Anesthesiology
59:127–131, 1983

A New Approach to Differential Peripheral Nerve Fiber Block: Na^+,K^+-ATPase Inhibition

B. Raymond Fink, M.D., F.F.A.R.C.S., and A. M. Cairns, Ph.D.†*

Differential block of peripheral nerve fibers was attempted *in vitro* by a new approach based on inhibiting the membrane pump with ouabain. Sequential concentration dependent extinction of the components of the compound action potential was obtained: C extinguished first, Aδ next, Aβ last. The sequence conforms to expectations based on axonal size.

Because pain is mediated by C and Aδ fibers, and block of these groups by ouabain was not reversed readily, further investigation of the practicability of the new approach seems warranted. (Key words: Metabolism: adenosine triphosphate. Nerve: conduction; potentials. Pharmacology: ouabain).

IN REGIONAL NERVE BLOCK one often wishes to stop peripheral nociception without blocking motor function or touch. This means interrupting conduction in unmyelinated (C) and small myelinated (Aδ) fiber groups and leaving conduction in the larger myelinated fibers (the Aαβ group) intact. In effect, one wants to block or spare peripheral nerve fiber groups according to size.

The Gasser-Erlanger principle,[1] advanced in 1929, proposed that susceptibility to block by local anesthetic was indeed a function of axonal size, specifically of the ratio of axonal surface area to axonal volume. It was argued that the protoplasm (axoplasm), which at that time was believed to be involved in the action of cocaine, was more accessible from the surface of small axons than of large ones. However, convincing evidence of a size-sensitivity relationship proved elusive,[1–4] and in retrospect this is hardly surprising because the size-sensitivity principle essentially is irrelevant to the molecular mode of action of local anesthetics, which now are believed to work at the molecular level by blocking sodium channels without regard to axonal diameter.

Nevertheless, if some other method of block that really did involve axonal volume could be found, the much-desired goal of a size-related differential block might be realizable yet.

The axoplasmic volume potentially functions as a reservoir for Na and K ions that leak across the surface membrane in opposite directions and are pumped back in the reverse direction to maintain axonal excitability. In small fibers there is less reservoir volume per unit of surface area than in large ones, and unimpaired pumping, therefore, might be more critical to continued excitability in small axons. Accordingly, we have investigated whether a differential block of small nerve fibers can be produced by inhibiting the pump.‡

Methods

The study was performed on 32 excised cervical vagus nerves of rabbits. This nerve contains Aβ, Aδ (or "B"), and C fiber groups. The de-sheathed nerve was fixed with agar to supports on an array of five platinum wires: a and b, the stimulating anode and cathode; c, the ground wire; and d and e, the recording electrodes. Distances were b–d, 35 mm; a–b and d–e, 5 mm. The array lay in a chamber that remained closed when the array was raised for recording. The nerve was maintained at 37° C in Ringer-glucose-bicarbonate solution continuously saturated with a mixture of 95% O_2–5% CO_2 as previously described in detail[5] and was exposed for 4 hours to ouabain (2×10^{-6}, 5×10^{-6}, 1×10^{-5}, or 2×10^{-5} M, n = 5 at each concentration, or 10^{-4} M (n = 1) or 10^{-3} M [n = 2] added to the solution. The compound action potentials were excited once every 5–10 minutes by supramaximal 0.15 ms pulses from a Grass stimulator (model S44) and were measured and recorded on magnetic disk, via a Nicolet digital storage oscilloscope. Time trends were observed with a microcomputer (Hewlett-Packard HP 85) programmed to graph keyboard entries. Reversibility of the ouabain effects was tested by washing, performed on five other nerves that had been exposed to ouabain 1×10^{-5} M (n = 2) or 2×10^{-5} M (n = 3) for 4 hours. For washing, the 50 ml of ouabain-containing solution in the chamber was drained and replaced by 50 ml of prewarmed, pregassed ouabain-free solution. The change occupied less than 1 minute and was effected without opening the chamber. In four of these experiments, concurrent con-

* Professor of Anesthesiology.

† Technical Assistant III.

