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Contrasting cardiovascular properties of the μ-opioid agonists morphine and methadone in the rat

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Morphine sulphate (PubChem CID: 5464280)
Methadone hydrochloride (PubChem CID: 4095)
Mepyramine maleate (PubChem CID: 6039)
Cimetidine (PubChem CID: 50963)
Endothelin-1 (PubChem CID: 44284481)
Naloxone hydrochloride (PubChem CID: 5464092)
Nω-nitro-l-arginine (PubChem CID: 440005)
Indomethacin (PubChem CID: 3715)
Nifedipine (PubChem CID: 4485)

ABSTRACT

Morphine and methadone share the property of μ-opioid receptor agonism yet have markedly different cardiovascular actions suggesting additional properties are at play. We investigated the i.v. dose–response relationships of the opioids on cardiovascular metameters in anaesthetised rats in the absence or presence of H_1- and H_2-receptor antagonism and the μ-opioid antagonist naloxone. *In vitro* tissue assays were employed to define more clearly cardiac and vascular mechanisms of action. Morphine (9, 30, 90 mg/kg i.v.) decreased heart rate (HR) and mean arterial pressure (MAP) – responses that were blocked by naloxone pretreatment (10 mg/kg i.v.). In contrast, methadone (3, 10, 30 mg/kg i.v.) caused dramatic short-lived (1–3 min) bradycardia, hypotension and lengthening of the QT interval before stabilising 5 min after i.v. dosing. Only the steady-state responses of HR and MAP were blocked by naloxone. Mepyramine (10 mg/kg i.v.) and cimetidine (100 mg/kg i.v.) also blocked the naloxone-sensitive components. In isolated small mesenteric arteries precontracted by K^+ 62 mM or endothelin-1, methadone (1–30 μM) relaxed vessels while morphine (1–100 μM) had no effect. Pretreatment with naloxone (10 μM), indomethacin (30 μM) or nitro-L-arginine (100 μM) did not affect the relaxation to methadone. In rat isolated left atria, morphine and methadone inhibited inotropic responses at high concentrations (100 μM). In rat papillary muscle and right atria, methadone was more than 30 times more potent at lengthening the refractory period and slowing the atrial rate than morphine. We conclude that methadone is a potent vasodilator agent, possibly through blocking L-type calcium channels.

1. Introduction

Morphine and methadone are μ-opioid receptor agonists. The μ-opioid receptors are subclassified into μ_1 and μ_2 for high and low affinity sites, respectively (Pasternak and Wood, 1986; Thompson et al., 1993; Wolozin and Pasternak, 1981). A third μ_3-subtype has been postulated to occur on endothelial cells to release nitric oxide (NO) that is sensitive to the μ-opioid receptor antagonist naloxone (Stefano et al., 1995). The opioid agonists cause varying degrees of hypotension and bradycardia caused by CNS actions, and direct and indirect actions on the heart and vasculature.

A major concern for the use of methadone to suppress the physical dependence in the heroin or morphine addict has been its association with potentially fatal ventricular arrhythmias, Torsades

Abbreviations: ECG, electrocardiogram; HR, heart rate; KPSS, isotonic potassium physiological salt solution; MAP, mean arterial pressure; NO, nitric oxide; NOS, nitric oxide synthase; pEC_{50}, the negative $\log_{10}$ of agonist concentration that caused 50% of the maximum response; PSS, physiological salt solution; QTC, QT interval of electrocardiogram corrected for heart rate

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de Pointes (TdP). This arrhythmia is caused by blockade of the rapid-rectifying K^+ channel that is encoded by the human ether-a-go-go-related gene, the hERG channel (Katchman et al., 2002). This channel is crucially responsible for the repolarization of the cardiac action potential (Zhou et al., 1998).

In this study, the dose-related hypotensive, bradycardic and ECG actions of morphine and methadone in anaesthetised rats were investigated. These opioid agonists were tested in the absence or presence of the μ-opioid antagonist naloxone or H_1- and H_2-histamine receptor antagonists to determine the role of μ-opioid receptors and potential for histamine release *in vivo*. To analyse the direct actions of these opioids, their activity was tested in isolated vascular rings of mesenteric small arteries, papillary and right and left atrial muscle.

These *in vitro* assays provided a reference for the likely concentration-dependent actions *in vivo*. Methadone was consistently 30 fold more potent than morphine in slowing the atrial rate and increasing the refractory period, accounting for the dramatic acute fall in heart rate and increase in QTc *in vivo* that were not sensitive to the μ-opioid receptor antagonist naloxone. The work supports the notion that methadone has significant L-type calcium channel blocking activity – an action not shared with morphine.

2. Materials and methods

Sprague Dawley rats (male; 285 ± 3 g) were used. Experiments were approved by the University of Melbourne Animal Ethics Committee in accordance with the *Australian code for the care and use of animals for scientific purposes* (8th edition, 2013, National Health and Medical Research Council, Australian Government, Canberra).

