Analgesia Produced by a Spinal Action of Morphine and Effects upon Parturition in the Rat

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Opiates administered into the lumbar spinal subarachnoid spaces of rats and rabbits through indwelling catheters produce dose-dependent analgesia. In the present experiments, such injections were made on the day of parturition in gravid female rats and rabbits. In rats, intrathecal injections of morphine sulfate, 15, 45, or 100 μg, were made at intervals such that significant analgesia, as measured by the tail-flick and hot-plate tests, would be maintained until parturition occurred. The injections had no detectable effect on onset of parturition, per cent of the litter alive after one hour, or respiratory rate of either the mother or the newborn. In contrast, morphine, 3, 10, or 20 mg/kg, administered subcutaneously, produced a dose-dependent decrease in newborn respiratory rates and an increase in the number of rat pups dead within the hour after birth. In rabbits, intrathecal injection of morphine, 80 mg, produced prolonged antinociception for 12–14 hours as measured by block of the skin twitch response. Such injections, however, failed to delay the onset of delivery or alter the interval between births in a litter. Respiratory rates and general appearances of newborn rabbits whose mothers had received intrathecal morphine were not different from those of controls. Plasma levels of morphine in mother and newborn were assayed at birth. Plasma morphine levels in the mothers ranged from 50 ng/ml (delivery occurring an hour after morphine injection) to 10 ng/ml (delivery occurring eight hours after morphine injection). Newborn plasma levels ranged from 0 to 2 ng/ml in the first four hours after intrathecal injection in the mother to 10 ng/ml when the delivery occurred eight hours after intrathecal injection in the mother. These data support previous work indicating the significant analgesia and the lack of effect upon respiratory rate and motor function of intrathecal morphine. They further demonstrate that intrathecal administration has no detectable effect on the initiation of gestation and the viability of the newborn or its respiratory rate. (Key words: Analgesia: measurement. Analgesics, narcotics: morphine. Anesthesia, obstetric. Anesthetic techniques, spinal. Pain: experimental, measurement.)

Recent experiments have demonstrated that opiates injected into the spinal subarachnoid space produce prolonged increases in the pain thresholds of rats,1–3 rabbits,4 cats, and primates.4 This effect is pharmacologically specific in that it is dose-dependent, stereospecific, produced by a broad family of opiate alkaloids and peptides, and is antagonized in a dose-dependent fashion by naloxone.4,5–7 Moreover, in contrast to the observed potent analgesic effects, no change has been detected in either voluntary motor function or the response to innocuous stimuli, such as light brush and touch. Such observations together suggest that these analgesic effects result from an action on opiate receptors known to be within the dorsal horn of the spinal cord,7 and that these receptors are associated with local spinal systems mediating the throughput of noxious somatosensory information. Of particular importance is that the observed analgesia is also unaccompanied by significant changes in either autonomic function (blood pressure, heart rate and respiration) or general behavior (depression, catalepsy, changes in feeding or drinking), which are commonly observed following the systemic administration of narcotics.8 This apparent selectivity is consistent with the fact that neural structures directly mediating these behavioral phenomena are located within the brain stem. The relevance of this spinal action of opiates, so clearly evident in the animal models, has been demonstrated clinically in man by Wang and colleagues9 and by Ventafridda.10 These investigators have shown that morphine administered via a lumbar puncture will produce remarkable relief for periods of 24 to 48 hours in patients who have metastatic cancer. As in the animal experiments, these effects were generally unaccompanied by alterations in voluntary motor function or autonomic side effects.

These results led us to consider the possibility that such a local action of opiates might serve as a useful clinical tool in controlling the pain of parturition. Two questions, however, are of prime importance in establishing whether such an intrathecal administration would be permissible in the gravid female at term. First, does intrathecal morphine hinder the delivery of the fetus? Secondly, does intrathecal morphine have any deleterious effect upon the viability of the newborn? To address these questions in an animal model, we examined the effects of intrathecal morphine on parturition in the rat and rabbit.

