Distribution of Morphine and Meperidine after Intrathecal Administration in Rat and Mouse

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Morphine and meperidine distribution in the neuroaxis were studied in rats after intrathecal injection through catheters ending at the lumbar level. 14C-Morphine and 3H-meperidine were injected with pharmacologic doses of each drug. Radioactivity was measured in spinal cord segments at different times. At 14 min the segment with maximum morphine concentration (T11–12) contained 8.6 ± 2.4 (mean ± SD) pmol/mg, a value 215 times higher than would be observed if distribution in the body were homogeneous. The ratio between concentration in the most rostral segment (C3–4) and in the segment with maximal concentration was 0.21 ± 0.10. At 14 min the segment with maximum meperidine concentration (T9–10) contained 161.4 ± 33.9 pmol/mg wet tissue, a value 75 times higher than would been seen with even distribution in the body. The ratio (C3–4 vs. T9–10) was 0.10 ± 0.04 at this time.

The distribution of 14C-morphine in the whole central nervous system (CNS) was studied in mice by whole body autoradiography after intrathecal injections of 5 μl at the L5–6 level. High levels of radioactivity were detected in the whole spinal cord and in brain regions close to the basal cisterns until 2 h after injection. At 4 h only the caudal part of the spinal cord had detectable levels of radioactivity.

The percent of the injected dose of morphine that was recovered from the spinal cord was 26.5 ± 4.5 at 14 min, 19.9 ± 8.8 at 44 min, and 4.5 ± 1.7 at 179 min after injection. The figure was 6.9 ± 1.8 at 14 min and 2.2 ± 0.7 at 44 min for meperidine. It could be postulated that the hydrophilic drug morphine persists for longer in the spinal cord, whereas the lipophilic meperidine is rapidly taken up by and eliminated, resulting in lower recovery. The spread of morphine into the basal cisterns is consistent with the clinical risk of ventilatory depression. (Key words: Analgesics: meperidine; morphine. Anesthetic techniques: spinal narcotics. Measurement techniques: autoradiography. Spinal cord: drug distribution.)

The demonstration of potent opioid analgesia after intrathecal drug administration in animals1,2 has led to extensive use of opioids for spinal analgesia (intrathecal and epidural) in humans.3,4 The duration of analgesia in both animals and humans seems to be inversely related to the lipophilic properties of the drugs.5,6 Central nervous side effects such as nausea, vomiting, and occasionally ventilatory depression have been observed in human hours after injection of epidural doses of morphine.7,8 The incidence of ventilatory depression seems to be higher for morphine than for more lipophilic drugs such as meperidine, fentanyl, and methadone, but convincing comparisons are lacking.5,8

Since spinal opioid analgesia is mediated by opioid receptors in the spinal cord, it is essential to characterize the uptake and elimination of the drugs from the spinal cord after intrathecal injections. A technique using intrathecal administration of radioactively labeled compounds in the rat made it possible to compare the uptake in the spinal cord of meperidine and morphine. The distribution of morphine in the whole central nervous system (CNS) was studied by whole body autoradiography after intrathecal injection in mice.

Materials and Methods

Distribution of Morphine and Meperidine

Surgery. Rats weighing 270–340 g (Sprague-Dawley, ALAB Sollentuna, Sweden) were implanted with a 8.5–9 cm PE 10 intrathecal catheter.9 To verify that the tip of the catheter was positioned in the lumbar subarachnoidal space, 4–6 days after surgery 10 μl lidocaine (Xylocaine® 50 mg/ml, Astra Läkemedel AB Södertälje, Sweden) was injected, followed by 15 μl 0.9% saline. All animals demonstrated a bilateral motor paresis of the hind legs were included in the study. Approximately 80% of the operated animals fulfilled this inclusion criterion.

Preparation of Radioactive Material. The specific radioactivity of 14C-morphine (Amersham Radiochemicals, Ltd., Amersham, England) was 56 mCi/mmole and the radiochemical purity 98%.

Meperidine–HCl was labeled with tritium under high pressure in the aromatic ring of the molecule (Amersham). The product was purified by thin-layer chromatography (TLC) with a mobile phase of methanol/chloroform 20:80% (v/v) with triethylamine added to a concentration of 1%. The identity of meperidine was confirmed by simultaneous chromatography of a reference control sample. The radioactive material then was extracted from silica plates by methanol/chloroform 20:80% (v/v) with...
triethylamine 1%. Using TLC with this mobile phase, meperidine was more than 95% pure.

