X-100 was eluted at the exclusion volume with the detergent micelles, while less than 0.1 percent was eluted at the exclusion volume when detergent was omitted from the DMB solution.

Our results indicate that the detergent concentrations to be used in assays which test for the inhibition of RIDP by rifamycin derivatives should lie between the concentration required for full RIDP activation and the one which gives micelle formation. The range of Triton X-100 concentrations which meets these requirements is very narrow, from 0.004 to 0.006 percent. These same limitations on appropriate detergent concentrations would apply to Nonidet P-40 (Shell Chemicals), a commonly used detergent similar to Triton X-100. However, Triton DN-65 is not subject to these restrictive limitations. Even though Triton DN-65 has approximately the same efficiency in solubilizing the RIDP in the extraction procedure as does Triton X-100 and activates as well as Triton X-100, it has a concentration range between full activation of the RIDP and micelle formation which is much wider, 0.004 to 0.023 percent. In addition to indicating the unusual appropriateness of Triton DN-65 for RIDP studies involving rifamycin derivatives, the wide range of Triton DN-65 concentrations between full RIDP activation and micelle formation has further implications in terms of the activation phenomenon. The mechanism of action must be a molecular one because the activation by the three different detergents occurs at comparable molar concentrations that are approximately an order of magnitude below those of micelle formation by Triton DN-65.

In summary, we have found that the nonionic detergent concentration is a significant variable in RIDP assays. The detergents not only are activators of the RIDP activity but also form micelles that interfere with RIDP inhibition by rifamycin derivatives.

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Electrical Responses of Insect Central Neurons: Augmentation by Nerve Section or Colchicine

Abstract. *Intracellular recording from the somata of central motor neurons in the cockroach* Periplaneta americana *normally shows little or no electrical response evoked by soma depolarization or by antidromic stimulation. Within 4 days after either cutting the axon or administration of colchicine, large action potentials can regularly be recorded from cell bodies of metathoracic motor neurons. Each experimental procedure evokes formation of a dense, perinuclear ribonucleic acid ring in the soma of neurons showing augmented electrical responses.*

Cellular communication may occur either by way of specific chemical substances or by electrical events. Because of the specialized electrical aspect of communication in the nervous system and the high level of metabolic activity, neurons provide an exceptional opportunity to examine the relation between biochemical and bioelectrical activity. The reaction of a nerve cell body to injury of its axon (axon reaction) is known to involve major changes in ribonucleic acid (RNA) and protein metabolism. In neurons of vertebrates, these metabolic changes are correlated with alterations in the organization of various cellular components, such as the disappearance of large basophilic Nissl aggregates of rough endoplasmic reticulum, enlargement and vesiculation of the Golgi apparatus, and an increase in nucleolar size (*1*). The above cytological changes associated with the axon reaction are correlated with an increased electrical excitability in cat motor neurons (*2*).

Central neurons of some insects also show marked morphological changes in response to axon injury. The most obvious component is a dense perinuclear ring of RNA that reaches its maximum

