FUNCTIONAL SEPARATION OF THE BIPHASIC ACTIONS OF RYANODINE

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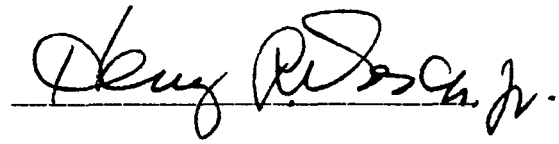
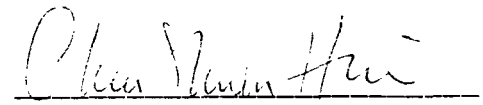
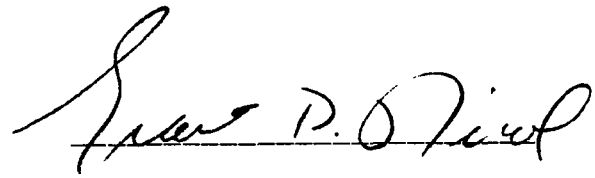


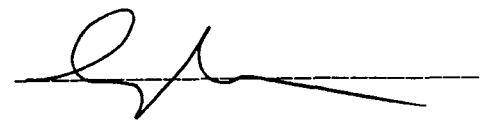
Henry R. Besch, Jr., Ph.D.



Chiu Shuen Hui, Ph.D.



Grant D. Nicol, Ph.D.



August M. Watanabe, M.D.

May 25, 1995

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Functional Separation of the Biphasic Actions of Ryanodine.

Ryanodine, an alkaloid purified from *Ryania speciosa* Vahl, exhibits two opposing actions on the sarcoplasmic reticular Ca^{2+} release channel (SR CRC) of striated muscle. Lower (nM to low μM) concentrations activate (open) the channel whereas higher (high μM) concentrations deactivate (close) the channel. Direct binding studies have failed to establish the number of binding sites on the SR CRC. The aim of the present study was to further characterize the structural and functional characteristics of the SR CRC at the whole-cell and subcellular levels.

The primary objective of the first section of the thesis was to identify and characterize a membrane current that is regulated by Ca^{2+} and could thus provide a potential means by which to evaluate the biphasic actions of ryanodine. The present study demonstrates that delayed rectifier K^+ current (I_{DR}) does not exhibit Ca^{2+} activation in guinea pig ventricular myocytes. However, ryanodine did enhance the amplitude of the slow inward Ca^{2+} current (I_{si}). Ryanodine also prolonged the action potential duration of the myocytes. A possible mechanism for these results, based on alterations in intracellular Ca^{2+}, is proposed.

At the subcellular level, the present study evaluated the effects of ryanoids and allosteric modulators of the SR CRC on Ca^{2+} fluxes across SR membrane vesicles. The results demonstrate that the concentration dependence of activation by ryanodine varies independently of deactivation when allosteric modulators of the SR CRC are present. The study also demonstrates that two C_{10}-O_{eq} semi-synthetic derivatives of ryanodine possess only activator effects on the SR CRC. The addition of allosteric modulators produces a leftward shift in their activation profile that is very similar to that observed with ryanodine. These results, together with evidence from previous studies, support the premise that functionally independent binding

sites on the SR CRC are responsible for the biphasic effects of ryanodine.

Finally, the present study demonstrated that the anti-cancer agent, suramin, functions both as an activator of the SR CRC and an inhibitor of the Ca^{2+}-ATPase of the SR membrane. Suramin exerts its effects on the SR CRC at a site distinct from the ryanodine binding site.

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