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EFFECTS OF PHENOLSULFONPHTHALEIN AND PROBENECID ON THE UPTAKE AND UTILIZATION OF CITRATE AND α-KETOGLUTARATE BY KIDNEY, *IN VITRO**

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Abstract—Guinea pig kidney cortical slices accumulate α-ketoglutarate† from the medium against a concentration gradient, whereas citrate is not accumulated against a gradient. The concentration of citrate in kidney slices is increased by 0·05–3·6 mM phenolsulfonphthalein. PSP also stimulates the accumulation of α-KG by kidney cortical slices, simultaneously inhibiting α-KG utilization and respiration. At 0° PSP does not affect the tissue concentration and utilization of citrate and α-KG. One to nine mM probenecid greatly decreases the concentration of citrate in kidney slices; 1–3 mM probenecid has no effect on the utilization of citrate, but 9 mM probenecid inhibits utilization by 25 per cent and respiration by 40 per cent. Probenecid inhibits the accumulation of α-KG in kidney slices, simultaneously inhibiting α-KG utilization and respiration. At 0° probenecid has only a slight decreasing effect on citrate concentration of the slices.

PSP inhibits the oxidation of α-KG and succinate by kidney mitochondria, whereas citrate oxidation is more resistant. Probenecid inhibits the oxidation of α-KG by kidney mitochondria, whereas citrate and succinate oxidations are more resistant to probenecid.

The results demonstrate that PSP and probenecid act both intracellularly at mitochondrial sites and at outer cell membrane.

IN THE dog most of the infused α-ketoglutarate[1–3] and citrate[4,5] are taken up by the kidneys and the liver. This may be due to selective permeability of the cell membranes of these organs to these organic substrates.[1] In the kidney a transcellular transport mechanism has been demonstrated, by which several foreign nonmetabolized organic anions, such as PAH and PSP are secreted into the urine.[8,9] The transcellular transport of PSP and PAH in the kidney tubule is blocked by probenecid.[9–11] *In vivo* most of the α-KG[2,3] and citrate[6,7] appearing in the glomerular filtrate is actively reabsorbed by the tubular cells. The fact that the PAH transport mechanism shows the same organ specificity as the uptake of α-KG and citrate,[8] and that the transport of α-KG across the antiluminar membrane in the renal tubular cell in the dog as well as the excretion of citrate in the chicken[12] are blocked by probenecid have led to the suggestion that α-KG, citrate, PSP and PAH are transported by partly identical

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† Abreviations used in this paper are: PSP: phenolsulfonphthalein, α-KG: α-ketoglutarate. PAH: *p*-aminohippurate, ATP: adenosine-5′-triphosphate, TCA: trichloroacetic acid, PCA: perchloric acid.

mechanisms.[1,3,12] From *in vivo* experiments, however, it is not possible to draw final conclusions about the transport of organic anions across cell membranes and its relation to their metabolism in the cell. This is why *in vitro* techniques have been used to give further information on those processes at the cellular level.

Experiments *in vitro* have given further evidence in support of the conclusion mentioned above, thus α-KG inhibits the accumulation of PAH by kidney cortical slices,[8] but stimulates its run-out from the slices.[13] Probenecid inhibits the accumulation of PAH in kidney cortical slices[13] and isolated kidney tubules.[14]

It has been found that guinea pig cortical slices accumulate exogenous α-KG against a considerable concentration gradient during aerobic incubation.[15,16] This accumulation is sensitive to anaerobiosis and cold,[15] showing its dependence on the aerobic energy metabolism. Kidney cortical slices also accumulate PSP against a concentration gradient.[17,18] This accumulation is inhibited by α-KG, but stimulated by citrate.[17]

We have used *in vitro* techniques to investigate the effects of PSP and probenecid on the uptake and utilization of α-KG and citrate in the kidney. The aim of our studies was to obtain further information on the transport of the organic anions, α-KG and citrate, which act as substrates in the energy metabolism in the kidney by demonstrating the modes of action of probenecid and PSP on the transport and utilization of α-KG and citrate.

MATERIALS AND METHODS

Materials

Chemicals. α-KG, citric acid and PSP were from E. Merck AG, Darmstadt, Germany, glutamic dehydrogenase and NADH, which were used in the determination of α-KG, and ATP from C. F. Boehringer & Soehne GmbH, Mannheim, Germany, and yeast hexokinase, type III, from the Sigma Chemical Company, St. Louis, Missouri, U.S.A. Probenecid was a gift from AB Astra, Södertälje, Sweden.

Animals. Male adult guinea pigs (Orion Oy, Mankkaa, Finland) weighing 500–700 g, and fed on guinea pig diet (Orion Oy, Mankkaa, Finland) ad libitum were used in all experiments.

Methods

Preparation of tissue slices. After killing the animal by a blow on the head and decapitation, the kidneys were quickly decapsulated, removed and placed in cold Krebs–Ringer phosphate solution.[20] The ice-cold slicing medium consisted of 0·14 M NaCl and 0·01 M KCl. Kidney cortical slices 0·4–0·5 mm thick were prepared with a Stadie–Riggs microtome.[19] The first slice of each hemikidney was discarded, and two slices containing only cortical tissue were obtained from each hemikidney. Each slice was blotted with filter paper, weighed on a torsion scale and placed in a Warburg flask containing Krebs–Ringer phosphate solution with glucose at room temperature. Two slices, total weight 100–130 mg, were used in each flask.

