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Influence of the Antiallergic Drug Oxatomide and Derivatives on Membrane Structures: Relation with Inhibition of Calcium Influx in Rat Basophilic Leukemia Cells

Jeannette J. C. Paulussen,† Marcel J. E. Fischer,* Nicolaas J. Zuidam,‡ J. Cees v. Miltenburg,§ Nico J. de Mol*‖ and Lambert H. M. Janssen**

*Department of Medicinal Chemistry and ‡Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Faculty of Pharmacy, Utrecht University; and §Chemical Interfaces and Thermodynamics Group, Faculty of Chemistry, Utrecht University, Utrecht, The Netherlands

ABSTRACT. Oxatomide is an H_1 antihistaminic drug that also inhibits mediator release from mast cells. From previous studies, it appeared that inhibition of the influx of extracellular calcium is the major cause of this inhibition of exocytosis. Here, we explored the role of drug–membrane interactions in the inhibition of mediator release. We investigated the effects on phase transition and fluidity of artificial membranes. All compounds studied distorted the phase transition in L-α-dipalmitoylphosphatidylcholine liposomes, which correlated with the drug-induced increase in membrane fluidity measured by fluorescence anisotropy of the bilayer interacting probe 1-[4-(trimethylamino)-phenyl]-6-phenylhexa-1,3,5-triene. Erythrocytes were used to study membrane effects on a cellular level. The hypotonic-induced haemolysis of erythrocytes was inhibited by the drugs. Compounds which increased membrane fluidity of liposomes to a greater extent were also more active in decreasing haemolysis. Drug-induced disturbance of the membranes is related to their effect on the activity of store-operated Ca^{2+} channels. The activity of these channels in rat basophilic leukemia cells, assayed as $^{45}Ca^{2+}$ influx, was most effectively inhibited by oxatomide derivatives, thereby inducing a more rigid membrane structure. Small changes in molecular structure affect the activity of the drugs and these structure–activity relations are discussed. BIOCHEM PHARMACOL **57**;5:503–510, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. antiallergic drug; membrane; calcium influx; mast cells; store-operated Ca^{2+} channels

Various studies with histamine H_1 receptor antagonists, like oxatomide, have shown that these compounds inhibit the exocytosis of preformed and newly formed mediators from mast cells and basophils *in vivo* and *in vitro* [1–3]. Along with H_1 antagonism, this effect contributes to the antiallergic activity. Recently, we demonstrated that the inhibitory effect of oxatomide on extracellular calcium influx via SOC channels¶ is a major cause of the inhibition of mediator release in RBL-2H3 cells [4, 5]. It has been suggested that the mechanism for inhibition of mediator release by lipophilic antiallergic H_1 antagonists is related to their effects on membrane structures [6, 7]. Perturbation of the membrane structure could affect the structural arrangement of membrane proteins such as the high affinity receptors for IgE [8] and plasma membrane Ca^{2+} channels [9]. Depolarization of the RBL cell membrane results in inhibition of Ca^{2+} influx and exocytosis [10].

In the present study, we aimed to explore the role of drug–membrane interactions in the inhibition of mediator release as observed in a mast cell model. The influence of a series of oxatomide derivatives on phospholipid phase behavior was examined, using artificial membranes and DSC. Membrane fluidity studies of phospholipid bilayers were performed by a fluorescence anisotropy assay with the fluorescent probe TMA-DPH. Haemolysis of erythrocytes was studied to detect membrane effects of the drugs on a cellular level. The ability of the compounds to affect membrane properties was interpreted in terms of the chemical structure of the drugs. The observed relation between effects on the membranes and inhibition of $^{45}Ca^{2+}$ influx strongly suggests that the drugs affect the SOC channels by affecting the bilayer structure. Drugs that are active in

† Present address: TNO Nutrition and Food Research, Department of Occupational Toxicology, P. O. Box 360, 3700 AJ Zeist, The Netherlands.

‖ Corresponding author: Dr. N. J. de Mol, Department of Medicinal Chemistry, Utrecht Institute for Pharmaceutical Sciences, Faculty of Pharmacy, Utrecht University, P. O. Box 80082, 3508 TB Utrecht, The Netherlands. Tel. (31) 30-2536989; FAX (31) 30-2536655.

