

The Effect of Inhalation Anesthetics on Repetitive Activity Generated at Motor Nerve Endings

Alan Van Poznak, M.D.*

ALTHOUGH cyclopropane is used in anesthesia to depress the central nervous system, a demonstration of its depressant action in clinically used concentrations on simple neuronal structures has not heretofore been made. In order to block conduction in peripheral axons, Carpenter¹ found it necessary to employ pressures of cyclopropane as high as 1.8 atmospheres. However, the work of Larrabee and Posternak² and others has shown that junctional structures are more sensitive than axons to the actions of anesthetics. In the present study, the motor nerve terminal preparation of Riker and his associates was chosen as the means for investigating the neural depressant action of cyclopropane.

Methods

The methods have previously been described by Riker *et al.*³ Adult cats were used. They were anesthetized with chloralose 80 mg. per kilogram. Five were subjected to transcollicular decerebration and allowed to recover from the anesthetic before the preparation was begun. The soleus muscle and its nerve were dissected in the popliteal fossa and a stimulating electrode placed through which supra-maximal rectangular pulses were administered. To monitor activity in the soleus motor axons a dorsal laminectomy was performed to expose the lumbar spinal cord. The ventral root of nerve L₇ was sectioned close to the cord and subdivided until a filament containing a single active soleus axon was obtained. This was placed on a platinum recording electrode. All exposed tissues were covered with pools of mineral oil, thermoregulated to 37° C. and bubbled with 95 per cent oxygen and 5 per cent carbon dioxide. Supramaximal rectangular

stimuli were applied at a rate of 0.4 cycles per second. At intervals of 5 minutes a 400 cycle per second stimulus was applied to the nerve for 10 seconds. The neural stimuli were 0.1 millisecond in duration (Fig. 1).

Soleus motor axons normally respond to a single stimulus with a single action potential, but following a period of high frequency stimulation the response to a single stimulus is repetitive. These post tetanic repetitive (PTR) potentials which are generated in the motor nerve terminal are conducted antidromically along the soleus axon and may be recorded from its ventral root.

The potentials were amplified and observed or photographed from an oscilloscope screen. Both the number of potentials per oscilloscope sweep and also the total time during which repetition was obtainable were recorded.

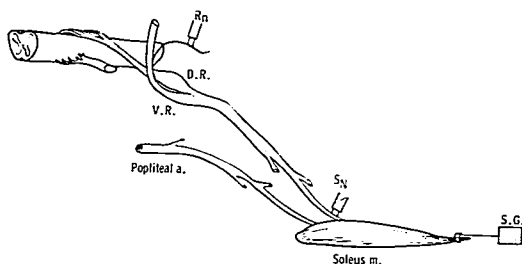
The animals were ventilated through a tracheostomy cannula with 5 liters per minute of gas supplied by a Takaoka positive-negative pressure respirator. The system was open (nonbreathing). In the control period the gas supplied was 97 per cent oxygen and 3 per cent carbon dioxide. For the experiments, part of the oxygen was displaced to achieve cyclopropane concentrations of 5, 10, 20, and 40 per cent in the inspired gas. Cyclopropane concentration was increased sequentially, the animal being exposed to each concentration for about 15 minutes. The carbon dioxide concentration was maintained at 3 per cent throughout the experiment. The gas mixtures were prepared with standard anesthesia rotameters whose accuracy was checked periodically by chromatographic analysis of the gases.

Results

There are no significant differences between the effects of cyclopropane on chloralose anesthetized and decerebrate preparations. Cyclo-

* Associate Professor of Anesthesiology in Surgery, Cornell University Medical College, New York City 10021.

FIG. 1. The L₂ ventral root has been detached from the spinal cord and progressively subdivided until it contains a single electrically active axon to the soleus muscle. Stimuli are introduced through electrode S_x on the soleus nerve in the popliteal fossa, and pass both orthodromically to the muscle and also antidromically to the recording electrode R_n on the ventral root filament. Mechanical activity of the muscle may be monitored by strain gage SG.



propane depresses the post tetanic repetitive activity in the soleus nerve. Both the number and frequency of the repetitive potentials in the train and also the time during which post tetanic repetition may be evoked is reduced. For convenience, the latter parameter was chosen as the measure of cyclopropane effects. Although cyclopropane affects all soleus motor nerve terminals, there is a considerable variation in the individual responses to the drug. The threshold appears to be at about 2½ per cent. All post tetanic repetitive activities is abolished at concentrations between 20 and 40 per cent. The initial cyclopropane effect on post tetanic repetition occurs within 5 minutes, but about 10 minutes are required to obtain maximum effect. The depression of post tetanic repetition is fully reversible, with recovery following an approximately exponential time course. The concentrations of cyclopropane used in this study have no discernible effects on the form or conduction velocity of the action potential conducted anti-dromically in the soleus nerve.

