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Inhibitory Postsynaptic Potentials in Grasshopper Muscle

Abstract. *Hyperpolarizing inhibitory postsynaptic potentials have been discovered in fibers of the "jumping" muscle of the grasshopper. These potentials attenuate the depolarizing excitatory postsynaptic responses. They are enhanced during depolarization of the muscle fiber with applied current and are diminished and then reversed during hyperpolarization. The electrogenesis appears to be caused by chloride-activation. Gamma-aminobutyric acid activates the inhibitory synaptic membrane and picrotoxin is an inactivator agent.*

The occurrence of inhibitory neuromuscular synapses in the Crustacea is well established, but evidence for peripheral inhibitory activity in the other large arthropod group, the Insecta, has been slight (*1*). However, during an examination of neuromuscular transmission in the metathoracic extensor tibiae muscle of the locust after section of its motor innervation ("denervation"), potentials were discovered (*2*) that appeared to be hyperpolarizing inhibitory postsynaptic potentials. Hyperpolarizing potentials had previously been recorded from this muscle by Hoyle (*3*), but they did not seem to attenuate the depolarizing excitatory potentials. In an attempt to resolve these conflicting observations the properties of the hyperpolarizing postsynaptic potentials of normal preparations have been reexamined.

The metathoracic extensor tibiae muscle of the lubber grasshopper, *Romalea microptera*, was used throughout this investigation. Recordings of inhibitory postsynaptic potentials were obtained from about 70 individual specimens. Most recordings were restricted to the closely packed, highly tracheolated, bundle of muscle fibers at the proximal end of the muscle. These fibers, most of which have smaller diameters than the rest of the fibers of the extensor muscle, are either triply innervated, receiving endings from a "fast" and a "slow" excitor axon as well as from the "hyperpolarizer" or inhibitor axon, or they are dually innervated, with no endings from the "fast" axon. Although the "slow" excitor and the inhibitor axons originate from the metathoracic ganglion (*3*) in the same fine nerve (No. 3b) and cannot be separated by dissection, the two axons usually have slightly different thresholds to electrical stimulation and can therefore be excited independently (Fig. 1*A*). Reflex excitation of the two axons obtained by stroking different parts of the animal with the tip of a fine brush has frequently been used as a further method of analyzing the two synaptic events, since by selective activation of different reflex pathways the two axons can be excited independently or simultaneously (Fig. 1, *B–D*).

The largest hyperpolarizing postsynaptic potentials (about 15 mv) are recorded from fibers with relatively low resting potentials (−35 to −45 mv), whereas only very small hyperpolarizing postsynaptic potentials are observed in fibers with resting potentials greater than −65 mv. The hyperpolarizing postsynaptic potentials summate (Fig. 1, *B* and *C*) and facilitate at high frequencies of stimulation, these effects being most obvious in fibers with low resting potentials. The "slow" excitatory potentials, which produce depolarizations between 1 and 30 mv, are modified by the hyperpolarizing electrogenesis. This excitatory potential is attenuated if it arises during the early phase of the hyperpolarization (Fig. 1*A*). Thus, such an attenuated potential may indeed be termed an inhibitory postsynaptic potential. However, if the excitatory potential arises during the declining phase of the inhibitory potential, the excitatory is usually slightly enhanced. Presumably, therefore, the active phase of the inhibitory electrogenesis is relatively brief compared with the duration of the inhibitory potential which it evokes, the declining phase of the inhibitory potential being passive. The discrepancies between the present results and those obtained by Hoyle (*3*) could be explained on the basis that he examined the effects of the passive and not the active phase of the inhibitory postsynaptic potential on the excitatory electrogenesis. This would account not only for the apparent lack of attenuation of the excitatory potentials by the inhibitory potential, but would also explain the slight enhancement of the "fast" excitatory response that he observed.

The inhibitory postsynaptic potential is augmented during depolarization of the muscle fiber by applied current (Fig. 2, *C* and *D*), while reversal of the response occurs during hyperpolarization of the muscle fiber (Fig. 2, *A* and *B*). The reversal or equilibrium potential for the inhibitory potential is usually between −65 and −75 mv, but in some

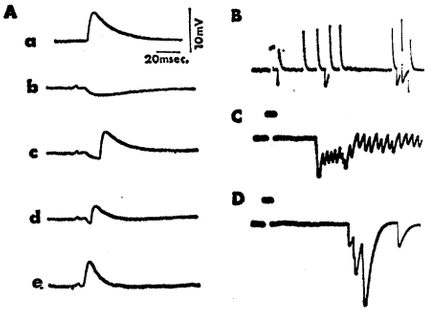

Fig. 1. Hyperpolarizing inhibitory and depolarizing excitatory postsynaptic potentials recorded from fibers of the metathoracic extensor tibiae muscle of *Romalea microptera*. *A*, Recordings from a single fiber. *a* and *b*, Control responses to reflex stimulation of the "slow" axon (*a*) and to electrical stimulation of the inhibitory axon (*b*). *c–e*, Interaction between excitatory and inhibitory postsynaptic potentials as intervals between responses are changed. The excitatory potential was not attenuated when it occurred after the peak of the inhibitory postsynaptic potential (*c*). Only the falling phase of the excitatory potential was affected when the inhibitory potential began later than did the excitatory potential (*e*). *B–D*, Postsynaptic potentials evoked by reflex stimulation recorded from three different fibers which had low resting potentials (−40, −42 and −45 mv, respectively). In *B* both excitatory and inhibitory potentials were evoked, the first response being an inhibitory potential which diminished an excitatory potential that followed it closely. Note marked summation of the inhibitory potentials in *C* and *D*. Calibrating pulses (5 mv and 100 msec) appear at the beginning of each trace.

fibers with low resting potentials values of the reversal potential close to −55 mv have been obtained. In contrast the excitatory potential is reversed by polarizing the fiber membrane inside-positive (*4*). Since the resting potentials of the fibers in the proximal bundle of the muscle rarely exceed −70 mv, it is not surprising that depolarizing postsynaptic potentials have not normally been recorded in response to stimulation of the inhibitory axon.

