Biochemical Pharmacology, Vol. 36, No. 4, pp. 481–487, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

THE INFLUENCE OF THE ANTIVIRAL DRUGS AMANTADINE AND RIMANTADINE ON ERYTHROCYTE AND PLATELET MEMBRANES AND ITS COMPARISON WITH THAT OF TETRACAINE

EDWIN DONATH, ANDREAS HERRMANN, W. TERENCE COAKLEY,* THOMAS GROTH, MARCEL EGGER and MARTIN TAEGER

Humboldt-Universitat zu Berlin, Sektion Biologie, Bereich Biophysik, 1040 Berlin, Invalidenstr. 42, German Democratic Replublic, and * University College, Department of Microbiology, Cardiff, CF2 1TA, Wales, U.K.

(*Received* 6 *May* 1986; *accepted* 9 *September* 1986)

Abstract—The influence of the antivirus drugs amantadine and rimantadine and of the anionic analogue l-adamantane-carboxylic acid on a range of properties of human erythrocyte membrane and of thrombocytes has been compared with the effect of the local anaesthetic tetracaine. At low antiviral drug concentrations the abilities of the drugs to induce erythrocyte shape change and suppress osmotic haemolysis were quantitatively proportional to their clinical potenty (rimantadine more effective than amantadine at the same concentration). Rimantadine was also more effective than amantadine in suppressing influenza virus-erythrocyte fusion and viral induced haemolysis. The antiviral drug effects were qualitatively similar to those induced by tetracaine. At the quantitative level, tetracaine was more efficient than the antiviral drugs in inhibiting osmotic haemolysis, virus membrane fusion and platelet aggregation. In the absence of any specificity of the antiviral drug effects we argue for a lysosomotropic mode of drug action, i.e. that the drugs modify virus–membrane interactions by changing the endosomal or lysosomal pH.

The derivatives of adamantane amantadine (l-amino-adamantane hydrochloride) and rimantadine (α-methyl-l-adamantanemethylamine hydrochloride) have been widely used clinically for the prevention of influenza and other virus infections [1]. However, at present there is still a variety of hypotheses about the mechanism of the antiviral effect. It has been reported that the drugs can affect the early stages of the adsorption of viruses on the membrane and their penetration into the cell [2] and can affect later stages involving the M-protein [3–7]. Recent studies with bilayer lipid membranes revealed that these drugs are indeed capable of modifying the interaction between virus proteins and lipid membranes [8]. It has also been shown by ESR-investigations that addition of antivirus drugs slightly influences the structure of the lipid phase of the virus envelope membrane [9]. Amphiphilic adamantane derivatives are capable of modifying the properties of artificial bilayer lipid membranes [10] and of thrombocyte membranes [11].

Summarising these findings it becomes clear that the adamantane derivatives have typical drug effects on membranes. It is also evident that we are far from understanding the mechanism of the prevention of virus infection [12]. Since the process of virus penetration and self-assembly includes several steps involving both intracellular and plasma membranes, we decided to investigate the influence of these antivirus drugs on biological membranes. As the molecular structure of amantadine and rimantadine is that of typical amphipaths, we compared their influence on human red cells and thrombocytes with the effects of the local anesthetic tetracaine.

MATERIALS AND METHODS

(i) *Observation of cell fragmentation during heating.* At 50°, the thermal denaturation temperature of the cytoskeletal protein spectrin [13], the erythrocyte shape becomes unstable. A surface wave grows on the rim of the cell and vesicles pinch from the crests of the growing waves [14]. Erythrocytes were obtained by finger prick and collected into 145 mM NaCl, 5 mM phosphate, pH 7.4. The cells were washed three times. The resulting cell suspension was held for 30 min at room temperature. The cells were then centrifuged and were resuspended in drug-containing solutions and held for approximately 10 min at room temperature. Observations of erythrocyte shape after drug addition showed that after 2 min the induced shape change remained nearly stable for 60 min.

The apparatus for heating cells at a known rate has been described by Crum *et al.* [15]. In the present work microcapillaries of 100 μm pathlength (Microslides, Camlab. Ltd.) were used. A thermocouple was inserted into a microslide filled with physiological saline which was then placed in the immediate neighbourhood of an empty microslide. The centres of both microslides were surrounded by external saline and a cover slip was placed on the saline. The whole system was heated at 0.5 K/sec by applying 20 kHz frequency current from an oscillator. When the temperature had reached about 30° a drop of the cell suspension was brought near an open end of the empty microcapillary and capillarity pulled the suspension into the microslide. Heating was continued and the process of cell fragmentation process at 50° was video recorded using Nomarksi differential

interference contrast microscopy. The number of wave crests per cell rim was scored and $\bar{W}$, the average number of waves for a given cell suspending phase, was calculated from observations on about 100 cells.

