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Inhibition of phosphatase by open-chain nucleoside analogues in insects

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Summary. (S)-9-(2,3-dihydroxypropyl) adenine (DHPA), D-eritadenine and some other open-chain nucleoside analogues, which exhibit adverse biological effects in microorganisms, plants and animals, cause pronounced inhibition of intestinal phosphatases in the hemipteran insect *Pyrrhocoris apterus*. The rate of p-nitrophenylphosphate hydrolysis by homogenates from intestinal epithelium and Malpighian tubules was inhibited up to 94% by 2–10 millimolar concentrations of these drugs. This effect is stronger than that of sodium fluoride, which is recognized as a common inhibitor of phosphatase. We conclude that inhibition of phosphatase activity in the digestive and excretory organs may be responsible for the previously reported massive excretion of phosphorylated derivatives of the nucleoside analogues after their oral administration to insects.
Key words. 9-Alkyladenines; D-eritadenine; SAH-hydrolase; acid phosphatase; alkaline phosphatase; *Pyrrhocoris apterus*.

Nucleoside analogues related to (S)-9-(2,3-dihydroxypropyl) adenine (DHPA) exhibit adverse biological effects in most living organisms including virus, plant and animal systems. The effects are manifested, for instance, by pronounced antiviral action [1], inhibition of growth in plant roots [2], sterilization or ovicidal action in insects [3, 13], teratogenic effects in chick embryos [4], or aspermatogenic effects in mice [5]. It is generally believed that these compounds interfere with diverse biological systems by means of inhibition of S-adenosyl-L-homocysteine hydrolase (SAH-hydrolase) [5], which is the key enzyme involved in the essential methylation reactions [6, 7].

In our previous studies on the dietary effects of these drugs in insects, we have found endogenous phosphorylation and rapid excretion of all of the nucleoside analogues administered in the diet in the form of the corresponding phosphates, including optical enantiomers of the natural nucleosides [7, 8]. This metabolic pathway, i.e. phosphorylation and excretion of the phosphate, is not very common, because any more extensive excretion of the phosphorylated metabolites would inevitably deprive the organism of energy and inorganic phosphate. Thinking about a possible selective advantage of this unusual phenomenon, we have assumed that it might perhaps help the organism to eliminate all atypical and therefore hazardous nucleotides from the endogenous pool of essential ones. The problem remains, however, of how the phosphorylated metabolites could pass through the epithelium of the excretory organs into the hind gut and, finally, into the excrements. The observed 'phosphonuria' can in fact be classified as a disease, because the alimentary duct and Malpighian tubules of insects contain extremely active phosphatase enzymes, which are capable of hydrolyzing a wide range of structurally unrelated esters of phosphoric acid over a broad range of pH values [9]. Physiologically, these enzymes are engaged mainly in the turnover and reutilization of inorganic phosphate and in the transport of molecules across the epithelial membranes. In this communication we report briefly on the in vitro and in vivo effects of the selected analogues of nucleosides on phosphate hydrolysis in the epithelium of the intestine and Malpighian tubules.

Materials and methods. The experiments were performed on adult females of *Pyrrhocoris apterus* L., fed with dry linden seeds and kept at 25 °C, as has been previously described [3]. The nucleoside analogues were obtained through the courtesy of Dr A. Holý; their purity and preparation have been described by Holý et al. [8]. The organs selected for the assays, i.e. intestine (actually the midgut portion of the intestine or midgut epithelium alone) and Malpighian tubules were dissected in ice-cold insect Ringer. After careful washing, the tissues were transferred into glass homogenizers and stored frozen at −20 °C until used (no more than 3 weeks). The phosphomonoesterase activity was determined by a common p-nitrophenylphosphate method, using the micromodification of Linhardt and Walter [10]. We used 5.5 mM substrate in 0.05 M citrate buffer, pH 4.8 (acid phosphatase of the intestine), or pH 5.2 (acid phosphatase of Malpighian tubules). For alkaline phosphatase we used 0.05 M glycine buffer, pH 9.3 for both tissues. The results are expressed in µmole equivalents of the hydrolyzed p-nitrophenylphosphate related to one organ, 1 min, at 30 °C. The values are averages from 8–10 separate measurements.

Results and discussion. In the adult females of *Pyrrhocoris*, the first reproduction cycle is terminated by oviposition at day 6 after adult emergence (25 °C). The maximum metabol-

