each) of 5.0 mM Tris-HCl buffer plus 50 mM sucrose, pH 7.4 at 0 °C plus 1% bovine serum albumin. Binding of [^{3}H] PCP to the tissue slice was quantitated by counting the tissue-laden slide fragment in 10 ml Aquassure scintillation cocktail (New England Nuclear). Specific binding was calculated as the difference in counts bound in the presence and absence of 0.1 mM PCP.

All data analyses comparing amantadine-treated and control [^{3}H] PCP binding were done using a t-test (student distribution) of mean differences.

Results and discussion. As indicated in the table, amantadine significantly increases the affinity (K_D) of [^{3}H] PCP for its binding site in a dose dependant manner. The number of sites (B_{max}) does not appear to be changed by the presence of amantadine in the incubation buffer. Interestingly enough, rimantadine, an analogue of amantadine with antiviral properties, but completely devoid of any CNS effects[10], is also totally inactive on the [^{3}H] PCP binding site.

It is tempting to speculate that may be some of the CNS effects of amantadine are related to an action of this drug on the PCP receptor complex. It is well known that PCP is able to induce the release of dopamine in various conditions[11-13] and acts as a 'non-amphetamine' stimulant of the dopaminergic system[14]. Since the mechanisms of action of amantadine appears to involve the release of dopamine from the pre-synaptic area[4-6], it is possible that both amantadine and PCP act on the same substrate to induce the release of dopamine in the brain. Also, this interaction of amantadine on the PCP binding site may explain some of the CNS effects of this antiviral drug. In fact, it had been reported that both PCP[15] and amantadine[7] induced hallucinations in humans. An other possibility might be the interaction of both PCP and amantadine with the cholinergic system[16], which may result in an increased release of dopamine induced by stimulation of nicotinic receptors[7]. Finally, our results show that [^{3}H] PCP binding sites in rat brain are different from those present in membranes of torpedo ocellata, since amantadine displaced [^{3}H] PCP from its binding site in this system which is probably related to the ion channel of the nicotinic receptor[16].

Effects of amantadine on [^{3}H] PCP binding parameters

Drug	[^{3}H] PCP K_D (nM)	B_{max} (fmole/slice)
Control	52±4.0	10.6±0.9
Amantadine (10 nM)	41±3.0*	11.0±0.8
Amantadine (100 nM)	36±3.0**	9.9±0.7
Rimantadine (100 nM)	54±5.0	11.4±1.0

Numbers are mean±SEM of 3 determinations each in triplicate. *$p<0.05$; **$p<0.01$.

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Drug modification of silver-induced sodium transport across toad skin[1]

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Summary. Stimulation of active Na^+ transport in the toad skin by antidiuretic hormone (ADH) and p-chloromecuribenzoate (P-CMB) was blunted by the presence of silver (Ag^+). Amiloride inhibited active Na^+ transport, equivalently, whether Ag^+ was present or not.

Silver ions (Ag^+) have been shown to stimulate electrical characteristics and ion transport across various epithelial preparations[2,3]. In fact, Walser[4] reported a stimulatory effect on short-circuit current (SCC) by Ag-AgCl electrodes immersed directly into the bathing solutions of isolated toad bladder. Curran concluded from studies of Ag^+ effects on permeability properties of frog skin that Ag^+ may increase skin shunt permeability and may affect the cation selectivity of the outer membrane, possibly by reacting with sulfhydryl groups. Gerencser et al.[6] suggested that silver chloride, or their complexes, bind to specific membrane groups in enhancing active sodium absorption across toad skin. The present work was therefore undertaken in order to assess possible mechanisms of silver-induced sodium transport across toad skin.

Materials and methods. Adult toads, *Bufo marinus*, of either sex were kept fasting at room temperature of 25 °C prior to experimentation. The transmural potential difference and SCC were measured across sheets of toad skin similar to those methods employed by Schultz and Zalusky[7] except that both Ag-AgCl and agar electrolytic bridges were used to apply external current to the system. These 2 methods were compared with one another and differences between them were analyzed statistically using the Student's t-test. The silver concentration of the bathing medium was determined using an atomic absorption spectrophotometer as described previously[6]. The skin was aerated and mounted between identical phosphate-buffered NaCl Ringer solution of the type described by Adrian[8].

Results. Using toad skin preparations from the same ani-

Table 1. Basal and drug-induced SCC's

	Basal SCC ($\mu A/cm^2$)	Change in SCC induced by: P-CMB ($\mu A/cm^2$)	ADH ($\mu A/cm^2$)	Amiloride ($\mu A\ cm^2$)
Electrolytic agar bridge	15.0±5.1 (7)	+35.7±4.2 (5)	+50.2±7.8 (7)	−13.1±2.5 (6)
Ag-AgCl electrode	35.2±5.7 (7)	+14.4±3.7 (5)	+13.2±2.1 (7)	−14.2±2.0 (6)
Probability	$p<0.01$	$p<0.01$	$p<0.01$	NS

Average values ± SEM are given for the number of experiments shown in parentheses. The polarity of SCC change is as follows: positive (+) refers to stimulation while negative (−) refers to inhibition.