Incubation of tissue slices. All incubations were carried out in a Warburg respirometer at a shaker speed of 100 cycles per minute, at 30° or 0°, as indicated, and with 100% oxygen as the gas phase. The incubation medium contained 25 μmoles glucose, usually 10 μmoles citrate or α-KG as indicated, and various amounts of PSP or probenecid in a total volume of 2·5 ml Krebs–Ringer phosphate buffer, gassed with

100% oxygen. The centre well of the flasks contained 0·2 ml 20% KOH. The organic acid and PSP or probenecid were added just before the flasks were connected to the respirometer. The flasks were gassed with 100% oxygen for 5 min, and after 5 min equilibration the respiration was recorded. After incubation the slices were quickly removed from the flasks, blotted on stiff filter paper, weighed and placed in a homogenizing tube containing 3·0 ml 10% TCA (for citrate) or 0·60 M PCA (for α-KG). A 1·0 ml aliquot of the incubation medium was taken into a centrifuge tube containing 2·0 ml TCA or PCA. Before homogenization the slices were allowed to stand for 30 min at room temperature. After homogenization the homogenate and the medium samples were centrifuged at 10,000 *g* for 15 min. The supernatants were analyzed for citrate or α-KG.

Preparation and incubation of kidney mitochondria. Kidney mitochondria were obtained by differential centrifugation of guinea pig kidney homogenate as described earlier,[37] except whole kidneys were used. The mitochondria from two kidneys were finally suspended in 4·0 ml 0·25 M sucrose, with EDTA and Tris·Cl.

Mitochondrial respiration was determined according to Warburg's direct method[20] at 30° in 15 ml Warburg flasks with a side arm. The medium contained, in a final volume of 2·0 ml, 50 μmoles potassium phosphate buffer, pH 7·4, 100 μmoles KCl, 16 μmoles $MgCl_2$, 2 μmoles ATP, 60 μmoles glucose, 1 mg hexokinase, 20 μmoles α-KG, citrate or succinate and various amounts of PSP or probenecid and 0·15 ml mitochondrial suspension. The reaction was started by adding the mitochondria to the flasks, and after 5 min thermoequilibration the oxygen consumption was recorded for 20 min.

Analytical methods. Citrate was determined by the pentabromacetone method modified from the methods of Jones[21] and Natelson *et al.*[22]: Citric acid to be analyzed in TCA solution was oxidized and brominated to pentabromacetone with vanadium pentoxide and $KBR–KBrO_3$ mixture as described by Jones. The pentabromacetone was extracted with petroleum ether. The yellow complex formed after extraction with thiourea-borax was measured at 440 mμ. Glucose and probenecid in the concentrations used did not affect the determination of citrate. The presence of PSP in the sample gave higher absorbance values for citrate. Because the increase in the absorbance was not linear with increasing PSP concentrations, the effect of PSP on the determination was eliminated by the following method: After deproteinization with TCA and centrifugation, the PSP concentration of the supernatant was determined by measuring the extinction of PSP at 560 mμ in 0·1 M bicarbonate solution. Then different amounts of PSP were added to the samples, so that before the oxidizing mixture was added, all test tubes, including the standard ones, contained equal amounts of PSP in equal volumes.

α-KG was determined by an enzymatic method described by Bergmeyer and Bernt.[23] PSP or probenecid in the concentrations used did not affect the determination.

α-KG recoveries of 97–100 per cent were obtained from the homogenates. The recovery of citrate was 98–100·5 per cent. PSP and probenecid did not affect the recoveries of these organic acids.

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

Terms. The term utilization is used to describe the total disappearance of the substrate anion (citrate or α-KG) from the medium and slices during incubation. The

concentration of the organic acid in the slices is expressed as μmoles per gram wet tissue weight. Slice to medium ratio (S/M ratio) is expressed as the ratio of the concentration of the organic acid in grams wet tissue weight to the ml medium.

RESULTS

Experiments with tissue slices

Pilot experiments with guinea pig cortical slices were performed by incubating the slices in media with various α-KG and citrate concentrations for different times. The following results were obtained:

The S/M ratio of α-KG in kidney cortical slices decreased with rising α-KG concentrations, while the utilization increased only slightly with α-KG concentrations higher than 3 mM. 4 mM α-KG was used in the subsequent experiments. In kidney cortical slices incubated in 4 mM α-KG the S/M ratio was found to increase sharply for 35–40 min, after which there was only a slight increase in the S/M ratio from 40 to 90 min. After 50–60 min incubation respiration decreased slightly. In the experiments with α-KG, kidney cortical slices were incubated for 40 min. When kidney cortical slices were incubated with 4 mM citrate, the S/M ratio increased continuously with time during 90 min. After 50–60 min incubation, however, respiration began to decline. The incubation time in further experiments with citrate was 40 min.