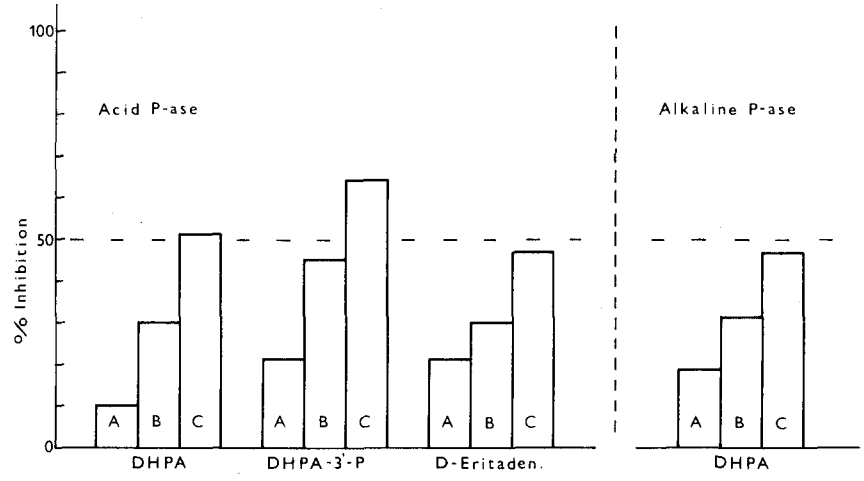

Figure 1. Inhibition of acid and alkaline phosphatases in the intestinal epithelium (midgut protion) of 3-day-old females of *Pyrrhocoris apterus*. Concentration of the inhibitors in the incubation mixture: A, 2 mM; B, 4 mM; C, 10 mM.

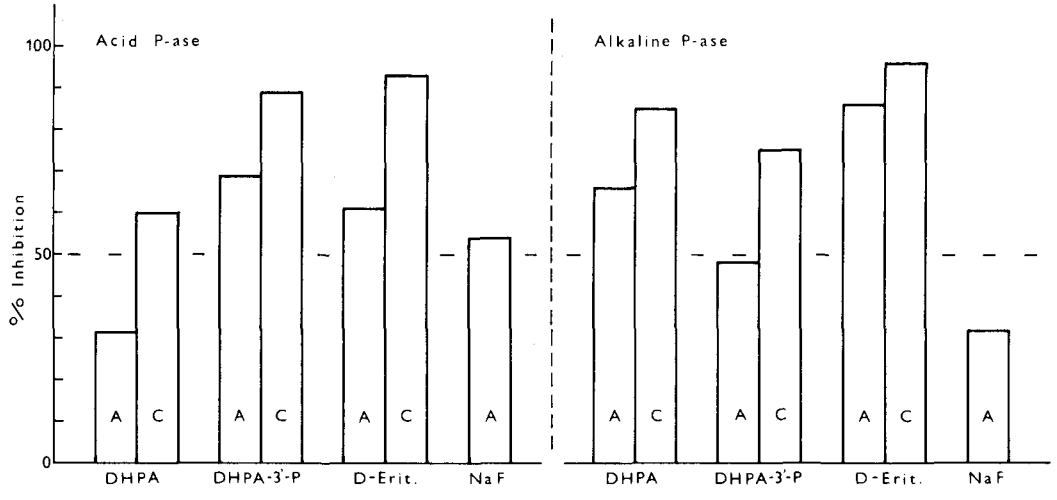

Figure 2. Inhibition of acid and alkaline phosphatases in the Malpighian tubules of 3-day-old females of *Pyrrhocoris apterus*. Concentration of the inhibitors in the incubation mixture: A, 2 mM; C, 10 mM.

ic intensity associated with the utilization of food is reached in the middle of the cycle. For this reason we used 3-day-old females for most of our enzyme assays. In order to simplify the discussions, we give the results in percentages of inhibition of phosphatase activity. The correct reference values for the untreated group of 3-day-old females (in µmole equivalents of hydrolyzed p-nitrophenylphosphate ± SD) are as follows: a) acid phosphatase in the midgut portion including the intestinal content – 186.3 ± 14.9; midgut epithelium alone – 94.4 ± 4.9; Malpighian tubules – 0.96 ± 0.15; b) alkaline phosphatase in the intestinal epithelium – 26.3 ± 12.7; Malpighian tubules – 0.58 ± 0.09. These values show that the substrate was hydrolyzed faster in the acidic pH range and, naturally, most of the enzyme activity was present in the intestinal epithelial cells (protein content of the intestinal epithelium used was 720 µg, that of Malpighian tubules 87 µg).

The results in figure 1 show that the activity of acid and alkaline phosphatases of the intestinal epithelium can be inhibited by millimolar concentrations of (S)-DHPA, (S)-DHPH-3′-phosphate and D-eritadenine. The (S)-DHPA-3′-phosphate has actually been determined as the chief metabolite of (S)-DHPA inside the body[7], as well as in the excrements[8]. Now we have found that it can cause even more pronounced inhibition of phosphatase in vitro than the parent compound itself. The results obtained with the homogenates from Malpighian tubules (see fig. 2) reveal basically similar relationships, although the degree of phosphatase inhibition is here somewhat higher. Thus, for instance, a 10 mM concentration of D-eritadenine inhibited hydrolysis of the phosphoric acid ester in Malpighian tubules by more than 94%. Moreover, at 2 mM concentrations, the nucleoside analogues tested elicited greater inhibition of phosphatase activity than did sodium fluoride, which is a common phosphatase inhibitor (cf. fig. 2).

