

Reversible Inhibition of Rapid Axonal Transport in Vivo by Lidocaine Hydrochloride

B. Raymond Fink, M.D.,* and Stephen J. Kish†

Rats were given standardized injections of ^3H -L-proline into the trigeminal ganglion and ^{14}C -lidocaine hydrochloride at the infraorbital foramen. The ^3H -L-proline was always injected 2.5 h before removal of the nerve. Lidocaine, 1, 2, and 4 per cent, produced a concentration-related inhibition of entry of ^3H -labeled rapid axonal transport into the distal portions of the nerve. Addition of epinephrine, 1:200,000, doubled the intensity of the effect. The time delay of recovery was also concentration-related, and with 4 per cent lidocaine recovery still seemed incomplete after 4.5 h. It is concluded that inhibition of rapid axonal transport is probably a usual byproduct of nerve block with local anesthetics such as lidocaine. The inhibition seems attributable in part to a disturbance of the energy metabolism of the nerve. (Key words: Anesthetics, local, axonal transport; Metabolism, axonal transport; Nerve, axonal transport).

CONSIDERABLE DISAGREEMENT presently exists concerning the effect of local anesthetics on rapid axonal transport (rapid axoplasmic transport, rapid intraaxonal transport). *In vitro*, Ochs and Hollingsworth¹ could not observe inhibition of fast transport in cat sciatic nerve by 1 per cent procaine, but Fink *et al.*² demonstrated interruption of rapid transport in rabbit vagus nerve by as little as 0.3 per cent lidocaine, and Aasheim *et al.*³ obtained arrest with 0.28 per cent procaine. The inhibition produced by 0.6 per cent lidocaine was not reversed by 4 h of washing. Byers *et al.*⁴ achieved analogous inhibition in trigeminal nerve in the living

rat by bathing the infraorbital branch of the trigeminal nerve in a pool of lidocaine at the exit of the nerve from the skull.

On the basis of such reports, Ngai *et al.*⁵ investigated the question of a possible "neurotoxic" effect of injected local anesthetics. They measured the axonal transport of three catecholamine-synthesizing enzymes in sympathetic efferent fibers of the sciatic nerve in the guinea pig and concluded that lidocaine and etidocaine did not inhibit the axonal transport of these enzymes. Bisby,⁶ however, was able to inhibit axonal transport in sympathetic and somatic motor nerve fibers *in vivo* by a two-day application of lidocaine and other local anesthetics dissolved in silicone; reversibility of the effect was not studied.

We have examined inhibition and reversibility in somatic sensory nerve fibers because such fibers are the principal targets in regional anesthetic procedures. This report describes dose-related results in the rat, obtained after labeling axonal transport in the infraorbital nerve via the trigeminal ganglion and injecting local anesthetic at the infraorbital foramen in a simulation of clinical nerve block.

Methods

The method of labeling the trigeminal axonal transport and the technique of standardized lidocaine injection have been described.^{7,8} In outline, pentobarbital-sedated rats (30 mg/kg, ip) were subjected to stereotaxic injection of L- ^3H -proline (specific activity 3.3 Ci/mmol, New England Nuclear) from a micropipette introduced into the right trigeminal ganglion. As a result, ^3H -labeled protein migrated into various branches of the right trigeminal nerve, transported at a rate of 192–384 mm/h, and reached the infraorbital foramen about 1.5 h after injection. Labeled transport was allowed to

* Professor of Anesthesiology.

† Research Technologist.

Received from the Anesthesia Research Center and the Center for Research in Oral Biology, University of Washington School of Medicine, RN-10, Seattle, Washington 98105. Accepted for publication October 20, 1975. Supported by Grants GM 15991 and DE 02600-08 from the National Institutes of Health, United States Public Health Service and a Grant from Astra Pharmaceutical Company.

