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Effect of Persantin on nucleoside metabolism of the perfused rabbit heart*

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PERSANTIN [2,6-bis(diethanolamine)-4,8-dipiperidinopyrimindo(5,4-*d*)pyrimidine] has been used clinically as a vasodilator.[1,2] The basis of this effect is not known; however, it has been suggested that the drug acts to spare adenosine, a potent vasodilator, from dismutation. Persantin inhibition of adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) is well recognized.[3] On the other hand, the vasodilator effect of administered adenosine is potentiated at dose levels of Persantin far below those required to inhibit adenosine deaminase activity (10^{-4} M).[3] Adenosine is avidly incorporated into the heart, and it has been suggested that its rapid uptake may be the basis for the termination of its vasodilator action.[4] Persantin inhibits the uptake of adenosine into red blood cells[5] and into the myocardium,[6] and it is this effect which may explain the potentiation of adenosine-induced vasodilation. Most of the studies on Persantin inhibition of adenosine uptake have dealt with the total radioactivity in the tissue. Thus, the effect of Persantin on the fate of adenosine taken up by the heart has not been previously reported. The present study is concerned with the effect of Persantin on the uptake of [8-^{14}C]adenosine and [8-^{14}C]inosine into the perfused rabbit heart and on the incorporation of the labeled nucleosides into the myocardial adenine nucleotides.

The methods used in this experiment have been described in a previous paper.[4] Rabbit hearts were perfused at 25 ml/min on a non-recirculating basis for 30–60 min with Ringer's solution equilibrated with 95% O_2 and 5% CO_2 at 31°. The perfusion medium was then changed to the Ringer's solution containing labeled adenosine or inosine, and the hearts were perfused for an additional period of 3 or 30 min. Persantin was made up to the desired concentrations in nucleoside containing Ringer's and perfused through the heart together with the labeled nucleoside. At the end of perfusion, the heart was freeze-pressed between the jaws of an aluminium clamp precooled in liquid N_2 and the radioactivity in the heart powder and in the perchloric acid extract was determined. Acid soluble nucleotides and their metabolites were separated by anion exchange column chromatography.[7] The concentration of nucleotides was measured with a spectrophotometer according to the procedure of Kalckar[8] and the radioactivity was determined with a liquid scintillation counter. Nucleotide specific activity is expressed as counts/minute per micromole of the nucleotide.

Persantin added to the perfusion medium at the concentration of 0·25–1·0 μg/ml greatly decreases the specific activity of adenine nucleotides determined after 30 min of perfusion with 0·2 μM of [8-^{14}C]adenosine (Fig. 1). The levels of myocardial adenine nucleotides are not affected. The lowest concentration (0·25 μg/ml) suffices to reduce the specific activity by 80 per cent. Concentrations of the drug greater than 0·25 μg/ml have little effect on the extent of inhibition of adenosine uptake. On the other hand, the order of magnitude of specific activity among adenine nucleotides (i.e. ADP ≥ ATP ≥ AMP) is not altered by Persantin. The total radioactivity recovered from the Persantin-treated hearts is consistently lower than that seen in the absence of the drug, while the distribution of the counts in the heart remains unchanged (Table 1). In both cases, the majority of the counts is found in the compartments extractable by perchloric acid, and the activity in the acid extract is mostly associated with the adenine nucleotides. The inhibition of adenosine uptake occurs so rapidly that lower specific activity of adenine nucleotides is observed even after 3 min of perfusion.

