

Investigation of Systemic Bupivacaine Toxicity using the In situ *Perfused Working Heart–Brainstem Preparation of the Rat*

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Background: **The inadvertent systemic administration of bupivacaine has been associated with fatal cardiovascular collapse. Systemic bupivacaine may affect neural control of the cardiovascular system in addition to having toxic actions on the heart. The study tested the hypothesis that systemic bupivacaine has toxic effects on brainstem cardiorespiratory control.**

Methods: **The working heart–brainstem preparation (WHBP) of rat was used to examine the actions of bupivacaine administered either by arterial injection or brainstem microinjection. The WHBP is a decerebrate rostral-half of a bisected rat, which is artificially perfused with a carbogenated Ringer solution *via* the aorta. Phrenic nerve activity, perfusion pressure, and electrocardiographic results were recorded.**

Results: **Systemic bupivacaine (3 μg/ml) evoked a prolonged pressor response (10.5 ± 5 mmHg) associated with marked bradycardia (−45 ± 22 beats/min) and prolonged the PR and QRS intervals of the electrocardiogram. The amplitude of respiratory sinus arrhythmia was attenuated (64 ± 15%) by bupivacaine without affecting activity recorded from the phrenic nerve. Bupivacaine selectively attenuated the baroreflex gain (55 ± 19%) but had no effect on the peripheral chemoreflex–evoked bradycardia. The bradycardia elicited by stimulation of the aortic depressor nerve was inhibited by bupivacaine, indicating baroreflex inhibition within the brainstem. Furthermore, bilateral microinjections of bupivacaine in the nucleus of the solitary tract reversibly inhibited the baroreflex.**

Conclusions: **These results demonstrate that arterial concentrations of bupivacaine that previously were shown to be cardiotoxic can selectively affect key cardiovascular control processes within the brainstem. Such impairment of neural cardiovascular control may contribute to the cardiovascular collapse associated with systemic bupivacaine.**

THE local anesthetic bupivacaine remains popular in clinical practice because of its efficacy and long duration. However, the inadvertent intravenous administration of bupivacaine can produce sudden cardiovascular collapse that is refractory to conventional cardiopulmonary resuscitation.[1,2] The malignant nature of systemic bupivacaine toxicity has received considerable attention since it was first described. Animal studies demonstrated systemic bupivacaine to have greater toxicity than other local anesthetics.[3] There is evidence supporting a direct toxic effect of bupivacaine on the heart, to reduce myocardial contractility and to slow conduction.[4] However, it has been suggested that the toxicity of systemic bupivacaine may be mediated in part by an action on the neural control of the cardiovascular system.[5,6] Specifically, it has been postulated that systemic bupivacaine may have a toxic action upon the brainstem[7] and that the cardiovascular collapse may reflect dysfunction of vital cardiorespiratory control systems.

Recent studies documented an inhibition of the baroreflex by systemic bupivacaine in anesthetized[8] and conscious rats.[9] The site at which bupivacaine acts to inhibit the baroreflex is uncertain but may reflect peripheral effects at the baroreceptors[10] and/or central effects within the brainstem. Using *in vivo* models it has been difficult to discriminate between the central nervous system and peripheral cardiotoxic effects of systemic bupivacaine. The interpretation of these *in vivo* experiments has been further complicated by the presence of general anesthetic agents or the occurrence of seizures following systemic bupivacaine. Therefore, we used the decerebrate *in situ* working heart–brainstem preparation (WHBP)[11] to distinguish between the direct effects of systemic bupivacaine on the heart and vasculature and any actions upon the brainstem.

Materials and Methods

All experiments conformed strictly to the United Kingdom Home Office guidelines regarding the ethical use of animals and were approved by our institutional ethical review committee. The experiments used the WHBP[11] from 35 male Wistar rats 4–6 weeks old (75–120 g). Rats were anesthetized with halothane until loss of paw withdrawal reflex. Following subdiaphragmatic transection, the rostral half of the animal was cooled to 10°C by immersion in a carbogenated Ringer solution. After craniotomy, the animal was decerebrated, and the cerebellum was removed for the brainstem microinjection experiments. No further general anesthetic agent was administered after decerebration. The diaphragm was reflected and the phrenic nerve identified. The preparation was moved to the recording chamber and a double-lumen cannula was inserted into either the cut end of the descending aorta or into the ascending aorta *via* the left ventricle. The preparation was perfused arterially with carbogenated Ringer solution containing ficoll 70 (1.25%) at 32°C using a peristaltic pump (Watson-Marlow, Falmouth, Cornwall, UK) at flow rates between 25

