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A COMPARISON OF THE EFFECTS OF ETHACRYNIC ACID AND A MERCURIAL DIURETIC (MERSALYL) ON SODIUM TRANSPORT ACROSS THE ISOLATED FROG SKIN

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The pharmacology of ethacrynic acid, 2-3-dichloro-4-(2-methylenebutyryl) phenoxy-acetic acid, a new diuretic drug, has been described by Baer, Michaelson, McKinstry & Beyer (1964) and Beyer, Baer, Michaelson & Russo (1965). Its diuretic activity is similar to that of the organic mercurials and it has been shown that when maximal diuresis is produced in dogs by meralluride and ammonium chloride, intravenous injection of ethacrynic acid does not enhance the effect (Beyer *et al.*, 1965).

A reduction in stainable protein-bound sulphydryl groups in dog kidney cells has been produced both by mercurials and ethacrynic acid (Cafruny & Farah, 1956; Komorn & Cafruny, 1964) and further studies have shown that ethacrynic acid may reduce the uptake of isotope-labelled chlormerodrin by both rat and dog kidney slices (Gussin & Cafruny, 1965). It has been suggested, therefore, that the mechanism of action of these diuretic drugs might be similar.

Further similarities have come from the work of Duggan & Noll (1965) who showed by experiments *in vitro* that ethacrynic acid inhibits the sodium–potassium-dependent adenosinetriphosphatase (ATP-ase) derived from renal cortex in the same way as organomercurials, although the concentrations employed were high. Hook & Williamson (1965) have shown that the diuretic activity of the drug may be unrelated to its inhibition of ATP-ase.

Evidence is accumulating, however, that the site of action in the kidney of mercurial diuretics and ethacrynic acid may be different. In stop-flow experiments mercurial diuretics appear to act predominantly on the proximal tubule (White & Rolf, 1963), but similar studies with ethacrynic acid suggest an action on the ascending limb of the loop of Henle (Cooke & Lindeman, 1965).

The effect of mercurial diuretics on the transport of ions across anuran membranes has previously been studied (Linderholm, 1952; Jamison, 1961). The purpose of the present investigation was to compare the effects of a mercurial diuretic (mersalyl) with those of ethacrynic acid on active sodium transport across the isolated frog skin.

METHOD

This was essentially that described in a previous communication (Baba & Smith, 1964). Frog skin was mounted in Ringer solution between Perspex half-cells and active sodium transport measured, with the skin potential reduced to zero, by the current generated ("short-circuit current") and simultaneously by the uni-directional passage of radioactive sodium (^{24}Na) through the skin (Ussing & Zerahn, 1951).

To one side of the bath 100-microcurie quantities of ^{24}Na were added and the rate of passage across the skin measured by sampling from the opposite side. After a control period, drugs were added to either the inside or outside of the skin surface and changes in short-circuit current were recorded. Alterations in active sodium transport were also measured by the change in rate of passage of ^{24}Na across the skin; both efflux and influx of ^{24}Na were measured in separate groups of experiments. The pH of the bath fluids was recorded and the addition of the drugs caused no significant change.

RESULTS

Effects on short-circuit current

Ethacrynic acid. This drug, obtained as the sodium salt, was dissolved in frog Ringer solution and administered as 1 ml. solution containing the selected dose. Although it was completely without effect on the short-circuit current when applied to the outside skin surface, ethacrynic acid consistently produced an increase in short-circuit current when applied to the inside skin surface (Fig. 1). This increase was observed with doses of 2, 5, 10 or 20 mg (concentration range $1.2 - 12 \times 10^{-4}$ M) and was followed by a more gradual decline in short-circuit current to, or below, base-line levels.

Mersalyl. This diuretic had no effect when added to the outside of the skin. In contrast to ethacrynic acid, mersalyl added to the fluid bathing the inside of the skin consistently produced a transient increase in short-circuit current followed, after a period

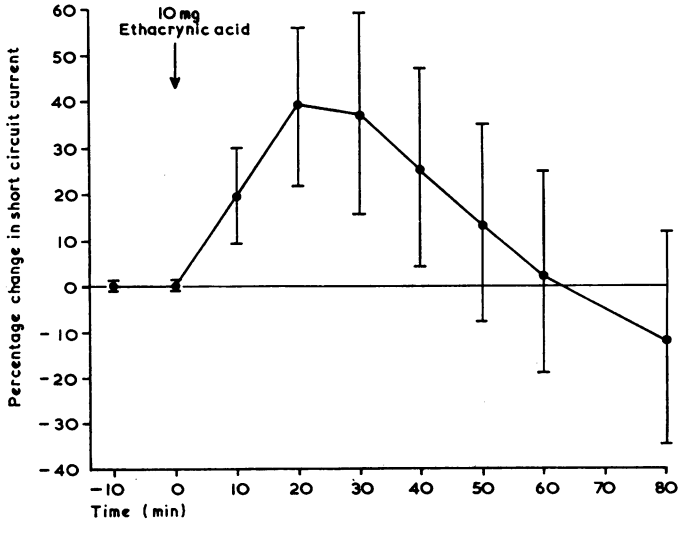

Fig. 1. Effect on short-circuit current of 10 mg ethacrynic acid applied to inside skin surface (mean ±S.D.: 10 expts.).