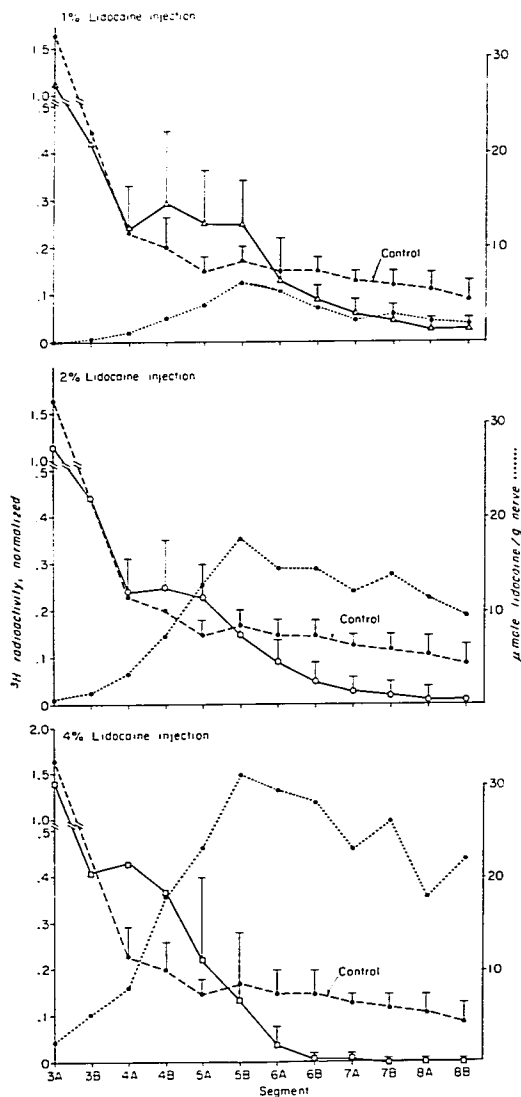
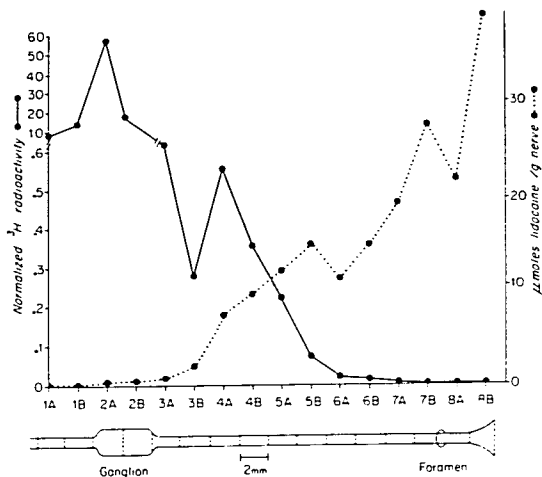


FIG. 1. Neural distribution of axonally transported ^3H radioactivity 1 h after injection of 0.2 ml lidocaine solution at the infraorbital foramen. Δ — Δ lidocaine 1 per cent, \circ — \circ 2 per cent, \square — \square 4 per cent, \bullet — \bullet physiologic saline control, \bullet — \bullet — \bullet segmental lidocaine content at time of sacrifice; ^3H -proline was injected into the trigeminal ganglion 2.5 h before removal of the nerve. Each point is the mean of seven experiments. Bars represent 1 SD, many omitted for the sake of clarity. SD for summed proximal (4A-5B) and distal (6A-8B) segments appear in table 1. SD of lidocaine content (not shown) averaged 0.4 of indicated means.

FIG. 2. Distribution in the infraorbital nerve of ^3H derived from L- ^3H -proline (●—●) injected into the trigeminal ganglion 2.5 h before sacrifice, and of ^{14}C representing lidocaine (●---●) injected at the infraorbital foramen 1 h before sacrifice. Lidocaine concentration: 2 per cent with epinephrine, 1:200,000; injected volume 0.2 ml.



proceed a further 1 h before the animals were sacrificed.

