Effects of RS-1893, a Novel Cardiotonic Agent, on Central and Peripheral Hemodynamics in Anesthetized Rats

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Abstract—After the use of two radionuclides in the tracer microspheres technique in the rat was verified, we examined the central and peripheral hemodynamic effects of RS-1893, an orally active cardiotonic agent. An intravenous infusion of RS-1893 at a dose of 3 µg/kg/min gradually decreased blood pressure and increased heart rate and cardiac output. Blood flows in the kidney and stomach were increased. There was no organ in which blood flow was decreased despite the fall in blood pressure. These data suggest that RS-1893 dilates blood vessels in the whole body, especially in the kidney and stomach.

Congestive heart failure is caused primarily by cardiac power failure of different pathophysiological origins and is characterized by underperfusion of the major organs. A decrease in renal blood flow causes water retention, and an increase in venous pressure causes pulmonary edema. These changes underlie symptoms associated with congestive heart failure: systemic edema, respiratory difficulty and easy fatigue (1).

Because of these hemodynamic changes in heart failure, not only cardiotonics but also vasodilators are used for the treatment of this disease (2, 3). For example inhibitors of angiotensin converting enzyme (ACE) improve symptoms associated with heart failure (4). In the case of ACE inhibitors, the increase of renal blood flow is believed to account for their beneficial effects in this situation. It is therefore important to know if a cardiotonic agent has beneficial hemodynamic effects. In the present study, we measured central and peripheral hemodynamic changes using the microsphere technique after a single intravenous administration of RS-1893 (2-[2-chloro-4-(2,3,4,5-tetrahydro-3-oxo-6-pyridazinyl)]-phenoxy-N-(2-morpholinoethyl)acetamide), an orally active cardiotonic agent (5, 6).

Male Wister-Imamichi rats weighing 365–400 g were anesthetized with thiobutabarbital-natrium (Inactin, Byk Gulden Konstanz, West Germany) at the dose of 100 mg/kg, i.p. The left femoral artery and vein were cannulated for the measurement of blood pressure and the injection of drugs, respectively. The right carotid artery was cannulated with a polyethylene catheter (No. 2, Imamura, Tokyo, Japan) filled with heparinized saline (50 units/ml), and it was advanced into the left ventricle for the injection of microspheres. The position of the left ventricular catheter was determined by the pressure wave detected by a pressure transducer (MPU 0.5, Nihon Kohden, Tokyo, Japan). The heart rate was measured using a tachometer triggered by the left ventricular pressure. These parameters were recorded on a rectiometer (Recti-Horiz-8K, NEC San-ei, Tokyo, Japan). At least 20 min was allowed for stabilization of cardiovascular parameters before the following experimental procedures.

Microspheres labelled with different radionuclides (¹⁴¹Ce and ⁵¹Cr, 3M, Minnesota, U.S.A.), with specific activities of approximately 10 mCi/g and with a diameter of 15±1 µm, were used. We measured regional blood flow according to the reference sample

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technique (7) as adapted for rats. The microspheres suspended with 10% dextran solution were thoroughly mixed by agitation and sonication (B-220, Branson, Connecticut, U.S.A.) immediately prior to each injection. About 100,000 to 200,000 microspheres were injected with 0.7 ml of saline into the left ventricle through the catheter within 30 sec. The first microspheres were injected during a 1.5 min period of reference blood sampling. Blood was withdrawn through the catheter in the femoral artery at a rate of 0.13 ml/min using a syringe pump (KN-204, Natume Seisakusyo, Tokyo, Japan). About 10 min after the first injection of microspheres, RS-1893 (3 μg/kg/min) or saline was infused into the femoral vein. During the infusion of the agent, 10 min after the start of infusion, the second batch of microspheres labelled with another nuclide was injected into the left ventricle as described above. At the end of the study, the animals were killed, and almost all organs and tissue samples were excised, blotted and weighed. The radioactivity in the tissues and reference blood sample (RBS) were counted for 3 min in a multichannel auto-gamma scintillation counter (Gamma 8000, Beckmann, California, U.S.A.). The total cardiac output and regional blood flows were calculated according to the following equations:

Cardiac output = Withdrawal rate × Counts of total microspheres injected / Counts of RBS
Regional blood flow = Withdrawal rate × Counts of tissue microspheres / Counts of RBS / Weight of tissue

RS-1893 was dissolved in 0.1 N HCl and diluted with physiological saline to make a 0.012% solution. The agent was infused at a rate of 3 μg/kg/min (0.066 ml/min) into the femoral vein. All values were expressed as the mean±S.E.M. The statistical significance was assessed by the paired Student’s t-test.

In the first series of experiments, we examined the validity of using the two nuclides in the rat. We injected microspheres labelled with 141Ce or 51Cr before and during saline infusion at a rate of 0.066 ml/min. The order of administration of 141Ce and 51Cr was randomized. Table 1 shows central and peripheral hemodynamic parameters before and during saline infusion. There was no difference in these parameters between the first and second microsphere injections, except in the lung. Blood flows in both the kidneys were almost identical.

