THE TRANSPORT OF NITRATE BY THE ISOLATED PERFUSED RECTAL GLAND OF *Squalus acanthias*.

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In experiments previously reported in the bulletin (Silva et al., MDIBL Bulletin 23:47, 1983) we observed that when chloride was replaced by nitrate in the perfusate the rate of secretion of sodium was substantially larger than that of chloride suggesting that sodium was being transported together with nitrate. In the present series of experiments we explored this this question more completely using a direct measurement of nitrate concentration in rectal gland secretions.

Rectal gland perfusions were performed as previously described (Silva et al., Am J Physiol 233:F298, 1977). All perfusions contained dibutyryl cyclic AMP 5×10^{-5}M and theophylline 2.5×10^{-4}M in order to stimulate maximal active secretion. Sodium concentration in the rectal gland secretion was measured by flame photometry. Chloride was measured by amperometric titration. Nitrate was measured using an ion selective electrode (Radiometer, Copenhagen). The electrode was calibrated against a series of nitrate solutions of the same electrolyte composition as that of the rectal gland secretion. The slope of the electrode was -50.1±0.72 mV per decade. Concentrations of chloride ranging from 0 to 500 mEq/l in the calibration solution resulted in an underestimation of the concentration of nitrate only when the latter was less than 5 mEq/l. Since the concentration of nitrate in the rectal gland secretion in the experiments reported was always higher than 5 mEq/l, the possibility of chloride interference with the nitrate electrode was negligible.

The concentration of nitrate in the rectal gland secretion, measured directly by the nitrate electrode did not differ significantly from that calculated from the difference between the concentration of sodium and chloride (slope 1.0±0.0, $p<0.001$). It is therefore possible to estimate nitrate concentration reasonably accurately in experiments on nitrate excretion in which nitrate cannot be measured directly.

When nitrate replaced all chloride in the perfusate, stimulated glands secreted nitrate at 512±71 μEq/h/g (n=9), about 30% of the rate at which they secreted chloride (1841±170) when shark Ringer solution containing chloride as the chief anion was used to perfuse the same glands. These experiments confirmed the capacity of the rectal gland to transport nitrate, though at a lower rate than chloride, and suggested that nitrate might interact with the Na: K: 2 Cl carrier.

The nature of this interaction was then investigated by kinetic analysis of experiments in which the effect of variations in nitrate concentrations in the perfusate upon nitrate secretion was studied, at different levels of perfusate chloride (25, 50 and 100 mM). In these experiments, chloride concentration in the perfusate was held constant, and gluconate served as a substitute for nitrate, to bring total anion concentration in the perfusing solution to 290 mEq/l. The results are summarized in Table I.

Hill plots of the data shown in Table I give Hill coefficients of 0.96±0.31, r=0.87, 0.75±0.13, r=0.96 and 0.73±0.15, r=0.94, for 25, 50 and 100 mM experiments. None of these coefficients was significantly different from 1 suggesting that nitrate interacts with its transport system at a single site.

Table I
Effect of chloride on nitrate secretion by the isolated perfused rectal gland

Nitrate concentration	Chloride concentration 25 mM	50 mM	100 mM
10	137.3±53.7	352.7±104	169.7±22.0
15	218.1±67.8	333.8±66.4	63.4±17.9
20	381.9±114.7	422.6±95.0	126.0±45.0
50	382.9±108.0	489.7±103.8	232.7±84.6
100	582.9±132.1	668.3±101.8	236.5±79.8
190	603.5±135.8	891.5±137.8	463.6±129.7

Values are μEq/h/g, mean ± SEM. N=4,3 and 4 for the 25, 50 and 100 mM experiments. Concentrations are mEq/L.

A Lineweaver-Burk plot of the data using the 50 and 100 mM concentrations of chloride indicates that chloride inhibits competitively the secretion of nitrate by the rectal gland.

Bumetanide 10^{-6}M had no effect on the secretion of nitrate at concentrations of chloride of 0 or 10 mM. At higher concentrations of chloride in the perfusate, on the other hand, bumetanide inhibited nitrate secretion by as much as 60%.

These experiments show that chloride has an interesting dual effect on the secretion of nitrate. At concentrations up to 50 mM, chloride clearly enhances nitrate secretion. At a higher concentration (100 mM), on the other hand, chloride inhibits nitrate secretion in a competitive manner. The observation that nitrate interacts with its transport system at only one site coupled with the dual effect of chloride on its secretion suggests that the interaction of nitrate is limited to one of the two chloride binding sites of the 2Cl: Na: K cotransporter, probably the low affinity binding site. When chloride is present at low concentrations, it occupies the high affinity binding site and increases the rate of operation of the carrier. At high concentrations it inhibits nitrate secretion by displacing it from the other binding site. The additional observation that bumetanide inhibits the secretion of nitrate only in the presence of chloride suggests that bumetanide interacts with the cotransport system at only one of the chloride binding sites, the one that cannot be occupied by nitrate.

The demonstration that nitrate can be transported by the Na: K: 2Cl cotransporter in rectal gland cells suggests that this mechanism may serve to move nitrate and perhaps other anions across plasma membranes of a variety of cells possesing the capacity for chloride cotransport.

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