Experimental Modelling of Spinal Anaesthesia in Rats

PhD Thesis

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INTRODUCTION

The sensation of pain is fundamental in the maintenance of the biological integrity of the body, but chronic pain status causes severe somatic and psychic impairments, which give rise to considerable social and economic burden not only on the individual but also on the society.

The International Association for the Study of Pain (IASP) defines pain as ‘an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage’.

Spinal and epidural anaesthetics and analgesics were introduced in the medical practice to reduce the potential complications of general anaesthesia.

Intrathecal catheterisation is a useful method for modelling spinal anaesthesia in animals. It allows the examination of the analgesic effect of certain substances in awake, freely moving rats. Physicochemical properties of drug injection, such as molecular weight and lipophilicity, should therefore be considered in assessing the efficacy of any drug, because these properties are likely to influence the penetration into the spinal cord, circulation, or both.

However, the location of the catheter can significantly influence the effect of the drugs.

Glutamate is the most common excitatory neurotransmitter in the central nervous system, which is released from the central terminals of nociceptive primary afferents. Glutamate acts on several ionotropic and metabotropic glutamate receptors. Ionotropic glutamate receptors are expressed in high density in the superficial spinal dorsal horn, particularly in the substantia gelatinosa. AMPA receptors mediate fast depolarization, while NMDA receptors mediate slower component of the excitatory postsynaptic potential, therefore they play an important role in the development and maintenance of central sensitization, a state of long-lasting increase in synaptic transmission, and neuropathic pain at several levels. Consequently, the blockade of spinal NMDA receptor elicits antinociception in various models of persistent pain.

Ketamine, a non-competitive NMDA receptor antagonist, exhibits analgesic and anaesthetic properties in rodents and humans. It is a chiral drug which has R- and S-isomers. It was first used in American soldiers during the Vietnam War. The racemic mixture was approved for general clinical use in 1970; its clinical usefulness has been limited because of its cardiovascular-stimulating properties and high incidence of disturbing emergence reactions.

The importance of stereochemistry in clinical pharmacology and therapeutics has been widely recognized in the past decade, particularly in the arena of new drug development. Most drugs are optically active and used clinically as the racemate (an equal mixture of optical isomers). Approximately 60% of anaesthetics are chiral drugs; some of them are administered as individual enantiomers. Although the R- and S-isomers contain the same substituent groups,

they occupy different positions in space. Consequently, the R- and S-enantiomers may form different three- dimensional relationships in the asymmetric environment of receptors and there may be significant differences in their pharmacodynamic and pharmacokinetic properties, i.e., individual enantiomers may differ in their receptor effects, dispositions, and toxicity. The analgesic effect of spinal administration of racemic ketamine was demonstrated by several studies, but there have been only a few published reports on the analgesic properties of the individual optical isomers of ketamine after systemic administration, and no reports are available on their analgesic properties after intrathecal administration.

AIMS

Experiment I.

The spinal cord is an important neuronal structure for pain transmission, and it is one of the pharmacological sites of action for the antinociceptive effects of different drugs. Subarachnoid administration of local anaesthetics is widely used for providing surgical anaesthesia, postoperative analgesia and in the treatment of chronic pain. A single injection of local anaesthetics into the epidural or intrathecal space has a limited duration of action. Catheterization techniques have been developed to prolong the anaesthetic effect. In our previous studies, we observed side differences after intrathecally administered drugs in pain tests of rats and only a few authors have noted that the position of the catheter tip in the transverse plane is an important factor governing the successful subarachnoid spread of a solution. The particular aims of our work were:

1. To observe the side differences in the motor and sensory disturbances after intrathecal administration of different doses of the local anaesthetic lidocaine in rats.
2. To determine the distribution of intrathecal catheter tips in the longitudinal and transversal plane.
3. To investigate the correlation between the location of the catheter tip and the motor and sensory disturbances after intrathecal administration of a small dose of lidocaine to a large number of animals. Such a correlation might be very important, especially if small doses of a drug are administered and their effects are investigated on both sides.

Experiment II.