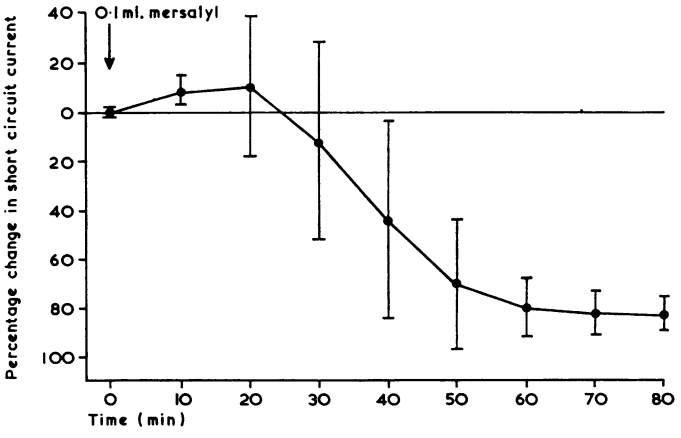

Fig. 2. Effect on short-circuit current of 0.1 ml. mersalyl applied to inside skin surface (mean ±S.D.: 5 expts.).

of 15–25 min, by a profound and irreversible fall (Fig. 2). The usual dose added to produce this effect was 0.1 ml. (4×10^{-4} M), though smaller doses were effective. This preparation contains theophylline (5% w/v) as a preservative.

Washing the skin with frog Ringer solution did not reverse the effects of mersalyl, nor could this be achieved by dimercaprol or diamino-ethane-tetra-acetic acid (EDTA) as the disodium salt applied before or after the introduction of the mercurial into the bath fluid.

Effects on sodium flux

Ethacrynic acid. The influx of sodium (expressed as μ-equiv Na/cm^2/30 min) was consistently increased in proportion to the increase in short-circuit current produced by the drug (Table 1).

Experiments to measure the efflux of sodium showed that ethacrynic acid produces no consistent change in the rate of outward passage of sodium ions through the skin. From the results of efflux experiments and simultaneous measurements of the short-circuit current, the electromotive force of the sodium-transporting mechanism (E_{Na}) and the partial resistance to the flow of sodium ions (R_{Na}) were calculated (Ussing, 1949). Ethacrynic acid produced little change in E_{Na} and only a small, though consistent, decrease in R_{Na} (Table 2).

Mersalyl. Sodium influx was reduced by this drug, the changes corresponding to those observed in the short-circuit current (Table 3).

By contrast, the efflux of sodium through the skin was increased by mersalyl, and similar calculations to those employed with ethacrynic acid showed a constant reduction in E_{Na} though the effects on electrical resistance were variable (Table 4).

Drug interactions

Experiments in which the two drugs were applied consecutively to the same skin showed that ethacrynic acid in a dose of 10 mg was ineffective in reversing the depressant

TABLE 1

SODIUM (^{24}Na) INFLUX: EFFECT OF 10 mg ETHACRYNIC ACID APPLIED TO INSIDE SKIN SURFACE

(30-min sampling periods)

Period	Mean S.C.C. (μA)	Na influx (μ-equiv/cm^2/ 30 min)	Na influx (mCoul/cm^2/ 30 min)	S.C.C. (mCoul)
(1) Control 1	75·0	0·686	66·2	47·7
Control 2	75·0	0·598	57·7	47·7
After drug 1	91·9	0·546	52·7	58·4
After drug 2	86·3	0·849	81·9	54·9
(2) Control 1	91·0	0·382	36·9	56·9
Control 2	85·0	0·709	68·4	53·1
After drug 1	129·3	0·948	91·5	80·8
After drug 2	150·0	0·821	79·1	93·8
(3) Control 1	106·0	0·742	71·6	67·4
Control 2	98·7	0·680	65·6	62·8
After drug 1	138·1	0·905	87·3	87·8
After drug 2	124·7	1·117	107·8	79·3
(4) Control 1	144·6	1·075	103·7	90·4
Control 2	135·2	0·808	77·9	84·5
After drug 1	220·1	1·327	128·0	137·6
After drug 2	162·3	1·319	127·3	101·4
(5) Control 1	166·4	1·520	146·7	105·8
Control 2	151·7	1·235	119·2	96·5
After drug 1	217·3	1·223	118·0	138·2
After drug 2	202·5	1·271	122·6	128·8

