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Abnormal Response of Tumor Vasculature to Vasoactive Drugs

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Abstract

The effects of the vasoconstrictor, phenylephrine, and the vasodilator, hydralazine, on blood flow to tumor were studied and compared to those on blood flow to normal tissues in vivo. Regional blood flow and cardiac output were measured with the use of radioactive microspheres in 150- to 250 g inbred Harlan F344 rats bearing subcutaneous nodules of two types of transplantable carcinoma ("hard" and "soft") with microscopically different vascular patterns. Three groups of rats were treated with hydralazine, saline, or phenylephrine, and regional blood flow was determined at the time of maximum blood pressure response. Results were correlated with quantitative morphometric analysis of arteriolar and capillary wall thickness in tumor and normal tissue. Phenylephrine decreased, and hydralazine increased, normal tissue perfusion as indicated by cardiac output. Tumor blood flow remained low and was not significantly influenced by drug treatment, except for the phenylephrine effect on hard tumors. Histological study of tumor vessel walls revealed· an absence of smooth muscle capable of responding to the vasoactive drugs by constriction or dilation. Evidently, by their selective action on normal vessels, vasoactive drugs can change the ratio of tumor to normal tissue perfusion. In particular, the increase of normal tissue vs. tumor blood flow by vasodilator drugs may enhance the selectivity of local heat therapy.

Abbreviation used: PAS=periodic acid-Schiff.

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Animals were maintained according to the guidelines set forth by the Purdue University Animal Care Committee.

INTRODUCTION

Changes in blood flow rate in and around tumor masses can significantly alter the effectiveness of chemotherapy, radiotherapy, and thermotherapy. The distribution of anticancer drugs to tumor tissue is influenced by blood flow to the tumor as compared with blood flow to other tissues (1). The efficacy of ionizing radiation therapy is known to be dependent on prevailing oxygen tension in irradiated tissues (2) and, in turn, tissue perfusion with oxygenated arterial blood. Destruction of cancer cells by hyperthermia is highly temperature-dependent, and since heat dissipation during local hyperthermia treatment depends largely on blood circulation (3), relatively low perfusion of tumor tissue is essential for selective heating of tumor masses.

Quite possibly, vasoactive drugs, which either constrict or dilate arterioles, can be used to alter the distribution of blood flow between tumor and normal tissues to therapeutic advantage. In pharmacoangiography, vasoactive drugs have been employed to amplify the radiographic contrast between normal and cancerous tissues (4-8). In radiation therapy such drugs have been investigated in hopes of increasing the oxygen tension within tumors during treatments (9-13). In chemotherapy, such drugs may help by preferentially channeling cytotoxic drugs into the tumor tissue (13). The success of each of these applications requires that the drugs in some way exert a differential effect on the tumor compared to normal vasculature.

The working hypothesis underlying the present research is that differential drug responses are possible because tumor vessels differ in structure and function from normal vessels. In particular, we have proposed that tumor vessels lack sufficient smooth muscle to constrict or to dilate in response to drugs that typically cause contraction or relaxation of normal resistance vessels (3). The objectives of the present study, therefore, are to investigate the effect of vasoactive drugs on tumor and normal blood flow rates with the use of the microsphere technique and to correlate the results with a quantitative histologic assay of vascular smooth muscle in normal and neoplastic tissues.

MATERIALS AND METHODS

Animals.-- Male inbred Harlan F344 rats weighing from 150 to 250 g were used. All rats were caged individually and given standard laboratory chow and tap water ad libitum. An automatic 12-hour light and dark cycle was maintained, and room temperature was maintained at 22°C.

