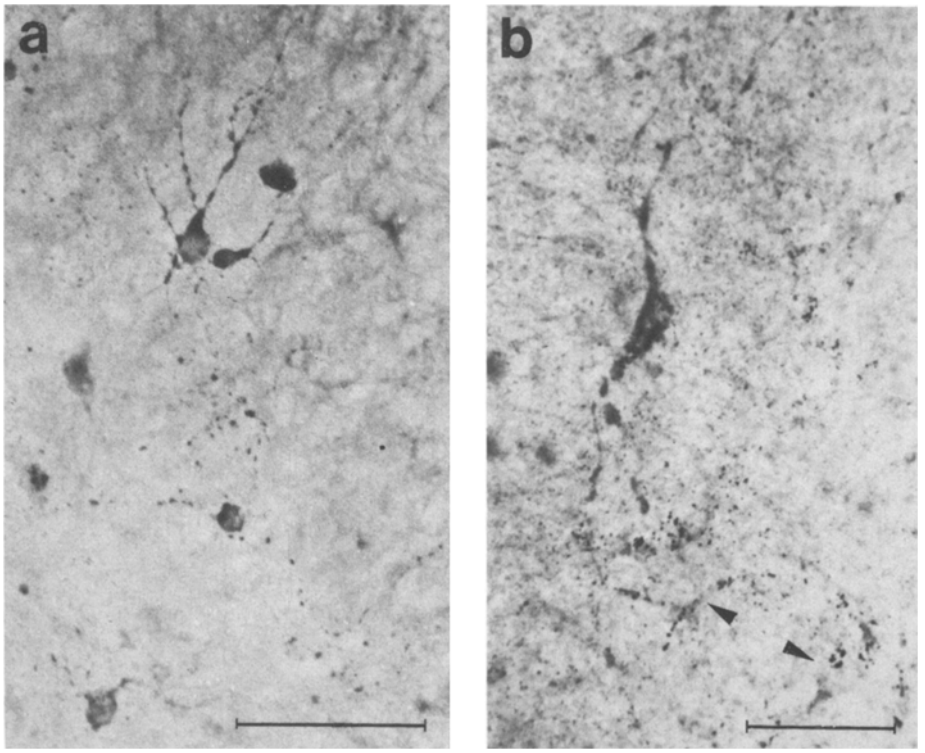

Figure 2. GABA-T cytochemistry in spinal cord cultures (21 days in vitro). *a* GABA-T-positive dorsal horn neurone and processes. *b* GABA-T-positive dorsal horn neurone showing processes which overlay unstained neurones (arrowed). Calibration bars indicate 50 µm.

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The inhibitory amino acid GABA hyperpolarizes motor axons: an intracellular study[1]

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Summary. The inhibitory amino acid γ-aminobutyric acid (GABA) hyperpolarized motor axons. This hyperpolarization was associated with an increase in the resting input conductance and with reduced action potential duration.

The putative central inhibitory transmitter γ-aminobutyric acid (GABA) has well documented actions on vertebrate primary afferent terminals[2], dorsal root ganglion cells[3,4] and motoneurones[5]. In addition extracellular recordings reveal that GABA can depolarize some peripheral axons[6] although it is not clear whether such an effect occurs on motor axons and what membrane mechanisms are associated with it.

In the course of some experiments on the frog spinal cord in vitro we encountered some units which we classified as motor axons according to the following criteria. Firstly, these cells exhibited very short latency spikes without an

initial segment component on antidromic stimulation and were able to follow antidromic stimulation rates ≥ 100 Hz. Secondly, the spikes did not show the typical afterpotentials which characterize spikes recorded from the frog motoneurone soma[7]. Thirdly, dorsal root stimuli which evoked EPSPs in motoneurone cell bodies did not do so in these units. Fourthly, visual inspection of the electrode penetration track showed a rather lateral locarion of the electrode placement. It was therefore thought interesting to assess whether GABA might affect these cells.

