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ROLE OF FATTY ACID METABOLISM ON RENAL TRANSPORT OF *p*-AMINOHIPPURATE *IN VITRO*

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SUMMARY

1. The accumulation of *p*-aminohippurate and oxygen consumption in cortical slices prepared from rabbit kidneys were measured in the presence of various fatty acids and inhibitors of fatty acid metabolism ((+)-palmitylcarnitine and 2-bromopalmitate). Furthermore, $^{14}CO_2$ production from radioactive labelled metabolites were estimated in the (+)-palmitylcarnitine and bromopalmitate experiments.

2. Palmitate, stearate, and oleate (bound to human albumin) were all readily oxidized by the renal tissue and gave rise to a significant stimulation of *p*-aminohippurate accumulation which was enhanced approx. 60% at 1 mM fatty acids in the incubation medium. However, at a fatty acid concentration of 5 mM the *p*-aminohippurate accumulation was reduced relative to that found at 1 mM. In accordance with previous reports octanoate (1 and 2 mM) drastically inhibited the uptake of *p*-aminohippurate.

3. Addition to the medium of (+)-palmitylcarnitine or 2-bromopalmitate caused a considerable decrease of *p*-aminohippurate accumulation and of $^{14}CO_2$ formation from [^{14}C]palmitate while oxygen consumption was almost unaffected. The unchanged oxygen consumption may be explained by a compensatory increase of carbohydrate metabolism, since a pronounced enhancement of $^{14}CO_2$ production from [^{14}C]glucose was observed.

4. Evidence based on the inhibitor-reversing effect of various metabolites support the view that in contrast to (+)-palmitylcarnitine, 2-bromopalmitate does not have a significant direct inhibitory effect on the *p*-aminohippurate transport through the cell membrane.

5. It is concluded that the metabolism of physiological long-chain fatty acids is necessary for accumulation of *p*-aminohippurate *in vitro*. However, at high concentrations a direct inhibitory action of the fatty acids on the transport of *p*-aminohippurate is apparent. It is suggested that the more pronounced inhibition of *p*-aminohippurate accumulation by short-chain fatty acids is due to a higher degree of water solubility of these compounds compared with that of the long-chain fatty acids.

INTRODUCTION

In a previous study it was suggested that metabolism of substances connected with carbohydrate degradation, probably pyruvate and L-lactate, is necessary for

maintaining renal transport of *p*-aminohippurate *in vitro*[1]. However, in several studies evidence has been brought forward that long-chain fatty acids are the principal fuel in renal cortex *in vitro*[2] as well as *in vivo*[3–5], while glucose catabolism only accounts for a small part of renal oxygen consumption[2,5–8]. This view is supported by observations showing a low respiratory quotient of cortical kidney slices[9,10]. Earlier investigations indicate that short-chain fatty acids (chain-length C_6–C_{12}) inhibit *p*-aminohippurate accumulation in kidney cortex slices to a variable extent[11–13], but no attempt has been made to evaluate the relation between active *p*-aminohippurate transport and metabolism of physiological long-chain fatty acids.

The present investigation was made in order to clarify the role of long-chain fatty acids and metabolism of these substances on active transport of *p*-aminohippurate in cortex slices from rabbit kidneys. The effect of addition of various fatty acids to the medium on *p*-aminohippurate accumulation and oxygen consumption was investigated. Furthermore, experiments were performed to evaluate the influence of inhibitors of fatty acid oxidation ((+)-palmitylcarnitine and 2-bromopalmitate) on *p*-aminohippurate accumulation and renal metabolism. The results show that physiological fatty acids participate as energy donors in active *p*-aminohippurate transport *in vitro*. However, the experiments with high concentrations of exogenous fatty acids indicate that fatty acids presumably have a direct inhibitory effect on the transport of *p*-aminohippurate through the cell membrane.

MATERIALS AND METHODS

Materials

The different fatty acids used in this study (palmitic-, stearic-, oleic-, and octanoic acid) were obtained from Fluka AG, Buchs, Switzerland, and were neutralized with NaOH before use. A sample of (+)-palmitylcarnitine was generously provided by Dr. A. Otsuka, Otsuka Pharmaceutical Factory, Osaka, Japan. A sample of 2-bromopalmitic acid was kindly supplied by Dr. R. A. Burges, Pfizer Ltd., Kent, England, and further purchased from Fluka AG, Buchs, Switzerland. Other materials were supplied from sources previously described[1]. All reagents were analytical grade.

