ACTIVE TRANSPORT OF ORGANIC ACIDS IN PROXIMAL RENAL TUBULES OF CAMPBELL RATS WITH INHERITED RETINAL DEGENERATION

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Campbell rats are affected by hereditary degeneration of the retina, also known as retinitis pigmentosa. The disease is due to homozygosity for a recessive autosomal gene [12]. Retinitis pigmentosa in Campbell rats is a model which can be used to study the corresponding disease in man. The writers showed previously [5] that both in Campbell rats and in human patients with retinitis pigmentosa the blood uric acid level is significantly raised, possibly as a result of its increased formation or delayed excretion. Uric acid is excreted in mammals by the kidneys with the participation of glomerular filtration and tubular secretion and, possibly, reabsorption [4, 11, 10]. Secretion of foreign organic acids and endogenous waste products is effected by a system of active transport of organic acids (SATOA), located in cells of the proximal tubules and evidently including three types of carriers with overlapping affinity: for acids of the hippuric type, the uric type, and the biliary type [4, 7, 10]. To assess the function of the SATOA in general, marker acids transported like those of the hippuric type are usually used: p-aminohippuric acid, phenol red, fluorescein [7, 10].

The object of this investigation was to study the system of active transport of organic acids in Campbell rats and to compare it with the analogous systems in Wistar and noninbred albino rats.

EXPERIMENTAL METHOD

Experiments were carried out on male Campbell and Wistar rats aged from 10 to 100 days and noninbred male albino rats aged 60-100 days. SATOA function was studied *in vitro* by a method of microfluorometric determination of the marker organic anion fluorescein, developed previously, in intact proximal tubules located on a natural surface of the organ [1-4]. Two temperatures of the incubation were used: 30°C, and when transport virtually ceased to depend on the Na^+ of the medium [3, 4]. Forty tubules were studied from each disk of the kidney, and kidneys from three or more animals were used in each experiment and in the control. The results of the measurements are given as the mean level of accumulation in the tubule ± the confidence interval at a 95% level of significance; accumulation was expressed as the ratio between fluorescein concentrations in tubule and medium (T/M).

When animals were to be used after unilateral nephrectomy the operation was performed on 60-day-old rats under combined anesthesia with pentobarbital and ether. The left kidney always was removed. Rats in which the left kidney was merely exposed and not subsequently removed, served as the control (mock operation). Activity of 5'-nucleotidase in the renal cortex [6] and the blood uric acid concentration [5] were determined.

EXPERIMENTAL RESULTS

Since the blood uric acid level is high in Campbell rats, the first problem to be studied was whether the presence of endogenous inhibitors affects fluorescein accumulation in surviving tubules. For this purpose, fluorescein accumulation was investigated in disks of kidney incubated in medium containing the marker acid immediately after sacrifice of the animal or after preincubation for 1 h in Ringer's solution. There was no difference in the level of fluores-

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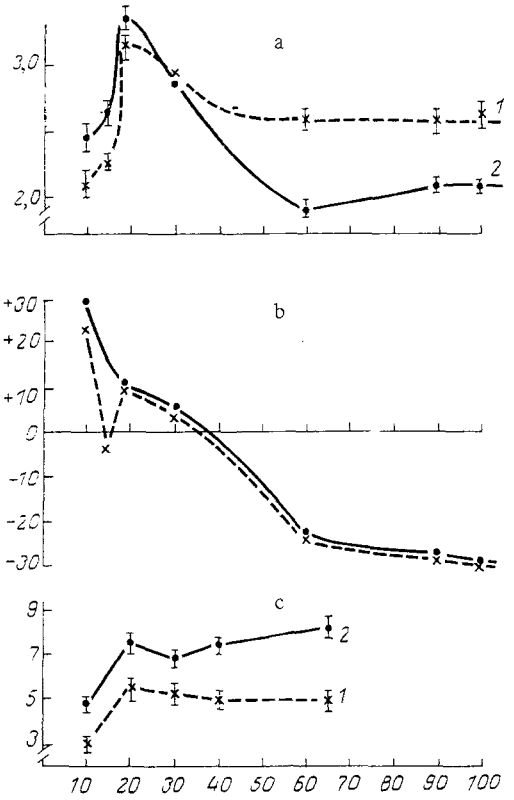

