Effects of Antihypertensive Agents on Intestinal Contractility in the Spontaneously Hypertensive Rat: Angiotensin Receptor System Downregulation by Losartan

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ABSTRACT

Hypertension is an inflammatory condition controlled by the renin angiotensin system and is linked to kidney disease, diabetes mellitus, and recently to dysfunction of the gut. The aim of this study was to determine what effect antihypertensive drug treatments may have on intestinal function of the spontaneously hypertensive rat (SHR). In the first experiment, SHRs were treated with enalapril, hydralazine, or with no treatment as a control. In the second experiment, SHRs were treated with losartan or with no treatment as a control. All drug treatments led to significant lowering of blood pressure after 16 weeks. At termination, intact tissue sections of the ileum and colon were induced to contract ex vivo by KCl; electrical stimulation; and agonists carbachol, angiotensin II, and prostaglandin E2 (PGE2). There were no differences in ileal or colonic contractility due to hydralazine or enalapril compared with no-treatment SHR control. However, for the ileum, the losartan group responded significantly more to KCl and carbachol while responding less to angiotensin II, with no difference for PGE2 compared with the no-treatment SHR control. In contrast, the colon responded similarly to KCl, electrical stimulation, and PGE2 but responded significantly less to angiotensin II. These results demonstrate that the ileum responds differently (with KCl and carbachol as agonists) to the colon after losartan treatment, whereas there is a reduced contractile response in both the ileum and colon following losartan treatment. Although there are few well documented major contraindications for angiotensin receptor blockers, the modulation of gut contractility by losartan may have wider implications for bowel health.

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ABBREVIATIONS: ACE, angiotensin-converting enzyme; Ang, angiotensin; ARB, angiotensin receptor blocker; AT1, angiotensin type 1; BP, blood pressure; PGE2, prostaglandin E2; RAS, renin-angiotensin system; SHR, spontaneously hypertensive rat.
for muscarinic or peptide-stimulated contraction of gut tissue (Patten et al., 2004, 2005). It is known that hypotension induced by the ACE inhibitor captopril involves a prostaglandin-dependent component (Pontieri et al., 1990). However, it is unknown to what extent the RAS interacts with prostanoid generation and how antihypertensive agents may influence contractility and motility of the gut.

The aim of this study was to examine the effects of long-term treatment with three classes of antihypertensive agents as: an ACE inhibitor, enalapril; an ARB, losartan; and the arterial relaxant hydralazine as a positive control on SHR gut contractility. Separate no-treatment SHRs were used as controls. To do this, we examined the effects of these antihypertensive agents on the ex vivo induced contractility of intact sections of ileum and colon by KCl; electrical stimulation; and muscarinic, prostanoid, and angiotensin receptor–dependent agonists (Patten et al., 2004, 2005, 2015a,b).

We hypothesize that antihypertensive drugs may affect intestinal contractility and motility.

Materials and Methods

Animal Experiments. Forty 12-week-old SHRs were delivered to the CSIRO small-animal facility from the Animal Resource Centre breeding facility in Western Australia (24 animals for experiment 1 and 16 animals for experiment 2). The animals were allowed 2 weeks to acclimatize in a 12-hour light/dark cycle at 22°C, and were ear-tagged and weighed. Animals were caged in groups of no more than five animals in the standard colony wire cages to decrease coprophagy and prevent the ingestion of bedding materials. Environmental enrichment was provided to allow animals to rest away from the wire-based floor. Animals were fed an AIN93M diet (ICN Biomedicals, Irvine, CA) (13.6% protein, 4.0%, fat, 4.7% crude fiber, and 15.1 MJ/kg digestible energy) ad libitum and were monitored daily and weighed weekly. For experiment 1, at 14 weeks of age, three groups of eight animals were randomly assigned on a weight basis to be administered the antihypertensive agents hydralazine (0.012%, w/v) or enalapril maleate (4 mg/kg per day) in the drinking water given ad libitum for 16 weeks or were assigned as no-treatment SHRs as the control group. For experiment 2, at 14 weeks, two other groups of eight animals were randomly assigned on a weight basis to be administered the antihypertensive losartan (10 mg/kg per day) in the drinking water given ad libitum for 16 weeks or were assigned as no-treatment SHRs as the control group. Sixteen weeks of treatment was undertaken to allow the animals to reach full maturity and stable blood pressure in the no-treatment SHR control groups. All drugs and fine chemicals were from Sigma Australia Pty. Ltd. (Sydney, Australia). All experimental protocols were undertaken in accordance with the Australian code for the care and use of animals for scientific purposes (8th edition, 2013, https://www.nhmrc.gov.au/guidelines-publications/ea28) and approved by the CSIRO Animal Ethics Committee and included power calculations.

