THE EFFECT OF ATRIOPEPTIN II ON THE CHLORIDE SECRETORY ACTIVITY OF THE KILLIFISH, Fundulus heteroclitus.

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Atriopeptin II is a peptide hormone found in cardiac tissue and functions in mammalian blood volume regulation by increasing blood flow and/or ion secretion (Maack, T. et al., Kid. Internat. 27:607-615, 1985). Stimulation of fluid and chloride secretion by ANF has been observed in the shark rectal gland (Solomon R. et al., Bull. Mt. Desert Island Biol Lab. 24:46-49, 1984). The opercular tissue of the seawater adapted killifish, Fundulus heteroclitus has a high density of chloride cells and secretes chloride (Zadunaisky, Fish Physiology, Vol. Xb:129-176), making this epithelium a good model for gill chloride secretion. Atriopeptin II was tested on both the isolated opercular epithelium and whole animal to ascertain hormonal action.

Opercular epithelium from seawater adapted killifish was dissected from freshly pithed fish. Opercular skin was gently teased away from the inside of the operculum and pinned onto a sylgard disc with an aperture of 0.178 cm^2 (Degnan et al., J. Physiol. 271:155-199, 1977). The discs containing the opercular epithelium were mounted in an Ussing-style chamber with teleost Ringers on both sides. Opercular epithelial short-circuit current (I_{scc}) was measured by an Iowa clamp with periodic voltage pulses monitoring the resistance (R_t). The transepithelial potential (TEP) was calculated from Ohm's law. Chemicals including atriopeptin II (synthetic, rat) were purchased from Sigma Chemical Co.

Atriopeptin II added serosally (10^{-7} M) to the isolated operculum of Fundulus heteroclitus resulted in a consistant, significant ($P<0.01$) stimulation of the short-circuit current (Table 1). Tissue resistance was significantly ($P<0.01$) decreased by the ANF addition, indicating an increase in chloride movement. The change produced by serosal addition of ANF was rapid (within one to 2 minutes) and in most tissues, a long lasting event. The action of ANF was specific to the serosal side only, mucosal ANF addition had no effect on the opercular electrical

Table 1. Comparison of the action of ANF (10^{-7} M) added serosally (n=24) or mucosally (n=7). Values represent the mean ± the standard error.

		Serosa		Mucosa	
		Control	ANF	Control	ANF
I_{scc}	($\mu A/cm^2$)	129.5 ± 12.3	153.5 ± 13.4*	127.5 ± 27.1	122.5 ± 24.9
R_t	($\Omega \cdot cm^2$)	114.9 ± 14.2	103.9 ± 11.8*	90.9 ± 7.0	93.0 ± 7.6
TEP	(mV)	13.2 ± 1.8	14.5 ± 1.7*	11.6 ± 2.8	11.0 ± 2.4

* significantly different from paired control values, $P<0.01$.

parameters. The addition of ANF was maximal at 10^{-7} M, although a consistant stimulation of the short circuit current was observed at 10^{-8} M (50-70% stimulation).

Since the 19% increase in current represents an increase of 0.9 ueq/cm^2·hr in the net flux, a change not easily discerned by radiotracer flux analysis, we tested the effect of ANF on the whole fish gill efflux. Whole animal efflux observations were on 3 g killifish injected with 1 to 3 µci ^{36}Cl. The fish were placed in a closed seawater flow system (total volume 23 ml), with the seawater circulation being driven by aeration (Figure 1). The appearance of ^{36}Cl was monitored over time. After 60 minutes, 1.7 X 10^{-7} M ANF was added to the seawater and the sampling procedure continued. The ^{36}Cl efflux was expressed as % ^{36}Cl lost from the total ^{36}Cl injected into the fish and half-times ($t_{0.5}$) were calculated graphically from semilog plots. The addition of ANF to the surrounding seawater results in an increase in the chloride efflux (Figure 2). The chloride secretory rate is increased greater than 2 times, resulting in a significant change in $t_{0.5}$. The peptide entry into the killifish was believed to be by the mouth or the gills.

