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Effect of nadolol, a β-adrenoceptor blocking agent, on myocardial metabolism in the dog ischaemic heart

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The effect of nadolol at a dose of 1 mg kg^{-1}, i.v. on the ischaemic myocardial metabolism has been examined in the dog. Ischaemia was induced by ligating the left anterior descending coronary artery for 3 min, and nadolol was injected 5 min before ligation. Ischaemia caused myocardial metabolic changes; it decreased energy charge potential and inhibited glycolytic flux through phosphofructokinase reaction. Pretreatment with nadolol lessened the decrease in energy charge potential and the inhibition of glycolytic flux being caused by ischaemia. Nadolol may have a beneficial effect on the ischaemic myocardium.

The β-adrenoceptor blocking agents reduce the severity of ischaemic injury in the myocardium because of decrease in myocardial oxygen consumption. Propranolol is a typical representative of this group of drugs and is used extensively in the treatment of angina pectoris. However, since propranolol is quickly metabolized in the liver and is bound to plasma proteins, it is difficult to maintain its effective concentration in plasma. In addition, propranolol easily penetrates into the brain, suggesting some side effects on the CNS (Heel et al 1980). Nadolol, also a β-adrenoceptor blocking agent, is not practically metabolized nor does it enter the CNS (Frishman 1981). The basic mechanism by which nadolol acts as an antianginal drug appears to be the same as that of propranolol.

We (Abiko et al 1979) have tried to evaluate the antianginal or anti-ischaemic effect of a drug by the use of an indicator of anaerobic metabolism in the ischaemic myocardium. Under these conditions, the ischaemia causes conversion of the myocardial metabolism from aerobic to anaerobic and a drug that reduces ischaemic injury can switch the metabolism back to aerobic. By this method, we have examined how ischaemic injury proceeds in the myocardium. We (Ichihara & Abiko 1975a; 1977) have found that glyceryl trinitrate and propranolol prevent anaerobic metabolism during ischaemia; that is, the myocardial metabolism appears to be aerobic metabolism in spite of ischaemia. The present study, therefore, was undertaken to examine whether nadolol, which has different physicochemical properties from propranolol, also has a beneficial effect on the ischaemic myocardial metabolism.

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METHODS

Thirty healthy mongrel dogs of either sex, 9–14 kg, were anaesthetized with sodium pentobarbitone (30 mg kg^{-1} i.v.), and endotracheally intubated and ventilated with a respirator. A left thoracotomy was performed between the fourth and fifth ribs to expose the left ventricular wall. After the heart was suspended in a pericardial cradle, a main trunk of the left anterior descending coronary artery was dissected free from the adjacent tissues distal to the first diagonal branch, and was loosely encircled with a 2-O silk thread ligature. Ischaemia was initiated by ligating the coronary artery. An ischaemic region of the myocardium was assessed by visible cyanosis and the elevation of the ST segment of ECG which was introduced by a wire electrode attached on the surface of the left ventricular wall. Arterial blood pressures were measured via a cannula introduced into the left carotid artery. Coronary blood flow was measured by an electromagnetic flow probe positioned just proximal to the ligature.

After control observations had been completed, approximately half the preparations received 1 mg kg^{-1} of nadolol (nadolol-treated), and the other half received an equivalent volume of saline intravenously (saline-treated). After 5 min the ligature around the coronary artery was tied in half the animals receiving nadolol and half those receiving saline. When the ischaemia had been present for 3 min a full thickness sample of the myocardium was taken from the centre of the ischaemic area. An equivalent sample was taken from the control animals that had not had the ligature tied around the coronary artery. The samples were immediately pressed and frozen with clamps previously chilled in liquid nitrogen in such a way that the endocardial

portion of the myocardium could be taken separately for analysis. The endocardial frozen tissue sample was used to determine the levels of glycogen, glucose, glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), fructose-1,6-diphosphate (FDP), lactate, adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), and creatine phosphate (CrP) in neutralized perchloric acid extract according to standard enzymatic procedures (Bergmeyer 1974). Energy charge potential (Atkinson & Walton 1967) was calculated from the concentration of ATP, ADP, and AMP to estimate the myocardial energy state according to the following equation:

$$([ATP] + 1/2[ADP])/([ATP] + [ADP] + [AMP])$$

The rate of glycolytic flux through phosphofructokinase (PFK) reaction was determined according to the equation ([G6P] + [F6P]/[FDP]) defined by Weishaar et al (1979).

