European Journal of Pharmacology 725 (2014) 55–63

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and analgesia

Lipopolysaccharide increases degradation of central monoamines: An *in vivo* microdialysis study in the nucleus accumbens and medial prefrontal cortex of mice

Floor van Heesch [a,*], Jolanda Prins [a], Jan Pieter Konsman [b], Gerdien A.H. Korte-Bouws [a], Koen G.C. Westphal [a], Joanna Rybka [a], Berend Olivier [a], Aletta D. Kraneveld [a], S. Mechiel Korte [a]

[a] Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Faculty of Science, Universiteitsweg 99, 3584 CG Utrecht, The Netherlands
[b] Psychoneuroimmmunology, Nutrition and Genetics, Victor Segalen Bordeaux 2 University, Bordeaux, France

ARTICLE INFO

Article history:
Received 24 September 2013
Received in revised form
24 December 2013
Accepted 8 January 2014
Available online 17 January 2014

Keywords:
Lipopolysaccharide
Serotonin transporter
Dopamine transporter
Norepinephrine transporter
Triple reuptake inhibitor
Microdialysis

ABSTRACT

Peripheral administration of lipopolysaccharide (LPS) in rodents induces anhedonia, i.e. the inability to experience pleasure. Recently, we reported that serotonin transporter (SERT) function is required for LPS-induced anhedonia. Less is known about the effect of LPS on the biological activity of dopamine transporters (DAT) and norepinephrine transporters (NET). Therefore, *in vivo* microdialysis was performed in the nucleus accumbens and medial prefrontal cortex of C57BL6/J mice exposed to saline or LPS (133 μg/kg i.p.). To investigate the possible involvement of different monoamine transporters, the triple reuptake inhibitor DOV 216,303 or saline was i.p. injected 30 min before the saline/LPS injection. The dose of LPS, shown to decrease responding for brain stimulation reward in mice, significantly increased extracellular levels of monoamine metabolites (5-HIAA, DOPAC and HVA) in the nucleus accumbens and medial prefrontal cortex. Remarkably, DOV 216,303 abolished LPS-induced DOPAC and HVA formation in the nucleus accumbens, suggesting that LPS increases DAT activity in this brain area. DOV 216,303 also inhibited LPS-induced DOPAC and HVA formation in the medial prefrontal cortex. Since DAT density is very low in this brain structure, reuptake of DA predominantly takes place via NET, suggesting that LPS increases DAT and NET activity in the medial prefrontal cortex. Furthermore, DOV 216,303 pretreatment prevented LPS-induced 5-HIAA formation only in the medial prefrontal cortex, indicating that LPS increases prefrontal SERT activity. In conclusion, the present findings suggest that peripheral LPS increases DAT activity in the nucleus accumbens and increases NET and SERT activity in the medial prefrontal cortex of mice.

1. Introduction

There is a growing body of evidence that inflammation plays an important role in the development of depression (Beumer et al., 2012; Dantzer et al., 2008; Konsman et al., 2002; Miller et al., 2013). For example, patients with different chronic inflammatory disorders have increased levels of serum proinflammatory cytokines (Komatsu et al., 2001; Tetta et al., 1990) and an increased risk to become depressed (Akay et al., 2002; Hauser et al., 2011; Isik et al., 2007; Loftus et al., 2011). Moreover, it has been demonstrated that treatment with interleukin 2 (IL-2) or interferon-α (IFN-α) highly increases the risk to develop depression in humans (Capuron et al., 2004; Heinze et al., 2010; Renault et al., 1987).

A core symptom of major depression is anhedonia, i.e. the inability to experience pleasure. In rodents, anhedonia is reflected by reduced responding for brain stimulation reward in the intracranial self-stimulation (ICSS) procedure (Kenny et al., 2003). Although only 0.025% of peripherally injected lipopolysaccharide (LPS) reaches the mouse brain (Banks and Robinson, 2010), peripheral LPS is known to increase both brain proinflammatory cytokines (Datta and Opp, 2008; Konsman et al., 2008) and anhedonia (Barr et al., 2003; Borowski et al., 1998; van Heesch et al., 2013a). The underlying mechanisms, however, are largely unknown.

