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PALMITATE UPTAKE AND OXIDATION BY KIDNEY CORTEX SLICES

EFFECTS OF PROBENECID, *p*-AMINOHIPPURATE AND PHENOLSULFONPHTHALEIN

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Abstract—To test the suggestion made earlier that the *p*-aminohippurate (PAH) transport system might play a role in the transport of free fatty acids (FFA) in the kidney, a study has been made of the effects of probenecid, *p*-aminohippurate and phenolsulfonphthalein (PSP) on the net uptake and oxidation of palmitate by guinea pig kidney cortex slices.

In Krebs–Ringer phosphate buffer with 2% albumin, 3–6 mM probenecid inhibited the oxygen consumption of the slices without affecting the uptake and oxidation of exogenous 1 mM palmitate. Twelve mM probenecid inhibited total oxygen consumption by 66 per cent, palmitate uptake by 43 per cent and palmitate oxidation by 87 per cent, and increased the respiratory quotient of the slices from the control value of 0·72 to 0·96. Probenecid in concentrations of 3–12 mM caused accumulation of endogenous lactate in the slices. PAH (1–9 mM) stimulated palmitate oxidation and (9 mM) the oxygen consumption of the slices, whereas the effects of PSP (0·4–3·6 mM) were negligible.

The results demonstrate that probenecid stimulates anaerobic glycolysis in kidney cortex slices, and do not support the suggestion that the PAH transport system is involved in the transport of FFA in the kidney.

THE UPTAKE of free fatty acids (FFA)* by the kidney has been demonstrated both *in vivo*[1–4] and *in vitro*.[5–8] FFA are probably the main endogenous substrates of the kidney.[3, 7] Other substrates utilized by this organ in significant amounts are lactate,[3, 9] pyruvate,[3, 9] citric acid cycle intermediates[3, 10, 11] and glutamine.[12, 13] On the other hand net glucose utilization is small.[14, 15]

In the removal of substrates from the blood the kidney shows some degree of specificity for certain organic acids, such as α-KG,[11] citrate[10] and palmitate.[2] This specificity suggests the existence of a separate mechanism for the transport of organic acid substrates in the tubule cell.[11, 16, 17] Evidence has been obtained in support of the hypothesis that α-KG[11, 16, 18] and citrate[17, 18] are transported by the PAH transport mechanism[19, 20] in the kidney. Barac-Nieto and Cohen[4] have suggested that FFA, in addition, may be transported by the same mechanism. They found that probenecid, an inhibitor of PAH transport, inhibits the uptake of infused FFA by the dog kidney. This suggestion is supported by the earlier studies of Cross and Taggart,[19] who

*Abbreviations used: FFA (free fatty acids), α-KG (α-ketoglutarate), PAH (*p*-aminohippurate), PSP (phenolsulfonphthalein), RQ (respiratory quotient).

demonstrated that medium-chain FFA inhibited PAH accumulation in kidney cortex slices, and by the report of Cohen,[21] which showed that *in vivo* the renal uptake of FFA can be inhibited by α-KG.

In this study, the effects of probenecid, PAH and PSP on the net uptake and oxidation of palmitate by guinea pig kidney cortex slices were studied, in order to test whether the PAH transport system is involved in the transport and/or utilization of FFA in the kidney. Because albumin was used in the incubation medium to solubilize the exogenous palmitate, it was further necessary to study the effect of probenecid on the binding of palmitate to albumin.

MATERIALS AND METHODS

Chemicals. All reagents were of analytical grade. Non-radioactive palmitic acid, *p*-aminohippuric acid and *n*-heptane were from Fluka AG., Buchs SG, Switzerland. Phenolsulfonphthalein and α-ketoglutaric acid were from E. Merck AG, Darmstadt, Germany, and L(+)lactic acid from the Sigma Chemical Company, St. Louis, Missouri, U.S.A. Bovine plasma albumin, fraction V, from Armour Pharmaceutical Company LTD, Eastbourne, England, was used. Palmitic acid-1-^{14}C in benzene solution, specific activity 55·2 mc/m-mole, was obtained from the Radiochemical Centre, Amersham, England, and the probenecid was a gift from AB Astra, Södertälje, Sweden. Hydroxide of Hyamine was purchased from the Packard Instrument Company, Illinois, U.S.A.

