Interactive report

Effects of neuroactive substances on the morphine-induced respiratory depression; an in vitro study

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Abstract

Effects of different neuroactive substances on morphine-induced respiratory depression were studied in medullary respiration-related structures using in vitro brainstem–spinal cord preparation from 1 to 4-day-old rats. Application of morphine (10 \textmu M) reduced respiratory rhythm (fR) as measured by C4 ventral root activity. The depressant effects of morphine were reversed by acetylcholine (10 \textmu M), substance P (50 nM), thyrotropin releasing hormone (TRH) (100 nM) and forskolin (10 \textmu M). The adenosine receptor antagonist, theophylline (100 \textmu M), the dopamine receptors antagonist, haloperidol (10 \textmu M), the cyclooxygenase inhibitor, indomethacin (10 \textmu M) and the phospholipase A\textsubscript{2} inhibitor, quinacrine (10 \textmu M) had no effect on morphine-induced respiratory depression.

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Topic: Opioids: anatomy, physiology, and behavior

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Morphine causes a G-protein mediated respiratory depression, a major drawback during its use as analgesic. Finding a way to reverse or counteract this depression of respiration without canceling analgesia is an important and clinically relevant goal. One of the putative action sites of morphine, in respiratory depression, is the medulla oblongata [4,25,26]. Some of its pharmacological effects seem to be due to the presynaptic modulation of transmitter release of substances such as acetylcholine, substance P, dopamine and adenosine [9,18,21,24]. These neuroactive substances have all been suggested to play an important role for respiratory activity in the medulla oblongata. Acetylcholine and Substance P stimulate while adenosine and dopamine depress respiration [3,8,14,22,29]. Moreover, in different cell types activation of G-protein coupled opioid receptors have been shown to induce changes in several second messenger-signalling systems. These include elevation of intracellular calcium, stimulation of inositol 1,4,5-trisphosphate (IP\textsubscript{3}) turnover, arachidonic acid mobilization and cAMP decrease) [5,6,17]. IP\textsubscript{3}, cAMP and arachidonic acid metabolites are involved in respiratory control [12,17]. However, it is not clear whether these substances interact with morphine-induced respiratory depression. Other drugs that have been reported to antagonize opioid-induced respiratory depression include; benzodiazepine receptor antagonist and thyrotropin releasing hormone (TRH) [10,15]. Hence, in the present study possible interactions in medullary respiration-related structures between morphine and these substances were examined in an in vitro brainstem–spinal cord preparation.

The head and upper thorax of SD-BKL57 rats (1–4 days old, \textit{n}=87) were dissected under ether anesthesia. Brainstem and spinal cord were isolated and perfused with artificial cerebrospinal fluid (aCSF) at 28.5°C as described previously [23,25]. Respiratory activity was measured at

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the C4 or C5 ventral root using suction electrodes. Recorded signals were amplified and band-pass filtered (10 Hz to 5 kHz, differential AC amplifier model 1700, A–M systems Inc.). The C4/C5 activity was rectified and integrated with a time constant of 100 ms. These activities were simultaneously monitored via an analogue–digital converter (Digidata 1200, Axon Instruments) and data acquisition software (Axotape, Axon Instruments). Data were stored on a computer for later off-line analysis.

**Experimental procedure:** After a 20 min control and stabilization period in which the preparation was superfused with aCSF alone, morphine (10 μM, Pharmacia, Stockholm, Sweden) was applied for 30 min. Subsequently morphine (10 μM) and a test agent (acetylcholine, substance P, forskolin, theophylline, indomethacin, quinacrine (all from Sigma, St. Louis, MO, USA), haloperidol (Janssen Pharmaceutica, Beersel, Belgium), flumazenil (Roche, Basel, Switzerland) or TRH (Fluka Chemi, Buchs, Switzerland) were co-applied for 30 min. In 5 preparations that were used as control, morphine (10 μM) was applied for 60 min after a 20 min control period. This was followed by the simultaneous application of morphine (10 μM) and naloxone (1 μM). According to previous investigations using this in vitro preparation or brain slices, the concentrations of test agents were determined as follows: acetylcholine, 10 μM [14]; atropine, 10 μM [14]; substance P, 50 nM [14,29] haloperidol, 10 μM [14]; theophylline, 100 μM [8]; forskolin, 10 μM [1]; indomethacin, 10 μM [19]; quinacrine, 10 μM [19]; flumazenil 1 μM [27]; TRH, 100 nM [14]. C4 ventral root activity was used to calculate respiratory frequency (fR) and peak amplitude substance P and TRH were not affected by atropine (Fig. 1), TRH (100 nM) and forskolin (10 μM) could partly reverse the morphine-induced fR reduction (Table 1). The fR stimulant effect of acetylcholine was abolished by atropine 10 μM (n=3/3) (Fig. 1) while the effects of substance P and TRH were not affected by atropine (n=4). Atropine (10 μM) alone had no respiratory effects (%fR 99.8±8.6% and %Int C4 99.5±3.7%, n=4). Apart from reversing the effects of morphine, Substance P, forskolin and TRH increased tonic motor activity of C4 and increased Int C4 (see Fig. 2).

