the stool. The spine was then severed at the base of the skull, and the intestine was removed as previously described.

The exsected intestine was immediately transferred to a beaker containing 50 ml of oxygenated saline (0.9% NaCl) at 0–4 °C, and the lumen was flushed through with the same solution delivered from a wash-bottle via the jejunal opening. The material was then everted on a surgical quality stainless steel rod (1.5 mm diameter, 40 cm length) and 4 everted sacs were prepared from the first 40 cm of the proximal jejunum[3]. Sacs were placed at random into 25 ml Erlenmeyer flasks and incubated for 30 min in 5.0 ml Krebs phosphate Ringer containing 28 m*M* glucose. The flasks were gassed continuously with 5% CO_2 in O_2 and maintained at 37 °C in a shaking incubator running at 100 oscillations per min. Total water transport was assessed by weighing the sacs empty and filled, prior to, and after incubation.

Results. The total fluid transport for each animal, expressed as ml per g initial wet weight per h, was derived as the mean value for 4 sacs, together with the standard error of the mean. The value for 7 animals sacrificed by ether anaesthesia was 0.37 ± 0.05 compared with 0.64 ± 0.06 for the 11 stunned and decapitated animals ($p < 0.01$).

Discussion. The removal of tissue from anaesthetized rather than freshly killed animals is usually justified on the grounds that it minimizes the period of arrested circulation prior to the isolation of the tissue and reduces the effects of shock induced by the trauma of sacrifice. Indeed some workers stress the importance of perfusing the intestine whilst the blood supply is still intact[4]. An obvious disadvantage of anaesthetics is their unknown effects on the metabolism of the intestine, and the necessity of working with tissue which has been exposed to unknown levels of potentially toxic materials.

In the present study it has been shown that the fluid uptake capacity of the isolated intestine is significantly lower in etherized as compared to stunned rats. The reasons for this remain obscure, but a reduction in glycolysis has been previously reported in everted sacs prepared from animals sacrificed with ether, and it seems possible that the effect is due to a direct toxic inhibition of energy-dependent processes in the mucosa[5].

It would be wrong to infer from the present results that stunning and decapitation is necessarily the ideal method of animal sacrifice, but it is suggested that minor aspects of the experimental technique used in this sort of work may have far-reaching effects on the viability of the tissue under study and this should be borne in mind when planning new research or comparing the accounts of other authors.

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Concerning the Ionic Basis of Presynaptic Inhibition

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Summary. The superfused rat cuneate nucleus has been used to investigate the sensitivity of primary afferent terminals and of evoked primary afferent depolarization (PAD) to alterations in extracellular K^+ and Cl^- ion levels. Results indicate that PAD is caused by an efflux of Cl^- from primary afferent terminals rather than by an increase in extracellular K^+.

Although it is generally accepted that depolarization of primary afferent terminals accompanies presynaptic inhibition in most vertebrate species, it is uncertain how this depolarization is brought about. The original suggestion[2] that primary afferent depolarization (PAD) is a secondary phenomenon dependent on an increase in extracellular potassium levels around afferent terminals has received support from recent studies using potassium-sensitive microelectrodes[3,4]. Another idea, strongly supported by electrophysiological and pharmacological observations, is that presynaptic inhibition is a primary synaptic event and that PAD is brought about by the direct action on afferent terminals of the presynaptic inhibitory transmitter, the amino acid GABA (γ-amino-*n*-butyric acid)[5,6]. Since the best established membrane actions of GABA are mediated by chloride ions rather than by potassium[7], the experiments reported here were designed to test the two hypotheses by investigating the sensitivity of primary afferent terminals and of evoked PAD to alterations in the extracellular levels of both potassium and chloride ions.

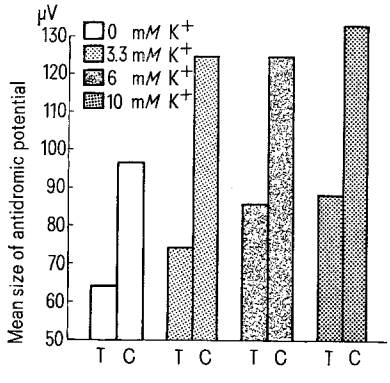

Fig. 1. The effect of increasing the potassium content of the cuneate superfusate in stages from 0 to 10 m*M* on both test (T) and conditioned (C) antidromic potential height. Testing and conditioning stimulus strengths remained constant throughout with conditioning stimulus strength supramaximal and interstimulus interval 15 msec.

