Amantadine: an antiviral and antiparkinsonian agent

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ABSTRACT: Amantadine is an antiviral agent that specifically inhibits influenza A virus replication at a micromolar concentration. This drug is also very effective in the treatment of human Parkinson’s disease. Other important clinical applications of this agent have been studied recently, ranging from viral infections, e.g. herpes, herpes zoster neuralgia to granulomatosis and from neuroleptic extrapyramidal movement disease to depression and cocain dependence. Biological and pharmacological activities of amantadine presented in this paper are focused on the explanation of the mechanism of amantadine antiviral and antiparkinsonian effects and on general use of this agent in medicine.

Keywords: influenza A; M2 protein; chemotherapy; chemoprophylaxis; Parkinson’s disease

List of abbreviations: AA – amino acid; AMA – amantadine; AMA-HCl – amantadine hydrochloride; AMA-S – amantadine sulphate; AZT – azidothymidine; DDI – dideoxyinosin; DNA – deoxyribonucleic acid; DOPC – 1,2-Dioleoyl-sn-glycero-3-phosphocholine; FTIR – infrared spectroscopy using the Fourier transformation; HA – haemagglutinin; M1 – matrix system; NA – neuraminidase; NA – nucleic acid; NMDA – N-methyl-D-aspartate; NMR – nuclear magnetic resonance; NP – nucleoprotein; PD – Parkinson’s disease; RNA – ribonucleic acid

1. INTRODUCTION

At present, viral diseases represent a considerable problem in medicine, which despite significant progress in the field of biophysics, biochemistry, molecular biology, pharmacology and medicine has not been satisfactorily coped with. Influenza, in comparison with other viral diseases such as AIDS, neoplastic diseases, hepatitis and others, is often perceived by the laic as well as professional public as a banal disease, however the opposite is true. Every year, thousands of people die of influenza or connected complications all over the world (Beran, 1999).

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AMANTADINE IN THE TREATMENT AND PREVENTION OF INFLUENZA A

Amantadine (Figure 1A) and its structural analogue rimantadine (Figure 1B) are applied in the chemotherapy of viral diseases. Amantadine is effective in the treatment of hepatitis C (Smith, 1996; Martin et al., 1999), herpes (Jáuregui et al., 1997), herpes zoster neuralgia (Douglas, 1990), Creutzfeldt-Jacob’s disease (König et al., 1996), Born’s disease (Hallensleben et al., 1997), influenza B (Douglas, 1990) and others. The studies on the therapeutical effects of amantadine on depression (Hubet et al., 1999) and cocainism (Volchow et al., 1990) are also known. Both medicaments, however, are preferentially intended for the treatment and prevention of influenza A because they specifically inhibit replication of its viruses already at the micromolar concentration (Douglas, 1990).

2.1. The use of amantadine in veterinary medicine

The viruses of influenza A are periodically transmitted from the reservoir animals – especially water birds to pigs, horses, domesticated poultry as well as to sea mammals and they cause infections. While in water birds the infection and circulating of the virus in the population have rarely clinical manifestations, transmission to domesticated poultry and mammals has usually bad consequences for a new host. There is a certain relationship between the human, swine and equine influenza, which are in the interrelation with avian influenza that is their common ancestor (Sabó, 1998). At present, the idea is supported that pigs and turkeys are mediators at the outbreaks of new epidemics or pandemic viruses of human influenza (Sabó, 1998). In 1997 in China there was an outbreak of avian influenza of the subtype H5N1 which was characterised by more specialities. Within the history of human influenza, this subtype had never occurred in man before and it was a case of direct interspecies transmission of influenza from birds (hens) to man. Overall 18 people got ill with this influenza, out of which 6 succumbed to this contagion. The disease broke out enormously fast and early administration of amantadine had a favourable therapeutic effect (Sabó, 1998). Therefore making diagnosis and monitoring the influenza disease in animals is of great importance (Sabó, 1998).

