Br. J. Pharmac. (1971), **41**, 552–557.

Effects of the diuretics, triamterene and mersalyl on active sodium transport mechanisms in isolated frog skin

L. A. SALAKO* AND A. J. SMITH

Department of Pharmacology and Therapeutics, University of Sheffield, Sheffield S10 2TN

Summary

1. Triamterene reduces the rate coefficients for sodium movement into the transporting system of the isolated frog skin. The isotopically measured 'active sodium transport pool' is also reduced.

2. Mersalyl reduces the rate coefficient for sodium and the calculated sodium flux from the transporting system to the inner bathing solution. The 'active sodium transport pool' is increased by this diuretic.

3. The action of triamterene closely resembles that of amiloride and both reduce the entry of sodium into the system. In contrast, mersalyl limits the exit of sodium ions from the skin.

Introduction

Several studies of the mode of action of the potassium-sparing diuretic amiloride have demonstrated its ability to reduce active sodium transport across isolated tissues (Eigler, Kelter & Renner, 1967; Baba, Lant, Smith, Townshend & Wilson, 1968; Bentley, 1968; Crabbé & Ehrlich, 1968; Ehrlich & Crabbé, 1968; Nagel & Dörge, 1970). It seems likely that the reduction in sodium transport in frog skin results from a diminished passive influx of sodium ions into the transporting system rather than a depression of the sodium pump itself (Salako & Smith, 1970a, b). In clinical practice the effects of amiloride are similar to those of the pteridine diuretic. triamterene, and these similarities extend to their actions in frog skin (Baba, Tudhope & Wilson, 1964; Baba *et al.*, 1968). In contrast the organomercurial, mersalyl, also effective in reducing net sodium transport, apparently affects sodium flux by a depression of sodium-pumping activity (Linderholm, 1962; Jamison, 1961; Baba, Smith & Townshend, 1966). We report here experiments in which we have examined in more detail the activity of triamterene and mersalyl on aspects of sodium transport in isolated frog skin.

Methods

Methods for measuring the 'active sodium transport pool' and the rate coefficients for sodium movement were as described in previous reports (Salako & Smith, 1970a, b). They were based on the work of Ussing & Zerahn (1951) and the three-

* Present address: Department of Pharmacology, University College Hospital, Ibadan, Western Nigeria.

TABLE 1. *Effects of triamterene* (2×10^{-7}M) *on net sodium transport, active sodium transport pool and rate coefficients for sodium in the frog skin*

Experiment number	Net sodium transport ((μEq/cm²)/h)		Active Na transport pool (μEq/g tissue water)		*k*12 ($h^{-1}\times10^{3}$)		*k*21 (h^{-1})		*k*23 (h^{-1})	
	Control	Triamterene	Control	Triamterene	Control	Triamterene	Control	Triamterene	Control	Triamterene
1	1·4	0·4	5·9	2·1	1·1	0·4	4·1	3·2	6·5	4·9
2	1·0	0·7	3·5	2·5	0·8	0·7	3·3	0·8	7·5	7·2
3	1·7	0·9	6·2	3·3	1·1	0·5	2·2	0·3	6·9	8·9
4	1·0	0·6	7·5	4·2	1·6	0·7	7·2	1·0	4·6	4·2
5	0·8	0·6	4·7	3·9	1·1	0·4	4·6	0·4	4·0	6·6
6	0·7	0·4	3·2	3·0	0·5	0·2	3·4	0·4	5·7	4·8
Mean±S.D.	1·1±0·4	0·6±0·2	5·2±1·7	3·2±0·8	1·0±0·36	0·5±0·17	4·1±1·7	1·0±1·1	5·9±1·4	6·1±1·8
Diff. ±S.E.M.	0·5±0·17		2·0±0·77		0·5±0·17		3·1±0·8		0·2±0·87	
P value	<0·05		<0·05		<0·05		<0·05		N.S.	

*k*12 etc.=rate coefficient from compartment 1→2.

compartment model used by Schoffeniels (1957) and Curran, Herrera & Flanigan (1963). All experiments were performed in pairs using symmetrical halves of the same skin. The drug concentrations in the fluid bathing the isolated ventral skin of the frog (*Rana temporaria*) were selected to produce a consistent and substantial fall in net sodium transport. Triamterene was effective at a concentration of 2×10^{-7}M in the outer bath fluid and mersalyl at 2×10^{-4}M in the inner bath fluid.

Results

Triamterene

This compound reduced net active sodium transport and the 'active sodium transport pool' of the skin. Rate coefficients ($k12$, $k21$) for sodium across the outer or permeability barrier were reduced as were the calculated fluxes in both directions at this site (ϕ 12, ϕ 21). A small but significant reduction in efflux at the inner barrier (ϕ 23) was also produced (Tables 1 and 2).