Tumors.-- The two types of tumors used in these studies have the same origin but differ greatly in their vasculatures. Their primary tissue source is not known, but they both diverged from a squamous cell carcinoma that appeared unexpectedly in the jaw of a rat being fed the carcinogen N-2-fluorenylacetamide (14). Divergence of the tumor types started after 1.5 years of transplant passage. To differentiate between the 2 tumors, we named them according to their gross textures: "hard" and "soft" tumors. When the rats reached the age of 6 weeks, we transplanted the tumors into their left hind limbs in the following manner: The donor rat was first killed with chloroform, the tumor was immediately exposed, and small non-necrotic sections were dissected from the edge and placed in balanced salt solution where they were minced. The recipient rat was sedated

with chloroform, and 4 pieces of tumor, each approximately 0.5 mm^3, were inoculated sc into the left hind limb of each rat with a trocar needle. Tumors matured in 2-3 weeks and then rats were randomly assigned to treatment groups.

Vasoactive drugs.-- Hydralazine hydrochloride (Apresoline, 20 mg/ml; Ciba-Geigy) was injected iv into 17 rats at a dose of 0.1 mg/l00 g body weight. This phthalazine derivative was chosen because it is a well-known, direct acting vasodilator (15). Phenylephrine hydrochloride (Neosynephrine; Winthrop), a vasoconstrictor, acts mainly on the α-adrenergic receptors of peripheral blood vessels with little effect on the β-receptors of the heart. Its predominant site of action is in peripheral vascular beds (15). A group of 17 rats received iv injections of 0.005 mg phenylephrine/l 00 g body weight. In control experiments, 0.3 ml of a 0.9% NaCI solution was administered to each of 11 animals.

Anesthetic.-- Ketamine hydrochloride (Vetalar; ParkeDavis) was selected for anesthesia because of its minimal effects on the cardiovascular system (16). A dose of 40 mg/kg was injected i.m. into the right leg. This dose was effective for 2 hours. If needed, a supplement of 0.25 times the original dose was given.

Catheters.-- All catheters used were made from PE 50 polyethylene tubing, except for the catheter placed in the left ventricle. This catheter employed a special design used by C. W. Song (personal communication). Two sizes of polyethylene tubing (PE 50 and PE 10) were joined together by nail polish. To standardize the length, which in turn governs the dead space, we connected a 6-cmlong PE 10 into a 5-cm-Iong PE 50 tubing. All catheters were connected to 23-gauge needles, which in turn were connected to syringes filled with heparinized Ringer's solution.

Animal preparation.-- Each rat was first weighed and anesthetized with ketamine. The femoral arteries were first exposed and cannulated. The right carotid artery was used for placement of the left ventricular catheter because it is closest to the straight portion of the ascending aorta. Then with a similar procedure the jugular vein was cannulated. So that clotting could be prevented, all catheters in position were flushed with heparinized Ringer's solution. The longer femoral catheter was connected to a motor-driven 1-ml syringe mounted on a Harvard withdrawal pump.

Pressure was monitored before and after drug administration with the use of a Harvard Apparatus pressure transducer model 377 connected to a chart recorder. The transducer was calibrated intermittently throughout the study, and the open air zero point was established each time it was connected to a rat. Pressure changes were monitored via the femoral artery. The complete preparation procedure took approximately 30-45 minutes. For the elimination of time-dependent variations, all microsphere injections were done 1.5 hours after the anesthetic was administered.

The drugs phenylephrine and hydralazine were dissolved in 0.9% NaCI solution, and the concentrations adjusted so that the total injected volume was approximately 0.1 ml to minimize the amount of fluid loading in the rat. All drugs were injected i.v. through the jugular vein and flushed with 0.2 ml saline. Microspheres were injected immediately after administration of

phenylephrine and 15 minutes after injection of hydralazine, at which times blood pressure changes produced by the drugs had stabilized.

Microsphere technique.-- Polystyrene microspheres (3M Co., Minneapolis, Minn.) labeled with ^{141}Ce, ^{85}Sr, or ^{46}Sc were used. These spheres averaged 15 microns in diameter and were suspended in a solution of 10% dextran and 0.5% Tween 80. Their activity was 5 mCi/50 ml solution. Before injection the spheres were shaken mechanically for 30 minutes and then sonicated for 5 minutes.