Data were evaluated using either the paired or unpaired Student's t-test and a P value of 0·05, or less, was considered significant.

Table 1. Haemodynamic data from animals in which the coronary arteries were ligated.

	Before injection	Maximum response	5 min after inj.	3 min after ligation
Systolic blood pressure (mmHg)				
Saline	142 ± 10	—	142 ± 10	126 ± 12‡
Nadolol	134 ± 9	123 ± 8**	129 ± 7	115 ± 7**,‡
Diastolic blood pressure (mmHg)				
Saline	99 ± 7	—	99 ± 7	88 ± 9†
Nadolol	98 ± 7	89 ± 7**	96 ± 6	88 ± 8*,†
Heart rate (beats min^{-1})				
Saline	136 ± 14	—	137 ± 15	138 ± 15
Nadolol	148 ± 10	122 ± 8**	122 ± 8**	120 ± 8**
Coronary flow (mL min^{-1})				
Saline	17 ± 4	—	17 ± 4	0**,‡
Nadolol	16 ± 1	14 ± 1	15 ± 1	0**,‡

Either saline, or nadolol (1 mg kg^{-1}) was injected intravenously 5 min before coronary artery ligation. Data are means ± s.e.m.
*$P < 0·05$; **$P < 0·01$ compared with 'Before injection'.
†$P < 0·05$; ‡$P < 0·01$ compared with '5 min after injection'.

RESULTS

Haemodynamics

Haemodynamic data from the animals in which the coronary arteries had been ligated are summarized in Table 1. Injection of nadolol significantly ($P < 0·01$) decreased heart rate within 1 min and sustained its lower level until ischaemia was initiated. Probably because of decreased heart rate, systemic arterial blood pressures significantly ($P < 0·01$) decreased immediately after nadolol injection, and then returned to their respective control levels 5 min later. There was no significant change in the coronary blood flow after nadolol injection. Ischaemia significantly decreased systolic blood pressure ($P < 0·01$) and diastolic blood pressure ($P < 0·05$) in both saline- and nadolol-treated animals, but did not modify heart rate.

Effect of nadolol on energy metabolism

The levels of adenine nucleotides and creatine phosphate are shown in Table 2. In either saline- or nadolol-treated heart, the level of ATP significantly ($P < 0·01$) decreased during 3 min of ischaemia, while the levels of ADP and AMP increased significantly (at least $P < 0·05$). The ATP level in nadolol-treated ischaemic heart (4·622 ± 0·084), however, was significantly ($P < 0·01$) higher than that in saline-treated ischaemic heart (3·323 ± 0·226). These values were used to calculate energy charge potentials which are illustrated in Fig. 1. In saline-treated heart, ischaemia decreased energy charge potential significantly ($P < 0·01$) from 0·916 ± 0·003 to 0·832 ± 0·005. In the nadolol-treated heart, energy charge potential was decreased by ischaemia from 0·911 ± 0·003 to 0·865 ± 0·007. Nadolol itself did not alter the value of energy charge potential in the non-ischaemic heart, while there was a significant difference ($P < 0·01$) in energy charge potential in the ischaemic heart between saline- and nadolol-treated heart. The level

Table 2. Effect of nadolol on changes in adenine nucleotides and CrP level during ischaemia.

	n	ATP	ADP	AMP	CrP
Saline-treated					
Non-ischaemia	8	5·007 ± 0·065	0·831 ± 0·036	0·091 ± 0·008	5·920 ± 0·510
Ischaemia	8	3·323 ± 0·226**	1·160 ± 0·083**	0·170 ± 0·013**	1·176 ± 0·246**
Nadolol-treated					
Non-ischaemia	7	5·620 ± 0·109‡	0·964 ± 0·034†	0·110 ± 0·007	5·800 ± 0·433
Ischaemia	7	4·622 ± 0·084**,‡	1·247 ± 0·049**	0·196 ± 0·032*	1·749 ± 0·249**

Data are expressed μmol g^{-1} wet tissue as means ± s.e.m.
*$P < 0·05$; **$P < 0·01$ compared with 'Non-ischaemia' in each group.
†$P < 0·05$; ‡$P < 0·01$ compared with 'Saline-treated'.

