Inhibitors and heavy metal ions. Incubating the 2 isoenzymes with 5×10^{-4} M EDTA Na_2 for 30 min at 25 °C lost all their activity which is not restored either with the addition of excess of $CaCl_2$ nor with dialysis against large volume of buffered solutions of $CaCl_2$. Thus Ca^{++} is necessary for the stability of both isoenzymes[1,3,4,15,16]. After preincubating the isoenzymes with 10^{-4} M $AgNO_3$, 10^{-4} M $CuSO_4$, 10^{-4} M $HgCl_2$ and 2×10^{-4} M p-chloromercuribenzoate-sodiumsalt (P-CMB) for 30 min at 25 °C, it was found that both amylases retained all their enzymic activity.

Substrate effect. After a long time of incubation of the 2 isoenzymes with soluble starch, the starch-iodine complex color disappeared. This property in combination with the other findings (calcium dependency, heat resistance, insensitivity to Ag^+ Cu^{++} and Hg^+) strongly indicates that both isoenzymes are of the α-type[1]. The 2 separated isoenzymes were also found to be inhibited by high substrate concentration (1%) in the same way, to exhibite practically the same K_m (0.8 mg/ml for the α1 and 0.7 mg/ml for the α2 isoenzyme) and to hydrolyze in the same way the amylose faster than amylopectin.

Subunit analysis. The α1 form was found to be composed of 2 different subunits of mol.wt 16,000 the one and 22,000 the other (mol.wt of α1 16,000 + 22,000 = 38,000 at least). Similarly the α2 form is composed of 2 different subunits of mol.wt 19,500 the one and 32,000 the other (mol.wt of α2 19,500 + 32,000 = 51,500 at least). The value for the α2 isoenzyme is apparently within the range (50,000–55,000) of the mol.wt found from other sources[3,7,11,17,18] while the value for the α1 isoenzyme seems low, although such isoenzymes have been reported in the literature[5,6,19]. Thus the 2 isoenzymes differ only in their mol.wt as well as in their electrophoretic mobility, and the occurrence in the living cell remains still without satisfactory explanation.

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Effect of aldosterone and methylprednisolone on cardiac NaK-ATPase

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Summary. Aldosterone (15 μg BID) and methylprednisolone (8 mg QD) administration to female guinea-pigs augmented both the total and the specific activity of NaK-ATPase but not the activity of adenylate cyclase in the cardiac sarcolemma. The rise in NaK-ATPase was due to increase in the number of enzyme molecules; catalytic activity and ouabain-sensitivity of individual molecules did not change.

In target tissues steroid hormones induce the synthesis of messenger and ribosomal RNA and subsequently that of specific proteins[2]. In the kidney aldosterone[3,4] enhances the synthesis of NaK-ATPase (EC 3.6.1.3), an enzymatic component of the Na^+-pump, and possibly of some Na^+-carrier also. In large doses methylprednisolone[5] and corticosterone[6] exert similar effects. In this paper we show that aldosterone and methylprednisolone preferentially increased the steady state level of NaK-ATPase also in the myocardium, without affecting the catalytic activity or ouabain-binding of the individual enzyme molecules.

Female Dunkin-Hartley guinea-pigs, weighing 337 ± 4 g initially, were randomly divided in 3 groups. Each group was injected daily as follows: 'controls' (24): 0.1 ml of 0.9% (w/v) NaCl i.p. twice daily for 14 or 24 days resp., 'aldosterone-treated' (16): 15 μg of aldosterone i.p. twice daily for 14 days; 'methylprednisolone-treated' (18): 8 mg of Depo-Medrol i.m. once a day for 14 or 24 days, resp. Previously similar experiments by other investigators were conducted on rats. We chose guinea-pigs because in this animal (unlike in the rat) the affinity of NaK-ATPase for ouabain is high and the number of the ouabain-binding sites can be measured directly and accurately by binding assay. After killing the animals, the heart ventricles were dissected free from pericardium and the atria, a piece of tissue was dried to constant weight at 120 °C, the rest of the ventricle was homogenized in ice-cold 0.33 M sucrose in 50 mM Tris-acetate (pH 7.2) containing 1 mM Tris-EGTA (ethyleneglycol-bis(α-aminoethylether)N,N-tetraacetic acid). Enzyme activities were monitored in this initial homogenate and in the sarcolemma, purified by extraction with 1 M KCl[7]. Protein was determined by the procedure of Miller[8]. NaK-ATPase was assayed as described earlier[9], with one modification: the reaction mixtures contained 1 mM Tris-EGTA and 2 mM NaN_3 also. The amount of phosphorylated NaK-ATPase in the sarcolemma was measured at 0.04 mM [γ^{32}P] ATP, ouabain-binding was measured in the presence of 0.05–1 μM [^{3}H] ouabain, dissociation constant of the enzyme-ouabain complex was calculated from Scatchard-plots (for details see Hegyvary[9]). Turnover number of NaK-ATPase was computed by dividing specific activity of the enzyme by the amount of phosphorylated NaK-ATPase at steady state. It was assumed that each enzyme molecule bound

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Effects of aldosterone and methylprednisolone treatment

