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Cardiac Output Redistribution Induced by Noradrenaline in Two Murine Tumor Models*†

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Abstract—*The response of tumor vessels to vasoactive substances could provide useful information on experimental tumor biology. We have studied the effects of noradrenaline (20 μg/kg i.v.) on cardiac output (%CO) distribution in C57BL/6J mice bearing syngeneic Lewis lung carcinoma (3LL) and BALB/c mice with JW sarcoma (JWS). Mice were studied at different stages during tumor growth using microspheres labeled with* ^{57}Co *(basal determination) or* ^{58}Co *(after noradrenaline or saline). In control C57BL/6J mice noradrenaline induced a redistribution of CO, with an increase in the heart and brain and a decrease in the kidneys and hind limb muscle CO fractions (%CO). In 3LL-bearing mice the %CO to the tumor was not changed by noradrenaline* 1 week *after implantation but was significantly less after* 2 *and* 3 weeks. *%CO to the total lung tissue or to isolated metastases did not change after noradrenaline. In control BALB/c mice noradrenaline increased the %CO to the brain and decreased that to the kidneys and hind limb muscle. In JWS-bearing mice the %CO to the tumor was reduced* 2 weeks *after implantation, was not changed after* 4 weeks *and was increased after* 6 weeks. *These results suggest that tumor vessel reactivity to a vasoactive substance may change markedly during various phases of tumor growth and may differ in different experimental models.*

INTRODUCTION

CHANGES in tumor blood flow in response to vasoactive agents have been reported in different experimental models in the hamster [1], rat [2, 3] and rabbit [4]. However, these studies were made at only one stage of tumor growth and did not therefore take into account that vascularization of tumors is a continuously changing process [5]. On the other hand, these studies concern only primary tumors, and apparently no data are available on the changes induced by vasoactive drugs on cardiac output distribution to metastatic tissues.

Since murine tumors are the most commonly used models for biological and pharmacological studies, we developed a method for measuring cardiac output distribution in mice [6]. With the use of radioactive microspheres we have studied the cardiac output distribution to two murine spontaneously metastasizing tumors, the Lewis lung carcinoma (3LL) and the JW sarcoma (JWS). We have found that blood supply to the tumor changes with its growth and differs markedly from the blood supply to the corresponding metastases [7].

The aim of this study was to investigate the effect of noradrenaline on tumor blood supply at various stages of growth in these two models.

MATERIALS AND METHODS

Animals and tumors

The Lewis lung carcinoma (3LL) was maintained by intramuscular passages in C57BL/6J mice every 3 weeks. Cell suspensions were prepared by mechanical homogenization in

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phosphate-buffered saline (Dulbecco) in a Virtis homogenizer, washed and resuspended in saline [8]. Animals were transplanted i.m. with 2×10^5 cells into the left hind limb. The characteristics of this spontaneously metastasizing lung tumor have been previously described [8].

The JW sarcoma (JWS) arose in 1974 as a spontaneous lung tumor in a BALB/c mouse [9] and was maintained in ascitic form by weekly passages of cells collected from the peritoneal cavity. When tumoral cells were implanted s.c. a solid tumor was obtained which metastasized to the lungs. Growth characteristics and hematological changes occurring in this tumor have been previously described [10]. In this study BALB/c mice were injected subcutaneously into the upper part of the back with 5×10^4 cells suspended in saline.

Experimental procedure

The effect of i.v. noradrenaline administration on cardiac output (CO) distribution was studied using the radioactive microsphere technique as previously described [6].

In brief, animals were anesthetized by i.p. injection of urethane (1.25 g/kg body wt), 120,000–160,000 ^{57}Co-labeled microspheres (15 ± 1.5 μm in diameter) mixed in 50 μl rat serum were injected into the cannulated left ventricle and the catheter flushed with an additional 50 μl saline. Ten minutes later noradrenaline (20 μg/kg body wt in 50 μl saline) was administered into the tail vein, and immediately afterwards a second injection of ^{58}Co-labeled microspheres was given. Four minutes later the animals were killed by an overdose of urethane and their organs were dissected, weighed and placed in counting vials for radioactivity measurement in a Packard well scintillation counter.

Heart, right and left kidneys, brain, lungs, small intestine, hind limb muscle, skin surrounding tumor and tumor were taken. In addition, in 3LL-bearing mice metastases were isolated from the surrounding pulmonary tissue after 3 weeks of tumor growth. The infiltrative growth of lung metastases in JWS and the associated inflammatory reactions made it very difficult to separate the nodules from the surrounding tissue; therefore radioactivity was measured in total lungs invaded by metastases.