The purpose of this paper is to report the effects of GABA on spike configuration, membrane potential and input conductance of central motor axons.

Methods. The details of the preparation have been described elsewhere[8]. Briefly, the spinal cord was removed from frogs *(Rana temporaria)* and a parasagittal slice prepared. The slice with 2 dorsal and 2 ventral lumbar roots was mounted in the central chamber (vol.0.2 ml) of a watercooled, 3 chambered bath. The roots, usually the 8th and 9th pairs, were led into the side chambers and drawn into suction electrodes for stimulation (0.33 Hz). Ringers and drug solutions were stored in separate, continuously gassed (O_2/CO_2:95/5%) reservoirs which led via cooled flow lines to the central bath compartment. The flow rate (5–10 ml min^{-1}) was adjusted in each experiment to maintain a maximum bath temperature of 7 °C to reduce the GABA uptake system[9]. The excess solution was led from the bath by a grounded wick.

Intracellular recordings were made with glass fiber microelectrodes, filled with either 3 M KCl or 3 MCsCl, connected to the input stage of a high impedance preamplifier equipped with capacity neutralization and bridge circuitry for current injection. The output of the preamplifier was displayed on an oscilloscope and potentiometric chart recorder and also stored on magnetic tape (DC–2.5 kHz) for later analysis.

Results. Intracellular recording from motor axons within the CNS is technically very difficult because of their small diameter. This fact, coupled with the need for a fast superfusion rate in order to avoid rapid desensitization to GABA, meant that intra-axonal impalements were of necessity shorter and less stable than recordings from motoneurone somata. These relatively short impalements also precluded detailed pharmacological studies involving dose-response curves and antagonist effects which can more conveniently be effected with extracellular recordings[10]. Thus although a number of units identified as axons were impaled only a few allowed a full analysis. The table summarizes the mean values recorded for several axonal characteristics. The mean resting potential and spike amplitude were −54 and 55 mV respectively: these values accord with those recently published for peripheral motor axons in the frog[11]. In 2 axons tested the current voltage relationship appeared linear within the range −50 to −80 mV.

As detailed in the table superfusion with GABA (3 mM) caused a small hyperpolarization (average 2.3 mV) of resting membrane potential, an increased input conductance (average 28%), little effect on the upstroke of the spike and a faster spike repolarization (average −1.25 msec). Figure 1 (bottom row) shows an example of GABA