Blood Pressure Measurements. For experiments 1 and 2, systolic blood pressure was measured in conscious rats from 14 weeks, every 2 weeks for the 16-week treatment phase. Blood pressure (BP) was measured using an indirect tail-cuff method at 30°C in measuring tubes using an electro-sphygmonanometer combined with a pneumatic pulse transducer/amplifier (model 6m22931, six-channel NIBP system, Mediquip Pty Ltd, Loganholme, Qld, Australia) using BpMonWin Monitor version 1.33 software (ITTC Life Science, Woodland Hills, CA). Rats were acclimatized in the measuring tubes twice before BP measurements were commenced.

Measurement of Ileal and Colonic Tissue Contractility Ex Vivo. Methodology for inducing contractility and magnitude measurements for both the ileum and colon have been described in detail previously (Patten et al., 2002, 2015; Bajka et al., 2010). Animals were euthanized via exsanguination while still under anesthesia (60 mg/kg Nembutal, sodium pentobarbitone, Sigma Chem Co, Sydney, Australia). Death of the animal was confirmed by the removal of the heart. In brief, sections of both the terminal ileum (4–5 cm) between 2 and 10 cm from the ileo-caecal junction and proximal colon (3–4 cm) were excised 2 cm from the caecal-colonic junction. Both the colon and ileum were mounted in tandem in organ chambers with a modified Krebs-Henseleit bicarbonate buffer, and the colon was excited by an electric field to induce contraction (Bajka et al., 2010). The gastrointestinal active compounds were then added sequentially to both ileal and colonic baths as follows: KCl (5–35 mM), carbachol (0.03–22 μM), angiotensin II (0.001–10 μM), and PGE2 (0.001–10 μM). Dose-response curves were generated by cumulative additions of a small bolus of KCl or agonist to the incubated tissue in the organ bath until contraction reached a plateau (normally after 3–5 minutes). The solution in the organ bath was washed out when maximal contraction was reached, ready for subsequent testing of contractility. No refractoriness was recorded after KCl, carbachol, angiotensin II, or PGE2 treatments. To assess this, 1 μM carbachol was added at the completion of the experiment, and only tissues with 100% recovery of contractile activity were used for experimental determinations.

Statistical Analysis. Data were summarized as the mean ± S.E.M. Comparisons between two groups were conducted using the unpaired Student’s t test, whereas comparison of groups of three was performed using one-way analysis of variance followed by the Bonferroni multiple comparison test using GraphPad Instat 3.0 (GraphPad Software, La Jolla, CA). Statistical significance was set at P < 0.05.

Results

Blood Pressure. For experiment 1, the initial and final BP for no-treatment SHR control, enalapril, and hydralazine SHR were 212.4 ± 3.7 and 221.5 ± 1.0, 212.3 ± 4.6 and 171.1 ± 1.8, and 203.2 ± 2.2 and 138.2 ± 2.5, respectively. For experiment 2, the initial and final BP for no-treatment SHR control and losartan SHR were 210 ± 3.6 and 220.9 ± 2.2, and 215 ± 3.3 and 186.4 ± 3.4, respectively. All drug treatments led to significantly lower blood pressure in the SHR (P < 0.05) compared with the no-treatment SHR control group. At the doses used, the hydralazine treatment group had a lower final blood pressure than the enalapril and losartan treatment groups (P < 0.05).

