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31. For complete protocols on figures our file numbers are as follows: Fig. 1 (Parsons 5); Fig. 2 (2–4^{IV}); Fig. 3 (2–4^{IV}); Fig. 4 (4–3^{IV}); Fig. 5 (Parsons 6A and B).

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Separation of Transducer and Impulse-Generating Processes in Sensory Receptors

Abstract. *New evidence is presented that spike and transducer processes in sensory receptors are independent events; impulse activity in the crustacean stretch receptor neuron and the mammalian pacinian corpuscle was selectively blocked by a compound (tetrodotoxin) without affecting any of the parameters of the generator potential.*

The two components of electrical activity in sensory receptors, the generator and the all-or-none action potential, have a number of distinctive features (*1*, *2*), suggesting that they may arise in different membrane components. It would be difficult, however, to provide direct evidence for such a segregation with the available techniques. Even in those favorable cases, such as the crustacean stretch receptor organ and the pacinian corpuscle, in which a coarse spatial segregation of the components appear to exist (*3*, *4*) there are uncertainties. In the first case, absence of impulse activity in the dendrites, where the generator potential arises (*5*), might be the consequence of geometrical factors; and in the latter case, spatial restrictions for excitations (*2*) and other factors (*6*) may possibly prevent all-or-none activity at the nonmyelinated nerve terminal. One has to rely, therefore, on the more indirect method of blocking selectively one of the two electrical components to demonstrate the independence of their underlying mechanisms. A variety of techniques have been used previously to this effect (*7*), but all have the disadvantage that in blocking the all-or-none component they also affect to some extent the generator potential. In the present experiments we used tetrodotoxin. This toxic substance obtained from the puffer fish has already been shown to block impulse activity in nerve and muscle fibers (*8*, *9*). Here, in two receptors, the crustacean stretch receptor neuron and the pacinian corpuscle, tetrodotoxin will be seen to act selectively on the all-or-none component, blocking the action potential, but leaving the generator potential unaffected.

The isolated crustacean neuron provided an appropriate preparation since, first, its dendrites are readily accessible to water-soluble molecules at least as large as sucrose (*10*); second, the generator potential is readily measured by intracellular recordings; and third, the stretch-generator potential relationship and the "equilibrium potential" of the generator potential are known (*3*).

The soma of neurons of the slowly adapting stretch receptor was impaled with microelectrodes (3*M* KCl) mounted on a bridge circuit to stimulate the cell directly and to measure membrane resistance (*3*). Potential changes (generator and action potentials) produced by different degrees of stretch were recorded. The bathing solution (van Harreveld) was then exchanged for one containing tetrodotoxin, and the measurements were reported.

Tetrodotoxin in concentrations of 1 to 5 g in 10^6 ml abolished the action potential produced by direct or antidromic stimulation, as well as the sustained impulse activity in response to a steady stretch (Fig. 1*A*). The resting membrane potential remained unchanged and the membrane resistance decreased only slightly. The amplitude of the initial and steady phases of generator potentials, just threshold for impulse initiation, was not affected by the drug, even when the concentration

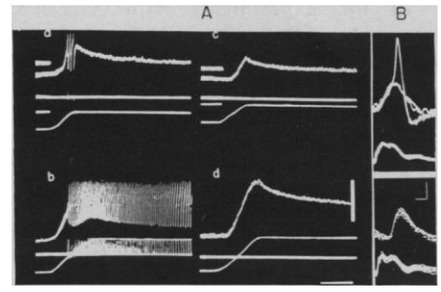

Fig. 1. (*A*) Crayfish slowly adapting stretch receptor organ. In each record from top to bottom: intracellular recording, record from the axon (2 mm or more from the soma), stretch. Response to two degrees of stretch before (*a*, *b*) and after (*c*, *d*) treatment with tetrodotoxin (5×10^{-6} wt/vol). Calibration: 2 sec; 10 mv. (*B*) Decapsulated pacinian corpuscle. Responses to a threshold stimulus before (upper record) and after (lower record) application of 5×10^{-4} wt/vol tetrodotoxin. Lower beam, photoelectric record of mechanical pulses. Calibration: 1 msec; 15 μv.

was increased by an order of magnitude above that sufficient to block impulse activity. The relationship between generator potential amplitude (steady phase) and applied stretch was similar to that of the untreated receptor (Fig. 2*A*). Moreover, in two receptors, in which delayed rectification was negligible, the equilibrium potential of the generator potential after application of tetrodotoxin was found to be similar to that previously obtained in untreated neurons (*3*).

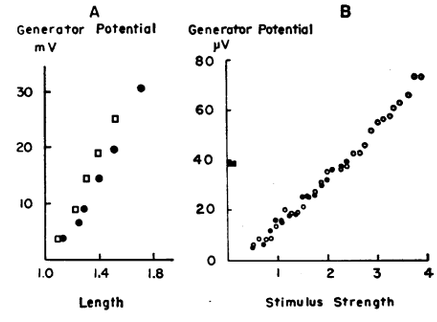

Fig. 2. (*A*) Relation between generator potential (steady phase amplitude) and relative length of the muscle bundle in two crustacean slowly adapting stretch receptors after treatment with tetrodotoxin. Maximum length at which no impulse activity was present was taken as unity. The relationship is simular to that previously described in normal receptors (compare with Fig. 1 of Ref. *3*). (*B*) Generator potential–stimulus strength relation in an intact pacinian corpuscle before (●) and after (o) application of tetrodotoxin. Bar on ordinate marks level for impulse initiation before drug application.

