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DISSIPATION BY TETRAETHYLAMMONIUM OF VERAPAMIL-STIMULATED *p*-AMINOHIPPURATE ACCUMULATION IN RAT KIDNEY CORTICAL SLICES

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Abstract—The stimulatory effect of verapamil on *p*-aminohippurate (PAH) accumulation was studied in rat kidney cortical slices. PAH accumulation was stimulated by the presence of 0.3 mM verapamil in the incubation medium, but this stimulatory action of verapamil was dissipated by adding tetraethylammonium (TEA) to the medium. TEA accumulation by the kidney cortical slices was inhibited by verapamil in a concentration-dependent fashion. In addition, kinetic studies revealed that verapamil resulted in an increase in the Michaelis constant (K_m) of TEA transport, with the V_{max} remaining constant, suggesting that verapamil competitively inhibited TEA transport. These results suggest that the transport system for verapamil is the same as that for TEA and that verapamil taken up within the cells may stimulate PAH transport.

It is well known that transport of organic anions such as *p*-aminohippurate (PAH) or of organic cations such as tetraethylammonium (TEA) in the renal tubules is active in nature [1–3]. We reported recently that PAH accumulation by rat kidney cortical slices was enhanced markedly by verapamil [4, 5]. This stimulatory action of verapamil cannot be explained by its action on Ca^{2+} [4]. In addition, verapamil stimulated PAH accumulation without affecting kidney microsomal $(Na^+ + K^+)$-ATPase activity or energy metabolism in the slices [5]. The marked stimulation of the PAH accumulation was also observed when the Na^+ gradient was dissipated by ouabain, suggesting that the action of verapamil on PAH accumulation was not due to an enhancement of the Na^+ gradient [5]. Moreover, it was shown that the PAH accumulation was stimulated in slices pre-incubated at 37° with verapamil, but not in slices pre-incubated at 0° [5]. These data suggest that verapamil may be accumulated by the slices.

In the present work, we have examined the effects of verapamil, an organic cation, on the kinetics of TEA uptake by rat kidney cortical slices. Competitive inhibition indicated that the transport system for verapamil is the same as that for TEA.

METHODS

Preparation of slices and incubation. Kidney cortical slices were prepared from adult male Sprague–Dawley rats as already described [6]. The slices were placed in an ice-cold medium that contained 137 mM NaCl, 5.9 mM KCl, 1.5 mM $CaCl_2$, 11.5 mM glucose and 5.8 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), titrated with NaOH to pH 7.4. The slices were transferred to 5 ml of an incubation medium that was identical to the above medium except that it also contained PAH (0.074 mM), *p*-amino[^{3}H]hippurate (50 nCi/ml) and [methoxy-^{14}C]inulin (30 nCi/ml). For the study of TEA uptake, the incubation medium had [^{14}C]TEA (5 nCi/ml), TEA and [methoxy-^{3}H]inulin (100 nCi/ml) instead of PAH, [^{3}H]PAH and [^{14}C]inulin. Unless otherwise stated, the concentration of TEA in the medium was 0.1 mM. Inulin was added to evaluate the extracellular space of the slices. Incubation was carried out at 37° with a gas phase of 100% oxygen.

Measurement of radioactivity. ^{3}H and ^{14}C radioactivities in the slices and media were estimated as already described [7]. After completion of incubation, the slices were weighed before and after desiccation at 110° for 18 hr. The dried tissues were digested in NaOH, and then scintillator was added. Supernatant fractions obtained by centrifugation of each incubation medium were mixed with scintillator. ^{3}H and ^{14}C radioactivities were counted in a liquid scintillation counter (Packard Tri-Carb 3255). Quenching was corrected with external standardization.

Chemicals and statistics. *p*-Amino[2-^{3}H]hippurate (374 mCi/mmole) was obtained from the Radiochemical Centre (Amersham, England). [1-^{14}C]Tetraethylammonium bromide (4.4 mCi/mmole), [methoxy-^{3}H]inulin (132 mCi/mmole) and [methoxy-^{14}C]inulin (19.6 mCi/g) were purchased from the New England Nuclear Corp. (Boston, MA). All other chemicals were of reagent grade.

Regression lines were calculated by the least-squares method. The values are expressed as mean ± S.E. Statistical analyses were performed by Student's *t*-test.

The amount of PAH or TEA in the slices was calculated as the difference between the content of each compound in the whole tissue and that in the inulin space of the slices. This quantity was then

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divided by the intracellular fluid (I.C.F) of the slices (wet wt of tissue minus fluid wt of inulin space minus dry wt of tissue) to obtain the intracellular concentration (S) of each compound. The accumulation of PAH or TEA (slice-to-medium ratio, S/M) was calculated.

