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Influence of Vasoactive Drugs on Local Tumor Blood Flow

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Abstract—*The influence of vasoactive drugs, including noradrenaline, isoprenaline and papaverine, on the local blood flow of a transplantable rat sarcoma and of normal subcutaneous tissue of the rat was studied by local [^{133}xenon] clearance technique. The microvascular bed of the tumour was studied by transmission electron microscopy (TEM). Vessels with one layer of contractile cells were found in the tumour vascular bed by TEM. A significant reduction of both tumour and normal subcutaneous tissue blood flow was found after administration of noradrenaline added to the injected xenon solution. In a dose–response experiment the tumour and subcutaneous vascular beds seemed to be equally sensitive to noradrenaline. Isoprenaline did not influence the blood flow in either subcutaneous tissue or tumour, while papaverine increased subcutaneous tissue blood flow significantly but did not influence tumour blood flow. The results of the present blood flow studies might suggest that the tumour vascular bed is normally in a state close to maximal dilatation.*

INTRODUCTION

IN A PREVIOUS study on two transplantable rat tumours, a 20-methylcholanthrene-induced sarcoma and a hepatoma, no adrenergic innervation was found related to the tumour vascular bed [1]. Similar observations were made in a later experimental study [2] and in a human renal adenocarcinoma [3]. These observations, however, do not preclude the possibility that a response to vasoactive drugs exists in some types of tumour; for example, in those in which the afferent vessels retain their original ultrastructure [4–6]. Endothelial cells have also been found to respond to a number of chemical mediators by change in shape [7].

In later studies on the two above-mentioned rat tumours in which the intra-tumour blood flow distribution was recorded by isotope technique, a significant change towards low tumour blood flow values was observed after intravenous administration of noradrenaline [8]. However, a change towards low blood flow values was also found in the normal tumour transplantation tissue, muscle, which suggested that a decreased tumour blood flow could be explained by an influence either directly on the tumour blood vessels or on normal afferent vessels in the transplantation area.

In order to differentiate between these two effects, another flow-recording technique was used in a recent study [9]. In this study the influence of noradrenaline on local tumour blood flow was examined by local [^{133}xenon] clearance. Noradrenaline was added to the [^{133}xenon] solution to ensure a direct effect of noradrenaline on the tumour vascular bed. A significant reduction of local tumour blood flow was found after administation of noradrenaline, suggesting an explanation by vasoconstriction of tumour vessels. In these experiments, in which 0.02 ml of a [^{133}xenon] solution was injected into tumour tissue for each flow recording, a high concentration of noradrenaline (0.01 mg of noradrenaline/ml of xenon in saline) was used.

A possibility of influencing the intra-tumour distribution of drugs and metabolites by vasoactive drugs is of great clinical interest. The aim of the present studies was to investigate further the influence on local tumour blood flow of different concentrations of noradrenaline and the influence of two other vasoactive drugs, isoprenaline and papaverine. Two different vasodilators were studied, based on their different mechanisms of action: papaverine with an unspecific action on smooth muscle cells, and isoprenaline with a specific β-stimulating effect. In addition, the tumour microvascular bed was studied by transmission electron microscopy (TEM) in an attempt to identify tumour vessels with contractile elements.

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MATERIALS AND METHODS

Animals and tumour

Inbred rats from a Lister strain with a mean body weight of 150 g were studied. A slightly differentiated 20-methylcholanthrene-induced fibrosarcoma in its 175th to 185th transfer generations was transplanted subcutaneously by trochar technique into one hindpaw of the rats. The experiments were performed 8 days after tumour transplantation with a tumour of about 5 mm diameter.

Isotope

Radioactive xenon ([^{133}xenon]), dissolved in saline (40 MBq/ml), was obtained from AB Kabi Diagnostica, Sweden.

Noradrenaline

Noradrenaline (Apoteksbolaget, Sweden) was added to the xenon solution in concentrations of 0.001, 0.005 and 0.01 mg/ml solution respectively.

Isoprenaline

Isoprenaline (Apoteksbolaget, Sweden) was added to the xenon solution in a concentration of 0.01 mg/ml solution.

Papaverine

Papaverine (ACO, Sweden) was added to the xenon solution in a concentration of 0.4 mg/ml solution.

