Br. J. Pharmacol. (1988), **93**, 723–728

Ventricular noradrenaline concentrations in naïve and morphine-treated rats subjected to acute myocardial ischaemia

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1 Ventricular noradrenaline concentrations in morphine-treated rats subjected to acute left coronary artery ligation were measured by high performance liquid chromatography with electrochemical detection.

2 In naïve rats, acute left coronary artery ligation induced a significant increase in right ventricular noradrenaline concentration at 5 min and significant decreases in left ventricular noradrenaline concentration at 3 and 10 min.

3 Acute morphine treatment did not significantly alter ventricular noradrenaline concentrations in rats subjected to acute coronary artery ligation.

4 Chronic morphine treatment caused significant declines in ventricular noradrenaline concentrations in rats subjected to acute coronary artery ligation. The reductions increased with duration of opiate treatment, and were reversed by opiate withdrawal.

5 These findings indicate that there is an increase in sympathetic activity during acute myocardial ischaemia. It is suggested that chronic morphine treatment may be able to retard this response, and consequently to lessen the occurrence of early ventricular arrhythmias resulting from acute myocardial ischaemia.

Introduction

It has been found in a recent study that rats undergoing chronic morphine-treatment exhibit significantly lower incidence and delayed onset of early ventricular arrhythmias during acute myocardial ischaemia (Chan *et al.*, 1987). However, the mechanism remains unclear.

Evidence has shown that increased sympathetic activity during acute myocardial ischaemia contributes, at least partly, to the production of early ventricular arrhythmias. Early activation of cardiac efferent sympathetic nerves and decreases in cardiac noradrenaline (NA) during experimentally-induced myocardial ischaemia have been demonstrated in animals (Russel *et al.*, 1961; Brown & Malliani, 1971; Mathes & Gudbjarnason, 1971; Gromova, 1977; Bosnjak *et al.*, 1979; Abrahamsson *et al.*, 1981; Abrahamsson *et al.*, 1982b). In addition, chronic cardiac sympathetic denervation, leading to depletion of myocardial NA stores, markedly prevents the development of ventricular fibrillation during myocardial ischaemia (Ebert *et al.*, 1968; Schaal *et al.*, 1969). It has been shown recently that chronic administration of morphine, besides producing tolerance and physical dependence, can also induce certain physiological changes, such as decreased cardiovascular responses to sympathetic nerve stimulation (Leung *et al.*, 1986). Therefore, it is possible that the observed decrease and delay in the development of early ventricular arrhythmias, in morphine-treated rats subjected to acute coronary artery ligation (Chan *et al.*, 1987), may be attributed to the reduced cardiovascular responses to sympathetic excitation.

The present investigation examines the above hypothesis by observing the changes in ventricular NA concentrations in response to acute myocardial ischaemia in naïve as well as in morphine-treated rats.

Methods

Animals

In all experiments, male Sprague Dawley rats were used. They were housed in groups of 4 per cage and

allowed free access to tap water and a standard laboratory diet of rat chow (Ralston Purina, U.S.A.). The animals were kept in an air-conditioned and humidity-controlled room (23 ± 1°C and 60–70% respectively), and were exposed to a 12-h day-night cycle.

Morphine treatment

For acute morphine treatment, rats weighing 400–500 g were injected i.p. with morphine sulphate (Macfarlan Smith, Middlesex) 8 mg kg^{-1}, expressed as the salt, 15 min before coronary artery ligation. Similar volumes (1 ml kg^{-1}) of vehicle, 0.9% w/v NaCl (saline), were given by the same route and at the same time as the controls.

In the chronic study, a previously described method for inducing opiate dependence (Badawy *et al.*, 1982; Chan *et al.*, 1987) was adopted for chronic administration of morphine. Rats, weighing 250–300 g, were used. They were given morphine sulphate in drinking tap water for 2, 3 or 5 weeks so that their weight at the end of the respective treatment periods was 400–500 g. The drug was given in increasing concentrations (48 h apart) of 0.1, 0.2, 0.3 and finally 0.4 mg ml^{-1}. The animals continued to receive the final concentration of morphine sulphate until the end of the respective treatment periods. Control animals received tap water without the opiate for similar treatment durations.

For studying the effects of opiate withdrawal, a group of rats was first given chronic morphine treatment in drinking water for 3 weeks and then tap water without the opiate to drink for 2 weeks before they were used for experiments.

