The activation of membrane ATPase by ouabain in several tissues of the golden hamster (*Mesocricetus auratus*) in the absence of potassium

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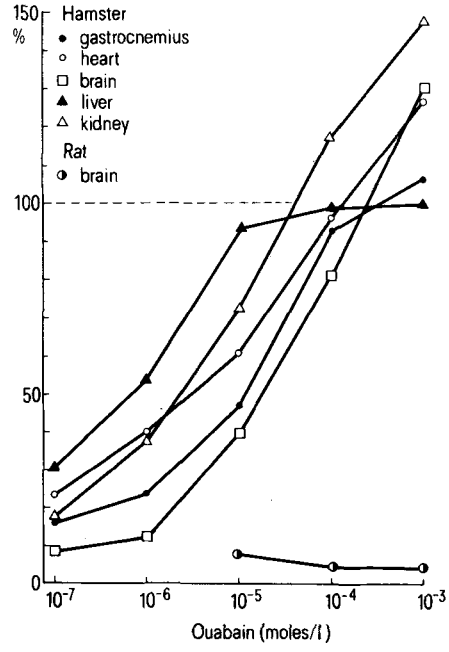
Summary. The activity of the membrane ATPase of 5 organs of the golden hamster was increased by 10^{-7}-10^{-3} moles/l ouabain in K^+-free medium. In similar experiments on rats no increase was observed.

It has recently been found that in a K^+-free medium, ouabain (10^{-4} mmoles/l) increases the activity of membrane-bound ATPase in crude membrane fractions isolated from golden hamster muscles[1]. This is in contrast to the inhibition usually produced by this drug. This unusual effect was also observed electrophysiologically in Na^+-enriched muscle fibres of the golden hamster diaphragm where it caused transient hyperpolarization of muscle fibres by several mV in a K^+-free muscle bath[1]. In contrast no stimulating or electrogenic effect was found in experiments on mouse muscle preparations[1].

In the present communication, we report the effect of 10^{-7} to 10^{-3} moles/l ouabain on membrane ATPase of skeletal muscle and several other tissues of the non-hibernating golden hamster (in winter) with the aim of checking whether the paradoxical effect of this drug is restricted to the muscle tissue, or whether it represents a general feature of the golden hamster membrane ATPase.

Membrane fractions were isolated according to standard isolation procedure[2] from skeletal muscle (gastrocnemius), heart, brain, liver and kidney taken from decapitated female golden hamsters (150-200 g body mass). Preparations from female Wistar rats of similar size were used as controls. The whole organs were isolated and freed of connective and fatty tissue before the isolation procedure.



The increase of membrane Na^+-ouabain ATPase activity (i.e. Na^+-Mg^{2+}-ouabain minus Mg^+-dependent activity) of 5 tissues of the golden hamster in the presence of several concentrations of ouabain. Values represents percentage of Na^+-K^+-dependent activity (i.e. Na^+-K^+-Mg^{2+} minus Mg^{2+}-dependent activity = 100%). For comparison, the absence of activating effect of ouabain on rat brain membrane fractions is also illustrated.

Membrane samples were stored at -20 °C for not longer than 3 weeks. Prior to use, the samples were depleted of inorganic ions by exhaustive ultrafiltration on a PM 10 Diaflo filter (Amicon, USA). Na^+-Mg^{2+} ATPase activity was determined[3] from the amount of inorganic phosphate (P_i) released from hydrolyzed ATP (Boehringer) at 37 °C. The protein content of the membranes was estimated according to Miller[4]. The reaction medium contained (mmoles/l) 130 (or none) Na^+, 5 Mg^{2+}, 30 (or none) K^+ as chloride salts, 1 ATP, 100 Tris, pH 7.5. For the estimation of the sodium content, muscles were extracted in 0.01 moles/l HNO_3 for 10 days and the extraction solution was analyzed for Na using absorption spectrophotometry. The extracellular space of muscles was estimated using inulin[5] and the intracellular Na^+ concentration was calculated according to Akaike[6].