Haloperidol (10 μM, n=5) did not reverse or potentate tolerance was observed 30–60 min after the application. As a few preparations responded weakly to morphine, the analysis of reversibility of the different test agents were performed on experiments where a clear-cut morphine induced respiratory depression was seen (>20% fR reduction) (67/80). Also the effect of a test agent on respiratory activity/morphine induced respiratory depression was evaluated 30 min after its application. The average values were calculated from the bursts recorded during 1–2 min and results expressed as percentage of the controls. All data are presented as means±standard error of the mean (S.E.M.). The influence of test agents on the morphine-induced respiratory depression were analyzed with one-way ANOVA repeated measures design with Scheffe’s F post hoc test. The effects of morphine, atropine and flumazenil on respiratory activity were analyzed with Student’s paired t-test. Probability values (P) below 0.05 were considered as statistically significant.

The main findings are summarized in Table 1. Morphine (10 μM) caused a significant reduction of fR (P<0.01) and RMA (P<0.05) while the effect on respiratory peak amplitude (Int. C4) was inconsistent. Morphine effects were completely reversed by naloxone (1 μM) (%fR: 53.5±13.9% to 99.2±5.3%, % Int. C4: 111.9±10.9% to 105.7±12.4% and %RMA: 56.7±11.7% to 116.3±11.7%, n=5).

Acetylcholine (10 μM) (Fig. 1), substance P (50 nM) (Fig. 2), TRH (100 nM) and forskolin (10 μM) could partly reverse the morphine-induced fR reduction (Table 1). The fR stimulant effect of acetylcholine was abolished by atropine 10 μM (n=3/3) (Fig. 1) while the effects of substance P and TRH were not affected by atropine (n=4).

<table>
<thead>
<tr>
<th>Test agents</th>
<th>n (%)</th>
<th>%fR (Morphine 10 μM)</th>
<th>%fR (M+a test agent)</th>
<th>%fR (Morphine 10 μM)</th>
<th>%fR (M+a test agent)</th>
<th>%Int C4 (Morphine 10 μM)</th>
<th>%Int C4 (M+a test agent)</th>
<th>%RMA (Morphine 10 μM)</th>
<th>%RMA (M+a test agent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Morphine alone)</td>
<td>5</td>
<td>55.5±10.1</td>
<td>53.5±13.9</td>
<td>111.8±8.6</td>
<td>119.0±10.9</td>
<td>61.0±9.2</td>
<td>56.7±11.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylcholine 10 μM</td>
<td>8</td>
<td>28.6±10.1</td>
<td>66.4±17.2*</td>
<td>106.0±2.7</td>
<td>111.9±11.0</td>
<td>27.3±14.2</td>
<td>80.9±20.3*</td>
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<tr>
<td>Substance P 50 nM</td>
<td>7</td>
<td>26.6±7.6</td>
<td>70.3±5.8**</td>
<td>98.1±5.0</td>
<td>116.8±4.6**</td>
<td>28.6±9.3</td>
<td>79.3±7.1*</td>
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<td></td>
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<tr>
<td>Forskolin 10 μM</td>
<td>8</td>
<td>34.8±9.4</td>
<td>65.9±9.4</td>
<td>89.1±9.8</td>
<td>113.8±6.4**</td>
<td>28.3±6.5</td>
<td>73.3±13.7*</td>
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<tr>
<td>TRH 100 nM</td>
<td>8</td>
<td>65.5±4.5</td>
<td>88.1±6.7**</td>
<td>116.8±6.8</td>
<td>133.9±4.5*</td>
<td>72.9±4.3</td>
<td>111.1±13.0*</td>
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<tr>
<td>Flumazenil 1 μM</td>
<td>6</td>
<td>62.1±5.3</td>
<td>47.6±6.8*</td>
<td>98.5±5.6</td>
<td>104.1±8.0</td>
<td>59.9±3.5</td>
<td>46.8±5.1**</td>
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<td></td>
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<tr>
<td>Haloperidol 10 μM</td>
<td>5</td>
<td>61.8±6.0</td>
<td>51.8±6.0</td>
<td>99.1±5.2</td>
<td>101.2±7.4</td>
<td>61.1±6.3</td>
<td>61.8±5.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Theophylline 100 μM</td>
<td>6</td>
<td>54.5±7.1</td>
<td>59.1±10.3</td>
<td>98.5±2.8</td>
<td>94.4±3.0</td>
<td>53.7±7.2</td>
<td>56.3±8.5</td>
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<tr>
<td>Indomethacin 10 μM</td>
<td>6</td>
<td>53.9±11.1</td>
<td>51.1±11.5</td>
<td>99.0±6.2</td>
<td>107.0±6.4</td>
<td>65.3±3.2</td>
<td>65.7±6.0</td>
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<tr>
<td>Quinacrine 10 μM</td>
<td>8</td>
<td>44.2±7.4</td>
<td>49.0±5.3</td>
<td>102.1±4.5</td>
<td>110.5±5.1</td>
<td>46.7±6.4</td>
<td>58.0±6.2</td>
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</table>