Solutions of α-ketoglutaric acid, lactic acid, probenecid, phenolsulfonphthalein and *p*-aminohippuric acid were neutralized to pH 7·4 with NaOH. Sodium palmitate was prepared from non-radioactive palmitic acid as described by Goodman.[22] Radioactivity was added to this as follows: A known amount of radioactive palmitic acid in benzene solution was dried by evaporating the benzene. A known volume of warmed non-radioactive sodium palmitate solution was added, and the clear solution was thoroughly mixed. Before being added to the incubation medium, the palmitate was warmed just sufficiently to render the solution clear. Albumin was purified from inherent fatty acids according to the method of Chen.[23] The purified albumin solution was dialyzed against distilled water at 4° for 1 day.

Animals. Male adult guinea pigs from Orion OY, Mankkaa, Finland, weighing 500–700 g, and fed on guinea pig diet (Orion OY, Mankkaa, Finland) *ad lib.*, were used in the studies with slices.

Preparation of tissue slices was performed as described earlier.[18]

Incubation of tissue slices was carried out in a Warburg respirometer at a shaker speed of 100 cycles/min at 30°, with 100% oxygen as the gas phase. 2·5 ml of the standard incubation medium consisted of Krebs–Ringer phosphate buffer, 2% albumin, 1 mM ^{14}C-palmitate and various concentrations of probenecid, PAH and PSP, as indicated. The centre well of the flasks contained 0·5 ml Hyamine. Two cortex slices, total weight 100–120 mg, were incubated in each flask. Palmitate and probenecid, PAH and PSP were added just before the flasks were connected to the manometers. The flasks were gassed and equilibrated as described earlier.[18] When not otherwise stated the incubation time was 90 min. After incubation the flasks were removed from the manometers for 1 min, in order to take a 0·1 ml sample from the incubation medium and to add 0·3 ml 6 N H_2SO_4 to the side arm. After the flasks had been reconnected to the manometers, the medium was acidified by tipping in the acid from the side arm.

The flasks were then equilibrated for 1 hr to allow absorption of all the CO_2 by the Hyamine, which was then transferred to Bray's scintillation solution.[24] Control experiments indicated that the removal of the flasks from the manometers for 1 min did not measurably decrease the amount of $^{14}CO_2$ in the Hyamine.

Studies on the binding of palmitate to albumin were performed by a modification of the equilibrium method of Goodman.[25] The experiments were conducted in 50-ml Erlenmeyer flasks, in a water bath at 23° ± 1° or 30° ± 1°, as indicated. The flasks were shaken to and fro at 68 cycles/min until equilibrium in the distribution of palmitate was attained after 48 hr. The volume of the aqueous phase was 20 ml and of the heptane phase 2·0 ml. The composition of the aqueous phase was varied, as indicated in the results. In some experiments, the aqueous phase contained in the buffer solution of Goodman[25] (sodium phosphate buffer, ionic strength 0·160 and pH 7·45), 0·05 mM albumin, various concentrations of ^{14}C-palmitate and probenecid, as indicated. The temperature was 23°. In the other part of these experiments the aqueous phase consisted of Krebs–Ringer phosphate buffer, pH 7·4, 0·2 or 2% albumin, 0·1 or 1mM ^{14}C-palmitate, with or without 1·2 or 12 mM probenecid. The temperature was 30°. After incubation the phases were transferred to centrifuge tubes, with care to avoid mixing of the phases. The tubes were centrifuged for 10 min at 500 r.p.m. Duplicate samples of 0·2 ml were taken from the heptane phase, which was then sucked off and duplicate samples of 0·1 ml were taken from the aqueous phase. Rat divactivity was measured in Bray's scintillation solution.[24] The concentrations of unbound and bound palmitate in the aqueous phase were calculated as described by Goodman.[25] The distribution of probenecid at various concentrations was also studied. It was found that even forceful shaking did not remove measurable amounts of probenecid from the aqueous phase.