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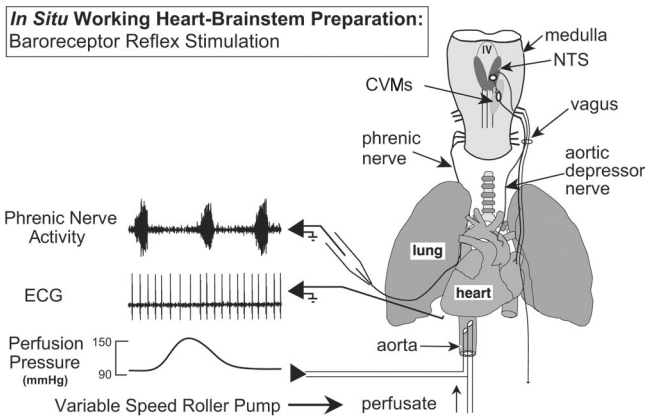

Fig. 1. Schematic of the working heart–brainstem preparation. The perfusion of the preparation is retrograde *via* a double lumen cannula inserted in the descending aorta. The perfusate is collected from the recording chamber and recirculated *via* a reservoir for carbogenation. Respiratory activity in the preparation gives an index of physiologic viability and is monitored with a suction electrode on the phrenic nerve. The baroreceptors are stimulated by increases in pump flow to cause a ramp change in perfusion pressure. The aortic arch baroreceptors signal *via* the aortic depressor nerve to the nucleus of the solitary tract (NTS). The NTS has an excitatory projection to the cardiac vagal motoneurones (CVMs), which in turn project in the vagus *via* the parasympathetic ganglia to the heart. ECG = electrocardiogram.

and 30 ml/min with a maintained circulating volume of 200 ml (fig. 1).

To examine the effect of systemic bupivacaine on lumbar sympathetic activity, the entire decerebrate rat was perfused artificially. To prepare the decerebrate artificially perfused rat (DAPR), the stomach, spleen, and intestine were ligated and removed *via* a midline laparotomy during deep halothane anesthesia. A midline sternotomy allowed access to the heart and phrenic nerve. The animal was cooled to 10°C in Ringer solution and decerebrated before transfer to the recording chamber. The perfusion cannula was inserted into the ascending aorta *via* the left ventricle.

In both the WHBP and the DAPR, the heart resumed beating as the perfusate flow rate (and thereby perfusion pressure) was gradually increased. Rhythmic respiratory muscle contractions recommenced after 2–5 min, typically when the mean perfusion pressure reached 65–80 mmHg. At this time, vecuronium bromide (4 μg/ml, Organon Teknica, Cambridge, UK) was added to the perfusion solution. The perfusion pressure was monitored *via* the second lumen of the cannula and was maintained at 60–100 mmHg by adjusting flow rate. Bupivacaine in carbogenated Ringer solution (25 ml) was perfused directly into the aorta from a separate reservoir. The first 25 ml of perfusate effluent was discarded to limit recirculation of bupivacaine.

Recording Techniques

A glass suction electrode (tip diameter, 0.2–0.3 mm) held in a micromanipulator was used to record from the phrenic nerve. Signals were AC amplified and band pass filtered (8 Hz-3kHz). Rhythmic, ramping phrenic nerve activity gave a continuous physiologic index of preparation viability. The electrocardiogram was visible on the phrenic nerve recording. A window discriminator was used to trigger the R-waves to derive heart rate. A suction electrode was used to record from the lumbar sympathetic chain at the L3 level.

Stimulation Methods

Baroreflex function was tested by applying ramp increases in perfusion pressure induced by increasing pump flow rate. The linear part of the baroreflex function curve was determined and the gain was calculated from the ratio of Δheart rate:Δperfusion pressure (beats · min^{-1} · $mmHg^{-1}$). Sodium cyanide (50–100 μl, 0.03%) was injected intraarterially to stimulate peripheral chemoreceptors. The chemoreflex responses were dose-dependent, and the doses used produced submaximal bradycardic reflex effects.

A bipolar Ag/AgCl stimulating electrode was attached to the peripheral end of the sectioned right vagus nerve in the neck. The electrode was insulated in low-melting-point paraffin wax. In two experiments, the aortic depressor nerve was identified in the neck during halothane anesthesia. A custom-made Ag/AgCl bipolar electrode was attached to the nerve and held in place with a silicone fixative (932, Wacker-Chemie, Munich, Germany). In a further two experiments, the aortic depressor nerve was identified in the WHBP and the proximal cut end was stimulated using a suction electrode. Electrical stimuli were applied from an isolated stimulator (1–2 s pulse train at 20–25 Hz, each pulse 5–40 V or 40–50 μA by 1 ms) to evoke a bradycardia.

