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EFFECTS OF CORONARY VASODILATOR DRUGS ON THE UPTAKE AND RELEASE OF ADENOSINE IN CARDIAC CELLS*

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Abstract—The effects of coronary vasodilator drugs on the uptake and release of adenosine due to hypoxia were studied in isolated cardiac cells maintained in culture. Isolated cardiac cells were used as a model in order to avoid the problems associated with intact heart models, i.e. cellular heterogeneity, and hormonal and neural influences. A K_m value of 2.5 μM and a V_{max} of 0.9 nmole/mg of protein/30 min were determined for the uptake of adenosine in this model. The ability of coronary vasodilator drugs to inhibit the uptake of adenosine (0.023 μM) into heart cells was in the following order: dipyridamole > dilazep > hexobendine > lidoflazine > papaverine. All of these agents blocked the uptake of adenosine in a competitive manner. Concentrations ranging from 10^{-3} to 10^{-9} M of aminophylline, carbochromen and nitroglycerine had no effect on the uptake of adenosine into the cardiac cells. None of the drugs had a significant effect on the release of adenosine from the heart cells under hypoxic conditions. It is concluded that the coronary vasodilator actions of dipyridamole, dilazep, hexobendine, papaverine and lidoflazine are related to a block in the uptake of adenosine.

Several long-acting coronary vasodilator drugs, dipyridamole, hexobendine, lidoflazine, dilazep and papaverine, have been reported to potentiate markedly the vasodilator action of adenosine in the heart [1–4]. Adenosine plays an important role in the regulation of coronary blood flow [5]. Action by vasodilator drugs on the uptake of adenosine may result in higher interstitial levels of this metabolite. The exposure of the coronary resistance vessels to this elevated concentration of adenosine would result in increased blood flow to the heart.

Most of the studies on the effects of vasodilator drugs on the metabolism of adenosine have been performed with isolated perfused, or *in situ*, heart preparations. These preparations have some disadvantages: (i) it is not certain whether the adenosine released from the hypoxic myocardium in an intact preparation arises from cardiac muscle cells, vascular smooth muscle cells, endothelial cells, or other cell types present in the myocardium; and (ii) it has been suggested [6] that local changes in the pO_2 around the resistance vessels may result in the production of adenosine and cause vasodilation. Thus, endothelial and vascular smooth cells rather than cardiac muscle cells may be the source of adenosine; for this reason the assumption made in the adenosine hypothesis [5] of coronary blood flow regulation, that adenosine is produced mainly by cardiac cells and not by other cells present in the myocardium, is in question. Also, it has been suggested that the production of adenosine is directly related to the metabolic status of the myocardium [7], which tends to rule out cells other than parenchymal cells as the main source of adenosine, due to the small contribution of non-cardiac muscle cells to the mass of the myocardium.

In order to understand the cellular mechanism(s) underlying the actions of coronary vasodilator drugs, it is necessary to know the effects of these drugs on the uptake and release of adenosine from cardiac muscle cells, which have been shown to be the main source of adenosine production in the myocardium [8]. The release and uptake of adenosine are the primary means which determine the amount of adenosine available for vasodilation in the heart. Also, the measured amounts of adenosine released into the perfusates of *in vivo* or *in vitro* heart preparations probably do not reflect the levels of adenosine *in situ*, since during its passage across the capillary membrane some of the adenosine is degraded to inosine and hypoxanthine.

Isolated cardiac cells offer certain advantages over intact heart models: (i) the direct effect of vasodilator drugs on a particular cell type can be determined wthout the influence of neural and hormonal factors; (ii) cardiac muscle cells can be studied without a significant contribution from other cell types present in the myocardium; and (iii) the transvascular diffusion barrier is nonexistent. This study is an evaluation of the effects of coronary vasodilator drugs, using isolated cardiac muscle cells maintained in culture as a model.

Kubler *et al.* [9], using dipyridamole, and Kukovetz and Poch [10], using dipyridamole, papaverine and lidoflazine, suggested that these drugs may actually inhibit the release of adenosine from the hypoxic myocardium of intact animals. This inhibitory action would serve to decrease extracellular concentrations of adenosine and would be incompatible with the adenosine hypothesis [5]. Therefore, a second objective of the investigation was to study the release and uptake of adenosine in the presence of various vasodilator drugs with the use of this model. Other coronary vasodilator

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drugs, nitroglycerine, carbochromen and aminophylline, were included in this investigation to determine their effects on the metabolism of adenosine.