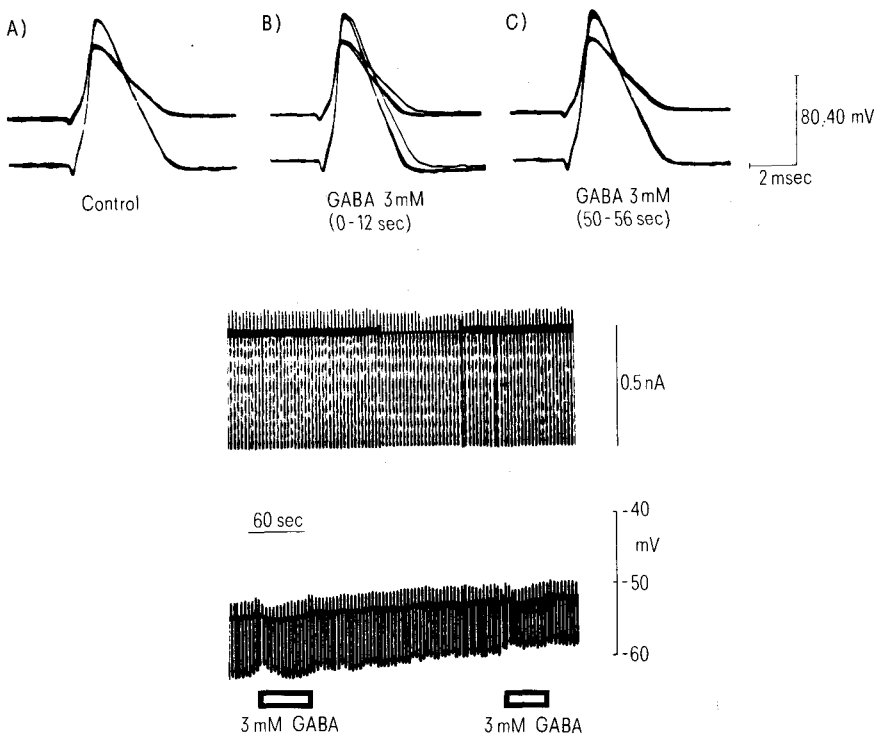

Figure 1. Effect of GABA on a frog motor axon in vitro. Top row: each panel shows D.C. low gain (top) and A.C. high gain (bottom) oscilloscope records. *A* 2 superimposed control spikes evoked directly by passing depolarizing current pulses through a KCl filled recording microelectrode. *B* 1st 3 responses superimposed during superfusion of GABA (3 mM); note the faster repolarization. *C* Fading of GABA effect during continued superfusion. Middle row: chart record of constant current pulses injected through the recording microelectrode. Upward deflections are truncated records of depolarizing pulses used to evoke spikes shown in top row. Downward deflections are non-truncated records of the long (> 600 msec) pulses used to evoke the membrane hyperpolarization for resting conductance measurements. Bottom row: chart record of membrane potential. Upwards deflections are truncated spikes and downwards are hyperpolarizing electrotonic potentials evoked by injection of the current shown in the middle row. Note the hyperpolarization and the increased conductance as shown by the decreased amplitude of the electrotonic potentials. Resting membrane potential: −53 mV. Resting input conductance: 63 nS.

The effect of GABA on resting and spike characteristics of central motor axons

Parameter	Control[a]	GABA[a]	ΔGABA[b]
Spike: amplitude: mV	55±5 (5)	50±7.0 (4)	−1.3±2.0
Spike: time to peak: msec	1.00±0.2 (5)	0.89±0.18 (4)	−0.08±0.07
Spike: time peak to baseline: msec	6.64±3.53 (5)	6.06±3.99 (4)	−1.25±0.47
Input conductance: nS	62.8±12.5 (5)	96.5±16.6 (4)	+22.5±9.8
Membrane potential: mV	−54±2.5 (5)	−55±2.7 (4)	−2.3±0.5

[a] Expressed as mean ± SEM with Nos in parenthesis. [b] Calculated as mean difference ± SEM of paired (GABA minus control) values.

evoked hyperpolarization and the increased input conductance: the latter is evidenced by the amplitude reduction of the hyperpolarizing electrotonic potentials. GABA had no effect on the upstroke or amplitude of the directly evoked spikes, but during the 1st few sec of superfusion the rate of spike repolarization increased (fig. 1,B) so that the time from peak to baseline decreased by 1.0 msec. With continued (60 sec) superfusion the effects of GABA on input conductance and spike repolarization (fig. 1,C) faded but could be reproduced by a 2nd application of the drug. Figure 2 shows the effects of GABA (3 mM) on another motor axon. Data from this cell was recorded with a relatively blunt microelectrode filled with CsCl, a K^+ channel blocker[12]. In figure 2,A an antidromic spike obtained shortly after impalement is shown. As the experiment continued a gradual membrane depolarization developed (maximum 9 mV) associated with a decrease in spike amplitude and prolonged spike repolarization such that the time from peak to baseline was increased from 6 to 20 msec (compare fig. 2,A and B). Presumably continuous intracellular leakage of Cs^+ was responsible for the loss of membrane potential and the pronounced tail on the falling phase of the spike. It is very unlikely that spontaneous decline of membrane potential and not Cs^+ was the cause of spike broadening because membrane depolarization is known to accelerate the non-inactivating outward K^+ current responsible for spike repolarization in frog axons[13]. Furthermore, since the membrane potential was within the linear range of the current-voltage curve it is unlikely that a potential dependent phenomenon, for example, anomalous rectification, influenced spike duration. In the presence of Cs^+ GABA produced a 2.5 mV hyperpolarization and a 55% increase in resting input conductance: again both effects of GABA faded during the 60 sec superfusion. Faster repolarization of the spike was evident (fig. 2,C) with the time from peak to base line decreasing by 3.0 msec and fading developing as the superfusion continued. As these experiments were carried out a low temperature to reduce GABA uptake[9], we presume that GABA response fading was due to desensitization rather than removal of the applied amino acid.

