Biochimica et Biophysica Acta 937 (1988) 135–144
Elsevier

BBA 73826

Comparative investigations on the uptake of phallotoxins, bile acids, bovine lactoperoxidase and horseradish peroxidase into rat hepatocytes in suspension and in cell cultures

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(Received 6 July 1987)
(Revised manuscript received 21 September 1987)

Key words: Phallotoxin; Bile acid; Peroxidase uptake; Endocytosis; Cell culture

Two alternative uptake mechanisms for phallotoxins by liver cells are debated: carrier-mediated uptake and receptor-mediated endocytosis. We have compared the properties of hepatocellular uptake of the phallotoxins, phalloidin and demethylphalloin, with the uptake of cholate as a substrate for carrier-mediated uptake and compared with iodinated bovine lactoperoxidase or iodinated horseradish peroxidase, as the latter are known to be taken up by vesicular endocytosis. Uptake of phallotoxins and [^{14}C]cholate uptake into isolated hepatocytes is independent of extracellular calcium but inhibited by A23187 or by monensin. Uptake of bovine lactoperoxidase strictly depends on external Ca^{2+}, was insensitive to A23197 and was not inhibited by monensin. No mutual uptake inhibition between phalloidin or cholate and peroxidases was seen, indicating independent permeation pathways in hepatocytes. However, high concentrations of cytochalasin B inhibited the uptake of either phalloidin, cholate or bovine lactoperoxidase. Horseradish peroxidase uptake, which was taken as an indicator for fluid pinocytosis, was low in isolated hepatocytes and could not account for the amount of phalloidin or cholate taken up. In cultured rat hepatocytes, uptake of phallotoxins decreased within 1 day to 10% of the uptake seen in freshly isolated hepatocytes. The results indicate different mechanisms for hepatocellular phallotoxin/bile-acid uptake and peroxidase internalization. As monolayer cultures of hepatocytes rapidly lost the carrier-mediated uptake of phallotoxins and bile acids, freshly isolated hepatocytes might be a more suitable experimental model than cultured cells for kinetic studies on this transport system.

Introduction

Phallotoxins are cyclic heptapeptides which are selectively taken up into hepatocytes. Two models for the uptake of phallotoxins into hepatocytes have been discussed. Previous results from our group suggested that phalloidin and demethylphalloin are taken up by the bile-acid transport system of liver parenchymal cells [1–3]. However, recent morphological studies of the uptake of a fluorescent phalloidin derivative, tetramethylrhodaminylphalloidin, in liver cell cultures [4,5] led to the assumption of an endocytotic pathway for this phallotoxin. Within 30 min, a patchy distribution of fluorescence was observed on 4-day hepatocyte cultures. The fluorescent pattern re-

Abbreviations: T_3, triiodothyronine; T_4, thyroxine.

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sembled that of endocytosed fluorescein concanavalin A [5] which was claimed to strongly indicate that this internalization was by receptor mediated endocytosis. Although, no patchy distribution was seen in isolated hepatocytes in suspension, endocytosis was also claimed to be the uptake mechanism for phalloidin into these cells [5].

With respect to the conclusions suggesting that phallotoxin uptake into hepatocytes was via endocytosis whereas many previous data suggested such uptake was carrier-mediated, we have studied the uptake of two peroxidases, bovine lactoperoxidase and horseradish peroxidase, as those are taken up into hepatocytes by vesicular internalization, e.g., uptake of bovine lactoperoxidase via receptor-mediated endocytosis [6] and uptake of horseradish peroxidase by fluid pinocytosis on hepatocytes [7,8]. We intended to find similarities or differences between hepatic phallotoxin/bile-acid uptake and the uptake of the peroxidases.

The controversal results mentioned above could be due to different procedures and methods. Carrier-mediated phallotoxin uptake has for example been studied in freshly isolated cells, whereas pinocytosis was described for hepatocyte cultures [4,5]. Consequently, we report here studies comparing phallotoxin and bile-acid uptake into cultured rat hepatocytes with their uptake into isolated hepatocytes in cell suspension.

Materials and Methods

Animals and experimental procedures

Hepatocytes were prepared from male Wistar rats weighing 230–260 g. The animals had free access to food and water and were housed in a room maintained at 22°C. Under urethane anaesthesia (1 g/kg body wt., i.p.) animals were heparinized with 1000 U liquemin immediately before laparotomy. The portal vein as well as the thoracal part of the vena cava caudalis were cannulated with polyethylene tubes. To remove blood, the liver was shortly perfused in situ with 37°C Krebs-Henseleit solution. Perfusion with collagenase, 0.05% in Ca^{2+}-free Krebs-Henseleit buffer, was performed for 15 min. in a temperature-controlled hood at 37°C by appropriate gassing of the perfusion buffer with oxygen/carbondioxyde (95%/5%). 80–90% of the isolated hepatocytes were viable, as judged by their staining with 0.2% Trypan blue. Prior to the uptake experiments, hepatocytes were equilibrated at 37°C for 30 min in Tyrode solution with 5.5 mM glucose as an energy source. Experiments were performed within 2 h with freshly isolated hepatocytes which had not been stored in a refrigerator.

