Postoperative Stroke in Patients After Spinal Anesthesia and Disparate Response Patterns of Carotid or Cerebral Blood Flow and Baroreflex Functionality to Spinal Bupivacaine in Rats

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**Postoperative stroke in patients after spinal anesthesia and
disparate response patterns of carotid or cerebral blood flow and
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Abstract

Spinal anesthesia is generally accepted as an effective and safe practice. Three rare incidents of postoperative cerebral infarction after surgery under spinal anesthesia with bupivacaine prompted us to assess whether spinal bupivacaine may impact carotid or cerebral blood flow and baroreflex functionality. We found that all three patients shared common pathology of stenosis or atheromatous lesions in the carotid or middle cerebral artery. In a companion animal study, we further observed that subarachnoid application of bupivacaine that reached low thoracic spinal cord in male Sprague-Dawley rats elicited an initial (Phase I) reduction in mean arterial pressure (MAP), carotid blood flow (CBF) and baroreflex-mediated sympathetic vasomotor tone, all of which returned to baseline in Phase II. Whereas heart rate (HR) exhibited sustained reduction, cardiac vagal baroreflex, baroreflex efficiency index (BEI) and tissue perfusion and oxygen in cerebral cortex supplied by middle cerebral artery remained unaltered during both phases. However, in one-third of animals studied, Phase II gave way to a Phase III characterized by secondary hypotension and depressed baroreflex-mediated sympathetic vasomotor tone, along with continuous decline in HR, sustained cardiac vagal baroreflex, decreased BEI, and reduction in CBF and tissue perfusion or oxygen in cerebral cortex. We conclude that carotid and cerebral blood flow can be compromised after spinal anesthesia, and impaired baroreflex-mediated sympathetic vasomotor tone that leads to hypotension plays a contributory role.

Introduction

Spinal anesthesia has been a widely used anesthetic technique since it was introduced to the surgical world by August Bier in 1898 ¹. It is generally considered a simple procedure with high successful rate; anesthesiologists master spinal anesthesia after only 40 to 70 supervised attempts ^{2,3}. Recent reports ⁴⁻⁶ showed that spinal anesthesia has advantages over general anesthesia in short term morbidity and mortality among the elderly who undertake total knee or total hip replacement.

The most reported common side effects of spinal anesthesia are hypotension and bradycardia ^{7,8} or drowsiness ^{9,10}, which are believed to be the cardiovascular consequences of induced preganglionic sympathetic paralysis ^{11,12}. Hypotension occurs from sympathetic block that leads to decreases in systemic vascular resistance and central venous pressure ¹³⁻¹⁶. Bradycardia occurs from shift in cardiac autonomic balance towards the parasympathetic system in high spinal anesthesia ¹⁷, or reverse Bainbridge reflex in low spinal anesthesia ¹⁸. Intraoperative drowsiness may be related to spinal anesthesia-induced hypotension or arterial oxygen desaturation ^{6,19}.

Baroreflex is the most fundamental feedback mechanism in short-term ²⁰ and long-term ²¹ regulation of blood pressure (BP) and heart rate (HR). Cerebral autoregulation is the intrinsic ability of the brain to maintain stable cerebral blood flow despite changing mean arterial pressure (MAP) ²². Of the limited literature on the effects of spinal anesthesia on these two fundamental regulatory processes on cardiovascular functions, Gratadour and colleagues ²³ reported no significant change in spontaneous cardiac baroreflex during spinal anesthesia with bupivacaine in patients scheduled for elective inguinal hernia repair. Small but statistically significant reduction of cerebral blood flow has been shown during spinal

anesthesia in the very elderly ²⁴ and preterm infants ²⁵. Perioperative stroke is a devastating complication associated with high morbidity and mortality ²⁶⁻²⁹, and postoperative stroke is uncommon in noncardiac or nonneurologic surgery ^{30,31}.

Three unanticipated incidents of patients who developed cerebral infarction after surgery under spinal anesthesia with bupivacaine prompted us to report these infrequent clinical cases. Their shared common pathology of stenosis or atheromatous lesions in carotid or middle cerebral artery further formed the impetus for our companion animal study with a primary aim to assess the guiding hypothesis that intrathecal administration of bupivacaine may impact carotid and cerebral blood flow and baroreflex dysfunction may play a contributory role.