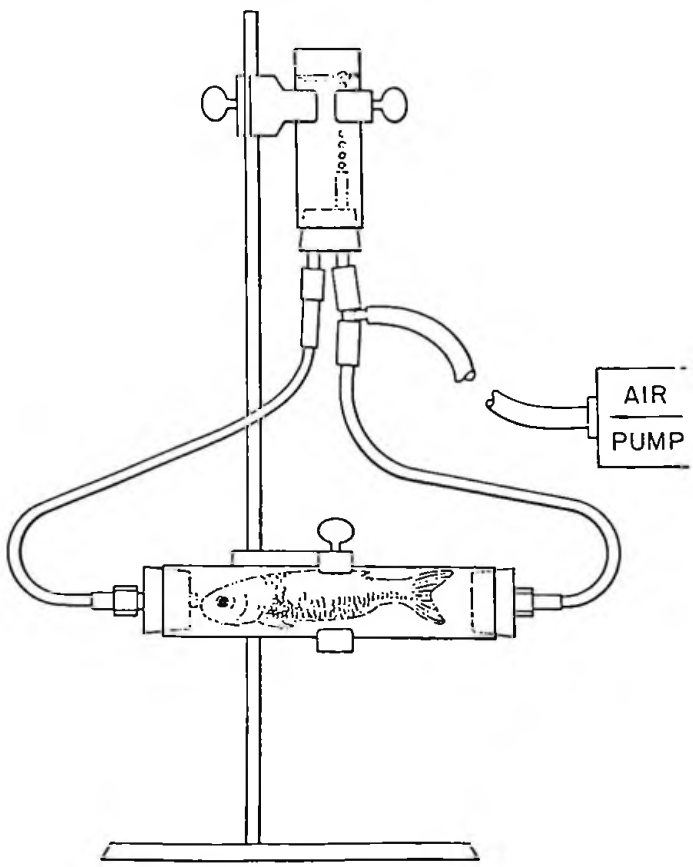

Figure 1. Representative schematic of the apparatus used to monitor the loss of ^{36}Cl in the intact killifish. The seawater flow was driven by aeration.

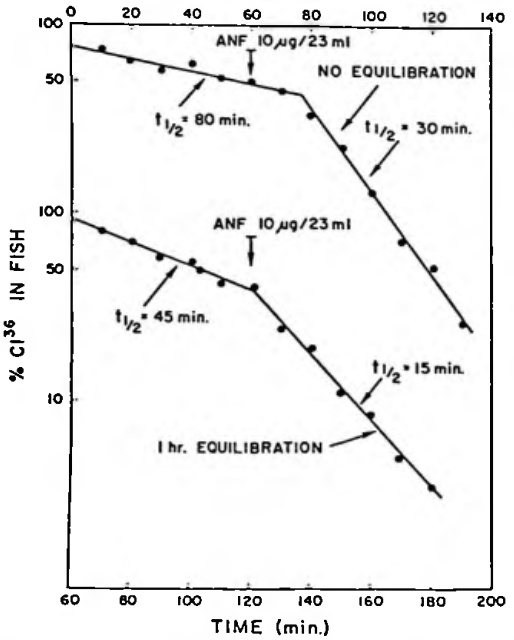

Figure 2. A semilog plot representing the loss of ^{36}Cl from 2 Fundulus heteroclitus over time.

A typical effect of ANF on the short circuit current is represented in Figure 3. The serosal addition of 10^{-8} M ANF increases the current with the decrease in tissue resistance indicated by the pulse bars (an increase in the pulse bar length represents a decrease in resistance). The stimulation of the current by ANF was not maximal, the serosal addition of 10^{-6} M isoproterenol additionally stimulates the current another 56% of control. The ANF stimulated current response was related to the isoproterenol response ($r=0.72$, $P < 0.001$, slope= 2.9, $n=21$). The addition of ANF after stimulation of the current by isoproterenol did not stimulate (increase) the current any further giving the appearance the two responses are linked.