(ii) *Erythrocyte preparation for hemolysis and fusion experiments.* Citrate blood 1–2 days old from the blood bank (Berlin-Lichtenberg) was centrifuged at 500 *g* for 5 min to remove plasma and the buffy coat. Two subsequent washings in phosphate buffered (5 mM, pH 7.4) physiological saline at 2000 *g* for 10 min followed.

(iii) *Osmotic hemolysis.* [$NaCl_{50}$], the sodium chloride concentration resulting in 50% hemolysis was determined by means of measuring hemolysis in a series of NaCl concentrations buffered with 5.8 mM phosphate (pH 7.4). Erythrocytes which had been resuspended (about 1% w/w) in isotonic phosphate-buffered NaCl containing drug were incubated for 5 min at room temperature. A volume of 5.8 mM phosphate buffer with the same drug concentration was added to reduce the sodium chloride concentration to [$NaCl_{50}$]. After 5 min the cells were centrifuged at 1500 *g* for 5 min. The extinction value of the supernatant was determined at 540 nm and expressed as a percentage of the extinction after total hemolysis.

(iv) *Virus-induced fusion.* Influenza virus A_0PR8 was kindly supplied by the Institut für Angewandte Virologie (Berlin-Schoneweide) at an initial concentration of 52 mg protein/ml (1.2×10^6 HAU/ml). Erythrocytes were resuspended in 150 mM NaCl, 20 mM Na-acetate, pH 5.2, containing various virus concentrations to a final haematocrit of 2.5%. The cells were incubated at 4° for 10 min and at 37° for 30 min. The sample was then diluted to a haematocrit of 0.1%. The particles were counted using phase contrast microscopy ensuring also counting of ghosts. The fusion index

$$\text{fusion index} = \frac{\text{particle count in control}}{\text{particle count after fusion}} - 1$$

[16] was calculated.

(v) *Virus-induced hemolysis.* Following the above incubation the cells were washed at 2000 *g* for 10 min. The supernatant (0.5 ml) was diluted in 2 ml of 0.5% NH_4OH and the extinction was measured at 540 nm. The reference value was determined by total hemolysis in NH_4OH.

(vi) *Thrombocyte preparation.* Venous blood was drawn using siliconised needles. The first 2–3 ml was immediately discarded. The next 9 ml was collected into a siliconised glass or plastic tube containing 1 ml 3.8% w/v sodium citrate. A very gentle shaking followed immediately. The blood was stored for 1 hr at room temperature. Platelet rich plasma (PRP, about 3.5×10^5 platelets/μl) was prepared by centrifugation at 4°, 200 *g* for 10 min. Another 10 min centrifugation at 300 g yielded platelet poor plasma (PPP) which was later used to prepare dilutions of PRP. The platelets were counted using a Thoma-Chamber. After 2 min gentle stirring at 37°, 20 μl of the aggregation-inducing solution was added and subsequently the change of light transmission was continuously recorded. We used either collagen (Kollagenreagenz Horm, Hormon-Chemie, Munchen) or Test-Kollagen (Behringwerke AG, Marburg) in concentrations of 0.25–4.0 μg/ml or ADP (Reanal, Budapest) in concentrations of 0.31–5.0 mM. Platelet aggregation was studied using a KZM-1 (MLW Labortechnik, Ilmenau). As a measure of aggregation we used the difference between light transmission of PRP and PPP. The measuring chamber contained 500 μl of suspension.

(vii) *EPR measurements.* Erythrocyte ghosts (in PBS at 4°) were prepared according to the procedure of Dodge *et al.* [17]. Proteolytic activity in ghost preparations was inhibited by addition of 1 mM PMSF. Forty micrograms of maleimide (MAL-6) spin label were added per mg of ghost protein and the ghosts were held for 2 hr at 4° [18]. The excess of spin label was removed by six washings. After this, no EPR signal could be detected in the supernatant. The EPR spectra were recorded by means of a ZWG 231 spectrometer with variable temperature adjustment using a flat quartz cell at 1 G modulation, 20 mW.

A typical EPR spectrum of human erythrocyte ghosts labelled with MAL-6 is given in Fig. 1. It consists of two components. There is a broad-line component, s_{+1} corresponding to a strongly immobilised site and a narrow-line component, w_{+1}, corresponding to a weakly immobilised site which is not caused by non-specific incorporation of the spin label into the membrane [19]. After Fung [20], the amplitude ratio w_{+1}/s_{+1} was used to characterise changes in the state of membrane proteins. Since this ratio is very sensitive to differences between various donors we compared for each donor the values before and after drug treatment. The drugs were added 5 min prior to the EPR measurements.