Results

Part 1. Patients

Shared pathology of patients who developed cerebral infarction after surgery under spinal bupivacaine anesthesia

We present here three patients who developed cerebral infarction 4-8 h after surgery under spinal bupivacaine anesthesia. Postoperative examinations revealed that a common pathology shared by all 3 patients is stenosis or atheromatous lesions in carotid or middle cerebral artery.

Patient A had experienced two cerebral ischemic attacks, 12 years and 1 year, prior to this admission. Preoperative transcranial ultrasound examination revealed high-grade stenosis in right internal carotid artery and atheromatous lesions in bilateral common carotid artery and left internal carotid artery. She developed right side weakness 5 h after PACU discharge. Diagnosis based on MRI was acute infarction of left fronto-parietal area with multiple vessel abnormality.

Patient B had no prior history of cerebrovascular incidence. He developed right side weakness 4 h after PACU discharge. Diagnosis based on MRI was small acute left corona radiate and basal ganglia infarcts. Carotid duplex study revealed atheromatosis in bilateral carotid artery bulb and left internal carotid artery.

Patient C had a history of over 50% stenosis in left middle cerebral artery based on transcranial ultrasound examination although no prior cerebrovascular episode was noted. He developed left side weakness 8 h after PACU discharge. Diagnosis based on MRI was acute infarction in right pons.

Part 2. Animals

The clinical observation that a common pathology shared by patients who develop cerebral infarction after surgery under spinal bupivacaine anesthesia is stenosis or atheromatous lesions in carotid or middle cerebral artery prompted us to assess in an animal study whether intrathecal administration of bupivacaine impacts carotid and cerebral blood flow and whether baroreflex dysfunction plays a contributory role.

Experimental set-up

Because of the special requirements for experimental set-up, male Sprague-Dawley rats that were instrumented with an indwelling catheter for intrathecal injection into the spinal subarachnoid space were assigned randomly into three groups (see relevant sections under Methods for detailed description). Group One animals received myelographic examination to establish the segmental distribution of intrathecally administered bupivacaine in the spinal cord. Our second and third groups were employed to determine the time-course alterations of mean arterial pressure (MAP), heart rate (HR), baroreflex-mediated sympathetic vasomotor tone, cardiac vagal baroreflex or baroreflex efficiency index (BEI), simultaneous with carotid blood flow (CBF) (Group Two) or tissue perfusion and oxygen in cerebral cortex supplied by middle cerebral artery (Group Three), in response to intrathecal administration of bupivacaine.

Distribution of contrast medium in spinal subarachnoid space after intrathecal administration

To set the stage for our animal study, we first established the segmental distribution of

intrathecally administered bupivacaine to induce spinal anesthesia in rats. For this purpose, we employed myelographic examination to determine the relationship between volume of administration and extent of dispersion in spinal cord using a contrast medium as the surrogate (Fig. 1B,C). Taking into consideration that the segments of spinal cord always lie higher than the corresponding vertebrae³², injection of 40 μ L of contrast medium via the tip of our catheter lodged at the subarachnoid space below L6 vertebra (Fig. 1A) manifested an enhanced roentgenological image at the S2 spinal cord (Fig. 1Bb,C) as compared with the pre-enhanced image (Fig. 1Ba). Increasing the volume of administered contrast medium to 60, 80 or 100 μ L extended the enhanced image to L1 (Fig. 1Bc,C), T10 (Fig. 1Bd,C), or T2 (Fig. 1Be,C) spinal cord. Based on those observations, we chose 80 μ L as the volume of bupivacaine injection in subsequent physiological experiments because its distribution in spinal cord closely mimics the targeted segmental levels of spinal anesthesia in our patients.

Common response pattern of carotid or cerebral blood flow and baroreflex functionality to spinal bupivacaine

Intrathecal administration of 80 μ L of bupivacaine resulted in two distinct phases of temporal changes in cardiovascular events, CBF and baroreflex functionality in 17 of 24 (70.8%) animals in Group Two studied (Figs. 2-4). Phase I, which endured 20 min, manifested a significant reduction in MAP and HR, accompanied by a significant decrease in CBF and baroreflex-mediated sympathetic vasomotor tone, without significant alterations in cardiac vagal baroreflex (Figs. 2A and 3). Phase II, which lasted the remainder of the 160 min observation period, exhibited a return of MAP, CBF or power density of BLF component to the basal level, alongside a progressive decrease in HR (Figs. 2A and 3). Of note is that BEI presented no significant alterations during Phases I and II as compared to its basal level (Fig.