Recently the papers investigating possibilities of chemotherapy of influenza in animals, namely in horses, have appeared (Chambers et al., 1995a, b; Oxford, 1997; Rees et al., 1997). Influenza is, for example, a highly significant acute disease in horses and at an equine influenza outbreak in Hong Kong in 1989, herds were destroyed within a month with losses of almost 100 million USD (Chambers et al., 1994). Therefore both the therapy and chemoprophylaxis with amantadine or rimantadine could be very useful because vaccination of horses is only partially effective. Clinical symptoms of the disease could be improved by an early treatment. Infected animals can be treated with appropriate doses of these drugs and at the same time vaccinated for the stimulation of the natural immunity development. Amantadine and rimantadine are effective against all the susceptible viral strains of influenza, whereas vaccines generally protect only against the strains derived from those being involved in the vaccine (Nahta and Brady, 1986).

Until influenza A in horses was reliably and fast diagnosed, its therapy was not practised. At present, there are new, fast and simple diagnostic methods such as e.g. Directigen FLU-A test (Chambers et al., 1995a) and others that make the antiviral treatment possible also in horses.

Evaluation of the pharmacological aspects and side-effects of amantadine at influenza A treatment in horses can be found in the paper of Rees et al. (1997). In these experiments amantadine and rimantadine were tested on the viruses of equine influenza of the subtype H3N8 of Miami strain from 1963 and Chinese equine influenza from 1989 which circulated in horses also in 1991–1994 in Kentucky. In vitro amantadine reliably inhibited these viruses at the concentrations of 30 ng/ml and less, with the exception of the strain KY/92, which was resistant.
also at the concentrations higher than 300 ng/ml (Rees et al., 1997). In comparison with amantadine, rimantadine is more effective. In vivo it was preliminarily tested at the treatment of horses at the dose of 10 mg/ml of live body weight administered intravenously, which did not induce any apparent side-effects, while the dose of 20 mg/ml l.b.w. or more caused different effects on the CNS, inclusive attacks. At the dose of 15 mg/ml l.b.w., the middle and temporary responses to the CNS in 3 out of 6 treated horses were observed. These signs appeared within 30 to 60 minutes following the drug administration. They involved stumbling, disharmonious position of limbs, dragging of hind limbs and weakness in the lower posterior muscles. Thus it has been concluded that administration of the individual dose of 15 mg/kg l.b.w. intravenously is connected with a significantly unfavourable response in horses (Rees et al., 1997). At the same doses administered orally, the efficacy of preparations depended on the ability of their absorption from the gastrointestinal tract, which was different in individual patients. Thus, intravenous administration of amantadine and rimantadine in the chemotherapy of equine influenza is more favourable than oral and the recommended effective dose is 10 mg/kg l.b.w. of the animal.

### 2.2. The use of amantadine in human medicine

Amantadine and rimantadine are suitable for the treatment of influenza A and other viral disease for many reasons:
- they are characterised by high selectivity of the effect
- they are effective in sufficiently low concentrations
- they are characterised by low toxicity to experimental cellular systems as well as to the organism – they have negligible side effects (Boreko et al., 1996)

*In vitro* doses from 0.4 µg/ml to 10 ng/ml (Douglas, 1990; Oxford, 1997) are high enough for the inhibition of most influenza viruses, specially of subtypes H3N2, H2N2, H1N1 (viral subtypes – see part 2.3.1.)

*In vivo* amantadine is well absorbed from the gastrointestinal tract. The maximum concentrations in the blood plasma after using a dose of 200 mg attain the values from 0.3 to 0.6 µg/ml. Almost the whole absorbed dose is excreted into urine in unchanged form. The drug is not almost metabolised. The half-time of excretion is 16 hrs, this value increases in older people and in patients with a decreased function of kidneys (Aoki and Sitar, 1988).

