PHYSOSTIGMINE ANTAGONIZES MORPHINE-INDUCED RESPIRATORY DEPRESSION BUT NOT ANALGESIA IN DOGS AND RABBITS

M. WEINSTOCK, D. ROLL, E. EREZ AND M. BAHAR

SUMMARY
The ability of physostigmine to antagonize the respiratory depressant effect of morphine was studied in conscious rabbits and ketamine-anaesthetized dogs pretreated with atropine methyl nitrate. Morphine 4 mg kg\(^{-1}\) increased \(P_{\text{aCO}_2}\) in the rabbit from 3.43 ± 0.16 to 4.95 ± 0.28 kPa, decreased arterial pH from 7.45 ± 0.01 to 7.31 ± 0.01 and decreased respiratory frequency by 36%. Physostigmine 0.1 mg kg\(^{-1}\) reduced \(P_{\text{aCO}_2}\) to control values within 10 min and significantly increased arterial pH and respiratory frequency. There was no antagonism of the analgesic effect of morphine. Neostigmine 0.1 mg kg\(^{-1}\) did not reverse the respiratory depressant effect of morphine. In dogs anaesthetized with ketamine, morphine 15 mg kg\(^{-1}\) caused loss of consciousness and marked analgesia, decreased the respiratory frequency by 47%, and increased \(P_{\text{aCO}_2}\) by 47%. Physostigmine 0.1 mg kg\(^{-1}\) antagonized the effect of morphine on respiration and restored consciousness in the dogs, but did not impair analgesia. It is concluded that physostigmine reverses morphine-induced respiratory depression by prolonging the effect of acetylcholine released from brain-stem neurones. The possibility should be considered of replacing opiate antagonists by physostigmine to reverse postoperative respiratory depression and drowsiness induced by opiates.

Physostigmine salicylate is an anticholinesterase which, unlike the commonly used agent neostigmine, is a tertiary amine, and therefore readily penetrates the blood–brain barrier. In decerebrate cats pretreated with physostigmine, application of acetylcholine (Ach) to the floor of the 4th ventricle markedly stimulates breathing (Miller, 1949).

Morphine and related narcotic analgesic drugs are known to produce respiratory depression by decreasing the sensitivity of the respiratory centre to carbon dioxide (Jaffe and Martin, 1975). The site of the respiratory depressant action of morphine has been localized to the lower brain stem (Florez, McCarthy and Borison, 1968). A number of studies in different species have demonstrated that opiates can inhibit the release of Ach from neurones in the central and peripheral nervous system (Jhamandas, Phillis and Pinsky, 1971; Weinstock, 1971; Domino and Wilson, 1973; Zsilla et al., 1977). It is possible that opiates depress respiration by reducing the availability of Ach in the vicinity of the respiratory centre (Schaumann, 1958). If this was so, it would be likely that physostigmine could reverse the respiratory depressant effect of morphine by preventing the hydrolysis of Ach. Since physostigmine itself (Flodmark and Wramner, 1945) and directly-acting cholinergic agonists such as oxotremorine and pilocarpine, have been shown to produce analgesia in animals and man (Ireson, 1970; Dayton and Garrett, 1973), it was considered unlikely that physostigmine would interfere with the analgesic activity of morphine.

MATERIALS AND METHODS
Male New Zealand rabbits, weight 2.5–3.0 kg, were trained to sit quietly in a restraining box. Blood samples (0.5 ml) for gas analysis were taken through a catheter (Quick Cath. 20 gauge Travenol Labs, Ireland Ltd) placed in the ear artery and filled with sterile saline containing heparin 25 u. ml\(^{-1}\). Drugs were administered through a 19 gauge butterfly needle placed in a marginal ear vein.

Rectal temperature was monitored with a thermometer (Yellow Springs Instruments) from a thermistor probe inserted to the rectum. Respiratory frequency was counted visually and blood-gas tensions and pH measured with a Corning automatic blood-gas analyser after correction for the appropriate body temperature.
Analgesia was assessed from the reaction (squeal or attempt at withdrawal) in response to one of four grades of pressure applied to the tip of the tail with a sponge holder clamp. A score of 1 indicated a positive reaction to the lowest grade of pressure, while 5 denoted that no reaction occurred to the highest grade of pressure.