Intrathecal morphine. A total dose of 13.3 nmol morphine (5 µg of morphine · HCl·(H₂O)₅) with 0.14 µCi ¹⁴C-morphine was administered through the intrathecal catheter with a standardized procedure. Isotope with parent compound was given in 10 µl 0.9% saline followed by a volume of 15 µl 0.9% saline to flush the catheter. The two solutions were separated by a 1 µl air bubble. The injection was performed over 20 s. The dose we used had shown anti-nociceptive effects in both the hot plate and tail-flick tests up to 60 min after injection, with a maximal effect at 30–45 min.² The rats were killed at 14 (n = 7), 44 (n = 8), or 179 (n = 8) min using diethyl ether inhalation. Death usually occurred within 2 min.

Intrathecal Meperidine. An intrathecal dose of 705 nmol meperidine (200 µg of meperidine · HCl with 0.13 µCi $^3$H-meperidine as tracer dose) was administered, since that dose had shown an anti-nociceptive effect in both the hot plate and tail-flick tests.² The injection procedure was as above. The rats were killed at 14 (n = 7) and 44 (n = 5) min after injection.

Tissue Preparation. The spinal cord was dissected free after bilateral laminectomy. The position of the catheter tip was confirmed by injecting 10 µl of a 1% trypan blue solution before the cord was excised. Since the aim was to quantify uptake of drugs to the spinal cord, it was rinsed to remove morphine not taken up. This was done twice in physiologic saline solution for a total of 10 s. The whole cord then was divided into 5 mm segments, each weighing 20–40 mg. A volume of 1 ml of Soluene-350® (Packard Instrument Co., Downersgrove, Illinois) was added to each tissue segment. The samples were digested at 50°C after which 10 ml of a toluene solution containing Permablen® (Packard) 5.5 g/l was added. The radioactivity was determined in a liquid scintillation spectrometer with external standard ratio correction of quenching (Packard model 1530). The counting efficacy was 85% for ¹⁴C and 45% for $^3$H.

The elapsed time between death and division of the segments was 18 ± 14 (mean ± SD) min for morphine and 22 ± 11 min for meperidine.

Calculations. The concentration (pmol/mg wet weight) for each cord segment was calculated on the basis of the specific radioactivity and expressed as mean ± SD.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>15</th>
<th>16</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Morphin (n = 7)</td>
<td>0.27 ± 0.15</td>
<td>0.22 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>Meperidine (n = 7)</td>
<td>0.18 ± 0.11</td>
<td>0.12 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>44</td>
<td>Morphin (n = 8)</td>
<td>0.28 ± 0.14</td>
<td>0.23 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>Meperidine (n = 5)</td>
<td>0.10 ± 0.03</td>
<td>0.09 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>179</td>
<td>Morphin (n = 7)</td>
<td>0.31 ± 0.09</td>
<td>0.30 ± 0.09</td>
</tr>
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</table>

Ratios of concentration in the most rostral (15, 16, 17) segments versus that of the segment with maximal concentration:

Means ± SD.

Statistical test was performed between ratios for meperidine and morphine at the same segmental level.
FIG. 2. Whole body autoradiogram of a mouse injected with 17.6 nmol $^{14}$C-morphine intrathecally at L5-6 level and killed at 15 min. Exposed area indicates isotope. The same section is stained with hematoxylin and eosin. Indicated structures are cisterna magna (1), foramen Magendie (2), falk cerebri with adjacent subarachnoid space (3), trigeminal nerve (4), olfactory bulb with striae (5), submandibular gland (6), lymphatic vessel (7), and liver (8).
Fig. 3. Details from whole body autoradiograms of mice injected with 17.6 nmol $^{14}$C-morphine intrathecally at L5–6 level and killed at 5, 15, 60, 120, or 240 min after injection. Details of interest arranged in two columns: brain and rostral part of the spinal cord (3A, opposite page) and lumbar cord (3B, above). Exposed area indicates isotope. Hematoxylin and eosin stained sections also are shown. Indicated structures are cisterna magna (1), foramen Magendie with fourth ventricle (2), lymphatic vessel (3), dura mater and subarachnoid space (4), central core of spinal cord (5), activity in central part of the brain (6), olfactory bulb with striae (7), place of injection (8), spinal ganglion with a nerve root (9), nerve roots (10).

