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THE RELEASE OF POTASSIUM IONS FROM RABBIT ERYTHROCYTES BY SOME STEROID ESTERS

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Abstract—Erythrocyte potassium was released into the plasma when fresh rabbit blood was incubated at 38° for 15 min with a hypnotic steroid, disodium 3α-hydroxy-5β-pregnane-11,20-dione 3-phosphate, at concentrations ranging from 9×10^{-4} M to 7×10^{-3} M. The release of erythrocyte potassium did not require hydrolysis of the ester link. The monosodium sulphate and diethylaminoacetate hydrochloride esters of the parent steroid named above had slight potassium-releasing activity. The sodium hemibut-2-enedioate may have been slightly active, but other esters, some of them hypnotics, were inactive. Other active steroids were disodium 3α-hydroxy-5β-pregnan-20-one 3-phosphate and disodium 3α-hydroxy-16α-methyl-5β-pregnane-11,20-dione 3-phosphate. Disodium 3β-hydroxy-5α-pregnane-11,20-dione 3-phosphate was slightly active. Of the steroid esters tested, 21-phosphates and 3- and 21-hemisuccinates did not release erythrocyte potassium.

THE claim by Schatzmann[1] that cardiac glycosides and aglycones at low concentrations can inhibit the active transport of sodium and potassium ions by erythrocytes has been amply confirmed.[2–5] The finding that the ability of a cardiac glycoside to promote this inhibition is proportional to its acute toxicity in the cat[6] suggests that a similar effect of such compounds on ion transport in the myocardium is fundamentally related to their cardiotonic action. Various metabolic inhibitors (iodoacetate,[7–9] fluoride,[7–9] arsenate,[9, 10] phloridzin[3]) also interfere with erythrocyte cation transport, but the cardiac glycosides have no effect on erythrocyte respiration or on glycolysis.[1]

Compounds of other classes possess this property. Many lactones inhibit erythrocyte sodium and potassium transport,[2, 3] and so do calcium ions at high concentration,[11] adenine, deoxyadenosine, ADP, ATP, cytidine, cytidine monophosphate, uridine and uridine monophosphate.[12] Adenosine[9, 10, 12] and inosine[12] support cation transport. Although it has been reported[13, 14] that hydrocortisone reduces the rate of erythrocyte cation flux without altering ion concentration in the cells,[13] Kumar and Sheth[5] claimed that the hemisuccinate was inactive. Likewise there have been disagreements over the effects of aldosterone[14–18] and deoxycorticosterone.[5, 19–21] Erythrocyte cation transport is affected by some but not all steroidal antagonists of aldosterone: SC 9420 (spironolactone) was inactive,[22] but SC 5233 inhibited sodium efflux from cold-stored erythrocytes.[5]

This report describes steroids of another class that affect cation transport by erythrocytes. We found that intravenous administration of a hypnotic steroid, disodium 3α-hydroxy-5β-pregnane-11,20-dione 3-phosphate, caused in a series of rabbits a

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transient increase in plasma potassium concentration (Fig. 1 shows a typical result); therefore we investigated the *in vitro* activities of this and related compounds by incubating them with fresh rabbit blood and measuring the changes produced in plasma potassium concentration.

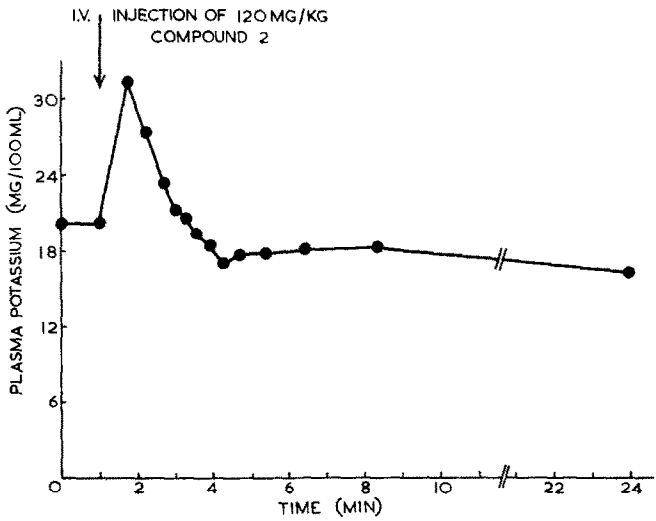

FIG. 1. Increase in plasma potassium concentration in a rabbit (3 kg body weight) given a single rapid intravenous injection of 360 mg disodium 3α-hydroxy-5β-pregnane-11,20-dione 3-phosphate (compound 2) as a 5% aqueous solution. The rabbit became anaesthetised at 2·5 min and recovered at 90 min.

METHODS

Potassium release from erythrocytes

Stoppered centrifuge tubes containing 5 ml samples of heparinised fresh rabbit blood were warmed in a water bath at 38° for 15 min. The steroids, dissolved in small volumes of vehicle, were then thoroughly mixed with the blood and incubated for 15 to 25 min. The tubes were centrifuged, and the plasma potassium concentrations were measured in duplicate with an EEL flame photometer.