Freshly prepared hepatocytes that had been incubated for 30 min under O_2/CO_2 atmosphere at 37°C in Tyrode buffer (pH 7.4) were plated on collagen(rat tail)-coated Petriperm dishes. The applied collagen was allowed to air-dry at room temperature during 60 min and the coated plates were stored at 37°C in a humidified incubator. Per dish $3 \cdot 10^6$ hepatocytes in 2 ml Tyrode buffer were transferred and allowed to settle down and attach to collagen during a 20 min incubation at 37°C. By addition of 4 ml Tyrode solution, the non-attached hepatocytes were washed off. The buffer was replaced by 3.5 ml Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 2 mM glutamin, penicillin/streptomycin (100 U/100 μg per ml), 10^{-6} M dexamethasone, 10^{-8} M T_3/T_4 and 5 μg/ml bovine insulin. Incubation was performed in an atmosphere of 5% CO_2/air during 5 days with daily changes of the culture medium.

Bovine leucocytes were isolated by a continuous-flow elution method using Szent-György & Blum equipment in connection with a Sorvall SS 34 rotor. Details of this method have been described [9]. From 3 l bovine blood, the yield was about 5 ml highly purified leucocytes containing 5 mg protein per ml. As with hepatocytes, uptake experiments were performed in Tyrode solution.

Uptake experiments were performed in cell cultures after substitution of the culture medium with Hepes-buffered Tyrode solution (10 mM Hepes) at 37°C. Culture dishes were placed on a shaker after addition of [^{3}H]demethylphalloin or [^{14}C]cholate, respectively. Quaduplicates of 50 μl of the supernatant were taken at the indicated time intervals. By this technique, the error of measurement was less than 10%. As total radioactivity which was added to a 3 ml culture dish represents 100%, the disappearance of radioactivity in the supernatant was regarded as being trapped by the cells. The amount of radioactivity

which was absorbed to collagen-coated, but cell-free Petriperm dishes was negligible. The decline of the radioactivity in the incubation medium at the end of an uptake experiment was between 7 and 15% of the added radioactivity. Non-specific binding to cultured hepatocytes was measured at 4°C. When an uptake experiment was finished, the culture medium was sucked off, replaced by 1 ml 0.1% Triton X-100 (in H_2O), and after 10 min incubation the cells were scraped off for the determination of their protein content.

Uptake of the radiolabelled compounds into hepatocytes in cell suspension was measured in Tyrode buffer at 37°C under O_2/CO_2 atmosphere. Aliquots of 100 µl were taken off at 15,45,75,105 and 135 s, and, as indicated in the figure legends, during the next 30 min. The extracellular fluid was separated from hepatocytes by the silicone-oil centrifugation method according to Refs. 10 and 11.

Radioactivity was either measured by liquid scintillation counting (lipoluma/lumasolve/water, 10:1:0.2 (v/v)) in a Packard Tri Carb 2660, or by counting in a W&W gamma counter type MR 1032. Per ml cell suspension about $(1-4) \cdot 10^5$ dpm of [^{3}H]demethylphalloin, [^{14}C]cholate, ^{125}I-labelled bovine lactoperoxidase and ^{125}I-labelled horseradish peroxidase as well as $(1-2) \cdot 10^6$ dpm of [^{3}H]insulin were added. The radiolabelled peroxidases were taken from a stock solution containing 0.2% albumin.

Determination of cell ATP was performed with the luciferin–luciferase method in a LUMAC biocounter M 2010 with a commercially available kit from Lumac corporation, Schaesberg. Cell samples, isolated hepatocytes and cultured hepatocytes which were scraped off, were boiled in 1 M $HClO_4$ for 10 min and stored at −70°C. $(Na^+ + K^+)$-ATPase activity was measured photometrically by the lactate dehydrogenase/ pyruvate kinase method according to Ref. 12 with 1 mM ouabain as inhibitor. $(Na^+ + K^+)$-ATPase activity amounted to 23% of the total ATPase activity in a cell lysate containing 0.1% Triton X-100.

Iodination of horseradish peroxidase and bovine lactoperoxidase was performed by the chloramine T method with 0.5 mCi $Na^{125}I$ for 60 s. Separation of free iodine wash achieved by a G-25 Sephadex column which was previously saturated with 500 µg non-labelled peroxidase. The efficiency of ^{125}I-incorporation, tested by trichloroacetic acid precipitation, was at least 95%. It was essential to store the iodinated peroxidases at −30°C to minimize the decay. The iodinated material was used within 1–2 weeks, during which free iodine increased from 5 to 20%. For this reason, the uptake experiments with iodinated peroxidases were performed in the presence of 1 mM sodium iodide. The applied protein concentration of the peroxidases was 0.5–1 µg/ml cell suspension.

All experiments were repeatedly performed with '*n*' different cell preparations as given in the figure legends.