Rabbits were allowed to rest for 1 h after cannulation before control readings were taken. Drugs were not given until two consistent values for blood-gas tensions were obtained. All rabbits were pretreated with atropine methyl nitrate (ATMN) 1 mg kg\(^{-1}\). In six rabbits, morphine 4 mg kg\(^{-1}\) was injected slowly i.v. over a period of 2–3 min. Respiratory frequency was counted and blood samples for gas analysis were collected at 30, 60 and 90 min after injection of morphine. In 12 other rabbits, physostigmine 0.1 mg kg\(^{-1}\) or neostigmine 0.1 mg kg\(^{-1}\) was injected i.v. immediately after the 30-min blood sample had been collected following morphine administration. Further blood samples for gas analysis were collected at 5, 10, 20, 30 and 60 min after physostigmine. At the end of the experiment, rabbits were returned to their cages.

**Ketamine anaesthetized dogs**

Five mongrel dogs of either sex, weight 10–13 kg, were used. Animals were re-used after an interval of 10 days. The dogs were anaesthetized with ketamine 20 mg kg\(^{-1}\) i.m. This agent was used in preference to a barbiturate, since these are known to inhibit strongly cholinergic neurones in the c.n.s. (Bradley and Dray, 1973; Weinstock et al., 1979). Furthermore, ketamine itself does not significantly depress respiration at anaesthetic doses (Waterman and Livingston, 1978). The femoral artery was cannulated (“Venflon” Viggo 18 gauge) under aseptic conditions. The cannula was filled with sterile saline containing heparin 50 u. ml\(^{-1}\). The brachial vein was also cannulated and sterile dextrose 5% was perfused continuously through the catheter at the rate of 2 ml min\(^{-1}\).

A rectal thermistor probe was inserted and body temperature monitored continuously with a Yellow Springs telethermometer. ATMN 0.5 mg kg\(^{-1}\) was injected i.v. Ten minutes later (30 min after induction of anaesthesia), respiratory frequency was counted and arterial blood samples were taken for the measurement of blood-gas tensions and pH.

Morphine was then administered by i.v. infusion at the rate of 0.3 mg kg min\(^{-1}\). Analgesia was assessed at 10-min intervals on a 1–5 scale as described in rabbits, by the reaction to pressure applied to the ear. Respiration rate, blood-gas tensions and pH were measured every 15 min.

We determined the dose of morphine which:
(a) prevented the animal from responding to sound or other external stimuli;
(b) increased the analgesic score to at least 4;
(c) reduced the respiratory frequency by at least 40%;
(d) increased \(P_{aCO_2}\) by 40%;
(e) maintained the above levels of analgesia and respiratory depression for at least 60 min after cessation of the morphine infusion.

Physostigmine 0.1 mg kg\(^{-1}\) was injected i.v. 60 min after the end of the infusion of morphine. Analgesia, respiratory frequency and blood-gas tensions were measured 15 and 30 min later. At the end of the experiment, cannulae were removed and the animal allowed to recover consciousness before returning to the animal house. Solutions of physostigmine were made up in sterile saline containing absorbic acid 1 mg ml\(^{-1}\) and sterilized by filtration before use.

**RESULTS**

**Conscious rabbits**

The mean control respiratory frequency of 18 conscious rabbits was 110 ± 8 b.p.m., mean \(P_{aCO_2}\) 3.43 ± 0.16 kPa and mean pH 7.45 ± 0.01. These values are within the normal range for rabbits. Body temperature was 38.9 ± 1.2 °C (Altman and Dittner, 1971).

I.v. injection of morphine 4 mg kg\(^{-1}\) markedly reduced respiratory frequency in the rabbits, peak depression occurring between 30 and 60 min after i.v. injection (table I). Maximum reductions in blood pH and \(P_{aO_2}\) and increases in \(P_{aCO_2}\) also occurred at this time (fig. 1). Good analgesia was obtained at 30 and 60 min after morphine as indicated by mean scores in six rabbits of 4.56 ± 0.22 and 4.50 ± 0.21, respectively, out of a total possible score of 5.0.

