

# Studies of Local Anesthetic Action on Natural Spike Activity in the Aortic Nerve of Cats

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The aortic nerve was used to study the blocking action of procaine and bupivacaine on natural spike activity. In anesthetized cats, a segment of the aortic nerve was placed in a perfusion chamber and exposed to increasing drug concentrations, varying pH, while temperature remained constant. Total nerve activity was recorded continuously, and its change was related to drug concentration. The half-time of recovery following drug wash-out was also determined. At pH 7.4, the minimal blocking concentration was  $0.5 \times 10^{-3}$  mol/l for procaine and  $0.05 \times 10^{-3}$  mol/l for bupivacaine, the half-times of recovery 1.4 and 3.0 min, respectively. Procaine and bupivacaine reversibly blocked natural spike activity at the same concentrations as they blocked electrically evoked activity. The aortic nerve, whose physiologic spike traffic can be followed continuously for hours, may be used to advantage for studying the long-term effects of local anesthetics *in vivo*. (Key words: Anesthetics, local: bupivacaine; conduction block; natural spike activity; procaine.)

THE PRESENT EXPERIMENTS aimed at the development of a nerve preparation for studying the time-dependent effects of local anesthetics in a functionally intact nerve. The aortic nerve appeared to be a promising choice. Its sensory nerve fibers, baroafferents of the A-delta and C-fiber group, are physiologically activated by the pulsatile changes in blood pressure *via* stretch receptors in the aortic wall so that their natural spike traffic can be followed continuously for hours. Because blocking concentrations were derived exclusively from electrically stimulated nerves in the past, it appeared necessary to determine if local anesthetics would block natural spike traffic at the same concentrations.

## Materials and Methods

Data were derived from 38 blocking experiments in ten anesthetized ( $\alpha$ -chloralose) cats, ventilated with ambient air *via* an endotracheal tube by a Starling pump. Blood gas tensions, pH, and rectal temperature were maintained within the normal range.

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The aortic nerve was approached from a midline neck incision and freed from the surrounding tissue with the aid of a microscope. The perineurium remained intact.

The following parameters were continuously recorded on chart and, for detailed analysis, on tape: spike activity (*via* bipolar platinum-iridium electrodes), averaged spike activity (root mean-square technique, analog modul BB 4341, rise time 150 ms, fall time 430 ms), arterial blood pressure, and heart rate.

As is shown in figure 1, a segment of the aortic nerve was placed in the perfusion chamber. The portion of the nerve inside the chamber was immersed in tyrode solution, that outside in paraffin oil at  $38 \pm 0.2^\circ$  C. Nerve activity, which originates from baroreceptors in the aortic arch and which correlates linearly with arterial pressure in the physiological range,<sup>1</sup> was recorded caudad to the chamber for control and cephalad to observe the drug effect. The modified tyrode solution ( $\text{Na}^+$  138,  $\text{K}^+$  2.7,  $\text{Mg}^{++}$  0.53,  $\text{Ca}^{++}$  1.82,  $\text{Cl}^-$  145.33, and glucose 5.56 mmol/l) was bubbled with carbogen (5%  $\text{CO}_2$ , 95%  $\text{O}_2$ ), and continuously flowed through the chamber at a rate of about 5 ml/min. The pH of the solution (7.2, 7.4, or 7.6) was adjusted by appropriate amounts of sodium bicarbonate. Procaine-HCl or bupivacaine-HCl were added from commercial stock solutions.

Following perfusion with tyrode solution for 10 min, the blocking experiments began with nonblocking concentrations, which were increased stepwise in 10-min intervals until nerve activity was blocked. The latter concentration was defined as minimal blocking concentration ( $c_m$ ).<sup>2</sup> Local anesthetics as well as the pH values were tested in random order. Once block was complete, drug wash-out was accomplished with plain tyrode solution for 30 min.

