

Isoproterenol (17 mg/kg/day) was administered to mice using ALZET osmotic minipumps (Durect Corp, Cupertino, CA). Prior to inserting the minipumps, mice will be anesthetized using avertin (0.2ml/10g BW) or chloral hydrate (400 mg/kg).

Isoproterenol is obtained from Sigma-Aldrich (St. Louis, MO) and is dissolved in phosphate buffered saline (PBS) containing 0.5mmol/L ascorbic acid. Sham treated animals received ALZET pumps that contained PBS and ascorbic acid only.

A small patch of hair in the dorsal interscapular area will be removed and the underlying skin will be prepped with betadine and alcohol. 0.1 cc of lidocaine without epinephrine will be injected into the dermal layer of the incision site. Using sharp dissecting scissors, a suitable incision (< 1cm) will be made, exposing the subcutaneous space. A hemostat will be inserted into the incision and a subcutaneous pocket (large enough to hold the pump and allowing free movement of the pump) will be made by opening and closing the hemostat jaws. The hemostat will be removed and an isoproterenol/angiotensinII filled (100ul) ALZET mini-pump (model 100-D) will be inserted into the pocket with the delivery portal first. The surgical incision will then be closed with two 4-0-dermalon sutures.

During and after the surgical procedure and up until the time of recovery, mice will be maintained on a 37°C temperature controlled pad.

Alzet pumps were maintained for 2 or 5-days. In vivo analysis of cardiac function (echocardiography and LV catheterization) were performed in anesthetized animals with the pumps still in place.