Pregnancy enhances the antinociceptive effects of extradural lignocaine in the rat

M. Kaneko, Y. Saito, Y. Kirihara and Y. Kosaka

SUMMARY
We have compared the antinociceptive effect of extradural lignocaine on somatic and visceral nociception in pregnant (n = 11) and non-pregnant rats (n = 9). Colorectal distension (CD) threshold and tail flick (TF) latency were measured as visceral and somatic nociception, respectively, for 60 min after extradural injection. On days 19, 20 and 21 of pregnancy, rats received lignocaine 200, 400 or 800 µg or normal saline via a chronically implanted lumbar extradural catheter. Extradural lignocaine produced dose-dependent antinociceptive effects on TF latency and CD threshold. Lignocaine 200 or 400 µg produced significantly greater peak effects in pregnant than in non-pregnant rats. Although the peak effects with lignocaine 800 µg were not different between groups, the duration of the effects were longer in pregnant compared with non-pregnant rats. We conclude that both somatic and visceral antinociceptive effects of extradural lignocaine were potentiated in pregnant rats near term compared with those in non-pregnant rats. (Br. J. Anaesth. 1994; 72: 657-661)

KEY WORDS

It has been demonstrated that the dose requirements of local anaesthetics for extradural anaesthesia are reduced during pregnancy [1]. Pregnant women need approximately 30% less local anaesthetic for extradural anaesthesia than non-pregnant women [1]. However, some investigators failed to demonstrate increased extradural spread in patients at term [2, 3]. It is not clear if pregnant women are more susceptible to conduction anaesthesia, and if they are if this alteration results from pregnancy-induced reduction in the concentration of local anaesthetic within the spinal canal (e.g. as a consequence of extradural vein distension). There is no experimental evidence to suggest that extradurally administered local anaesthetics produce greater analgesia during pregnancy, although peripheral nerves have been shown to be more susceptible to local anaesthetic block during pregnancy [4-6].

On the other hand, most basic research on analgesic effects of extradural anaesthesia have focused on somatic nociception. Relatively little attention has been given to visceral nociception, in spite of its obvious clinical importance. Widely used visceral pain models, such as writhing tests or electrical stimulation, suffer from problems of reliability and reproducibility in pregnant animals. Recently, colorectal distension has been reported as a useful method for the quantitative study of visceral nociception in rats [7]. We have assessed visceral nociception in pregnant rats using colorectal distension as the noxious visceral stimulus [8].

The purpose of the present study was to evaluate the antinociceptive effects of extradural lignocaine on both somatic and visceral noxious stimuli during late pregnancy in rats and to compare them with those in non-pregnant animals.

MATERIALS AND METHODS
The study was approved by the Animal Research and Use Committee of Shimane Medical University.

Animals and surgical preparation
Female Sprague-Dawley rats (mean body weight 302.4 (range 280-324) g, before conception) were housed individually in a room with a light period of 12 h per day (08:00-20:00) with free access to food and water. All animals were handled and trained in the test situation for at least 5 days before the experiments, so that they became familiar with the researchers, and to avoid non-specific stress. Rats were time-mated and the first day in which sperm were detected in the vaginal smear was designated as day 1 of pregnancy.

On day 14 of pregnancy, a catheter was implanted into the lumbar extradural space using a sterile technique. Animals were anaesthetized with halothane in oxygen and a saline filled catheter (PE-10:1.5 cm connected to PE-20:12 cm) was inserted with the PE-10 part passed caudally into the lumbar extradural space through an incision at the T13-L1 intervertebral space. The free end of the catheter was passed subcutaneously to the nape. At the end of surgery, all animals were given cephalixin 100 mg...
i.m. Animals exhibiting any neurological deficits, infection or other health problems such as complications of surgery, were excluded from further experiment. The location of the distal end of the catheter was verified at the end of an experiment by injection of indigocarmine dye and postmortem examination of the spinal cord. Data obtained from animals in which the dye failed to stain the lumbar extradural space or in which the spinal cord had observable damage were not included in the data analysis.

