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Myocardial Opiate Receptors

M. E. SAXON, G. R. IVANITSKY, F. F. BELOYARTSEV, V. G. SAFRONOVA, Yu. M. KOKOZ and A. A. FREYDIN

Institute of Biological Physics, Academy of Sciences of the USSR, 142292 Pushchino, Moscow Region, USSR

Abstract. The effect of morphine and met-enkephaline on the isometric contraction of rabbit papillary muscles was studied. The tested compounds produced a dose-dependent, stereospecific and naloxon-reversible negative inotropic effect indicating the existence of opiate receptors in cardiac muscle. A pretreatment of papillary muscles with the β-blocking agent atenolol attenuated the negative inotropic action of morphine and met-enkephaline suggesting a presynaptic localization of cardiac opiate receptors.

Key words: Opiate receptor — Cardiac muscle — Contraction — Opiate and opioid peptide — Naloxone — Dextrorphane

Introduction

The opiate receptors and their endogenous ligands, enkephalins and β-endorphin, have already been identified in the central and peripheral nervous systems of many animal species (Kosterlitz and Huges 1978; Kosterlitz 1980). The presence of one of these opiod peptides, met-enkephaline, has been also detected in mammalian myocardial tissue (Hughes et al. 1977). However, specific opiate receptors have not yet been identified in the previous studies of the effect of morphine on different cardiac preparations. (Carnie et al. 1961; Kennedy and West 1967; Kosterlitz and Taylor 1959; Montel and Starke 1973; Hughes et al. 1977; De Silva et al. 1978). In the present work the question concerning myocardial opiate receptors has been approached by studying the inotropic effects of morphine and met-enkephaline on a more simple bioassay system — rabbit papillary muscle.

Methods

Adult New Zealand rabbits of both sexes weighing 2.0 to 3.0 kg were used for the experiments. Papillary muscles, 0.7—1.0 mm in diameter, were rapidly excised from the right ventricle and placed into a bath designed by the "chamber method" of stimulation (Kamiyama and Matsuda 1966; Saxon et al. 1981). Tyrode solution of the following composition was used (in mmol/l): Na^+ 150.8, K^+ 4.0, Cl^- 148.4, Ca^{2+} 2.7, Mg 1.0, HCO_3^- 12, $H_2PO_4^-$ 1.8, glucose 10; pH 7.4; $t = 36 \pm 1$ °C. This solution was gassed with 4% CO_2 + 96% O_2. The preparation was stimulated by 3 ms 2.0-threshold square pulses

applied through Ag-AgCl electrodes placed into both chambers of the bath. The muscles were equilibrated for 1h before the experiments started at 1 Hz driving rate.

The transmembrane potential was recorded differentially between intracellular and extracellular microelectrodes using standard electrophysiological technique. The signals were led through a high impedance unity gain amplifier, displayed on an oscilliscope screen and photographed on film.

The isometric tension was measured by a transducer (6M×2B, USSR) and recorded on an oscillograph. The resting tension was adjusted to 0.3 g.

The drugs used were: morphine sulfate, methionine-enkephaline (Serva), atenolol hydrochloride (Merck), dextrorphane tartrate (FRG). Fresh solution of the above compounds were applied. All drugs were dissolved in distilled water. Morphine or met-enkephaline was added to the bathing medium at cumulative concentrations. The effect of naloxone was tested after a 30 min exposure to the narcotic or the peptide. The results shown in the text and the figures are expressed as mean values ± SEM. *P* values of 0.05 or less were considered to be significant.

Results

The administration of morphine or met-enkephaline induced a dose-dependent decrease of contractility in rabbit papillary muscles. The inhibition of the mechani-

