RESPONSIVENESS AND HIGH-ENERGY PHOSPHATE METABOLISM OF ISOLATED DOG CORONARY ARTERIES

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Accepted February 26, 1979

Abstract—The relationship between responsiveness and high-energy phosphate metabolism was observed in isolated dog coronary arterial strips before and after equilibration, under aerobic conditions. The strips responded slowly to potassium, noradrenaline, isoproterenol and 5-hydroxytryptamine before being equilibrated but did respond quickly to these drugs after being equilibrated for 1 hour under aerobic conditions. The contents of ATP, creatine phosphate (CP) and lactate were not significantly altered before and after equilibration for 1 hr. It is proposed that, before being equilibrated under aerobic conditions, coronary arteries may be unable to utilize ATP and CP due to injury of contractile or relaxant mechanisms resulting in a weak vascular responsiveness.

It has been reported that oxygen tension (P02) of the bathing solution influences the contractility of vascular smooth muscle (1–5) and that high-energy phosphate compounds provided by anaerobic glycolysis are insufficient to maintain active tension of the isolated coronary artery (6). Generally, in experiments using isolated vascular strips, it is usual for the strips to be allowed to equilibrate for 1–2 hr before any treatment is initiated, presumably to acquire a good response in the preparations. This presumption has not, however, been adequately substantiated. We investigated the relationship between responsiveness and high-energy phosphate metabolism of isolated dog coronary arteries before and after equilibration under aerobic conditions.

MATERIALS AND METHODS

Forty-one male and female dogs weighing 8–15 kg were anesthetized with sodium pentobarbital 30 mg/kg i.v. Before starting the experiments, it was confirmed in all animals that norepinephrine 5 μg/kg i.v. and 5-hydroxytryptamine 10 μg/kg i.v. produced elevations of systemic blood pressure while isoproterenol 5 μg/kg i.v. produced a decrease in pressure. Thereafter, the heart was isolated under conditions of artificial ventilation. Within 10 min after isolating the heart, the circumflex branches of the left coronary arteries were cut into helical strips (2.0 mm wide and 20 mm long) in Krebs-Ringer bicarbonate solutions bubbled with room air and maintained at 37°C. These strips were suspended in a 20-ml muscle chamber filled with Krebs-Ringer bicarbonate solutions maintained at 37°C. The room air was continuously bubbled through the muscle chamber. The composition of the solution, expressed in millimoles per liter, was as follows: NaCl 117.7, KCl 4.7, CaCl2 2.5, KH2PO4 1.2, MgSO4 1.2, NaHCO3 24.4 and dextrose 10.0. When measured by a blood-gas analyzer
(Instrumentation Laboratory Micro-13), the PO$_2$ of the solution was 150 mmHg and the pH was 7.35. To analyze the vascular responsiveness, the strips were attached to an isometric force-displacement transducer (Nihonkoden SB-1T), and the tension was recorded on a polygraph (Nihonkoden RJG-3024). Initial tension was adjusted to 1.0 g. Five min after suspending the strips, the drug solutions were added dropwise to the bath and changes in tension development were observed. After washing out the drug solutions, a gas mixture of 95% O$_2$ and 5% CO$_2$ was continuously bubbled through the chamber. The PO$_2$ was averaged at 650 mmHg and the pH was 7.40. After 1 hr, administration of the drugs was repeated, and the effects were monitored.

For biochemical analysis, two helical strips were cut longitudinally from the same coronary artery to obtain paired vascular preparations. Each coronary arterial strip weighing 50-90 mg (wet weight), before and after being equilibrated for 1 hr under aerobic conditions, was placed into liquid nitrogen. The frozen tissue was crushed under cooling with dry-ice. The powdered sample was mixed with 0.5 ml of 0.3 M HC10$_4$. After 20 min gentle stirring of the mixture under ice-cooling, 2.5 ml of distilled water was added to the mixture, and the resultant mixture was centrifuged at 10,000 g for 10 min at 0°C. The supernatant was employed for determination of adenosine triphosphate (ATP), creatine phosphate (CP) and lactate (LA) contents. ATP and CP contents were measured by a modification of the firefly luminescence method of Strehler (7) using a liquid scintillation counter and LA by an enzymatic method (8). The precipitate obtained by the centrifugation was digested with 10 ml of 1 M KOH for 15 min at 100°C. The resultant solution was used for measurement of protein concentration, using the method of Lowry et al (9). As a standard protein, bovine serum albumin was used.

Drugs employed in this study included dl-norepinephrine hydrochloride (Sankyo), dl-isoproterenol hydrochloride (Nikken-Kagaku) and 5-hydroxytryptamine creatinine phosphate complex (Wako Pure Chem.). All doses of the drugs were referred to final concentration of the salts and expressed in terms of g/ml. The drugs were dissolved with the Krebs-Ringer bicarbonate solution and administered in a volume of 0.2 ml. As indicators for vascular responsiveness, the peak tension(mg) of coronary arterial strips and the velocity of tension development (dT/dt, mg/sec) were used. In the case of potassium, the peak tension was measured 5 min after administration of the potassium solution.

Tests of statistical significance were determined by Student’s t-test.

