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Effects of Dipyridamole in vivo on ATP and cAMP Content in Platelets and Arterial Walls and on Atherosclerotic Plaque Formation

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Summary. In rabbits receiving an atherogenic diet for 2 months, the ATP content of platelet rich plasma (PRP) and arterial tissue was significantly elevated as compared to normal rabbits. This increase in ATP levels of platelets from atherosclerotic rabbits was paralleled by higher basal as well as PGI_2-induced cAMP levels. In arterial tissues, an increase was only obtained in PGI_2-stimulated cAMP content.

Treatment with dipyridamole (DPD) for 4 weeks resulted in a reduction of the ATP content in platelets and arterial tissue from atherosclerotic rabbits to values seen in normal animals. Again, the reduction of ATP content was reflected in a decrease of basal as well as PGI_2-induced cAMP levels in platelets, whereas in arterial tissue a decrease was only obtained in PGI_2-induced cAMP content. At the same time, DPD treatment enhanced atherosclerotic plaque formation in the aortic wall.

The enhanced atherosclerotic plaque formation seen in DPD treated atherosclerotic rabbits may be linked to the inhibition of adenosine uptake, resulting in a decrease of the adenine nucleotide pools of arterial wall cells. The decrease also caused a reduction in PGI_2-induced cAMP content. This effect may be linked to altered proliferative activity, since in many cell types, stimulation of cAMP levels results in reduced proliferation rates.

Key words: Atherosclerotic plaque formation – Dipyridamole in vivo – ATP content – cAMP content – PGI_2 – Platelets – Aortic tissue.

Introduction

Experimental and human atherosclerosis is associated with a decrease in prostacyclin (PGI_2) formation by arterial walls (Dembinska-Kiec et al., 1977; D'Angelo et al., 1978; Gryglewski et al., 1978) and an increase in aggregability of platelets (Breddin et al., 1974; Zmuda et al., 1977). Increased platelet aggregation is suggested to be a causative factor for the development of atherosclerosis, since aggregating platelets release arachidonic acid (AA) metabolites such as proaggregatory and vasoconstrictory thromboxane A_2 (TXA_2) (Hamberg et al., 1975) and factors which enhance the proliferative activity seen in atherosclerotic plaque formation (Harker et al., 1976).

Increased proliferation of connective tissue cells has been shown to be associated with a decrease in cAMP levels (Pastan et al., 1975). Decreased cAMP content was found in atherosclerotic arteries (Numano et al., 1976; Numano, 1977). Therefore, compounds which increase cAMP levels in platelets and in arterial walls may affect the development of atherosclerosis. PGI_2 formed by vascular endothelium (Moncada et al., 1977) has been described to stimulate platelet cAMP levels (Tateson et al., 1977). PGI_2 was also shown to increase cAMP levels in bovine coronary arteries and in rabbit aortic rings in vitro (Dembinska-Kiec et al., 1979a).

Dipyridamole (DPD) was described to potentiate the antiaggregatory action of endogenous PGI_2 in vivo and this effect was linked to an inhibition of phosphodiesterase activity in platelets (Moncada and Korbut, 1978). An other action of DPD in vitro is the inhibition of adenosine uptake into platelets (Born and Mills, 1969; Sixma et al., 1976) and arterial endothelium (Bono et al., 1976; Dieterle et al., 1978).

In previous studies from this laboratory it was shown that DPD treatment for 4 weeks resulted in a reduction of platelet loss in rabbits with advanced atherosclerosis (5 months atherogenic diet). Surprisingly, an increase of atherosclerotic plaque formation was observed in aortic tissue of these rabbits (Dembinska-Kiec et al., 1979b). Therefore, the influence of DPD treatment in vivo was studied on ATP

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and cAMP content in platelet rich plasma (PRP) and aortic tissue in developing atherosclerosis of rabbits (2 months atherogenic diet).

Materials and Methods

Materials were obtained from following sources: Dipyridamole (DPD) (Persantin® Ampullen) from Thomae AG (Biberach, FRG); adenosine triphosphate (ATP) and cyclic 3′:5′-adenosine monophosphate (cAMP) from Boehringer (Mannheim, FRG): 3-isobutyl-1-methylxanthine (IBMX) from Aldrich Chemicals (Milwaukee, USA). All other chemicals were of reagent grade from the usual commercial sources.

Prostacyclin (PGI_2) was kindly supplied by Dr. W. Bartmann (Hoechst AG, Frankfurt, FRG) and Dr. J. Pike (Upjohn C., Kalamazoo, USA).

Induction of Atherosclerosis and Treatment

Four groups of bastard male rabbits (3–4 kg) were used, each group consisting of 5 animals. Normal controls were maintained on standard rabbit food ad libitum during 8 weeks. The second group was maintained under identical conditions as the control animals, but received 3 × 10 mg/day DPD intramuscularly for the last 4 weeks. The third group received an atherogenic diet by addition of 1% cholesterol and 3% olive oil to the standard pellet food for 8 weeks. The fourth group consisted of rabbits receiving the atherogenic diet for 8 weeks, but being treated with 3 × 10 mg/day DPD i.m. for the last 4 weeks. The animals were sacrificed at the end of the experimental series 10 h after the last dose of DPD.