Experimental groups

Due to differences in the pattern of growth and survival time of the two models, the effect of noradrenaline was studied at different time intervals. 3LL-bearing mice were studied at 1, 2 and 3 weeks and JWS-bearing mice at 2, 4 and 6 weeks after tumor implantation. As control groups, normal, tumor-free C57BL/6J or BALB/c mice were used.

In order to test the possible effects of the injection *per se*, isotonic saline (0.9%, 50 μl) was administered into the tail vein, just before the second microsphere injection, in 5 mice bearing 3LL (11 days after implantation) and 5 mice bearing JWS (18 days after implantation).

Statistical evaluation and expression of results

The effect of noradrenaline on the CO fraction to any organ was calculated as the ratio:

$$\frac{\%\text{CO after noradrenaline}}{\%\text{CO before noradrenaline}} = r.$$

As a ratio is not normally distributed, to evaluate $\bar{r} \neq 1$ Student's t test was used after logarithmic transformation of each individual r value [11].

In view of the large number of organs of the various experimental groups to be studied, the results were expressed by means of columns representing the distance of $\bar{r}$ values from 1 (i.e. % change in respect to basal values, measured before noradrenaline injection). An example of this calculation is the following: when a ratio (e.g. in 3LL tumor at 2 weeks) was 0.62, with 95% confidence limits between 0.50 and 0.76, the value on the graph was expressed as −38% (0.62–1). A statistically significant difference was indicated by the black columns.

RESULTS

Effect of saline injection on tumor-bearing mice

No significant differences were found between basal values of CO distribution and those obtained after i.v. injection of 50 μl of isotonic saline in 3LL-bearing mice (11 days after implantation; Fig. 1A). The administration of saline slightly modified the CO distribution in JWS-bearing mice; indeed, a slight but significant increase in the %CO to the heart was observed (Fig. 1B).

Effect of noradrenaline in 3LL-bearing and control mice

In contrast to saline, i.v. bolus injection of noradrenaline induced a change in distribution of CO in all experimental groups, although of a different direction and degree (Fig. 2).

In the control group (tumor-free animals) the CO fraction to the heart, small intestine and brain increased, while those to the kidneys and hind limb muscle decreased. One week after tumor implantation an increase in the %CO to the skin surrounding the tumor was observed. At this time of tumor growth there was also an increase in the

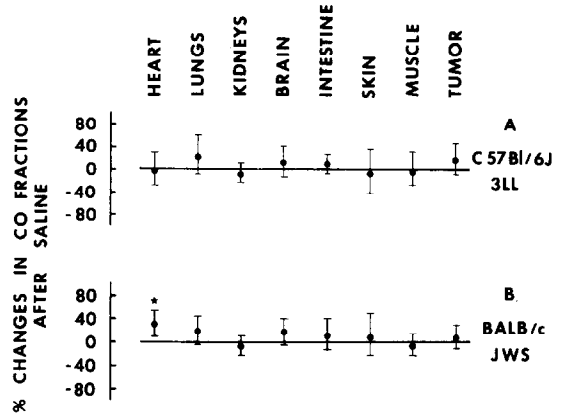

Fig. 1. Effect of saline (50 µl i.v.) on CO distribution in (A) 3LL-bearing mice and (B) JWS-bearing mice. The means (black points) and the 95% confidence limits (vertical bars) of the % changes in CO fractions with respect to basal (before saline) values are shown. Statistically significant differences are indicated by asterisks.