2.1. In vivo experiments

Rats were initially lightly anaesthetised with 5% halothane (Veterinary Companies of Australia; Artarmon, NSW, Australia) in O_2 and air, then surgical anaesthesia was induced with pentobarbitone (60 mg/kg i.p.). Atropine (1 mg/kg s.c.) was given to inhibit bronchial secretions. After infiltration with a long-acting local anaesthetic (0.5% ropivacaine HCl; Naropin, AstraZeneca, Sydney, Australia), a tracheotomy was performed for mechanical ventilation (O_2 and room air; initial settings 6.5 ml/kg stroke volume and 75 breaths/min; Rodent ventilator 7025, Ugo Basile, Comerio, Italy) and a carotid artery cannulated for the measurement of phasic and mean arterial pressure (MAP) via a calibrated blood pressure transducer (Cobe, Argon Medical, Athens, TX, USA) connected to a Powerlab data acquisition system (8SP; ADInstruments, Sydney, Australia). Heart rate (HR) was computed from phasic blood pressure. The right jugular vein was cannulated for drug administration. Electrocardiogram (ECG) leads (lead II), connected to a BIO Amp (ML136; ADInstruments), were inserted under the skin; parameters were analysed using the Chart ECG Analysis Module (v2.0; ADInstruments). Regular arterial blood gas analyses were performed (ABL5, Radiometer Medical A/S, Copenhagen, Denmark) to monitor blood pH (7.43 ± 0.01), CO_2 and O_2 levels prior to the start of the experiment; optimum blood gas parameters were maintained by adjusting ventilator stroke volume and rate. Body temperature was maintained at 38 °C by a homoeothermic blanket with a rectal probe (Harvard Apparatus, Holliston, MA, USA). Stable cardiovascular parameters and anaesthetic state were maintained for 10 min prior to the start of the 62 min experiment protocol. Thereafter, the anaesthetic agent (s) was not readministered to obviate its effects on cardiovascular variables (depth of anaesthesia was adequate for the typically 72 min experimental period).

2.1.1. In vivo protocols

2.1.1.1. Effects of μ-opioid agonist alone. Rats were injected (i.v.) with 3 volumes of saline (each 2.5 ml/kg) or cumulative doses of morphine (9, 30 and 90 mg/kg) or methadone (3, 10 and 30 mg/kg) at 21 min intervals. This allowed sufficient time for MAP and HR to stabilise after each dose, according to observations from pilot studies. Since the plasma half-life for morphine is 1.5–2 h and for methadone more than 20 h, doses were given in a cumulative manner; doses shown are the total in each case. To control for effects of bolus volumes, all drug doses were standardized to 2.5 ml/kg as an i.v. infusion over 3.5 min.

2.1.1.2. Effects of μ-opioid receptor antagonism. Rats were pre-treated with the μ-opioid receptor antagonist naloxone (10 or 20 mg/kg i.p.) 20 min prior to the administration of the 3 doses of saline, morphine or methadone, as described above.

2.1.1.3. Effects of histamine $H_1 \pm H_2$ receptor antagonism. Rats were pre-treated with the histamine H_1-receptor antagonist mepyramine (10 mg/kg i.p.) or with the histamine H_1- and H_2-receptor antagonists mepyramine and cimetidine (10 and 100 mg/kg, respectively, i.p.) 20 min prior to the administration of the 3 doses of saline, morphine or methadone, as above. These doses of mepyramine and cimetidine were shown to inhibit responses to histamine (10–100 μg/kg i.v.).

2.1.1.4. ECG analyses. During the protocols described above, the ECGs of 4 consecutive heartbeats were acquired and averaged at each time point. The QT interval – time (ms) between the start of the QRS complex and the end of the T wave – was measured. Since the QT interval is highly dependent on the heart rate (RR interval), it must be corrected, giving the QTc interval. For this purpose, we used the formula of Fridericia ($QTc = RR \cdot QT^{-0.33}$) (Fridericia, 1920), considered to be the more suitable choice than the Bazett correction (EMEA, 2006).

2.2. In vitro experiments

Male Sprague Dawley rats were anaesthetised with 5% halothane in O_2 and killed by decapitation. The heart and a loop of intestine with mesenteric arteries attached were immediately excised and placed in cold physiological salt solution (PSS) of the following composition (in mM): NaCl 119; KCl 4.69; $MgSO_4$ 1.17; KH_2PO_4 1.18; glucose 11 (5.5 for arteries); $NaHCO_3$ 25; EDTA 0.026; and $CaCl_2$ 2.5. The PSS was continuously saturated with carbogen (95% O_2; 5% CO_2) at pH 7.4.

2.2.1. Rat mesenteric arteries

Rat second or third order small mesenteric arteries were carefully dissected from surrounding tissues under a microscope; the endothelium remained intact. Each vessel segment (2 mm long) was mounted on 40 μm diameter wires in an isometric myograph (Danish Myo Technology, Aarhus, Denmark) containing oxygenated PSS at 37 °C. One wire was connected to a force transducer and the other to a micrometre; force was recorded on a Powerlab data acquisition system. From a computer-fitted curve, parameters were determined to stretch each vessel to $0.9L_{100}$, where L_{100} is the circumference of the artery when distended at a transmural pressure of 100 mmHg (Angus and Wright, 2000; Mulvany and Halpern, 1977). After an equilibration period of 20 min, each vessel was exposed to potassium-depolarising solution (KPSS; PSS with an equimolar substitution of KCl for NaCl; K^+ 124 mM) for 2 min to obtain a reference maximum contraction (100% KPSS tone). The myographs were then washed out and vessels pre-contracted with endothelin-1 (1–3 nM) to approximately 70–100% KPSS tone. In another series of experiments,