Discussion. To our knowledge this is the first report, using intracellular techniques to describe the effects of GABA on motor axons within the vertebrate spinal cord. Our inability to demonstrate an EPSP following stimulation of dorsal roots suggests that impalements were several space constants distant from the region of synaptic input. The data presented show that GABA consistently hyperpolarized axon membranes. This effect, unlike that on Müller cell axons[14], cannot be attributed to electrical coupling with nearby cells because it was associated with a conductance increase implying a direct effect on axon membranes. We cannot, however, exclude the possibility that the actions described result from release of an endogenous compound(s) by GABA. This issue may be addressed with further experiments using Ca^{2+} antagonists to block release mechanisms.

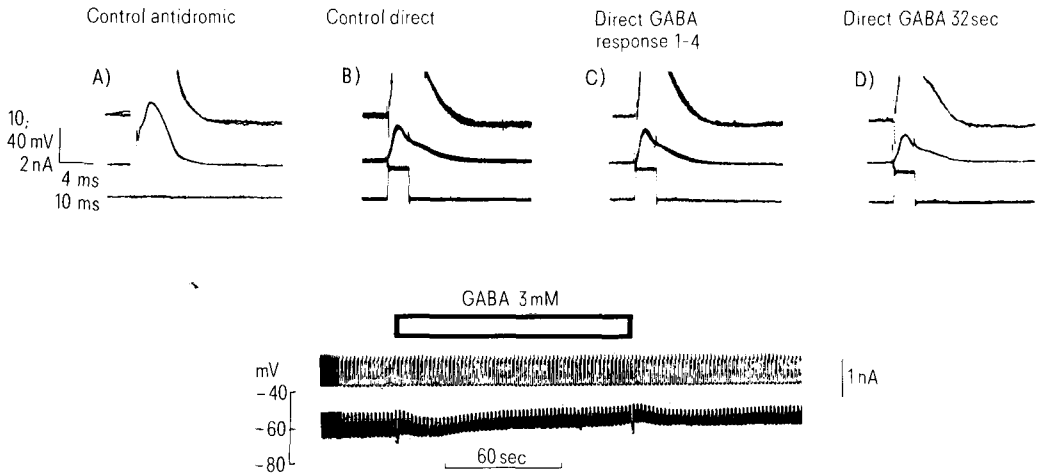

Figure 2. Effect of GABA on a frog motor axon in vitro. Recordings made with a 3 M CsCl filled microelectrode. Top row: oscilloscope records showing in each panel (A–D) from above downwards high gain A.C., low gain D.C. and injected current traces (in A no current is injected). Horizontal time calibration is 4 msec for A and 10 msec for B–D. *A* Control antidromic spike (evoked by stimulation of 9th ventral root shortly after microelectrode impalement). *B* Directly evoked control spikes (note broader spike caused by intracellular leakage of Cs^+). *C* 1st 4 superimposed direct spikes during application of GABA (3 mM), note faster repolarization. *D* Fading of GABA effect during continued GABA superfusion. Middle row: chart record of injected current. Bottom row: chart record of membrane potential. Note the hyperpolarization and increased input conductance during GABA application. The small preceding depolarization is a flow artifact. Resting membrane potential: −57 mV. Resting input conductance: 97 nS. Other details as in figure 1.