Contractility of Ileum and Colon Ex Vivo, Experiment 1. In the ileum, there were no differences in contraction due to KCl for the enalapril and hydralazine groups compared with no-treatment SHR controls (Table 1). For the colon, there were no differences in contraction due to KCl or electrical stimulation for the enalapril- and hydralazine-treated groups compared with no-treatment SHR controls (Table 2). For both the ileum (Fig. 1) and colon (Fig. 2), there were no differences in maximal contractility (mm/g) or sensitivity (EC50) in response to concentration dose curves of carbachol, angiotensin II, and PGE2. There was a depressed response to PGE2 in the ileum and colon compared with what we have found previously for control Wistar Kyoto rats (results not shown) (Patten et al., 2004, 2005).

Contractility of Ileum and Colon Ex Vivo, Experiment 2. For the ileum, the contractility responses to 10 and 40 mM KCl were higher for the losartan-treated group compared with the no-treatment SHR control group (Table 3). For the colon, however, there were no differences in response to KCl or electrical stimulation between the no-treatment SHR control or losartan groups (Table 3). For the ileum, with respect to losartan treatment, there was a higher contractile response to carbachol (Fig. 3A; Table 3) and lower responses to angiotensin II (Fig. 3B; Table 3), which also demonstrated...
lower sensitivity (higher EC50) (Fig. 3B; Table 3), with no difference noted for PGE2 (Fig. 3C; Table 3) compared with the no-treatment SHR control group. For the colon, with respect to losartan treatment, there were no differences in contractile responses to carbachol (Fig. 4A; Table 3) or PGE2 (Fig. 4C; Table 3), with lower responses noted for angiotensin II (Fig. 4B; Table 3) with concomitant lower sensitivities (higher EC50) (Table 3) compared with the no-treatment SHR control group. For experiment 2, there were no differences noted in the mean tissue densities of ilea or colons from the losartan-treated group compared with the no-treatment SHR control group (Table 3).

**Discussion**

The RAS is involved in the development of essential hypertension, which is an inflammatory condition that not only influences the cardiovascular system but dysregulates glucose control and kidney disease and can also influence gut microbiota and the functionality of the gut (Kuba et al., 2013; Yang et al., 2015; Zhu et al., 2016). We examined the effects that three classes of antihypertensive drugs may have on SHR gut function after 16 weeks of treatment. In the first experiment, SHRs were treated with the ACE inhibitor enalapril and the direct-acting smooth muscle arteriole relaxant hydralazine, which resulted in no significant differences in gut contractility in response to all agents tested ex vivo compared with no-treatment SHR controls. In human patients undergoing hypertension treatment, complications to the gut due to ACE inhibitors are uncommon. However, they have been reported to induce intestinal angioedema that may lead to unnecessary invasive procedures, such as exploratory laparotomy (Weingärtner et al., 2009), but to our knowledge, contraindications for gut motility have not been reported. Indeed, in animal models such as the mouse, enalaprilat has been shown to reduce the severity of dextran sulfate sodium–induced colitis and reduce tumour necrosis factor-α levels and epithelial cell apoptosis (Spencer et al., 2007), whereas enalapril was shown to attenuate the upregulation of IκBα phosphorylation and reduced the severity of colitis as assessed by histologic examination (Lee et al., 2014).

In this study, we used hydralazine, a non-nucleoside DNA methyltransferase inhibitor and a potent arterial vasodilator (Knowles et al., 2004), which is approved for the treatment of severe hypertension and heart failure (Graça et al., 2014). Hydralazine is not routinely used as a primary drug for treating human hypertension because it elicits a reflex sympathetic stimulation of the heart (the baroreceptor reflex). The sympathetic stimulation may increase heart rate and cardiac output and, in patients with coronary artery disease, may cause angina pectoris or myocardial infarction. However, in animal models, this class of drug can inhibit gastric emptying in rats and inhibit gastrointestinal propulsion in mice (Chiba et al., 1981), but this dysmotility was not evident for ex vivo intestinal contractility in hydralazine-treated SHRs used in this study.