The averaged activity during the control was arbitrarily set to 100, and to zero during complete nerve block. Thus, nerve activity is expressed in per cent of control for the same blood pressure levels. Concentration/effect curves were then plotted by relating the per cent changes in averaged nerve activity against drug concentration. Recovery after drug wash-out was expressed as half-time.<sup>3</sup>

The blocking concentrations and recovery times are presented as mean values ( $\pm$ SE). For each drug, the effect of pH on the minimal blocking concentrations was tested by analysis of variance, and the differences in the recovery times between procaine and bupivacaine regardless of pH were tested by Student's *t* test for unpaired data. Significance was assumed when  $P < 0.05$ .

**Results**

The time course and an example of data analysis are shown in figure 2. The inserted oscilloscope recordings show the typical discharge bursts of aortic nerve baroafferents at the cephalad (= drug effect [R<sub>E</sub>]) and caudad (= control [R<sub>C</sub>]) recording electrode. The burst height and the averaged nerve activity decrease in parallel during perfusion with increasing concentrations of procaine, reaching a minimum when spike activity is blocked. No such effects are seen in the nerve caudad to the perfusion chamber. The recording also demonstrates the rapid onset of action and the establishment of steady states within a few minutes after drug exposure and the rapid recovery after drug wash-out.

The individual concentration/effect curves derived from such experiments are illustrated in figure 3. The minimal blocking concentrations and the interpolated EC<sub>50</sub> values extracted from them are given in table 1. These results show that bupivacaine is approximately ten times more potent than procaine. Over the range of pH tested there is no significant effect of pH on drug potency.

Recovery time was short with either agent, but procaine-blocked nerves recovered significantly (*P* = 0.02) faster (1.4 ± 0.4 min, n = 10) than bupivacaine blocked nerves (3.0 ± 0.5 min, n = 16). Recovery times were not affected by pH. In 12 out of 38 experiments the nerve did not fully recover because of local anesthetic trapped in the silicone seal that could not be removed by flushing with tyrode solution. However, because the time course and also the concentration dependency of these 12 experiments corresponded entirely with the others, these data were included for analysis of blocking behavior, but omitted for the analysis of recovery. The mean diameter of the nerves was 190 μm (range 160–220 μm).

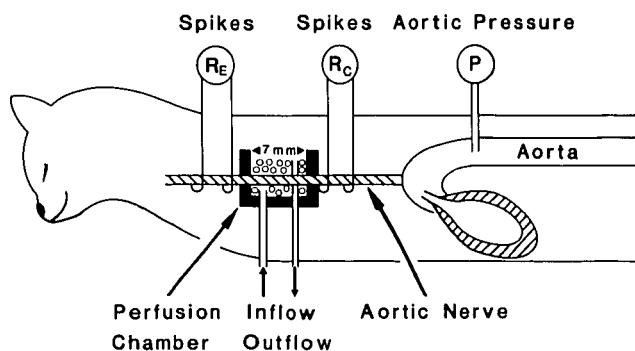


FIG. 1. Experimental set-up. A segment of the nerve was placed in the perfusion chamber, i.e., it was inserted through narrow slits and sealed by silicone gel (Sil Gel® 604 A und B, Wacker-Chemie, Muenchen, FRG). Spike activity, which originates from baroreceptors in the aortic arch, was recorded caudad to the chamber for control (R<sub>C</sub>), and cephalad to the chamber to observe the drug effect (R<sub>E</sub>) so that the activity is compared against simultaneous control. Blood pressure (P) was also recorded.

