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Antiviral effects of dihydroxypropyladenine ((*RS*)-DHPA) and bromovinyldeoxyuridine (BVDU) on plant viruses

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Summary

Two established antiviral agents, dihydroxypropyladenine (*RS*)-DHPA and bromovinyldeoxyuridine (BVDU) were evaluated for their inhibitory effects on 4 plant viruses: Tobacco mosaic virus, potato virus X (PVX), eggplant mosaic virus (EMV) and a potyvirus isolated from *Solanum palinacanthum* (Poty-Sp). Using the leaf disc incubation test, BVDU proved virtually inactive while (*RS*)-DHPA efficiently inhibited EMV and Poty-Sp when applied at concentrations as low as 5 mg/l. TMV was less susceptible to the chemical while PVX could be inhibited at drug concentrations of 100 mg/l. To achieve a similar inhibitory effect in the leaf spray test, concentrations up to 250 and 500 mg/l were required. Using these tests no phytotoxicity was observed with (*RS*)-DHPA at any of the concentrations used.

Dihydroxypropyladenine ((*RS*)-DHPA); Bromovinyldeoxyuridine (BVDU); Plant viruses, Tobacco mosaic virus (TMV); Potato virus X (PVX); Eggplant mosaic virus (EMV); Potyvirus

Introduction

Virus diseases are the major limiting factor of many agricultural crops, especially perennial crops, in which they may cause chronic losses (Dawson, 1984; Schuster, 1983; Vicente and De Fazio, 1987). According to recent literature there

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are few effective and promising antiviral chemicals which may be considered for agricultural purposes (Pennazio and Martelli, 1983; Stevenson and Monette, 1983; White and Antoniw, 1983). However the control of replication of human and animal viruses achieved a great progress in basic and applied research with the discovery of suitable enzymatic targets for antiviral agents (Galasso, 1981; Helgstrand and Oberg, 1980). In plants, the study of antiviral drugs is still of a substantially empirical nature. Thus, investigations on the potential antiphytoviral activity of chemicals which show a promising activity against animal viruses have only recently been initiated (Dawson, 1984).

Dihydroxypropyladenine ((*RS*)-DHPA), an aliphatic adenosine analog, has shown a potent antiviral action against some DNA and RNA animal viruses, particularly ss (−) RNA and ds (±) RNA viruses (De Clercq et al., 1984). BVDU, another nucleoside analog, is a promising antiviral agent for the treatment of DNA viruses, in particular herpesviruses (De Clercq, 1984). Dawson (1984) found that quite surprisingly compounds which are inhibitory to DNA viruses in animal systems may also inhibit RNA plant viruses. Therefore, both (*RS*)-DHPA and BVDU were selected for evaluation against 4 different groups of plant viruses: Tobamovirus, Potyvirus, Potexvirus and Tymovirus (Matthews, 1982).

Materials and Methods

Antiviral substances

(*RS*)-DHPA [9-(2,3-Dihydroxypropyl)adenine] and BVDU [(*E*)-5-(2-bromovinyl)-2-deoxyuridine] were synthesized by Dr. Antonin Holý (Czechoslovak Academy of Sciences, Prague, Czechoslovakia) and Dr. Roger Busson (Rega Institute, Leuven, Belgium), respectively. For the antiviral assays dilutions of the drugs were made in distilled water. Initially dilutions of 125, 250 and 500 mg/l were used. When these concentrations proved to be highly inhibitory, lower doses such as 5,10, 50 and 100 mg/l were used. Controls consisted of distilled water.

Plant hosts and viruses

Nicotiana tabacum 'White Burley' was chosen as systemic host for tobacco mosaic virus (TMV) and potato virus X (PVX). As local hosts, *Nicotiana glutinosa* or *Datura stramonium* were used for TMV and *Gomphrena globosa* for PVX. *N. glutinosa* was used as the systemic and local host for eggplant mosaic virus (EMV) and for a potyvirus isolated from *Solanum palinacanthum* (Poty-Sp).

The TMV and PVX inoculum consisted of partially purified virus preparations (1 mg/ml) and the EMV and Poty-Sp inoculum consisted of a crude sap preparation in the proportion of 1 g infected leaf tissue to 5 ml sodium sulphite solution at 0.5%.

