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Gamma-Aminobutyric Acid: Role in Primary Afferent Depolarization

Abstract. *The effects of putative transmitters on the primary afferent terminals were studied in the magnesium-treated, isolated spinal cord of the frog. Gamma-aminobutyric acid and glutamic acid reversibly depolarized primary afferent terminals and increased their excitability, whereas glycine produced weak and variable effects. Bicuculline and picrotoxin, which reduce primary afferent depolarization, reversibly antagonized the gamma-aminobutyric acid–mediated responses but had little effect on those produced by either glutamic acid or glycine. The glutamic acid- and the gamma-aminobutyric acid–induced depolarizations remained in the absence of external chloride but disappeared in the absence of external sodium. These results support the hypotheses that gamma-aminobutyric acid is the transmitter mediating the synaptic depolarization of primary afferent terminals and that sodium is the predominant ion involved.*

The prolonged inhibition of the monosynaptic excitation of motoneurons by orthodromic volleys in muscle and cutaneous afferent fibers is generally attributed to a presynaptic depolarization of the primary afferent terminals that presumably decreases both the amplitude of the presynaptic action potential and the quantity of transmitter released (*1*). The depolarization of the primary afferent terminals (primary afferent depolarization) is electrotonically conducted along the dorsal root where it can be recorded as the dorsal root potential (*1, 2*). The depolarization and the coincident increase in excitability of the terminals suggests that the last step in the generation of primary afferent depolarization is mediated by an excitatory transmitter. Since picrotoxin and bicuculline reduce the dorsal root potential (*3–5*) and selectively antagonize synaptic events mediated by gamma-aminobutyric acid (GABA) (*5, 6*), GABA (or a closely related substance) has been proposed as the transmitter mediating primary afferent depolarization (*1*). However, in all other systems, GABA is exclusively involved in inhibitory events, decreasing excitability by increasing subsynaptic membrane conductance, predominantly to chloride (*7*). We have used the isolated spinal cord of the frog to investigate this paradox since this preparation is suitable not only for studying the action of putative transmitters on primary afferent terminals (*8*), but also for studying the ionic mechanisms underlying these actions. We now report (i) that GABA mimics the action of the natural transmitter by depolarizing and increasing the excitability of primary afferent terminals, (ii) that this action is selectively blocked by both picrotoxin and bicuculline, and (iii) that it is de-

