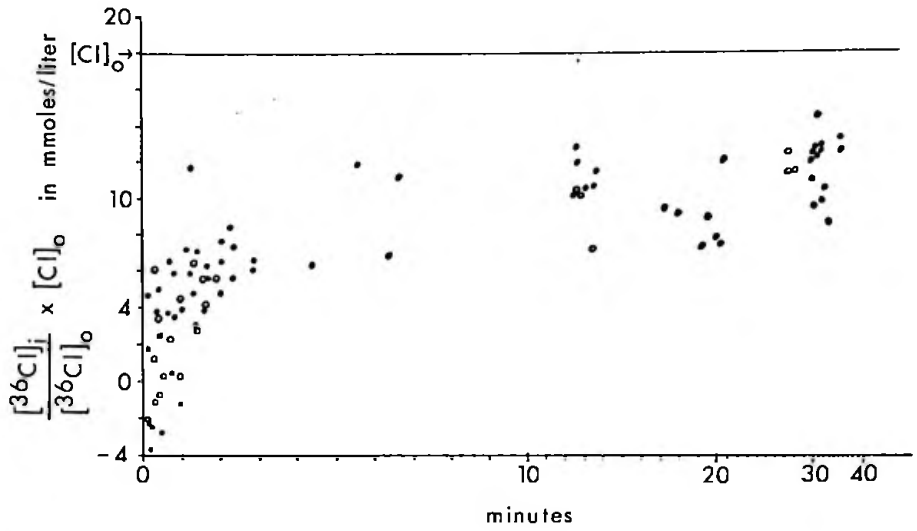

Figure 2. Uptake of ^{36}Cl by dispersed rectal gland tubules. Extracellular marker and furosemide added 20 min before ^{36}Cl. Open circles: furosemide (1 mM); closed circles: control.

of the isolated tubules is not known (-80 mV in isolated, perfused glands--see Duffey et al., this volume). No inhibition of Cl^- uptake by furosemide could be detected. Since the scatter was large, however, a small effect could easily have been missed.

We conclude from these studies that viable suspensions of isolated rectal gland tubules can be prepared by proteolytic digestion but that such preparations are not especially well suited for determinations of initial rates of uptake of Na^+ and Cl^-. This work was supported by NIH grant AM-21345.

THE POSITIVE INOTROPIC ACTION OF CATECHOLAMINES ON ISOLATED ATRIUM AND VENTRICULAR MYOCARDIUM OF THE ELASMOBRANCH, *Raja erinacea*

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There is no anatomical or physiological evidence to suggest that there is a direct sympathetic innervation of the elasmobranch heart. J. Z. Young in a comprehensive study of the autonomic nervous system of selachians, that involved 10 species including 3 Rajiformes, found none after a systematic search of all possible paths including the ductus Cuvieri and the walls of the esophagus. Also, he found no ganglion cells in the walls of the atrium and ventricle (Q. J. Micr. Sci. 75:571-624, 1933). Inhibitory "parasympathetic" cardiac nerves, however, arise from the last branchial branch and from the visceral branch of the vagus, but B. R. Lutz also was unable to find either anatomical or physiological evidence of cardiac sympathetic adrenergic nerves in the little skate (*Raja erinacea*), 2 other species of *Raja* or in *Squalus acanthias* (Am. J. Physiol. 93:669, 1930).

A close connection between β-adrenergic stimulation and taurine fluxes has been reported in the rat heart (Huxtable, R. and J. Chubb, Science 198:409-411, 1977). We chose to test this hypothesis by characterizing taurine uptake in an in vitro skate atrium preparation because of this elasmobranch's complete lack of sympathetic adrenergic innervation to the heart and found that indeed no β-adrenergic stimulation of taurine transport could be demonstrated. The active carrier-mediated, Na- and energy-dependent β-amino acid transport system was not affected by isoproterenol nor dibutyryl cyclic AMP

