Metabolism of Adenosine by the Isolated Anoxic Cat Heart.* (26739)

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In extracts of heart muscle the most active pathway of nucleotide synthesis is *via* direct phosphorylation of adenosine(1). In support of these observations, experiments on the isolated intact perfused cat heart have demonstrated a rapid incorporation of labelled adenosine into cardiac nucleotides in the presence of oxygen(2). The present study indicates that adenosine 8-C^{14} is also incorporated into myocardial nucleotides in the absence of oxygen.

Methods. Cat hearts were isolated and perfused with Tyrode's solution as described previously(2) and aerobic control collections of 200 ml of perfusate were made. The hearts were then perfused with Tyrode's solution equilibrated with a gas mixture of 95% nitrogen and 5% carbon dioxide for about 2 minutes before adenosine 8-C^{14} was added to the perfusion fluid. Adenosine was added in concentrations of 3.8×10^{-3} to 4.2×10^{-3} μM per ml, and the hearts were perfused with 200 ml of the solution. After a single passage through the heart the perfusates were collected in flasks immersed in ice.

The neutralized perchloric acid extracts of the hearts were analyzed for nucleic acid derivatives by a modification of the method of Cohn and Carter(3). The nucleotides were adsorbed on Dowex-1 resin, the columns were washed with 0.1 M ammonium chloride in 0.1 M ammonium hydroxide, and the nucleotides were eluted with 0.1 N hydrochloric acid in one fraction. The counts per minute in wash and eluate were determined. Nucleotide derivatives recovered in the perfusates were separated by paper chromatography, assayed enzymatically and counted. Details of the analytical procedures have been previously described(2).

Results. Perfusion of the cat heart with deoxygenated Tyrode's solution containing adenosine 8-C^{14} resulted in extensive conversion of the adenosine to myocardial adenine nucleotides during a single passage of the solution through the heart (Table I). In Experiment 1, 44% of the recovered counts were found in the acid soluble supernatant of the heart homogenate, whereas in Exp. 2 and 3 (Table I) about 54% of the recovered counts were present in this fraction. The balance of the recovered counts were present in the perfusates. A loss of 16-23% of the total counts occurred during the experimental procedures (Table I), and a maximum of 2% of the counts in the heart homogenates were found in the non-nucleotide eluates from the columns.

Characterization of the nucleotides in the 0.1 N hydrochloric acid column eluates by paper chromatography indicated that the greatest amounts of radioactivity, determined with a paper strip counter, were in the ATP, ADP, and AMP regions. Further characterization of the nucleotides in the 0.1 N hydrochloric acid eluates by paper chromatography of their acid hydrolysis products indicated that at least 85 to 90% of the radioactivity was in the adenine region. A small peak was observed in the ITP region of one of the hydrochloric acid column eluates. Some activity was also observed at the solvent front, suggesting the presence of adenine.

Analysis of the perfusates revealed the presence of most of the radioactivity in the inosine and hypoxanthine regions of paper chromatograms in all 3 experiments. A small radioactive peak was seen in the adenosine region in Exp. 3, and a small peak was also observed at the origin in this experiment, although no ultraviolet absorbing spot was visible.

It was previously observed(2) that enzy-

* This research supported, in part, by Research Grants from Nat. Heart Inst., and from Life Insurance Medical Research Fund.

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TABLE I. Distribution of Perfused Adenosine 8-C^{14} between Myocardium and Perfusate.

Exp. No.	Adenosine 8-C^{14} perfused		CPM recovered		% of CPM recovered	Heart wt, g
	CPM	μM	Perfusate	Heart		
1	186,000	.84	88,000	68,000	84	12.3
2	182,000	.77	64,000	77,000	77	18.4
3	176,000	.76	69,000	76,000	82	19.9

matic assay detected no striking differences between amounts of inosine and hypoxanthine recovered during control and experimental periods when low concentrations of adenosine were perfused in the presence of oxygen. Similarly, no difference was observed in amounts of inosine and hypoxanthine recovered during the period of anoxia when adenosine was perfused. However, it should be emphasized that prolonged perfusion of anoxic Tyrode's solution (by recirculation) does produce significant increases in amount of inosine and hypoxanthine recovered in the perfusates compared to amount in oxygenated control perfusates. In Exp. 1 the perfusate contained a larger part of the radioactivity than the myocardium, in contrast to Exps. 2 and 3 (Table I). The heart stopped beating for about one minute during the perfusion period in Exp. 1, indicating severe anoxia. In the other 2 experiments the rhythm became irregular for varying periods of time but the heart beat was maintained.

Anoxia or perfusion with adenosine (4×10^{-3} μM/ml) produces coronary vasodilation. However, addition of adenosine to anoxic Tyrode's solution did not induce a greater coronary flow than that observed with anoxia alone.

Discussion. The average incorporation of adenosine 8-C^{14} into adenine nucleotides in the anoxic heart (51%) compares favorably with that observed when the adenosine is perfused in oxygenated Tyrode's solution (57%)(2). Since knowledge of the rate limiting step in incorporation of adenosine into cardiac nucleotides and the size and nature of precursor pools are not available in the present study, the reason for similar degrees of incorporation in presence or absence of oxygen is not apparent. It is possible that in the non-working heart energy derived from glycolysis is sufficient to maintain totle cardiac adenine nucleotides at normal levels and perhaps even maintain normal ratios of ATP, ADP, and AMP. The glucose present in the perfusion medium would be made more readily available under the conditions of the experiments since anoxia enhances glucose transport across the myocardial cell membrane and rate of phosphorylation in the isolated heart(4). The extent and rapidity of incorporation of the labelled adenosine into adenine nucleotides suggest active synthesis or turnover of myocardial nucleotides.

Summary. The anoxic isolated perfused cat heart incorporates approximately 50% of labelled adenosine into myocardial nucleotides with one passage of the adenosine-enriched perfusion fluid through the heart.

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Received April 4, 1961. P.S.E.B.M., 1961, v107.