In all tissues of the golden hamster and the rat, the lowest levels of ATPase activity were found in solutions containing Mg^{2+} only (table 1). The addition of Na^+ and K^+ increased the ATPase activity and addition of ouabain to the K^+-Na^+-Mg^{2+} solution reduced the activity. The levels in ouabain were similar to those found in the Mg^{2+} solution. In Na^+-Mg^{2+} solution the activity was somewhat greater than in the Mg^{2+} solution and in the golden hamster this was further increased by ouabain in a concentration dependent manner (table 1, figure). In all tissues the activity of the enzyme rose with the concentration of

Table 1. The effect of ouabain (OUA) on the activity of membrane ATPase of gastrocnemius muscle (GM), heart, brain, liver and kidney membrane fractions

Ions present	GM	Heart	Brain	Liver	Kidney
Golden hamster					
Mg, Na, K	196±2	402±8	325±8	200±5	85±6
+OUA 10^{-5}	58±3	220±9	185±6	85±4	40±6
10^{-4}	36±2	199±9	117±8	82±3	26±4
10^{-3}	33±2	125±5	116±8	70±3	25±3
Mg	32±3	111±5	86±7	56±4	29±3
+OUA 10^{-3}	33±4	132±4	95±3	70±3	33±3
Mg, Na	34±2	133±4	97±4	71±3	33±5
+OUA 10^{-7}	59±3	180±5	104±5	102±5	39±4
10^{-6}	72±3	227±9	120±4	134±4	50±6
10^{-5}	109±4	289±6	181±7	196±5	70±4
10^{-4}	185±5	400±9	281±8	191±4	95±5
10^{-3}	206±3	482±8	400±9	202±7	112±6
Rat					
Mg, Na, K	160±9	417±5	320±9	267±6	118±6
+OUA 10^{-4}	81±5	116±7	136±6	86±6	42±6
Mg	54±5	98±7	68±9	88±2	31±4
Mg, Na	77±4	117±5	69±3	90±5	34±2
+OUA 10^{-5}	86±4	127±6	76±4	92±5	30±6
10^{-4}	77±2	116±7	72±4	80±4	33±4
10^{-3}	78±4	89±2	72±7	81±4	30±4

Results are means ± SEM of 10 experiments. Concentration of ions, if they are present (mmoles/l): Mg^{2+} 5.0, Na^+ 130, K^+ 30. Activity is expressed in μmoles of P_i released in the presence of 100 mg of membrane protein/h at 37 °C. Values are means ± SEM. Concentrations of ouabain (G-strophantin, BDH) are given in moles/l.

ouabain and, except in the liver, it finally exceeded the control values by 7-48% (gastrocnemius muscle and kidney, respectively) in the presence of 10^{-3} moles/l ouabain.
Although the ouabain-induced increase of activity was similar in all tissues, the tissue sensitivity to the drug was different. The concentrations of ouabain which activated the membrane ATPase to one half of the Na^+-K^+-Mg^{2+}-dependent values were (in moles/l) 1.2×10^{-5} for the gastrocnemius muscle, 3×10^{-6} for the heart, 1.8×10^{-5} for the brain, 7×10^{-7} for the liver, and 2.3×10^{-6} for the kidney. The liver membrane fractions were thus the most sensitive.
The susceptibility of membrane Na^+-K^+-Mg^{2+} ATPase to the blocking effect of ouabain is the most common and characteristic feature of this enzyme[7,8]. In concentrations as low as 10^{-7} to 10^{-6} moles/l, ouabain inhibits the enzyme by combining with the outer part of the enzyme complex[9]. Potassium ions compete with the binding of ouabain on the outer surface of the cell membranes[10]. In the golden hamster, however, the relationship between ouabain and potassium seems to be more complicated and probably reflects some unusual characteristics of the enzyme in this animal. When K^+ is present, ouabain has a 'normal' blocking effect; the ouabain stimulation occurs only in the absence of K ions in the reaction medium. This suggest that 2 distinct binding sites may exist on the external surface of the enzyme complex, one for K^+ and another for ouabain. Separate occupation of either of them stimulates the enzyme, whereas the simultaneous occupation of both sites results in enzyme inhibition.
In the whole diaphragm of the golden hamster, we observed sodium extrusion from the muscle fibres during activation of membrane ATPase by ouabain (table 2) in the absence of K^+ in the incubation medium. 20 min after the application of ouabain, the intracellular sodium concentration decreased from 20.8 to 16.0 mmoles/l. This indicates that a) ouabain probably stimulates the enzyme from the outer side of the membrane, b) the ouabain-stimulated enzyme apparently corresponds to the ion transporting ATPase and c) ouabain activation of this enzyme is not coupled with Na^+-Na^+ exchange, but rather with the net outward transport of sodium ions.
Further comparative studies on other mammals are necessary to check whether the unusual effect of ouabain is due to unique properties of the golden hamster membrane ATPase per se or whether this phenomenon is related to the ability of the animal to hibernate.