Bupivacaine Microinjections

Bupivacaine (0.5%, 200–350 ng) was pressure microinjected from a glass micropipette (tip diameter, 20 μm). Micropipettes were placed in the nucleus of the solitary tract (NTS) under direct visual control with use of a binocular microscope at 0.5 mm rostral and 0.5 mm lateral to calamus scriptorius. Sequential bilateral microinjections were made 0.5 mm below the surface of the dorsal medulla. The volume of injectate (40–70 nl) was assessed from the decrease in meniscus level using a binocular microscope with an eyepiece graticule.

Data Recording and Analysis

Perfusion pressure, electrocardiogram, heart rate, and phrenic and sympathetic nerve activity were recorded using Neurolog amplifiers and filters (Digitimer, Welwyn Garden City, Hertfordshire, UK) and collected *via* an A:D interface (1401micro, CED, Cambridge, UK) on a computer using Spike2 software (CED). Custom scripts were used for data acquisition and analysis in Spike2.

Statistical Analysis

Data are expressed as mean ± SD. To quantify respiratory sinus arrhythmia, data were collected during eight respiratory cycles and the peak-to-trough heart rate changes were averaged; n refers to the number of preparations. A two-tailed paired *t* test was used to establish statistical significance (Statview for Windows V5, SAS, Cary, NC). Statistical significance was defined as $P < 0.05$.

Materials

The composition of the modified Ringer solution was (in mM): NaCl, 125; $NaHCO_3$, 24; KCl, 5; $CaCl_2$, 2.5; $MgSO_4$, 1.25; KH_2PO_4, 1.25; $dextrose_{10}$; pH 7.3 after carbogenation. Ficoll (1.25%) was added to the perfusion solution. Bupivacaine hydrochloride, hexamethonium bromide, and prazosin were dissolved in carbogenated Ringer solution. All chemicals from Sigma (Poole, Dorset, UK).

Results

Bupivacaine was applied to the preparation by aortic perfusion at concentrations between 1 and 6 μg/ml (3.1–18.5 μM) for a period of 1 min. All concentrations of bupivacaine evoked a pressor response, typically lasting 3–5 min, associated with bradycardia (fig. 2A, table 1). Increasing doses of bupivacaine were accompanied by progressive changes in electrocardiogram morphology (prolonged PR & QRS intervals; table 1). Doses of bupivacaine greater than 3 μg/ml were frequently associated with the loss of sinus rhythm and typically produced second or third degree heart block, which prevented the assessment of vagally mediated brainstem reflex responses. Hence, the experiments described herein used bupivacaine at a dose of 3 μg/ml to examine its effect on brainstem function. Systemic bupivacaine at this concentration was without significant effect on either the respiratory cycle length or burst amplitude of phrenic nerve activity (table 1, fig. 2A). However, it reduced the amplitude of the respiratory sinus arrhythmia (RSA) by 64 ± 15% (n = 8, fig. 2B, table 1).

Cardiovascular Reflex Sensitivities to Systemic Bupivacaine

Bupivacaine caused an attenuation (55 ± 19%, n = 9) of the baroreflex bradycardia evoked by increasing perfusion pressure (fig. 3A, table 1). In contrast, the peripheral chemoreflex bradycardia evoked by sodium cyanide was not attenuated by bupivacaine (table 1, fig. 3B). The bupivacaine block of the baroreflex was slowly reversible over a period of 5–10 min.

Evidence Suggesting Bupivacaine Inhibits the Baroreflex within the Brainstem

The responses to electrical stimulation of the efferent (vagus) and afferent (aortic depressor nerve) limbs of the baroreflex were examined. Stimulation of the distal end of the sectioned right vagus nerve evoked a bradycardia (70 ± 24 beats/min) that was not significantly attenuated by bupivacaine (−13 ± 20%, n = 4, fig. 4A). In contrast, stimulation of the aortic depressor nerve evoked a bradycardia (65 ± 42 beats/min) that was reversibly inhibited by bupivacaine (−82 ± 14%, n = 4, fig. 4B). These observations are consistent with an action of systemic bupivacaine within the brainstem. Furthermore, the bilateral microinjection of bupivacaine (0.5%, 40–70 nl) into the NTS reversibly blocked the baroreflex bradycardia (fig. 5, n = 3). These NTS microinjections of bupivacaine were without effect on perfusion pressure, heart rate, electrocardiogram morphology, respiratory pattern, or respiratory sinus arrhythmia.