*% of respiratory activity during control conditions (respiratory frequency (fR) and peak amplitude of integrated C4 discharge (Int. C4), RMA=respiratory minute activity) (Morphine 10 μM=M). Data are presented as means±S.E.M. Significant differences from the values before the application of a test agent are indicated with *P<0.05 or **P<0.01.
Fig. 1. The effects of acetylcholine on the morphine-induced respiratory depression. (A) C4 activity (upper traces) and integrated C4 activity (lower traces) recorded from the in vitro preparation in standard solution. (B) 30 min after perfusion with morphine (10 μM). (C) 30 min after perfusion with morphine (10 μM) and acetylcholine (10 μM). (D) 10 min after perfusion with morphine (10 μM), acetylcholine (10 μM) and atropine (10 μM).

morphine induced respiratory depression. Flumazenil (1 μM), a benzodiazepine-antagonist, alone had no respiratory effects (%fR 96.3±3.3% and %Int. C4 95.9±6.1%) (n=3). However, flumazenil potentiate morphine induced fR reduction. Theophylline (100 μM), indomethacin (10 μM) and quinacrine (10 μM) did not reverse or potentate the morphine-induced respiratory depression (Table 1).

We show in the present study that TRH, Ach, SP and Forskolin may partly reverse the respiratory depression induced by morphine action in the medulla oblongata. Several in vivo studies have shown that anticholinesterases and TRH can antagonize or reverse opioid-induced respiratory depression see ex. [10,28]. Our data are in agreement with these in vivo findings and indicate that their effects are mediated through direct action in the medulla oblongata. We furthermore suggest that activating SP-receptors may partly reverse respiratory depression induced by morphine.

Muscarin, Tachykinin and TRH receptors are all coupled to G-proteins. μ-opioid and NK₁-receptors are present on respiratory neurons in the proposed respiratory rhythm generating centers in rostral ventrolateral medulla oblongata [7]. Also, postsynaptic Muscarin and TRH receptors are present on respiration-related neurons in the medulla oblongata [16,20].

All of the agents that reverse the effect of morphine have been shown to induce depolarization in respiratory neurons (TRH, Ach, forskolin and SP) [7,11,16,20]. Thus, a possible explanation to our data could be that morphine induced postsynaptic hyperpolarization of brainstem respiratory neurons (Shinhiro Takeda et al., unpublished data) may be reversed by agents/modulators depolarizing the affected neurons.

Opioids act presynaptically to inhibit the release of acetylcholine and substance P, and enhance the release of dopamine and adenosine [9,12,18]. Our present results do not support the idea that the morphine-induced fR reduction was mainly due to inhibition of substance P and acetylcholine release. Because firstly, atropine alone did not affect fR and Int C4; and secondly, acetylcholine and substance P could not completely reverse the morphine-induced fR reduction. Neither does enhancement of the release of adenosine and dopamine seem to play a role in the respiratory depression induced by morphine because an adenosine receptor antagonist like theophylline and a dopamine receptors antagonist like haloperidol did not reverse the morphine-induced respiratory depression. Hence, it is not likely that the morphine effects found in this study were due to presynaptic effects.

The increased noise in the interburst period observed during TRH, SP and forskolin application is in accordance with previous studies [16]. TRH has been shown to induce
Fig. 2. The effects of substance P on the morphine-induced respiratory depression. (A) C4 activity (upper traces) and integrated C4 activity (lower traces) recorded from the in vitro preparation in standard solution. (B) 30 min after perfusion with morphine (10 μM). (C) 5 min after perfusion with morphine (10 μM) and substance P (50 nM). D: 20 min after perfusion with morphine (10 μM) and substance P (50 nM). Note the rapid reversal of morphine effect and the increased non-respiratory ventral root activity in C.

Depolarization of motor neurons [16] and SP is well known to be able to similarly induce such increased excitation also in non-rhythm generating and non-respiratory neurons in the spinal cord.

Arachidonic acid metabolites are involved in respiratory control [2]. Administration of an intravenous cyclooxygenase inhibitor, ketoprofen, may have an antagonistic action on the morphine-induced respiratory depression in humans [13]. Based on the results in the present study it was not possible to support these earlier results. Thus, arachidonic acid metabolites as prostaglandins and leukotrienes are probably not mediators of respiratory depression via opioid receptors in the medulla oblongata of newborn rat.

Our present data of the inability of benzodiazepine antagonist to reverse the effects of morphine are in accordance with a recent study showing that GABA-A receptors are not crucially involved in μ-receptor induced depression of respiratory frequency [7].

In summary; agents like acetylcholine, substance P, TRH and forskolin, which are known to depolarize respiration-related neurons, reversed morphine-induced fR reduction. Adenosine, dopamine agonists/antagonists, a cyclooxygenase and a phospholipase A2 inhibitor did not reverse morphine-induced respiratory depression.

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