4A).

In a separate series of experiments (Figs. 2B, 3 and 4A), intrathecal administration of 80 μ L of bupivacaine in 16 of 24 (66.7%) animals in Group Three studied resulted in phasic changes in MAP, HR, BEI, baroreflex-mediated sympathetic vasomotor tone or cardiac vagal baroreflex that were comparable to those depicted above. Of interests is that local blood flow, tissue oxygen tension or temperature in cerebral cortex remained similar to their basal levels during both Phases I and II.

Anomalous response pattern of carotid or cerebral blood flow and baroreflex functionality to spinal bupivacaine

We also encountered in 15 of 48 (31.3%) Groups Two and Three animals studied an unanticipated anomalous response pattern to intrathecal administration of 80 μ L of bupivacaine. Whereas the biphasic response patterns of MAP, HR, BLF power, BRS or BEI in those rats were comparable to those depicted in Figs. 2A, 3 and 4A, Phase II gave way to a Phase III after sustaining for less than 40 min (Figs. 4B, 5A and 6). Phase III, which persisted until the end of our 160 min observation period, was characterized by a significant secondary hypotension and baroreflex-mediated sympathetic vasomotor tone, alongside continuous decline in HR, sustained cardiac vagal baroreflex and significant reduction in BEI. Of note is that the waxing and waning alterations in MAP, BLF power and BEI were manifested in a correlated manner. CBF also underwent a secondary decrease (Figs. 5A and 6) in 7 of 24 (29.2%) animals in Group Two studied. Likewise, 8 in 24 (33.3%) Group Three rats exhibited a significant decrease in local blood flow or tissue oxygen tension in cerebral cortex (Figs. 5B and 6) during Phase III.

Discussion

Spinal anesthesia is generally accepted as an effective and safe technique by anesthesiologists and surgeons because the anesthetic effect can be confined to lower thoracic or lumbar dermatomes of the body and interference to cardiovascular functions is usually benign and readily correctable. With the exception of infants²⁵ or elderly²⁴, it is also generally conceived that spinal anesthesia would not have discernible effect on cerebral blood supply. Based on complementary observations from patients and animals, our study revealed that those notions may have to be modified because carotid and cerebral blood flow can indeed be compromised after spinal anesthesia with bupivacaine and baroreflex dysfunction plays a contributory role.

Steel observed in 1925³³ that “A fall of blood pressure accompanies each spinal anesthesia....Its low point is usually ten minutes after the injection, and most fatalities have occurred at that time. After fifteen minutes, one is working away from the danger point, not toward it as in other general anesthetics”. Amazingly, this almost century-old observation is still valid today, and results from our animal study offer a plausible underlying physiological scenario. We demonstrated that on intrathecal administration of 80 μ L of bupivacaine that reached T10 spinal cord, there was indeed a transient decrease of MAP (Phase I) in all rats from Groups Two and Three. As a consequence of this hypotension, CBF underwent a transitory reduction. On the other hand, cerebral blood flow and tissue oxygen tension remained stable, indicating that cerebral autoregulation was operational. Our results further indicated that the two arms of baroreflex play differential roles. The essentially unaltered BRS and BEI during Phase I implied that, as reported previously²⁴, cardiac vagal baroreflex in low spinal anesthesia is not responsible for the induced bradycardia. Instead, the transient decrease in HR is possibly related to reverse Bainbridge reflex¹⁸ because of dilatation of

veins by the bupivacaine-induced preganglionic sympathetic paralysis ¹¹. On the other hand, the elicited hypotension may be accounted for by the temporary curtailment of the baroreflex-mediated vasomotor tone to exert its tonic actions on systemic vascular resistance because of the elicited sympathetic block ¹³⁻¹⁶. During Phase II, “....working away from the danger point” takes place on resumption of baroreflex-mediated vasomotor tone, leading to significant return of MAP and CBF towards baseline, amid sustained bradycardia.