Received from the Department of Anesthesiology and Pain Research Center, University of Washington School of Medicine, Seattle, Washington. Accepted for publication January 27, 1983. Supported by USPHS Grant GM 27678-02. Presented at the 1982 Annual Meeting of the American Society of Anesthesiologists.

Address reprints to Dr. Fink: Department of Anesthesiology, RN-10, University of Washington School of Medicine, Seattle, Washington 98195.

‡ ANESTHESIOLOGY 57:A183, 1982.

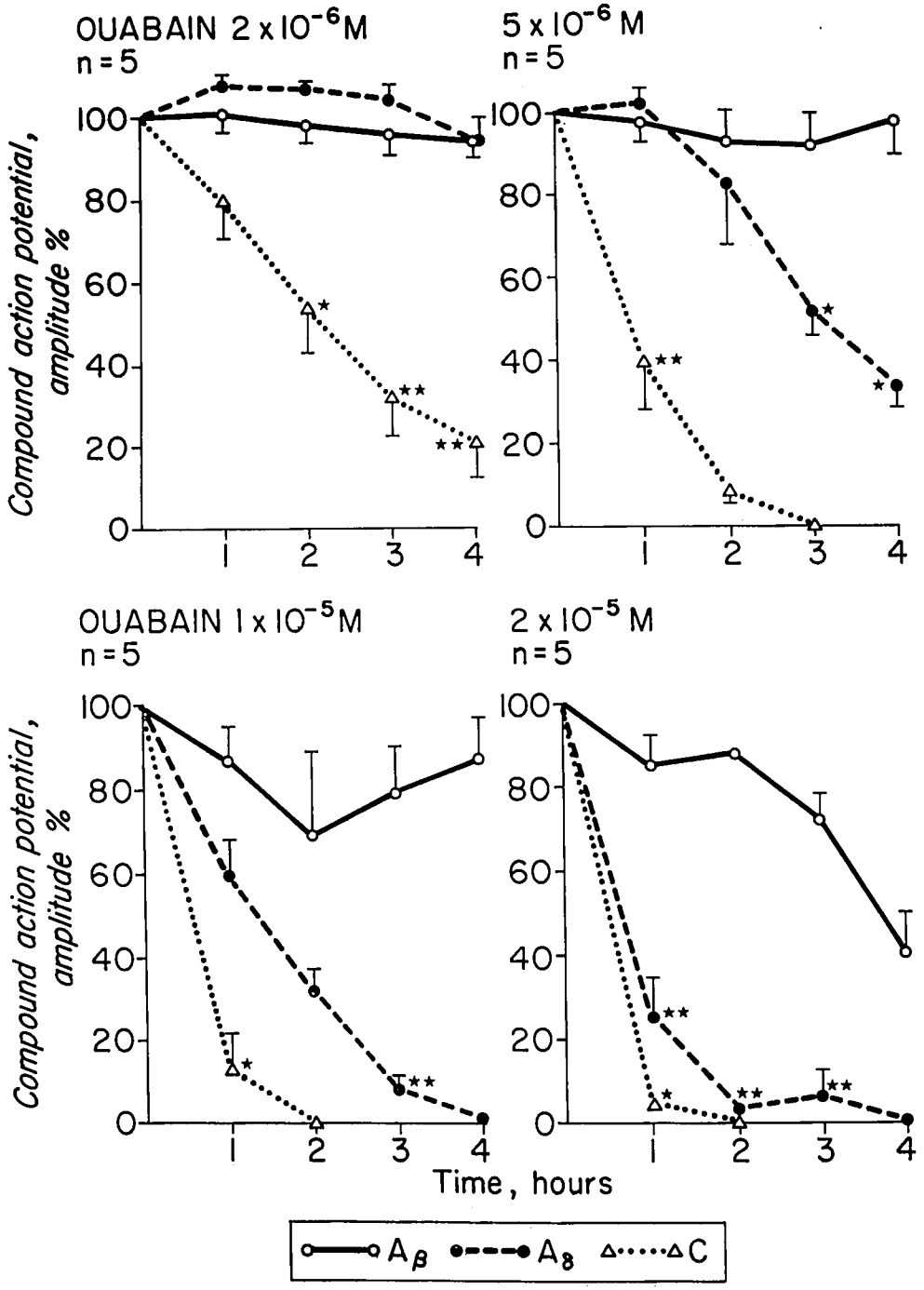

FIG. 1. Amplitude (mean and SEM) of Aβ, Aδ, and C components of the compound action potentials of desheathed vagus nerves exposed to ouabain. Aβ = ○——○, Aδ = ●- - -●, C = △···△. Only one concentration of ouabain was tested per nerve. Asterisks denote result of *t* test for two nerves in comparison with corresponding nearest less-affected component at the same concentration; $*P < 0.01$. $**P < 0.001$.

trol observations were made on the contralateral nerve, incubated continuously in ouabain-free solution.[6]

Results

In ouabain-free medium, all components remained at control amplitude, showing little or no deterioration in 6 hours. A relatively low concentration of ouabain, 2×10^{-6} M, progressively depressed the C component to 20% of the control amplitude in the space of 4 hours, while the Aβ and Aδ components apparently remained unaffected (fig. 1). A somewhat higher concentration, ouabain 5×10^{-6} M (figs. 1 and 2), extinguished the C component in 3 hours and depressed the Aδ component