The serotonin transporter (SERT), dopamine transporter (DAT) and norepinephrine transporter (NET) critically regulate the duration of cellular actions of serotonin (5-HT), dopamine (DA) and norepinephrine (NE), respectively. These monoamines and its

* Correspondence to: Utrecht University, Faculty of Science, Department of Pharmaceutical Sciences, Division of Pharmacology, Universiteitsweg 99, 3584 CG Utrecht, The Netherlands. Tel.: +31 6 202 74 192; fax: +31 30 253 7900.
E-mail address: F.vanHeesch@uu.nl (F. van Heesch).

0014-2999/$ - see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejphar.2014.01.014

transporters have been shown to play an important role in major depression (Albert et al., 2012; Prins et al., 2011a). Recently, it has been shown that LPS and proinflammatory cytokines increase SERT activity (Mossner et al., 1998; Zhu et al., 2006; Zhu et al., 2010) and that LPS-induced anhedonia is abolished in SERT knockout rats (van Heesch et al., 2013a), suggesting that increased SERT function is needed to provoke LPS-induced anhedonia. Whether LPS also increases DAT and NET activity is still under debate.

Both the medial prefrontal cortex and nucleus accumbens are innervated by monoaminergic neurons and play an important role in the pathophysiology of major depression. The medial prefrontal cortex is known for its role in overall cognitive functioning and suppression of negative affect (Robbins and Arnsten, 2009), whereas the nucleus accumbens, i.e. the reward center of the brain, has been hypothesized to be crucial for the development of anhedonia (Nestler and Carlezon, 2006). This makes the nucleus accumbens and medial prefrontal cortex interesting brain areas to analyze LPS-induced alterations in extracellular levels of monoamines, monoamine metabolites and monoamine transporter function.

Two microdialysis experiments were performed to investigate whether and how LPS affects monoamine transporter function in the nucleus accumbens and medial prefrontal cortex of mice. In the first study, the effects of LPS on DA, DOPAC and HVA levels (metabolites of DA) and on 5-HT and 5-HIAA levels (metabolite of 5-HT) were measured. In the second study, it was investigated whether blockade of DAT, SERT and NET by the triple reuptake inhibitor DOV 216,303 could prevent the LPS-induced effects on extracellular monoamine and monoamine metabolite levels in the nucleus accumbens and medial prefrontal cortex.

2. Material and methods

2.1. Animals

Forty-six male C57BL/6 J mice (Charles River, Maastricht) arrived at the age of 9–10 weeks and were socially housed, eight to ten mice per cage on a 12 h light/dark cycle with lights on at 6:00am and off at 6:00pm. Food and water were available ad libitum. Mice had one week to acclimate to their new environment. Both studies were conducted according to the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 and were approved by the Ethical Committee for Animal Research of Utrecht University, The Netherlands. During the experiments all efforts were made to minimize animal pain, distress and discomfort.