Amantadine is used in the form of amantadine hydrochloride salt (AMA-HCl) with the trade name Virosol, Virofral, Symadine or Symmetrel – it was registered under these names for human use in Europe and in the USA 34 years ago. In this country it is registered under the name Viregyt-K; Symmetrel and Symadine are not registered. The preparations Contenton and PK-Merz on the basis of amantadine sulphate (AMA-S) are also known. In the countries of the former USSR rimantadine is used that, however, does not have a licence in Western Europe and in the USA. It is produced as capsules and also as syrup. A daily dose for children from 1 to 9 years is from 4.4 to 8.8 mg/kg of body weight and it must not exceed 150 mg per day. For older children and adults the dose of 200 mg per day or better 100 mg twice a day is suitable. In older people and in the patients with a decreased function of kidneys the dose should be reduced to 100 mg per day. The drug should be applied during 5 days (Douglas, 1990).

Its side-effects manifest sporadically at the recommended doses, at the dose of 200 mg daily in 1–5% of patients with the normal function of kidneys some smaller neurological symptoms (insomnia and troublesome concentration) were recorded (Dolin et al., 1982). Excessively high doses when the amantadine concentration in the blood plasma is from 1 to 5 µg/ml are connected with the toxic effects of the drug on the central nervous system, namely nervousness, hallucinations, attacks and coma. Persons with psychiatric diseases, epilepsy or pregnant and nursing women should not be treated with amantadine (Dolin et al., 1982).

Preventive effects of amantadine are very significant. A high number of clinical studies proved the efficacy of amantadine in the prevention of influenza A (Dolin et al., 1982; Blake, 1990; Monto, 1994; Tamblyn, 1997). The efficacy of the agent in the prevention shows a frequency within the range of 70–90% (Tamblyn, 1997), which is comparable with vaccination. Its utilisation is recommended especially in various social facilities where there is a high potential of contagion spreading.

Vaccination is a preferred method in the prevention of influenza A. Unlike the use of vaccine, amantadine has an advantage that it is recommended for protection of patients of any age at the moment when the influenza incidence was proved in the given community. It is not necessary to administer it in advance before the influenza season. The treatment with amantadine should start immediately after the outbreak of influenza epidemics and continue during its persistence (usually 5–6 weeks). Since the drug does not weaken the immune response to vaccine, the vaccinated patient could discontinue it after 2 weeks (Douglas, 1990).

### 2.3. Mechanism of the amantadine effect on the influenza A virus

#### 2.3.1. Characteristics of the influenza virus

The influenza virus, discovered in 1932, belonging to the family Orthomyxoviridae, is an enveloped RNA virus 80–120 nm in size. The virion nucleus contains a
The structure and function of M2 protein

High amounts of M2 protein occur in the plasma membrane of influenza virus-infected cells, but there is only about 16–64 molecules in one virion (Lamb et al., 1985; Zebedee et al., 1985; Zebedee and Lamb, 1988; Ciampor et al., 1998). It is coded by segment 7 of viral RNA (Surgue and Hay, 1991; Pinto et al., 1992). This segment also codes the matrix protein M1, coded by almost a complete transcript of segment 7, while the smaller protein M2 is coded by a transcript originating by cutting of seg-
ment 7. Both proteins co-operate in the process of viral replication (Čiampor et al., 1998).

Ninety-seven AAs form the primary structure of M2 protein (Hay et al., 1985) and its amino acids sequence is steady for all the viral strains coming from various sources (Wang et al., 1993). The M2 protein is an integral protein of the IIIrd type in the protein topology with uncleaved signal anchor sequence and Ntermin-C terminus orientation (von Heijine, 1988; Parks and Lamb, 1991). It spans the membrane once and is orientated in such a way that it has 23 N-terminal extracellular residues and a 54 residue C-terminal cytoplasmic domain. In the transmembrane region it forms homo-tetramers secondarily aligned as the α-helix (Sugrue and Hay, 1991). Tetramers are con-
nected by disulphide bridges at the site of cysteins C_{17} and C_{19} in the extracellular region (Figure 2) (Sugrue and Hay, 1991).