Experimental procedures

Two types of experiments were carried out:

Incubation of leaf discs in different concentrations of the chemicals

Two expanded leaves of the systemic host were mechanically inoculated with the virus and after 1 h 12 mm discs were punched out from the interveinal area of the leaves. The antiviral solutions or distilled water (control) were distributed in 5 cm diameter Petri dishes containing 5 ml of liquid. The discs, 10 per plate, were randomly distributed in the plates, floating on the solution with the lower surface in contact with the liquid. The plates were then incubated for 72 h in a 25°C incubator with a 16 h photoperiod. After incubation, the discs were thoroughly rinsed, blotter-dried and grounded with 0.01 M (pH 7) phosphate buffer in the proportion of 0.1 ml per disc. The sap obtained was mechanically inoculated in the local host (recovery test) using the half-leaf method for TMV, EMV and Poty-Sp and the opposite leaves of *G. globosa* for PVX. In all cases 35 µl juice from leaf discs were inoculated in each half leaf or opposite leaf of the local host plant. The other half or opposite leaf received the control sap. The inoculated plants were maintained in a glasshouse until local lesions could be counted (5–7 days, according to the virus-host combination).

To evaluate the antiviral effect, the number of lesions obtained in the treated half or opposite leaf was counted and compared with the controls. To calculate the inhibition percentage (IP) the following formula was used: $IP = 100 - (\frac{A}{B} \times 100)$ where (A) = number of local lesions in the treated groups, (B) = number of local lesions in the control groups.

For each concentration, at least 9 leaves were used and the experiments were repeated 5 times. Data were submitted to statistical analysis. The least significant difference (LSD) was calculated by the Tukey test: $P=0.05$.

Spray of host leaves with different concentrations of the chemicals, before and after inoculation

This test was done only with (*RS*)-DHPA, because BVDU proved ineffective in the leaf disc incubation test.

Concentrations of 125, 250 or 500 mg/l were sprayed onto all leaves of the local host until dripping. Approximately 2 h later, when the leaves were dry, 3 leaves per plant were mechanically inoculated with the virus and immediately thereafter a second spray of the same solution was applied. Controls were sprayed with water. To all solutions, including controls, one drop of Tween-20/100 ml liquid was added as a wetting agent.

The plants were transferred to a glasshouse until local lesions could be counted. For each concentration 5 plants were used and the calculation of the inhibition percentage was based on the above mentioned formula. The experiment was repeated twice.

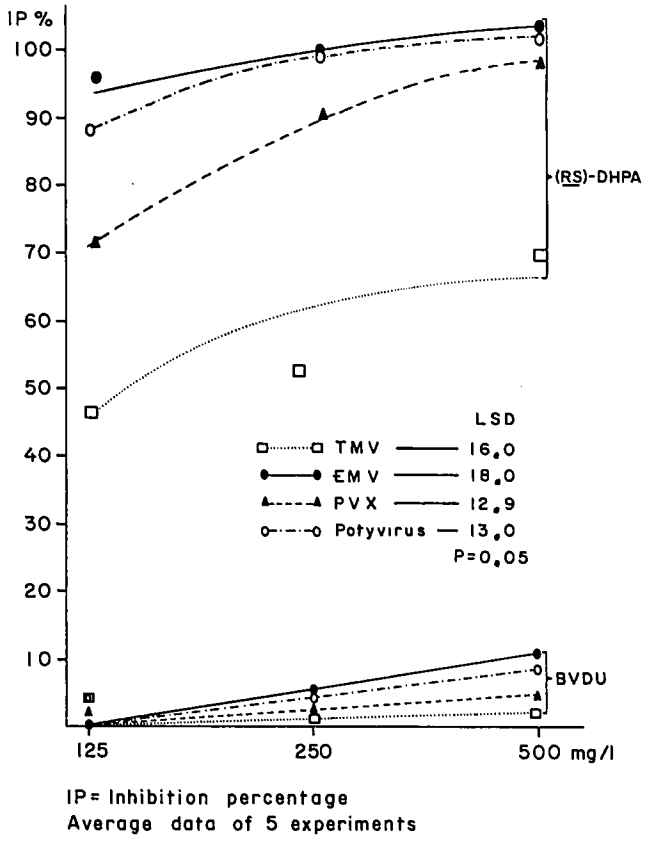

Fig. 1. Antiviral activity of high doses of (*RS*)-DHPA and BVDU against four plant viruses in the leaf disc incubation tests.