Analytical methods. FFA were extracted from the samples of medium by Dole's method.[26] A sample of the extracted FFA was taken for determination of radioactivity.

The lipids and FFA of the slices were extracted by the method of Folch *et al.*[27] This extraction was performed only in a number of control experiments to check the recovery of the total radioactivity in the incubation flasks.

The albumin concentration was determined by the method of Lowry *et al.*[28] Probenecid was determined as described by Berndt,[29] and lactate by the method of Hohorst.[30]

Oxygen consumption and the RQ of the slices were determined by Warburg's direct method.[31]

The radioactivity measurements were performed with a Packard Tri-Carb liquid scintillation spectrometer. The counts were corrected for quenching, using internal standards and on the basis of changes in channel ratios.

Statistical analyses were performed using Student's *t*-test. Unless otherwise stated, the values presented are arithmetic means ± S.D. (standard deviation).

The recovery of the total radioactivity in the slice studies (medium, slices plus Hyamine) was 90–95 per cent. In the studies on the binding of palmitate to albumin the recovery of the radioactivity ranged from 96 to 101 per cent.

Terms. The term uptake of palmitate by the slices is used to describe the disappearance of the extractable radioactivity from the incubation medium, and is expressed as micromoles per gram wet tissue weight.

RESULTS

Incubation conditions

Oxygen consumption of the slices in various incubation conditions. In Krebs–Ringer

phosphate buffer, the endogenous oxygen consumption of the slices was about 150 μatoms/g/hr. Addition of 1 mM palmitate increased the oxygen consumption by about 6 per cent. Further additions of 2–8% albumin to the medium increased the oxygen consumption by 16–19 per cent, respectively. The omission of Ca^{2+} from the medium did not affect the oxygen consumption of the slices.

In earlier studies in this laboratory,[18] it was found that probenecid inhibits the respiration of kidney cortex slices, when α-KG, for example, was used as exogenous substrate. To study whether albumin counteracts this effect of probenecid, the slices were incubated with probenecid and with or without albumin. With 4 mM α-KG as exogenous substrate, 9 mM probenecid inhibited respiration by 63 per cent. When 4% albumin was added, the inhibition of respiration by probenecid was only 17 per cent. This protective effect of albumin is probably due to its ability to bind probenecid.

In the later experiments, unless otherwise stated, the albumin concentration was 2%.

Various palmitate concentrations and incubation times. When the slices were incubated for 90 min, the uptake of ^{14}C-palmitate increased linearly with increasing palmitate concentrations from 0·025 to 2·0 mM, and $^{14}CO_2$ production increased linearly, when the palmitate concentration was varied from 0·5 to 2·0 mM (Fig. 1).

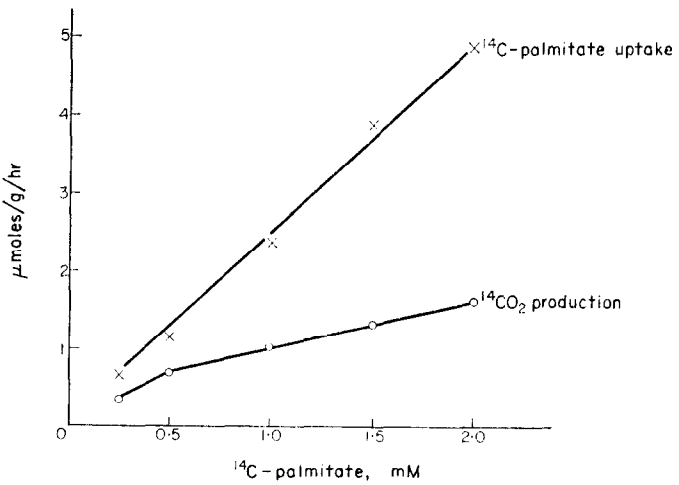

FIG. 1. Dependence of ^{14}C-palmitate uptake and $^{14}CO_2$ production on palmitate concentration. Slices incubated in 2·5 ml medium consisting of Krebs–Ringer phosphate buffer with 2% albumin and the concentrations of ^{14}C-palmitate indicated in a Warburg respirometer at 30° for 90 min, with 100% oxygen as the gas phase. Each point is the mean of three parallel determinations.