Mersalyl

Net sodium flux across the skin was reduced by this compound but a considerable increase occurred in the 'active sodium transport pool'. Rate coefficients and sodium fluxes at the outer permeability barrier were not consistently affected by mersalyl but the rate coefficient for sodium efflux at the inner barrier (k 23) and the calculated sodium flux at this site (ϕ 23) were both considerably reduced (Tables 3 and 4).

Discussion

The results presented here enable a comparison to be made of the actions of amiloride, triamterene and mersalyl on isolated frog skin. All reduce active sodium transport but the dominant effects of both amiloride and triamterene appear to be a reduction in the rate of entry of sodium ions into the cellular transport mechanism and of the 'active sodium transport pool' within the isolated tissue. The action of mersalyl, by contrast, appears to be centred on the inner transport barrier where sodium efflux from the skin is reduced. A concomitant increase in the 'active sodium transport pool' also occurs.

TABLE 2. *Effects of triamterene* (2×10^{-7}M) *on unidirectional sodium fluxes in frog skin*

Experiment Number	ϕ12		ϕ21		ϕ23	
	Control	Triamterene	Control	Triamterene	Control	Triamterene
1	61·2	16·4	24·2	6·7	38·4	10·3
2	36·2	20·0	11·2	2·0	25·5	18·0
3	54·5	30·4	13·6	1·0	42·8	29·4
4	87·3	21·6	54·0	4·2	34·5	17·6
5	40·4	27·2	21·6	1·6	18·8	25·7
6	26·2	14·9	10·9	1·2	18·2	14·4
Mean (±S.D.)	51·0±21·8	21·8±6·1	22·6±16·4	2·8±2·2	29·7±10·4	19·2±5·6
Diff (±S.E.M.)	29·2±9·2		19·8±6·7		10·5±4·8	
P value	<0·05		<0·05		=0·05	

Sodium fluxes expressed as (microequivalents/gram tissue water)/hour. ϕ12 etc.=Flux from compartment 1→2.

TABLE 3. *Effects of mersalyl* (2×10^{-4}M) *on net sodium transport, active sodium transport pool and rate coefficients for sodium on frog skin*

Experiment number	Net sodium transport ((μEq/cm²)/h)		Sodium pool (μEq/g tissue water)		K_{12} ($h^{-1}\times10^{3}$)		K_{21} (h^{-1})		K_{23} (h^{-1})	
	Control	Mersalyl	Control	Mersalyl	Control	Mersalyl	Control	Mersalyl	Control	Mersalyl
1	1·5	0·1	3·4	8·4	0·8	0·4	2·7	4·2	5·5	1·5
2	1·7	0·1	5·3	10·5	1·3	0·9	4·8	4·5	6·9	1·1
3	1·6	0·4	5·2	7·8	1·9	1·2	9·8	8·2	5·9	2·7
4	1·3	0·3	7·5	12·3	1·3	1·5	3·8	5·7	4·0	0·9
5	1·2	0·1	6·2	10·8	1·3	0·6	6·3	4·3	5·6	1·3
6	2·1	0·4	5·9	9·4	1·2	0·9	1·6	2·7	7·3	1·0
Mean (±S.D.)	1·6±0·3	0·2±0·2	5·6±1·4	9·7±1·7	1·3±0·3	0·9±0·4	4·8±2·9	4·9±1·9	5·9±1·2	1·4±0·7
Diff. (±S.E.M.)	1·4±0·14		4·1±0·88		0·4±0·22		0·1±1·4		4·5±0·55	
P value	<0·001		<0·01		N.S.		N.S.		<0·001	

TABLE 4. *Effects of mersalyl* (2×10^{-4}M) *on unidirectional sodium fluxes in frog skin*

Experiment number	ø12		ø21		ø23	
	Control	Mersalyl	Control	Mersalyl	Control	Mersalyl
1	27·8	23·9	9·2	17·6	18·7	6·3
2	60·7	56·8	25·6	47·3	36·7	12·0
3	81·4	85·0	51·0	64·0	30·7	21·1
4	58·1	81·0	28·5	70·1	30·0	11·1
5	70·2	59·0	39·0	46·4	34·7	14·0
6	50·5	34·9	9·4	25·8	43·1	9·4
Mean (±S.D.)	58·1±18·3	56·8±24·3	27·1±16·4	45·2±20·6	32·3±8·2	12·3±1·0
Diff. (±S.E.M.)	1·3±12·4		18·1±10·8		20·0±3·9	
P value	N.S.		N.S.		<0·01	

Sodium fluxes are expressed as (microequivalents/gram tissue water)/hour.