More than 50% of currently used drugs are chiral compounds and about 90% of them have been marketed and clinically administered as racemates, which contain an equal mixture of two enantiomers. Enantiomers are well known to show differences in pharmacodynamics, which originate from the stereo structure-specific actions. One enantiomer may be preferred over its enantiomeric counterpart or a racemic mixture because it may increase beneficial activity and may decrease adverse toxicity, indicating the clinical advantage of using a single enantiomer. The particular aims of our work were:

1. To investigate the antinociceptive effect of intrathecal racemic ketamine on carrageenan-induced thermal hyperalgesia in the paw withdrawal test (PWD).
2. To investigate the antinociceptive effect of intrathecal S(+)- and R(-) ketamine enantiomer on carrageenan-induced thermal hyperalgesia.
3. To investigate the antinociceptive effect of intrathecal S(+)- and R(-) ketamine enantiomer on acute heat pain sensitivity in tail-flick (TF) test.
4. To investigate the antinociceptive effect of intrathecal S(+)- and R(-) ketamine enantiomer on acute heat pain sensitivity in hot-plate (HP) test.

Animals

After institutional approval had been obtained from the Animal Care Committee of the University of Szeged, Faculty of Medicine, male Wistar rats (weighing 160–330 g) were studied (n = 448). All experiments were performed during the same period of the day (8:00 AM to 1:00 PM) to exclude diurnal variations in pharmacological effects. The animals were randomly assigned to treatment groups, and the observer was blinded to the treatment administered. In order to reduce the stress of animals, they were habituated to the hand before experiments.

Intrathecal catheterization

For spinal drug administration, the rats were surgically prepared under ketamine and xylazine anaesthesia (72 and 8 mg/kg intraperitoneally, respectively). An intrathecal catheter (PE-10: I.D. 0.28 mm; O.D. 0.61 mm) was inserted through a small opening in the cisterna magna and passed 8.5 cm caudally into the intrathecal space, as described previously. After surgery, the rats were allowed to recover for at least 3 days before use. Rats exhibiting postoperative neurologic deficits (approximately 10%) were excluded.

Drugs

- ketamine hydrochloride (Ketalar; Pfizer Med-Inform, Vienna, Austria)
- xylazine hydrochloride (Rompun; Bayer, Leverkusen, Germany)
- phenobarbital sodium (Hungaropharma Rt., Budapest, Hungary)
- lidocaine 2% and 10% (Egis, Budapest, Hungary)
- carrageenan-λ (Sigma-Aldrich Kft, Budapest, Hungary)
- racemic ketamine [SR(+)-ketamine], S(+)- ketamine, and R(-)-ketamine (all ketamines were Parke-Davis; a generous gift from Gödecke Ltd., Vienna, Austria).

Intrathecal administration

Intrathecally applied drugs were dissolved in sterile, physiological saline freshly prepared on the day of the experiment and given in single (experiment I.) or cumulative (experiment II.) doses. Drugs were injected intrathecally over 30 s in a volume of 5 μl, followed by a 10 μl flush of physiological saline.

Carrageenan-induced inflammation

Unilateral inflammation was induced by intraplantar injection of 3 mg of carrageenan in 0.1 ml of physiological saline into the right hind paw. The carrageenan solution was prepared freshly on the day of the experiment.

Pain tests

1. Tail flick test

The rats were covered with a towel on the measuring surface and were held at rest. time measurement was started with a footswitch when the lower 5 cm portion of their tail was immersed in hot water (51.5°C) and was stopped when the animals reacted by flicking their tail (cut off time: 20 s).

2. Hot-plate test

A clear Plexiglas chamber was placed on the surface of a hot plate. The latency of licking one of the hind paws or jumping was measured. The surface temperature of the HP was maintained at 52.5 °C; cut off time was 60 seconds. Animals that failed to react to the thermal stimulus within 60 seconds were removed from the HP surface and assigned 60 seconds response latency.

3. Paw withdrawal test

Nociceptive sensitivity of the hind paws was determined by studying the unilateral PWD latency in response to radiant heat stimulation. A movable radiant heat source powered by a constant current supply was directed on the glass from below. The cut off time was set at 20 s to avoid any thermal injury.

Assessment of motor impairment

1. Determination of motor paralysis

Motor impairment was determined after lidocaine administration. Clubbing of the hind paw and the inability to stand on one of the hind limbs were indicative of motor block (n = 376).

2. Grip-strength test

We used an apparatus designed for the measurement of unilateral hind limb grip strength. The device consisted of a horizontally mounted, 6-mm mesh stainless-steel (0.6-mm diameter)

screen attached to an electronic force-gauge dynamometer. For each test, the rat's tail was pulled backward in a smooth but firm manner until the rat's grip on the screen was broken. The mean of the results of three consecutive attempts (the duration of the test was approximately 20 s) was used for statistical analysis. Grip-strength testing was performed before and within 5 min after lidocaine administration (n = 23).