(vii) *Drugs.* The effects of the antivirus drugs, amantadine (Serva) and rimantadine (Inst. Organic Chemistry, Acad. Sci. U.S.S.R., Riga), and of an analogue without therapeutic effect (sodium and potassium salts of 1-adamantane-carboxylic acid (Inst. Electrochem., Acad. Sci. U.S.S.R., Moscow)) were tested. The effects were compared with those of tetracaine (Sigma). In some cases the influence of chlorpromazine (Sigma) and indomethacin (Sigma) were also studied.

RESULTS

(i) *The influence of drugs on the fragmentation pattern of heated erythrocytes.*

It has recently been shown [21] for a number of anionic and cationic drugs, that the heat-induced fragmentation pattern of red cells can be used to quantify the effects of drugs on cell shape. If $\bar{W}$, the average number of waves per cell rim, is measured as a function of the drug concentration, one obtains an exponential decrease (with an associated constant of proportionality, k) to a limiting value for cationic drugs and an increase for anionic drugs [21]. Since we had previously found that the antivirus drugs amantadine and rimantadine both induce stomatocytes under physiological conditions [22] we were interested in comparing the values of k for these drugs with those for anesthetics. Figure 2 shows the experimental data and the best exponential fits for the two cationic antivirus drugs amantadine and

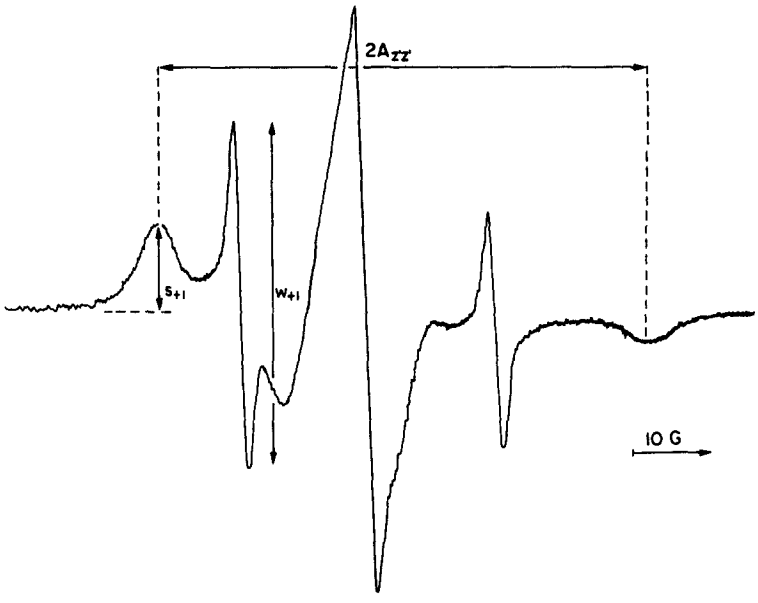

Fig. 1. Typical EPR spectrum of MAL-6 labelled human erythrocyte ghosts in 150 mM NaCl, pH 7.4. $2A_{z'z'}$ is the external peak distance of the strongly immobilised component. w_{+1} and s_{+1} refer to the amplitudes of the weakly and strongly immobilised components.

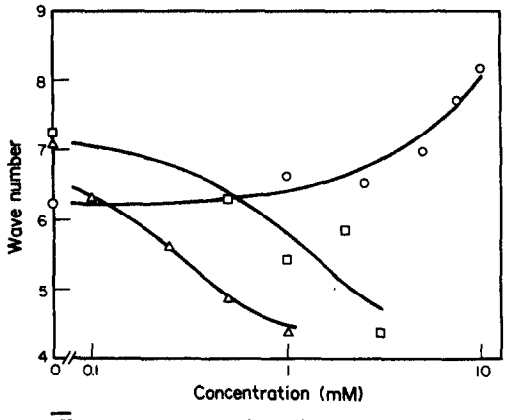

Fig. 2. $\overline{W}$, the average number of surface waves on fragmenting heated erythrocytes as a function of drug concentration: △, rimantadine; □, amantadine; ○, l-adamantane-carboxylic acid. The solid lines represent the best exponential fits; N = 3; SD approximately 0.6. Saturation values of $\overline{W}$ were set at 4.4 and 12.5. The cells were heated in phosphate buffered physiological saline, pH 7.4.