Table 2. Sodium fluxes in amphibian Ringer

	J_{OI} nEq/cm²/min	J_{IO} nEq/cm²/min	J_{OI}^{NET} nEq/cm²/min	SCC nEq/cm²/min
Ag-AgCl electrodes	19.31±3.16 (4)	1.78±0.21 (4)	17.53±3.06	19.81±5.05

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

mal, the mean basal SCC driven by electrolytic bridges was 15.0±5.1 $\mu A/cm^2$ tissue (control) while those driven by Ag-AgCl immersion electrodes was 35.2±5.7 $\mu A/cm^2$ tissue (table 1). These values were significantly different from one another ($p < 0.01$).

The average concentration of silver measured in the bathing solutions in which the Ag-AgCl electrodes were immersed was 5×10^{-7} M (N = 6).

The SCC response to a compound containing sulfhydryl groups was tested and it was shown that 5 mU/ml antidiuretic hormone (ADH), added to the inside bathing solution, stimulated SCC significantly greater using the electrolytically-driven system ($p < 0.01$) as opposed to the Ag-AgCl driven system (table 1).

The sulfhydryl-binding compound p-chloromecuribenzoate (P-CMB) was added to the inside bathing solution (0.5 mM) and stimulated SCC in both electrolytic and Ag-AgCl driven systems; however, the increase in SCC was much greater ($p < 0.01$) for the electrolytically-driven compared to the Ag-AgCl driven SCC (table 1).

In contrast, when 1.0 µM amiloride was added to the outside bathing solution, equivalent inhibitory SCC responses were noted in both electrolytically-driven and Ag-AgCl driven systems (table 1).

In order to discern the ionic nature of the Ag-induced SCC, determination of the unidirectional outside to inside (J_{OI}) and inside to outside (J_{IO}) Na^+ fluxes using $^{22}Na^+$ in paired preparations when their respective Ag-AgCl driven short circuit currents matched were performed. As shown in table 2 there was no significant difference between the mean J_{OI}^{NET} of Na^+ and the mean SCC.

Discussion. The present results indicate that Ag^+ induces Na^+ transport across the toad skin and that this effect may be mediated via changes in tissue sulfhydryl status. The finding that Ag-AgCl immersion electrodes themselves stimulate SCC (table 1) and that this enhanced SCC is identical to the active Na^+ flux (table 2 and Gerencser et al.[6]) across the toad skin suggests that the Ag^+-induced effect is relatively Na^+-specific. This is in contrast to what Curran[5] observed for the effects of Ag^+ on frog skin.

The previous findings of Gerencser et al.[6] that $AgNO_3$ addition to a sulfate-Ringer caused no change in the electrical characteristics of toad skin in a salt-bridge driven system and that the dose-response relationship of Ag^+ concentration to SCC change reached a maximum and minimum at 10^{-5} M and 10^{-7} M Ag^+, respectively, in a chloride-Ringer strongly suggested that Ag^+Cl^- complexes induced the electrical effects. This is based upon the empirical finding that Ag^+ placed in a high Cl^- aqueous medium has a solubility product constant of 1.56×10^{-10} at 25 °C. Therefore any higher concentration of Ag^+ in a similar high Cl^- aqueous medium will be manifested as Ag^+Cl^- precipitates or complexes[6]. Strengthening this argument is the present result where 5×10^{-7} M Ag^+ was present in the bathing solution where Ag-AgCl electrodes were immersed. However, this observation still does not negate the possibility that the Ag^+, despite its low concentration in an aqueous medium, plays a role, though minor, in effecting Na^+ permeability of the toad skin epithelium.

The finding that P-CMB stimulated SCC (therefore active Na^+ transport) to a greater extent with an electrolytically-driven system as opposed to a Ag-AgCl driven system strongly suggests that Ag^+, or more appropriately Ag^+Cl^- complexes[6] inhibit P-CMB binding to tissue sulfhydryl groups for it is widely known that P-CMB and Ag^+ are avid sulfhydryl group binders[9].

The observation that ADH stimulated SCC to a greater extent with an electrolytically-driven system compared to a Ag-AgCl-driven system indicates that Ag^+ diminishes this effect by possibly binding to the sulfhydryl group of the ADH molecule, thereby partially or wholly inactivating it. The polarity of Ag^+ action implied in this conclusion is based upon the previous observation of Gerencser et al.[6] who showed that the greatest electrical effects and shortest response times induced by Ag^+ were obtained by the addition of Ag^+ to the inside bathing solution.

Amiloride is a diuretic[10] that is neither sulfhydryl-binding in its action or sulfhydryl containing in its nature. The observation that this compound inhibited active Na^+ transport in an equivalent fashion whether the tissue was being short-circuited by a electrolytic bridge system or a Ag-AgCl system strongly suggests (when coupled with the previous observations) that Ag^+ or Ag^+Cl^- complexes[6] stimulate Na^+ transport across the toad skin by interaction with tissue sulfhydryl groups.

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