	Control	Aldosterone (after 14 days)	Methylprednisolone (after 24 days)
Final b.wt (g)	375 ± 5 (24)	395 ± 4 (14)	412 ± 22 (10)
Mean aortic blood pressure (mm Hg)	75 ± 5	78 ± 7	115 ± 6
		NS	$p < 0.005$
Relative heart weight	2.84 ± 0.04 (24)	2.93 ± 0.19 (14)	3.29 ± 0.11 (10)
(mg ventricle)/(g final b.wt)		NS	$p < 0.005$
Dry weight of heart (%)	17.82 ± 0.25 (24)	18.53 ± 0.61 (14)	18.95 ± 0.50 (10)
		NS	$p < 0.025$
Protein content of heart	99.8 ± 2.8 (20)	98.4 ± 3.7 (14)	139.6 ± 6.2 (10)
(mg protein)/(g ventricle)		NS	$p < 0.005$
NaK-ATPase			
Total activity	193.9 ± 5.9 (18)	276.5 ± 13.9 (14)	332.0 ± 22.1 (10)
(μmoles P_i)/(g ventricle × h)		$p < 0.005$	$p < 0.005$
Specific activity (μmoles P_i)/(mg protein × h)			
In homogenate	1.97 ± 0.12 (18)	2.79 ± 0.29 (14)	2.33 ± 0.08 (10)
		$p < 0.005$	$p < 0.005$
In sarcolemma	3.96 ± 0.23	6.72 ± 0.47 (14)	5.38 ± 0.17
		$p < 0.025$	$p < 0.025$
Turnover number	9371 ± 137	9112 ± 56	10475 ± 634
		NS	NS
Dissociation constant for ouabain (nM)	79.1 ± 5.5 (8)	90.8 ± 9.2 (8)	73.4 ± 10.9 (10)
		NS	NS
Adenylate cyclase (pmoles of cyclic AMP)/			
(mg protein × min) Basal activity	51.7 ± 5.9 (12)	52.2 ± 4.3 (8)	50.9 ± 2.6 (10)
		NS	NS
With 8 mM NaF	134.4 ± 14.5 (12)	127.5 ± 10.6 (8)	140.6 ± 12.8 (10)
		NS	NS

one mole of phosphate covalently. Adenylate cyclase of the homogenate was measured according to Drummond and Duncan[10], with one modification: instead of an ATP-regenerating system 8 mM Tris-ATP was added to the reaction mixture. Adenosine 3′5′-monophosphate (cyclic AMP) was determined by competitive binding assay[11]. Mean aortic blood pressure was measured with the pressure transducer of a Grass physiograph. The animals were anesthetized with pentobarbital (30 mg/kg) before cannulating the aorta. Statistical significance was calculated by Student's unpaired t-test. All measurements were done at least in triplicates. The table shows the results as mean ± SE, with the number of animals studied in parentheses. NS stands for 'not significant' ($p > 0.05$). During the period of observation the 2 hormones did not alter the growth rate of the animals with respect to the controls. In accord with previous investigations[12] aldosterone did not raise the blood pressure and did not cause cardiac hypertrophy. In another species (rat) and at this dose level aldosterone caused hypertension only if the animals were 'sensitized' by unilateral nephrectomy and salt loading[12]. In our experiments methylprednisolone induced moderate hypertension and, probably as a consequence, cardiac hypertrophy. This cardiac hypertrophy was not due to tissue edema because the dry weight and protein content of the hearts also increased.

The 2 hormones increased both the total and the specific activity of NaK-ATPase but did not affect adenylate cyclase, another enzyme of the sarcolemma. The rise of NaK-ATPase therefore did not result from a general increase in sarcolemmal enzyme proteins. Aldosterone was more potent than methylprednisolone in raising NaK-ATPase because aldosterone a) was effective in low, near physiological dose; b) increased specific activity of NaK-ATPase to a higher level; c) its effect could be detected earlier (after one week; not shown). In 14 days methylprednisolone raised only the total but not the specific activity of NaK-ATPase, i. e. the activity of the enzyme changed in parallel with the protein content of the cell.

Though neither hormone altered NaK-ATPase in vitro measurement of enzyme activity alone did not determine whether these hormones enhanced the catalytic activity of the enzyme or whether they increased the number of enzyme molecules in the myocardium. Since the catalytic activity (i.e. turnover number, the ratio of specific activity and the number of enzyme molecules) remained unchanged during hormone treatment, the 2 hormones obviously increased the number of active enzyme molecules per cell. Another characteristic property of the enzyme, its affinity for the specific inhibitor ouabain, did not change either. The number of ouabain binding sites was equal to that of the phosphorylation sites, i.e. one mole of phosphoenzyme bound one mole of ouabain (not shown). These data only demonstrated the action of these hormones on cardiac NaK-ATPase but did not explain the underlying mechanism. Elevation of the steady-state level of the enzyme molecules in vivo might have resulted from enhanced synthesis or decreased breakdown or from activation of latent molecules. These possibilities need to be tested directly. Finally though the heart contains receptor protein(s) for gluco- or mineralocorticoids[13] synthesis of NaK-ATPase may not be stimulated directly by these hormones, but rather may be secondary to changes in plasma electrolyte concentration due to aldosterone-induced sodium retention and kaliuresis. Such an adaptation of NaK-ATPase to changes in extracellular sodium and potassium concentration has been observed[14].

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