At various times, as specified below, a standardized 0.2-ml injection of ^{14}C -lidocaine solution was made at the infraorbital foramen with the aid of a palatal jig. The jig guided a hypodermic needle to the foramen. Physiologic saline solution (0.2 ml) was injected at the contralateral foramen. Abolition of the aversive response to pinching the lip on the right side with preservation of the response on the left was used as the criterion of successful block of nerve impulse conduction. Complete block developed within 45 sec in 80 per cent of the animals. The other 20 per cent were excluded from the study. The radioactive lidocaine solution was prepared by adding 1 μCi lidocaine hydrochloride labeled with ^{14}C at the carbonyl carbon atom (specific activity 4.94 mCi/nmol, New England Nuclear) to 1 ml of unlabeled 1, 2, or 4 per cent lidocaine (Astra Pharmaceutical Co.) (about 37, 74 or 148 mM) in sodium chloride solution, free of preservative, isotonic, pH adjusted to 7.3 with hydrochloric acid or sodium hydroxide. The purity of the radioactive proline and lidocaine was verified by chromatography and found to exceed 99 per cent. One and 2 per cent lidocaine

solutions containing epinephrine (1:200,000) were also studied. In some animals a second lidocaine injection was made 1 h after the first.

Groups of animals were sacrificed 1, 3.5, or 4.5 h after the last lidocaine injection, though always 2.5 h after the ^3H -proline injection, as mentioned above. Seven replicates were included in each time and concentration group, unless otherwise stated. The trigeminal root and ganglion, the maxillary division and the infraorbital nerve were removed in continuity on both sides and divided into 2-mm segments, starting at the ganglion, numbered as shown in figure 2. The site of lidocaine injection was between segments 7B and 8A. The ^3H and ^{14}C radioactivity in each segment was determined by liquid scintillation counting after digestion in Soluene (Packard). Counts, expressed as disintegrations per minute (dpm), were corrected for background, for quenching determined by automatic external standardization, and, on the labeled side, for any activity in the corresponding contralateral segment. In control experiments physiologic saline solution was injected on both sides. Significance of differences between control and lidocaine measurements was estimated by two-tailed t-test.

The effect on axonal transport was evaluated by calculating the ratio of the total ^3H radioactivity in four relatively proximal nerve segments (4A-5B) to the total ^3H radioactivity in the next six distal segments (6A-8B), after normalizing the individual measurements by expressing them as a fraction of the total radioactivity in the nerve. The total neural ^3H -radioactivity varied relatively little in homologous sets of nerves. In the control series, the total tritium dpm in right-side nerves averaged $2.941 \times 10^6 \pm 0.542 \times 10^6$ (SD).

Results

ONE HOUR AFTER SINGLE LIDOCAINE INJECTION

Physiologic saline control nerves contained a gradient of ^3H -radioactivity (representing transported protein), shown by the interrupted lines in figure 1, decreasing progressively from segments 4A to 8B; the ratio (activity in segments 4A-5B): (activity in segments 6A-8B) averaged 1.0 (table 1).

Injection of 0.2 ml of 1, 2, or 4 per cent lidocaine resulted in the neural distribution of ^3H -radioactivity shown by solid lines in figure 1. A dose-related accumulation of radioactivity was evident in the proximal segments (4A-5B) together with a roughly corresponding

deficiency in the distal segments (6A-8B). The means (\pm SD) and the ratios of the normalized (activity in segments 4A-5B): (activity in segments 6A-8B) are listed in the first three columns of table 1. Compared with the physiologic saline controls, the ratio of proximal activity (4A-5B) to distal activity (6A-8B) was always significantly elevated by lidocaine and showed a definite concentration dependence. Addition of epinephrine to lidocaine considerably increased the magnitude of the effect. A peak of ^3H -radioactivity was present in one of the proximal segments (4A-5B) in at least 70 per cent of the nerves, but this fact is obscured by averaging in figure 1, because the segments containing the peaks varied in different specimens. Figure 2 shows the tritium peak in a typical experiment with 2 per cent lidocaine with epinephrine, 1:200,000, and the gradient of ^{14}C -lidocaine radioactivity in the same nerve.

The dotted curves in figure 1 indicate the segmental levels of ^{14}C -radioactivity (representing lidocaine), expressed as μmol lidocaine/g nerve, at the time of sacrifice. The highest level was found in segment 5B at all concentrations studied (1, 2, and 4 per cent), but the large standard deviation of the means deprived this of statistical significance. The true location of the ^{14}C peak was thus indeterminate.

