Table 4.--$K^+$ induced vascular smooth muscle contraction

| Bath concen. mEq $K^+$/ml | Isolated Arterial Strips % Δ in Resting Tension mg + SE | n |
|---|---|---|
| Saline | -11 ± 6 | 10 |
| 0.0003 | 52 ± 51 | 7 |
| 0.0017 | 90 ± 44* | 6 |
| 0.0067 | 119 ± 23** | 6 |
| 0.0133 | 208 ± 30** | 6 |
| 0.003 (before phentolamine) | 284 ± 98** | 10 |
| 0.003 (after phentolaine) | 212 ± 49** | 9 |

Elasmobranch saline contains, in addition to NaCl and urea, small amounts of KCl, CaC12 and NaHC03.
*P < 0.05. **P < 0.01.

two lines, Table 4), the strips were exposed to an increment of 0.003 mEq $K^+$/ml. A highly significant increase in tension was observed. After saline washing and re-equilibration, phentolamine (0.17 mg/ml) was added to the bath and $K^+$ added as before. This resulted once again in a highly significant increase in tension, but there was no significant difference between the tension generated before and after phentolamine treatment.

Phentolamine did not inhibit the direct contractile effect of $K^+$ on arterial strips in the isolated muscle bath. However, phentolamine did inhibit the in vivo pressor response to $K^+$. The pressor response, therefore, cannot be due solely to a direct vasoconstrictor effect of $K^+$ on resistance vessels. Adrenergically-mediated factors must be involved also. The released E and NE would augment any direct vasoconstrictor effect of $K^+$ by further increasing arterial blood flow resistance and decreasing venous capacitance (which would increase venous return). E also increases myocardial contractility and, in conjunction with an increase in venous return, increases ventricular stroke volume, as evidenced by the greater arterial pulse pressure which invariably accompanies pressor responses occurring after $K^+$, E, NE and angiotension II administration. Phentolamine blocks alpha adrenergic catecholamine-mediated increase in arteriolar resistance and decrease in venous capacitance, but not an increase in myocardial contractility which is beta adrenergically-mediated. Lacking alpha adrenergic effects, the direct contractile effect of $K^+$ along on resistance and capacitance blood vessels is not great enough, apparently, to generate a pressor response.

## METHYLAMINE SECRETION BY ISOLATED PERFUSED RENAL TUBULES OF THE WINTER FLOUNDER, PSEUDOPLEURONECTES AMERICANUS

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### INTRODUCTION

Methylamine (MA) is a weak organic base which at physiological pH exists predominantly in protonated form. Its use as an indicator of intracellular pH is based on the premise that the lipid portion of the cell membrane is permeable to the

uncharged species alone. Others found that giant barnacle muscle fibers are permeable to the ionic form of MA (Boron and Ross, Am. J. Physiol. 231(3): 799-809, 1979). The situation in the kidney, which possess a secretory mechanism for organic cations, is not known. Clearance studies on the Winter Flounder indicated that the kidney secretes MA (Booz, MDIBL Bull. 18:25). The primary objective of the present study on isolated perfused renal tubules was to establish whether MA is handled as an organic cation. In addition, preliminary experiments were carried out to ascertain the role played by pH gradients, as suggested for the transepithelial transport of weak electrolytes (Hogben et al., J. Pharmacol. Exp. Ther. 125: 275-282, 1959).

METHODS

Renal tubules were prepared and selected for in vitro perfusion as described elsewhere (Beyenbach et al., This Bulletin). Composition of bath and perfusate medium was: 145 mM NaCl, 20 mM $NaHCO_3$, 5 mM KCl, 1.65 mM $Na_2HPO_4$, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 0.3 mM $NaH_2PO_4$, gassed with 1% $CO_2$/99% $O_2$ (pH 7.8). MA secretion was measured with ($^{14}$C)MA (New England Nuclear). Radioactivity was assayed by liquid scintillation spectrometry.

RESULTS

Net secretory flux. No MA was present in the initial perfusate. 50 $\mu$M ($^{14}$C)MA was present in the bath. Results are summarized in Table 1. Flux was linearly related to collection rate ($V_c$) at higher rates. The mean ratio of MA concentration in the final perfusate to bath is greater than one, indicating that MA was secreted. This ratio exhibited good correlation with $V_c$ over the entire range of collection rates (the mean coefficient of determination for the 4 tubules = 0.77 $\pm$ 0.05 SD).

