A METHODOLOGICAL STUDY OF SPINAL (SUBARACHNOID) ANAESTHESIA IN THE RAT AND THE MOUSE

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Experimental models for spinal (subarachnoid) anaesthesia have been described in various animal species, including sheep (Lebeaux, 1975) and dog (Feldman and Covino, 1981). A technique in the monkey has been developed (Denson et al., 1981, 1982) which is of value in studying primate physiology and the pharmacology of spinal anaesthesia. There is a need, however, for a technique in small animals to permit the study of the efficacy of new local anaesthetic agents in relation to controls, and the basic problems of spinal anaesthesia. The present report describes the spinal anaesthetic effect of some commonly used local anaesthetics in the rat and mouse, following modifications to the techniques for intrathecal injection described originally by Yaksh and Rudy (1976) and Hylden and Wilcox (1980). The two methods may prove to be complementary to techniques in larger animals.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Anticimex, Sweden) weighing 300–400 g were anaesthetized with methohexitone 40 mg kg\(^{-1}\) i.p. After removal of hair on the neck, an incision was made in the midline extending caudally about 1 cm from the occipital crest. A slit was made in the exposed cisternal membrane, followed by the insertion of a polyethylene catheter filled with lactated Ringer's solution. The catheter was advanced caudally in the subarachnoid space to the level of the lumbar enlargement, as described by Yaksh and Rudy (1976) and Yaksh and Wilson (1979). To avoid damage to spinal tissue, the diameter of the portion (9 cm) of the catheter (PE10) to be implanted had been decreased further by immersion in hot water and extension. The catheter was sutured to surrounding tissue. The skin incision was sutured or closed with Locktite. The external part of the catheter extended freely (7–8 cm) and its end was closed by heating.

Following the operation, and throughout the experimental period, the animals were housed singly in Macrolon cages on Torrax bedding (aspen wood cuttings, Anticimex, Sweden) with free access to food (rat pellets, Ewos AB, Sweden) and water. The animals were kept in a temperature-controlled (10–22 °C), ventilated (17 air changes per hour) and artificially lighted room with a relative humidity not less than 55%. All experiments described below were performed at room temperature (22 °C) between 8 a.m. and 3 p.m. The studies were approved by the Committee for Ethical Issues of the Swedish Board of Agriculture.

SUMMARY

Rat and mouse were utilized as models to study the spinal (subarachnoid) anaesthetic effects of five commonly used local anaesthetic agents. Duration and frequency of motor and sensory block, and onset time were determined after injection of 5.0% lignocaine, 0.75% bupivacaine and 1.0% amethocaine to the same groups of rats with chronically implanted catheters in the lumbar subarachnoid space. Dose–response curves for lignocaine, mepivacaine, bupivacaine, amethocaine and cinchocaine were obtained after single intrathecal injection to the mouse. The relative potency and other characteristics of the compounds investigated were in agreement with results obtained in other species, including man. The techniques described may provide useful adjuncts to methods in larger animals for the evaluation of potential new spinal anaesthetic agents, and the study of various factors that may influence spinal anaesthesia.

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Rat model

After a recovery period of 1 week, the rat was slightly restrained in a plastic holder and 5.0% lignocaine 20 μl injected at a rate of approximately 1 μl s⁻¹ followed by lactated Ringer's solution 10 μl to flush the catheter of its content of drug. The anterior end of the holder was elevated to allow the injected solution to flow down the subarachnoid space, thereby avoiding rostral spread and possible toxic reactions. One minute after the end of the injection the animal was removed from the holder and the degree of local anaesthesia assessed. Complete motor blockade was defined as inability to stand on the hind-limbs. Complete sensory blockade was considered present if the animal did not vocalize or attempt to withdraw the hind-legs in response to pinching of the foot-pads with Allis tissue forceps. The time of onset and the duration of complete blockade, and the time taken to the return of normal motor and sensory function, were measured at regular intervals. In the present study, two of 10 operated rats displayed unilateral blockade and were excluded from further trials. Bilateral, reversible motor and sensory blockade followed the injection of lignocaine in the remaining eight rats and, at post-mortem, was found to be directly correlated with proper positioning of the tip of the catheter in the subarachnoid space. In six rats, the injection of lignocaine was followed at 5-day intervals by the administration of 0.75% bupivacaine and 1.0% amethocaine and cinchocaine. All agents were obtained from Astra Lakemedel AB, Sweden. Solutions of the hydrochloride salts were made in physiological saline on the day of the experiments with adjustment of pH to 6.5–7.0. Commercial lignocaine 50 mg ml⁻¹ heavy spinal solution was used in the experiment in the rat.

Statistical significance was determined by means of Student's t test (P ≤ 0.05).

Mouse model

Intrathecal injection in the mouse was carried out as described by Hylden and Wilcox (1980). Before spinal anaesthesia in the conscious animal (male NMRI mice, 20–25 g, Anticimex, Sweden), 0.1 ml of prilocaine 5 mg ml⁻¹ was injected intracutaneously to produce dermal analgesia of the sacral region before a 1.0-cm cutaneous incision was made perpendicularly to the vertebral column. A 30-gauge, 0.5-inch needle (B.D. Yale) connected to a 20-μl luer tip syringe (Hamilton) was inserted to the sub-arachnoid space. The local anaesthetic solution was injected to L5–6 in a volume of 5 μl. Inability to stand on the hind legs was taken as the criterion of complete motor blockade, and the mouse was considered recovered when it could walk and grip normally with the toes. After the time of onset of motor blockade was recorded, the skin incision was closed and the presence of blockade tested at regular intervals until normal function had returned. Signs of adverse reactions to the injection were recorded. Each compound was tested at varying concentrations in groups of eight or 10 mice.

