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Original article

Objective markers of the analgesic response to morphine in experimental pain research

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ABSTRACT

Introduction: In experimental pain research the effect of opioids is normally assessed by verbal subjective response to analgesia. However, as many confounders in pain assessment exist, objective bed-side assessment of the effect is highly warranted. Therefore, we aimed to assess the effect of morphine on three objective pharmacodynamic markers (pupil diameter, prolactin concentration and resting electroencephalography (EEG)) and compare the changes from placebo with subjective analgesia on experimental muscle pain for convergent validation.

Methods: Fifteen healthy male participants received placebo or 30 mg rectal morphine at two separate sessions. At baseline and several time points after drug administration, the central effects of morphine were assessed by experimental muscle pain, pupil diameter, prolactin concentration and resting EEG.

Results: Morphine increased tolerance to muscle pain, together with significant reductions in pupil diameter and increase in prolactin concentration (all $P < 0.001$). Miosis was induced simultaneously with the onset of analgesic effect 30 min after dosing, while a significant increase in prolactin concentration was seen after 45 min. The change in pupil diameter was negatively correlated to change in tolerated muscle pressure ($r = -0.40$, $P < 0.001$), whereas the increase in prolactin concentration was positively correlated ($r = 0.32$, $P = 0.001$). The effect of morphine on EEG was seen as a decrease in the relative theta (4–7.5 Hz) activity ($P = 0.03$), but was not significant until 120 min after dosing and did not correlate to the increase in tolerated muscle pressure ($r = -0.1$, $P = 0.43$).

Discussion: Prolactin concentration and pupil diameter showed similar temporal development, had good dynamic ranges and were sensitive to morphine. Thus, both measures proved to be sensitive measures of morphine effects. EEG may give additive information on the brain's response to pain, however more advanced analysis may be necessary. We therefore recommend using pupil diameter in studies where a simple and reliable objective measure of the morphine-induced central activation is needed.

1. Introduction

Opioids, typified by morphine, are potent analgesics producing analgesia via receptors primarily in the central nervous system (CNS). The analgesic effect can be assessed in animals and in humans with experimental pain models, which have the advantage that stimulus intensity, modality and duration can be controlled (Olesen, Andresen, Staahl, & Drewes, 2012). The evaluation of analgesic response has traditionally been based on the use of a subjective assessment quantified via e.g., visual analog scales (VAS) (Olesen et al., 2012). Subjective pain assessments can be combined with objective methods to acquire detailed information regarding the pain pathways or the central effect of opioids. Several such measures are available, however no previous studies have assessed them in combination and it is not yet known which is the most

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sensitive to monitor the effects of opioids. Following a sufficient dose of an opioid drug, pupillary miosis is induced and pupil diameter has therefore been a frequently used objective index of CNS effects of opioids in humans (Macleod et al., 2012; Matouskova, Slanar, Chytil, & Perlik, 2011; Setnik, Sommerville, Goli, Han, & Webster, 2013; Skarke, Darimont, Schmidt, Geisslinger, & Lotsch, 2003; Slanar, Nobilis, Kvetina, Idle, & Perlik, 2006; Stoops, Glaser, & Rush, 2013; Weinhold & Bigelow, 1993). Thus, one objective approach is to use pupillometry. It is also well recognized that opioids generally stimulates the endocrine system (Vuong, Van Uum, O'Dell, Lutfy, & Friedman, 2010). Numerous studies, in both animals and humans, have documented the effect on prolactin levels, which therefore provides a second objective measure (Delitala, Grossman, & Besser, 1983; Devilla et al., 1985; Lo Dico et al., 1983; Vuong et al., 2010). In general, acute opioid administration stimulates prolactin secretion, while the effect of chronic opioid administration is less clear. It has been indicated that activation of opioid receptors on prolactin secretion is mediated via doperminergic neurons in hypothalamus (Chang et al., 2011; Delitala et al., 1983; Vuong et al., 2010). Thus, elevated prolactin levels may also be used as a measure of opioid effects mediated via the CNS. Finally, a third available objective approach is to utilize electroencephalography (EEG), which has been widely used as biomarker of opioid effects on the CNS (Malver et al., 2014). EEG is a non-invasive method that reflects the electrical brain activity. It is either recorded as spontaneous EEG or as evoked potentials. Spontaneous EEG measures the neural activity at rest (or during painful tonic stimulations) and this method has mainly been used to identify altered brain activity following pharmacological intervention (Malver et al., 2014).

We hypothesized that pupil diameter, prolactin concentrations and resting EEG would yield different objective sensitivity to morphine. Therefore, we aimed to assess the effect of morphine administration on these three measures and compare the changes in these objective parameters to subjective analgesia using mechanical evoked muscle pain for convergent validation.