Topical applications of γ-aminobutyric acid in concentrations $>10^{-5}M$ activate the inhibitory synaptic membrane, the membrane resistance and time constant decreasing markedly within a few seconds after applying the drug. If the fiber which is impaled with a microelectrode has a low resting potential the drug also causes hyperpolarization. Picrotoxin (10^{-5} to $10^{-3}M$) abolishes the inhibitory potentials and also antagonizes the activation by γ-aminobutyric acid, but it has little or no effect on the excitatory potentials of the muscle fibers.

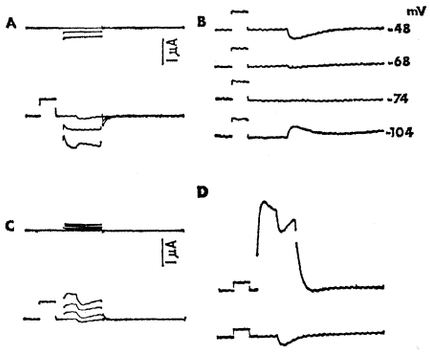

Fig. 2. Dependence of amplitude and polarity of inhibitory postsynaptic potentials on the level of membrane polarization in four different fibers of the extensor tibiae muscle. *A* and *B*, Reversals of hyperpolarizing postsynaptic potentials with increasing membrane polarization. *A*, Three superimposed sweeps with simultaneous registration of the currents applied through another intracellular microelectrode (upper trace) and the membrane potential (lower trace). A hyperpolarizing inhibitory postsynaptic potential which was evoked by electrical stimulation of the inhibitor axon seemed to disappear when the stimulus was applied during a brief hyperpolarization of the muscle fiber by about 8 mv. This inhibitory potential reappeared inverted in sign and of larger amplitude when the muscle fiber was hyperpolarized still further. *B*, The inhibitory potentials were evoked at different levels of polarization of the muscle fiber membrane. At the resting potential (−48 mv) the inhibitory potential was hyperpolarizing. As the membrane was hyperpolarized the inhibitory potential at first diminished, then seemed to disappear, and then reappeared reversed in sign. *C* and *D*, Enhancement of hyperpolarizing inhibitory potentials during depolarization of the membrane. *C*, Recording as in *A*, but of four superimposed sweeps, and with depolarizing (outward) current of different strengths delivered through an intracellular microelectrode. *D*, Two successive traces of the inhibitory postsynaptic potential at the resting potential of the fiber (lower) and during a large depolarization (upper). Calibrating pulses at the beginning of each voltage trace represent 10 mv and 50 msec (*A* and *B*) and 2 mv and 50 msec (*D*).

The sign of the inhibitory potential is inverted from hyperpolarization to depolarization by removing chloride from the medium that is bathing the muscle and substituting an impermeant anion, such as propionate. This suggests that the inhibitory potential arises from an increase in chloride-permeability during activation of the inhibitory postsynaptic membrane. Thus, with respect to both its pharmacological properties and the electrochemical nature of its electrogenesis, the inhibitory synaptic membrane of the muscle fibers resembles that of the inhibitory synapses of crayfish (*5*) and lobster (*6*). The occurrence of inhibitory postsynaptic potentials in *Romalea*, even though they may be confined to specific muscle bundles, may have implications for data on the evolutionary relations of Crustacea and Insecta (*6*).

As already noted, the inhibitory postsynaptic potentials have thus far been observed only in a particular bundle of the extensor muscle. Since the effects of inhibitory electrogenesis on the mechanical responses of the extensor tibiae muscle have not yet been examined, it would be unwise to speculate on the possible functional role of the inhibitory axon. However, if the coupling between the electrogenic and contractile activity of insect muscle is graded, the presence of an inhibitory axon capable of diminishing or regulating the mechanical response by attenuating the depolarizing excitatory postsynaptic potentials would be of value. This applies especially to an animal such as the locust or grasshopper in which the normal electrogenesis of the electrically excitable membrane of the muscle fiber is also a graded response (*4*).

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Atmospheric Aldehydes Related to Petunia Leaf Damage

Abstract. *Snowstorm petunias grown in the greenhouse developed a necrotic banding of the actively expanding foliage characteristic of injury ascribed to various photochemically produced pollutants in the atmosphere. In this case the damage appeared to be related to the high aldehyde content of the ambient air. Each time the aldehyde concentration exceeded 0.20 parts per million for 2 hours, injury appeared within a day or two. From July to September 1963 such plant injury was observed on seven occasions.*

During the summer of 1963 it became apparent that, among the experimental plants growing in our greenhouse, a particular variety of white petunia (Snowstorm) was extremely susceptible to a toxicant in the ambient air. After exposure to certain atmospheric conditions, the leaves were marked in a manner similar to that which Taylor *et al.* (*1*) found after exposure of leaves to the polluted ambient air of California. Leaves that were rapidly expanding in size appeared water-soaked between the veins, and after several hours of exposure to sunlight the upper surface developed typical necrotic bands and the lower surface had a glazed appearance (Fig. 1). The youngest leaves were marked only slightly, if at all, at the apex; and the oldest leaves entirely escaped injury. Taylor *et al.* reproduced such symptoms by exposing plants to irradiated mixtures of NO_2 and hexene. More recently Stephens and his group (*2*) induced this same damage to petunias not only with