Biochimica et Biophysica Acta 903 (1987) 248–256
Elsevier

BBA 73687

Physiological assay of liposome-mediated transport of a drug across *Xenopus* intestine: cell–liposome interaction

Tadashi Kashiwagura, Kazuhisa Sakurai and Noriaki Takeguchi

Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Toyama (Japan)

(Received 18 May 1987)

Key words: Liposome; Drug delivery; Atropine methyl bromide; Intestinal absorption; Muscle contraction; *(Xenopus)*

(1) The antagonistic effect of atropine methyl bromide entrapped in liposomes on contraction of *Xenopus* intestine in vitro induced by acetylcholine was studied. The results provided some insight into cell-liposome interaction. (2) Acetylcholine (0.1 mM) was added to the medium in the bath (serosal solution), while liposomes containing atropine methyl bromide in their internal and external phases were added on the mucosal side of the intestine. Large multilamellar liposomes were prepared from egg lecithin (phosphatidylcholine, PC) and cholesterol in various molar ratios. Atropine methyl bromide had most effect in liposomes composed of PC and cholesterol in a ratio of 7:3, less in those with a ratio of 4:5, and none in those with a ratio of 9:1. These effects were parallel with the sizes of these liposomes, determined by quasi-elastic light-scattering; that is, the larger the liposomes, the greater was their effect. Addition (to the liposomes) of phosphatidic acid, the negative charge of which increases the distance between the lamellar layers, increased the effect, indicating that atropine methyl bromide in the space between lamellar layers was effective. Another type of liposomes in which atropine methyl bromide was present only in the external phase of liposomes was as effective as liposomes in which atropine methyl bromide was present in both the internal and external phases. (3) From these results the following new model for liposome-mediated stimulation of transport of atropine methyl bromide is proposed. Large multilamellar liposomes have structural defects in their external lamellae through which atropine methyl bromide in the mucosal solution can penetrate into the space between the external lamellar layers and move into intestinal cells through regions of fusion between the outermost layers of the liposomes and the cell membrane.

Introduction

There are several advantages in use of liposomes for in vivo administration of drugs. For example, when encapsulated in liposomes, bioactive polypeptides such as interferon are protected against inactivation by proteases such as trypsin [1], and encapsulation of coagulation factor VIII in liposomes increased gastrointestinal absorption of the drug [2]. However, details of liposome–cell interaction, particularly at the level of the cell surface, are still unknown. The interactions of liposomes with cells have been classified into several types, such as stable adsorption, including both specific and non-specific adsorption, endocytosis, fusion, cell-induced leakage of liposome contents and lipid transfer [3–6]. However, the occurrence of a given mechanism in an actual

Abbreviations: PC, egg phosphatidylcholine; PA, DL-dipalmitoylphosphatidic acid; DCP, dicetyl phosphate.

Correspondence: N. Takeguchi, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Toyama, 930–01, Japan.

system is difficult to determine unequivocally because it cannot usually be determined in a single experiment. In particular, physiological methods to evaluate the efficiency of liposomes for administration of a given compound and for characterizing liposome–cell interaction have not yet been developed, although many biochemical methods have been applied in studies on liposomes.

This paper reports a new physiological method for evaluating the efficiency of liposomes in transport of entrapped material into intestinal cells. By this method, we studied liposome-mediated transport of atropine methyl bromide into the intestine of *Xenopus laevis* in vitro. We also propose a new model for the mechanism of liposome-mediated transport of the agent into intestinal cells.

Materials and Methods

Measurement of isometric tension of intestine

The jejunum was isolated from denervated *X. laevis* (African clawed aquatic frog). Both ends of the isolated intestine of 2 cm length (not inverted) were tied to polyethylene tubes with silk thread. The intestine was immersed in 20 ml of a solution consisting of (in mM) 105 Na^+, 5 K^+, 1 Mg^{2+}, 2 Ca^{2+}, 97 Cl^-, 18 HCO_3^-, 1 $H_2PO_4^-$ and 11 glucose (pH 7.4) in a bath. The solution was stirred by bubbling it with air. The internal space of the intestine was filled with mucosal solution of the same composition as that of the bath solution, unless otherwise noted. Then the ends of the two polyethylene tubes were closed with stopcocks. The diameter of the intestine was kept normal, that is, neither expanded nor deflated, by addition of an appropriate volume of the mucosal solution; the external diameter of the intestine ranged from 0.4 to 0.6 cm. The centre of the intestine was hooked to a strain transducer by a wire, and the signal from the transducer was amplified and recorded. Anticholinergic agents such as atropine sulfate and atropine methyl bromide were added to either bath solution or the mucosal solution as indicated. Acetylcholine was added to the bath solution. All experiments were done at 25 °C.