Effect of PSP on citrate uptake by kidney cortical slices. The results of these experiments are illustrated in Fig. 1. Citrate was not accumulated in the slices against

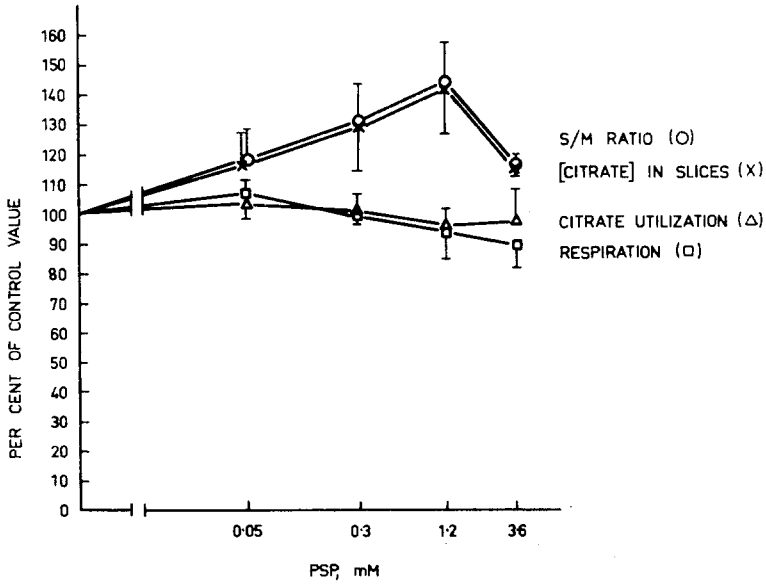

FIG. 1. Effects of PSP on respiration and the accumulation and utilization of citrate in kidney cortical slices. Slices incubated in Krebs–Ringer phosphate buffer with 10 mM glucose and 4 mM citrate in a Warburg respirometer at 30° for 40 min with 100% oxygen as gas phase, as explained under methods. Control values (=100%): Respiration 223 ± 17 μatoms O/g/hr, citrate utilization 30·8 ± 2·4 μmoles/g/hr, [citrate] in slices 1·95 ± 0·18 μmoles/g and S/M ratio 0·663 ± 0·076. Each point is the mean ± S.D. of four to six parallel determinations.

a concentration gradient, the S/M ratio being 0·663 ± 0·076 in the control. The citrate content of the tissue after 40 min was 1·95 ± 0·18 μmoles/g. The utilization of citrate was 30·8 ± 2·4 μmoles/g/hr and the oxygen consumption 223 ± 17 μatoms 0/g/hr. Added PSP in 0·05–3·6 mM concentrations increased the tissue content of citrate, maximally 40 per cent with 1·2 mM PSP ($P < 0·01$). Citrate utilization was not affected by 0·05–3·6 mM PSP; thus there was also an increase in the S/M ratio, about 40 per cent with 1·2 mM PSP, whereas with 3·6 mM the increase was only about 15 per cent ($P < 0·01$). The respiration of the slices was slightly inhibited by the highest PSP concentration used ($P < 0·05$).

Effect of PSP on α-KG uptake by kidney cortical slices. Guinea pig kidney cortical slices have previously been shown to accumulate α-KG from the medium against a concentration gradient.[15,16] We obtained the S/M ratio of 3·80 ± 0·23 with 4 mM α-KG in the medium (Fig. 2). The tissue concentration of α-KG at the end of the

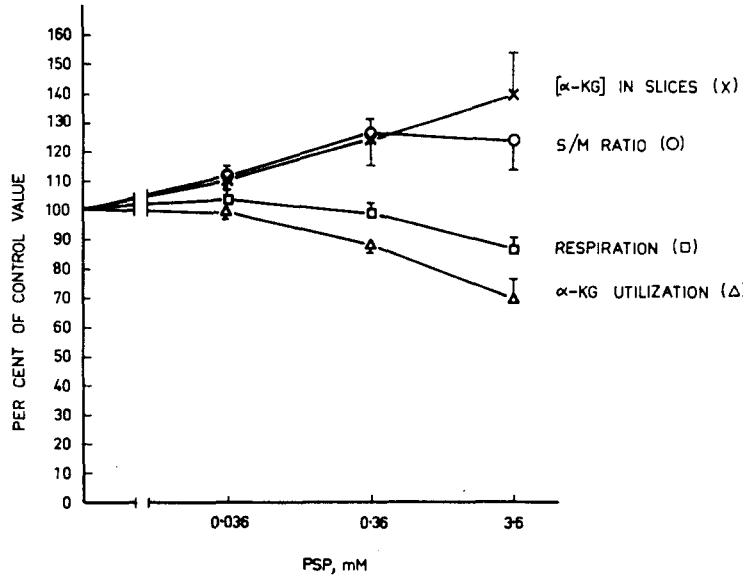

FIG. 2. Effects of PSP on respiration and the accumulation and utilization of α-KG in kidney cortical slices. Incubation conditions as in Fig. 1, except that 4 mM α-KG was used instead of citrate. Control values (=100%): Respiration 235 ± 15 μatoms O/g/hr, α-KG utilization 50·1 ± 4·5 μmoles/g/hr, [α-KG] in slices 8·02 ± 0·78 μmoles/g and S/M ratio 3·80 ± 0·23. Each point is the mean ± S.D. of four parallel determinations.

incubation was 8·02 ± 0·78 μmoles/g. The utilization of α-KG was faster than that of citrate (50·1 ± 4·5 μmoles/g/hr), and the oxygen consumption of about the same magnitude as with citrate as substrate (235 ± 15 μatoms/g/hr). PSP in concentrations of 0·036–3·6 mM increased the tissue concentration of α-KG ($P < 0·01$), maximally 40 per cent. Unlike citrate utilization, the utilization of α-KG was inhibited by PSP concentrations of 0·36–3·6 mM ($P < 0·01$). With 3·6 mM PSP the inhibition was about 30 per cent. As with citrate, only 3·6 mM PSP significantly inhibited the oxygen consumption (about 14 per cent, $P < 0·01$).

Effect of PSP on α-KG and citrate uptake by kidney cortical slices at 0°. When the

slices were incubated with 4 mM α-KG or citrate at 0° for various times, the tissue contents and the S/M ratios of these acids did not vary significantly with incubation times from 30 min to 120 min, the mean S/M ratio being about 0·26 for α-KG and about 0·44 for citrate.