Localization of the catheter tip

After experimental use, rats (n = 376) were killed with an overdose of phenobarbital sodium, and laminectomies were performed between vertebrae C7 and L5 without removing the spinal cord from the vertebrae. Before laminectomies 5 μl of methylene blue was injected into the cannula, and the level and side positions of the catheter tip were determined. This method also helped us to visualize whether the catheter tips were placed epidurally, subdurally, or inside the spinal cord. Catheter tip positions in the longitudinal plane were identified by the level of the vertebral body. In the transverse plane, the subarachnoid space was subdivided into four parts — left, right, dorsal, and ventral. It has been postulated that if the tip of the catheter is closer to one side, the effect of the drug should be more intensive on that side. We used the dorsal or ventral categories only when the tip of the catheter was very close to the midline.

Data analysis

Data are given as means ± SEM. Data sets were examined with analysis of variance and correlation analysis by using STATISTICA software (StatSoft Inc., Tulsa, OK).

The statistical significance of different enantiomers (treatment) and doses was assessed by two-way analysis of variance (ANOVA). One-way ANOVA was used to compare treatment effects, with the Newman-Keuls test for post hoc comparison for differences between means. A level of $P < 0.05$ was considered significant.

RESULTS

Experiment I

The difference between the two sides of baseline PWD was not significant.

The motor paralysis of rat hind paw was observed almost immediately after intrathecal injection of 100 and 500 μg of lidocaine. 100 μg lidocaine caused temporary paralysis only on one side, in contrast 500 μg lidocaine induced paralysis on both sides in 90% of cases. 500 μg lidocaine increased the PWD latency significantly.

As regards the 8.5-cm length of the catheter, it served to place the tip between vertebrae T12 and L2. The level of the tip correlated significantly with body weight ($r = 0.4$, $P < 0.05$)**.** The catheter tip was located on the left side in 39.6 %, on the right side in 39.4 %, dorsally in 14.4 %, and ventrally in 4.84% of the 376 rats. Therefore, in 79 % of the cases, the catheter tip was situated on one side (left or right)**.** It has been observed that the side on which the catheter tip was located correlated significantly with the side on which motor paralysis was observed and also with the PWD latencies. There was also close correlation between the motor paralysis and the PWD latencies ($n = 193$; $r = 0.75$ for the right and $r = 0.82$ for the left hind paw, respectively). A significant correlation was observed between the side position of the cannula tip and the grip strength after 100 μg lidocaine administration ($r = 0.50$ and $r = 0.51$ for the left and the right hind limb, respectively; $P < 0.05$).

Experiment II

There were no significant differences in paw withdrawal responses to noxious thermal stimuli between the hind paws before the intraplantar injection of carrageenan. The repeated administration of racemic and S(+)-ketamine increased PWD latency significantly in a dose dependent manner after carrageenan inflammation. Racemic ketamine had significant effects after the second (50 μg) and third dose (100 μg), and S(+)-ketamine had significant effects only at the largest dose (100 μg) compared with saline. Although indicating a clear trend toward a dose-dependent reversal of thermal hyperalgesia, R(-)- ketamine did not achieve statistical significance compared with saline.

In the hot plate test, the largest dose of both isomers (500 μg) caused a significant increase in licking latency at 10 min, although this dose induced supraspinal effects as well; for example, the animals demonstrated circling movements and head weaving.

Neither S(+)- nor R(-)-ketamine influenced the tail flick latency significantly in the applied doses (10 μg, 100 μg and 500 μg).

DISCUSSION

Experiment I

Our results demonstrated that the intrathecal catheter tips were always at an appropriate level, corresponding to the spinal segments that innervate the hind paw. The side position of the catheter tips, however, exhibited large variability, and they significantly influenced the anaesthetic effect of the small dose of lidocaine. The anaesthetic potency of lidocaine correlated with the location of the catheter tip.

There was little rostrocaudal diffusion of the methylene blue dye injection along the spinal axis and the spread depended on the volume applied. The spread of the dye in a volume of 5 μl was limited to approximately 0.5–1.5 cm from the catheter tip. Furthermore, even for compounds such as naloxone, which can permeate neural tissues rapidly, the levels that appear in the brain after spinal subarachnoid administration of the drug were low. In contrast, our earlier results demonstrated that the supraspinal, mydriatic effect of intrathecally administered lipophilic α2-adrenoceptor agonist, dexmedetomidine, appeared at a smaller dose than that required for its antinociceptive effect. Thus, the properties of the drug and the longitudinal position of the catheter tip clearly play major roles in the effect of the compound, whereas few authors have noted that the position of the catheter tip in the transverse plane is also important as concerns successful subarachnoid spread.