Liposomes

Multilamellar liposomes were prepared by the method of Bangham et al. [7]. Briefly, a lipid mixture in chloroform was deposited on the wall of a round-bottomed flask by removal of the organic solvent in a rotary evaporator. In most experiments, 3 ml of the mucosal solution supplemented with 0.1 mM atropine methyl bromide were added to 60 μmol of total lipids, and the suspension was shaken by hand for 1 h and then used as the mucosal suspension without dilution.

When indicated, atropine methyl bromide was removed from the external phase of liposomes by passing the liposomes at a concentration of 60 μmol lipid/ml through a Bio-Gel A-50 m (50–100 mesh) column (0.9 × 22 cm). After elution, the lipid concentration of the suspension was adjusted to 10 μmol/ml.

When smaller liposomes were required, the liposome suspension was sonicated in a Branson B-42 bath for 1 min at 25 °C, and then cooled on ice for 30 s. This process of sonication-cooling was repeated 30 times.

Phosphatidylcholine (PC), DL-dipalmitoylphosphatidic acid (PA), and dicetyl phosphate (DCP) were used as lipids. Crude PC was prepared from egg yolk as described elsewhere [8] and was purified further on a silicic acid column. The purified PC gave a single spot on thin-layer chromatography with chloroform/methanol/water (65 : 25 : 4, v/v) as solvent. Cholesterol, DCP and PA were obtained from Sigma.

Determination of the diameter of liposomes

The average diameter of liposomes was determined by quasi-elastic light-scattering as described elsewhere [9].

Statistical analysis

Values are expressed as mean ± S.E. Acetylcholine-induced contraction of *Xenopus* intestine was measured on preparations from three frogs. The significance of differences between values were assessed by Student's *t*-test.

Results

Contraction of Xenopus intestine by acetylcholine in the presence and absence of anticholinergic agents in the bath solution

First, experiments were made without liposomes. Addition of 0.1 mM acetylcholine to the

bath solution caused contraction of the intestine. The mucosal solution did not contain a cholinergic or anticholinergic agent. The first or second contraction was maximal and then the response decreased progressively with time. The average maximal response in 20 experiments was 1.85 ± 0.13 g (mean ± S.E.). The maximal response was taken as the index of the extent of contraction under different conditions. Because the maximal response was always observed within 3 min after addition of acetylcholine to the bath solution, the response was measured for 3 min. Then, acetylcholine in the bath solution was washed out. When the process of stimulation-washing was repeated, the maximal response was constant for at least 4 h (three observations). Hereafter, we use the word 'response' in place of maximal response.

The response as a percentage of the maximum response at high doses of acetylcholine was measured as a function of the acetylcholine concentration. The half-maximal concentration of acetylcholine for stimulation was 1.0 μm. The addition of atropine sulfate and atropine methyl bromide at 0.66 μM to the bath solution shifted the half-maximal concentration from 1 μM to 0.1 mM and 0.7 mM, respectively. On the other hand, the concentration of atropine methyl bromide for 50% inhibition of the response caused by 0.1 mM acetylcholine was 0.17 μM.

Effects of atropine sulfate and atropine methyl bromide in the mucosal solution

Fig. 1 shows the experimental protocol. After measuring the control response to 0.1 mM acetylcholine, the bath solution was changed to fresh acetylcholine-free solution. Then, atropine sulfate or atropine methyl bromide was added at 0.1 mM to the mucosal solution (without liposomes). The presence of atropine sulfate rapidly caused complete inhibition of the acetylcholine-induced response, as shown in Fig. 2. This rapid antagonistic effect indicated that atropine sulfate quickly penetrated into the serosal intercellular solution, where it bound competitively to cholinergic receptor sites of intestinal muscle cells.

In the presence of 0.1 mM atropine methyl bromide in the mucosal solution, the acetylcholine-induced response decreased to 73% of the control within 30 min and then remained steady

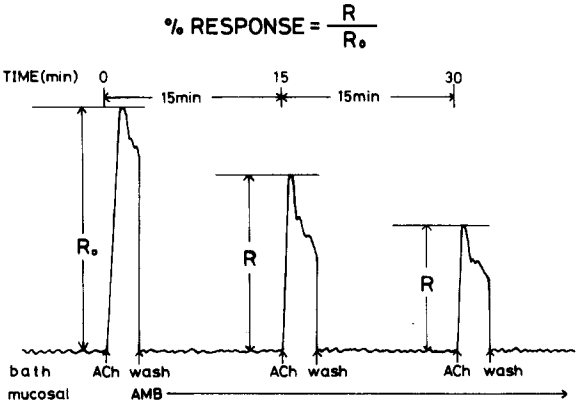

Fig. 1. Protocol for the results shown in Figs. 2–5. Acetylcholine (ACh) (0.1 mM) was added to the bath solution every 15 min. Contraction was measured for 3 min after addition of acetylcholine. Then the bath solution was changed to acetylcholine-free solution. Liposomes and anticholinergic agents were added on the mucosal side as indicated in the legends to Figs. 2–5.