Electron microscopy

Subcutaneous tissue and tumour was studied in an animal 8 days after transplantation and with a tumour diameter of 4 mm. The lower part of the rat body, including the tumour-bearing leg, was perfusion-fixed by injection into the abdominal aorta under ether anaesthesia of a cacodylate-buffered glutaraldehyde solution (3.5% glutaraldehyde, pH 7.2). The specimens to be studied were kept in the perfusion fluid for two hours. The specimens were postfixed in 1% OsO_4 with cacodylate buffer for two hours at 4°C.

After dehydration of the specimens they were embedded in Epon 812. Ultrathin sections were taken from the surface and central parts of tumour and from subcutaneous tissue by an ultratome fitted with a diamond knife (LKB Ultratome III). The sections were stained in a saturated solution of uranylacetate in 30% ethanol and in lead citrate according to Venable and Coggeshall [10]. They were examined in a transmission electron microscope (Philips 300).

Recording of local blood flow by [^{133}xenon] disappearance rate

Animals were studied under light ether anaesthesia. The [^{133}xenon] solution with or without the addition of vasoactive drugs was injected into tumour or subcutaneous tissue in a volume of 0.02 ml through a fine-bore injection needle with a diameter of 0.3 mm. At repeated injections into the same tissue, an attempt was made to inject the isotope into the same limited tissue area, by standardizing the direction and introduction of the injection needle. This technique was recently found to give a comparatively low spread between repeated control tumour flow recordings [9].

Radiation from [^{133}xenon] at the injection site was detected with a 3.8-cm NaI crystal in a cylindrical thick lead collimator directly over the tumour or normal tissue to be studied. The detector signals were registered in a pulse height analyser and a rate-meter. The disappearance curve was written on a linear recorder. Activity was plotted in a semilogarithmic diagram vs time and the $t_{1/2}$ for disappearance was calculated. The local capillary blood flow in ml/min/g tissue $= \lambda \times \ln 2/t_{1/2}$, where λ is the partition coefficient for xenon between tissue and blood. The partition coefficients for tumour blood and subcutaneous tissue blood were earlier found to be 0.56 ± 0.10 and 1.2 ± 0.6 respectively [9].

Administration of vasoactive drugs

In a first experiment, the local blood flow in tumours and in subcutaneous tissue of three groups of animals was recorded by injection of xenon without adding any vasoactive drug. After these control recordings, xenon solution with noradrenaline added in a concentration of 0.001 mg/ml was injected into tumours and subcutaneous tissue of one group of animals. A second group of animals was injected with xenon, with noradrenaline added in a concentration of 0.005 mg/ml, and a third group of animals was injected with xenon, with noradrenaline added in a concentration of 0.01 mg/ml.

In a second experiment on three groups of animals, xenon control clearance recordings in tumour and in subcutaneous tissue were primarily performed in all groups of animals. After these, one group of animals was studied with xenon, with noradrenaline added in a concentration of 0.01 mg/ml, a second group of animals with isoprenaline added to xenon in a concentration of 0.01 mg/ml, and a third group with papaverine added in a concentration of 0.4 mg/ml xenon solution.

