Comparison of the effects of intrathecal administration of levobupivacaine and lidocaine on the prostaglandin E₂ and glutamate increases in cerebrospinal fluid: a microdialysis study in freely moving rats

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Background. Bupivacaine has a lower incidence of transient neurological symptoms than lidocaine after intrathecal (i.t.) injection. The increased toxic potential of lidocaine does not support its use in the clinical setting and could be related to augmented levels of spinal prostaglandin E₂ (PGE₂). We tested whether levobupivacaine leads to lower PGE₂ levels than lidocaine. Moreover, we compared the release of PGE₂ and glutamate after i.t. injections of levobupivacaine or lidocaine.

Methods. Rats were anaesthetized for implantation of an i.t. dialysis catheter. This allowed sampling dialysates of cerebrospinal fluid (CSF) for measuring PGE₂ and glutamate levels. The microdialysis setting included baseline sampling and was followed by an i.t. injection of levobupivacaine 250 mg, 100 mg, or saline. PGE₂ and glutamate levels in CSF were analysed for 4 h. In addition, the residual effect of a second i.t. injection on, respectively, of PGE₂ and glutamate changes was compared after injection of either 250 or 100 µg levobupivacaine, 1000 or 400 µg lidocaine, or saline.

Results. Prolonged spinal PGE₂ increases lasting 50–120 min were observed after levobupivacaine injection. Higher PGE₂ concentrations were observed after the second lidocaine 1000 µg injection. Glutamate release after the second injection did not vary between the local anaesthetic groups.

Conclusions. Spinal PGE₂ levels are similarly increased after i.t. levobupivacaine injection of 250 and 100 µg. A higher PGE₂ response was observed after a second i.t. injection in the animals receiving 1000 µg lidocaine than those receiving 400 mg lidocaine or either dose of levobupivacaine.


Keywords: measurement techniques, microdialysis; pharmacology, lidocaine, levobupivacaine, prostaglandins, glutamate

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In humans, intrathecal (i.t.) lidocaine administration has been reported with a seven-fold increased incidence of transient neurological symptoms (TNS) when compared with bupivacaine (7.4 per 10,000 vs 1 per 10,000).¹ Furthermore, lidocaine has the potential to cause more neurotoxicity than other local anaesthetics when given at higher i.t. concentrations and doses than those used clinically.²–⁶ The mechanisms underlying this reduced margin of safety are still obscure.⁷ Recently, we demonstrated in a rat microdialysis spinal cord study that i.t. lidocaine administration was accompanied by transiently increased prostaglandin E₂ (PGE₂) levels in cerebrospinal fluid (CSF) and mechanical hyperalgesia, suggesting a short-lasting period of spinal sensitization.⁸ Whether other local anaesthetics with a reduced incidence for TNS after i.t. administration would have less influence on increases of
PGE₂ levels still needs to be further explored. In this setting, we tested levobupivacaine, the preservative-free \( \text{t}-\text{enantiomer} \) of the racemate bupivacaine. Levobupivacaine is considered to be a safer analogue than racemic bupivacaine with reduced cardiovascular and neurological side-effects.\(^\text{10–12} \) Except for an i.t. rat cauda equina model in which similar histopathological neurotoxicity between bupivacaine and its enantiomers was shown,\(^\text{13} \) literature on the spinal cord effects of levobupivacaine after i.t. administration is scarce.\(^\text{14} \)

TNS incidence does not change with dilution of the local anaesthetic dose.\(^\text{15,16} \) Neither does it change when comparing i.t. 60 and 75 mg doses.\(^\text{17} \) This contrasts with the incidence of more neurotoxic lidocaine effects, such as the cauda equina syndrome (CES) that tends to be associated with higher i.t. lidocaine doses. A different identity was suggested for the two clinical manifestations. Some reject a different identity and believe that TNS is at the lower end of a spectrum of lidocaine toxicity scale, whereas the more neurotoxic events of lidocaine are at its higher end.\(^\text{18} \)

We measured dialysate level changes in CSF of glutamate and PGE₂ as changes in these may be considered as surrogates for assessing local-anaesthetic-induced nociception or central hypersensitivity.\(^\text{3,8} \) To further examine whether a relationship exists between potential transient PGE₂ changes and levobupivacaine dose, we investigated two doses corresponding to a normal and a large clinical i.t. injection and measured glutamate and concentrations in CSF.