(Fig. 2). The response was found to be equivalent to that in the presence of 0.1 μM atropine methyl bromide in the bath solution.

These results show that the rate of transport of atropine sulfate was too rapid to allow measurement of liposome-mediated transport of the drug, but that the rate of transport of atropine methyl bromide was appropriate for detection of the ef-

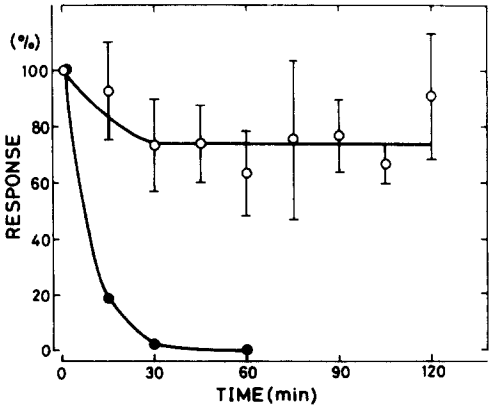

Fig. 2. Contraction of *Xenopus* intestine induced by 0.1 mM acetylcholine in the absence of liposomes. At zero time atropine sulfate (●) or atropine methyl bromide (○) at 0.1 mM was added to the mucosal solution (without liposomes). Open circles and bars show mean ± S.E. for three experiments and closed circles show data for single experiments.

fect of liposomes when the bath solution was renewed every 15 min. Therefore, we used atropine methyl bromide in subsequent studies.

Effects of liposomes per se on intestinal contraction by acetylcholine

Fig. 3 shows the effect of addition of 0.1 mM acetylcholine to the bath solution when the mucosal suspension contained empty liposomes (the word 'empty' means anticholinergic agent-free). With liposomes composed of PC and cholesterol in a molar ratio of 7 : 3, the addition of acetylcholine induced an average of 110% of the response of the control without liposomes, while with those composed of DCP, PC and cholesterol in a ratio of 3 : 7 : 3, it induced a 100% response. Liposomes composed of PA, PC and cholesterol in a ratio of 1 : 4 : 5 induced 108% of the control response (Table I). Since these differences from the control were not significant, liposomes per se did not affect contraction of the intestine.

Effects of atropine methyl bromide encapsulated in liposomes on intestinal contraction

The above experiments showed that the present system with *Xenopus* intestine could be used for physiological evaluation of the efficiency of liposome-mediated transport of atropine methyl

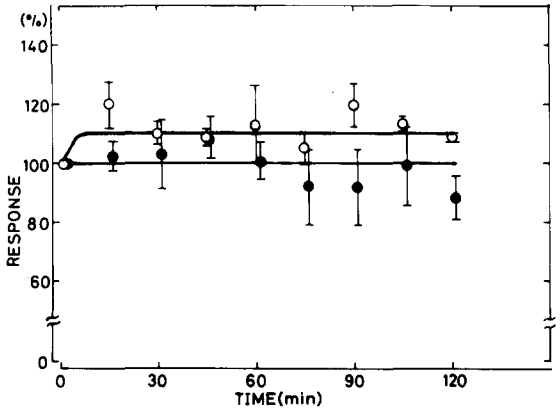

Fig. 3. Contraction of intestine induced by 0.1 mM acetylcholine in the presence of empty liposomes. The mucosal suspension contained empty multilamellar liposomes composed of lecithin (PC) and cholesterol in a ratio of 7 : 3 (○) or of DCP, PC and cholesterol in a ratio of 3 : 7 : 3 (●). No anticholinergic agents were added. Points and bars are mean ± S.E. for three experiments.

TABLE I

EFFECT OF LIPOSOME COMPOSITION ON INTESTINAL CONTRACTION

Unless specified, lipid concentration was 20 μmol/ml aqueous solution. Chol, cholesterol; PA, dipalmitoylphosphatidic acid; DCP, dicetyl phosphate. Unless specified, atropine methyl bromide (AMB) was present both inside and outside liposomes. H.S. means hand shaking. 'empty' means AMB-free liposomes. Values are average ± S.E. for observations on three different intestines.