ATP and cAMP Levels in Platelet Rich Plasma (PRP)

Blood samples were collected from the ear artery into 3.8% sodium citrate 10 h after the last dose of DPD. PRP was prepared by centrifugation of the citrated blood at 200 × g for 10 min at room temperature. Platelet counts were performed by standard procedures (Thrombosol, Türk's chamber). For determination of ATP and cAMP levels in platelets, 1.0 ml samples of PRP were incubated with or without PGI_2 (0.3–30 μM) at 37° C for 1 min. For determination of ATP levels, aliquots of 0.2 ml were removed from the incubations and pipetted into 1.0 ml boiling 0.05 M glycine buffer pH 11.0. ATP was measured by adding 0.5 ml aliquots to 5 ml luciferin-luciferase solution in 40 mM $MgSO_4$ and 0.2 M sodium arsenate buffer (pH 7.4) and recording of the immediate photon emission in a Nuclear Chicago 720 Liquid Scintillation Counter (Kalbhen and Koch, 1967). Results are expressed as nmoles ATP/2 × 10^8 cells.

For determination of cAMP levels, 0.5 ml aliquots of the incubations were pipetted into 0.25 ml 15% trichloroacetic acid (TCA). Following removal of TCA by ether extraction, cAMP was measured by use of the protein binding procedure (Gilman, 1970). Values are expressed as pmoles cAMP/2 × 10^8 cells.

ATP and cAMP Content in Aortic Rings

Aortas were carefully dissected, transferred into cold Krebs-Henseleit solution, cut into rings (25–30 mg) and immediately used for incubations. For determination of ATP content, aortic rings (25–30 mg) were incubated in a final volume of 0.5 ml Krebs-Henseleit solution in the presence and absence of 0.3–30 μM PGI_2 at 37° C for 3 min. Reactions were stopped by immersing the rings into 1.0 ml boiling 0.05 M glycine buffer pH 11.0. The tissue was homogenised by sonification and diluted 10-fold in glycine buffer. Aliquots (0.5 ml) were added to 5 ml luciferin-luciferase solution for ATP determination as described above. Protein was determined according to Lowry et al. (1951). Results are expressed as nmoles ATP/mg protein.

For determination of cAMP content, incubations were performed under identical conditions as for ATP content. 1 mM IBMX was added 1 min before addition of PGI_2, when cAMP content was studied in the presence of a phosphodiesterase inhibitor. Incubations were stopped by immersing the rings into liquid nitrogen and adding 0.5 ml 15% TCA in the moment when liquid nitrogen was about to evaporate. The tissue was homogenized by sonification. Aliquots (0.3 ml) of the homogenate were used for cAMP assay following removal of TCA by ether as described above. Results were expressed as pmoles cAMP/mg protein.

Determination of Atherosclerotic Plaque Formation

Rings of the ascending aorta (2 cm long) were dissected, suspended in saline and fixed between slides. Visible atherosclerotic plaques were copied on transparent papers. Pieces of transparent paper corresponding to the total aortic tissue and to the atherosclerotic plaques were carefully cut out and weighed. The percentage of aortic wall occupied by atherosclerotic plaques was calculated from the weight of the papers. Lipid deposits in the aortic tissue were confirmed by use of standard hematoxylin-eosine and Sudan IV staining techniques.

Statistics. Statistical significance was calculated by means of the Student's *t*-test.

Results

ATP Content in Platelets and Aortic Rings

Platelets from atherosclerotic rabbits contained more ATP than platelets from normal rabbits (Fig. 1). DPD treatment did not significantly affected ATP levels in platelets from normal animals. However, DPD treatment significantly reduced ATP levels in platelets from atherosclerotic rabbits. Addition of PGI_2 in vitro for 1 min did not significantly alter platelet ATP content under all conditions.

Similar results were obtained with ATP content in aortic rings. Aortic rings from atherosclerotic rabbits contained significantly more ATP than rings from normal rabbits (Fig. 2). DPD treatment did not significantly affected ATP content in rings from normal rabbits, but significantly reduced ATP content in aortic rings from atherosclerotic rabbits, resulting in ATP content similar to that obtained in normal rabbits. Again, addition of PGI_2 in vitro for 3 min did not significantly alter the ATP content of arterial tissue under all conditions.

cAMP Content in Platelets and Aortic Rings

PGI_2 dose-dependently stimulated cAMP levels in platelets. Platelets from untreated atherosclerotic rabbits showed significantly higher cAMP levels following addition of PGI_2 as compared to platelets from untreated normal rabbits (Fig. 3). The DPD treatment reduced PGI_2 stimulated cAMP levels both in platelets from normal and atherosclerotic rabbits, but was much more effective in platelets from atherosclerotic rabbits,