*David S. Warner, M.D., Editor*

# Positively Active

## *How Local Anesthetics Work*

Hugh C. Hemmings, Jr., M.D., Ph.D.,* Paul Greengard, Ph.D.†

On the Active Structure of Local Anesthetics. By J. Murdoch Ritchie and Paul Greengard. J Pharmacol Exp Ther 1961; 133:241–5.

**Abstract:** The action of local anesthetics, containing a tertiary nitrogen, on mammalian nonmyelinated fibers of the rabbit's vagus nerve has been analyzed to determine whether the uncharged or the positively charged form of these compounds is responsible for their ability to block impulse conduction. The compounds studied were dibucaine, tetracaine, chlorpromazine, imipramine, and procaine. Impulse conduction was restored, in fibers in which it had been blocked by pretreatment with a local anesthetic, by increasing the pH of the perfusing solution from approximately 7.0 to 9.5; block was rapidly reestablished when the fibers were again perfused with the solution of pH approximately 7.0. From the way in which the size of the action potential varied with pH in nerve fibers pretreated with a local anesthetic, it has been concluded that the active form of the local anesthetic is the cation.

THE importance of local anesthetics to the practice of medicine is inestimable. From the precisely placed image-guided injections by anesthesiologists to their use in cardiology as antiarrhythmic drugs, along with uses by nonspecialists and even consumers as topical anesthetics, local anesthetics are, with the general anesthetics, the essential and defining drugs in the practice of anesthesia. Great progress has been made in understanding the molecular mechanisms of action of local anesthetics, including their critical interactions with voltage-gated $Na^+$ channels, their principal target of action in blocking the action potential and thereby nerve conduction. This Classic Publication Revisited[1] concerns an important chapter in defining the mech-

* Professor of Anesthesiology and Pharmacology, Department of Anesthesiology, Weill Cornell Medical College, New York, New York. † Professor and Head, Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, New York.

Address correspondence to Dr. Hemmings: Department of Anesthesiology, Box 50, Weill Cornell Medical College, 1300 York Avenue, New York, New York 10065. hchemmi@med.cornell.edu.This article may be accessed for personal use at no charge through the Journal Web site, www.anesthesiology.org.

**Fig. 1.** Paul Greengard, Ph.D., in the 1950s at the time of his nerve conduction studies.

anism of local anesthesia that began 50 yr ago as an early project led by one of us (P.G.), a pharmacologist probably unknown to most anesthesiologists.

Paul Greengard traveled to the National Institute of Medical Research in Mill Hill, London, in the late 1950s as a postdoctoral fellow after his graduate training in neurophysiology at Johns Hopkins University (fig. 1). There he met J. Murdoch Ritchie, Ph.D. (1925–2008), who went on to take a faculty position at Albert Einstein School of Medicine in New York, New York. Greengard accepted a job as research director of a new research facility established by the Geigy Laboratories, Ardsley, NY, with the promise of considerable resources, including the opportunity to continue basic research in the laboratory of Ritchie 1 day a week. Such an arrangement is similar to the research commitments of many clinical investigators. Ritchie's laboratory studied the pharmacology of $Na^+$ channels, and together Ritchie and Greengard began a collaboration to clarify some conflicting evidence regarding the site of action of local anesthetics on action potential conduction in nerves.

Most local anesthetics are tertiary amines, and therefore at normal pH, they exist in equilibrium between cationic (protonated) and neutral forms. This raised the question of which form is responsible for their nerve conduction blocking actions. Nerve conduction studies performed in multifiber preparations over a range of pH had shown that potency increased with increasing pH, leading to the conclusion that the neutral form was responsible for the anesthetic action.[2,3] The work by Ritchie *et al.*[1,4,5] published in three papers between 1961 and 1965, as well as in an influential review published in 1966,[6] turned this conclusion around by showing that it is the cationic form of local anesthetics that blocks the action potential. They used desheathed unmyelinated C fibers from rabbit cervical vagus nerve to show that local anesthetics were more potent at lower than at higher pH in the desheathed nerve, a critical maneuver. By alternating the pH bathing the nerve, they observed that complete block occurred at neutral pH and was relieved when the pH became alkaline (fig. 2). Subsequent studies showed that such pH dependence was not present for uncharged local anesthetics such as *n*-butanol,[4] and that alkaline anesthetic solutions were more effective in sheathed preparations, whereas neutral anesthetic solutions were more effective in desheathed preparations.[5] This result was consistent with the nerve sheath representing a diffusion barrier selectively permeable to the uncharged form of the anesthetic predominating at basic pH, which must be protonated after diffusion into the nerve to form the active cationic form of the anesthetic. This forms the scientific basis for the practical application of neutralized local anesthetic solutions to increase the speed of onset of nerve blocks.[6]

The differential effects of local anesthetics on sheathed and desheathed nerve preparations led Ritchie and Greengard to conclude that the active form of local anesthetics is the cation. This observation has been supported by many subsequent studies, including the elegant demonstration that
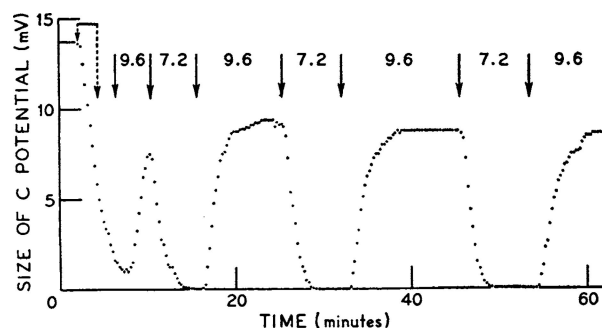