rimantadine. The increase in waviness induced by the anionic analogue l-adamantane-carboxylic acid is also shown. The values of k were: $k_{am} = 0.71 \pm 0.26\,mM^{-1}$, $k_{rim} = 3.34 \pm 0.26\,mM^{-1}$, and $k = 0.035 \pm 0.007\,mM^{-1}$ for amantadine, rimantadine and l-adamantane-carboxylic acid respectively. The neutral analogue adamantanol was also tested. Since this compound is insoluble in water ethanol was used as a solvent for the 80 mM stock solution. Up to final adamantanol concentrations of 1 mM no effect on the fragmentation pattern of the cells was detected. At higher adamantanol concentrations the ethanol started to influence the red cell fragmentation pattern and stability.

In summary, we found that the fragmentation pattern of both, anionic and cationic derivatives of adamantane was very similar to those induced by anaesthetics [21]. Rimantadine has an effect comparable to that of tetracaine ($k = 3.9\,mM^{-1}$ [21]) while l-adamantane-carboxylic acid was much less efficient.

(ii) *Osmotic haemolysis protection.*

It is known that the majority of anaesthetics and tranquilisers protect erythrocytes from colloid-osmotic haemolysis in hypotonic media [23]. Figure 3 shows this effect for the drugs of the present study. Since (i) rimantadine has an approximately 10-fold effect on bilayer membranes [24], (ii) its therapeutic concentration is one-tenth that of amantadine, and (iii) the k_{rim} from the fragmentation experiments was five times larger than k_{am}, one might expect a lower optimum haemolysis protection concentration for rimantadine compared with amantadine. On the contrary, Fig. 3 shows that while at low concentrations rimantadine affords better protection than amantadine, both antiviral drugs have nearly the same maximum protection concentration around 5 mM. The optimum haemolysis protection concentration for tetracaine is below 0.05 mM.

(iii) *Virus-induced haemolysis and fusion inhibition.*

The human erythrocyte (while of course not being the influenza virus target cell) has proved a useful system for studying virus cell interaction [25, 26]. Virus-induced erythrocyte fusion is always accompanied by haemolysis [27]. Consequently, both effects were measured with rimantadine and amantadine and compared with the influence of tetracaine. Figure 4 shows the virus-induced haemolysis inhibition, and Fig. 5 gives the data for the fusion index. We used concentrations of drugs which

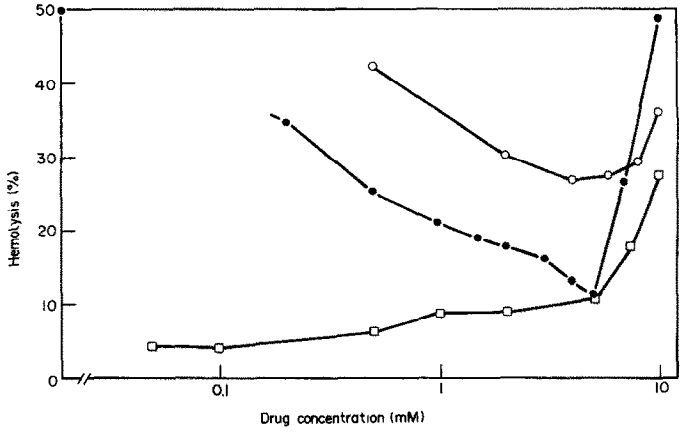

Fig. 3. Human erythrocyte osmotic haemolysis protection as a function of drug concentration: ○, amantadine; ●, rimantadine; □, tetracaine. Note that haemolysis in the absence of drugs was 50%.

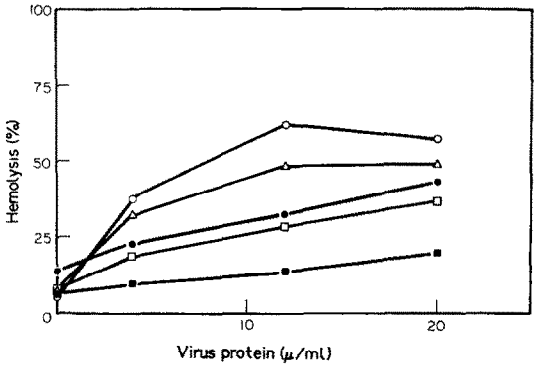

Fig. 4. Virus-induced haemolysis of human erythrocytes in the presence of drugs: ○, control cells; △, 0.05 mM chlorpromazine; ●, 5 mM rimantadine; □, 5 mM amantadine; ■, 5 mM tetracaine, N = 3.

