Comparison of the effects of intrathecal administration of levobupivacaine and lidocaine on the prostaglandin E\(_2\) and glutamate increases in cerebrospinal fluid: a microdialysis study in freely moving rats

V. J. Umbrain\(^1\)*, M.-H. Lauwers\(^1\), L. Shi\(^1\), I. Smolders\(^2\), Y. Michotte\(^3\) and J. Poelaert\(^1\)

\(^1\)Department of Anaesthesia, \(^2\)Department of Pharmacology and \(^3\)Department of Pharmaceutical Chemistry and Drug Analysis, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium

*Corresponding author. E-mail: vincent.umbrain@uzbrussel.be

**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 E\(_2\) (PGE\(_2\)). We tested whether levobupivacaine leads to lower PGE\(_2\) levels than lidocaine. Moreover, we compared the release of PGE\(_2\) 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 PGE\(_2\) and glutamate levels. The microdialysis setting included baseline sampling and was followed by an i.t. injection of levobupivacaine 250 \(\mu\)g, 100 \(\mu\)g, or saline. PGE\(_2\) and glutamate levels in CSF were analysed for 4 h. In addition, the residual effect of a second i.t. injection on, respectively, of PGE\(_2\) and glutamate changes was compared after injection of either 250 or 100 \(\mu\)g levobupivacaine, 1000 or 400 \(\mu\)g lidocaine, or saline.

**Results.** Prolonged spinal PGE\(_2\) increases lasting 50–120 min were observed after levobupivacaine injection. Higher PGE\(_2\) concentrations were observed after the second lidocaine 1000 \(\mu\)g injection. Glutamate release after the second injection did not vary between the local anaesthetic groups.

**Conclusions.** Spinal PGE\(_2\) levels are similarly increased after i.t. levobupivacaine injection of 250 and 100 \(\mu\)g. A higher PGE\(_2\) response was observed after a second i.t. injection in the animals receiving 1000 \(\mu\)g lidocaine than those receiving 400 mg lidocaine or either dose of levobupivacaine.


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

Accepted for publication: January 25, 2009

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 E\(_2\) (PGE\(_2\)) 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 l-enantiomer of the racemate bupivacaine. Levobupivacaine is considered to be a safer analogue than racemic bupiva-
caine with reduced cardiovascular and neurological side-
effects. Except for an i.t. rat cauda equina model in which similar histopathological neurotoxicity between bupiva-
caine and its enantiomers was shown, literature on the spinal cord effects of levobupivacaine after i.t. admin-
istration is scarce.

TNS incidence does not change with dilution of the local anaesthetic dose. Neither does it change when comparing i.t. 60 and 75 mg doses. 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.

We measured dialysate level changes in CSF of glutamate and PGE2 as changes in these may be considered as surro-
gates for assessing local-anaesthetic-induced nociception or central hypersensitivity. 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. 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 ±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. 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 μl s.c. injection of buprenorphine (Temgesic® 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 surround-
ings. 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 CaCl2 2.3 mM) at a flow rate of 7.5 μl 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 μl (“Levo 100 μg”) or 50 μl of levobupivacaine (“Levo 250 μg”) (Chirocaine®, Abbott, Solbaevegen, Norway). A third, control group, received 50 μl 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 μl (“lido 400 μg”) or 50 μl (“lido’ 1000 μg) was injected i.t. (Linisol® 2% pro-injection, B. Braun, Melsungen, Germany). These experiments were performed as described previously. 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 μl 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°C for sub-
sequent 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 PGE₂ levels and 20 min glutamate levels was based on the magnitude and duration of glutamate and PGE₂ changes observed in previous studies.¹ ² ³

Assay of PGE₂ and glutamate in CSF

The concentration of PGE₂ in the microdialysate samples was quantified using a commercially available Correlate-EIA PGE₂ (competitive immunoassay) kit in accordance with the manufacturer’s protocol (Assay Design, Inc., USA). The concentration of PGE₂ 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.²⁰ The intra- and inter-assay coefficients of variation were 5.2% and 6.4% for PGE₂, 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 PGE₂ and glutamate baseline responses and set this average to 100%. Drug effects were expressed as % change of baseline values. The glutamate and PGE₂ 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 PGE₂ and glutamate, data were analysed using the Wilcoxon test. Between-group global differences of PGE₂ 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 PGE₂ 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 PGE₂ were stable. Measured baseline PGE₂ 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 μg, and the lido 400 and 1000 μg 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) μM, and for, respectively, the saline, the levo 100 and 250 μg, and the lido 400 and 1000 μg groups (n=40 for each group).

Taking into account the individual range variability observed in baseline values for PGE₂, we expressed our results as a percentage of the individual mean baseline values for further analysis.

Levobupivacaine-induced changes in PGE₂ and glutamate

In both the levo 100 and the 250 μg groups, spinal PGE₂ 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 μg, glutamate levels increased by 170% and 10% (Fig. 1B) within 10 min after i.t. administration. The glutamate concentrations rapidly returned to baseline within 20 min after levobupivacaine administration (Fig. 1B).

Repeated injection setting

We observed in the lido 1000 μg group, an increased (P=0.035) PGE₂ release in CSF after the second injection (ratio: 1.70). In the lido 400 μg group, a tendency (P=0.20) (Lido 400 μg) of increased PGE₂ release after the second injection was observed (ratio: 1.65). In contrast, the 60 min PGE₂ 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 μg) and lidocaine (1000 or 400 μg) (Fig. 2B).