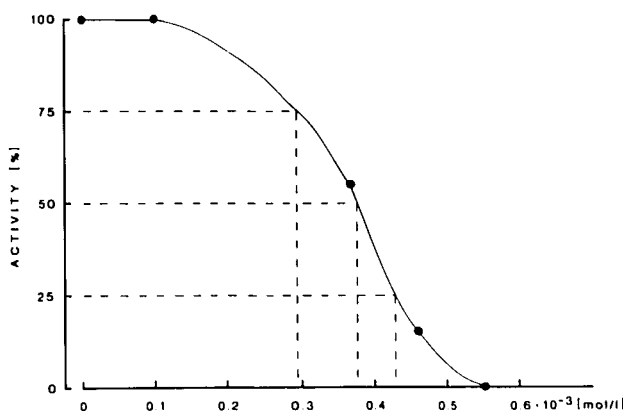
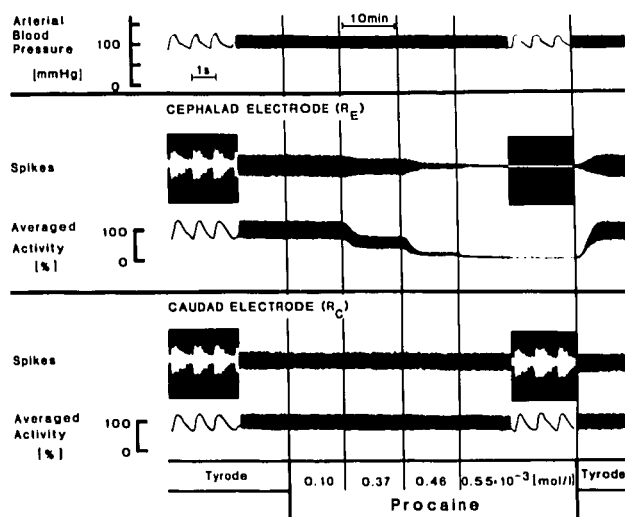


FIG. 2. Experimental protocol and time course of blocking action of procaine on natural spike activity. Cat aortic nerve, pH 7.4, 38° C. Drug effects shown at the cephalad electrode (R<sub>E</sub>), controls at the caudad electrode (R<sub>C</sub>). Original recordings. *Inserts*: oscilloscope recording at an expanded time scale. Burst height and also averaged nerve activity decrease in a concentration-related manner until complete nerve block at 0.55 × 10<sup>-3</sup> mol/l. Note also the rapid onset of action with early plateaus in the nerve activity recordings and the rapid recovery during drug wash-out. *Lower*: Concentration/effect curve of procaine. Data derived from the recordings shown above. Nerve activity expressed as per cent of control. Blocking concentrations for various degrees of block (25, 50, 75%) were interpolated as shown by the dashed lines.

**Discussion**

The aortic nerve offers several advantages over other nerve preparations used in the past for studying the actions of local anesthetics. First, the spike activity of its baroafferents, which is evoked physiologically, not electrically, can be followed continuously for hours. Second, the baroafferents of the aortic nerve are sensory fibers and belong, similar to those in the pain conducting system, to the A-delta and C-fiber category. Third, the thinness of the aortic nerve (diameter range between 160–220 μm) fosters a rapid establishment of a concentration equi-

