Vascular Reactivity to Norepinephrine of 7,12-Dimethylbenz(a)anthracene-induced Rat Mammary Tumors and Normal Tissue as Studied in Vitro

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ABSTRACT

Vascular reactivity to norepinephrine has been studied in dimethylbenz(a)anthracene-induced rat mammary neoplasia and compared with that of skeletal muscle, salivary gland, kidney, and uterus by means of an artificial perfusion technique. Perfusion of tissues and organs was measured with the microsphere tracer technique during smooth muscle relaxation and infusion of norepinephrine at two different dose levels. This procedure makes possible a dose-response analysis of several tissues under controlled conditions without confounding endogenous vasoregulation.

The tumor vascular bed has a low perfusion capacity during smooth muscle relaxation and responds rapidly with an increased resistance to norepinephrine infusion. The results indicate a hypersensitivity, although the relative maximal constrictor response is equal to or less than that of other vascular beds studied.

INTRODUCTION

Contradictory results on vascular reactivity of tumor tissue as compared to various normal tissues can partly be ascribed to the use of different tumor models and methods of analysis (1). Most studies are moreover performed in vivo, either during anesthesia or under conscious conditions. The central and peripheral hemodynamics thus vary greatly (2, 3), complicating interpretation of the findings. The vascular tone in various organs and tissues also varies greatly within one and the same animal at the time of experimentation and no baseline vascular tone to which the tumor vasculature can be related can be achieved.

An in vitro perfusion technique was therefore developed as a further elaboration of the procedure described by Folkow et al. (4). Since it was recently shown that the blood flow in DMBA-induced mammary tumors of the rat was relatively high, but with a drastic reduction upon norepinephrine infusion (5), it was considered of interest to elucidate whether this finding is due only to the assumed maximal dilation of the tumor vascular bed at rest or whether there are other characteristics of the vascular response specific to tumors.

MATERIALS AND METHODS

Tumor Model. Female Sprague-Dawley rats (Anticimex, Stockholm, Sweden), 50–55 days old, were fed by gavage with 16 mg DMBA dissolved in 1 ml of olive oil (6, 7). Multiple mammary tumors became overt from the sixth wk after induction and experiments were performed 4 wk later.

Perfusion Technique. The rats were anesthetized with Nembutal (50 mg/kg body weight) i.p. The abdomen was opened in the midline by cauterization, and the stomach and intestines were removed, and the vessels and cut ends of viscera were carefully ligated. The thorax was cut open by cauterization and the aortic root was rapidly connected to a pump system. The right cardiac ventricle was cut open, a cannula was inserted for free outflow, and the perfusate was started. The caudal and one femoral artery were cannulated for measurement of pressure and reference flow, respectively. The femoral vein was cannulated for measurement of venous pressure. Pressures were continuously recorded on a Grass polygraph.

The oxygenated perfusate, kept at 38°C, consisted of 6% dextran (mean M, 70,000; Macrodex; AB Pharmacia, Uppsala, Sweden) and 100 ml of horse serum (normal serum; SBL, Stockholm, Sweden) in 1000 ml of a salt solution with Na+, 143 mM; K+, 4.3 mM; Ca2+, 2.5 mM; Mg2+, 0.83 mM; Cl−, 141 mM; HCO3−, 13.3 mM; H2PO4−, 0.46 mM; and glucose, 5.6 mM (8). The perfusion system is illustrated in Fig. 1.

The pump was a peristaltic constant flow type Ismace MP 4 set to a constant flow of 20–30 ml/min. Norepinephrine was injected into the perfusate proximal to the pump by means of an adjustable infusion pump. To monitor possible fluid retention, the animal was placed on a balance throughout the experiment. The temperature of the preparation was kept constant at 37°C by means of a heating lamp mastered by a rectal thermistor.

Regional Blood Flow Determination. Papaverine was used to obtain maximal smooth muscle relaxation. During this condition, the first measurement of regional blood flow took place, whereupon norepinephrine infusion was started and gradually increased, resulting in a raised perfusate perfusion pressure. At two different levels of norepinephrine infusion, injections of microspheres were given. The polystyrene spheres (3M Co., St. Paul, MN), diameter, 15 ± 3 (SD) μm were injected into the tubing connected to the aortic root. The spheres were labeled with 54Ce, 85Sr, and 51Cr, respectively, and given approximately 150,000 at a time. During and immediately after microsphere injection (90 s), a reference perfusate sample was drawn from the femoral artery at a rate of 0.3 ml/min. After the perfusion experiment, the tumors, psoas muscle, salivary gland, uterus and kidneys were dissected out, weighed, and placed in vials for activity measurement in a Packard Autogammaspectrometer Model 5019. From the activity in the reference samples, the perfusate flow in the tissue samples could be calculated (9, 10). Care was taken to perform the experiments in a standardized way and as rapidly as possible since prolongation leads to edema and increased venous pressure. Experiments exhibiting such artifacts were rejected.

