Pharmacological Research xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Pharmacological Research

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

Indole and indoxyl sulfate, gut bacteria metabolites of tryptophan, change arterial blood pressure via peripheral and central mechanisms in rats

Tomasz Huć, Artur Nowinski, Adrian Drapala, Piotr Konopelski, Marcin Ufnal*

Department of Experimental Physiology and Pathophysiology, Laboratory of the Centre for Preclinical Research, Medical University of Warsaw, Warsaw, Poland

ARTICLE INFO

Article history:
Received 31 August 2017
Received in revised form
21 December 2017
Accepted 22 December 2017
Available online xxx

Names of chemical compounds studied in the article:
indole (1H-indole)
indoxyl sulfate (potassium 1H-indol-3-yl sulfate)
pizotifen
ondansetron
cis-(Z)-flupenthixol dihydrochloride

Keywords:
Indole
Gut bacteria
Metabolites
Arterial blood pressure
Serotonin receptors

ABSTRACT

Arterial blood pressure (BP) is regulated by a complex network of peripheral and central (brain) mechanisms. Research suggests that gut bacteria-derived compounds may affect the circulatory system. We evaluated hemodynamic effects of indole, a gut bacteria-derived product of tryptophan, and indoxyl sulfate (indoxyl), a liver metabolite of indole.

BP and heart rate (HR) were recorded in anesthetized, male, Wistar rats at baseline and after the administration of either a vehicle, indole, or indoxyl into the femoral vein (IV) or into the lateral ventricle of the brain (ICV). Besides, we evaluated the effect of pretreatment with flupentixol, a non-selective D_1, D_2, α_1 and 5 HT_{2A} receptor blocker; pizotifen, a non-selective 5-HT_1, 5-HT_{2A} and 5HT_{2C} receptor blocker; and ondansetron, a 5-HT_3 blocker, on hemodynamic responses to indole and indoxyl.

Vehicle infused IV and ICV did not affect hemodynamics. Indole administered IV produced a dose-dependent increase in BP but not HR. In contrast, the ICV infusion of indole produced a decrease in BP and HR. Indoxyl infused IV produced an increase in BP and HR, whereas indoxyl infused ICV did not affect BP and HR. The hemodynamic effects of indole and indoxyl were inhibited by pretreatment with ondansetron and pizotifen but not flupentixol.

In conclusion, indole and indoxyl sulfate affect arterial blood pressure via peripheral and central mechanisms dependent on serotonin signalling. We propose that indole and indoxyl sulfate may be mediators in the interaction between gut bacteria and the circulatory system.

1. Introduction

Arterial blood pressure (BP) is regulated by a complex network of peripheral and central (brain) mechanisms to provide effective blood perfusion of tissues [1,2]. Accumulating evidence suggests that gut bacteria-derived molecules may be involved in BP control [3–5] Gut bacteria metabolites may affect the circulatory system homeostasis via a few pathways. First, they may enter the circulation and as blood-borne molecules influence the functioning of vasculature, the heart or glands involved in BP regulation [6,7]. Second, blood-borne molecules may target the circumventricular organs (CVOs) that are located in the walls of the brain ventricular system [8]. The CVOs lack the blood-brain barrier and serve as an interface between the brain and the periphery, integrating hormonal and nervous regulations of animal body systems, including the circulatory system. Finally, small and lipophilic molecules may cross the blood-brain barrier and affect the deep brain centers controlling BP via the autonomic nervous system [1,8].

Indole is a gut bacteria-produced molecule, formed in intestines from tryptophan, an essential amino acid and a precursor for several pivotal mediators including tryptamine, serotonin, melatonin, kynurenines, and nicotinic acid [9,10]. The majority of indole is transformed by the colonic epithelium and the liver into indoxyl sulfate (indoxyl). Both indole and indoxyl sulfate were suggested to exert biological effects and to be involved in cardiorenal syndrome [11–14]. However, to the best of our knowledge, the effects

Abbreviations: BP, arterial blood pressure; b.w., body weight; CVOs, circumventricular organs; ICV, intracerebroventricular; Indoxyl, indoxyl sulfate; IV, administered into the femoral vein; MABP, mean arterial blood pressure; HR, heart rate; OND, ondansetron; PIZ, pizotifen; FLU, flupentixol.

