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PARAMETERS OF HORMONAL REGULATION OF FLUID-ELECTROLYTE EXCHANGE AND cAMP RECEPTION IN THE RAT RENAL PAPILLA DURING ADAPTATION TO COLD

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Under the influence of moderately low temperatures the diuresis and excretion of chlorides with the urine increase in man [3]. In experiments on animals exposed for several days to cold, changes in diuresis and sodium excretion also have been observed [9], and the antidiuretic activity of the blood is reduced [8]. A state of adaptation is known to arise in rats 30-40 days after the begining of exposure to cold [12] and it is characterized by a persistently raised level of metabolism [2]. Accordingly it is important to study the coordinated changes in parameters of fluid-electrolyte exchange and of its regulators, such as antidiuretic hormone (ADH) and aldosterone, in rats adapted to cold.

Since the effectiveness of action of hormones depends not only on their concentration in the plasma, but also evidently on the state of the intracellular systems which mediate their action [1], it was decided to study the intracellular reaction of cAMP, which is an important functional component of the action of ADH in target tissues, in the renal papilla.

EXPERIMENTAL METHOD

Experiments were carried out on mature male Wistar rats weighing 200-250 g. Animals of the experimental group were kept at a temperature of 4-5°C, rats of the control group at 19-21°C. The 24-hourly urine was collected from the animals 7 days after the beginning of the

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TABLE 1. Effect of Adaptation to Cold on Diuresis, Excretion of Sodium and Aldosterone with the Urine, and Plasma ADH Concentration in Rats (M ± m)

Experimental conditions	Diuresis, ml/day	Sodium in urine, meq/day	Aldosterone in urine, ng/day	ADH in plasma, pg/ml
Control	5,1±0,4 n=11	0,61±0.07 n=4	46,9±2,2 n=4	19,0±2,1 n=6
Adaptation to cold	6,5±0,6 n=12	2,00±0.13 n=4	25,5±2,5 n=4	13,0±0,6 n=6
P	<0,06	<0,001	<0,001	<0,05

Legend. n) Number of animals.

experiment, its volume measured, its sodium concentration deteremined by flame photometry on an AAS 1N spectrophotometer (Carl Zeiss, East Germany) and its aldosterone concentration determined by radioimmunoassay, using ALDOK kits (from CIS International, France). The rats were decapitated, and the ADH concentration in this blood determined by the use of Vasopressin-RIA kits (from LTD, Switzerland). The kidneys were removed into cold physiological saline and the papillae were isolated in the cold. Papillary tissue from three animals was pooled and homogenized in buffer 1, containing 100 mM Tris-HCl (pH 7.5), 1 mM EDTA, and 5 mM 2-mercaptoethanol in the ratio (w/v) of 1:10. This and subsequent procedures were carried out in the cold. To obtain cytosol and homogenate was centrifuged for 1 h at 105,000g. From each sample of cytosol two aliquots, each of 0.4 ml, were taken, a concentration of $0.5 \cdot 10^{-8}$ M of ^{3}H-cAMP was created in each, cAMP was added to one aliquot up to a concentration of 10^{-6} M to determine nonspecific binding, and both were incubated for 19 h at 4°C. The resulting cAMP–receptor complexes were separated from unbound ligands on Synpor filters (pore diameter 0.4 μ), after which each filter was washed with 4 ml of cold buffer [1]. The filters were dried and their radioactivity determined in toluene scintillator. Specific binding was calculated by subtracting nonspecific binding from the total.

EXPERIMENTAL RESULTS

Prolonged exposure to cold caused significant changes in renal function. As will be clear from Table 1, in rats adapted to cold, the vasopressin concentration in the plasma was reduced, as also was aldosterone excretion with the urine. Meanwhile the diuresis of the animals was increased, and sodium excretion with the urine was considerably increased. The fall in the concentration of hormones responsible for sodium and water reabsorption in the renal tubules was evidently adaptive in character in the presence of an increased level of metabolism during adaptation of the animals of cold.

The decrease in the 24-hourly aldosterone excretion and increase in sodium excretion discovered in these experiments point to some depression of function of the renin–angiotensin–aldosterone system under conditions of adaptation to cold.

It might be supposed that during adaptation to cold not only the hormonal regulation of fluid-electrolyte homeostasis would be changed, but also the target organ itself and the intracellular mechanisms of action of these hormones. We know that the action of ADH in the epithelial cells of the collecting tubules of the kidney is linked with adenylate cyclase activation and, consequently, with an increase in the cAMP concentration, leading to increased activity of cAMP-dependent protein kinases which, in turn, induces phosphorylation of intracellular substrates and a change in permeability of the epithelium for water [5]. An essential role in this chain is played by interaction between cAMP and the intracellular receptors which are mainly regulatory subunits of cAMP-dependent protein kinases [6].

These experiments showed that specific binding of ^{3}H-cAMP in the cytosol of the renal papilla in animals adaptated to cold was 3.2 ± 0.6 femtomoles/kg protein (n = 4), compared with 1.2 ± 0.04 femtomoles/mg protein in the control rats (n = 3; n denotes the number of tests, each on pooled papillae from three animals).

Thus during adaptation of animals to cold substantial changes evidently take place in the tissue of the renal papilla in the intracellular mechanisms that participate, in particular, in realization of the effect of ADH, the specific hormone for that tissue.

An increase in receptor activity may be linked either with a marked increase in the number of receptors or with the appearance of receptors with increased affinity for cAMP. In either case induction of synthesis of the corresponding proteins must take place. The role of inducers may be played by catecholamines, glucocorticoids, and thyroid hormones, changes in the secretion of which during adaptation of animals to cold have been demonstrated in a number of investigations [4, 10]. Probably these hormones can exert their influence on the state of the kidney, for there is evidence that specific reception of glucocorticoids [7] and of thyroid hormones [11] is possible in the kidney.

In the present experiments a significant fall in the ADH concentration was observed in the blood plasma of the experimental animals. It can be tentatively suggested that the increase in receptor activity in the papilla is of compensatory importance for the epithelium of the collecting tubules, the target tissue for the hormones, and which constitutes a large part of the material of the papilla.

The decrease in the concentration of hormones participating in the regulation of fluid–electrolyte exchange discovered in these experiments, and the corresponding changes in excretion of water and electrolytes, can be regarded as a homeostatic reaction aimed at maintaining the normal level of hydration under conditions of cold-induced hypermetabolism.

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