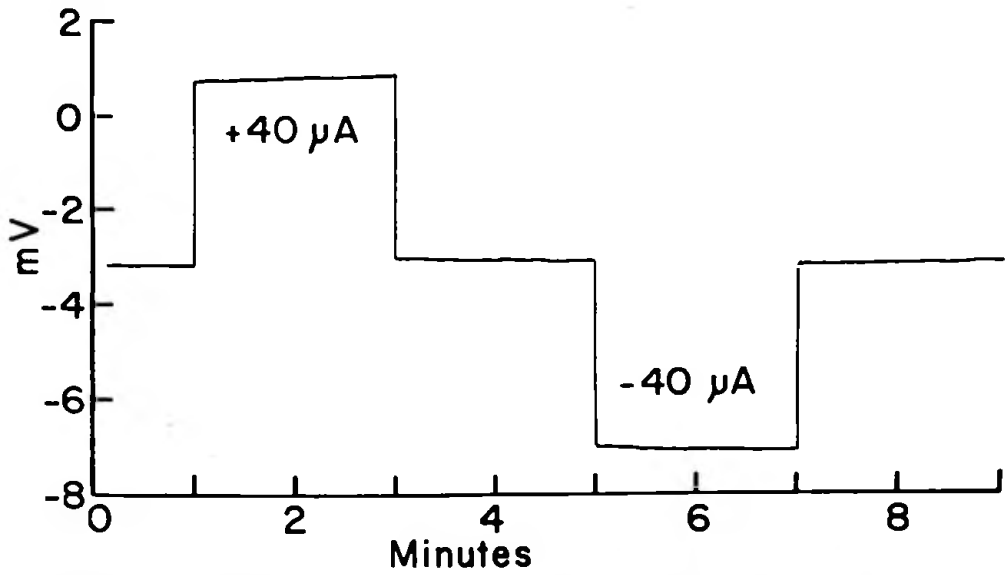

Figure 2. The effect of passing a square current pulse across dogfish gastric mucosa. No long time-constant transient responses (τ = 30 sec.) can be found. Current density 40 μA for 3.14 cm^2 tissue.

COUPLED NaCl INFLUX ACROSS THE MUCOSAL MEMBRANE OF *Pseudopleuronectes americanus* INTESTINE: EFFECTS OF FUROSEMIDE AND CYCLIC AMP

Raymond A. Frizzell, Philip L. Smith and Michael Field, Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA; Department of Medicinal Chemistry and Pharmacology, Northeastern University, Boston, MA; and Departments of Medicine and Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL

Previous studies of ion transport by flounder small intestine under short-circuit conditions suggested an obligatory interaction between the absorptive fluxes of Na and Cl (Field, Karnaky, Smith, Bolton and Kinter J. Membrane Biol. in press). Support for this notion rests on the observations that replacement of either Na or Cl in the bathing media with nontransported ions abolishes both electrolyte absorption and the spontaneous (serosa-negative) transepithelial electrical potential difference (PD). In addition, ouabain reduces active Cl absorption and the PD to zero when added to the serosal bathing solution. Thus, coupling between Na and Cl absorption appears to result from a direct interaction of both ions with cellular transport mechanism(s).

To reconcile these findings with the fact that the rate of active Cl absorption exceeds that of Na under short-circuit conditions, Field et al. (J. Membrane Biol. in press) proposed that one-for-one transcellular NaCl transport might be obscured by the perm-selective properties of the paracellular pathway. In essence, much of the Na transported into the lateral intercellular spaces could recycle to the mucosal solution via Na-selective tight junctions, thereby reducing transepithelial Na transport to a fraction of that for Cl. According to this model, the processes responsible for coupled, transcellular NaCl transport would be similar to those of rabbit gallbladder (Frizzell, et al. J. Gen. Physiol. 65:769, 1975) and small intestine

(Nellans, et al. Am. J. Physiol. 225:467, 1973), wherein the obligatory coupling between Na and Cl absorption results from the presence of a neutral uptake process at the mucosal membrane which mediates t entry of Na and Cl from the mucosal solution into the cell in a one-for-one manner.

In the present study, direct determinations of unidirectional Na and Cl influxes from the mucosal sol tion into the epithelium (J_{me}) disclose the presence of a coupled NaCl influx process at the mucosal membrane of flounder intestine. This process appears to be solely responsible for Na and Cl absorption a is inhibited by furosemide and cyclic AMP.