Materials and Methods

Multiparous female Sprague-Dawley rats (220–250 g) and New Zealand Albino rabbits (2–5 kg)
obtained from the breeding colony were employed in these experiments. Groups of rats were housed with a single male, and vaginal smears were made every morning at 0800. Twelve days following the observation of sperm and with development of visible signs of pregnancy, intrathecal catheters were implanted in these rats as described below. Following catheter implantation the rats were placed in individual maternity cages with wire screen floors until delivery. On the day of delivery, litter for nest building was provided. Animals that conceived at the same time were treated as a group, in that all animals received catheter implants within the same six-hour period (on the twelfth day). Rats were housed with a single male and a minimum of three episodes of intromission observed within a three-hour period was taken as evidence of mating. Eighteen days later these mated rabbits had intrathecal catheters implanted as described below and were housed separately. On the projected day of delivery a nesting box was provided in the cage.

To permit the intrathecal administration of drugs into the spinal subarachnoid spaces of the unanesthetized and unrestrained rat and rabbit, polyethylene catheters (pe-10) were placed down the subarachnoid space through an incision made in the atlanto-occipital membrane. The catheters extended 8.5 and 30 cm in the rat and the rabbit, respectively, depths shown to correspond by roentgenograms and dissection to the rostral portions of the lumbar enlargements. In the rat, these catheters were secured to the skull by placing a ligature through the two holes drilled in the parietal bone, and in the rabbit, by skull screws and cranioplast cement. Following closure of the incision, the catheters were flushed with sterile saline solution and the animals permitted to recover. Other details of the implantation procedure for the rat and rabbit are given elsewhere. In preliminary experiments, the operative procedure, carried out at this time, failed to have any effect upon the size or viability rate of control litters.

Morphine sulfate dissolved in sterile saline solution was administered intrathecally in volumes of 15 and 300 μl to the rat and rabbit, respectively, followed by an injection of saline solution, 10 μl (rat), or 100 μl (rabbit), to clear the catheter of remaining drug and disperse the injectate over the surface of the cord. These injection techniques have been shown to distribute dye and radiolabel uniformly over a distance of 3-4 cm, effectively reaching the lumbar and sacral portions of the cord without affecting cervical or brain-stem regions.

The hot-plate and tail-flick tests were used to assay for the analgesic action of intrathecal morphine in the rat. In the hot-plate test, the rat was placed on a 55-C aluminum surface surrounded by a plexiglass wall. The measured endpoint was the characteristic, species-specific, response of licking the hindpaw. In the tail-flick test, the rat's tail was placed over a slit through which showed a 300-watt projection bulb. The endpoint was removal of the tail. Lack of any response after 30 sec on the hot plate or 5 sec on the tail flick was cause to terminate the trial, and the animal was assigned that value as a response time. To assay the antinociceptive effects of intrathecal morphine in the rabbit, the latency of the skin twitch reflex evoked by a 70-C heat probe (surface area of 1 cm²) touched lightly to a depleted region of the flank was measured. This is a local reflex, characterized by a regional contraction of the skin in the vicinity of the probe. In the absence of a response, the heat probe was removed at 15 sec to prevent blistering. To permit comparisons, a normalizing procedure was employed where the measures were converted to maximum percentage effect (MPE), where:

\[ MPE = \frac{\text{postinjection response latency} - \text{predrug response latency}}{\text{cut-off time} - \text{predrug response latency}} \times 100 \]

Cut-off times are equal to 5 and 30 sec for the tail-flick and hot-plate test responses, respectively.

To assess the time course of the analgesic effect in female rats, morphine, 15, 45, and 100 μg was administered intrathecally to nongravid animals and the tests repeated at hourly intervals until a return to baseline level was observed (3.5 to 6.5 hours). The lowest dose employed in these experiments (15 μg) was sufficient to produce a reliable blockade of the hot-plate and tail-flick test responses (100 per cent effective dose, \( ED_{100} \)). For comparison, nongravid female rats in a second group were given morphine 3, 10, or 20 mg/kg, subcutaneously, and the time courses similarly examined. Results obtained from the intrathecal and subcutaneous morphine experiments were then used to estimate the maximum interdose interval that would maintain a minimum 40 per cent increase in the nociceptive threshold. In addition to changes in the nociceptive thresholds, respiratory rates and general behavior were recorded.