Expt. No.	Lipid composition	Type of liposome	Steady Response (% of control)
1	liposome-free	AMB on the mucosal side	73 ± 5
2	PC/Chol (7 : 3)	H.S. (empty)	110 ± 2
3	DCP/PC/Chol (3 : 7 : 3)	H.S. (empty)	100 ± 3
4	PA/PC/Chol (1 : 4 : 5)	H.S. (empty)	108 ± 4
5	PC/Chol (7 : 3)	H.S.	33 ± 8
6	PC/Chol (4 : 5)	H.S.	57 ± 10
7	PC/Chol (9 : 1)	H.S.	72 ± 5
8	PA/PC/Chol (1 : 4 : 5)	H.S.	28 ± 3
9	PA/PC/Chol (1 : 4 : 5)	sonication	63 ± 4
10	PC/Chol (7 : 3)	sonication	40 ± 9
11	PC/Chol (7 : 3)	H.S. + phlorizin	60 ± 6
12	liposome-free [a]	AMB on the mucosal side	63 ± 5
13	PC/Chol (7 : 3) [b]	sonication	41 ± 5
14	PC/Chol (7 : 3) [b]	sonication (AMB only inside)	100 ± 3
15	PC/Chol (7 : 3) [b]	sonication (AMB only outside)	43 ± 6

[a] Intestines were previously treated with empty liposomes composed of PA, PC and cholesterol in a ratio of 1 : 4 : 5 for 2 h as described in text.

[b] 10 μmol lipids/ml aqueous solutions.

bromide. Furthermore, studies showed that in the absence of liposomes, atropine methyl bromide on the mucosal side antagonized the effect of acetylcholine about 27%. Therefore, an acetylcholine-induced response of less than 73% of the control was judged to be due to the effect of liposome-mediated transport of atropine methyl bromide.

In following studies, suspensions of liposomes containing atropine methylbromide in their inter-

nal and external phases were used, unless otherwise noted. The experimental protocol was the same as that for Fig. 1, except that liposome suspensions were used in place of a mucosal solution of atropine methyl bromide.

The effect of liposomes was found to depend on their lipid composition. The response induced by 0.1 mM acetylcholine first decreased with time and then became steady (Fig. 4), and the steady value was a function of the lipid composition; that is, it was 72, 57 and 33% of the control value with liposomes composed on PC and cholesterol in ratios of 9 : 1, 4 : 5 and 7 : 3, respectively (Table I). The times for attaining the steady values were about 30, 60 and 70 min, respectively. Liposomes with PC and cholesterol at a ratio of 7 : 3 were more effective than those with a ratio of 4 : 5, and those with a ratio of 9 : 1 had no effect. We previously reported determination of the sizes of liposomes by the method of quasi-elastic light-scattering [10]. The average diameters of multilamellar liposomes composed of PC and cholesterol in ratios of 7 : 3, 1 : 1 and 9 : 1 were found to be 1.2, 0.9 and 0.8 μm, respectively. Thus, the order of efficiency of the liposomes decreased with decrease in their size.

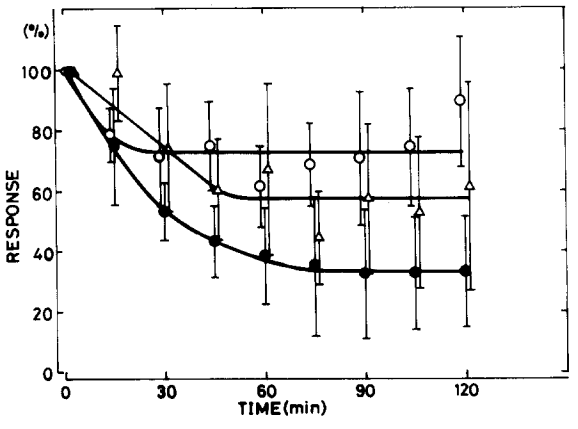

Fig. 4. Contraction of intestine caused by 0.1 mM acetylcholine in the presence of loaded PC-cholesterol liposomes. Multilamellar liposomes were prepared in mucosal solution supplemented with 0.1 mM atropine methyl bromide as described in Materials and Methods and used without dilution as the mucosal suspension for the experiments shown in fig. 4 and 5. Here, results are for liposomes composed of PC and cholesterol in ratios of 7 : 3 (●), 4 : 5 (△) and 9 : 1 (○). Points and bars are mean ± S.E. for three experiments.

Effects of incorporation of phosphatidic acid into liposomes and sonication of multilamellar liposomes on transport of atropine methyl bromide

Liposomes composed of PA, PC and cholesterol in a molar ratio of 1 : 4 : 5 have been used extensively by other studies, such as on lymphatic transport of liposomes-encapsulated agents [11], since the presence of negatively charged phospholipids increase the distance between lamellar layers, resulting in increase in the extent of drug encapsulation.