Human serum albumin (Cohn's Fraction V, 96%, AB Kabi, Stockholm, Sweden) was dialysed for 48 h against 0.0075 M phosphate buffer (pH 7.4) before use for the removal of acetate and other water-soluble compounds from the albumin. In a few experiments the albumin was further purified by treatment with charcoal as described by CHEN[14] in order to remove the content of fatty acids. The albumin solutions were concentrated by pressure filtration through Diaflo membranes (UM10, Amicon N.V., Oosterhout, Holland) and the concentration of albumin determined by measuring the extinction at 278 nm.

Experimental procedure

The general procedures were as previously described[1]. Adult rabbits (2–4 kg) of either sex were used as experimental animals. Thin slices of kidney cortex were prepared with a razor blade and incubated in Warburg cups containing 3 ml of 75 μM *p*-aminohippurate, 0.097 M NaCl, 0.040 M KCl, and 0.0075 M phosphate buffer (pH 7.4). The oxygen consumption was measured manometrically by the conventional Warburg techniques under the following conditions: Gas phase, 100% O_2; temp.,

25°; incubation period, 60 min. The center well contained KOH or hyamine for $^{14}CO_2$ determinations. The kidney slices were weighed after the incubation following a brief blotting on filter paper.

In radioactive experiments the incubation medium contained D-[6-^{14}C]glucose (or D-[U-^{14}C]glucose giving the same results) or [1-^{14}C]palmitate at concentrations of 0.07 μC/ml and 0.04 μC/ml, respectively.

Chemical analyses

The concentration of *p*-aminohippurate in trichloroacetic acid filtrates of tissue and incubation media was determined by the diazotation method of BRATTON AND MARSHALL[15] as modified by SMITH *et al.*[16]. S/M_{PAH} indicates the ratio between the amount of *p*-aminohippurate taken up per g final wet tissue weight and the amount of *p*-aminohippurate per ml final incubation medium. Accumulation of *p*-aminohippurate was expressed as (S/M_{PAH} —1) in order to correct for nonactive uptake of *p*-aminohippurate into the tissue slices.

The fatty acid content of the tissue slices and albumin preparations were estimated by microtitration after extraction with isopropanol–heptane as described by DOLE[17]. It was found that the unpurified albumin contained 1.1–1.3 moles of fatty acid per mole of albumin whereas the charcoal treated albumin contained less than 0.1 mole of fatty acid per mole of albumin. When the albumin was treated two times by charcoal no more fatty acids were removed.

Radiochemical analyses

The radioactivity of tissue extracts, media, and of CO_2 in hyamine were counted in a Packard liquid scintillation spectrometer as previously described[1].

The heptane phase (0.5 ml) from the isopropanol–heptane tissue extracts prepared for determinations of nonesterified fatty acids was extracted with 2 ml 0.1 M NaOH. Following 30 min with frequent shaking the content of esterified [^{14}C]-palmitate was determined by counting 100 μl of the upper phase (heptane phase) in 10 ml toluene scintillator fluid. The amount of nonesterified [^{14}C]palmitate was measured by counting 500 μl of the lower phase (NaOH phase) in 10 ml of the scintillator fluid described by BRAY[18] to which was added 100 μl formic acid (98–100%).

TABLE I

EFFECT OF (+)-PALMITYLCARNITINE ON THE CONCENTRATION OF NONESTERIFIED FATTY ACIDS IN KIDNEY CORTEX SLICES AND THE INCORPORATION OF [^{14}C]PALMITATE INTO KIDNEY TISSUE LIPIDS

All incubation media contained 50 mg/100 ml D-glucose, 1 mM palmitate, 4.5 g/100 ml dialyzed human albumin, and 0.04 μC/ml [1-^{14}C]palmitate. Furthermore 4 mM (+)-palmitylcarnitine was added to some of the vessels as indicated in the table. The figures in parentheses show the percentage changes induced by the addition of (+)-palmitylcarnitine. The results represent the average of duplicate determinations from 6 experiments.