In this series of experiments distribution of microspheres in the lung amounted to 3.6±

| Table 1. Central and peripheral hemodynamic changes in saline treated rats |
|----------------------------------------------------------|
| Mean blood pressure (mmHg) | Before infusion | During saline infusion | % change |
|----------------------------|-----------------|------------------------|----------|
| Heart rate (beats/min)     | 329±13          | 325±13                 | -2%      |
| Cardiac output (ml/min/kg)  | 296±11          | 304±8                  | +3%      |
| Total peripheral resistance (mmHg·kg·min⁻¹) | 395±21          | 375±13                 | -4%      |
| Regional blood flows (ml/min/100 g) | | | |
| Brain                      | 92±9            | 85±7                   | -7%      |
| Heart                      | 528±47          | 545±44                 | +3%      |
| Lung*                      | 149±14          | 96±11**                | -36%     |
| Liver                      | 7.2±1.1         | 7.5±1.1                | +4%      |
| Spleen                     | 81±11           | 70±6                   | -13%     |
| Kidneys                    | 578±28          | 575±30                 | -1%      |
| Stomach                    | 136±15          | 133±10                 | -2%      |
| Small intestine            | 232±14          | 227±7                  | -2%      |
| Large intestine            | 150±18          | 138±7                  | -8%      |
| Skeletal muscle            | 6.6±0.7         | 6.2±0.7                | -6%      |
| Testes                     | 29±2            | 31±2                   | +6%      |

*: Bronchial arterial flow+peripheral arteriovenous anastomotic flow. **: Significant by the paired t-test, P<0.05, N=6.
0.4 and 2.3±0.4% of the total microspheres injected on the first and second injections, respectively. Of these, 1% is derived from the bronchial artery (8) whose blood flow can not have changed during the two injections of microspheres because blood flows in the other organs remained unchanged. The remainder of the microspheres may have originated from arteriovenous anastomosis in the whole body. The reason for the decrease in anastomotic flow on the second injection is not clear, but some alterations in the vascular tone in the skin may account for this decrease because a major portion of arteriovenous anastomosis in the whole body lies in the skin (9).

After the validity of the method was confirmed, we carried out the second series of experiments. Microspheres labeled with 141Ce or 51Cr were injected into the left ventricle. During infusion of RS-1893, at a rate of 3 μg/kg/min, i.v., microspheres labelled with a nuclide different from that on the first microspheres were again injected. Intravenous infusion of RS-1893 caused a gradual fall in blood pressure and an increase in heart rate. These variables reached a steady state within 10 min: mean blood pressure decreased from 123±5 to 111±5 mmHg, heart rate increased from 332±7 to 352±6 beats/min, total peripheral resistance decreased from 443±18 to 331±17 mmHg·kg·min/l and cardiac output increased from 279±11 to 340±16 ml/min/kg.

Figure 1 shows the effect of RS-1893 on regional blood flows. Data for the lung were not taken because the first series of the present study demonstrated that the method did not allow accurate measurement of lung blood flow. RS-1893 markedly increased blood flows in the stomach and kidneys. The agent caused about a 20% increase of blood flow in the both side of the kidneys. Regional blood flows in the liver, heart and skeletal muscles tended to increase, and blood flows to other organs remained unchanged. Baseline blood flows in all organs were in good agreement with those reported elsewhere for the rat (10-12).

Changes in central hemodynamics produced by RS-1893 in anesthetized rats were similar to those obtained in anesthetized dogs (5). We did not measure left ventricular pressure in the present study, because the diameter of the left ventricular catheter was too small for this measurement. However, it was confirmed in a separate series of experiments that RS-1893 at the same dose as used in the present study increased cardiac contractile force: left ventricular dP/dtmax was increased from 7900±400 to 8800±600 mmHg/sec, N=3.

We have previously shown that RS-1893 dilates arterial and venous blood vessels in the hindlimb of dogs (6). However, in the previous study, we did not examine vaso-
dilator action in other vascular beds. In the present study, there was no organ where blood flow was decreased by RS-1893 despite the fall of blood pressure. This fact suggests that the agent dilates blood vessels in the whole body. The decrease of total peripheral resistance supports this notion. The vasodilator action of RS-1893 may be caused by a specific inhibition of phosphodiesterase-III (PDE-III) (5), because it is well-known that PDE-III inhibitors have both cardiotonic and vasodilator actions (13).

Specific increase of renal blood flow, however, seems to be not common to all PDE-III inhibitors: milrinone increases renal blood flow (12), but pimobendan (14) and piroximone (15) do not. The mechanisms underlying the organ selectivity of the vasodilator action of RS-1893 remains to be solved, but the increase in renal blood flow may have beneficial effects on excretory function of the kidney in patients with heart failure.

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