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Interactive Effects of Ethanol and Silver on Sodium Transport across Toad Skin (41868)

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Abstract. Both ethanol and silver ions have been shown to affect ion transport across various epithelia. This investigation was principally undertaken to further define mechanisms of silver ions and ethanol, and their possible interactions, on sodium transport across toad skin. Isolated toad skin, mounted between identical oxygenated amphibian bicarbonate Ringer solutions, maintained stable transepithelial potential differences (serosa positive) and short-circuit currents for several hours at 25°C. It was observed that (1) ethanol inhibited the active transcellular component of sodium absorption and this effect was reversible; (2) inhibition of sodium transport by ethanol was directly proportional to the applied concentration; (3) pretreatment with silver ions prevented any ethanol effects; and (4) pretreatment with ethanol prevented any silver ion effects. It was concluded from these results that ethanol induced its inhibitory effects on membrane phospholipids thereby perturbing the function of a sulfhydryl ligand, while silver ion or silver chloride complex binding to this ligand would maintain its function in sodium transport despite the presence of ethanol.

Silver–silver chloride (Ag–AgCl) electrodes immersed directly or connected via electrolytic bridges in the bathing media of isolated epithelia have been extensively used in the measurement of transepithelial bioelectric phenomena such as transepithelial potential difference (PD) and short-circuit current (SCC). The first observation of silver on these bioelectric characteristics was made by Nedergaard and Ussing (1). They demonstrated a silver-induced SCC across frog skin. Using direct immersion Ag–AgCl electrodes, Clarkson and Toole (2) described a transient increase in SCC across rat ileum that gradually decreased over a 1-hr period. They proposed that Ag^+ from the Ag–AgCl electrodes increased mucosal membrane permeability of the rat enterocytes to Na^+ which resulted in an increased Na^+ transport pool and, consequently, an increased active Na^+ efflux across the basolateral membrane. Walser (3) demonstrated a stimulation of SCC by Ag–AgCl immersion electrodes in isolated toad bladder using electrolytic bridge systems as controls. In fact, SCC was increased when a non-current-carrying Ag–AgCl wire was immersed in the mucosal bathing solution of the control system. Curran (4) demonstrated that Ag^+ addition to the outside bathing medium of isolated frog skin resulted in a sharp transient rise in SCC followed by a decrease. Through mannitol, Na^+, and K^+ flux studies, Curran concluded that Ag^+ affected both shunt and cellular permeability properties for Na^+. Boyett and Van Bruggen (5) demonstrated a concentration-dependent inhibition of ethanol on Na^+ transport across isolated frog skin. The present study was therefore undertaken to further define mechanisms of Ag^+ and ethanol, and their possible interactions, on Na^+ transport across toad skin.

Materials and Methods. Toads, *Bufo marinus,* of either sex were caught around Manoa Valley (Honolulu, Hawaii) and around Lake Alice (Gainesville, Fla.). They were kept fasting at room temperature (25°C) prior to experimentation. Adult animals were used in these experiments.

The toad was stunned by a blow on the head and its ventral skin was removed and mounted between two Lucite chambers as a flat sheet having an area of 3.14 cm^2 for the determination of electrical characteristics. The skin was aerated and bathed by amphibian bicarbonate Ringer of the type described by Gerencser *et al.* (6) having a total osmolality of 235 mosmol/liter and a pH of 7.8 at 25°C. The chambers employed were equipped with two sets of electrodes and were similar in design to those described by Ussing and Zerahn

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(7). PD and SCC were measured by methods similar to those employed by Schultz and Zalusky (8) except that both Ag–AgCl and agar salt bridges were used to apply external current to the system. A voltage clamp device was used to maintain short-circuit conditions as described by Rothe *et al.* (9). The electrolyte content of the agar salt bridges was identical to that of the bathing solution. The agar bridges from the potential-sensing electrodes contained saturated KCl. To minimize potential offset between these electrodes, the ends of these bridges were preequilibrated with the bathing solution for several hours before the experiments. Offset between the potential-sensing electrodes was measured at the beginning of the experiment and again at the end of the run following removal of the tissue and replacement of the bathing fluid.

Each mirror-image compartment of the chamber had a volume of 20 ml. The addition of 0.48 ml of a 95% ethanol solution (20.62 *M*) to one compartment gave a final concentration of 0.50 *M* ethanol, whereas 0.24 ml of the same solution gave a final concentration of 0.25 *M* ethanol. The opposite compartment of the chamber received an equivalent volume of amphibian Ringer solution, thereby preventing the development of a hydrostatic gradient across the tissue.