Postoperative stroke is rare but its consequence is devastating. It is generally considered that postoperative stroke is multifactorial, and intraoperative hypotension may aggravate the pathological process by increasing the infarction size ^{26,27}. Furthermore, a decrease of MAP by more than 30% from baseline is significantly associated with the occurrence of a postoperative stroke ³⁴, and watershed infarct is associated with hypotension following spinal anesthesia ³⁵. By postoperative MRI examination, we confirmed that watershed infarct is absent in the three patients. On the other hand, we are aware that the secondary decline in CBF during Phase III in Group Two animals is reminiscent of the potentially impeded blood flow in their carotid or middle cerebral artery because of stenosis or atheromatous lesions in these vessels. Judging from the significant and correlative waxing and waning alterations in MAP, BLF power and BEI, it is conceivable that the retarded effectiveness of baroreflex-mediated sympathetic vasomotor tone during Phase III underpins the secondary hypotension that leads to the reduction in CBF. It follows that, coupled with dysfunctional cerebral autoregulation, the significant decrease in local tissue blood flow or tissue oxygen tension in cerebral cortex supplied by the middle cerebral artery offers a plausible *modus operandi*, albeit differences in time-windows (minutes versus hours), for the unanticipated cerebral infarction in our three reported cases. This notion is in line with the observation ³⁶ that dynamic autoregulation is impaired in a subgroup of patients with carotid artery

stenosis and are at risk from subsequent stroke, as demonstrated by their inability to maintain middle cerebral artery blood flow in response to a rapid reduction of BP. Because of the maintained cardiac vagal baroreflex, it is also likely that the secondary decrease in HR is sustained by reverse Bainbridge reflex ¹⁸.

We are cognizant that our study design does not allow us to offer a mechanistic explanation for the transition between the phases in Groups Two and Three animals. Nevertheless, four pieces of information from the physiological and anatomical literature afford some speculations. First, it is well-known that sympathetic vasomotor tone is generated by a tonic excitatory action from the rostral ventrolateral medulla onto the intermediolateral nucleus, where 85% of the sympathetic preganglionic neurons (SPNs) take origin, subjected to modulation by inputs from the baroreceptor afferents via the nucleus tractus solitarii ³⁷. Second, superimposed on this well-known phenomenon, we reported previously ³⁸ that both nitric oxide synthase I and II (NOS I and NOS II) are present in the thoracic spinal cord and are tonically active. In particular, the endogenous NO generated by NOS I-containing SPNs exerts a tonic excitatory action on vasomotor tone mediated by norepinephrine released from the adrenal medulla and sympathetic nerve terminals. On the other hand, NO derived from NOS II-containing fibers that originate from the rostral ventrolateral medulla and impinge on SPNs exerts a tonic inhibitory action on sympathetic outflow that targets primarily the blood vessels. Third, the spinal origin of SPNs to the celiac ganglion in the rat, which regulates splanchnic circulation, is T4 to T13; and that to the adrenal gland is T4 to T12 ³⁹. Interestingly, more than 60% of the SPNs that project to the celiac ganglion exhibit NOS I-immunoreactivity ⁴⁰. Fourth, sympathetic input to the splanchnic vasculature is not critical for normal functions of the splanchnic organs, but instead participates mainly in overall regulation of the circulation ⁴¹. Speculatively, the initial

brunt of intrathecal administration of bupivacaine is paralysis of the NOS I-containing SPNs located below T10, resulting in the acute withdrawal of the tonic excitatory action on the splanchnic vasculature during Phase I. During Phase II, baroreflex-mediated vasomotor tone triggered by hypotension utilizes the intact NOS I-containing SPNs located above T10 as its output component via their innervation of the celiac ganglion and adrenal gland. The inadvertent shift of dominance towards NOS II activity that exerts an inhibitory action on SPNs may account for the events observed during Phase III. The correlative waxing and waning alterations in MAP, BLF power and BEI may in effect reflect the result of reciprocal balance between NOS I and NOS II activity. These speculations, however, are subject to further validation.