For the calibration of regional blood flow measurements, an arterial blood sample was collected in the 1ml syringe mounted on the Harvard withdrawal pump at a rate of 0.4 ml/minute. The pump was turned on 10 seconds before the microsphere injection and continued to run for 1 minute. A microsphere suspension (0.3 ml) was injected over 20 seconds as a series of 0.I-ml pulses. This pulsed injection technique was used especially to promote mixing of the microspheres with blood in the left ventricle. Residual microspheres in the catheter were flushed into the left ventricle with 0.2 ml of Ringer's solution.

Arterial blood collected in the 1-ml syringe was then transferred to a vial for counting, and any microspheres left inside the syringe were rinsed into another vial. The rat was killed by the injection of air into its jugular vein. Afterward, the animal was shaved, and tissue samples were taken from different sites to determine their radioactivity. These sites included the tumor, leg muscle under tumor, if any, skin covering tumor, abdominal muscle, stomach, brain, and both kidneys. Whenever possible, tissue samples greater than 1 g were extracted. The heart was opened to confirm proper placement of the catheter.

The counting system used for this study was an NaI(T1) crystal counter connected to a multichannel analyzer (Canberra Industries, Meridien, Conn.). We prepared a standard for each isotope used by putting 0.1 ml of the well-sonicated microsphere suspension into a vial with 5 ml of saline. All standards were counted each time with the samples to eliminate the effect of decay. Samples with cpm less than 10 times background cpm were counted for 10 minutes, and those with cpm greater than 100,000 cpm were counted for 30 seconds. All other samples were counted for 1 minute. Window width was set to include approximately 95% peak area. Flow rate to each organ was determined from the following equation: flow rate/g tissue = (cpm/sample x syringe withdrawal rate)/(sample weight (g) x cpm of reference blood sample). Student's t-test for unpaired data was used to test the null hypothesis that blood flow in either drug treatment group was the same as that in the saline treated control group. The null hypothesis was rejected for values of $P<.05$.

Histology. -- Slices (9 microns thick) were prepared with the use of standard histologic techniques. Twelve tumor-bearing rats, sedated with ketamine, provided tissue specimens for study. The thoracic cavity of each animal was quickly opened, and the tissues were perfused with 10% formalin via an 18-gauge needle inserted into the left ventricle. Whole-body perfusion was maintained under constant pressure, and the perfusion rate was approximately 150 ml/10 minutes, exiting from the cut right atrium. Tissues under investigation were then removed and soaked in formalin for a minimum of 24 hours before being dehydrated, embedded, sliced, and mounted. Dried slices were then stained, one set with Masson trichrome technique and another

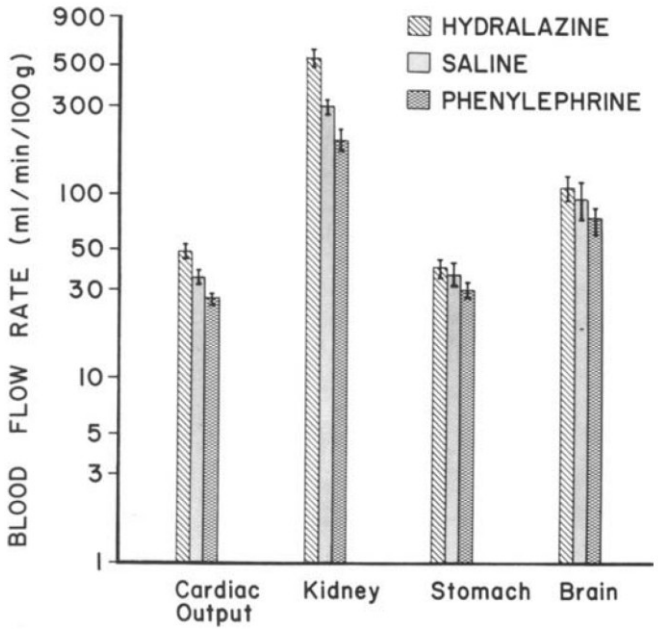
with the PAS method--the former to identify connective tissues and smooth muscle and the latter to identify endothelial lining. Since tumor vasculature did not have much smooth muscle, the PAS stain was essential for differentiating a vessel from a fixation artifact.