Table 1. Relative increase of the ratio of the amplitudes of the narrow (w_{+1}) and broad (s_{+1}) line components in drug-treated human erythrocyte ghosts compared to untreated (control) ghosts

Drug	Conc. (mM)	$(w_{+1}/s_{+1})_d/(w_{+1}/s_{+1})_c$ At 20°	SD	$(w_{+1}/s_{+1})_d/(w_{+1}/s_{+1})_c$ At 37°	SD
Amantadine	2.5	1.10	0.04	1.13	0.07
Amantadine	5.0	1.16	0.08	1.18	0.02
Rimantadine	2.5	1.16	0.04	1.14	0.11
Rimantadine	5.0	1.21	0.05	1.14	0.08
AdCOONa	2.5	1.14	0.04	1.08	0.03
AdCOONa	5.0	1.18	0.06	1.14	0.06

Standard deviation (SD) is included; N = 3. AdCOONa is the sodium salt of 1-adamantane-carboxylic acid.

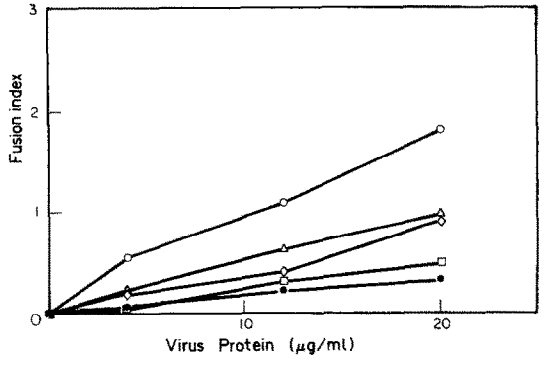

Fig. 5. Virus-induced human erythrocyte fusion in the presence of drugs: ○, control cells, △, 0.05 mM chlorpromazine; ◇, 1 mM tetracaine; □, 5 mM amantadine; ●, 5 mM rimantadine. At 5 mM tetracaine no fusion occurred; N = 3.

corresponded to the range of osmotic haemolysis protection concentrations. The main results were that the qualitative effects of the antivirus drugs did not differ from the effect observed with the anaesthetic tetracaine. Additional studies with other amphipaths (e.g. chloropromazine) showed that generally these substances seem to inhibit virus-induced haemolysis and fusion (cf. Figs. 4 and 5). However, we found that there was the same order of efficiency as in the osmotic haemolysis protection experiment [28].

(iv) *EPR-measurements*

The primary action of anaesthetics is directed towards membrane proteins in order to inhibit excitation. However, most of the membrane actions of anaesthetics occur also in non-excitable membranes, such as the human erythrocyte membrane. It is generally assumed that an important part of the anaesthetics action is to cause some conformational changes of the membrane proteins either directly, or more probably through modified lipid–protein interactions. The conformational changes of the membrane proteins can be easily demonstrated using covalently bound spin labels [19, 20]. With the use of maleimide (MAL-6) we determined the ratio of the amplitudes of the narrow-line and the broad-line component. This ratio represents a measure of conformational alterations of membrane proteins [20]. Table 1 presents this ratio for drug-treated membranes and for control membranes at two different temperatures. The external peak distance $2A_{z'z'}$ of the strongly immobilised component was not affected by the drug treatment. It followed that

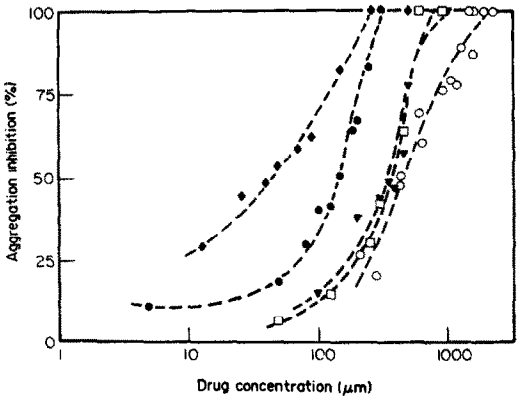

Fig. 6. Inhibition of platelet aggregation as a function of drug concentration: ♦, tetracaine; ●, chlorpromazine; ▼, indomethacin; □, rimantadine; ○, l-adamantane-carboxylic acid. SD approximately 10%; N = 10 ± 3. Aggregation induced by 2.5 μM ADP.

independent of the sign of the charge of adamantane derivatives these changes were similar. Sinha and Chignell [29] found almost the same values with antitumour drugs. The increase of the ratio w_{+1}/s_{+1} indicated that the proteins became more mobile and, consequently, were probably less ordered leading to disfunction in the presence of drugs.