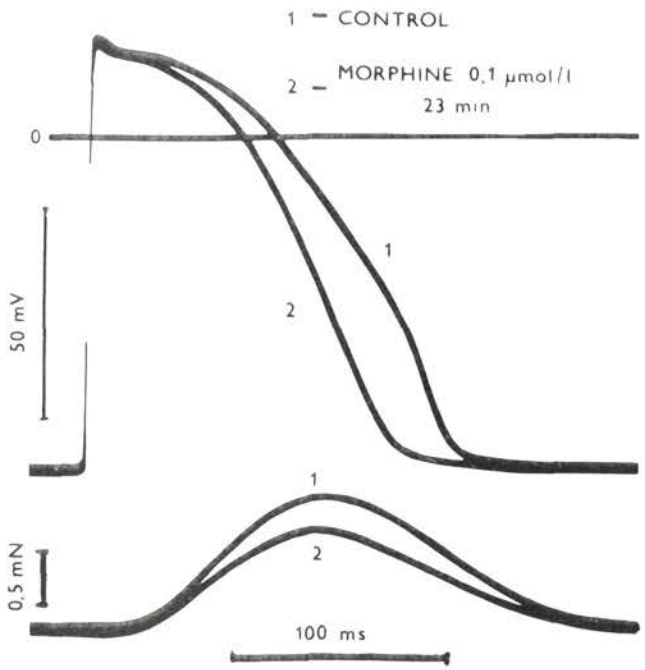

Fig. 1. Example of morphine cardiodepressive action. The superposition of the action potentials and isometric contractions registered in rabbit papillary muscle before (1) and after (2) morphine exposure for 23 min. Stimulation 1 Hz.

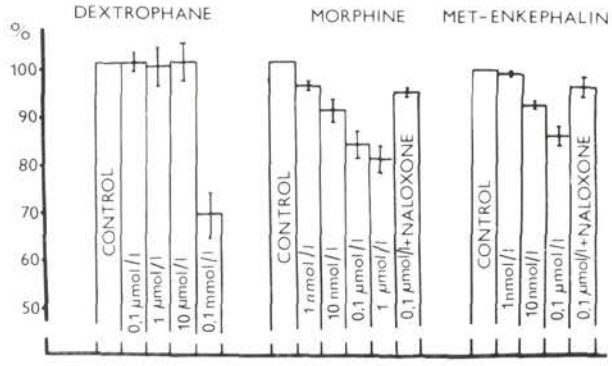

Fig. 2. Dose-dependent negative inotropic effect of dextrorphane, morphine and met-enkephaline on electrically-driven rabbit papillary muscles and the antagonism of naloxone. The data are expressed as per cent of control values of the isometric contraction amplitude. Bar represents ± SD, n = 7.

cal activity was accompanied by shortening of the action potential duration. Fig. 1 presents an instance of such a cardiodepressive effect of morphine. Identical results were obtained with the peptide. Fig. 2 demonstrates a dose dependence of the negative inotropic action of the opiate and opioid peptide.

It is interesting to note, that the papillary muscles showed a greater depressive response to morphine than to met-enkephaline. The inhibitory effect of both compounds with peaks 15—20 min after the administration was reversible. The time dependence of the negative inotropic effect of morphine and met-enkephaline is shown in Fig. 3.

It should be emphasized that a statistically significant inhibitory effect was observed at low concentrations of morphine (10^{-9} mol/l) and met-enkephaline (10^{-8} mol/l), whereas even a 1000 fold higher concentration of nonactive (+)− enantiomer dextrorphane was without any effect (Fig. 2). In order to determine whether the cardiodepressive action of morphine and met-enkephaline is due to the activation of opiate receptors, the effect of naloxone, the specific opiate antagonist, was tested. Naloxone, at a concentration uneffective per se ($10^{-7} - 10^{-6}$ mol/l), restored the amplitude of the isometric contraction altered by morphine or met-enkephaline (Fig. 2, 3). The antagonistic effect of naloxone was partial (Fig. 2A, B) or complete in different preparations and was observed 15 to 20 min after the administration.

The inhibitory influences associated with opiate and opioid were not antagonized by atropine (at 10^{-6} mol/l) or phentolamine (at 10^{-6} mol/l).

To localize the pre- or post-synaptic effect of narcotics, the β-blocking agent, atenolol, was used. As seen in Fig. 4, 10^{-6} mol/l atenolol has its own negative inotropic effect. Besides atenolol markedly reduces the inhibitory effect of