Discussion

Surprisingly, this investigation shows that two different doses of i.t. administered levobupivacaine are accompanied by 50–90 min PGE₂ increases in CSF. These PGE₂ 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 levobupiva-

caine 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,8 we observed different durations of PGE 2
increases after i.t. injection of saline, levobupivacaine 100 µg (Levo 100), or
levobupivacine 250 µg (Levo 250). Data are reported as mean (s.d) change of baseline. *P<0.05
vs baseline.

Fig 1 (A) PGE 2 concentrations in CSF dialysates after i.t. injection of saline, levobupivacaine 100 µg (Levo 100), or levobupivacine 250 µg (Levo 250). Data are reported as mean (s.d) change of baseline. *P<0.05
vs baseline. (B) Glutamate concentrations in CSF dialysates after i.t. injection of saline, levobupivacaine 100 µg (Levo 100), or
levobupivacine 250 µg (Levo 250). Data are reported as mean (s.d) change of baseline. *P<0.05
vs baseline.

The physiological background behind our model can be
explained as follows: i.t. lidocaine or levobupivacaine
induces dorsal horn neuronal circuitry activation in which
PGE 2 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 A 2 (PLA 2). PLA 2
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.21

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 levobu-
ivacaine and to observe possible residual PGE 2 effects
after a first i.t. dose. Using this approach, we found
that the 60 min AUC of PGE 2 values were higher after

Effects of i.t. administration of levobupivacaine and lidocaine

Fig 2 (A) Comparative 60 min AUC of PGE 2 response after both i.t.

injections in the levobupivacaine 100 µg (Levo 100) or levobupivacine
250 µg (Levo 250) and lidocaine 400 µg (Lido 400) and lidocaine 1000 µg
(Lido 1000), and saline groups. Data are reported as mean (s.d).
*P<0.05 vs baseline. (B) Comparative 20 min AUC of glutamate
response after two i.t. injections of levobupivacaine 100 µg (Levo 100)
and 250 µg (Levo 250), lidocaine 400 µg (Lido 400) and 1000 µg (Lido
1000), and saline. Data are reported as mean (s.d). *P<0.05 vs baseline.
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 abnor-
mal pain hypersensitivity, we believe that this finding may
corraborate 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 investiga-
tion, 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 inci-
dence 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 injec-
tion of methylene blue. The rats also did not receive a
high-dose continuous infusion of local anaesthetics
through the catheter,13 but two single i.t. injections. We
therefore think it is highly unlikely that the observed
PGE2 changes were related to CES, but rather believe that
the observed PGE2 changes after i.t. levobupivacaine
and lidocaine may reflect initiating reversible white matter
damage25 and a subsequent self-limiting inflammation
induced. The resulting effect after i.t. injection might also
be dependent on previous nervous inflammation, on exist-
ing vasa nervosum atherosclerosis, and on the extent of
rostral spread and distribution of the anaesthetic after i.t.
 injection. Further studies are therefore needed to establish
the relationship between morphological changes induced
in vitro and the occurrence of clinical symptoms of TNS
or CES and PGE2 changes.

Whether the syndrome of TNS is to be considered as a
manifestation of mild, transient lidocaine neurotoxicity is
still controversial, because TNS does not seem to be as
closely associated with either a high dose or sacral pooling
of lidocaine.26

We did not compare exact equipotent i.t. doses. Taking
into account the 1:4.70 (95% CI 3.65–7.07) lidocaine/
bupivacaine potency ratio for i.t. injection in rats6 and the
suggested equipotent potency ratios between bupivacaine
and levobupivacaine,27 we should have given 470 and
1175 µg of lidocaine to achieve equipotent doses.
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 con-
trast 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.

References

1 Zaric D, Christiansen C, Pace NL, Punjasawadwong Y. Transient
neurologic symptoms after spinal anesthesia with lidocaine versus
other local anesthetics: a systematic review of randomized, con-
2 Li DF, Bahar M, Cole G, Rosen M. Neurological toxicity
of the subarachnoid infusion of bupivacaine, lignocaine or
3 Drasner K, Sakura S, Chan VW, Bollen AW, Ciriales R. Persistent
sacral sensory deficit induced by intrathecal local anesthetic infu-
sion in the rat. Anesthesiology 1994; 80: 847–52
4 Yamashita A, Matsumoto M, Matsumoto S, Itoh M, Kawai K,
Sakabe T. A comparison of the neurotoxic effects on the spinal
cord of tetracaine, lidocaine, bupivacaine, and ropivacaine
administered intrathecaly in rabbits. Anesth Analg 2003; 97:
512–9
7 Eisenach JC, Yaksh TL. Safety in numbers: how do we study toxicity of spinal analgesics? Anesthesiology 2002; 97: 1047–9
10 McLeod GA, Burke D. Levobupivacaine. Anesthesia 2001; 56: 331–41
15 Hampf KE, Schneider MC, Margger H, Gut J, Drewe J, Drasner K. A similar incidence of transient neurologic symptoms after spinal anesthesia with 2% and 5% lidocaine. Anesth Analg 1996; 83: 1051–4
24 Johnson ME, Saenz JA, DaSilva AD, Uhl CB, Gores GJ. Effect of local anesthetic on neuronal cytoplasmic calcium and plasma membrane lysis (necrosis) in a cell culture model. Anesthesiology 2002; 97: 1466–76
27 Alley EA, Kopicz DJ, Mc Donald SB, Liu SS. Hyperbaric spinal levobupivacaine: a comparison to racemic bupivacaine in volunteers. Anesth Analg 2002; 94: 188–93