ACUTE AND CHRONIC EFFECTS OF INTRATHECAL MORPHINE IN MONKEYS

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SUMMARY
In a double-blind study, seven Macaca fascicularis monkeys receiving intrathecal (i.t.) morphine in saline, 0.07 mg kg⁻¹, were compared with a control group of four monkeys receiving either lumbar puncture alone (n = 1) or i.t. saline (n = 3). Neither morphine nor saline solutions contained preservatives. Arterial blood gas tensions, respiration, arterial pressure, e.g., state of consciousness and motor function were recorded for 24 h. The control group was sacrificed 42 days later and the study group was sacrificed at 6 (n = 2) or 42 days (n = 5) after injection. The central nervous system, meninges, nerve roots and dorsal root ganglia were examined macroscopically and microscopically. Respiratory depression did not occur in either the control or the study groups. There were moderate but statistically significant decreases in systolic and diastolic arterial pressures following i.t. morphine. In both groups, the pathological findings were localized to the cauda equina region and characterized by mononuclear cell infiltration. In neither group was there evidence of demyelination, arachnoiditis or necrosis. Focal endoneurial fibrosis was found in only one animal in the control group following multiple lumbar punctures associated with paraesthesia. The features appeared to correlate with the physical trauma associated with lumbar puncture rather than with the injectate.

The safety of intrathecal (i.t.) morphine has not been established unequivocally. In rats, rabbits, cats and one primate, neither cardiovascular nor respiratory depression was observed with i.t. morphine (Yaksh and Rudy, 1976; Wang, 1977; Yaksh, 1978; Yaksh et al., 1979). Similar results were observed in man using morphine 1-20 mg (Cousins et al., 1979; Samii et al., 1979; Wang, Nauss and Thomas, 1979; Nelson and Katz, 1980). However, serious cardiovascular and respiratory depression have been reported (Liolios and Anderson, 1979; Baskoff, Watson and Muldoon, 1980; Davies, Tolhurst-Cleaver and James, 1980; Murphy, 1980). The i.t. administration of a drug may produce toxic reactions such as demyelination (Watanabe, Hodges and Dworzack, 1979), necrosis (Rubinstein et al., 1975), arachnoiditis and vascular changes (Hurst, 1955). Such reactions may progress only slowly.

This study was designed to resolve the controversy about the acute effects of i.t. morphine on cardiovascular and respiratory function and to examine possible chronic deleterious effects on the nervous system.

METHODS
Eleven monkeys (Macaca fascicularis), mean weight 5.4 ± 0.7 (SEM) kg, were studied. The control group consisted of four monkeys: one received lumbar puncture without any drug and three were injected i.t. with normal saline. All were sacrificed 42 days after lumbar puncture. The study group of seven monkeys received morphine sulphate in normal saline i.t.; two of these were sacrificed 6 days later and five 42 days later.

The investigators were unaware of the nature of the injectate until 5 days after the study, while the neuropathologist was not informed of the treatment and the sacrifice interval until the end of the study.

The morphine powder without a preservative (Mallinkrodt Chemical Company) was dissolved in preservative-free normal saline to produce a final concentration of morphine sulphate 2 mg ml⁻¹. The solution was filtered through a 0.22-μm filter, packed in aliquots of 1 ml in sterile vials, autoclaved at 120°C for 15 min and then stored in a refrigerator. To prepare the saline vials, sterile 0.9% sodium chloride solution without a preservative was repacked under aseptic conditions.
technique, autoclaved and refrigerated. Each vial contained 1 ml of solution.

To increase the chance of detecting a toxic reaction to morphine, the dose selected was five times the human therapeutic dose, 0.014 mg kg\(^{-1}\), recommended by Wang, Nauss and Thomas (1979). Therefore, the i.t. dose of morphine in this study was 0.07 mg kg\(^{-1}\) (0.035 ml kg\(^{-1}\)). To compensate for the deadspace in the needle used for lumbar puncture, 0.05 ml of the solution was added to the calculated volume of the injectate.

**Experimental procedure.** The monkeys, which had been in quarantine, were fasted overnight and the next morning weighed and injected with ketamine 10 mg kg\(^{-1}\) i.m. A peripheral venous cannula and a femoral arterial catheter were inserted. When the monkey showed signs of recovery from anaesthesia, it was placed in a primate chair (Plas-Labs, Inc., Lansing, MI) and the study commenced 6 h after ketamine injection. During this period e.c.g. leads were attached, and arterial pressure was recorded continuously via a pressure transducer (Statham T23-ID, Gould, Inc.) with an eight-channel Grass polygraph (MP-7, Grass, Inc., Quincy, MA). Arterial blood-gas tensions and \(pH\) were measured intermittently using a trielectrode blood-gas analyser (model 165, Corning). Respiratory rates were obtained by visual inspection of chest movements over 1-min periods.