In conclusion, we present in this study three rare cases of postoperative stroke in patients who received surgery under spinal anesthesia with bupivacaine. Based on their shared common pathology of stenosis or atheromatous lesions in carotid or middle cerebral artery, our companion animal study revealed the presence of two disparate response patterns of carotid or cerebral blood flow and baroreflex functionality in rats that received spinal bupivacaine. In all animals studied, intrathecal administration of bupivacaine that reached the low thoracic (T10) spinal cord exhibited an initial (Phase I) reduction in MAP, CBF and baroreflex-mediated sympathetic vasomotor tone, all of which returned to basal levels in Phase II. Whereas HR exhibited a sustained reduction, cardiac vagal baroreflex, BEI and local blood flow, tissue oxygen tension or temperature in cerebral cortex remained unaltered during both phases. In approximately one-third of the animals studied, Phase II gave way to a Phase III characterized by a significant secondary hypotension and depressed baroreflex-mediated sympathetic vasomotor tone, alongside continuous decline in HR, sustained cardiac vagal baroreflex, decrease in BEI, and secondary reduction in CBF and local

blood flow or tissue oxygen tension in cerebral cortex. These complementary observations from patients and animals post a cautionary note that carotid and cerebral blood flow can indeed be compromised after spinal anesthesia with bupivacaine and impaired baroreflex-mediated sympathetic vasomotor tone that leads to hypotension plays a contributory role.

Methods

Ethics statement

Retrospective report of the three clinical cases was approved by the Institutional Review Board of the Chang Gung Memorial Hospital (IRB approval number: 202000449B0C501). The Board also waived informed consent from the patients because they have been de-linked. All experimental procedures carried out on laboratory animals were approved by the Institutional Animal Care and Use Committee of the Kaohsiung Chang Gung Memorial Hospital (IACUC approval number: 2019062001). Methods were performed in accordance with the ARRIVE guidelines and were in compliance with the Animal Protection Law set forth by the Council of Agriculture, Taiwan and AAALAC-International Guide for the Care and Use of Laboratory Animals. All efforts were made to reduce the number of animals used and to minimize animal suffering during the experiment.

Patients

As indicated under Introduction, the immediate impetus of this study arises from three incidents between February, 2014 and January, 2018 when patients who received surgery under spinal anesthesia developed cerebral infarction 4-8 h after discharge from the postanesthetic care unit (PACU). Both patient A, a 64-year-old woman and Patient B, a 77-year-old man, suffered from knee osteoarthritis and were scheduled for total knee arthroplasty. Patient C, a 72-year-old man, suffered from left inguinal hernia and was scheduled for herniorrhaphy. Spinal anesthesia was performed with 3 ml of 0.5% bupivacaine injected through the L4-L5 interspace into the lumbar subarachnoid space. Intraoperative course was smooth and patients were returned to the wards after one-hour

observation in the PACU. The three patients were not instructed to remain bed rest after returning to the wards ⁴².

Animals

Experiments were carried out on specific pathogen-free adult male Sprague-Dawley rats (body weight: 300-350 g; age: 9 -10 weeks; n = 55) purchased from BioLASCO, Taiwan, Republic of China. They were housed 2 per cage under 12:12-h light-dark cycle and temperature control (24–25°C) in an AAALAC International-accredited Center for Laboratory Animals. Standard laboratory rat chow and tap water were available ad libitum.

Sample size calculation

We determined that the minimal number of animals of each of the three groups was 7 based on the equation: $n = 2\sigma^2(Z_{\alpha/2} + Z_{1-\beta})^2 / (\mu_1 - \mu_2)^2$; where the α level is 0.05; the pooled standard deviation (σ^2) is 0.64; the normal deviate at 5% significance ($Z_{\alpha/2}$) is 1.96, the normal deviate at 80% statistical power ($Z_{1-\beta}$) is 0.84, and the estimated difference between two means ($\mu_1 - \mu_2$) is 1.2.

Subarachnoid catheterization

Animals were instrumented with an indwelling catheter into the lower lumbar spinal subarachnoid space by modifying our previously devised method⁴³ for the thoracic spinal cord in the rat. Briefly, animals were anesthetized in an induction chamber with an induction dose of 4% isoflurane (Abbot Laboratories, Abbot Park, IL, USA) using a Matrx VIP 3000 vaporizer (Midmark, Orchard Park, NY, USA). After induction, rats were placed on an

operating table, and anesthesia was maintained with 2% isoflurane in 50% oxygen through a nose piece to carry out surgery. We removed the spinous process of L6 vertebra and drilled a hole through the cut portion of the lamina until the dura was exposed. A slit was made by traversing the surface of the dura with the tip of a 30-gauge needle, resulting in leakage of clear cerebrospinal fluid (CSF). A PE-10 catheter (Clay Adams, Parsippany, NJ, USA) was then inserted into the slit and advanced cephalically until 0.5 cm of the catheter was lodged in the subarachnoid space, placing its tip below the middle portion of L6 vertebra (Fig. 1A). Animals were returned to the animal room for postoperative recovery in individual cages after they received sodium penicillin (1000 IU; YF Chemical Corporation, Taipei, Taiwan) given intramuscularly to prevent postsurgical infection, and were allowed free access to standard rat chow and water. Only animals that showed full recovery after 7 days were used in subsequent experiments.

