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p-AMINOHIPPURATE (PAH) TRANSPORT AND Na–K–ATPase ACTIVITY IN RAT RENAL CORTICAL SLICES DURING POSTNATAL MATURATION AND DRUG-INDUCED STIMULATION

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Abstract—PAH transport and Na–K–ATPase activity markedly increase during the first month of postnatal life. Pretreatment of rats with PAH or cyclopenthiazide induces a stimulation of *in vitro* PAH accumulation in renal cortical slices, whereas Na–K–ATPase activity is unchanged in comparison to saline-pretreated controls. 5 mM ouabain in the incubation medium reduces PAH accumulation. Developmental pattern and stimulation effects are pronounced as in controls. The ouabain-insensitive component of net PAH accumulation progressively increases with age and is significantly enhanced following drug pretreatment, whereas the ouabain-sensitive component of net PAH accumulation shows relatively slight modifications. Consequently, Na–K–ATPase seems not to be linked with postnatal maturation or drug-induced stimulation in tubular PAH transport.

In vitro PAH uptake in renal cortical slices from newborn rats is low compared with that from adult ones [1]. Following 4-day-pretreatment of adult rats with various drugs such as PAH, phenol red, probenecid, sulfamethoxypyridazine or cyclopenthiazide, PAH uptake in renal cortical slices is significantly enhanced in comparison to saline-pretreated controls [2].

An involvement of Na–K–ATPase in tubular PAH transport has been postulated since ouabain produces a marked decrease of in vitro PAH accumulation in renal cortical slices [3–5] and of PAH secretion in microperfusion experiments on separated proximal tubules of rabbits [6]. Marked age-related differences in Na–K–ATPase activity has previously been documented [7, 8]. It was the aim of the present study to elucidate whether or not Na–K–ATPase activity is linked with the postnatal development and with the drug-induced stimulation in tubular PAH transport.

MATERIAL AND METHODS

Wistar rats of different ages from our institute's colony breed were used. Newborn and infant rats of both sexes were kept with their dams up to the time of experimentation. Adult female rats were fed with standard pellets (type R, VEB Versuchstierproduktion, Schönwalde) and tap water *ad libitum* up to the beginning of the studies.

In a series of experiments adult rats were pretreated with saline (control) or relatively high single doses of PAH (300 mg/100 g b.wt.) or cyclopenthiazide (5 mg/100 g b.wt.) twice a day for 4 days [9]. Sixteen to twenty-four hours after the last intraperitoneal administration, studies were performed with renal cortical slices from 55-day-old rats. Na–K–ATPase (EC 3.6.1.3): The activity of ouabain-sensitive, Na–K–stimulated, Mg-dependent ATPase (full name) was measured in homogenate of kidney cortex from rats of different ages.

Na–K–ATPase activity was defined as the difference in inorganic phosphate (Pi) split from ATP by total ATPase and ouabain-insensitive ATPase activities. Total ATPase activity was determined in a reaction mixture (1.5 ml) containing 5 mM ATP (Ferak, Berlin-West), 5 mM $MgCl_2$, 100 mM NaCl, 20 mM KCl, 0.5 mM EDTA, 20 mM imidazole and 20 mM glycylglycine (pH 7.4). Ouabain-insensitive ATPase activity was measured in a reaction mixture which contained 5 mM ATP, 5 mM $MgCl_2$, 120 mM NaCl, 0.5 mM EDTA, 5 mM ouabain, 20 mM imidazole and 20 mM glycylglycine. The reaction was started by adding 0.1 ml homogenate ($\approx$ 1 mg protein). The tubes were incubated for 15 min (25°) and then the reaction was stopped by adding 2.5 ml of 0.5 M perchloric acid. After centrifugation the supernatant was analysed for inorganic phosphate using the method of Fiske and Subbarow [10]. An aliquot of homogenate was analysed for protein using a modified biuret procedure [11]. Blanks contained 5 mM ATP, 5 mM $MgCl_2$, 120 mM NaCl, 0.5 mM EDTA, 20 mM imidazole and 20 mM glycylglycine. These samples remained in ice and were handled as described above.