Results

Leaf disc incubation test

In this type of experiment the 2 tested drugs did not show any phytotoxicity.

Figs. 1 and 2 present the inhibition percentages obtained in the recovery tests. These results indicate that (*RS*)-DHPA was efficient in controlling EMV and Poty-Sp infections at concentrations above 50 mg/l, inducing high inhibition percentages (above 75%). The inhibitory effect of (*RS*)-DHPA on EMV and Poty-Sp infections gradually decreased at concentrations below 50 mg/l but was still noticeable at a concentration of 5 mg/ml (≈40% IP). TMV was less susceptible to the chemical while PVX could be efficiently inhibited at a concentration of 100 mg/l or higher. At concentrations of 125, 250 or 500 mg/l the drug was more effective against PVX than TMV. At a dose of 5 mg/l the drug was completely ineffective against PVX and TMV.

In contrast with (*RS*)-DHPA, BVDU was ineffective in protecting the plants

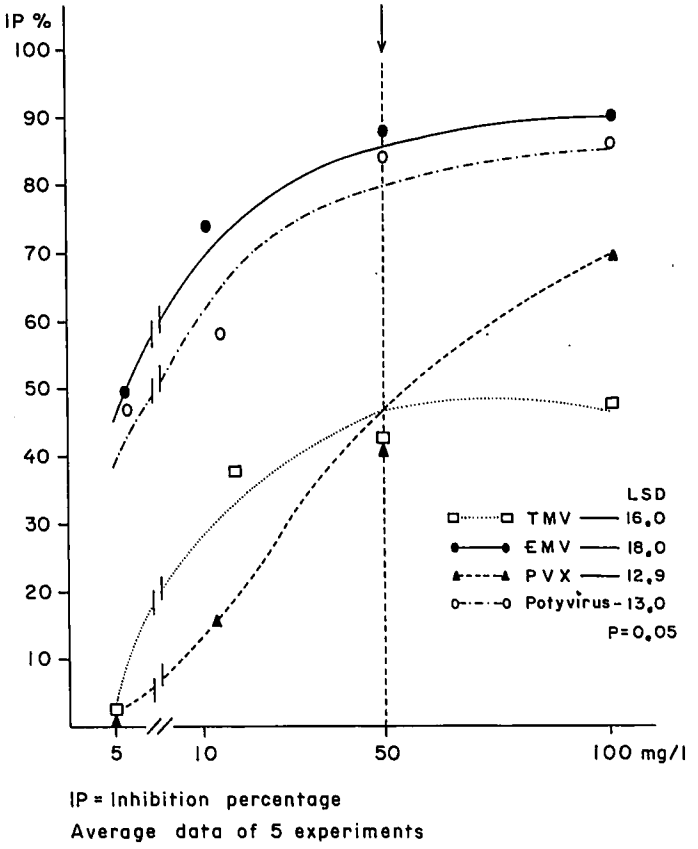

Fig. 2. Antiviral activity of low doses of (*RS*)-DHPA against four plant viruses in the leaf disc incubation test.

against any of the 4 viruses studied, even at a concentration up to 500 mg/l.

Leaf spray experiments

Leaf sprays, an adequate method for agricultural purposes, were applied to all leaves of the local host to further establish the inhibitory effect of (*RS*)-DHPA on the viruses studied. As the leaf epidermis is a barrier for the penetration of chemicals, in this set of experiments only high doses of the drug were used (125, 250 and 500 mg/l) in combination with the wetting agent Tween-20.

After spraying (*RS*)-DHPA twice, before and after virus inoculation, no phytotoxicity could be observed, even at the higher concentration of 500 mg/l. Using this method IP values for (*RS*)-DHPA were much lower than those obtained in the leaf disc incubation test (Fig. 3). At 500 mg/l, the effect of (*RS*)-DHPA was similar for all viruses, with IP values of approximately 40%. At 125 mg/l, the drug was ineffective against EMV, TMV and Poty-Sp, but at this concentration it was still effective against PVX, with an IP value of 35%.