Losartan is an orally active, nonpeptide AT1 receptor antagonist which provides a more specific and complete blockade of the actions of angiotensin II than renin or ACE inhibitors (Simpson and McClellan, 2000) and is effective in controlling BP and long-term renal damage in hypertensive patients. Although it has been reported that a specific ARB, olmesartan, may interfere with gut immune homeostasis, is known to cause rare cases of sprue-like enteropathy in predisposed individuals, and is associated with an increased risk of hospitalization for intestinal malabsorption and celiac disease (Scioliom et al., 2015; Basson et al., 2016), losartan has been reported to be well tolerated alone and in combination, with only limited reports of gastrointestinal side effects, such as constipation (Weber, 1997; Gokhale et al., 2002), and generally no association with increased risk of cancer for ARBs (Bhaskaran et al., 2012).

In our study with SHRs, losartan treatment led to increased receptor-independent KCl-induced depolarization-driven ileal contractility that was not evident for the colon. There was also an increased ileal contractility after losartan treatment with the muscarinic-mimetic carbachol with no significant change.
in sensitivity (EC50) and no effects noted for the colon. Electrical-stimulated colon also demonstrated no difference in the response to losartan treatment. Although the prostanoid response is depressed in SHRs (Patten et al., 2004, 2005), there was also no change in ileal or colonic response to the prostanoid PGE2. However, losartan treatment led to a large suppression of both ileal and colonic response to angiotensin II. For the ileum, there was a concomitant decrease in sensitivity to angiotensin II. The colon showed decreased sensitivity to angiotensin II. The changes in contractility due to losartan treatment could not be explained by changes in either ileal or colonic tissue density or muscle mass, which were not significantly different compared with no-treatment SHR controls. It is to be determined if other ARBs currently used in clinical settings have similar effects.

These differences in intestinal tissue responsiveness are difficult to explain, so the knowledge gap requires the investigation of the
mechanisms for the losartan-induced changes in intestinal contractility. It is known that, in animal models of disease, transcription and translation of elements of the RAS may be regulated, and that ARBs such as losartan can modulate these effects (Sim and Chen, 2006). In a recent study, cardiac expressions of ACE and Mas were decreased in the hypertrophied left ventricle of SHRs, whereas those of the AT1 receptor and ACE2 were unchanged. Continuous perinatal losartan treatment reduced left ventricular weight but did not influence the altered cardiac RAS expression (Klimas et al., 2015). Since the ileal and colonic preparations undergo extensive washout prior to stimulation, it is very unlikely that residual losartan would explain the decreased angiotensin II–induced contractile responses observed in this study. It is more likely that the AT1 receptor density within the contractile tissue was decreased following the 14-week losartan treatment; however, there is a need to undertake receptor binding studies of ileal and colonic tissue after losartan treatment to determine muscarinic receptor density of: 1) ileal tissue and 2) the profile of AT1 receptors of both the ileum and colon (Leifert et al., 2009). We have previously shown that there is a depressed prostanoid response in young rats (Patten et al., 2006) and SHRs, but have not measured prostanoid receptor profiles (Patten et al., 2004, 2005). In earlier studies, we also demonstrated that dietary fish oil supplementation led to higher receptor-induced contractility in normal (Patten et al., 2002) and hypertensive rats (Patten et al., 2005) that was not explained by changes in receptor number or sensitivity (Patten et al., 2005), although it was determined fish oil could lead to altered sensitivity of muscarinic 1 receptor subtype (Patten et al., 2006). We have recently shown that dietary resistant starch can alter colonic contractility in healthy adult rats, and demonstrated alterations in colonic expression of genes related to systems that influence gastrointestinal contractility using genomic microarray techniques (Patten et al., 2015b) that may be used to discern losartan effects shown in this study on the intestinal genome. It is also to be determined if other ARBs, such as olmesartan, which has been reported to have some evidence
contractility of the ileum, whereas the angiotensin-receptor system was severely depressed in both ileal and colonic tissue by mechanisms that are to be determined. This is the first description of altered smooth muscle contractility induced by losartan in a hypertensive animal model, and we may speculate that this phenomenon in part explains some adverse side effects of ARB treatment of high blood pressure.

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Authorship Contributions

Participated in research design: Patten, Abeywardena.

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