A mucosal suspension containing liposomes composed on PA, PC and cholesterol in a ratio of 1 : 4 : 5 significantly decreased the acetylcholine-induced response to 28% of the control (Table I), which was less than that with liposomes composed of PC and cholesterol in a ratio of 4 : 5 (57% of the control). The diameter of liposomes containing PA was 1.1 μm. Of the liposomes tested, those composed of PA, PC and cholesterol were the most effective.

Sonication of multilamellar liposomes (PA : PC : cholesterol = 1 : 4 : 5) reduced their diameter from 1.1 to 0.13 μm, resulting in inhibition of their effect (Table I).

On the other hand, sonication of multilamellar liposomes composed of PC and cholesterol in a ratio of 7 : 3 decreased their diameter from 1.2 to 0.95 μm (averages for two different preparations), but this final size was sufficient to stimulate transport of atropine methyl bromide, judging from the results in Fig. 4 and, in fact, the efficiency of the sonicated multilamellar liposomes was not significantly different from that of unsonicated liposomes (Table I).

Effects of phlorizin and of glucose-free mucosal medium

Phlorizin is a competitive inhibitor of active transport of glucose through brush-border membranes [12]. The presence of 0.5 mM phlorizin in the mucosal solution containing multilamellar liposomes composed of PC and cholesterol in a ratio of 7 : 3 inhibited the antagonistic effect of atropine methyl bromide 60 min after the start of experiment (Fig. 5), indicating that glucose carrier in the brush-border membrane was not directly involved in the uptake of atropine methyl bromide.

The removal of glucose from the mucosal sus-

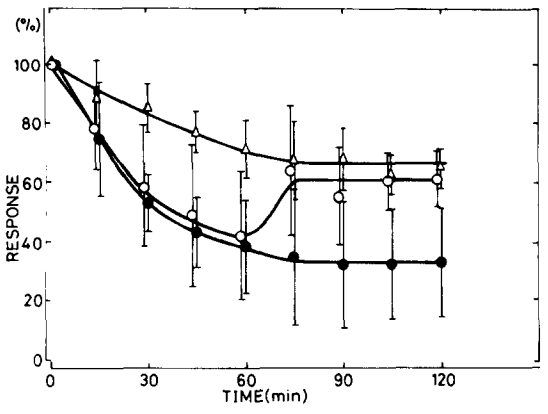

Fig. 5. Effect of glucose on the contraction of intestine induced by 0.1 mM acetylcholine in the presence of loaded PC-cholesterol liposomes. The mucosal suspension included multilamellar liposomes composed of PC and cholesterol in a ratio of 7:3. Phlorizin (0.5 mM) was added (○) or glucose was removed (△) from the suspension. Control values (●) without phlorizin or with glucose are also shown. Points and bars are mean ± S.E. for three experiments.

pension significantly inhibited the antagonistic effect of atropine methyl bromide (Fig. 5). Thus, some energy-requiring process in absorptive cells participates in transport of atropine methyl bromide.

Effects of empty liposomes on the tight junction of intestine

It may be possible that liposomes affect the tight junction of the intestine (for example, by binding of Ca^{2+} to phospholipids), and thus favor the paracellular pathway. For demonstrating this possibility, intestine was treated first with the mucosal suspension containing empty liposomes composed of PA, PC and cholesterol in a molar ratio of 1:4:5 for 2 h. The average response was 108%. Then it was treated with the mucosal solution containing 0.1 mM atropine methyl bromide (without liposomes) for 2 h. The effects of atropine methyl bromide became steady within 30 min and the steady value was 63%. This response was less than that of the control (73%), but greater than that of 28%, in which experiment atropine methyl bromide was present both inside and outside the liposomes. The time necessary for attaining the steady value was the same as that of the control (Fig. 2) but shorter than those in liposome-mediated effects (it took about 1 h). This difference in time indicated that the faster response found here was likely due to the increased paracellular pathway, although the effect was not enough to explain the liposome-mediated effects with the response less than 60%.

Effects of atropine methyl bromide inside and outside liposomes

In the studies described above, atropine methyl bromide was present both inside and outside liposomes. Next, we examined the effects of three different types of suspensions of sonicated multilamellar liposomes (PC/cholesterol = 7 : 3); namely, those with atropine methyl bromide both the inside and outside the liposomes, only inside the liposomes, and mainly outside the liposomes. The second type of liposomes was prepared by passing the first type through a gel column, as described in Materials and Methods. The third type was prepared by adding atropine methyl bromide to a suspension of empty liposomes just before experiments. We determined the rate of leak from the second type and their captured volume of atropine methyl bromide. In experiment, liposomes were prepared in a solution containing 10 mM atropine methyl bromide, 67 mM Na_2SO_4, 18 mM $NaHCO_3$, 3 mM K_2SO_4, 1 mM KH_2PO_4 and 1 mM $MgSO_4$ (pH 7.4) and were passed through the column. The amount of Br^- leaked from the liposomes into the medium was determined by the Mohr method [13] and the total content of Br^- in liposomes was determined after solubilizing liposomes with *n*-octyl glycoside. The results showed that atropine methyl bromide leaked slowly at a rate of 1% of the total content per h at 20 °C during the experimental period of 6 h. The captured volume was 5.5 μl per mg of lipid, which was nearly the same as that of multilamellar liposomes reported by others [14].