The net uptake of added 1 mM ^{14}C-palmitate was linearly correlated with the incubation time, when the latter was varied from 30 to 120 min and the $^{14}CO_2$ production was linear from 60 to 120 min (Fig. 2).

In the subsequent experiments, unless otherwise stated, palmitate concentration of 1 mM and an incubation time of 90 min were used.

The uptake and oxidation of palmitate by the slices in the standard incubation conditions. In the standard incubation conditions the uptake of the exogenous ^{14}C-

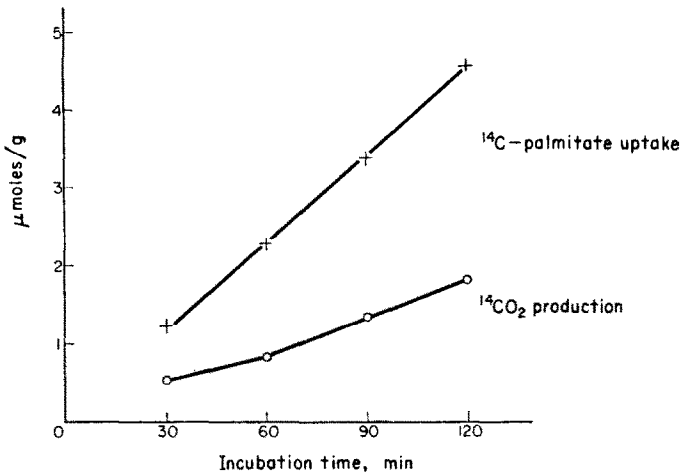

FIG. 2. Time course of ^{14}C-palmitate uptake and $^{14}CO_2$ production. Incubation conditions as in Fig. 1, except that 1 mM ^{14}C-palmitate was used, and the incubation time varied as indicated. Each point is the mean of three parallel determinations.

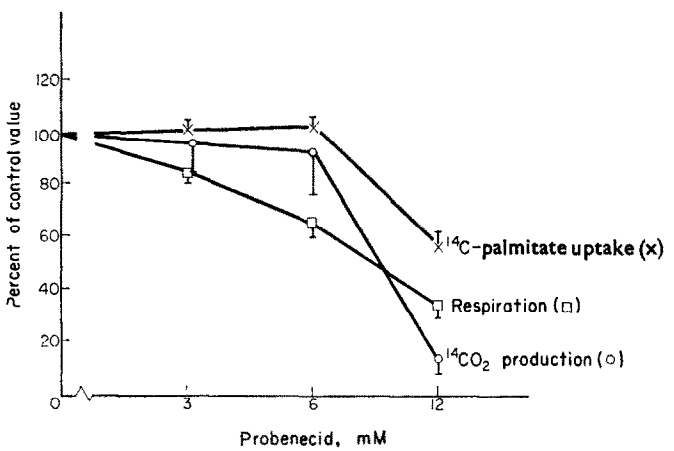

FIG. 3. Effects of probenecid on respiration, ^{14}C-palmitate uptake and $^{14}CO_2$ production of kidney cortex slices. Incubation conditions as in Fig. 1, except that 1 mM ^{14}C-palmitate was used. Control values (=100%): ^{14}C-palmitate uptake 2·50 ± 0·16 μmoles/g/hr, $^{14}CO_2$ production 0·98 ± 0·14 μmoles/g/hr and oxygen consumption 196 ± 7 μatoms O/g/hr. Each value is the mean ± S.D. of four to five parallel determinations.

palmitate was 2·34 ± 0·27 μmoles/g/hr. Thus, during a 90-min incubation, the slices removed 14–17 per cent of the added palmitate. The rate of $^{14}CO_2$ production was 0·78 ± 0·22 μmoles/g/hr. Lee *et al.*[7] found that the rate of $^{14}CO_2$ production from palmitate-1-^{14}C and palmitate-11-^{14}C closely agreed with each other in the slices of rabbit kidney cortex, kidney medulla and liver. It is thus probable that the rate of ^{14}C-1-O_2 production represents the rate of oxidation of palmitate to CO_2. If this assumption is true, about one third of the palmitate that disappeared from the medium was oxidized to CO_2.