To compare quantitatively the tumor vessels to normal vessels, we constructed density plots of vessels. One cross sectional slide from each rat that best represented tumor and different normal tissues was selected for the microscopic study. Since the focus of our study was vascular smooth muscle, only those vessels with a wall thickness greater than 1 micron were counted. We determined the area studied by tracing sections onto graph paper. Vessel wall thickness was measured with the use of a micrometer eyepiece on a microscope (Leitz, Wetzlar, Federal Republic of Germany). The wall thickness of oblique cross sections of vessels was measured along the smallest diameter of the vessel profile. Then the number of vessels per unit area was determined by being counted and was plotted as a function of wall thickness to display graphically differences between tumor and normal vasculature.

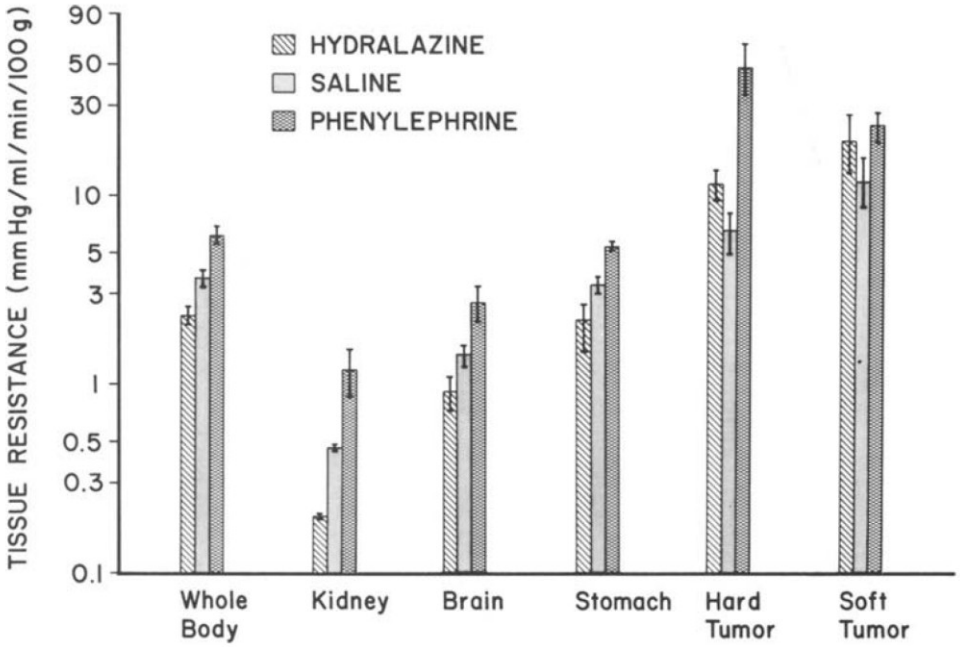
RESULTS

Blood Flow and Vascular Resistance

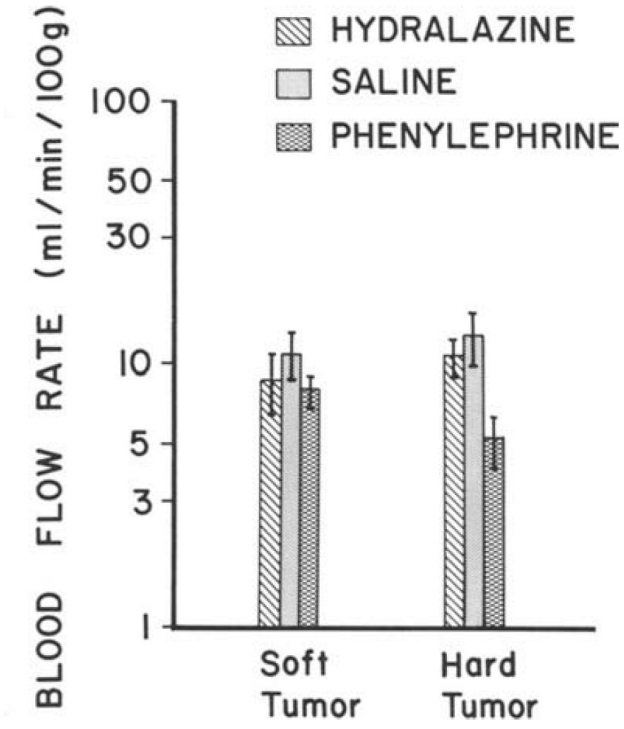
Intravenous infusion of hydralazine caused an overall increase in cardiac output of 30% compared to the output in saline-treated controls, whereas phenylephrine caused a 28% decrease. Similar results were obtained for the following major organs: kidney, brain, and stomach (text-fig. 1). For the control rats given the saline injection, the average cardiac output was 34.34 ml/minute/ 100 g body weight ± 12.52 SD. With hydralazine the average