The secondary and tertiary structures of M2 protein were studied in greater detail by Sugrue and Hay (1991), Duff et al. (1992), Kovacs and Cross (1997), Kukol et al. (1999). Duff et al. (1992) found by means of circular dichroism that the transmembrane region of M2 protein incorporated in 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) liposomes exists in the α-helix form while this structure is not altered by either higher temperature or AMA addition. The α-helix parameters in four bundles of M2 protein were determined by the NMR (Kovacs and Cross, 1997) and FTIR method (Kukol et al., 1999). Using the NMR method, the tilt of the helix with respect to the bilayer normal was determined to be 33° ± 3° as well as the orientation round the helix axis. These results imply that the tertiary arrangement of tetrameric protein in the membrane is a left-handed four-helix bundle (Figure 3). Only with such a large tilt angle are the hydrophilic residues aligned to the channel axis, which forms the M2 tetramer in the membrane (Kovacs and Cross, 1997).

Kukol et al. (1999) confirmed the helix angle described by Kovacs and Cross (1997) by the FTIR method. They found the precise position of AAs in the α-helix, and based upon this finding with respect to the lateral chains it follows that a pore is closed by His-37 residues (Figure 4).

It can bee seen in Figure 4 that the His-37 residues from four monomers play a role of the regulator that conducts protons in one direction. Protonation of one N His-37 atom from the outside of the virus could induce deprotonation of another N atom of the imidazole part of His-37, because a positive charge is distributed through the π-electron circular system. The initial status could be renewed by tautomerization or a ring flip. At the low pH value due to multiple protonation a conformation change could occur that would allow the passage of cations (Shimbo et al., 1996).

As it was mentioned above, the M2 protein forms an ion channel whose function is the passage of protons from
endosome to virion. Due to a decrease in pH, the matrix M1 protein is separated from the nucleocapsid and is released to the cytoplasm – this process is often called uncoating (Hay et al., 1985; Pinto et al., 1992; Hay, 1992). In the process of virus replication (Figure 5) it is so-called early effect or early permeabilization of the membrane by M2 protein. In the late stage of the replication cycle, M2 protein fulfills the function of a proton channel again when it regulates pH in the transport vesicles of HA, whereby it ensures the formation of right conformation of newly synthesized HA during its transport to the cytoplasmic membrane of the cell (Čiampor et al., 1992a, 1998). This process occurs shortly after the outlet of HA from the Golgi complex during the passage of HA through the trans-Golgi network (Čiampor et al., 1992a). The M2 protein recognises and binds itself to the cytoplasmic part of the HA trimer. Subsequently, the virion is assembled and released from the cell by budding through the plasmic membrane (Figure 5) (Čiampor et al., 1998). The M2 protein has the same function in both stages of viral infection, namely to regulate pH by the formation of proton channel and to create a suitable environment for viral replication.

2.3.4. Inhibition of M2 protein by amantadine

The activity of some alicyclic amines was compared with the influenza viruses from various sources in vitro (Table 1) (Hay et al., 1985). It follows from the results that the effect of the tricyclic amine AMA is highest in most viral subtypes from various sources. Cyclooctylamine exhibits activity similar to amantadine.

In the cell cultures, amantadine manifests two concentration-dependent inhibitory effects against the viral replication (Hay and Zambon, 1984) that are connected with an early and late stage of viral replication. First, it is a non-specific inhibition at the concentrations higher than 0.1 mM (Daniels et al., 1985). At these concentrations it has an effect on the influenza viruses of B type and on a number of other enveloped viruses, e.g. paramyxoviruses, togaviruses, retroviruses (Skehel et al., 1978), herpesviruses, viruses of rubella (Douglas, 1990), hepatitis C (Martín et al., 1999). Amantadine concentrations from 0.1 to 5 µM specifically inhibit only the influenza A virus (Hay et al., 1985) while the substance efficacy is different for different strains of viruses and depends on the time of administration (Table 2). The resistance of viral particles against AMA could originate. It is evident from the data in Table 2 that replication of amantadine-resistant mutants was not stopped either by the concentrations higher than 50 µM (Hay et al., 1985).