We hypothesized that i.t. levobupivacaine injection will be accompanied by lower PGE₂ levels in CSF than levels observed after i.t. lidocaine injection.\(^\text{8} \) We also investigated the exact time relationship between PGE₂ and glutamate changes in CSF. We compared residual PGE₂ and glutamate effects after two identical separated i.t. injections of either levobupivacaine or lidocaine, the second injection being given after full motor and sensory block recovery and 4 h after the first dose. The aim of the second i.t. injection was to evaluate the effect of a higher cumulative dose of levobupivacaine or lidocaine on spinal PGE₂ and glutamate changes without compromising the physical integrity of the animal after a single administration of an excessive dose.

**Methods**

The Bioethical Committee of animal experimentation of the Vrije Universiteit Brussel approved the experimental protocol, which complied with the guidelines for animal experimentation of the International Association for the Study of Pain and with the guidelines of the Belgian Ministry of Agriculture.

**Implantation of the i.t. triple lumen loop catheter**

Male Wistar rats of \( \pm 300 \) g (B&K Universal Limited, UK) were anaesthetized using i.p. sodium pentobarbital (60 mg kg\(^{-1} \)) for implantation of an i.t. triple lumen catheter with a single loop and three outlets (Marsil Scientific, San Diego, CA, USA). This allowed i.t. injection and dialysate sampling of CSF. The catheter was introduced via the atlanto-occipital membrane as described previously.\(^\text{19} \) Briefly, the loop of the catheter was placed at the rostral margin of the lumbar enlargement and its free ends were externalized through the skin at the top of the skull. Surgery ended with a 50 \( \mu l \) s.c. injection of buprenorphine (Temgesic\(^\text{®} \) 0.3 mg ml\(^{-1} \), Schering-Plough, Brussels, Belgium) for postoperative analgesia.

**Microdialysis experiment**

After surgery, the rats were allowed to recover for 5 days. Rats showing neurological impediments were not used and were killed appropriately.

The rats were placed in a microdialysis cage (Freely Moving System BAS/Microdialysis, West Lafayette, IN, USA) in the microdialysis room the evening before the experiment to allow them to adjust to their new surroundings. On the day of the experiment, the dialysis probes were connected to a microdialysis pump (CMA 100, CMA/Microdialysis, Stockholm, Sweden) and perfused with a modified Ringer’s solution (NaCl 147 mM, KCl 4 mM, and CaCl\(_2\) 2.3 mM) at a flow rate of 7.5 \( \mu l \) min\(^{-1} \) for at least 60 min.

**Intrathecal injections**

**Levobupivacaine setting**

Baseline measurements (three samples of 10 min for PGE₂ and five samples of 10 min for glutamate) were followed by i.t. injection of either 20 \( \mu l \) (‘Levo 100 \( \mu g \)) or 50 \( \mu l \) of levobupivacaine (‘Levo 250 \( \mu g \)) (Chirocaine\(^\text{®} \), Abbott, Solbaevegen, Norway). A third, control group, received 50 \( \mu l \) i.t. saline (‘Saline’). Dialysates of CSF were sampled at 10 min intervals for the first hour after injection and at 30 min intervals for another 3 h.

**Repeated injection setting**

Two groups of rats received lidocaine where 20 \( \mu l \) (‘lido 400 \( \mu g \)) or 50 \( \mu l \) (‘lido’ 1000 \( \mu g \)) was injected i.t. (Linisol\(^\text{®} \) 2% pro-injection, B. Braun, Melsungen, Germany). These experiments were performed as described previously.\(^\text{8} \) The identical second i.t. dose was given after checking for total motor and sensory recovery of the rat, 4 h after the first dose. All solutions were injected manually by bolus injection at a rate of \(~10 \) \( \mu l \) per 40 s. Dialysates of CSF were sampled at 10 min intervals for the first hour after injection and at 30 min intervals for another 3 h. Additionally dialysates of CSF were sampled at 10 min intervals for another hour after the second injection. All samples were collected on ice and stored at \(-70^\circ C\) for subsequent analysis. We calculated the 20 min glutamate and 60 min PGE₂ area under the curve. Differences in 60 min PGE₂ and 20 min glutamate release after a first i.t. injection and the second injection were analysed.
The decision to compare 60 min PGE$_2$ levels and 20 min glutamate levels was based on the magnitude and duration of glutamate and PGE$_2$ changes observed in previous studies.\textsuperscript{\textcopyright}8

**Assay of PGE$_2$ and glutamate in CSF**

The concentration of PGE$_2$ in the microdialysate samples was quantified using a commercially available Correlate-EIA PGE$_2$ (competitive immunoassay) kit in accordance with the manufacturer’s protocol (Assay Design, Inc., USA). The concentration of PGE$_2$ in the microdialysate samples was calculated from the measured optical density by means of four-parameter logistic regression. A standard curve was constructed between 39.4 and 5000 pg ml$^{-1}$. The concentrations of glutamate were analysed by narrow-bore liquid chromatography with fluorescence detection after pre-column derivation with ortho-phthalaldehyde and beta-mercaptoethanol, as described elsewhere.\textsuperscript{\textcopyright}20 The intra- and inter-assay coefficients of variation were 5.2\% and 6.4\% for PGE$_2$, and 2.1\% and 6.4\% for glutamate, respectively.