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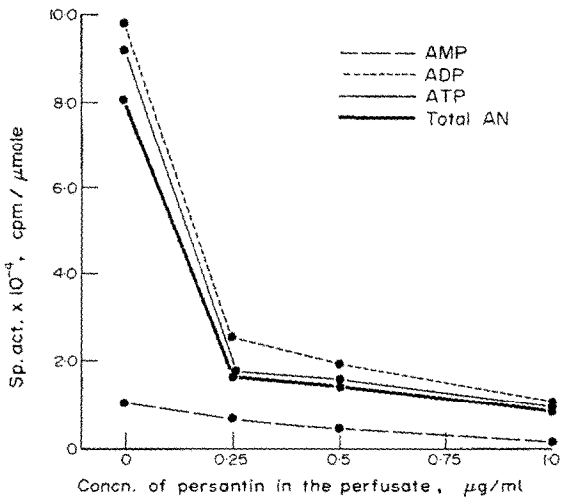

FIG. 1. Effect of Persantin on the specific activity of adenine nucleotides of rabbit heart after perfusion of 0·2 μM of [8-^{14}C]adenosine (sp.act. = 8·89 × 10^7 counts/min/μmole) for 30 min. Each value represents the average of two hearts.

TABLE 1. DISTRIBUTION OF RADIOACTIVITY IN THE PERFUSED HEART AFTER 30-MIN PERFUSION WITH 0·2 μM OF [8-^{14}C]ADENOSINE AND DIFFERENT LEVELS OF PERSANTIN*

Concn of Persantin (μg/ml)	^{14}C/g Heart (counts/min × 10^{-4})		
	Heart powder	Acid extract	Ad. nucleotides
0	13·70	12·85	9·95
0·25	4·56	3·29	2·48
0·50	4·27	3·28	1·86
1·0	2·29	2·22	1·59

* Each value represents the average of two hearts. Specific activity of [8-^{14}C]-adenosine perfused = 8·89 × 10^7 counts/min/μmole.

A constant level of Persantin (1 μg/ml) was perfused with various concentrations of adenosine (0·2–20 μM) for 30 min and the specific activity of adenine nucleotides was determined. As the specific activity of adenosine in the perfusate is different with each concentration, the specific activity of adenine nucleotides is divided by the specific activity of perfused adenosine; this value is designated as the relative specific activity and is used to compare the uptake of adenosine of different concentrations. Adenosine incorporation into adenine nucleotides in the presence and the absence of the drug can be described by a modified Michaelis–Menten plot (the reciprocal of the relative specific activity vs the reciprocal of the adenosine concentration (Fig. 2). The apparent K_m for adenosine incorporation is 7 × 10^{-6} M.

Persantin at a concentration of 1 μg/ml also prevents the incorporation of radioactivity from [8-^{14}C]inosine (0·3 μM) into the adenine nucleotides during a 30-min perfusion period. However, the extent of inhibition (60 per cent) is considerably less than that seen in the case of adenosine.

An earlier study showed that perfused adenosine is readily incorporated into myocardial adenine nucleotides without prior deamination or loss of the ribose moiety.[4] It is suggested that adenosine incorporation is mainly mediated by myocardial adenosine kinase (ATP: adenosine 5′-phosphotransferase, EC 2.7.1.20).[9,10] Radioactivity from perfused [8-^{14}C]inosine is also recovered in adenine nucleotides;[4,11] however, the extent of uptake is one-fifteenth that of adenosine, and it is likely that inosine is first converted to hypoxanthine prior to incorporation into nucleotides.[4] The present

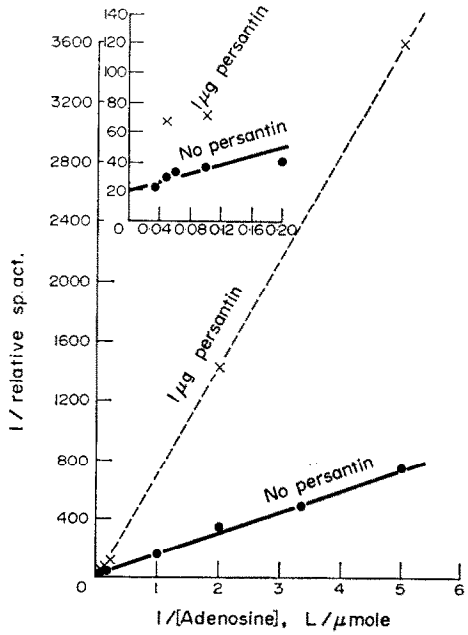

FIG. 2. Plot of the reciprocal of the relative specific activity (specific activity of adenine nucleotide/specific activity of adenosine) and the reciprocal of adenosine concentration in the perfusate. The regression lines are obtained by least squares analysis. The inset shows the distribution of values at higher adenosine levels.

study shows that Persantin added to a perfusate containing labeled adenosine or inosine leads to a decrease in the uptake of radioactivity into the perfused heart. These findings are in agreement with the observations of Kolassa *et al.*[6] but extend the observation to the drug effect on the intracellular metabolism of the nucleosides.