The effect of PSP on the uptake of α-KG at 0° is seen in Fig. 3. PSP did not significantly affect the tissue content of α-KG. After incubation with 3·6 mM PSP there was slightly more α-KG left in the incubation medium than in the case of the control ($P < 0·05$), so that the S/M ratio was slightly depressed by 3·6 mM PSP ($P < 0·01$). The effect of PSP on the uptake of citrate by kidney cortical slices at 0° was investigated in three experiments. In the control experiments the mean [citrate] in the slices was 1·40 μmoles/g. With 0·036, 0·36 and 3·6 mM PSP the [citrate] in the slices averaged 102·7 per cent, 101·4 per cent and 102·7 per cent of the respective controls. Neither [citrate] in the medium nor the S/M ratio were affected by PSP.

Effects of PSP on the oxygen consumption of kidney cortical slices incubated with and without added α-KG. It has previously been found that the respiration of kidney cortical slices is stimulated by addition of α-KG to the saline incubation medium.[24] In our experiments at 30°, 4 mM α-KG stimulated respiration on guinea pig kidney cortical slices by about 30 per cent (Table 1). The endogenous respiration of the

TABLE 1. EFFECT OF PSP ON RESPIRATION OF KIDNEY CORTICAL SLICES INCUBATED WITH AND WITHOUT ADDED α-KG

Exp. No.	α-KG (mM)	PSP (mM)	Respiration (μatoms O/g/hr)	Inhibition of respiration by PSP (%)	Endogenous respiration stimulated by added α-KG (%)
1	0	0	148·5		
2	0	0	166		
3	0	0	167		
1	0	3·6	119	19·9	
2	0	3·6	144	13·3	
3	0	3·6	138	17·4	
1	4	0	195		31·3
2	4	0	212		27·7
3	4	0	217		29·9
1	4	3·6	159	18·5	
2	4	3·6	164	22·6	
3	4	3·6	168	22·6	

Slices incubated in Krebs–Ringer phosphate buffer with 10 mM glucose with and without 4 mM α-KG in a Warburg respirometer at 30° for 40 min with 100% oxygen as gas phase.

slices was inhibited by 3·6 mM PSP 13·3–19·9 per cent in three experiments, and the respiration in the presence of α-KG to about the same extent (18·5–22·6 per cent). This might indicate that the inhibition of respiration by PSP was not solely due to the inhibition of α-KG oxidation, although the inhibition of α-KG utilization by PSP roughly parallelled the inhibition of respiration (Fig. 3). It seems probable that α-KG substitutes the endogenous substrates,[25] most of which are fatty acids.[26,27]

Effect of PSP on the tissue content of α-KG and citrate when kidney cortical slices were incubated with pyruvate plus malate or succinate. Tables 2 and 3 show some of the results of the experiments in which we studied the effects of added glucose and of some Krebs cycle acid intermediates on the α-KG and citrate content of kidney cortical

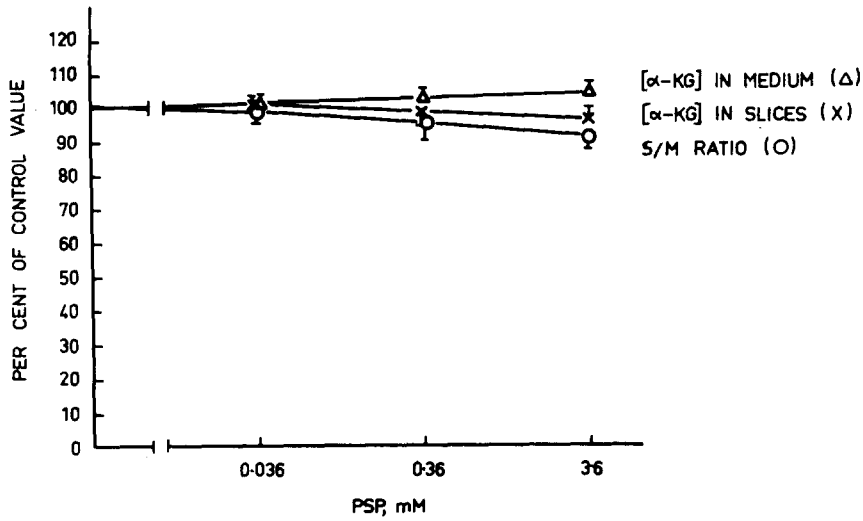

FIG. 3. Effect of PSP on the accumulation of α-KG in kidney cortical slices at 0°. Incubation conditions as in Fig. 1, except that 4 mM α-KG was used instead of citrate and the temperature was 0°. Control values (=100%): [α-KG] in medium 3·78 ± 0·06 μmoles/ml, [α-KG] in slices 0·978 ± 0·059 μmoles/g and S/M ratio 0·259 ± 0·015. Each point is the mean ± S.D. of four parallel determinations.

TABLE 2. EFFECT OF PSP ON TISSUE [α-KG] IN KIDNEY CORTICAL SLICES INCUBATED WITH PYRUVATE PLUS MALATE

Pyruvate + Malate (mM)	PSP (mM)	[α-KG] in slices (% of control)	Respiration (% of control)
4 + 4	0	100	100
4 + 4	0·036	94·3 (87·8 – 98·1)	106·8 (104·8 – 109·6)
4 + 4	0·36	96·3 (94·7 – 99·3)	94·2 (85·7 – 99·0)
4 + 4	3·6	130·5 (102·8 – 152·5)	85·3 (83·5 – 88·9)

Incubation conditions as in Table 1, except that 4 mM pyruvate plus 4 mM malate were used as substrate. Control values (=100%): [α-KG] in slices 1·67 (1·30–2·06) μmoles/g and respiration 256 (249–265) μatoms O/g/hr. The results expressed as means and ranges of three parallel determinations.

