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Iodipamide uptake by rat liver plasma membrane vesicles enriched in the sinusoidal fraction: evidence for a carrier-mediated transport dependent on membrane potential

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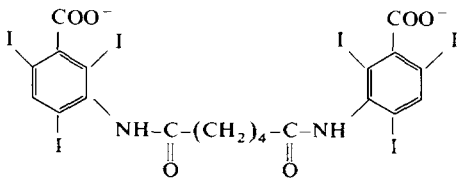
Iodipamide, a cholecystographic agent, is known to be taken up by isolated hepatocytes by a mechanism similar or identical with the inward transport of bile salts (Petzinger, E., Joppen, C. and Frimmer, M. (1983) Naunyn-Schmiedeberg's Arch. Pharmacol. 322, 174–179). To elucidate its mode of transport, uptake of iodipamide was studied by rapid-filtration techniques on plasma membrane vesicles enriched in the sinusoidal fraction. Uptake was found to be dependent upon the temperature, the intravesicular volume, a gradient of monovalent cations (Na^+, K^+ or Li^+) and the substrate concentration (saturation kinetics with respect to iodipamide: apparent $K_m = 70\ \mu M$, $V_{max} = 0.31$ nmol per mg protein per min at 100 mM NaCl and 25°C). Countertransport and transstimulation in tracer exchange experiments indicate that in vesicles, iodipamide uptake rather than binding occurs. Na^+ could be replaced by K^+ or Li^+ in our system without any effect. However, in the presence of choline chloride a slight, but distinct reduction occurred. Iodipamide uptake was inhibited by cholate, phalloidin, 4,4′-diisothiocyanato-1,2-diphenylethane-2,2′-disulfonic acid and by bromosulfophthalein with inhibition being competitive in the case of cholate and non-competitive in the case of bromosulfophthalein. Alteration of the membrane potential by addition of NO_3^-, SCN^- or SO_4^{2-} modified the uptake rate for iodipamide. The above results support our earlier hypothesis that the hepatocellular uptake of iodipamide is due to a carrier-mediated transport, probably similar to that of bile acids. However, translocation of iodipamide is assumed to be driven by the membrane potential only and not by Na^+ cotransport.

Introduction

Iodipamide, a conventional cholecystographic agent, is a weak acid with high content of iodine.



It is cleared from the circulating blood by the liver and subsequently excreted into the bile duct [1]. The rapid accumulation of iodipamide is usually explained either by binding to cytoplasmatic proteins, e.g., ligandin or z-protein [2], or by a transport system present in the sinusoidal membrane of hepatocytes [3,4].

Earlier studies on isolated hepatocytes supported the latter explanation [5]. In addition, iodipamide was found to be a competitive inhibi-

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Abbreviations: H_2DIDS, 4,4′-diisothiocyanato-1,2-diphenylethane-2,2′-disulfonic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

tor of the hepatocellular inward transport of both cholate and of phallotoxins [6,7]. These and other findings suggest that all the above compounds might be translocated by an identical transporter with heterospecific properties [8–10]. If this assumption is correct, iodipamide should be a more suitable substrate for transport studies than bile acids because of its water solubility.

In the following report we present transport studies on plasma membrane vesicles enriched in the sinusoidal fraction with [^{125}I]iodipamide as the substrate. The use of vesicles complements earlier work on whole cells [5,6] and has the advantage of excluding the influence of intracellular binding, simultaneous excretion and metabolic changes. Iodipamide uptake into plasma membrane vesicles was characterized in order to find further evidence for the postulated identity of the transporter involved in the uptake of bile acids, phallotoxins and cholecystographic agents.

Materials and Methods

Materials. ^{125}I-iodipamide (sodium salt) (1.5 Ci/mmol), H_2-DIDS and phalloidin were gifts of Dr. A. Speck, Schering AG, Berlin, and Professor Dr. H. Fasold, Frankfurt am Main and Professor Dr. T. Wieland, Heidelberg, respectively. ^{22}NaCl (22.8 mCi/mmol) was obtained from New England Nuclear (Boston, MA), [1-^{14}C]glucose (55 mCi/mmol) from Amersham-Buchler, Braunschweig. Cholic acid and bromosulfophthalein were purchased from Sigma, Munich. All other chemicals were of at least analytical grade purity.

Preparation of rat liver plasma membranes enriched in the sinusoidal fraction. Plasma membranes were isolated from rat liver according to Inoue et al. [11]. Storage of membranes at −196°C for up to two months did not alter the transport properties for iodipamide. Protein was determined according to Lowry et al. [12] using bovine serum albumin as a standard.

Marker enzyme assays. $(Na^+ + K^+)$-ATPase and Mg^{2+}-ATPase activities were measured by the method of Kinne et al. [13]. 5′-Nucleotidase was determined according to Michell and Hawthorne [14] and glucose-6-phosphatase according to Harper and Bergmeyer [15].

Transport assay. Uptake of ^{125}I-labeled iodipamide by membrane vesicles was measured by a rapid filtration technique as described previously [16]. Unless otherwise indicated, the assay mixture contained 0.25 M sucrose/0.2 mM $CaCl_2$/10 mM $MgCl_2$/10 mM Hepes-Tris (pH 7.4) and varying concentrations of [^{125}I]iodipamide and 1.1 mg vesicle protein in a final volume of 0.55 ml, and the incubation was carried out at 25°C. Other conditions are indicated in the individual experiments. Frozen membrane samples were thawed at 37°C and kept on ice until use. Transport was initiated by adding 50 μl of membrane vesicles to 500 μl incubation medium, which had been preincubated for 4 min at 25°C. Aliquots of 50 μl were removed at timed intervals and diluted with 1 ml of ice-cold stop solution with the same composition as the incubation medium, but containing unlabeled iodipamide. The diluted samples were immediately filtered through a Millipore filter (GSWP 0.22 μm), followed by washing with 5 ml of ice-cold stop solution. Radioactivity on the filters was measured in a γ-counter (MR 1032 W + W electronic).

Variations in transport activity with different vesicle preparations were small, and all experiments were repeated at least three times with similar results (n = number of experiments with at least two different vesicle preparations). Initial uptake rate, V_0, was determined using the uptake mesurements at 10 and 40 s, and from this the uptake per min was calculated.