There are several methods for determining the effect of a drug on both hind paws after unilateral inflammation or nerve injury. In these cases it cannot be excluded that the effects of small doses of such drugs might be influenced by the location of the catheter tip. Because our results revealed that the location of the catheter tip correlated with the anaesthetic potency of small-dose lidocaine, we suggest that studies of the effects of drugs might well be predicted by preliminary determination of the potency of lidocaine on both sides. If the goal of a study is to investigate the effect of a drug on the injured side, the injury should be performed on the side where lidocaine is more effective.

Experiment II

Originally, Marietta et al. reported differences in pharmacological potencies of the optical isomers of ketamine in rats after intravenous injection. The (+)-enantiomer had a higher therapeutic index than the racemate or the (-)-enantiomer, and at equihypnotic doses, the (+)-isomer caused less locomotor stimulation than the (-)-isomer. In human studies, the S(+)-enantiomer has 4 times the potency of the R(-)- enantiomer of ketamine and may have significant clinical advantages in comparison with the racemic drug. Furthermore, S(+)-ketamine possesses superior efficacy with fewer side effects compared with the racemate. There are also differences in the pharmacokinetics of the ketamine enantiomers. Administered individually, the plasma clearance of S(+)-ketamine is greater than that of the R(-)-enantiomer. A series of clinical studies has suggested potent analgesia after epidural or spinal administration of racemic ketamine. Ketamine has been administered epidural to humans for pain relief without side effects, such as respiratory depression, urinary retention, or pruritus, frequently observed after epidural opioids.

Our results are consistent with the observation that NMDA receptor antagonists have a particular role in mediating persistent pain and hyperalgesia. In behavioural tests, NMDA receptor antagonists attenuate or reverse the thermal hyperalgesia evoked by local inflammation or peripheral neuropathy, without any analgesic effect on the response to noxious stimulation of the contralateral, non-affected limb. Furthermore, when tested in rats without nerve injury or tissue damage, NMDA receptor antagonists usually do not produce significant behavioural analgesia until the doses used result in visible motor dysfunction. Ketamine interacts with not only NMDA receptors, but also with opioid, monoaminergic, and muscarinic receptors and voltage-sensitive calcium channels. In addition, it has been shown that in the spinal cord, acute NMDA produced thermal hyperalgesia is mediated by activation of the constitutive neuronal form of nitric oxide synthase and the production of nitric oxide. Therefore, several neuronal receptor systems may be involved in the depressant action of ketamine on the excitation of wiled dynamic range neurons in the dorsal horn of the spinal cord. The antinociceptive effects of subanaesthetic doses of ketamine are likely to occur at concentrations sufficient to block the NMDA channels. At larger (anaesthetic) doses, other receptors, in particular opioid δ-receptors and μ-receptor sites, may contribute to the pharmacological effects of ketamine.

SUMMARY

Experiment I

Many previous studies suggest that the correct location of the catheter tip is crucial concerning the effect obtained. However, previous studies did not suggest a reliable method for the prediction of the location of the catheter tip before experiments on living animals.
We revealed that:

1. the tips of chronic intrathecal catheters were found to be variously located in the transverse plane in the rat spinal subarachnoid space.
2. the position of the intrathecal catheter tip influences the pharmacological effects of the local anesthetic lidocaine.
3. the differences between the effects of the drug on the two sides might be very important, especially if small doses of the drug are applied and their effects are investigated on both sides.
4. the paralytic and/or antinociceptive effect of a small dose of lidocaine before the experiments is a simple and reliable method for prediction of the side position of the catheter tip before rats are used in further studies of spinal systems that mediate nociception and antinociception.

Experiment II

This was the first study to examine the antinociceptive efficacy of the optical isomers of ketamine after intrathecal administration.
We demonstrated that:

1. Both racemic and S(+)-ketamine significantly increased paw withdrawal latencies in the inflamed paw.
2. The R(-)-ketamine was ineffective in reducing thermal hyperalgesia.
3. Conversely, acute pain tests did not reveal any differences between ketamine enantiomers; i.e. only the largest dose (500 μg) caused a nonstereospecific, significant increase in hot plate latency, but this dose caused supraspinal effects as well. Neither S(+)-nor R(-)-ketamine showed any significant effect in tail flick test in applied doses.

Our result suggest that the potential advantages of using pure enantiomers rather than a racemate include a less complex and more selective pharmacodynamic profile, a higher therapeutic index, less complex pharmacokinetics, less complex drug interactions, and less complex concentration-response relationships.

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