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EFFECT OF EXOGENOUS ANGIOTENSIN-II ON LOCAL BLOOD FLOW IN KIDNEYS WITH NEOPLASM

Experiments in the rat

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Neoplastic vessels may lack the ability to react to vasoactive agents, depending on tumor type, the state of tumor development, and normal reactive vessel inclusion. This may probably account for the somewhat disparate results obtained with respect to the effects of vasoactive drugs on the tumor blood flow.

In kidneys with neoplasm, bolus injection of angiotensin-II (Ang-II) in the renal artery has been found to give improved angiographic demonstration of tumor vessels in man (EKELUND et coll. 1972). This might well be due to selective reduction of flow in the normal renal tissue.

An investigation has therefore been carried out to ascertain whether continuous infusion of Ang-II would produce such flow distribution, and whether it could be maintained over a period of time sufficient to direct circulating agents selectively to the tumor. For repeated parallel measurements of local flow in tumors and intact renal tissue the H_2 gas washout method was chosen.

Methods

Blood flow in control kidneys. Seventeen male Wistar rats with a body weight of 370 to 440 g were kept fasting with free access to water for one day before the experiment. The animals were anesthetized with pentobarbital-Na, tracheostomized and placed on a heating table with thermostatic control via a rectal thermometer keeping the body temperature between 37 and 38°C. A polyethylene catheter was introduced via the left carotid artery to the aorta and connected to a Hewlett-Packard transducer and recorder for arterial blood pressure and heart rate registrations. Another catheter was introduced into a femoral vein for infusion of Ang-II. The animals were laparotomized and the left kidney was gently immobilized. Two to four platinum electrodes, active tip diameter 0.1 mm and length 0.5 to 1.0 mm, were placed with the tip 1 to 3 mm deep into the renal tissue. Hydrogen concentration around the electrode tip was determined polarographically at a potential of +0.17 V versus an Ag/AgCl electrode placed against the inner abdominal wall in the right iliac fossa. The amplified electrode current was recorded on a 6-channel recorder (Rikadenki Kogyo Co Model B-64). The animals were given inhalation air with 5% H_2 gas to the tracheal cannula until constant H_2 concentration was recorded from the different electrode sites. The gas was then suddenly withdrawn and the washout curves were recorded.

The blood flow per gram tissue was calculated from the slope of the semilogarithmically plotted washout curves. For the principle of and details on the method, cf. AUKLAND (1968). Two or more

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consecutive washout curves were recorded at each electrode position for calculation of the control blood flow. Then angiotensin-II-val-5-amide (Hypertensin, Ciba, Switzerland) dissolved in isotonic saline was infused intravenously at rates of 50, 250, 500, 1 000 or 2 000 ng/kg/min with an infusion volume of 0.5 ml/min. The infusion was maintained for 15 min or more and one to three washout curves were recorded at steady mean arterial blood pressure (BP). In 3 of the animals, measurements were made at two different infusion rates of Ang-II separated by a control measurement at normalized BP. In the other animals, only one infusion rate of Ang-II was used.

The flow at each electrode position during control conditions and Ang-II infusion, respectively, was calculated as the mean of the flow values from the consecutive measurements.

Blood flow in kidneys with neoplasm. Ten inbred Lister rats of both sexes with a body weight of 173 to 196 g were used. Seven days before the blood flow measurements, the animals were anesthetized with ether and laparotomized. Transplantable tumor tissue was obtained from donor rats with 20-methylcholanthrene-induced sarcoma (delivered by Sahlgrenska sjukhuset, Gothenburg). The sarcoma was developed on this strain in 1968 and is now in its 172 transfer generation (KJARTANSSON 1976). The tumor was exposed in the donor rat and approximately 1 mm^3 of tumor tissue was sampled and deposited by a trochar technique into the lower pole of the left kidney in the recipient rats. The abdominal wall was closed and the animals recovered without complications.

The animals were prepared for blood flow measurements as described. One or two electrodes were placed in the intact renal tissue and one or two other electrodes were placed in the tumor. The mean tumor diameter was 4 mm (range 3–5 mm). Blood flow in tumor and normal kidney tissue was determined from 2 or more H_2 washout curves before and during infusion of Ang-II. Five animals received 500 and another 5 received 2 000 ng/kg/min by intravenous infusion.