The following local anaesthetics were used: lignocaine, mepivacaine, prilocaine, bupivacaine, amethocaine and cinchocaine. All agents were obtained from Astra Lakemedel AB, Sweden. Solutions of the hydrochloride salts were made in physiological saline on the day of the experiments with adjustment of pH to 6.5–7.0. Commercial lignocaine 50 mg ml⁻¹ heavy spinal solution was used in the experiment in the rat.

Statistical significance was determined by means of Student's t test (P ≤ 0.05).
TABLE I. Spinal anaesthesia in the rat. Time (min) of onset and duration of blockade after subarachnoid injection of 20 µl of the test solution into a group of six animals. Each value is the mean ± SEM from 12 legs. Blockade was 100% except *11/12. Significant differences: † v. bupivacaine and amethocaine; ‡ v. amethocaine; ‡‡ v. bupivacaine

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concen (%)</th>
<th>Motor blockade</th>
<th>Sensory blockade</th>
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<tr>
<td></td>
<td></td>
<td>Complete block</td>
<td>Duration to recovery</td>
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<tr>
<td>Lignocaine</td>
<td>5.0</td>
<td>1.2 ± 0.2</td>
<td>20.0 ± 1.1†</td>
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<tr>
<td>Bupivacaine</td>
<td>0.75</td>
<td>1.9 ± 0.3*</td>
<td>29.6 ± 2.7‡</td>
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<tr>
<td>Amethocaine</td>
<td>1.0</td>
<td>1.6 ± 0.2</td>
<td>40.4 ± 4.2</td>
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RESULTS

The subarachnoid injection of 5.0% lignocaine 20 µl, 0.75% bupivacaine 20 µl or 1.0% amethocaine 20 µl to rats produced spinal anaesthesia in a readily reproducible manner. The duration of motor blockade increased in the order lignocaine, bupivacaine and amethocaine (table I), the duration of motor blockade with amethocaine being about twice as long as that with lignocaine. In regard to sensory blockade, bupivacaine gave the longest duration of action, and lignocaine the shortest.

The onset of blockade with amethocaine and bupivacaine was similar, but took somewhat longer than lignocaine. All blocks were completely reversible and there were no overt adverse reactions.

The local anaesthetics studied were of two categories in regard to spinal anaesthetic potency (motor block) in mice (fig. 1). One group consisting of lignocaine and mepivacaine had the minimum blocking concentration at 2.0%, which resulted in blockade of a short duration. Only a short prolongation of the duration of action was seen when the concentration was increased to 6.0%. The other group of compounds, consisting of bupivacaine, amethocaine and cinchocaine, displayed higher activity with minimum blocking concentrations at 0.125–0.25%. The compounds showed profound differences in their dose-duration curves (fig. 1).

Cinchocaine was the only agent that showed observable side-effects on spinal injection in mice. The animals reacted by jumping and scratching as well as biting the hind-quarters as signs of local irritation. With 1.0% cinchocaine, CNS-toxicity in the form of a transient loss of the righting reflex was recorded.

DISCUSSION

The relative potency and other characteristics of the compounds used in the present study in the rat and mouse compare well with results obtained with subarachnoid anaesthesia in other species, such as sheep (Adams and Doherty, 1977), dog (Feldman and
Covino, 1981) and man. In the mouse, cinchocaine required the lowest dose for satisfactory blockade, closely followed by amethocaine and bupivacaine, while lignocaine and mepivacaine were less potent. The duration of motor blockade was longest with cinchocaine, followed in order of decreasing duration by amethocaine, bupivacaine, lignocaine and mepivacaine. A relatively short-acting agent such as lignocaine had a more rapid onset than the more long-acting compounds. Another similarity with clinical experience was the propensity for sensory blockade with bupivacaine, as shown by the experiments in the rat. Thus, the spinal anaesthetic properties in the rat and mouse appear qualitatively similar to those in other species, despite differences in absolute values, for example for latencies and durations.

The technique in the rat has the advantage that comparative studies can be performed in one and the same animal, allowing evaluation of both sensory and motor blockade. With the rat preparation, the tip of the catheter is not always localized dorsally in the subarachnoid space. For example, unilateral blockade by a local anaesthetic was mostly associated with a lateral position of the tip of the catheter at postmortem. It is difficult completely to avoid immediate failures such as unilateral blockade, but in our experience the frequency of successful blockade is around 80%. We have found that the injection of lignocaine which results in a bilateral, reversible blockade is a good test of a successful preparation before rats are used in further studies of spinal systems that mediate nociception and antinociception (Åkerman, Rosell and Folkers, 1982). Rats tolerate the chronic catheter well. The animals used in the present study had useful functioning catheters over a period of 4 weeks, during which time test solutions were injected four times without problem.

The technique in the mouse seems to be of particular value for the rapid determination of blocking properties of potential new spinal local anaesthetics relative to reference compounds, and structure-activity relationships. Information about sensory blockade was gained in the rat, but not in the mouse, from the degree of inhibition of the withdrawal response to pressure to the foot-pads. Results of preliminary studies (Åkerman et al., unpublished observations) indicate that, in both species, inhibition of the tail-flick response to painful heat can be used as an alternative to pressure as an indication of blockade of sensory modalities by local anaesthetic agents.

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REFERENCES