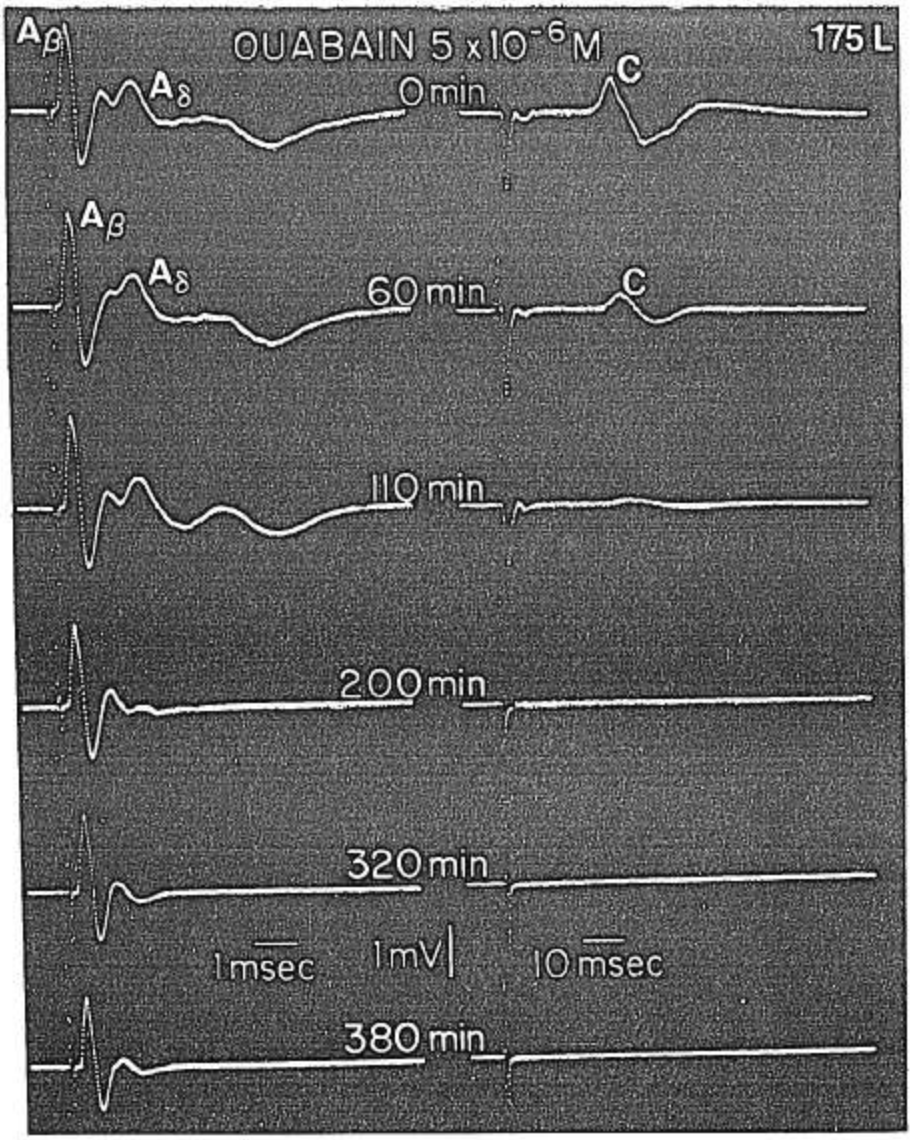

FIG. 2. Action potentials from a nerve incubated with ouabain 5×10^{-6} M. The numbers show elapsed time (minutes). Ab = Aβ component, Ad = Aδ component.

to 40% of control in 4 hours but left the Aβ component at control amplitude. Ouabain 1×10^{-5} M extinguished the C component in 2 hours and the Aδ component in 4 hours but had a biphasic effect on the Aβ component. In two of the five nerves tested with this concentration, the amplitude of the Aβ peak decreased to 20% of control in 2 hours and then gradually recovered to 80%. A relatively high concentration, ouabain 2×10^{-5} M, effected 95% extinction of the C component in 1 hour and the Aδ component in 2 hours, with only 10% extinction of the Aβ component, which, at the end of 4 hours, still was at 40% of control amplitude (fig. 1).

One-way analysis of variance of the amplitudes of the three component potentials after 1 hour incubation in ouabain solutions revealed that a probably significant difference already had developed even in the weakest concentration: $F = 5.25$, $P < 0.05$ with 2×10^{-6} M ouabain, $F \geqslant 11.5$, $P < 0.01$ with 5×10^{-6}, 1×10^{-5} and 2×10^{-5} M ouabain. Scheffé's F test for multiple comparisons was applied to the measurements made at 4 hours of exposure to ouabain solutions. The test indicated that, except for the difference between Aβ and Aδ with 2×10^{-6} M ouabain, all differences between nonzero means at each of the four concentrations were significant at the 0.01 level of confidence.