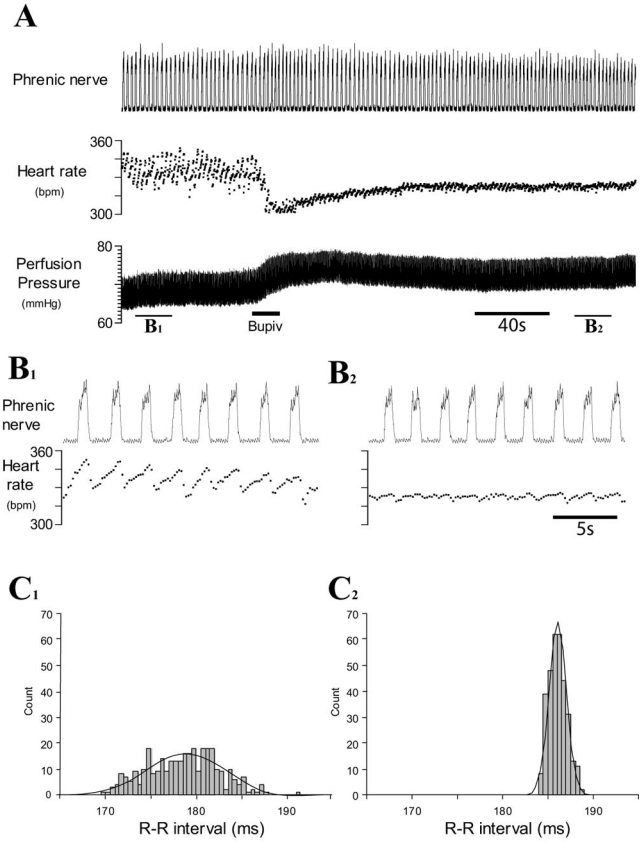

Fig. 2. Cardiovascular response to systemic bupivacaine. (*A*) Arterial perfusion of bupivacaine (3 μg/ml, *bar*) evoked a prolonged pressor response associated with bradycardia. It was without significant effect on the integrated phrenic nerve activity. (*B*) Expansion of the phrenic nerve and heart rate traces demonstrates respiratory sinus arrhythmia before (*B1*) and in the presence of bupivacaine (*B2*). (*C*) Frequency distribution of the electrocardiogram R-R interval before (*C1*) and after (*C2*) bupivacaine with a fitted normal distribution (*solid line*). bpm = beats/min.

Mechanisms of Pressor Response and Cardiac Slowing Evoked by Systemic Bupivacaine

The pressor effect of bupivacaine was seen when perfusion was retrograde *via* the cut descending aorta and

Table 1. Effects of Systemic Bupivacaine on Cardiorespiratory System

	Control	Bupivacaine	Wash (5 min)	n	*P*
Mean perfusion pressure (mmHg)	88.0 ± 10.3	98.5 ± 11.6	89.9 ± 9.6	11	<0.0001
Heart rate (beats/min)	298 ± 22	253 ± 26	289 ± 27	11	<0.0001
Electrocardiogram–PR interval (ms)	60 ± 5.0	83 ± 10.6	63 ± 4.6	11	<0.0001
Electrocardiogram–QRS duration (ms)	32 ± 7.3	53 ± 7.3	36 ± 8.6	11	<0.0001
Baroreflex gain (beats · min^{-1} · $mmHg^{-1}$)	−1.60 ± 0.90	−0.79 ± 0.57	−1.33 ± 0.66	9	<0.0005
Chemoreflex bradycardia (beats/min)	152 ± 45	142 ± 50		7	0.67
Respiratory cycle length (s)	3.2 ± 1.1	2.8 ± 0.8	2.8 ± 0.7	8	0.2
Sinus arrhythmia–Peak to trough (beats/min)	17.4 ± 9.9	6.0 ± 4.0	13.2 ± 7.4	8	<0.003

All results expressed as mean ± SD. *P* values indicate significance of bupivacaine effects compared with control using paired Student *t* test. Bupivacaine was given at 3 μg/ml.

when the perfusion was anterograde into the ascending aorta. As anterograde perfusion bypasses the heart, then the bupivacaine pressor action is independent of the cardiac output and, therefore, reflects an increase in systemic vascular resistance. To further investigate this pressor action, we used the DAPR preparation and showed that the characteristic pressor, bradycardic responses to bupivacaine were associated with a reduction of lumbar sympathetic activity (fig. 6A, n = 4). The addition of the autonomic ganglion blocker hexamethonium (330 μM, fig. 6B, n = 4) to the perfusate caused a decrease in perfusion pressure, increase in heart rate, loss of sinus arrhythmia, and loss of the baro- and chemoreflex bradycardia. This blockade of autonomic ganglia was without effect on the pressor effect of bupivacaine (fig. 6B), indicating that it was independent of centrally generated sympathetic activity. Prazosin (0.5–1 μM), an α_1-adrenoceptor antagonist, was added to the perfusate and blocked the pressor effect of bupivacaine (n = 3).