**Nociceptive tests**

**Tail flick (TF) test.** The tail flick test was performed to measure the response to a noxious somatic stimulus. The time between stimulus onset and withdrawal of the tail from the heat source (an 100-W projector lamp) focused on a distal segment of the tail was defined as the response latency (TF latency). The apparatus (model DS20, Ugo Basile, Cameri-Varese, Italy) was calibrated to give an average baseline latency of approximately 4.0 s. A maximum latency of 10 s was used to prevent tissue damage.

**Colorectal distension (CD) test.** To assess visceral nociception, we used the CD test, modified from Ness and Gebhart [17]; this involved inflation of a 8-cm long, flexible latex balloon with air. The balloon consists of two parts, a 7-cm long stimulating balloon (proximal part) and a 1-cm long sensing balloon (distal end). The pressures in both parts were monitored continuously via in-line pressure transducers and recorded (Rectigraph, San-ei, Tokyo, Japan). The balloon was passed into the rectum and the descending colon under light halothane anaesthesia and tested while awake after recovery from anaesthesia. The pressure within the stimulating balloon was increased steadily at a rate of 2.5 mm Hg s\(^{-1}\), beginning with 0 mm Hg until contraction of abdominal musculature occurred and repeated; a rapid increase in pressure (spike-like waves) in the sensing balloon was detected. The minimal pressure at which these signs occurred was defined as the threshold for visceral nociception in this test (CD threshold). A maximum distension pressure of 60 mm Hg was used to prevent tissue damage.

**Measurements**

Nociceptive thresholds were determined during the course of pregnancy. After determining baseline values for both the TF and CD tests before conception, one group of rats (group P, n = 11) was mated and re-tested on days 7, 14 and 19 of pregnancy. The other group (group N-P, n = 9), in which rats were mated but did not become pregnant, was tested in the same manner at the same intervals as non-pregnant rats.

**Extradural injections.** On days 19, 20 and 21 of pregnancy, each rat received an extradural injection of normal saline or lignocaine 200, 400 or 800 μg after baseline measurements for TF latency and CD threshold. All injections were made up in a volume of 40 μl, manually over 30 s, followed by a 15-μl flush of normal saline with a 50-μl Hamilton microsyringe. TF latency and CD threshold were measured at 5, 10, 15, 20, 30, 40, 50 and 60 min after injection. Animals in group N-P were tested in the same manner as those in group P.

**Statistical analysis**

The antinociceptive effects of extradural lignocaine were assessed by transforming the response latency or threshold to the percentage maximum possible effect: \(\%\text{MPE} = \frac{\text{post-drug value} - \text{pre-drug value}}{\text{cut-off value} - \text{pre-drug value}} \times 100\%\). The area under the curve (AUC) of \(\%\text{MPE vs time} \) was calculated using the trapezoidal rule in order to express the overall magnitude and duration of effect for each test. Differences in \(\%\text{MPE and AUC between groups after extradural lignocaine were assessed by analysis of variance for repeated measures and Dunnet's test was used for post hoc comparison. Within-group differences between baseline values and those after extradural administration were assessed by analysis of variance for repeated measures and Student's paired t test was used for post hoc comparison. Data were analysed using the Statview II statistical package (Abacus Concepts Inc., Berkeley, CA, U.S.A.) on a Macintosh IIci (Apple Computer Inc., Cupertino, CA, U.S.A.). Differences were considered statistically significant when \(P < 0.05\).

**RESULTS**

Changes in nociceptive thresholds during the course of pregnancy (fig. 1)

In pregnant rats, there were significant increases in both TF latency and CD threshold compared with baseline measurements (\(P < 0.001\)) and compared with those in group N-P at the same times (\(P < 0.05\)). Mean TF latency increased from a baseline value of 4.0 (95% confidence interval 3.75–4.25) to 4.8 (4.51–5.09) s on day 14 and to 5.4 (5.03–5.77) s on day 19 of pregnancy. Mean CD threshold increased from a baseline value of 20.2 (19.66–20.74) mm Hg on day 7, to 24.8 (23.53–26.07) mm Hg on day 14 and to 27.0 (25.97–28.03) mm Hg on day 19 of pregnancy. In group N-P, statistically significant changes were not observed in either TF latency or CD threshold.