The degree of consciousness was scored arbitrarily using the following point system: 3 = alert, eyes open and appropriate response to threats and surrounding activity; 2 = sleepy, but can be aroused by gentle stimulation; 1 = sleepy, aroused only with painful stimuli; and 0 = comatose, cannot be aroused. By attempting to pull each of the animal’s four limbs, the motor function was scored using the following point system: 2 = normal motor power; 1 = paresis, but still pulled back with moderate force; and 0 = paralysis, no motor power.

Beginning 6 h after ketamine injection, baseline measurements were made at 60, 30 and 5 min before lumbar puncture. Lumbar puncture without barbotage was performed with the monkey in the sitting position using a 22-gauge hypodermic needle under aseptic conditions. Following verification of successful lumbar puncture by withdrawal of blood-free cerebrospinal fluid (c.s.f.), the unknown solution was injected and the needle withdrawn. Thereafter, all indices were recorded at 30 and 60 min, hourly for the next 5 h and every 2 h up to 24 h. The data were compared using Student’s \(t\) test with a minimum \(P\) value of 0.05 for statistical significance.

**Histopathological analysis of the nervous system.** On the day of sacrifice, each monkey was anaesthetized with i.m. ketamine hydrochloride 10 mg kg\(^{-1}\); the trachea was intubated and the lungs were ventilated mechanically with a positive end-expiratory pressure of 5 cm H\(_2\)O. Thoracotomy was performed at the fourth intercostal space, the left ventricle was perfused with 500 ml of Karnovsky’s solution (4% paraformaldehyde, 5% glutaraldehyde in Millonig’s phosphate buffer at pH 7.4) to sacrifice the animal and simultaneously fix the tissues. Brain, spinal cord, dorsal root ganglia and a 1–2 cm portion of dorsal and ventral roots including dura and arachnoid were removed and immersed in Karnovsky’s solution for 7–10 days.

The brain of each monkey was weighed. Gross examination of coronal sections of the brain at 0.8-cm intervals, transverse sections of the brain stem and cerebellum at 0.5-cm intervals and cross-sections of spinal cord at 0.5-cm intervals were made carefully. Representative blocks were submitted for paraffin embedding and histological processing for light microscopic examination. Tissue blocks adequately fixed were available for ultrastructural study. Histological sections 5–8 \(\mu\)m thick were stained with haematoxylin and eosin and Masson’s trichrome for light microscopic study of any evidence of inflammatory reaction, cellular proliferation, necrosis, fibrosis or demyelination. The examined regions included sections of the basal ganglia, thalamus, hypothalamus, hippocampus, midbrain, pons, medulla, cerebellum and spinal cord at the cervical, thoracic, lumbar and sacral levels, in addition to sections of the dorsal root ganglia and nerve roots of the cauda equina.

**RESULTS**

Preinjection respiration rate in morphine- and saline-injected monkeys was about 30 b.p.m., resulting in \(P_{A\text{O}}\) about 12 kPa and \(P_{A\text{CO}}\) about 4 kPa (fig. 1). Injection of morphine or saline did not result in any significant changes between groups or within groups before and after injection.

Significant differences in systolic and diastolic arterial pressures were observed between morphine- and saline-injected monkeys (fig. 2). In
both groups, systolic arterial pressure was in the range of 110–120 mm Hg and diastolic pressure in the range 80–90 mm Hg at a heart rate of approximately 200 b.p.m. After injection of saline, systolic arterial pressure increased from 120 to 140 mm Hg approximately, but gradually returned towards control within 8 h. In the morphine-injected monkeys, systolic arterial pressure decreased from preinjection values of approximately 115 mm Hg to 95 mm Hg. Systolic arterial pressures in morphine-injected monkeys were significantly less than the preinjection values and less than saline-treated controls during the first 16 h after injection. Diastolic pressure was not significantly altered after injection in the control group. In the morphine-injected group, the diastolic pressure was lower than in the period before injection. It was also lower than in the control group during the first 16 h after injection. The heart rates were not significantly different before or after injection or when comparing the two groups.

In the morphine-injected group, the consciousness score was reduced to 2 in six of seven monkeys during the period between the 2nd and 4th h after injection. Before and after this period in the morphine group, and throughout the study in the saline group, the consciousness score was 3. None of the monkeys vomited or showed paresis or paralysis. All the animals were normally active without any gross neurological deficit until the time of sacrifice.

The specific gravity of the monkeys’ c.s.f. was 1.008 ± 0.0007 g ml⁻¹ and those of the morphine sulphate solution and normal saline, 1.0068 ± 0.0003 g ml⁻¹ and 1.0066 ± 0.0003 g ml⁻¹, respectively, using gravimetric procedures at 24 °C.