METHODS

Preparation of heart cell cultures

Cardiac cells were isolated from hearts taken from 16-day-old chick embryos according to the procedure described by Mustafa *et al.* [8]. The hearts were dissected, using sterile techniques, from 6 to 8 dozen chick embryos and placed in a mixture of 50% modified Hank's and 50% Puck's medium. Each heart was cut into four pieces to facilitate the removal of intracardiac blood. The hearts were minced and transferred to a 50-ml sterile conical flask. About 10–12 ml of a mixture consisting of 0.1% collagenase and 0.2% hyaluronidase made up in 50% N-16 Puck's medium and 50% of normal modified Hank's medium (containing 3.06×10^{-3} M Ca^{2+} and 0.81×10^{-3} M Mg^{2+}) was added to the flask and stirred for 10 min at low speed. The supernatant fraction which contained mostly broken cells and blood was discarded. This process was repeated once more and the supernatant fraction discarded again. A fresh solution of collagenase and hyaluronidase was added to the remaining tissue and the process of tissue digestion was repeated five to six times to obtain a sufficient number of cardiac cells. The supernatant fractions were centrifuged and the pellet was resuspended, washed, and resuspended in about 80 ml of growth medium (15% horse serum + 40% N-16 Puck's + 44% modified Hank's + 1% penicillin-streptomycin). Portions of this suspension were transferred to 25 cm^2 plastic culture dishes and sealed under sterile conditions. The cultures were then left in an incubator for 24 hr with medium containing 10^{-4} M adenosine, as a means of restoring cellular ATP values of the cultures to control levels [8, 11, 12].

The viability of the preparation was assessed by examining the monolayer cultures with a phase contrast microscope. The cells in culture, observed after 24 hr of plating, showed spontaneous contractions and were revealed to be cardiac muscle cells [13]. Cultures older than 48 hr were not used because the ratio of fibroblasts to muscle cells increased as the cultures aged.

Effects of vasodilator drugs on the incorporation of adenosine into cardiac cells

Varying the drug concentrations. The cultures (maintained between 24 and 48 hr) were washed several times, without disturbing the cell layer, with modified Hank's solution to remove cellular debris and growth medium. The incubation mixture consisted of 0.05 ml [U-^{14}C]adenosine (0.023×10^{-6} M, sp. act. to 2.71 mCi/m-mole; 26,665 cpm) with various amounts of drug (10^{-3} to 10^{-9} M), and modified Hank's solution containing glucose-free phosphate buffer (pH 7.4) in a total volume of 2.0 ml. A control, with vehicle (modified Hank's solution) substituted for the drug, was run simultaneously. The dishes were incubated at 37° with shaking for 30 min in room air. At the end of the incubation, the cells were scraped off and immediately transferred to polyethylene centrifuge tubes. These tubes with the samples were immersed in ice. Two dishes were used per experiment; each was washed once with 1 ml of modified Hank's solution and the washings were added to the same tube. The tubes were then immediately centrifuged at 4° at 10,000 *g* for 5 min. After centrifugation, the supernatant fraction was decanted into a separate tube. The pellet was resuspended and washed once with 0.5 ml of modified Hank's solution, centrifuged, and added to the previous supernatant fraction. The supernatant fraction will hereafter be referred to as "medium" and the pellet as "cells".

Perchloric acid (0.5 N) (1 ml) was added to the cell fractions to inactivate proteins. The cells were then homogenized with a polytron homogenizer (Brinkman, NY) for 90 sec at half maximum speed and centrifuged for 10 min at 10,000 *g* in the cold (4°) to remove the protein precipitate. The precipitate obtained from the cell fraction was washed once with perchloric acid (0.2 ml) and used for protein measurement [14]. The results of the uptake and release of adenosine were expressed on the basis of cellular protein concentrations.

The supernatant fraction containing perchloric acid was brought to pH 7.0 by the addition of potassium hydroxide, then placed in the refrigerator overnight, and the perchlorate subsequently removed by centrifugation.

The medium (discussed earlier) fractions were boiled for 5 min to denature any remaining protein, followed by centrifugation at 10,000 *g* for 5 min to collect the supernatant fraction. An aliquot from each fraction (medium and cells) was removed for measurement of radioactivity in a Beckman liquid scintillation counter. The scintillation mixture consisted of 5.5 g of 2,5-diphenyloxazole (PPO), 0.1 g of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzene (dimethyl POPOP), 333 ml of Triton X-100 and 667 ml of toluene. The counting efficiency of ^{14}C was unchanged from sample to sample.

Varying the extracellular concentration of adenosine. The washed cultures were incubated with various concentrations of extracellular adenosine at 37° for 30 min. The final concentration of adenosine ranged between 1 and 30×10^{-6} M. Individual solutions contained 9.22 pmoles [U-^{14}C]adenosine (5.42 mCi/m-mole; 13,332 cpm) with the balance made up of unlabeled adenosine to achieve the required concentration. In experiments where the effect of drug on adenosine uptake was studied, a constant volume of dissolved drug (0.1 ml) at a specified concentration (10^{-5} to 10^{-8} M) was included in the assay mixture (2.0 ml). At the end of incubation, the samples were processed for counting radioactivity according to the procedure described earlier.