Discussion

For a discussion of the biological significance of PTR, the interested reader is referred to Standaert. It is sufficient to state at this point that these experiments demonstrate that clinically used concentrations of cyclopropane affect motor nerve terminals. This effect is most readily recognized in the depression of post tetanic repetition. Since post tetanic repetition is a manifestation of nerve terminal activity, its depression is good evidence for drug

action on the nerve terminal. The nerve terminal is far more susceptible to cyclopropane action than is the parent axon. We have noted that the threshold for PTR depression is about 2½ per cent cyclopropane. In contrast, 40 per cent cyclopropane has no discernible effect on the axonal potential and, as has previously been mentioned, Carpenter's experiments indicate that about 1.8 atmospheres of cyclopropane are required to block conduction in rats sciatic nerve. Many authors have been impressed by the potency of anesthetic drugs on peripheral presynaptic structures and have suggested that peripheral drug actions are important to central nerve systems actions of these drugs. There have been several recent attempts to examine anesthetic drug action on presynaptic terminals in spinal monosynaptic reflex arcs.^{4, 5}

These experiments have documented the sensitivity of the motor nerve terminal to cyclopropane action. It seems reasonable that neurons within the central nervous system may be similarly affected at their fine processes by the action of cyclopropane, and if so, the phenomena herein described are directly relevant to the mechanism of cyclopropane action in the central nervous system. Other substances used for their ability to depress the central nervous system have also shown depressant effects in the exquisitely sensitive motor nerve terminal model system. These include diethyl ether whether given by inhalation or by close intra-arterial injection,⁶ barbiturates,³ and diphenylhydantoin.⁷ The study of the central nervous system action of cyclopropane and

other depressant substances may be facilitated by studying the drug action on the more accessible peripheral nerve terminals.

References

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DISCUSSION

Dr. Ngai: The neuromuscular junction is not the only junction that can be depressed by the drugs we use. I subscribe to the idea that when you administer cyclopropane to obtain relaxation, something higher up becomes depressed. Obviously, the central nervous system is also sensitive to cyclopropane.

Dr. Salmoiraghi: I am puzzled by the difference you find with regard to gastrocnemius and the soleus. If I understand you correctly, you see action potentials in one case but not in the other. Is this so?

Dr. Van Poznak: That is right. This is a physiological difference between the two types of muscles. In soleus, PTP is a sequence of neural repetition, but in gastrocnemius, PTP is almost entirely a muscle phenomenon.

Dr. Salmoiraghi: Could you tell us what are the mechanisms of action of cyclopropane, for these two muscles?

Dr. Van Poznak: This is something for a physiologist to explain. Why is it that post tetanic potentiation in gastrocnemius is not accompanied by repetition in the nerve, but in soleus post tetanic potentiation it is? This is a basic difference in the physiology of the two muscles.

Dr. Salmoiraghi: What are your thoughts on the possible mechanism.

Dr. Van Poznak: Cyclopropane depresses neural activity in both preparations, but abolishes post tetanic potentiation only in the soleus.

Dr. Salmoiraghi: Would you explain what you mean by neural activity?

Dr. Van Poznak: It depresses the ability to evoke post tetanic repetition.

Dr. Salmoiraghi: What is the mechanism that causes the repetitive discharge in the nerve?

Dr. Van Poznak: I do not know, except that it exists; Standaert has postulated that this is related to the setting up of a generator potential at the

motor nerve terminal. This generator potential is related to a prolonged negative after-potential which arises at the point where the axon suddenly narrows and loses its myelin sheath, so that the post tetanic potential is different. This sets up a condition favorable to the establishment of repetitive discharge.

Dr. Salmoiraghi: Right. Could you now discuss what causes build up of this negative potential?

Dr. Van Poznak: This involves basic physiology, and I should like to refer this to experts in the field.

Sir John Eccles: The explanation of this repetitive firing, as I remember it, is that acetylcholine is probably accumulating and acting on receptor sites in the nerve terminal; furthermore I think that, if you give an anticholinesterase in quite low doses, the gastrocnemius will show this effect also. I suspect, too, that tetanic stimulation will give rise to considerable local concentrations of acetylcholine. Acetylcholine, in fact, if injected intrarterially does produce the same firing. This might help you in your story. Tubocurarine in quite low doses does block this repetitive firing, which you would expect if it were generated by acetylcholine.

