Contribution of Cardiopulmonary Nerves and Blood PaCO_2 to the Coronary Flow in a Dog Heart-Lung Preparation

Naohisa ISHIKAWA, Motoshi KAINUMA, Itaru ICHIMURA
and Tatsuro SHIGEI

Department of Pharmacology, Nagoya University School of Medicine,
Showa-ku, Nagoya 466, Japan

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Heart-lung preparation (HLP) is a useful preparation for studying the effects of cardioactive drugs on the heart. Since the time of Starling (1), various aspects of these preparations have been improved. However, it is difficult to evaluate the effect of drugs which act on the coronary beds because coronary blood flow increases spontaneously and gradually (2–5) during the course of HLP experiments. In an attempt to prevent the increase in coronary flow, Imai et al. developed a HLP supported by a donor dog, and in this preparation, the gradual increase in coronary blood flow was no longer observed (2). They suggested that the mechanism responsible for the increase in coronary blood flow in HLP might be an accumulation of prostaglandin I_2 (pro-stacyclin) in the circulatory blood.

In the course of our recent study on the graphical analysis of drug actions in HLP (6), we noticed that the degree of increase in coronary blood flow seemed to depend on the presence or absence of intact cardiopulmonary nerves (efferent and afferent nerves which coursed to and from the heart and lungs) and also on the composition of the respiratory gas used for ventilation of the lungs (N. Ishikawa et al., unpublished observation). This experience led us to the present study. We found that the progressive increase in coronary blood flow occurs only when the cardiopulmonary nerves are intact and the PaCO_2 of circulating blood is high.

We anesthetized mongrel dogs of either sex, weighing 6–10 kg, with sodium pentobarbital at 25–35 mg/kg i.p. HLP's were prepared according to the Krayer-Mendez modifications of the original Starling method,

the details of which were described elsewhere (6). Heparin sodium was used to prevent coagulation of the blood. For priming of the extracorporeal circuit, larger dogs anesthetized with ketamine (10 mg/kg i.m.) and sodium pentobarbital (20 mg/kg i.v.) were exsanguinated. Collected blood was diluted with a 10% solution of dextran 40 so that hemoglobin content was in the range of 8–10 g/dl. Outflow from the coronary sinus (CSBF) was euded with a Morawitz cannula and measured by a square-wave electromagnetic flowmeter (Nihon Kohden, MF25) which utilized a cannulating-type flow probe. Coronary sinus blood was returned to the preparation via a cannula inserted into the superior vena cava. Systemic cardiac output (SOP), right and left atrial pressures (RAP, LAP) and heart rate were recorded. Systemic arterial pressure was adjusted to 70 mmHg with a Starling resistor. The height of blood in the venous reservoir was 100 mm from the level of the opening of the superior vena cava to the right atrium. A uniform initial condition was selected by the following procedure: The RAP and SOP signals were fed into the X- and Y-axes of an X-Y recorder (Yokogawa, Type 3077), and the equilibrium point denoted by A.C. Guyton, the crosspoint of two curves (i.e., cardiac output and venous return curves), was recorded on the RAP-CO relation diagram. At the beginning of an experiment, the venous resistance was adjusted so as to place the equilibrium point on the straight line which intersected the abscissa at 100 mmH₂O and the ordinate at 500 ml/min (6).

Experiments were performed under the following four conditions, one condition for

each animal: I) cardiopulmonary nerves severed and respiratory gas consisting of 95%O₂+5%CO₂, II) cardiopulmonary nerves severed and respiratory gas consisting of 50%O₂+50% room air, III) cardiopulmonary nerves intact and respiratory gas consisting of 95%O₂+5%CO₂ and IV) cardiopulmonary nerves intact and respiratory gas consisting of 50%O₂+50% room air. In groups I and II, we cut all the cardiopulmonary nerves found at the sites just peripheral to the junctions of the superior vena cava and the brachiocephalic and left subclavian arteries with the right atrium and the aortic arch, respectively, to the depth of the trachea or esophagus, soon after the thoracotomy. The vagus, phrenic and recurrent laryngeal nerves were also severed. We ventilated the lungs artificially with the respiratory gas stored in a large bag (1 m³ in volume). The significance of differences between the means was determined by Student's *t*-test.

The partial pressures of blood gases, pH and base excess (BE), which were measured by an automatic blood gas analyzer (Radiometer, ABL2), depend upon the composition of respiratory gas. The means and corresponding standard errors (*n*=5) for PaO₂ and PaCO₂ were 157.6±9.1 and 14.3±0.5 mmHg in the low CO₂ gas group and 250.1±51.3 and 34.4±1.6 mmHg in the high CO₂ gas group, respectively. A significant difference was seen in the PaCO₂ levels (*P*<0.05). There was also a significant difference in pH (*P*<0.05): 7.6±0.02 in the low CO₂ gas group and 7.32±0.03 in the high CO₂ gas group. No difference was seen in base excess: -4.68±0.38 mEq/l in the low CO₂ gas group and -5.95±0.66 mEq/l in the high CO₂ gas group.

