Supporting Material for:

Autocrine A2 in the T-System of Ventricular Myocytes Creates Transmural Gradients in Ion Transport: A Mechanism to Match Contraction with Load?

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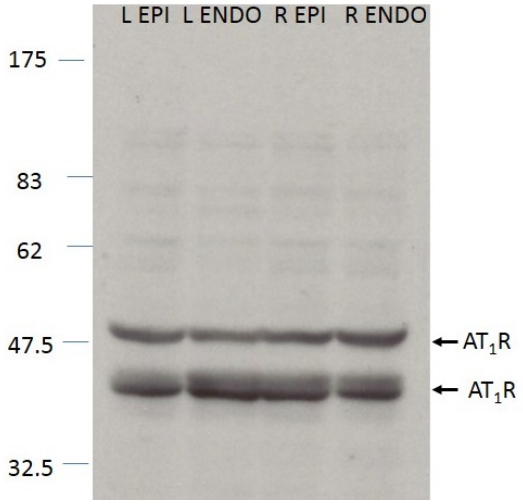

Figure S1. Western blots of the AT_1Rs in EPI and ENDO of the canine left (L) and right (R) ventricles show no differences. The antibody was AT1(N10) (Santa Cruz Biotechnology). The AT_1R generally runs at two molecular weights, possibly representing phosphorylated vs not phosphorylated receptors.

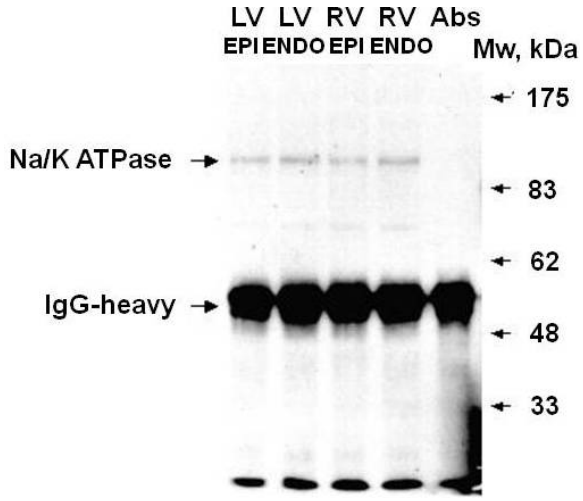

Figure S2. The Na/K ATPase co-immuno-precipitates with the AT_1R in canine ventricular myocytes. As described in the INTRODUCTION, the AT_1R and associated proteins are internalized following activation of the receptor and its association with β-arrestins, so the simplest hypothesis was that the Na/K pump is associated with the AT_1R in canine ventricular myocytes and is therefore internalized along with the activated receptor/β-arrestin complex. The results of immunoprecipitation with mouse monoclonal anti AT_1R antibody, then probing with a mouse monoclonal antibody for the α1-isoform of the Na/K pump are consistent with this hypothesis. The presence of a band for the Na/K pump in EPI and ENDO of both right (RV) and left (LV) ventricle suggests the AT_1R and Na/K pump are indeed associated, hence activation of AT_1R and its internalization might bring the Na/K pump with it, and thus reduce the amount of pump protein in the T-system membrane. These observations in connection with work on I_{to} have led to the internalization hypothesis.

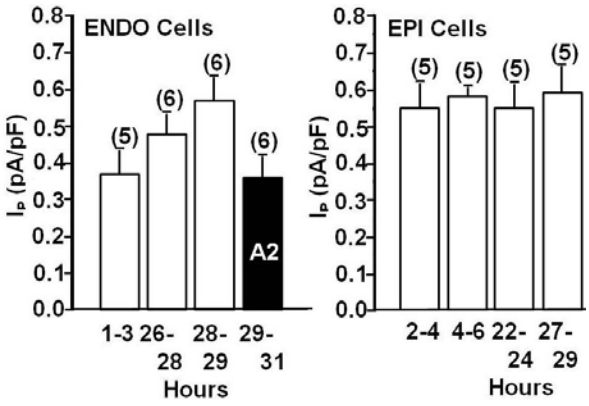

Figure S3. The time course of AT_1R inactivation in isolated myocytes. The Na/K pump current I_P in ENDO myocytes runs up to EPI levels over a time period of 28-29 hours, presumably because autocrine A2 secretion into the T-system lumen is running down. Consistent with this interpretation, external application of 5 μM A2 brings the current back down to its original value. In EPI myocytes, the pump current remains constant over a period of 27-29 hours, presumably because A2 secretion is absent in the T-system of these myocytes. An alternative interpretation is that high affinity AT_1Rs in ENDO are slowly being modified into low affinity AT_1Rs like those in EPI. Either interpretation is possible, but as described in the text, some experiments favor the model in which A2 secretion creates the differences between EPI and ENDO.

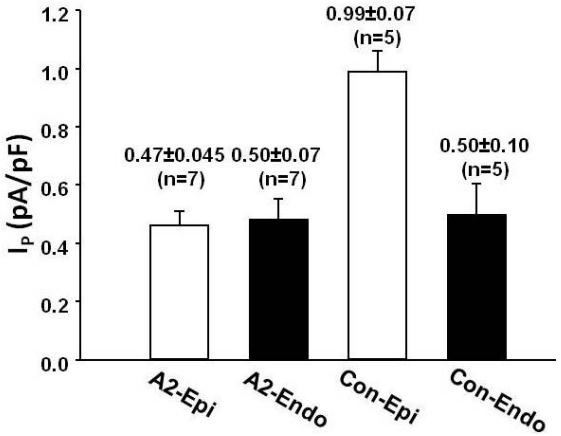

Figure S4. In situ delivery of A2 at 37° C. Two chunks of left ventricle were canulated through a coronary artery and both perfused for 1 hour with Normal Tyrode or Normal Tyrode containing 5 μM A2. At the end of this period, cells were isolated as described in METHODS and immediately used for whole cell patch clamp characterization of I_P. Throughout this process the cells were maintained at 37° C. In the absence of A2, Control myocytes from EPI had about twice the amplitude of I_P as ENDO myocytes. However, when both groups were perfused with A2, I_P in both EPI and ENDO were reduced to Con-ENDO level. These data are consistent with results presented in the text, where the studies were done at room temperature and A2 applied directly to the isolated myocytes for 2 hours.

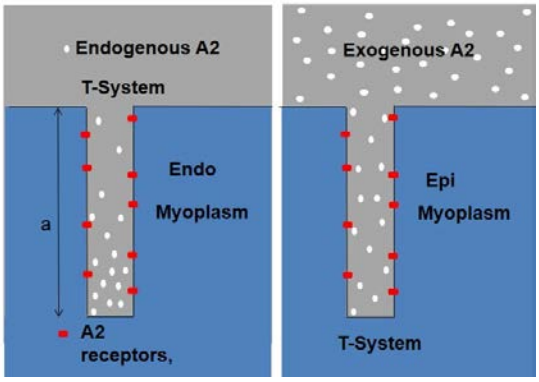

Figure S5. A sketch of the difference between the spatial distributions of A2 when endogenously generated vs when exogenously added to the bathing solution