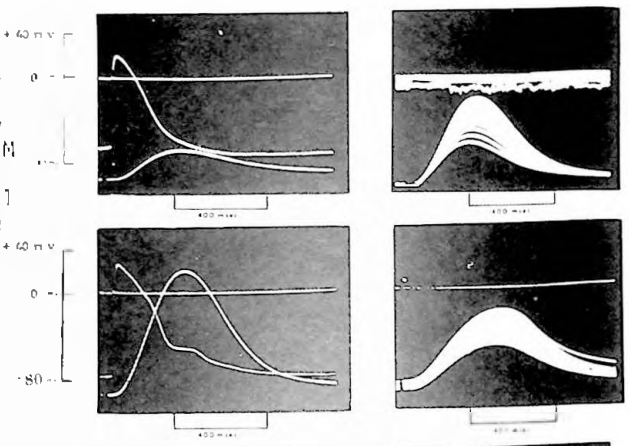

Figure 1. Upper left panel, action potential and contraction recorded from a skate ventricular myocardial fiber under control conditions. Upper right, progressive increase in tension immediately following replacement of control medium with 10^{-6} M of the β-agonist, isoproterenol, added to medium. Lower left panel, steady state reached with maximal stimulation of tension and action potential by the β-agonist. Bottom right, addition of 10^{-6} M β-blocker, propanolol progressively diminishes the increased tension produced by isoproterenol.

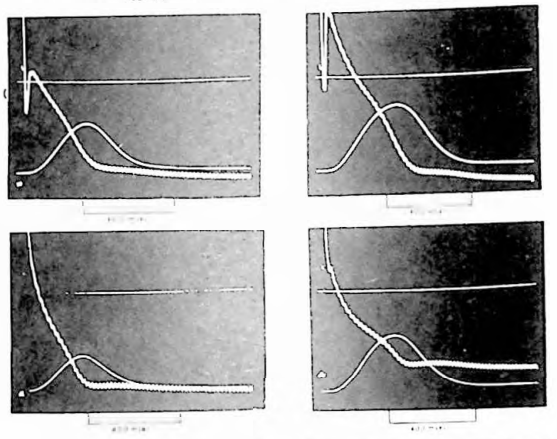

Figure 2. Atrium. Left upper panel is control isometric tension and part of the action potential recorded from single skate atrial fiber, 100-200 microns diameter, approximately 0.5 cm long. Upper right, increased tension in presence of 5 x 10^{-6} M epinephrine. Lower left panel, reversal of augmented contraction following removal of epinephrine. Lower right, again addition of epinephrine following reversal of the catecholamine stimulation.

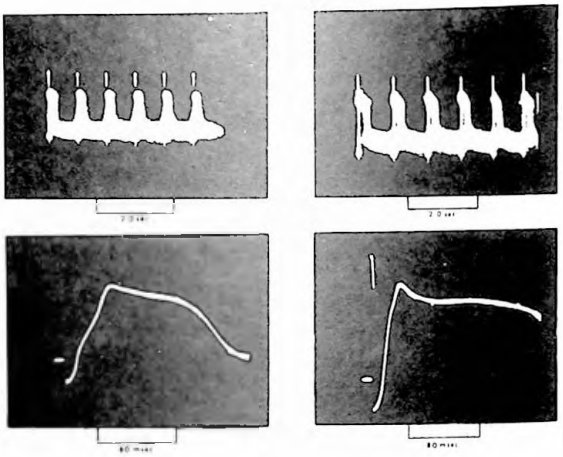

Figure 3. Skate atrial fiber, transgap recording of summed action potential generated by external stimulation. Upper left panel is control and upper right is following stimulation with 10^{-6} M epinephrine. The effect of the catecholamine was originally greater than shown here. Apparently the effects of epinephrine decrease rather rapidly with time. Note Figure 2 also for diminished response following reversal of initial stimulation. Bottom left panel, shows shape of single atrial action potential with expanded time scale. Note relatively slow phase 0 (depolarization) and down sloping plateau. Lower right, following 10^{-6} M epinephrine there is increased size of action potential indicating that increased contraction is likely due to increased electrical excitation. Note the more rapid depolarization and the prolonged plateau.