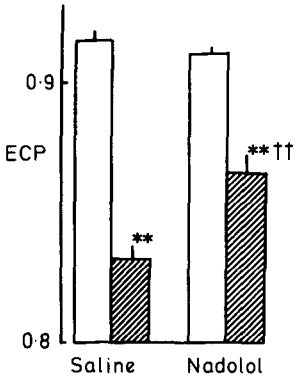

FIG. 1. Effect of nadolol on energy charge potential (ECP) in non-ischaemic (□) and ischaemic (▨) myocardium. Energy charge potential was calculated according to the equation of ([ATP] + 1/2[ADP])/([ATP] + [ADP] + [AMP]) by the use of individual values of ATP, ADP, and AMP. Data are means ± s.e.m. of 7–8 observations in each group. $^{**}P < 0.01$ compared with non-ischaemia. $‡P < 0.01$ compared with saline-treated ischaemia. Saline = saline-treated myocardium.

of CrP also decreased during ischaemia (Table 2). Again, nadolol appeared to lessen the decrease in CrP due to ischaemia.

Effect of nadolol on carbohydrate metabolism

Table 3 shows the levels of glycogen and glucose in the non-ischaemic and ischaemic myocardium. In the saline-treated heart, the level of glycogen decreased significantly ($P < 0.05$), whereas the level of glucose increased significantly ($P < 0.05$) during ischaemia. In the nadolol-treated heart, ischaemia tended to decrease the glycogen level and to increase the glucose level, but the differences in those levels between non-ischaemia and ischaemia were not significant.

The levels of G6P, F6P, and lactate increased significantly ($P < 0.01$) and the level of FDP appeared to decrease during ischaemia in saline-treated heart (Table 4). Nadolol inhibited the increase in G6P, F6P, and lactate, and the decrease in FDP caused by ischaemia. We calculated the ratio of [G6P] + [F6P] to [FDP] to estimate the glycolytic flux through PFK reaction. Results are illustrated in Fig. 2. This ratio was significantly ($P < 0.01$) increased by ischaemia, suggesting the inhibition of glycolytic flux at the level of PFK. Nadolol lessened the inhibition of glycolytic flux due to ischaemia.

Table 3. Effect of nadolol on glycogen and glucose level in the ischaemic myocardium.

	Glycogen	Glucose
Saline-treated		
Non-ischaemia	36·420 ± 5·455	1·593 ± 0·311
Ischaemia	16·997 ± 4·390*	2·794 ± 0·448*
Nadolol-treated		
Non-ischaemia	38·540 ± 5·680	2·088 ± 0·163
Ischaemia	28·860 ± 5·699	2·677 ± 0·340

Data are expressed μmol g^{-1} wet tissue as means ± s.e.m.
$^{*}P < 0.05$ compared with 'Non-ischaemia' in each group.

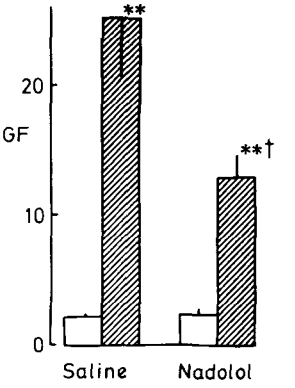

FIG. 2. Effect of nadolol on glycolytic flux (Gf) in non-ischaemic (□) and ischaemic (▨) myocardium. Glycolytic flux was estimated by the ratio of [G6P] plus [F6P] to [FDP]. Data are means ± s.e.m. of 7–8 observations in each group. $^{**}P < 0.01$ compared with non-ischaemia. $†P < 0.05$ compared with saline-treated ischaemia. Saline = saline-treated myocardium.

Table 4. Effect of nadolol on hexose phosphates and lactate level in the ischaemic myocardium.