With high concentrations of ouabain ($2 \times 10^{-3} - 10^{-4}$ M) conduction in all fiber groups was blocked within 60 minutes. The progressive decrease in ampli-

TABLE 1. Effects of Washing of One to Four Hours on Amplitude of Aβ, Aδ, and C Potentials Following Exposure to Ouabain (Initial = 100)

Exposure	Ouabain 1–2 Hours			Ouabain 4 Hours			No Ouabain 2 Hours*		
	Aβ	Aδ	C	Aβ	Aδ	C	Aβ	Aδ	C
Ouabain 1×10^{-5} M†									
End of exposure	72–98	47–82	2–4	47–87	0	0	90–100	87–99	93–100‡
End of 2–4 hour wash	80–102	40–91	9–21	36–81	5–7	0–5	85–86	79–93	85–95
Ouabain 2×10^{-5} M§									
End of exposure	90–93	0–20	0–4	40–54	0	0	85–100	88–100	82–87‡
End of 2–4 hour wash	51–55	64–75	10–13	18–32	0–76	0	100–104	95–102	82–82

* Four control nerves.
† Two nerves.
‡ Two control nerves.
§ Three nerves.

tude of a given peak with ouabain was accompanied by little obvious change in latency (fig. 2). Conduction extinguished by high concentrations of ouabain showed little or no recovery during washing for as long as 20 hours. Recovery from lower concentrations (2×10^{-5} and 1×10^{-5} M) depended on the duration of exposure and washing. Washing that started after 2 hours of exposure to 2×10^{-5} M ouabain produced partial recovery in all groups but when started after exposure for 4 hours, recovery of the C component did not occur (table 1). After similar exposures to the 1×10^{-5} M concentration, the C group did show a little recovery, and the nerves in the other groups recovered somewhat more than the nerves that had been exposed to the 2×10^{-5} M concentration.

Retention of the perineurial sheath slowed the rate of extinction but did not alter the differential susceptibility to ouabain.