¶ *Abbreviations:* SOC channel, store-operated Ca^{2+} channel; RBL-2H3, rat basophilic leukemia cell line; DSC, differential scanning calorimetry; TMA-DPH, 1-[4-(trimethylamino)phenyl]-6-phenylhexa-1,3,5-triene; DPPC, L-α dipalmitoylphosphatidylcholine; EPC, egg phosphatidylcholine; log P, logarithm of the partition coefficient in an n-octanol/water system; T_m, temperature at the peak of the phase transition as measured by DSC; $T_{1/2}$, temperature range spanned at half the height of the peak of phase transition as measured by DSC; and T_S, onset temperature of phase transition as measured by DSC.

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inhibiting Ca^{2+} influx induce a more rigid bilayer than nonactive drugs.

MATERIALS AND METHODS

Materials

DPPC was donated by Nattermann Phospholipid GmbH. EPC was a gift from Lipoid GmbH. As determined by Lipoid, the phosphatidylcholine content of the EPC was 98.6% and contained traces of lysophosphatidylcholine (<0.2%), sphingomyelin (<0.5%), cholesterol (0.3%) and α-tocopherol (0.1%). TMA-DPH was purchased from Molecular Probes. Bovine erythrocytes were supplied by Biotrading. Oxatomide, (1-{3-[4-(diphenylmethyl)-1-piperazinyl]propyl}-1,3-dihydro-2*H*-benzimidazol-2-one), and its derivatives were generously provided by Janssen Pharmaceutica.

Preparation of Liposomes

DPPC or EPC was dissolved in chloroform/methanol (1:1) in a round bottom flask. The organic solvent was evaporated under vacuum. The thin lipid film obtained was hydrated with a buffer of 60°. A Tris/HCl buffer (0.1 M, pH 7.4) was used in calorimetric measurements, and in fluorescence measurements a Tyrode's buffer (137 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.31 mM NaH_2PO_4, 5.6 mM D-glucose, 10 mM HEPES, 0.1% $NaHCO_3$, pH 7.4) was used. Multilamellar vesicles were prepared by adding glass pearls to the suspension and shaking vigorously. The resulting liposomes remained stable at 4° under N_2. The final lipid concentration for DSC was 2.7 mM and for anisotropy 0.6 mM.

Calorimetric Measurements

The effects of oxatomide and related compounds on the phase behavior of DPPC were examined by DSC. Samples of 100 μM drug (or 4% ethanol as a control) and 2.7 mM liposomes in Tris buffer resulting in a drug to phospholipid molar percentage of 3.7% were used. The thermotropic phase behavior of the dispersions was measured using a Setaram DSC 111. Indium was used to calibrate the apparatus. A 100 μL sample was scanned at a rate of 1°/min.

Fluorescence Anisotropy

Anisotropy of the fluorescent probe TMA-DPH in RBL-2H3 cells and EPC or DPPC liposomes was measured as an indication for membrane fluidity. TMA-DPH was dissolved in dimethylformamide at a concentration of 1 mM. The RBL cells (2×10^5 cells/mL) were labeled with the fluorescent probe at a final concentration of 0.4 μM. Furthermore, EPC and DPPC liposomes were used at a concentration of 0.4 or 0.6 mM, with a probe concentration of 0.2 and 0.4 μM, respectively. After incubation for 5 min at 37°, drug (or 0.3% DMSO as a control) was added at a final concentration of 33 μM [drug to phospholipid molar percentage of 8.25% (EPC) or 5.5% (DPPC)]. The anisotropy measurements were performed with an LS-50 spectrofluorimeter (Perkin-Elmer) after 5 min of incubation. The excitation and emission wavelengths were 360 and 435 nm, respectively. Anisotropy values (r) were computed after correction for optical and electronic differences in the parallel and perpendicular channels (G-factor). Influence of scattering could be excluded by extrapolation of the anisotropy values to a liposome concentration of zero as proposed by Litman and Barenholz [11]. To compare the effects of the drugs, one concentration of liposomes was used without correction for scattering. From a control, it appeared that the antiallergic agents did not affect TMA-DPH fluorescence intensity.

Haemolysis of Erythrocytes

Bovine erythrocytes were centrifuged and suspended in physiological saline (10 mM NaH_2PO_4 and 154 mM NaCl, pH 7.4) containing 1.2×10^9 cells/mL. An aliquot of 100 μL erythrocytes was added to 1.4 mL hypotonic PBS containing 69 mM NaCl, which gave about 50% haemolysis. As a control for spontaneous lysis, cells in saline with 154 mM NaCl were used. To determine the protection against haemolysis, erythrocytes in hypotonic saline were incubated with 30 μM compound for 5 min. After centrifugation, supernatants were measured spectrophotometrically at 543 nm. Results were expressed as the percentage inhibition of haemolysis corrected for spontaneous lysis according to Lau and Pearce [6].

