

## Tachyphylaxis to Local Anesthetics Does Not Result from Reduced Drug Effectiveness at the Nerve Itself

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Possible development of tachyphylaxis to local anesthetics in the nerve itself (time-dependent change in axonal conduction properties) was studied in the aortic nerve of eight rabbits anesthetized with urethane. The nerve was immersed in Tyrode solution with or without bupivacaine at pH 7.4 and 38° C in a trough molded from the surrounding tissues. After control measurements the nerve was exposed to increasing bupivacaine concentrations until complete nerve block at minimal blocking concentration. Subsequently, bupivacaine concentrations were reduced and kept constant for 4 h (partial block). Finally, intact nerve function was confirmed after bupivacaine washout with Tyrode solution. For quantification total nerve activity was recorded continuously and related to drug concentrations. Two findings argue against the occurrence of tachyphylaxis at the nerve itself: 1) nerve activity decreased rather than increased over time in the presence of constant bupivacaine concentrations during partial block; and 2) for the same bupivacaine concentration, nerve activity during partial block was always lower than during the initial blocking experiments. Thus, drug effectiveness increased rather than decreased over time, which cannot be reconciled with the theory that tachyphylaxis might be mediated by changes in axonal conduction properties. (Key words: Anesthetics, local: bupivacaine. Nerve: conduction block; natural spike activity. Tachyphylaxis: local anesthetics.)

POSSIBLE EXPLANATIONS for tachyphylaxis to local anesthetics include a decrease in drug effectiveness or a decrease in drug concentration at the site of action. The first alternative relates to pharmacodynamics and implies a time-dependent change in axonal conduction properties,<sup>1</sup> which should be demonstrable also in isolated nerves. The second relates to pharmacokinetics and implies a reduced drug concentration at the axon<sup>1</sup> in which case tachyphylaxis would occur only in intact animals or humans. Either hypothesis could explain the need for larger doses to maintain a given degree of nerve block.

The first alternative has not been tested unequivocally. The claim that tachyphylaxis is not a phenomenon of decreased drug effect rests on only one report using isolated frog sciatic nerves in which "no diminution of blockade (electrically evoked activity) occurred during exposures

(5 mmol/l lidocaine or mepivacaine) that lasted as long as 8 hours."§ However, in view of the rather steep concentration/effect relationship of local anesthetics,<sup>2</sup> it is questionable whether restoration of nerve function could have been expected in the presence of concentrations twofold to fivefold higher than the minimal blocking concentrations.<sup>3,4</sup> Furthermore, tachyphylaxis has been observed only in warm-blooded species including the rabbit<sup>5</sup> so that nerves of frogs do not seem particularly suited to evaluate this phenomenon. We speculated that a time-related increase in nerve activity during partial block at constant drug concentrations or a time-related shift in concentration/effect relationship toward higher drug concentrations would indicate development of tachyphylaxis. Accordingly, we studied the effects of constant as well as repeated bupivacaine concentrations on natural spike activity of the aortic nerve of rabbits for up to 6 h. We concluded that tachyphylaxis does not result from changes in axonal conduction properties.

### Materials and Methods

Following approval by the regional government (Regierungspräsidium Düsseldorf), eight experiments in eight rabbits (weight: 3.2 kg; range: 2.8–3.7 kg) were performed. The animals were anesthetized with urethane (2.5 g/kg intravenously) and received supplemental urethane as needed. Via a tracheostomy, they breathed oxygen-enriched room air. Blood gas tensions and pH were maintained within the normal range, and rectal temperature was kept between 37.1° and 39.5° C.

### EXPERIMENTAL PREPARATION

With the animals in the lateral position, the aortic nerve was dissected in the neck over a length of approximately 5 cm, and under a microscope freed from epineurial tissue with the perineurium remaining intact.

A trough, in which the nerve was immersed in a carbogenated Tyrode solution either with or without bupivacaine (Carbostesin®, Astra), was molded from the surrounding tissues. The trough held between 15 and 20 ml and was perfused continuously at a rate of 5 ml/min so

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§ Poppers PJ: Tachyphylaxis to local anesthetic agents, *Advances in Regional Anaesthesia. Proceedings of the International Symposium on Local Anaesthetics and Regional Anaesthesia, The Hague*. Edited by Poppers PJ, van Dijk B, 1977, pp 77–85.