Coronary artery ligation

Acute left coronary artery ligation was carried out in various groups of morphine-treated rats and their controls with a previously described technique (Dai, 1986; Chan *et al.*, 1987).

Under general anaesthesia with pentobarbitone sodium (Abbott) 60 mg kg^{-1} i.p., the rats were subjected to left thoractomy and artificial ventilation (82 strokes min^{-1}, 1 ml 100 g^{-1}) with room air using a respirator (Palmer, U.K.). A 6/0 braided silk suture attached to a 10 mm micro-point reverse cutting needle (Mersilk W812, Ethicon) was placed under the main left coronary artery of the rat heart. The silk ligature was tied after allowing a period of 10 min for equilibrium. Sham-operated rats were subjected to the same experimental procedure except that the ligature was not tied. At 3, 5 or 10 min following coronary artery ligation, the whole heart was rapidly removed, the entire right ventricle and the anterior wall of the left ventricle were dissected out, weighed, instantly frozen and then stored in liquid nitrogen until determination of the tissue NA concentration 10–14 days later.

Measurement of ventricular NA concentration

Ventricular tissue NA concentration was assayed by high performance liquid chromatography (h.p.l.c.) with electrochemical detection according to the method described by Zaczek & Coyle (1982), with slight modifications.

After thawing, the tissue samples were homogenised in 10 volumes (weight/volume) of ice-cold 0.2 N perchloric acid (PCA) (BDH) which contained 0.5 μM 3,4-dihydroxybenzylamine (DHBA) (Sigma) as internal standard. The homogenate was centrifuged at 15,000 *g* at 4°C for 20 min to precipitate protein. Fifty μl of the PCA extract was injected into the h.p.l.c. system.

Separation was achieved with a Bondapac C-18 column (Waters), the mobile phase being acetate buffer (0.08 M, h.p.l.c. grade, Fisher), pH 4.0, containing EDTA (300 μM, Sigma), heptane sulphonate (4.5 mM, Eastman) and acetonitrile (935 : 65, h.p.l.c. grade, Merck). The flow rate was adjusted to 1.0 ml min^{-1}. Quantitative analysis was performed with an electrochemical detector (Bioanalytic System LC-4) with a glassy carbon electrode (detector setting: +0.80 V).

A standard curve, constructed with NA (Sigma) standards ranging from 375 to 1000 ng ml^{-1} and 0.5 μM DHBA as the internal standard, was used to calculate the NA concentration of the ventricular tissue samples.

Statistical analysis

Data are expressed as means ± s.e. mean and analysed by Student's *t* test.

Results

Left coronary artery ligation in naïve rats

Figure 1 illustrates typical chromatograms obtained from PCA extract of rat ventricles at 5 min after acute coronary artery ligation. At a solvent flow rate of 1 ml min^{-1}, NA and the internal standard, DHBA, were eluted at 5 min 30 s and 7 min 20 s, respectively. The chromatogram patterns of ventricle

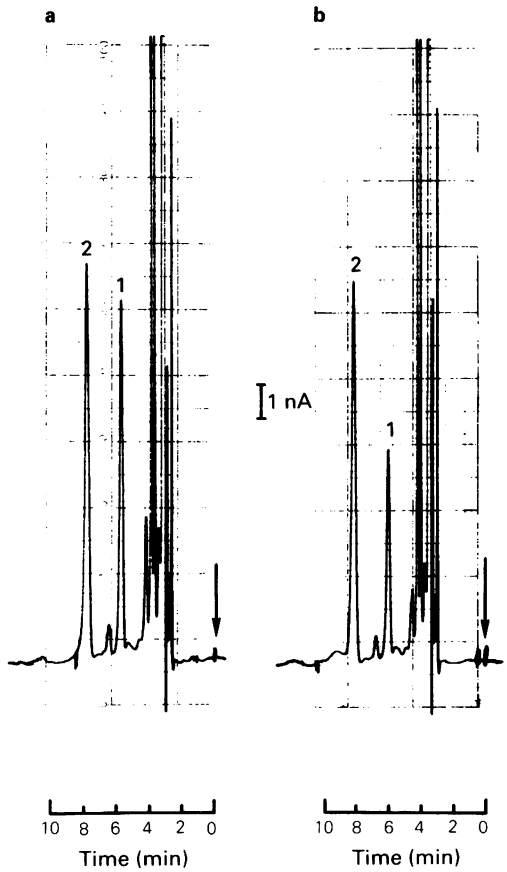

Figure 1 Chromatograms of perchloric acid extract of rat ventricles at 5 min after acute left coronary artery ligation. (a) Right ventricle; (b) left ventricle; 1 = noradrenaline; 2 = DHBA; ↓ = injection.

extracts of the various test groups were essentially similar.