Effects of probenecid

Effect of probenecid on the uptake and oxidation of palmitate. As shown in Fig. 3, added 3 and 6 mM probenecid inhibited the total oxygen consumption of the kidney cortex slices by 15 and 35 per cent, respectively, but the uptake of ^{14}C-palmitate and $^{14}CO_2$ production were not affected. 12 mM probenecid inhibited the uptake of ^{14}C-palmitate by 43 per cent and the total oxygen consumption of the slices by 66 per cent, whilst the decrease in $^{14}CO_2$ production was 87 per cent. Thus, at this concentration probenecid decreased the ratio of $^{14}CO_2$ production to ^{14}C-palmitate uptake.

Effect of probenecid on the RQ and the endogenous [lactate] of the slices. The effect of probenecid on the aerobic metabolism of the slices, described above, prompted the study of its effects on the RQ and the endogenous lactate concentration of the slices.

In the standard incubation conditions the RQ of the slices was 0·72 ± 0·03. Twelve mM probenecid increased the RQ to 0·96 ± 0·10 ($P < 0·01$).

In Krebs–Ringer phosphate buffer, without albumin, probenecid inhibited the endogenous respiration, and simultaneously increased the endogenous lactate concentration of the slices (Fig. 4).

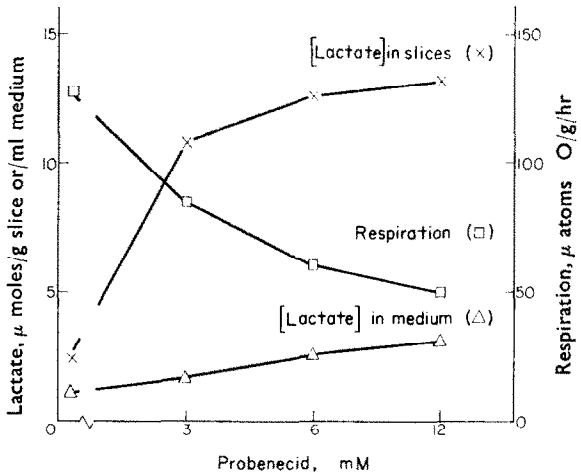

FIG. 4. Effect of probenecid on endogenous [lactate] in kidney cortex slices. Slices incubated in Krebs–Ringer phosphate buffer, with 10 mM glucose, at 30° for 60 min. Each value is the average of two parallel determinations.

These results probably indicate a change in the energy metabolism of the slices. The RQ near one, the decreased oxygen consumption of the slices and the accumulation of endogenous lactate suggest that anaerobic glycolysis is stimulated by probenecid.

Effect of probenecid on the binding of palmitate to albumin. Probenecid combines with tissue particles.[29, 32] Thus it seemed possible that probenecid might affect the binding of the added palmitate to the medium albumin in the studies with cortical slices, and thereby cause the effects observed in these experiments. For this reason it was necessary to study the effect of probenecid on the binding of palmitate to albumin. The results are presented in Fig. 5 and Tables 1 and 2. As seen from Fig. 5, the number of moles

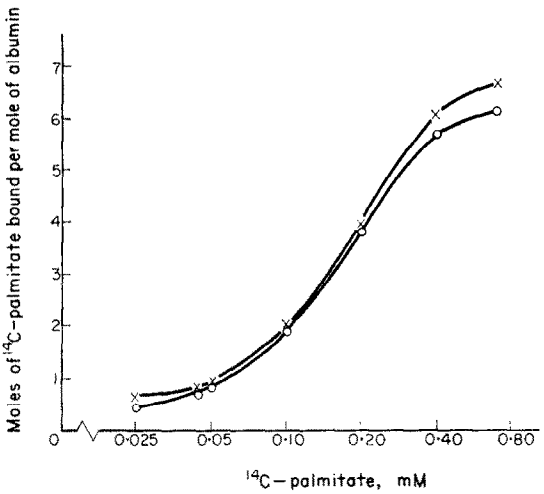

FIG. 5. Effect of probenecid on the binding of various concentrations of palmitate to albumin. The incubations were conducted in 50-ml Erlenmeyer flasks at 23° for 48 hr. Twenty ml of the aqueous phase consisted of sodium phosphate buffer, ionic strength 0·160 and pH 7·45, 0·05 mM albumin and various concentrations of ^{14}C-palmitate. Two ml of *n*-heptane was used as the organic phase. The concentration of probenecid was 0·113 mM. Each point is the average of two parallel determinations, — × — without probenecid, — ○ — with probenecid.