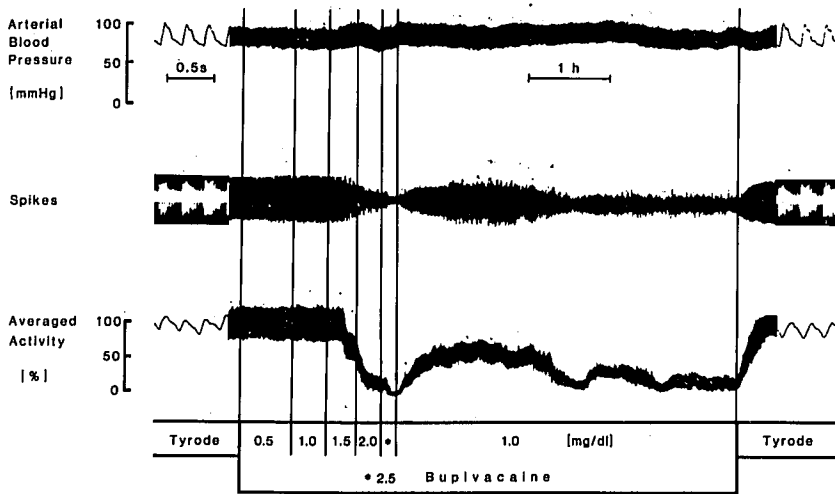


FIG. 1. Time course of a blocking experiment (original recording). Following complete conduction block at 2.5 mg/dl bupivacaine, the nerve is exposed to 1.0 mg/dl for 4 h. Inserts show, at an expanded time scale, the typical spike activity during control and after drug washout. Activity decreases toward the end of the period of partial block. For the same bupivacaine concentration, activity during the period of partial block is always lower than during the initial blocking period. Note also that arterial pressure remains constant only fluctuating within physiologic limits.

that the volume/flow ratio yielded time constants between 3 and 4 min for the perfusing system. Accordingly, the drug concentration within the trough reached that of the perfusate after five time constants, *i.e.*, after 15 to 20 min. This was verified further by measuring the time course of Evans blue concentrations perfused through the trough in bench experiments. By using an appropriate suction device, the level of fluid within the trough was regulated. The temperature of the solution was kept between 36° and 39.5° C by surface heating.

To avoid diffusion of local anesthetic into the silicone or oil compartment, a problem associated with conventional spike recording techniques under an oil cover and to simulate more physiologic conditions spike activity was recorded bipolarly within the Tyrode solution by way of two suction electrodes of our own design, approximately 1.5 cm apart. The glass tubes, each with an interior platinum-iridium electrode near the distal opening, were filled with normal Tyrode solution. By means of a slight negative pressure, the nerve was sucked gently against the opening, which had an inner diameter of approximately 200  $\mu$ m, similar to the nerve diameter. Thus, the recording site was isolated from the surrounding Tyrode solution, and due to the large electrical impedance, measurable voltages between the electrode and a reference in the surrounding Tyrode pool resulted.

Nerve activity was determined by converting natural spike activity burst by burst into an effective voltage (root mean-square technique using an analog module BB 4341). The resulting averaged nerve activity, together with arterial pressure (measured from a catheter placed in the thoracic aorta), and ECG were recorded continuously, along with the original nerve activity on tape.

#### EXPERIMENTAL PROTOCOL

Control spike activity during perfusion with Tyrode solution was recorded for at least 20 min.

The nerve was then exposed to increasing bupivacaine concentrations until complete nerve block just resulted. Each concentration was maintained for at least 15 min until averaged nerve activity had either reached a plateau or activity was blocked completely (determination of the minimal blocking concentration =  $c_m$ ). Following complete block the bupivacaine concentration was reduced to an intermediate concentration, which resulted in a partial block. This concentration was kept constant for 4 h. To confirm intact nerve function, bupivacaine was washed out by Tyrode solution.