Our results confirm the striking similarity which exists between the two potassium sparing diuretics, amiloride and triamterene, and the quite dissimilar behaviour of mersalyl. These conclusions are based on the model for frog skin originally proposed by Koefoed-Johnsen & Ussing (1958) and on the model for a three-compartment system of Schoffeniels (1957). The validity of this model has recently been challenged by Zerahn (1969) and the interpretation we have put on our findings will need revision if his views are confirmed—in particular in relation to the site and nature of the 'active sodium transport pool'. It seems unlikely, however, that subsequent revision will invalidate our demonstration of the close similarity between the two potassium retaining diuretics or of their fundamental difference in mode of action from mersalyl.

We thank Mrs. S. Holmes, Mrs. J. Harlow, Mrs. S. Smith and Mr. D. Gow for technical help. L.A.S. was in receipt of a Boots Research Fellowship and radioisotopes were purchased with a grant from the United Sheffield Hospitals Endowment Research Fund. We thank Mrs. J. Leicester and Miss M. Weeds for secretarial help.

REFERENCES

BABA, W. I., LANT, A. F., SMITH, A. J., TOWNSHEND, M. M. & WILSON, G. M. (1968). Pharmacological effects in animals and normal human subjects of the diuretic amiloride hydrochloride (MK-870). *Clin. Pharmac. Ther.*, **9**, 318–327.

BABA, W. I., SMITH, A. J. & TOWNSHEND, M. M. (1966). A comparison of the effects of ethacrynic acid and a mercurial diuretic (mersalyl) on sodium transport across the isolated frog skin. *Br. J. Pharmac.*, **28**, 238–245.

BABA, W. I., TUDHOPE, G. R. & WILSON, G. M. (1964). Site and mechanism of action of the diuretic triamterene. *Clin. Sci.*, **27**, 181–189.

BENTLEY, P. J. (1968). Amiloride: a potent inhibitor of sodium transport across the toad bladder. *J. Physiol., Lond.*, **195**, 317–330.

CRABBÉ, J. & EHRLICH, E. N. (1968). Amiloride and the mode of action of aldosterene on sodium transport across toad bladder and skin. *Pflügers Arch. ges. Physiol.*, **304**, 284–296.

CURRAN, P. F., HERRERA, F. C. & FLANIGAN, W. J. (1963). The effect of Ca and antidiuretic hormone on Na transport across frog skin. II. Sites and mechanisms of action. *J. gen. Physiol.*, **46**, 1011–1027.

EIGLER, J., KELTER, J. & RENNER, E. (1967). Wirkungscharakteristika eines neuen acylguanidins—amiloride—HCl (MK-870) an der isolierten haut von amphibien. *Klin. Wschr.*, **45**, 737–738.

EHRLICH, E. N. & CRABBÉ, J. (1968). The mechanism of action of amipramizide. *Pflügers Arch. ges. Physiol.*, **302**, 79–96.

JAMISON, R. L. (1961). The action of mercurial diuretic on active sodium transport, electrical potential and permeability to chloride of the isolated toad bladder. *J. Pharmac. exp. Ther.*, **133**, 1–6.

KOEFOED-JOHNSEN, V. & USSING, H. (1958). The nature of the frog skin potential. *Acta physiol. scand.*, **42**, 298–308.

LINDERHOLM, H. (1952). Active transport of ions through frog skin with special reference to the action of certain diuretics. *Acta physiol. scand.*, **27**, Suppl. 97, 1–144.

NAGEL, W. & DÖRGE, A. (1970). Effect of amiloride on sodium transport of frog skin. I. Action on intracellular sodium content. *Pflügers Arch. ges. Physiol.*, **317**, 84–92.

SALAKO, L. A. & SMITH, A. J. (1970a). Effects of amiloride on active sodium transport by the isolated frog skin: evidence concerning site of action. *Br. J. Pharmac.*, **38**, 702–718.

SALAKO, L. A. & SMITH, A. J. (1970b). Changes in sodium pool and kinetics of sodium transport in frog skin produced by amiloride. *Br. J. Pharmac.*, **39**, 99–109.

SCHOFFENIELS, E. (1957). An isolated single electroplax preparation. II. Improved preparation for studying ion flux. *Biochim. biophys. Acta*, **26**, 585–596.

USSING, H. & ZERAHN, K. (1951). Active sodium transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta physiol. scand.*, **23**, 110–127.

ZERAHN, Z. (1969). Nature and localisation of the sodium pool during active transport in the isolated frog skin. *Acta physiol. scand.*, **77**, 272–281.

(*Received August* 18, 1970)