Mucosal sheets, free of muscle, were prepared as previously described (Nellans et al. Am. J. Physiol. 226:1131, 1974) and mounted in a chamber permitting ready access to the mucosal surface of the tissue. U directional influxes of Na and Cl (J^{Na}_{me} and J^{Cl}_{me}) were determined by isotopic techniques as described by Schultz et al. (J. Gen. Physiol. 50:1241, 1967) with ^{3}H-PEG (1000 MW) as the extracellular marker. All influx measurements were carried out under short-circuit conditions (Frizzell and Turnheim, J. Membrane Bi in press) with appropriate correction for fluid resistance. Tissues were preincubated 20-30 min with mucosal and serosal media whose composition was identical to that subsequently employed for the influx determination. The solutions were bubbled with 1% CO_2 in O_2 and were maintained at 15°C with a thermo-electric cooling plate (Cambion, Cambridge, Mass.), the temperature of the bathing media being continuousl monitored with a thermistor probe.

Several preliminary experiments indicated that the uptakes of ^{22}Na or ^{36}Cl were linear with time for a least one min, and that ^{3}H-PEG was an adequate marker of the extracellular space during this interval. Therefore, reliable estimates of J^{Na}_{me} and J^{Cl}_{me} were obtained using a 45 sec exposure of the mucosal sur-face to isotopic media.

Other preliminary studies indicated that furosemide rapidly inhibited the short-circuit current acros flounder intestine when added to the mucosal solution alone. The effect of various furosemide concentrat on I_{sc} was explored to select a maximally-effective level of the diuretic (Figure 1). Near-complete inhibition of I_{sc} was obtained at a concentration of 10^{-3} M. The decline in I_{sc} produced by addition furosemide to the mucosal solution was complete within one min, suggesting a direct interaction with the process(es) responsible for Na and/or Cl transport across the mucosal membrane.

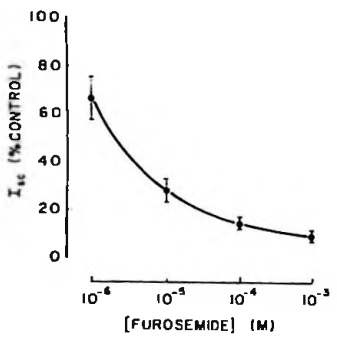

Figure 1. Short-circuit current as a function of mucosal solution furosemide concentration. Each point represents the mean ± SEM of 7 determinations.

The effects of furosemide and Na-free media on J^{Cl}_{me}, short-circuit current, I_{sc}, and tissue conductan G_t, are given in Table 1. The inhibition of J^{Cl}_{me} and I_{sc} elicited by furosemide or Na-free solutions were approximately equal. In addition, the inhibitory effect of furosemide on J^{Cl}_{me} was abolished in the absence of Na. Similar results were obtained when the effects of furosemide or Cl-free media on Na influ were determined (Table 2). Exposure to furosemide or Cl-free solutions decreased J^{Na}_{me} to the same exten

Table 1

EFFECT OF FUROSEMIDE AND Na-FREE MEDIA ON CHLORIDE

INFLUX IN FLOUNDER INTESTINE

	J^{Cl}_{me}	I_{sc}	G_t
Control	8.1 ± 0.5	-4.0 ± 0.5	24 ± 1
+1 mM Furosemide	4.3 ± 0.5*	-0.6 ± 0.2*	25 ± 2
Na-free	5.2 ± 0.5*	-0.3 ± 0.1*	6 ± 1*
Na-free +1 mM Furosemide	4.3 ± 0.8*	-0.4 ± 0.1*	9 ± 1*

All values expressed in $\mu Eq/cm^2$, hr except G_t in $mmhos/cm^2$. Tissues preincubated in Na-free media for 30 min prior to influx determination and with furosemide added to the mucosal solution alone for 5 min prior to the influx measurement. The composition of teleost Ringer (containing 20 mM HCO_3) given by Field and Smith (MDIBL Bull., Vol. 15) except that a half-normal Cl Ringer was employed as the control solution throughout in order to reduce diffusional Cl fluxes while maintaining near-normal values of I_{sc} (Field, et al., MDIBL Bull., Vol. 16). In this solution Cl concentration was reduced to 75 mM by isosmotic replacement with SO_4 and mannitol. Na-free media prepared by substituting choline Cl and $(Tris)_2\ SO_4$ for NaCl and Na_2SO_4, and choline HCO_3 for $NaHCO_3$. All values are mean ± SEM from 8 determinations, * different from control value, $p < 0.05$.

