The effect of intrauterine growth restriction on the Ca^{2+}-sensitivity and maximum Ca^{2+}-activated force of β-escin skinned mesenteric arteries of 5-week old male Wistar-Kyoto rats

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An analysis of epidemiological cohort studies by Barker and colleagues (1990) found intrauterine growth restricted (IUGR) infants had a significantly increased risk of developing hypertension in adulthood. A later study by Anderson *et al*. (2006), examining the sensitivity of the mesenteric artery smooth muscle from both young male and female rats to the vascular agonist phenylephrine, found that in IUGR animals vascular smooth muscle had significant increase in sensitivity. However, it is unclear whether these observed changes in vascular sensitivity are driven by changes in receptor number or by changes in downstream events that lead to force development, specifically, the Ca^{2+}-regulated activation of the contractile apparatus. The aim of this study is to therefore examine the effects of IUGR on both the Ca^{2+}-sensitivity and maximum Ca^{2+}-activated force in young (5-week old) male Wistar-Kyoto (WKY) rat mesenteric arteries.

Pregnant WKY rats were randomly assigned to a control (*ad libitum* diet; $n = 9$) or nutrient restriction (NR; 40% of control diet from day 15-21 of pregnancy, $n = 8$) as described previously (Williams *et al*., 2005). Following birth all dams/pups were fed *ad libitum*. At 5 weeks of age, male pups were euthanised by an overdose of isoflurane (4% v/v). Single segments of intact mesenteric artery were then dissected and mounted on a single wire myograph system (320A, ADInstruments) and placed in physiologic saline solution (PSS; containing in mM: 10 HEPES, 150 NaCl, 3 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$ and 5.5 glucose; pH 7.3) while constantly aerated with 100% O_2. Segments were normalized to achieve optimised internal circumference for development of tension described previously (Mulvany and Halpern, 1977). Extracellular force responses to both KCl (150mM) and phenylephrine (10^{-8} to 10^{-4}M) were measured. The same artery segment was then skinned with β-escin (30μM in Ca^{2+}-free EGTA solution (that mimics the normal intracellular environment) containing in mM: 136 K^+, 36 Na^+, 90 HEPES, 50 EGTA, 10.3 Mg^{2+}, 8 ATP, 10 creatine phosphate; pH 7.1) using a modified procedure described previously (Satoh *et al*., 1994). Skinned artery segments were then exposed to a series of highly buffered Ca^{2+}-EGTA solutions containing increasing levels of free [Ca^{2+}] (between 0.1μM and 20μM) that were made by combining in different ratios the Ca^{2+}-free EGTA solution above to a similar solution that contained (mM): 136 K^+, 36 Na^+, 90 HEPES, 48.5 Ca^{2+}, 50 EGTA, 8.12 Mg^{2+}, 8 ATP, 10 creatine phosphate; pH 7.1.

Phenylephrine induced significantly larger force responses in IUGR rats compared to controls ($P<0.05$) consistent with previous observations by Anderson *et al*. (2006). Maximum responsiveness to KCl was significantly larger in IUGR rats (Control: 3.16±0.25 mN/mm, $n = 5$; NR: 3.82±0.21 mN/mm, $n = 6$; $P<0.05$; Mean±SEM). In β-escin permeabilized mesenteric arteries the Ca^{2+}-sensitivity of the contractile apparatus was unchanged between experimental groups ($P>0.05$). However, the maximum Ca^{2+}-activated force response was found to be significantly increased in IUGR male pups in comparison to controls ($P < 0.05$). This is an original result that demonstrates that the increased extracellular responsive changes to phenylephrine associated with IUGR may be due to intracellular alterations to the Ca^{2+}-activated contractile machinery. This result still requires further biochemical experiments to fully unveil the underlying cause.

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