TABLE 1. Proximal-Distal Distribution of Axonally Transported ^3H Radioactivity in the Infraorbital Nerve as a Function of Time Following One Injection of Lidocaine at the Infraorbital Foramen

	One Injection					
	One Hour			3.5 Hours		
	Proximal Mean \pm SD	Distal Mean \pm SD	Ratio	Proximal Mean \pm SD	Distal Mean \pm SD	Ratio
Lidocaine, g/100 ml						
0	.75 \pm .14	.74 \pm .15	1.0 (7)	.72 \pm .10	.52 \pm .17	1.4 (7)
1	1.02 \pm .23*	.38 \pm .13†	2.7 (7)	.54	.43	1.3 (2)
1 + E	.73 \pm .19	.13 \pm .06†	5.6 (7)	.65 \pm .10	.53 \pm .15	1.2 (9)
2	.87 \pm .19	.22 \pm .16†	4.0 (7)	.68	.55	1.2 (2)
2 + E	1.41 \pm .42	.15 \pm .07†	9.4 (7)	.68 \pm .12	.46 \pm .20	1.5 (7)
4	1.16 \pm .31	.07 \pm .04†	16.6 (7)	1.01 \pm .37	.42 \pm .23	2.4 (7)

Radioactivity was normalized by expressing dpm measurements in the proximal segments (4A-5B) and the distal segments (6A-8B) of a nerve as fractions of the total dpm in the nerve.

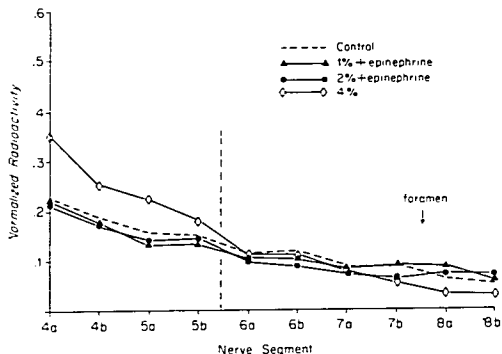
E = epinephrine, 1:200,000.

Numbers in parentheses = numbers of replicates.

* $P < 0.05$.

† $P < 0.005$.

FIG. 3. Neural distribution of axonally transported ^3H radioactivity 3.5 h after a single injection of 0.2 ml lidocaine solution at the infraorbital foramen. ^3H -proline was injected into the trigeminal ganglion 2.5 h before removal of the nerve.



At 1 h after lidocaine, response to noxious stimulation of the upper lip on the side that received a lidocaine injection was always absent, whereas response to stimulation of the other side remained unaffected.

THREE AND A HALF HOURS AFTER SINGLE LIDOCAINE INJECTION

Reversibility of the lidocaine-produced impairment of rapid transport was evaluated by deferring sacrifice until 3.5 h after the injection of local anesthetic (or saline solution in controls). The distribution of ^3H -radioactivity 3.5 h following 1 per cent or 2 per cent lidocaine with epinephrine (fig. 3) was similar to that in physiologic saline controls and was devoid of peaks, but following 4 per cent lidocaine the proximal-distal ratio was sometimes slightly elevated (table 1) and a peak of ^3H -radioactivity was present in two of the nerves. ^{14}C -lidocaine radioactivity in the proximal segments (4A-5B) did not exceed $3 \mu\text{mol/g}$, and response to noxious stimulation had been restored in all these animals at the time of sacrifice, save one after exposure to 4 per cent lidocaine.

DOUBLE LIDOCAINE INJECTION

In these experiments a second lidocaine injection identical to the first was administered 1 h following the first. The second injection was given in order to prolong the inhibition

of rapid transport and to determine whether it affected subsequent recovery.

Decreased entry of rapid axonal transport into the distal segment was still detectable in this series 3.5 h after the last injection of 2 or 4 per cent lidocaine (table 2). With 4 per cent lidocaine, an inhibitory effect apparently persisted for more than 4.5 h. Insensitivity of the lip to pinching was still present in two of five animals 4.5 h after 2 per cent lidocaine-epinephrine, and also after 4 per cent lidocaine. In animals in which the behavioral response had recovered the neural level of lidocaine was $0.7 \mu\text{mol/g}$ or less. Only one nerve of the series showed a peak of radioactivity in the proximal segments.

