dry wt compared to 632.2 n moles/mg dry wt in P. spiralis (PS) (sum of columns, Table 1). Such a species difference in the pool size of free amino acids may reflect a greater dependence on free amino acids for cell volume regulation in P. spiralis. Of particular significance were the major differences in the pool sizes of the individual free amino acids between species (Table 1). The major free amino acids in C. arenarius were asparagine, proline and alanine which made up 61.6% of the total free amino acid pool. However, in P. spiralis, taurine, aspartic acid, glycine and arginine comprised 88.1% of the free amino acis. Of these, it is interesting that the levels of proline, alanine, taurine and glycine are most commonly reported to be affected during regulatory volume decrease in marine invertebrates. Hence, it may be expected that C. arenarius utilizes asparagine, proline and/or alanine during regulatory volume decrease, whereas in P. spiralis taurine, glycine and/or arginine would be most available. This work was supported by NIH grant 5 RO1 AM15973-09.

INHIBITION OF CYCLIC AMP-STIMULATED CHLORIDE TRANSPORT IN THE RECTAL GLAND OF SQUALUS ACANTHIAS BY A RELATED SERIES OF "LOOP" DIURETICS

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Coupled NaCl cotransport systems have been suggested to occur in a number of epithelial tissues (for review, see Frizzell, Am. J. Physiol. 236:F1, 1978) including dogfish rectal gland, where they may contribute to active Cl transport across the tissue layer. In many of these cases active Cl movements are susceptible to inhibition by the diuretic furosemide. However, high concentrations (~1 mM) of this drug are frequently needed to block transport, and this has raised questions as to the specificity of furosemide's action. Recently, a series of benzoic acid derivatives related to furosemide has been synthesized (Neilsen and Feit, Am. Chem. Soc. Symp., 83:12, 1978), several of which have been shown to be potent diuretics in vivo in dogs. It has also been shown that representatives of this group of agents are highly active as specific inhibitors of a cyclic AMP activated Cl-dependent NA^+ plus K^+ contransport system in avian erythrocytes (Palfrey, Feit and Greengard, Am. J. Physiol., in press, 1979). The relative potency of these compounds in erythrocytes correlated well with their diuretic efficacy, measured as Na^+ excretion, in dogs; some analogs being totally ineffective in both systems. We tested various members of this series on total fluid and Cl secretion by the perfused dogfish rectal gland with the purpose of: (a) comparing the relative potency of the compounds in this tissue with that already established in erythrocytes, and (b) finding inhibitors that would be more potent, and possibly more specific, than furosemide.

Dogfish rectal glands were perfused and volume and Cl analyses performed as previously described (Soloman et al., Bull. MDIBL., 17:59, 1977). Dibutyryl cyclic AMP (0.05 mM) and theophylline (0.1 mM) were routinely added to the perfusate to obtain maximal secretory rates. The diuretic compounds used in this study were obtained from Dr. P.W. Feit, Leo Pharmaceutical Products, Ballerup, Denmark and their structures are indicated in Table 1. Stock solutions were made up in DMSO at 1000X the final concentration in the perfusion medium; 0.1% DMSO alone was found to have no effect on fluid or electrolyte secretion by the rectal gland. Ten minute collection periods were employed, the usual experimental protocol being 30 min control (no drug), 30 min drug, 30 min control. Occasionally more samples were collected when incomplete effects of a drug or reversal were suspected. The results for total fluid and Cl secretion by the rectal gland are compiled in Table 1.

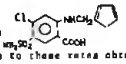
The most potent analog tested was 3-benzylamino-4-phenyl-5-sulfamoylbenzoic acid (Compound I), greater than half-maximal inhibition (IC_50) with this compound being obtained at $< 10^{-6}$ M. This agent was thus more than two hundred times as potent as furosemide ($IC_{50} > 5 \times 10^{-4}$ M). The relative efficacy of the series of compounds closely matched that found previously in the avian erythrocyte (Table 1), although the sensitivity of the rectal gland trans-