Intrathecal administration

During the recording sessions, the sealed end of the catheter was retrieved through a small skin incision under local anesthesia, and was first flushed with 10 μ L of artificial CSF (aCSF) to ensure patency. This was followed by intrathecal administrations of either a contrast medium or bupivacaine into the spinal subarachnoid space, delivered at a rate of 10 μ L/min by an infusion pump (CMA/102; CMA Microdialysis, Stockholm, Sweden).

Myelogram

Myelographic examination was performed in Group One animals under 1.5% isoflurane anesthesia on 7 rats with the implanted subarachnoid catheter, using the Ultimax-IDREX-

U180 digital x-ray system (Toshiba Medical Systems Corporation, Otawara, Japan). Four successive intrathecal administrations of a contrast medium (Opaque; GE Health, Cork, Ireland) were delivered at incremental volumes of 40, 60, 80 and 100 μ L. Radiographs were taken in the prone position 2 min after each dosing, and the distribution of the contrast medium in the subarachnoid space was recorded.

General preparation for physiological experiments

Animals in Groups Two and Three similarly received an induction dose of 4% isoflurane, placed on an operating table and were maintained under 2% isoflurane in 50% oxygen through a nose piece to carry out preparatory surgery. This included cannulation of the right femoral artery with a PE-20 catheter (Clay Adams) to measure arterial pressure and isolation of the right common carotid artery for measurement of blood flow. After surgery, animals were placed in a supine position on a platform with a heating pad and their rectal temperature was maintained at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$.

Measurement of blood pressure, heart rate and spontaneous baroreflexes

During the recording sessions, anesthesia was maintained at 1.5% isoflurane in 50% oxygen, delivered via a rat anesthesia mask. The recorded BP signals from the femoral artery were processed by an arterial BP analyzer (APR31a, Notocord, Croissy-Sur-Seine, France) to obtain systolic BP (SBP), HR, and pulse interval (PI). SBP signals were simultaneously subjected to online and real-time spectral analysis (SPA10a, Notocord) to detect the power density of the low-frequency component of SBP spectrum (BLF; 0.25-0.8 Hz). The power density of this spectral band has been demonstrated to be a valid experimental index for spontaneous

baroreflex-mediated sympathetic vasomotor tone ^{38,44}. To evaluate the spontaneous cardiac vagal baroreflex, we employed a baroreflex sequence analyzer (BRS10a, Notocord) to determine baroreflex sensitivity (BRS) based on online computerized scanning in search of reflex changes of PI in response to spontaneous sequences of consecutive increases or decreases in SBP ⁴⁵. We also calculated the baroreflex effectiveness index (BEI), which reflects the number of times changes in HR are evoked in response to BP fluctuations over a time-window of 60 s. Concurrent temporal changes in SBP, MAP and HR were continuously recorded, alongside power density of the BLF band, BRS or BEI. To avoid potential bias created by nonstationary disturbances, only stationary segments of data from spectral analysis were used for statistical analysis.

Measurement of carotid blood flow

We measured CBF in Group Two animals using a transit-time blood flowmeter (TS420, Transonic, Ithaca, NY, USA). The transonic flow probe (1.0 mm V-series; Transonic) was placed around the right common carotid artery to record the flow volume in ml/min. Anesthesia was maintained at 1.5% isoflurane during the recording session.