(v) *Platelet aggregation studies*

It is known that a number of drugs inhibit platelet activation [30]. Colman *et al.* [11] showed that the cationic antivirus drugs of the adamantane group inhibit platelet aggregation induced by ADP. We partly repeated that [11] study and compared the influence of amantadine and rimantadine with tetracaine and additionally with chloropromazine and indomethacin. We confirm the platelet aggregation inhibition in Fig. 6 and also show that the anionic analogue of amantadine (l-adamantane-carboxylic acid) was an efficient inhibitor. Figure 6 demonstrates the platelet aggregation inhibition occurred almost with the same efficiency independently of the sign of the charge of the particular compound used.

DISCUSSION

Although the action of anaesthetics is primarily directed towards excitable membranes, it is known that most, if not all, membranes can be used to detect the basic membrane-perturbing influences of anaesthetics [23]. We selected the human erythrocyte, which has long been used in anaesthetics research because its membrane structure and functions are much better known than those of other cells, to investigate the influence of the antivirus drugs amantadine and rimantadine.

Generally, all experiments performed did not exhibit any special feature of the antiviral drugs compared with anaesthetics (Figs 2–6, Table 1). Consequently one might expect, during therapeutic treatment with these drugs, effects similar to those induced by anaesthetics. Indeed, that there are side effects of antiviral treatment, particularly on the central nervous system [31] and on muscle excitation [32]. Amantadine has also been used to treat central nervous disorders, e.g. Parkinson's disease [1, 33].

The similarities in the effects of rimanatadine, amantadine and tetracaine arises from the amphipathic nature of the drugs. No dramatic effects of antiviral drugs on the virus envelope have been detected [9]. Neither did we find in this study effects which were significantly qualitatively different from those of other amphiphilic molecules. On the other hand it is highly improbable that the amphipathic character of the molecule itself is the primary explanation for its antiviral effect. Otherwise one would expect that other amphipaths (e.g. anaesthetics) would generally also show antiviral activity. However, to our knowledge, such an effect has not been reported to date. It may well be that because of their dramatic effect on excitable cell membranes anaesthetics cannot be applied in the necessary dosage to prevent virus infections.

However, one advantage of the antivirus drugs is that they are not metabolised and are relatively harmless to membranes. Amantadine and, especially, rimantadine as shown in Fig. 3 can be applied to the red cell membrane in rather high concentrations without causing destruction of the cell membrane integrity. With rimantadine we found a well pronounced stomatocytic effect below 1 mM [22] and also a significant reduction of the wave number (Fig. 2), but even at more than a 10-fold greater concentrations (10 mM) the degree of osmotic haemolysis was not higher than in control cells. Tetracaine, as shown in Fig. 3, is also well tolerated by membranes. In the concentration range investigated tetracaine-induced lysis was not observed. This is not the case with all amphipaths where haemolysis concentrations may not be much higher than the concentration range where significant membrane effects are observed. It has been suggested that the methyl group of rimantadine may be responsible for the greater tolerance to rimantadine as compared with amantadine during medical treatment [34]. The inhibition of fusion and haemolysis (Figs 4 and 5) is consistent with the conclusion of Bashford *et al.* [35] that osmotic swelling caused by virus-induced permeability changes is an important step in virus-induced fusion of erythrocytes [27]. Since the membrane surface area increases due to incorporation of amphipaths less membrane tension arises when osmotically-induced volume increase occurs and swelling take place. Remarkably, we observed the same order of drug efficiency in virus-induced fusion and haemolysis inhibition and in osmotic haemolysis. We conclude that the effect of the antivirus drugs on the virus-induced phenomena is related to inhibition of swelling and, consequently, not a special antivirus effect of rimantadine and amantadine.

Another question to be discussed is what might be the significance of the sign of the charge of the antivirus drugs. To our knowledge all adamantane derivatives used for virus infection prevention form cations around the physiological pH-range. In our studies above with an especially synthesised anionic analogue (l-adamantane-carboxylic acid) we have

examined the influence of drug charge on the membrane effects. The thermal fragmentation pattern (Fig. 2), EPR-results and thrombocyte activation inhibition did not differ qualitatively from the other drugs. Of course, anionic drugs caused externalisation (Fig. 2) of erythrocytes at physiological concentrations and pH [36].

Recently, Simonova *et al.* [37] found that only neutral rimantadine is capable of penetrating planar lipid bilayers. When drugs pass through biological membranes in the neutral form they will accept protons at the inner membrane surface becoming again positively charged. If the membrane is not permeable to protons this will lead to a continuous decrease of the proton concentration of the interior until equilibrium of drug distribution with the resulting electrical gradient is reached.