(+)-Palmityl-carnitine (mM)	*Nonesterified [^{14}C]palmitate (disint./min per mg tissue)*	*Esterified [^{14}C]palmitate (disint./min per mg tissue)*	*Nonesterified fatty acids (μmoles/100 g tissue)*
0	18.2	192	400
4	20.4 (+ 12%)	173 (− 10%)	451 (+ 13%)

In order to evaluate if the inhibitors of fatty acid metabolism used in this study caused any alterations in the specific activity of the labelled palmitate in the kidney tissue, the effect of (+)-plamitylcarnitine on the incorporation of [^{14}C]palmitate into the tissue lipids and the concentration of fatty acids in the kidney slices was examined. Table I shows that [^{14}C]palmitate taken up by the tissue slices is incorporated into tissue lipids to a high extent, since the concentration of esterified radioactive palmitate is about 10 times higher than that of the nonesterified palmitate. Furthermore, the table shows that addition of (+)-palmitylcarnitine at a concentration of 4 mM (the highest concentration used in the experiments) causes approximately the same percentage increase of the concentration of the nonesterified [^{14}C]palmitate as found for nonesterified unlabelled fatty acids in the tissue slices. Thus, the fatty acid inhibitor induces no significant changes in the specific activity of the nonesterified fatty acids and therefore the formation of $^{14}CO_2$ expressed as disint./min from [^{14}C]palmitate has been used directly as a measure of the fatty acid degradation.

RESULTS

Effect of different fatty acids on p-aminohippurate accumulation

In order to illustrate the role of fatty acid metabolism on active transport of *p*-aminohippurate the effect of addition to the incubation medium of palmitate, stearate, oleate, and octanoate on the accumulation of *p*-aminohippurate (S/M_{PAH} —1) and oxygen uptake in kidney cortex slices has been examined. It is seen from Table II that 1 mM of palmitate, stearate, or oleate, bound to human albumin causes a significant increase of *p*-aminohippurate uptake and oxygen consumption, but no further effect on the *p*-aminohippurate accumulation is discernible when

TABLE II

EFFECT OF DIFFERENT FATTY ACIDS AND OF ALBUMIN ON *p*-AMINOHIPPURATE ACCUMULATION AND OXYGEN CONSUMPTION IN KIDNEY CORTEX SLICES

The incubation media contained fatty acids (1 or 2 mM), and dialyzed human albumin (4.5 g /100 ml) as indicated in the table. Preformed fatty acids had previously been removed from the albumin by treatment with charcoal (see MATERIALS AND METHODS). The results represent the average of duplicate determinations from 6 experiments. (S/M_{PAH} —1) = *p*-aminohippurate accumulation. Q_{O_2} = μl O_2 consumption per mg final wet weight per h.

Fatty acid added	*Concn. (mM)*	*Albumin*	$(S/M_{PAH} - 1)$	Q_{O_2}
0	0	0	3.1	0.99
0	0	+	4.6	0.99
0	0	+*	7.2	1.31
Palmitate	1	+	7.6	1.37
	2	+	8.3	1.38
Stearate	1	+	6.3	1.28
	2	+	5.7	1.35
Oleate	1	+	7.6	1.31
	2	+	7.4	1.47
Octanoate	1	0	1.0	1.86
	2	0	0.4	2.08

* In this case preformed fatty acids were not removed from the albumin by charcoal treatment.

increasing the fatty acid concentration from 1 to 2 mM. In contrast to incubation with long-chain fatty acids, addition of octanoate to the medium gives rise to a pronounced inhibition of the accumulation of *p*-aminohippurate accompanied by an approx. 2-fold stimulation of the oxygen uptake. A stimulation of *p*-aminohippurate accumulation and oxygen consumption to the same degree as that found by addition of long-chain fatty acids is observed when incubating the slices with albumin which has not been treated with charcoal. This is probably due in part to the fatty acids bound to the albumin which were found to be about 0.8 mM in the medium. In agreement with this view, titration of albumin-bound fatty acids before and after a 60-min incubation period showed a distinct decrease (15%) of the amount of fatty acids bound to the non-treated albumin. However, *p*-aminohippurate accumulation was stimulated approx. 50% in the presence of charcoal-treated albumin although the oxygen consumption remained unaltered. The stimulating effect of long-chain fatty acids on *p*-aminohippurate accumulation mentioned above is noticeable in contrast to the potent inhibitory action of octanoate.

The effect of a wider range of concentrations of palmitate in the medium on *p*-aminohippurate accumulation and oxygen consumption is shown in Fig. 1. It appears from the figure that at a palmitate concentration of 5 mM the *p*-aminohippurate accumulation is reduced relative to that found at 1 and 2 mM while there is a further increase of the oxygen uptake. This observation may indicate some direct inhibitory effect of palmitate on the transport of *p*-aminohippurate through the cell membrane, but it should be noted that at the highest concentration of fatty acid there is still a net stimulation of *p*-aminohippurate uptake.