Results

Marker enzymes and electron microscopy

The activity of $(Na^+ + K^+)$-ATPase, a marker of sinusoidal plasma membrane [17], was enriched 11-fold in our membranes compared with the crude homogenate, whereas Mg^{2+}-ATPase, a marker of canalicular plasma membrane, was increased 4-fold only. The activity of 5′-nucleotidase, which is located on sinusoidal, lateral and canalicular plasma membranes, was enriched 6.4-fold. The above enzyme assays indicated minor contamination by intracellular organelles, such as the endoplasmic reticulum, as determined by the activity of glucose-6-phosphatase, which was increased 3-fold. Consequently, we isolated plasma membranes enriched in the sinusoidal fraction. Electron mi-

croscopy revealed that membrane samples consisted of vesicles with diameters of approx. 0.3–0.4 μm. There was not detectable contamination by organelles.

Iodipamide transport and binding

The time-course for uptake of iodipamide by our rat liver plasma membrane vesicles is shown in Fig. 1. The initial rates for iodipamide uptake increased, varying the temperature from 4 to 37°C in the presence of a NaCl gradient. This temperature dependence points to facilitated transmembranal transport rather than binding and free diffusion, although in some cases binding to membranes is temperature-dependent as well [18].

Generally, it is important to discriminate between transport and binding. Therefore the effect of the medium osmolarity on iodipamide uptake was determined. The equilibrium uptake (after 40 min) decreased linearly as the osmolarity (concentration of raffinose) increased and the vesicles diminished in size (Fig. 2A). This finding is indicative of iodipamide transport into an osmotically sensitive intravesicular space. By extrapolation to infinitely high osmolarity (theoretical zero intravesicular space) it was estimated that at least 51% of total equilibrium uptake represents binding.

The same degree of binding was determined by hypotonic lysis of the vesicles. Diluting the membranes 20- to 50-fold with deionized water decreased the uptake to 51 ± 3%.

To discriminate between binding to the inner and outer surfaces of the membrane vesicles, the membranes were diluted and washed with ice-cold buffer solution containing a 100-fold excess of unlabeled iodipamide. No detectable amount of iodipamide could be removed, suggesting preferential binding of iodipamide to the inside of the vesicles.

From our osmotic data (Fig. 2A) we calculate that about 49% of equilibrium uptake represents free iodipamide in the intravesicular space. In our plasma membrane vesicles the intravesicular space was determined to be 1.3 μl per mg protein by measuring the equilibrium (30 min) uptake of [^{14}C]glucose. This agrees with data on other membrane vesicle preparations obtained from the liver [11,19]. From our osmotic data the intravesicular

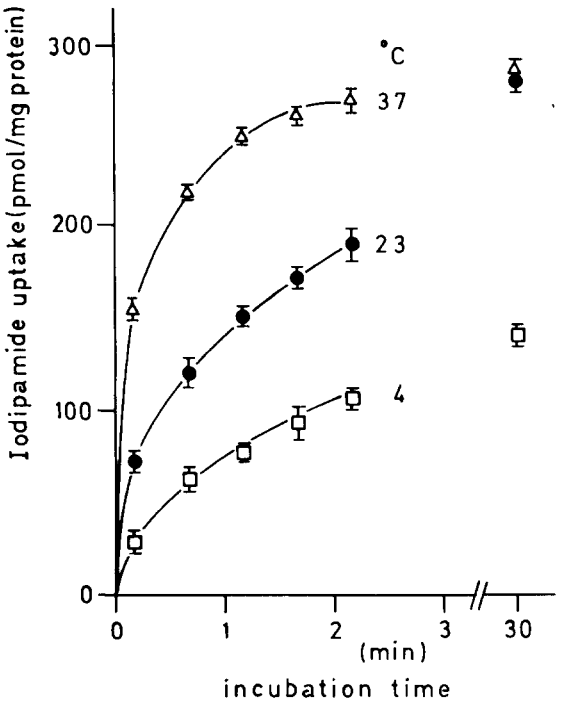


Fig. 1. Influence of incubation temperature on iodipamide uptake. Iodipamide uptake was measured in an incubation medium containing 0.25 M sucrose/0.2 mM $CaCl_2$/10 mM $MgCl_2$/10 mM Hepes-Tris buffer (pH 7.4)/20 μM [^{125}I]iodipamide/100 mM NaCl, in a final volume of 0.55 ml at 4°C (□), 23°C (●) and at 37°C (△). Transport was started by adding membrane vesicles (1.3 mg protein) and 50 μl aliquots were removed at timed intervals. Details are described in Materials and Methods. Each point represents the mean value of four experiments ($n = 4$) (bars represent S.D.).

concentration of free iodipamide was calculated to be 5-fold higher than that in the external incubation medium, 1 min after initiating transport. In this case the portion due to binding was subtracted from total iodipamide uptake.

In short-time experiments the initial rate of iodipamide uptake decreased with the size of the vesicles (Fig. 2B). Therefore we assume that the initial uptake of iodipamide would represent predominantly transmembranal transport and not binding.

Another support for the presence of transport rather than binding is the demonstration of countertransport for iodipamide uptake. Preloading the vesicles with unlabeled iodipamide (0.2 mM saturable concentration) and subsequent dilution