Figure 2 shows the ventricular NA concentrations in pentobarbitone-anaesthetized naïve rats at various periods following acute left coronary artery ligation. In the sham-operated controls, the amine concentrations in the right and left ventricles were not significantly different from each other. Following left coronary artery ligation, NA concentrations of right ventricle became significantly and consistently higher than those of left ventricle ($P < 0.001$ for ali groups). When compared with the corresponding values in the sham-operated controls, NA concentrations of the right ventricle significantly increased at 5 min ($P < 0.001$) and those of the left ventricle significantly decreased at 3 and 10 min ($P < 0.05$ for both) after coronary artery ligation.

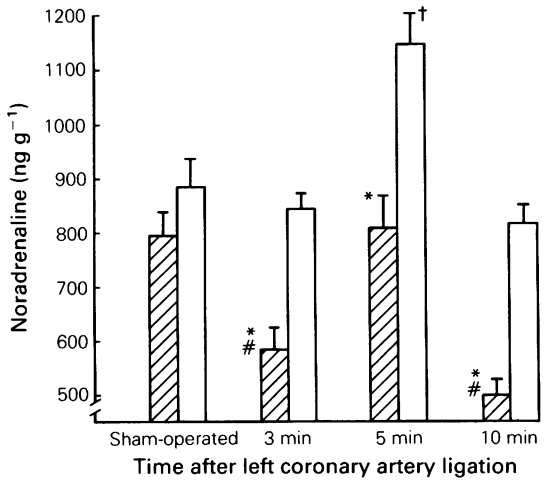

Figure 2 Effects of acute left coronary artery ligation on ventricular noradrenaline concentrations in pentobarbitone-anaesthetized naïve rats. The values plotted are the means and vertical lines indicate s.e. mean. Hatched columns represent left ventricle and open columns, right ventricle. $n = 8$ for each group. $^{*}P < 0.001$ when compared with the right ventricle of the same treatment group. $^{\#}P < 0.05$ when compared with the left ventricle of the sham-operated group. $\dagger P < 0.001$ when compared with the right ventricle of the sham-operated group.

Morphine treatment in sham-operated rats

In rats subjected to sham operation, neither acute nor chronic administration of morphine caused significant changes in either right or left ventricular NA concentrations (Figure 3). The 3- and 5-week morphine-treated groups appeared to have lower

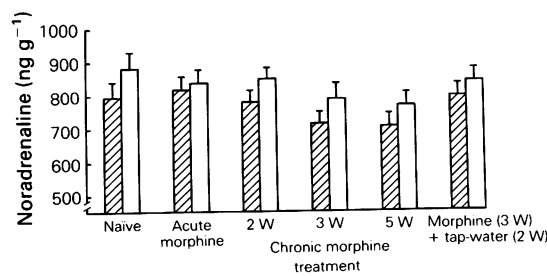

Figure 3 Effects of morphine treatment on ventricular noradrenaline concentrations in sham-operated rats. The values plotted are the means and vertical lines indicate s.e. mean. Hatched columns represent left ventricle and open columns right ventricle; W = weeks. $n = 8$ for each group.

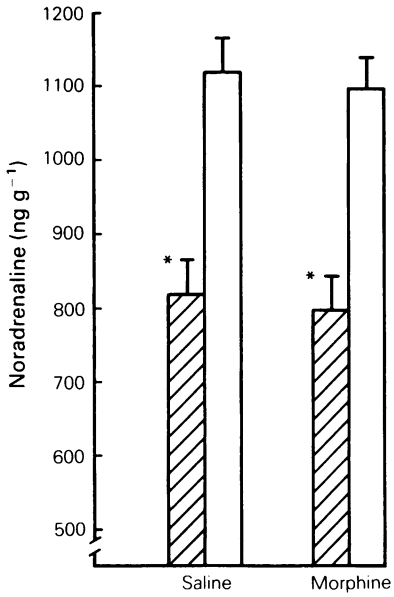

Figure 4 Ventricular noradrenaline concentrations in acute morphine-treated (8 mg kg^{-1}) rats at 5 min after acute left coronary artery ligation. The values plotted are the means and vertical lines indicate s.e. mean. Hatched columns represent left ventricle and open columns, right ventricle. $n = 9$ for each group. $^{*}P < 0.001$ when compared with the right ventricle of the same treatment group.

