Venom of box jellyfish, *C. fleckeri,* causes vasoconstriction and induces an increase in cytoplasmic calcium in cardiomyocytes, the latter likely through poorly-selective cation channels

V. Suturin,[1] J. Chaisskul,[2] M. Tare,[1] H.A. Coleman,[1] W.C. Hodgson,[2] N. Konstantakopoulos[2] and H.C. Parkington,[1] [1]Department of Physiology, Monash University, Clayton, VIC 3800, Australia and [2]Monash Venom Group, Department of Pharmacology, Monash University, Clayton, VIC 3800, Australia.

The box jellyfish, *Chironex fleckeri*, inhabits the oceans of northern Australia and is responsible for numerous envenomations each year, with some lethal outcomes. Envenomation causes intense pain and, in severe cases, further symptoms can include cardiovascular, respiratory, neurological and renal effects. The venom of *C. fleckeri* causes cardiovascular collapse in anaesthetized rats and induces a marked increase in cytoplasmic calcium (Ca^{2+}_i) in isolated cardiomyocytes, though the mechanisms by which the venom acts at the level of the tissue are poorly understood. The aim of the present study was to further investigate the effects of *C. fleckeri* venom on cardiac and vascular smooth muscle cells.

Adult female and male rats were anaesthetized with pentobarbitone (80 mg/kg ip) and, in some studies, were fitted with carotid artery and jugular vein catheters for blood pressure measurement and venom administration, respectively. In other studies the heart was rapidly removed and mounted in a Langendorff apparatus for either left ventricular (LV) function studies, or perfusion with collagenase solution for cardiomyocyte isolation. Ca^{2+}_i was measured in the isolated cells following 1 hr incubation with the Ca^{2+} indicator, fluo-4-AM.

In anaesthetized rats, 5 μg/kg venom (i.v. bolus administration over 10 s) induced a rapid 40 mmHg increase in blood pressure, which persisted for 1-2 min (n=7). This was then followed by a decrease in blood pressure to 40-50 mmHg, followed by recovery that was complete in about 15 min. 10 μg/kg venom was lethal in about 4 min. Heart rate was unaffected in these studies. Venom (2 μg in 200 μl over 10 s) infused into isolated Langendorff hearts induced an initial 10 mmHg reduction in LV developed pressure and a prompt 65% drop in coronary flow rate. Following 1-2 min, LV developed pressure fell from 70 to 22 mmHg (n=6) but recovered over about 15 min. 4 μg of venom was lethal. Again, there was no effect on heart rate. Venom caused constriction (110% of high K^+ constriction, n=4) in isolated coronary, mesenteric and renal arteries. It also induced an increase in Ca^{2+}_i in isolated aortic smooth muscle cells. Venom application, 2 μg/ml over 30 s, induced an increase in Ca^{2+}_i in isolated cardiomyocytes and this did not occur in the absence of extracellular Ca^{2+}. The increase in Ca^{2+}_i was not prevented by prior emptying of the endoplasmic reticulum store (n=7), blockade of L-type Ca^{2+} channels with nifedipine (n=6), or a combination of both treatments (n=4) but it was reduced by blockers of poorly-selective cation channels, to 23% (n=6) by SKF96365 and to 36% (n=4) by 2-APB.

This study demonstrates that the venom of *C. fleckeri* causes significant vasoconstriction that, in the heart, blunts the ability of the ventricle to develop pressure. The venom also facilitates Ca^{2+} influx in cardiomyocytes, likely *via* a poorly selective cation channel, possibly of the TRPC family.