Effects of drugs on the release of radioactive adenosine from hypoxic cardiac cells

During the change of growth medium 24 hr after plating, 0.05 ml [U-^{14}C]adenosine (0.023×10^{-6} M; 2.71 mCi/m-mole; 26,665 cpm) was added to each culture dish along with unlabeled adenosine to label the nucleotide pool(s) of the cells. These cultures, after a total incubation of 48 hr, were used to assess the release of radioactivity under hypoxic conditions. The medium was decanted and the adhering cell layer washed several times with modified Hank's solution to remove cellular debris and growth media which also contained labeled adenosine and its metabolites.

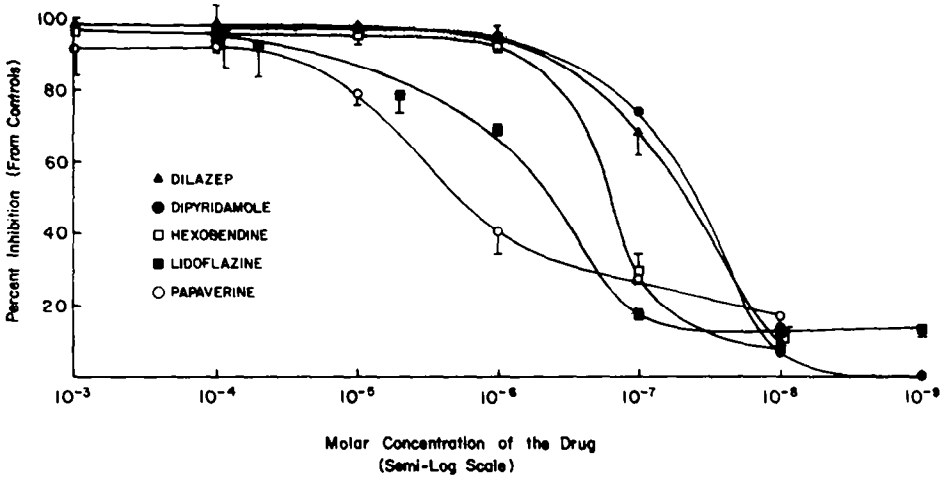

Fig. 1. Dose–response relationships between various vasodilator drugs and percent inhibition in the uptake of intracellular [U-^{14}C]adenosine into cardiac cells. The assay mixture (2 ml) contained 0.05 ml of radioactive adenosine (0.023 μM; sp. act. 2.71 mCi/m-mole; 26,665 cpm), an appropriate amount of drug, and the balance made up of modified Hank's solution with no glucose (pH 7.4). Values are means ± S.E.M. from four to six experiments. Other details are given in the text.

An assay mixture containing 0.2 ml of drug (in modified Hank's solution), and 1.8 ml of modified Hank's solution (pH 7.4; without glucose) was placed over the cells. In control experiments the drug was replaced by an additional 0.2 ml of modified Hank's solution. The dishes were mixed by swirling and then incubated at 37° for 30 min in an incubator with a continuous inflow of 95% N_2 + 5% CO_2 atmosphere to accelerate the breakdown of adenine nucleotides. At the end of incubation, the samples were processed for radioactivity according to the procedure described earlier. The appearance of radioactivity in the incubation medium demonstrated the effects of these agents on the release of adenosine (or degradation products) from the cells.

The drugs were obtained from the following companies: dipyridamole (Boehringer Ingelheim, New York, NY); papaverine–HCl (Marion Laboratories, Inc., Kansas City, MO); aminophylline (Sigma Chemical Co., St. Louis, MO); nitroglycerine (Eli Lilly & Co., Indianapolis, IN); dilazep dihydrochloride (Asta Werke, AG, Bielefld, West Germany); hexobendine (Chemie Linz, AG, Austria); lidoflazine (Janssen, New Brunswick, NJ); and carbochromen (Cassella, Frankfurt, West Germany).

RESULTS

Effects of vasodilator drugs on the uptake of adenosine in the cardiac cells

The dose–response relationship at concentrations ranging from 10^{-3} to 10^{-9} M for dipyridamole, papaverine, hexobendine, dilazep and lidoflazine are given in Fig. 1 and the calculated ID_{50} values in Table 1. The data are expressed on the basis of percent inhibition of the uptake of adenosine in comparison to controls on a per mg protein basis. Dipyridamole (10^{-4} to 10^{-6} M) exerted an inhibition of almost 97 per cent which fell to 74 and 7 per cent at 10^{-7} and 10^{-8} M, respectively, with no effect seen at 10^{-9} M. The inhibition by papaverine of the uptake of adenosine dropped steadily from 92 per cent at 10^{-3} to 10^{-4} M to 17 per cent at 10^{-8} M. Accordingly, dipyridamole (based on the ID_{50} value) was found to be more potent than papaverine in its ability to block the uptake of adenosine into heart cells.