The non-specific inhibition of the virus replication by AMA is ascribed to the early stage of viral replication. The natural environment of endosome is acid – pH is about 5 (Ohkuma and Poole, 1981). The proton channel formed by M2 protein transports protons from the endosome inside the virion. This pumping is necessary for the interruption of macromolecular interactions which keep together the virion coat (Hay et al., 1985; Pinto et al., 1992; Wang et al., 1993). Amantadine behaves as a lysosomotropic substance that passes easily through the endosome membrane (Duff et al., 1993) and accumulates in it. As a weakly basic substance it binds protons in the endosome to itself, whereby making them impossible to flow inside the virion. In this way, both the interruption of the matrix M1 protein and release of the virion nucleus into the host cell environment are prevented (Hay et al., 1985; Pinto et al., 1992; Wang et al., 1993). A more detailed explanation of the lysosomotropic features of amantadine is presented in Chapter 3.1. Higher concentrations of amantadine (above 0.1 mM) are necessary for viral inhibition in this stage than for inhibition in the later stage of viral replication. Amantadine is also effective in the early stage against the other above-mentioned enveloped viruses.

At the low concentrations (from 0.1 to 5 µM, see Table 2) amantadine blocks the late stage of viral matura-

<table>
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<th>Compound</th>
<th>Singapore</th>
<th>Rostock</th>
<th>Weybridge</th>
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<tr>
<td></td>
<td>5 µM</td>
<td>5 µM</td>
<td>50 µM</td>
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<tr>
<td></td>
<td>0.5 µM</td>
<td>5 µM</td>
<td></td>
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<tr>
<td>Amantadine</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Cyclooctylamine</td>
<td>5</td>
<td>5</td>
<td>5</td>
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<tr>
<td>Cyclohexylamine</td>
<td>60</td>
<td>70</td>
<td>30</td>
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<tr>
<td>Cyclooctanol</td>
<td>95</td>
<td>100</td>
<td>100</td>
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<tr>
<td>Octylamine</td>
<td>100</td>
<td>100</td>
<td>ND</td>
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<td>ND = not determined</td>
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The M2 protein plays a role in inhibition in this stage. Due to its blocking by amantadine, reduction of pH in the transport vesicles occurs that induces a conformational change in HA (Čiampor et al., 1992a). So amantadine is supposed to induce a premature conformational change in HA that occurs in the trans-Golgi network during the HA transport on the cell surface. This change in HA blocks a release of virions out of the host cell (Sugrue et al., 1990; Čiampor et al., 1992b). Viral particles with conformation HA originate at pH 5, they aggregate on the cell surface after maturation and do not separate from the infected cell surface (Čiampor et al., 1998). The need for a hundred fold lower concentration of the drug in this stage, compared to so-called early permeabilization, results from different mechanisms of the AMA effect in both stages. At inhibition in the process of virus uncoating, the ratio of proton concentration inside the endosome and in cytoplasm is 1 : 100 because the pH in endosome is 5 and in cytoplasm 7. At non-specific inhibition the amount of amantadine has to be such (about 0.5 mM) to balance this ratio and to prevent the proton passage through the virion coat by means of M2 channel. In the second stage, however, the interior of the transport vesicle of Golgi apparatus is neutral because the M2 channel pumps protons from it into the cytoplasm. Its evidence is the fact that viral infection causes an increase in the acidity in the trans-Golgi region (Čiampor et al., 1993). These results confirm the contrary orientation of M2 protein together with HA. Thus, it pumps protons from a vesicle into the cytoplasm to ensure the optimal environment for obtaining the proper HA conformation. Therefore AMA does not behave as a lysosomotropic

Table 2. Concentration dependence of the inhibition of virus production by amantadine (Hay et al., 1985)