The injection sequence for the pregnant animals was begun three to four hours after the initiation of nest-building by the individual animals. Subsequent injections were made at intervals corresponding to
the maximum interdose intervals for that dose and route of administration of the drug. This was continued until delivery. As mentioned above, three to five pregnant rats with the same projected delivery date were received at the same time and operated upon together. To prevent any consistent bias from being associated with a single dose, an effort was made to give at least one of the three intrathecal doses to one rat of the group. Animals were checked every one and a half to two hours after the start of the injections to determine the approximate time of onset of delivery and to record the numbers of live and dead pups. Respiratory rates, appearances, and general behavior of the pups were recorded at this time.

The time course of the antinociceptive effects of intrathecal injection of morphine, 80 µg, was assessed in four nongravid female rabbits. This dose has been shown to produce a prolonged increase in the nociceptive threshold of the cat and primate. On the projected day of delivery, a nesting box was provided for each animal and the rabbits observed for signs of chest-hair pulling, a preliminary sign generally preceding the onset of delivery by 8–12 hours. An intrathecal injection of morphine was then made. Within 5 min of delivery, the respiratory rates and general appearances of the newborns were assessed. At this time, the newborns were quickly anesthetized with ether and a blood sample taken by cardiac puncture. A blood sample from the mother was taken just after the last birth by an ear-vein puncture, and the plasma frozen. Morphine levels were ascertained by radioimmunoassay. The absolute sensitivity of this assay is 300–500 pg.

Statistical comparison were made by use of the Student t test, and differences of P < 0.05 were considered significant.

Table 1. Approximate Times of Onset of Delivery after the Initiation of Nest-building as a Function of Treatment with Saline Solution or Intrathecally or Subcutaneously Administered Morphine Sulfate

<table>
<thead>
<tr>
<th>Group Treatment*</th>
<th>Time of Onset of Parturition (Hour)</th>
<th>Median Number of Injections</th>
<th>Total Amount of Morphine Group Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls, no injection</td>
<td>5, 8, 10, 11</td>
<td>8.5</td>
<td>—</td>
</tr>
<tr>
<td>Controls, saline-injected</td>
<td>3, 6, 8, 9</td>
<td>6.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Intrathecal morphine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 µg</td>
<td>4, 6, 6, 10</td>
<td>6.8</td>
<td>3</td>
</tr>
<tr>
<td>45 µg</td>
<td>3, 8, 9, 12</td>
<td>8.0</td>
<td>3</td>
</tr>
<tr>
<td>100 µg</td>
<td>2, 5, 7, 10</td>
<td>6.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Subcutaneous morphine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>6, 6, 9</td>
<td>7.0</td>
<td>7</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>6, 9, 11</td>
<td>8.7</td>
<td>7</td>
</tr>
<tr>
<td>20 mg/kg</td>
<td>10, 12, 13</td>
<td>11.7</td>
<td>5</td>
</tr>
</tbody>
</table>

* Injection schedule described in the text.
† Mean weights in the three groups receiving subcutaneous injections of morphine were 255, 270, and 260 g, respectively.
Fig. 2. Histogram plot showing mean percentages with SE of litters surviving during the hour after parturition. The bottom number in each histogram block gives the number of litters, while the top number of each pair indicates the total number of rat pups living and dead. Histogram A presents the control group, which consists of unimplanted control rats and rats implanted with intrathecal catheters who received intrathecally administered saline solution. Histograms B, C, and D indicate groups that received morphine intrathecally in doses of 15, 45, or 100 µg as indicated. Histograms E, F, and G represent groups that received morphine subcutaneously in doses of 3, 10, and 20 mg/kg. The schedule governing the times of injections for each group is given in the text. (+) indicates P < 0.05 difference from control.