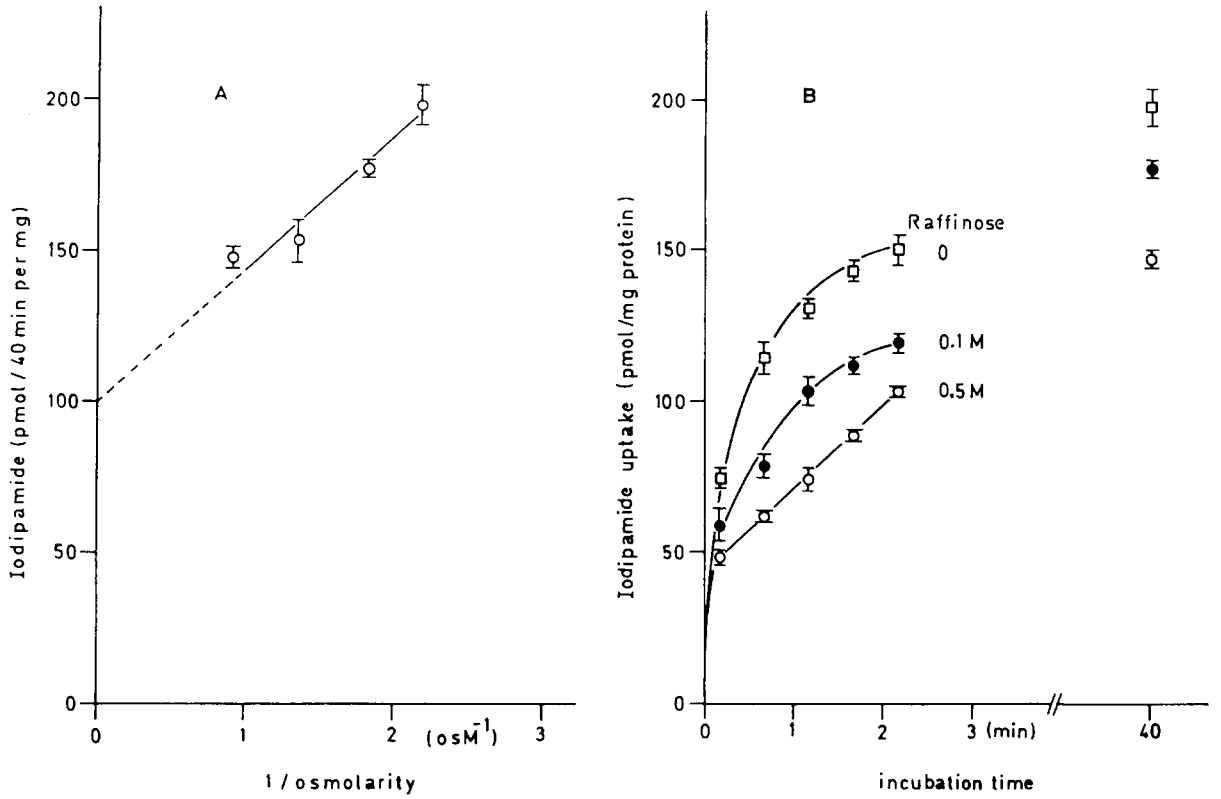


Fig. 2. (A and B) Effect of medium osmolarity on the uptake of iodipamide. Membrane vesicles were preincubated for 30 min at 25°C in 0.25 M sucrose containing 0.2 mM $CaCl_2$/10 mM Hepes-Tris buffer (pH 7.4) in the presence of varying concentrations of raffinose (in B: without raffinose (□), 0.1 M raffinose (●) and 0.5 M raffinose (○)). Transport was started by adding 100 mM NaCl/10 mM $MgCl_2$/10 μM [^{125}I]iodipamide. 50 μl aliquots were removed at timed intervals and at equilibrium (40 min). In the case of vesicles pretreated with raffinose, washing of the vesicles was performed with the same stop solution as described in the legend to Fig. 1, except for 0.3 M NaCl. Other conditions were the same as described in the legend to Fig. 1. Each point represents the mean value of four experiments ($n = 4$).

of vesicles enhanced the initial uptake rate of labeled iodipamide 1.4-fold (Table I).

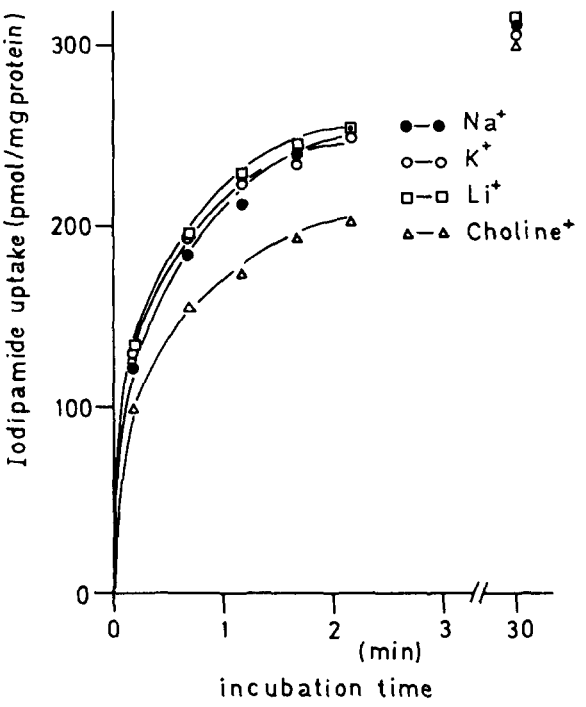
Furthermore the results of transstimulation of iodipamide are consistent with carrier mediated transport. Loading the vesicles with unlabeled iodipamide (0.1 mM) stimulated the uptake of radioactively labeled iodipamide 1.6-fold by tracer exchange (Table I).

Effect of sodium on iodipamide uptake

In the following the effect of various cations on iodipamide uptake in vesicles was studied. Replacement of sodium by choline reduced the initial uptake rate of iodipamide by 15% (Fig. 3). On the other hand, potassium and lithium did not influence the initial uptake, suggesting that iodipamide uptake in vesicles might be dependent in fact upon monovalent cations and not exclusively upon sodium.

Uptake of iodipamide as a function of external sodium concentration demonstrates Na^+-dependent and Na^+-independent uptake (Fig. 4A). The latter probably represents simple diffusion (compare uptake at 4°C in Fig. 1 and Fig. 7A). The extent of iodipamide uptake and initial rate of uptake increased with the Na^+-gradients imposed. Thus iodipamide uptake was found to be stimulated by Na^+-gradient and was saturated in the range 50–100 mM NaCl (Fig. 4B).

The stimulatory action of Na^+ gradient could be due to the generation of a vesicle inside positive diffusion potential or to a Na-iodipamide cotransport system in the membrane. To distinguish between these possibilities, iodipamide uptake was



determined in the absence of a monovalent cation gradient, under tracer exchange conditions. The vesicles were preloaded with sodium, potassium or choline and unlabeled iodipamide, and afterwards the uptake of labeled iodipamide was measured. The initial uptake rate of labeled iodipamide was the same in sodium or potassium medium (8.5 and 8.3% cpm · mg^{-1} · min^{-1}, respectively), but reduced by 10% in choline (7.5% cpm · mg^{-1} · min^{-1}).

Sodium uptake was as fast as iodipamide uptake suggesting similar membrane permeabilities (Fig. 5). Considering the range of standard deviation equilibrium uptake of sodium was attained at nearly the same time as that of iodipamide.