* Corresponding author at: Medical University of Warsaw, Banacha 1B, 02-097 Warsaw, Poland.
E-mail address: mufnal@wum.edu.pl (M. Ufnal).

https://doi.org/10.1016/j.phrs.2017.12.025
1043-6618/

of indole and indoxyl on arterial blood pressure have not yet been reported. Therefore, in this study we evaluated hemodynamic effects of indole and indoxyl in rats.

2. Methods

2.1. Animals

The experiments were performed according to Directive 2010/63 EU on the protection of animals used for scientific purposes and approved by the Local Bioethical Committee. Rats were housed in groups (3–4) in polypropylene cages with environmental enrichment, 12 h light/12 h dark cycle, temperature 22–23 °C, humidity 45–55%.

Measurements were performed on 14-week-old, male (n = 137), Wistar rats, (Mossakowski Medical Research Center Polish Academy of Sciences, Warsaw, Poland) fed a standard laboratory diet *ad libitum*. The measurements were performed under general anesthesia with urethane at a dose of 1.5 g/kg of body weight (b.w.). Before the measurements rats were implanted with a polyurethane arterial catheter which was inserted through the femoral artery into the abdominal aorta and connected to the BP recording system. For intravenous treatment, a catheter was implanted into the femoral vein.

2.2. Hemodynamic effects of indole and indoxyl sulfate administered intravenously

The measurements were started 40 min after the induction of anesthesia, and 10 min after connecting the arterial and venous catheters. Hemodynamics were recorded for 20 min at baseline and for 120 min after the IV administration of either (1) a vehicle (0.5 ml of 20% DMSO) (n = 7) or (2) indole at a dose of 0.0085 mmol/kg/b.w. (1 mg/kg/b.w.) (n = 6), 0.085 mmol/kg/b.w. (10 mg/kg/b.w.) (n = 6), 0.5 mmol/kg/b.w. (60 mg/kg/b.w.) (n = 6) or (3) indoxyl at a dose of 0.25 mmol/kg/b.w. (60 mg/kg/b.w.) (n = 7), 0.5 mmol/kg/b.w. (120 mg/kg/b.w.) (n = 6).

In separate series of experiments rats were IV infused with either indole at a dose of 0.5 mmol/kg/b.w. or indoxyl at a dose of 0.5 mmol/kg/b.w. after the pre-treatment with either (1) flupentixol, a non-selective D_1,D_2, α_1 and 5 HT_{2A} receptor blocker at a dose of 0.3 mg/kg/b.w., or (2) pizotifen, a non-selective 5-HT_1, 5-HT_{2A} and 5HT_{2C} receptor blocker at a dose of 0.5 mg/kg/b.w., or (3) ondansetron, a 5-HT_3 blocker at a dose of 1 mg/kg/b.w. Specifically, the following experimental series were performed: the administration of indole after the pre-treated with either (1) flupentixol (n = 7), (2) pizotifen (n = 7), or (3) ondansetron (n = 6), and the administration of indoxyl after the pre-treatment with either (1) flupentixol (n = 6), (2) pizotifen (n = 5), or (3) ondansetron (n = 6).

In addition, hemodynamic effects of flupentixol (n = 4), pizotifen (n = 4), and ondansetron (n = 4) administered alone were evaluated.

2.3. Hemodynamic effects of indole and indoxyl administered into the cerebroventricular system

Rats were implanted with a polyurethane arterial catheter under general anesthesia as described above. Next, the rats were implanted with a stainless steel cannula (ID 0.7 mm × OD 0.9 mm) that was inserted into the lateral ventricle of the brain 1.2 mm posterior to the bregma and 1.8 mm laterally to the midsagittal suture. The tip of the cannula was located 4.0 mm below the skull surface and secured with a dental cement. The intracerebroventricular infusions (ICV) were performed by means of a stainless steel infusion tube (ID 0.5 mm × OD 0.6 mm) which was inserted into the previously implanted cannula.