The inhibition of the uptake of adenosine by hexobendine (10^{-3} to 10^{-6} M) was almost 95 per cent and identical to that of dipyridamole at 10^{-4} and 10^{-6} M concentrations. The ID_{50} for the uptake of adenosine was 10^{-7} M. This ID_{50} value is 2.5-fold higher than for dipyridamole. The dose–response curve for dilazep is almost identical to the curve for dipyridamole, but differs from the curve for hexobendine in its potency at concentrations lower than 10^{-6} M. The ID_{50} in this case was found to be 4.5×10^{-8} M. The dose–response curve for lidoflazine was somewhat comparable to papaverine. Lidoflazine was not as potent a blocker of adenosine uptake as were dipyridamole, hexobendine and dilazep. The inhibition dropped from 95 per cent at 10^{-4} M lidoflazine to 17 per cent at 10^{-7} M.

Aminophylline, nitroglycerine and carbochromen, in final concentrations ranging from 10^{-3} to 10^{-6} M, were tested for their effects on the uptake of adenosine. These drugs were found to have no significant effects on the uptake of adenosine into heart cells at any concentration (10^{-3} to 10^{-8} M) tested.

Effects of vasodilator drugs on the uptake of adenosine in cardiac cells at various extracellular concentrations of adenosine

The effects of several concentrations of vasodilator drugs were tested on carrier-mediated transport (the major phase of the uptake process and most likely operative at low substrate concentrations) of adenosine into the cardiac cells. The results of such an experiment with dipyridamole are illustrated in Fig. 2A. Dipyrida-

Table 1. Summary of the ID_{50} and K_m values*

Drug	Concn (M)	Apparent K_m ($\times 10^{-6}$ M)	ID_{50} (M)
Control		2.5	
Dipyridamole			4×10^{-8}
	1×10^{-5}	100	
	1×10^{-6}	50	
	5×10^{-7}	20	
Papaverine			2×10^{-6}
	1×10^{-5}	9.0	
	1×10^{-6}	5.8	
	1×10^{-7}	2.3	
Hexobendine			1×10^{-7}
	5×10^{-6}	100	
	5×10^{-7}	33.3	
	5×10^{-8}	5.8	
			4.5×10^{-8}
Dilazep			
	1×10^{-6}	86.4	
	1×10^{-7}	7.5	
			4.5×10^{-7}
Lidoflazine			
	1×10^{-5}	66.6	
	1×10^{-6}	2.5	

* The ID_{50} values represent the concentration of the drug which resulted in 50 per cent inhibition in the uptake of adenosine into cardiac cells, compared with controls. The values are obtained from Fig. 1. The K_m values were obtained from the double reciprocal plots (Lineweaver–Burk) of the data in Figs. 2–4 and each represents the reciprocal of the intercept on the X-axis.

mole at final concentrations of 10^{-5}, 10^{-6} and 5×10^{-7} M significantly depressed the uptake of adenosine. A Lineweaver–Burk double reciprocal plot (Fig. 2B used as an example for the other drugs) of the data in Fig. 2A resulted in a K_m value of 2.5×10^{-6} M and a V_{max} of 0.9 nmole/mg of protein/30 min for the control experiment. Apparent K_m values of 100, 50 and 20×10^{-6} M from this plot were determined in the presence of dipyridamole at concentrations of 10^{-5}, 10^{-6} and 5×10^{-7} M respectively. From these plots, it is likely that the inhibition in the uptake of adenosine is competitive in nature.

The effects of papaverine on the uptake of adenosine at various extracellular concentrations of adenosine are shown in Fig. 3A. The extent of inhibition due to papaverine (Fig. 3A) was comparable to the dose–response curve in Fig. 1. In order to determine the type of inhibition, these data were plotted on Lineweaver–Burk double reciprocal coordinates (similar to Fig. 2B) and the apparent K_m values of 9.0 and 5.8×10^{-6} M were obtained at 10^{-5} and 10^{-6} M concentrations of the drug respectively. No appreciable change in the V_{max} of the uptake was evident. These data demonstrate that papaverine blocked the uptake of adenosine into cardiac cells in a competitive manner.

Figure 3B shows the effects of hexobendine on the uptake of extracellular adenosine. Apparent K_m values of 100, 33.3 and 5.8×10^{-6} M were obtained from Lineweaver–Burk double reciprocal plots (similar to Fig. 2B) of the data in Fig. 3B at final concentrations of 5×10^{-6}, 5×10^{-7} and 5×10^{-8} M, respectively, of hexobendine without changes in V_{max}. Hexobendine appeared to produce a similar type of inhibition in the uptake of adenosine.