Influenza and other similar virus infections at the single cell level occur in distinct steps. The final uncoating of the virus and release of the genome is thought to take place at the inner lysosomal or endosomal membrane [38–41]. One of the necessary requirements for this fusion process is a sufficiently low pH-value. At pH 5.2 the exposing of hydrophobic parts of the virus envelope proteins is at a maximum and can mediate the fusion process [42, 43]. Weak bases perturb the lysosomal and endosomal pH [40, 44, 45]. Cassell *et al.* [44] showed that the infection of BHK-21 cells by sindbis virus was inhibited by weak bases and Helenius *et al.* [41] demonstrated inhibition of Semliki forest virus penetration. So, a possible explanation of the mechanism of rimantadine and amantadine action is that these substances cross the lysosome or endosome membrane in a neutral form and subsequently accept protons from the interior of the lysosome or endosome. In this way both the inner surface pH and internal pH of lysosomes increases. Such an increase would act to block the influenza virus-lysosome membrane fusion process. A simple calculation supports this mechanism. At pH 5.0 a lysosome of diameter 0.5 μm contains approximately 400 protons. We recently estimated the charge density of human erythrocyte membranes due to rimantadine incorporation as being 4×10^{-4} C/m^2 [22]. Assuming that this value would be of the same order with the endosomal or lysosomal membrane we estimate that there are about 10^4 molecules of rimantadine associated with the intracellular vesicles. That is, there would be sufficient proton acceptors to increase the internal pH of lysosomes or endosomes, even taking into account a certain buffer capacity of the intrasomal content.

The efficiency of this suggested mechanism of virus penetration inhibition essentially depends on the surface to volume ratio of the particles under consideration. With large particles, e.g. cells, there would be no significant pH increase. The suggested mechanism may explain why not all kinds of enveloped viruses, even not all influenza viruses, are sensitive to these antivirus drugs. It may be that the pH-sensitivity differs for different viruses.

Acknowledgements—We are grateful to colleagues from the Inst. of Electrochem. Acad. Sci. U.S.S.R. for making available adamantane derivatives. We are also indebted to Prof. J. Tigyi and Prof. K. Hideg (Pecs) for the gift of the spin label and to Dr G. Lassmann (Berlin), for providing the facility for EPR measurements. A significant part of this work was supported by a British Council grant to one of us (E.D.). We also thank Dr V. A. Tverdislov for initiating this work and Prof. R. Glaser for his continuous encouragement and support.