The application of radiotracer technique for the determination of the unidirectional fluxes of $^{22}Na^+$ under short-circuited conditions was as described by Quay and Armstrong (10).

All data are reported as means ± SE. Differences between means were analyzed statistically using Student's *t* test.

Results. When ethanol was added to the outside bathing solution of isolated toad skin (0.5 *M*, final concentration), the SCC immediately decreased to a new level and remained steady for several hours. Rinsing and replacing ethanol with an ethanol-free Ringer solution promptly restored the SCC of the skin to control levels (Fig. 1). The SCC response to the addition of ethanol was proportional to the concentration of ethanol (Table I). Maximal inhibition of SCC was obtained with 0.5 *M* ethanol while 0.25 *M* ethanol elicited 50% inhibition. The skin resistance (PD/SCC) was not changed significantly by ethanol addition to the outside bathing solution. The addition of ethanol (0.5 *M*, final concentration) to the inside bathing medium had no significant effect on SCC ($N = 3$). The addition of sucrose (0.5 *M*, final concentration) to the outside ($N = 3$) or inside ($N = 2$) bathing solutions had no significant effect on SCC.

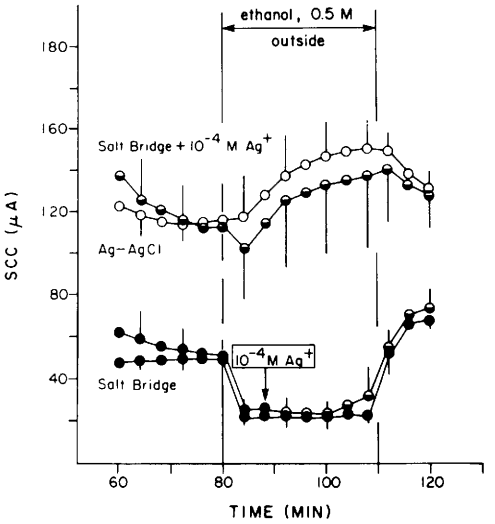

FIG. 1. Comparison between the inhibitory effect of ethanol on SCC in a salt bridge-driven system with and without 10^{-4} *M* Ag^+ in the medium and Ag–AgCl immersion electrodes. Open circles (○) represent Ag–AgCl electrode, closed circles (●) represent salt bridge-driven systems, whereas half-closed circles (◒) represent salt bridge + 10^{-4} *M* Ag^+. Each point represents the mean of seven experiments. Vertical bars represent the SEM.

The next series of experiments examined the interactive effects of Ag^+ and ethanol on SCC. Figure 1 shows matched comparisons of SCC using three skin preparations from the same animal. As can be seen ethanol had no significant effect on SCC in the skin preparations exposed to Ag^+ (salt bridge + 10^{-4} *M* $AgNO_3$ or Ag–AgCl current-driving system). The Ag^+-induced increase in mean SCC, however, had considerable variation due to each skin responding differently to the given concentration of Ag^+. Rinsing and replacing the outside bathing medium with ethanol-free Ringer solution had no significant effect on SCC in those preparations exposed to Ag^+. Similarly, replacing Ag^+ with 0.5 m*M* *p*-chloromercurobenzoate (*p*-CMB) in the inside bathing solution prevented any subsequent SCC inhibition by 0.5 *M* ethanol added to the outside bathing solution ($N = 4$). The addition of 10^{-4} *M* $AgNO_3$ to a 0.5 *M* ethanol-inhib-

TABLE I. BASAL AND ETHANOL-INHIBITED SCCs

	SCC (μA/cm^2)		
	Basal	0.25 *M* ethanol	0.5 *M* ethanol
Electrolytic agar bridge	14.3 ± 2.5 (5)	8.9 ± 1.8 (5)	2.7 ± 0.5 (5)

Note. Average values ± SEM are given for the number of experiments shown in parentheses.

ited SCC had no effect on the reduced SCC (Fig. 1).

In order to discern the mechanism and ionic nature of the ethanol-induced SCC, an assessment of the unidirectional outside-to-inside (J_{OI}) and inside-to-outside (J_{IO}) Na^+ fluxes was made using $^{22}Na^+$ in three preparations from the same animal when their respective Ag–AgCl or salt bridge-driven short-circuit currents matched. As shown in Table II the mean J_{OI}^{NET} ($J_{OI} - J_{IO}$) of Na^+, in both Ag^+-exposed and Ag^+-free tissues, respectively, was not significantly different from the mean SCC in the presence of ethanol (0.5 *M*). Ethanol had no significant effect on J_{IO} of Na^+ whether the tissue was exposed to Ag^+ or not. However, ethanol significantly reduced ($P < 0.05$) the J_{OI} of Na^+ in the Ag^+-free preparation but had no significant effect on the J_{OI} of Na^+ in the Ag^+-exposed skin preparations.