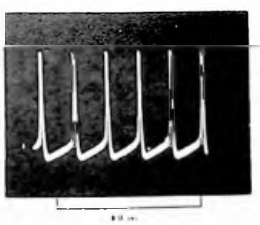

Figure 4. This resumption of a spontaneous atrial beat 4 hours after the fiber was isolated, mounted, and subjected to repeated experimentation (see Figure 3) attests to the viability of the skate in vitro preparation. Subsequent experiments to determine whether epinephrine and taurine had a chronotropic effect on this spontaneous diastolic depolarization were negative.

(Forster, R.P., J.A. Hannafin and J.S. Shiffrin, this issue). This led to the following experiments in which we tested the direct action of catecholamines, especially the β-adrenergic agonist isoproterenol, on excitation and contraction of skate atrial and ventricular myocardial fibers.

The preparation used to measure action potential and contraction of isolated heart muscle fibers was similar essentially to that used by Morad, M. and Y. Goldman (Bull. MDIBL 13:82-84, 1973) for their excitation-contraction coupling studies recorded from ventricular strips of the dogfish, *Squalus acanthias*. Muscle fibers approximately 100-200 μ in diameter and 0.5-1 cm long were drawn through small holes in 2 latex membranes which separated the preparation into 3 compartments. Isosmotic sucrose solution in the central compartment electrically isolated the extracellular spaces of the fiber in the 2 outer compartments. The left compartment contained a balanced isotonic elasmobranch medium of the following mM composition: NaCl 280, KCl 6, $CaCl_2$ 5, $MgCl_2$ 3, Na_2SO_4 0.5, NaH_2PO 1, urea 350, glucose 5 and $NaHCO_3$ 8. In the left compartment glass microelectrodes measured action potential and association of the fiber was made here with an isometric tension transducer. The right compartment contained a depolarizing solution of isotonic KCl.

Figures 1-4 show that the myocardial fibers of both atrium and ventricle respond positively to direct catecholamine administration despite a total absence of sympathetic adrenergic innervation to the skate heart. The positive inotropic response to various agents is indicated directly by the recordings of isometric contraction, and indirectly by contour changes in the action potential recordings. Details are provided in the legends under each figure.

These experiments demonstrate a direct positive inotropic action of β-agonists (isoproterenol) and other catecholamines on isolated skate myocardial fibers where there is no anatomical or physiological evidence of a sympathetic adrenergic innervation, but actually there may not be the difference between rat and skate hearts we originally sought in designing our experiment testing the relationship between β-adrenergic stimulation and taurine fluxes. It is reasonable to believe that extrinsic control of the heart in elasmobranchs may represent an intermediate situation in the evolution of that in higher vertebrates. There is evidence of intrinsic chronotropic responses of the fish myocardium to increased perfusion pressure, and there exists also a fully developed extrinsic inhibitory cholinergic "parasympathetic" innervation. The absence of an adrenergic sympathetic innervation to complete the autonomic dual control of the elasmobranch heart may be compensated for by a unique strategically situated pair of whitish 'axillary bodies' lying in the posterior cardinal sinus just behind the heart (Young, op. cit.). They contain sympathetic ganglia and a mass of chromaffin tissue shown to be rich in norepinephrine and other catecholamines (Shepherd, D.M. et al. Nature (Lond.) 112:509, 1953). When released into the blood stream these would quickly pass to the heart, in effect functioning thereby as neurotransmitters would in stimulating β-receptors within the elasmobranch myocardium. This work was supported by NIH grant HL 04457-20.

FURTHER STUDIES ON THE PHYLOGENY OF VERTEBRATE CARBONIC ANHYDRASE IN RED CELLS AND SECRETORY ORGANS

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We are trying to learn the pattern of the development of carbonic anhydrase in vertebrates. Last year (Maren and Rittmaster, Bull. MDIBL 17:35-39, 1977) we showed that the enzyme in elasmobranch red cells is of low specific activity, and that there is a striking increase in teleosts. Indeed, the teleost red cell carbonic anhydrase has a turnover number $\frac{V_{max}}{E}$ K_{cat} as high as primate carbonic anhydrase C (also called II), the greatest of any known enzyme.