Dr. Van Poznak: This is one of the possibilities considered by Dr. Standaert, that it is due to liberated acetylcholine returning to excite the nerve terminal. However, neither he nor Dr. Riker (Riker, W. F., and Standaert, F. G.: *Ann. N. Y. Acad. Sci.* 135: 164, 1966, and Standaert, F. G., and Riker, W. F.: *Ann. N. Y. Acad. Sci.*, in press) believes that reflux of acetylcholine has a role in the generation of this phenomenon. I hope I have not been negligent in not discussing the drug story. I omitted it because I am incompetent to discuss it. Acetylcholine will give repetitive firing, and the anticholinesterases will do this; one can obtain it with physostigmine and neostigmine, and there are many drugs which can evoke the repetitive

firing. We have confined ourselves for the purposes of this discussion, only to the electrical situation.

Dr. Krnjević: There is much evidence that what is happening is an accumulation of acetylcholine, and what is very important is the action of cholinesterase at the nerve endings. There is evidence that certain anesthetics potentiate the action of cholinesterase. That would explain what you are observing, without necessarily postulating any change in excitability to acetylcholine of the nerve ending. If cholinesterase is more active than normal, acetylcholine would not have a chance to act on the nerve ending.

Dr. Van Poznak: Do all anesthetics share this ability?

Dr. Krnjević: This has been shown for ether, I am not sure about others (Bacq and Brown: *J. Physiol.* 89: 45, 1937).

Dr. Van Poznak: This is an interesting point. I have tried to stay clear of acetylcholine and acetylcholinesterase, and to stay only with the one drug that I am familiar with.

Dr. Karis: Dr. R. Kitz at Columbia Presbyterian could not measure any appreciable change in activity of acetylcholinesterase produced by diethyl ether.

Dr. Krnjević: Was this *in situ* or *in vitro*?

Dr. Karis: This was a test tube experiment.

Dr. Krnjević: That may not be quite the same thing.

Dr. Winters: Alpha chloralose has actions on the central nervous system which are probably excitatory (Winters, W. D., and Spooner, C. E.: A neurophysiological comparison of alpha-chloralose with gamma-hydroxybutyrate in cats, *Electroenceph. Clin. Neurophysiol.* 20: 83, 1966). Have you done experiments using cyclopropane as the anesthetic to perform the surgical preparation, then continued using cyclopropane to see the effects on this preparation, so that you do not have the introduction of another variable.

Dr. Van Poznak: If you use cyclopropane for the preparation, you have to allow for elimination

of cyclopropane. If you do this, it may take 45 minutes to 90 minutes before the effect is gone, but then you will again obtain repetitive activity, so that the chloralose and the decerebration as additional variables, do not bother me. Even if chloralose introduces an excitatory influence, the fact remains that this is a neural phenomenon that we are examining, a phenomenon that can be depressed by cyclopropane. Whether one has done it with chloralose or not, does not seem to make much difference. The fact that you have a reproducible system which you can modify is enough to permit one to state that even in this artificial situation, cyclopropane can depress the motor nerve terminal.

Questioner: Is the repetitive firing in the nerve accompanied by repetitive firing in the muscle?

Dr. Van Poznak: It is. As a matter of fact, that is how the phenomenon was first discovered; repetitive firing was noticed in the muscles, then tracked back, bit by bit into the nerve and into the ventral roots. When you time this in the preparation with electrodes at the ventral root, and in the muscle, and make appropriate correction for the time for antidromic conduction, the source of the firing is shown to be at the motor nerve terminal.

Dr. Fink: Dr. Akamatsu and I, some time ago, were studying nitrous oxide as an analgesic in man and thought there might be a peripheral as well as a central element. We administered an analgesic concentration of nitrous oxide, to volunteers who were not aware of what we were trying to do. They were stimulated in both legs by electrical stimuli, that seemed of equal intensity. On one leg there was a pneumatic cuff, to occlude the blood flow, and prevent entrance of anesthetic to that leg. The subject had to compare the stimuli and report which disappeared sooner. A majority of the subjects reported disappearance of feeling in the leg which did not have the cuff, but remained aware of a weak electrical stimulus in the leg to which access of nitrous oxide had been prevented.