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Effect of Naphthalene on Sodium Active Transport in the Frog Skin

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Isolated abdominal frog skin, when mounted on an in vitro chamber, actively transports sodium from the pond-side bathing solution to the serosal-side. Active transport of sodium causes an electrical potential difference (PD), up to 140 mv, that is proportional to the log of the pond-side sodium concentration (Koefoed-Johnsen and Ussing 1958). When the solution bathing each side of the skin is identical and enough external current is applied to change the PD to zero, the skin is "short-circuited". Under short-circuit conditions, no driving force for ions or water exists across the skin. Thus, any net flux of ions (or water) must be due to processes internal to the skin and equal to the short-circuit current (ISC). Ussing and Zerahn (1950) showed that the ISC correlated well with the measured net sodium flux. ISC is abolished by low concentrations of amiloride, a diuretic, which blocks the entry of sodium from the pond-side, thus causing the disappearance of Na+ transport and a dramatically reduced ISC. The currently accepted model for sodium active transport in the frog skin is that proposed by Koefoed-Johnsen and Ussing (1958) and modified through the years (Ussing and Windhager 1964) to include the paracellular pathway. This model proposes that sodium enters through a passive channel in the pond-side membrane of the first layer of living cells. Sodium diffuses through the cytoplasm of all of the epithelial cells of the epithelium and is pumped out in exchange for potassium by Na/K ATP'ases on the basolateral membranes. Net transport of sodium is accomplished by the differential permeabilities of the pond-side membrane and the basolateral membrane resulting in no net potassium transport and a measurable net sodium transport towards the basolateral side of the skin.

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The pond-side membrane is mainly permeable to sodium whereas the basolateral membrane is mainly permeable to potassium. Thus the next flux of potassium is near zero and the net flux of sodium differs considerably from zero (typically 20 microamps/cm ^{2}). Some isolated frog skins exhibit considerable chloride conductance which can be abolished by exchanging the chloride in the bathing solution for sulfate.

Naphthalene, a polycyclic aromatic hydrocarbon (PAH), occurs in oil by-products and in crude oil (Boylan and Tripp 1971). Naphthalene and similar compounds have been identified as acutely toxic petroleum products (Anderson et al. 1974). Naphthalene has been shown to modify Na/K ATP'ase of aquatic invertebrates (Darville et al. 1983). Mitochondrial respiration is inhibited at levels of 15 mg/L naphthalene (Harmon and Sanborn 1982). We have investigated the effect of naphthalene on the active transport of sodium by the frog skin.

MATERIALS AND METHODS

Frogs, Rana pipiens, were purchased from Wm. Lemberger (Oshkosh, Wisconsin) and kept, as is normal in transport experiments, unfed until use. Frogs were rinsed daily with tap water (pH 7, 20^{o} C., 146 mg/L hardness). Generally the frogs were used within 2 wk after arrival. Frogs were anesthetized, using AVMA guidelines on anesthesia, by injection of 10% urethane into dorsal lymph sac. The abdominal skin was excised and placed in frog Ringer's solution which contained 110 mM NaCl, 2.5 mM KCL, 1.0 mM $CaCl_2$, 2.5 mM TRIS buffer and were adjusted to pH 8.3. Some solutions were made with $NaSO_4$ replacing NaCl. Solutions containing naphthalene were freshly prepared for each experiment. Naphthalene was dissolved at the final concentration by agitation and heating at 70 deg C overnight. A modified Ussing chamber made of glass was used to mount the skin. The PD was measured by two 3% agar-frog Ringer's bridges; current (e. g., ISC) was passed by Ag-AgCl electrodes placed so the current density was uniform across the skin. An automatic voltage clamp was used to maintain the PD at preset values. Values of PD and ISC were read from a digital panel meter. A chart recorder (Schlumberger, Benton Harbor, Michigan) was used to obtain time based records.

RESULTS AND DISCUSSION

Experiments described in Figure 1 showed the effect of naphthalene when applied in separate doses. The initial dose (1 mg/L) is near the minimal dose at which we could detect a response. Often the initial 1