2.2. Microdialysis

2.2.1. Microdialysis surgery

Mice were anesthetized by inhalation of a mixture of isoflurane gas (2%) and oxygen and placed in a stereotaxic instrument. The microdialysis study comprised of two consecutive experiments. In the first microdialysis study mice were pretreated with saline. In half of the saline pretreated animals, cuprofane microdialysis probes (MAB 4.6.1 CU, 1 mm membrane length) were implanted in the nucleus accumbens ($n=14$), whereas in the other half of the saline pretreated animals probes (MAB 4.6.2. CU, 2 mm membrane length) were implanted in the medial prefrontal cortex ($n=16$). In the other microdialysis study mice were pretreated with the triple reuptake inhibitor DOV 216,303. In this experiment two probes per mouse were implanted; one probe in the nucleus accumbens, the other in the medial prefrontal cortex ($n=16$). The coordinates of the nucleus accumbens and medial prefrontal cortex were anterioposterior +1.5 mm; mediolateral +0.8 mm (under a 0° angle) from bregma; dorsoventral −5.0 mm from skull surface and anterioposterior +1.9 mm; mediolateral +0.9 mm (under a 8° angle) from bregma; dorsoventral −3.3 mm from skull surface, respectively (Paxinos and Franklin, 2001). Probes were anchored with nonacrylic dental cement on the scull. After implantation of the microdialysis probes, mice were housed individually and placed in the microdialysis room until the end of the experiment.

2.2.2. Pretreatment: DOV 216,303

The triple reuptake inhibitor DOV 216,303 [(±)-1-(3,4-dichlorophenyl)-3-azabicyclo-[3.1.0]hexane hydrochloride] (Sepracor Inc., Marlborough, USA) was dissolved in saline and prepared freshly on test days (0.5 mg/ml). DOV 216,303 was administered intraperitoneally (i.p.), 5 mg/kg in a volume of 10 ml/kg, 30 min before exposure to saline or LPS. This dose was based on observations described earlier in mice (Caldarone et al., 2010). Control animals received i.p. injections of saline in a volume of 10 ml/kg.

2.2.3. Treatment: LPS

Escherichia coli derived lipopolysaccharide (LPS) (Sigma, 0127: B8) was dissolved in saline and prepared freshly on test days from the stock solution (0.5 mg/kg dissolved in saline, stored at −80 °C). To induce a cytokine response, 133.33 μg/kg LPS was administered i.p. in a volume of 10 ml/kg. This dose is known to induce anhedonia in C57BL/6J mice as measured in an ICSS paradigm 1 h and 4 h after exposure to LPS (van Heesch et al., 2012). Control animals received i.p. injections of saline in a volume of 10 ml/kg.

2.2.4. Microdialysis study

The microdialysis studies were performed in conscious freely moving mice, one day after implantation of the microdialysis probes. A pump (KdScientific Pump 220 series, USA) perfused the system with Ringer solution (147 mM nucleus accumbensl, 2.3 mM KCl, 2.3 mM $CaCl_2$ and 1 mM $MgCl_2$) at a constant flow rate of 0.02 ml/h. During microdialysis, the flow rate was set at 0.07 ml/h. At 8:00 am mice were connected to a channel swivel (type 375/D/22QM) which allowed them to move freely. Three hours after connection, 30-minute samples were manually collected in vials containing 11.7 μl of 0.1 M acetic acid and frozen at −80 °C until analysis with HPLC. From 11:00 am until 1:00 pm four baseline samples were collected. Subsequently, animals in the saline pretreatment microdialysis study were i.p. injected with saline (nucleus accumbens: $n=14$; medial prefrontal cortex: $n=16$), whereas animals in the DOV 216,303 pretreatment microdialysis study were i.p. injected with 5 mg/kg DOV 216,303 (nucleus accumbens and medial prefrontal cortex: $n=16$). Thirty minutes later, half of the animals in each pretreatment group received an i.p. saline injection, whereas the other half of each group received an i.p. LPS injection (nucleus accumbens saline pretreatment: $n=7$, medial prefrontal cortex saline pretreatment: $n=8$ and nucleus accumbens and medial prefrontal cortex DOV 216,303 pretreatment: $n=8$, respectively). After the last injection samples were collected for an additional 4 h. In each microdialysis study 13 30-minute samples were collected in total. Immediately after collection of the last microdialysis sample animals were sacrificed. The brains were dissected and stored in formaldehyde to verify probe localization later on.

2.2.5. HPLC

Microdialysis samples were stored at −80 °C until analysis. Neurotransmitters, dopamine (DA) and serotonin (5-HT) and their metabolites, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) respectively