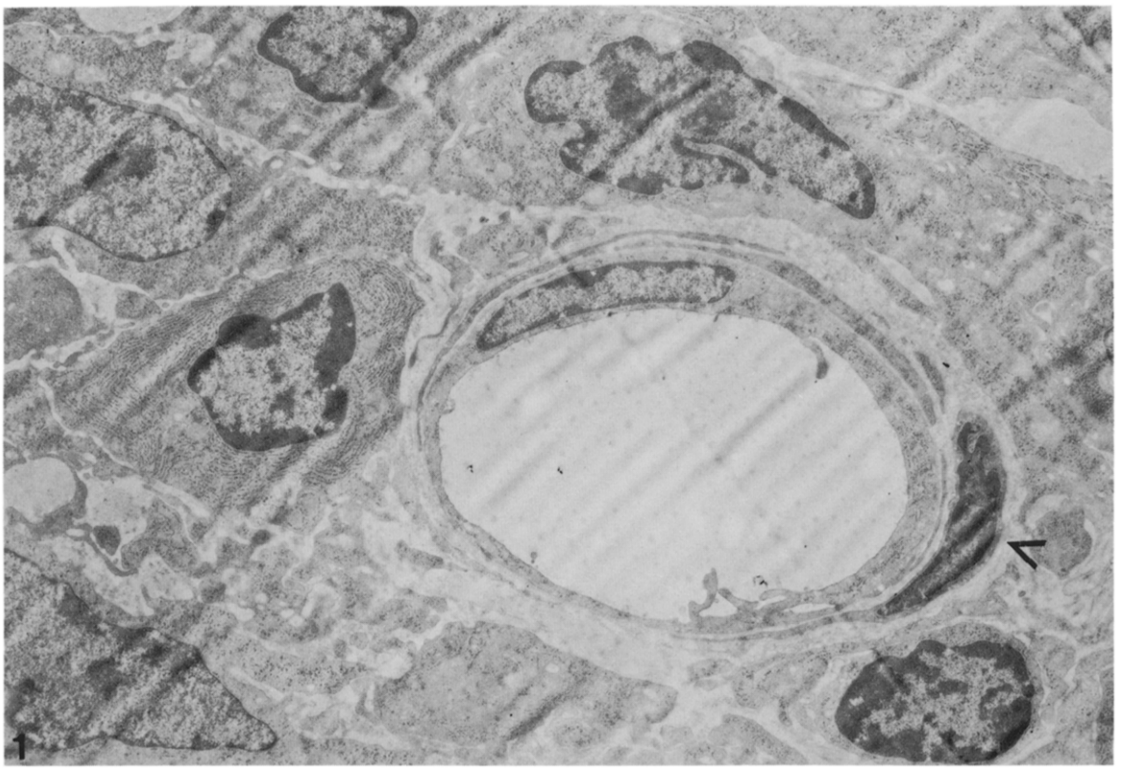

Fig. 1. *Vessels from tumour tissue. Immediate perivascular tissue intact. Note possible contractile cell embracing the vessel. Magnification* ×6750.

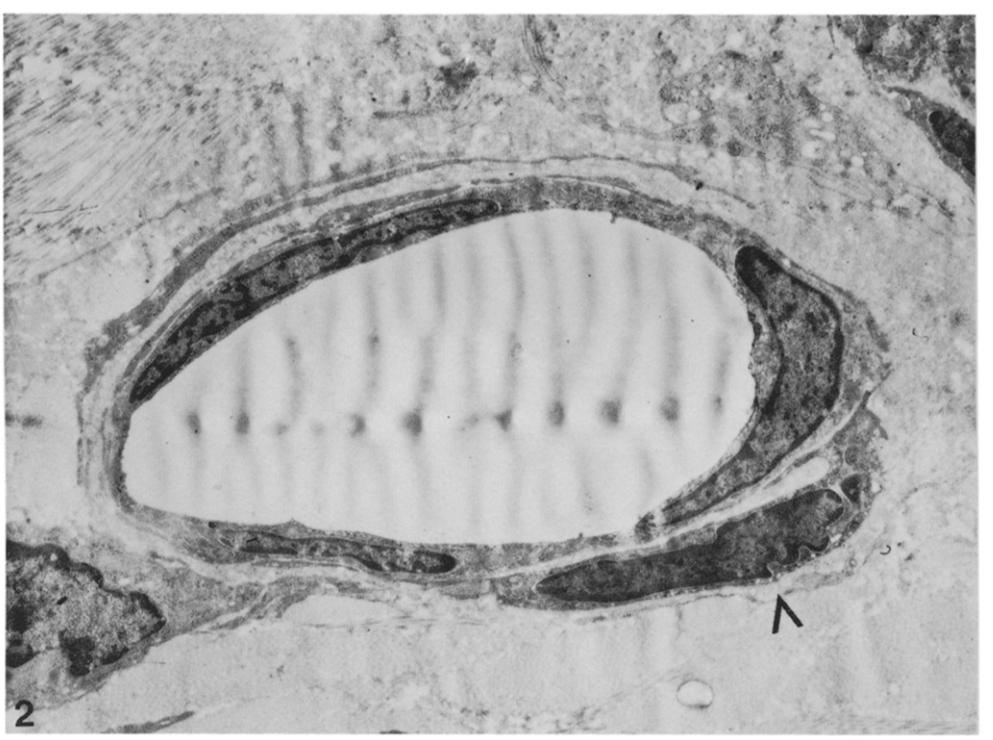

Fig. 2. *Vessel in subcutaneous tissue. Note perivascular similarity to tumour vessel. Magnification* ×6750.