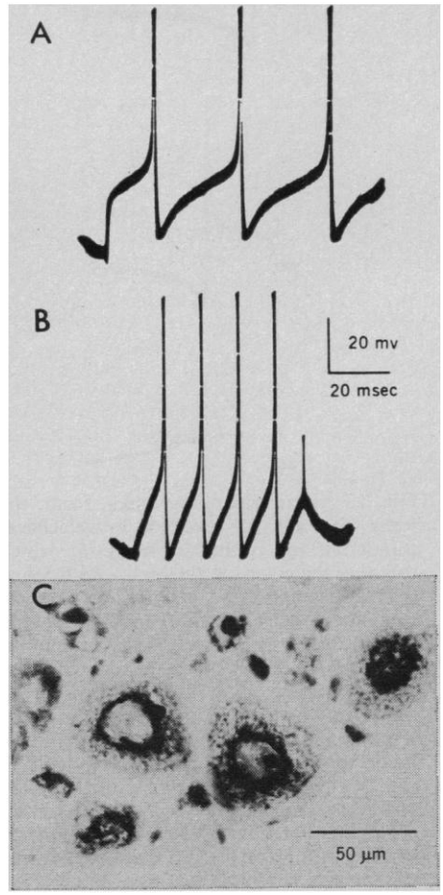

Fig. 1. Intracellular recording of responses in the soma of cell 28 evoked by depolarization of the soma membrane. (A) Six days after cutting the axon of cell 28 in nerve 5. The depolarizing pulse applied across the soma membrane is maintained for the duration of the record. (B) Four days after treatment with 5 percent colchicine. The depolarizing pulse is maintained during the burst of activity and its termination is indicated by the small vertical deflection at the extreme right. Note the large overshooting action potentials present in both records. Normally little or no response to intracellular depolarization can be recorded from the soma of this cell. (C) Section stained with Pyronine-malachite green showing motor neuron cell bodies in the metathoracic ganglion 4 days after treatment with 5 percent colchicine. The dense perinuclear basophilic ring in the two large central cells is similar to the RNA ring seen after nerve section (*3*).

by about 4 days after injury and is dispersed at approximately 10 days (*3*). There is a general increase in rough endoplasmic reticulum (Nissl substance) at the time the RNA ring is present (*4*). The response can be interpreted as an initial increase in Nissl substance followed by subsequent dispersal. Therefore, changes in quantity and distribution of rough endoplasmic reticulum are a common factor of the axon reaction in both vertebrate and invertebrate neurons, even though the sequence of events may vary from one type of neuron to the next (*1*).

It is generally difficult to detect signs of electrical activity from the somata of insect central neurons by means of intracellular recording (*5*). Only a specific group of dorsal neurons in the ventral cord ganglia of the cockroach (*6*) and some motor neurons in *Drosophila* (*7*) have consistently shown overshooting action potentials that can be recorded from the soma. We, and others (*8*), have occasionally seen small spikes (2 to 10 mv) in some large ventral motor nerve cell bodies in the cockroach; this has also been reported for the locust (*5*). We have examined the electrical responses recorded from the somata of axotomized central neurons in the cockroach and find an increase in the electrical responsiveness similar to that seen in injured vertebrate neurons. Overshooting action potentials can be readily evoked and recorded from the somata of a number of axotomized motor neurons in this insect.

Vertebrate central neurons treated with colchicine show many of the cytological changes found in neurons that have had their axons cut (*9*). We administered colchicine to the cockroach and found that it evokes the major cytological changes seen in axotomized insect central neurons. In addition, these colchicine-treated neurons show greatly increased electrical responses when intracellular recordings are made from the cell body.

Neurons in the metathoracic ganglion of the adult, male cockroach (*Periplaneta americana*) were used. We examined primarily the fast coxal depressor motor neuron numbered 28 by Cohen and Jacklet (*10*). This appears to be identical with the motor neuron D_f of Pearson and Iles (*11*). This cell activates the coxal depressor muscles 177d, 177e, 178, and 179 (*11, 12*). For studies on the motor neurons alone the metathoracic ganglion was removed from the body, desheathed, and submerged in circulating oxygenated saline (*6*). For recording muscle activity evoked by depolarizing the motor neuron cell body, we used an isolated metathoracic segment. This preparation was obtained by transverse cuts through the entire body anterior and posterior to the segment, leaving the metathoracic legs attached. The gut was immediately removed, the ganglion exposed, and the entire preparation submerged in saline. In order to record from coxal muscle fibers, some of the exoskeleton covering the muscles was removed. Colchicine in concentrations ranging from 1 to 10 percent was made up in gelatin. A colchicine-gelatin pellet of approximately 0.2 mm in diameter was implanted into the leg through an incision made in the exoskeleton of the coxa overlying nerve 5. The colchicine pellet was left in the animal for the duration of the experiment.