Discussion. The present results indicate that ethanol inhibits active Na^+ transport across the toad skin and are similar to the results of Boyett and Van Bruggen (5) in frog skin. The finding that ethanol inhibited SCC proportional to the dose administered (Table I) attests to its inhibiting the active Na^+ transport pathway because the mean SCC was identical to the mean net J_{OI}^{NET} of Na^+ in the absence or presence of ethanol (Table II). The finding that 0.5 *M* ethanol or 0.5 *M* sucrose, added to either compartment, had no significant effect on the J_{IO} of Na^+ or SCC, respectively, strongly suggests that the ethanol-induced effect was not osmotic in origin (11).

The previous findings by Gerencser *et al.* (6, 12, 13) and the present observation (Table II) that Ag^+ addition to the bathing medium, or Ag–AgCl immersion electrodes, stimulates SCC that is identical to the active Na^+ absorptive flux across the toad skin indicates that the Ag^+-induced effects are Na^+ specific. The effects observed by Ag^+ addition are probably caused by Ag^+Cl^- complexes, rather than the Ag^+, as suggested by Gerencser *et al.* (6). Previous observations also demonstrated that Ag^+ addition had no effect on J_{IO} of Na^+, therefore eliminating any paracellular effects of this ion on Na^+ transport (6, 12). The finding that there was no significant change in the Ag^+ or *p*-CMB-induced SCC by ethanol addition (Fig. 1) or in either J_{OI} or J_{IO} of Na^+ during ethanol administration (Table II) suggests that the ethanol-induced inhibition of active Na^+ transport acts on the same site as does Ag^+ or Ag^+Cl^- complexes in stimulating active Na^+ transport across the toad skin. Buttressing this argument is the observation that

TABLE II. SODIUM FLUXES IN TOAD RINGER

	J_{OI} (neq/cm^2/min)	J_{IO} (neq/cm^2/min)	SCC (neq/cm^2/min)
Salt bridge			
Before ethanol addition	10.2 ± 3.1 (5)	1.9 ± 0.7 (5)	10.3 ± 2.6 (5)
After ethanol addition	4.3 ± 1.6 (5)	2.1 ± 0.6 (5)	3.4 ± 1.6 (5)
Ag–AgCl electrode			
Before ethanol addition	21.6 ± 4.2 (5)	2.8 ± 0.7 (5)	19.8 ± 4.6 (5)
After ethanol addition	23.2 ± 5.6 (5)	3.6 ± 1.6 (5)	18.1 ± 5.3 (5)

Note. Average values ± SEM are given for the number of experiments shown in parentheses.

Ag^+ or Ag^+Cl^- complexes could not alter the inhibition of Na^+ transport induced by ethanol. These conclusions concur with those of Boyett and Van Bruggen (5) and Curran (4) who speculated that ethanol and Ag^+, respectively, had effects on cellular ionic permeability in frog skin.

Gerencser *et al.* (6, 12, 13) suggested that Ag^+ or Ag^+Cl^- complexes and *p*-CMB induced their effects on toad skin by interacting with sulfhydryl groups of the cells that permit active transport of Na^+ which was in concert with the conclusions of Curran (4) and Benos *et al.* (14) in frog skin. Since ethanol had no effect on Na^+ transport in the presence of Ag^+ or *p*-CMB, it is not unreasonable to speculate that all three chemical species affect the same sulfhydryl ligand, Ag^+ or Ag^+Cl^- complexes and *p*-CMB binding to it directly, whereas ethanol perturbing it indirectly by its effects on surrounding membrane phospholipids as postulated by Gutknecht and Tosteson (15). These chemically induced effects, both direct and indirect, probably alter Na^+ channel conductive properties (16). It seems more feasible that ethanol affected Na^+ translocation at the outer membrane of the transporting cell rather than affecting ($Na^+ + K^+$) ATPase at the inner membrane because ethanol applied to the inside bathing medium had no significant effect on SCC. These conclusions are in accordance with those of Boyett and Van Bruggen (5) for ethanol effects on frog skin and are in contrast with those studies on a partially purified ($Na^+ + K^+$) ATPase preparation (17).

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