TABLE 2

SODIUM (^{24}Na) EFFLUX: EFFECT OF 10 mg ETHACRYNIC ACID APPLIED TO INSIDE SKIN SURFACE

(30-min sampling periods)

Period	Mean S.C.C. (μA)	Na efflux (μ-equiv/cm^2/ 30 min)	Na efflux (mCoul/ cm^2/30 min.)	S.C.C. (mCoul)	E_{Na} (mv)	R_{Na} (Ω/cm^2)
(1) Control 1	138·1	0·0212	2·046	87·8	97·1	1,989
Control 2	129·2	0·0545	5·259	82·2	71·5	1,566
After drug 1	153·3	0·0470	4·537	97·5	80·0	1,477
After drug 2	135·0	0·1042	10·051	85·9	57·9	1,214
(2) Control 1	149·0	0·0090	0·872	94·8	120·6	2,291
Control 2	130·0	0·0319	3·073	82·7	85·4	1,859
After drug 1	174·1	0·0104	0·999	110·7	121·0	1,967
After drug 2	159·3	0·0204	1·971	101·3	101·6	1,805
(3) Control 1	96·2	0·0399	3·845	60·1	72·2	2,162
Control 2	97·0	0·0255	2·457	60·6	83·3	2,472
After drug 1	134·7	0·0335	3·232	84·2	84·6	1,809
After drug 2	128·3	0·0484	4·665	80·2	74·5	1,672
(4) Control 1	112·5	0·0213	2·021	70·3	91·8	2,350
Control 2	110·0	0·0112	1·062	68·8	107·5	2,815
After drug 1	149·8	0·0140	1·350	93·6	109·2	2,100
After drug 2	212·6	0·0192	1·802	132·9	110·8	1,501
(5) Control 1	35·3	0·0161	1·357	22·1	73·1	5,962
Control 2	36·0	0·0402	3·874	22·5	49·2	3,936
After drug 1	93·9	0·0477	4·606	58·7	67·3	2,064
After drug 2	84·0	0·1700	1·640	52·5	89·7	3,075

TABLE 3

SODIUM (^{24}Na) INFLUX: EFFECT OF 0·1 ml. MERSALYL APPLIED TO INSIDE SKIN SURFACE

(30-min sampling periods)

Period	Mean S.C.C. (μA)	Na influx (μ-equiv/cm^2/ 30 min)	Na influx (mCoul/cm^2/ 30 min)	S.C.C. (mCoul)
(1) Control 1	123·3	0·531	51·2	77·1
Control 2	110·3	0·507	48·9	68·9
After drug 1	112·0	0·830	80·0	70·0
After drug 2	28·4	0·300	29·0	17·8
After drug 3	10·0	0·119	11·4	6·3
(2) Control 1	132·5	0·719	69·4	84·3
Control 2	123·9	0·793	76·5	78·8
After drug 1	121·1	1·780	171·8	77·0
After drug 2	92·4	1·522	146·9	58·8
After drug 3	15·7	0·618	59·6	10·0
(3) Control 1	72·5	0·587	56·6	45·3
Control 2	67·2	0·482	46·5	42·0
After drug 1	80·3	0·560	54·1	50·2
After drug 2	46·6	0·403	38·9	29·1
After drug 3	19·4	0·100	9·7	12·1

TABLE 4

SODIUM (^{24}Na) EFFLUX: EFFECT OF 0·1 ml. MERSALYL APPLIED TO OUTSIDE SKIN SURFACE

(30-min sampling periods)

Period	Mean S.C.C. (μA)	Efflux Na (μ-equiv/cm^2/ 30 min)	Efflux Na (mCoul/ cm^2/30 min.)	S.C.C. (mCoul)	E_{Na} (mv)	R_{Na} (Ω/cm^2)
(1) Control 1	100·0	0·0319	3·078	63·6	78·9	2,232
Control 2	98·0	0·0206	1·991	62·3	89·2	2,576
After drug 1	100·4	0·0304	2·934	63·9	80·2	2,262
After drug 2	45·5	0·0421	4·058	28·9	53·8	3,343
After drug 3	12·0	0·0849	8·193	7·6	16·9	3,974
(2) Control 1	121·8	0·0284	2·743	77·5	86·7	2,013
Control 2	122·0	0·0220	2·118	77·6	93·1	2,160
After drug 1	141·0	0·0557	5·370	89·7	73·8	1,481
After drug 2	58·5	0·1500	14·434	37·2	31·7	1,534
After drug 3	12·1	0·0863	8·327	7·7	16·8	3,932
(3) Control 1	78·2	0·0423	4·079	48·9	65·8	2,425
Control 2	68·1	0·0352	3·400	42·6	66·9	2,827
After drug 1	54·8	0·0498	4·808	40·5	57·6	3,026
After drug 2	44·2	0·0962	9·281	27·6	35·4	2,307
After drug 3	34·0	0·0850	8·204	21·3	32·9	2,782