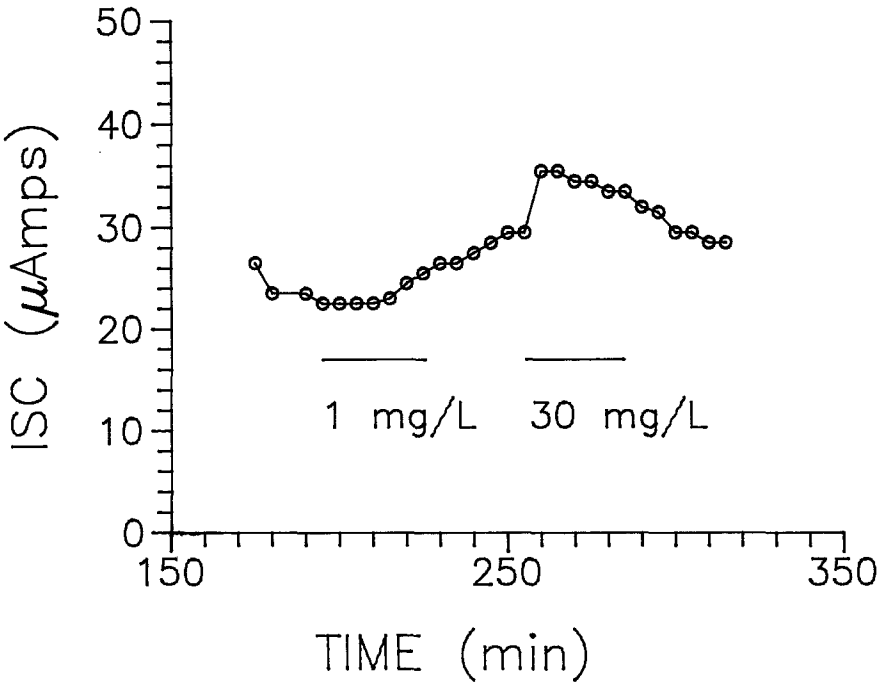

Figure 1 demonstrates the effect of 1 mg/L naphthalene and 30 mg/L naphthalene solution upon the ISC of the frog skin. After the ISC stabilized, 1 mg/L of naphthalene was added to the pond-side for 30 minutes. After rinsing with control frog Ringer's the pond-side skin was then exposed to a 30-min dose of 30 mg/L naphthalene solution.

mg/L dose did not elicit a measurable response. Also shown in Figure 1 is a probable long term effect of the low dose of naphthalene, the steady rise in ISC during the 220 to 250 minute record. Subsequent application of the 30 mg/L dose produced a strong response in ISC and a recovery to near control ISC.

Once we established the effect of naphthalene on the ISC, we then asked whether a dose-response relationship existed. Figure 2 records the results of an experiment using sequential doses of varying concentrations of naphthalene. In this figure the initial 1 mg/L dose of naphthalene caused a drop in ISC (compare to the rise in Figure 1). Subsequent applications of naphthalene evinced more predictable responses with the final stepwise naphthalene administration producing graded responses of ISC.

Amiloride blocks the passive entry of sodium. In the experiment described in Figure 3, we tested the

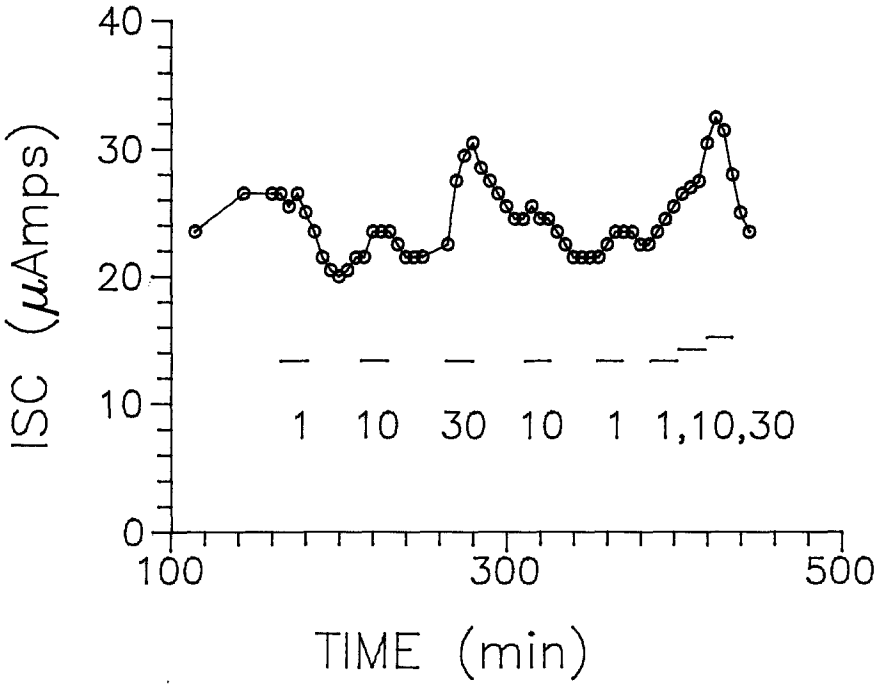

Figure 2 is the result of a stepwise experiment using 1 mg/L, 10 mg/L, and 30 mg/L solution of naphthalene. The first set of doses were administered and the skin allowed to recover in frog Ringer's before the next 15 minute exposure of naphthalene. The last naphthalene sequence (385 min) is an increase from 1 mg/L to 30 mg/L naphthalene without rinsing with frog Ringer's solution.

interaction of amiloride and naphthalene. If naphthalene caused the entry of sodium via a separate route, amiloride should not block the napthalene-mediated sodium entry. As seen in Figure 3, amiloride causes a rapid drop in ISC. In the latter section of the figure (i.e. 360 minutes), combined amiloride and naphthalene solution also produced a rapid drop in ISC, showing that the entry path enhanced by naphthalene is probably the same pathway through which sodium normally enters the skin.

