Effects of morphine on visceral nociception evoked by colorectal distension in rats: comparative examinations of electrophysiological and behavioral responses

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Abstract: The purpose of this study was to compare the effects of intravenously administered morphine on electrophysiological and behavioral responses to colorectal distension (CRD) and to examine the influence of noxious stimuli applied to another part of the body (a laminectomy) on the visceromotor response to CRD. The effects of morphine (0.1–6.4 mg·kg⁻¹) were examined in rats anesthetized with pentobarbital. Electrophysiological (n = 16) and behavioral experiments (n = 47) were done. Electrophysiological experiments were conducted to examine the effects of morphine on the responses of visceral dorsal horn neurons to CRD; behavioral studies were conducted to compare the effects of morphine with and without a laminectomy (intact group: n = 24; laminectomy group: n = 23). Morphine suppressed the evoked activities of the visceral dorsal horn neurons in a dose-dependent manner. Similar suppression of the behavioral visceromotor response was observed. Visceromotor thresholds were significantly lower in the intact group than in the laminectomy group during the control study. When morphine was administered, the visceromotor thresholds in both groups increased to a similar level. Behavioral and neurophysiological responses were suppressed in a similar fashion by morphine. Although laminectomy affected the threshold values of CRD for visceromotor responses, the laminectomy per se plays an insignificant role when adequate morphine is administered.

Key words: Visceral dorsal horn neuron, Visceromotor response, Colorectal distension, Nociception, Morphine, DNIC, Nocigenic inhibition

Introduction

Spinal analgesia utilizing analgesic agents has been a part of clinical practice over the past two decades. Our laboratory has an ongoing interest in spinal sensory processing of information about visceral pain. We are particularly interested in examining spinal neuronal responses to noxious visceral stimuli and the ability of analgesic drugs to modify those responses, and in comparing the effects of drugs on neuronal responses with that on behavioral responses.

There are reports about the effects of morphine on sensory responses of dorsal horn convergent neurons activated by thermal, mechanical or chemical stimuli [1-4], and some reports about the effects of morphine on visceromotor responses [5] and on visceral responses of dorsal horn neurons to colorectal distension (CRD) [6-8]. There are, however, no reports comparing the effects of morphine sulfate on visceral responses of visceral dorsal horn neurons as defined by Ness and Gebhart [6,8] and visceromotor responses [5] elicited by visceral stimuli such as CRD in similar conditions.

There has been evidence that noxious stimuli associated with surgical preparation for an acute neurophysiological study of the spinal cord could alter both the neuronal responses to a noxious visceral stimulus as well as the impact of analgesics on those neuronal responses [9,10]. Noxious stimuli in distant parts of the body have been shown to suppress noxiously evoked activity in the spinal dorsal horn [9-11]. This phenomenon has been termed “nocigenic inhibition” [9,10] or “diffuse noxious inhibitory controls (DNIC)” [11]. On the other hand, increasing levels of surgical trauma have been reported to enhance reflex responses to mechanical pinch stimulus [12].

The purpose of this study was to compare the effects of intravenously administered morphine sulfate on electrophysiological and behavioral responses to CRD and to examine the influence of noxious stimuli applied to another part of the body (a laminectomy) on a visceromotor response elicited by the same noxious CRD.
Materials and methods