Table 2

EFFECT OF FUROSEMIDE AND Cl-FREE MEDIA ON SODIUM

INFLUX IN FLOUNDER INTESTINE

	J^{Na}_{me}	I_{sc}	G_t
Control (15)	22 ± 1	-3.1 ± 0.5	26 ± 2
+1 mM Furosemide (11)	17 ± 1*	-0.5 ± 0.1*	27 ± 2
Cl-free (12)	15 ± 1*	0.5 ± 0.2*	20 ± 2
Cl-free +1 mM Furosemide (8)	16 ± 2*	0.4 ± 0.1*	24 ± 3

All values in $\mu Eq/cm^2$, hr except G_t in $mmhos/cm^2$. Tissues preincubated in Cl-free media for 30 min prior to the influx determination and with furosemide in the mucosal solution alone for 5 min prior to the influx measurement. Cl-free media prepared by substituting Na_2SO_4 and mannitol for NaCl. All values are mean ± SEM, the number of observations is given in parentheses, * different from control value, $p < 0.05$.

Again, furosemide did not affect J^{Na}_{me} in the absence of Cl. These results strongly suggest that approxi mately 4-5 μEq/cm^2 hr of the influxes of Na and Cl are coupled and that the inhibitory effect of furosemi is restricted to the coupled entry process. It is unlikely that these results are due to changes in the electrical potential difference across the mucosal membrane, which would alter Na and Cl influxes in an opposite manner, or to alterations of tissue conductance since furosemide had no effect on G_t.

Previous studies (Field et al. MDIBL Bull. Vol. 16 and unpublished observations) indicated that cyc AMP and theophylline reduce I_{sc} and inhibit Cl absorption by increasing the undirectional flux of Cl f serosa-to-mucosa (J^{Cl}_{sm}). In other epithelia where coupled NaCl influx processes have been described, cyc AMP reduces Cl absorption by inhibiting the coupled entry process which is manifest as a decrease in muco to-serosa Cl flux, J^{Cl}_{ms} (Frizzel et al. J. Gen. Physiol. 65:769, 1975 and Nellans et al. Am. J. Phys 226:1131, 1974). However, Field et al. (MDIBL Bull. Vol. 16) have observed a cyclic AMP-mediated increa in transepithelial Cl permeability which could obscure any effect of this agent on J^{Cl}_{ms}. Therefore, fur semide was employed in evaluating the effect of cyclic nucleotides on J^{Cl}_{me} in flounder intestine since furosemide acts as a specific inhibitor of coupled NaCl influx. These results are presented in Table 3.

Table 3

EFFECT OF FUROSEMIDE AND DBC PLUS THEOPHYLLINE

ON CHLORIDE INFLUX IN FLOUNDER INTESTINE

	J^{Cl}_{me}	I_{sc}	G_t
Control	8.9 ± 0.4	-4.2 ± 0.7	27 ± 2
+1 mM Furosemide	4.8 ± 0.3*	-0.3 ± 0.1	25 ± 1
+0.25 mM DBC +5 mM Theophylline	7.5 ± 0.5	-0.9 ± 0.3*	28 ± 1
+1 mM Furosemide +0.25 mM DBC +5 mM Theophylline	6.0 ± 0.6*	0.2 ± 0.1*	29 ± 2

All values expressed in μEq/cm^2, hr, except G_t in mmhos/cm^2. Tissues were preincubated in DBC plus theophylline added to serosal media for 30 min prior to the influx determination, and preincubated with furosemide added to the mucosal solution 5 min prior to the influx measurement. All values are mean ± SEM from 8 determinations, * different from control value, $p < 0.05$.

Dibutyryl cyclic AMP (DBC) and theophylline reduced I_{sc} as previously reported, but the apparent decrea in J^{Cl}_{me} was not statistically significant. However, the results obtained using furosemide in combinatio with DBC and theophylline suggest that the coupled NaCl influx process is inhibited by cyclic nucleotides. Under control conditions furosemide reduced J^{Cl}_{me} by 4.1 μEq/cm^2 hr, but in the presence of DBC and theo- phylline, furosemide inhibited J^{Cl}_{me} by only 1.5 μEq/cm^2 hr. Thus, DBC and theophylline partially inhibi coupled NaCl influx leading to a reduction in Cl absorption, as previously observed.