Results

Blood flow in control kidneys. From 53 different electrode positions in 16 animals 146 single washout curves were obtained for determination of the control flow. During Ang-II infusion, 93 washout curves were recorded from the same positions. The semilogarithmic washout curves were linear to a H_2 concentration of less than 10 per cent of the concentration at the beginning of the linear slope in 139 single curves, to 10 to 20 per cent in 61 curves, and to 20 to 30 per cent in 39 curves. One experiment was excluded as none of the curves were linear to less than 35 per cent.

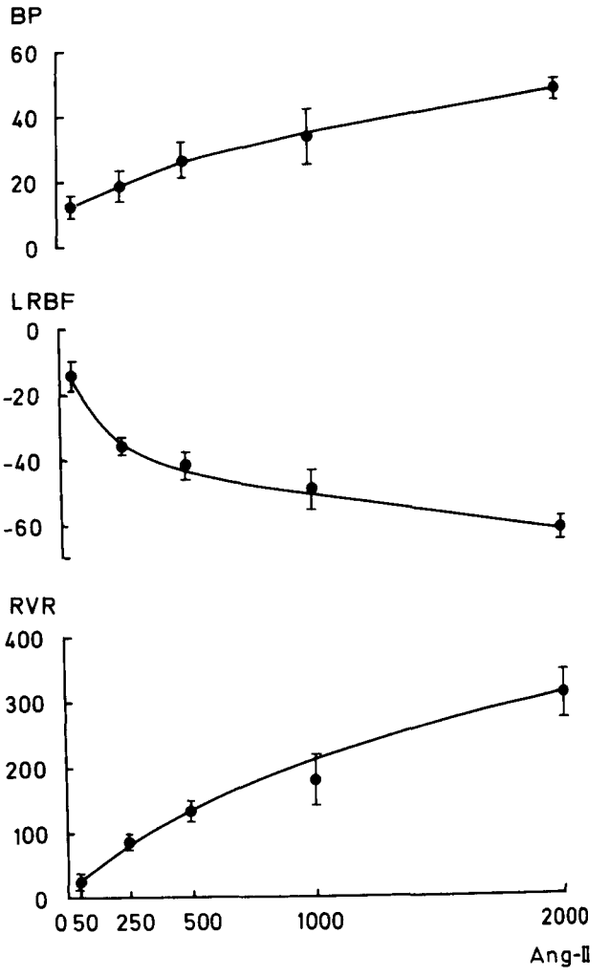

Fig. 1. Effects of intravenous infusion of angiotensin-II (Ang-II) on mean arterial blood pressure (BP), local renal blood flow (LRBF) and the corresponding renal vascular resistance (RVR), expressed as per cent of control levels.

The range of control BP was 88 to 145 mmHg. Total range of control local renal blood flow (LRBF), including cortical and outer medullary flow, was 1.30 to 6.18 ml/min/g. The coefficient of variation for consecutive recordings from a single electrode position was 6.9 per cent in control conditions and 7.5 per cent during Ang-II infusion.