2. Methods

The protocol and informed consent were approved by The North Denmark Region Committee on Health Research Ethics, Denmark (N-20110077), the Danish Health and Medicines Authority (EudraCT identifier: 201100516920) and registered in clinicaltrialsregister.eu. The study was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) guidelines. The study was monitored by the GCP unit at Aarhus University Hospital.

2.1. Study protocol

The assessments in this paper consider two of four treatment arms from a randomized, double-blind, placebo-controlled four-way cross-over study. The treatment regimen included rectal administration of morphine and placebo at two separate study days with a washout period of minimum one week. Results from a previous population pharmacokinetic/pharmacokinetic study of rectally administered morphine suggest that the drug is well absorbed; the bioavailability after rectal administration corresponds with that seen after oral administration (Kreilgaard et al., 2014). The study was carried out at Mech-Sense, Department of Gastroenterology & Hepatology, Aalborg University Hospital in a quiet laboratory setting with dimmed light conditions and without interruptions. Prior to the first study day, at a separate screening session, the participants were instructed in and accustomed to the laboratory settings and the comprehensive procedures including the use of pain ratings (Fig. 1). Pain assessments were performed with a combined electronic visual analog scale (VAS) for measurement of non-painful (0 to 5) and painful (5 to 10) sensations. The VAS was combined with anchor words to describe the sensations: 0 = no perception, 1 = vague perception of mild sensation, 2 = definite perception of mild sensation, 3 = vague perception of moderate sensation, 4 = definite perception of moderate sensation, 5 = the pain threshold, 6 = mild pain, 7 = moderate pain, 8 = pain of medium intensity, 9 = intense pain, and 10 = unbearable pain. The VAS has proven to be robust and valid in assessment of experimental visceral pain and is described in detail elsewhere (Drewes & Gregersen, 2006). Moderate pain (VAS = 7) was chosen as the stimulation endpoint for the mechanical muscle stimulation.

The participants fasted for a minimum of 8 h prior to each experiment. To reduce the nausea associated with the fast, intravenous administration of 5% glucose (Fresenius Kabi, Copenhagen, Denmark) was started before initiation of each experiment. Approximately 1/2 L of glucose solution was administered during the experiment. Since the effect of rectally administered morphine was evaluated, the participants had their bowels cleansed with an enema (Toilax 2 mg/ml (Bisacodyl) Orion Corporation, Espoo, Finland) prior to the experimental procedure. The participants were asked to lie in the left lateral position and remained lying like this throughout the study. The experiment had duration of 180 min and the different experimental measures were performed by a trained investigator at preset times before treatment (baseline) and after treatment, and in the same order (1. blood sampling, 2. pupillometry, 3. resting EEG and 4. mechanical muscle stimulation) (Fig. 1).

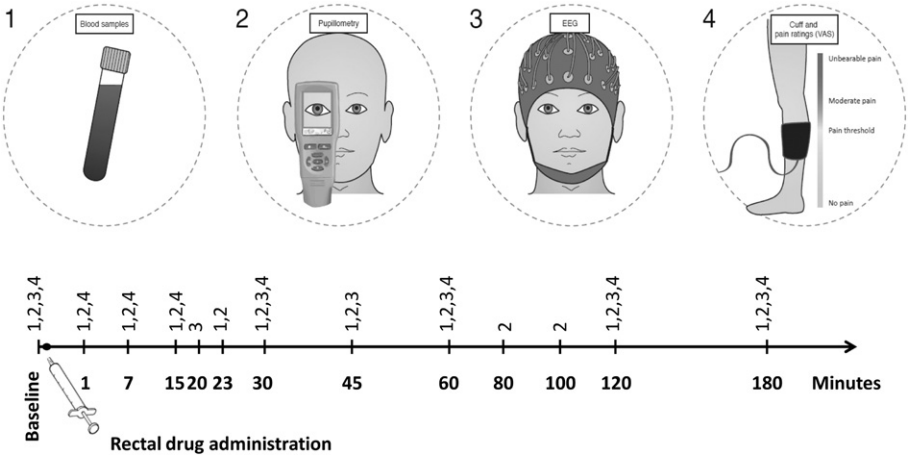

Fig. 1. Flowchart of the experimental setup: During each experiment the subjective measure and the three objective measures were performed before drug administration (baseline) and up to 11 time points after drug administration (1, 7, 15, 23, 30, 45, 60, 80, 100, 120 and 180 min). They were applied in the indicated order: 1) blood sampling (prolactin), 2) pupillometry, 3) resting EEG and 4) mechanical muscle stimulation.