**Verification of probe positioning**

The animals were killed after each experiment with an overdose of pentothal. That part of the spinal cord containing the i.t. catheter membrane was dissected. The position of the catheter in the spinal cord was confirmed by injecting methylene blue.

**Data analysis**

To evaluate the effects of the i.t. drug injections, we averaged the PGE$_2$ and glutamate baseline responses and set this average to 100\%. Drug effects were expressed as \% change of baseline values. The glutamate and PGE$_2$ data are presented as \% change (SD). Statistical significance of differences was accepted at $P<0.05$. Data were analysed with Statistica System Reference 2001 (Statsoft Inc., Tulsa, OH, USA).

Within group differences of PGE$_2$ and glutamate, data were analysed using the Wilcoxon test. Between-group global differences of PGE$_2$ and glutamate were analysed using one-way ANOVA. Least significant difference test was used for post hoc comparisons. The Mann–Whitney test was performed to detect between-group differences at fixed time intervals and to detect differences in AUC of the PGE$_2$ after i.t. injection.

**Results**

Forty rats without neurological sequelae from spinal microdialysis catheter insertion were studied ($n=8$ per group).

**Baseline levels**

The investigation was started when baseline values of glutamate and PGE$_2$ were stable. Measured baseline PGE$_2$ concentrations [mean (SD)] were: 97 (33), 89 (38), 102 (21), 109 (46), and 106 (54) pg ml$^{-1}$ for, respectively, the saline, the levo 100 and 250 $\mu$g, and the lido 400 and 1000 $\mu$g groups ($n=24$ for each group).

Baseline glutamate levels [mean (SD)] were: 0.34 (0.02), 0.35 (0.03), 0.37 (0.02), 0.34 (0.02), and 0.37 (0.02) $\mu$M, and for, respectively, the saline, the levo 100 and 250 $\mu$g, and the lido 400 and 1000 $\mu$g groups ($n=40$ for each group).

Taking into account the individual range variability observed in baseline values for PGE$_2$, we expressed our results as a percentage of the individual mean baseline values for further analysis.

**Levobupivacaine-induced changes in PGE$_2$ and glutamate**

In both the levo 100 and the 250 $\mu$g groups, spinal PGE$_2$ levels increased reaching peak values of, respectively, 410\% and 500\% of their baseline value after 40 and 30 min. A gradual return to normal baseline was seen after 90 min in both groups (Fig. 1A).

After i.t. injection of levobupivacaine 100 or 250 $\mu$g, glutamate levels increased by 170\% and 10\% (Fig. 1n) within 10 min after i.t. administration. The glutamate concentrations rapidly returned to baseline within 20 min after levobupivacaine administration (Fig. 1n).

**Repeated injection setting**

We observed in the lido 1000 $\mu$g group, an increased ($P=0.035$) PGE$_2$ release in CSF after the second injection (ratio: 1.70). In the lido 400 $\mu$g group, a tendency ($P=0.20$) (Lido 400 $\mu$g) of increased PGE$_2$ release after the second injection was observed (ratio: 1.65). In contrast, the 60 min PGE$_2$ values steadied in the levobupivacaine groups after the second injection (ratio <1) (Fig. 2A).

Glutamate release in CSF was equal in all treatment groups. A second injection of lidocaine or levobupivacaine did not influence glutamate release. Comparable glutamate values were found after the second injection of levobupivacaine (100 or 250 $\mu$g) and lidocaine (1000 or 400 $\mu$g) (Fig. 2n).

**Discussion**

Surprisingly, this investigation shows that two different doses of i.t. administered levobupivacaine are accompanied by 50–90 min PGE$_2$ increases in CSF. These PGE$_2$ increases happen simultaneously with glutamate within the first 10 min. Our microdialysis setting where dialysates were collected at fixed time intervals did not allow us, however, to determine the exact timing.
of release. The observed glutamate releases with levobupivacaine in CSF are not different in time or magnitude from those previously reported with bupivacaine in rabbits. Compared with our previous investigation with i.t. lidocaine, we observed different durations of PGE2 increases after i.t. levobupivacaine when compared with baseline levels: 120 min in the lido 1000 mg group vs 50 min for the lido 400 mg group and 90 min for both the levo groups. We also observed a different time to peak of PGE2 levels with a slight rightward shift of PGE2 increase after i.t. levobupivacaine. We believe that these differences are related to the different PD and PK profiles and onsets, peaks of action, and duration times of lidocaine and levobupivacaine.