#### DATA ANALYSIS

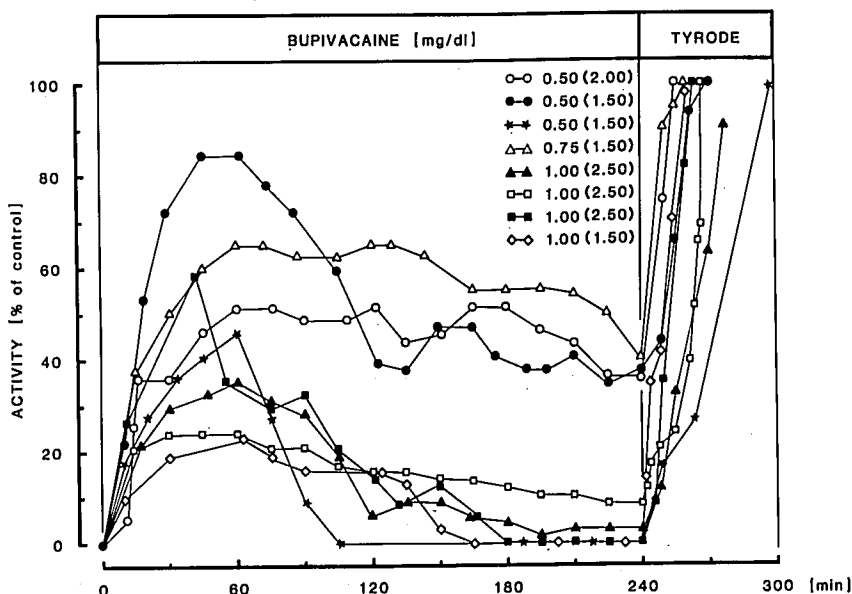
Averaged nerve activity before blocking (perfusion with Tyrode solution) was arbitrarily set 100, and 0 during complete block. Thus, nerve activity is expressed in percent of control measurements for the same arterial pressure levels which is important because aortic nerve activity correlates linearly with arterial pressure in the physiologic range.<sup>4</sup> Concentration/effect curves were plotted by relating the percent changes in averaged nerve activity against bupivacaine concentrations.

#### Results

The experimental protocol is exemplified by an original recording in figure 1. After control measurements with Tyrode solution the nerve was exposed to increasing bupivacaine concentrations. Spike activity and averaged nerve activity decreased with each concentration until complete conduction block ensued at 2.5 mg/dl ( $c_m$ ). At each concentration the blocking effect reached a plateau within 11–15 min (average for all experiments: 15 min; range: 8–20 min), indicating an equilibrium of drug concentrations outside and inside the nerve at the site of action.

When after complete block drug concentration was reduced from 2.5 to 1.0 mg/dl, nerve activity initially in-

FIG. 2. Time course of nerve activity at constant concentrations of bupivacaine. Nerve activity in percent of control measurements. At time zero perfusion started with the listed intermediate bupivacaine concentrations, which were reduced by 30–75% when compared to the minimal blocking concentrations (values given in parentheses). After the initial increase (lowered drug concentration) nerve activity decreased during the following 3 h in the presence of a constant concentration and returned to control during the final washout period.



creased reaching its maximum activity of 35% within 60 min, but during the following 3 h slowly decreased to an activity of only 5% before drug washout despite maintenance of a similar arterial pressure.

Nerve activity during partial block was lower than at the same concentration during the initial blocking period when at 1.0 mg/dl bupivacaine activity remained unimpaired. During the final washout, however, activity recovered within 40 min to 90% of control. That nerve activity always decreased over time at constant bupivacaine concentrations is apparent from figure 2. In response to the lowered bupivacaine concentrations nerve activity increased over the first 30–60 min (average: 50 min) but then decreased during the following hours in spite of constant bupivacaine concentrations and arterial pressure. During the final washout nerve activity recov-

ered within 15–60 min (average: 30 min) to the controls excluding nerve damage as a reason for the preceding decrease in nerve activity.

The bupivacaine concentrations used for partial block in figure 2 reduced nerve activity to a substantially greater extent than in the initial blocking experiments as is shown by the differences in activity during either period (table 1). Thus, nerve activity decreased over time in the presence of a constant concentration of bupivacaine, and the effect for a given concentration increased with repeated drug application.

### Discussion

The results from this study suggest that tachyphylaxis apparently does not originate from a loss of drug effec-

TABLE 1. Nerve Activity (percent of control measurements) for a Given Bupivacaine Concentration Applied at the Beginning of an Experiment and Subsequently during the Period of Partial Block

Experiment	Bupivacaine Concentration (mg/dl)	Initial Nerve Activity (%)	Nerve Activity during Constant Drug Administration for 4 Hours			
			Maximum		Minimum	
			(%)	Δ	(%)	Δ
1	0.50	100	51	-49	36	-64
2	0.50	85	85	±0	34	-51
3	0.50	45	45	±0	0	-45
4	0.75	80	65	-15	40	-40
5	1.00	100	35	-65	5	-95
6	1.00	67	24	-43	9	-58
7	1.00	100	59	-41	0	-100
8	1.00	48	23	-25	0	-48