TABLE 3. EFFECT OF PSP ON TISSUE [CITRATE] IN KIDNEY CORTICAL SLICES INCUBATED WITH PYRUVATE PLUS MALATE

Pyruvate + Malate (mM)	PSP (mM)	[Citrate] in slices (% of control)	Respiration (% of control)
4 + 4	0	100	100
4 + 4	0·036	89·1–111·0	90·0–105·1
4 + 4	0·36	84·8–101·4	82·7–93·7
4 + 4	3·6	89·2–89·9	79·1–85·4

Incubation conditions as in Table 1, except that 4 mM pyruvate plus 4 mM malate were used as substrate. Control values (=100%): [citrate] in slices 2·18–2·30 μmoles/g and respiration 253–277 μatoms O/g/hr.

slices. After the slices had been incubated 40 min with 10 mM glucose, the concentration mostly used in these experiments, no measurable amount of α-KG was found in the slices or in the medium. After incubating the slices with 4 mM pyruvate plus 4 mM malate in Krebs–Ringer glucose solution, the mean tissue content of α-KG was 1·67 μmoles/g in three experiments (Table 2). There was also a small amount of α-KG in the incubating medium.

Four mM succinate also caused an increase in the α-KG content of the slices, the concentration averaging 2·31 μmoles/g as the mean of three experiments (range 2·04–2·67 μmoles/g).

3·6 mM PSP increased the tissue content of α-KG when the slices were incubated with pyruvate plus malate (Table 2). The mean increase was 30·5 per cent. Lower PSP concentrations (0·036 and 0·36 mM) did not seem to affect the tissue content of α-KG.

Pyruvate plus malate in the medium also increased the tissue content of citrate with respect to the control (Table 3). The citrate concentration in two determinations was 2·18–2·30 μmoles/g. PSP maybe slightly decreased the tissue content of citrate and inhibited the respiration.

It is evident, that the effects of PSP found on [α-KG] and [citrate] in slices after incubating with precursors do not account for the effects of PSP on the tissue content of these organic anions in the experiments described earlier, because only 3·6 mM PSP clearly increased the tissue content of α-KG from the precursors and the [citrate] in the slices was not affected at all. It may be that the increasing effect of PSP on [α-KG] in slices is due to the competitive inhibition of the utilization of the intracellularly formed α-KG.

Effect of probenecid on citrate uptake by kidney cortical slices. The results of these experiments are illustrated in Fig. 4. One to nine mM probenecid greatly decreased

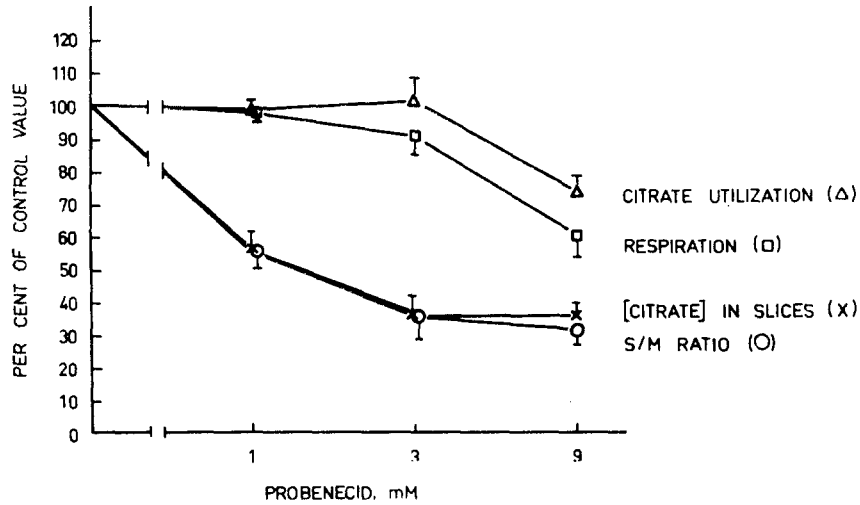

FIG. 4. Effects of probenecid on respiration and the accumulation and utilization of citrate in kidney cortical slices. Incubation conditions as in Fig. 1. Control values (=100%): Respiration 245 ± 19 μatoms O/g/hr, citrate utilization 34·9 ± 4·3 μmoles/g/hr, [citrate] in slices 2·24 ± 0·09 μmoles/g and S/M ratio 0·791 ± 0·050. Each point is the mean ± S.D. of five parallel determinations.

the tissue content of citrate with respect to the control ($P < 0.01$). With 1 mM probenecid the decrease was about 45 per cent. One mM and 3 mM probenecid did not affect the utilization of citrate, and 9 mM probenecid gave about 25 per cent inhibition ($P < 0.01$). The S/M ratio was greatly depressed with 1–9 mM probenecid and the respiration slightly with 3 mM ($P < 0.01$) and more with 9 mM probenecid ($P < 0.01$). These results clearly show that the citrate content of kidney cortical slices can be reduced by probenecid without affecting the utilization of the substrate.