Materials

[^{14}C]Cholate, specific activity 50 mCi/mmol and [^{3}H]inulin, specific activity 1–5 Ci/mmol were from Amersham. [^{3}H]Demethylphalloin, spec. act. 3.5 Ci/mmol was a gift from Professor Dr. Wieland, Heidelberg. Horseradish peroxidase (EC 1.11.1.7) type II and bovine lactoperoxidase (EC 1.11.1.7) with 80–100 units per mg protein, and A23187 were from Sigma, Munich. Collagenase from *Clostridium histolyticum* was purchased from Boehringer, Mannheim. Monensin was a gift from Eli Lilly Co., Bad Homburg. All other chemicals were from Merck, Darmstadt.

Results

Phallotoxins as well as cholate are rapidly taken up into isolated rat hepatocytes. Over 5 min incubation, 9.3% of [^{3}H]demethylphalloin and 55% of [^{14}C]cholate is accumulated in intact hepatocytes (Fig. 1). This uptake is blocked by the presence of 10 µg/ml of the sodium ionophore monensin (Fig. 2), indicating the importance of the sodium gradient as a prerequisite for the uptake of both these compounds. In the absence of $CaCl_2$ in the incubation buffer, no significant uptake reduction was seen (Fig. 3). This was, however, in contrast to the uptake of ^{125}I-labelled bovine lactoperoxidase (see below). Although, these results indicate that phalloidin and cholate uptake are not dependent on extracellular Ca^{2+}, it was dependent on the intracellular Ca^{2+} concentration. When $(Ca^{2+})_i$ was elevated by the addition of the ionophore A23187, both phalloidin

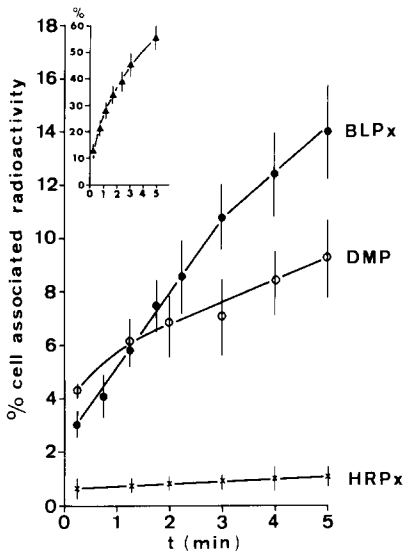

Fig. 1. Uptake kinetics of bovine lactoperoxidase (BLPx), demethylphalloin (DMP), horseradish peroxidase (HRPx) and cholate (inset) on isolated rat hepatocytes in cell suspension. $2 \cdot 10^6$ hepatocytes per ml were incubated for 5 min in the presence of 0.5–1.0 μg ^{125}I-labelled bovine lactoperoxidase (●); 0.5–1.0 μg ^{125}I-labelled horseradish peroxidase (x); 1.0 μg demethylphalloin per 45 ng [^{3}H]demethylphalloin (○) and 0.5 μg [^{14}C]cholate (▲). $n = 3$–4; mean ± S.D.

and bile-acid uptake were inhibited (Fig. 3). Both phallotoxin and cholate uptake were markedly lower in isolated hepatocytes which were maintained in cell cultures (Fig. 4). When hepatocytes were cultured for 4 h, the uptake of [^{14}C]cholate within 10 min decreased by 61% as compared to the uptake rate seen in freshly isolated cells. For phallotoxin uptake, the decrease was 74%. After 4 h, the attached cells started to spread and to form monolayers (Fig. 5). After 8 h, monolayers were nearly confluent (Fig. 5), while the loss in uptake rate was 75% for [^{14}C]cholate and 84% for [^{3}H]demethylphalloin. Monolayer and bile canaliculi formation was complete by approx. 20 h (Fig. 5). Phallotoxin and cholate uptake at this time were reduced to only 7 and 11%, respectively, of the uptake levels seen in freshly isolated hepatocytes in cell suspension (Fig. 4). This loss was not due to energy deprivation, as neither ATP content nor ($Na^+ + K^+$)-ATPase activity was reduced in 1-day cultures (Table I).

Uptake of ^{125}I-labelled horseradish peroxidase into isolated rat hepatocytes was very small when compared with phallotoxin/bile acid or lactoperoxidase uptake (Fig. 1). Only 1–2% of the total added radioactivity was internalized by