In an additional group of experiments we have tried to evaluate the nature of the biphasic effect of palmitate on *p*-aminohippurate accumulation by measuring the effect of different metabolites on *p*-aminohippurate transport in the presence of a high palmitate concentration. The metabolites selected for this purpose are known to

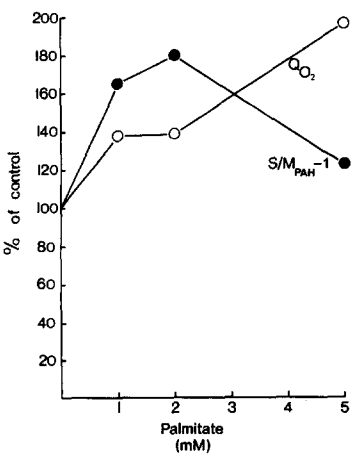

Fig. 1. Biphasic effect of palmitate on *p*-aminohippurate accumulation in kidney cortex slices. All incubation media including that of the control contained 4.5 g/100 ml dialyzed human albumin treated with charcoal before use (see MATERIALS AND METHODS). Each point denotes the average of duplicate determinations from 3 experiments. All values are represented as percentage changes of those without addition of palmitate. ($S/M_{PAH} - 1$) = *p*-aminohippurate accumulation. Q_{O_2} = μl O_2 uptake per mg final wet weight per h.

stimulate the accumulation of *p*-aminohippurate under various conditions as described by MAXILD AND MØLLER[1]. The results are presented in Table III. The table shows that the *p*-aminohippurate uptake is markedly reduced when palmitate (5 mM) is added to the medium containing pyruvate, L-lactate, or acetate, whereas there is a pronounced enhancement of the oxygen uptake. Furthermore, it is seen from Column 2 that pyruvate, L-lactate, and acetate have a very slight stimulating effect only on *p*-aminohippurate accumulation when palmitate is present in the medium even though the oxygen consumption is significantly increased*. The finding that pyruvate, lactate and acetate do not stimulate the *p*-aminohippurate accumulation in the presence of palmitate in spite of a marked increase of the oxygen uptake suggests that in addition to a stimulatory metabolic effect palmitate may have a direct inhibitory action on the *p*-aminohippurate transport system itself.

TABLE III

EFFECT OF PYRUVATE, L-LACTATE, AND ACETATE ON *p*-AMINOHIPPURATE ACCUMULATION IN THE PRESENCE AND ABSENCE OF PALMITATE

Metabolites were added to the incubation media as indicated in the table. The concentration of pyruvate, L-lactate, and acetate was 10 mM, and that of palmitate 5 mM. Dialyzed human albumin (4.5 g/100 ml) was added when palmitate was present in the medium. Control incubations contained no pyruvate, lactate, or acetate. The results are the average of duplicate determinations from 3 experiments. ($S/M_{PAH} - 1$) = *p*-aminohippurate accumulation. Q_{O_2} = μl O_2 consumption per mg final wet weight per h.

	$(S/M_{PAH} - 1)$		Q_{O_2}	
	No palmitate	*Palmitate (5 mM)*	*No palmitate*	*Palmitate (5 mM)*
Control	—	11.1	—	2.23
Pyruvate	17.5	12.2	1.55	2.52
L-Lactate	19.6	12.5	1.58	2.80
Acetate	20.8	12.4	1.87	2.90

p-Aminohippurate accumulation and renal metabolism in the presence of inhibitors of fatty acid metabolism

In this section we present the results of experiments which were designed to show the effect of (+)-palmitylcarnitine and 2-bromopalmitate on *p*-aminohippurate accumulation and renal energy metabolism. These substances have been demonstrated to act as competitive inhibitors of fatty acid oxidation[19,20]. Fig. 2 illustrates the effect of (+)-palmitylcarnitine on *p*-aminohippurate accumulation, oxygen consumption, and formation of $^{14}CO_2$ from [^{14}C]palmitate and [^{14}C]glucose. The values of *p*-aminohippurate accumulation and of the metabolic parameters at different concentrations of (+)-palmitylcarnitine (0–4 mM) are expressed as percentage values of those of the controls (with no addition of inhibitor). It is seen from the figure that *p*-aminohippurate accumulation is progressively inhibited by increasing the concentration of (+)-palmitylcarnitine in the medium, (S/M_{PAH} —1) being reduced to 19%