This study protocol was approved by the Yale Animal Care and Use Committee, and institutional, state and federal guidelines for humane care and use of laboratory animals were observed during all aspects of this study. The experiments were performed on 63 adult male Sprague-Dawley rats (CAMM) weighing 240–480 g at the start of the experiments. Two kinds of experiments were done: behavioral experiments (n = 47) and electrophysiological experiments (n = 16). The conditions were very similar in all of the experiments. The one important difference was the presence or absence of a laminectomy. The behavioral group was divided into two groups, one without laminectomy and one with laminectomy. Animals were anesthetized with intraperitoneal pentobarbital 40 mg·kg⁻¹. After 10 min of observation, if animals responded to initial surgical incision, an additional 20 mg·kg⁻¹ pentobarbital was administered intraperitoneally. Among the three groups (behavioral study with laminectomy, behavioral study without laminectomy, and electrophysiological study with laminectomy), the average doses of intraperitoneal pentobarbital were 46.9 mg, 46.7 mg, and 47.5 mg, respectively. An intravenous pentobarbital infusion was started approximately 30 min after intraperitoneal injection at a rate of 4–6 mg·kg⁻¹·h⁻¹. The level of anesthesia described by Sandkühler and Gebhart [13] was monitored for 10 min to assure the absence of spontaneous movement and the presence of corneal, auricular, pinal, and limb flexion reflexes. If spontaneous movement was present, the dose of intravenous pentobarbital was increased by 0.35 mg·kg⁻¹·h⁻¹ and that rate was continued. If reflexes were absent, the intravenous pentobarbital dose was decreased by 0.35 mg·kg⁻¹·h⁻¹. This level of anesthesia was monitored and maintained throughout the experiments. In the behavioral experiments with or without laminectomy, the animals were breathing spontaneously. This level of anesthesia was sufficient to maintain the animals without spontaneous movement. In the electrophysiological study, the same level of anesthesia was maintained throughout except for a 1-h period when actual neuronal recording was made. During this period, pancuronium was administered intravenously to record stable single neuron activity, and the animals were mechanically ventilated. We therefore assumed that a similar proper level of anesthesia was present during the single neuron recording.

Behavioral studies

Behavioral studies were done with and without a laminectomy (laminectomy group and intact group, respectively). The purpose of the laminectomy in the behavioral experiment was to make comparable animal preparations between the behavioral study and the electrophysiological study which necessitated laminectomy. Following tracheostomy, an external jugular vein and an internal carotid artery were cannulated for fluid and drug administration and for monitoring of arterial blood pressure. In the laminectomy group, a laminectomy from T12 to L1 was performed. No muscle relaxant was used for the behavioral study. Intravenous pentobarbital was started approximately 30 min after intraperitoneal injection. The behavioral study was begun within 1 h after completion of laminectomy. At this point and throughout the behavioral study, the animals presented no spontaneous movement with this level of anesthesia. A light level of anesthesia described by Sandkühler and Gebhart [13] with corneal, auricular, pinal, and limb flexion reflexes present in the absence of spontaneous movement was maintained throughout the duration of the experiment by continuous intravenous infusion of pentobarbital (4–6 mg·kg⁻¹·h⁻¹). End-tidal CO₂ was maintained around 40 mmHg for the duration of the experiment (Capnometer Model 2200 Traverse Medical Monitors, CA, USA). Body temperature was monitored with an esophageal probe and maintained within normal limits.

CRD was used as a noxious visceral stimulus. The method of CRD we used is similar to that described by Ness and Gebhart [5]. Distension of the descending colon and rectum was achieved by a pressure-controlled, air inflation of a 7-cm-long distension balloon inserted intra-anally [5–8]. The distension balloon was connected to a pressure-controlled balloon inflator [14] through a balloon catheter, and was inflated at a rate of 4 mmHg·s⁻¹ beginning at 0 mmHg until a maximum of 80 mmHg was reached. A small detection balloon (1–1.5 cm long, flexible, made of latex) was attached distal to the tip of the distension balloon catheter to monitor changes in intraluminal pressure [15]. It was filled with 0.6 ml of air to monitor intraluminal pressure which was found to be stable at this level of anesthesia. This intraluminal pressure was set at zero pressure on the recording chart. Abdominal muscle contraction in response to CRD (viserosomatic response), which were defined as visceromotor responses by Ness and Gebhart [5] is signaled by the detection balloon with a sudden rise in pressure. The pressures within the detection and distension balloon were recorded on a chard recorder simultaneously. A sudden rise in pressure of 1 mm or greater followed by continual rise can be detected easily on the recording chart. At the same time, muscular contraction was observed which verified the rise in the intraluminal pressure seen on the recording chart. Our decision to determine positive visceromotor response was based on the long lasting rise in pressure following an initial 1-mm rise and observation of abdominal