TABLE 1. EFFECT OF VARIOUS PROBENECID CONCENTRATIONS ON THE BINDING OF ^{14}C-PALMITATE TO ALBUMIN

Probenecid (mM)	Moles of palmitate bound per mole of albumin	Freeing of palmitate by probenecid (%)
0	3·93	—
0·03	3·86	1·8
0·06	3·86	1·8
0·11	3·86	1·8
0·23	3·82	2·8
0·45	3·72	5·3
0·90	3·66	6·9

Incubation conditions as in Fig. 5, except that the concentration of ^{14}C-palmitate was 0·20 mM and the concentration of probenecid was varied. Each value is the average of two parallel determinations.

TABLE 2. EFFECT OF PROBENECID ON THE BINDING OF ^{14}C-PALMITATE TO ALBUMIN IN KREBS–RINGER PHOSPHATE BUFFER

Albumin (%)	Palmitate (mM)	Probenecid (mM)	Moles of palmitate bound per mole of albumin	Freeing of palmitate by probenecid (%)
0·2	0·1	0	2·88 (2·82 – 2·93)	—
0·2	0·1	1·2	2·43 (2·42 – 2·43)	15·6
2·0	1·0	0	3·42 ; 3·44	—
2·0	1·0	12	2·91 ; 2·91	15·3

Incubation conditions as in Fig. 5, except that the temperature was 30° and the aqueous phase consisted of Krebs–Ringer phosphate buffer, with the concentrations of albumin, ^{14}C-palmitate and probenecid as indicated in the table. The results are expressed as the mean and the ranges of three parallel experiments or as the values of two parallel experiments.

of palmitate bound per mole of albumin increased with increasing palmitate concentration. This figure and Table 1 show that, in the conditions indicated, when palmitate and probenecid concentrations were varied, probenecid only slightly decreased the amount of palmitate bound, maximally by 6–7 per cent. Table 2 shows that in an incubation medium identical with that used in the slice studies, the highest probenecid concentration, 12 mM, decreased the amount of palmitate bound by about 15 per cent.

From these results it is concluded that the freeing of the albumin-bound palmitate by probenecid is probably too small to contribute essentially to the results obtained in the studies with kidney slices.

Effects of PAH and PSP

Effect of PAH on the uptake and oxidation of palmitate. 0·1–9 mM PAH in the incubation medium did not significantly affect the uptake of ^{14}C-palmitate by the slices, whereas 1–9 mM PAH stimulated $^{14}CO_2$ production by 12–16 per cent (Table 3). Nine mM PAH also slightly increased the total oxygen consumption of the slices.

Effect of PSP on the uptake and oxidation of palmitate. Earlier studies in this laboratory[18] indicated that PSP affects the transport and utilization of α-KG and citrate in kidney cortex slices. In the present study, 0·4–3·6 mM PSP did not significantly affect

TABLE 3. EFFECTS OF PAH ON RESPIRATION, ^{14}C-PALMITATE UPTAKE AND $^{14}CO_2$ PRODUCTION IN KIDNEY CORTEX SLICES

PAH (mM)	^{14}C-palmitate uptake (% of control)	$^{14}CO_2$ production (% of control)	O_2 consumption (% of control)
0	100	100	100
1	102 ± 12	116 ± 8*	103 ± 6
3	112 ± 10	115 ± 9†	101 ± 3
9	105 ± 8	112 ± 7*	107 ± 3†

Incubation conditions as in Fig. 1, except that 1 mM ^{14}C-palmitate was used. Each value is the mean ± S.D. of three to four parallel determinations. Control values (=100%): ^{14}C-palmitate uptake 2·22 ± 0·29 μmoles/g/hr, $^{14}CO_2$ production 0·73 ± 0·23 μmoles g/hr and oxygen consumption 182 ± 7 μatoms O/g/hr.
*P < 0·025.
†P < 0·05.

