Compound 48/80-induced permeability change in liposomal membrane

Takashi Katsu, Kenji Tasaka and Yuzaburo Fujita

Faculty of Pharmaceutical Sciences, Okayama University, Tsushima, Okayama 700, Japan

Received 18 October 1982; revision received 6 December 1982

The action of compound 48/80 (a mixture of condensation products of *p*-methoxy-*N*-methylphenethylamine with formaldehyde) on liposomal membranes was studied by means of K^+-loaded liposomes and a K^+ ion-selective electrode. Prompt efflux of K^+ was detected when 48/80 was added to the negatively charged liposome suspension, while the monomer of 48/80, *p*-methoxy-*N*-methylphenethylamine, did not release K^+ from the same liposomes. The mechanism for the action of 48/80 is discussed in comparison with that of a polymyxin, well known as an antibiotic acting on bacterial membranes.

Compound 48/80 *Membrane permeability* *Liposome* *Ion-selective electrode* *K^+ marker* *Polymyxin B*

1. INTRODUCTION

Compound 48/80 produces a potent histamine release from mast cells [1–3]. This compound is synthesized by condensing *p*-methoxy-*N*-methylphenethylamine with formaldehyde [4], and is thought to be a mixture of linear copolymers. Dialysis and gel filtration [5], and ^{13}C NMR studies [6] have shown that the most active polymers are probably the hexamer (fig.1). Although the detailed molecular mechanism of histamine release due to 48/80 has not yet been clarified, recent ESR studies using spin-labelled 48/80 have shown that an initial interaction may take place between 48/80 and anionic proteins on the surface membrane [7]. Aside from mast cells, several reports have described the effects of 48/80 not related to histamine secretion; one of the interesting actions of 48/80 is an antimicrobial activity, exhibited as the inhibition of the growth of protozoa, bacteria, and fungi [8]. Such action of 48/80 is similar to that of a polymyxin, well known as an antibiotic [9]. The mechanism of the antimicrobial activity of the polymyxin has been discussed in detail [9], and the polymyxin is also known to release histamine, as in the case of 48/80 [10]. The polymyxin molecule interacts preferentially with acidic phospholipid and lipopolysaccharide, and then alters the structure and permeability of the bacterial membranes [9].

We were interested in the structure-resemblance between 48/80 and polymyxin; both are polycation

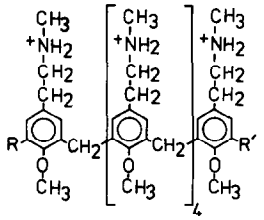

Fig.1. Structure of active constituent of 48/80.

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; CHOL, cholesterol; DCP, dicetylphosphate; SA, stearylamine; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid

Published by Elsevier Biomedical Press
00145793/83/0000–0000/$3.00

with an appropriate lipophilic character. This suggests that 48/80 may also interact with negatively charged lipid constituents of the membrane to induce the permeability changes. In this communication, we report on the permeability change of liposomal membrane induced by 48/80. We reported the liposomes entrapped K^+ as a marker, and the release of K^+ was measured with a K^+ ion-selective electrode (K^+ ISE). It was found that the rapid efflux of K^+ was observed when 48/80 molecules interacted with negatively charged liposomes.

2. MATERIALS AND METHODS

Liposomes were prepared as previously described [11]. In brief, a dried thin film prepared with 10 μmol DPPC and appropriate quantities of various other substances (CHOL, DCP or SA), with molar ratios as indicated in the text, was swollen at 55°C in 0.15 M KCl solution buffered with 5 mM HEPES adjusted to pH 7.4 by addition of KOH. To remove the untrapped K^+ marker, the resulting multilamellar liposomes were washed with a fresh HEPES-buffered isotonic salt solution (0.15 M NaCl plus 5 mM HEPES adjusted to pH 7.4 with NaOH) and centrifuged (22000 × g for 10 min) at 15°C, 6-times. The final pellet of liposomes was dispersed in 1 ml of NaCl–HEPES buffer to yield 10 mM phosphatidylcholine liposome suspension. The background level of K^+ was reduced to 10^{-5} M.

A K^+ ISE was constructed as in [11]. Fig.2 shows the response of K^+ ISE in NaCl–HEPES buffer. The electrode provided a Nernstian response from 0.1–10^{-5} M of K^+. The interference of 48/80 on the electrode was negligible within the concentration range studied.

3. RESULTS AND DISCUSSION

Fig.3 shows the effect of 48/80 on the K^+ release from the liposomes prepared with DPPC, CHOL and DCP in a molar ratio of 1:0.75:0.1. Experiments were carried out at room temperature in a beaker (10 ml) containing 1 ml NaCl–HEPES buffer. Addition of a liposome aliquot (0.2 ml) produced a slight increase of the K^+ concentration due to the untrapped and remaining K^+ in the suspension. Addition of 0.1 ml of 48/80 (5×10^{-6} g) induced a rapid increase of the K^+ concentration.