REFERENCES

1. J. W. Tilley and M. J. Kramer, *Progr. Med. Chem.* **18**, 1 (1981).
2. W. Koff and V. Knight, *J. Virol.* **31**, 261 (1979).
3. J. Cupp, M. Klymkowski, J. Sanos, A. Keith and W. Snipes, *Biochim. biophys. Acta* **389**, 345 (1975).
4. M. D. Lubeck, J. L. Schulman and P. Palese, *J. Virol.* **28**, 710 (1978).
5. J. J. Skehel, A. V. Hay and V. A. Armstrong, *J. gen. Virol.* **38**, 97 (1977).
6. A. J. Hay, N. C. T. Kennedy, J. J. Skehel and G. Appleyard, *J. gen. Virol.* **42**, 189 (1979).
7. R. A. Narmanbetova, S. G. Tulkes, N. K. Vorkunova, G. K. Vorkunova and A. G. Bukrinskaya, *Vopros. Virol.* (in Russian) **27**, 23 (1982).
8. V. A. Tverdislov, S. El-Karadaghi, D. J. Bucher, J. A. Zakomirdin and I. G. Kharitonenkov, *Biochim. biophys. Acta* **778**, 276 (1984).
9. I. G. Kharitonenkov, V. I. Poltorak and E. K. Ruuge, *Molek. Biol.* (in Russian) **13**, 1035 (1979).
10. P. D. Morse, D. M. Lusczakoski-Nesbitt and R. B. Clarkson, *Chem. Phys. Lipids* **31**, 257 (1982)
11. R. W. Colman, J. Kuchibhotla, M. H. Jain and R. K. Murray Jr., *Biochim. biophys. Acta* **467**, 273 (1977).
12. Y. Becker and J. Hadar, *Prog. Med. Virol.* **26**, 1 (1980).
13. J. F. Brandts, L. Erickson, K. Lysko, A. T. Schwartz and R. D. Taverna, *Biochemistry* **16**, 3450 (1977).
14. W. T. Coakley and J. O. T. Deeley, *Biochim. biophys. Acta* **602**, 355 (1980).
15. L. A. Crum, W. T. Coakley and J. O. T. Deeley, *Biochim. biophys. Acta* **554**, 76 (1979).
16. H. Peretz, Z. Toister, Y. Laster and A. Loyter, *J. Cell Biol.* **63**, 1 (1974).
17. J. T. Dodge and C. Mitchell, *Archs biochem. Biophys.* **100**, 119 (1963).
18. L. W. M. Fung, *Biophys. J.* **33**, 253 (1981).
19. O. A. Butterfield and W. R. Markesbery, *Biochem. Int.* **3**, 517 (1981).
20. L. W.-M. Fung, *Ann. N.Y. Acad. Sci.* **414**, 162 (1983).
21. A. Nwafor and W. T. Coakley, *Biochem. Pharmac.* **34**, 3329 (1985).
22. V. A. Tverdislov, S. El-Karadaghi, I. G. Kharitonenkov, R. Glaser, E. Donath, A. Herrmann, P. Lentzsch and J. Donath, *Gen. Physiol. Biophys.* **5**, 61 (1986).
23. P. Seeman, *Pharmac. Rev.* **24**, 583 (1972).
24. S. El-Karadaghi, J. A. Zakomirdin, C. Shimane, D. J. Bucher, V. A. Tverdislov and I. G. Kharitonenkov, *Biochim. biophys. Acta* **778**, 269 (1984).
25. G. Poste and C. A. Pasternak, *Cell Surface Rev.* **5**, 305 (1978).
26. T. Maeda and S. Ohnishi, *FEBS Lett.* **122**, 283 (1980).
27. A. Loyter and A. Lalazar, in *Membrane–Membrane Interactions* (Ed. N. V. Gilula), p. 11. Raven Press, New York (1980).
28. R. Motais, A. Baroin, A. Motais and S. Baldy, *Biochim. biophys. Acta* **599**, 673 (1980).
29. B. K. Sinha and C. F. Chignell, *Biochem. biophys. Res. Commun.* **86**, 1051 (1979).
30. M. B. Feinstein, J. Fiekers and C. Fraser, *J. Pharmac. exp. Ther.* **197**, 215 (1976).
31. J. T. Pearlman, A. H. Kadish and J. C. Ramseyer, *J. Am. med. Assoc.* **237**, 1200 (1977).

32. E. X. Albuquerque, A. T. Eldefrawi, M. Eldefrawi, N. A. Mansour and M. C. Tsai, *Science* **199**, 788 (1978).
33. R. S. Schwaab, A. C. England, D. C. Poskanzer and R. R. Young, *J. Am. med. Assoc.* **208**, 1168 (1969).
34. R. B. Couch and G. G. Jackson, *J. Infect. Dis.* **134**, 516 (1976).
35. C. L. Bashford, K. J. Micklem and C. A. Pasternak, *Biochim. biophys. Acta* **814**, 247 (1985).
36. B. Deuticke, *Biochim. biophys. Acta* **163**, 494 (1968).
37. M. W. Simonova, V. Cerny, E. Donath, W. F. Sokolov and W. F. Markin, *Biologitscheskie Membrany*, in press.
38. R. Dourmashkin, S. Patterson, D. Shah and J. S. Oxford, *J. Virol. Meth.* **5**, 27 (1982).
39. A. Yoshimura, K. Kuroda, K. Kawasaki, S. Yamashine, T. Maeda and S. Ohnishi, *J. Virol.* **43**, 284 (1982).
40. A. Yoshimura and S.-I. Ohnishi, *J. Virol.* **51**, 497 (1984).
41. A. Helenius, M. Marsh and J. White, *J. gen. Virol.* **58**, 47 (1982).
42. J. J. Skehel, P. M. Bayley, E. B. Brown, S. R. Martin, M. D. Waterfield, J. R. White, T. A. Wilson and D. C. Wiley, *Proc. natn. Acad. Sci. U.S.A.* **79**, 968 (1982).
43. S. Sato, K. Kawasaki and S.-I. Ohnishi, *Proc. natn. Acad. Sci. U.S.A.* **80**, 3153 (1983).
44. S. Okhuma and B. Poole, *Proc. natn. Acad. Sci. U.S.A.* **75**, 3327 (1978).
45. T. Bachi, N. Gerhard and J. W. Yewdell, *J. Virol.* **55**, 307 (1985).
46. S. Cassell, J. Edwards and D. Brown, *J. Virol.* **52**, 857 (1984).