The Effect of Glycyrrhetic Acid on the Action of Aldosterone in Stimulating Sodium Transport in Frog Skin

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Abstract

In order to elucidate the mechanism of action of glycyrrhetic acid in causing the mineralocorticoid excess syndrome, the effect of the agent on the active transport of sodium in frog skin was examined. The rate of active transport was evaluated by a short circuit current using a Ussing's chamber. The short circuit current increased significantly after the addition of 10^{-8} M aldosterone to the incubation media. It remained unchanged or suppressed when either glycyrrhizin or glycyrrhetic acid was added. The addition of 10^{-6} M glycyrrhetic acid in the presence of 10^{-8} M aldosterone stimulated the short circuit current significantly as compared with the control skin which was treated with aldosterone alone. From these results, glycyrrhetic acid is thought to potentiate the action of aldosterone and facilitate the active transport of sodium in amphibian epithelium. It is suggested that the pseudoaldosteronism induced by the administration of glycyrrhizin in man may in part be due to the potentiation of aldosterone action by this drug.

The administration of licorice or its active ingredient, glycyrrhizin, causes elevation of blood pressure, hypokalemia and metabolic alkalosis in man (Conn *et al.*, 1968). The symptoms which were referred to as licorice-induced pseudoaldosteronism resemble the clinical findings observed in cases of mineralocorticoid excess such as primary aldosteronism (George *et al.*, 1970). The pathogenesis of this syndrome induced by glycyrrhizin is not fully understood. Three possible modes of action of this agent have been considered. The first is that glycyrrhizin itself has a mineralocorticoid action on the renal tubule (Groen *et al.*, 1952; Ogata *et al.*, 1977). The facilitation of active transport of sodium at this site would

be the main effect in this case. The second possibility is potentiation of aldosterone action by this agent on the renal tubule (Elmadjian *et al.*, 1956). These two possibilities involving renal function seem to be supported by the recent report showing the presence of receptors for glycyrrhetic acid in renal tubule (Ulmann *et al.*, 1975). The third is that the glycyrrhizin may have an extrarenal action such as decreasing the clearance of aldosterone *in vivo* or stimulating aldosterone from the adrenals (Kumagai *et al.*, 1957; Girerd *et al.*, 1960; Mattingly *et al.*, 1970).

The present *in vitro* experiments were performed to elucidate the mechanism of action of glycyrrhizin in causing the mineralocorticoid excess syndrome. Frog skin, which responds to aldosterone by facilitating active transport of sodium across the membrane, was utilized as an *in vitro* model

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to study sodium transport of epithelium (Ussing *et al.*, 1951).

Materials and Methods

Frogs (*Rana Pipiens*) were kept in 0.6% saline solution for at least 24 hours. This maneuver is known to suppress the endogenous aldosterone and increase the sensitivity of frog skin to it (Saito *et al.*, 1973).

The abdominal skin of frog was resected and cut vertically along the midline into two pieces. Thus two identical pieces of hemiskin were obtained from the same animal, one of which was used for the test and the other for the control. The rate of active transport of sodium was determined by the method of short circuit current in the Ussing's chamber as described previously (Saito *et al.*, 1973). Ringer solution used in the present studies consisted of 110.0 mM NaCl, 2.4 mM KHCO_3 , 1.0 mM CaCl_2 and 10.0 mM glucose (222 mosmol/kg H_2O).

In order to evaluate the effect of glycyrrhizin or glycyrrhetic acid on the active transport of sodium in frog skin, four series of experiments were performed as follows.

Experiment I: Direct action of aldosterone, glycyrrhizin or glycyrrhetic acid was determined. The two resected hemiskins were kept separately in the following solutions aerated by bubbling at room temperature for 18 hours. The test skin was kept in the Ringer's solution containing 10^{-6} M aldosterone, 10^{-6} M or 10^{-5} M glycyrrhizin or 10^{-6} M or 10^{-5} M glycyrrhetic acid. The other piece of hemiskin was kept in the Ringer's solution without these solutes and served as a control. After keeping for 18 hours, the skins were mounted on the Ussing's chambers and each filled with 20.0 ml of the Ringer's solution. The solution was aerated and the short circuit current was determined 30 min after the skins were mounted.