Data Processing. Response to norepinephrine was measured as changes in perfusate flow, being in turn the result of changes in the cross-sectional area of the vascular bed suitably expressed as organ vascular resistance per unit weight by dividing the perfusion pressure by the perfusate flow. Narrowing of the vascular lumen may be assumed to occur due to shortening of effector cells around the vessel. According to Poiseuille's Law, flow is proportional to the fourth power of the vessel radius and, in thin-walled vessels, the circumference. The vascular resistance is thus inversely proportional to the fourth power of the effector cell length. It is thus obvious that the shape of the dose-response relationship is different when measured at the cellular level or as vascular resistance; but if the latter is plotted against the common logarithm of the norepinephrine concentration, a sigmoid curve is obtained which can be further straightened if the ordinate, resistance, is also logarithmically transformed. A linear regression line was therefore calculated based on the logarithmically transformed norepinephrine dose and vascular resistance for each organ and tissue, norepinephrine concentration being less than 2.0 μg/ml perfusate. Vascular resist-
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Caudal art prest.

Fig. 1. Schematic illustration of the perfusion set-up (modified from Weiss et al. (21)). Fem. art. ref., femoral artery reference; art. press., arterial pressure.

Tumor

Fig. 2. Mean dose-response curves for various tissues. As the upper halves of these curves are indirectly obtained (see text), the curves are dotted. Bars, SEs of the means for organ vascular resistance at maximal relaxation and constriction, respectively, and for the norepinephrine concentration required to produce 50% of the maximal response. *, significant differences (P < 0.05) from the mean tumor dose-response curve. R, right; L, left.

The dose-response curve has been constructed and the following three characteristics have been considered of interest: (a) vascular resistance at maximal dilatation; (b) vascular resistance at maximal constriction; and (c) norepinephrine concentration at half-amplitude.

Statistics. Analyses of significant differences between tumor and organ vascular resistance at maximal dilation and at highest norepinephrine dosage were made by means of Student's t test, pairing design, and by means of Wilcoxon's signed rank test for paired samples (11). By covariance analysis residual variance for norepinephrine concentration at half-amplitude could be calculated and a Student's t test group comparison was performed.

RESULTS

Ten successful perfusion experiments were performed but a larger number of experiments were rejected owing to technical failures resulting in edema, increased venous pressure, or uneven distribution of microspheres between the two kidneys. In the 10 animals having a mean weight of 277 g before evisceration and 257 g when perfused, 68 tumors were analyzed, with a mean weight of 2.3 g.

In seven experiments blood flow was measured at two dose levels of norepinephrine less than 2.0 µg/ml perfusate. In three experiments norepinephrine was infused at a concentration above 2.0 µg/ml during the third microsphere injection. The mean of the vascular resistance from these measurements constitute the assumed maximal constriction. In total, 17 dose-response values were available for each organ for norepinephrine concentrations between 0.15 and 2.0 µg/ml perfusate.

The mean perfusate flow to the preparation was 8.21 ml/min x 100 g and the total resistance to flow during maximal smooth muscle relaxation was 2.52 ± 0.14 mm Hg x 100 g x min x ml⁻¹, at a mean perfusion pressure of 21 mm Hg. Mean dose-response curves are illustrated in Fig. 2. Vascular resistance at maximal vasodilation is at least four times higher for the tumor vascular bed representing a low flow capacity as compared to other vascular beds at the perfusion pressure used. The vascular resistance at maximal constriction is also very high for tumor; thus, the curve amplitude is great, indicating that the vessels to or within the tumors are able to constrict; however, the relative resistance increase for the tumor vascular bed is not particularly high, approximately 10 times, being in the same range as that in the psoas muscle. The uterus increases its resistance approximately 20 times and the kidneys are capable of a 60-fold increase. Salivary gland has the lowest relative increase, approximately 5-fold. It is of interest to note that for the tumors alone, the three experiments where norepinephrine was infused at a concentration higher than 2 µg/ml showed a lower organ (tumor) vascular resistance than was noted after infusion of 1–2 µg/ml. The maximal response for tumors in Fig. 2 is derived from results of experiments in the latter concentration range. This might reflect a collapse of contractile elements at high distending pressures, specific to tumor tissue. The norepinephrine concentration required to produce one-half of the maximal constriction is lowest for the tumors and is significantly different from that required to produce the same response in other tissues studied. This finding might be taken as an indication of hyperreactivity of the tumor vascular bed to norepinephrine.