Table 1.--Methylamine secretion by isolated perfused renal tubules

| | Net secretory flux |
|---|---|
| tubule length, mm | 0.949 $\pm$ 0.208 |
| $V_c$, nl $\cdot$ min$^{-1}$ $\cdot$ mm$^{-1}$ | 7.17 $\pm$ 1.23 |
| J, pmole $\cdot$ min$^{-1}$ $\cdot$ mm$^{-1}$ | 0.63 $\pm$ 0.04 |
| (P/B) | 2.76 $\pm$ 0.45 |
| N;n | 4;22 |

Abbreviations: $V_c$, collection rate; J, net secretory flux; (P/B), final perfusate to bath concentration ratio. Values are MEAN $\pm$ SE for (n) number of measurement on a total of (N) number of tubules. 50 $\mu$M ($^{14}$C) methylamine was present in the bath. No methylamine was present in the initial perfusate.

Self-inhibition and effect of methazolamide. 50 $\mu$M ($^{14}$C)MA was present in the bath and initial perfusate. Results are summarized in Table 2. The presence of 10 mM unlabelled MA in the bath and perfusate reduced the excretory flux of ($^{14}$C)MA by 38%, and the final perfusate to bath concentration ratio by 57%. Such results indicate that MA secretion occurs by a saturable, i.e., carrier-mediated, transport process.

The effect of the carbonic anhydrase inhibitor, methazolamide (courtesy of T.H. Maren), on ($^{14}$C)MA secretion was tested. 0.1mM methazolamide in the bath and perfusate reduced efflux of MA by 25% and the final perfusate to bath concentration ratio by 36%. This suggests that proton gradients may play a role in MA secretion. However, the effect of methazolamide was not observed with all tubules.

Table 2.--Methylamine secretion by isolated perfused renal tubules

| | Self-inhibition and effect of methazolamide | | |
|---|---|---|---|
| | Control | 10 mM methylamine | 0.1 mM methazolamide |
| tubule length, mm | 1.061 ± 0.162 | 0.715 ± 0.130 | 0.930 ± 0.078 |
| $V_c$, nl · $min^{-1}$ · $mm^{-1}$ | 6.78 ± 0.79 | 7.16 ± 0.79 | 6.33 ± 0.62 |
| J, pmole · $min^{-1}$ · $mm^{-1}$ | 0.76 ± 0.08 | 0.47 ± 0.10 | 0.57 ± 0.05 |
| (P/B) | 2.87 ± 0.32 | 1.24 ± 0.11 | 1.85 ± 0.13 |
| N;n | 5;22 | 4/14 | 4/26 |

Abbreviations: $V_c$, collection rate; J, excretory flux; (P/B), final perfusate to bath concentration ratio.

Values shown are MEAN ± SE for (n) number of measurements on a total of (N) number of tubules. 50 μM ($^{14}C$) methylamine was present in the bath and initial perfusate.

## DISCUSSION

Results presented here establish that the flounder kidney secretes MA against a concentration gradient by a saturable mechanism. Hence, MA cannot be used as an indicator of intracellular pH in the kidney.

To minimize backflux of MA from lumen to bath, tubules were perfused at the highest rates possible without causing damage to the epithelium. That backflux did occur, and that the epithelium is highly permeable to MA, is suggested by: 1. The final perfusate to bath concentration ratio was the same whether MA was present or absent from the initial luminal perfusate; and 2. net secretory flux was linearly related to collection rate. Concerning the latter, it must be pointed out that collection rate is an approximation of perfusion rate, since the renal tubule might secrete or reabsorb fluid. No attempt was made to measure fluid movement. Clearance studies on whole fish indicate that MA secretion is inversely related to fluid reabsorption (Booz, unpublished observations). The present finding that the final perfusate to bath concentration ratio was linearly related to collection rate may reflect that fact.

Carbonic anhydrase has been implicated in proton secretion by renal tubules. The inhibitory effect of methazolamide on MA secretion suggests that pH gradients influence MA secretion. Note, however, that rapid perfusion -- as in the present study -- of a buffered solution through relatively short tubular segments would have precluded development of a transepithelial pH gradient.

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## XENOBIOTIC METABOLIZING SYSTEMS IN AQUATIC SPECIES; ACTIVITIES IN EXTRAHEPATIC TISSUES AND EFFECTS OF ELLIPTICINE ON HEPATIC MIXED-FUNCTION OXIDASES IN AQUATIC AND MAMMALIAN SPECIES

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Xenobiotic metabolism in aquatic species not only aids in the clearance of chemicals from tissues but can also produce toxic metabolites (carcinogens/mutagens) from ingested chemicals. Recently much work has been done on these systems in