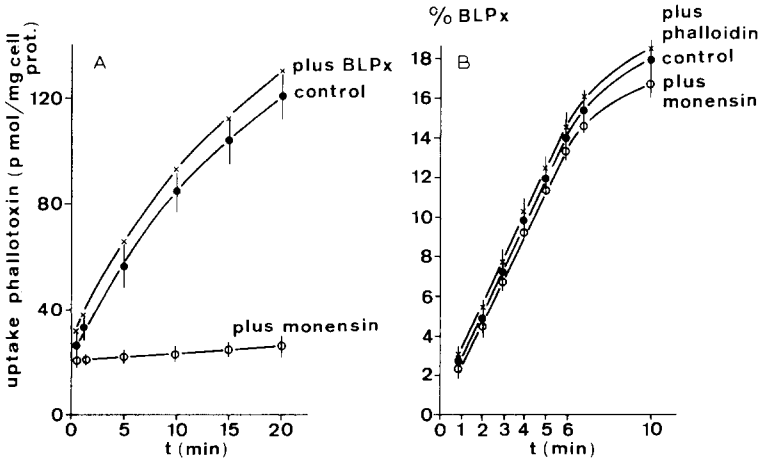

Fig. 2. Differential effect of monensin on the uptake of phallotoxin and bovine lactoperoxidase in isolated rat hepatocytes. Lack of mutual inhibition for bovine lactoperoxidase and phallotoxin uptake. (A) Uptake of phallotoxin (5 μg phalloidin per 45 ng [^{3}H]demethylphalloin) (●) in isolated rat hepatocytes in the presence of 10 μg monensin per ml (○) and 1.0 mg bovine lactoperoxidase per ml (x). $n = 3$ and 5; mean ± S.D. (B) Uptake of ^{125}I-labelled bovine lactoperoxidase (0.5–1.0 μg/ml) (●) in isolated rat hepatocytes in the presence of 10 μg monensin per ml (○) and 50 μg phalloidin per ml (x). $n = 4$; mean ± S.D.

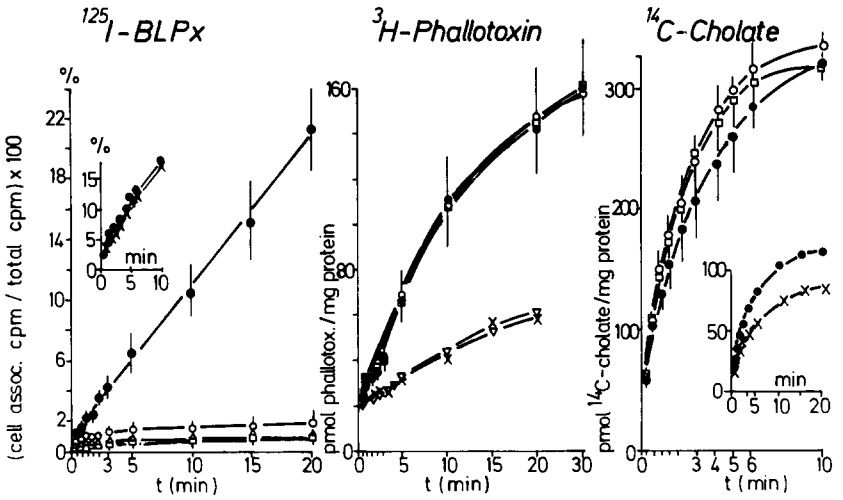

Fig. 3. Effect of Ca^{2+} on the uptake of phallotoxin, cholate and bovine lactoperoxidase in isolated rat hepatocytes. $2 \cdot 10^6$ hepatocytes per ml were incubated in Tyrode buffer containing 1.8 mM Ca^{2+} 1.05 mM Mg^{2+} (control, ●); in the absence of Ca^{2+} and Mg^{2+} (○); in Tyrode buffer with Mg^{2+} containing 1.8 mM Ba^{2+} instead of Ca^{2+} (△); or in Ca^{2+}/Mg^{2+}-free buffer substituted with 1 mM EGTA (□). $n = 3$–5; mean ± S.D. The effect of the Ca^{2+} ionophore A23187 is shown (insets). 1 μM A23187 (▽) or 10 μM A23187 (x) was added 5 min before the uptake of phallotoxin, cholate or bovine lactoperoxidase was measured (data from a representative experiment).

