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Effects of morphine: an electrophysiological study on guinea-pig papillary muscle

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Abstract—Intracellular microelectrodes were used to study the electrophysiological effects of morphine on guinea-pig papillary muscle. Morphine (5×10^{-4} M) caused a significant decrease in the maximum rate of depolarization. At high concentrations (5×10^{-3} M) morphine induced a decrease in the action potential amplitude and a prolongation of the action potential duration. The administration of naloxone (10^{-7} M) partially antagonized the cardiac electrophysiological effects of morphine. These results suggest that the electrophysiological effects of morphine may be due to an interaction with opioid receptors.

The depression of myocardial function by morphine has been suggested to be due to interaction with myocardial opioid receptors (Riggs et al 1986). Furthermore, the electrophysiological and mechanical effects of opiates on isolated heart muscle could be due to their interaction with myocardial opioid receptors (Helgesen & Refsum 1987). However, it has been demonstrated in different heart muscle preparations from mammalian species, including man, that opiates do not influence cardiac function directly (Nawrath et al 1989).

In order to elucidate the possible mechanisms involved in the cardiac electrophysiological effects of morphine we have investigated the cardiac electrophysiological effects of morphine, in the absence or in the presence of naloxone, on guinea-pig ventricular muscle.

Materials and methods

Experiments were performed on guinea-pig right ventricular papillary muscles. Guinea-pigs, 350–400 g, were stunned by a blow on the head and immediately decapitated. The chest was opened with a midsternal incision and the hearts were rapidly excised. Papillary muscles, 2–3 mm in length and less than 1 mm in diameter, were isolated from the right ventricle. The muscles were pinned to the silastic base of a recording chamber and superfused continuously at a constant rate of 6 mL min^{-1} with Tyrode solution equilibrated with 95% O_2–5% CO_2 at a temperature of 37 °C (pH 7·4). The composition of the Tyrode solution was (mM): NaCl 136·9, KCl 5·0, $MgCl_2$ 1·05, NaH_2PO_4 0·4, $NaHCO_3$ 11·9, $CaCl_2$ 1·8 and glucose 5·0.

Rectangular pulses, 2 ms in duration and twice threshold voltage, delivered through a bipolar silver electrode connected to a multipurpose programmable stimulator (Cibertec CS-20) were used to stimulate the preparation at a rate of 1 Hz. Transmembrane action potentials were recorded using conventional microelectrode techniques. The variables measured were resting membrane potential (RMP), action potential amplitude (APA), duration at 50, 75 and 90% of repolarization (APD50, APD75 and APD90, respectively). The maximal rate of depolarization (V_{max}) of the action potential was obtained by electronic differentiation. The differentiator used had an upper limit of linearity of 1000 V s^{-1} and possessed variable imput filters (3 Hz–30 kHz). Both action potential and V_{max} were displayed on a storage oscilloscope (Tektronix 2230) and the oscilloscope traces were recorded on a plotter (Hewlett–Packard 7457 A).

In all cases, the preparations were allowed to equilibrate for 1 h before exposure to the drugs. To observe drugs effects on preparations, 6–10 action potentials were recorded before and 30 min after cummulative addition of morphine (5×10^{-4}, 10^{-3} and 5×10^{-3} M) or morphine plus naloxone (10^{-7} M) added to the organ bath 30 min before morphine. The drugs were dissolved in reservoirs of gassed Tyrode to obtain the final bath concentrations.

Drugs. The drugs used in this study were: morphine hydrochloride (Alcaliber Laboratories, Spain) and naloxone hydrochloride (Abello Laboratories, Spain).

Statistics. Data from the electrophysiological studies are expressed as mean values ± s.e.m. The data were analysed by analysis of variance and Student's *t*-test. Differences of $P < 0.05$ were considered significant.