Fig. 3. Effect of cation replacement on iodipamide uptake. The same incubation medium as described in the legend to Fig. 1 was used for transport, except that 100 mM NaCl (●) was replaced by the same concentration KCl (○), LiCl (□) or by choline chloride (△). The concentration of [^{125}I]iodipamide was 20 μM. Other conditions were the same as described in the legend to Fig. 1. The result of a typical experiment is shown ($n = 3$).

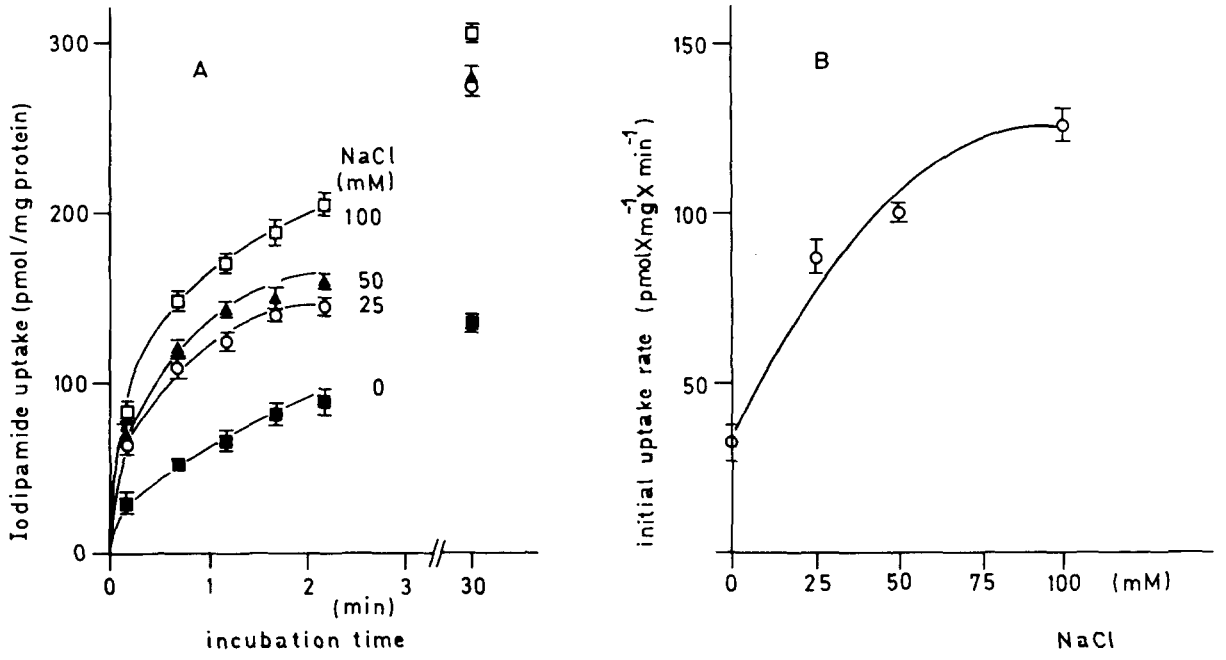


Fig. 4. (A and B) Sodium concentration dependency of iodipamide uptake. Incubation mixtures contained 20 μM [^{125}I]iodipamide/0.25 M sucrose/0.2 mM $CaCl_2$/10 mM Hepes-Tris buffer (pH 7.4) and varying concentrations of NaCl (in A: without NaCl (■), 25 mM NaCl (○), 50 mM NaCl (▲), 100 mM NaCl (□)). Isoosmotic adjustments were performed with raffinose. Other conditions were the same as described in the legend to Fig. 1. Each point represents the mean value of six experiments ($n = 6$).

TABLE I

COUNTERTRANSPORT (A) AND TRACER EXCHANGE (B) OF IODIPAMIDE IN PLASMA MEMBRANE VESICLES

(A) Membrane vesicles were preincubated for 15 min at 25°C in the presence of 0.2 mM unlabeled iodipamide in 0.25 M sucrose containing 0.2 mM $CaCl_2$/10 mM $MgCl_2$/10 mM Hepes-Tris buffer (pH 7.4)/100 mM NaCl. Countertransport was started by diluting the membranes with medium by factor 10 and by adding 20 μM [^{125}I]iodipamide. Other conditions were the same as described in the legend to Fig. 1. Each value represents the mean value ± S.D. of six experiments ($n = 6$). (B) Membrane vesicles were preincubated for 15 min at 25°C in the presence of 0.1 mM unlabeled iodipamide in the same medium as described above. Tracer exchange was started by adding 4 μM [^{125}I]iodipamide. Other conditions were the same as in (A).

	Conditions inside vesicles	Initial uptake rate V_0 of labeled iodipamide (% cpm in vesicles of total cpm per mg protein per min)
(A) Counter-transport	control	8.9 ± 0.6
	0.2 mM unlabeled iodipamide	12.5 ± 0.5
(B) Tracer exchange	control	6.9 ± 0.5
	0.1 mM unlabeled iodipamide	11.3 ± 1.0

Effect of the membrane potential on iodipamide uptake

In the translocation of solutes the potential difference across biological membranes (inside negative) might represent an important component of the driving force for rheogenic solute uptake (charge transfer). Rheogenic Na^+-dependent trans-

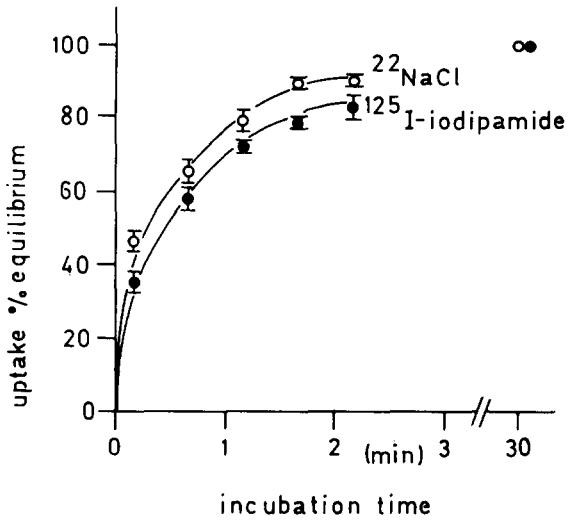


Fig. 5. Time-course of iodipamide and sodium uptake. The same incubation medium as described in the legend to Fig. 1 was used for transport. The concentration of [^{125}I]iodipamide (●) was 35 μM and of ^{22}NaCl (○) 100 mM. For the study of ^{22}Na-uptake NaCl in the stop solution was replaced by 0.1 M $MgCl_2$. Each point represents the mean value of four experiments ($n = 4$).