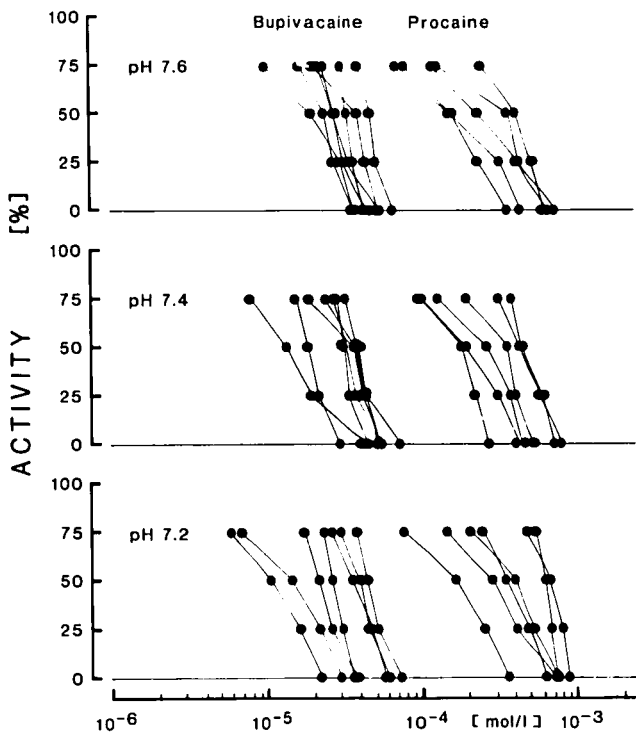


FIG. 3. Concentration/effect curves for procaine and bupivacaine at various pH values. Aortic nerve of 10 cats studied at 38°C, nerve activity in per cent of control. The concentration/effect curves for both agents are clearly separated and are not influenced by pH. There is tendency for the bupivacaine curves to be shifted to the left with decreasing pH, but this is not statistically significant.

librium between its surface and its interior. This was demonstrated by the rapidity with which a plateau in the averaged nerve activity occurred following drug exposure and drug wash-out. Finally, by recording spike activity caudad and cephalad to the site of drug action, the drug effect can be evaluated against a simultaneous control recording.

The aortic nerve activity reflects mainly the functional state of the myelinated fibers for the following reasons: the myelinated fibers far outnumber the unmyelinated fibers<sup>4</sup>; the activity of A-delta fibers predominates at normal arterial blood pressure, whereas C-fibers are mostly silent and are activated mainly at higher blood pressures

than the myelinated ones<sup>5</sup>; A-delta fibers generate spikes of much higher amplitude and frequency than the C-fibers, and therefore are much more easily detected by the recording technique employed than are C-fibers.

The minimal blocking concentrations for A-delta and also C-fibers that were derived from isolated, electrically stimulated nerves under conditions similar to ours are summarized in table 2. For procaine, these concentrations agree reasonably well with ours and also with those (approximately  $0.74 \times 10^{-3}$  mol/l) found in the cerebrospinal fluid of humans<sup>10,11</sup>, cows,<sup>8</sup> and dogs<sup>12</sup> during spinal or epidural block. In addition, similar concentrations for 50% block of A-fibers ( $0.41 \times 10^{-3}$  mol/l) and C-fibers ( $0.71 \times 10^{-3}$  mol/l) were reported for desheathed vagus nerves of rabbits.<sup>13</sup> In the case of bupivacaine, our values are also similar to those in the cerebrospinal fluid during spinal block in humans ( $0.06$  and  $0.1 \times 10^{-3}$  mol/l, respectively).<sup>14</sup> But, for unknown reasons, they are lower than reported values for isolated nerves even though our potency ratio of 10:1 between bupivacaine and procaine is close to 8:1 for rat sciatic nerve.<sup>15</sup> Curiously, our  $EC_{50}$  values for bupivacaine ( $0.031 \times 10^{-3}$  mol/l; see table 1) are similar to those reported for A-fibers ( $0.048 \times 10^{-3}$  mol/l),<sup>16</sup> but are again lower than  $EC_{50}$  values for C-fibers ( $0.201 \times 10^{-3}$  mol/l).<sup>16</sup> Changes in pH between 7.2 and 7.6 did not influence the drug action in the present study.

Recovery was more rapid from procaine than from bupivacaine. For isolated nerves, recovery times on the order of minutes were reported for procaine,<sup>3,6</sup> but much longer recovery times ranging from 17.5 min<sup>17</sup> up to 30–45 min<sup>8</sup> have been reported for bupivacaine: recovery times by and large correlate with the drug concentration present at the start of drug wash-out.<sup>2</sup> We observed short recovery times by looking at recovery from minimal blocking concentrations. Other authors however, who found much slower recovery,<sup>8,17</sup> had exposed their preparations to higher drug concentrations. Therefore, it appears justified to say that the aortic nerve, when exposed to the minimal blocking concentrations of either procaine or bupivacaine,

§ Numans SR, Havinga E: Die Wirkungsweise von Lokalanästhetika bei der Epiduralinjektion. Recueil des Travaux Chimiques des Pays-Bas 62:497–502, 1943.