TABLE 1

DOSE-DEPENDENCE OF DIURETIC EFFECTS ON FLUID AND CHLORIDE SECRETION FROM PERFUSED RECTAL GLANDS

COMPOUND	R_1	R_2	% Control Secretion Rate [a]								IC_{50} (M) TURKEY ERYTHROCYTES [b]
			3×10^{-7} M		10^{-6} M		10^{-5} M		10^{-4} M	10^{-3} M	
			FLUID	Cl	FLUID	Cl	FLUID	Cl	FLUID Cl	FLUID Cl	
I	$-NHCH_2$	-O-	57.3 (1)	68.6 (1)	30 (2)	28.7 (2)	22 (3)	19.2 (3)	—	—	6.1×10^{-8}
II	$-NH(CH_2)_3CH_3$	-S-	100 (1)	100 (1)	72 (2)	72.5 (2)	22.3 (4)	26 (3)	—	—	8.2×10^{-8}
III (SO_2NH_2 replaced by SO_2CH_3)	$-NHCH_2$	-O-	— [c]		64.4 (2)	—	16.6 (2)	14.5 (2)	—	—	3.7×10^{-7}
IV (Bumetanide)	$-NH(CH_2)_3CH_3$	-O-	100 (1)	100 (1)	84.5 (3)	83.2 (3)	47.8 (6)	43.2 (6)	22 (2) 20 (2)	—	2.5×10^{-7}
V (Piretanide)		-O-	—		100 (1)	100 (1)	87.7 (2)	86.9 (2)	16.3 (2) 14.9 (2)	—	2.8×10^{-6}
VI (-COOH replaced by SO_3Na)	$-NH(CH_2)_3CH_3$	-O-	—		—		69.5 (2)	—	29.2 (3) —	—	4.8×10^{-6}
VII	$-NHCH_2$-(o)-CH_3	-O-	—		—		85.5 (2)	85.5 (2)	33 (1) —	—	1×10^{-4}
VIII (Furosemide)	[d]	[d]	—		—		98 (1) 97 (1) (5×10^{-30}) 70 (2) 63 (1)		35 (1) 31 (1)	—	2.3×10^{-5}
IX	$-NH(CH_2)_3CH_3$	Cl	—		—		94 (1)	—	93 (1)	—	3.9×10^{-5}
X (-COOH replaced by $CH_2NHCH_2C_6H_5$)	$-NH(CH_2)_2CH_3$	-O-	—		—		—		100 (1) 100 (1)	95 (1) 87 (1)	$> 10^{-3}$
XI	$-NH_2$	-O-	—		—		—		—	97 (1) 97.5 (1)	$> 10^{-3}$

[d] The structure of furosemide is



[a] Control secretion rates refers to those rates obtained in the presence of 0.05 mM DbcAMP and 0.1 mM theophylline. In this series of experiments this ranges from 1.11-5.48 (mean 2.71) ml/hr/g wet weight total fluid secretion and from 469-2753 (mean 1259) μeq/hr/g wet weight rectal gland.

[b] Taken from Palfrey, Feit and Greengard, Am. J. Physiol., in press, 1979.

[c] A dash indicates not tested.

port system appears to be approximately 10-fold lower. The same arguments regarding the structural requirements for inhibition of transport that were applied to the erythrocyte system (Palfrey et al., op. cit.), thus also apply to the rectal gland. These can be summarized as follows: (1) the nature of the substitutent at the 3 position on the benzene ring is important. For instance, the unsubstituted amine (Compound XI; Table 1) is totally ineffective as an inhibitor of rectal gland secretion (and of Na plus K contransport in the avian erythrocyte) whereas the substituted amines (Compounds, I, II and III etc). are all effective. Moreover, a comparison of Compounds VII and I reveals that slight structural modification of the side chain (i.e., the addition of a methoxy group to the benzylamino moiety) markedly reduces the degree of inhibitory activity. (2) A phenyl- or phenylthio-substituent at position 4 on the benzene ring is superior to the sister halogenated compound (cf. Compound II and IV with Compound IX). (3) The sulfonamide group at position 5 is not essential for activity, for the corresponding methylsulfonate is about as active (compare Compounds III and IV). (4) An anionic group at position 1 (either carboxyl or sulfonyl, although the former is more potent) appears to be necessary for activity as a corresponding substituted compound (X) is not active.

All compounds acted reversibly in inhibiting Cl transport, as they have been shown to do in the avian erythrocyte. However, the rate of onset and reversal of inhibition were not "instantaneous." Usually full inhibition or washout of all the agents used in this study was not achieved until the second or third collection period (i.e., 10 or 20 min after changing solutions). This lag indicates a finite time for the diuretics to reach their sites of action. Whether this could, for example, mean that these sites are intracellular is at present unknown. Evidence accumulated to date in the avian erythrocyte model suggests that these agents act directly on the transport system at the membrane lelve, and not by interfering with some antecedent cellular metabolic step (e.g., a cyclic nucleotide-dependent protein kinase) which may be essential for cyclic AMP-activated cation cotransport. These agents also had no effect on other membrane transport systems in the erythrocyte, e g, the Na/K pump, at concentrations which fully inhibited Na^+ plus K^+ cotransport. Similarly, preliminary experiments with shark rectal gland homogenates showed no inhibitory effects

of these compounds on Na/K ATPase activity at concentrations which inhibited Cl secretion. It seems reasonable to propose, therefore, that the action of diuretics of this chemical series on rectal gland secretion may be via blockade of the putative NaCl contransport system localized at the serosal surface of the tissue (see Silva et al., Am. J. Physiol. 233:F298, 1977).

