**Product Name: Tocrifluor 1117** **Cat. No. 2540**

**Visualisation of vascular cannabinoid receptors and their potential interaction with α1-adrenergic receptors.**

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The role of cannabinoid receptors (CB1 & CB2) within the cardiovascular system is unclear. The endogenous cannabinoid anandamide (AEA) mediates vasodilation *in-vitro* [1] whilst *in-vivo* a triphasic blood pressure response comprising pressor and depressor components has been reported [2]. A possible role for both CB1 and CB2 receptors exists within vascular tissue. However, non-CB mediated responses in the vasculature have been observed and a role for the orphan receptor GPR55 has been postulated [3]. The aims of this study were to a) investigate the role of endogenous cannabinoids in mouse tail artery, a thermoregulatory vessel rich in α1-adrenoceptors and b) examine, for the first time, the binding of a novel fluorescent ligand for CB receptors (Tocrifluor 1117).

Tail arteries were removed from 4 month old male C57 black mice. Vessel segments were either mounted in a wire myograph for functional studies or incubated in Tocrifluor 1117 (0.5µM) & QAPB (1µM, fluorescent α1-adrenoceptor antagonist) for confocal analysis. Concentration response curves (CRC) to noradrenaline (NA) were performed in the presence and absence of the endocannabinoids AEA (1µM) and 2-arachidonylglycerol (2-AG, 1µM). Tocrifluor 1117 and QAPB were imaged under 543nm and 488nm excitation respectively. Tocrifluor 1117 binding was also examined in HEK293 cells stably expressing GPR55.

AEA 1µM caused a transient contraction when applied to isolated tail artery segments (0.08g). The NA CRC was shifted to left in the presence of 1µM AEA (Log $EC_{50}$ control –6.80 vs –7.82, $p<0.05$). The maximum contractile response was unchanged. In the presence of 2-AG (1µM) a small leftward shift of the NA CRC was observed which failed to reach significance. However, comparison of the effect of 2-AG alone (Log $EC_{50}$ –7.36) and 2-AG plus indomethacin (10µM, Log $EC_{50}$ -6.33) revealed a significant difference ($p>0.05$). Tocrifluor 1117 binding was observed on all 3 vascular tunics with particularly strong fluorescence evident on perivascular fat, adventitial cells, nerves and smooth muscle. In several areas of media, colocalisation of Tocrifluor 1117 and QAPB was observed. In live HEK293 GPR55 cells, Tocrifluor 1117 generated a rise in $Ca^{2+}$ and promoted receptor clustering, visible as punctate fluorescence which developed over time.

Tocrifluor 1117 (fluorescent analogue of the CB1 antagonist AM251) is a potentially very powerful tool for identifying the cellular location of cannabinoid receptors, including GPR55 in living tissues. AM251 has been shown to activate GPR55 [4]. The results of the study suggest that endogenous cannabinoids potentiate the actions of noradrenaline in tail artery possibly via co-localised cannabinoid and α1-adrenergic receptors in vascular smooth muscle. The importance of the fluorescent binding in perivascular fat and adventitia requires further study.

**References**

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