PAH transport studies: Renal cortical slices from 5-, 15-, 33- and 55-day-old rats were prepared as previously described [1]. Pooled renal cortical slices (200 mg) were incubated in 3.0 ml Krebs-Ringer phosphate buffer (pH 7.4) containing 8.5×10^{-5} M PAH. All incubations were carried out in a Warburg apparatus at 25° under a gas phase of 100% oxygen

for 120 min. The incubation medium did not contain any respiratory fuels such as nonesterified fatty acids, pyruvate, acetate or Krebs cycle intermediates. The viability of renal cortical slices is not impaired during an incubation time up to 150 min. If respiratory fuels are added to the incubation medium, the viability is not impaired up to 240 min and more [12]. For inhibition of Na–K–ATPase in renal cortical slices, 5 mM ouabain was added to the incubation medium. In the rat, a species whose Na–K–ATPase is relatively insensitive to ouabain, nearly complete inhibition is achieved with 5 mM, whereas 0.1 mM ouabain completely inhibits Na–K–ATPase in the rabbit [13]. In our own studies the maximum inhibition of PAH accumulation was nearly achieved using an ouabain concentration of 2.5 M (0.1, 1.0, 2.5, 5.0 and 10.0 mM ouabain was tested).

After incubation the slices were removed from the medium, blotted, homogenized and denaturated with 10% trichloroacetic acid. PAH was determined in the supernatant fraction as well as in the incubation medium using the Bratton–Marshall reaction [14]. PAH accumulation was expressed as the ratio of slice to medium concentration ($Q_{S/M}$).

PAH efflux: PAH efflux was measured as previously described [15].

Data analysis: Arithmetic means ± S.E.M. are given. Differences between means were statistically analysed using Student's *t*-test ($P \leq 0.05$).

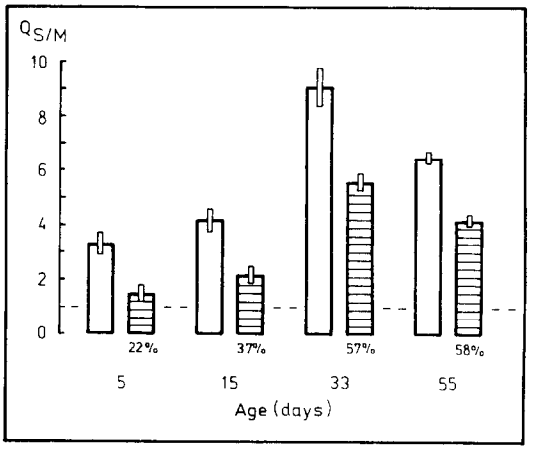

Fig. 2. Effect of ouabain on PAH accumulation in renal cortical slices from rats of different ages. PAH slice to medium ratio ($Q_{S/M}$) is given. Columns represent means ± S.E.M. of 3–4 samples. Symbols: ▭ control, ▤ ouabain (5 mM). Below the columns representing $Q_{S/M}$ in presence of ouabain, the percentage of ouabain-insensitive net PAH accumulation related to total net PAH accumulation is given. Net PAH accumulation (energy-dependent uptake) is defined as $Q_{S/M} > 1$. A value of one or slightly less than one is interpreted to mean that PAH in the tissue got there by diffusion. Ouabain-sensitive component of net PAH accumulation is defined as the difference in $Q_{S/M}$ between the control and the sample containing ouabain.

RESULTS

First, total ATPase, ouabain-insensitive ATPase, and Na–K–ATPase activities in homogenate of kidney cortex from rats of different age were quantified. Age-related differences are most pronounced in Na–K–ATPase activity. Na–K–ATPase activity is 3-fold in homogenate of 55-day-old rats compared with that in homogenate of 5-day-old ones (Fig. 1). Pretreatment of rats with PAH or cyclopenthiazide did not alter Na–K–ATPase activity in comparison to saline-pretreated controls (64 ± 5, 52 ± 8 and 55 ± 14 nmol Pi/mg protein min were liberated in homogenate of 55-day-old controls, PAH-treated and cyclopenthiazide-pretreated rats, respectively).