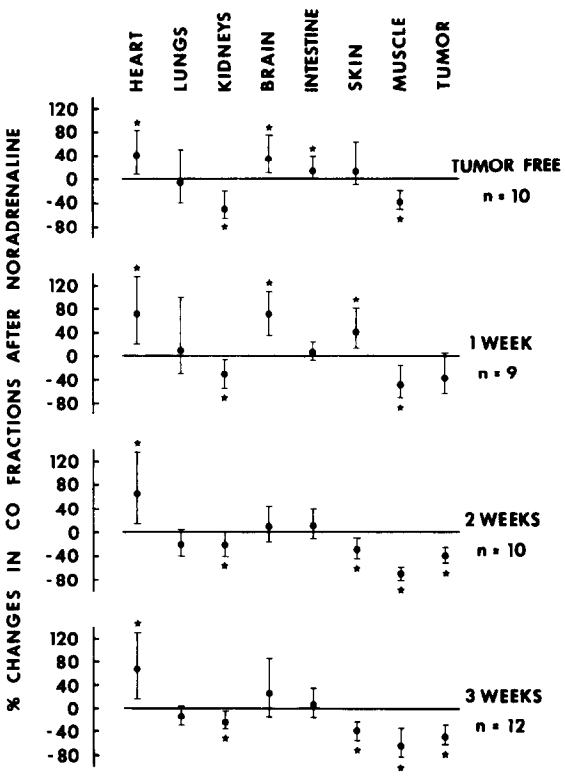

Fig. 2. Effect of i.v. noradrenaline administration on CO distribution in 3LL-bearing and control C57BL/6J mice. The means [black points] *and the 95% confidence limits (vertical bars) of the % changes in CO fractions with respect to basal (before noradrenaline) values are shown. Statistically significant differences are indicated by asterisks.*

%CO to the heart and brain and a decrease in that to the kidneys and hind limb muscle.

At 2 and 3 weeks a similar picture was observed, with the exception that the %CO to the brain was not increased and the %CO to the skin surrounding the tumor was decreased.

The %CO to the 3LL tissue was decreased after noradrenaline administration at all the stages of growth, although the difference was not significant at the first week ($0.01 > P > 0.05$). Noradrenaline did not modify the %CO to the lungs of control or 3LL-bearing mice at any instance; the %CO to the isolated lung metastases also remained unchanged (Table 1).

Table 1. Effect of noradrenaline on the CO fractions to 3LL primary and metastatic tissues (3 weeks after tumor implantation) expressed as the ratio %CO after/%CO before noradrenaline (95% confidence limits are given in parentheses)

	Primary 3LL	Metastatic lungs	Isolated metastases
Ratio	0.52 (0.40–0.68)	0.85 (0.62–1.08)	0.82 (0.63–1.07)

Effect of noradrenaline in JWS-bearing and control mice

The modifications in CO distribution induced by the administration of noradrenaline in control and JWS-bearing mice are shown in Fig. 3.

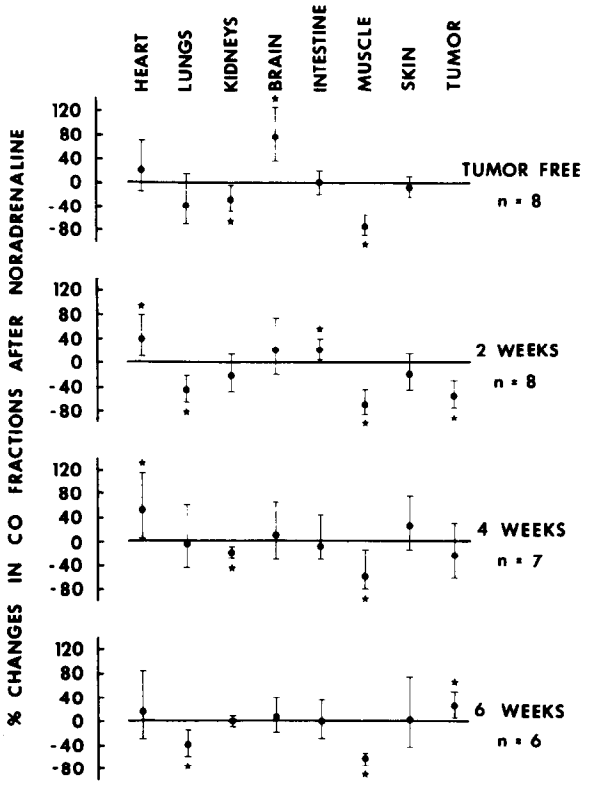

Fig. 3. Effect of i.v. noradrenaline administration on CO distribution in JWS-bearing and control BALB/c mice. The means (black points) and the 95% confidence limits (vertical bars) of the % changes in CO fractions with respect to basal (before noradrenaline) values are shown. Statistically significant differences are indicated by asterisks.

In control (tumor-free) mice there was a significant decrease in the %CO to the kidneys and hind limb muscle, while the %CO to the brain was increased. Such an increase in the cerebral CO fraction was not observed in JWS-bearing mice at any stage of tumor growth. As shown in Fig. 3, the most remarkable noradrenaline-induced effects were the constant strong reduction in blood supply to the hind limb muscle and the changes in the response of tumoral vessels: the %CO to the JWS was decreased after 2 weeks and significantly increased after 6 weeks of tumor growth. The %CO to the lungs was unchanged after noradrenaline in tumor-free mice and in JWS-bearing mice after 4 weeks of primary tumor growth, which corresponds to the initial stage of metastasis growth. In contrast, there was a significant decrease in the %CO to the lungs at the initial and final stages of primary tumor growth.

