Asymmetric dimethylarginine may mediate increased heat pain threshold in experimental chronic kidney disease

Jan T. Kielstein1,*, Mayuren Suntharalingam1,*, Ronny Perthel1, Song Rong1, Jens Martens-Lobenhoffer2, Kristin Jäger3, Stefanie M. Bode-Böger2 and Heike Nave3

1Department of Internal Medicine, Division of Nephrology and Hypertension, Medical School Hannover, Hannover, Germany, 2Institute for Clinical Pharmacology, Otto-von-Guericke University, Magdeburg, Germany and 3Department of Anatomy and Cell Biology, Faculty of Medicine, Martin Luther University Halle-Wittenberg, Halle, Germany

Correspondence and offprint requests to: Jan T. Kielstein; E-mail: kielstein@yahoo.com

*J.T.K. and M.S. contributed equally to the manuscript and are both considered first authors.

Abstract

Background. Thermal sensitivity in uraemia is decreased. Non-selective synthetic nitric oxide synthase (NOS) inhibitors significantly attenuate thermal hyperalgesia in preclinical models. The aim of our study was to evaluate the effect of experimental uraemia, which is associated with an increase of the endogenous NOS inhibitor asymmetric dimethylarginine (ADMA), on thermal sensitivity in rats. Furthermore, we intended to study the effect of chronic ADMA infusion alone on thermal sensitivity.

Methods. Male Sprague–Dawley rats (n = 54), 10 weeks old, weight 370–430 g, were randomly assigned to three groups receiving either (i) isotonic saline or (ii) ADMA via osmotic mini pumps or (iii) underwent 5/6 nephrectomy (Nx). After 14 days, 50% of all animals from all groups underwent thermal sensitivity testing and terminal blood draw. After 28 days, the remaining animals underwent the same procedures. Thermal sensitivity examination was performed by the hot-plate test, measuring time from heat exposition to first paw licking or jumping of the animal.

Results. While the median [interquartile range] latency time between heat exposition to first paw licking or jumping of the animal in the NaCl infusion group remained unchanged between Day 14 (8.4 [6.75–11.50] s) and Day 28 (7.35 [6.10–7.90] s) both, ADMA infusion and 5/6 nephrectomy tended to increase the thermal pain threshold at Day 14 (9.25 [6.55–12.18] s) and (9.50 [5.8 ± 11.0] s), respectively, compared to NaCl on Day 14 (8.4 [6.75–11.50] s). This difference became statistically significant at Day 28 where the median latency time in the ADMA group (13.10 [11.85–15.95] s) and in the 5/6 Nx group (13.50 [10.85–17.55] s) were significantly higher than in the NaCl group (7.35 [6.10–7.90] s).

Conclusions. Induction of progressive renal failure in rats by 5/6 nephrectomy, which is accompanied by a marked increase of the serum levels of the endogenous NOS inhibitor ADMA, leads to a significantly increased heat pain threshold at 28 days. The sole infusion of ADMA into healthy rats leads to the same increase in heat pain threshold.

Keywords: ADMA; brain function; nociception; SDMA; uraemia

Introduction

Humans exhibit a highly variable sensitivity to pain, including distinct responses to identical injuries. Although patients with chronic kidney disease (CKD) frequently complain about pain adversely affecting their quality of life [1], the possible contribution of impaired renal function has only been poorly studied. Up to two thirds of patients with CKD report chronic pain [2], yet it has been known for a long period of time that the thermal sensitivity in uraemia is decreased [3], a finding that was confirmed recently [4]. It suggests that, among others, unmyelinated C fibres are affected in uraemia. The exact mechanism for these clinical findings is, however, only scantily studied. It is known that the release of excitatory amino acids leads to activation of the glutamatergic N-methyl-D-aspartate receptor. One essential downstream effect of its activation is the increase of neuronal nitric oxide synthase (NOS). The subsequent production of NO has some pro-analgesic properties. Accordingly, L-NG-nitro arginine methyl ester (L-NAME), a non-selective synthetic NOS inhibitor, but not L-NIO (a selective endothelial NOS inhibitor), significantly attenuated thermal hyperalgesia in mice [5]. In a different preclinical model of pain modulation, it was conformingly shown that the synthetic NOS inhibitor L-NAME significantly enhanced the pain threshold and potentiated morphine-induced analgesia [6]. The role of the endogenous NOS inhibitor asymmetric dimethylarginine (ADMA) in this process is, however, unclear although it has been speculated that ADMA might be involved in the modulation of pain perception [7]. Interestingly,
Cardounel and Zweier have shown that the intrinsic neuronal ADMA concentration is sufficient to profoundly inhibit neuronal NOS function [8]. As the naturally occurring NOS inhibitor ADMA is highly elevated in CKD patients [9], we aimed to elucidate its potential role in the modulation of pain in CKD by comparing the long-term (4 weeks) effect of ADMA infusion on thermal sensitivity in healthy animals to the effects of 5/6 nephrectomy (Nx) using a hot-plate test which characterizes the phenomenon of pain perception at thalamic level.