Alterations in the uptake of adenosine with various extracellular concentrations of adenosine in the presence of dilazep (10^{-6} to 10^{-7} M) are given in Fig. 4A. The inhibition of the uptake of adenosine from this curve was comparable to the inhibition noted from the dose–response curve in Fig. 1. The data in Fig. 4A were plotted on Lineweaver–Burk double reciprocal coordinates (similar to Fig. 2B). Since the Y-intercept was unchanged in the presence of dilazep, it was concluded that dilazep competitively blocked the uptake of adenosine. Apparent K_m values of 86.4 and 7.5×10^{-6} M were obtained from this plot at 10^{-6} and 10^{-7} M concentrations respectively. Finally, the data obtained on the uptake of adenosine in the presence of lidoflazine are given in Fig. 4B. The Lineweaver–Burk double reciprocal plots of the data in Fig. 4B illustrate competitive inhibition. Thus, lidoflazine, like other drugs, competitively blocked the uptake of adenosine into the heart cells. Apparent K_m values of 66.6 and 2.5×10^{-6} M were obtained at a lidoflazine concentration of 10^{-5} and 10^{-7} M respectively.

When the cardiac cells were incubated at a 10^{-4} M concentration of extracellular adenosine, the uptake in the presence of the vasodilator drugs at their lowest concentration (see Figs. 2A, 3 and 4) was identical to the control value, thus confirming the competitive nature of the inhibition in the uptake of adenosine obtained from the Lineweaver–Burk plots for these drugs. These data suggest that it is the carrier-mediated portion of the uptake process which is affected by these drugs.

Effects of vasodilator drugs on the release of radioactive adenosine from hypoxic cardiac cells

Cardiac cells (prelabeled with [U-^{14}C]adenosine), when made hypoxic, release radioactivity into the incu-

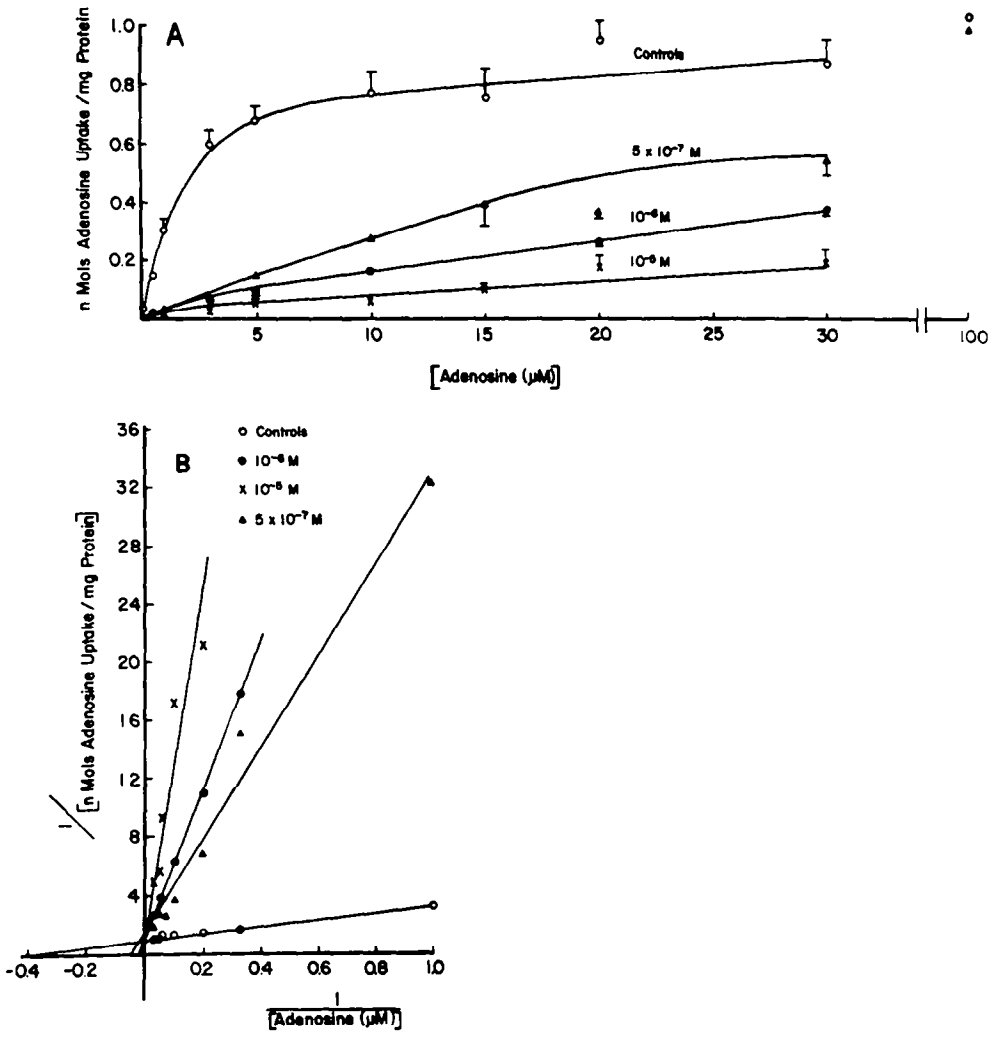

Fig. 2. Relationships between the uptake of intracellular adenosine and various concentrations of extracellular adenosine both in the presence and absence of dipyridamole (Graph A). The various concentrations of adenosine had different specific radioactivities. Values are means ± S.E.M. from four to six experiments. Graph B shows the Lineweaver–Burk double reciprocal plots for dipyridamole of the data in Graph A (used as an example for other drugs). Other details are given in the text.