effect of 0.1 ml. mersalyl on the short-circuit current. When the order of administration was reversed, the stimulatory effect of ethacrynic acid was overcome by the fall in short-circuit current induced by mersalyl (Fig. 3).

Treating the skin beforehand with actinomycin D (50 μg in 50 ml. bath fluid) did not alter the response to ethacrynic acid.

Theophylline increases the short-circuit current generated by isolated toad bladder (Orloff & Handler, 1962). The addition of mersalyl to the inside surface of a skin

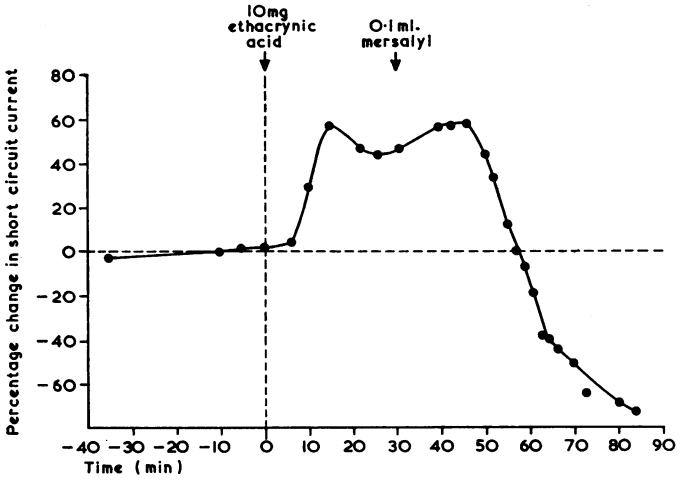

Fig. 3. Effect of 0.1 ml. mersalyl applied to inside skin surface after preliminary application of 10 mg. ethacrynic acid.

previously stimulated with 25 mg theophylline resulted in a delayed fall in short-circuit current which was not preceded by the usual brief stimulatory phase.

DISCUSSION

The results presented demonstrate opposite effects of ethacrynic acid and mersalyl on active sodium transport across the frog skin. The effects of both drugs were not reversed by washing the skin and it proved impossible to increase the short-circuit current with ethacrynic acid when once mersalyl had been added.

The initial transient increase in short-circuit current produced by mersalyl was probably a response to the small amount of theophylline present in the mersalyl solution as it was abolished by pretreatment of the skin with theophylline.

Actinomycin D is known to inhibit the stimulatory effect of aldosterone upon sodium transport across toad bladder, while the response to vasopressin is little affected (Edelman, Bogoroch & Porter, 1963; Crabbé & De Weer, 1964). In this respect ethacrynic acid behaved similarly to vasopressin as its effect could not be blocked by actinomycin D.

Thus, despite the evidence of other workers that the two drugs act in a similar manner, our results suggest that mersalyl and ethacrynic acid have distinct and dissimilar actions upon active transport mechanisms in the isolated frog skin. This is supported by the action of ethacrynic acid and mersalyl on potassium excretion in human subjects. Dale and Sanderson (1954) demonstrated a potassium conserving effect of mersalyl in normal subjects which was abolished if hyperaldosteronism was induced. Ethacrynic acid consistently increases potassium excretion in both normal subjects and oedematous patients (Dollery, Parry & Young, 1964).

More recently, however, Lipson & Hays (1966), in a short communication, claim that both ethacrynic acid and the mercurial diuretic chlormerodrin reduce the short-circuit

current across toad bladder, although their effects are produced by different mechanisms. It is not possible, at present, with the data available, to reconcile our results with those which they present.

SUMMARY

1. In experiments using the isolated frog skin preparation, ethacrynic acid consistently increased the short-circuit current and the influx of sodium ions.

2. Mersalyl consistently reduced the short-circuit current and the influx of sodium ions.

3. The electromotive force of the sodium-transporting mechanism (E_{Na}) and the partial resistance to the flow of sodium ions were calculated from measurements of short-circuit current and sodium efflux.

4. Ethacrynic acid produced little change in E_{Na} and a small decrease in resistance; mersalyl produced a considerable fall in E_{Na}.

5. The results suggest different modes of action of these diuretic drugs on active sodium transport.

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