soleus (slow oxidative) and the extensor digitorum longus (fast oxidative glycolytic) skeletal muscles[8], both in rats and hamsters. The data further demonstrate that there may be a significant difference in catalase activity between the regions of a single muscle. Such is the case with the lateral and medial heads of the rat gastrocnemius muscle. Failure to take such differences into account might result in erroneous conclusions. These data demonstrate that samples for catalase assays must be taken not only from the same muscle, but from the same muscle region if comparisons are to be made in skeletal muscles.

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Irreversible inhibition of sodium transport by the toad urinary bladder following photolysis of amiloride analogs

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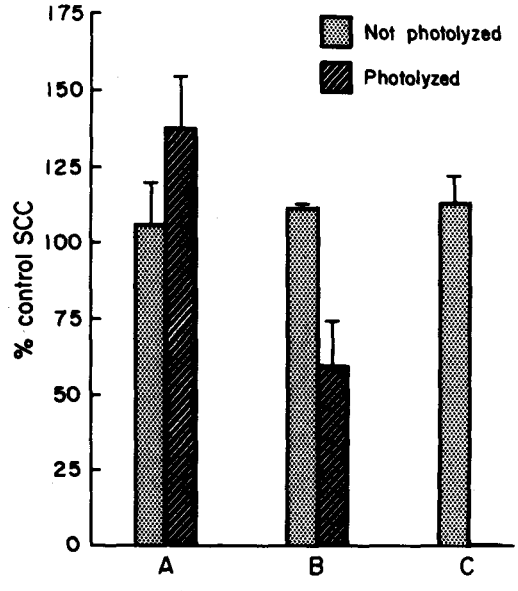
Summary. Active sodium transport was completely and irreversibly inhibited in toad urinary bladders photolyzed in the presence of iodoamiloride.

Identification of the membrane components involved in the specific entry of sodium into mucosal cells is essential for the elucidation of the biochemical mechanisms associated with the active transcellular transport of the ion by epithelial tissues. Amiloride (N-amidino-3,5-diamino,6-chloropyrazine-carboxamide)[1], a rapidly reversible inhibitor of sodium transport, evidently acts by binding to the site in the apical membrane responsible for sodium permeation[2]. The apparent specificity of amiloride for the sodium entry site is such that the amount of amiloride bound by tissues has been used to determine the number of sodium channels[3]. An amiloride analog that could be used as an affinity label would be an elegant tool for the study of apical membrane sodium permeability; a radioactive amiloride affinity label might be used to identify, isolate and characterize the membrane components comprising the passive sodium entry mechanism.

The design of an effective amiloride-based affinity label has been hampered by the fact that even relatively simple derivatization of amiloride is associated with a loss of drug activity. For example, although results from in vivo studies of amiloride analogs as antagonists of the effects of deoxycorticosterone in the dog suggest that analogs with derivatized guanidine amino groups are potent diuretics[1], in isolated epithelial tissues such as the toad bladder these analogs retain no more than 1% of the sodium transport inhibitory activity of amiloride (Cobb, unpublished data). The discrepant results from the intact animal studies are probably due to the conversion of analogs to more active forms in vivo, since the parent compound, amiloride, can be recovered in the urine of these animals. Benos and Mandel[4] found that bromoamiloride (N-amidino-3,5-diamino,6-bromo-pyrazinecarboxamide), an amiloride analog in which the chlorine atom is replaced by bromine, will, after photolytic activation, irreversibly inhibit approximately 30% of the short circuit current (SCC) across the frog skin. In the following study we report the complete irreversible inhibition of the SCC across the toad urinary bladder by iodoamiloride, an amiloride analog which offers the potential for preparation with radioiodine to yield a photoaffinity label with very high specific activity.

Hemibladders excised from Dominican toads were mounted in a 2-section Ussing chamber in Ringer's solution of the following composition: 85 mM NaCl, 4 mM KCl, 17.5 mM $NaHCO_3$, 0.8 mM $MgSO_4$, 0.8 mM KH_2PO_4, 1.5 mM $CaCl_2$ and 10.5 mM glucose. The SCC was measured in both halves of the hemibladders, which were then incubated for 30 min in Ringer's solution alone or containing 500 µM bromoamiloride or 20–200 µM iodoamiloride. One half of each hemibladder was photolyzed, with a Hanovia mercury lamp through a skylight filter (> 320 nm), for 3 times for 30-sec intervals in a period of 10 min. The hemibladders



Irreversible inhibition of sodium transport by photolysis of amiloride analogs. After measurements of the SCC were made, hemibladders were treated for 30 min with Ringer's solution, *A* without amiloride analogs, *B* with 500 µM bromoamiloride, or *C* with 100 µM iodoamiloride. Following photolysis of one half of each tissue as described in the text, both halves of the tissues were rinsed with 3 changes of Ringer's solution for 30 min after which the SCC was measured again. Photolyzed tissue, right bar; unphotolyzed tissue, left bar.