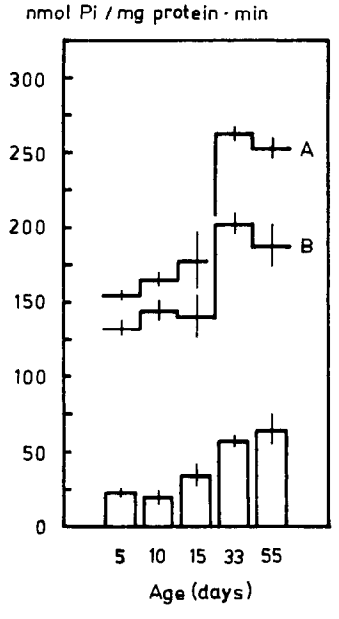

Fig. 1. Total ATPase (A), ouabain-insensitive ATPase (B), and Na–K–ATPase (columns) activities as a function of age in homogenate of rat kidney cortex. Na–K–ATPase activity was defined as the difference in inorganic phosphate (Pi) split from ATP by total ATPase and ouabain-insensitive ATPase activities. Means + S.E.M. are given (n = 4–8). Asterisks indicate values which differ significantly from the value of 5-day-old rats ($P \leq 0.05$).

In a second series of experiments the effect of an inhibition of Na–K–ATPase on PAH accumulation was quantified in renal cortical slices from 5-, 15-, 33-, and 55-day-old rats. PAH accumulation progressively increases with age up to a maximum in slices from 33-day-old rats. 5 mM ouabain in the incubation medium partially reduces PAH accumulation in renal cortical slices from rats of all ages (Fig. 2).

If ouabain is added the pattern of postnatal development in PAH accumulation is similar to that seen in controls. In detail the ouabain-insensitive component of net PAH accumulation markedly increases as a function of age. Thus, the ouabain-insensitive component of net PAH accumulation is 9 or 6 times higher in renal cortical slices from 33- and 55-day-old rats, respectively, than in slices from 5-day-old rats. In contrast, age-related modification in the ouabain-sensitive component of net PAH accumulation are low in comparison with the ouabain-insensitive component. Thus, the ouabain-sensitive component of net PAH accumulation in slices from 33-day-old rats is only twice as high as that in slices from 5-day-old ones. The ratio of ouabain-insensitive

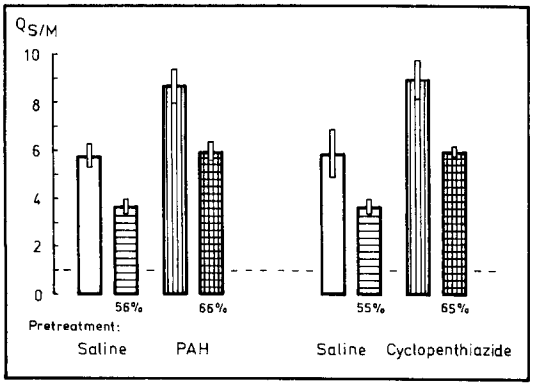

Fig. 3. Effect of ouabain on drug-induced stimulation of PAH accumulation in renal cortical slices from 55-day-old rats. Rats were pretreated with PAH or cyclopenthiazide for 4 days before the experiments were performed (see in methods). Symbols: ▭ control, ▤ ouabain (5 mM), ▥ pretreated rats, ▦ pretreated rats, ouabain (5 mM). For further details see legend of Fig. 2.

to ouabain-sensitive net PAH accumulation shifts from 0.28 in slices from 5-day-old rats to 1.39 in slices from 55-day-old ones.

In further experiments the effect of ouabain on the drug-induced stimulation of PAH accumulation was studied. Repeated administrations of PAH or cyclopenthiazide to rats are followed by about 50% increase in PAH accumulation compared with values of saline-pretreated controls (Fig. 3). 5 mM ouabain in the incubation medium partially inhibits PAH accumulation in renal cortical slices from saline pretreated controls as well as in slices from rats pretreated with PAH or cyclopenthiazide. Figure 3 also shows that the ouabain-insensitive component of net PAH accumulation in renal cortical slices from PAH- or cyclopenthiazide-pretreated rats is nearly twice that in slices from saline-pretreated controls. In contrast, the ouabain-sensitive component of net PAH accumulation in renal cortical slides from drug-pretreated rats is only by about 1.3 fold higher than in slices from controls.