RESULTS

Before being equilibrated, the coronary arterial strips responded slowly to potassium, norepinephrine and 5-hydroxytryptamine, with no response whatever to isoproterenol (Fig. 1A). When equilibrated for 1 hr under aerobic conditions, the same strips responded quickly to these drugs and the peak tensions of these strips were larger (Fig. 1B). The velocity of tension development induced by each drug before and after equilibration under aerobic conditions was as follows: potassium 30 mM, from 13.0±2.3 to 22.4±2.3 (n=20, p<0.01); norepinephrine 10$^{-5}$ g/ml, from 1.8±1.4 to 16.8±7.3 (n=7, NS); iso-
proterenol 10^{-5} g/ml, from 0±0 to −26.3±5.2 (n=4, p<0.01); 5-hydroxytryptamine 10^{-5} g/ml, from 2.8±0.9 to 30.0±5.9 mg/sec (n=10, p<0.001), respectively. The peak tension before and after equilibration was changed as follows: by potassium 30 mM, from 588.8±100.6 to 1137.5±101.0 (n=20, p<0.001); by norepinephrine 10^{-5} g/ml, from 21.4

**FIG. 1.** Typical responses of coronary arterial strips to potassium 30 mM (K⁺), norepinephrine 10^{-5} g/ml (NE), isoproterenol 10^{-5} g/ml (IPA) and 5-hydroxytryptamine 10^{-5} g/ml (5-HT). A=before equilibration; B=after equilibration for 1 hr under aerobic conditions.

**FIG. 2.** Changes in the velocity of tension development (dT/dt) and the peak tension (Tension) of coronary arterial strips. K⁺=potassium 30 mM; NE=norepinephrine 10^{-5} g/ml; 5-HT=5-hydroxytryptamine 10^{-5} g/ml; IPA=isoproterenol 10^{-5} g/ml; A=before equilibration; B=after equilibration for 1 hr under aerobic conditions; n=number of preparations. Each point represents the means±SE. *Significantly different from values before equilibration (p<0.01). **Significantly different from values before equilibration (p<0.001).
±13.4 to 258.6±127.9 (n=7, NS); by isoproterenol 10⁻⁵ g/ml, from 0±0 to -375.0±43.3 (n=4, p<0.001); by 5-hydroxytryptamine 10⁻⁵ g/ml, from 37.0±10.6 to 395.0±72.1 mg (n=10, p<0.001), respectively. Figure 2 illustrates the changes in the velocity of tension development (dT/dt) and the peak tension induced by each drug before and after equilibration under aerobic conditions.

The contents of ATP, CP and LA in the coronary arterial strips varied on a large scale as shown in Fig. 3. When the mean values of contents were calculated, the mean ATP content before and after equilibration was changed from 2.42 to 2.32 (n=17), the mean CP content, from 2.35 to 1.98 (n=17) and the mean LA content, from 18.40 to 17.83 µmol/g protein (n=11), respectively. Differences between these contents before and after equilibration were not statistically significant.

FIG. 3. Changes in adenosine triphosphate (ATP), creatine phosphate (CP) and lactate (LA) contents of coronary arterial strips. A=before equilibration; B=after equilibration for 1 hr under aerobic conditions. Open circles represent the mean values in each case. No significant difference between A and B.

DISCUSSION

There is always the possibility that the contractile or relaxant mechanism of isolated vasculature will be injured during isolation and when the strips are being cut helically. One possibility is the mechanical damage and the other metabolic disturbances and/or these two. Metabolic disturbances of isolated vessels should be restored when those vessels are allowed to equilibrate for a few hours under aerobic conditions.

In the present experiments, the isolated dog coronary artery responded slowly to potassium, norepinephrine, isoproterenol and 5-hydroxytryptamine before being equilibrated under aerobic conditions. When being equilibrated for 1 hr under aerobic conditions, these strips responded quickly to these drugs. This means that the responsiveness of coronary arteries is restored by equilibration under aerobic conditions. Though differences between the two pH values of the solution before and after equilibration have to be considered, the grade of the velocity of tension development and the peak tension induced by norepinephrine,
isoproterenol and 5-hydroxytryptamine was about ten times compared with that before equilibration. On the other hand, the vascular responsiveness to potassium reached twice the value seen before equilibration. According to the difference between these two values of the vascular responsiveness (ten times and two times), it is considered that the receptors to norepinephrine, isoproterenol and 5-hydroxytryptamine may severely be damaged before equilibration though it is obvious that the contractile and relaxant mechanisms are injured to some extent before equilibration.

It is well known that the vascular contractility is influenced by oxygen tension of the bathing fluid (1–5). The weakness of the vascular responsiveness to the drugs, as observed in this experiment, is due to the low oxygen tension of the solution. However, Gellai et al. (3) concluded that the contractility of vascular smooth muscle is not affected by the oxygen tension of the solution when it is over 100 mmHg. In our data, even before equilibration with a gas mixture of 95% O₂ and 5% CO₂, the oxygen tension of the bathing solution was 150 mmHg. Therefore, the possible low oxygen tension factor can be ruled out. Additionally, consideration of the possible effects of residual pentobarbital need not be considered since it was confirmed before start of the experiment that norepinephrine i.v. and 5-hydroxytryptamine i.v. produced hypertensive actions and isoproterenol i.v. a hypotensive one.

If high-energy phosphate compounds were decreased before equilibration, it goes without saying that the coronary responsiveness should be lowered. We have reported that there are significant correlations between high-energy phosphate compounds contents and tension developments of isolated dog coronary arteries (6). However, it was shown in the present study that the coronary contents of ATP and CP were not significantly changed before and after equilibration. Fukuda and Shibata (10) reported that tissue sodium concentration of guinea pig taenia cecum increased and the potassium concentration decreased when the tissue was kept in cold medium for 5–6 days. Though the tissue ion contents and ionic process were not examined in this study, it is likely that, before being equilibrated under aerobic conditions, the isolated coronary artery may be unable to utilize ATP and CP due to tissue ionic alterations induced by mechanical injuries thus resulting in a weak vascular responsiveness.

Acknowledgement: This work was supported by a grant from the Ministry of Education, Science and Culture of Japan. We are indebted to Mrs. Naoko Ueno for invaluable technical assistance.

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