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SHORT COMMUNICATION

ADRENALINE AND BRANCHIAL NERVE STIMULATION INHIBIT ^{45}Ca INFLUX INTO THE GILLS OF RAINBOW TROUT, *SALMO GAIRDNERI*

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The tissues in the core of the gill filaments of *Salmo gairdneri* are innervated by cholinergic and adrenergic nerves (Donald, 1984, 1986, 1987). It is unlikely that the nerves are involved in vasomotor regulation, because the wall of the filament venous sinus of *S. gairdneri* is a simple endothelium, lacking any muscular elements (Donald, 1986). Since many of the nerves are closely associated with the filament epithelium (Donald, 1986), they might be involved in the regulation of some aspect of epithelial function.

Adrenaline and acetylcholine affect the movement of water and electrolytes across the branchial epithelium in teleosts (see Isaia, 1984; Rankin & Bolis, 1984) and chloride cell function in opercular membranes (see Zadunaisky, 1984). Autonomic nerves have been shown to be involved in the regulation of water and electrolyte transport in tissues of other vertebrates, e.g. mammalian intestine (Sjövall, 1984; Tapper, 1983). The possibility of neural involvement in epithelial function in fish is suggested by the finding (Mayer-Gostan & Hirano, 1976) that transection of the glossopharyngeal and vagus nerves disturbed water and electrolyte balance in the eel, *Anguilla anguilla*. Although these authors attributed the effect to changes in ion and water fluxes in the stomach, it is possible that concomitant changes in fluxes through the gills may have contributed to the overall result.

In this paper the possibility of autonomic mechanisms affecting an aspect of epithelial function has been examined using the uptake of ^{45}Ca into the gills as a marker of epithelial activity. The inhibitory effects of adrenaline and stimulation of the branchial nerves on the influx of ^{45}Ca into the gills of *S. gairdneri* are reported.

Rainbow trout, *Salmo gairdneri* Richardson (100–300 g), were obtained from the Silverstream Trout Farm, Buxton, Victoria. The fish were kept in a recirculating

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freshwater tank at 17–18 °C with a sand/charcoal filter, and a Ca^{2+} concentration of between 0·9 and 1·1 mmol l^{-1}. The fish were equilibrated for 1 week before experimentation, and were held for no longer than 4 weeks.

The experiments were performed using ^{45}Ca, as $CaCl_2$ (0·17 μCi μmol^{-1}, Amersham) and [^{3}H]mannitol (New England Nuclear). Mannitol was used as a marker of external space to enable the calculation of the amount of calcium taken up by the gills. Preliminary experiments showed that sufficient ^{45}Ca influx occurred in 5 min and that the influx of mannitol was negligible over this time.

Trout were anaesthetized with MS 222 (tricaine methane sulphonate, Rural Chemicals) dissolved in the freshwater medium, and the heart and bulbus arteriosus were exposed by a ventral midline incision. A loose cotton ligature was placed around the bulboventricular junction. The bulbus arteriosus was cannulated with polyethylene tubing (i.d. 0·86 mm, o.d. 1·27 mm, Dural Plastics) *via* an incision in the ventricle, and was perfused at constant pressure with heparinized (5 i.u. ml^{-1}) Hepes-buffered physiological saline [composition in mmol l^{-1}: NaCl, 138·0; KCl, 5·0; $MgCl_2.6H_2O$, 0·5; $CaCl_2$, 2·5; glucose, 5·6; Hepes (Sigma), 10·0; NaOH, 7·0; bubbled with O_2] that had been passed through a 0·2 μm filter (Sartorius). Immediately after commencement of perfusion, irrigation of the gills with recycled fresh water was begun. The perfusion rate was measured using a photoelectric drop-counting apparatus and was recorded on a Grass 79D polygraph. The perfusion rate was initially adjusted by altering the height of the reservoir, until it approximated the *in vivo* cardiac output of rainbow trout, approximately 37 ml kg^{-1} min^{-1} (Wood & Shelton, 1980).

After 10 min of perfusion, the first and second gill arches from both sides were removed and placed in a dish of physiological saline. The branchial nerve supply of the first and second gill arches from both sides was exposed and harnessed with a fine cotton ligature. Each arch was then attached to a jig and the nerve was passed through platinum ring electrodes in a small reservoir of physiological saline attached to the jig. The gill on its jig was immersed for 5 min in 100 ml of magnetically stirred distilled water containing 20 μCi of ^{45}Ca and 5 μCi of [^{3}H]mannitol. The nerves to the gills from one side only were stimulated with 1 ms, 20 V pulses at 10 Hz delivered from a Grass S9 stimulator throughout the period of immersion. The contralateral gills acted as an unstimulated control. Prior to addition of the gills, isotope activities in the bath were measured. The gill arches were placed in 4 ml of 0·1 mol l^{-1} nitric acid for 24 h to elute the radioactivity. A 0·5 ml sample was added to 10 ml of scintillation cocktail (Insta-gel, Packard) for determination of ^{45}Ca and ^{3}H activities (L'Annunziata, 1979). The effect of nerve stimulation was also tested in trout which had received an intraperitoneal injection of the adrenergic neurone-blocking drug, bretylium (1 mg kg^{-1}), 2 h before the experiment.