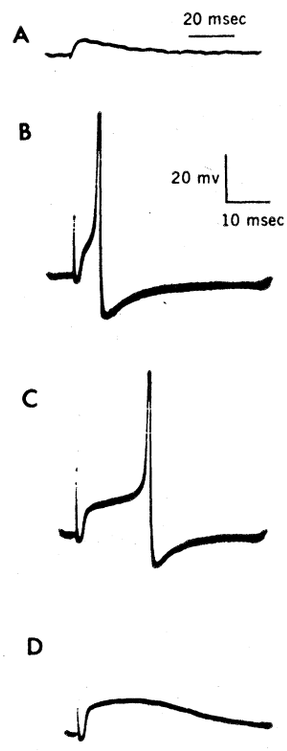

Fig. 2. Intracellular responses from the soma of cell 28 evoked by electrical stimulation of ipsilateral nerve 5, which contains the axon of this cell. (A) Slow-graded response in normal preparation stimulated at 1 hertz. This is probably synaptic activity evoked by afferents in nerve 5. No large spikes were seen even with maximum stimulus strengths. (B to D) Overshooting antidromically evoked responses seen 4 days after treatment with 5 percent colchicine. Stimulation at 1, 5, and 10 hertz, respectively. Initial deflection is a stimulus artifact. Note separation of action potential into two components and the failure of the late, fast component at the higher stimulation rate in (D).

Normally it is difficult to record an electrical response from the nerve cell body when current is passed through a microelectrode impaling the soma of cell 28. Extraordinarily large transmembrane currents (10^{-6} amp) are required to elicit responses in the innervated muscles. The axon of cell 28 leaves the ganglion in nerve trunk 5 (*10*). Approximately 4 to 10 days after severing nerve 5 in the proximal region of the coxa, a relatively small depolarizing current applied across the soma membrane evokes a train of overshooting action potentials that may reach an amplitude of 70 to 90 mv. The resting potential is between 60 and 70 mv with acetate-filled electrodes. Figure 1A shows such a train of spikes evoked 6 days after section of nerve 5. This increased electrical response of the soma disappears by approximately 2 weeks after nerve section.

Colchicine-treated animals show a similar increase in the electrical responsiveness to intracellular depolarization (Fig. 1B). This enhanced electrical activity appears approximately 4 days after the application of colchicine. The colchicine-treated animals showing heightened electrical responses were observed prior to dissection and their locomotion appeared to be normal. In axotomized or colchicine-treated animals, as in normal preparations, electrical stimulation of contralateral nerve 5 or the posterior connectives evokes depolarizing synaptic potentials in the soma of cell 28.

A dense, perinuclear accumulation of cytoplasmic RNA is seen in the somata of neurons in the metathoracic ganglion by 4 days after the application of colchicine (Fig. 1C). Such RNA rings can frequently be seen both ipsilateral and contralateral to the leg treated with colchicine. This suggests that the drug enters the circulatory system and can act at regions somewhat distant from the site of administration.

A slow, small (up to 15 mv) depolarization was recorded in the soma of motor neuron 28 when ipsilateral nerve 5 was electrically stimulated through a suction electrode in a normal preparation (Fig. 2A). We believe that this is a synaptic potential because of its relatively long duration, its graded nature, and the resemblance to synaptic input evoked through other pathways. Although so sign of an antidromically evoked action potential is usually recorded from the normal soma of cell 28, occasionally, spikes of 2 to 5 mv are seen in the cell body during

stimulation of nerve 5. These probably reflect the electrotonic spread of an action potential that is blocked at some distance from the soma. However, in animals treated with colchicine, antidromic stimulation of ipsilateral nerve 5 evokes overshooting action potentials that can be recorded in the soma of cell 28. Figure 2, B to D, shows the effect of increasing frequencies of nerve 5 stimulation on the response recorded from the soma of cell 28. In Fig. 2B, a single electrical stimulus was applied to nerve 5. The evoked overshooting action potential has a noticeable inflection in the rising phase. With increasing frequency of stimulation, this inflection becomes progressively lengthened until the rapidly rising late component disappears (Fig. 2D).