Fig. 1. Changes in level of equilibrium accumulation of fluorescein in proximal renal tubules (a, b) and in serum uric acid concentration (c) of Wistar (1) and Campbell (2) rats. Abscissa, age of animals (in days); ordinate: a) T/M, b) relative level of fluorescein accumulation in tubules of Campbell rats (in % of accumulation in Wistar rats at the same period), c) uric acid concentration (in mg%). Fluorescein concentration in medium 5×10^{-5} M; temperature: a) 30°C, b) 20°C (broken line) and 30°C (continuous line); duration of incubation: a) 22.5 min, b) 30 min.

cein accumulation in Campbell rats 60 days old at both 20 and 30°C between experiments with and without preincubation. Similar results also were obtained on Wistar rats. However, the T/M ratio was about 30% lower in Campbell than in Wistar rats. SATOA activity in 60-day-old Campbell rats was thus lower than in Wistar rats and the decrease was not due to the presence of endogenous inhibitors in the blood.

The equilibrium level of fluorescein accumulation in the tubules is determined by the ratio between the rates of its inflow and outflow [4, 11]. The study of the rate of fluorescein outflow from tubules loaded with it beforehand showed that in the course of incubation for 15 min at 20°C in medium without fluorescein 46% of this anion left the tubules in the case of Campbell rats compared with 35% from Wistar rats; the corresponding figures for incubation at 30°C were 48 and 42%. Consequently, in 60-day-old Campbell rats a low level of equilibrium accumulation was due both to delayed inflow and accelerated outflow of fluorescein from the tubules.

Fluorescein accumulation (T/M) during postnatal development is characterized in rats of both strains by phasic fluctuations, which are particularly pronounced in medium at a temperature of 30°C (Fig. 1a): after the age of 10 days T/M rises to reach a maximum between 20 and 30 days, after which it falls and stabilizes after 60 days. A similar type of change in the

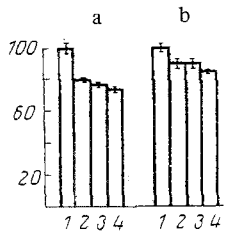

Fig. 2

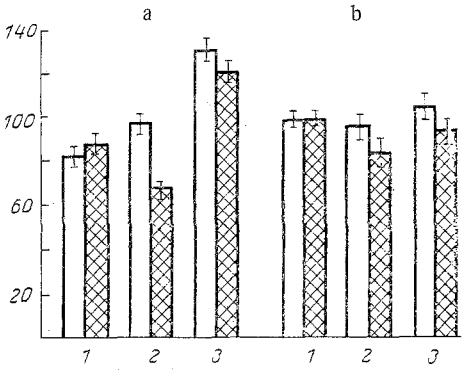

Fig. 3

Fig. 2. Action of AMP and adenosine on fluorescein accumulation in renal tubules of 60-day-old Wistar (a) and Campbell (b) rats. Abscissa: 1) Control (5×10^{-5} M fluorescein); 2) fluorescein + AMP (1 mM); 3) fluorescein + adenosine (1 mM); 4) fluorescein + AMP + adenosine (1 mM of each). Ordinate, fluorescein accumulation in tubules (in % of control). Temperature of medium 30°C, duration of incubation 30 min.

Fig. 3. Action of left-sided nephrectomy on fluorescein accumulation in tubules of right kidney in noninbred rats (a) and Campbell rats (b). Abscissa: 1, 2, 3) 1, 15, and 30 days respectively after operation; ordinate: accumulation in tubules (in % of accumulation in rats at the same time after the mock operation). Fluorescein concentration in medium 5×10^{-5} M, duration of incubation 25 min. Empty columns — 20°C; shaded columns — 30°C.

level of accumulation of organic acid in the renal cortex during the postnatal period has been described for several laboratory animals [4, 10]. On the 10th-20th day of postnatal development the level of accumulation at both 20 and 30°C was higher in Campbell rats, but from 60 days and later it was higher in Wistar rats (Fig. 1b). The blood lactic acid concentration in Campbell and Wistar rats differs in the course of postnatal development with effect from the 10th day, and the differences reach a maximum by 60-70 days (Fig. 1c). During postnatal development complete correlation thus does not exist between SATOA activity, measured by fluorescein accumulation, and the blood lactic acid level in Campbell rats. This can be partly explained on the grounds that in postnatal development the carriers for acids of hippuric, uric, and biliary types behave differently [10].