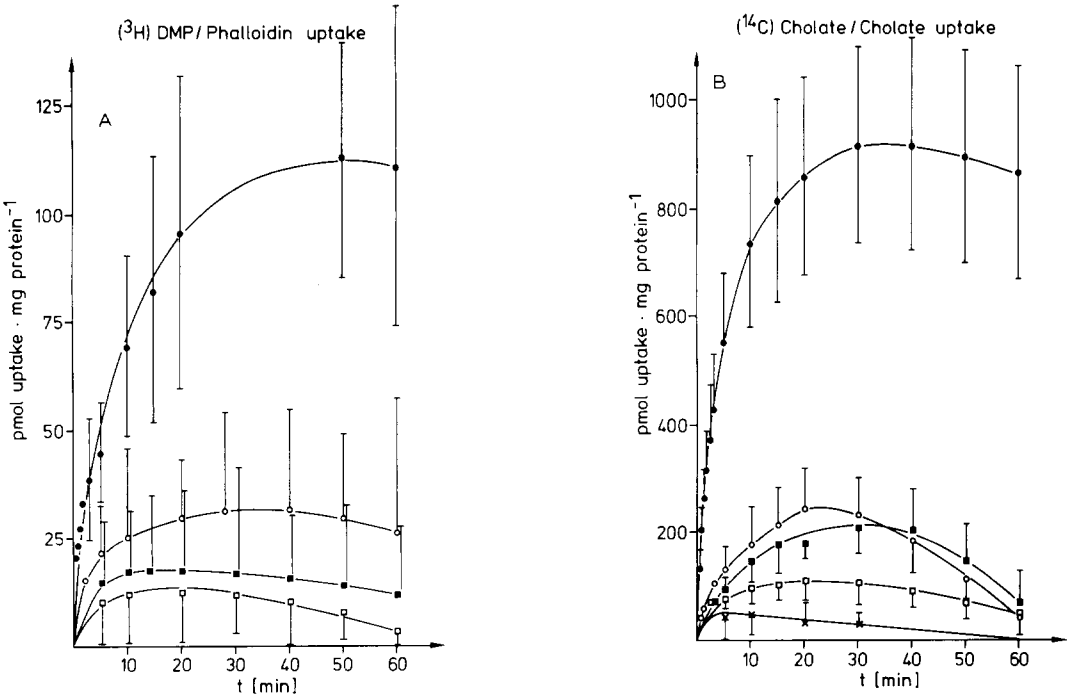

Fig. 4. Loss of uptake properties for phallotoxin and cholate in isolated rat hepatocytes grown in monolayer cultures. Isolated rat hepatocytes were cultured for 5 days. Uptake of phallotoxin (5 μg phalloidin per 50 ng [^{3}H]demethylphalloin per ml) and 1 μM [^{14}C]cholate/6 μM cholate was measured in 4-h cultures (○), $n = 8$ and 12; 8 h (■), $n = 9$ and 7; 20 h (□), $n = 7$ and 13; and 48 h (x), $n = 7$. For comparison, uptake of both compounds in isolated hepatocytes in cell suspension was measured prior to culture conditions (●), $n = 4$ and 24; mean ± S.D.