cardiac output was 47.81 ml/minute/l00 g ± 12.11 SD, and with phenylephrine it was 26.3 ml/minute/ 100 g ± 6.48 SD. Total peripheral vascular resistance of the animal under the influence of drug was found to be 2.4 mm Hg/ml/minute/100 g ± 0.23 SE. with hydralazine and 6.96 mm Hg/ml/minute/100 g ± 0.60 SE with phenylephrine. The control rats had an average resistance of 3.71 mm Hg/ml/minute/l00 g ± 0.40 SE. These together with the individual organ and tumor resistance are shown in text-figure 2. Blood flow rates to the 2 tumor types are displayed in text-figure 3. Except for the effect of phenylephrine on hard tumors, there was no significant influence of these drugs on tumor blood flow.



TEXT-FIGURE 1.-Cardiac output and regional blood flow (ml/min/100 g body wt) of normal tissues in rats under the influence of hydralazme (0.1 mg/100 g, i.v.), 0.9% NaCl, and phenylephrine (0.005 mg/100 g, i.v.). Note log scale; vertical bars indicate ± 1 SE.



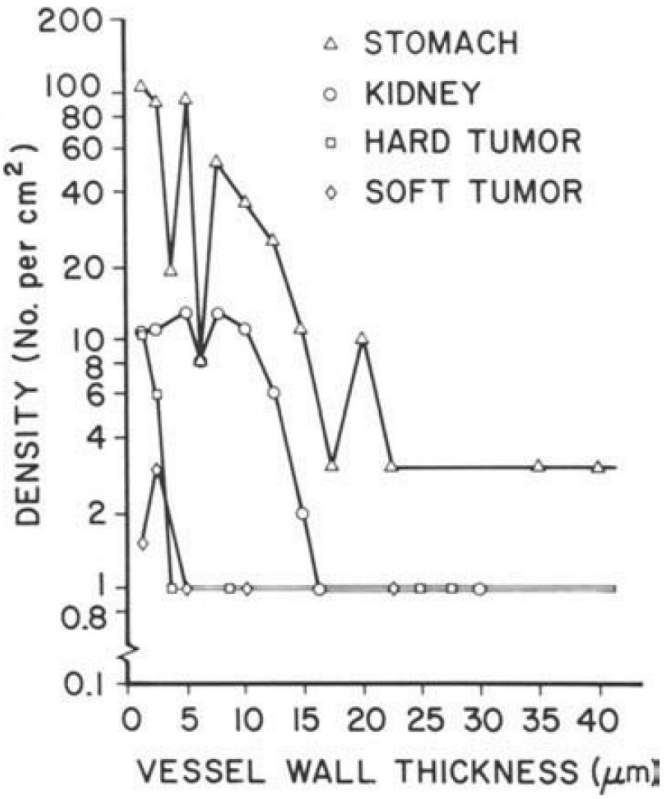
TEXT-FIGURE 2.-Effects of hydralazine, saline, and phenylephrine on the peripheral vascular resistance on the peripheral vascular resistance and resistances of individual organs and tumors. Note log scale; vertical bars indicate ± 1 SE.



TEXT-FIGURE 3.-Tumor blood flow rate (ml/min/ I00 g body wt) under the influence of hydralazine (0.1 mg/100 g, i.v.), 0.9% NaCl, and phenylephrine (0.005 mg/100 g body wt, i.v.. Note log scale; vertical bars indicate ± 1 SE.