Neuropathological findings. In morphine-injected monkeys, no gross or microscopic lesion was found in three of five animals sacrificed 42 days after injection (table I). The other four animals injected with morphine (two sacrificed after 2 days and two after 42 days) showed minimal reaction in the form of occasional mononuclear cell infiltration surrounding the blood vessels in the leptomeninges, nerve roots or dorsal root ganglia.
**Table I. Injectate, lumbar puncture (LP) and pathological findings.** *Pathological findings in cauda equina region only; + = occasional; ++ = moderate; +++ = marked*

<table>
<thead>
<tr>
<th>Group</th>
<th>Injectate</th>
<th>No. attempts at LP</th>
<th>No. days before sacrifice</th>
<th>Pathological findings*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>1</td>
<td>42</td>
<td>Perivascular mononuclear cells surrounding nerve roots</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>2</td>
<td>42</td>
<td>Mononuclear cells in spinal root ganglia</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>1</td>
<td>42</td>
<td>Mononuclear cells around nerve roots and dorsal root ganglia</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>Multiple</td>
<td>42</td>
<td>Focal endoneurial fibrosis of nerve roots with mononuclear infiltrate</td>
</tr>
<tr>
<td>Study</td>
<td>+</td>
<td>1</td>
<td>6</td>
<td>Mononuclear cells in leptomeninges</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>3</td>
<td>6</td>
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<tr>
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<td></td>
<td>+</td>
<td>1</td>
<td>42</td>
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<td>42</td>
<td>Perivascular mononuclear infiltrate in leptomeninges</td>
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<td></td>
<td>+</td>
<td>1</td>
<td>42</td>
<td>No gross or microscopic lesion</td>
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**Fig. 3. Usual microscopic findings of the cauda equina 6 weeks following i.t. injection of morphine or saline (haematoxylin and eosin stain).** 1 = Dorsal root ganglion (minimal mononuclear infiltrate); 2 = leptomeninges (minimal mononuclear cell infiltrate); 3 = nerve root (normal). Horizontal bar represents 0.1 mm.

**Fig. 4. Microscopic findings of the cauda equina region 6 weeks following i.t. injection of saline (haematoxylin and eosin stain).** 1 = Nerve root showing endoneurial fibrosis and fibroblastic reaction. 2 = Normal nerve fibres. Horizontal bar represents 0.1 mm.
of the cauda equina (fig. 3). None of the animals injected with morphine showed demyelination, necrosis, vascular changes or fibrosis.

All the animals in the control group injected with normal saline showed mononuclear cell infiltration similar to the four monkeys in the morphine-injected group. However, one monkey in the control group, having had multiple lumbar puncture attempts associated with paraesthesia, showed focal endoneurial fibrosis in the nerve roots and extensive mononuclear cell reaction in the cauda equina region (fig. 4). The extent of the pathological findings appeared to correlate with the physical trauma induced by the lumbar puncture rather than with the injectate (table I).

DISCUSSION

The sedation and decrease in arterial pressure following i.t. injection of morphine were indicative of the pharmacological activity of the drug. We do not know if the sedation was the result of the drug reaching the reticular formation of the brain stem directly through the c.s.f. or indirectly via the blood stream.

The increase in arterial pressure in the control group following lumbar puncture was probably a result of excitement initiated by the manipulations. This increase was contrasted by a decrease in the arterial pressure in the study group. The decreases in systolic and diastolic pressures associated with i.t. morphine could have been a result of a direct effect on the vasomotor centre, a vasodilator effect on the blood vessels, histamine release or orthostatic hypotension (Jaffe and Martin, 1980). In other reports (Liolios and Anderson, 1979; Baskoff, Watson and Muldoon, 1980; Davies, Tolhurst-Cleaver and James, 1980; Winnie, 1980), hypotension was associated with respiratory depression and bradycardia about 6-12 h after i.t. injection indicating direct depression of the cardiovascular centres. In contrast, the decrease in arterial pressure in our study was moderate, early in onset, and associated with neither respiratory depression nor bradycardia.

Acute cardiovascular and respiratory depression following i.t. morphine in man is a rare but serious complication (Glynn et al., 1979; Liolios and Anderson, 1979; Baskoff, Watson and Muldoon, 1980; Davies, Tolhurst-Cleaver and James, 1980; Murphy, 1980). Most of these subjects had received excessive doses of i.t. morphine (10-15 mg), were elderly (60 yr or more), had received i.t. morphine and parenteral narcotics or i.t. local anaesthetic drugs, and underwent abdominal or thoracic surgery.