Nerve activity in column 3 was determined during the initial blocking period when concentrations were stepwise increased until complete nerve block. The activity in columns 4 and 6 resulted at the same

concentration during the period of partial block. Δ in columns 5 and 7 = differences from initial nerve activity (column 3).

tiveness at the nerve itself. Our experiments rest on the premise that alterations in axonal susceptibility to local anesthetics should be detectable if nerve activity could be evaluated in relation to the drug concentration at the site of action over sufficiently long time periods. The aortic nerve, a pure sensory nerve, which carries only baroafferents originating from the aortic arch, appeared to be a good experimental choice. Its natural spike activity, evoked by the pulsatile arterial pressure changes coincident with each heartbeat, can be recorded for hours and nerve function assessed reliably from changes in averaged nerve activity. Furthermore, the aortic nerve has a diameter of only 200  $\mu\text{m}$ , which, in agreement with de Jong,<sup>4</sup> fosters rapid equilibrium of the drug concentrations outside and inside the nerve within 10 min.<sup>2</sup> That a steady state in averaged nerve activity was reached during the blocking period only after 20 min almost certainly results from the time required for attaining a concentration equilibrium between perfusate and the nerve with the perfusion system used. In agreement with this observation, it takes 15–20 min for the given time constant of the system until the concentrations around the nerve reach those of the perfusate (see "Methods"). Thus, after washin is complete a constant bupivacaine concentration at the site of action can be maintained for hours.

Finally, the experimental protocol with the period of partial block extending 4 h preceded by complete blocking experiments should have been adequate for detecting tachyphylaxis if present. In animals as well as in humans, tachyphylaxis has been reported to occur within 4 h regardless of the mode of local anesthetic application, *i.e.*, with continuous infusions,<sup>7–11,¶</sup> as well as repeated injections of single doses.<sup>12–14</sup> In this context it should be stressed that 90 min was needed to determine concentration/effect curves that preceded the period of partial block so that the nerves were in contact with bupivacaine for up to 6 h.

Continuous drug application was mimicked by the period of partial block when the nerves were exposed to constant bupivacaine concentrations. The effects of repeated exposures to equal concentrations were tested by comparing effects during blocking period and partial block (table 1). We thus tried to account for observations in humans,<sup>14</sup> demonstrating the need for higher doses to reestablish adequate analgesia after partial or complete recovery (interanalgesic interval).

Because we could assess nerve function in relation to drug concentration at the site of action over time periods of hours, our experimental approach should have been appropriate to detect tachyphylaxis if present. However, no tachyphylaxis was demonstrated.

The increase in spike activity during the initial period of partial block resulted from the washin of lower concentrations of bupivacaine. Because during this period drug concentrations in the pool approached slowly within 20 min that of the perfusate, the time lag to maximum nerve activity up to 60 min was not unexpected. The recurrence of nerve activity with decreasing drug concentrations usually lags behind the onset of block, a phenomenon resembling hysteresis.<sup>15</sup> It should be noted also that at the same drug concentrations nerve activity during partial block was substantially lower than that during the preceding blocking experiments (table 1). Most importantly, after the early maximum during partial block nerve activity decreased over the following 3 h in spite of constant drug concentrations of bupivacaine around the nerve and, in all likelihood, at the site of drug action.

Nerve fiber degeneration suggested to result from prolonged drug exposure<sup>12</sup> is excluded as a possible reason for the time-related decrease because normal spike activity was restored completely upon final drug washout. Changes in *pH*, which were suggested to be responsible for tachyphylaxis<sup>16</sup> as well as changes in temperature which also might influence drug effectiveness, are excluded because both variables were kept constant. Whatever the reasons may be, the decrease in spike activity is drug-induced and promptly reversible.

These observations cannot be reconciled with the theory that tachyphylaxis might be mediated by a reduced effect of local anesthetics at the nerve itself.<sup>1</sup> Provided that these results are transferable to humans, tachyphylaxis either is a consequence of reduced drug concentration at the axon, and therefore related to pharmacokinetics or, as proposed recently,<sup>17</sup> a phenomenon of increased central nervous input during chronic conduction block. In addition, these two alternatives would support the widely held but hitherto unproven view that tachyphylaxis occurs only in intact animal or humans.<sup>18,19</sup>

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