**Fig. 2.** The effect of pH on the action potential of a mammalian nerve pretreated with a local anesthetic. The *ordinate* is the height of the C elevation of the monophasic compound action potential of the rabbit's cervical vagus nerve elicited every 15 s. During the brief period between the *broken arrows*, the perfusing solution contained 1 mM dibucaine. From the beginning of the experiment until the *first solid arrow* the pH of the perfusing solution was 7.2. Thereafter, the pH of the perfusing solution was alternated, at the *arrows*, between 9.6 and 7.2. The pH during each perfusion period is indicated on the figure. The temperature of the nerve was 28°C. Reproduced with permission of the American Society for Pharmacology and Experimental Therapeutics, from Ritchie JM, Greengard P: On the active structure of local anesthetics. J Pharmacol Exp Ther 1961; 133:241–5.

acidification of axoplasmic pH increases local anesthetic potency[7] and that the quaternary forms of local anesthetics are only effective if applied internally.[8] At the time of these studies, the molecular nature of the target for local anesthetics was unknown, and a lipid theory of action was in vogue by which drugs altered the physicochemical properties of the membrane to reduce ion permeability.[6] Moreover, it was thought that the principal diffusion barrier was the nerve sheath and that the receptor was on the axon surface, but it is now apparent that lowering the external pH must also have reduced the pH of the axoplasm. The pharmacology of local anesthetics has advanced considerably since then with the identification of voltage-gated $Na^+$ channels as the principal molecular targets for local anesthetic depression of action potential amplitude.[9] The nature of the interactions between local anesthetics and $Na^+$ channels,[10] as well as other targets,[11] has been revealed at much greater resolution. But these simple studies involving little more than manipulating the pH of the bath while recording action potentials in isolated nerves revealed one of the most important, and clinically relevant, features of local anesthetic pharmacology.

Shortly after performing these landmark experiments, one of us (P.G.) was scheduled for an elective surgical procedure at the most highly regarded medical center in New York City at the time. While chatting with the anesthesiologist before the induction of general anesthesia, Greengard was asked about his work. He replied that he was a pharmacologist and had found that local anesthetics act in their charged forms to block action potentials. The anesthesiologist begged to differ, citing the old view that the uncharged form was the active species. As Greengard protested and attempted to ex-

**Fig. 3.** Hugh C. Hemmings, Jr., M.D., Ph.D., Paul Greengard, Ph.D., and Jean-Antoine Girault, M.D., at the Nobel Ceremony, December 10, 2000, Stockholm, Sweden. Photo courtesy of Jean-Antoine Girault. Color version of this figure is available at www.anesthesiology.org.

plain his work, he recalls a large black mask descending over his face that quickly ended the disagreement, to the detriment of the unenlightened anesthesiologist's education. This anecdote illustrates a number of important lessons for anesthesiologists, such as always listen to your patients, they are usually right; you owe it to your patients to keep up to date on the scientific basis of the medicine you practice; and general anesthesia might be an effective way to end an argument, but not to build confidence in your profession.

After this important foray into anesthetic neuropharmacology, Greengard left the industry and took a position in the Yale Department of Pharmacology in which Ritchie had recently arrived to become chair and replace the father of cancer chemotherapy, Arnold D. Welch, M.D., Ph.D. (1909–2003). Just before coming to Yale, Greengard took a sabbatical as a visiting professor at Vanderbilt University (Nashville, Tennessee) with Earl W. Sutherland, Jr. (1915–1974) to study cyclic adenosine monophosphate during which he discovered neurotransmitter-sensitive adenylyl cyclase. His subsequent work at Yale extended these landmark studies to the brain, leading to his well-known work showing that protein phosphorylation is an important regulator of cellular signaling and synaptic transmission in the nervous system, for which he was awarded the Nobel Prize in Physiology or Medicine in 2000 (fig. 3).[12] But one of the Greengard's earliest contributions to pharmacology involved the application of classic approaches to elucidate a guiding principle in the pharmacology of local anesthetic drugs and leading to a truly classic publication.

## References

1. Ritchie JM, Greengard P: On the active structure of local anesthetics. J Pharmacol Exp Ther 1961; 133:241–5
2. Rud J: Local anesthetics. An electrophysiological investigation of local anesthesia of peripheral nerves, with special reference to xylocaine. Acta Physiol Scand Suppl 1961; 51:1–171
3. Wilson A, Schild HW: Clarke's Applied Pharmacology, 9th edition. London, Churchill, 1959, p 336
4. Ritchie JM, Ritchie B, Greengard P: The active structure of local anesthetics. J Pharmacol Exp Ther 1965; 150:152–9
5. Ritchie JM, Ritchie B, Greengard P: The effect of the nerve sheath on the action of local anesthetics. J Pharmacol Exp Ther 1965; 150:160–4
6. Ritchie JM, Greengard P: On the mode of action of local anesthetics. Annu Rev Pharmacol 1966; 6:405–30
7. Narahashi T, Frazier T, Yamada M: The site of action and active form of local anesthetics: I. Theory and pH experiments with tertiary compounds. J Pharmacol Exp Ther 1970; 171:32–44
8. Frazier DT, Narahashi T, Yamada M: The site of action and active form of local anesthetics: II. Experiments with quaternary compounds. J Pharmacol Exp Ther 1970; 171:45–51
9. Yanagidate F, Strichartz GR: Local anesthetics. Handb Exp Pharmacol 2007; 177:95–127
10. Nau C, Wang GK: Interactions of local anesthetics with voltage-gated $Na^+$ channels. J Membr Biol 2004; 201:1–8
11. Strichartz GR: Novel ideas of local anaesthetic actions on various ion channels to ameliorate postoperative pain. Br J Anaesth 2008; 101:45–7
12. Greengard P: The neurobiology of slow synaptic transmission. Science 2001; 294:1024–30

H. C. Hemmings, Jr. and P. Greengard