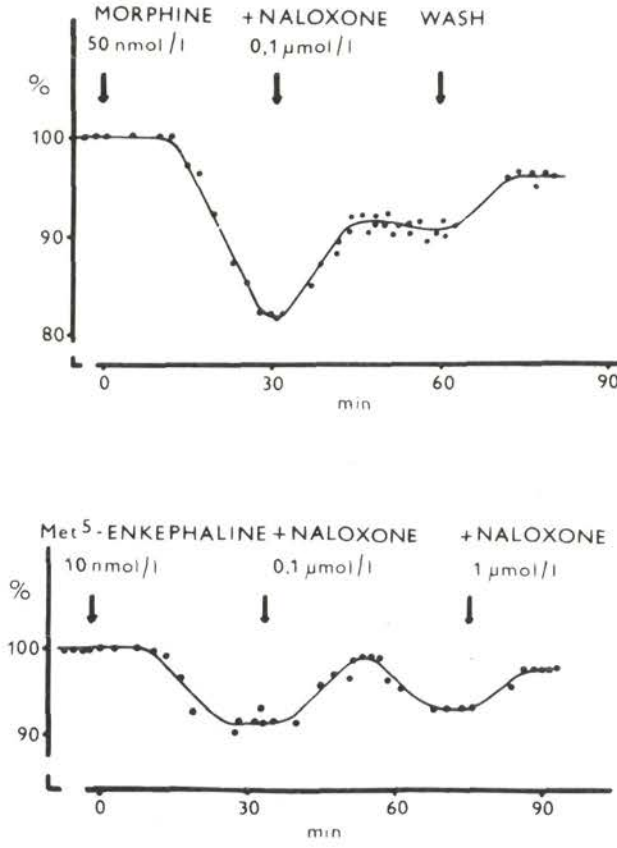

Fig. 3. Time-dependent inotropic effects of morphine and met-enkephaline and subsequent administration of naloxone on rabbit papillary muscles. Stimulation — 1 Hz.

morphine. Similar results were found with met-enkephaline in the presence of atenolol.

Discussion

Morphine and met-enkephaline produce a stereospecific, naloxone-reversible negative inotropic action suggesting the existence of specific opiate receptors in rabbit cardiac muscle. This pharmacological conclusion was recently substantially supported by Burnie (1981). The author demonstrated the stereospecific displacement of ^{3}H-diprenorphine from the binding sites of rat cardiac membranes by levorphanol.

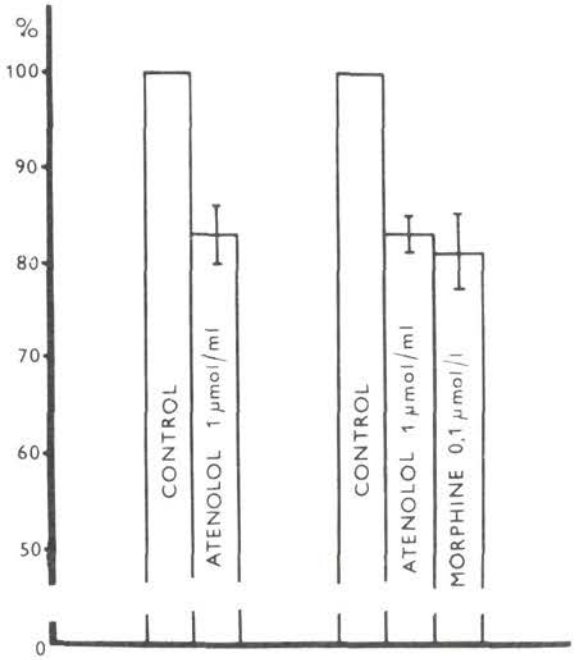

Fig. 4. Protective action of β-blocking agent atenolol against morphine negative inotropy in rabbit papillary muscles. A. The own negative inotropic effect of atenolol at 10^{-6} mol/l, 30 min (n = 5) B. (10^{-7} mol/l) morphine, added consequently after atenolol failed to produce a marked inhibitory action (n = 5). Stimulation — 1 Hz.

In rabbit papillary muscle morphine was found to be more potent (about 10 times) than met-enkephaline. This suggest that the effect of these compounds is mediated by currently postulated μ-receptors. However further biochemical evidence is required to identify the subtype of the myocardial opiate receptors.

The protective action of the β-blocking agent atenolol found in our experiments indicates a presynaptic localization of the opiate receptors in the rabbit heart and suggested an indirect effect of morphine and met-enkephaline on myocardial contractility.

These findings correlate well with the viewpoint that morphinomimetic agents depress the sympathetic transmission through activation of presynaptic opiate receptors which inhibit the noradrenaline release at different levels of the nervous system (Taube et al. 1976; Gothert and Wehking 1980).

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