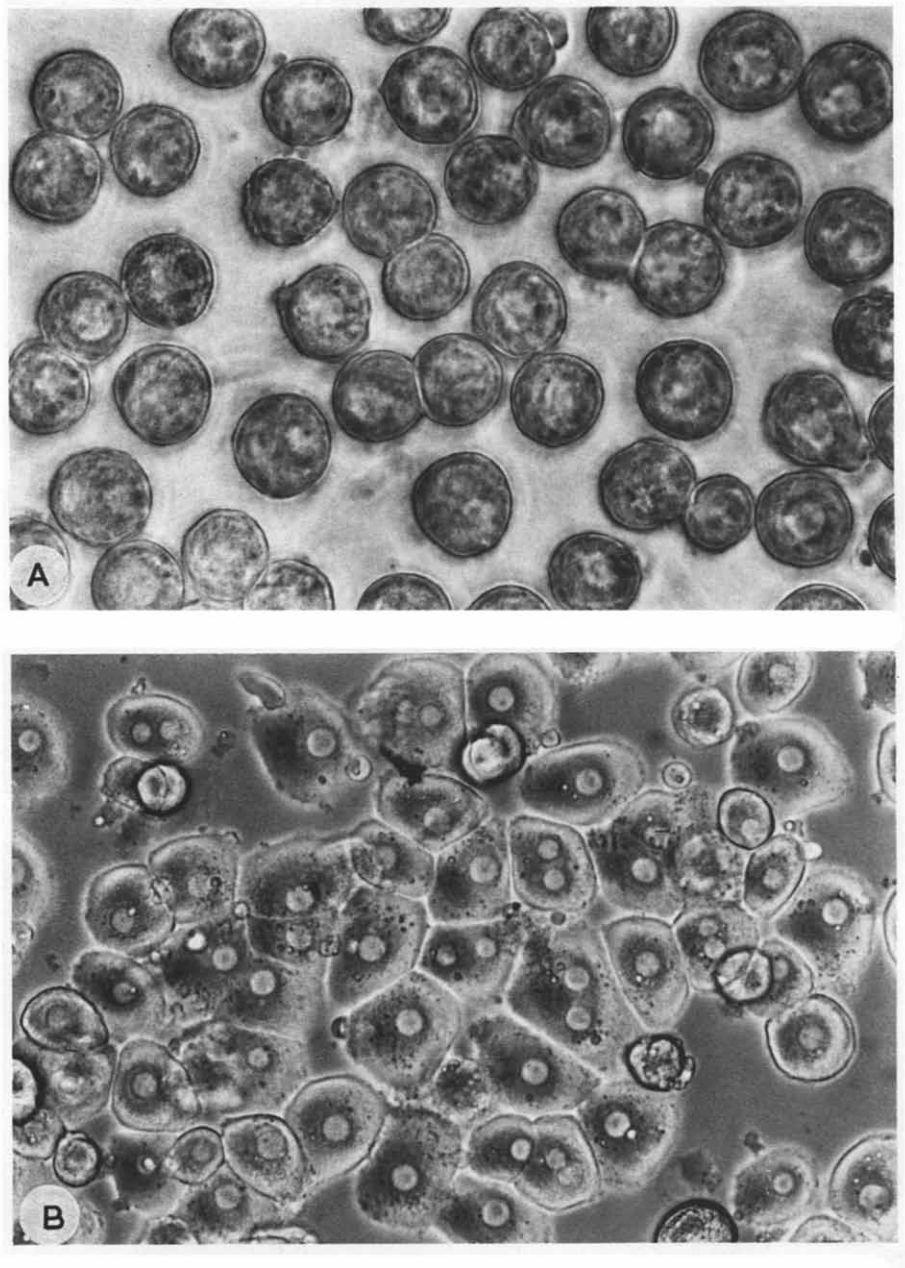

Fig. 5. Monolayer formation of isolated rat hepatocytes during culture conditions. Isolated rat hepatocytes were grown on collagen-coated gas-permeable Petriperm dishes under air 5% CO_2 for 20 h. Flattening of cells and formation of bile canaliculi was observed within the first 12 h. (A) Isolated rat hepatocytes in cell suspension; (B) cultured rat hepatocytes for 8 h; (C) monolayer of rat hepatocytes after 20 h. Arrows indicate bile canaliculi. Magnification × 400.

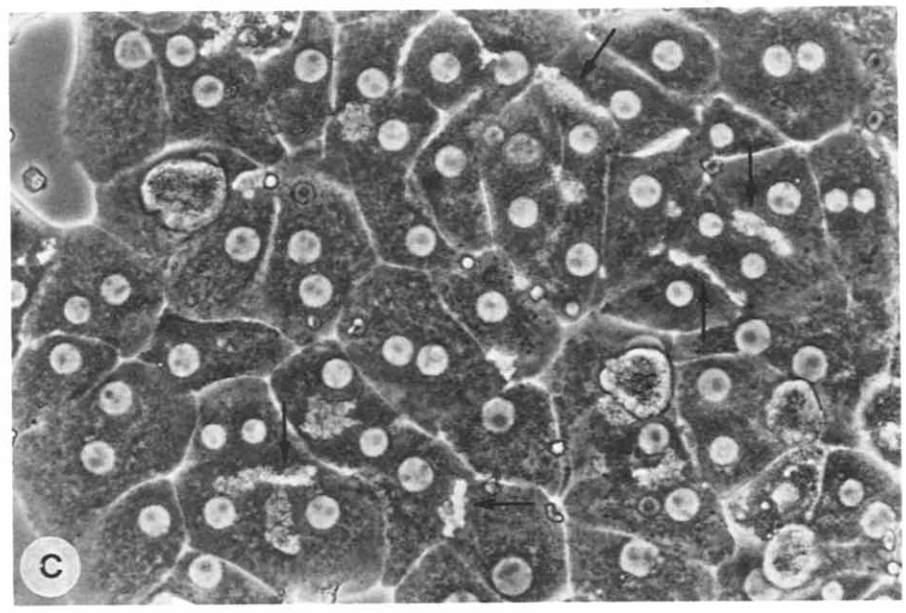

Fig. 5 (continued).

hepatocytes over a 30 min period. [^{3}H]Inulin uptake was even smaller and amounted to only 0.1–0.2% in isolated hepatocytes and 0.8–1.1% in purified bovine leucocytes (Fig. 6). From such observations it was concluded that the pinocytotic capacity of isolated rat hepatocytes is too low to account for the observed level of either bile-acid or phalloidin uptake activity. However, when the uptake of bovine lactoperoxidase into hepatocytes was measured, a rapid and significant internalization was observed (Fig. 1). Within 5 min approx. 14% of the added radioactivity was associated with cells incubated at 37°C, whereas only 1–2% of bovine lactoperoxidase was found in the cell pellet at 4°C. This amount was regarded as representing binding to the cell surface. The uptake at

TABLE I

AMOUNT OF ATP AND ACTIVITY OF ($Na^+ + K^+$)-ATPase OF ISOLATED RAT HEPATOCYTES IN CELL SUSPENSION AND IN CELL CULTURE FOR 3 DAYS

$n = 5$; mean ± S.D.; n.d., not determined.

	0 h (cell suspension)	4 h	20 h	48 h	72 h
ATP (ng/mg protein)	947 ± 428	821 ± 250	1125 ± 343	1509 ± 908 [a]	4255 ± 1872 [b]
($Na^+ + K^+$)-ATPase (μmol P_i per mg protein per h)	0.18 ± 0.06	n.d.	0.18 ± 0.12	0.17 ± 0.13	0.12 ± 0.09

Student's t-test:
[a] $P \leq 0.05$.
[b] $P \leq 0.001$.