The physiological background behind our model can be explained as following: i.t. lidocaine or levobupivacaine induces dorsal horn neuronal circuitry activation in which PGE2 is involved. Glutamate is released by i.t. lidocaine or levobupivacaine injection and induces a post-synaptic depolarization. The post-synaptic depolarization then leads indirectly to an increase of intracellular calcium, which in turn results in activation of a number of intracellular enzymes, including phospholipase A2 (PLA2). PLA2 activation then induces an increase in cytosolic arachidonic acid, which will enter the cyclooxygenase cascade leading to the synthesis of a variety of prostaglandins that gain access to the extracellular space. Prostanoids then affect presynaptic prostanoid E receptors that further increase intracellular calcium in sensory afferents and depolarize dorsal horn neurones and increase spinal excitability.

The second i.t. injection given after full motor and sensory block recovery made it possible to evaluate the effects of higher cumulative doses of lidocaine and levobupivacaine and to observe possible residual PGE2 effects after a first i.t. dose. Using this approach, we found that the 60 min AUC of PGE2 values were higher after
a second 1000 µg dose of lidocaine than after a second dose of 400 µg lidocaine or after both levobupivacaine administrations. This interesting finding may point to a more important anaesthetic dose-related cumulative PGE2 effect after a double injection of 1000 µg of lidocaine than after lidocaine 400 µg or levobupivacaine 250 or 100 µg. As high PGE2 levels in CSF21 22 have repeatedly been linked with persisting central pain sensitization and abnormal pain hypersensitivity, we believe that this finding may corroborate the higher incidence of reported TNS with lidocaine. The high PGE2 lidocaine levels found are in agreement with previously demonstrated levels, which were accompanied with transient mechanical hyperalgesia and increased heat sensitization.

It has been hypothesized that TNS reflects transient local anaesthetic neurotoxicity, which is reversible at low dose and interacts with multiple incompletely characterized factors that modulate pain perception.18 Interestingly, our observed PGE2 changes may, as suggested before,8 run parallel with this hypothesis. Similarly, the transient PGE2 changes after i.t. lidocaine or levobupivacaine may mirror the transiently increased calcium changes in cytoplasmatic (lidocaine) or endoplasmatic (bupivacaine) reticulum observed after local anaesthetic administration in cell line cultures.23 24 In line with these in vitro investigations, where lidocaine in the higher dose range gave more marked calcium increases than for other local anaesthetics, we found that in our investigation, increasing lidocaine dose by a second injection was accompanied by higher PGE2 increases. This was in contrast with levobupivacaine, where no dose-related PGE2 increases were found after a second injection.

There might be some evidence for CES in this setting as the inserted spinal microdialysis catheter may be coiled near the insertion site or be directed caudally with the tip of the catheter in the dural sac. In addition, injecting through an i.t. catheter may result in an increased incidence of localized drug misdistribution in the dural sac with subsequent increased incidence of CES. However, we killed all our rats at the end of surgery and checked the position of the probe by meticulous dissection after injection of methylene blue. The rats also did not receive a high-dose continuous infusion of local anaesthetics with subsequent increased incidence of CES. Nevertheless, we believe that the i.t. administration of exact equipotent doses of lidocaine would probably not alter our conclusions. Similarly, we believe as found by others that decreasing the concentration by dilution or increasing the concentration but keeping the same dose is unlikely to change our results.15 16

There are practical implications of this rat laboratory investigation for the clinical arena, though confirmation in humans is needed. Our results suggest a lidocaine dose-related incidence of PGE2 increase and possibly TNS. Furthermore, though the clinical safety margin after single administration of i.t. bupivacaine or its analogues is reported to be improved in comparison with i.t. lidocaine,1 the effect on spinal PGE2 release suggests caution, as the ‘safety’ margin we observed in PGE2 release after single spinal levobupivacaine administration was narrower than expected. We suggest reducing the i.t. lidocaine dose and tailoring its use to the clinically requested block. Though a dose-dependent effect on PGE2 release does not seem to apply after i.t. levobupivacaine administration, caution should still be exercised.

To summarize, we showed, using an in vivo analysis system, that i.t. injections of levobupivacaine transiently raised both PGE2 and glutamate levels in the CSF. In contrast to levobupivacaine, higher PGE2 responses after a second injection of 1000 µg lidocaine may suggest an increased level of local anaesthetic toxicity on the spinal cord with lidocaine.

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