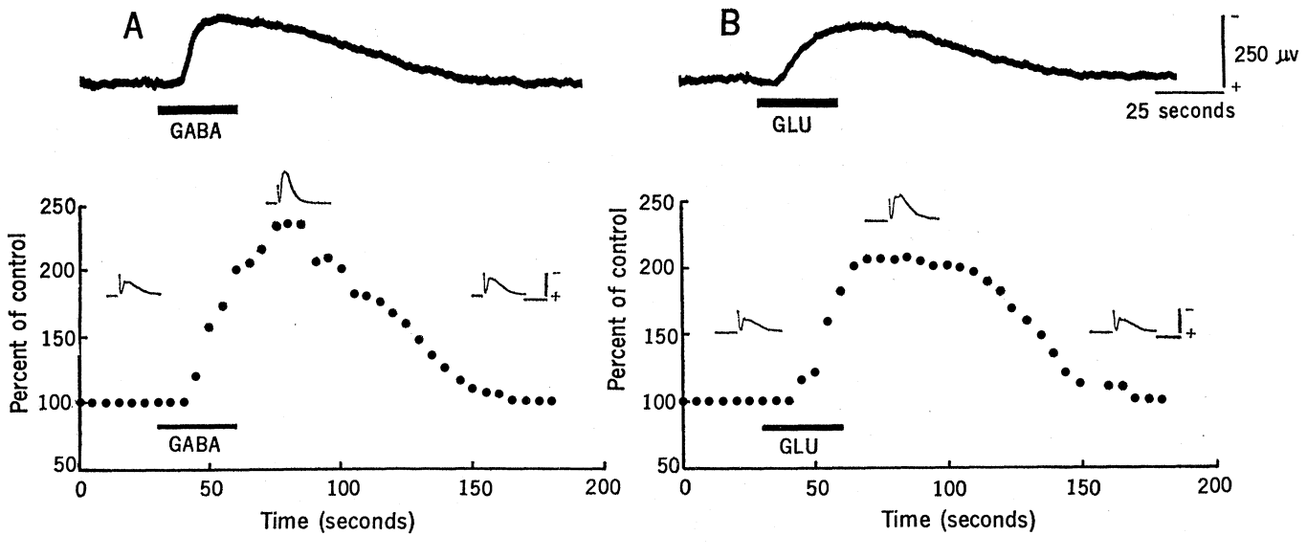

Fig. 1. Gamma-aminobutyric acid and glutamic acid increase the excitability of primary afferent terminals and depolarize the dorsal root of the isolated spinal cord of the frog. Synaptic activity was eliminated 2 hours prior to the above records by the addition of 20 m*M* $MgSO_4$ to the perfusing Ringer solution. The upper trace in each figure is a d-c recording of the polarization level of the dorsal root. The lower part of each figure is a graph of the size of the antidromically conducted dorsal root volleys (recorded a-c every 5 seconds) before, during, and after application of drugs. The insets are examples of these volleys. The excitability testing and the d-c recordings were made within 5 minutes of each other. (A) Application of GABA ($5 \times 10^{-3} M$) for 30 seconds (solid bar, upper trace, and lower graph) depolarizes the dorsal root and increases primary afferent excitability with a similar time course. (B) Application of glutamic acid ($5 \times 10^{-3} M$) for 30 seconds (solid bar, upper trace, and lower graph) also depolarizes the dorsal root and increases excitability with similar time courses. For insets in (A) and (B), vertical calibration is 1 mv and horizontal, 1 msec.

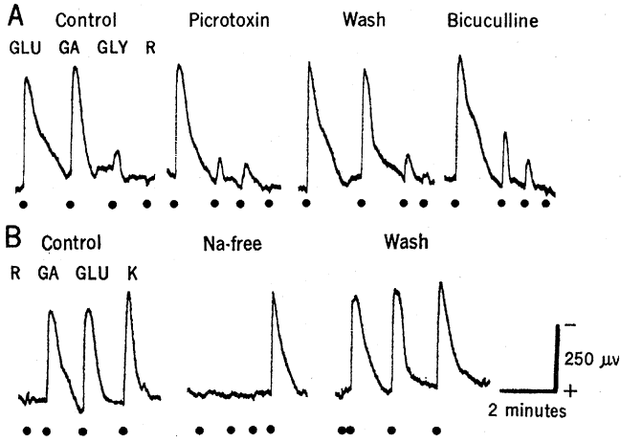

Fig. 2. All traces are d-c recordings of the polarization level of the dorsal root in the magnesium-treated, isolated spinal cord of the frog. Each trace is a continuous record. The dots underneath the traces represent 5-second injections (by syringe) of various substances directly into the bath. (A) Picrotoxin and bicuculline antagonize the GABA response. Injection of either $5 \times 10^{-3}M$ GABA (*GA*), $5 \times 10^{-3}M$ glutamic acid (*GLU*), or $5 \times 10^{-3}M$ glycine (*GLY*) reversibly depolarizes the dorsal root (*Control*). A control injection with Ringer solution (*R*) has little effect. Perfusion of the spinal cord with $1.7 \times 10^{-4}M$ picrotoxin for 5 minutes greatly diminishes the depolarizing response to GABA but does not affect either the glutamic acid or the glycine response (*Picrotoxin*). Washing of the spinal cord with picrotoxin-free Ringer solution for 1 hour restores the GABA-mediated depolarization (*Wash*). Perfusion of the spinal cord with Ringer solution containing $10^{-4}M$ bicuculline attenuates the GABA response but does not noticeably affect the other drug responses (*Bicuculline*). (B) Sodium-free Ringer solution eliminates the depolarizing responses to GABA and glutamic acid. Injection of either $5 \times 10^{-3}M$ GABA (*GA*), $5 \times 10^{-3}M$ glutamic acid (*GLU*), or 25 m*M* KCl (*K*) in Ringer solution depolarizes the dorsal root (*Control*), whereas Ringer solution (*R*) has little effect. Perfusion of the spinal cord for 30 minutes with sodium-free saline (choline substitute) eliminates both GABA- and glutamic acid–induced depolarizations (*Na-free*) but has little effect on the depolarizing response to potassium. Washing of the spinal cord with normal Ringer solution for 45 minutes restores both the drug responses (*Wash*).

pendent on the presence of external sodium.

Frogs (*Rana pipiens*) were chilled in ice to an anesthetic state and then were decapitated. The spinal cord and roots were removed. The spinal cord was hemisected sagitally and placed in a trough [modified after Tebecis and Phillis (*8*)], which was continuously perfused with oxygenated Ringer solution (*9*). Lumbar roots 8 and 9 were led out through partitions sealed with Vaseline into paraffin pools and each was placed on two Ag-AgCl wires. The d-c polarization levels of the roots were recorded differentially between the two wires on the same root and were displayed on an oscilloscope and pen recorder. (This recording arrangement permitted better stability than did one employing differential recording between bath and roots, although the potentials so recorded were considerably reduced in size.) The excitability of primary afferent terminals was tested by stimulating the fibers through a low-resistance, glass microelectrode (filled with 4*M* NaCl) after the method of Wall (*10*), and the conducted antidromic volley was recorded monophasically. The results are based on observations from 60 experiments.