The bupivacaine bradycardia was unaffected by bilateral vagotomy (n = 3) or by autonomic ganglion blockade with hexamethonium (330 μM, n = 3), suggesting that it was mediated by a direct slowing of pacemaker activity within the heart.

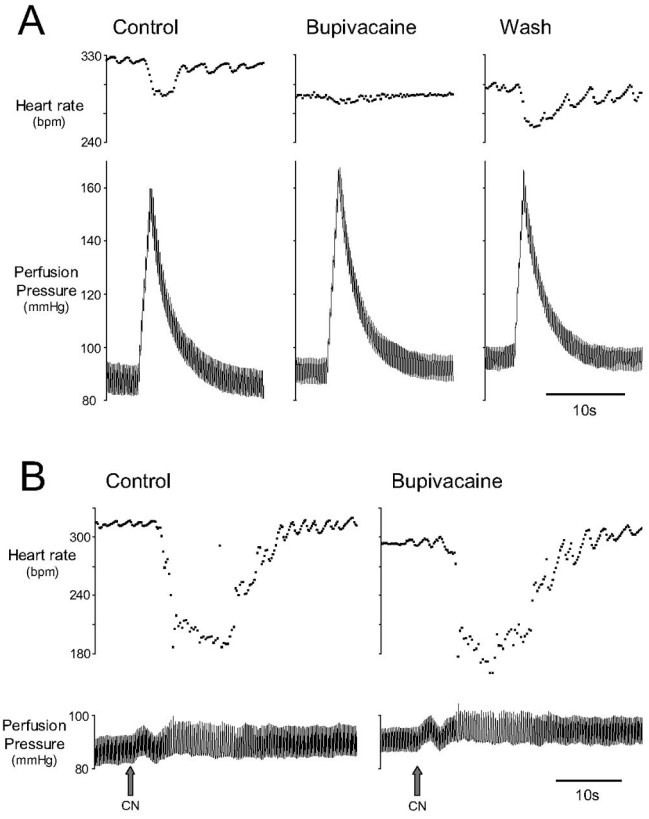

Fig. 3. Effect of systemic bupivacaine on baro- and chemo-reflex bradycardia. (*A*) Ramp increases in perfusion pressure evoked a baroreflex bradycardia (*A1*). This was inhibited by bupivacaine (3 μg/ml, *A2*). The response recovered after 10 min of washing (*A3*). (*B*) The injection of sodium cyanide (100 μl × 0.03%, CN) to stimulate peripheral chemoreceptors evoked a bradycardia (*B1*) in the same preparation as *A*. This chemoreflex bradycardia was not attenuated by bupivacaine (*B2*).

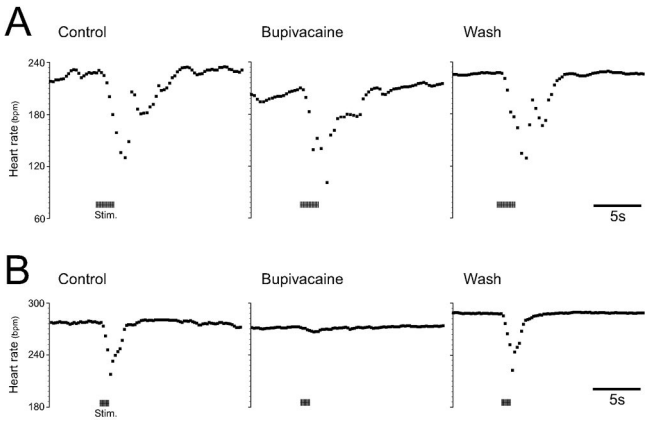

Fig. 4. Effect of bupivacaine on the vagal and aortic depressor nerve evoked bradycardia. (*A*) Electrical stimulation (8 V × 1 ms, 20 Hz, 2 s) of the distal end of the cut right vagus in the neck evoked a reproducible bradycardia. The application of bupivacaine (3 μg/ml) was associated with a pressor, bradycardic response. However the bradycardia evoked by vagal stimulation was unaffected at the same time the baroreflex gain was reduced by 78%. (*B*) Electrical stimulation of the aortic depressor nerve (10 V × 1 ms, 25 Hz for 1 s) evoked a reproducible bradycardia. The systemic application of bupivacaine (3 μg/ml) attenuated the aortic depressor nerve evoked bradycardia and reduced the baroreflex gain by 44%. Both the baroreflex and the evoked aortic depressor nerve bradycardia recovered to control after 5 min of washing.