Assay of $^{45}Ca^{2+}$ Influx

This assay was performed as previously described [4]. After sensitizing with IgE, RBL cells were washed with Tyrode's buffer supplemented with 0.1% BSA. Oxatomide (30 μM) and antigen (40 ng/mL) were added in Tyrode's buffer with pH 7.0, 7.5 or 8.0. After 5 min of activation, radioactivity was measured. Results were expressed as the percentage of $^{45}Ca^{2+}$ influx after stimulation without drug.

Molecular Surface and Volume

The calculations of the molecular surface and volume of the antiallergic agents were performed using the Biosym Insight II programme (version 2.3.5). The compounds were first energy minimized with DISCOVER 2.96. Calculations were performed using a Connolly surface, with a probe of 1.4 Å.

Log P Determinations and Calculations

Determinations were performed at Janssen Pharmaceutica with the double extraction method as the partition coefficient of compound between an aqueous and organic phase.

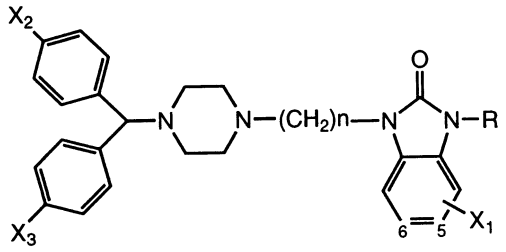

FIG. 1. Basic structure of oxatomide-related benzimidazolones.

The octanol phases were analyzed by UV-spectroscopy. Log P values were calculated using substituent constants according to Hansch and Leo [12], with oxatomide as the parent structure whose log P value was experimentally determined.

Statistical Analysis

Statistical significance was determined by a Student's *t*-test. Correlation analyses were performed using NCSS software (Number Cruncher Statistical System, version 5.01).

RESULTS

The structures of oxatomide and derivatives used in this study (basic structure in Fig. 1) are listed in Table 1.

Phase Transition in DPPC Liposomes

Measurement of thermodynamic properties of DPPC membranes using DSC provides information about the gel-to-liquid crystalline phase transition of lipids by measuring the heat capacity at different temperatures. The drugs were added to the DPPC liposomes in a 4% (v/v) solution of ethanol (final concentration). Figure 2A shows the thermograms of pure DPPC, DPPC with 4% ethanol, and DPPC with oxatomide. DPPC shows a T_m of 41.2° (Table 2), which is in agreement with values reported in the literature [13, 14]. The transition was initiated at a lower temperature when 4% ethanol was added, but the transition width, the $T_{1/2}$, remained the same. A decrease in T_m by approximately 1.5° upon addition of 4% ethanol is in accordance with observations of Simon and McIntosh [15]. At this ethanol concentration, the packing of the DPPC bilayer is still intact and interdigitation only occurs at higher ethanol concentration [15]. Addition of oxatomide decreased the transition temperature further from 39.8° to 39.3° and the transition width increased from 0.78 to 1.24° (Fig. 2A).

TABLE 1. Structures of analogues of oxatomide

Compound	R	n	X_1	X_2	X_3
Oxatomide	H	3	H	H	H
R34058	H	3	H	F	F
R35873	H	* $-CH_2-\underset{\displaystyle CH_3}{\underset{\vert}{CH}}-CH_2-$	H	F	F
R35918	H	4	H	H	H
R36262	$-C(=CH_2)-CH_3$	3	H	H	H
R36415	H	3	5Cl	H	H
R36599	H	3	6Cl	H	H
R37477	H	5	H	H	H
R37685	H	6	H	H	H
R37907	H	4	H	F	H

*Replaces $(CH_2)_n$.

TABLE 2. Influence of oxatomide and analogues on the phase transition of DPPC liposomes in a drug to phospholipid molar percentage of 3.7% in 0.1 M Tris buffer, pH 7.4

Compound	T_S (°)	T_m (°)	$T_{1/2}$ (°)
DPPC (−EtOH)	40.04 ± 0.19	41.16 ± 0.09	0.83 ± 0.02
DPPC	38.34 ± 0.39	39.84 ± 0.09	0.78 ± 0.04
+R36599	36.56 ± 0.35	39.35 ± 0.08	1.40 ± 0.08
+R36415	36.61 ± 0.04	39.23 ± 0.01	1.50 ± 0.06
+R35873	36.64 ± 0.17	39.42 ± 0.16	1.32 ± 0.16
+R36262	36.80 ± 0.31	39.28 ± 0.03	1.20 ± 0.05
+R34058	36.81 ± 0.21	39.34 ± 0.02	1.32 ± 0.02
+Oxatomide	36.86 ± 0.29	39.30 ± 0.11	1.24 ± 0.12
+R37685	36.97 ± 0.06	39.53 ± 0.01	1.14 ± 0.03
+R37477	37.32 ± 0.37	39.44 ± 0.13	1.03 ± 0.10
+R35918	37.42 ± 0.13	39.51 ± 0.02	0.96 ± 0.03