Results

Fig. 1 shows the effect of increasing concentrations of morphine in the absence (top traces) and in the presence of naloxone (bottom traces) on the action potential configuration recorded from a papillary muscle driven at 1 Hz. It is apparent that morphine induces a marked lengthening of the action potential duration. Concentration-response curves for morphine in the presence of naloxone were constructed to determine whether naloxone antagonizes the effect of morphine. Naloxone (10^{-7} M) was devoid of effect on the action potential recorded from the guinea-pig papillary muscle. Naloxone partially blocks the lengthening of the action potential duration induced by morphine. Table 1 summarizes the effect of morphine in the absence and in the presence of naloxone on the action potential characteristics. It is clear that morphine influenced several parameters depending on the concentration used: at 5×10^{-4} M there was a statistically significant ($P < 0.001$) decrease in the V_{max}. At 10^{-3} M the V_{max} also was significantly ($P < 0.001$) reduced, as well as the APA. At the highest concentration used (5×10^{-3} M) morphine also significantly reduced the amplitude of the action potential and significantly ($P < 0.001$) increased the action potential duration (APD50, APD75 and APD90). At this high concentration we observed a significant ($P < 0.001$) decrease of the V_{max} (from 244·28 ± 6·3 to 64·32 ± 4·3 V s^{-1}).

In the presence of naloxone (10^{-7} M), morphine (5×10^{-4} M and 10^{-3} M) did not modify the characteristics of the action potential when compared with the control values. However, 5×10^{-3} M morphine significantly decreased the V_{max} and also increased the duration of action potential (APD75 and APD90) without modifying the amplitude of the action potential (APA). Nevertheless, the decrease of V_{max} induced by the highest concentration of morphine in the presence of naloxone was significantly lower than that obtained with morphine alone. Furthermore, the effect of morphine on the duration of the action potential was less marked in the presence of naloxone (Table 1).

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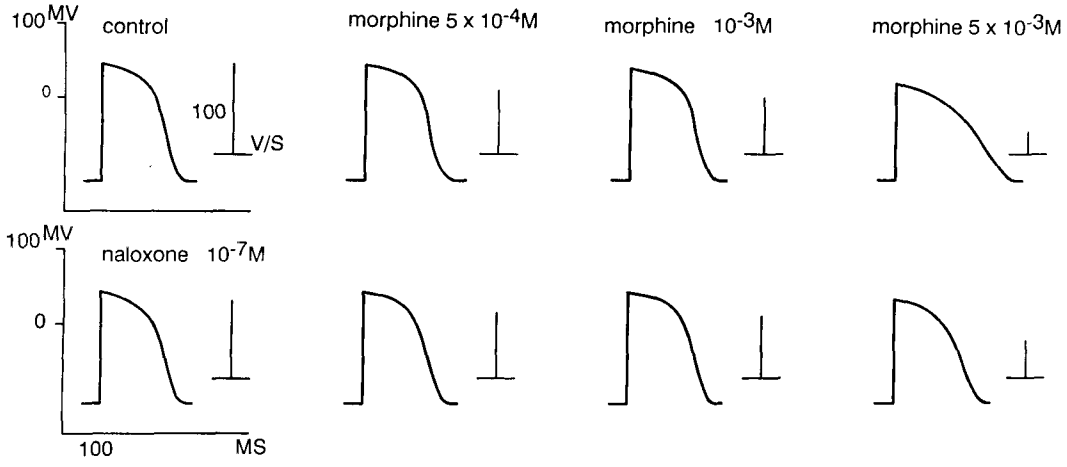

FIG. 1. Effect of morphine in the absence (top traces) and in the presence of naloxone 10^{-7} M (bottom traces) on the action potential configuration of ventricular papillary muscles. n = 6 for each set of experiments.

Table 1. Effects of morphine, in the absence and in the presence of naloxone, on the action potential characteristics of guinea-pig ventricular muscle.