Table I shows that the second type of suspension did not affect the acetylcholine response, indicating that atropine methyl bromide encapsulated in liposomes was not effective. The first and third types of liposomes are equally effective in decreasing the acetylcholine response (the response was 41–43%, Table I). These observations suggest that for the response liposomes need not be taken up into intestinal cells, but that the interaction of liposomes with the cell surface is important.

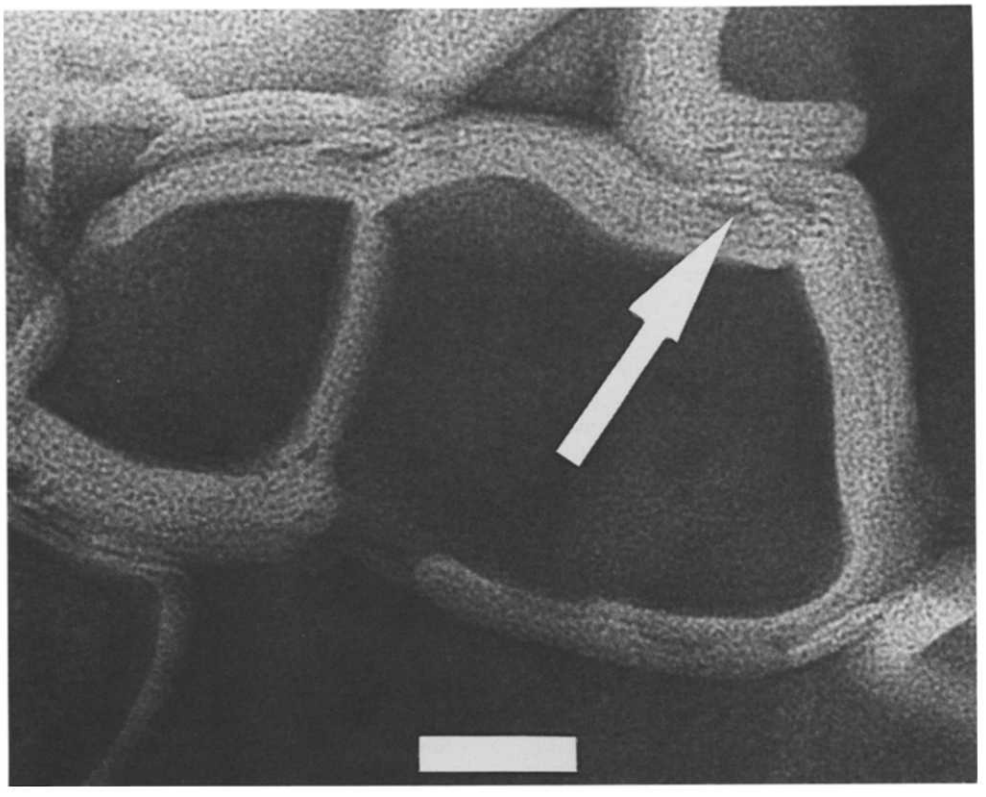

Fig. 6. Electron micrograph of multilamellar liposomes composed of PC and cholesterol in a ratio of 2:1. The liposome suspension was mixed with an equal volume of 2% potassium phosphotungstate. The arrow shows one of dislocation. Scale bar, 0.1 μm.

Electron micrograph of multilamellar liposomes composed of PC and cholesterol in a ratio of 2 : 1

Multilamellar liposomes have been indicated to have some structural defects [15,16]. The arrow in Fig. 6 shows one edge dislocations around the regions where two adjacent liposomes contact.

Discussion

The present results on acetylcholine-induced contraction of the intestine of *Xenopus* in vitro and the antagonistic effects of atropine methyl bromide indicate that our system is useful for physiological evaluation of liposome-mediated transport of atropine methyl bromide into intestinal cells.

Two main mechanisms of cell-stimulated transport of liposome-encapsulated drug into cells have been studied: endocytosis, or fusion, and leakage caused by liposome–cell interaction. Reported examples of the former mechanism are receptor-mediated endocytosis of antibody-opsonized liposomes by tumor cells [17], endocytosis by, or fusion of liposomes with amoebae [18], fusion of liposomes with the plasma membrane of plant tonoplasts [19] and endocytosis of negatively charged liposomes by coated vesicles in a cell line of African Green Monkey kidney [20]. Reported examples of the latter mechanism are cell-induced leakage of liposome contents of isolated hepatocytes and Zajdela ascites hepatoma cells [6], a cell line of lymphocytes (L1210) [5] and transport-deficient cell lines of mouse mammary tumor (EMT6) and Balb/c mouse T-cell lymphoma (S49) [21].