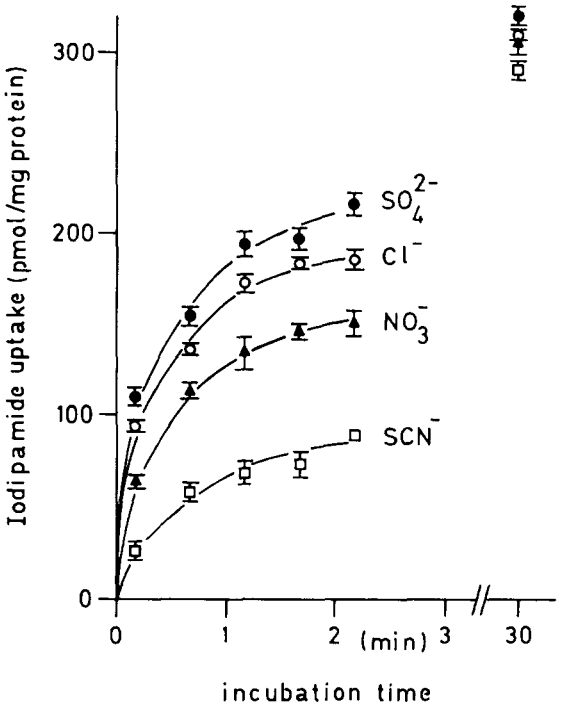


Fig. 6. Effect of anion replacement on iodipamide uptake. The same incubation medium as described in the legend to Fig. 1 was used for transport, except that 100 mM NaCl (○) was replaced by the same concentration of NaSCN (□), $NaNO_3$ (▲) and Na_2SO_4 (●). Other conditions were the same as described in the legend to Fig. 1. Each value represents the mean value of six experiments ($n = 6$).

port of sugars and amino acids was demonstrated in studies with brush-border membrane vesicles isolated from small intestine and kidney proximal tubule (for a review, see Ref. 20).

Therefore the effect of artificially imposed diffusion potentials on iodipamide uptake was analysed using the anion replacement method (Fig. 6). In the presence of a Na^+-gradient, replacement of Cl^- by the more permeant anions, NO_3^- and SCN^-, decreased the initial rate of iodipamide uptake to 88% and 52%, respectively (Table II). Total uptake was also reduced. When a less permeant anion, SO_4^{2-}, was used instead of Cl^-, the initial rate of uptake increased to 106% and total uptake was enhanced too. Since SCN^- usually diffuses into vesicles more rapidly and SO_4^{2-} more slowly than Na^+, our data might indicate that uptake of iodipamide is inhibited by the more negative intravesicular membrane potential. Thus, in the presence of Na^+, iodipamide might be taken up as an anion, which is consistent with rheogenic solute uptake.

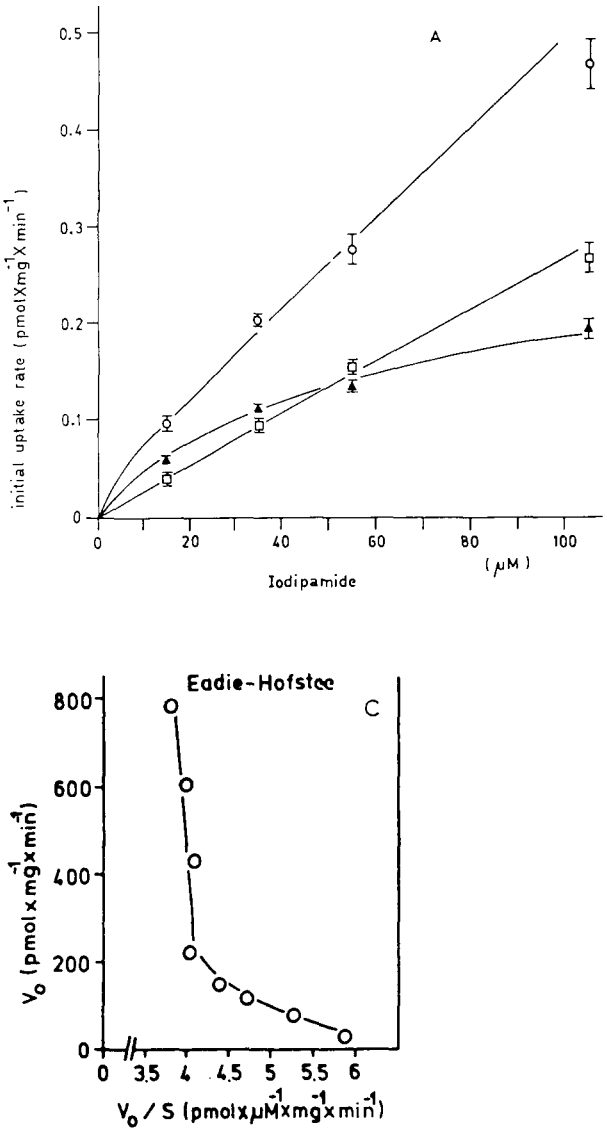


Fig. 7. (A and B) Saturation of iodipamide uptake. The same incubation medium as described in the legend to Fig. 1 was used for transport, except that varying concentrations of [^{125}I]iodipamide were used. Uptake was measured in the presence of 100 mM NaCl gradient at 4°C (□), at 25°C (○). The saturable iodipamide uptake (▲) was calculated by subtracting values obtained at 4°C from those obtained at 25°C. Other conditions were the same as described in the legend to Fig. 1. Each point represents the mean value of triplicate experiments ($n = 3$) with three different vesicle preparations. (C) shows the results of a typical experiment in an Eadie-Hofstee-type plot. Initial rate of uptake V_0 was plotted versus V_0/S, where S represents the concentration of iodipamide.

TABLE II

EFFECT OF ANION REPLACEMENT ON IODIPAMIDE UPTAKE

Conditions of incubation were the same as described in the legend to Fig. 6. Initial uptake rates V_0 were calculated as described under 'Materials and Methods'. Each value represents the mean value ± S.D. of six experiments ($n = 6$).