Figure 1 shows an experimental recording obtained under condition III: that is, the condition where the cardiopulmonary nerves were intact and where respiratory gas consisted of 95%O₂+5%CO₂. CSBF increased without a marked heart failure, as shown by the fact that the SOP, RAP and LAP did not change. Heart rate tended to decrease slightly. The same tendency was also noted in other groups of experiments where CSBF was unaltered. These results suggest that the CSBF increase may involved

Fig. 1. Typical recordings of hemodynamics in an experiment under condition III (cardiopulmonary nerves intact and respiratory gas consisting of 95%O₂+5%CO₂). The numbers in squares at the top indicate the time after the preparation was completed. PAP: pulmonary arterial pressure, LAP: left atrial pressure, RAP: right atrial pressure, SOP: systemic cardiac output, CBF: outflow from the coronary sinus. The chart speed in each recording is shown between SOP and CBF.

some substance, as discussed by Imai et al. (3), that dilates only the coronary vessels without affecting the pacemaker and the heart muscle.

Figure 2 shows the time courses of CSBF under four different experimental conditions. All of the points and bars indicate the means and the corresponding standard errors of 5 experiments. CSBF increased during only one of the four conditions; that is, the case in which the cardiopulmonary nerves were intact and the PaCO₂ level was relatively high. There was no statistical difference among the initial CSBF, as shown at the beginning of the experiment, when the one-way analysis of variance was used. The paired *t*-tests between the means of CSBF, at the beginning and at 2 hr of experiment, revealed that in only one con-

Fig. 2. Change in coronary blood flow (outflow from the coronary sinus, divided by the body weight) under four experimental conditions. Each point and bar indicate the mean and its S.E. of 5 experiments. Condition I: C.P.N. (cardiopulmonary nerves) were severed and 95%O₂+5%CO₂ (high PaCO₂) was used for the respiration gas. Condition II: C.P.N. were severed and 50%O₂+50% room air (low PaCO₂) was used. Condition III: C.P.N. were intact and 95%O₂+5%CO₂ was used. Condition IV: C.P.N. were intact and 50%O₂+50% room air was used.

dition (condition III), CSBF at 2 hr was greatly increased, compared with that at the beginning ($P < 0.05$). It is important to note that the CSBF increases under the condition that is most similar to the physiological condition. As described above, we found a slight difference in PaO₂ between the animals in which two different respiratory gases were used for the lung ventilation. Generally speaking, coronary blood flow increases with low PaO₂, but we cannot attribute the change in CSBF in this experiment to the difference in PaO₂, since the PaO₂ in each of the two different respiratory

gas groups was higher than the physiological range. As a rule, at the end of experiments in which an increase in CSBF was observed, 1 mg of indomethacin (Sigma) was added to the blood reservoir, by which the elevated level of CSBF returned to the original level in about 10 min. This observation confirmed the finding by Imai et al. (3), suggesting that some prostaglandin was mediating the coronary vasodilatation. Therefore, a prostaglandin-like substance might be produced in a close relationship with both the function of the cardiopulmonary nerves and the PaCO₂ or pH level. There is another possibility that a gradual change in sensitivity of the coronary beds to the released substance results in an increase in CSBF.

Recent investigators have reported two different results in the sympathetic influences on coronary flow. Vatner et al. (7) reported that in acute conscious dog experiments, the resistance against the coronary flow, measured with a Doppler ultrasonic flow probe on the left circumflex coronary artery, decreased with the electrical stimulation of the carotid sinus nerve. They suggested that the sympathetic constrictor tone is present in the resting conscious dog. This idea was supported by other investigators (8, 9). Meanwhile, Cobb et al. (10) reported that in chronic conscious dog experiments, the resistance to coronary flow, measured with an electromagnetic flow probe positioned on the proximal left circumflex artery, was largely increased after cardiac sympathectomy. They ascribed their results to the reduced cardiac oxygen consumption and the reduced sensitivity to catecholamines. The former result was mediated by an α -receptor, whereas the latter by a β -receptor. In our results, the coronary flow levels at the beginning of experiments were not influenced by the presence or absence of the intact cardiopulmonary nerves. Since α - and β -receptors blockade did not inhibit the gradual increase in CSBF, it seems more likely that the cardiopulmonary nerves play some role in triggering a release of coronary vasodilator substance.

Originally, the surgical elimination of cardiopulmonary nerves was performed for the purpose of avoiding any possible influence

of cerebral ischemia. It remains to be elucidated whether such a factor is related to the gradual increase in CSBF. The results of the present study indicate, however, that we should be more careful in analyzing the effects of coronary vasodilators, or in developing new drugs, since the pharmacological activity or the physiological release of some active substance may change during the course of study under different experimental conditions.

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