Injection of physostigmine 0.1 ml kg\(^{-1}\) significantly increased respiratory frequency and blood pH, slightly increased \(P_{aO_2}\), and decreased \(P_{aCO_2}\) to control values, within 5–10 min. The reversal of the respiratory depressant effects of morphine by physostigmine lasted at least 60 min (fig. 1 and table I). There was no reduction in the level of analgesia induced by morphine following the in-
MORPHINE REVERSAL BY PHYSOSTIGMINE

TABLE I. The effect of physostigmine and neostigmine on respiratory frequency and analgesia induced by morphine in the rabbit. *P < 0.05 by paired t test compared with morphine alone; **P < 0.01 by paired t test compared with control alone.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Time after morphine (min)</th>
<th>Time after physostigmine or neostigmine (min)</th>
<th>Respiratory frequency (b.p.m. ± SEM)</th>
<th>Analgesia scores (± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (before drug)</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>110 ± 8</td>
<td>1.29 ± 0.12</td>
</tr>
<tr>
<td>Morphine 4 mg kg⁻¹</td>
<td>6</td>
<td>30</td>
<td>0</td>
<td>69 ± 9**</td>
<td>4.48 ± 0.24**</td>
</tr>
<tr>
<td>Morphine 4 mg kg⁻¹ + physostigmine 0.1 mg kg⁻¹</td>
<td>6</td>
<td>35</td>
<td>5</td>
<td>98 ± 10*</td>
<td>4.91 ± 0.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>10</td>
<td>77 ± 10</td>
<td>4.80 ± 0.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>20</td>
<td>78 ± 14</td>
<td>4.51 ± 0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>30</td>
<td>80 ± 12</td>
<td>4.12 ± 0.27</td>
</tr>
<tr>
<td>Morphine 4 mg kg⁻¹ + neostigmine 0.1 mg kg⁻¹</td>
<td>6</td>
<td>35</td>
<td>5</td>
<td>70 ± 10</td>
<td>4.40 ± 0.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>20</td>
<td>68 ± 9</td>
<td>4.21 ± 0.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>30</td>
<td>67 ± 8</td>
<td>4.01 ± 0.23</td>
</tr>
</tbody>
</table>

Fig. 1. Effect of physostigmine 0.1 mg (○ — ○) and neostigmine 0.1 mg (△ — △) (right-hand arrows, 30 min) on changes in arterial blood gases and pH induced by morphine 4 mg kg⁻¹ (□ — □) (left-hand arrows, 0 min). Each point represents the mean value ± SEM from six rabbits. **P < 0.01; ***P < 0.001; compared with morphine alone by one-tailed test.

Injection of physostigmine (table I). There were also no signs of peripheral cholinergic effects such as lachrymation, miosis, salivation, defaecation or micturition following physostigmine after pretreatment with ATMN 1 mg kg⁻¹. In one rabbit in which \( P_{aCO_2} \) was increased from 3.33 to 6.52 kPa by morphine 8 mg kg⁻¹, physostigmine 0.1 mg kg⁻¹ reduced \( P_{aCO_2} \) to 4.52 kPa and a further dose of 0.1 mg kg⁻¹, given 10 min later, reduced \( P_{aCO_2} \) to 3.46 kPa.

Neostigmine 0.1 mg kg⁻¹ injected 30 min after morphine 4 mg kg⁻¹, to six other rabbits, did not significantly alter any of the respiratory variables depressed by morphine. Larger doses of neostigmine (0.2–0.3 mg kg⁻¹) caused marked fasciculations and increased the respiratory depressant effect of morphine.

Dogs anaesthetized with ketamine

Pretreatment of the dogs with ketamine prevented the usual manifestations of excitement, "sham rage", hyperventilation and howling, that normally occur when morphine is given i.v. to conscious dogs (preliminary findings in this laboratory, and Fennessy and Ortiz, 1968). The mean respiratory frequency in dogs before ketamine was 24 ± 2. Thirty minutes after ketamine, it increased to 30 ± 4. Results for blood-gas tensions, pH and analgesia after ketamine are shown in table II. The normal \( P_{aCO_2} \) for unanaesthetized dogs is 5.05 kPa (Altman and Dittner, 1971).