Morphology

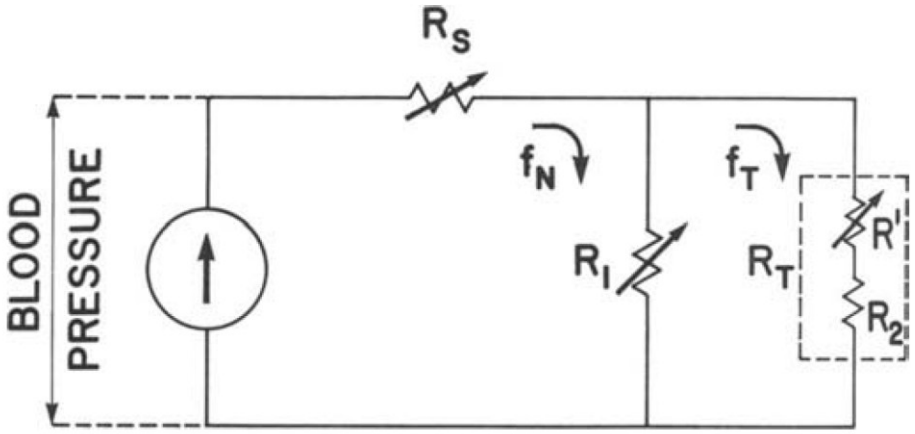
Histologic examination showed morphologic features characteristic of squamous cell carcinomas. The majority of the vessels in both tumor types were sinusoids with very thin walls. Normal vessels recruited by the tumors were easily seen along the periphery. However, due to the striking lack of smooth muscle in tumor vessels, traditional methods for classifying blood vessels do not apply, and most vessels could not be recognized, except under PAS stain, which marks the endothelial lining. Hard tumors had more vessels with measurable wall thickness than did soft tumors. As in kidneys and stomach, the vascular distribution for hard tumors decreased exponentially as a function of wall thickness, whereas the distribution for soft tumors was clearly abnormal with a paucity of large vessels. Compared to normal stomach and kidney, both tumor types have a very limited number of vessels with wall thicknesses greater than 7 microns (text fig. 4).



TEXT-FIGURE 4.-Distribution of vessel wall thicknesses In stomach, kidney, hard tumor, and soft tumor.

DISCUSSION

Results in this study confirm previous reports describing tumor blood vessels as sinusoidal capillary beds that are maximally dilated during tumor growth (17-22). Instead of having a normal microvasculature, these tumors are nourished by a sinusoidal capillary bed connected both in parallel and in series with normal vessels at the periphery of the tumor. These sinusoids in general have very poorly developed smooth muscle walls (20), which quite logically show little response to vasoactive drugs like hydralazine and phenylephrme that act mainly on vascular smooth muscle.



TEXT-FIGURE 5.-Electrical analogue to tumor and normal tissue blood flow. R_s variable resistance in major arteries that later branch into the different organs and tumor; R_1, resistance of normal organs; R_T, resistance of tumor; R_2, resistance of tumor vessels; R', resistance of normal vessels recruited by the tumor in the periphery; f_N, blood flow rate through normal tissues; f_T blood flow rate through tumors.

The overall effects of vasoactive drugs on the distribution of blood flow can be predicted with the use of an electrical analogy. In text-figure 5, the heart is represented by a current source that provides blood to different parts of the body, depending on the peripheral vascular resistance of the organ. A variable series resistor R_s represents the major arteries that later branch into different organs and tumor, which are connected m parallel. The normal tissues or organs, nourished by the healthy vessels capable of responding to vasoactive drugs, are represented by the variable resistor R_1. Although the tumor has recruited some normal vessels, R′, near its periphery, the majority of tumor vessels, R_2, are not capable of further dilation. When hydralazine is infused into the circulation, it will be distributed throughout the body and it will dilate all muscular arterioles. As a result, resistance in these vessels will decrease, causing more blood to flow through them. Since the number of vessels in the tumor that can respond to vasodilator drugs is limited, the increase in tumor flow will be small compared to that in normal tissues. Furthermore, if arterial blood pressure drops as a result of drug treatment while tumor resistance remains constant, there will be a drop in the tumor blood flow rate.