Blood pressure recording

Systolic blood pressure was recorded under ether anaesthesia in separate animals by a polyethylene catheter (PE50) in the left carotid artery, connected with a Grass polygraph recorder with a Statham pressure recorder.

Statistical method

Student's *t*-test was used for testing the significance of the differences between control and experimental blood flow recordings in tumours and subcutaneous tissue.

RESULTS

In the TEM study a dense network of vessels was found both in tumour and in the surrounding subcutaneous tissue. Invasion of tumour cells into the lumen of tumour vessels was observed. The ultrastructure of the microvasculature in tumour and in subcutaneous tissue was similar. Thus vessels in tumour (Fig. 1) and in the adjacent tissue (Fig. 2) were found to be surrounded by a single layer of cells embracing the endothelial lining of the vessel lumen. These cells had few ribosomes and scattered profiles of rough endoplasmic reticulum. The mitochondria were few in number. In the cytoplasm there were filaments with occasional condensation against the cell membrane opposing the endothelial cells. A few vesicles were also identified along this cell membrane. Based on these characteristics, the observed cells were interpreted as pericytes or primitive smooth muscle cells.

In the blood flow studies the recorded semilogarithmic disappearance curves were all linear except for the first 1–2 min after local xenon injection. The slope of the curves was constant throughout the recordings with the exception of 4 tumour recordings, two with noradrenaline added at a concentration of 0.01 mg/ml and two with noradrenaline added at a concentration of 0.005 mg/ml to the xenon solution. In these registrations the curves became steeper 4–5 min after the injection.

In the first blood flow experiment (Fig. 3) the local blood flow in subcutaneous tissue and in tumour was significantly ($P < 0.01$) reduced by noradrenaline at a concentration of 0.01–0.005 mg/ml. Noradrenaline at a concentration of 0.001 mg/ml did not significantly influence the local blood flow in either subcutaneous tissue or tumour. The mean tumour blood flow values (± S.E.M.) after injection of noradrenaline 0.001 mg/ml, 0.005 mg/ml and 0.01 mg/ml xenon solution were 31 (± 5), 11 (± 2) and 6 (± 1) ml/min/100 g tumour tissue respectively. The mean corresponding control values were 27 (± 3), 21 (± 2) and 22 (± 2).

The mean flow values in subcutaneous tissue after administration of noradrenaline at concentrations of 0.001 mg/ml, 0.005 mg/ml and 0.01 mg/ml were 45 (± 7), 13 (± 5) and 8 (± 2) ml/min/100 g tissue respectively. The mean corresponding control values were 58 (± 7), 47 (± 7) and 40 (± 4).

In the second experiment (Fig. 4) the local blood flow in tumour and in subcutaneous tissue was significantly ($P < 0.01$) reduced by