A total of morphine 15 mg kg⁻¹ was given to obtain the required increase in analgesia and respiratory depression. The effects produced 30–90 min after this dose of morphine are shown in table II.
TABLE II. Effect of physostigmine on changes in respiratory frequency, arterial blood-gas tensions and analgesia induced by morphine in the dog. Values are mean ± SEM from five dogs given either morphine alone or morphine followed 60 min later by physostigmine. Compared with morphine alone, 60 min or 90 min: *P < 0.05; **P < 0.01

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time after morphine (min)</th>
<th>Respiratory frequency (b.p.m. ± SEM)</th>
<th>pH</th>
<th>(P_{\text{aCO}_2}) (kPa ± SEM)</th>
<th>(P_{\text{aO}_2}) (kPa ± SEM)</th>
<th>Analgesic score (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (ketamine 20 mg kg(^{-1}) + ATMN 0.5 mg kg(^{-1}))</td>
<td>-20</td>
<td>30.0 ± 4.0</td>
<td>7.37 ± 0.03</td>
<td>4.55 ± 0.23</td>
<td>12.63 ± 0.53</td>
<td>2.0 ± 0.4</td>
</tr>
<tr>
<td>Morphine 15 mg kg(^{-1})</td>
<td>30</td>
<td>16.0 ± 0.4</td>
<td>7.21 ± 0.04</td>
<td>6.60 ± 0.24</td>
<td>10.11 ± 0.27</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>15.5 ± 0.8</td>
<td>7.20 ± 0.04</td>
<td>6.69 ± 0.29</td>
<td>10.37 ± 0.27</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>16.5 ± 0.8</td>
<td>7.21 ± 0.03</td>
<td>6.44 ± 0.25</td>
<td>10.91 ± 0.27</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>Morphine 15 mg + physostigmine 0.1 mg kg(^{-1})</td>
<td>75</td>
<td>20.0 ± 0.9**</td>
<td>7.25 ± 0.02</td>
<td>5.21 ± 0.31**</td>
<td>10.91 ± 0.27*</td>
<td>4.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>18.0 ± 1.0</td>
<td>7.22 ± 0.02</td>
<td>5.85 ± 0.32</td>
<td>10.91 ± 0.40</td>
<td>3.7 ± 0.4</td>
</tr>
</tbody>
</table>

When physostigmine 0.1 mg kg\(^{-1}\) was given 60 min after the end of the morphine infusion, the dog sat up, looked around the room and responded to sounds within 10 min. After a further 5 min, there was a significant increase in both the rate and depth of respiration which was accompanied by an increase in pH and \(P_{\text{aO}_2}\) and a decrease in \(P_{\text{aCO}_2}\) (table II). However, there was no difference in the level of analgesia from that obtained before physostigmine was given, as indicated by the virtual absence of a response to pressure on the ear. Thirty minutes after physostigmine, the dogs became sedated once again, and the respiratory depressant effect of morphine also returned. In one of the dogs, a further injection of physostigmine 0.1 mg kg\(^{-1}\) restored respiratory frequency and \(P_{\text{aCO}_2}\) to pre-morphine values.

This study demonstrated that physostigmine can cause reversal of the respiratory depressant effect of morphine in conscious rabbits. Although physostigmine altered both the frequency of respiration and the blood \(P_{\text{aCO}_2}\), significantly, more importance can be attached to the latter measurement, since we have observed that marked changes in spontaneous respiratory frequency can occur in conscious rabbits with little change in blood-gas tensions. The depth of respiration, which was seen to change but not measured, would doubtless explain alterations obtained in blood-gas tensions.

ATMN did not prevent the effect of physostigmine and morphine reversal was not achieved with neostigmine, which shows that central cholinergic neurones are involved in the interaction between morphine and physostigmine.