After first assessing the general condition of the cord by recording dorsal root potentials, we added 20 m*M* $MgSO_4$ to the perfusate and monitored the dorsal root potentials until they were markedly reduced or abolished (5 to 15 minutes). Under such conditions synaptic events that might occur during drug application should be reduced, thus permitting us to better investigate the effects of putative transmitters on primary afferent terminals. Perfusion of the magnesium-treated spinal cord with GABA or glutamic acid (in concentrations of $10^{-3}M$ to $10^{-2}M$) invariably depolarized the primary afferent terminals (Fig. 1, top traces). These depolarizations were always associated with an increase in the excitability of primary afferent fibers that was of a similar time course (Fig. 1, lower graphs). By constructing curves of antidromic volley size as a function of stimulus strength before, during, and after drug applications [a more precise method of quantitating excitability change (*11*)], we have found that GABA ($5 \times 10^{-3}M$) and glutamic acid ($5 \times 10^{-3}M$) produce a 10 to 20 percent increase in excitability. The present findings confirm several earlier reports (*3*, *12*) but contradict the isolated case in which GABA is reported to decrease the excitability of afferent terminals (*13*).

To examine the effects of putative transmitters on primary afferent terminals further, we applied drugs rapidly by direct injection with a syringe into the bath (*14*). Injection of either GABA or glutamic acid consistently produced reversible depolarizations of the dorsal root (Fig. 2A, *Control*). Similar applications of glycine ($10^{-3}M$ to $10^{-2}M$) caused small responses that varied from spinal cord to spinal cord (Fig. 2A, *Control*). Since the convulsants picrotoxin and bicuculline have been reported to reduce dorsal root potentials (*3–5*) and block GABA-mediated synaptic events (*5*, *6*), we perfused the spinal cord with Ringer solution containing either picrotoxin or bicuculline. Picrotoxin ($10^{-5}M$ to $10^{-3}M$) rapidly antagonized the GABA depolarization but had little effect on either the glutamic acid or glycine responses (Fig. 2A, *Picrotoxin*). When we washed the spinal cord with picrotoxin-free Ringer solution the depolarizing response to GABA returned (Fig. 2A, *Wash*). Bicuculline (saturated solution, approximately $10^{-4}M$) also attenuated the GABA response (*Bicuculline*). Thus, both bicuculline and picrotoxin specifically antagonized only the GABA response.

In order to investigate the ionic mechanism(s) underlying the depolarizing action of GABA and glutamic acid in this system, we examined the drug responses in the absence of either external chloride or sodium (*15*). The depolarizing responses to GABA and glutamic acid consistently remained after 5 hours of chloride-free perfusion, which indicates that chloride is unlikely to be the main ion causing depolarization of the terminals (*16*). However, perfusion with sodium-free Ringer solution for 20 to 30 minutes resulted in the loss of both depolarizing responses to GABA and glutamic acid (Fig. 2B, *Na-free*). The primary afferent terminals were still capable of being depolarized in sodium-free Ringer solution since the depolarization to a tenfold increase in external potassium remained under these conditions (Fig. 2B, *Na-free*). Both the GABA and glutamic acid depolarizations were restored on

returning to normal Ringer solution, thus suggesting that sodium is the predominant ion mediating the GABA and glutamic acid depolarizations. The GABA response usually returned within 10 minutes of washing whereas the glutamic acid response often took much longer to return (approximately 1 hour).

In summary, we have treated the isolated spinal cord of the frog with magnesium in order to minimize synaptic activity and permit better investigation of the effects of putative transmitters on primary afferent terminals. Both GABA and glutamic acid increased the excitability of the terminals and concomitantly depolarized the dorsal root. Furthermore, both bicuculline and picrotoxin antagonized the GABA-induced depolarizations but had little effect on either the glutamic acid or glycine responses. Since both bicuculline and pictrotoxin attenuate the synaptically developed dorsal root potential (*3–5*) and block GABA-mediated events elsewhere in invertebrate and vertebrate nervous tissue (*5, 6*), our evidence supports the hypothesis that GABA is the transmitter mediating primary afferent depolarization (*1*).