Another effect of GABA on motor axons is the faster repolarization of the action potential. Since this effect persisted in the presence of the K^+ channel blocker Cs^+, it may be suggested that GABA was not modulating a voltage-gated K^+ conductance. In cultured dorsal root ganglion cells GABA reduces action potential duration by a selective decrease in a voltage sensitive Ca^{2+} conductance[15]. Several lines of evidence suggest that a similar mechanism is not readily applicable to our data, since, for example, in squid axons the late Ca^{2+} current cannot be detected under voltage clamp[16]. In any event calcium spikes disappear from *Xenopus* neurites during development[17] and transmitter release at the frog neuromuscular junction, a process exquisitely sensitive to calcium, is not modified by GABA[18]. One possible explanation for our data is that the decreased input resistance caused by GABA, presumably mediated by chloride ions like most other GABA responses[2], reduced the membrane time constant and consequently shortened the spike. Clearly this hypothesis requires further investigation.

The physiological significance of GABA receptive sites on motor axons is unclear because the synaptic input does not extend beyond the axon hillock[19] and we used rather a high concentration of this amino acid to elicit axonal responses. It might well be that GABA-sensitive sites are widely present on nerve membranes during development and that they have only a vestigial role in the adult animal. Perhaps more important are the pharmacological implications of our results since attempts to treat neurological conditions characterized by a deficit of GABA (e.g. Huntington's chorea) with systemic administration of GABA agonists[20] may affect extrasynaptic sites (for instance on axons) and contribute to side effects such as muscle hypotonia[21].

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Experimental catalepsy: Influences of cholinergic transmission in restraint-induced catalepsy

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Summary. Possible cholinergic mechanisms in experimental catalepsy were evaluated by using the 'pinch-induced' model in mice. In control, saline-injected mice, the median number of attempts needed to achieve a criterion level of catalepsy was 6. All 3 dose levels of physostigmine reduced this median to about 2 trials; neostigmine did not significantly reduce the number of trials. Opposite effects were obtained with atropine, with which all 3 doses tested increased the number of trials needed to cause catalepsy, and at the higher doses (5 and 10 mg/kg) most of the mice (80%) became insusceptible; atropine methyl bromide had no such effects. Thus, this kind of catalepsy may be mediated by cholinergic mechanisms that are central and not peripheral.

Catalepsy, which is commonly defined[1] as an immobile condition of waxy flexibility where the subject tends to remain in any imposed position, has been studied experimentally in various ways. One method which does not involve the use of drugs, such as neuroleptics or opiates[2,3], is a restraint-induced catalepsy (RIC). A technique for inducing RIC in mice, which is probably the same or akin to the reflex immobility state popularly known as 'animal hypnosis'[4,5], has recently been described by Amir et al.[6].

RIC is especially useful for studying cataleptic mechanisms because it can be produced without the confounding influence of drugs; one can therefore evaluate the effect of drugs which have known actions on specific neurotransmitter systems. This report describes the effects of drugs which alter function of the transmitter acetylcholine. The results strongly indicate that RIC is a central nervous system phenomenon that is dependent upon acetylcholine's action on muscarinic receptors.

RIC was produced and scored as previously described[6]: young adult outbred mice (25–30 g) were held on a flat surface by the scruff of the neck, using the thumb and index finger. After being kept immobile for 5 sec, the mouse was placed in an abnormal posture, with both forefeet placed on the top of a 5-cm wall. Initially, mice quickly removed their feet from the wall, but after about 6 such trials, with 20-sec inter-trial intervals, all of the undrugged mice would remain motionless with feet elevated for a criterion duration of 20 sec (fig. 1). The number of trials needed to reach criterion immobility, as well as the percentage of susceptible animals, provided the frame of reference for evaluating the cholinergic drugs.

Each test group contained 10 mice. Solutions were in concentrations that permitted the same volume to be injected irrespective of drug. The observor who scored the RIC did not know which drug, or saline, had been injected. Normal cholinergic function can be augmented by inhibit-