Morphine (M)	Naloxone (M)	RP (mV)	V_{max} ($V\ s^{-1}$)	APA (mV)	APD50 (ms)	APD75 (ms)	APD90 (ms)
		112·8±0·7	244·2±6·3	156·8±2·8	173·9±9·5	190·8±9·9	198·4±10
5×10^{-4}	—	113·9±1·4	169·9±2·7***	152·5±2·1	170·4±7·2	190·8±9·4	199·9±10
10^{-3}	—	114·2±2·6	139·9±10·5***	149·4±1·8*	171·4±7·4	192·8±10	204·5±10
5×10^{-3}	—	110·8±3·0	64·32±4·3***	131·1±1·8*	218·3±11·6***	257·1±9·6***	289·2±8·0***
5×10^{-4}	10^{-7}	115·4±2·7	177·1±10·1	151·9±3·2	168·8±8·5	195·4±9·5	203·5±10·7
10^{-3}	10^{-7}	115·1±2·1	167·1±6·8	149·7±3·7	168·8±7·6	193·3±10	203·5±12·5
5×10^{-3}	10^{-7}	113·9±2·4	97·1±14·1*	147·4±4·0	177·5±8·2	208·1±9·7***	229·8±7·2***

Data are presented as means±s.e.m. n = 6 for each group of experiments; *$P<0.05$; ***$P<0.001$ vs control. RP = resting potential; APA = action potential amplitude; APD = action potential duration measured at 50% (APD50), 75% (APD75) and 90% (APD90) of repolarization.

Discussion

Our results demonstrate that morphine exerts electrophysiological effects on ventricular muscle. The main effect of morphine was an increase in action potential duration and a decrease in V_{max}. These effects were partially blocked by naloxone. It is possible that the effects of morphine may be related or secondary to its opioid receptor occupancy.

The depressant effects of V_{max} and action potential amplitude induced by morphine in our results, are qualitatively similar to those reported for buprenorphine on sheep Purkinje fibres and rat papillary muscle and may be due to a blockade of fast Na^+ channels, the opening of which are responsible for the upstroke of action potential in ventricular tissue (Boachie-Ansah et al 1989). Moreover, it has been demonstrated (Saini et al 1989) that fentanyl (a selective μ-agonist) increases cardiac electrical stability. DeSilva et al (1978) described a reduction in ventricular vulnerability by morphine. These studies suggest that opiates exert significant effects on cardiac electrical properties.

The mechanism underlying the action potential prolongation induced by morphine in guinea-pig papillary muscle, remains unknown. The fact that this effect was more pronounced on terminal repolarization suggests a possible block of delayed outward K^+ conductance, but further studies are required to provide a definitive answer. This effect is similar to those obtained for meptazinol and naloxone (Fagbemi et al 1983; Cerbai et al 1989).

The cardiac electrophysiological effects of morphine shown in this study (depression of V_{max} and prolongation of the action potential duration) are in contrast with those of other authors (Helgesen & Refsum 1987; Nawrath et al 1989) who reported that morphine did not produce changes of the intracellulary recorded action potential in ventricular heart muscle of guinea-pig, rabbit, rat or man. This discrepancy could be related to the different doses used. The concentrations of morphine used in those studies were lower than those in our experiments, as we used maximal doses of morphine (5×10^{-3} M) in order to obtain a full effect of this opiate.

On the other hand, the electrophysiological effects induced by morphine were similar to those described for antiarrhythmic drugs. Thus, the ability to block fast Na^+ channels (leading to depression of V_{max}), is thought to be essential for the antiarrhythmic drugs such as lignocaine (Carmeliet 1984). Similarly, prolongation of the duration of the cardiac action potential is thought to be the basis of antiarrhythmic drugs like quinidine (Campbell 1989).

It remains to be resolved, however, whether the observed antiarrhythmic and cardiac electrophysiological effects of morphine are indeed mediated via specific opioid receptor activation. The high concentrations of morphine required to modify cardiac action potential characteristics could be used to argue against the possible involvement of opioid receptor activation. However, a low concentration of naloxone partially antagonized the cardiac electrophysiological actions of morphine. Thus, it is possible that the electrophysiological actions of morphine were mediated via opioid receptors; μ-opioid receptors have been detected in the heart muscle (Gautret & Schmitt 1984).