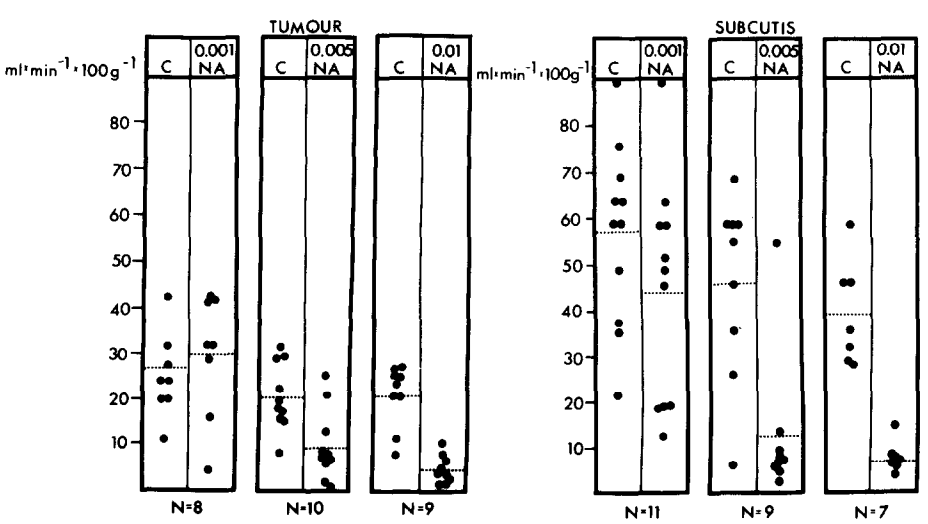

Fig. 3. Local blood flow in ml/min/100 g *tissue recorded by local xenon clearance in tumour and subcutaneous tissue.* N = *number of animals. Recordings where noradrenaline was added to the xenon solution in concentrations of* 0.001, 0.005 *and* 0.01 mg/ml (*NA*) *with corresponding control recordings* (*C*). *Note the significant* ($P < 0.01$) *reduction of both tumour and subcutaneous tissue blood flow from a noradrenaline concentration of* 0.005 mg/ml.

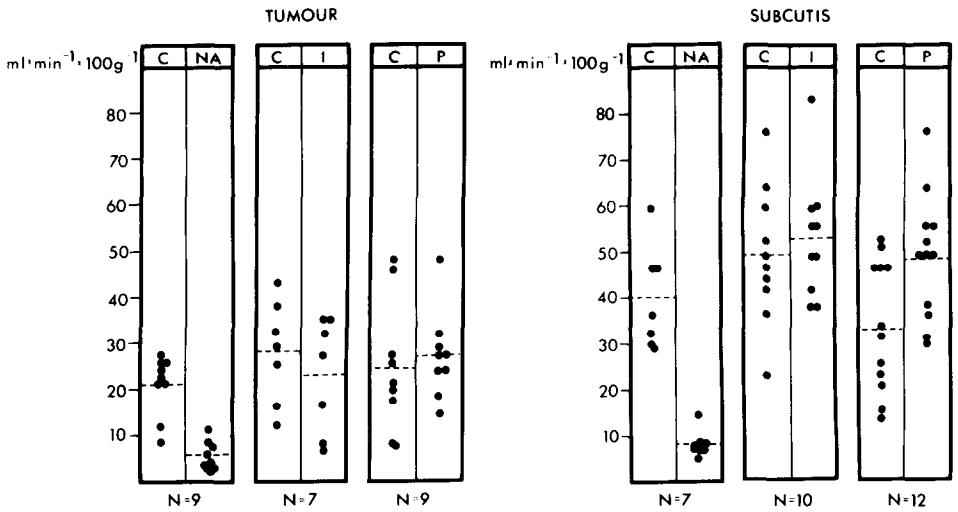

Fig. 4. Local blood flow recordings in tumour and subcutaneous tissue. Control recordings (C) and recordings where isoprenaline (I) or papaverine (P) was added to the xenon solution. Note the significantly increased blood flow ($P < 0.01$) *in subcutaneous tissue after administration of papaverine. Noradrenaline (NA) in a concentration of* 0.01 mg/ml *xenon reduced the tumour and subcutaneous tissue blood flow significantly* ($P < 0.01$), *as in the previous experiment.* N = *number of animals.*

noradrenaline at a concentration of 0.01 mg/ml xenon solution. Isoprenaline did not significantly influence the local blood flow in either subcutaneous tissue or tumour. A significant change ($P < 0.01$) towards higher blood flow values was found in subcutaneous tissue after adding papaverine to the xenon solution. The local tumour blood flow was, however, not significantly influenced by papaverine.

The mean tumour blood flow values in the second experiment (± S.E.M.) were after noradrenaline 6 (± 1) ml/min/100 g with corresponding control recordings 22 (± 2), after isoprenaline 23 (± 5) with corresponding control recordings 28 (± 4), and after papaverine 28 (± 3) with corresponding control recordings 25 (± 5) ml/min/100 g tumour tissue.