TABLE 4. EFFECTS OF PSP ON RESPIRATION, ^{14}C-PALMITATE UPTAKE AND $^{14}CO_2$ PRODUCTION IN KIDNEY CORTEX SLICES

PSP (mM)	^{14}C-palmitate uptake (% of control)	$^{14}CO_2$ production (% of control)	O_2 consumption (% of control)
0	100	100	100
0·4	107 ± 17	98 ± 25	99 ± 6
1·2	90 ± 7	103 ± 11	97 ± 4
3·6	92 ± 3*	104 ± 11	97 ± 7

Incubation conditions as in Fig. 1, except that 1 mM ^{14}C-palmitate was used. Each value is the mean ± S.D. of three to five parallel determinations. Control values (=100%): ^{14}C-palmitate uptake 2·35 ± 0·27 μmoles/g/hr, $^{14}CO_2$ production 0·61 ± 0·04 μmoles/g/hr and oxygen consumption 179 ± 9 μatoms O/g/hr.
*P < 0·025.

$^{14}CO_2$ production or the respiration of the slices when ^{14}C-palmitate was used as exogenous substrate (Table 4). Slight inhibition of ^{14}C-palmitate uptake by 3·6 mM PSP was found. It is evident that albumin binds PSP,[33, 34] and this binding may have prevented PSP from exerting any effect on the parameters studied. The effects of higher PSP concentrations were not studied, because of the slight solubility of PSP in aqeuous solutions.

DISCUSSION

The aim of these experiments was to shed more light on the problem of whether the PAH transport mechanism participates in the transport and/or metabolism of FFA in the kidney.

No attempt was made to demonstrate active transport of palmitate into the tubule cells, e.g. by showing accumulation of palmitate in the slices against a concentration gradient, because the numerous fates of the added palmitate and its binding to cellular structures[35, 36] would have made interpretations difficult. However, the preference shown by the kidney for palmitate over other long-chain FFA[2] and the fact that FFA are bound to cell membranes[35] and intracellular particles[36] suggest that palmitate penetrates into cells by a mechanism that is not mere diffusion.[4] Probably an active process is involved.

In the standard incubation conditions, calculated on the basis of $^{14}CO_2$ production, the mean rate of exogenous palmitate oxidation was 0·78 μmoles/g/hr. This corresponds to an oxygen consumption of 36 μatoms/g/hr. However, the total oxygen consumption of the slices was 180–200 μatoms/g/hr, which means that in these conditions the substrates oxidized by the kidney cortex are mostly endogenous.

Effects of probenecid. One widely accepted argument for the assumption that a substance is transported by the PAH transport mechanism in the kidney is that its transport can be inhibited by probenecid.[20, 37]

Probenecid has been reported to suppress the renal excretion of a great number of substances,[17, 38, 39] to inhibit some chemical reactions,[40, 41] and to bind to tissue structures.[29, 32] This variety of probenecid effects has induced several hypotheses about the mechanism of probenecid action in the renal transport of organic anions: Weiner *et al.* indicated that probenecid is secreted into the urine by the tubule cells. This supports the hypothesis that probenecid inhibits the excretion of some organic anions by competing with them for the common transport pathway into the tubule cells.[42] On the other hand, the inhibition of some chemical reactions, such as acetylation[40] and conjugation,[41] by probenecid, supports the idea that it may affect the PAH transport system by inhibiting some of the enzymic reactions of the transport process. Despopoulos suggested that by binding to tissue proteins, probenecid may unspecifically block the access of substrates to receptor sites at the kidney cell surface, thus inhibiting their transport.[37]

In this study lower probenecid concentrations, 3 mM and 6 mM, inhibited the oxygen consumption of the slices, while not affecting the uptake of ^{14}C-palmitate or the $^{14}CO_2$ production (Fig. 3). Thus, at these concentrations probenecid evidently acts at intracellular sites which involve the aerobic oxidation of endogenous substrates, but not of exogenous palmitate. On the other hand, the highest probenecid concentration, 12 mM, inhibited $^{14}CO_2$ production much more than ^{14}C-palmitate uptake. Thus, it decreased the ratio of the amount of palmitate oxidized to that removed from the in-

cubation medium. This may show that the main action of probenecid on palmitate metabolism is the intracellular inhibition of palmitate oxidation. The decrease in palmitate uptake by probenecid may be a secondary effect.