Anion	Initial uptake rate V_0 of iodipamide (pmol per mg protein per min)	Percentage
Cl^-	79 ± 3	100
NO_3^-	70 ± 5	88 ± 3
SCN^-	41 ± 6	52 ± 6
SO_4^{2-}	84 ± 5	106 ± 2

Saturability of iodipamide uptake

The initial uptake rate of iodipamide depends on the concentration of iodipamide in the extravesicular fluid (Fig. 7A). Experiments were performed in the presence of a Na^+ gradient at 4°C and 25°C. It can be seen that the initial uptake rate of iodipamide at 4°C increased linearly up to 100 μM iodipamide. This points to an unsaturable uptake, probably simple diffusion. By subtracting the unsaturable part from the uptake rate at 25°C, a saturable iodipamide transport was found. It follows simple Michaelis-Menten kinetics and double-reciprocal plots of initial uptake rate, V_0, vs. iodipamide concentration (Fig. 7B) gave a straight line, from which an apparent K_m of 70 μM and V_{max} of 0.31 nmol per mg protein per min at 25°C in the presence of 100 mM Na^+ gradient were derived.

These results suggest two different transport systems for iodipamide at 25°C, an unsaturable one, which presumably represents simple diffusion, and a saturable one, which might be due to carrier mediated transport. This view is supported by Eadie-Hofstee analysis of saturation kinetic data, since a curvilinear plot (Fig. 7C) suggests the presence of multiple transport systems for iodipamide uptake in our plasma membrane vesicles.

Specificity of the transport system

The specificity of the iodipamide transport in our plasma membrane vesicles was examined by the addition of cholate, known to be a competitive inhibitor of iodipamide uptake in hepatocytes [6]. Cholate inhibited the initial rate of iodipamide uptake by 19% at an inhibitor to substrate ratio of 10 : 1 and by 42%, if the inhibitor concentration was doubled (Table III). If the initial rate of

TABLE III

EFFECT OF SOME INHIBITORS ON UPTAKE

Conditions of incubation were the same as described in the legend to Fig. 1. In case of an inhibition with sodium cholate or phalloidin the inhibitor was added simultaneously with 10 or 12 μM [^{125}I]iodipamide without preincubation. In other experiments vesicles were preincubated 10 min with H_2DIDS and 30 s with bromosulfophthalein, before iodipamide uptake was started by adding 100 mM NaCl and 15 μM [^{125}I]iodipamide. Each value represents mean value ± S.D. of six experiments ($n = 6$).

Inhibitor	Concentration of inhibitor (μM)	Initial uptake rate of iodipamide		Concentration ratio $C_{inhibitor}/C_{substrate}$
		(pmol per mg protein per min)	Inhibition (%)	
Control	0	78 ±4	0	
Sodium cholate	100	63 ±5	19 ±2	10 : 1
Sodium cholate	200	45 ±1	42 ±2	20 : 1
Control	0	90.0 ± 0.2		
Phalloidin	6.7	70.6 ± 0	21.5 ± 0	0.53 : 1
Phalloidin	35.6	65.2 ± 0.6	27.5 ± 0.5	2.8 : 1
Phalloidin	68.9	60.2 ± 0.5	33.1 ± 0.6	5.5 : 1
Control	0	96.5 ± 2.5		
H_2DIDS	400	20.3 ± 3.7	79 ±3	
Control	0	82 ±6		
Bromosulfophthalein	100	46 ±2	44 ±2	

iodipamide uptake in the absence and presence of cholate was plotted in a Lineweaver-Burk diagram, cholate increased the Michaelis constant K_m, but did not change V_{max} (Fig. 8A). These results suggest that the uptake of iodipamide in our plasma membrane vesicles is also competitively inhibited by cholate as already demonstrated for hepatocytes [6]. From the Lineweaver-Burk diagram an inhibition constant (K_i) of cholate was calculated to be 58 μM.

Phalloidin also inhibited initial iodipamide uptake in vesicles in a concentration dependent manner (Table III). However, the effect of phalloidin seems to be stronger than that of cholate. 4,4'-Diisothiocyanato-1,2-diphenylethane-2,2'-disulfonic acid (H_2DIDS), which is an irreversible inhibitor of anion transport in red cells [21], also blocks the uptake of bile acids and of some foreign substrates (e.g., phalloidin) in isolated hepatocytes [9]. H_2DIDS strongly inhibited initial uptake rate of iodipamide in our vesicles (Table III).

Bromosulfophthalein, an organic anion, whose uptake was found not to be sodium dependent [22], was previously shown to inhibit iodipamide uptake in isolated hepatocytes in a mixed-order fashion [6].