Discussion

Extinction in succession of the C, Aδ, and Aβ components of the compound action potential by ouabain is consistent with our hypothesis that inhibition of the membrane pump (Na^+,K^+-ATPase) will deplete transmembrane gradients sequentially according to the size of the axons.

Ouabain is known to be a potent inhibitor of brain Na^+,K^+-ATPase, but whether the enzyme is the pharmacologic receptor for the drug is not yet clear.[7] Many other Na^+,K^+-ATPase inhibitors are available but have been studied less extensively. As regards effects of ouabain on peripheral nerve, little previous evidence is available for comparison. Landowne and Ritchie[8] measured the binding of ^{3}H-ouabain 2×10^{-5} M in rabbit de-sheathed cervical vagus nerve at 20°C. They estimated the degree of pump block by measuring the amount of posttetanic hyperpolarization and concluded that ouabain 2×10^{-5} M produced nearly complete block of the pump in unmyelinated axons in 80 minutes. No mention was made of myelinated axons. In our experiments, at 37°C, this concentration appeared to block C-fiber conduction in about 60 minutes (fig. 1), and washing was ineffective in reversing the block (table 1).

Although there was relatively little apparent change in the latency of the compound potentials (fig. 2), evidence from single axons will be needed to determine whether the apparent differential extinction is real or merely reflects differential slowing of conduction.

Attempts to produce differential block of large and small axons by means of local anesthetics have had variable results. On the one hand, Nathan and Sears[2] claimed to have found a critical concentration of procaine that blocked unmyelinated axons and small myelinated fibers without blocking large myelinated fibers. This was called an absolute differential block, although equilibrium conditions probably were not established.

Franz and Perry,[3] on the other hand, were unable to find a procaine concentration that blocked only the small myelinated axons but did produce such a block by manipulating the length of nerve exposed to the drug. Neither of these groups of investigators nor Gissen *et al.*[4] achieved an absolute differential block of C axons. However, no contradiction need be seen between the present results and any of those cited above, because the mechanisms studied are different: block by inhibition of the sodium pump in the present case, block by inhibition of the sodium conductance in the others. It is well established that the action of the sodium pump is independent entirely from the Na and K excitability channels.[9] The present results suggest that, as a potential approach to management of pain by selective block of C and Aδ axons, inhibition of the axonal sodium

pump with ouabain warrants evaluation now for antinociceptive effectiveness in animals, as well as for cardiotoxicity and neurotoxicity.§

References

1. Gasser HS, Erlanger J: The role of fiber size in the establishment of a nerve block by pressure or cocaine. Am J Physiol 88:581–591, 1929
2. Nathan PW, Sears TA: Some factors concerned in differential nerve block by local anesthetics. J Physiol (Lond) 157:565–580, 1961
3. Franz DN, Perry RS: Mechanisms for differential block among single myelinated and unmyelinated axons by procaine. J Physiol (Lond) 236:193–210, 1974
4. Gissen AJ, Covino BG, Gregus J: Differential sensitivities of mammalian nerve fibers to local anesthetic agents. ANESTHESIOLOGY 53:467–474, 1980
5. Fink BR, Cairns AM: A bioenergetic basis for peripheral nerve fiber dissociation. Pain 12:307–317, 1982
6. Ferguson GA: Statistical analysis in psychology and education, 3rd edition. New York, McGraw-Hill, 1971, pp 270–271
7. Schwartz A, Collins JH: Na^+/K^+-ATPase. Structure of the enzyme and mechanism of action of digitalis. Membranes and Transport, vol 1. Edited by Martonosi AN. New York, Plenum Press, 1982, pp 521–527
8. Landowne D, Ritchie JM: Optical studies on the kinetics of the sodium pump in non-myelinated nerve fibers. J Physiol (Lond) 212:483–502, 1971
9. Keynes RD: Organization of ionic channels in nerve membranes. The Nervous System, vol. 1. The Basic Neurosciences. Edited by Tower DB, Bardy RO. New York, Raven Press, 1976, pp 165–175

§ For a preliminary note, see ANESTHESIOLOGY 57:A183, 1982.