Effect of probenecid on α-KG uptake by kidney cortical slices. The results are expressed in Fig. 5. Probenecid inhibited the accumulation of α-KG. With 9 mM

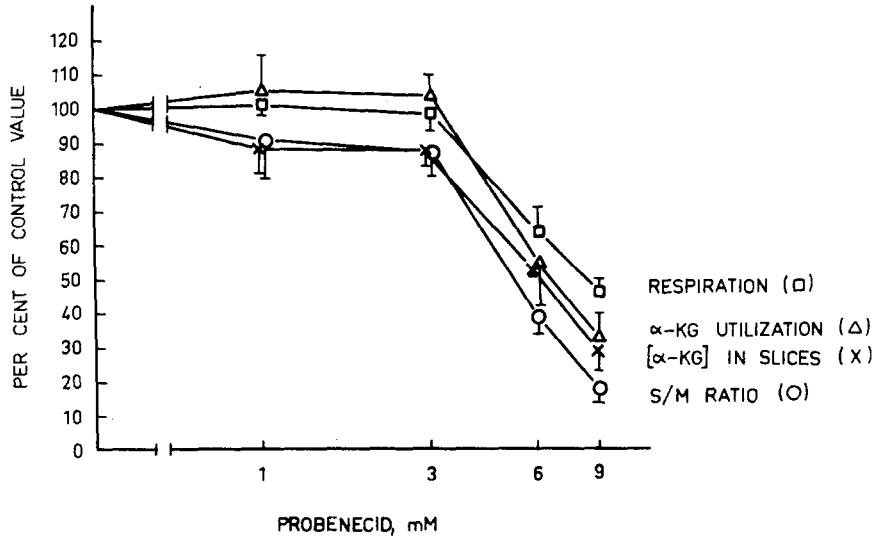

FIG. 5. Effects of probenecid on respiration and the accumulation and utilization of α-KG in kidney cortical slices. Incubation conditions as in Fig. 1, except that 4 mM α-KG was used instead of citrate. Control values (=100%): Respiration 234 ± 18 μatoms O/g/hr, α-KG utilization 48·5 ± 4·4 μmoles/g/hr, [α-KG] in slices 9·68 ± 1·30 μmoles/g and S/M ratio 4·47 ± 0·55. Each point is the mean ± S.D. of four to six parallel determinations.

probenecid the S/M ratio decreased about 80 per cent. One to three mM probenecid slightly depressed the tissue content of α-KG ($P < 0.025$ for 1 mM and <0.01 for 3 mM probenecid) without affecting the utilization of the substrate or respiration. With higher probenecid concentrations (6–9 mM) all the four parameters measured were strongly affected ($P < 0.01$), and no significant difference in sensitivity was found between the tissue accumulation and the utilization of α-KG.

Effect of probenecid on citrate uptake by kidney cortical slices at 0°. In the cold probenecid had only a small effect on citrate concentration in the slices, as can be seen from Fig. 6. In the cold the [citrate] in the slices was only slightly lower than at 30°. The small effect of probenecid on citrate accumulation found in the cold may indicate that the effect of probenecid is energy dependent. Thus probenecid seems to inhibit the transport of citrate and does not seem to reduce the passive influx of the substrate, because it seems probable that the effects of probenecid on the passive influx would also have been apparent in the cold.

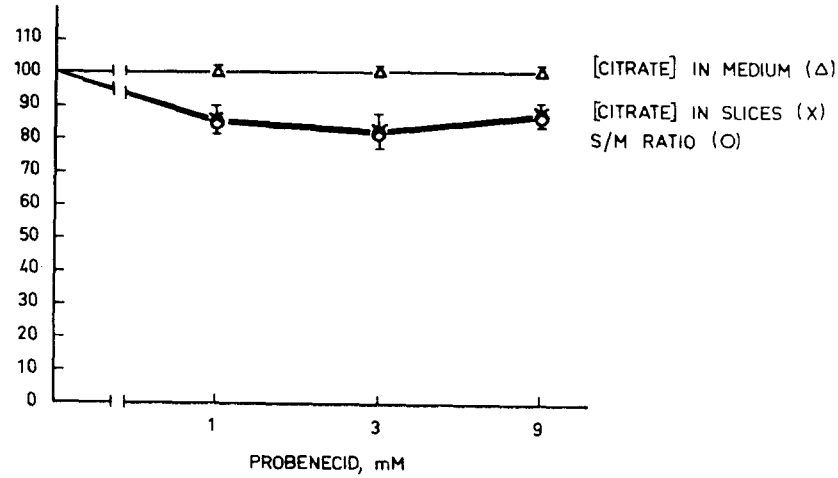

FIG. 6. Effect of probenecid on the accumulation of citrate in kidney cortical slices at 0°. Incubation conditions as in Fig. 1, except that the temperature was 0°. Control values (= 100%): [Citrate] in medium ± 0·03 μmoles/ml, [citrate] in slices 1·65 ± 0·16 μmoles/g and S/M ratio 0·417 ± 0·040. Each point is the mean ± S.D. of four parallel determinations.

