamphetamine challenge dose administration. Both these findings are in accordance with our previous experimental experiences (Landa et al. 2006a,b) as well as suggestions of some others (e.g.: Cadoni et. al. 2001; Wolf et al. 2002; Tanda & Goldberg 2003; Chiang & Chen 2007; Wiskerke et al. 2008; Panlilio et al. 2010).

Neurobiological mechanisms underlying phenomenon of behavioural cross-sensitization are believed to increase vulnerability for use of other drugs of abuse (Steketee & Kalivas 2011). In the case of psychostimulants (including methamphetamine) and cannabinoids it is believed that they induce increase in dopamine activation in the mesolimbic reward pathway. The stimulation of specific cannabinoid CB1 receptor relieves suppression upon dopaminergic neurons, leading to dopamine release and thus facilitates responses to administration of psychostimulants. However, all outcomes of studies oriented towards involvement of CB1 receptor in effects of amphetamines have not been consistent (e.g.: Ellgren et al. 2004; Solinas et al. 2007; Thiemann et al. 2008; Panlilio et al. 2010).

The part of the present study dealing with relationship between cannabinoid CB1 receptor agonist methanandamide and methamphetamine effects on the level of CB1 receptor mRNA expression brought rather controversial results, too. Neither single nor repeated methamphetamine dose of 2.5 mg/kg caused significant increase in relative expression of CB1 receptor mRNA in the mouse mesencephalon (just a trend to stimulation
of expression was registered after the repeated treatment. Increased CB1 receptor expression across rat brain regions including medial prefrontal cortex, striatum, amygdaloid complex and hippocampal formation was reported after the exposure to methamphetamine treatment, however, with the dosing regimen (4 mg/kg, subcutaneously × 4 injections, 2 h apart), inducing neurotoxic effects (Bortolato et al. 2010).

On the contrary, there was measured a decrease in numbers of CB1 receptor (both Bmax and mRNA) in the mouse nucleus accumbens after the repeated chronic methamphetamine administration (4 mg/kg/day) developing behavioural sensitization while microinjection of CB1 antagonist into the nucleus accumbens suppressed the behavioural sensitization to methamphetamine (Chiang & Chen 2007). The activation of the CB1 receptor was evaluated as a cause facilitating adaptive responses to psychostimulants, such as reduction of dopamine and serotonin turnovers resulting in sensitization (Thieman et al. 2008). However, the density of cannabinoid CB1 receptor mRNA-positive neurons was significantly lower in Cannabis sativa users (Villares 2007).

Thus the mechanisms that regulate CB1 receptor modifications are far from being completely understood. Moreover adaptations vary by brain region (Sim-Selley 2003) and the results of studies dealing with CB1 receptor density are dependent also on the method used (e.g. receptor binding, mRNA expression, immunofluorescence). There is also evidence that internalization of CB1 receptors following agonist treatment can occur (Coutts et al. 2001). This could be a reason for discrepant results we have obtained in the present study using PCR evaluation of the relative expression of CB1 mRNA comparing to another one with immunofluorescence detection of receptors the intensity of which was assayed by image analysis (Sulcova et al. 2007). The latter one showed on the surface of VTA neuronal membranes in rats sensitized to methamphetamine I.V. self-administration decreased density of cannabinoid CB1 receptors while in the present study a trend to the increase in expression of CB1 receptors in methamphetamine sensitized mice was found.

In spite that the increased expression of CB1 receptor was associated in the present study with methanadamide cross-sensitization to methamphetamine effects on mouse locomotion, there was measured after the drug challenge dose significantly lower expression of CB1 receptor but significantly higher than under the influence of vehicle treatment and with no difference from the level in mice pretreated repeatedly with methamphetamine. Nevertheless, increased expression of CB1 receptor in mesencephalon was associated with higher sensitivity to methamphetamine psychostimulatory effects.

This is in agreement with findings that CB1 knockout mice as well as wild type mice pre-treated with CB1 receptor inverse agonist AM 251 were less sensitive to the psychomotor stimulant as well as locomotor sensitizing effects of amphetamine (Thieman 2008), and to some extent are also consistent with our earlier study (Vinklerova et al. 2002) in which self-administration of methamphetamine was reduced by AM 251, and increased by methanadamide.

In conclusion, the results of the present study brought further evidence that modulation of CB1 receptor expression may play an important role in behavioural responses to methamphetamine. Pharmacological support of CB1 receptor activity may increase expression of CB1 receptor mRNA associated with sensitization to methamphetamine stimulatory effects what supports the hypothesis on increased vulnerability to methamphetamine abuse after neuroplastic changes induced by cannabinoid CB1 receptor agonists including delta-9-tetrahydrocannabinol from marijuana.

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