The results of this study indicate that absorption of Na and Cl by flounder intestine can be attributa to the presence of a coupled NaCl influx process at the mucosal membrane which is inhibited by furosemide. This appears to be a neutral mechanism involving one-for-one uptake of Na and Cl from the mucosal solutio

since the inhibitions of J_{me}^{Cl} and J_{me}^{Na} elicited by Na- or Cl-free media or by furosemide are approximately equal. Humphreys (Am. J. Physiol. 230:1517, 1976) has reached similar conclusions with regard to the inhibition of NaCl absorption elicited by furosemide using in vivo rat ileum. Indeed, neutral NaCl transport processes have been suggested for gastrointestinal epithelia of a variety of species, ranging from arthropods through mammals, and direct evidence for a coupled NaCl influx process at the mucosal membrane has been obtained for rabbit gallbladder and small intestine (Frizzel et al., J. Gen. Physiol. 65:769, 1975 and Nellans et al., Am. J. Physiol. 225:467, 1973). Thus, as suggested for rabbit gallbladder, the energy required for active Cl absorption by flounder intestine may be derived from the Na gradient across the mucosal membrane. Information on the electrical potential profile and cellular Na and Cl activities is necessary to evaluate this possibility. In agreement with the findings reported here, cyclic nucleotides inhibit neutral NaCl influx mechanisms in gallbladder and small intestine; in flounder intestine, an increase in transepithelial Cl permeability also occurs.

Finally, these results lend further support to the notion (Field et al., J. Membrane Biol. in press) that a dissociation of neutral transcellular NaCl transport occurs at the level of the paracellular pathways leading to a preponderance of transepithelial Cl over Na absorption under short-circuit conditions. In this regard, it is of interest that a ouabain and furosemide-inhibitable active Cl pump has been postulated for the thick ascending limb of the loop of Henle which also displays a lumen-positive PD (Burg and Green, Am. J. Physiol. 224:659, 1973 and Burg, Kidney Internat. 9:189, 1976). The possibility of neutral NaCl transport modified by the paracellular pathway seems worthy of investigation in this nephron segment in view of the common physiologic and pharmacologic characteristics of these epithelia.

Supported by grants from the NIH (AM-18199 and AM-21345).

FURTHER EVIDENCE THAT *Squalus acanthias* LACKS ANGIOTENSIN II ACTIVATED VASCULAR RECEPTORS

D. F. Opdyke, R. F. Holcombe and D. W. Wilde. Department of Physiology, CMDNJ-New Jersey Medical School, Newark, New Jersey

Previous work from our laboratory reported that perfusion with perfusates containing angiotensin II (AII) did not result in an increase in blood flow resistance in the isolated dogfish gut preparation (Opdyke and Holcombe, MDIBL Bulletin 16:84-86, 1976). However, marked increase in flow resistance resulted when either epinephrine or norepinephrine was perfused in the same preparations. These results were interpreted as additional evidence that the pressor response to AII observed in the intact dogfish results solely from release of adrenergic catecholamines by AII (Opdyke and Holcombe Am. J. Physiol. 231:1750-1753, 1976). We have extended this study by observing the effect of AII, norepinephrine and isoproterenol on blood flow resistance in the gill and systemic circulations of dogfish whose hearts were replaced by a calibrated mechanical pump which perfused the circulation of the otherwise intact fish at known rates of flow.

Methods

Male and female dogfish averaging 3.2 Kg in weight were anesthetized with 20 mg/Kg pentobarbital sodium through an indwelling catheter introduced into the dorsal aorta via the caudal artery. The catheter served also for the recording of dorsal aortic pressure (DAP). The fish were placed belly up in a V-shaped trough and the gills bathed with cold running sea water through tubes inserted into both spiracles. The pericardial sac was carefully opened and, following heparinization (8 mg/Kg), a large polyethylene catheter (PE 280), which was filled with dogfish blood and connected to the perfusion pump, was tied into the conus arteriosus. Another large catheter (PE 320) was introduced into the atrium retrograde from the ventricle and tied