The absence of respiratory depression in our study could be attributed to species differences, the use of preservative-free morphine and saline (Craig and Habib, 1977), the absence of other medications such as narcotics or local anaesthetics, and the relatively smaller dose of morphine compared with some other reports (Liolios and Anderson, 1979; Baskoff, Watson and Muldoon, 1980).

Until more data are available from studies in man, the dose of i.t. morphine should not exceed 1 mg, the patient should be observed closely for 24 h in a recovery room, and the means of supporting the circulation and respiration, including naloxone, should be readily available.

No consistent deleterious effect of morphine on nervous tissue was found. Trauma was the primary cause in producing the pathological findings; manual dexterity is extremely important when performing lumbar puncture.

ACKNOWLEDGEMENTS

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REFERENCES


**EFFETS CHRONIQUES ET AIGUS DE LA MORPHINE ADMINISTREE PAR VOIE INTRATHECALE A DES SINGES**

RESUME

Au cours d'une étude à double inconne, sept singes *Macaca fascicularis* ayant reçu 0,07 mg kg⁻¹ de morphine dans une solution saline administrée par voie intratécale (i.t.) ont été comparés à un groupe de quatre singes ayant reçu une ponction lombaire seule (*n* = 1) ou une injection saline i.t. (*n* = 3). Ni les solutions de morphine, ni les solutions salines ne contenaient de préservatifs. La tension du sang, l'air, la respiration, la pression artérielle, l'électrocardiogramme, l'état de conscience et la fonction motrice ont été enregistrés pendant 24 heures. Le groupe témoin a été sacrifié 42 jours plus tard et le groupe faisant l'objet de l'étude a été sacrifié soit 6 jours après l'injection (*n* = 2), soit 42 jours après l'injection (*n* = 5). On a examiné macroscopiquement et à l'aide d'un microscope le système nerveux central, les meninges, la racine des nerfs et les racines des ganglions dorsaux. Il n'a produit aucune dépression respiratoire ni dans le groupe témoin ni dans le groupe faisant l'objet de l'étude. Il y a eu une diminution modérée, bien qu'ayant une importance statistique, de la pression artérielle systolique et de la pression artérielle diastolique, après l'injection i.t. de morphine. Dans les deux groupes, les constatations pathologiques ont été localisées à la région de la queue de cheval et caractérisées par une infiltration de cellules mononucléées. Dans aucun des deux groupes on n'a constaté de démyélinisation, d'arachnoïdite ou de nécrose. On n'a trouvé de fibrose focale de l'endomètre que sur un seul animal du groupe témoin après de multiples ponctions lombaires associées à la paraesthésie. Les caractéristiques semblent être en corrélation avec les traumatismes physiques associés aux ponctions lombaires plutôt qu'au produit injecté.

**AKUTE UND CHRONISCHE AUSWIRKUNGEN VON INTRATHEKALEM MORPHIN BEI AFFEN**

ZUSAMMENFASSUNG


**EFFETOS CRONICOS Y AGUDOS DE LA MORFINA ENTRATECAL EN LOS MONOS**

**SUMARIO**

Durante un estudio de doble anonimato se compararon 7 monos *Macaca fascicularis*, los cuales recibieron morfina intratecal (i.t.) en una solución salina de 0.07 mg kg⁻¹ con un grupo de control de cuatro monos que recibieron tan sólo un pinchazo lumbar (*n* = 1) o una solución salina i.t. (*n* = 3). Ni la morfina ni las soluciones salinas contenían preservativos. Se registraron por espacio de 24 horas las tensiones del gas de la sangre arterial, la presión arterial, el electrocardiograma, el estado conciente y la función motriz. El grupo de control fue sacrificado 42 días después y el grupo de estudio fue sacrificado a los 6 (*n* = 2) ó a los 42 días (*n* = 5) de la inyección. Se examinaron macroscópicamente y microscópicamente el sistema nervioso central, las meninges, las raíces nerviosas y las raíces de los ganglios dorsales. No tuvo lugar depresión respiratoria en el grupo de control ni en el de estudio. Hubo disminuciones moderadas, pero estadísticamente significativas, en las presiones arteriales sistólica y diastólica a raíz de la morfina i.t. Los resultados patológicos se concentraron, en ambos grupos, en la región equina caudal y se caracterizaron por la infiltración de células mononucleares. No hubo evidencia de desmielinización, aracnoditis ni de necrosis en ninguno de los grupos. Se encontró fibrosis endoneurial focal en un animal tan sólo, perteneciente al grupo de control, a raíz de los pinchazos lumbares múltiples asociados con la paraestesia. Las características parecieron correlacionar con el trauma físico asociado con el pinchazo lumbar y no con la inyección.