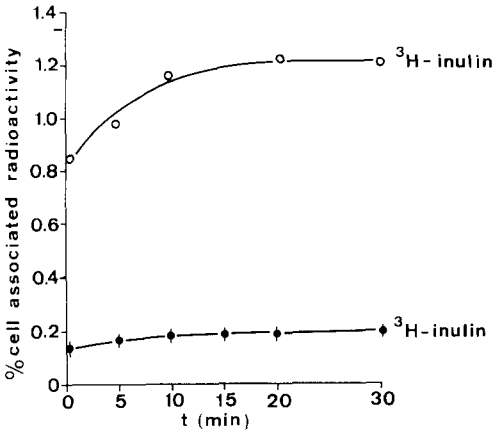

Fig. 6. Pinocytosis of [^{3}H]inulin by isolated rat hepatocytes and purified bovine leucocytes incubated in Tyrode solution without albumin. 3.8 mg cell protein per ml of isolated rat hepatocytes (●) ($n = 3$; mean ± S.D.) and 5 mg cell protein per ml of purified bovine leucocytes (○), $n = 2$, were incubated at 37 °C in the presence of 0.2 μCi [^{3}H]inulin during 30 min. Cell-associated radioactivity was determined after pelleting the cells through silicone oil.

TABLE II

INHIBITION OF THE UPTAKE OF PHALLOTOXIN, CHOLATE AND BOVINE LACTOPEROXIDASE BY CYTOCHALASIN B ON ISOLATED RAT HEPATOCYTES

Hepatocytes were preincubated for 30 s with 100 μM cytochalasin B when 50 ng [^{3}H]demethylphalloin per 5 μg phalloidin or 0.5 μg [^{14}C]cholate or 0.5–1.0 μg ^{125}I-labelled lactoperoxidase (BLPx) was added. V_i: initial rate of uptake between 15–135 s, $n = 4$.

Time period	% inhibition of cell associated radioactivity		
	[^{3}H]phallotoxin	[^{14}C]cholate	^{125}I-labelled BLPx
V_i (Δ 15–135 s)	66 ± 5	50 ± 7	58 ± 3
Δ 10 min uptake	60 ± 9	47 ± 7	50 ± 6

37 °C could be prevented completely by the presence of an excess (1000-fold) of non-labelled bovine lactoperoxidase (Fig. 7), but not by phalloidin or monensin (Fig. 2). In contrast to phalloidin and cholate uptake, lactoperoxidase internalization at 37 °C was dependent on extracellular Ca^{2+} and not inhibited by A23187 (Fig. 3). Bovine lactoperoxidase receptors were absent on AS-30 D ascites hepatoma cells (Fig. 7) which also lack the multispecific bile-acid uptake system [20,21].

The uptake of all three compounds, namely bovine lactoperoxidase, phalloidin and cholate, was inhibited in the presence of 100 μM cytochalasin B (Table II), but there was no mutual

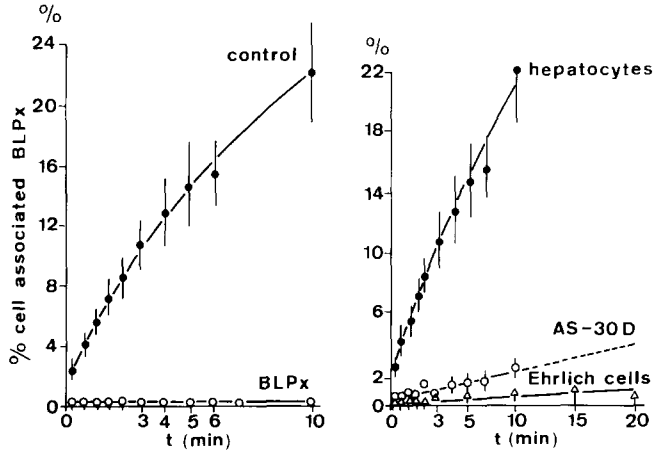

Fig. 7. Substrate- and cell specificity of bovine lactoperoxidase uptake into isolated rat hepatocytes in cell suspension. (A) Inhibition of ^{125}I-labelled lactoperoxidase (0.5–1.0 μg/ml) in the presence of 1 mg/ml non-labelled bovine lactoperoxidase (○). $n = 3$; mean ± S.D. (B) Comparison in ^{125}I-labelled lactoperoxidase uptake in isolated rat hepatocytes (●) (3.8 mg cell protein per ml), ascites hepatoma cells AS 30D (○) (4.2 mg cell protein per ml) and Ehrlich ascites tumor cells (△) (4.0 mg cell protein per ml). $n = 4$–6; mean ± S.D.

inhibition of bovine lactoperoxidase internalization by phalloidin or of phalloidin uptake by bovine lactoperoxidase (Fig. 2).