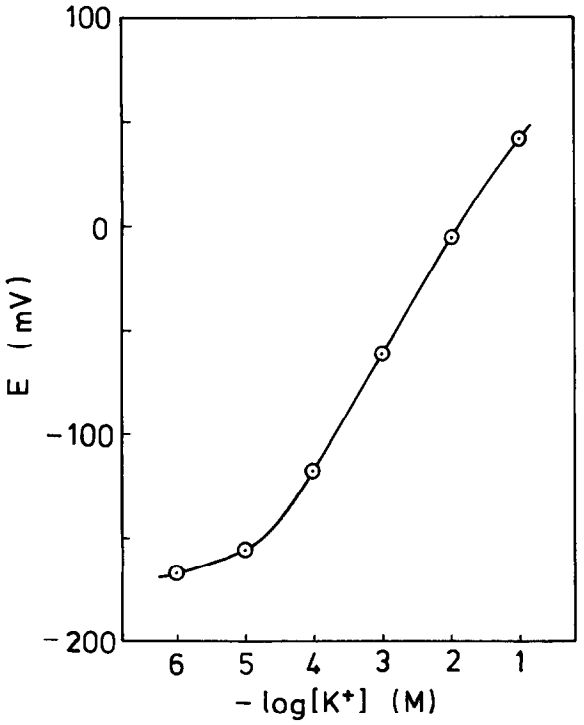

Fig.2. Response of K^+ ISE in NaCl–HEPES buffer (pH 7.4).

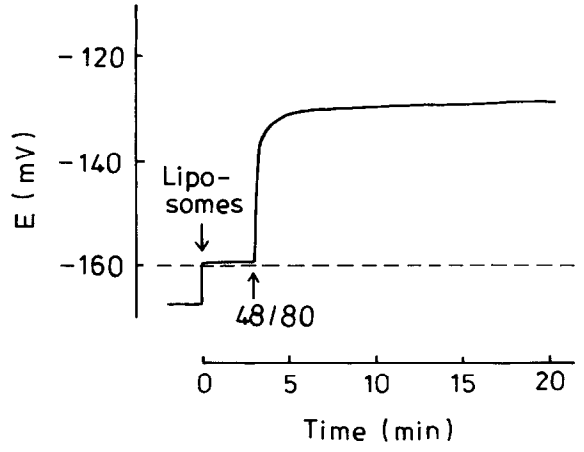

Fig.3. Potential change due to K^+ release upon addition of 48/80. At zero time, 0.2 ml of liposome aliquot (prepared from DPPC, CHOL and DCP in a molar ratio 1:0.75:0.1) was added to 1 ml of NaCl–HEPES buffer. After 3 min, 0.1 ml of 48/80 (containing 5×10^{-6} g) was added.

The dose response curve of 48/80 is shown in fig.4, along with those of the other two types of liposomes, prepared respectively with either stearylamine as a positively charged or uncharged lipid components. The results clearly demonstrate that liposomes made of lipid with negative charge are sensitive to 48/80, while the other liposomes made of positively charged lipid do not respond to 48/80. The figure also shows that the monomer of 48/80, *p*-methoxy-*N*-methylphenethylamine, did not provode any release of K^+ from the liposomes prepared with dicetylphosphate.

In the range of temperature tested (8–38°C), the action of 48/80 showed the same rapid efflux of K^+, though the rate became slightly slower at lower temperatures. In addition, K^+ release was also observed in the liposomes without cholesterol at room temperature. These facts seem to indicate that 48/80 does not act as a carrier-type ionophore, facilitating the transport of ions by diffusion across the membrane, since the carrier-type ionophore shows a marked temperature dependence [12]. In accordance with this assumption it has been demonstrated that when 48/80 coated Sepharose beads were exposed to mast cells, histamine release was provoked, similarly to the application of 48/80 in the solution [13].