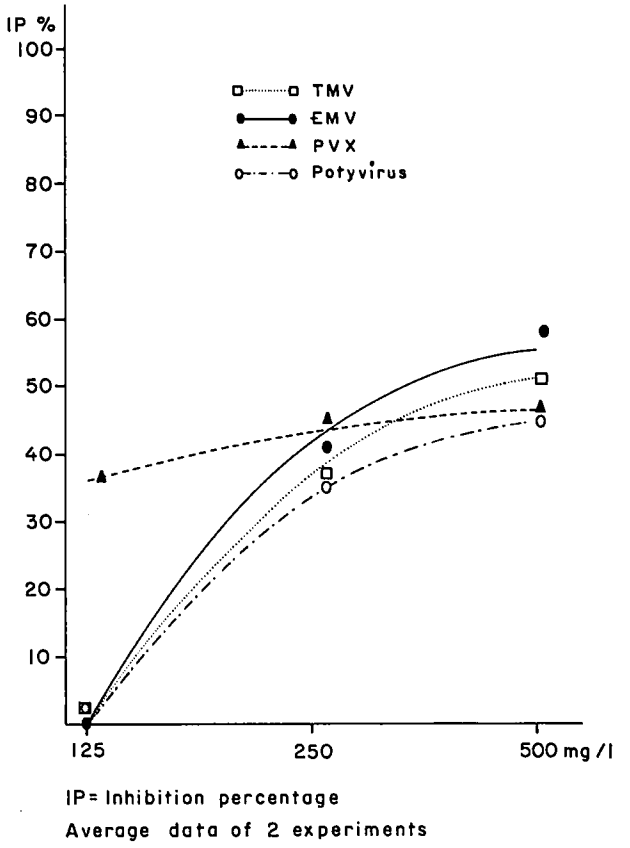

Fig. 3. Antiviral activity of high doses of (*RS*)-DHPA and BVDU against four plant viruses in the leaf spray experiments.

Discussion

(*RS*)-DHPA showed a selective inhibitory effect on some plant viruses, being more efficient against EMV and Poty-Sp than PVX and TMV. Similar results were obtained by Dawson (1984) who noticed that the compound inhibited the replication of cowpea chlorotic mottle virus (CCMV) but was substantially less active against TMV. It should be pointed out that (*RS*)-DHPA, which is active against animal (−)RNA and (±)RNA viruses, can also inhibit single stranded (+) RNA viruses which represent the majority of the plant viruses. BVDU did not exhibit any activity against any of the 4 plant viruses studied, probably because it is specific for the herpes virus group (De Clercq, 1984).

In the experiments with (*RS*)-DHPA no phytotoxic effect was observed. However, Beneš et al. (1984) found that when this drug was applied through the roots of *Vicia faba*, in nutrient solution, it causes a strong inhibition of root development.

Similar results were obtained when applying (*RS*)-DHPA through the roots of several other host plants (tobacco, *N. glutinosa*, bean, tomato) at concentrations above 100 mg/l (our unpublished results). Stevenson and Monette (1983) also observed a high phytotoxicity of (*S*)-DHPA at concentrations as low as 10 mg/l when applied to *Vitis vinifera* tissue cultures, infected with grapevine leafroll virus.

According to Pennazio and Martelli (1983), selected antiviral chemicals may be regarded as promising antiphytoviral compounds, if fulfilling the agricultural conditions for their use, such as low phytotoxicity, ecological neutrality and high therapeutic index (maximum tolerated dose/minimum effective dose). Thus, the practical application of (*RS*)-DHPA in agriculture will depend on appropriate studies of its phytotoxicity, toxic residues and metabolism in the plant, as well as the proper fomulation which would make it suitable for soil application.

The biochemical mechanisms of antiviral action of (*RS*)-DHPA in plants was not investigated. Nevertheless, its antiviral effect is presumably connected with an inhibition of S-adenosyl-L-homocysteine hydrolase, a key enzyme involved in the maturation of mRNA through S-adenosyl-L-methionine-dependent transmethylation reactions (De Clercq, 1987).

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