Discussion

From figure 1 it is clear that the inhibitor solution was not restricted to the immediate region of the infraorbital foramen but spread proximally along the nerve sufficiently to inhibit axonal transport in the region of segments 4A-5B. The presence of transport inhibition at this distance from the foramen tends to exclude injection trauma as a contributory factor. The negative results with physiologic saline solution prove that the trauma of injection was by itself insufficient to affect rapid transport.

The tritium levels in segments 3B-5A an hour after the single injection of lido-

TABLE 2. Proximal-Distal Distribution of Axonally Transported ^3H Radioactivity in the Infraorbital Nerve as a Function of Time Following Two Injections of Lidocaine at the Infraorbital Foramen

	Two Injections					
	3.5 Hours			4.5 Hours		
	Proximal Mean \pm SD	Distal Mean \pm SD	Ratio	Proximal Mean \pm SD	Distal Mean \pm SD	Ratio
Lidocaine, g/100 ml						
0	.82 \pm .31	.51 \pm .17	1.6 (6)			
1 + E	.67	.40	1.7 (2)			
2 + E	.74 \pm .22	.31 \pm .22	2.4 (5)	.90 \pm .22	.51 \pm .15	1.8 (5)
4	.59	.18	3.3 (2)	1.02	.43	2.4 (2)

Radioactivity was normalized by expressing dpm measurements in the proximal segments (4A-5B) and the distal segments (6A-8B) of a nerve as fractions of the total dpm in the nerve.

E = epinephrine, 1:200,000.

Numbers in parentheses = numbers of replicates.

caine, listed in table 3, show that with 4 per cent lidocaine, there was an accumulation of ^3H -radioactivity in both segments 4A and 4B but not in segment 3B. One may deduce that with this concentration of local anesthetic inhibition resulting in accumulation of rapidly transported material began in segment 4A, and continued there until the lidocaine level in that segment fell below the critical inhibitory value of approximately 4-10 $\mu\text{mol/g}$.¹ Rapidly transported material could then advance into segment 4B, but was held there because at the time of sacrifice the level of lidocaine in this segment had not yet receded below the critical inhibitory value. Similar arguments explain the presence of progressively more distal accumulation fronts in the experiments with 2 per cent and 1 per cent lidocaine.

The results clearly indicate that lidocaine inhibits at least some of the elements of rapid axonal transport in the rat trigeminal nerve *in vivo*. They show that inhibition increases with duration of exposure to lidocaine and with the concentration of lidocaine injected, and that recovery from the effects of the inhibitor is correspondingly delayed. As expected, addition of epinephrine accentuates the *in-vivo* inhibition produced by lidocaine, though not inhibition *in vitro* (Fink, B. R., unpublished observations).

¹ The limits of the inhibitory range are the lidocaine levels shown in segment 4A in figure 1, middle and lower panels, respectively.

The cumulative effect of higher concentration and longer exposure to the drug is probably explained by slow penetration of lidocaine through the nerve sheath into the axon. Equilibration of the intraneural and extraneural levels of lidocaine is known to require several hours *in vitro*,^{2,3} and should be slower in larger than in smaller fibers. Accordingly, an anesthetic level sufficient to inhibit transport would not have been reached simultaneously in all the fibers of the infraorbital nerve and, with the lower lidocaine concentrations at least, may never have been reached in some.

The present observations *in vivo* are consistent with *in-vitro* data from this laboratory² showing inhibition and recovery of axonal transport dependent on both the concentration of and duration of exposure to lidocaine. The results are also consistent with the inhibition of fast axonal transport *in vivo* by chronic exposure to lidocaine observed by Bisby.⁶ In Bisby's study local anesthetics were applied in very high concentrations, dissolved in a Silastic cuff that enclosed the sciatic nerve for two days. The concentration actually bathing the nerve, however, was indeterminate.