This model can be used in conjunction with the histologic observations to explain subtle differences in the experimental results. Recall that the amount of vascular smooth muscle seen in the soft tumor was essentially zero, whereas some vascular smooth muscle was seen in the hard tumor. In terms of the model, then, when resistance in other organs decreases after hydralazine treatment, more blood will be channeled away from the soft tumor than from the hard tumor, which has some series reactive vessels, R′. With phenylephrine treatment major blood vessels constrict, causing R_s to increase and decreasing the flow through R_1 and R_T. Comparatively, R_T in hard tumors will increase more than that in soft tumors since R′ is essentially zero in soft tumors. As a result, there will be a relatively larger decrease in the flow rate to the hard tumor when a vasoconstrictor drug is given.

This correlation of vascular structure and function is reflected in the vessel density plots (text-fig. 4) and in the change in tumor vascular resistance (text-fig. 2). Thus it seems logical to assume that the differential response to drugs between tumor and normal tissues is explained by differences in the structure of small blood vessels.

In earlier studies with catecholamines, Gullino and Grantham (10, 23) observed a decrease in blood flow to implanted tumors in kidneys and ovaries after i.v. administration of epinephrine. On the contrary, Abrams et al. (4) observed that injection of epinephrine can cause an increase in tumor blood flow. With the use of a similar technique, however, Rockoff et al. (8) concluded that response to epinephrine is highly tumor type-dependent. Our results suggest that changes in tumor perfusion caused by vasoactive drugs depend on the microvasculature of both the tumor and the surrounding normal tissues. In the studies of Gullino and Grantham (10, 23) flow was measured in ovaries totally replaced by tumor and not in any parallel channels of normal tissues. Evidently, as in our hard tumor type, there was some capacity for vasoconstriction in these tumors, so that when epinephrine was injected selectively into the tumor circulation, tumor flow decreased. Alternatively, Abrams (24) gave epinephrine systemically, so that the same concentration of the drug perfused both normal and tumor tissues in parallel. His observation of increased tumor perfusion is explainable if the vasoconstrictive response of normal vessels was greater than in tumor vessels and if, as would be expected after systemic epinephrine administration, arterial blood pressure increased.

Mattsson, Lilja, and Peterson (20) used the xenon clearance method to study the influence of vasoactive drugs on blood flow to normal subcutaneous tissue and transplanted fibrosarcomas in rats. They injected 0.02-ml volumes of xenon solution containing either the vasoconstrictor noradrenalin or the direct-acting vasodilator papaverine. After injection of noradrenaline, subcutaneous tissues showed a greater absolute and relative decrease in blood flow than did tumor tissue. Conversely, papaverine caused no change in tumor blood flow, compared to the flow seen in saline treated controls, but it caused a roughly 40% increase in flow to subcutaneous tissue. These results are entirely consistent with those of the present study and with the hypothesis that tumor vessels are relatively less reactive to vasoactive drugs than are normal vessels in normal tissues.

The relative effects of vasoactive drugs on tumor versus normal perfusion are worthy of further study because of several potential clinical implications. In radiotherapy, for example, vasoconstrictors might be used to decrease normal tissue perfusion and oxygenation relatively more than in tumor, in order to reduce formation of oxygen-based free radicals in the normal tissue and enhance therapeutic gain. Similarly, in chemotherapy, such drugs may be used to increase the ratio of tumor perfusion to normal tissue perfusion, especially during regional intra-arterial chemotherapy. In hyperthermia treatment, vasodilators such as hydralazine that act mainly on smooth muscles can be very useful in increasing blood cooling of normal tissues, allowing greater overall power input and temperature rise in the tumor (25). Each of these applications is based on the selective structural and functional abnormalities of tumor vasculature in relation to the surrounding normal vasculature, which provide a potential target for more selective cancer therapy.

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