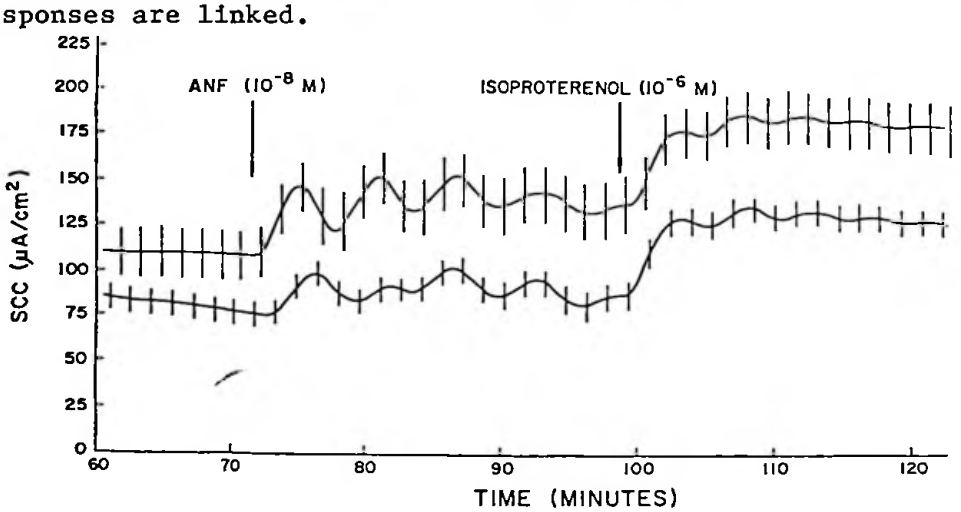

Figure 3. Two individual current (SCC) tracings of the killifish opercular epithelium. The addition of ANF (in this figure 10^{-8} M, serosal) results in a stimulation of the current. Adding isoproterenol serosally additionally stimulates the current.

Stimulation of the opercular current by 10^{-7} M ANF was observed in the presence of 10^{-5} M propranolol (a B-adrenergic antigonist), at a concentration that was inhibitory to isoproterenol (Table 2). The effect of ANF in stimulating the current must be convergent to the isoproterenol response at a point later than the receptor.

Table 2. Effect on the opercular I_{scc} ($\mu A/cm^2$) by serosal 10^{-7} M ANF then serosal 10^{-6} M isoproterenol in the presence of serosal 10^{-5} M propranolol or 10^{-6} / 10^{-5} M tetrodotoxin. Values represent the mean $\pm$ the standard error of the mean.

		CONTROL	ANF	ISOPROTERENOL
Propranolol	5	101.1 $\pm$ 12.8	115.1 $\pm$ 13.8*	108.7 $\pm$ 12.9
Tetrodotoxin	6	80.7 $\pm$ 8.4	108.3 $\pm$ 11.3*	166.9 $\pm$ 20.9**

* significant stimulation above paired control values, $P<0.01$.
** significant stimulation above paired ANF values, $P<0.01$.

The ANF effect (stimulation of the opercular current) was evident in the presence of either 10^{-6} M or 10^{-5} M tetrodotoxin. Since the observations were the same at either TTX concentration, the data were combined. The isoproterenol stimulation of the current was also observed in the presence of TTX. Atriopeptin II appears to be directly stimulating the current and was not the result of stimulating other neural activity.

Atriopeptin II stimulation of the current and decrease in the tissue resistance acts in a manner similar to isoproterenol in the opercular epithelium. The ANF effect was not a maximal one indicating that either the stimulatory activity was a subset of the chloride secretion regulated by isoproterenol or not entirely specific due to the use of rat ANF not teleost.

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