<table>
<thead>
<tr>
<th>Amantadine concentration (µM)</th>
<th>Virus yield (% of control)</th>
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<tr>
<td></td>
<td>Rostock</td>
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<tr>
<td>Amantadine concentration (µM)</td>
<td>a</td>
</tr>
<tr>
<td>0.05</td>
<td>95</td>
</tr>
<tr>
<td>0.5</td>
<td>21</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>500</td>
<td>17</td>
</tr>
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amantadine was added: a = 30 min after infection; b = 60 min after infection
BEL R = amantadine resistant virus strain

Figure 6. Location of histidine residues and possible mode of interaction of the M2 protein transmembrane region with amantadine (Gandhi et al., 1999)
3. AMANTADINE – THE AGENT IN THE TREATMENT OF PARKINSON’S DISEASE

Unlike other drugs used in the treatment of Parkinson’s disease (PD) the effect of amantadine on the PD symptoms were discovered randomly. In April 1968 a patient with PD cured by the neurologist R.S. Schwab was preventively taking amantadine against influenza twice a day. The PD symptoms such as akinesia, rigidity, tremor disappeared. After discontinuing amantadine the PD symptoms returned. This finding was verified many times, and so amantadine has become an effective antiparkinsonian agent (Danielczyk, 1995). It has a good effect mainly at akinetic crises and in combination with the conventional preparation L-Dopa it suppresses the main symptoms of PD if L-Dopa alone is not effective (Schwab and Poskanzer, 1972; Greulich and Fenger, 1995).

Monotherapy of PD with amantadine is indicated in the early stage of PD and in the cases of weaker symptoms (Greulich and Fenger, 1995). It is also effective after the prolonged treatment. Long-term studies on a great population of patients indicate the effects of AMA for a lot of years.

Two forms of amantadines are used in the therapy of PD. It is already mentioned AMA-HCl used in the treatment and prevention of influenza, and amantadine sulphate (AMA-S). In the Central Europe AMA-S is preferred, which is practically unknown in most parts of the western world – it was registered only in 12 countries (Danielczyk, 1995). While AMA-HCL has not been used for example in Austria, in the other parts of the world the maximal doses of 200–300 mg are used. The disadvantage of this form is that this dose cannot be elevated in the case of disease progression because the side-effects of the drug manifest to a greater extent (Danielczyk, 1995). On the other hand, AMA-S offers the possibility to increase the dose up to 600 mg daily because the level of AMA-S in the blood rises much more slowly than in AMA-HCl. Thus, the side-effects, especially those induced by circulation, are less pronounced. At the states of acute akinesia that is accompanied by akinetic crises and in combination with the conventional preparation L-Dopa its suppressing the main symptoms of PD if L-Dopa alone is not effective (Schwab and Poskanzer, 1972; Greulich and Fenger, 1995). Precautionous doses are necessary in older patients. Among the side-effects of amantadine at the treatment of PD belong: insomnia, oedema, nausea, anxiety, confusion as well as pains of stomach, muscles, pruritus, livedo reticularis, halucinations, nightmares (Schwab et al., 1972; Zeldowicz and Huberman, 1973; Bauer and McHenry, 1974; Hayden et al., 1983; Duvoisin, 1991; Pfeiffer, 1996).
3.1. Mechanism of amantadine effect on suppressing the symptoms of Parkinson’s disease

The mechanism of the AMA effects as an antiparkinsonian drug is not precisely known, or better expressed, is less explained than in the case of influenza. It refers to the ability of the preparation to block the neuromuscular transmission, which has an influence on the synthesis and excretion of dopamine (Grelak et al., 1970). Amantadine likewise at influenza manifests as a blocker of these channels and blocks:

– serotonin-activated ion channel
– ion channel of nicotine-acetylcholine receptors
– ion channel of N-methyl-D-aspartate (NMDA) receptors (Kornhuber and Streifler, 1992).

There are other biochemical and pharmacokinetic characteristics of amantadine:

– increase in fluidity of cellular membranes
– increase in electrically stimulated excretion of dopamine and serotonin

– inhibition of monoaminooxidase A
– loss of manifestation for a direct effect on the dopaminergic receptors (Kornhuber and Streifler, 1992) that can explain why there is no akinesia at its application (Danielezyk, 1995).