* The control *p*-aminohippurate accumulation of 11.1 is relatively much higher than the *p*-aminohippurate accumulation in the presence of palmitate presented in Table II when the biphasic effect of palmitate is taken in consideration (Fig. 1). However, as earlier pointed out[1] there may be a remarkable variation in *p*-aminohippurate accumulation between the individual animals under the same experimental conditions. Thus, it is not possible to compare directly the absolute *p*-aminohippurate accumulation in the different tables.

of the control value at an inhibitor concentration of 4 mM, whereas the production of $^{14}CO_2$ from [^{14}C]palmitate decreases to only 60% of that of the control. Besides, it is observed that the oxygen consumption remains practically constant at all concentrations of the inhibitor while $^{14}CO_2$ production from [^{14}C]glucose rises steadily and reaches a value of 192% of that of the uninhibited slices at 4 mM (+)-palmitylcarnitine. Thus, the unaffected oxygen uptake may be explained by an increase in the metabolism of substances other than fatty acids as evidenced by the stimulation of $^{14}CO_2$ production from [^{14}C]glucose.

The effect of 2-bromopalmitate (0–4 mM) on *p*-aminohippurate accumulation and the different parameters of renal metabolism is shown in Fig. 3. It appears from the figure that *p*-aminohippurate accumulation is reduced to about the same extent as found in the (+)-palmitylcarnitine experiments. However, $^{14}CO_2$ liberation from radioactive palmitate is decreased to 29% of the control value, indicating that 2-bromopalmitate is a more potent inhibitor of palmitate oxidation than is (+)-palmitylcarnitine. The similar course of the two curves shows that there is a good correlation between the magnitude of *p*-aminohippurate accumulation, and that of fatty acid metabolism. In spite of the marked decrease of fatty acid degradation in the presence of 2-bromopalmitate, Q_{O_2} is only reduced to 85% of the control value, but as shown by the figure a drastic stimulation of carbohydrate metabolism sets in as indicated by an increase of $^{14}CO_2$ production from [^{14}C]glucose of 332% at 4 mM bromopalmitate.

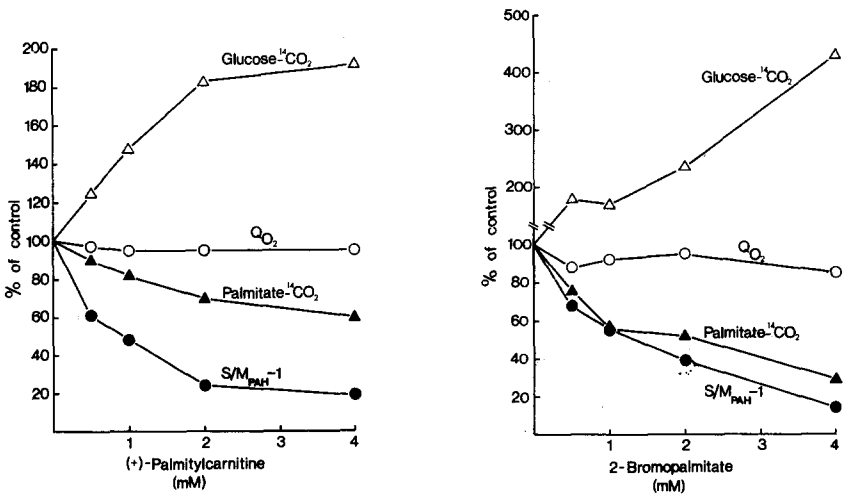

Fig. 2. Effect of (+)-palmitylcarnitine on *p*-aminohippurate accumulation, Q_{O_2}, and formation of $^{14}CO_2$ from [^{14}C]palmitate or [^{14}C]glucose. The incubation media contained 1 mM of palmitate, 4.5 g/100 ml dialyzed human albumin, and 50 mg/100 ml D-glucose. Each point denotes the average of duplicate determinations from 6 experiments. All values are represented as percentage changes of those without addition of (+)-palmitylcarnitine. ($S/M_{PAH} - 1$) = *p*-aminohippurate accumulation. Q_{O_2} = μl O_2 uptake per mg final wet weight per h.