In dogs anaesthetized with ketamine and given large doses of morphine, physostigmine produced a prompt recovery of consciousness lasting at least 15 min without reducing analgesic activity. It is unlikely that the reversal of the narcosis by physostigmine resulted from antagonism of ketamine rather than morphine. Although physostigmine has been shown to reduce ketamine-induced sleeping time in rats (Lawrence and Livingston, 1979) and sedation in humans (Balmer and Wyte, 1977), an interval of at least 2 h had elapsed in the experiments between the injection of ketamine and that of physostigmine. In dogs, the duration of anaesthetic effect of ketamine 20 mg kg\(^{-1}\) alone was found to be only 20-30 min.

It is therefore reasonable to conclude that physostigmine can antagonize the hypnotic and respiratory depressant effects of morphine without impairing its analgesic activity.

DISCUSSION

Central cholinergic mechanisms have often been implicated in various actions of morphine (Weinstock, 1971). While morphine has been shown to inhibit Ach release from neurones in the brain (Jhamandas, Phillis and Pinsky, 1971; Domino and Wilson, 1973), other studies have shown that the analgesic activity (Saxena, 1958), toxicity (Brister and Davis, 1974) and morphine withdrawal syndrome can be modified by drugs that stimulate or block cholinergic receptors (Jhamandas, Sutak and Bell, 1973).

Apart from an early study in rats anaesthetized with urethane (Schaumann, 1958), a detailed investigation of the action of anticholinesterases on the respiratory depressant action of opiates does not appear to have been carried out.
We suggest that physostigmine be used in place of conventional narcotic antagonists for the reversal of postoperative respiratory depression induced by opiates. It may be possible to maintain the desired level of analgesia, while the patient remains fully awake and breathing normally, by suitably spaced injections or an infusion of physostigmine.

ACKNOWLEDGEMENTS

Supported by a VSPHS grant from NIDA, RO1 DA 02150-01 and by the joint Research Fund of the Hebrew University and Hadassah, and the Herbert Singer Funds for medical research.

REFERENCES


Die Fähigkeit von Physostigmine, die atmungseinschränkende Wirkung von Morphin zu bekämpfen, wurde bei vollem Bewusstsein bei Kaninchen und bei ketaminanästhetisierten Hunden, die mit Atropinmethylnitrat vorbehandelt worden waren, studiert. Morphin 4 mg kg⁻¹ verursachte eine Zunahme der PaCO₂ beim Kaninchen von 3,43 ± 0,16 auf 4,95 ± 0,28 kPa, eine Abnahme des arteriellen pH-Wertes von 7,45 ± 0,01 auf 7,31 ± 0,01 und eine Verringerung der Atmungsfrequenz um 36%. Physostigmine 0,1 mg kg⁻¹ reduzierte PaCO₂ auf Kontrollwerte innerhalb 10 Minuten und führte zu einer bedeutenden Zunahme des arteriellen pH-Wertes und der Atmungsfrequenz. Es fand keine Bekämpfung der analgesischen Wirkung von Morphin statt. Neostigmine 0,1 mg kg⁻¹ hat die atmungseinschränkende Wirkung von Morphin nicht rückgängig gemacht. Bei Hunden, die mit Ketamin anästhetisiert waren, führte Morphin 15 mg kg⁻¹ zu Bewusstseinsverlust und ausgeprägter Analgesie, einer Vermindерung der Atmungsfrequenz um 47% und einer Zunahme der PaCO₂ um 47%. Physostigmine 0,1 mg kg⁻¹ bekämpfte die Wirkung von Morphin auf die Atmung und stellte das Bewusstsein der Hunde wieder her, beeinträchtigte die Analgesie aber in keiner Weise. Es wird geschlossen, dass Physostigmine die morphininduzierte Atmungseinschränkung rückgängig macht, indem es die Wirkung von Acetylcholine verlängert, die von den Hirnstammeinheiten abgegeben wird. Man sollte die Möglichkeit erwägen, Opiatbekämpfungsmittel durch Physostigmine zu ersetzen, um die durch Opiate induzierte postoperative Atmungseinschränkung und Schlaffrigkeit rückgängig zu machen.