Table 2.

Control	13.0±0.9
K^+-free	20.8±1.3
+OUA	16.1±0.7
+5 K^+	15.0±0.8
+5 K^+, OUA	20.6±2.2

Sodium content of golden hamster diaphragm in mmoles per l of muscle fibre water after immersion in standard Liley muscle bath[11] containing 5 mmoles/l K^+ for 6 h (control) or on K^+-free muscle bath before (K^+-free) and 20 min after application of 5 mmoles/l K^+ (+5 K^+), ouabain 10^{-4} moles/l (+OUA) and simultaneous application of potassium and ouabain (+5 K^+, OUA), respectively. Mean values±SEM from 6 absorption spectrophotometry estimations in each group (with correction for inulin space of diaphragm[6]) are given.

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Platelet antiaggregating activity in the salivary secretion of the blood sucking bug *Rhodnius prolixus*

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Summary. The salivary secretion of *Rhodnius prolixus* inhibits both ADP and collagen induced platelet aggregation in human platelet-rich plasma. Fractionation studies show that at least 3 different inhibitors are present in *Rhodnius* saliva.

It has been observed among blood sucking insects that most species studied have developed mechanisms for preventing blood from clotting in the food cannals while feeding is in progress. Most of the anticoagulant activity is found in the salivary glands or in the gut contents after the meal[1-3]. The blood sucking bug *Rhodnius prolixus* presents an anticoagulant activity in its salivary glands[4] that was characterized as an antifactor VIII[5]. We recently described a powerful apyrase activity (ATP → AMP+2 orthophosphate) in the salivary secretion of *Rhodnius*[6] and suggested that this activity was physiologically important in preventing host platelet aggregation during the parasite meal. In the work recorded here we provide evidence that *R. prolixus* saliva has indeed an antithrombotic activity that is distinct from the anticoagulant activity. This is the first time the presence of such activity in blood sucking insects has been described.
Material and methods. All experiments were carried out with salivary secretion obtained from 1000-2000 adults and 5th instar nymphs starved for 30 days, which probed for 5 min a heated artificial feeder apparatus containing 10 ml water[6]. The solution yielded about 8 mg of protein; this was freeze-dried and stored at -20 °C until used. Human platelet-rich plasma (PRP) was obtained as described before[7]. Platelet aggregation was monitored by the method of Born and Cross[8]. ATPase and ADPase activities were assayed as described before[6]. 1 enzyme unit is the amount of enzyme that produces 1 µmole of orthophosphate per min at pH 7.5 and 30 °C. Agarose slab gel electrophoresis was performed in Tris Cl buffer 0.025 M pH 7.5[9]. Protein was determined by the technique of Lowry et al.[10] using bovine serum