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Inhibition by diltiazem of left ventricle collagen proliferation during renovascular hypertension development in rats

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Abstract—Diltiazem administered in drinking water (0·7 mg mL^{-1}) to Goldblatt two kidney-one clip rats over 16 weeks did not prevent the development of hypertension and left ventricular hypertrophy (LVH). When the collagen content of the left ventricles was assayed (as hydroxyproline), it was found that the fibrosis, characteristic of LVH, was inhibited by diltiazem treatment, despite the fact that hypertension and LVH had developed. This study provides some indirect evidence for the notion that the collagen and myocyte compartments of the myocardium are under separate influences during LVH development in renovascular hypertension.

It seems likely that the altered mechanical properties of the hypertrophied myocardium in hypertension and the subsequent development of heart failure are due, at least in part, to a progressive perivascular and interstitial fibrosis (Weber et al 1990). Several laboratory and clinical studies have shown that antihypertensive treatment with the calcium antagonist diltiazem can induce regression of established left ventricular hypertrophy (LVH) (Grellet et al 1988; Szlachcic et al 1989). Furthermore, it has been reported recently that LVH regression with diltiazem in a salt model of hypertension involves parallel regression of the myocyte and collagen compartments of the myocardium (Baxter 1991).

Brilla et al (1990) have suggested that during LVH development, myocytes and fibroblasts are under separate haemodynamic and humoral controls. If this is the case, it may be possible to modify the response of each compartment to hypertension. In the present study, the effects of diltiazem treatment on hypertrophy development were investigated in a rat model of renovascular hypertension (the Goldblatt two kidney-one clip model) (Robertson et al 1986). The influence of this treatment on blood pressure elevation, left ventricle mass and left ventricle collagen content (assayed as hydroxyproline) was assessed.

Materials and methods

Animals. Renovascular hypertension was induced in male Sprague-Dawley rats, 6–8 weeks old, by placing a silver clip (internal gap width, 0·2 mm) on the left renal artery. Sham operated animals underwent the same surgical procedure but no clip was placed on the renal artery.

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Drug treatment. Two days after surgery, a sham operated (i.e. normotensive) group and a renal artery-clipped group were assigned to receive diltiazem hydrochloride (Sigma, Poole, Dorset, UK) in distilled water as drinking fluid. The concentration of the drug was increased gradually over one week to 0·7 mg mL^{-1} and maintained at this concentration for a further 15 weeks. Fluid intake was monitored daily and the dose taken by each animal was estimated. During the first three weeks after the concentration was increased to 0·7 mg mL^{-1}, the mean dose ($\pm$s.e.m.) was 89 ± 3 mg kg^{-1} day^{-1} and during the final three weeks it was 72 ± 3 mg kg^{-1} day^{-1}. Corresponding normotensive and clipped control groups received distilled water as drinking fluid.

Blood pressure measurement. Systolic blood pressure was measured by tail-cuff plethysmography (PE-300 programmed electrosphygmomanometer; Narco Biosystems, Houston, TX) after 15 min pre-warming at 37°C.

Tissue processing and hydroxyproline assay. Sixteen weeks after surgery, hearts were excised under pentobarbitone sodium anaesthesia. Each heart was dissected into right ventricle free wall (right ventricle) and left ventricle free wall plus interventricular septum (left ventricle). The tissues were washed, blotted and weighed to give wet weight and then dried to constant weight at 45°C to give the dry weight and water content. The left ventricle samples were hydrolysed by heating with 5 M HCl for 3 h at 130°C in sealed Pyrex ampoules. The resulting tissue digests were neutralized with NaOH, filtered and diluted. Each solution was assayed in duplicate for hydroxyproline by a modified method of Woessner (1961). In brief, after oxidation with chloramine-T, the solution was treated with *p*-dimethylaminobenzaldehyde and the absorbance of the chromophore was measured at 557 nm. The concentration of hydroxyproline in the left ventricle samples (mg g^{-1} dry weight) was calculated.

Differences between group means were evaluated using Student's unpaired *t*-test with Dunnett's modification for multiple comparisons, and they were considered significant when $P < 0.05$.

Results and discussion

The data are presented in Table 1. The large oral dose of