Measurement of microvascular perfusion, tissue oxygen level and temperature in cerebral cortex

Because of the special positions for measuring probes, measurement of microvascular perfusion, tissue oxygen level and temperature in cerebral cortex simultaneous with CBF determination is not feasible and has to be carried out in separate experiments (Group Three). A combined oxygen/temperature/blood flow probe designed for simultaneous and

continuous measurement of tissue oxygen tension (PtO_2), blood flow and temperature (BF/OF/E; Oxford Optronix, Abingdon, UK) was stereotaxically positioned in an area of the cerebral cortex that exhibited severe infarction on occlusion of the middle cerebral artery ⁴⁶. Real-time microvascular red blood cell perfusion in tissue was processed by an OxyFlo monitor (Oxford Optronix). The laser Doppler signals from the tissue were recorded in blood perfusion units (BPU), which is a relative unit defined against a controlled motility standard. Instantaneous changes in local oxygen tension, compensated for fluctuations in tissue temperature, were processed by an OxyLite monitor (Oxford Optronix).

Statistical analyses

Data were analyzed as previously described ⁴⁷. Briefly, all values are expressed as means $\pm$ SEM. The averaged values calculated in each response phase (see Results section) were used for statistical analyses using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Two-way analysis of variance with repeated measures was first used to assess the group means, followed by the Dunnett or Tukey multiple-range test for post hoc assessment of individual means. $P < 0.05$ was taken to indicate statistical significance.

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Study conception: YYP, SHHC, CYT.

Study design: YYP, YWL, SHHC, CYT.

Data collection: YYP, YHH, YWL.

Data analysis: all authors.

Writing, revising and approving the manuscript: all authors.

Declaration of interest

The authors declare that they have no conflicts of interest.

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Fig. 1. **(A)** Demonstration of lodging of the tip of the catheter (orange arrow) in the subarachnoid space below the middle portion of L6 vertebra. **(B)** Representative examples of myelographic examination before (a) and after intrathecal administration of 4 successive doses of contrast medium, given at 40 (b), 60 (c), 80 (d) or 100 (e) μ L. Note that all demarcations denote levels of the spinal cord, the lower yellow arrows mark the location of the tip of the catheter, and the upper yellow arrows indicate the highest points reached by the enhanced roentgenological images. CM: conus medullaris. **(C)** Scattered plots showing extent of dispersion in spinal cord of contrast medium on intrathecal administration at 40, 60, 80 or 100 μ L. Values are mean $\pm$ SEM of 7 animals.

Fig. 2. Illustrative examples of real-time and on-line recording of common phasic changes in mean arterial pressure (MAP), heart rate (HR), power density of the low-frequency component in systolic blood pressure spectrum (BLF), baroreflex sensitivity (BRS) or baroreflex effectiveness index (BEI), simultaneous with carotid blood flow (CBF) in Group Two animals **(A)**; or concurrent with tissue perfusion (Tissue Flow), tissue oxygen tension (PO_2) or tissue temperature in cerebral cortex in Group Three animals **(B)** on intrathecal administration of 80 μ L of bupivacaine (at arrow).

Fig. 3. Common response patterns of MAP, HR, BLF, BRS, CBF, or tissue perfusion, PO₂ or temperature in cerebral cortex to intrathecal administration of 80 µL of bupivacaine. Note values for MAP, HR, BLF and BRS are mean ± SEM of 33 animals (17 from Group Two plus 16 from Group Three); values for CBF are mean ± SEM of 17 animals from Group Two; and values for tissue perfusion PO₂ or temperature in cerebral cortex are mean ± SEM of 16 animals from Group Three. *P < 0.05 versus data obtained under basal conditions in post hoc Dunnett multiple-range analysis; *P < 0.05 versus data obtained during Phase I in post hoc Tukey multiple-range analysis.

Fig. 4. Common (**A**) and anomalous (**B**) response patterns of BEI to intrathecal administration of 80 µL of bupivacaine. Values for (**A**) are mean ± SEM from 33 animals (17 from Group Two plus 16 from Group Three); and values for (**B**) are mean ± SEM of 15 animals (7 from Group Two plus 8 from Group Three). *P < 0.05 versus data obtained under basal conditions in post hoc Dunnett multiple-range analysis.

Fig. 5. Illustrative examples of real-time and on-line recording of anomalous phasic changes in MAP, HR, BLF, BRS or BEI, simultaneous with CBF in Group Two animals (**A**); or concurrent with tissue perfusion, PO₂ or temperature in cerebral cortex in Group Three animals (**B**) to intrathecal administration of 80 µL of bupivacaine (at arrow).