DISCUSSION

This study was prompted by conflicting reports on tumor vascular reactivity to various vasoactive drugs (e.g. Refs. 12 and 13). Most morphological studies do not reveal a tissue substrate believed to be capable of vasoconstriction (14) whereas most
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authors using functional measurements have found blood flow reduction in tumor tissue upon catecholamine exposure (e.g., Refs. 15 and 16), some of these measurements showing a powerful response, greater than that of normal tissues (5, 15, 17). All of these studies have, however, been performed in live tumor-bearing animals, either conscious or under various types of anesthesia; thus, resting conditions are ill-defined and the various vascular beds are subject to undefined and different vasoconstrictor tone, making reliable estimates of drug reactivity in various vascular beds impossible. An in vitro perfusion model was therefore considered useful for the present purposes. A similar perfusion system was used to characterize the vascular bed of rat hind quarters (4); however, only bulk flow as derived from the pump settings was recorded, giving information on dose-response characteristics of the whole preparation but not on regional perfusion. It was therefore decided to combine the perfusion procedure with the labeled microsphere technique generally used in the in vivo situation. This combination opens up possibilities of obtaining dose-response curves for an unlimited number of tissues but has certain practical limitations. Owing to a tendency towards edema formation, perfusion experiments should be completed within a short time (18); otherwise, the vascular resistance will gradually increase. The venous outlet must not be compromised; otherwise, the venous pressure will increase.

The microsphere tracer technique involves some complications when used in this system. Since the aortic root is cannulated, there is a risk of insufficient mixing of spheres within the perfusate, especially at low flow rates. Even mixing of spheres was ensured by rejecting experiments with more than 20% difference in perfusate flow between the two kidneys. The number of spheres used in the consecutive injections should be limited since vascular plugging will otherwise give rise to increased vascular resistance. It seems that the in vitro situation is more susceptible to this artifact than the in vivo situation.4 The number of observations along the dose-response curve will therefore be limited in this work to three points: at maximal vascular relaxation and at two different dose levels of norepinephrine. Construction of average dose-response curves is complicated by the fact that except for maximal relaxation only two points along the curve are obtained for each sample, but since norepinephrine dosages vary between the 10 experiments, data processing as described in “Materials and Methods” has made these measurements possible.

As an alternative to the fixed flow perfusion used in this work, fixed pressure perfusion could be considered. At maximal vasodilation, the pressure must be fairly low in these experiments, approximately 20 mm Hg, to prevent edema formation. Upon norepinephrine infusion, the perfusate flow would then decrease to such an extent that it cannot be measured, at least not with the microsphere technique; thus, a constant perfusate flow was used in each experiment and upon norepinephrine infusion the perfusion pressure was increased as a result of increased total vascular resistance. Since the various organ vascular beds can be considered to be parallel coupled, a redistribution within the preparation of the unchanged bulk flow will occur. Vascular beds with high contractile capacities will thus decrease their perfusion at the expense of other vascular beds with less contractility, where perfusion will increase to varying extent; in fact, a vascular bed with low contractile capacity might be forced to increase its perfusion proportionally more than the increase in perfusion pressure, demonstrating a decreased vascular resistance upon norepinephrine infusion. As shown by the pump flow setting and measured perfusion pressure, the total vascular resistance could be increased approximately 4.5 times. Salivary gland showed a similar increase, but all other organs and tissues studied showed greater increases. The renal vascular bed exhibited the greatest increase, approximately 60 times, a value much greater than that obtained in an isolated kidney preparation (19) probably reflecting the fact that in the latter isolated preparation perfusion could not be redistributed. The tumor vascular bed had the capacity to increase its vascular resistance approximately 10 times, demonstrating the existence of intense contractility of vessels to or within the tumors. This increase is in the same range as for most other organs studied here, which is in contrast to results of in vivo reactivity studies in the same tumor model (5) in which vascular resistance increased much more than in other organs studied. This finding might be due to a maximally dilated tumor vascular bed during resting in vivo conditions, in contrast to the varying basal tone in most other tissues.

The norepinephrine concentration required to produce one-half the maximal vascular resistance response is dependent not only upon smooth muscle sensitivity but also on the wall to lumen ratio and the maximal pressor response to norepinephrine (4). It will be seen from Fig. 2 that the tumor vascular bed seems to be the most reactive. This shift to the left of the dose-response curve might also explain the in vivo reactivity findings discussed above.

The prevailing increased interstitial fluid pressure within these tumors (20) must also be considered in the interpretation of the hemodynamic data. It has recently been shown (21) that a change in perfusion pressure influences the tumor vascular bed differently from other tissues in that an increase in perfusion pressure seems to open up tumor vessels more than vessels elsewhere. A pharmacologically induced perfusion pressure increase may possibly open up certain vessels (22), thereby masking the pharmacological contractile response. If this phenomenon is present, it means that the "true" dose-response curve for tumors should be displaced further to the left; on the other hand, it has also been shown that infusion of norepinephrine in vivo decreased the interstitial fluid pressure (23). If the precapillary resistance is increased proportionally more than the perfusion pressure, the hydrostatic pressure of the capillaries will decrease and the high interstitial fluid pressure could cause occlusion of presumably central parts of the tumor capillary network. These events might potentiate the vasoconstrictive effect of norepinephrine and shift the dose-response curve to the left.

The dose-response curves for norepinephrine obtained with an in vitro perfusion system thus indicate the existence of contractility of vessels to or within the DMBA-induced mammary tumors and a possible hyperreactivity, tentatively a phenomenon which warrants further investigation because active intervention to tumor perfusion may be of importance in various therapeutic situations.

REFERENCES

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