Results

In non-gravid female rats the duration of the effect of intrathecally administered morphine was dose-dependent, with the approximate times needed for the effect to diminish to the 40 per cent level of analgesia being 2.5, 3.5, and 6.5 hours, respectively for the three increasing doses of the drug (fig 1). Intrathecally administered morphine at these doses had no effect on respiration. With the highest dose of morphine only, there was some evidence of motor rigidity and an enhanced startle reflex, with exaggerated sensitivity to light touch in three of seven animals. All of the effects produced by intrathecally administered morphine were completely antagonized by naloxone, 1 mg/kg, intraperitoneally. The subcutaneous injections of morphine also produced a dose-dependent increase in the nociceptive threshold. The times needed for the analgesic levels to return to 40 per cent were 0.7, 1.3, and 2.4 hours, respectively. Unlike the effects of intrathecally administered morphine, subcutaneously administered morphine produced characteristic signs of catalepsy (tonic immobility) and exophthalmos, particularly at the highest dose. Also of significance was a biphasic change in the respiratory rate, with the lowest dose producing a slight increase in rate (+6 ± 2) while the two higher doses decreased rate (−7 ± 2 and −13 ± 4).

Neither saline solution nor morphine given intrathecally altered the time of onset of birth from that occurring in the control group (table 1). Estimates of possible effects of intrathecal administration of morphine on the duration of the birth process are difficult to make, as litter sizes differ and, in many cases, the exact time of onset of delivery or when the last fetus was expelled is unknown. The amount of morphine necessary to produce a sustained increase in nociceptive threshold is significantly greater when morphine is given subcutaneously as compared with intrathecally (table 1). For example, to produce an increase in the nociceptive threshold for two and a half hours, the systemic-to-intrathecal dose ratio was 480:1. This large difference is accounted for not only by the fact that smaller doses were given with each intrathecal injection, but because the total number of intrathecal injections was less (median numbers of doses given were three for the 15-µg dose versus seven and five for the 10 and 20 mg/kg doses, respectively) reflecting the longer duration of action for even the lowest intrathecal dose.

The percentages of the litters that were alive 60 to 150 min after birth were not different for controls without implanted catheters and saline-injected controls. In this combined control group, 98 per cent of the newborns of each litter survived the first hour after birth (fig. 2). No significant change in the viability index was produced by any dose of intrathecally administered morphine. In contrast, morphine given subcutaneously produced a dose-dependent decrease in the viability index. Likewise, intrathecal administration of morphine produced no detectable effect upon newborn respiratory rate, as compared with saline-injected controls or controls receiving no injec-
tion, while all doses of subcutaneously injected morphine produced some evidence of newborn respiratory depression (fig. 3). In accord with the respiratory effects, morphine given subcutaneously produced a clear depression of the behavioral reactivity of the newborn rat pup. These pups were often ashen in color and showed little vocalization. In contrast, the appearances and behaviors of newborn rat pups whose mothers had received morphine intrathecally were indistinguishable from those of controls. In general, these animals showed considerable vocalization (meowing) and arching of the back in response to touching with a probe and turning.

Morphine, 80 μg, given intrathecally produced a prolonged blockade (10–14 hours) of the skin twitch response in four non gravid does (fig. 4). During this interval the animals evidenced no sign of discomfort or change in respiratory rate. Newborn rabbits of five does given morphine, 80 μg, intrathecally did not show any change in respiratory rate or the intervals between births in individual litters (fig. 5), regardless of the time interval from injection to birth. There is no evidence that morphine either delayed the onset of parturition or increased the birth interval. Maternal blood levels of morphine following intrathecal injection did not exceed 30 ng/ml at birth, with a decline after four hours to about 10 ng/ml. Fetal levels were near zero until eight hours, when they became equilibrated with the levels in maternal blood. Consistent with the low levels of morphine, newborn rabbits whose mothers had received intrathecally administered morphine were behaviorally and physically indistinguishable from control animals.

Discussion

The results of the present experiments indicate that morphine, in doses that were as much as seven times that required to block completely the animal's behavioral response to strong thermal stimuli, had no detectable effect upon the initiation of gestation in the gravid rat or rabbit, on the viability of the newborn, or on respiratory rate or apparent voluntary motor function in the newborn.8,3 Even the highest dose of intrathecally administered morphine did not depress the mean respiratory rate of the newborn rat and rabbit. These observations are consistent with the normal appearance of the newborns, as well as their general level of behavioral reactivity. These observations stand in contrast to the effects produced by systemically administered morphine. Even at a dose that failed to exert a reliable ED50 effect (3 mg/kg), there was a clear depression in respiratory rate of the rat newborn. Higher systemic doses necessary to produce maximum antinociceptive effects also produced significant decreases in both respiration and the viability index at parturition.

