Gamma Aminobutyric Acid Induced Changes in the Spontaneous Firing Rates of Insect Neurons

Previous work has shown that GABA (γ-aminobutyric acid) is found in nervous tissue[1,2], and that it has an inhibitory influence on neurons[3,4] and on some skeletal muscle cells in insects[5]. A factor which inhibits crayfish stretch-receptors, concentrated from beef brain extracts, was found to be GABA[4].

Experiments by USHERWOOD and GRUNDFEST on peripheral inhibition in insect muscles showed that GABA mimics the effects of stimulating an inhibitory axon[5]. VERESHTCHAGIN et al. also reported that GABA depressed bioelectrical activity in ganglia of the Pine Moth caterpillar[6]. We therefore expected that spontaneous neural firing in isolated insect ganglia would be inhibited by perfusing with saline containing varying concentrations of GABA. We selected GABA for this work partly because we wanted an inhibitory substance to test an 'activity clamp' technique being developed for quantitative pharmacological investigations of these neurons.

Methods. Figure 1 shows a block diagram of the apparatus. A ganglion dissected from a cockroach (male, species *Periplaneta americana*) was attached to an electrode assembly by sucking a connective into a glass capillary tube where it contacted 1 electrode. The ganglion itself rested on the other electrode. The preparation was immersed in a flow of Ringer-Hoyle solution passing through a T-tube. A motor-driven syringe injected GABA solution into the flow system just ahead of the preparation in such a way that total flow rate was constant; thus, GABA concentration was proportional to syringe rate. The spontaneous pulses were amplified and recorded on one channel of stereo magnetic tape; the other channel was used for verbal monitoring of time and experimental conditions.

The amplified pulses were also fed into a pulse rate tachometer which gave a dc output proportional to the number of pulses per unit time. This dc output was used in 2 ways: (1) to make a chart recording of a ganglion's pulse rate, and (2) to control the syringe rate. The purpose of the latter was to see if an 'activity clamp' could be achieved by injecting GABA at a rate proportional to the neural firing rate. At low pulse rates the pulse rate tachometer drives the syringe slowly or not at all. With a rise in activity, injection of GABA into the system increases. If GABA were inhibitory, this should reduce the activity level, and the cycle should repeat with activity oscillating about some constant level. The syringe was sometimes driven at constant rates by external dc signals to insure that the feedback system was not producing extraneous effects.

Results. When placed in flowing Ringer-Hoyle solution and left on its own, a ganglion would emit spontaneous nerve pulses having amplitudes from 10–200 μV for up to 6 h. In these experiments with GABA applied periodically, spontaneous activity lasted 2 or 3 h on the average, with runs as short as $^1/_2$ h or as long as 6 h. 12 different recordings were made representing 38 h of activity and about 100 GABA injections at concentrations from $0.1–25 \cdot 10^{-4} M$ around the ganglion. In 80% of the cases, injection of GABA was followed by a drop in activity level. The response most frequently observed began with a sharp rise, followed by a quick drop, and often complete cessation of activity. An example is seen in the section of chart recording in Figure 2(A). After such a response where cut-off occurred, the syringe was usually turned off; if recovery ensued, it would take from 10–20 min. A less frequently seen response was an immediate drop from the initial activity level. This varied from a sharp, rapid drop to a slow, sometimes delayed decline.

In the range of concentrations tried, we could not establish an overall threshold concentration above which GABA had an effect on all ganglia. Throughout the entire range of concentrations all types of responses were observed. However, individual ganglia did exhibit thresh-

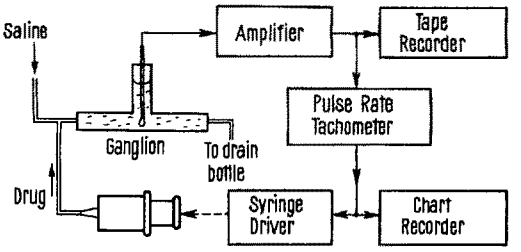

Fig. 1. Block diagram of experimental apparatus.

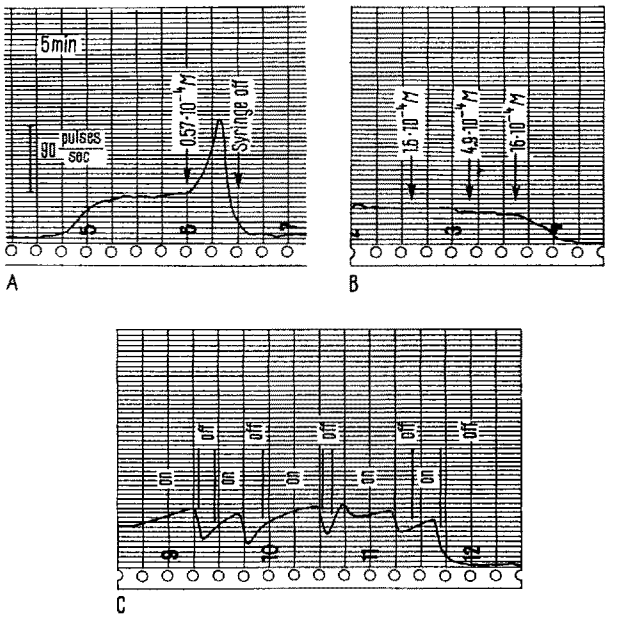

Fig. 2. (A) Recovery of activity from previous injection followed by injection of GABA. The response was an increase in activity, then complete cessation. Recovery again ensued 15 min after turning off the syringe. (B) Threshold effect. There was no significant response to GABA below a concentration of $16 \cdot 10^{-4} M$. The syringe was turned off after cessation of activity and the ganglion recovered 10 min later. (C) Syringe was driven at a rate proportional to the ganglion's activity. The pulse rate signal turned the syringe 'on' and 'off' as activity oscillated around an impulse frequency which caused an average GABA concentration of about $1 \cdot 10^{-4} M$. The final drop represents dying of this preparation. Calibrations in (A) apply to all 3 records.