Summarizing the effect of naphthalene on frog skin, we found that naphthalene has an EC_{50} using the maxinal change in ISC at 30 min after naphthalene application of 4.4 mg/L determined from the eleven frogs. Two of those frogs did not respond to naphthalene or amiloride and were not used in the data set. The response of the skins to 10 mg/L naphthalene was 17 $\pm$ 4 (SEM) which was significant at the 1% level. Naphthalene had no effect when administered to the

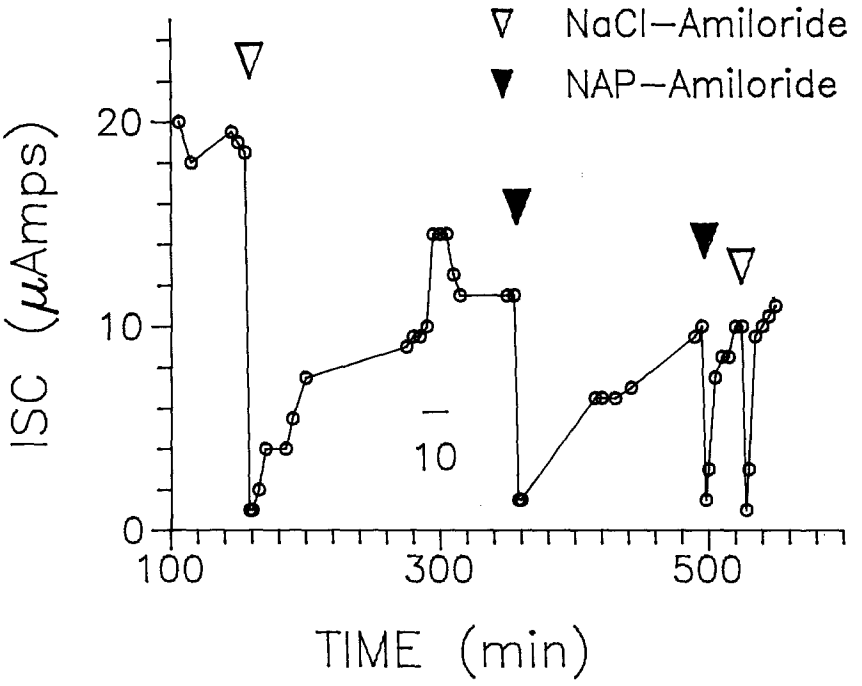

Figure 3 shows the effect of the interaction of naphthalene and amiloride on the ISC. The first application shows the typical response of amiloride in frog Ringer's. After a period of recovery, the pond-side bathing solution was changed to 10 mg/L naphthalene for 15 minutes. After another period of recovery the pond- side bathing solution was changed to a 10 mg/L naphthalene solution containing 10 micromolar amiloride.

blood side of the frog skin. The experiments reported here demonstrate an effect of naphthalene on sodium active transport in frog skin. Figure 1 shows a dose-response relationship exists. It is also important to note that the 1 mg/L effect did not appear until the end of the 30-min dose. Therefore the overall conclusion from these experiments is that at all concentrations of naphthalene tested, the ISC increased corresponding to an increase in sodium transport. The only exception is demonstrated in Figure 2. The initial dose of 1 mg/L naphthalene caused the ISC response to decrease . Subsequent doses of naphthalene, regardless of concentration or time administered, caused an increase in ISC. Similar results to these have also been observed in our experiments using ethanol as a solvent for naphthalene (unpublished data). Since the frog skin is a widely accepted model for vertebrate sodium active transport (e.g., mammalian kidney, human fetal

skin, mammalian bladder) inferences can be drawn about one of the modes of action of naphthalene (and possibly other PAH) on sodium active transport. Although the earlier work of Darville et al (1983) reported effects of naphthalene on Na/K ATP'ase isolated from cells of Chironomus, the effects reported here were on intact cells and on an intact epithelial preparation. It is surprising then that the epithelial preparation is more sensitive to naphthalene than the isolated components. We can also infer from the rapidity of response (see Figure 3) that the mode of action in these experiments was probably on the entry of sodium into the cell. Regardless of the bathing solution used, whether frog Ringer's or naphthalene solution, amiloride caused the ISC to drop dramatically. Though the possibility exists that naphthalene acts on the Na/K ATP'ase, the data support a primary effect on the sodium entry pathway.

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