As the rank order of potency of these chemically related derivatives is strikingly similar in the inhibition of chloride transport by the rectal gland, cation cotransport by the avian erythrocyte, and salt transport by the dog kidney it suggests these differing tissues may have pharmacologically similar diuretic-binding sites and possibly physiologically similar transport mechanisms. This analogy can be extended to other active Cl transporting tissues where it has already been shown that one of the most effective compounds of the series (bumetanide; Compound IV) potently inhibits a similar process in the amphibian cornea (McGahan et al., J. Pharm. Exp. Ther., 203:97, 1977) and in the squid giant axon (Russell, Ann. N.Y. Acad. Sci., in press, 1979). This agent was also effective in reducing Cl transport in isolated kidney tubules of rat and rabbit (Imai, Eur. J. Pharm., 41:409, 1977) where the site of action was shown to be the thick ascending limb of Henle's loop. In agreement with the present observations this compound was effective at much lower levels than furosemide in these tissues. If these transport systems do involve a common element, the new agents described here may be useful tools to selectively inhibit this component in physiological studies. Aided by NIH grant AM18078 and NSF grant PCM-77-01146.

CELL VOLUME REGULATION IN THE RECTAL GLAND OF SQUALUS ACANTHIAS: EFFECTS OF SODIUM AND CHLORIDE FREE MEDIA AND UREA FREE HYPOTONIC MEDIA

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We have extended our previous studies (Booz et al. Bull. MDIBL 18:23, 1978) of cell volume regulation in slices of the rectal gland of the dogfish shark (Squalus acanthias). The objective of the present work was to determine changes in cell volume and tissue electrolyte content in the rectal gland following aerobic incubation in media rendered free of either sodium or chloride or made hypotonic by the absence of urea.

Slices cut perpendicular to the long axis of the gland (mean thickness 0.3-0.4 mm) were employed. The slices were prepared free-hand by the method of Deutsch (J. Physiol. 87:568, 1936) or by a mechanical slicer, and the fibrous capsule was trimmed off. Slices were incubated in either: (1) standard dogfish Ringers (Forrest et al, Bull. MDIBL 18:10, 1978); (2) sodium free media containing choline; (3) chloride free media prepared by replacement of all chloride with nitrate salts ; (4) urea free hypotonic media. All Ringer's solutions contained ^{3}H-polyethylene glycol (PEG) (1 mg/ml; 1μci/ml) as a marker of the extracellular space. Usually 10-12 slices, each weighing approximately 10 mg. wet wt., were incubated in 2.5 ml saline for 60-120 minutes following gassing with 99% O_2, 1% CO_2. Tissue cations (Na^+ and K^+) were determined by flame photometry, Cl^- by potentiometric titration, and the activity corresponding to tissue PEG was assayed by scintillation spectrometry. Tissue concentration of urea was determined by the method of Fawcett et al. (J. Clin. Path. 13:156, 1960). The means and S.E. for each experimental group were determined and data are expressed in kg H_2O, meq. electrolytes per kg tissue D.W. and μ moles urea per g wet wt. The extracellular space E is given in kg/kg tissue W.W.

The effects of omitting all sodium (choline Ringers) an actual shrinkage (13%) of the tissue was observed compared to normal Ringers with a loss of 0.45 kg H_2O/kg D.W. at 60 minutes and 0.48 kg H_2O/kg D.W. at 120 minutes (both $p < 0.001$). A marked decline in the tissue content of sodium and potassium and a modest decline in tissue chloride occurred. Whereas the calculated intracellular sodium concentration $[Na^+]_i$ decreased markedly (58.2 ± 6.2 to 21.9 ± 4.2, $p<0.01$), $[Cl^-]_i$ declined only slightly (mean 91.3 ± 2.8 vs 84.2 ± 3.6, $p<0.05$) suggesting replacement of intracellular sodium by choline. In contrast to the omission of sodium, the absence of chloride from the media was