DISCUSSION

It is well known that catecholamines can influence heart function and induce changes in hemodynamic status which may well affect the regional distribution of cardiac output and almost certainly affect regional blood flow [12]. This study shows that noradrenaline induces changes in cardiac output distribution in mice with experimental malignancies.

In order to correctly evaluate the results presented here one has to consider that the method used in this study is only semi-quantitative. Indeed, in mice it is not possible to measure cardiac output in absolute values, but only to express fractions (%CO) of its distribution to the various organs. Changes in these fractions may reflect three different types of response: (1) decrease in %CO due to moderate to strong vasoconstriction; (2) no change in %CO due to weak vasoconstrictory response; or (3) increase in %CO due to absence of vasoconstriction, or to vasodilatory response balanced with a reduction in blood supply to other tissues.

The systemic administration of noradrenaline produces vasocontriction of vessels in some tissues (muscle, skin, kidneys) by direct activation of the α-adrenergic receptors and vasodilation of some other districts by readaptative reflex due to increased arterial pressure and metabolic changes [12]. Thus we can put forward the hypothesis that a direct stimulation of α-adrenergic receptors may explain the strong vasocontriction which we observed in the hind limb muscle and kidneys of 3LL-bearing as well as of control C57BL/6J mice.

The increase in the %CO to the heart in both models could be secondary to a rise in arterial pressure and/or to vasodilation due to the increase of heart work and subsequent metabolic changes [13].

The loss of autoregulation resulting from carotid artery occlusion in the experimental conditions used might account for the increased %CO to the brain observed in some instances after noradrenaline [14].

A strong response to noradrenaline, which could be ascribed to vasoconstriction, was observed in the vessels of the 3LL primary tissue similar to that of muscular vessels. Noradrenaline-induced vasoconstriction has been described in other experimental tumor models of the rat [2, 3], hamster [1] and rabbit [4]. In contrast, apparently no information is so far available about cardiac output distribution to metastatic tissues [7] and their response to vasoactive agents. In this study we found no significant changes in the %CO to 3LL lung metastases after noradrenaline. One could speculate that a lower amount of α-adrenergic receptors are found in vessels of metastatic as compared to primary 3LL tumor. Alternatively, generation of higher amounts of vasodilatory substances (i.e. prostaglandins) could decrease the reactivity of metastatic tissue to vasoactive agents [15]. We have recently shown that metastatic lung tissues and isolated metastases of 3LL produced significantly higher amounts of vasodilatory prostacyclin than the corresponding primary tumor tissue [16]. A change in the local production of this type of mediator might also explain the changes in response to noradrenaline observed in some tissues (e.g. skin) during 3LL development.

In BALB/c control mice, similarly to C57BL/6J, noradrenaline decreased the %CO to the hind limb muscle and kidneys and increased the cerebral blood supply. In JWS-bearing mice a decrease in the %CO to hind limb muscular vessels was the most consistent change.

Hypothetically, the decrease in %CO observed after 2 weeks could be due to the fact that JWS tumor vessels still possess α-adrenergic receptors at early stages; in contrast, vessels developing at later stages of tumor growth could be deprived of these receptors, thus accounting for lack of response to noradrenaline. Alternatively, α-adrenergic receptors could well be present in all stages of tumor growth, but their reactivity could be controlled by local vasodilatory substances (like prostaglandins), the production of which may change, conceivably, during tumor development.

A study of the response to other vasoactive agents and to prostaglandin-synthesis inhibitors in these conditions might give a more conclusive answer to these questions.

Since the metastatic growth of JWS extensively

invaded lung tissues, isolated nodules could not be separated from the surrounding tissue; it was thus impossible to establish whether the observed decrease in the %CO to the lungs after noradrenaline should be ascribed to the response of intact lung, metastatic vessels or both. In view of the variable amount of inflammatory reaction and malignant tissue in the lungs of JWS-bearing mice, it appears questionable to draw conclusions from these lung studies.

From our results we may conclude that: (1) the i.v. administration of noradrenaline changes the distribution of cardiac output in tumor-bearing mice in a way which may be different in different stages of tumor development; (2) in 3LL-bearing mice the response to noradrenaline differed in primary and metastatic tumoral vessels; and (3) the microsphere method appears to be a useful procedure for studying the effect of vasoactive agents on mouse tumoral vessels.

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