Materials and methods

Animals

All applicable institutional and governmental regulations concerning the ethical use of animals were followed during this research. The research and animal care procedures were approved by the Lower Saxony district government (Hannover, Germany) and followed the guidelines set by the European Communities Council Directive of 24 November 1986.

Male Sprague–Dawley rats (n = 54), 10 weeks of age, weighing 370–430 g, were obtained from Charles River GmbH, Sulzfeld, Germany. The rats were held in cages of up to five rats for the first 3 days covered with standard cage bedding. Consecutively, they were individually placed in plastic cages bedded in cellulose in an air-conditioned facility under a 14-h light–10-h dark cycle (lights on at 07:00 a.m.) at an ambient temperature of 24°C. Standard carbohydrate chow (50% carbohydrate, 19% protein, 12% water, 4% fat and 2.1 kcal/g; Altromin, Lage, Germany) and water were available ad libitum.

Experimental design

Fifty-four rats were randomly divided into six groups. On Day 1, Alzet-pumps (model 2ML4 with a flow rate of 2.5 μL/h) (Durect Corporation, Cupertino, CA) were subcutaneously implanted under inhalation anaesthesia (isoflurane). Groups 1 and 2 received 0.9% NaCl, Groups 3 and 4 received 250 μmol/kg/day ADMA (Alexis Biochemicals, Lausen, Switzerland) which was dispensed in 0.9% NaCl and subsequently sterile filtered. The hot-plate test was performed on Day 14 in Groups 1 and 3 and on Day 28 in Groups 2 and 4. Rats in Groups 5 and 6 underwent 5/6 nephrectomy (Nx) as previously described [10]. In brief, we performed selective ligation of the extrarenal renal artery branches of the left renal artery under ketamine and xylazine anaesthesia to obtain a two-thirds renal infarction. The opposite kidney was then removed. In Groups 5 and 6, the hot-plate test was also performed after 14 and 28 days, respectively.

Hot-plate test

The hot-plate test (model no 1440-E–34; Coulbourn Instrument, Columbus, OH) is used to measure nociception-reflexes on medullar and brain level [11]. The rat was placed on the plate, heated to a temperature of 51 ± 1°C. The time to elicit paw licking or jumping was measured and the animal was removed from the hot-plate immediately. The cut-off time was 30 s to prevent tissue damage. All hot-plate experiments were conducted in the light cycle (08:00–12:00 a.m.)

Results

Both, ADMA infusion as well as 5/6 nephrectomy, lead to a significant elevation of ADMA levels as compared to NaCl infusion (Table 1). ADMA infusion yielded higher ADMA levels than 5/6 nephrectomy (Table 1). Both, ADMA infusion and 5/6 nephrectomy lead to a comparable increase in pain threshold (Figure 1), which was significant after Day 28 as compared to Day 14 in the same group or compared to Days 14 and 28 in the NaCl group (Figure 1). The infusion of 0.9% NaCl had no effect on pain threshold.

Renal function in the 5/6 nephrectomy group was severely impaired as judged by the increase in SDMA, creatinine and urea as compared to both, the NaCl-treated animals as well as the ADMA infusion animals (Table 1). As expected, animals that underwent 5/6 nephrectomy exhibited a weight loss as compared to the NaCl and ADMA infused animals at Days 14 and 28 (Table 1).

Discussion

The pertinent findings of our study were that (i) uraemia due to 5/6 nephrectomy increases the heat perception threshold in rats and (ii) chronic ADMA administration induces a rise in the heat-perception threshold in otherwise healthy rats.