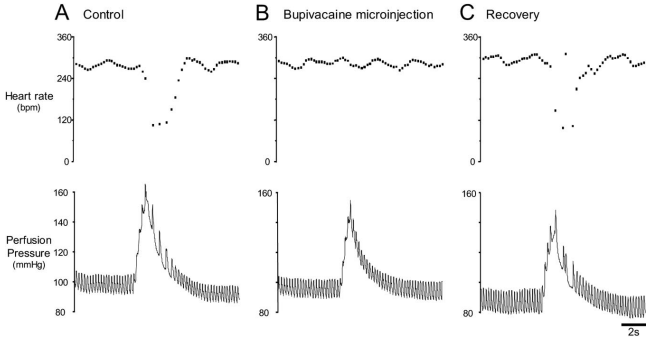

Fig. 5. Bilateral microinjection of bupivacaine to the nucleus of the solitary tract (NTS) reversibly blocked the baroreflex bradycardia. (*A*) A baroreflex bradycardia (gain −2.4 beats · min^{-1} · mmHg) was evoked using perfusion pressure ramps. (*B*) Microinjection of bupivacaine (60 nl × 0.5%) bilaterally into the NTS completely blocked the baroreflex bradycardia. (*C*) The baroreflex bradycardia recovered to control after 12 min of washing.

Discussion

Using the decerebrate *in situ* WHBP of rat and a novel perfused entire rat preparation, we were able to dissect the direct cardiovascular *versus* central nervous system effects of systemic bupivacaine. This approach has allowed the responses to be examined in the absence of general anesthetic agents or complicating seizure activity. Furthermore the artificial perfusion of the preparation has allowed the actions of known arterial concentrations of bupivacaine on the brainstem to be examined independently of its depressant effects on cardiac output. We showed a selective inhibition of the baroreflex and an attenuation of respiratory sinus arrhythmia, both mediated by bupivacaine acting within the brainstem. In addition, we demonstrated a pressor effect mediated *via* α_1-adrenoceptors and a bradycardic action mediated directly on the heart.

Our study has predominantly used a bupivacaine concentration of 3 μg/ml applied by intraarterial perfusion. This dose was chosen as it was found to be the largest dose of bupivacaine that consistently left the heart in sinus rhythm, hence allowing the effect of the vagal outflow to the heart to be monitored. Previous *in vitro* studies documented cardiac toxicity to occur at doses of bupivacaine between 1 and 5 μg/ml.[12–15] Hence the brainstem toxicity observed in our study is seen at doses of bupivacaine that cause cardiac toxicity *in vitro*.

There are difficulties in extrapolating to the clinical situation from the results of our *in situ* study of bupivacaine toxicity because of the complex pharmacokinetics of bupivacaine following intravenous administration *in vivo*. In particular there is likely to be little protein binding of bupivacaine in the perfusate in our study. However, there is little evidence to indicate the relevance of protein binding in determining bupivacaine toxicity, hence most human studies quote total plasma concentrations of bupivacaine. Human intravenous infusion studies showed that the first central nervous system symptoms, such as circumoral tingling, tinnitus, and light-headedness, occur at venous bupivacaine concentrations of 2.25 μg/ml[16] and 2.1 μg/ml[17] (with a corresponding arterial concentration of 4 μg/ml). Therefore, our study used a total dose of bupivacaine that is at the low end of the human clinical toxicity spectrum. A strength of our study methodology is that we applied a known arterial concentration of bupivacaine. Previous studies demonstrated a marked arterial-to-venous concentration gradient for bupivacaine and, hence, the arterial concentration may better reflect toxic end-organ effects.[4]

The intravenous administration of bupivacaine has been shown to inhibit the baroreflex response in pentobarbital anesthetized[8] and conscious rats[9] to pressor (phenylephrine) and depressor (sodium nitroprusside) agents. This baroreflex inhibition was observed at an arterial plasma bupivacaine concentration of 1.8 μg/ml.[9] A previous study from the same investigators showed that the discharge of baroreceptors recorded from an *in vitro* isolated aortic arch preparation of rat was inhibited by clinically relevant concentrations of bupivacaine (1.6–16 μg/ml).[10] Although these doses may affect pe-