Injecting morphine into the mothers at fixed intervals, prior to a complete diminution of the effect, produced a cumulative dose effect. The present paradigm should maximize the negative aspects of the route of administration over time by increasing the effective drug levels. This, therefore, serves to emphasize the apparent safety factor associated with the intrathecal route as compared with the systemic route of administration.

The lack of effect of intrathecally administered morphine on fetal viability in general and respiratory rate in particular correlates with the fact that morphine, having a low lipid partition coefficient, is cleared only slowly from tissue.13 In the present experiments, blood levels following intrathecal administration did not exceed 20–30 ng/ml at delivery. These results are in agreement with findings in other experiments carried out by us in male rabbits and cats. In these experiments, the blood levels observed following an equianalgesic dose of morphine given subcutaneously are on the order of 50 to 100 times higher than those found with intrathecal injection (unpublished observations). Thus, potent analgesia can be obtained by intrathecal administration, which is accompanied
by a low blood level of morphine. As there is no evidence that the fetus concentrates opiates in its circulation, it is reasonable to expect that its blood level will be low, not exceeding that of the mother. This speculation is borne out by the results of rabbit experiments in which plasma levels following intrathecal morphine were examined in the newborn. In any case, in the clinical work reported by Wang and colleagues and by Ventafredda, morphine sulfate, 0.5 mg, is an adequate dose for providing appreciable analgesia. By comparison, for pain relief in obstetrics as much as 20 times that amount is needed following intrathecal injection (6-10 mg). Thus, even should all of intrathecally injected morphine gain access to the circulation, the final dose would still be less than that normally employed systemically.

The slow clearance of morphine from the intrathecal space contributes to its prolonged duration of action. Exposure of the drug to peripheral metabolism, the distribution of the drug throughout the entire body mass, and its excretion account concurrently for the high doses needed and relatively shorter duration of the effect following systemic administration.

We believe that the effects produced by morphine following intrathecal injection result from an action on a specific receptor entity located within the spinal cord, and not a general action on nerve membranes.

Fig. 5. The effects of either morphine, 80 μg (closed circles), or saline solution (open circles) administered intrathecally to five rabbits on respiratory rates and birth intervals of litters. The abscissa is the time after the intrathecal injection when the birth of each litter occurred with the times at which the several measures were taken. The bottom panel shows the blood levels of morphine (ng/ml) of the mother (●) and her newborn (○) immediately postpartum. The blood sample taken from one newborn delivered at the eight-hour time was deleted from these results due to contamination.
such as occurs with local anesthetics. Although the underlying neural systems associated with this effect are not yet known, it has been established that opiates, by a direct effect on the spinal cord, will produce a dose-dependent decrease in the discharge from spinal nociceptive neurons which is stereospecific and antagonized by naloxone.15-18 The presence of opiate binding in the substantia gelatinoa of the dorsal horn,7 presumably reflecting opiate receptor sites on the primary afferent terminals,18,25 suggests that this region may be the locus of the action. This region is also of particular significance inasmuch as anatomically it has been shown to be the site of termination of primary afferents.21 It also has a high concentration of substance P, a small peptide thought to be associated with high-threshold primary afferents mediating the input of nociceptive information,22-24 and whose release in vitro is blocked by morphine.25

Whether the intrathecal action of opiates will serve to attenuate the pain of labor in man cannot be answered by the present experiments. The sources of pain during the three stages of labor are complex. Nevertheless, it is certain that a major route of pain sensation in labor is via somatic nerve pathways that lead through synaptic connections within the spinal cord. It is known that morphine can depress the potentials evoked by splanchnic-nervous stimulation,26 and in recent experiments we have found that intrathecal administration of morphine can antagonize the whirling response observed in rats following the intraperitoneal injection of phenylquinone (Yaksh, unpublished data). These findings strongly suggest that opiates with an action limited to the spinal cord can in fact antagonize the nociceptive input derived from visceral sources.

In sum, the present experiments have shown that intrathecal administration of opium in doses that produce prolonged analgesia in the gravid rat and rabbit has no detectable effect upon the onset of parturition, the respiratory rate of the mother, or the viability, respiratory rate, or general appearance of the neonate.

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