The inability of Ngai *et al.* to observe inhibition of axonal transport of tyrosine hydroxylase and aromatic amino acid decarboxylase might be explained by the slow rate of transport of these enzymes, about 1 mm/h. Dopamine- β -hydroxylase (DBH), which migrates at a rate of 6-7 mm/h,

gave equivocal results. It is possible that slowly moving elements may be relatively less susceptible to local anesthetic inhibition than rapidly moving ones. These investigators, however, applied local anesthetic to the sciatic nerve via an indwelling catheter. In our experiments deposition of local anesthetic was standardized in relation to fixed points on the skeleton and was effected in tissue undisturbed by dissection; standardized specimens were removed for examination in all experiments. The ratio method of evaluation discriminated in favor of the more rapidly transported labeled elements since application of inhibitor took place at the time that the labeled fast transport first approached the infraorbital foramen.⁷ Slower elements that had not entered the distal segments in controls would have little effect in changing the ratio in any of our experiments.

The data obtained 3.5 h and 4.5 h after injection of lidocaine indicate that the speed of recovery from the inhibition is a function of both the duration of exposure to lidocaine and the concentration of lidocaine injected. Delay in recovery is greatest with the highest concentration of lidocaine, and is more noticeable after double than after single injection. Delay is also markedly accentuated with the addition of epinephrine. It is apparent that with an agent such as lidocaine the level of anesthetic in nerve fibers during clinical block does not maintain a plateau, but is probably waxing or waning continuously.

The distribution profiles of ¹⁴C-lidocaine 1 h after injection of the 2 and 4 per cent solutions, averaged in figure 1, show levels of approximately 4 and 10 $\mu\text{mol/g}$, which in turn are about 1.3–3 times greater than the external concentration necessary to block propagation of the compound action potential *in vitro* in 10 minutes.¹⁰ There is thus fair agreement between the *in-vivo* and *in-vitro* values of the respective blocking concentrations. Consequently, if impairment of rapid transport is accepted as a bellwether of neurotoxicity, as suggested by other investigators,^{2,6} the data in this report provide an interesting first estimate of the neurotoxicity margin of safety available in clinical nerve block. The safety margin is the difference between the minimum neural level of a local anesthetic required for noci-sensory block (approximately 1 $\mu\text{mol/g}$

TABLE 3. Normalized ³H Radioactivity One Hour after Exposure to Physiologic Saline Solution or Lidocaine (Mean \pm SD)

Segment	Saline Control	Lidocaine		
		1 Per Cent	2 Per Cent	4 Per Cent
3B	.44 \pm .21	.42 \pm .20	.44 \pm .23	.41 \pm .11
4A	.23 \pm .06*	.24 \pm .09	.24 \pm .07	.43 \pm .24
4B	.20 \pm .06*	.29 \pm .15	.25 \pm .10	.37 \pm .18
5A	.15 \pm .03	.25 \pm .09	.23 \pm .07	.22 \pm .18

* $P < .05$, t test.

nerve in the case of lidocaine)⁸ and the minimum neural level required for block of rapid axonal transport (approximately 4–10 $\mu\text{mol/g}$ nerve). It appears that under clinical conditions part of this margin is utilized to secure a neural level of local anesthetic high enough for a useful duration of regional anesthesia. Inhibition of rapid axonal transport may therefore be an important limiting factor of the duration of block safely attainable with a single injection of local anesthetic.

The question whether longer-acting anesthetics such as bupivacaine have a correspondingly persistent action on rapid transport in sensory fibers is not answered by these experiments, and requires further investigation. Studies of longer-term reversibility are in progress.

The mechanism of the inhibition of rapid transport by lidocaine is not yet clear. Neuronal microtubules, which are believed to be involved in the normal mechanism of rapid transport, are not always depolymerized at a time when lidocaine has inhibited the transport.¹¹ High-energy phosphate is a known requisite of rapid axonal transport,¹ and depletion of high-energy phosphates may be partly responsible, since other studies¹² have shown that lidocaine hydrochloride is a potent depressant of mitochondrial oxidative metabolism, the EC_{50} being about 8 mM or approximately 0.2 g/100 ml. The structural changes in anoxic vagus nerve *in vitro*, however, differ from those produced by lidocaine.¹¹

In view of the above, one can provisionally visualize the injection of a local anesthetic as occasioning a deficiency of oxidative energy in affected nerve fibers, doubtless intensified if the solution contains a vasoconstrictor.

