THE POTENTIAL ROLE OF SPINAL KETAMINE IN MULTI-COMPONENT ANTINOCICEPTION

Ph.D. Thesis

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Introduction

The spinal cord is an important site for modulation of pain sensations, where several mechanisms, such as interconnections between nociceptive and nonnociceptive afferent pathways, different type of interneurons, descending fibers by the released neurotransmitters can control the transmission of nociceptive information to higher centers in the brain.

Glutamate is a major excitatory neurotransmitter in the central nervous system. The laminar localization of the ionotropic glutamate receptors concentrates in the superficial dorsal horn of the spinal cord. Excitation of the N-methyl-D-aspartate - type (NMDA) glutamate receptor system plays an important role in the sensitization processes (central sensitisation and wind-up). Therefore blocking the NMDA receptor produces only weak or no antinociception against acute thermal or mechanical stimuli in uninjured rats, but causes significant antinociception in various models of persistent pain .

Ketamine, a non-competitive antagonist at the NMDA glutamate receptors has been used as an anaesthetic drug in clinical use for almost 40 years. The effect of ketamine, the so called "dissociative anaesthesia" has been proven to be advantegous in several cases, although undesired phychomimetic effects (hallucinations, vivid dreams) may accompany the recovery phase in some patients. Intrathecal racemic ketamine produces weak or no antinociception against acute thermal or mechanical stimuli in uninjured rats, but causes antinociception in inflammation-induced persistent pain and after nerve damage.

Racemic ketamine is a mixture of two optical isomers: the left-handed optical enantiomer, S(+)-ketamine and the right-handed one, the R(-)-ketamine. Recent data has proved that S(+)-ketamine has four times the anaesthetic potency of the R(-)-enantiomer and may have significant clinical advantages in comparison with the racemic drug. The incidence of psychotomimetic phenomena appeared to be less common after S(+)-ketamine in comparison to racemic ketamine, besides their quality was described as far less unpleasant. Moreover, increasing experimental evidence supports a remarkable neuroprotective effect of S(+)-ketamine, which may become a promising drug for new therapeutic approaches to neuroprotection.

Following these facts our aim was to examine the antinociceptive effect of intrathecally administered racemic ketamine and the pure enantiomers alone and in combination with other spinally active antinociceptive agents, on acute pain sensation in rats. We have chosen μ-opioid and α_2-adrenoceptor agonist drugs for drug combination studies with ketamine, since both of these systems modulate greatly the transmission of pain at the spinal level.

Opioid receptors – μ, δ and κ - are widely distributed throughout the central nervous system, including the spinal dorsal horn, especially laminae I and II. At spinal sites, ligands for each of the three opioid receptors can produce analgesic effect. Morphine administered via either intrathecal or epidural route is widely used to treat chronic, postoperative and labor pain, however due to its several side effects the

limitations of spinal opioids are considerable. Endomorphin-1 is a novel endogenous μ-opioid receptor ligand with high affinity and selectivity for this receptor. It is a potent antinociceptive agent acting spinally-, supraspinally- and peripherally therefore it might have potential clinical significance, especially in combination with different drugs.

The noradrenergic descending inhibitory control is mediated at the spinal level by α_2-adrenoceptors. α_2-Adrenoceptor-mediated spinal analgesia has been extensively investigated both in animal and human studies. Dexmedetomidine is a new, highly selective and potent α_2-adrenoceptor agonist, which causes significant behavioral antinociception after low subsedative intrathecal doses in rats. Besides, it was found to reduce the need for general anaesthetics and analgesics in patients undergoing minor surgery. Dexmedetomidine has been recently licensed (in 2000) in the USA for postoperative sedation in intensive care units.

Specific aims

The thesis examines the spinal antinociceptive effect of racemic ketamine and its pure enantiomers and their possible, advantegous combinations with morphine, endomorphin-1 and dexmedetomidine on acute pain sensations (using the tail flick test) in rats. Our aims were the following:

1. To examine the antinociceptive effect of racemic ketamine and its enantiomers at the spinal level.
2. To examine the effect of both ketamine enantiomers on morphine-induced antinociception.
3. To examine the effect of S(+)-ketamine on endomorphin-1-induced antinociception.
4. To examine the effect of both ketamine enantiomers on antinociception induced by dexmedetomidine.
5. To examine the possible interaction of endomorphin-1 and dexmedetomidine after intrathecal administration.
6. Furthermore, the thesis investigates the possible, advantegous triple combination of endomorphin-1 and dexmedetomidine with S(+)-ketamine.