Experiment II: Effect of glycyrrhizin or glycyrrhetic acid in the presence of aldosterone was determined. In this series, 10^{-8} M aldosterone was added to the incubation solutions of both hemiskins. The glycyrrhizin or glycyrrhetic acid of 10^{-5} M to 10^{-7} M in concentration was added to the solution bathing the skin of the test group. The other hemiskin served as a control. Experimental procedure was the same as described in Experiment I. Following the incubation for 18 hours under these conditions, the short circuit current was determined 30 min after the skins were mounted on the Ussing's glass chambers.

Experiment III: Effect of glycyrrhetic acid on the serosal or mucosal side of the skin was determined. Aldosterone is well known to facilitate the active transport of sodium across the frog skin only

when it is added to the solution bathing the serosal side of the skin (Saito *et al.*, 1973). In order to determine whether the addition of glycyrrhetic acid to the solution bathing serosal or mucosal side of the skin modifies this action of aldosterone, the following experiments were performed. Immediately after the skin was resected from the animal, both skin pieces were mounted in the Ussing's chamber, and the chamber was filled with 20.0 ml of the Ringer's solution. Aldosterone in 10^{-8} M concentration was added to the serosal side of both skins. After incubation for 30 min, the short circuit current was measured. Glycyrrhetic acid in a 10^{-6} M concentration was then added to the solution bathing the serosal side of the test skin, and no glycyrrhetic acid was added to the control skin. Following the incubation for 18 hours, the two incubation solutions were renewed. The incubation was continued for a further 30 min, and the short circuit current was measured again. In the same manner, glycyrrhetic acid in a 10^{-6} M concentration added to the solution bathing the mucosal side of the skin was studied. Thus, the effect of glycyrrhetic acid on the serosal or mucosal side of the skin was evaluated.

Experiment IV: Effect of glycyrrhizin and glycyrrhetic acid in the presence of excess aldosterone was determined. Both hemiskins were incubated in the same Ringer's solution as described in Experiment II in the presence of 10^{-6} M aldosterone. The effect of glycyrrhizin or glycyrrhetic acid was then examined by adding either agent to one of the hemiskins according to the same procedure as described in Experiment II.

Statistical analysis: The effect of aldosterone, glycyrrhizin or glycyrrhetic acid was evaluated by Student's paired t-test (Snedecor *et al.*, 1967). The ratio of the test to control skins was calculated. In Experiment III, the increase in the short circuit current was evaluated by subtracting the value before incubation from that after incubation. The test was applied to examine whether this difference was significant.

Results

Experiment I

The results of estimation of the short circuit current in frog skin in the presence of aldosterone, glycyrrhizin or glycyrrhetic acid are shown in Table 1. The addition of aldosterone resulted in an increase to 204% in the initial value of the short circuit current. The addition of 10^{-6} M or 10^{-5} M glycyrrhizin or 10^{-6} M glycyrrhetic acid alone did not cause a significant change

Table 1. Effect of aldosterone, glycyrrhizin and glycyrrhetic acid on the short circuit current in frog skin.

	Concentration (mol/l)	n	Short Circuit Current ($\mu\text{A}/\text{cm}^2$)		$\frac{\text{Test}}{\text{Control}} \times 100$	P
			Control	Test		
Aldosterone	10^{-6}	8	18.4 ± 1.3	37.4 ± 2.2	204 ± 9	<.02
Glycyrrhizin	10^{-6}	8	17.4 ± 1.4	18.6 ± 1.6	107 ± 5	ns
Glycyrrhizin	10^{-5}	8	17.0 ± 1.4	16.8 ± 1.4	99 ± 5	ns
Glycyrrhetic Acid	10^{-6}	8	18.1 ± 1.2	15.4 ± 1.0	85 ± 5	ns
Glycyrrhetic Acid	10^{-5}	5	22.9 ± 1.5	17.4 ± 1.5	76 ± 4	<.05

Test hemiskins were kept in the Ringer's solution containing aldosterone, glycyrrhizin or glycyrrhetic acid. The control hemiskins were kept in the Ringer's solution. After 18 hours, the hemiskins were mounted in Ussing's chamber and short circuit current was determined.