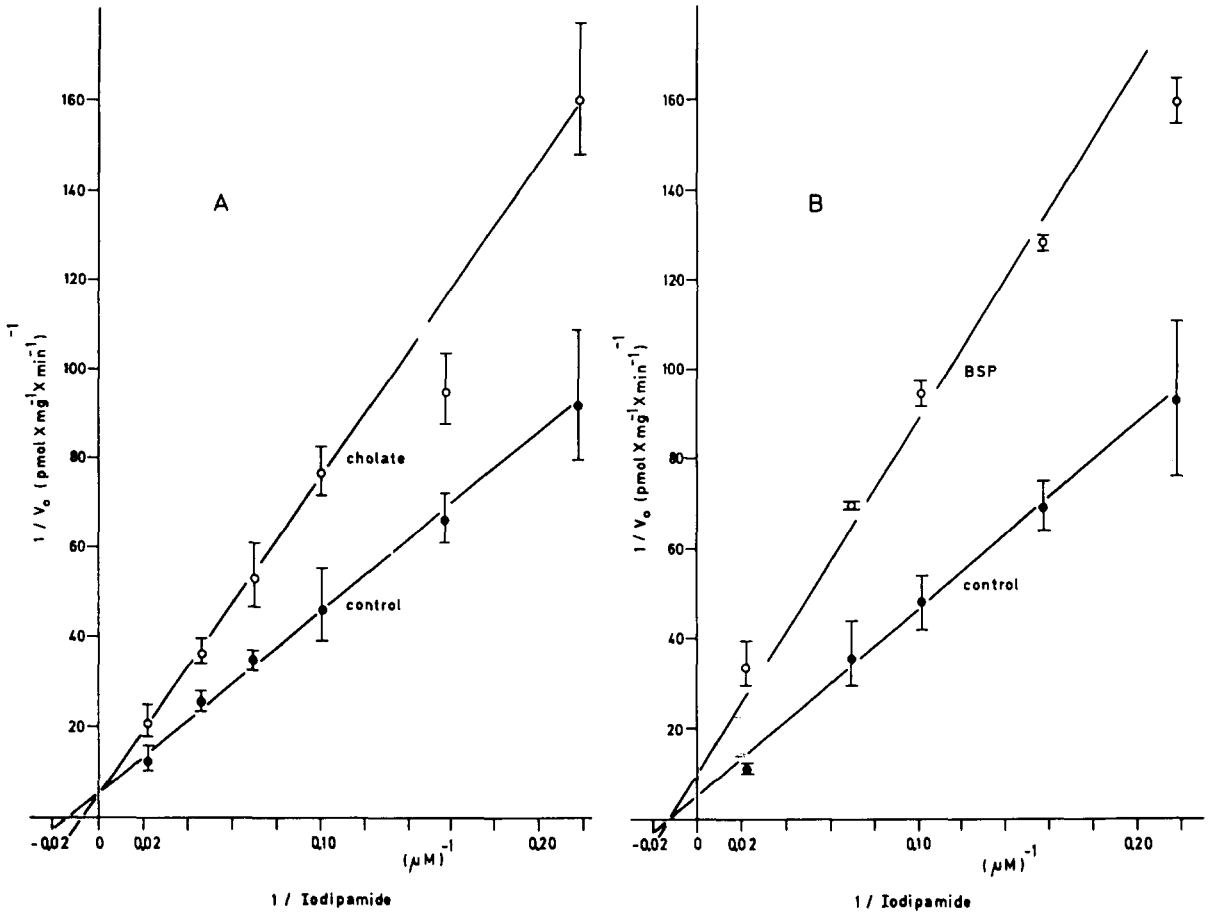


Fig. 8. Lineweaver-Burk diagram of the inhibition of iodipamide uptake by cholate and bromosulfophthalein. Iodipamide uptake was measured in the absence and presence of 166 μM cholate (A) and of 150 μM bromosulfophthalein (BSP) (B) at 25°C and at 4°C. Cholate and bromosulfophthalein were added simultaneously with [^{125}I]iodipamide. The initial rate of uptake V_0 was calculated from the uptake at 10 and 70 s. Other conditions were the same as described in the legend to Fig. 7. Each point represents the mean value of six experiments ($n = 6$) with three different vesicle preparations.

In our plasma membrane vesicles a kinetic analysis according to Lineweaver and Burk indicated a non-competitive type inhibition of the iodipamide uptake by bromosulfophthalein (Fig. 8B). From this diagram an inhibition constant (K_i) of bromosulfophthalein was calculated to be 150 μM.

Discussion

Various studies on isolated hepatocytes have provided some evidence that cholecystographic agents such as iodipamide and phallotoxins are translocated by the bile acid carrier system [5,6,8–10]. In the present study with plasma membrane vesicles, enriched in the sinusoidal fraction, competitive inhibition of iodipamide transport by cholate is a supporting argument for the above hypothesis. Further findings are also consistent with this view: iodipamide uptake in our plasma membrane vesicles is assumed to be carrier mediated and to be driven by the membrane potential related to monovalent cations.

A problem in studying iodipamide transport in isolated vesicles is binding to the membranes, however, iodipamide binding at equilibrium (51%) was found to be significantly lower than taurocholate binding (75%) in comparable plasma membrane vesicles [11]. The more hydrophilic character of iodipamide can explain this difference. Some hints for translocation were obtained from the different rates of initial iodipamide uptake, which were dependent on the size of the vesicles, and from the demonstration of countertransport and transstimualtion. Countertransport is not observed if only binding occurs. In this case the binding sites are saturated by preloading with unlabeled iodipamide and decreased uptake of labeled compound is expected; however, the opposite results were obtained.

The uptake of iodipamide in our plasma membrane vesicles, enriched in the sinusoidal fraction, satisfies the accepted criteria for carrier-mediated transport (saturability with respect to iodipamide, transstimulation and substrate specificity). The specificity of iodipamide uptake was demonstrated via competitive inhibition by cholic acid and noncompetitive inhibition by bromosulfophthalein.

Iodipamide uptake in vesicles was also inhibited by phalloidin, which is thought to be translocated by the bile acid carrier of hepatocytes [9,10]. For 30–40% inhibition of the initial iodipamide uptake in vesicles a 5-fold excess of phalloidin or a 20-fold excess of cholate over that of iodipamide was required (Table III). This suggests that phalloidin might have higher affinity for the iodipamide transport system than cholate.

H_2DIDS, an irreversible inhibitor of bile acid and phallotoxin uptake in isolated cells [9], was found to inhibit iodipamide uptake in isolated hepatocytes [5] as well as in vesicles.

Our observations concerning the inhibitors and their mode of inhibition in vesicles are fully consistent with previous studies concerning iodipamide transport in isolated hepatocytes [5,6]. However, the inhibitory potency of phalloidin on cholate uptake is significantly smaller in hepatocytes [9].

We found noncompetitive inhibition of iodipamide uptake by bromosulfophthalein in our plasma membrane vesicles. A mixed type inhibition of iodipamide uptake by bromosulfophthalein could be demonstrated in hepatocytes [6]. This leads to the assumption that hepatocellular iodipamide and bromosulfophthalein uptake are performed by different carriers.

According to previous evidence, bromosulfophthalein is probably not transported by the bile acid carrier but by another transport system for organic anions, involved in the physiological transport of bilirubin [23–26]. Since the two sulfonic acid groups of bromosulfophthalein are ionized at physiological pH, bromosulfophthalein might exert its inhibition on iodipamide uptake by nonspecific binding to the plasma membrane, thereby increasing the density of negatively charged groups at the cell surface [27]. This would lower the accessibility of the plasma membrane to other anions and lower the rate of uptake of these anions, e.g., iodipamide [28].

With respect to the driving forces of iodipamide uptake, our studies are suggestive of a membrane potential dependent uptake of iodipamide. A more negative intravesicular potential was found to inhibit, whereas a more positive intravesicualar potential stimulated iodipamide uptake. These data agree with the results obtained with hepatocytes [5] and suggest that in the presence of a Na^+-gradient (in vivo), iodipamide might be taken up

as an anion. (Iodipamide bears two negative charges at physiological pH (see introduction).