The decrease in specific activity of adenine nucleotides after Persantin could be due to the blockade of adenosine permeation or to the interference with iutracellular metabolic processes for adenine nucleotide formation. Since Persantin has been implicated as an inhibitor of adenosine deaminase, the decrease in the specific activity of adenine nucleotides could also be a result of endogenous accumulation of adenosine which acts to repress further adenosine uptake or to dilute the specific activity of perfused labeled adenosine.

The last assumption is unlikely because the concentration of Persantin used is much lower than that reported to be effective for adenosine deaminase inhibition.[3] It is possible that Persantin acts by inhibiting adenosine kinase, and since cell membranes are permeable to nucleosides, inhibition of adenosine kinase would lead to the ready efflux of adenosine that enters the heart. The finding that most of the activity in the heart is in the adenine nucleotides and that the order of specific activity of adenine nucleotides is not altered after Persantin suggests that adenosine kinase and/or the enzymes involved in the conversion of adenosine to adenine nucleotides are apparently not affected. The lower specific activity of AMP than that of ADP and ATP has been explained on the basis that a rapid equilibrium occurs among adenine nucleotides and that separate pools of AMP occur in the myocardium.[4] Recently, we have isolated a soluble adenosine kinase from rabbit and dog heart and have shown that the addition of Persantin has no effect on the phosphorylation of adenosine *in vitro*.[12] The failure of Persantin to inhibit nucleoside kinase while preventing nucleoside uptake was also found in rat hepatoma cell cultures[13] and in murine leukemic cells.[14]

The observation that inosine incorporation is inhibited by Persantin provides further support for the suggestion that the drug acts mainly by affecting nucleoside permeation into the myocardium. Inosine, while readily incorporated into myocardium, requires further metabolism to hypoxanthine before conversion to nucleotides. Hypoxanthine permeation into myocardium is not affected by Persantin.[6] Thus, the inhibition of inosine incorporation into the nucleotides is likely to be a result of

the inhibition of inosine permeation and a consequent decrease in the availability of inosine as a substrate of conversion to hypoxanthine.

The present study leads to the conclusion that, in the functioning heart, as in isolated cells, the primary effect of Persantin at a low concentration is on the permeation of nucleosides rather than on the metabolism of nucleosides in the cell.

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Subcellular location of the kininogenase in the coagulating gland of the guinea-pig*

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THE COAGULATING glands of the guinea-pig are paired accessory sex glands situated near the base of the seminal vesicles.[1] The secretion from these glands was first shown to be highly "toxic" to laboratory animals by Freund *et al.*[2,3] They also observed that it increased vascular permeability, possessed arginine esterase activity, and coagulated the secretion of the seminal vesicles. In further studies, it was shown that the substances responsible for these effects were proteins and that the coagulating action was due to a different protein from that responsible for the other effects of the secretion.[3,4]

Bhoola *et al.*[5] and Moriwaki and Schachter[6] later found that the accessory sex glands of the guinea-pig, and the coagulating gland in particular, contain a potent kininogenase. They showed that this kininogenase, called CGK, was reponsible for the hypotensive, permeability-enhancing, and esterolytic actions of extracts of the coagulating gland of the guinea-pig. They also showed that CGK released the nonapeptide, bradykinin.

The main purpose of the present experiments was to determine the subcellular location of this kininogenase. Recent studies of this nature have been done for the kininogenases of the submaxillary gland,[7–9] pancreas[10] and kidney.[11,12] In the submaxillary gland and pancreas of several mammals studied, the enzyme was found mainly in zymogen-type granules.[7–10] In the kidney of the rat, it has

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