**Effects of extradural lignocaine**

Extradural lignocaine produced dose- and time-dependent increases in TF latency (fig. 2A, c, e) and CD threshold (fig. 2B, d, f), in both groups P and N-P, while saline had no significant effects. The peak effect with lignocaine 800 μg did not differ between groups, but statistically significant differences in \(\%\text{MPE between groups were observed at 15 and 20 min in the TF test and at 20 and 30 min in the CD test (fig. 2A, e), indicating that the duration of antinociceptive effects was longer in group P. Lignocaine 400 μg produced significantly greater peak effects in group P than in group N-P in both TF latency and CD threshold. Mean peak \% MPE for TF latency was 89 (61–117) in group P and 62 (27–97) in group N-P (\(P < 0.05\)) (fig. 2C). Mean peak \% MPE for CD threshold was 97 (92–102) in group
ANTINOCEPTION BY EXTRADURAL LIGNOCAINE IN PREGNANCY

Fig. 1. Changes in TF latency (a) and CD threshold (b) during pregnancy (mean, sn) in group P (●) (n = 11) and group N-P (○) (n = 9). ***P < 0.001 compared with pre-gestational (Pre.) value; †P < 0.05 compared with non-pregnant rats.

Fig. 2. Changes in percentage maximum possible effect (% MPE) (mean, SEM) for TF latency and CD threshold after extradural injection of lignocaine in group P (●) (n = 8–9 for each dose) and group N-P (○) (n = 6 for each dose) or after saline in group P (▲) and group N-P (△). a: TF test, lignocaine 800 µg or normal saline. b: CD test, lignocaine 800 µg or normal saline. c: TF test, lignocaine 400 µg. d: CD test, lignocaine 400 µg. e: TF test, lignocaine 200 µg. f: CD test, lignocaine 200 µg. *P < 0.05, **P < 0.01 compared with pre-injection (Pre.) value; †P < 0.05 compared with group N-P.

P and 70 (41–99) in group N-P (P < 0.05) (fig. 2d). With lignocaine 200 µg, there were statistically significant differences between groups only in CD threshold; mean peak %MPE in group P, 69 (51–87) vs 41 (10–72) in group N-P (P < 0.05) (fig. 2f).

AUC values tended to be greater in group P than in group N-P for both TF latency and CD threshold with all doses of lignocaine, but a statistically significant difference between groups was observed only with lignocaine 200 µg for CD threshold (P < 0.05) (fig. 3).

FIG. 1. Changes in TF latency (A) and CD threshold (B) during pregnancy (mean, sn) in group P (●) (n = 11) and group N-P (○) (n = 9). ***P < 0.001 compared with pre-gestational (Pre.) value; †P < 0.05 compared with non-pregnant rats.

FIG. 2. Changes in percentage maximum possible effect (% MPE) (mean, SEM) for TF latency and CD threshold after extradural injection of lignocaine in group P (●) (n = 8–9 for each dose) and group N-P (○) (n = 6 for each dose) or after saline in group P (▲) and group N-P (△). a: TF test, lignocaine 800 µg or normal saline. b: CD test, lignocaine 800 µg or normal saline. c: TF test, lignocaine 400 µg. d: CD test, lignocaine 400 µg. e: TF test, lignocaine 200 µg. f: CD test, lignocaine 200 µg. *P < 0.05, **P < 0.01 compared with pre-injection (Pre.) value; †P < 0.05 compared with group N-P.