Another characteristic of AMA is lysosomotropism or also acidotropism that probably cause its high preservation in the cerebral tissue (Kornhuber et al., 1995). Concentration of AMA in the brain is high if referring to the CNS and blood serum – the ratio is 20 : 1. Particularly interesting is a slow accumulation of the drug in the brain. Approximately 65 mg of AMA is necessary to reach the concentration of AMA free base 217 µM in 1.5 l (the brain volume). Taking into consideration that AMA accumulates not only in the brain but also in other organs (Aoki and Sitar, 1988), and at oral treatment most AMA is excreted as non-metabolised (more than 85%), then it is clear that treatment with AMA has to persist at least for several days so that such high concentrations in the brain may be reached.

A high preservation and slow accumulation of AMA could have two reasons that follow from the nature and characteristics of the amantadine molecule: a) its high lipophilicity, and b) already mentioned lysosomotropism (Kornhuber et al., 1995). The AMA lipophilicity has also been confirmed by other authors investigating the interaction of AMA free base with 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) using X-ray diffraction who found that the ability of non-protonated form of AMA to penetrate through the bilayer is higher than in other compounds (Duff et al., 1993). A direct evidence for lysosomotropism of AMA comes from several in vitro studies (Okhuma and Poole, 1981; Johnson et al., 1981; Richardman et al., 1981) and can be explained by a few sentences. The intracellular subcompartments such as lysosomes and endosomes have an acid nature with pH of about 5 (Okhuma and Poole, 1981). Weakly basic substances, to which a non-protonated form of AMA also belongs, have a tendency to accumulate in these compartments. While plasmic and lysosomal membranes are permeable for neutral forms of weak bases, the same membranes are impermeable for protonated forms of these substances. Weak bases are captured by protonation inside the lysosomes and accumulate there (Figure 7) (Okhuma and Poole, 1981).

The ratio of intra/extra lysosomal concentrations of these substances is equal to the ratio of concentration of hydrogen ions in lysosomes and in their vicinity, i.e. 1 : 100 if we suppose that pH in lysosomes is 5 and in cytoplasm 7. The amount of the permeable form of weak base passing through the membrane depends on the substance pKa value and pH value of solution. The higher the pKa value, the lower the permeable form ratio. Therefore the drugs with high pKa values similar to AMA (pKa = 10.14 at 37°C (Perrin and Hawkins, 1972)) have a slow speed of penetration into lysosomes (de Duve et al., 1974).
When the concentration of the base inside the lysosome reaches isotonia, water starts to enter the lysosome osmotically, which enlarges its volume and forms a large vacuole (de Duve et al., 1974).

Many effects of lysosomotropic drugs on the cell function are not fully studied. These effects involve a direct inhibition of lysosomal functions by an increase in the intralysosomal pH. It may be expected that besides lysosomes the biochemical processes of all other intracellular organelles (whose normal function is conditioned by the acid environment) are also disturbed. (Goldstein et al., 1985; Mehlman et al., 1986). A well-known example is the transport of monoamines into the sympathetic vesicles. The vesicle membrane contains reserpine – a sensitive transporter that can mediate an exchange of cytoplasmic amines for internal protons. The vesicular monoamine transporter non-selectively accumulates biogenic amine transmitters such as serotonin, dopamine and noradrenaline. Lysosomotropic substances such as e.g. AMA influence the intravesicular increase in pH whereby they disturb the accumulation of amines (Kornhuber et al., 1995).

4. SUMMARY

The aim of the present paper was to provide available information about the antiviral and antiparkinsonian drug amantadine. Here belongs the knowledge of its biological and pharmacological activities as well as information about amantadine use in medicine. Since some attempts to employ this drug also in veterinary medicine have appeared recently, the study of its properties, mechanisms of effects and interactions can be interesting also in this field.

5. REFERENCES


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