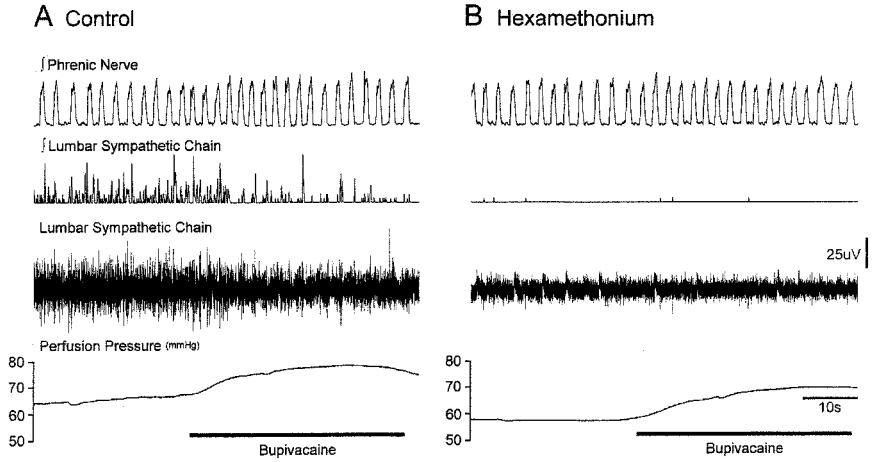

Fig. 6. The pressor response to bupivacaine is unaffected by the autonomic ganglion blocker hexamethonium. (*A*) Bupivacaine (3 μg/ml) evokes a prolonged pressor response. Recording from the lumbar sympathetic chain indicates that the pressor response is associated with a decrease in sympathetic activity. (*B*) The addition of hexamethonium (330 μM) to the perfusate caused a decrease in perfusion pressure, an increase in heart rate, and a loss of sinus arrhythmia associated with a blockade of activity recorded in the lumbar sympathetic chain. Systemic perfusion of bupivacaine still evoked a pressor response.

ripheral baroreceptor transduction, our data, using a relatively low dose, supports a central site of action for bupivacaine within the brainstem. Furthermore, it would be difficult to explain the reported selectivity of bupivacaine for depression of the vagal component of the baroreflex[9] by a peripheral block of the baroreceptor endings alone.

We showed bupivacaine to cause a comparable degree of baroreflex inhibition to that reported in previous rat studies.[8,9] We demonstrated that this inhibition is reflex selective, because the peripheral chemoreflex bradycardia is not affected by the same concentration of bupivacaine. Furthermore, we showed, with electrical stimulation, that the vagus nerve–mediated bradycardia is not blocked by bupivacaine (3 μg/ml), indicating that the entire peripheral efferent limb of the baroreflex pathway is functional. This is consistent with the report that a 50% block of vagal transmission requires 134 μM (44 μg/ml) bupivacaine.[18] Our observation that bupivacaine reversibly inhibited the bradycardic response to stimulation of baroreceptor afferents in the aortic depressor nerve indicates a site of action within the brainstem.

The inhibition of baroreflex function was mimicked by microinjection of bupivacaine bilaterally into the NTS. We therefore speculate that the NTS is a likely central site of action for bupivacaine in attenuating the baroreflex. Such baroreflex blockade was without effect on RSA and, interestingly, we observed minimal cardiovascular changes. In particular there were no electrocardiographic changes or malignant arrhythmias consequent on bupivacaine microinjection to the NTS. This is in contrast to a previous NTS microinjection study in anesthetized rats *in vivo*[7] which documented falls in mean arterial pressure and heart rate that were associated with increased PR and QRS intervals on the electrocardiogram. These authors also reported the delayed development of malignant arrhythmias 20 min after microinjection. These marked cardiovascular effects may reflect their injection of a 160-fold greater dose of bupivacaine than was used in our study. Indeed the total dose of bupivacaine (40 μg) injected into the brainstem by Thomas *et al.*, 1986 was approximately half the dose (75 μg) administered systemically in our study. This suggests that the observed arrhythmias could have resulted from widespread diffusion of the local anesthetic to produce effects analogous to those reported to follow intracerebroventricular administration of local anesthetics.[6]

An inhibition of cell firing by systemic bupivacaine in the NTS in anesthetized[19] and conscious[20] rats has been reported. These recordings were made from physiologically uncharacterized NTS neurons. The interpretation of these findings is complicated by the depressor effect of bupivacaine on blood pressure, which would reduce the firing rate of baroreceptors and hence barosensitive NTS neurons. Our demonstration of a selective block of baroreflex but not chemoreflex bradycardia by bupivacaine argues against a generalized depressant action of systemic bupivacaine within the NTS, as both reflexes are integrated at this site.