The inhibitory action of probenecid on the aerobic metabolism of the slices prompted the study of its effects on the RQ and on the endogenous lactate concentration of the slices. With palmitate as an exogenous substrate, the RQ of the slices was 0·72. This agrees with earlier reports, which showed that the RQ of the kidney cortical tissue was about 0·7–0·8.[43–45] Added 12 mM probenecid changed the RQ to 0·96. The RQ near one, the accumulation of endogenous lactate and the decreased oxygen consumption, indicate that anaerobic glycolysis is stimulated by probenecid. This stimulation can be explained as follows: Probenecid inhibits the aerobic oxidation of substrates by blocking their transfer into the mitochondria of the tubule cells, or by inhibiting some of the oxidative enzymes in the mitochondria, thus decreasing the energy state, which stimulates glycolysis by activating the key glycolytic enzymes, e.g. phosphofructokinase.[46] It may be suggested that this extra glycolytic capacity is an additional way to produce energy to maintain the essential functions of the kidney in hypoxia.

A high concentration of probenecid was needed to demonstrate its effects on the uptake and oxidation of the added palmitate. However, because of the evident binding of probenecid to the albumin of the medium, it was not possible to determine the real concentration that accounted for the effects. In kidney cortex slices probenecid inhibits the uptake of PSP[47] and citrate[18] without affecting oxygen consumption, whereas higher concentrations of probenecid inhibit oxygen consumption as well.[18, 48] In the present study it was found that higher probenecid concentrations are needed to inhibit the uptake and oxidation of the exogenous palmitate than to inhibit the respiration of the slices. It is possible that the action of probenecid at low concentrations (which do not inhibit oxygen consumption) are more or less specific for the PAH transport system, but that high concentrations, in addition, cause nonspecific effects by influencing the energy metabolism of the tubule cells. Thus it may be unwarranted to conclude that the effects of probenecid found in the present study are specific for the PAH transport mechanism, since it is possible that by decreasing energy production in the citric acid cycle, e.g. from FFA, probenecid may inhibit several different energy dependent transport processes in the kidney.

Effects of PSP and PAH. PSP and PAH are evidently transported by a common mechanism in the kidney.[20, 47] PSP had no effect on the parameters studied, except for the slight inhibition of palmitate uptake by 3·6 mM PSP. However, PSP combines with albumin,[33, 34] and this binding may prevent most of the effects of PSP. A consideration of this phenomenon must precede any further discussion of the possible effects of PSP.

If FFA were transported by the same mechanism as PAH, competitive inhibition of palmitate uptake by PAH would be expected. However, the only effects exerted by PAH were the slight stimulation of palmitate oxidation and of the total oxygen consumption of the slices. It is improbable that this reflects competition for a common transport mechanism. It could be, that a stimulated energy production for the transport of PAH into the slices is reflected in the increased oxidation of the added palmitate and in the increased oxygen consumption. If this interpretation is correct, palmitate does not show specific affinity for the PAH transport system, but is only one of the several substrates that may feed the citric acid cycle.

CONCLUSIONS

Probenecid exerts its effects intracellularly, inhibiting the oxidation of exogenous palmitate and endogenous substrates, and stimulating anaerobic glycolysis in the kidney tubule cell. It is assumed that these effects of probenecid are *not* specific for the PAH transport system. The enhanced oxidation of the added palmitate by PAH is suggested to be due to an increased energy demand for PAH transport. These results do not support the suggestion[4] that FFA were transported by the PAH transport mechanism in the kidney.

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