were then washed in 3 changes of Ringer's solution and the SCC in each half was measured. The SCC of half-hemibladders photolyzed under control conditions, i.e. without having been treated with an amiloride analog, was 137% of the SCC prior to photolysis (figure, A). The SCC of the other half-hemibladders, those portions not irradiated, was 106% of the previous value. This indicates that photolysis under these conditions does not inhibit the SCC, while the half-hemibladders protected from irradiation serve as acceptable controls for the irradiated halves. Since bromoamiloride was reported to inhibit irreversibly 30% of the SCC after UV-activation[4], we examined the effect of bromoamiloride on the SCC following photolysis as described above. The SCC of half-hemibladders exposed to bromoamiloride during photolysis was 60% of the SCC value preceding photolysis (figure, B). The unphotolyzed halves had an SCC of 112% of the previous value, indicating noncovalently bound bromoamiloride had been washed out of the preparations. Thus 40% of the SCC was irreversibly inhibited by this treatment, as reported by Benos and Mandel[4]. Although the concentration of bromoamiloride used to achieve this degree of irreversible inhibition was much higher than that used by Benos and Mandel, two aspects of our experimental design should make it superior to theirs. First, the radiation used in their study is of a wavelength generally destructive to proteins and second, the length of exposure to irradiation was shorter than in their study, with a resultant decrease in the amount of SCC remaining.

Using the same protocol, the effects of iodoamiloride were examined. The SCC of half-hemibladders photolyzed in the presence of iodoamiloride (100 µM and greater) was completely abolished in all trials (figure, C). At lower concentrations with equal lengths of photolysis, the inhibition was reduced. In the tissue-halves treated with iodoamiloride without photolysis, the SCC attained 114% of the pretreatment SCC following removal of the drug, indicating the reversible action of unphotolyzed iodoamiloride. Although the inhibitory potency of unphotolyzed iodoamiloride is less than that of bromoamiloride, the iodinated analog is more promising for photoaffinity labeling studies for at least two reasons: 1. Iodoamiloride appears to form a more highly reactive intermediate. This is important because relatively unreactive species are likely to attach covalently to numerous sites on the membrane. On the other hand, highly reactive species are likely to react with their specific binding sites before they dissociate and diffuse to other less specific sites. Furthermore, for derivatives which form highly reactive species, shorter times of photolysis will probably inactivate more sodium sites thus reducing the necessary amount of irradiation, which could cause considerable damage to proteins. 2. Monitoring the photoaffinity labeled molecule, which might be a relatively minor component of the membrane, requires a radioactive label of high sp. act. Iodoamiloride offers an excellent prospect, since it can be prepared with radioactive iodine of high (^{125}I, 2500 Ci/mmole) sp. act.

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The effects of sodium and amiloride on the motility of the caudal epididymal spermatozoa of the rat[1,2]

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Summary. The forward motility of the rat caudal epididymal spermatozoa has been studied in different Na^+ concentrations. When spermatozoa were suspended in a completely Na^+-free solution, the forward motility suffered a progressive fall and after 3 h was completely suppressed. This effect was fully reversible on resuspending the spermatozoa in a solution containing Na^+. Amiloride caused a fall in motility and the effect was similar to that of Na^+ removal. The inhibition by amiloride of the motility was concentration dependent and the dose response curve showed an IC_{50}-value of about 5×10^{-5} M. The role of Na^+ influx in the maintenance of sperm motility was discussed.

In the rat when the spermatozoa reach the cauda epididymidis, they are fully mature but are maintained in a quiescent state during storage. The caudal epididymal fluid contains a high concentration of potassium and a low concentration of sodium[3,4]. This results from the active absorption of sodium and secretion of potassium by the epididymal epithelium[5]. The high K^+/Na^+ ratio in the epididymal fluid may be responsible for maintaining the spermatozoa in an inactive state hence reserving energy for the vital processes of capacitation and fertilization. It has been reported that monovalent cations like sodium and potassium affect the motility of spermatozoa of many species[6,7]. However, the precise relationship between ion transport across the sperm membrane and sperm motility has not been explored. In this work, we have studied the effect of different sodium ion concentrations on the forward motility of the rat caudal epididymal sperm. Amiloride, a drug which is known to block Na^+ influx into cells[8] has also been used as a tool to investigate the role of sodium influx in sperm motility.

Methods. Spermatozoa were collected by flushing out the contents of the cauda epididymidis with sodium-free tris buffer after cannulation of the cauda epididymidis[5]. This solution contained (mM): Choline chloride, 138, KCl, 4.7; $CaCl_2$, 2.56; $MgSO_4$, 1.13; Tris (pH 7.2), 5; osmolarity, 300 mosmol/l. The spermatozoa were diluted with the same buffer to a final concentration of 43×10^6 per ml and incubated at 35 °C. This represented the stock suspension from which aliquots (10 µl) were taken up into assay solutions (420 µl) containing normal (138 mM Na^+) or different concentrations of sodium (0 to 143 mM). The ionic compositions of these solution were the same as that of the sodium-free tris buffer except that sodium chloride and choline chloride were present in different proportions