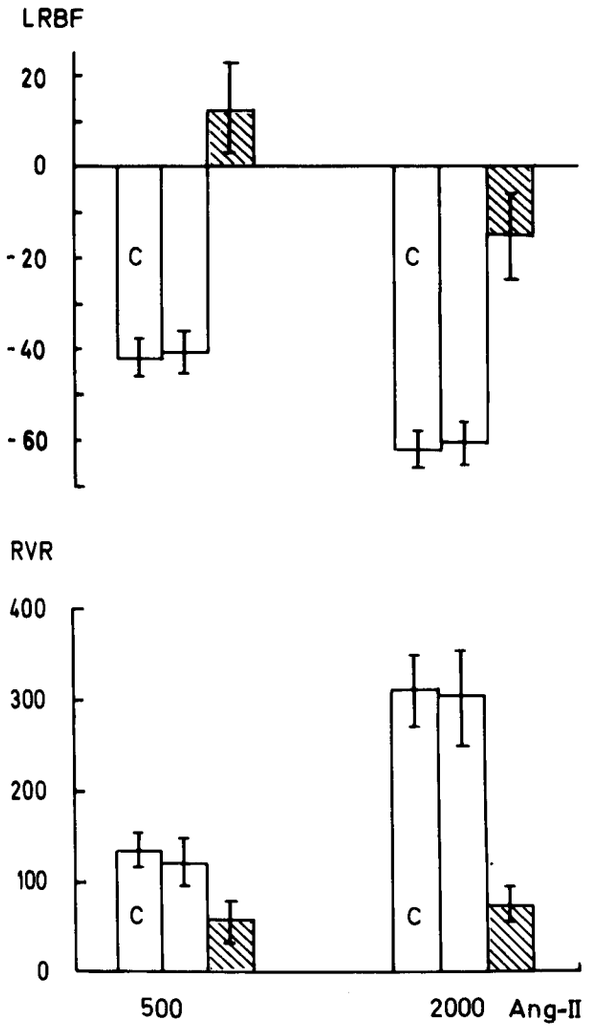

Fig. 2. Changes of local renal blood flow (LRBF) and renal vascular resistance (RVR) induced by intravenous infusion of angiotensin-II (Ang-II), ng/kg/min. Open columns: intact tissue. Hatched columns: tumor. Control group results (C) shown for comparison.

The results obtained in the control group appear in Fig. 1. In all animals a higher BP and a lower LRBF steady state level were established during Ang-II infusion. No significant correlations between control levels and the changes of BP or LRBF were obtained.

Increasing infusion rates gave increasing renal vascular resistance (RVR) but relatively less at the higher rates (Fig. 1). In fact, RVR increased in proportion to the log of Ang-II infusion rate.

Blood flow in kidneys with neoplasm. In the tumors, 32 control washout curves and 28 curves during Ang-II infusion were obtained from 16 electrode positions with concomitant intact tissue recordings.

The extent of linearity of semilogarithmic washout curves from intact tissue was similar to that of the control Wistar rats. Of the 60 single curves from the tumors, only 16 were linear to less than 10 per cent H_2 saturation, 23 to 10 to 20 per cent, 14 to 20 to 30 per cent, and 7 were linear to 30 to 35 per cent H_2 saturation. The coefficient of variation was 8.4 per cent for consecutive measurements in intact parenchyma, and 9.3 per cent in the tumors. Control LRBF averaged 2.25 ml/min/g (range 0.40–6.38) whereas the average local flow in the tumors was 0.66 ml/min/g (range 0.20–1.52), i.e. only 29 per cent of the level in intact parts of the kidneys. No significant correlation was found between control BP (range 95–150 mmHg) and control blood flow in the tumors.

Infusion of Ang-II, 500 ng/kg/min, increased BP by 35 per cent, while infusion of 2000 ng/kg/min gave an increase of 38 per cent, i.e. not significantly different (Fig. 2). The coefficient of variation at consecutive flow measurements during Ang-II infusion was 4.0 per cent in intact tissue and 6.0 per cent in the tumors.

At the higher Ang-II dosage, the flow reduction in the intact renal tissue averaged 41 per cent while the flow reduction in tumors was markedly less, 16 per cent (Fig. 2). Also at the lower dosage the flow in intact tissue was markedly reduced, while in contrast a modest average increase of local tumor blood flow was observed. No significant correlation was obtained between control BP and the change in local tumor blood flow during Ang-II infusion.

Considering all the animals together, paired analysis showed that the infusion of Ang-II gave markedly less reduction of local blood flow in the tumor than in intact tissue ($p<0.0005$).

Fig. 2 also demonstrates the corresponding changes in RVR. At 500 ng/kg/min the vascular resistance in the tumors increased by 61 per cent, although a small decrease was obtained for 3 of 8 electrode positions.

Discussion

The purpose of the control material of Wistar rats was to test the H_2 gas washout method in the rat kidney and to assess the dose response of Ang-II on LRBF.

The degree of linearity of the semilogarithmic

washout curves and the reproducibility of the recordings from the different electrode sites is satisfactory for reliable flow estimates at high and low flow levels in both tissue types, but is no guarantee to that effect. Tissue H_2 washout rate might underestimate local blood flow depending on the rate of arterial blood H_2 desaturation. However, a prolonged arterial desaturation due to impaired alveolar ventilation would cause underestimation of the relative flow reduction in a high flow as compared with a low flow tissue. Thus, this possible error would tend to produce disparate flow changes in intact tissue and tumor, but in the opposite direction of that observed.