Ethanol was used to dissolve 3α-hydroxy-5β-pregnane-11,20-dione (compound 1,* insoluble in water). Before the experiment it was ascertained that 1 mg of this steroid in 0·025 ml ethanol was soluble in 2 ml plasma.

The technique for adding the steroids to the blood was varied to suit the different compounds. Before being added to the blood the water-soluble compounds were dissolved in 0·9% saline. Compounds 4, 5, 7 and 10, which are acids insoluble in water, were dissolved in warm saline and a few drops of 0·1 N NaOH. Compound 9 was similarly dissolved with the aid of 0·1 N HCl.

Some of the steroids could be added to blood at high concentrations without harming the cells. Other were haemolytic, but only in concentrations substantially greater than those used in the experiments. Nevertheless, precautions were taken to prevent

* For brevity the steroids are usually referred to in the text by the numbers assigned to them in Table 1.

lysis when the steroid solutions were added to the blood; samples suspected of being haemolysed were rejected, and the experiments were repeated with more stringent precautions.

No special precautions were necessary with compounds 2, 14, 15, 18 or 19 or the inorganic phosphates. Various volumes of the same saline solution of compound 3 were added to the blood to obtain the required different steroid blood concentrations.

TABLE 1. LIST OF STEROIDS TESTED

Compound number	Chemical name
1	3α-Hydroxy-5β-pregnane-11,20-dione
	Esters of compound 1:
2	3-phosphate disodium
3	3-hemisuccinate sodium
4	3-hemibut-2-enedioate
5	3-hemiphthalate
6	3-hemisulphate sodium
7	3-hemioxydiacetate
8	3-hemiglutarate sodium
9	3-diethylaminoacetate
10	3-carbobenzyloxyglutamate
11	3α-Hydroxy-5β-pregnan-20-one 3-phosphate disodium
12	3α-Hydroxy-5α-pregnane-11,20-dione 3-hemisuccinate sodium
13	3β-Hydroxy-5α-pregnane-11,20-dione 3-phosphate disodium
	Esters of 3α-hydroxy-16α-methyl-5β-pregnane-11,20-dione:
14	3-phosphate disodium
15	3-hemisuccinate sodium
	Esters of 21-hydroxy-5β-pregnane-3,20-dione:
16	21-phosphate disodium
17	21-hemisuccinate sodium
18	Hydrocortisone 21-phosphate disodium
19	Prednisolone 21-phosphate disodium

Small volumes of saline solutions of compounds 6, 8, 11, 12, 13 and 16 were pipetted into separate tubes and warmed; rapid mixing was attained by first adding the warmed blood samples to the steroid solutions and then pouring the mixture back and forth. For the most haemolytic compounds 1, 4, 5, 7, 9, 10 and 17, the 5 ml blood samples were centrifuged, but the plasma was not removed from the tube; after the initial 15 min warming period the steroid solutions were added to the supernatant plasma, and the contents of each tube were rapidly mixed.

Each experiment was controlled by the inclusion of a tube containing 5 ml blood and the appropriate vehicle.

Hydrolysis of compound 2

The rate of *in vitro* hydrolysis of the 3-phosphate, compound 2, was determined by incubating at 38° 25 ml fresh heparinised rabbit blood with 25 mg of the steroid dissolved in 1·0 ml 0·9% saline. Aliquots were removed immediately and at 6 min intervals for 1 hr; the whole blood inorganic phosphate was determined on each by the method of Fiske and Subbarow.[24] Because control studies showed that insignificant changes in the blood inorganic phosphate occurred in the absence of steroid, the amounts of unhydrolysed steroid were calculated from the increases in blood inorganic phosphate.

TABLE 2. POTASSIUM RELEASE FROM ERYTHROCYTES DURING INCUBATION OF RABBIT BLOOD AT 38° WITH VARIOUS STEROIDS

Compound number	Added to 5 ml blood		Incubation time (min)	Plasma potassium (mg/100 ml)	
	mg compound	ml vehicle		Test	Control
1	1	0·025	20	14	16
2	1	0·1	15	19	19
	2	0·1	15	23	19
	4	0·1	15	29	19
	8	0·1	15	49	19
	16	0·1	15	99	19
3	5	0·2	15	15	12
	10	0·4	15	14	13
	20	0·8	15	15	13
4	8	0·2	15	24	20
5	8	0·2	15	20	20
6	8	0·1	25	17	15
	16	0·1	25	23	16
7	8	0·2	15	20	18
8	8	0·1	25	15	15
9	8	0·4	15	23	18
10	8	0·2	15	22	19
11	8	0·1	15	27	19
	8	0·1	25	21	15
	16	0·1	25	46	16
12	8	0·4	15	16	15
13	8	0·1	15	18	14
	8	0·1	25	16	15
	16	0·1	25	21	16
14	8	0·1	15	31	14
15	8	0·1	15	15	14
16	5	0·1	15	19	19
17	6	0·1	15	22	20
	8	0·1	15	22	20
	10	0·1	15	22	20
18	8	0·1	15	18	19
19	8	0·1	15	18	19
Na_3PO_4	3	0·1	15	18	19
Na_2HPO_4	3	0·1	15	18	19