T_S is the onset temperature of the phase transition, T_m represents the temperature at the peak of the thermogram, and $T_{1/2}$ indicates the width of half the height of the thermogram. Values are means ± SD from at least three independent experiments. The melting characteristics of all liposome dispersions containing a drug were significantly different ($P < 0.05$) from the liposome dispersions without a drug.

We investigated a series of oxatomide derivatives to determine their effect on DSC thermograms in relation to the chemical structure of these compounds. The thermograms of R36415 and R35918, which affect phase transition the most and least, respectively, are shown in Fig. 2B. Table 2 presents the parameters derived from the thermograms. The effects on T_m were in a rather narrow range, with small differences compared to the control (DPPC with 4% ethanol). The peak width showed more variety, with a $T_{1/2}$ of 0.96° for R35918, the least active compound, and 1.50° for R36415 (Fig. 2B). The enthalpy (ΔH) of the phase transition of DPPC alone, 29.0 ± 2.2 kJ/mol, was not changed by the addition of ethanol or drug.

The gel-to-liquid crystalline phase transition of DPPC liposomes represents a change: phospholipid acyl chains, rather than being rigid and packed in an ordered manner, exhibited more free rotational movement around their carbon bonds and less dense packing [16, 17]. The shift of the T_m and the onset temperature (T_S) of DPPC towards lower values indicates that the drugs prefer the liquid crystalline phase over the gel phase. The transformation from a narrow to a lower and broadened peak indicates a reduction in the cooperativity of the transition, resulting in a less abrupt phase transition [16]. The transition enthalpy remained constant, as has also been reported for flunarizine [16], some other antiallergic drugs [7], and several antiinflammatory agents [18].

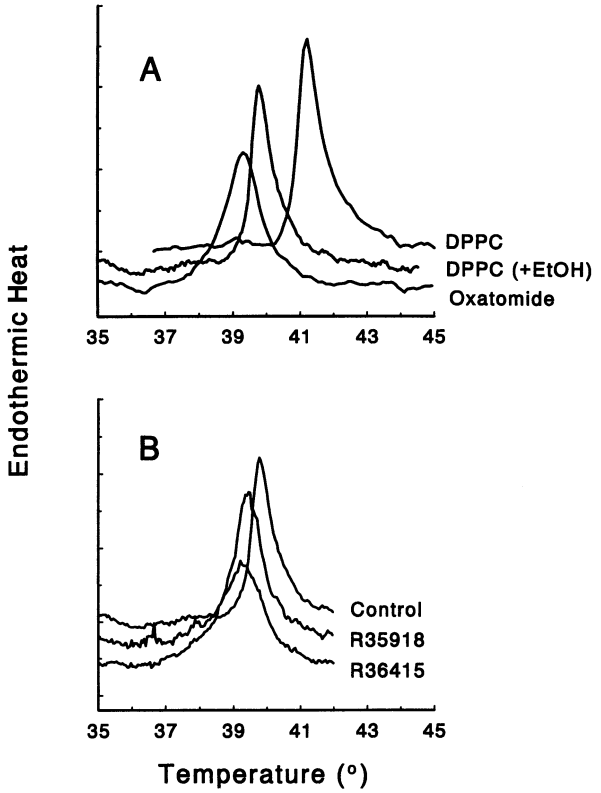

FIG. 2. DSC thermograms of DPPC liposomes (final lipid concentration 2.7 mM) in 0.1 M Tris buffer, pH 7.4. (A) In the absence of ethanol (DPPC), in the presence of 4% ethanol (control), and in the presence of oxatomide. (B) In the presence of R35918 and R36415. The drug to phospholipid molar ratio is 3.7%. These tracings are representative of at least three independent experiments.