In the present study, the effectiveness of liposomes containing atropine methylbromide depended on the lipid composition. Liposomes composed of PC and cholesterol in a ratio of 7 : 3 (Fig. 4) were more effective than those composed of PC and cholesterol in a ratio of 4 : 5, while

those with a ratio of 9:1 had no effect. The effectiveness of the liposomes increased with increase in their size. Furthermore, the presence of PA, which is negatively charged and increases the distance between the lamellar layers, increased the effectiveness of liposomes: liposomes composed of PA, PC and cholesterol in a ratio of 1:4:5 were more effective than those composed of PC and cholesterol in a ratio of 4:5 (Table I). Reduction in size of liposomes composed of PA, PC and cholesterol in a ratio of 1:4:5 by sonication significantly decreased their effectiveness (Table I). These results suggest that the effectiveness of liposomes may depend on the amount of atropine methyl bromide in their interlamellar space.

Since non-entrapped drug was not removed for the results shown in Figs. 4 and 5, and listed in Table I (No. 5–11 and 13), even if cell-induced leakage of atropine methyl bromide from the liposomes occurred, it would not have substantially increased the local concentration of the drug near the cell surface, i.e., the leakage would not have contributed to liposome-mediated transport of atropine methyl bromide. On the other hand, a liposome suspension in which the nonentrapped drug was removed by passing the liposomes through a column was not effective (Table I), suggesting that endocytosis of liposomes is not a main reason for the stimulation by liposomes of transport of atropine methyl bromide. Furthermore, liposomes in which atropine methyl bromide was present only in the exterior spaces of the liposomes were as effective as liposomomes in which drug was present in both the exterior and interior spaces of the liposomes (Table I). These results suggest that the presence of the drug in the interior of liposomes is not necessary for stimulation of its transport, although liposome–cell interaction is necessary. This conclusion seems to be contrary to the finding that the effect of liposomes depended on the amount of drug between the lamellar layers.

The present results cannot be explained by either cell-induced leakage, fusion, endocytosis or increased paracellular pathway. Therefore, we propose a new model for the mechanism of this liposome-mediated transport to explain these conflicting facts. Fig. 7 shows models in which the outer lamellae of liposomes have some structural defects, such as edge dislocation (Fig. 6 and Ref. 15). These simplified diagrams of the defects are based on following evidence. When Na^+ permeability through membranes of multilamellar and unilamellar liposomes composed of dipalmitoylphosphatidylcholine was compared, the permeability in the former liposomes was larger than that in the latter liposomes [16], suggesting the presence of some structural defects in multilamellar structure. Many studies have shown that structural defects were caused by several different mechanisms, such as contact of two liposomes (Fig. 6), adsorption of liposomes to the cell surface [5,6, 18,21–23], fusion of liposomes with the cell membrane (reviewed in Ref. 24). Therefore, the present

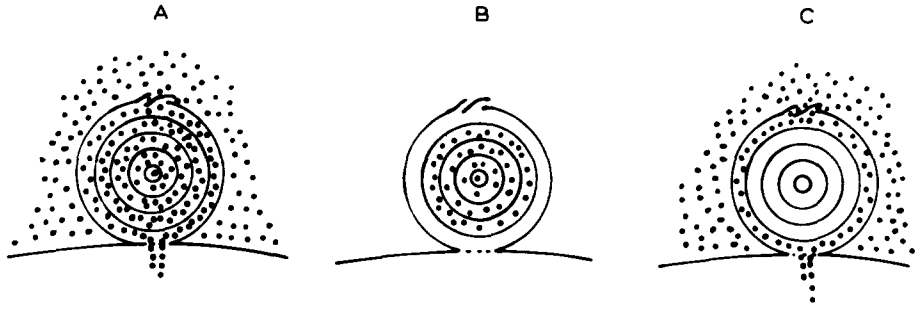

Fig. 7. Models for cell–liposome interaction. Each circle shows one bilayer. (A) Atropine methyl bromide, indicated by dots, is in the external and internal aqueous phases of the liposome. Fusion of the liposome with the cell membrane allows penetration of atropine methyl bromide into the space between the external bilayers. (B) Atropine methyl bromide is in some internal phase of the liposome. When liposomes were passed through a column, atropine methyl bromide in the space between disconnected bilayers was removed. On fusion, the drug does not penetrate into the cell. (C) Atropine methyl bromide in the external phase penetrates into the space between external bilayers through disconnected bilayers and into the cell through the fused region.