RESULTS

The maximal stimulation of PAH accumulation by kidney cortical slices had been observed in the presence of 0.3 mM verapamil in the medium [4]. Figure 1 shows the effect of TEA on PAH accumulation in the presence or absence of 0.3 mM verapamil. The PAH accumulation was not affected by TEA concentrations from 0 to 30 mM in the absence of verapamil. TEA is known to have no effect on organic anion transport [2, 3]. The stimulation of PAH accumulation by verapamil was observed, as already shown [4, 5]. The stimulatory effect of verapamil on PAH accumulation was reduced by TEA in a concentration-dependent manner, and it was nearly abolished by 30 mM TEA (Fig. 1).

The effect of verapamil on the uptake of TEA was studied. Figure 2 shows the effect of various verapamil concentrations on TEA accumulation by the slices. Verapamil inhibited TEA accumulation by the slices in a concentration-related fashion. Incubation with 0.03 mM verapamil reduced TEA accumulation to half the control value.

The effect of verapamil on the kinetic parameters of TEA uptake was studied. Uptake of TEA by renal cortical slices is a saturable process exhibiting Michaelis–Menten kinetics [8]. The uptake of TEA at medium concentrations up to 0.8 mM was examined in the presence and absence of 0.03 mM verapamil (Fig. 3). It was confirmed that TEA uptake was linear up to 15 min under both conditions (data not shown). Thereafter, slices were incubated at 37° for 15 min. From a Lineweaver–Burk plot an apparent Michaelis constant (K_m) was obtained (Fig. 3B). Figure 3B reveals that the K_m value of TEA uptake was increased by verapamil from 446 ± 26 to 784 ± 54 μM ($P < 0.002$), while the V_{max} remained constant (control, 7.31 ± 0.48; verapamil, 6.64 ± 0.31 mmoles per kg I.C.F per 15 min). These data indicate that verapamil competitively inhibited the

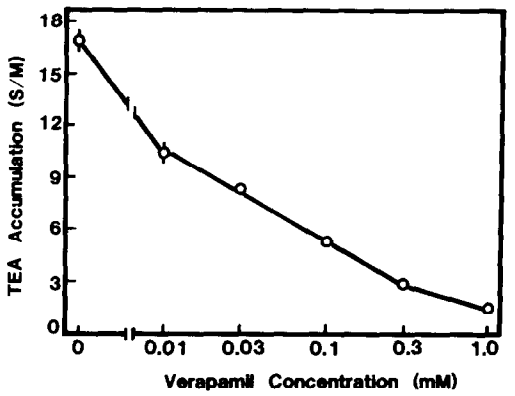

Fig. 2. Dose–response curve for the effect of verapamil on TEA accumulation by slices. Slices were incubated at 37° for 30 min in medium containing the indicated concentrations of verapamil. Each point represents the mean ± S.E. of four experiments. Where no vertical line appears, the S.E. lies within the symbol used to designate the mean.

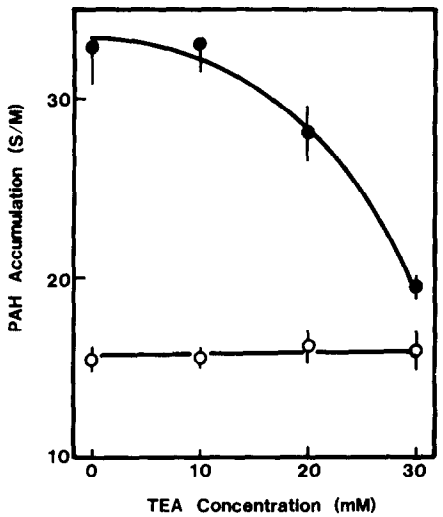

Fig. 1. Influence of TEA on the stimulation of PAH accumulation induced by verapamil. Slices were incubated at 37° for 45 min in medium containing the indicated concentrations of TEA with (●) or without (○) 0.3 mM verapamil. Values shown are the mean of four experiments. Vertical lines indicate S.E.