bation medium. In two separate experiments, the medium was analyzed for adenine nucleosides using a thin-layer chromatographic system [15] to characterize the radioactivity. Hypoxia of cardiac cells (with prelabeled nucleotide pools) resulted in approximately a 50 and 60 per cent increase in the amount of radioactive adenosine and inosine released respectively. The radioactivity in the hypoxanthine fraction was unchanged due to hypoxia. The appearance of radioactive inosine in the medium was probably due to its rapid conversion from adenosine, sine no significant radioactivity in the inosine and IMP fractions of the cardiac cells was detected. Based on these observations, the effects of various drugs on the release of adenosine were assessed by measuring only the release of total radioactivity into the incubation medium. All of the vasodilator drugs (10^{-3} to 10^{-9} M) tested in this study were without an effect on the release of radioactivity into the incubation medium in comparison to controls. These data, therefore, suggest that dipyridamole, papaverine, hexobendine, dilazep, lidoflazine, aminophylline, nitroglycerine and carbochromen are without an effect on the release of adenosine due to hypoxia.

DISCUSSION

In the present study, dipyridamole, papaverine, hexobendine, dilazep and lidoflazine showed significant inhibition of uptake of adenosine into cardiac cells. Aminophylline, nitroglycerine and carbochromen, on the other hand, were without effect on the uptake of adenosine. These drugs have been reported [16–18] to have a different mechanism of action in relation to coronary vasodilation.

The ID_{50} values (Table 1) for inhibition of adenosine uptake show the following relationships between the

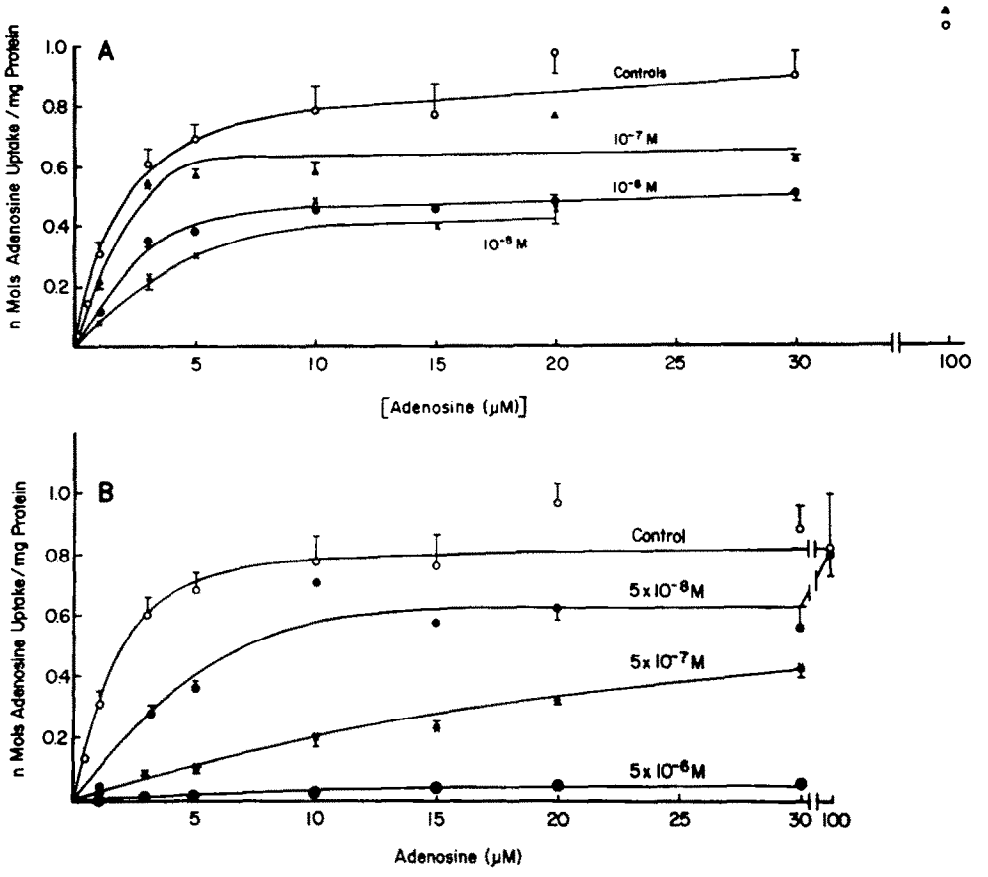

Fig. 3. Relationships between the uptake of intracellular adenosine and various concentrations of extracellular adenosine, both in the presence and absence of papaverine (A) and hexobendine (B). The control curves have been redrawn from Fig. 2. Values are means ± S.E.M. from four to six experiments.