The mean subcutaneous tissue blood flow values in the second experiment (± S.E.M.) were after noradrenaline 8 (± 2) with corresponding control recordings 40 (± 4), after isoprenaline 53 (± 4) with corresponding control recordings 49 (± 5) and after papaverine 49 (± 4) with corresponding control recordings 33 (± 5) ml/min/100 g subcutaneous tissue. A significant difference ($P < 0.05$) was found between control recordings in the isoprenaline and the papaverine groups.

Mean systolic blood pressure was not changed by local injection into tumour or subcutaneous tissue of the vasoactive drugs when studied in 4 separate animals for each drug. After injection of noradrenaline (0.01 mg/ml) the mean systolic blood pressure (± S.E.M.) was 105 ± 6 mm Hg with corresponding control 108 ± 4 mm Hg, after isoprenaline (0.01 mg/ml) 113 ± 4 mm Hg with corresponding control 113 ± 4 mm Hg, and after papaverine (0.4 mg/ml) 111 ± 2 mm Hg with corresponding control 110 ± 4 mm Hg.

DISCUSSION

In the TEM study, vessels with one layer of perivascular cells were found in the tumour tissue. The ultrastructure of these cells was similar to that of pericytes or primitive smooth muscle cells [11]. Thus in the fibrosarcoma studied in the present investigation there were effector cells in the microvasculature which could react to vasoactive stimuli.

The local blood flow of the transplanted fibrosarcoma as well as of normal subcutaneous tissue was significantly reduced by noradrenaline in concentrations from 0.005 mg/ml xenon solution as studied by local xenon clearance. Papaverine significantly increased the blood flow in subcutaneous tissue but had no significant effect on tumour blood flow. Isoprenaline did not influence either subcutaneous tissue or tumour blood flow.

The intra-tumour blood flow distribution is characterized by a wide heterogeneity [12]. This means that single recordings of tumour blood flow by local isotope clearance techniques will reflect the local blood flow of very limited tissue areas and will most probably not give a good description of the mean tumour blood

flow. The difference between two control groups in the second experiment of the present study might illustrate this, even if other parameters such as slight changes of body temperature (controlled by an electric pad under the animal) might have influenced the recordings.

However, with the aim of studying the direct influence of vasoactive drugs on the tumour vascular bed, the local clearance method has the advantage that the active drug could be administered added to the isotope solution injected into tumour tissue.

The volume of the injected dose of isotope and vasoactive drug was 0.02 ml, which should be related to the calculated mean tumour volume of 0.10 ml in the present study. Each experimental blood flow value was also related to a control blood flow value from the same limited tissue area.

The localization of the injected bolus was separately studied after local ink injections into tumours, and was found to be limited to tumour tissue. The recorded isotope disappearance curves did not indicate any simultaneous isotope injection into two or more different tissues. This would have given non-linear curves with their slope decreasing with time from injection. In four tumour recordings with noradrenaline the curves became steeper after 4–5 min, and this was interpreted as an escape from the noradrenaline effect [13].

The influence of noradrenaline on experimental tumour blood flow has been examined by different techniques but with similar results. Thus a marked reduction of total tumour blood flow was observed by venous outflow recording technique [14], and marked changes in recorded tumour oxygen tension [15] and significantly reduced tumour blood flow in tumours transplanted into the liver and compared to normal liver tissue by recording of blood flow with a microsphere technique [2]. In some previous studies it was suggested that tumours are more sensitive to circulating amines than normal tissue [15, 16]. In the present study, normal subcutaneous tissue blood flow reacted to the same concentration of noradrenaline as tumour blood flow. The difference between the results of the present study and of previous studies might be explained by different tumours studied and by different flow recording techniques.

Vasodilating drugs such as isoprenaline and papaverine did not significantly influence local tumour blood flow, while papaverine significantly increased the blood flow in subcutaneous tissue. Isoprenaline did not influence the blood flow in subcutaneous tissue. The concentrations of all vasoactive drugs in the present study were tested and chosen not to influence the systemic blood pressure, which might indirectly modify the tumour blood flow [17].

The failing influence of vasodilating drugs on tumour blood flow might suggest that the tumour vascular bed is normally in a state of close to maximal dilatation. The experimental technique used in the present study and the observation of cells with contractile properties [7, 11] related to tumour vessels suggest a direct influence by vasoconstricting drugs on the tumour vascular bed.

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