Fig. 3. Effect of 2-bromopalmitate on *p*-aminohippurate accumulation, Q_{O_2}, and formation of $^{14}CO_2$ from [^{14}C]palmitate or [^{14}C]glucose. The incubation media contained 1 mM of palmitate, 4.5 g/100 ml dialyzed human albumin, and 50 mg/100 ml D-glucose. Each point denotes the average of duplicate determinations from 5 experiments. All values are represented as percentage changes of those without addition of bromopalmitate. ($S/M_{PAH} - 1$) = *p*-aminohippurate accumulation. Q_{O_2} = μl O_2 uptake per mg final wet weight per h.

The metabolic effect of (+)-palmitylcarnitine and 2-bromopalmitate (as shown in Figs. 2 and 3) indicate that the inhibition of fatty acid metabolism elicits an increased degradation of glucose and probably also of other metabolites as well which leads to little or no changes in over-all renal metabolism as indicated by the unaltered oxygen consumption. It is rather surprising, then, that the accumulation of *p*-aminohippurate is affected to such an extent by these inhibitors of fatty acid metabolism.

Effect of various metabolites on metabolic inhibition of p-aminohippurate transport

The experiments presented below were performed mainly to clarify if (+)-

TABLE IV

EFFECT OF DIFFERENT METABOLITES ON *p*-AMINOHIPPURATE ACCUMULATION IN THE PRESENCE AND ABSENCE OF (+)-PALMITYLCARNITINE

The incubation media contained metabolites, albumin, and (+)-palmitylcarnitine as indicated in the table. The concentration of D-glucose, pyruvate, L-lactate, and acetate was 10 mM; the concentration of palmitate, stearate, and oleate was 2 mM; the concentration of dialyzed human albumin was 4.5 g/100 ml, and that of (+)-palmitylcarnitine 1 mM. The results are the average of duplicate determinations from 5 experiments. ($S/M_{PAH} - 1$) = *p*-aminohippurate accumulation. Q_{O_2} = μl O_2 consumption per mg final wet weight per h.

	$(S/M_{PAH} - 1)$		Q_{O_2}	
	No inhibitor	*(+)-Palmitylcarnitine*	*No inhibitor*	*(+)-Palmitylcarnitine*
No metabolite	5.3	2.3	0.92	0.94
Glucose	7.1	2.5	1.01	1.05
Pyruvate	14.8	3.8	1.37	1.67
L-Lactate	15.7	4.1	1.22	1.66
Acetate	19.8	4.4	1.52	1.61
Albumin	8.9	4.5	1.11	1.11
Albumin + palmitate	8.6	3.5	1.43	1.31
Albumin + stearate	8.8	3.1	1.25	1.25
Albumin + oleate	9.5	4.7	1.47	1.23

TABLE V

COUNTERACTING EFFECT OF SOME METABOLITES ON *p*-AMINOHIPPURATE ACCUMULATION IN THE PRESENCE OF 2-BROMOPALMITATE

All incubation media contained 4.5 g/100 ml dialyzed human albumin. D-Glucose (10 mM), pyruvate (10 mM), L-lactate (10 mM), acetate (10 mM), and 2-bromopalmitate (2 mM) were added as shown in the table. The results are the average of duplicate determinations from 6 experiments. ($S/M_{PAH} - 1$) = *p*-aminohippurate accumulation. Q_{O_2} = μl O_2 consumption per mg final wet weight per h.

	$(S/M_{PAH} - 1)$		Q_{O_2}	
	No inhibitor	*2-Bromopalmitate*	*No inhibitor*	*2-Bromopalmitate*
Albumin	10.3	1.9	1.16	0.84
Albumin + glucose	11.3	3.8	1.14	0.94
Albumin + pyruvate	9.7	8.8	1.35	1.27
Albumin + L-lactate	12.3	8.6	1.35	1.38
Albumin + acetate	14.7	9.6	1.50	1.62

palmitylcarnitine or 2-bromopalmitate exert an inhibition on the *p*-aminohippurate accumulation by interfering directly with the transport of *p*-aminohippurate through the membrane. To this end we have examined the reversing effect of various metabolites on *p*-aminohippurate accumulation in kidney slices in the presence of inhibitors as previously described[1]. Furthermore, the ability of fatty acids to sustain accumulation of *p*-aminohippurate during reduced carbohydrate metabolism was investigated.