Membrane Fluidity of DPPC and EPC Liposomes, and RBL Cells

Additional information on the effect of oxatomide and its derivatives on bilayers was obtained from measurement of membrane fluidity by means of polarization fluorescence using the fluorescent probe TMA-DPH, which has been widely used to study membrane fluidity [19, 20]. TMA-DPH is located at the interface of the phospholipid headgroup and the acyl chains of membranes [21]. The anisotropy value (r) of the probe reflects the packing and rigidity of membrane lipid fatty acid chains, with $r = 0.4$ for completely rigid systems [22]. We used fluorescence anisotropy to determine the rigidity of DPPC and EPC liposomes as well as of RBL cell membranes.

The r values in EPC and DPPC liposomes were 0.236 and 0.348, respectively at 37° when corrected for scattering (extrapolated to zero). Under these conditions, r is independent of the membrane concentration [11]. These results indicate that EPC liposomes have a more fluid membrane compared to DPPC liposomes, due to the heterogeneous character of the acyl chains and the unsaturated fatty acyl chains of EPC. The r value for TMA-DPH in RBL cells was 0.285 when corrected for scattering. Apparently, the membrane of the RBL cells has a more fluid character compared to the homogeneous, densely packed DPPC membranes.

No effect of the drugs on the anisotropy of EPC membranes was observed at a molar drug percentage of 5.5%. A slight increase in the anisotropy value could be observed for some compounds when the molar drug percentage was increased to 8.25%. However, the compounds did show an effect on the fluidity in the homogeneous DPPC membranes (0.6 mM). Results are shown in Table 3. Oxatomide decreased the r value, which indicates that the membrane fluidity increased. All other compounds also decreased the anisotropy of DPPC liposomes significantly, except for compound R35918. No significant effects of the drugs on fluorescence anisotropy in the intact RBL cells could be detected. It appears that more fluid membranes, like those of EPC and RBL cells, are less sensitive to detection of drug-induced fluidity changes than the more rigid DPPC vesicles.

The good linear correlation between the effect of the drugs on the fluorescence anisotropy and the phase transition in DPPC liposomes, shown in Fig. 3 ($N = 9$, $r^2 = 0.81$, $F = 29.22$, $P = 0.001$), indicates that both assays reflect the same molecular events, namely the perturbation of the packing and interactions between the acyl chains in the DPPC membrane. Drugs effective in decreasing the r value had the largest increase in $T_{1/2}$, which indicates that these drugs are most effective in increasing membrane fluidity.

Haemolysis of Erythrocytes

To study the drug–membrane interaction on a cellular level, we measured the effect of the antiallergic compounds on the haemolysis in erythrocytes [6]. From a concentration effect curve with oxatomide, it appeared that at 10 μM

TABLE 3. Influence of oxatomide and derivatives on fluorescence anisotropy r values of TMA-DPH in DPPC liposomes (0.6 mM lipid) in Tyrode's buffer, pH 7.4

Compound	r value* (DPPC)	Log P_{calc}	Log P_{exp}
DPPC (control)	0.262 ± .002		
+R35918	0.258 ± .004	5.25	3.79
+R37685	0.257 ± .002†	6.29	4.68
+Oxatomide	0.256 ± .002†	4.73	4.73‡
+R37477	0.255 ± .001†	5.77	4.70
+R36262	0.251 ± .003†	5.92	ND§
+R34058	0.241 ± .002	5.01	ND
+R35873	0.238 ± .002‖	5.53	ND
+R36599	0.236 ± .001‖	5.44	ND
+R36415	0.231 ± .005‖	5.44	ND

Drug to phospholipid molar percentage is 5.5%. Also included are calculated and experimentally determined log P values. Values are means ± SD from at least three independent experiments.

†$P < 0.05$ or ‖$P < 0.001$ versus control.

*Not corrected for light scattering.

‡Calculated log P values are based on this value.

§Not determined.

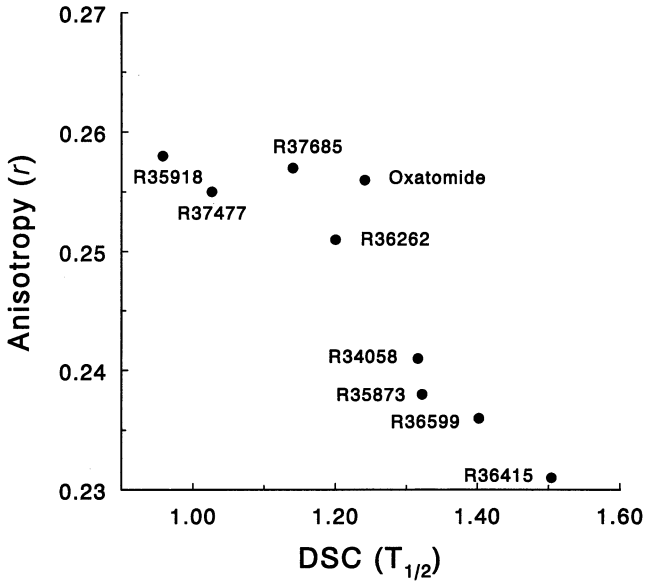

FIG. 3. Relation between width at half height ($T_{1/2}$) from DSC thermograms and *r* value from anisotropy measurements.

