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CONTRACTILITY IS INCREASED IN THORACIC AORTAE OF APOLIPOPROTEIN E KNOCK-OUT MICE PRIOR TO LESION DEVELOPMENT

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High plasma cholesterol has been implicated as a risk factor in the development of atherosclerosis and hypertension. The removal of cholesterol from cellular membranes using methyl-β-cyclodextrin has been shown to reduce contractility in the vascular smooth muscle in response to a range of agonists. The mechanism for this has been linked to the disruption of caveolae and the associated signalling micro-domains. In particular it has been shown that disruption of caveolae can reduce store-operated calcium entry which, in some vascular tissues, can contribute to contraction. There is currently limited evidence showing the effects of high serum cholesterol on vascular function. In the current study, thoracic aorta contractility was assessed in apolipoprotein E knock-out (ApoE−/−) and control (C57/Bl6) mice fed a standard chow diet for 8 weeks. Contraction was stimulated through depolarisation (100mM KCl) and the use of the agonists phenylephrine (PE; 10 μM) and serotonin (5-HT; 10 μM), on vessel segments mounted on a wire myograph. In addition, fluorescence microscopy was employed to measure global intracellular calcium concentrations in INDO-1 (20 μM) loaded segments of thoracic aorta. Store operated calcium entry was induced by removal of [Ca2+]e and incubation with cyclopiazonic acid (CPA; 10 μM) followed by re-introduction of calcium (2mM). Data are represented as means±S.E.M., compared using one-way ANOVA. In response to a depolarising stimulus, there was no change in contractility observed between chow fed control and ApoE−/− mice (1.98±0.17 mN vs 2.08±0.20 mN increase in tension; n=11,4, respectively). However, contractility in chow fed ApoE−/− mice when compared to the chow fed age and strain matched controls was significantly increased after exposure to both phenylephrine (47.2±5.5 vs. 82.1±9.7% normalised contraction; $p<0.05$, n=11,4) and serotonin (168.7±4.7 vs. 250.3±11.1% normalised contraction; $p<0.01$, n=11,4). Stimulation of store-operated calcium entry showed no difference in the rise in [Ca2+]I between control and ApoE−/− animals (1.07±0.12 vs. 1.020±0.08 ΔF400: F500; n=3,3). Assessment of contractility in response to induced store-operated calcium entry was negligible (0.17±0.03mN, n=4) in this tissue. These data show that contractility is increased in ApoE −/− mice after chow feeding for 8 weeks. The direct mechanism for this increase in contractility is yet to be elucidated but is independent of store-operated calcium entry. As previously stated the removal of cholesterol from membranes leads to disruption of caveolae and inhibition of agonist contractility. Therefore the increased agonist contractility seen in the ApoE−/− mice may result from an effect of increased plasma cholesterol on vascular membranes, possibly through augmentation of caveolar signalling.