model of dislocation is a representative of the above non-exclusive mechanisms. Another important feature of present model is the fusion of liposomes with intestinal cells. In this model, atropine methyl bromide in the exterior of liposomes can penetrate into the interlamellar space through disconnected regions of the lamellae and enter the cell through the fused membrane area (Fig. 7(A), (C)). Atropine methyl bromide taken up by these cells is transported to the serosal intercellular space where the drug competitively binds to the acetylcholine receptor. Since muscle contraction was greater when phlorizin was added to the mucosal suspension or glucose was omitted than in normal medium (Fig. 5), the effect in medium without glucose or with phlorizin was not due to decreased energy supply to the muscle cells, but to a decreased rate of transport of atropine methyl bromide in some energy-requiring cellular process (es).

When the liposome suspension was passed through a gel column, atropine methyl bromide on the exterior of liposomes and in the spaces between outer lamellae that had structural defects (Fig. 7(B)) would be removed. Thus, in this liposome suspension the drug did not reach the fusion area and so would not be effective.

Thus, our results on the physiological assay of liposome-mediated transport of atropine methyl bromide into intestinal cells can be explained by a new, fusion model in which structural defects in the lamellae are important. Although this model is not indisputable at present, it provides an interesting working hypothesis for future study, in which the mechanism of drug transport across the basolateral membrane of the intestinal cells also has to be clarified.

References

1 Eppstein, D.A., Marsh, Y.V., Van der Pas, M., Felgner, P.L. and Schreiber, A. (1985) Proc. Natl. Acad. Sci. USA 82, 3688–3692

2 Hemker, H.C., Hermens, W.T., Muller, A.D. and Zwaal, R.F.A. (1980) Lancet i, 70–71

3 Gregoriadis, G. (1977) Nature 265, 407–411

4 Pagano, R.E. and Weinstein, J.N. (1978) Annu. Rev. Biophys. Bioeng. 7, 435–468

5 Szoka, F.C. Jr., Jacobson, K. and Papahadjopoulos, D. (1979) Biochim. Biophys. Acta 551, 295–303

6 Van Renswoude, J. and Hoekstra, D. (1981) Biochemistry 20, 540–546

7 Bangham, A.D., Standish, M.M. and Watkins, J.C. (1965) J. Mol. Biol. 13, 238–252

8 Faure, M. (1950) Bull Soc. Chem. Biol. 32, 503–508

9 Morii, M., Ishimura, N. and Takeguchi, N. (1984) Biochemistry 23, 6816–6821

10 Morii, M., Abu-Zaid, S.S. and Takeguchi, N. (1981) Yakugaku Zasshi 101, 1023–1029

11 Hirano, K. and Hunt, A. (1985) J. Pharm. Sci. 74, 915–921

12 Hopfer, U., Nelson, K., Perroto, J. and Isselbacher, K. (1973) J. Biol. Chem. 248, 25–32

13 Kolthoff, I.M., Sandell, E.B., Meehan, E.J. and Bruckenstein, S. (1969) in Quantitative Chemical Analysis, 4th Edn., pp. 796–797, MacMillan, London

14 Szoka, F. Jr. and Papahadjopoulos, D. (1978) Proc. Natl. Acad. Sci. USA 75, 4194–4198

15 Hui, S.W., Stewart, T.P., Boni, L.T. and Yeagle, P.L. (1981) Science 212, 921–923

16 Papahadjopoulos, D., Jacobson, K., Nir, S. and Isac, T. (1973) Biochim. Biophys. Acta 311, 330–348

17 Leserman, L.D., Weinstein, J.N., Blumenthal, R. and Terry, W.D. (1980) Proc. Natl. Acad. Sci. USA 77, 4089–4093

18 Batzri, S. and Korn, E. (1975) J. Cell. Biol. 66, 621–634

19 Lurquin, P.F. and Sheehy, R.E. (1982) Plant Sci. Lett. 25, 133–146

20 Straubinger, R.M., Duzgunes, N. and Papahadjopoulos, D. (1985) FEBS Lett. 179, 148–154

21 Allen, T.M., McAllister, L., Mausolf,, S. and Gyorffy, E. (1981) Biochim. Biophys. Acta 643, 346–362

22 Pagano, R.E. and Takeichi, M. (1977) J. Cell Biol. 74, 531–546

23 Bouma, S.R., Drislane, F.W., and Huestis, W.H. (1977) J. Biol. Chem. 252, 6759–6763

24 Poste, G. (1980) in Liposomes in Biological Systems (Gregoriadis, G. and Allison, A.C., eds.), pp. 101–151, John Wiley and Sons, New York