	G6P	F6P	FDP	Lactate
Saline-treated				
Non-ischaemia	0·206 ± 0·039	0·037 ± 0·009	0·122 ± 0·028	1·617 ± 0·189
Ischaemia	1·347 ± 0·211**	0·319 ± 0·052**	0·068 ± 0·011	12·300 ± 0·660**
Nadolol-treated				
Non-ischaemia	0·131 ± 0·021	0·037 ± 0·004	0·083 ± 0·010	0·959 ± 0·300
Ischaemia	1·098 ± 0·212**	0·273 ± 0·042**	0·111 ± 0·022	9·008 ± 1·042**,†

Data are expressed μmol g^{-1} wet tissue as means ± s.e.m.
$^{**}P < 0.01$ compared with 'Non-ischaemia' in each group.
$†P < 0.05$ compared with 'saline-treated'.

There was a significant difference ($P < 0.05$) in the ratio of [G6P] + [F6P] to [FDP] in the ischaemic myocardium between the saline-treated (25·27 ± 4·80) and nadolol-treated (12·93 ± 1·79) heart.

DISCUSSION

We have examined the metabolic changes in the endocardial portion obtained after 3 min of ischaemia. The reason for this is because the endocardial portion of the myocardium is more vulnerable to ischaemia (Winbury 1975), and because the ischaemic changes occurring within a few minutes after the onset of ischaemia are of importance for the patient with angina pectoris. Anginal attack must occur immediately after the coronary blood flow has been interrupted. In our experiments (Ichihara & Abiko 1975b), the most potent changes in myocardial metabolism were observed in the endocardium 3 min after coronary artery ligation in dogs.

It has been demonstrated that β-adrenoceptor blocking agents, such as propranolol, reduce the abnormalities of ischaemic myocardium (Epstein & Braunwald 1966). We reported that propranolol inhibited myocardial metabolic changes caused by coronary ligation (Ichihara & Abiko 1977), and attenuated myocardial acidosis caused by ischaemia (Ichihara & Abiko 1982a) in the dog. These findings suggest that propranolol protects the myocardium against ischaemic injury. This effect is due largely to its β-adrenoceptor blocking action, by which the heart rate and myocardial contractility are reduced. However, because propranolol has a membrane stabilizing action, it is possible that a direct action on myocardial cell membrane contributes to prolong the viability of ischaemic myocardium. Abiko & Sakai (1980) reported that (+)-propranolol, as well as (−)-propranolol, attenuated myocardial acidosis during ischaemia. Welman (1979) has demonstrated that a direct membrane effect of propranolol may partly be involved in the mechanism by which propranolol salvages ischaemic myocardium. At the present time, it is hard to say how important a direct effect is in the protection of ischaemic myocardium. Although nadolol has no direct effect on the membrane, it preserved the ATP store in the myocardium during ischaemia, leading to a high value of energy charge potential in the ischaemic myocardium (Table 2, Fig. 1). This result by β-adrenoceptor blocking agents confirms that the main mechanism of myocardial protection is β-adrenoceptor blockade.

Usually, oxygen deficiency accelerates the rate of glycolytic flux through activation of PFK to produce ATP anaerobically. Ischaemia, however, inhibits the glycolytic pathway at the level of PFK (Opie 1976; Ichihara & Abiko 1982b). In the ischaemic myocardium where there is no coronary flow, glycolysis may be inhibited to prevent accumulation of the metabolic end products. Weishaar et al (1979) have demonstrated that it is possible to estimate the severity of myocardial injury or energy state of the ischaemic myocardium by studying glycolytic flux through PFK reaction. As the deleterious effect of ischaemia becomes worse, glycolytic flux through PFK is inhibited more severely. Weishaar et al (1979) calculated the ratio of ([G6P] + [F6P])/[FDP] as the index indicating glycolytic flux at the PFK reaction. This ratio does not directly mean the rate of glycolytic flux, but it may reflect the degree of PFK inhibition during ischaemia. When PFK is inhibited by ischaemia, the levels of G6P and F6P increase and the level of FDP decreases. The ratio of ([G6P] + [F6P])/[FDP] increased more than 10 times during ischaemia (Fig. 2). Nadolol significantly ($P < 0.05$) reduced the increase in this ratio being caused by ischaemia. This indicated that nadolol lessened the inhibition of PFK during ischaemia, suggesting a reduction in the deleterious effect of ischaemia. In addition, nadolol inhibited glycogen breakdown and lactate accumulation being caused by ischaemia.

From the foregoing results, it is concluded that nadolol, which differs from propranolol in physicochemical and pharmacological properties, can protect the myocardium against ischaemic injury.

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