The writers showed previously [6] that 5'-nucleotidase activity in the retina of Campbell rats is lower than in Wistar rats. This enzyme is known to catalyze the conversion of AMP into adenosine. The study of the action of AMP and adenosine on various tissues of Campbell rats thus seemed to be particularly interesting. AMP and adenosine acted identically on fluorescein accumulation in 60-day-old rats of the same strain (Fig. 2), but summation of their inhibitory action did not take place if they were administered together. Meanwhile both AMP and adenosine inhibited fluorescein transport in the renal tubules of Campbell rats much less strongly than in Wistar rats (Fig. 2). 5'-Nucleotidase activity (in nanomoles inorganic phosphate/mg protein/min) in renal cortical homogenate from Wistar and Campbell rats amounted on the 17th day to 134.0 ± 12.1 and 120.0 ± 8.8, on the 39th day to 105.8 ± 6.9 and 124.9 ± 5.1, and on the 62nd day to 171.1 ± 19.4 and 166.8 ± 20.3, respectively, i.e., it was the same in animals of both strains. Since the effects of AMP and adenosine are equal in magnitude and direction, it is logical to suggest that the substance which acts on transport is in fact adenosine. Consequently, SATOA in the proximal renal tubules of Campbell rats is less sensitive to the action of adenosine than in Wistar rats.

The study of the response of SATOA in the proximal tubules to unilateral nephrectomy in Campbell and noninbred rats showed considerable differences between them (Fig. 3). In noninbred rats the level of accumulation on the 1st and 15th days after the operation was appreciably lower than in the control, and 30 days after the operation there was a significant increase in fluorescein accumulation. In Campbell rats at these same times there was practical-

ly no change in fluorescein accumulation compared with the control. In noninbred rats 30 days after the operation the volume of the residual kidney was 65 ± 5% of the volume of both kidneys in animals undergoing the mock operation, compared with 50 ± 5% in Campbell rats. SATOA in the proximal renal tubules of Campbell rats thus does not respond to humoral stimuli associated with unilateral nephrectomy at all times of the investigation, and the residual kidney shows no signs of compensatory hypertrophy 1 month after the operation.

The results thus indicate that SATOA in the proximal renal tubules of Campbell rats is defective both in power and in reactivity. Since SATOA plays an important role in purification of the internal medium of the body from endogenous waste products and foreign substances and since similar systems exist not only in the proximal renal tubules but also in structures of several other organs, including the eye [4, 7, 8, 9], the role of this defect (or defects) in the pathogenesis of retinitis pigmentosa requires further study.

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FUNCTIONAL AND STRUCTURAL CHANGES IN THE SMALL INTESTINE IN THE COURSE OF EXPERIMENTAL HYPERCHOLESTEROLEMIA

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The mechanisms of disturbances of cholesterol metabolism, subsequently leading to the development of hypercholesterolemia (HCh) are not yet clearly understood. Under these circumstances the study of the role of the digestive system and, in particular, the intestine and liver, which play a decisive role in cholesterol (Ch) metabolism, in this process assumes great importance [5-7, 9, 11]. It was concluded from the results of experiments on dogs [2, 3, 5, 10] that important factors preventing elevation of the blood Ch level are an intensification of its excretion with the intestinal secretion, the rapid removal of remnants of chylomicrons (CHM) from the circulation, and an increase in excretion of fatty acids.

This paper gives data on functional and structural changes in the digestive organs arising at different stages of experimental HCh in rabbits, which are known to have low resistance to exogenous Ch.

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