agents studied: dipyridamole > dilazep > hexobendine > lidoflazine > papaverine. These data are qualitatively similar to the inhibitory activity observed during incubation at various concentrations of adenosine with several concentrations of each drug (Figs. 2–4). Similar findings with regard to potency have been reported by others [19]. Dipyridamole, dilazep and hexobendine (Fig. 1) were closely related in potency when inhibiting the uptake of adenosine at higher concentrations ($>10^{-6}$ M). The coronary-dilating potencies of hexobendine, dipyridamole and lidoflazine (based on their abilities to inhibit the uptake of adenosine), when extrapolated to *in vivo* conditions, compare favorably to the data reported by Hopkins [20], using isolated perfused guinea pig hearts. Huang and Drummond [19], using guinea pig ventricular slices, reported an 80–90 per cent inhibition of the uptake of adenosine with dipyridamole and hexobendine at concentrations of 10^{-5} M. These workers found an insignificant effect of lidoflazine on the uptake of adenoine, contrary to our present findings. However, several workers [6, 7, 21, 22], employing isolated heart and intestinal muscle preparations [23], have reported inhibition by lidoflazine of the uptake of adenosine.

Aminophylline, nitroglycerine and carbochromen (10^{-3} to 10^{-9} M) had no effects on the uptake of adenosine, a finding reported by Huang and Drummond [19], and by us [8] using theophylline in concentrations as high as 10^{-3} M. Kukovetz and Poch [10] reported similar data using isolated perfused guinea pig hearts in the presence of sodium nitrate. Papaverine (10^{-3} to 10^{-4} M) has been reported [19, 24] to block the uptake of adenosine, which is consistent with the present findings. Dilazep has also been reported [4] to enhance the vasodilator action of adenosine in both guinea pig and dog heart preparations.

In the present experiments, cardiac cells displayed Michaelis–Menten kinetics when incubated with various concentrations of adenosine ($1–30 \times 10^{-6}$ M), a finding similar to those reported in freshly isolated cardic cells [15], red cell ghosts [25], and canine heart preparations [26, 27]. Also, in the control curve (Fig. 2–4), there was an increase in the uptake of adenosine at higher extracellular concentrations (10^{-4} M). These data indicate that the uptake of adenosine is a combination of carrier mediated (major phase) possibly at lower concentrations and a simple diffusion (major phase) across the gradient at higher concentrations, as reported by other workers [15, 25, 26].

The addition of dipyridamole, papaverine, hexobendine, dilazep and lidoflazine at various extracellular adenosine concentrations in the present investigation resulted in a significant depression of the dose–response curve. Since none of these drugs had a significant effect