Table 2. Effect of glycyrrhizin and glycyrrhetic acid on the short circuit current in the presence of aldosterone.

	Concentration (mol/l)	n	Short Circuit Current ($\mu\text{A}/\text{cm}^2$)		$\frac{\text{Test}}{\text{Control}} \times 100$	P
			Control	Test		
Glycyrrhizin	10^{-6}	8	32.9 ± 1.4	34.4 ± 1.6	105 ± 5	ns
Glycyrrhizin	10^{-5}	8	28.5 ± 1.5	28.5 ± 1.5	99 ± 4	ns
Glycyrrhetic Acid	10^{-7}	9	26.8 ± 2.1	27.6 ± 1.7	103 ± 7	ns
Glycyrrhetic Acid	10^{-6}	14	26.9 ± 1.8	34.4 ± 2.0	128 ± 6	<.02
Glycyrrhetic Acid	10^{-5}	8	29.9 ± 1.6	35.3 ± 1.8	118 ± 7	<.05

Aldosterone 10^{-8} M was added to the solution for both control and test hemiskins. The glycyrrhizin or glycyrrhetic acid was added to the test hemiskin. After incubation for 18 hours, the control and test hemiskins were used for the determination of short circuit current.

in the short circuit current. The glycyrrhetic acid in 10^{-5} M concentration caused a slight but significant fall in the short circuit current.

Experiment II

Among the experiments in this group in which the effect of glycyrrhizin or glycyrrhetic acid was evaluated in the presence of 10^{-8} M aldosterone, the addition of 10^{-6} M glycyrrhetic acid did stimulate the short circuit current as compared with the control skin which was treated with aldosterone alone. This indicates that the glycyrrhetic acid potentiated the aldosterone action to facilitate the active transport of sodium (Table 2).

Experiment III

The potentiation of aldosterone action

to facilitate the active transport of sodium by glycyrrhetic acid was again observed in this experiment. This action of glycyrrhetic acid, however, was seen only when it was added to the solution bathing the inner surface of the skin. No significant effect was observed when it was added to the media on the mucosal side of the skin (Fig. 1).

Experiment IV

In the presence of aldosterone in a 10^{-6} M concentration, the short circuit current was stimulated to a level of $40.5 \pm 2.4 \mu\text{A}/\text{cm}^2$. No additional increase was seen even when 10^{-6} M glycyrrhizin or glycyrrhetic acid was added (Table 3).

Table 3. Effect of glycyrrhizin and glycyrrhetinic acid on the short circuit current in the presence of aldosterone.

	Concentration (mol/l)	n	Short Circuit Current ($\mu\text{A}/\text{cm}^2$)		$\frac{\text{Test}}{\text{Control}} \times 100$	P
			Control	Test		
Glycyrrhizin	10^{-6}	10	40.5 ± 2.4	43.7 ± 2.6	108 ± 6	ns
Glycyrrhetinic Acid	10^{-6}	8	42.1 ± 2.6	42.9 ± 2.4	102 ± 5	ns

Fig. 1. Effect of glycyrrhetinic acid on the short circuit current in the frog skin treated with aldosterone when it is added to the serosal (inner) or mucosal (outer) side of the membrane. SCC_1 and SCC_2 represent short circuit current in the frog skin before and after incubation of 18 hours respectively. The incubation was started after addition of 10^{-6}M glycyrrhetinic acid to the solution bathing serosal or mucosal side of the frog skin in the presence of 10^{-8}M aldosterone in the media on the serosal side.

