# ATRIOPEPTIN RELEASE FROM THE ISOLATED PERFUSED HEART OF SQUALUS ACANTHIAS: THE EFFECTS OF PRESSURE AND CHLORIDE CONCENTRATION

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We have previously demonstrated in Squalus acanthias that rectal gland secretion of chloride and water in vivo is stimulated by extracellular volume expansion but not by an increase in extracellular osmolality [Solomon, et al., Am. J. Physiol. 248:R638-R640, 1985]. Furthermore, the stimulation of secretion in vivo is mediated by a humoral factor(s) [Solomon et al., Am. J. Physiol. 246:R62-R66, 1984]. Both in vivo and in vitro evidence suggests that atriopeptin (ANP), a cardiac peptide with natriuretic effects, is one such humoral factor [Solomon et al., Am. J. Physiol. 249:R348-R354, 1985]. In mammals, the major stimulus for ANP secretion is an increase in atrial pressure such as occurs during extracellular volume expansion [eg. Sonnenberg, Fed. Proc. 45: 2106-2110, 1986]. The following experiments were undertaken to determine the role of atrial pressure, ventricular pressure, and perfusate chloride on the release of atriopeptin from cardiac myocytes of *Squalus acanthias*. The isolated perfused heart preparation was employed permitting precise control of perfusate composition and preload and afterload pressure in the absence of neuronal influences. Alterations in atriopeptin metabolic clearance rates are also avoided in this preparation.

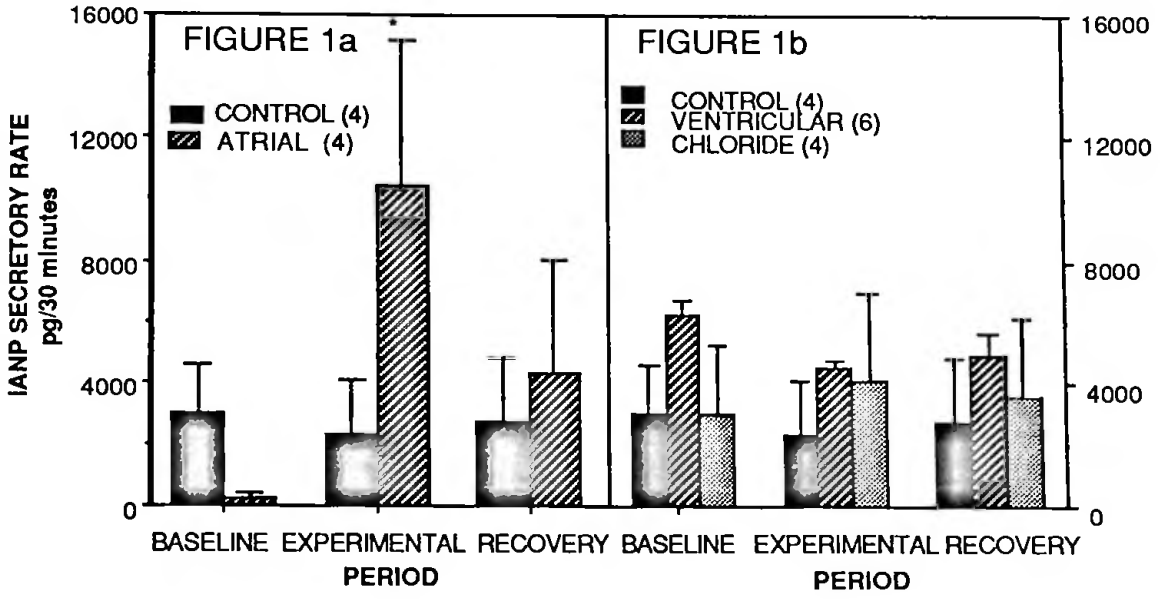
The beating heart, including conus and sinus venosus, was removed from pithed dogfish and perfused in an antegrade direction with oxygenated, shark's Ringers solution containing 0.1% albumin at 15°C and pH=7.60. Perfusate flowed into the sinus venosus via gravity from a reservoir and preload pressure was regulated by the height of the reservoir. Conus outflow pressure was regulated by a thumb screw clamp placed on the outflow tubing. Both preload and afterload pressures were monitored continuously via pressure transducers. All hearts continued to beat spontaneously throughout the experiments. Approximately 100 ml of perfusate was recirculated through the heart. The entire perfusate was exchanged for fresh perfusate every 30 minutes for three exchanges during the experiment. The three collections corresponded to the BASELINE, EXPERIMENTAL, and RECOVERY phases of the protocol. The collected perfusate was filtered, extracted on Sep-pak C-18 cartridges, lyophilized, and reconstituted in RIA buffer. Assay of the perfusate for immunoreactive atriopeptin (iANP) was carried out as described previously [Epstein et al. Bull. MDIBL 27:72-73, 1987].

The following experimental procedures was applied during the EXPERIMENTAL period of perfusion: an increase in inflow pressure (ATRIAL), an increase in outflow pressure (VENTRICULAR), an increase in chloride concentration without a change in total osmolality (CHLORIDE), accomplished by a reduction in urea concentration in the perfusate.

During CONTROL perfusions with preload pressures at 2.3 mmHg, afterload pressures at 15 mmHg, and a cardiac output of 20 ml/min, the heart secreted atriopeptin at a rate of 4000 pg/30 minutes. The iANP secretory rate

was constant over the three 30 minute collection phases of the study (Figure 1a).

When the preload was increased to 6.5 mmHg without altering afterload or cardiac output (ATRIAL), the rate of ANP secretion increased 3 fold ($p \leq 0.05$). Return to the baseline level of preload led to a reduction in iANP secretory rate (Figure 1a).




(n)= the number of heart perfusions in each group

Increases in afterload from 18 to 37 mmHg, without changes in preload or cardiac output (VENTRICULAR), were not associated with increased iANP secretory rate. Increases in perfusate chloride concentration from 270 to 301 (CHLORIDE) without a change in osmolality, did not affect iANP release (Figure 1b).

These preliminary results confirm that the isolated perfused heart releases iANP in vitro during perfusion at normal preload and afterload pressure. Cardiac output under these conditions is similar to in vivo measurements. The secretory rate of iANP in this denervated preparation is not stimulated by increases in either afterload or chloride concentration. In contrast, changes in preload rapidly and reversibly affect ANP secretion. The increase in preload to 6.3 mmHg mimics the effects of infusion of 30 ml/kg of saline in vivo [unpublished results]. We have previously observed an increase in plasma iANP levels in animals infused with a similar load [Epstein et al, loc. cit.]. Thus, the present results are consistent with the hypothesis that the major stimulus to release of atriopeptin is an increase in preload, i.e. atrial pressure, as occurs during extracellular volume expansion. These observations further define the important volume regulatory role of atriopeptin in this species.

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