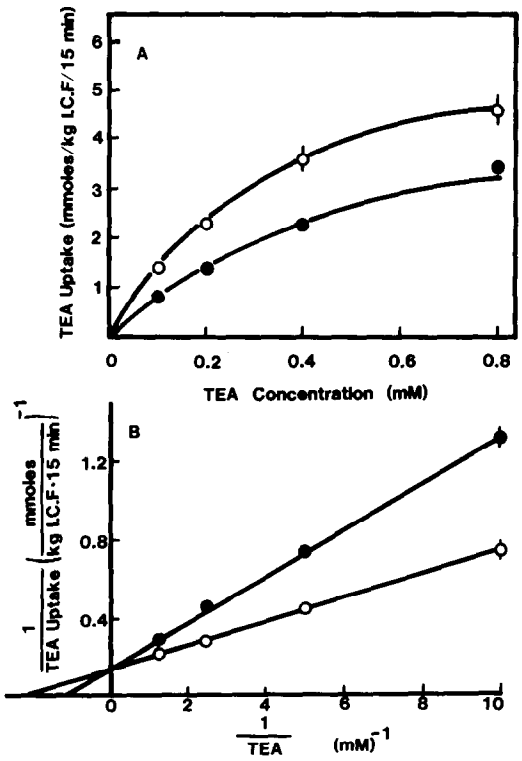

Fig. 3. (A) Effect of verapamil on TEA uptake by slices with different concentrations of TEA. Slices were incubated at 37° for 15 min with (●) or without (○) 30 μM verapamil. Each point represents the mean ± S.E. of four experiments. (B) Lineweaver–Burk plots of TEA uptake against TEA concentration in the medium with (●) or without (○) verapamil.

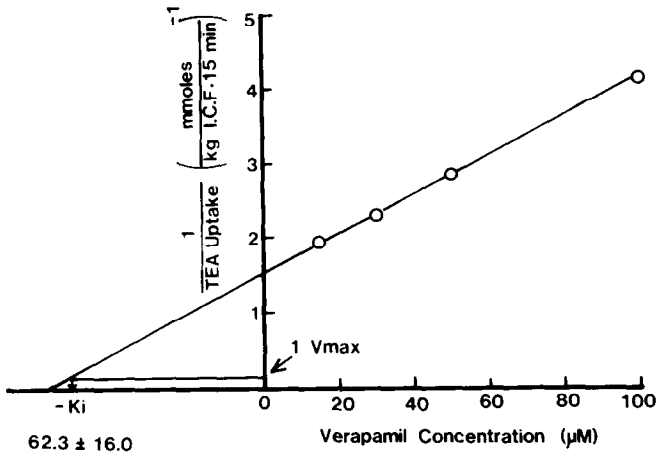

Fig. 4. Determination of apparent K_i of verapamil by the method of Dixon [9]. V_{max} was calculated from the data in Fig. 3 to be 7.31 ± 0.48 mmoles per kg I.C.F per 15 min. Slices were incubated at 37° for 15 min in the medium containing 0.1 mM TEA and the indicated concentrations of verapamil. Each point represents the mean ± S.E. of four experiments.

TEA transport. The inhibitory constant (K_i) for verapamil was calculated to be 62.3 ± 16 μM by the method of Dixon [9] (Fig. 4). Thus, the affinity of the organic cation transport system for verapamil seems to be about seven times higher than that for TEA. The Dixon plot also reveals that the inhibition of TEA uptake by verapamil is competitive in nature because a straight line for the dependence of TEA uptake on inhibitor concentration is obtained [9].

DISCUSSION

The experimental data presented in this paper extend our recent observations about the existence of an intracellular effect of verapamil on PAH accumulation. In addition, the transport system of verapamil is shown to be the same as that of TEA in rat kidney cortical slices.

The addition of verapamil to the incubation medium resulted in a decrease in TEA accumulation by slices in a concentration-dependent fashion (Fig. 2). Kinetic analyses revealed that verapamil increased the apparent Michaelis constant (K_m) with the V_{max} remaining constant (Fig. 3), i.e. verapamil competitively inhibited TEA transport in kidney slices. The affinity of the organic cation transport system for verapamil seems to be higher than that for TEA because the inhibitory constant (K_i) for verapamil was higher than the apparent Michaelis constant for TEA (Figs. 3 and 4). These data suggest that verapamil may be actively secreted into the urine. Schomerus *et al.* [10] studied the physiological disposition of [^{14}C]verapamil in man and found that the drug was rapidly changed to various metabolites which were largely eliminated into the urine. Moreover, it has been shown that some amounts of [^{14}C]verapamil and its derivatives can be excreted into the urine in rats and dogs [11].

We have shown recently that aerobic preincubation of the slices with verapamil increased PAH accumulation [5]. On the other hand, the PAH accumulation in the slices was unaffected by pretreating the slices at 0° for 90 min in the medium containing verapamil [5]. Moreover, the present data (Fig. 1) show that the addition of TEA to the medium reduced the stimulatory action of verapamil on the PAH accumulation. These results suggest that verapamil taken up by the slices stimulates PAH accumulation.

The present results suggest that verapamil may be transported by the organic cation transport system in rat kidney and that verapamil taken up by the slices may stimulate PAH accumulation. However, one cannot exclude the possibility that the metabolites of verapamil affect the PAH accumulation in the slices. Further experiments must be carried out to clarify the mechanism of stimulatory action of verapamil on the PAH transport system.

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