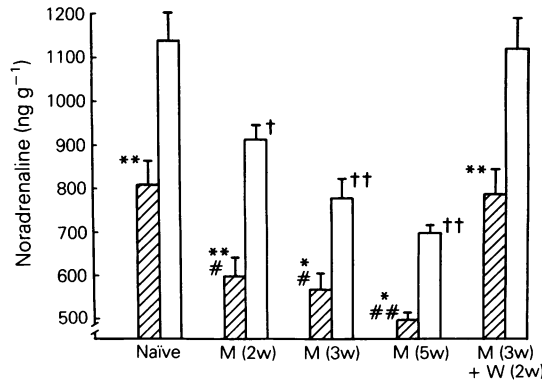

Figure 5 Ventricular noradrenaline concentrations in rats undergoing chronic morphine-treatment at 5 min after acute left coronary artery ligation. The values plotted are the means and vertical lines indicate s.e. mean. Hatched columns represent left ventricle and open columns, right ventricle. M = morphine treatment; W = tap-water treatment; w = weeks. $n = 8$ for each group. $^{*}P < 0.01$; $^{**}P < 0.001$ when compared with the right ventricle of the same treatment group. $^{\dagger}P < 0.01$; $^{\dagger\dagger}P < 0.002$ when compared with the right ventricle of the naïve group. $^{\#}P < 0.01$; $^{\#\#}P < 0.002$ when compared with the left ventricle of the naïve group.

ventricular NA levels, but the differences in comparison with the naïve animals were not statistically significant. The NA concentrations of the right and left ventricles were not significantly different from each other in all the test groups.

Acute morphine treatment and coronary artery ligation

At 5 min following acute left coronary artery ligation, NA concentrations of the left ventricles in both saline- and morphine-treated rats were significantly lower than those of their right ventricles ($P < 0.001$ for both) (Figure 4). Intraperitoneal injection of morphine sulphate 8 mg kg^{-1} did not cause remarkable changes in tissue NA levels. The values of NA concentrations of either the right or left ventricles in morphine-treated animals were not significantly different from those in the saline-treated controls.

Chronic morphine treatment and acute coronary artery ligation

The effects of chronic morphine treatment on the ventricular NA concentration at 5 min after acute left coronary artery ligation are shown in Figure 5. Chronic morphine treatment caused a duration-dependent decline in NA concentrations in both ventricles. Significant decreases were already observed in rats which had received opiate treatment for 2 weeks ($P < 0.01$ for both ventricles). There were further reductions in the 3- ($P < 0.002$ and $P < 0.01$ for right and left ventricles respectively) and 5-week ($P < 0.002$ for both ventricles) morphine-treated animals. At 2 weeks after opiate withdrawal, however, the ventricular NA concentrations in rats previously given 3-week morphine-treatment increased and returned to levels which were not significantly different from those in the naïve group. In all treatment groups, the NA concentrations of the left ventricles were significantly lower than those of the right ventricles at 5 min following acute left coronary artery ligation ($P < 0.01$ for 3- and 5-week morphine-treated groups, $P < 0.001$ for naïve, 2-week morphine-treated and post opiate withdrawal groups).

Discussion

Enhanced sympathetic activity due to central reflexes has been found to occur during acute myocardial ischaemia (Kliks *et al.*, 1975), resulting in an acceler-

ation of the biosynthesis as well as the release of NA (Kopin, 1977). It was found in the present study that, in naïve rats, a significant increase in right ventricular NA concentration was observed only at 5 min after coronary artery ligation. If NA concentration does reflect the degree of sympathetic activity, then the current findings indicate that sympathetic excitation was most pronounced at about 5 min after coronary artery ligation. It was also found in the present investigation that the left ventricular NA concentration progressively decreased at 3 and 10 min following acute coronary ligation. This could be due to a reflex-independent local release of NA in the ischaemic myocardium as a consequence of hypoxia, acidosis and increased extracellular K^+ (Holmgren *et al.*, 1981; Abrahamsson *et al.*, 1982a; Hirche *et al.*, 1985). It may also have resulted from destruction of postganglionic sympathetic nerve terminals due to tissue necrosis. However, at 5 min after coronary artery ligation left ventricular NA concentration increased to a level which was not significantly different from that of the sham-operated controls. This may be attributed to enhanced NA synthesis caused by an increase in sympathetic activity compensating for the NA loss.