The results obtained in the case of (+)-palmitylcarnitine are summarized in Table IV, the inhibitor being added to the incubation medium at a concentration to give a reduction of *p*-aminohippurate uptake of approx. 50%. It is seen from the table that there is a poor reversing influence on the accumulation of *p*-aminohippurate of all metabolites added, while the metabolites induce an enhancement of the oxygen uptake to a variable extent in presence of the metabolic inhibitor. Therefore, it is reasonable to assume that (+)-palmitylcarnitine exerts some direct inhibition on the transport of *p*-aminohippurate. It is of interest that addition of (+)-palmitylcarnitine causes a pronounced increment of the oxygen uptake when pyruvate or L-lactate is present in the medium, indicating a stimulation of the metabolism of the two substances (*cf.* the stimulatory effect of (+)-palmitylcarnitine on glucose degradation as shown in Fig. 2).

Table V shows the counteracting effect of some substrates on *p*-aminohippurate transport inhibited by 2-bromopalmitate at a concentration resulting in a marked reduction of the accumulation of *p*-aminohippurate. In these experiments it was necessary to add albumin to the medium because of the hydrophobic characteristics of bromopalmitate. It is seen that in the presence of bromopalmitate, addition of pyruvate, L-lactate, or acetate gives rise to a considerable increase of the *p*-aminohippurate uptake, accompanied by a pronounced stimulation of the oxygen consumption. In the case of pyruvate the inhibitor is almost completely counteracted, while glucose only slightly reverses the inhibitory effect of bromopalmitate. These findings indicate that in contrast to (+)-palmitylcarnitined bromopalmitate may not significantly interfere with the *p*-aminohippurate transport system itself.

In another series of experiments it was examined if palmitate was able to stimulate the *p*-aminohippurate accumulation inhibited about 50% by addition of F^- to the medium. Table VI shows that albumin-bound palmitate induces a distinct enhancement of *p*-aminohippurate accumulation as well as of oxygen uptake in the presence of F^-. In contrast to the oxygen uptake the accumulation of *p*-aminohippurate is not

TABLE VI

STIMULATORY EFFECT OF PALMITATE ON F^--INHIBITED *p*-AMINOHIPPURATE ACCUMULATION

The incubation media contained palmitate (2 mM), dialyzed human albumin (4.5 g/100 ml), and F^- (2 mM) as indicated in the table. The results are the average of duplicate determinations from 4 experiments. ($S/M_{PAH} - 1$) = *p*-aminohippurate accumulation. Q_{O_2} = μl O_2 consumption per mg final wet weight per h.

Inhibitor	*Palmitate*	*Albumin*	$(S/M_{PAH} - 1)$	Q_{O_2}
o	o	o	6.3	1.01
o	+	+	8.6	1.40
F^-	o	o	3.4	0.86
F^-	+	+	5.8	1.37

reversed completely to the control level which seems curious, since as earlier reported, F^- presumably has no direct inhibitory influence on the *p*-aminohippurate transport system[1]. However, the table illustrates that metabolism of fatty acids to a certain extent is able to replace carbohydrate metabolism in maintaining active *p*-aminohippurate transport.

DISCUSSION

Effect of exogenous fatty acids on p-aminohippurate accumulation and renal metabolism

The experiments described in the present study show that metabolism of palmitate and other long-chain fatty acids plays an important role for active transport of *p*-aminohippurate in kidney cortex slices as indicated by a pronounced stimulation of *p*-aminohippurate accumulation as well as oxygen consumption following addition of albumin-bound fatty acids to the incubation medium under various conditions. The accumulation of *p*-aminohippurate at 1–2 mM long-chain fatty acids in the medium was increased, although not to the same extent as that observed by addition of pyruvate, L-lactate, or acetate which is known to stimulate *p*-aminohippurate accumulation to a maximal extent[1]. This finding is remarkable, since it has been previously shown that short-chain fatty acids (C_6–C_{12}) inhibit active transport of *p*-aminohippurate[11–13]. This is also apparent from Table II of our study which shows that 1 mM octanoate almost completely abolishes the *p*-aminohippurate accumulation.

It is of interest that addition of palmitate to the medium at a concentration of 5 mM resulted in a decrease of *p*-aminohippurate accumulation compared with that observed at 1 and 2 mM. However, there was still some net stimulation of *p*-aminohippurate transport. It is remarkable that under conditions of a high palmitate concentration in the medium, further addition of pyruvate, L-lactate, or acetate did not increase the accumulation of *p*-aminohippurate. Therefore, it appears probable that apart from the metabolic effect palmitate also exerts a direct inhibitory action on the *p*-aminohippurate transport of the membrane which is prominent at high concentrations of palmitate. The inhibitory effect appears to have an inverse relationship to the number of carbon atoms in the fatty acid chain which is probably due to differences in water solubility, resulting in less contact between the long-chain fatty acid molecules and the *p*-aminohippurate transport system than in the case of fatty acids possessing a shorter chain length.