Discussion

The enhanced increase in the short circuit current after the administration of glycyrrhetinic acid to frog skin treated with 10^{-8}M aldosterone strongly suggests the potentiation of aldosterone action by this agent. Elmadjian *et al.* (1956) reported that in adrenalectomized patients the administration of monoammonium glycyrrhizinate was ineffective in maintaining normal sodium balance. When hydrocortisone was added, the agent enhanced the action of hydrocortisone. The results of the present experiment in frog skin seem to be consistent with their results that glycyrrhizin potentiated the action of adrenal steroids. Although the results of *in vitro* experiments using frog skin cannot be extrapolated directly to the mechanism of action of a drug in man, the close correlation between the action on the renal tubule and that in amphibian epithelium has been repeatedly shown with several mineralocorticoids (Leaf *et al.*, 1957; Koefoed-Johnsen *et al.*, 1958; Saito *et al.*, 1973). It therefore seems possible that the formation of licorice-induced pseudoaldosteronism in man may in part be due to the potentiation of the action of aldosterone.

The present experiments also suggest that mineralocorticoid excess syndrome may not be the result of the direct action of glycyrrhetinic acid because the short circuit current did not change when it was added alone to the frog skin. These findings are consistent with the report that this agent

had no effect on sodium retention in patients with Addison's disease (Borst *et al.*, 1953).

The mechanism by which the action of aldosterone is potentiated by glycyrrhetic acid was not elucidated in the present experiments. The fact, however, that this action of glycyrrhetic acid was observed only when it was added to the solution bathing the serosal side of the skin where aldosterone also exerts its effect seems to suggest a similarity in the actions of the two substances. This similarity was also indicated by the finding that effect of glycyrrhetic acid was eliminated in the presence of 10^{-6} M aldosterone at which concentration the maximal action of this hormone is exerted.

It is recently reported that a renal cytosol receptor for glycyrrhetic acid which competitively binds mineralocorticoid is demonstrated and glycyrrhetic acid acts directly on the renal tubules to cause active transport of sodium through aldosterone binding receptors (Ulmann *et al.*, 1975). In consideration of their report which indicates the action of this agent on the renal tubule, we feel that further studies are necessary and emphasize the need to define the action of glycyrrhetic acid in the mineralocorticoid excess syndrome.

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References

- Borst, J. G., S. P. Holt, L. A. DeVries and J. A. Molhuysen (1953). *Lancet* 1, 657.
- Conn, J. W., D. R. Revner and E. L. Cohen (1968). *JAMA* 205, 492.
- Elmadjian, F., J. M. Hope and G. Pincus (1956). *J. Clin. Endocrinol.* 16, 338.
- George, J. M., L. Wright, N. H. Bell and F. C. Bartter (1970). *Am. J. Med.* 48, 343.
- Girerd, R. J., G. L. Rassaert, G. DiPasquale and R. L. Knoc (1960). *Am. J. Physiol.* 198, 718.
- Groen, J., H. Pelser, M. Frenkel, C. E. Kamminga and A. F. Willebrands (1952). *J. Clin. Invest.* 31, 87.
- Koefoed-Johnsen, V. and H. H. Ussing (1958). *Acta Physiol. Scand.* 42, 298.
- Kumagai, A., S. Yano, M. Otomo and K. Takeuchi (1957). *Endocrinol. Japon* 4, 17.
- Leaf, A. and A. Renshaw (1957). *Biochem. J.* 65, 82.
- Mattingly, D., C. Tyler and E. Bilton (1970). *Br. Med. J.* 3, 498.
- Ogata, E., K. Nishiki, N. Kugai and T. Kishikawa (1977). *Am. J. Physiol.* 232, E401.
- Saito, T., A. Essig and E. R. Caplan (1973). *Biochim. Biophys. Acta* 318, 371.
- Snedecor, G. W. and W. G. Cochran. Statistical Methods. Iowa State University Press, Ames, Iowa (1967).
- Ulmann, A., J. Menard and P. Corvol (1975). *Endocrinology* 97, 46.
- Ussing, H. H. and K. Zerahn (1951). *Acta Physiol. Scand.* 23, 110.