Methods and Materials

Intrathecal catheterization

To examine the biological action of the drugs at the spinal level in the behaving animal we have inserted a chronic indwelling polyethylene canule into the spinal subarachnoid space of the rats. Accordingly, after institutional approval had been obtained from our animal care committee, male Wistar rats weighing 250-350 g were studied (n=666). After the rats were surgically prepared under ketamine-xylazine anaesthesia (87 and 13 mg/kg intraperitoneally, respectively), an intrathecal catheter (PE-10 tubing) was inserted through a small opening in the cisterna magna and passed 8.5 cm caudally into the intrathecal space. Following surgery the rats were housed individually, had free access to food and water and were allowed

to recover for at least 3 days before use. Rats exhibiting any postoperative neurological deficit (about 10%) were excluded from further studies. After experimental use, each rat was killed with an overdose of pentobarbital and 1 % methylene blue was injected to confirm the position of the catheter and the probable spread of the injectate. The position of the tip of the intrathecal catheter following the above-mentioned method was always at the proper place - vertebral T13-L3 level-, corresponding to the spinal segments, which innervate the tail.

Nociceptive testing

Application of noxious heat to the rat's tail generates a quick withdrawal response that is called the tail flick reflex. This reflex has been used frequently to study pain mechanisms; it is clearly visible and occurs at a consistent latency if a constant heat intensity is used. Accordingly, the reaction time in the tail flick test was determined by immersing the lower 5 cm portion of the tail into hot water (51.5 ^{o}C water) until the typical tail-withdrawal response was observed (cut-off time: 20 s). Baseline latencies were obtained immediately before and then 10, 30, 60 and 90 min after the drug injections. All experiments were performed during the same period of the day (8:00 to 11:00 AM) to exclude diurnal variations in pharmacological effects. The animals were randomly assigned to treatment groups (n=5-13 rats/group).

Drugs

The employed drugs were the following:

- ketamine hydrochloride (Ketalar, Parke-Davis, Vienna, Austria),
- xylazine hydrochloride (Rompun, Bayer, Leverkusen, Germany),
- racemic ketamine hydrochloride, R(-)-ketamine hydrochloride, S(+)-ketamine hydrochloride (all ketamines for intrathecal administration: Parke-Davis; generous gifts from Gödecke Ltd., Vienna, Austria),
- morphine hydrochloride (Alkaloida, Tiszavasvari, Hungary),
- dexmedetomidine (a generous gift from Orion-Farmos, Finland),
- endomorphin-1 (was synthetized by a solid-state method and purified by means of high performance liquid chromatography in the Isotope Laboratory of the Biological Research Centre of the Hungarian Academy of Sciences).

Drugs were dissolved in sterile, physiological saline. The drugs were administered intrathecally over 30 s in a volume of 5 μl, followed by a 10 μl flush of physiological saline.

Statistical analysis

Analgesic latencies obtained from the acute pain test were converted to the percentage of the maximal possible effect (%MPE) by using the formula:

%MPE=([observed latency - baseline latency]/[cut off - baseline latency])*100

Data were presented as means ± SEM. Time course and dose-effect curves were constructed for each drug or drug combinations. The ED_{50} (the dose that yielded 50% of the maximum possible effect) values were calculated by linear regression. Isobolographic analysis was performed to study the interaction between dexmedetomidine and endomorphin-1. Data sets were examined by one- and two-way analyses of variance. Post-hoc comparison was carried out with the Newmann-Keuls test. A *P*-value less than 0.05 was considered significant.

Series of experiments

1. Single-drug studies

The first series of experiments was planned to evaluate the time-response and dose-response effect of intrathecally administered:

a.) racemic ketamine, S(+)-ketamine and R(-)-ketamine: 30, 100 and 300 μg

b.) morphine: 0.1, 0.3, 1 and 3 μg

c.) dexmedetomidine: 0.1, 0.3, 1, 3, 6 and 10 μg

d.) endomorphin-1: 0.6, 2, 6, 18 and 50 μg

2. Drug interaction studies

The second series of experiments was planned to examine the possible interaction of the following drug combinations:

a.) 30 or 100 μg racemic, S(+)-, R(-)-ketamine with 0.1, 0.3, 1 and 3 μg morphine

b.) 30 or 100 μg racemic, S(+)-, R(-)-ketamine with 0.3, 1 and 3 μg dexmedetomidine

c.) 30 or 100 μg S(+)-ketamine with 2, 6 and 18 μg endomorphin-1

d.) endomorphin-1 and dexmedetomidine in a 4:1 fixed dose ratio: 1, 2, 4, 8 μg endomorphin-1 + 0.25, 0.5, 1, 2 μg dexmedetomidine.

e.) triple combination : S(+)-ketamine with a fixed 4:1 dose ratio of endomorphin-1 and dexmedetomidine: 0.04, 0.12, 0.4, 1 μg endomorphin-1 + 0.01, 0.03, 0.1, 0.25 μg dexmedetomidine + 100 μg S(+)-ketamine.

Results and discussion

There was no significant difference in the baseline tail flick latencies between the different treatment groups (7.0±0.10 sec) before any drug administration. The tail flick latencies of the control group did not change significantly during the time of the investigation.