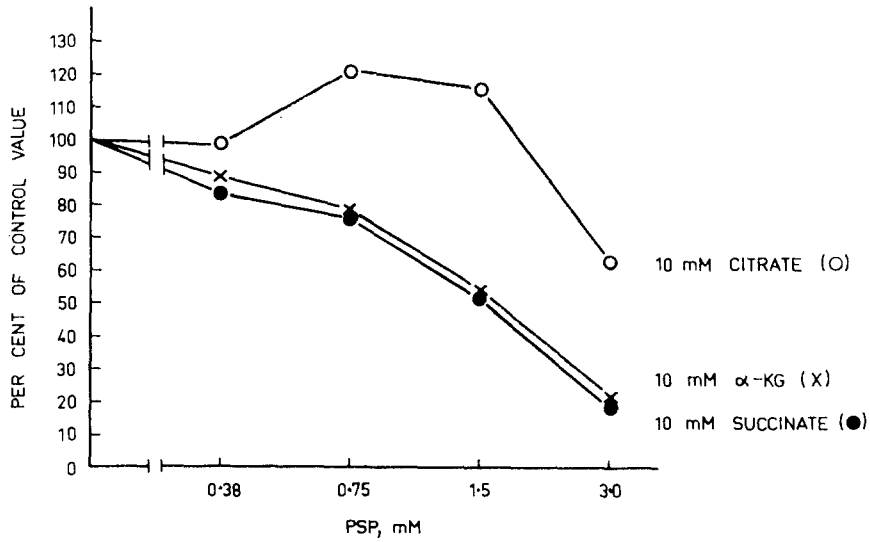

FIG. 7. Effect of PSP on the respiration of kidney mitochondria oxidizing α-KG, citrate and succinate. The respiration was determined in a Warburg respirometer at 30° with air as gas phase, as explained under methods. Control values (= 100%): Oxygen consumption with α-KG 27·9 μatoms O/hr, with citrate 8·64 μatoms O/hr and with succinate 32·3 μatoms O/hr. Each point is the mean of three parallel determinations.

Experiments with kidney mitochondria

Effects of PSP on respiration of kidney mitochondria oxidizing α-KG, citrate and succinate. As can be seen from Fig. 7, PSP inhibited the oxidation of α-KG and succinate to about the same extent. Fifty per cent inhibition was obtained with 1·5 mM PSP. The oxidation of citrate was much more resistant to PSP than the oxidation of α-KG or succinate. In three experiments 0·75 and 1·5 mM PSP caused an increase

in oxygen consumption with citrate as substrate, whereas 3·0 mM PSP gave about 40 per cent inhibition. The stimulating effect of 0·75 and 1·5 mM PSP on citrate oxidation was not further studied, but may have been due to increased membrane permeability or to an uncoupling of oxidative phosphorylation by PSP at these concentrations.

Effects of probenecid on respiration of kidney mitochondria oxidizing α-KG, citrate and succinate. The effects of probenecid on respiration of kidney mitochondria differ somewhat from those found with PSP (Fig. 8). About 0·3 mM probenecid caused 50 per cent inhibition of α-KG oxidation, which is thus more sensitive to probenecid than to PSP, but, in contrast to PSP, probenecid only slightly inhibited succinate respiration.

Corresponding results were obtained in the slice experiments: Citrate utilization in kidney cortical slices is more resistant to the effects of PSP and probenecid than α-KG utilization.

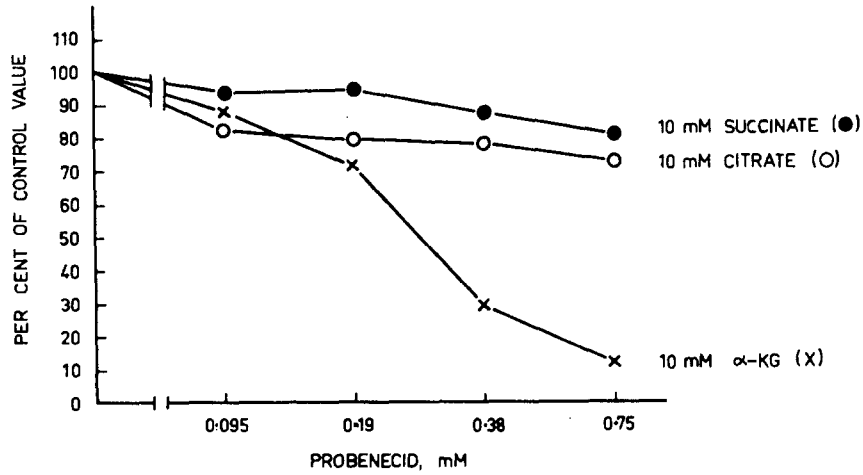

FIG. 8. Effect of probenecid on the respiration of kidney mitochondria oxidizing α-KG, citrate and succinate. Incubation conditions as in Fig. 7. Control values (=100%): Oxygen consumption with α-KG 25·9 μatoms O/hr, with citrate 10·9 μatoms O/hr and with succinate 32·4 μatoms O/hr. Each point is the mean of three parallel determinations.

DISCUSSION

Uptake and utilization of α-KG and citrate by kidney slices. Guinea pig kidney cortical slices accumulate α-KG against a concentration gradient.[15,16] We obtained an S/M ratio of about 4 by incubation at 30° for 40 min. The accumulation of α-KG by kidney cortical slices is evidently an active process that requires energy. This is evidenced by the high S/M ratio. α-KG utilization in the slices was about 50 μmoles/g/hr.

However, citrate is not accumulated against a concentration gradient, and an S/M ratio of 0·6–0·8 was found. Thus on the basis of these results it is not known, if the tubular cells take up citrate by an active process. Citrate utilization was 30–40 per cent lower than α-KG utilization. Simpson has shown that calcium in the incubation medium greatly reduces citrate utilization by liver slices and much less by kidney

cortical slices.[28] In spite of this finding, we did not omit calcium from our incubation medium, because we wanted to keep the conditions more physiological.