CKD and heat perception

Impaired renal function in the 5/6 nephrectomy group could be confirmed by an increase of SDMA, a new marker

<table>
<thead>
<tr>
<th></th>
<th>NaCl (0.9%)a</th>
<th>ADMA infusiona</th>
<th>5/6 Nephrectomya</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 14</td>
<td>Day 28</td>
<td>Day 14</td>
</tr>
<tr>
<td>ADMA (μmol/L)</td>
<td>0.65 (0.60–0.71)</td>
<td>0.57 (0.52–0.60)</td>
<td>1.33* (0.95–1.52)</td>
</tr>
<tr>
<td>SDMA (μmol/L)</td>
<td>0.24 (0.22–0.25)</td>
<td>0.30 (0.25–0.38)</td>
<td>0.28 (0.25–0.39)</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>36.7 (34.2–39.1)</td>
<td>37.4 (35.0–39.6)</td>
<td>35.0 (33.0–37.7)</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>424 (404–442)</td>
<td>464 (449–476)</td>
<td>432 (400–444)</td>
</tr>
</tbody>
</table>

*Data are presented as median (interquartile range).

P < 0.05 versus NaCl Day 14 and NaCl Day 28 in analysis of variance confirmed by Tukey’s multiple comparison test.
of acute and chronic impairment in renal function in humans [14–16] and experimental animals (as recently reviewed in [17]) as well as an increase in established markers or renal function, i.e. creatinine and urea. As the duration of the Alzet pump is limited to 28 days, our study was limited to 4 weeks to avoid a second surgical procedure with anaesthesia even though the full (histological) picture of CKD usually occurs after 6–8 weeks. Our chronic renal failure model was associated with an increase of pain threshold using the hot-plate test. This is in line with the scarcity of studies examining thermal sensation in CKD patients (non-diabetic haemodialysis patients) where warm detection was shown to be abnormal in 13% [4] and 30% [3]. Interestingly, impaired thermal discrimination was the first sign of neuropathy in 15% of the patients. In their study, there was no significant correlation between measures of thermal sensation and measures of large fibre dysfunction, indicating that a small-fibre uraemic neuropathy may exist as a separate entity [3]. It is important to point out that due to the lack of established animal models, we did not evaluate cold-detection thresholds, which have been reported to be abnormal in up to 60% of non-diabetic haemodialysis patients [4] Cold sensation is mediated by thin myelinated A delta fibres and heat by unmyelinated C fibres. Therefore, our finding only suggests that unmyelinated axons are affected by both chronic ADMA infusion and 5/6 nephrectomy.

**ADMA and pain**

Using the endogenous nitric oxide (NO) inhibitor ADMA, we found that latency increased significantly by 80%. In the study by Moore et al. [18], the acute single-dose administration of the NO synthase inhibitor L-NAME prolonged the time for foot withdrawal in the hot-plate test by ~80%. However, the authors used a dose of 75 mg/kg L-NAME in an acute setting, while we used ~10 mg/kg/day ADMA for 4 weeks. Moreover, the effect of ADMA in comparison to L-NAME on several physiological parameters has been shown to be about one order of magnitude less potent than that of L-NAME [19]. Several animal studies have unanimously shown that inhibition of NO synthesis can considerably reduce both inflammatory and neuropathic pain, as recently reviewed [20]. While there are no reports on the relationship of the endogenous NOS inhibitor ADMA and pain, the synthetic NOS inhibitor L-NAME has long been known to elicits dose-related and long-lasting antinociception in rodents [18]. Thus, it has been used in various pain models showing an increase in the nociceptive threshold for thermal stimuli (hot-plate) [21]. The precise site of the antinociceptive effect of ADMA in our rat model remains unclear. As ADMA is antinociceptive in the hot-plate test, it is likely that it might act supraspinally. But other levels can of course not be excluded. In that regard, it is of interest that one key component of pain perception, the transient receptor potential vanilloid type 1 (TRPV1) channel, can also be modified by NOS inhibition [16]. One interesting finding that comes to mind is also the fact that ADMA is low in the state of acute inflammation [22]. Whether this would then make patients more susceptible to pain is at this point pure (but tempting) speculation.

Is ADMA a main player in the pathophysiology of pain perception in CKD?

The complex changes of progressive renal failure can never be explained by a single substance or a single pathophysiological pathway vividly illustrates by the multitude of compounds described by the EUTox group [23]. Nevertheless, it is interesting how the alteration of a single parameter in progressive renal failure, i.e. the threshold for thermal pain, can be reproduced by the infusion of a single uraemic toxin, i.e. ADMA. In summary, the present results show that uraemia is associated with a severely altered pain perception and/or response to pain, raising the intriguing possibility that ADMA might be of pathophysiological relevance in this process. Further clinical and preclinical studies have to elucidate this neglected part of uraemia.

**Acknowledgements.** This study is supported by a grant of the Werner Jackstädt Stiftung to J.T.K. and H.N.

**Conflict of interest statement.** Dr Kielstein owns and hosts the website www.adma.com.

**References**


Received for publication: 20.7.11; Accepted in revised form: 26.9.11