Single-drug studies

1. Neither the racemic-ketamine nor its enantiomers alone had significant antinociceptive effect on influencing the acute pain sensation after intrathecal administration. Only the highest dose of racemic ketamine caused significant change compared to the control group, but this was accompanied with transient motor disorder.
2. Intrathecal adiministration of morphine, endomorphin-1 or dexmedetomidine alone resulted in a dose-dependent increase in the thermal withdrawal latency. The rank order of potency was morphine > dexmedetomidine > endomorphin-1.

Our results are in agreement with those already published in the literature (about other NMDA antagonists as well), since we have found that neither the racemic ketamine nor its enantiomers caused antinociception on acute pain sensation.

Drug combination studies

1. The high dose of intrathecal S(+)-ketamine and racemic ketamine (100 μg) synergistically enhanced the antinociception caused by intrathecal morphine, while the R(-)-ketamine had no such effect. Both the racemic-, and the S(+)-ketamine not only potentiated, but also prolonged the antinociceptive effect of morphine.
2. All of the ketamine treatments significantly enhanced the antinociception of dexmedetomidine and reduced its ED_{50} value, although the high dose of S(+)-ketamine and racemic ketamine were the most effective. This dose of racemic-, and S(+)-ketamine not only potentiated, but also prolonged the antinociceptive effect of dexmedetomidine.
3. Co-administration of S(+)-ketamine potentiated, but did not prolong the antinociceptive effect of endomorphin-1.
4. Co-administration of endomorphin-1 with dexmedetomidine showed synergistic interaction.
5. Co-administration of endomorphin-1 and dexmedetomidine with S(+)-ketamine caused a dose-dependent antinociception and a prolonged effect, which be observed at higher doses. The ED_{50} value of the triple combination was significantly reduced compared to the ED_{50} value of the endomorphin-1 + dexmedetomidine combination.

Various studies have already shown the beneficial antinociceptive effect of opioids and NMDA receptor antagonists co-administered both in acute and chronic pain by either systemic or intrathecal route. Powerful synergism arises from combinations of threshold doses of morphine with low doses of NMDA antagonists. *Our results with morphine/endomorphin-1 + ketamine are in agreement with the previous results, since we have showed that this kind of advantageous, synergistic interaction can also be performed with ketamine at the spinal level. Moreover the S(+)-enantiomer proved to be more efficacious in some cases.*

Systemic (intramuscular) co-administration of the α_2-adrenoceptor agonists xylazine and racemic ketamine is a widely used combination in veterinary anesthesia to provide adequate analgesia, muscle relaxation and complete immobilization. Others have shown that the intrathecal co-administration of clonidine with MK-801 resulted in a dose-dependent increase in the mechanical pain threshold in a peripheral neuropathic pain rat model. *Our results with dexmedetomidine + ketamine contribute to the above-mentioned data, namely that the combination with ketamine also produce effective antinociception after intreathecal route of administration. Moreover the S(+)-enantiomer proved to be more efficient in some cases in potentiating the effect of dexmedetomidine than either the racemate or the R(-)-enantiomer.*

There are several possible explanations for the observed synergistic interaction between ketamine and morphine, endomorphin-1 or dexmedetomidine. Since opioid-, glutamate- and α_2-adrenergic receptors are all abundant in the spinal cord, co-activation or antagonisms of these receptors could have a beneficial effect on inhibiting the pain sensation at low doses which cause minimal side effects, in spite of the differences in the mechanism of action between the applied drugs. Ketamine decreases the activation of dorsal horn neurons - by inhibiting the ionotropic glutamate receptor opening -, while the major mechanism of spinal opioid and α_2-adrenoceptor agonist analgesia is the inhibition of transmitter release from the C-fibre primary afferent terminal, besides having inhibitory effect on both interneurons and projecting neurons. A presynaptic action of these latter drugs contrasts with the postsynaptic location of the NMDA receptor. The dual actions at both pre- and postsynaptic sites allows for possible synergy.

In some cases (ketamine plus morphine/dexmedetomidine) we have found a prolonged antinociception besides potentiation. Our results showed that the antinociception after the ketamine combinations was longer-lasting than it was after the single morphine or dexmedetomidine treatments. The explanation for this phenomena might hide in the different pharmacokinetic properties of the co-applied drugs. Since S(+)-ketamine did not prolong the antinociceptive effect of endomorphin-1, it suggests a mainly pharmacodynamic interaction between these drugs.

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Publications

Papers involved in the thesis:

Joo G., Horvath G., Klimscha W., Kekesi G., Dobos I., Szikszay M., Benedek G. The effects of ketamine and its enantiomers on the morphine- or dexmedetomidine-induced antinociception after intrathecal administration in rats. *Anaesthesiology* 2000; **93**: 231-241
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