In the experiments with pacinian corpuscles, the toxin was applied to single intact or decapsulated corpuscles (*4*) isolated from the cat's mesentery. The preparation was set up in a drop of Krebs's solution surrounded by mineral oil. The solution-oil interface, which served as peripheral recording electrode (another electrode was placed on the central stump of axon), was adjusted to lie at the point of axon emergence from the intact corpuscle; in the decapsulated preparation, the solution-oil interface was lying at the level of the first Ranvier node. For tetrodotoxin application, the Kreb's fluid was exchanged for one containing the compound. Generator and action potentials were elicited by mechanical pulses from a piezoelectric crystal (*4*).

Tetrodotoxin in concentration of 1 g in 10^5 ml blocked the action potential in both the intact and decapsulated corpuscles, leaving no detectable all-or-none residue (Fig. 1*B*). The generator potential was not appreciably affected. The generator potential–stimulus strength relation and the rates of rise and fall of the potential were the same as in the untreated receptor. It is noteworthy that the generator potential is Na^+ dependent in both the pacinian (*11*) and stretch receptor neuron (*10*).

The above-described findings indicate that spike and generator potential in the two receptors examined are independent events being subserved by different mechanisms. If so, these are likely to reside in separate regions of membrane, although the spatial arrangement may vary in different receptors (coarsely discernible regions of the cell, separate membrane patches within a region, and submicroscopic mosaic).

Tetrodotoxin has also been shown to block the action potential in muscle fibers (*8*) and electroplates (*12*), without affecting the endplate potential or the depolarizing action of externally applied acetylcholine. These findings and the present results are taken here as lending support to Grundfest's generalization that synaptic and generator processes are analogous transducer actions (*1; 13*).

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13. The experiments reported here were designed following a suggestion by Dr. H. Grundfest to one of us (C.A.T.) to apply tetrodotoxin to the crustacean stretch receptor. Tetrodotoxin was purchased from the Sankyo Co., Tokyo, Japan.

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Mitomycin C: Chemical and Biological Studies on Alkylation

Abstract. *The presence of an aziridine ring in mitomycin C suggests that the mechanism of action of the antibiotic is like that of the antitumor alkylating agents. However the compound is unexpectedly stable during aerobic incubation with rat liver homogenates although rapidly metabolized anaerobically. Mitomycin is not reactive with γ-(4-nitrobenzyl)pyridine and reacts only slowly at acid pH with thiosulfate. It is proposed that mitomycin is activated in vivo, possibly by a reduction which "unmasks" the potential activity of the fused aziridine ring.*

Mitomycin C is an antibiotic and also a potent inhibitor of transplantable rodent tumors (*1*). The structure of the agent (*2*) suggests possible relations between its chemical configuration and biological activity (Fig. 1). The aziridine ring is an alkylating group, similar to those present in antitumor mustards (*3*), and the quinone and carbamate moieties are like those found in some mitotic inhibitors (*4*). The carbamate and the aziridine groups, and the possibility that an *o*-aminophenol derivative may be formed by reduction of the aminoquinone, are indications that mitomycin may be a carcinogen (*5*). Biotransformation of mitomycin might occur by reduction, by O-demethylation, by deamination, or by any change which increases the reactivity of the aziridine ring.

On the basis of the known properties of the antitumor alkylating agents, which include numerous ethylenimines (*3*), it is reasonable to propose that the aziridine group is primarily responsible for the biological effects of mitomycin. This is consistent with the antitumor actions, with the toxicity which is characterized by lesions in hematopoietic tissues and intestinal epithelium and by delayed deaths (*6*), and with the observation that mouse ascites sarcoma made resistant to alkylating agents is also resistant to mitomycin (*7*). It is also consistent with mutagenesis (*8*) and phage-activation (*9*) in *Escherichia coli*, and with a primary inhibition of DNA synthesis in *E. coli* (*10*), in mammalian cells in culture (*11*), and in proliferative tissues of rats (*12*).

However, the high potency and specificity of action of mitomycin in vivo are unexpected, for such properties are not usually found in monofunctional alkylating agents (*3*). [Mitomycin C is more potent in laboratory animals than most polyfunctional alkylating agents, with the possible exception of triethylene melamine (*13*).] Moreover, mitomycin C does not alter virus infectivity (*11*) or transforming activity (*14*), viscosity and melting-out characteristics (*14, 15*), or x-ray diffraction patterns (*16*) of isolated DNA, even though these may be directly affected by antitumor alkylating agents (*3*).

Another curious property of mitomycin is its relative stability in tissue breis under aerobic conditions. Our previous studies (*17*) showed that the antibiotic is rapidly metabolized anaerobically by rat liver homogenates. The anaerobic metabolism, measured by changes in ultraviolet absorption at 363 mμ, was proportional to loss of biological potency which was assayed with *Bacillus subtilis* spores. These earlier studies have now been extended to a comparison of the changes in biological activity with spectral changes under aerobic conditions (Table 1). The results agreed within the reliability of the bioassay (20 percent), although recoveries were slightly higher by the spectro-