Short communications

Adrenoceptors and adenyl cyclase in gills

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The content of cyclic 3',5'-adenosine monophosphate (cAMP) in the gills of the mullet (*Mugil capito*) has been measured. The basal level was found to be 2·9±0·3 pmol/mg wet weight. When fish were first anaesthetized with MS222 (tricaine) and the gills perfused to remove blood, the concentration of cAMP was raised to 14·3±1·9 pmol/mg wet weight.

Adrenaline caused a significant increase in cAMP content, an effect that was blocked by propranalol, a β-adrenoceptor antagonist. In the presence of the α-adrenoceptor blocking agent phentolamine, adrenaline still caused an increase in nucleotide content.

The results are discussed in relation to adrenaline and the adenyl cyclase system.

Adrenoceptors are known to be present in a number of epithelia which transport sodium and water; these receptors have been classified by the use of specific antagonists and the effects of catecholamines on transport processes have been interpreted in terms of interactions with the adenyl cyclase system (Handler, Bensinger & Orloff, 1968; Rajerison, Montegut, Jard & Morel, 1972; Turtle & Kipnis, 1967; Wong, Bedwani & Cuthbert, 1972).

This paper reports investigations into the type of adrenoceptors present in the epithelia of a teleost gill with particular reference to the activation or inhibition of adenyl cyclase, and bears directly on the proposition that adenyl cyclase is a component of the adrenergic receptor (Mayer, 1970).

Methods.—Cyclic 3',5'-adenosine monophosphate (cAMP) has been measured in the gill filaments of the seawater mullet (*Mugil capito*). The protein-kinase binding assay of Gilman (1970) was used throughout. Acetic acid extracts of gill filaments were dried down at 70–80° C and the cAMP content of the extracts determined from a standard curve prepared at the same time as the extracts were assayed. Extracts were assayed either in duplicate or triplicate. Two checks were used to confirm that the activity measured was due to cAMP. These were that small quantities of cAMP added to the extracts were recovered quantitatively on assay and that the activity present in the extracts was destroyed by incubation with phosphodiesterase.

To prepare the extracts the usual procedure was as follows. Fish were injected intraperitoneally with adrenaline, an antagonist or simply with solvent underwater in the aquarium. Thirty minutes later, the fish were anaesthetized by placing in seawater containing MS222 (tricaine, 0·1 g/litre). The gills were freed from blood by perfusion wih fish-Ringer via a cannula tied into the conus arteriosus. The gill arches were then removed and placed on ice. The gill filaments were removed, blotted dry and weighed, and then homogenized in 50% acetic acid (50 μl/mg tissue).

In a few experiments fish were removed from the aquarium and dropped into liquid nitrogen within 2 seconds. Gill filaments were then dissected, but without freeing from blood. Acetic acid extracts were prepared from these filaments as before.

The fish-Ringer used had the following composition, mM: NaCl, 141; $CaCl_2$, 1·8; $MgCl_2$, 8·6; KCl, 3·4; $(NH_4)_2SO_4$, 0·4; Na_2HPO_4 1·6 and KH_2PO_4, 0·36. Heparin (2·5 u/ml) was added to this solution. Before use the animals were kept in a running seawater aquarium. Adrenaline was dissolved in distilled water made to pH 3 with HCl, phentolamine and propranalol were dissolved in distilled water. Doses of blocking drugs are expressed in terms of the salts propranolol hydrochloride and phentolamine mesylate.

Results.—Experiments were performed on 50 small mullet with a mean weight of 12·7±0·6 g. In experiments in which the fish were killed within 2 seconds of removal from the aquarium by immersion in liquid nitrogen the cAMP content of the gills was found to be 2·9±0·3 pmol/mg wet weight. This value is presumably close to the basal level of nucleotide in mullet gills.

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TABLE 1. *Effects of adrenaline on cyclic 3′, 5′-adenosine monophosphate (cAMP) levels (pmol/mg wet weight) in gill filaments of* Mugil

	[cAMP]	ΔcAMP	*P*
Liquid nitrogen controls	2·9±0·3 (6)	−18·1	<0·001
Non-injected controls	14·3±1·9 (5)	−6·7	<0·1
Injected controls (water at pH 3)	21·0±2·2 (4)	—	—
Adrenaline, 5μg	30·2±2·9 (8)	+9·2	<0·05
Adrenaline, 5μg plus propranalol, 100μg	18·3±0·9 (5)	−2·7	ns
Adrenaline, 5μg plus phentolamine, 100μg	32·6±4·8 (10)	+11·6	<0·05
Injected controls (water)	16·9±1·2 (4)	—	—
Propranalol, 100μg	12·6±0·3 (4)	−4·3	<0·01
Phentolamine, 100μg	14·7±1·8 (4)	−2·2	<0·1

Mean ± standard errors are shown. Values in parentheses refer to the number of measurements. Differences are given with respect to the injected controls. *P* values were calculated by Student's *t* test.