Further, we found that the transport in vesicles was dependent upon a gradient of monovalent cations. If the sodium gradient was substituted by a gradient of potassium or lithium, iodipamide uptake was not altered, as previously shown with hepatocytes [5]. However, under the same conditions uptake of cholic acid and demethyphalloidin was inhibited more than 50% [29]. In this case the pronounced inhibition by K^+ might be due to its side effect on the membrane potential [30]. Choline reduced iodipamide uptake in vesicles as well as in hepatocytes by only 15% or 20%, respectively [5]. Choline also slightly inhibited tracer exchange of iodipamide in vesicles. These findings suggest that the stimulatory action of a monovalent cation gradient might be due to the generation of a diffusion potential inside the vesicle. This means an influence on the electrochemical membrane potential. Choline had a slightly reduced effect presumably because of its low ability to permeate the plasma membrane.

We assume that the hepatocellular iodipamide uptake might be driven mainly by the membrane potential but not by Na^+ cotransport. Support for this hypothesis comes from the findings on isolated hepatocytes that valinomycin and gramicidin A reduced iodipamide uptake considerably [5]. Monensin, a carboxylic carrier ionophore, also inhibited iodipamide uptake in hepatocytes [5]. By dissipating the Na^+ gradient monensin presumably prevented the generation of a diffusion potential.

An Na^+-cotransport system has been postulated for bile acids based on kinetic analysis in hepatocytes [31,32] and sinusoidal membrane vesicles [11,33–35]. However previous evidence suggested that besides the Na^+ gradient, the membrane potential might also contribute equally to the energization of bile acid transport in hepatocytes. As demonstrated on isolated liver cells dissipation of the membrane potential by valinomycin and gramicidin reduced cholate uptake considerably [30]. Therefore, a possible interpretation of our results is that the bile acid carrier might be at least partially involved in the hepatocellular uptake of iodipamide. Arguments for this hypothesis are:

(1) cholate was found to be a competitive inhibitor;

(2) the characteristics of iodipamide transport were found to be similar to that of bile acids in that DIDS and phalloidin inhibited the uptake, while bromosulfophthalein inhibited noncompetitively.

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References

1 Berk, P.N., Loeb, P.M. and Ellzey, B.A. (1977) in Radiographic Contrast Agents (Miller, R.E., Skucas, I., eds.), University Park Press, Baltimore
2 Levi, A.J., Gatmaitan, T. and Arias, I.M. (1969) J. Clin. Invest. 48, 2156–2167
3 Barany, E.H. (1972) Acta Physiol. Scand. 86, 12–27
4 Felder, E. and Tanara, C.B. (1976) Farm. Ed. Prat. 31, 283–294
5 Joppen, C., Petzinger, E. and Frimmer, M. (1985) Naunyn-Schmiedeberg's Arch. Pharmacol. in the press
6 Petzinger, E., Joppen, C. and Frimmer, M. (1983) Naunyn-Schmiedeberg's Arch. Pharmacol. 322, 174–179
7 Frimmer, M., Petzinger, E. and Ziegler, K. (1980) Naunyn-Schmiedeberg's Arch. Pharmacol. 313, 85–89
8 Frimmer, M. (1982) Trends Pharmacol. Sci. 3, 395–397
9 Petzinger, E. and Frimmer, M. (1980) Arch. Toxicol. 44, 127–135
10 Petzinger, E. (1981) Naunyn-Schmiedeberg's Arch. Pharmacol. 316, 345–349
11 Inoue, M., Kinne, R., Tran, Th. and Arias, I.M. (1982) Hepatology 2, 572–579
12 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
13 Kinne, R., Schmitz, J.-E. and Kinne-Saffran, E. (1971) Pflügers Arch. 329, 191–206
14 Michell, R.H. and Hawthorne, J.N. (1965) Biochem. Biophys. Res. Commun. 21, 333–338
15 Harper, A.E. and Bergmeyer, H.U. (1962) Methoden der enzymatischen Analyse, p. 788, Verlag Chemie, Weinheim
16 Evers, J., Murer, H. and Kinne, R. (1976) Biochim. Biophys. Acta 426, 598–615
17 Blitzer, B. and Boyer, J.L. (1978) J. Clin. Invest. 62, 1104–1108

18 Accatino, L. and Simon, F. (1976) J. Clin. Invest. 57, 496–508
19 Sips, H.J., Van Amelsvoort, J.M.M. and Van Dam, K. (1980) Eur. J. Biochem. 105, 217–224
20 Murer, H. and Kinne, R. (1977) in Biochemistry of Membrane Transport (Semenza, G. and Carafoli, E., eds.), pp. 292–304, Springer Verlag, Berlin
21 Cabantchik, Z.J. and Rothstein, A. (1972) J. Membrane Biol. 10, 311–330
22 Schwenk, M., Burr, R., Schwarz, L. and Pfaff, E. (1976) Eur. J. Biochem. 64, 189–197
23 Paumgartner, G. and Reichen, J. (1975) Experientia 31, 306–308
24 Anwer, M.S. and Hegner, D. (1978) Hoppe-Seyler's Z. Physiol. Chem. 359, 1027–1030
25 Minder, E. and Paumgartner, G. (1979) Experientia 35, 888–890
26 Lunazzi, G., Tiribelli, C., Gazzin, B. and Sottocasa, G. (1982) Biochim. Biophys. Acta. 685, 117–122
27 Burr, R., Schwenk, M. and Pfaff, E. (1974) Hoppe-Seyler's Z. Physiol. Chem. 355, 1182
28 Duszyński, J. and Wojtczak, L. (1974) FEBS Lett. 40, 72–76
29 Petzinger, E. (1981) Naunyn-Schmiedeberg's Arch. Pharmacol. 316, R 21
30 Petzinger, E. and Frimmer, M. (1984) Biochim. Biophys. Acta 778, 539–548
31 Schwarz, L.R., Burr, R., Schwenk, M., Pfaff, E. and Greim, H. (1975) Eur. J. Biochem. 55, 617–623
32 Scharschmidt, B.F. and Stephens, J.E. (1981) Proc. Natl. Acad. Sci. USA 78, 986–990
33 Ruifrok, P.G. and Meijer, D.K.F. (1982) Liver 2, 28–34
34 Duffy, M.C., Blitzer, B.L. and Boyer, J.L. (1983) J. Clin. Invest. 72, 1470–1481
35 Meier, P.I., St. Meier-Abt, A., Barrett, C. and Boyer, J.L. (1984) J. Biol. Chem. 259, 10614–10622