The ratio of ouabain-insensitive to ouabain-sensitive net PAH accumulation shifts from 1.3 in slices from saline-pretreated controls to 1.9 in slices from PAH-pretreated rats. Similarly, the ratio shifts from 1.2 in controls to 1.7 following cyclopenthiazide pretreatment of rats. PAH efflux from renal cortical slices of 55-day-old rats back into the incubation medium is not modified by ouabain (ke/min^{-1} is 0.115 for controls and 0.110 if efflux media contain 5 mM ouabain).

DISCUSSION

Kinetic analysis of tubular PAH transport in renal cortical slices from rats of different ages shows age-related differences in maximum PAH uptake, whereas apparent Michaelis constant (K_m) is equal in all ages [16]. Likewise, drug-induced stimulation of tubular PAH transport is connected with an increase in maximum PAH uptake, whereas apparent K_m is unchanged [15]. Kinetic data permit the assumption that for both the pattern of postnatal maturation and for the drug-induced stimulation an increase in the number of functional transport sites in the basolateral plasma membrane or an enhancement in turnover rate of the individual carriers may be responsible. A modification in carrier-substrate affinity does not probably occur. The turnover rate of the carrier might be linked to Na–K–ATPase activity. Thus, Na–K–ATPase could either indirectly drive PAH transport by providing a Na^+ gradient, or could directly energize PAH carrier by a linkage to the phosphorylated form of the enzyme [17].

PAH transport studies in basolateral plasma membrane vesicles from adult rabbit kidney did not provide an evidence of the existence of a Na^+–PAH co-transport system [18, 19]. Recently, it was postulated that PAH transport comprises both a Na^+ gradient- and a metabolism-dependent component [20–22].

The age-related differences in Na–K–ATPase activity demonstrated in this paper agree with findings previously noted for enzyme activity in homogenate of rabbit kidney during postnatal maturation [7, 8]. Schmidt and Horster [23] have demonstrated that Na–K–ATPase activity related to μm^2 of basolateral membrane surface is constant during the post-natal differentiation of proximal tubular cells. Consequently, in early postnatal life the increase in Na–K–ATPase activity reflects an enhancement in the surface of the basal labyrinth since Na–K–ATPase is located in this membrane area of the tubular cells [24]. In addition, Schwartz *et al.* [25] previously suggested that most of the increase in PAH transport seen in the developing kidney is the result of an enhancement in intrinsic carrier transport whereas only about 33% is the consequence of the rise in surface area. After unilateral nephrectomy, followed by a compensatory growth of the remaining kidney, both the secretory capacity for PAH [26] and Na–K–ATPase activity [27, 28] are significantly enhanced.

In contrast, 4-day-pretreatment of rats with PAH or cyclopenthiazide has no effect on Na–K–ATPase activity in homogenate of rat kidney cortex. A similar result was obtained following penicillin pretreatment of rabbits which also induces a stimulation in tubular PAH transport without detectable alterations in Na–K–ATPase activity [8]. Thus, an increase in tubular PAH transport seems not to be necessarily related to an increase in Na–K–ATPase activity or in the surface area of the transporting membrane.

The addition of ouabain to the incubation medium reduces PAH accumulation in renal cortical slices from rats of all ages. It has been demonstrated that maximum PAH uptake is decreased, whereas apparent K_m is unchanged [5].

In the presence of ouabain the developmental pattern and the stimulation effects are pronounced as in controls. It is surprising, however, that most of net PAH accumulation is ouabain-sensitive in kidney cortical slices from 5-day-old rats, whereas most of net PAH accumulation is ouabain-insensitive in slices from adult ones. In detail, the ratio of ouabain-insensitive to ouabain-sensitive net PAH accumulation shifts from 1 : 4 to 3 : 2 during postnatal development and from 3 : 2 to 2 : 1 following drug

pretreatment. The ouabain-insensitive component of net PAH accumulation absolutely increases with age and is significantly enhanced following drug pretreatment. In contrast, the ouabain-sensitive component of net PAH accumulation shows only slight modifications as compared with the ouabain-insensitive component. Consequently, another factor, not Na–K–ATPase activity must be linked with the postnatal development and with the drug-induced stimulation in tubular PAH transport. This factor seems not to be the ouabain-insensitive ATPase activity either because age-related differences are relatively small and following drug pretreatment no changes were found. Finally, the age-related and the drug-induced increase in ouabain-insensitive (Na–K–ATPase-independent) PAH transport might include an enhancement in carrier protein concentration as previously suggested [2, 28].

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