haemolysis was almost at a maximum (results not shown). Table 4 shows the effects on hypotonic-induced haemolysis in erythrocytes. Oxatomide and derivatives all demonstrated a strong inhibitory activity in a rather narrow range, from 76% inhibition of haemolysis for R37477 to 93% for R36599.

The $T_{1/2}$ values from the DSC analysis (Table 3) and the inhibition of haemolysis from erythrocytes (Table 4) appear to be correlated ($N = 7$, $r^2 = 0.59$, $F = 7.20$, $P = 0.04$). The larger the membrane disturbance measured in liposomes, the more inhibition of hypotonic-induced haemolysis from erythrocytes is observed. However, it must be noted that the differences in inhibition of haemolysis were in a very narrow range.

TABLE 4. Influence of some oxatomide analogues on hypotonic-induced erythrocyte haemolysis and Ca^{2+} influx

Compound	Inhibition of haemolysis	Ca^{2+} influx*
R37477	76.6 ± 4.9	11.2 ± 2.6
R37907	80.9 ± 2.5	10.9 ± 0.3
Oxatomide	82.0 ± 3.1	16.1 ± 6.0
R35918	83.1 ± 3.1	13.7 ± 2.7
R34058	85.6 ± 2.7	29.6 ± 6.7
R35873	86.4 ± 0.6	43.1 ± 5.7
R36415	88.5 ± 3.3	39.3 ± 10.6
R36599	92.9 ± 3.3	54.9 ± 5.5†

Effect of 30 μM compound on the inhibition of erythrocyte haemolysis and on the $^{45}Ca^{2+}$ influx in RBL cells upon antigen activation. $^{45}Ca^{2+}$ influx is expressed as a percentage of control (no drug). Values are means ± SD from at least four determinations.

*Data from Ref. 5.

†Thapsigargin activation of RBL cells.

TABLE 5. Influence of pH on inhibitory activity of oxatomide (30 μM) on the release of β-hexosaminidase (β-hexo) and $^{45}Ca^{2+}$ influx upon activation of RBL cells with antigen

Assay	pH 7	pH 7.5	pH 8
β-Hexo (%)*	35.0 ± 1.3	28.6 ± 1.7	19.3 ± 1.50
Control $^{45}Ca^{2+}$ influx (dpm)	1760 ± 170	2500 ± 300	3300 ± 500
$^{45}Ca^{2+}$ influx (%)	22.2 ± 2.0	16.4 ± 3.2	11.9 ± 0.2

Values are expressed relative to control (no drug present). Also included are control values for $^{45}Ca^{2+}$ influx in dpm.

*Data from [7]. No effect of pH on control release value (59.1 ± 2.3% of total β-hexo content).

Effect of pH on Oxatomide Activity

If the cellular effects of oxatomide are primarily caused by interference of the drug at the membrane level, it would be expected that these effects are pH-dependent. The two piperazinyl nitrogens, which are potential protonation sites, are located in the hydrophobic moiety of the molecule. This moiety is assumed to interact with the acyl chains of the membrane bilayer [7]. The pK_a value of oxatomide in water is 7.1.* The ionization state in the bilayer environment is not necessarily identical to that in bulk water, because the apparent pK_a is affected by both the dielectric constant and the pH at the surface of the bilayer, with the pK_a possibly being 1–2 units lower [16].

We performed $^{45}Ca^{2+}$ influx and β-hexosaminidase release experiments in a pH range from 7 to 8. From previous control experiments and comparison with experiments assaying the intracellular Ca^{2+} concentration, we conclude that the $^{45}Ca^{2+}$ influx assay is representative for influx of extracellular Ca^{2+} into the cell for the short applied time intervals [4]. Control values for β-hexosaminidase did not change within the applied pH range. The $^{45}Ca^{2+}$ influx under control conditions (no drug present) increased with pH (Table 5).

It appears that upon an increase in pH from 7 to 8, the inhibition by oxatomide of both the antigen-triggered exocytosis, as well as the $^{45}Ca^{2+}$ influx in RBL cells, is increased (Table 5). This suggests that the uncharged species of oxatomide is the active form.