When mullet were anaesthetized with MS222 and the gills perfused to remove blood the cAMP content was found to be 4·3±1·9 pmol/mg wet weight. The gill content of cAMP was found to be even larger in animals receiving solvent injections (Table 1).

Thus the effect of adrenaline on the gill content of cAMP has to be judged with respect to the levels found in control injected fish. Adrenaline (5 μg per fish) caused a significant increase in the cAMP content measured 30 min after injection (Table 1). A series of experiments was devised to determine the nature of the adrenoceptors involved by using blocking agents. In addition the effects of blocking agents *per se* were investigated. The results are shown in Table 1.

When adrenaline was given with the β-adrenoceptor blocking drug, propranalol, the cAMP levels were not significantly different from the injected controls. By contrast, simultaneous injection of adrenaline and phentolamine, an α-adrenoceptor blocking drug, produced a slightly larger increase in cAMP content than did adrenaline alone.

Phentolamine given alone had no effect on the nucleotide levels, compared to injected controls, while propranalol alone caused a small but significant reduction in cAMP content.

Discussion.—As far as we are aware there has been no other report on the cAMP content of fish gills. In *Mugil* we have found values of about 15 pmol/mg wet weight for the perfused gills. These values are high compared to most of those reported for mammalian and amphibian tissues (1–2 pmol/mg wet weight: Steiner, Parker & Kipnis, 1970). However, the values found for gills taken from fish killed by liquid nitrogen are within the normal range.

In two recent reports (Pic, Mayer-Gostan & Maetz, 1972; Pic, 1972) it was shown that adrenaline inhibited the branchial efflux of sodium (and chloride) while at the same time there was an increased diffusional water efflux and osmotic water loss across the gill. The inhibition of sodium transport by adrenaline was evidently mediated by α-adrenoceptors since this effect was blocked by tolazoline and by phentolamine, while the effect on water movement was blocked by propranalol and was evidently due to an activation of β-adrenoceptors. In the discussion of the paper by Pic *et al.* (1972) evidence was put forward to suggest that both of the effects of adrenaline were mediated on cells of the branchial epithelium rather than on the vasculature of the gills. More precisely it was held that the effects on water flow were exerted through the respiratory epithelial cells of the gills, whereas the effects on salt movement were via the so called 'chloride cells'. These cells are undoubtedly the site of active sodium transport (Conte, 1969; Kamiya, 1972). If the arguments presented by these authors are correct it seems that adrenaline simultaneously increases water flow by an action on β-receptors situated in the respiratory epithelial cells and increases the cAMP concentration of the gills. Activation of β-receptors is associated with adenyl cyclase activation in a number of other systems (see Mayer, 1970). Activation of α-receptors may be associated with an inhibition of adenyl cyclase activity in some tissues, for example, pancreatic islets and adipose tissue (Turtle & Kipnis, 1967). If this were true for the mullet gill then it might be expected that adrenaline given together with an α-receptor blocking agent would increase cAMP levels more than

with adrenaline alone. That is, the nucleotide concentration in the respiratory cells would rise by excitation of β-receptors, while the levels in the chloride cells would not fall, due to blockade of stimulation of α-receptors. Although when adrenaline was given with phentolamine the cAMP levels rose somewhat higher than with adrenaline alone, the difference was not significant. This does not necessarily mean the expectation is unjustified, but may reflect the paucity of chloride cells in the gills.

The increase in cAMP content consequent on anaesthetization, with or without control injections may be due to the stressful release of endogenous catecholamines from the chromaffin tissue (Mazeaud, 1964; Nakano & Tomlinson, 1967). Propranalol given alone reduced the levels of nucleotide in gills compared to the injected controls, however, the levels did not fall to those found in fish killed instantly by liquid nitrogen. This may mean that substances which affect adenyl cyclase, other than catecholamines are released by stress, or alternatively adrenaline is released and acts to raise cAMP levels before the antagonist has penetrated to the gills.

Our intention in these experiments was to answer the question whether or not permeability changes in gills following adrenaline involved adenyl cyclase. We have used a high concentration of adrenaline (5 μg/fish) and measured the cAMP concentrations 30 min afterwards. Our reasons for this are as follows. The flux studies reported by Pic *et al.* (1972) used the same conditions and the effects on sodium, chloride and water fluxes were fully developed at 30 minutes. It was also found that control injections caused a three-fold increase in the circulating adrenaline levels in small mullet (Pic, unpublished observations), so that adrenaline levels had to be raised above those of the injected controls. Finally there is no information on the efficiency of absorption of adrenaline in mullet from an intraperitoneal site.

We suggest that the results show clearly that adrenaline increases cAMP content of gills. Furthermore, this effect is mediated by β-receptors, probably situated on the respiratory epithelial cells, and is associated with an increase in diffusional and osmotic permeability for water. Thus the gill epithelium shows responses to adrenaline not unlike other epithelial structures, for example frog skin (Rajerison *et al.*, 1972) There is insufficient evidence at present to know if the relationship between permeability increase and cAMP concentration is a causative one.

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