DISCUSSION

This is the first study to provide clear evidence that pregnant rats are more susceptible to the effects of extradural anaesthesia than non-pregnant rats. These results are consistent with the clinical findings of Bromage [1] who observed increased spread of extradural anaesthesia in pregnant women, although our study was not designed to determine dermatomal levels. The results of the present study show increased antinociceptive effects produced by
extradural lignocaine in pregnant rats earlier (5 and 10 min after injection) with smaller doses (200 and 400 μg) and later (15, 20 and 30 min) with larger doses (800 μg), indicating that pregnancy reduced the concentration of local anaesthetic required to inhibit nociceptive responses.

There has been relatively little attention given to visceral nociception, in spite of its obvious clinical importance. Recently, colorectal distension was developed in a rat model by Ness and Gebhart [7] and has been reported as a useful test that is more quantitative and reproducible and closely related to human pathology than those used previously, for example chemically induced writhing or electrical stimulation. In the present study, we have demonstrated that extradural lignocaine produced dose- and time-dependent antinociceptive effects during visceral nociception and also during somatic nociception. In addition, the CD test performed in the present study did not interfere with the process of pregnancy. The smaller dose of lignocaine used in the present study produced significantly greater peak effects in pregnant than in non-pregnant rats on visceral but not on somatic nociception. While it is difficult to compare the relative activity of a given agent on different nociceptive tests, the difference in antinociceptive effects of extradural lignocaine between the TF and CD tests suggests that the visceral is more susceptible to extradural anaesthesia than the somatic nociceptive system during late pregnancy. This finding is relevant to clinical observations that extradural infusion of relatively small concentrations of local anaesthetics produce satisfactory analgesia for labour pain [9, 10], one of the most intense pains studied [11,12]. The mechanism of facilitated spread of extradural anaesthesia in pregnant women has not been elucidated. Mechanical factors, such as elevated intraabdominal pressure and inferior vena cava compression from the pregnant uterus or exaggerated lumbar lordosis of pregnancy have been proposed. However, neural causes have been suggested in some investigations [4–6, 13]. Fagraeus, Urban and Bromage [13] demonstrated increased spread of extradural analgesia even during the first trimester, comparable in magnitude with those reported for parturients at term, at a time when mechanical factors are unlikely to play a significant role. The space occupying effects of distended extradural veins and exaggerated lumbar lordosis at term are not likely to occur in rats because of their natural semi-prone position. Thus our data support a neural cause.

The present study did not reveal the underlying mechanism of increased antinociceptive effects. One possible explanation is that pregnancy increases the susceptibility of spinal nerve fibres to local anaesthetic-induced conduction block and thus reduces the concentration of local anaesthetics required to inhibit spinal nerves and also peripheral nerves, as demonstrated in several studies [4–6]. Datta and colleagues [4] and Flanagan and colleagues [5] showed that vagus nerves removed from pregnant rabbits were more susceptible to local anaesthetic-induced conduction block. Butterworth, Walker and Lysate [6] demonstrated that the median nerve of pregnant women in the third trimester is more susceptible to conduction block induced by lignocaine than that of non-pregnant women. Increased plasma or cerebrospinal fluid concentrations of progesterone during pregnancy have been attributed to alteration in excitability of the nervous system [14–16]. Another possibility is an interaction between local anaesthetic and endogenous spinal opioid receptors. Increases in pain thresholds have been observed during gestation in laboratory animals [17] and humans [18] and this analgesia is mediated via activation of the spinal opioid system. Intrathecal administration of naltrexone to pregnant rats reduces pregnancy-induced antinociception [19] and intrathecal administration of the kappa-selective opioid antagonist, norbinaltorphimine, or dynorphin antisera during gestation, has implicated the involvement of spinal kappa receptors [20,21]. On the other hand, it has been demonstrated that intrathecal or extradural administration of both morphine and lignocaine or bupivacaine interact to produce synergistic antinociceptive effects in rats [22–24] and that the synergism is related, at least in part, to spinal kappa receptor subtypes [25].

ACKNOWLEDGMENT

This work was supported by a grant-in-aid for scientific research from the Ministry of Education, Science and Culture in Japan to Y. Saito (No. 0285 7212).

REFERENCES