TABLE 1.  $EC_{50}$  and Minimal Blocking Concentrations (mean values  $\pm$  SE) in mol/l for Procaine and Bupivacaine at Various pHs—Isolated Aortic Nerves of Cats at 38°C

	Procaine						Bupivacaine					
	pH 7.2	n	pH 7.4	n	pH 7.6	n	pH 7.2	n	pH 7.4	n	pH 7.6	n
$EC_{50}$	$0.43 \times 10^{-3}$ $\pm 0.08 \times 10^{-3}$	6	$0.33 \times 10^{-3}$ $\pm 0.05 \times 10^{-3}$	6	$0.27 \times 10^{-3}$ $\pm 0.05 \times 10^{-3}$	5	$0.028 \times 10^{-3}$ $\pm 0.005 \times 10^{-3}$	7	$0.031 \times 10^{-3}$ $\pm 0.004 \times 10^{-3}$	7	$0.032 \times 10^{-3}$ $\pm 0.004 \times 10^{-3}$	7
$c_m$	$0.69 \times 10^{-3}$ $\pm 0.07 \times 10^{-3}$	6	$0.55 \times 10^{-3}$ $\pm 0.08 \times 10^{-3}$	6	$0.57 \times 10^{-3}$ $\pm 0.07 \times 10^{-3}$	5	$0.048 \times 10^{-3}$ $\pm 0.007 \times 10^{-3}$	7	$0.052 \times 10^{-3}$ $\pm 0.005 \times 10^{-3}$	7	$0.050 \times 10^{-3}$ $\pm 0.004 \times 10^{-3}$	7

TABLE 2. Minimal Blocking Concentrations for Procaine and Bupivacaine from Electrically Stimulated Nerves

Author	Species	Number of Experiments	$C_m$ ( $10^{-5}$ mol/l)	Nerve Preparation	Fibers	pH	Temperature
<i>Procaine</i>							
Nathan, Sears <sup>6</sup>	Cat	?	2.2	Anterior roots	A-delta (18–35 m/s)	7.0–7.4	39° C
Nathan, Sears <sup>6</sup>	Cat	?	0.37	Posterior roots	A-delta (9.6–11.6 m/s)	7.0–7.4	39° C
Nathan, Sears <sup>6</sup>	Cat	?	0.74	Posterior roots	C	7.0–7.4	39° C
Franz, Perry <sup>7</sup>	Cat	1	0.74	Saphenous nerve filaments, perineurium intact	myelinated +C	7.0–7.2	37° C
Own results	Cat	17	0.60 (range 0.28–0.92)	Aortic nerve, perineurium intact	mainly A-delta	7.2–7.6	37.8–38.2° C
<i>Bupivacaine</i>							
Rosenberg <i>et al.</i> <sup>8</sup>	Rabbit	3	0.2	Cervical sympathetic trunk, sheath intact	C	7.4	35.7–37.2° C
Curtis, Scurlock <sup>9</sup>	Frog	3	0.4	Sciatic nerve, epineurium removed	A-delta (20 m/s)	7.2	?
Own results	Cat	21	0.05 (range 0.023–0.077)	Aortic nerve, perineurium intact	mainly A-delta	7.2–7.6	37.8–38.2° C

Values in parentheses = conduction velocities.

usually regains its normal activity within minutes after the start of drug wash-out.

In conclusion, procaine and bupivacaine at pH values between 7.2 and 7.6 blocked the natural spike traffic of the isolated aortic nerve at concentrations similar to that which blocked the electrically evoked activity in isolated nerves. These results document the reliability of the aortic nerve for testing the potency and, in particular, the time course of local anesthetic action.

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