Discussion

Our results do not support the studies of Faulstich [4,5] which suggest that phallotoxins are taken up into hepatocytes, either in cultured or in cell suspension, by endocytosis. In our studies with isolated hepatocytes, the uptake of phalloidin and cholate was different from that of the peroxidases which were taken either as markers for fluid-phase endocytosis or receptor-mediated endocytosis. The total amount of horseradish peroxidase and inulin uptake, markers for fluid-phase endocytosis, was comparably small. Only 0.1–1% of the added compounds was taken up into isolated hepatocytes compared to 55% of cholate and 9% of phallotoxin. With bovine lactoperoxidase, the uptake of which was regarded as marker for receptor-mediated endocytosis, completely different uptake characteristics were found. These differences include the dependence on external Ca^{2+}, the insensitivity to the calcium ionophore A23187, and the lack of inhibition by monensin which was exactly the reverse of phalloidin/cholate uptake. The only common feature for phalloidin/cholate uptake on the one hand and the peroxidase uptake on the other was the sensitivity of cytochalasin B (Table II). As in these experiments, 100 μM cytochalasin induced membrane blebs on hepatocytes, during the 30 min for transport studies, this effect is likely to reflect cell damage (not observed under all other experimental conditions, except with phalloidin). Phalloidin (50 μg/ml) also induced cell blebbing, but bovine lactoperoxidase uptake was, however, not affected. This is of particular interest, as it agrees with earlier observations that phalloidin-treated hepatocytes maintain 'vitality', despite their morphological alterations [13]. In addition, bovine lactoperoxidase had no effect on phalloidin uptake, although the applied concentration was sufficient to block ^{125}I-labelled bovine lactoperoxidase completely.

The original conclusion regarding endocytosis of phalloidin was drawn from experiments on hepatocytes cultured for 3 and 4 days [5], and was claimed to hold true also for freshly isolated hepatocytes. However, cultured hepatocytes lost approx. 90% of the transport capacity for phalloidin/cholate within the first day of culture. This rapid loss was not regained during the next 4 days. With 5-day-old cultures, we were unable to obtain 'uptake curves' for phalloidin. Cholate, for instance, was not able to inhibit the cell binding of [^{3}H]demethylphalloin in these cultures. This phenomenon was not simply due to a loss of driving forces for phalloidin uptake, as the cultured cells had levels of ATP and $(Na^+ + K^+)$-ATPase enzyme activity similar to those seen in freshly isolated hepatocytes. These observations make it unreasonable to conclude that identical uptake mechanisms are involved in both cell systems.

Whether the patchy distribution of fluorescence within hepatocytes, which was found with tetramethylrhodaminylphalloidin treatment, is of endocytotic origin remains controversial. Suchy et al. [14] have shown that an iodinated bile acid at a very early stage of uptake into hepatocytes in vivo was not found to be present in submembranous vesicles. However, these authors could demonstrate the presence of radioactivity in Golgi- and smooth endoplasmic reticulum-derived vesicles within 5 min of incubation. The authors thus concluded from their observations that bile acids permeate into hepatocytes via integrated channel-forming carrier proteins with subsequent inclusion into Golgi and smooth endoplasmic reticulum membrane vesicles. Their conclusions are consistent with numerous observations on the carrier kinetics of bile-acid uptake into isolated rat hepatocytes [11,15–19]. The patchy distribution of the phalloidin fluorescence was, however, seen after 30 min exposure to this molecule. Within this period, a marked redistribution of tetramethylrhodaminylphalloidin within the hepatocytes should have occurred. In analogy to the results with iodinated bile acids, we would assume that the patchy fluorescence of tetramethylrhodaminylphalloidin seen after 30 min does not proof the hypothesis of an endocytotic uptake process.

Of particular relevance to this studie is whether the tetramethylrhodamine derivative of phalloidin is comparable to phalloidin with respect to its physicochemical and some biological properties. The molecular weight of the analogue is for exam-

ple almost twice that of phalloidin and is positively charged in contrast to phalloidin which is neutral. Tetramethylrhodaminylphalloidin is up to 50-times less toxic than phalloidin and its affinity to F-actin is only 15% of that of phalloidin or demethylphalloin [4]. Thus tetramethylrhodaminylphalloidin varies considerably from the original peptide with respect to a number of important characteristics. Conclusions drawn from results with the fluorescent derivative are not necessarily valid for phalloidin.

The results presented in this paper are thus not inconsistent with vesicular uptake of phalloidin by cells which lack the bile-acid transport system. For example, vesicular uptake of another fluorescent phalloidin derivative was seen in myoblasts [22], which certainly have no bile-acid carrier. The slow velocity of this process, similar to that observed with the horseradish peroxidase on hepatocytes in this study, led Barak et al. [22] to conclude that pinocytosis was involved in non-liver cell uptake of phallotoxins. Pinocytotic uptake in freshly isolated hepatocytes, however, does not contribute significantly to the overall uptake of either, phallotoxins and bile acids. In contrast, fluid-phase endocytosis seems to be markedly enhanced in cultured hepatocytes [23]. Thus, whereas the high capacity uptake for phallotoxins and bile acids seen in suspended isolated hepatocytes is lost during culture, the patchy fluorescence of tetramethylrhodaminylphalloidin in cultured hepatocytes might stem either from Golgi/smooth endoplasmic reticulum-derived vesicles or even from pinocytotic vesicles containing this phallotoxin.

Acknowledgement

This work was supported by the German Research Foundation, grant PE 250, 5–1.

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