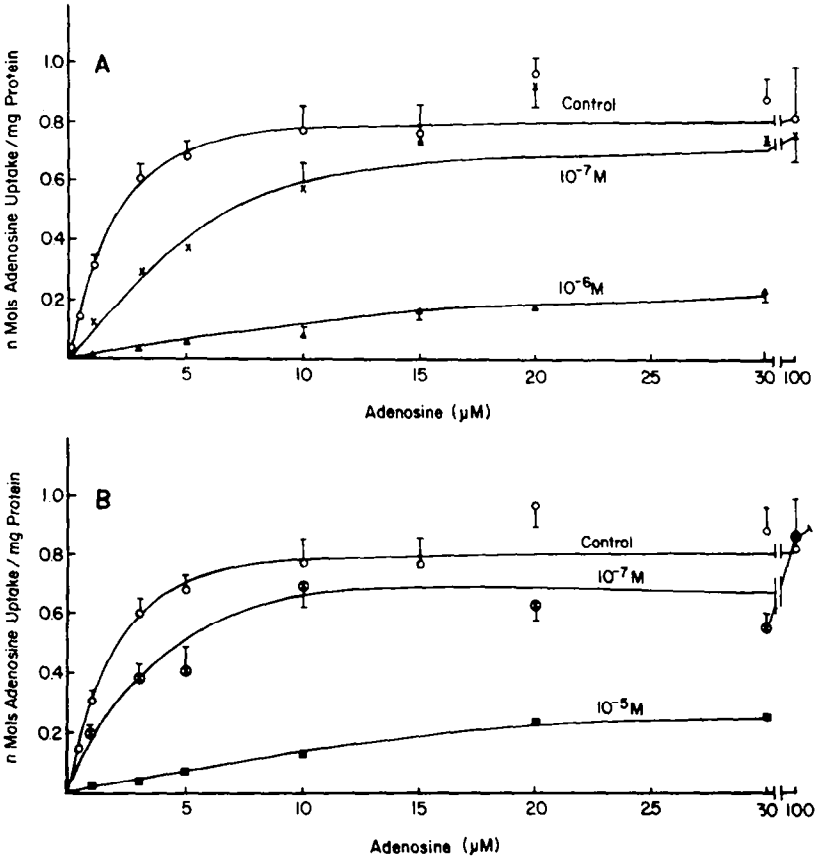

Fig. 4. Relationships between the uptake of intracellular adenosine and various concentrations of extracellular adenosine, both in the presence and absence of dilazep (A) and lidoflazine (B). The control curves have been redrawn from Fig. 2. Values are means ± S.E.M. from four to six experiments.

on the uptake of adenosine at a concentration of 10^{-4} M, it is suggested that these drugs most likely affect the carrier-mediated portion of the uptake phase and not the simple diffusion process. Huang and Drummond [19] found no significant effect of dipyridamole, papaverine and hexobendine on the uptake of adenosine in ventricular slices of the guinea pig after raising the concentration of adenosine from 10^{-5} to 10^{-4} M, a finding similar to the present data.

The double reciprocal plots in the presence and absence of coronary vasodilator drugs resulted in a similar V_{max} (0.9 nmole/mg of protein/30 min) for the uptake of adenosine but different K_m (apparent) values (Table 1). These data, together with the observation that the uptake of adenosine in the presence of vasodilator drugs at 10^{-4} M adenosine (extracellular) was unchanged, confirms the competitive nature of these drugs.

Kubler *et al.* [9] have suggested that dipyridamole blocks not only the uptake of adenosine into myocardial cells but also its release due to hypoxia. Similar findings were reported by Kukovetz and Poch [10] using dipyridamole and hexobendine. These findings are not compatible with the adenosine hypothesis [5]. According to this hypothesis, adenosine must be present extracellularly, and not intracellularly, in order to exert its vasodilator action. Also, as reported earlier [15], adenosine could not be detected intracellularly in isolated cardiac cells even with extracellular concentrations as high as 10^{-3} M. In the present study, none of the vasodilator drugs tested demonstrated a significant effect on the release of adenosine from hypoxic cardiac cells. This is a direct demonstration of the effect of these drugs in a simple homogeneous cell system which does not take into account the existence of an interstitial space. Thus, the observed differences between the effects of dipyridamole in this study and the studies of others [9, 10] may be explained by the hypothesis of Degenring *et al.* [28] who suggested that dipyridamole may influence the transport of adenosine across endothelial cells. It was further suggested that a dipyridamole-induced compartmentalization may exist, whereby adenosine is unable to enter the myocardial cell and is degraded before reaching the vascular adenosine receptor sites to exert its vasodilator action. Evidence of such a compartmentalization of adenosine comes from the studies of Schrader and Gerlach [29].

The basis for the action of these coronary vasodilator drugs cannot be attributed to their inhibitory effects on adenosine deaminase, due to the fact that the concentrations of these drugs which significantly alter the uptake of adenosine are without an effect on adenosine deaminase [6, 19, 30]. In addition, the cardiovascular actions of these drugs cannot be attributed to phosphodiester-

ase inhibition since the concentrations of the drugs which decrease the uptake of adenosine are lower than those required [31, 32] for phosphodiesterase inhibition. Although it is true that isolated cardiac cells do not represent vascular tissue in a few details, there may be some similarities between the myocardial and vascular smooth muscle cells as outlined by Huang and Drummond [19]. It has been suggested, though, that in intact heart preparations, one of the more important actions of vasodilator drugs is the inhibition of the uptake of adenosine. Herlihy *et al.* [33], employing hog carotid artery strips, found increased levels of cyclic AMP only at extremely high concentrations of extracellular aminophylline and adenosine. Aminophylline and carbochromen, which have been reported [31, 32] to be potent phosphodiesterase inhibitors, had no effects on the uptake of adenosine in the present experiments, even at concentrations as high as 10^{-3} M. It does not appear that the ability of these drugs to inhibit phosphodiesterase is related to their ability to increase coronary blood flow. Conversely, it appears to be the inhibition in the uptake of adenosine which is responsible for the dilation of coronary vessels, by providing more extracellular adenosine.

In the experiments of Hopkins [20], a 20 per cent inhibition in the uptake of adenosine by dipyridamole and hexobendine ($3–5 \times 10^{-8}$ M) resulted in a 2-fold increase in the degree of potentiation of the action of adenosine on isolated atria of the guinea pig. This concentration of the drug is too low to exhibit any phosphodieterase inhibition. Thus, even a small but significant inhibition of the uptake of adenosine in the heart can lead to a several-fold increase in sensitivity to adenosine.

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