In order to test a possibility of in vivo effects of the nucleoside analogues on phosphatase activity, we administered 1 mg · ml^{-1} of (S)-DHPA orally to the freshly emerged females of *Pyrrhocoris* (the compound was given in drinking water from day 0 to day 3 after emergence; the calculated daily dose was 1 mg · g^{-1} of body weight). According to our previous observations, administration of this dose would cause a 60% reduction of female egg laying and 100% inhibition of larval hatching from the eggs[3]. Analysis of phosphatase activity in the intestinal epithelium and Malpighian tubules of these 3-day-old females revealed a substantial de-

crease in the enzyme activity, mostly below 50% of that found in the same organs of equally old untreated control females. In certain cases phosphatase activity of the treated 3-day-old females averaged only about 30% of the control values. These results clearly show that the dietary supply of sublethal doses of (S)-DHPA interferes with physiological functions of the digestive and excretory phosphatase enzymes. We believe, therefore, that the previously reported massive excretion of the phosphorylated metabolites of unnatural nucleosides supplied in the diet[7,8] was most probably due to the inhibition of phosphatase activity in the intestine and in the Malpighian tubules.

Females of *Pyrrhocoris* which were sterilized by oral administration of 1 mg · ml^{-1} of (S)-DHPA also showed considerable suppression of the activity of SAH-hydrolase in the ovaries[7]. We concluded that the inhibition of SAH-hydrolase and consequently a decrease in the methylation index might be responsible for the induction of sterility in this system. Now, with these results, we can see that an inhibition of phosphatase activity in the visceral organs can be involved in female sterility as well. Phosphatase is a common hydrolytic enzyme that functions in all living organisms, including viruses. Inhibition of this enzyme is obviously an important factor which must be considered in calculations concerning the pharmacobiological action of any chemical compound. We still cannot decide whether inhibition of phosphatase would also be involved in causing the adverse effects of these drugs in biological systems other than insects. The general concept explaining the action of nucleoside analogues on the basis of inhibition of SAH-hydrolase[5-7,11] has some weak points. Thus, for example, recent studies have shown that certain open-chain nucleoside analogues (3'-O-phosphonyl-9-(S)-(2,3-dihydroxypropyl) adenine) can exhibit strong ovicidal and sterilization effects, although they do not inhibit SAH-hydrolase from rat liver[12]. Moreover, in the case of the aspermatogenic effect in mice[4], phosphatase inhibition would certainly be a more vulnerable biological target than inhibition of the SAH-hydrolase, because testicular tissues and accessory sexual glands of vertebrates are always linked with enormously large phosphatase activities. Correlations between biological activity and phosphatase functions can be found in various other target systems of the nucleoside analogues. They exist in insect reproduction[3,13] or in the effects of these drugs on the development of the seedling root system in plants[2]. Characteristically, in all these processes the phosphatase mediated extra-or intracellular transport of molecules plays a vital role.

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Dopamine-induced relaxation in human pulmonary arteries

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Summary. Dose-dependent relaxations were induced by dopamine in human pulmonary arteries that had been contracted with prostaglandin $F_{2\alpha}$ without α-adrenergic blocking agents. The dopamine-induced relaxation was inhibited by haloperidol and fluphenazine, but not by domperidone, suggesting that this relaxation was mediated via DA_1 receptors.
Key words. Dopamine; vasorelaxation; human; pulmonary arteries; DA_1 receptors.

It is well known that in some species dopamine has a specific vasodilator effect on several vascular beds, particularly in the renal and gastro-intestinal circulation. This vasodilator effect is generally ascribed to the stimulation of specific vascular dopamine receptors[1-4].

The subtype of dopamine receptors in vascular beds such as the dog renal arteries[2] and rabbit mesenteric[3] and splenic arteries[4] appears to be DA_1 receptors. In human vascular beds, dopamine-induced relaxation occurs in the renal[5] and cerebral arteries[6,7], but the basilar artery is the only one known to have DA_1 receptors. Recently, Hoshino et al. reported the relaxant effect of dopamine via DA_1 receptors in isolated rabbit pulmonary arteries[8]. However, there have been no reports on the relaxant effects of dopamine in the isolated human pulmonary artery, though the disorders in pulmonary circulation are clinically important[9-11].

The present study was undertaken to examine the effects of dopamine on human pulmonary arteries, and the subtype of receptors in the arteries was investigated.

Methods. Five pulmonary arteries (artery segments, 3 to 5 mm in diameter) were obtained from pneumonectomized or lobectomized specimens of lung tumors. The 5 patients (two men, 58 and 75 years old; and 3 women, 53, 57 and 59 years old) did not suffer from pulmonary or systemic hypertension and were not treated with drugs acting on adrenoceptors or dopamine receptors. The arteries were cut into helical strips, approximately 10 mm long. The strips were fixed vertically under a resting tension of 1.5 g in a cuvette containing 3 ml of Krebs-Henseleit buffer solution maintained at 37 ± 0.5 °C and aerated with a gas mixture of 95% O_2 and 5% CO_2. Preparations were allowed to equilibrate for 90 to 120 min before the start of experiments. Isometric