DISCUSSION

In previous studies, we have shown the major role of the inhibition of Ca^{2+} influx via SOC channels by oxatomide and derivatives in the exocytosis process [4, 5]. SOC channels have only recently been described and are not yet well characterized [23]. Here, we studied drug-induced membrane distortions and their role in the inhibition of Ca^{2+} influx. The membrane effects of a series of oxatomide derivatives was studied by fluorescence anisotropy. We were not able to observe significant differences in membrane fluidity induced by the compounds in RBL cells. Aloui *et al.*

* Tollenaere JP, Janssen Research Foundation, Beerse, Belgium, personal communication.

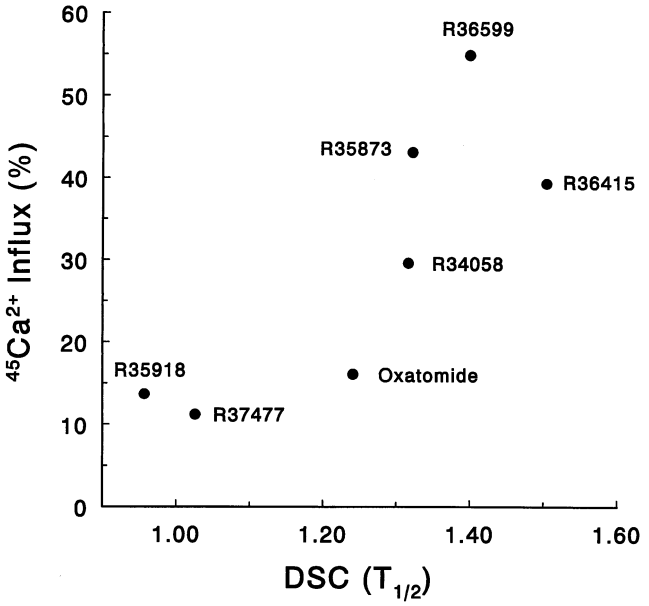

FIG. 4. Relation between width at half height ($T_{1/2}$) as determined with DSC in DPPC liposomes and the inhibition of the antigen-induced $^{45}Ca^{2+}$ influx in RBL cells.

[24] have reported that effects of anaesthetics on anisotropy values in leukocytes were only visible at 1 mM. In RBL-2H3 cells, Chang *et al.* [8] were not able to detect differences in anisotropy after changing the lipid composition of the cellular membrane. From this, we conclude that the cellular model cannot be used to study effects of the drugs on the membrane.

Instead, we used artificial membranes (liposomes) to study the influence of the compounds by DSC and fluorescence anisotropy assays. A good correlation was observed between distortion of the phase transition and membrane fluidity of DPPC liposomes (Fig. 4). From these two independent assays, we conclude that the drugs influence interactions between the acyl chains in the DPPC bilayer, which facilitates transitions from the gel-to-liquid crystalline phase and increases the fluidity of the membrane. The changes in the DSC thermograms upon addition of drug are in agreement with changes due to localization of the drugs in the vicinity of the first eight carbon atoms of the acyl chain [25]. Such location is in agreement with the amphiphilic properties of oxatomide and its derivatives. In DPPC liposomes the compounds are assumed to penetrate into the membrane. The more polar benzimidazolone moiety is expected to be located near the hydrophilic head groups of the phospholipids, and the lipophilic unprotonated diphenylmethyl piperazinyl moiety is expected to be located at the upper part of the hydrophobic acyl chains. Insertion of the uncharged diphenylmethyl piperazinyl moiety into the membrane is also in agreement with the observed pH dependence (Table 5).

We have calculated log P values (Table 3), the molar volume, and the water-accessible surface (Connolly surface) of the drugs (results not shown). Although there was an excellent correlation between calculated log P values and the molecular surface and volume, this was not correlated with the disturbance of the membranes (e.g. for log P_{calc} versus DSC ($T_{1/2}$) $r^2 = 0.05$). An extrathermodynamic relation between partition in organic bulk phase/buffer systems and biological membrane/buffer systems is frequently observed and forms the basis for quantitative structure–activity relationship (QSAR) analysis [26, 27]. However, the antiallergic drugs studied here have extremely high log P values (Table 3). Jain and Wu [25] showed that an increase in the log P value above 3.5 did not affect the $T_{1/2}$ of the phase transition. Therefore, we do not ascribe changes in the thermograms to changes in lipophilicity and the amount of penetration into the membrane, but rather to differences in structural effects of the drugs on the membrane bilayer.