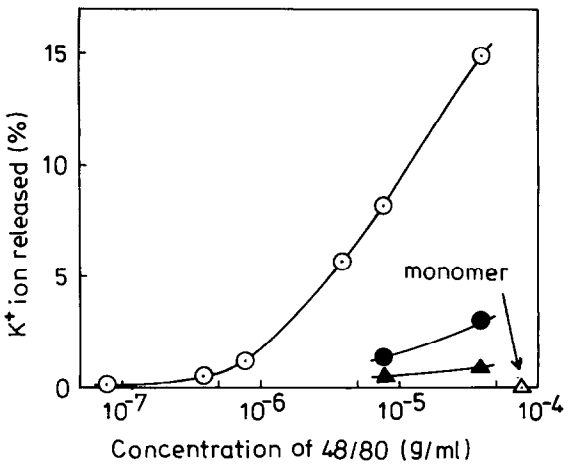

Fig.4. Release of K^+ from various liposomes by the action of 48/80. The percentage of K^+ release was determined from the marker efflux within 10 min after 48/80 addition. The total amount of the K^+ marker was determined by disrupting the liposome with an ultrasonicator (Bransonic B-220, 125W) for 15 min at 55°C. Liposome composition (molar ratios): (○—○) DPPC, CHOL and DCP (1:0.75:0.1); (●—●) DPPC and CHOL (1:0.75); (▲—▲) DPPC, CHOL and SA (1:0.75:0.1). By addition of the monomer of 48/80 (10^{-4} g), K^+ was not released at all from the liposomes composed of DPPC, CHOL and DCP in a molar ratio of 1:0.75:0.1.

The following reason for the K^+ release is considered. 48/80 may easily be incorporated into the hydrophilic region of the liposomal membrane since it provides high solubility in alcoholic solutions and this process may be accelerated by the electrostatic interaction with negatively charged lipid molecules. Insertion of such highly ionized polymer into the lipid bilayers may cause some region in which lipid arrangement in the membrane will be disordered, thus results in the K^+ release from the liposomes. The membrane disorder as a result of the interaction with negatively charged lipids has also been discussed in the action of polymyxin with biological and artificial membranes [9,14]. However, the most remarkable difference in the action of both compounds depends on the composition of liposomes. Little K^+ release was observed with 7.7×10^{-6} g/ml of polymyxin B from the liposomes with the same composition (DPPC, CHOL and DCP in a ratio of 1:0.75:0.1); this result is consistent with the observation carried out with glucose as a marker [15]. Without cholesterol, polymyxin disrupts the lipid membrane effectively [15]. It seems to be reasonable to assume that the cholesterol molecule filled in the space between the lipid molecules may hinder the penetration of polymyxin molecule into membranes. Fig.5 shows the permeability change of the liposome composed of DPPC and DCP in a molar ratio of 1:0.1. The time sequence of K^+ release by polymyxin B was gradual compared with 48/80. This difference may be due to much lower solubility of polymyxin in phospholipid bilayer; the lower solubility of polymyxin in alcoholic solution may imply the poor incorporation of polymyxin into the hydrophilic region. Accordingly, the action of polymyxin is considered to be initiated by the electrostatic interaction with the negatively charged lipid molecules. Then a hydrophobic paraffin tail penetrates gradually into the lipid bilayer, possibly inducing a deformation of the bilayer structure.

It may be worthwhile to note here in order to explain the mechanism of the action of 48/80 fur-

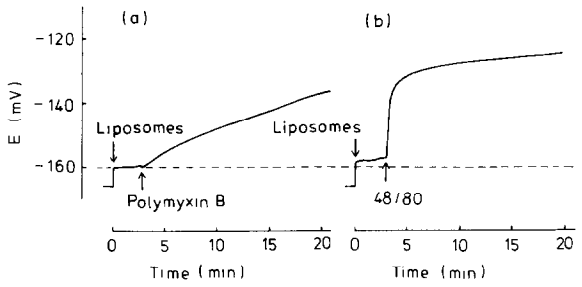

Fig.5. Potential change due to K^+ release upon addition of polymyxin B (a) and 48/80 (b). Liposomes were composed of DPPC and DCP in a molar ratio of 1:0.1. Liposomes, and polymyxin B or 48/80 were added in the manner shown in fig.3. The same concentration of 48/80 and of polymyxin B was applied (7.7×10^{-6} g/ml).

ther, that the addition of 10 μg/ml of 48/80 has only induced 10% efflux of K^+ marker (fig.4). This percentage corresponds to the amount of K^+ entrapped in the outermost envelope of multilamellar liposome [16], indicating that 48/80 interacts only with the outermost membrane surface, while inner membranes are not effectively attacked. This observation is of interest in relation to the initial stimulation of 48/80 on the mast cells. Below the concentration of 10 μg/ml, the stimulation of 48/80 on mast cells is known as a non-cytotoxic reaction, which is different from cytotoxic action, as produced by some detergents, for instance, Triton X-100 [17]. The finding that Triton X-100 disrupts the multilamellar liposomes, releasing the majority of K^+ entrapped, is in accordance with the toxic action of this compound on mast cells. In the case of mast cells, the non-cytotoxic action of 48/80 has been initiated by the interaction with the acidic parts of proteins on the cytoplasmic membrane [7].

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