RESULTS AND DISCUSSION

Of the esters of compound 1, only the disodium phosphate, compound 2, had marked activity in releasing potassium ions from erythrocytes (Table 2). It was effective under our experimental conditions at a concentration as low as $8{\cdot}6 \times 10^{-4}$ M. Though it

is often misleading to compare one set of conditions with another, it is probable that this steroid is more active than the lactones and less active than the cardiac glycosides and aglycones reported by Kahn.[3]

The sodium hemisulphate and diethylaminoacetate of compound 1, and possibly the sodium hemibut-2-enedioate, were weakly active. Such differences as were found between test and control samples for the other esters of the same steroid were negligible. Compound 11, the 11-deoxy derivative of compound 2, likewise released potassium ions from erythrocytes, but it was less active than compound 2. A low activity was found for compound 13, the 3,5-isomer of compound 2, whereas compound 2 itself and its 16α-methyl derivative (compound 14) showed approximately the same activity. Thus, marked ability to affect erythrocyte potassium was found only in the three phosphates of 3α-hydroxy steroids. The steroid 21-phosphates (16, 18 and 19) were inactive, and so were the two inorganic phosphates. The other steroid esters, of which hemisuccinates were most extensively tested, had little or no activity. The most active of the non-phosphate esters, compound 6, was also derived from an inorganic acid.

As it was essential to ensure that the effect of the steroid phosphates on erythrocytes was a property of the whole molecule, and not just of the steroid moiety, the rate of hydrolysis of compound 2 was determined. It was found that hydrolysis in rabbit blood proceeded exponentially (Fig. 2), with a half-life of 68 min; thus, in 15 min

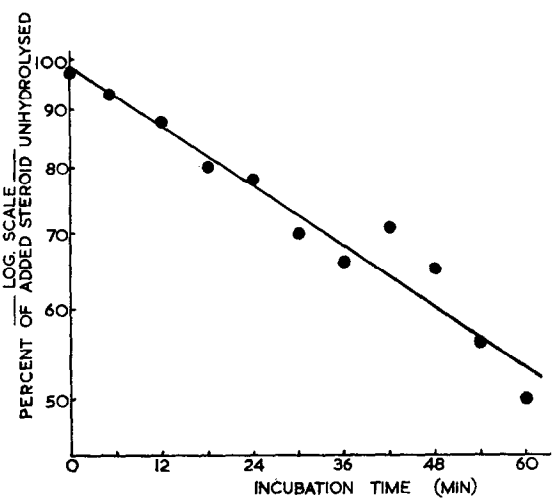

FIG. 2. Hydrolysis of disodium 3α-hydroxy-5β-pregnane-11,20-dione 3-phosphate (initial concentration 1 mg/ml) at 38° in rabbit blood. The degrees of hydrolysis were estimated from the increases in blood inorganic phosphate.

approximately 15 per cent of the added steroid phosphate was hydrolysed. Because 1 mg compound 1 (equivalent to the total steroid alcohol released in 15 min from 9 mg compound 2) did not increase the plasma potassium concentration when incubated with blood, it is concluded that compound 2 is active *per se*.

The potassium-releasing effects of these steroids were not related to their hypnotic properties. Compound 17 (hydroxydione), a well-known steroid hypnotic, did not release potassium from erythrocytes. Further, of the remaining compounds, only 4, 5,

6, 18 and 19 have no hypnotic properties in mice (unpublished observations). Nor, we believe, do these compounds act as mineralocorticoids: the results of experiments by our colleague Mrs. L. Jenkins (unpublished) showed that compound 2, injected subcutaneously in single 8 mg doses into saline-loaded adrenalectomised male rats, had no effect over 5 hr on urinary sodium excretion; not surprisingly perhaps, potassium excretion was increased. The possibility remains that of this group of steroids those effecting potassium ion release from erythrocytes have also cardiotonic properties. This we hope to test in due course.

There is no obvious structural relationship between the active steroids of this series and the cardiac glycosides and aglycones previously reported as interfering with cation transport in the erythrocyte. As our potassium-releasing steroids each contain only one hydroxyl group, it may be thought that, being less active than the cardiac glycosides, they support the hypothesis of Kahn and Acheson[2] that inhibition of erythrocyte cation transport depends on the number of hydroxyl groups on the steroid nucleus. Although originally Kahn believed that the steroid nucleus of a cardiac glycoside could only modify the fundamental ability of its lactone moiety to inhibit cation transport, he later concluded[3] that a steroid could confer activity on an otherwise inactive lactone. We suggest that, because simple steroid esters can have the same kind of activity on erythrocytes as cardiac glycosides, studies with the steroid nuclei of the latter might prove rewarding.

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