Effects of PSP. PSP was found to increase the accumulation of α-KG by kidney cortical slices, simultaneously inhibiting the utilization of α-KG as well as respiration (Fig. 2). The inhibition of α-KG oxidation in mitochondria by PSP could have caused the increased accumulation of α-KG found. 0·036 mM PSP increased the [α-KG] in the slices (110 ± 1·9% of the control), while α-KG utilization was not affected (99·9 ± 1·9% of the control). This difference is significant, but so small that it is not legitimate to conclude that the increased accumulation of α-KG is due to increased influx or decreased efflux of the substrate. The strong inhibition of α-KG oxidation by PSP in mitochondria (Fig. 7) would tend to increase the accumulation assuming a constant influx rate and the effects on α-KG transport across the cell membrane may then be masked, since indeed a net effect on influx and utilization was measured. At 0° PSP does not affect the concentration of α-KG in the slices or α-KG utilization (Fig. 3), as it does at 30°, suggesting the dependence of the PSP effect on intact energy metabolism.

The effects of PSP on the [citrate] in kidney slices and citrate utilization are somewhat different from the effects on these parameters with α-KG as substrate: PSP in concentrations that increase the tissue concentration of citrate and the S/M ratio (0·05–1·2 mM) has no significant effect on citrate utilization and respiration (Fig. 1). This finding differs somewhat from that in our preliminary report: In the preliminary experiments[30] we found a slight increase in citrate utilization at low PSP concentrations, while the [citrate] in the slices increased. It has been found that the stimulation of citrate utilization by PSP then reported was not statistically significant.

We observed that isolated kidney mitochondria are rather resistant to the inhibiting effect of PSP on the oxidation of citrate (Fig. 7). This finding may explain the lack of effects of PSP on citrate utilization which was found in kidney cortical slices. On the basis of these results, we propose that the increased citrate concentration of the slices observed is due to either stimulation of the influx and/or reduction of the outflux.

1·2 mM PSP maximally increased the tissue [citrate] and the S/M ratio, whereas 3·6 mM PSP gave a smaller increase. This may indicate that the effect of PSP on citrate uptake by kidney slices is not an increase in the permeability of the cell membrane promoting passive diffusion of the substrate, since this effect would probably have been evident with 3·6 mM PSP as well.

After incubating kidney cortical slices with exogenous citrate in the cold the citrate concentration of the slices was lower than after incubation at 30°. PSP had only a small effect on the tissue content of citrate and the S/M ratio in the cold. This may also indicate that PSP does not increase the permeability of the cell membrane to passive influx of citrate, but affects the active transport of this organic acid across the cell membrane.

Thus PSP increases the intracellular concentration of α-KG and citrate and inhibits their oxidation in the mitochondria. The inhibition of α-KG oxidation could account for the increase in the tissue α-KG content. With citrate, however, it is evident that the increase in the tissue content of this substrate cannot be due to the inhibition of its oxidation because citrate utilization in the slices was not inhibited by PSP. Furthermore isolated mitochondria were rather resistant to PSP. PSP evidently affects the

transport of citrate and possibly also of α-KG across the tubular cell membrane by regulating the permeability of the cell membrane. We suggest that the effect at the cell membrane may be as follows: PSP accumulates in kidney cortical slices against a concentration gradient.[17,18] By following out of the cell it could competitively inhibit the efflux of citrate and α-KG, causing their accumulation in the cell. Another possibility is that the efflux of the intracellular PSP would stimulate the influx of the extracellular organic anions. This would parallel the transport mechanism called "flow induced by counterflow", which has been described for amino acids.[31,32] PSP would thus "competitively" stimulate the transport of organic anions across the cell membrane.

Effects of probenecid. Probenecid inhibits the oxidation of α-KG at the mitochondrial level (Fig. 8). However, in spite of the inhibition of α-KG utilization in the cells by probenecid we found a simultaneous decrease in [α-KG] in the slices and the S/M ratio. Thus it seems evident that probenecid inhibits the influx or stimulates the efflux of α-KG at the tubular cell membrane, simultaneously inhibiting its utilization by mitochondria.

The concentration of citrate in kidney cortical slices is much more sensitive to probenecid than citrate utilization and oxygen consumption (Fig. 4). This probably indicates that probenecid affects the cell membrane by inhibiting the transport of citrate through the cell membrane into the cell. In the cold, probenecid only slightly reduces the [citrate] in the slices (Fig. 6). This may indicate that the effect of probenecid on citrate transport is not to inhibit the passive influx of citrate into the cell, but rather to inhibit the active transport of citrate.

Citrate probably penetrates the tubular cell membrane at a slower rate than α-KG, as can be seen from the lower slice concentration of citrate in spite of slower utilization. This slow penetration does not seem to limit the rate of utilization of citrate, because an increase in the tissue content of citrate by PSP does not significantly stimulate its utilization, and the decrease in tissue citrate content by 1–3 mM probenecid does not decrease the utilization of citrate.

With α-KG we obtained an S/M ratio of about 4 in the kidney. Thus the intracellular concentration of α-KG may already be so high that its increase by PSP cannot stimulate its utilization. The inhibition of α-KG oxidation in mitochondria by PSP may also be a significant factor.

CONCLUSIONS

PSP and probenecid affect the kidney tubular cell at the cell membrane and at the mitochondrial level regulating the transport and metabolism of citrate and α-KG. The present results support the idea that the PAH transport mechanism participates in the transport of citrate and α-KG in the kidney.

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