The RSA in the WHBP is a centrally generated rhythmic discharge transmitted by the vagus to the heart.[21] It reflects an inhibitory input from the respiratory network to cardiac vagal motoneurons such that vagal outflow is inhibited during inspiration.[22] We demonstrated that bupivacaine attenuates RSA. It would seem likely that bupivacaine is acting centrally to block RSA given that it was without effect either on phrenic nerve activity or vagal efferent transmission to the heart. Interestingly, bilateral microinjections of bupivacaine into the NTS were without effect upon RSA suggesting the site of action is outside the NTS. One possible site for the inhibition of RSA by systemic bupivacaine may be at the level of the cardiac vagal motoneurons.

Intravenous infusion of the local anesthetic lidocaine has been reported to inhibit RSA in humans.[23] There is increasing evidence that loss of R-R variability and baroreflex function in cardiovascular diseases is associated with an increased risk of malignant arrhythmias and sudden death in humans.[24,25] The loss of RSA after systemic bupivacaine may hence contribute to the development of refractory arrhythmias that characterize cardiac arrests associated with local anesthetic toxicity.[1]

The vasoconstriction caused by systemic bupivacaine observed in our study has been reported previously in isolated arteries[26,27] although no mechanism has been proposed to explain this action. A similar pressor effect in response to bupivacaine has been reported in the conscious rat.[9] This study documented significant rises in circulating epinephrine and norepinephrine which were suggested to be responsible for the pressor action and reflected a central sympatho-activation by bupivacaine. Several human studies have also shown intravenous bupivacaine infusion to be associated with a pressor effect.[16,17,28] One of these studies[28] showed a small increase in circulating epinephrine (not norepinephrine) but concluded that this was not sufficient to account for the pressor effect of bupivacaine.

We used the decerebrate artificially perfused rat to show that the pressor effect of systemic bupivacaine is associated with a decrease in activity in the lumbar sympathetic chain. Furthermore, we showed that the pressor effect is maintained in the presence of the autonomic ganglion blocker hexamethonium indicating that it is not a result of central sympatho-activation. This finding is different from that seen when bupivacaine is administered intracerebroventricularly, this causes a pressor effect mediated by central sympatho-activation that is blocked by hexamethonium.[29] We suggest that the mechanisms underlying the pressor effect seen after systemic *versus* intracerebroventricular bupivacaine administration are different. We also showed that the pressor effect is inhibited by prazosin an α_1-adrenoceptor

antagonist. These data indicate that bupivacaine is acting *via* α_1-adrenoceptor on the arterial tree to cause vasoconstriction. This bupivacaine vasoconstrictor action could be *via* an agonist action at the α_1-adrenoceptor or by an indirect action to release norepinephrine from sympathetic terminals or to block catecholamine reuptake.

Our study has indicated that bupivacaine has a selective toxic action on the brainstem with inhibition of baroreflex gain and RSA without affecting the chemoreflex or respiratory activity in the phrenic nerve. The mechanism for this apparent selectivity may reflect a differential distribution of bupivacaine or selective targeting of specific neuronal or synaptic populations within the brainstem. Further studies using single cell recording within the brainstem will be required to distinguish between these possibilities. It also remains to be established whether or not the L- and D-stereoisomers of bupivacaine have differential effects on baroreflex gain and respiratory sinus arrhythmia.

In summary, using the perfused *in situ* WHBP and DAPR preparation, we demonstrated multiple actions of bupivacaine upon the cardiovascular system, including an increase in vascular resistance mediated *via* α_1-adrenoceptor but independent of central sympathetic activity, a direct slowing of heart rate, an inhibition of baroreflex function, and loss of respiratory sinus arrhythmia. The latter two effects were both mediated within the brainstem. During bupivacaine toxicity, these changes, allied to a reduction in myocardial contractility, may be envisaged to put the heart under considerable strain. A decrease in cardiac output, because of a reduction in stroke volume (reduced contractility and increased afterload) and a decrease in heart rate, is compounded by a loss of the compensatory baroreflex. Furthermore, the loss of the RSA removes a vagal input to the heart that is believed to be antiarrhythmic. This combination of cardiovascular system and central nervous system toxic actions may account for the dramatic cardiovascular collapse associated with bupivacaine toxicity.

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