In a further group of experiments, the effect of adrenaline on the influx of ^{45}Ca into the gill was tested. The right-side gills were perfused with physiological saline for 10 min and the left-side for a further 10 min with physiological saline containing adrenaline (10^{-6} mol l^{-1}). The gills from one side were placed together in 100 ml of

Table 1. *Effect of nerve stimulation (10 Hz, 1 ms, 20 V) and adrenaline (10^{-6} mol l^{-1}) on the influx of ^{45}Ca (μmol h^{-1} g gill mass^{-1})*

Control	Influx	Test	Influx
No stimulation	0·34 ± 0·05	Stimulation	0·19 ± 0·04 $N = 9$, $0{\cdot}05 > P > 0{\cdot}01$
Bretylium treatment (1 mg kg^{-1})			
No stimulation	0·33 ± 0·05	Stimulation	0·27 ± 0·04 $N = 5$, NS, $P = 0{\cdot}515$
Adrenaline treatment (10^{-6} mol l^{-1})			
Ringer's solution	0·32 ± 0·03	Adrenaline	0·14 ± 0·03 $N = 6$, $0{\cdot}002 > P > 0{\cdot}001$

Comparison of control and test by Student's *t*-test. Mean ± S.E.

water containing ^{45}Ca and [^{3}H]mannitol for 5 min, and then placed in 0·1 mol l^{-1} nitric acid. The samples were processed as in the nerve stimulation experiments.

The amount of ^{45}Ca entering the gills will be the total amount in and on the gills minus the expected amount in the external water adhering to the gills. Since the external water space is marked by the amount of ^{3}H, the amount of ^{45}Ca entering the gills can be determined by:

$$^{45}Ca(\text{inside}) = {}^{45}Ca(\text{total}) - (R \times {}^{3}H) ,$$

where R is the ratio of ^{45}Ca and ^{3}H and in the bath sample.

The influx of ^{45}Ca was expressed as μmol Ca^{2+} h^{-1} g gill wet mass^{-1}.

The ^{45}Ca influxes are shown in Table 1. Stimulation of the branchial nerves throughout the 5 min exposure to ^{45}Ca significantly reduced the influx of ^{45}Ca into the gills, compared with the unstimulated contralateral gills. In preparations taken from fish injected 2 h previously with bretylium, there was no significant difference in ^{45}Ca influx between stimulated and unstimulated gills (Table 1).

Addition of adrenaline (10^{-6} mol l^{-1}) to the perfusion fluid of the test gill arches significantly reduced the influx of ^{45}Ca compared with the influx observed in the contralateral control gills perfused with normal physiological saline (Table 1).

In the present experiments the inhibition of calcium influx by adrenaline was in contrast to the results of Payan *et al.* (1981), who found that adrenaline increased the uptake of calcium into the perfused head of rainbow trout. The conflicting results may be due to differences in experimental design. In the perfusion experiments of Payan *et al.* (1981), adrenaline infusion would have altered the haemodynamic state of the gills, which in turn could have altered the 'functional surface area' of the gills available for molecular exchanges. In the nonperfused gill preparations used in this study, the inhibition of calcium influx by adrenaline and nerve stimulation was considered to be independent of their effect on gill haemodynamics.

Perry & Wood (1985) provided evidence that Ca^{2+} uptake into the gills of freshwater fish is mediated by active transport – it showed saturable Michaelis–

Menten kinetics – and they concluded that either a Ca^{2+} carrier and/or a selective ion channel was involved. However, there is no clear evidence to show which epithelial cells are involved in the Ca^{2+} movement. Ultrastructural studies have shown that the chloride cells are the only cells in the gills that appear to be morphologically specialized for active transport, since they are the only epithelial cells to have the numerous mitochondria, surface microvilli and cytoplasmic tubulovesicular extensions that characterize ion-transporting cells (see Laurent *et al.* 1985).

Substantive evidence that neurotransmitters can affect chloride cell function comes from the studies on isolated opercular membranes of teleost fish, in which adrenaline has been shown to inhibit the transport of chloride by the chloride cells (see Zadunaisky, 1984). It is possible that in this study nerve stimulation and adrenaline were affecting the ability of chloride cells in the filament epithelium to inhibit the uptake of ^{45}Ca into the gills. Thus, in addition to vasomotor regulation, autonomic mechanisms could be involved in the regulation of molecular exchanges across the filament epithelium of the gills.

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