The calculated concentration (pmol/mg) in each segment was divided by the dose per body weight. This ratio gives the spinal cord concentration compared with that expected if distribution of the drug were homogeneous.

Ratios between the concentrations of the most cranial segments versus those with maximal concentrations were calculated after administration of drug. A one-sided Mann-Whitney U-test$^{10}$ was used to test if the ratios for morphine were higher than those for meperidine at the same segmental level. Level of significance was taken to be $P < 0.05$.

**Whole-body Autoradiography**

*Animal Experiments.* $^{14}$C-Morphine (see above) was dissolved in physiologic saline to a concentration of 1.32 mg/ml. Five male albino mice (NMRI, Anticimex, Stock-holm, Sweden) weighing 22.8–24.7 g (mean 24.1 g) were given an intrathecal injection of 17.6 nmol (6.6 μg in 5 μl) of the $^{14}$C-morphine (0.98 μCi/animal) between vertebrae L5 and L6, according to an established single-injection technique.$^{11}$ The animals were anesthetized in diethyl ether and killed by immersion in hexane cooled to $-70^\circ$ C with solid CO$_2$ at 5, 15, 60, 120, or 240 min after injection.

*Autoradiography.* Conventional whole body autoradiographic techniques were employed.$^5$ The animals were embedded in a 2.5% aqueous solution of carboxymethylcellulose and frozen for 10 min at $-70^\circ$ C. Sagittal sec-

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tions (20 µm) at different levels were cut at −20 °C with a cryomicrotome (LKB 2258 PMV, LKB-Produkter, Sweden). Each section was mounted on tape (No. 810, Minnesota Mining and Manufacturing Co.) and dried at the same temperature for 2–3 days. The tape mounted sections were pressed against x-ray film (Structurix D7®, Agfa-Gevaert, Belgium) and stored at −20 °C for 7 days. The exposed film plates were developed in Agfa-Gevaert G 250 x-ray developer for 5 min at 20 °C and fixed in Agfa-Gevaert G-305.

Of the 40 sagittal sections cut for each mouse, one section was chosen to represent the uptake of drug into brain and rostral part of the spinal cord and one for the lumbar area.

Hematoxylin and Eosin Staining. After autoradiography, the selected sections were stained in Mayer’s hematoxylin solution and then contrast stained with 0.5% solution of eosin. Afterward they were mounted and dried.

Results

Distribution of Intrathecal Morphine and Meperidine in Rats

The tip of the catheter had been placed at the level corresponding to vertebrae T12–13 or T13–L1 (the rat has 13 thoracic vertebrae and five lumbar).

Morphine was distributed fairly evenly from the lumbar to the middle of the thoracic level (T5–6) at all times after injection (fig. 1A). The segment with maximal concentration (mean) of morphine contained 8.6 ± 2.4 pmol/mg at 14 min and 6.8 ± 5.4 pmol/mg at 44 min. The total amount of the injected dose recovered in the spinal cord was 26.5 ± 4.5% at 14 min, 19.9 ± 8.8% at 44 min, and 4.5 ± 1.7% at 179 min.

Meperidine was distributed less uniformly in the spinal cord, as shown in figure 1B. The segment with maximal concentration (mean) of meperidine contained 161 ± 34 pmol/mg at 14 min and 47 ± 16 pmol/mg at 44 min. At 14 min, only 6.9 ± 1.8% of the meperidine dose was recovered from the spinal cord and 2.2 ± 0.7% at 44 min.

The ratio of morphine concentration in the most rostral segment (no. 17) compared with that in the segment with maximal concentration was 0.21 ± 0.10 at 14 min and 0.21 ± 0.07 at 44 min (table 1). For meperidine, the corresponding figures were 0.10 ± 0.04 and 0.06 ± 0.02, respectively. These ratios were significantly higher for morphine than those for meperidine (P < 0.05, table 1).

The maximal mean concentration of morphine in the cord was 215, 170, and 40 times higher at 14, 44, and 179 min than if the drug had been homogeneously distributed in the body at zero time. The corresponding figures were lower for meperidine, being 75 and 30 times higher at 14 and 44 min, respectively.

Whole Body Autoradiography

Morphine disposition in the brain and thoracic part of the spinal cord is shown at 15 min after injection (fig. 2). The spinal cord and the ventral parts of the brain contained high radioactivity. Low levels of radioactivity were found in all other parts of the body.