The measurements were started 60 min after the induction of anesthesia, and 10 min after connecting the arterial catheter and the ICV infusion tube. Hemodynamics were recorded for 20 min at baseline and for 90 min after the ICV administration of either (1) a vehicle (0.01 ml of 20% DMSO) (n = 4) or (2) indole at a dose of 0.001 mmol/kg/b.w. (0.125 mg/kg/b.w.) (n = 5), 0.002 mmol/kg/b.w. (0.25 mg/kg/b.w.) (n = 6), 0.004 mmol/kg/b.w. (0.5 mg/kg/b.w.) (n = 7) or (3) indoxyl at a dose of 0.004 mmol/kg/b.w. (1 mg/kg/b.w.) (n = 6).

In separate series of experiments rats were ICV infused with indole at a dose of 0.004 mmol/kg/b.w. after the pre-pretreatment with either (1) flupentixol, at a dose of 0.1 mg/kg/b.w., (n = 6), or (2) pizotifen at a dose of 0.06 mg/kg/b.w., (n = 5), or (3) ondansetron at a dose of (0.04 mg/kg/b.w.) (n = 5). In addition, the hemodynamic effects of flupentixol (n = 4), pizotifen (n = 4), and ondansetron (n = 4) ICV administered alone were evaluated.

Urethane, indole, indoxyl sulfate, DMSO, flupentixol, pizotifen and ondansetron were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.4. Data analysis and statistics

Mean arterial blood pressure (MABP) and heart rate (HR) were calculated on the BP tracing by AcqKnowledge 4.3.1 Biopac software (Biopac Systems, Goleta, USA).

For the evaluation of MABP and HR response to the treatment, the average over 5 min baseline was compared with the averages over 5 min for 90-min (ICV infusions) or 120- min (IV infusions) period after the treatment by means of one-way analysis of variance (ANOVA) for repeated measures. Differences between the experimental series were evaluated by multivariate ANOVA, followed by Tukey's post hoc test or *t*-test, when appropriate. The Kolmogorov-Smirnov test was used to test normality of the distribution. A value of two-sided $p < 0.05$ was considered significant. Analyses were conducted using Dell Statistica, version 13 (Dell Inc, Tulsa, USA).

3. Results

3.1. Hemodynamic effects of indole and indoxyl sulfate administered intravenously

At baseline, there was no significant difference between the experimental series in MABP and HR, (Table 1).

3.1.1. Indole

The administration of the vehicle did not affect hemodynamics. Indole at a dose of 0.0085 mmol/kg/b.w. ($F_{5,50} = 2.72$, $p < 0.05$), 0.085 mmol/kg/b.w. ($F_{5,50} = 3.62$, $p < 0.05$) and 0.5 mmol/kg/b.w. ($F_{5,50} = 9.89$, $p < 0.05$) produced a significant increase in MABP (Fig. 1A). A significant difference between the series in MABP changes was found ($F_{3,20} = 6.50$, $p < 0.05$, analyzed from 15 to 30 min after infusions). The changes in MABP were accompanied by not significant changes in HR in all the series (Fig. 1B).

Hemodynamic effects of indole were inhibited by the pretreatment with ondansetron and pizotifen but not flupentixol. Namely, rats infused with indole after the pretreatment with flupentixol showed a significant increase in MABP ($F_{5,50} = 4.55$, $p < 0.05$), (Fig. 2C). In contrast, rats pretreated with ondansetron and pizotifen showed no significant hemodynamic response to indole, (Fig. 2A and B).

3.1.2. Indoxyl

Indoxyl at a dose of 0.25 mmol/kg/b.w. produced a transient significant increase in MABP ($F_{5,40} = 2.43$, $p < 0.05$). Rats infused with indoxyl at a dose of 0.5 mmol/kg/b.w. showed a two-phase significant increase in MABP [from 15 to 30 min ($F_{7,56} = 15.23$, $p < 0.05$),

Please cite this article in press as: T. Huć, et al., Indole and indoxyl sulfate, gut bacteria metabolites of tryptophan, change arterial blood pressure via peripheral and central mechanisms in rats, Pharmacol Res (2017), https://doi.org/10.1016/j.phrs.2017.12.025