Palmitate, stearate, and oleate were all readily oxidized by the kidney slices. This finding does not agree with studies of GOLD AND SPITZER[4] who report that, in contrast to palmitate, no significant amounts of oleate were taken up by dog kidneys *in vivo*. In the present investigation a remarkably high exchange between nonesterified and esterified fatty acids in the tissue lipids was observed as is apparent from the fact that approx. 90 % of the radioactive palmitate taken up by the slices was incorporated into the tissue lipids. In this connection WEIDEMANN AND KREBS[21] found an incorporation of [U-^{14}C]oleate into the lipids of kidney slices of about two-thirds of the oleate removed from the medium. In agreement with previous observations[1] determinations of the concentration of long-chain fatty acids in the slices in this study (see Table I) indicated a noticeable large tissue fatty acid concentration which is approx. 8–10 times higher than that of plasma. In contrast, the endogenous glucose concentration in kidney cortex is not greater than that of plasma[1,22,23].

Effect of inhibitors of fatty acid metabolism on p-aminohippurate accumulation and renal metabolism

It is well-documented that in mitochondrial preparations from rat heart muscles (+)-palmitylcarnitine is able to produce a competitive inhibition of palmitate oxidation[19]. Similarly, inhibition of palmitate metabolism by 2-bromopalmitate in rat heart homogenates and in the isolated perfused rat heart has been described[20]. In the present study (+)-palmitylcarnitine and 2-bromopalmitate caused a decreased liberation of $^{14}CO_2$ from [^{14}C]palmitate which was accompanied by a reduction of *p*-aminohippurate accumulation. The decrease of *p*-aminohippurate transport, relative to the inhibition of fatty acid oxidation, was more pronounced with (+)-palmitylcarnitine than with bromopalmitate. However, in the case of (+)-palmitylcarnitine practically no counteracting effect on the accumulation of *p*-aminohippurate could be detected by addition of various metabolites to the medium, suggesting that (+)-palmitylcarnitine like palmitate has a direct inhibitory action on *p*-aminohippurate accumulation. In contrast, pyruvate, L-lactate, and acetate were able to reverse the accumulation of *p*-aminohippurate to a considerable degree in the presence of bromopalmitate. Thus, it would appear that at least part of the reduction of *p*-aminohippurate accumulation by bromopalmitate is due to the inhibition of fatty acid metabolism.

It is noticeable that addition of the two metabolic inhibitors caused a drastic increase of the glucose degradation. Recently, a great stimulation of the oxidation of [U-^{14}C]glucose to $^{14}CO_2$ by 2-bromopalmitate in heart muscles was also observed by BURGES *et al.*[20], especially when insulin was simultaneously added. Although the regulatory mechanisms and enzyme systems involved are obscure[24–27], it is reasonable to assume that the tremendous stimulation of glucose metabolism in kidney slices observed in the present investigation may be a direct consequence of the decrease of fatty acid metabolism induced by (+)-palmitylcarnitine and bromopalmitate. This view is supported by the finding that the more pronounced reduction of the fatty acid metabolism in the case of bromopalmitate, compared with that of (+)-palmitylcarnitine, was accompanied by a higher stimulation of the carbohydrate degradation in the bromopalmitate experiments.

In summary, as indicated by the experiments with exogenous nonesterified fatty acids, it is concluded that metabolism of long-chain fatty acids exerts a stimulatory effect on renal *p*-aminohippurate transport. Furthermore, it has been found that the fatty acids are able to compensate a F^--inhibited carbohydrate metabolism to a certain extent. Long-chain fatty acids added to the incubation medium play a less important role as energy donors for active *p*-aminohippurate transport than previously found for pyruvate and L-lactate[1], because the direct inhibitory effect of fatty acids on the transport through the cell membrane is a limiting factor for the accumulation of *p*-aminohippurate. Under endogenous conditions, however, the results obtained with (+)-palmitylcarnitine and 2-bromopalmitate suggest that intact fatty acid metabolism is necessary for providing optimal conditions for active transport of *p*-aminohippurate in kidney tissue.

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