Figure 3 depicts regions of interest over time. High levels of radioactivity were observed in the cisterna magna and in adjacent brain regions at 5 and 15 min. In the more central parts of the brain, radioactivity still was found at 1 and 2 h after injection (cf arrow 6) but not at 4 h. There was isotope in the lumbar region as well as in individual nerve roots at 4 h after injection. The volume of the urinary bladder increased over time, with maximal size at 4 h.

Discussion

High concentrations of both morphine and meperidine in the spinal cord were produced after intrathecal administration compared with those that would have been attained with the drugs distributed evenly in the body. However, the percentage recovery of morphine in the spinal cord was higher than for meperidine at all times after injection. The differences in distribution are most probably related to physicochemical characteristics, with morphine being less lipophilic than meperidine. A lipophilic compound would be expected to rapidly be removed from the spinal cord, and the opposite is expected for a hydrophilic drug. Early removal from the brain has been demonstrated previously for lipophilic opioids injected intraventricularly.

An inverse relationship between lipophilicity and duration of experimental analgesia after intraventricular administration has been shown, although the time of onset was shorter for the more lipophilic compounds. This was considered to be the result of rapid penetration and corresponding rapid removal from the brain tissue of lipophilic opioids such as fentanyl and etorphine. In contrast, morphine diffused so slowly that more than 4 h was required for maximal penetration into the brain. The present study indicates that the same considerations could be valid after intrathecal injection of opioids.

As morphine was distributed more uniformly along the neuroaxis than meperidine (cf. figs. 1A–B, table 1) it suggests that there is a higher risk of supraspinal side effects with morphine. The lipophilic meperidine is more likely to be absorbed systemically at a higher rate than morphine, leaving a lower amount to be rostrally distributed. As we always performed the injection in 20 s, bulk cerebrospinal fluid due to the injection per se would influence the distribution of morphine and meperidine to an equal extent. The observed differences therefore cannot be explained by technical differences in drug administration.
In interpreting the results, it is important to recall that the assayed radioactivity in the spinal cord should represent unchanged drug and not metabolites. In vitro experiments using human and monkey brain showed that morphine was not metabolized to the extent that the main 3- and 6-glucuronide metabolites were detected.** We therefore infer that the radioactivity in the spinal cord should represent unchanged drug. N-demethylating enzymes have been identified in the rat brain. Since the capacity for the cytochrome P-450 system of the brain to metabolize meperidine was only 1.3% of that in the liver, it is reasonable to assume that the assayed radioactivity in the spinal cord in the present investigation represents unchanged drug.

Whole body autoradiography showed that morphine distributed within the entire CNS. This spread probably occurred through the CSF, since maximal uptake was observed in tissues adjacent to the CSF system. The finding that high radioactivity of morphine was present in the trigeminal nerve (cf. arrow 4, fig. 2 and ventral parts of the autoradiograms at 15 and 60 min after injection in fig. 3A) is interesting because itching on the nose after epidural administration has been observed clinically. This side effect might result from a high uptake into the nerve. As late as 1–2 h after injection, activity was found in the brain in line with the long-lasting duration of side effects with spinally applied opioids. An intrathecal volume of 5 μl in a mouse is large compared with the total CSF volume of 50 μl. Some volume displacement therefore can occur but would be minor since local anesthetic drugs in this volume gave a pharmacologic effect restricted to the hind legs. The autoradiographic study should, therefore, in a qualitative way, describe the rostral spread of morphine.

The urinary bladder was found to increase in size over time after the injection of morphine. This probably reflects the urinary retention commonly observed after spinal administration of morphine. Such effects have not been observed after intrathecal injections of local anesthetic agents, indicating that this is a specific effect of morphine. It is unlikely that spinal cord damage after the intrathecal injection could explain this effect, since all the mice had normal motor function.

In conclusion, the recovery of morphine in spinal cord was greater than that of meperidine in accordance with data on brain uptake after intraventricular injection. Morphine also was distributed more evenly in the spinal cord than meperidine. Thus, not only intrinsic activity and receptor affinity but also variation in distribution from the spinal cord determines differences in analgesic effect after injection of spinal opiates. The technique described in this article also would be useful for the evaluation of take and elimination of adrenergic and serotonergic drugs, where analgesic effects are presumed to be mediated in the spinal cord.18

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