In order to examine spike propagation and neuromuscular transmission in animals treated with colchicine, simultaneous intracellular recordings were made from the soma of cell 28 and from a fiber in muscle 177d, 177e, 178, or 179 (*11*). The top trace in Fig. 3 shows an action potential recorded in the soma of cell 28 in response to soma depolarization. The bottom trace is the intracellular record from a fiber in muscle 177d showing the fast muscle potential produced by the spike evoked in cell 28. This shows that orthodromic propagation of the action potential and neuromuscular transmission remain intact after treatment with colchicine.

Our results indicate that there is a close relation between the metabolic state of a nerve cell and its electrical properties. We have demonstrated that treating insect neurons with colchicine induces the same type of perinuclear RNA rings that are produced by cutting the axon of these nerve cells. We have also found that the perinuclear RNA accumulation induced by each procedure is temporally correlated with an increased electrical response of these neurons. It is likely that the same factor is responsible for initiating the changes seen after both colchicine treatment and axotomy because the cytological and electrical responses evoked by each procedure are similar. It is well known that colchicine causes a decrease in the microtubules of nerve cells and that a colchicine-binding protein exists in microtubules (*13*). Schmitt (*14*) suggests that neuronal microtubules might be concerned with the fast component of axoplasmic flow, and indeed this factor is decreased in some neurons by colchicine (*15*). It

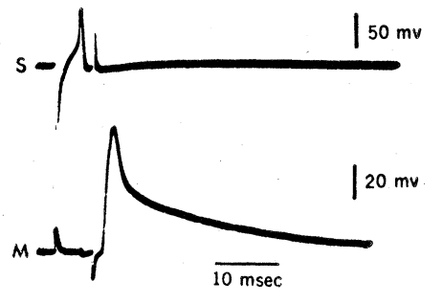

Fig. 3. Neuromuscular transmission 4 days after treatment with 5 percent colchicine; *S*, action potential recorded in the soma of cell 28 in response to soma depolarization; *M*, intracellular record from a fiber in muscle 177d showing the "fast" muscle potential evoked by the action potential generated in cell 28.

may be that both nerve section and colchicine evoke their common cytological and electrical changes in the soma by way of some link with the intracellular transport system. However, the effect of colchicine on microtubules and intracellular transport appears to vary from one type of neuron to the next [see (*1*) for review]. Colchicine can interfere with axoplasmic flow without affecting the structure of the mircrotubules (*15*). The evidence that colchicine could be binding directly to membrane fractions of the cell (*16*) broadens still further the possible routes through which colchicine and axotomy may bring about their common effects.

After repetitive antidromic stimulation in animals treated with colchicine, the action potential recorded in the soma is seen to consist of two components. The initial, slowly rising phase probably represents electrotonic spread of the action potential from the initial process into the cell body. The late, rapidly rising phase of the action potential is thought to represent an active invasion of the soma. A similar division of the action potential into two components has been described in the neurons of *Aplysia* (*17*). If this interpretation is valid and the action potential does indeed invade the soma, then colchicine causes a portion of the neuronal membrane that was electrically inexcitable to become electrically excitable. This appears similar to the changes seen in axotomized cat motor neurons where chromatolysis seems to induce areas of dendritic membrane that were electrically inexcitable to become excitable and generate partial spikelike responses (*2*). The increased electrical response of the insect neuron soma and the correlated cytological changes induced by colchicine should permit examination of the link between cell metabolism and the electrical properties of membranes.

The individual motor neurons and muscles involved in such discrete bits of insect behavior as walking, flying, and singing have been studied in detail (*18*). However, the interactions between units in any particular circuit have not been completely determined, largely because of the difficulty in stimulating and recording from the neuron soma. Therefore, the heightened electrical activity of the insect nerve cell body treated with colchicine should also aid in defining the interaction between units in the neural circuits generating these specific behavioral acts.

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