The intention was to measure LRBF in cortical and outer medullary portions of the kidney, as the tumors later taken into consideration most likely receive their blood supply from different levels of the renal arteries. For methodologic reasons it would also be an advantage to compare flow changes induced in intact tissue and tumor at similar flow levels. Thus, the electrodes were placed at random, some of them relatively deep into the kidney, i.e. into low flow outer medullary regions. This explains the low average and wide range of LRBF values obtained from different electrode sites and animals.

Correlation analysis showed that control LRBF level did not significantly affect the degree of flow change induced. This may indicate that the effect of Ang-II is much the same in cortical and medullary regions of the rat kidney.

In general, the present effects of Ang-II on BP and LRBF correspond well with previous results obtained in the rat by other methods (FINBERG & PEART 1972, RENTSCH et coll. 1976, ARENDSHORST & FINN 1977) as well as in the dog by the present technique (AUKLAND).

In the Lister rats with renal neoplasm, the changes in BP, LRBF and RVR in the intact part of the kidneys were quite similar to those obtained in the control rats (Fig. 2), indicating that the tumor had not affected intact tissue responsiveness to the vasoconstrictor.

The semilogarithmic washout curves from the tumors had a somewhat lower degree of linearity as compared with the curves from intact parenchyma. This observation could in principle reflect variable local flow or different flow rates in the immediate vicinity of the electrode tips in the tumors.

In any case, the present data leave no doubt that the local blood flow was significantly less reduced in the tumors than in the intact tissue. At doses of 500 ng/kg/min even a slight although statistically not significant increase of tumor blood flow occurred (Fig. 2). A maintained or increased LRBF during Ang-II infusion may have two explanations: (1) The elevated arterial pressure may increase the blood flow through the tumor vessels, provided these vessels lack mechanisms for autoregulation of blood flow and responsiveness to Ang-II. (2) The tumor flow (0.2–1.5 ml/min/g) is markedly less than the intact tissue flow (0.4–6.38). This might suggest that intact tissue exerts a blood pressure stealing effect on the tumor. Pressure stealing would tend to disappear or become negative when intact tissue resistance is raised, and thereby tend to increase the tumor flow.

The vascular resistance in the tumors increased during systemic Ang-II infusion. This may be due mainly to constriction of the feeding vessels and of normal vessels included in the growing tumor (MATTSSON et coll. 1979). Whether a variable pressure stealing effect plays a significant role in controlling the tumor flow also depends on the tumor feeding vessels, i.e. on their specific blood flow rather than on tumor flow per gram tissue, and on their level of origin in the renal vascularity.

The blood flow in experimental tumors is assumed to be more susceptible to blood pressure changes than the flow in normal tissue (ALGIRE et coll. 1954, VAUPEL 1975, ZANELLI & FOWLER 1977). The present lack of correlation between the control BP and the change in tumor blood flow over a wide BP range (95–150 mmHg) does not oppose this concept but may merely reflect a wide range of local tumor flow.

The present experiments demonstrate that Ang-II infusion has a lower flow-reducing effect on the tumors than on the intact renal tissue. This flow pattern can be maintained by continuous Ang-II infusion for up to 45 min (longest period tested) without tendency to change. Thus, infusion of Ang-II might offer an opportunity to direct a larger fraction of cytostatic drugs given selectively into the renal artery to the tumor. However, the possible usefulness of such attempts, using Ang-II, or a more potent constrictor (MATTSSON et coll. 1980), will depend upon several factors, for instance whether tumor or intact tissue uptake of a particular drug is flow limited, and on its blood to tissue partition coefficient. Such information seems at present to be rather limited for commonly used cytostatics.

SUMMARY

The effect of angiotensin-II on the local renal blood flow and arterial blood pressure was investigated in the rat with the H_2 gas washout technique. Increasing intravenous infusion rates gave a decreasing blood flow and increasing blood pressure, renal vascular resistance being close to proportional to the log of infusion rate. In kidneys with experimental neoplasm, the flow reduction was proportionally less in the tumor than in intact renal tissue. The respective flow levels were maintained for 10 to 45 min without tendency to change.

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