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olds. A ganglion would seem unaffected by low concentrations, but as higher concentrations were tried, one would appear at which an effect was noticed. For example, in Figure 2(B) there is no response to $1.6 \cdot 10^{-4} M$ or $4.9 \cdot 10^{-4} M$ GABA, but a drop in activity follows application of $16 \cdot 10^{-4} M$. Subsequent recovery with the syringe off showed this was not due to deterioration of the ganglion. Comparison with the pronounced effect of $0.57 \cdot 10^{-4} M$ GABA seen in Figure 2(A) indicates the wide variability in sensitivity of these neurons to GABA. In general, complete cessation of activity occurred more often at concentrations $> 10 \cdot 10^{-4} M$. For lower concentrations the usual response was a decrease in activity but not complete cessation.

Figure 2(C) shows an example where the syringe was left on, driven by the pulse rate signal. The oscillations about a level demonstrate that an activity clamp can be achieved.

The initial increase in neural firing rate which usually preceded the decline in activity in our experiments was not anticipated, since most reports on effects of GABA emphasize its inhibitory action. With the large electrodes we used, the pulse rate from a ganglion is a sum of the individual pulse rates of many neurons. The rise in activity at the start of GABA injection might come from some neurons which are stimulated by GABA, with the subsequent decline indicating the more commonly observed inhibition. Another possibility is that the excitatory and inhibitory phases are due to action of the drug on different parts of the neurons. As GABA diffused into a ganglion from the saline solution, it would reach the peripherally-disposed cell bodies before passing into the neuropile region. KUFFLER and EDWARDS[3] proposed that a mechanism for action of GABA is to cause increased conductance in dendrite and cell body membranes. Perhaps the observed excitatory effect comes from increased conductance in the cell body, while the inhibitory effect results from a similar action on the dendrites[7].

Zusammenfassung. γ-Aminobuttersäure (GABA) vermindert die Impulsfrequenz der spontan-aktiven Nervenzellen beim Insekt. Wirksame Konzentrationen betragen $0{,}1\text{–}25 \cdot 10^{-4} M$. Ein unerwartetes Ergebnis war die Beobachtung, dass nach Applikation von GABA eine Erhöhung der Impulsfrequenz vor der Verminderung stattfindet.

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Über einen die Samenbläschen hemmenden Stoff aus Luzerne

Dass Fütterung von Luzerne die Fertilität der Haus- und Laboratoriumstiere negativ beeinflussen kann, beweisen mehrere Forschungsergebnisse (ZADINA und GEISSLER[1], FOLTYN[2], ADLER und TRAININ[3], CHURÝ und PÁNEK[4]). Der Mechanismus dieser Wirkung infolge Luzernefütterung konnte bisher nicht näher abgeklärt werden. ADLER und TRAININ[3] nehmen zum Beispiel an, dass diese Wirkung eng mit dem Phytoöstrogengehalt dieser Pflanze zusammenhängt; ZADINA und GEISSLER[1] glauben, dass es sich um den Effekt eines Glykosids oder antithyreoidalen Stoffes handle. Nach den Angaben von ZADINA und GEISSLER[1] ähnelt die Wirkung von Luzernefütterung sehr dem Effekt von Lithospermum-Arten; bei diesen wurde der antigonadale Effekt von verschiedenen Autoren festgestellt (CRANSTON[5], DRASHER[6], PLUNKET und NOBLE[7], JURAND[8], KEMPER und LOESER[9,10], BRENEMAN et al.[11]). Nach GASSNER et al.[12] soll für den Effekt bei Lithospermum-Arten eine Polyphenolsäure verantwortlich sein. Untersuchungen von Luzerne zeigen, dass diese Pflanze östrogen sowie auch antiöstrogen wirksame Stoffe enthält (CHENG et al.[13], ANDREWS[14], CHURÝ[15], ADLER[16], ROLINSKI[17]). Man könnte darum diesen Effekt von Luzernefütterung auf den Gehalt der genannten Stoffe zurückführen. Da aber in der Luzerne die Menge von Phytoöstrogenen verhältnismässig stark schwankt (CHURÝ[18]) und weil dieser antifertile Effekt der Luzernefütterung auch nach Entfernung von Phytoöstrogenen und Antiöstrogenen erhalten bleibt (PÁNEK und CHURÝ[19]), muss angenommen werden, dass ausserdem noch ein unbekannter Stoff mit dem antifertilen Effekt der Luzerne zu tun hat. Für eine solche Annahme sprechen auch die Angaben von KARG[20]. Anknüpfend an unsere Voraussetzungen und früheren Versuche bemühten wir uns, die antifertile Wirkung der Luzerne genauer abzuklären.

Als Ausgangsmaterial diente uns trockene Luzerne, welche von verschiedenen Orten stammte. Das Pflanzen-

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