Evidence has shown that increased sympathetic activity during acute myocardial ischaemia contributes, at least partly, to the genesis of early ventricular arrhythmias. However, reports on an association between the changes in NA levels and the onset of serious ventricular arrhythmias are conflicting (Hirche *et al.*, 1980; Abrahamsson *et al.*, 1982b; Daugherty *et al.*, 1986). The present study revealed that the increase in sympathetic activity was most pronounced at 5 min after coronary artery ligation, during which severe ventricular arrhythmias most often occur (Dai, 1986; Chan *et al.*, 1987). These findings support the theory that increased sympathetic activity may play a role in the causation of early ventricular arrhythmias during acute myocardial ischaemia and suggest that the onset of ventricular tachycardia and fibrillation may be associated with the changes in myocardial NA concentration in rats.

It was revealed in the present investigation that acute administration of morphine sulphate $8\,mg\,kg^{-1}$ did not significantly alter the ventricular NA concentration in rats subjected either to sham operation or acute myocardial ischaemia. This is not at variance with the findings of Addicks *et al.* (1987), and may explain the past observations that acute morphine treatment failed to affect the occurrence of early ventricular arrhythmias resulting from acute myocardial ischaemia (Addicks *et al.*, 1987; Chan *et al.*, 1987).

Rats receiving chronic morphine-treatment and subjected to acute coronary artery ligation were found in this study to have significantly lower ventricular NA concentrations than naïve animals subjected to the same operation. However, this phenomenon was not seen when the animals were subjected to sham operation. Therefore, it is not unreasonable to infer that chronic morphine treatment affects ventricular NA content only under conditions of acute ischaemia. The degree of NA reduction increases with the duration of chronic opiate treatment and is reversible following opiate withdrawal. If NA concentration indeed reflects sympathetic activity, the present findings indicate that chronic morphine treatment could lessen the intensity of sympathetic excitation in response to acute myocardial ischaemia in a treatment duration-dependent manner. This is in agreement with previous findings that chronically morphine-treated rats have significantly less cardiovascular responses to sympathetic nerve stimulation (Leung *et al.*, 1986). If the increased sympathetic activity during acute myocardial ischaemia is contributory to the production of early ventricular arrhythmias, the current results on ventricular NA concentration may explain the previously observed phenomenon that chronically morphine-treated rats have significantly lower incidence and slower onset of early ventricular arrhythmias following acute coronary artery ligation (Chan *et al.*, 1987). This concept is further supported by the observation that, while after morphine withdrawal the ventricular NA concentration response to ischaemia is similar to that of naïve rats, the incidence and time of onset of arrhythmia in this group were also comparable to that of naïve rats (Ko *et al.*, 1988).

Several endogenous substances such as prostaglandins and thromboxanes (Coker, 1982), cyclic AMP (Podzuweit, 1982), endorphins (Fagbemi *et al.*, 1982) and histamine (Dai, 1986) have also been found to be involved in the genesis of early ventricular arrhythmias resulting from acute myocardial ischaemia. It is possible that chronic opiate administration may induce changes in some of these substances which contribute to the decreased occurrence of early ventricular arrhythmias during acute myocardial ischaemia. Further studies have to be carried out before any firm conclusions can be drawn.

This study was supported by a University of Hong Kong Research Grant and the Wing Lung Bank Medical Research Fund. The author wish to thank Dr Michael Y.P. Chan and Mr Simon T.S. Siu for their invaluable advice on the h.p.l.c. analysis for noradrenaline, Miss S.Y.N. Lee and Mr G.S.K. Man for their technical assistance, Professor C.W. Ogle for his constructive criticism of the manuscript, and Miss Wynne W.L. Lau for typing this manuscript.

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(Received June 10, 1987
Revised August 20, 1987
Accepted November 9, 1987)