With the injection of a sufficiently high concentration the safe duration of application may be as limited as that of a mechanical tourniquet.

References

- Ochs S, Hollingsworth D: Dependence of fast axoplasmic transport in nerve on oxidative metabolism. *J Neurochem* 18:107-114, 1971
- Fink BR, Kennedy RD, Hendrickson AE: Lidocaine inhibition of rapid axonal transport. *ANESTHESIOLOGY* 36:422-432, 1972
- Aasheim C, Fink BR, Middaugh M: Inhibition of rapid axoplasmic transport by procaine hydrochloride. *ANESTHESIOLOGY* 41:549-553, 1974
- Byers MR, Fink BR, Kish SJ, et al: Arrest of rapid axoplasmic transport in a sensory nerve *in vivo* by ligature or lidocaine treatment. *Molecular Mechanisms of Anesthesia*. Edited by BR Fink. New York, Raven Press, 1975, pp 25-38
- Ngai SH, Dairman W, Marchelle M: Effects of lidocaine and etidocaine on the axoplasmic transport of catecholamine-synthesizing enzymes. *ANESTHESIOLOGY* 41:542-548, 1974
- Bisby MA: Inhibition of axonal transport in nerves chronically treated with local anesthetics. *Exp Neurol* 45:481-489, 1975
- Fink BR, Kish SJ, Byers MR: Rapid axonal transport in trigeminal nerve of rat. *Brain Res* 90:85-95, 1975
- Fink BR, Aasheim GM, Kish SJ, et al: Neurokinetics of lidocaine in the infraorbital nerve of the rat *in vivo*: Relation to sensory block. *ANESTHESIOLOGY* 42:731-736, 1975
- Strobel GE, Bianchi CP: The effects of pH gradients on the uptake and distribution of C¹⁴-procaine and lidocaine into intact and desheathed sciatic nerve trunks. *J Pharmacol Exp Ther* 172:18-32, 1970
- de Jong RH: *Physiology and Pharmacology of Local Anesthesia*. Springfield, Ill., Charles C Thomas, 1970, p 90
- Byers MR, Hendrickson AE, Fink BR, et al: Effects of lidocaine on axonal morphology, microtubules, and rapid transport in rabbit vagus nerve *in vitro*. *J Neurobiol* 4:125-143, 1973
- Haschke RH, Fink BR: Lidocaine effects on brain mitochondrial metabolism *in vitro*. *ANESTHESIOLOGY* 42:737-740, 1975

Neuroanesthesia

HYPOCAPNIA AND INTERNAL CRANIAL COMPLIANCE Hyperventilation to decrease intracranial pressure is an accepted procedure. In order to predict the effect of lowering cerebral blood flow, and thus diminishing intracranial volume, the concept of intracranial compliance must be considered. In five patients who had chronic increased CSF pressure, voluntary hyperventilation lowered P_{aCO_2} from 38.4 to 29.8 torr. This was accompanied by a decrease of ventricular fluid pressure from 24.2 to 13.2 torr. At the same time intracranial compliance more than doubled in four of the five patients studied. The observed change in compliance was proportional to the change in intracranial pressure. Similar findings resulted from studies in baboons with elevated intracranial pressures. The data suggest that while hypocapnia decreases intracranial pressure and increases intracranial compliance, it does not alter the shape of the overall volume-pressure curve.

They further suggest that if it is desired to alter this curve, the use of mannitol or steroids to reduce water content of the brain might be more efficacious. (*Rouved DW, and others: Hypocapnia and intracranial volume-pressure relationship. A clinical and experimental study. Arch Neurol* 32:369-373, 1975.)
ABTRACTER'S COMMENT: It must be pointed out that the effect of hyperventilation may vary depending on whether the patient is on the flat portion (small change in pressure resulting from a given change in volume) or steep portion (large change in intracranial pressure resulting from a small change in intracranial volume) of the compliance curve. In the latter case, even small decreases in intracranial volume will be extremely useful while, conversely, small increases in volume (resulting, perhaps, from an increase of just a few torr in P_{aCO_2}) could be detrimental.