The effect on the membrane disturbance can be qualitatively explained by the structure of the drug. Compounds with a longer alkyl chain (R35918, R37477, R37685) are expected to fit well between the lipid acyl chains. These drugs make the bilayer relatively rigid (Fig. 3). On the other hand, a structure with a methyl side chain in the alkyl moiety (R35873), which will not fit as well between the acyl chains, increases the fluidity of the DPPC membrane. The drugs with the bulkier chlorine substituents in the benzimidazolone moiety (R36599 and R36415) show a remarkably increased membrane disturbance. However, introduction of a bulky substituent on the nitrogen of the benzimidazolone head group (R36262) hardly affects the membranes.

Remarkably, the experimental log P value for compounds with a longer alkyl chain ($N \geq 4$) are lower than the calculated value starting from oxatomide as a parent compound (Table 3) (e.g. for R35918, $N = 4$, log P_{calc} is 5.25, log P_{exp} 3.79). A likely explanation for this is the facilitation of the folding of the molecule due to a longer flexible alkyl chain. Preliminary molecular mechanics calculations *in vacuo* demonstrated that van der Waals interactions between the benzimidazolone moiety and a phenyl of the benzhydryl moiety are possible. Modeling studies of oxatomide show that various low energetic conformations of the alkyl chain may exist [28].

Membrane effects of the drugs on a cellular level, studied as haemolysis of erythrocytes, correlated with effects in DPPC liposomes. This suggests that effects measured in artificial membranes are relevant for cellular membranes. Erythrocytes originate from the same haemopoietic stem cell as basophils such as RBL cells, and the lipid composition of the plasma membranes of these cells shows similarity [8, 29].

Having demonstrated that the drugs affect the membrane bilayer structure, we examined a possible role for membrane disturbance in regulating Ca^{2+} influx, which is responsible for inhibition of mediator release [4, 5]. The relation between antigen-induced $^{45}Ca^{2+}$ influx and disturbance of DPPC membranes is shown in Fig. 4. Linear regression of this relation yields an r^2 of 0.85 ($N = 7$, $F = 34.33$, $P = 0.001$), notwithstanding the differences between model

membranes and cells. This demonstrates that the inhibition of the Ca^{2+} influx can be largely explained by effects of the drugs on membranes. Compounds active in inhibiting Ca^{2+} influx induce a more rigid bilayer structure than less active drugs. Membrane fluidity as such is not sufficient to explain the effect on Ca^{2+} influx, as without drug the membrane is most rigid (Table 5). Therefore, other consequences of insertion of the drugs in the bilayer also have to be taken into account, e.g. membrane expansion [26].

Membrane distortion is not sufficient for inhibition of Ca^{2+} influx as appears from the antiallergic drug meclozine, which is structurally related to oxatomide in its having a methyl head group [7]. This drug does not inhibit Ca^{2+} influx, but has a large effect on the DSC thermogram. This indicates that in addition to aspecific membrane interactions, specific interactions of the benzimidazoline head group are also involved. The decreased activity of compounds with a chlorine in the head group (R 36599 and R 36415) is in line with this conclusion.

Based on the results of this and previous studies [4, 5], we conclude that oxatomide is located in the membrane bilayer with the uncharged diphenyl piperazinyl moiety between the upper part of the acyl chains and the benzimidazolone head group between the polar lipid head group. This location of the drugs induces membrane distortion, e.g. membrane expansion in combination with changes in rigidity. This membrane distortion might affect the dynamics and activity of the SOC channel. In addition, as pointed out above, specific interactions of the benzimidizalone moiety of the drugs contribute to inhibition of Ca^{2+} influx. The SOC channel appears to be sensitive to changes in the membrane environment as membrane depolarization inhibits Ca^{2+} influx [10].

As the concentration needed to induce inhibition of Ca^{2+} influx and distortion of membranes seem to be rather high compared to clinical applied concentrations [30], direct comparison of the effects *in vitro* to those *in vivo* is limited. However, decrease in histamine release by oxatomide is observed *in vivo* and is considered to contribute to the antiallergic effect of oxatomide [31, 32]. This and our previous studies [3–5] demonstrate that on a cellular level the inhibition of mediator release is mainly a consequence of inhibition of SOC channels by the drugs for which distortion of membrane structures seems to be important.

Aloui *et al.* [24] reported the interesting observation that leukocyte membranes of allergic patients are more sensitive to drug-induced changes than those of a control group. This increased membrane fluidity, in line with our observation, will activate Ca^{2+} influx and subsequent mediator release in mast cells.

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