Fig. 6. Anomalous response patterns of MAP, HR, BLF, BRS, CBF, or tissue perfusion, PO₂ or temperature in cerebral cortex to intrathecal administration of 80 µL of bupivacaine. Note values for MAP, HR, BLF and BRS are mean ± SEM of 15 animals (7 from Group Two plus 8 from Group Three); values for CBF are mean ± SEM of 7 animals from Group Two; and values for tissue perfusion, PO₂ or temperature in cerebral cortex are mean ± SEM of 8 animals from Group Three. *P < 0.05 versus data obtained under basal conditions in post hoc Dunnett multiple-range analysis; *P < 0.05 versus data obtained during Phase I in post hoc Tukey multiple-range analysis.

Figure 1

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Figures

Figure 1

(A) Demonstration of lodging of the tip of the catheter (orange arrow) in the subarachnoid space below the middle portion of L6 vertebra. (B) Representative examples of myelographic examination before (a) and after intrathecal administration of 4 successive doses of contrast medium, given at 40 (b), 60 (c), 80 (d) or 100 (e) µL. Note that all demarcations denote levels of the spinal cord, the lower yellow arrows mark the location of the tip of the catheter, and the upper yellow arrows indicate the highest points reached by the enhanced roentgenological images. CM: conus medullaris. (C) Scattered plots showing extent of dispersion in spinal cord of contrast medium on intrathecal administration at 40, 60, 80 or 100 µL. Values are mean ± SEM of 7 animals.

Figure 2

Illustrative examples of real-time and on-line recording of common phasic changes in mean arterial pressure (MAP), heart rate (HR), power density of the low-frequency component in systolic blood pressure spectrum (BLF), baroreflex sensitivity (BRS) or baroreflex effectiveness index (BEI), simultaneous with carotid blood flow (CBF) in Group Two animals (A); or concurrent with tissue perfusion (Tissue Flow), tissue oxygen tension (PO₂) or tissue temperature in cerebral cortex in Group Three animals (B) on intrathecal administration of 80 µL of bupivacaine (at arrow).

Figure 3

Common response patterns of MAP, HR, BLF, BRS, CBF, or tissue perfusion, PO₂ or temperature in cerebral cortex to intrathecal administration of 80 μ L of bupivacaine. Note values for MAP, HR, BLF and BRS are mean $\pm$ SEM of 33 animals (17 from Group Two plus 16 from Group Three); values for CBF are mean $\pm$ SEM of 17 animals from Group Two; and values for tissue perfusion PO₂ or temperature in cerebral cortex are mean $\pm$ SEM of 16 animals from Group Three. *P < 0.05 versus data obtained under basal conditions in post hoc Dunnett multiple-range analysis; +P < 0.05 versus data obtained during Phase I in post hoc Tukey multiple-range analysis.

Figure 4

Common (A) and anomalous (B) response patterns of BEI to intrathecal administration of 80 μ L of bupivacaine. Values for (A) are mean $\pm$ SEM from 33 animals (17 from Group Two plus 16 from Group Three); and values for (B) are mean $\pm$ SEM of 15 animals (7 from Group Two plus 8 from Group Three). * $P < 0.05$ versus data obtained under basal conditions in post hoc Dunnett multiple range analysis.

Figure 5

Illustrative examples of real-time and on-line recording of anomalous phasic changes in MAP, HR, BLF, BRS or BEI, simultaneous with CBF in Group Two animals (A); or concurrent with tissue perfusion, PO₂ or temperature in cerebral cortex in Group Three animals (B) to intrathecal administration of 80 μ L of bupivacaine (at arrow).

Figure 6

Anomalous response patterns of MAP, HR, BLF, BRS, CBF, or tissue perfusion, PO₂ or temperature in cerebral cortex to intrathecal administration of 80 μ L of bupivacaine. Note values for MAP, HR, BLF and BRS are mean $\pm$ SEM of 15 animals (7 from Group Two plus 8 from Group Three); values for CBF are mean $\pm$ SEM of 7 animals from Group Two; and values for tissue perfusion, PO₂ or temperature in cerebral cortex are mean $\pm$ SEM of 8 animals from Group Three. *P < 0.05 versus data obtained under basal conditions in post hoc Dunnett multiple-range analysis; +P < 0.05 versus data obtained during Phase I in post hoc Tukey multiple-range analysis.