The investigation of the ionic mechanism(s) underlying the amino acid–induced depolarization of the dorsal root has provided evidence to support the hypothesis that sodium is the predominant ion involved in these responses (*5*). This sodium-dependent, GABA-induced depolarization of primary afferent terminals differs from its well-established action as a chloride-dependent inhibitory transmitter and suggests that GABA may mediate synaptic events by utilizing different ions at different sites.

Note added in proof: Since this report was submitted for publication Davidoff has reported an irreversible, specific antagonism of dorsal root potentials and both GABA-induced increases in primary afferent excitability and depolarizations of the dorsal root (*17*). This evidence complements our report and further strengthens the notion that GABA or some closely related analog is the natural transmitter mediating the presynaptic inhibition.

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9. The Ringer solution consisted of: 114 m*M* NaCl, 2.5 m*M* KCl, 1.9 m*M* $CaCl_2$, and 2.5 m*M* dextrose buffered to *p*H 7.4 with 10 m*M* tris-maleate-NaOH. The $MgSO_4$ (20 m*M*) was added to this solution after assessing the synaptic potentials present. The flow was adjusted so that approximately tenfold the bath volume was exchanged every minute. The ambient temperature of the experimental room and preparation was monitored by a thermister in the bath and was maintained at 15°C. A calomel-cottonwick electrode in the bath served as a ground.
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14. By direct injection through a syringe we were able to supply three times the chamber volume within 5 seconds. This permitted a quick examination of pharmacologic responses. Repeated applications produced responses that were within 15 percent of each other, which indicates a good degree of reliability by this technique. Since this procedure occasionally resulted in small artifacts, Ringer solution was also injected with each series of drug tests. Ringer-produced artifacts that were large enough to alter the responses eliminated the drug responses from further study. An alternative method consisted of switching from the Ringer solution to a drug-containing solution merely by turning a stopcock. Since the flow was controlled between the stopcock and the bath, the flow rate was constant. Results obtained by this technique were similar to those derived from syringe injection. The drug concentrations used in the present study did not alter the *p*H of the solutions. The dorsal root responses to application of the amino acids were similar in normal Ringer solution and in high magnesium Ringer.
15. Chloride-free Ringer solutions consisted of either 114 m*M* sodium isethionate, or 57 m*M* Na_2SO_4 and 57 m*M* sucrose, 1.25 m*M* K_2SO_4, 1.9 m*M* $CaSO_4$, 2.5 m*M* dextrose, and 10 m*M* tris-maleate-NaOH buffered to *p*H 7.4. Sodium-free solutions consisted of: either 114 m*M* choline chloride, 114 m*M* LiCl, 114 m*M* tris-Cl, or 57 m*M* $MgCl_2$ and 57 m*M* sucrose, 2.5 m*M* KCl, 1.9 m*M* $CaCl_2$, 2.5 m*M* dextrose, and 10 m*M* tris-HCl buffered to *p*H 7.7. The 20 m*M* $MgSO_4$ was added to each solution in order to reduce synaptic activity.
16. The synaptically developed depolarization of primary afferent terminals remained and was markedly prolonged after 5 hours of chloride-free bathing, thus confirming earlier work [B. Katz and R. Miledi, *J. Physiol. London* **168**, 389 (1963)]. The amino acid–induced depolarizations might possibly reflect an outward chloride current. If 5 hours of chloride-free washing is not sufficient to entirely remove internal chloride, then part of the remaining depolarization might be due to a residual chloride current. These chloride-free experiments thus do not rule out the possibility, but those with sodium-free solutions would appear to eliminate chloride as the predominant ion involved in the drug-induced depolarizations.
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18. We thank F. F. Weight for suggestions and encouragement, F. E. Bloom and A. L. F. Gorman for their criticisms of the manuscript, and J. C. Eccles for his comments on the final draft.

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Depression and Later Enhancement of the Critical Flicker Frequency during Prolonged Monocular Deprivation

Abstract. *One eye was visually deprived for 1 day, and the critical flicker frequency in the other eye was determined at the start of the deprivation period and then at intervals of 3, 6, 9, 15, and 24 hours. There was an initial depression in performance, followed by an enhancement effect. No significant changes in the critical flicker frequency were observed in the occluded eye at corresponding times; thus the depression-enhancement phenomenon is specific to the nonoccluded eye.*

In 1923, Allen (*1*) reported that 3 hours of monocular light deprivation produced a decrease in the critical flicker frequency (CFF) of the nonoccluded eye, a result confirmed by Hollenberg (*2*). In contrast, we demonstrated (*3*) a negatively accelerating improvement in the CFF of the nonoccluded eye during 1 week of monocular deprivation, the first measurement being taken 8 hours after deprivation was begun and the remainder at daily intervals. Furthermore, a sizable aftereffect in this eye was still present 1