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Pharmacological Responses of the Microvasculature of Transplanted Cardiac Tissue

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CORNISH, K. G., W. L. JOYNER AND J. P. GILMORE. *Pharmacological responses of the microvasculature of transplanted cardiac tissue.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1285–1286, 1982.—Neonatal cardiac tissue was transplanted onto the cheek pouch membrane of hamsters. After vascularization, the response of the coronary microvasculature to various vasoactive drugs was tested. Of those drugs tested, adenosine caused the greatest dilatation while angiotensin II was the most potent vasoconstrictor.

Pharmacological responses Microvasculature Transplanted cardiac tissue

THE direct effect of vasoactive substances upon the microvasculature of the heart has been difficult to measure directly in situ. Observations of the coronary microcirculation have been limited and normally involve the use of high speed cinephotography [2,12]. The objective of the present study was to provide a model of the coronary microvasculature which permits the direct manipulation of the coronary microcirculatory environment while observing the vascular responses.

METHOD

Sixty female hamsters weighing 100–200 g were prepared by inserting a plastic plate into the cheek pouch as described by Greenblatt *et al.* [9] and modified by Click *et al.* [4]. Atrial or ventricular tissue obtained from neonate hamsters was placed on the exposed cheek pouch membrane and then the chamber was sealed with a plastic window. At least seven days were allowed to elapse after the surgery before the transplants were examined. The criteria used to determine the acceptability of the transplant for study were: (1) the presence of contractile activity in the transplant, (2) an infection free chamber and (3) the vessels used must respond repeatedly to angiotensin since the cheek pouch vessels exhibit immediate tachyphylaxis to angiotensin while transplant vessels do not [5]. The transplanted tissue has spontaneous contractile activity ranging from 20 to 300 beats/min. The contractile activity decreases and eventually stops completely as the transplant is gradually rejected by the cheek pouch. Thus, tissue that showed no spontaneous contractile activity or vessels that were tachyphalactic to angiotensin were not used in this study. The time required for adequate vascular development for any transplant varied from seven to fourteen days. Therefore, transplants were studied during this period. Due to our selection criteria only 19 transplants were used in this study.

At the beginning of the experiment, the animal was anesthetized with chloralhydrate (400 mg/100 g of body weight, intraperitoneally) and cannulae were inserted into the trachea, the femoral artery and femoral vein. These permitted adequate ventilation of the animals, the monitoring of blood pressure and a route for the administration of supplemental anesthetic, respectively. With the chamber secured to a movable stage, a Plexiglas rod with a polished beveled tip (45°) was inserted below the chamber and illuminated at the distal end with a 100-W mercury lamp to permit transillumination of the transplant. A Zeiss optics system (Collins Microscope Co.) permitted visualization of the microvasculature of the transplant. The cheek pouch chamber was then suffused with Ringer's bicarbonate solution containing either 2% gelatin or 2–5% dextran (Polysciences, Inc.). The temperature of the solution was controlled at 36–37.5°C with a pH of 7.28–7.87. The transplant was exposed by removing the saran part of the chamber which had been used to cover the cheek pouch membrane and the transplant tissue.

When a suitable vessel was located within the transplant, the micropipette (tip diameter 7–15 μm) containing the test drug was placed in close approximation to the vessel. Drugs were dissolved in Ringer's bicarbonate solution so that the dose to be tested was contained in 2.25 μl. The drugs tested were angiotensin I (AI), angiotensin II (AII), norepinephrine (NE), adenosine (ADENO), isoproterenol (ISO), acetylcholine (ACh) and nitroglycerine (NTG).

Routinely, the chamber was suffused (10 ml/min) for two to five minutes between each drug application and a minimum of five minutes between each drug. The suffusion was discontinued during drug application.

The vascular response was determined by measuring the vessel diameter with a shearing eyepiece (Vickers) before, during and after the application of the drug. The output from the shearer was recorded on a Gould strip-chart recorder. The response to the drug was calculated as the percent of

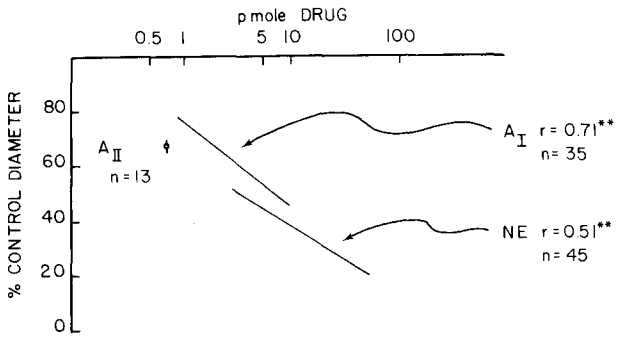

FIG. 1. Percent decrease in vascular diameter as the result of the direct application of AI, AII or NE to the coronary microvasculature. r=regression coefficient; $^{**}p<0.01$.

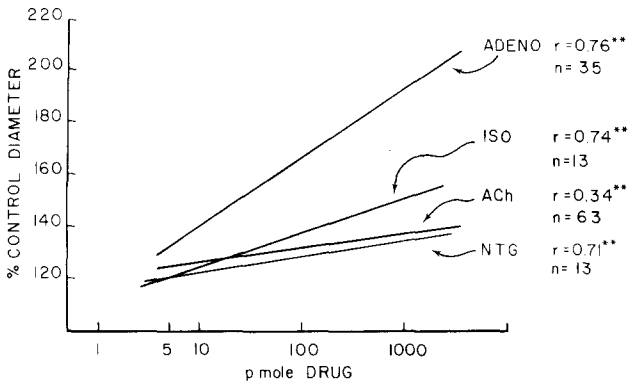

FIG. 2. Percent increase in vascular diameter as the result of the direct application of ADNEO, ISO, ACh, or NTG to the coronary microvasculature.

control diameter (vessel diameter at maximal response/control vessel diameter × 100).

The data were analyzed using a linear regression to test the hypothesis that the slope=0. A t-value was determined from the regression coefficient with a $p<0.05$ considered significant.

RESULTS

The application of ADENO, ISO, ACh or NTG to the vessels of the transplanted cardiac tissue resulted in vasodilatation which began within one to two seconds and was complete by five seconds. There was no discernible difference in the time course of response between any of those vasodilators tested or within the dose range for any drug. Angiotensin I, AII and NE all produced a rapid vasoconstriction which began within one second or less and was essentially complete at the end of three seconds. The vasoconstriction was more rapid in onset and persisted for a longer period than the vasodilatation. Figure 1 shows the dose response curves for AI and NE. Since AII was tested at only one dose, a dose response curve was not determined. The curves are drawn to extend over the range of doses used. Figure 2 presents the cumulative data obtained for ADENO, ISO, ACh and NTG. The application of vehicle did not measurably alter vascular diameter.

DISCUSSION

In a previous study [5], we showed that the microcirculation of the transplanted heart tissue converted AI to AII. The present study was done to further characterize the microvasculature of the transplanted cardiac tissue. From the results presented, it is observed that ADENO, ACh, ISO and NTG all cause vasodilatation of the coronary microvasculature while AI, AII and NE cause coronary vasoconstriction. This is in accordance with the work of others [1, 3, 6, 7, 8]. It is of interest to note that of all the substances tested, adenosine was the most potent vasodilator which is in accordance with the hypothesis of Rubio and Berne [10,11] that adenosine is of major importance in coronary blood flow regulation.

The results of this study further support the use of the transplanted myocardial tissue as a model for studying the normal coronary circulation.

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