Materials and methods. The exposed cuneate nucleus in chloralose-urethane anaesthetized rats was superfused

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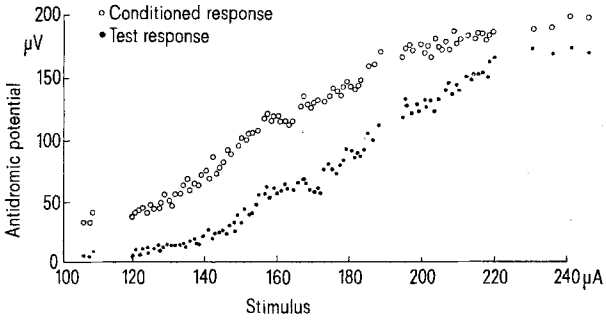

Fig. 2. Dependence of conditioned antidromic response amplitude on size of test response. Graph shows increase in size of test response (●) as testing stimulus strength is increased from just above threshold. Conditioning stimulus strength remains constant throughout at supramaximal with interstimulus interval 15 msec. Conditioned antidromic response height (▲) increases approximately in parallel with the test response.

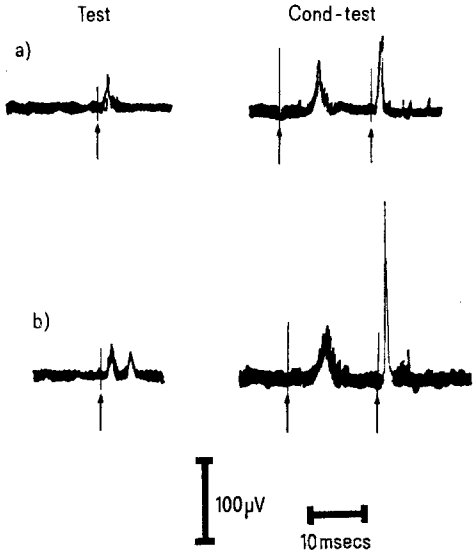

Fig. 3. Sample records of test and conditioned antidromic responses from the superfused cuneate nucleus recorded in the ipsilateral ulnar nerve. Variation between individual responses indicated by standard deviations in Figure 4. Superfusate in a) is control CSF and in b) low chloride CSF. Records in b) taken after 5 min low chloride superfusion. The small additional response evoked by the conditioning stimulus in both a) and b) is a dorsal column reflex which does not interfere with the following antidromic response. Arrows indicate stimulus artefacts. Stimulus strengths remain constant as in Figure 1.

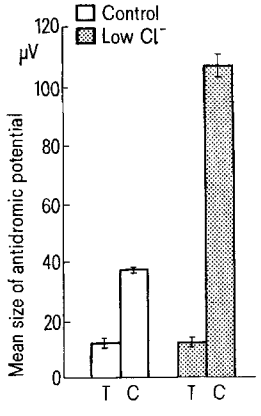

Fig. 4. Histogram summarizing data of Figure 3. T and C as in Figure 1. Bar height equals 1 standard deviation. Test response remains unchanged after low chloride superfusion but conditioned response increases by almost 200%.

with various artificial cerebrospinal fluid (CSF) solutions ranging from potassium-free to high potassium (10 m*M*), low chloride (155 m*M* sodium isethionate replacing sodium chloride) and a control solution[8]. WALL's[9] antidromic excitability testing technique was used to measure both the resting excitability of afferent terminals and evoked PAD in the superfused cuneate nucleus as described by DAVIDSON and SOUTHWICK[6]. PAD was evoked by supramaximal conditioning stimulation of the ipsilateral median nerve and antidromic potentials were recorded from the ipsilateral ulnar nerve.

Results and discussion. When the potassium content of the cuneate superfusate was increased in stages from 0 to 10 m*M*, afferent terminal excitability increased as indicated by the augmented test antidromic potential height. Conditioned antidromic potential height – a generally accepted measure of PAD[9] – also increased (Figure 1), but there is no indication that varying the potassium content of the superfusate above or below control values had any specific effect on evoked PAD. This conclusion is supported by the data shown in Figure 2. In this experiment the test antidromic response was gradually increased in size by increasing the size of the test stimulus. The cuneate nucleus was superfused with control CSF and the conditioning stimulus, which preceded the test stimulus by 15 msec, remained unchanged throughout at supramaximal. The graph shows clearly however, that the conditioned antidromic response height did not remain constant but steadily increased as the test antidromic response height increased, in a manner similar to that seen in Figure 1, when the test response height was increased by raising superfusate potassium levels.

In contrast, superfusion with low chloride CSF had little effect on resting afferent terminal excitability but produced large increases in evoked PAD. Figure 3 shows records of test and conditioned antidromic responses during superfusion of the cuneate nucleus with control (a) and low chloride CSF (b). Figure 4 shows the data in histogram form. Evoked PAD increased by almost 200% after low chloride superfusion, an effect which cannot be explained by any alteration in resting afferent terminal excitability during the experiment.

It is difficult to reconcile these data with the hypothesis that PAD is caused by an elevation in extracellular potassium levels. Because of the well-established GABA-nergic pharmacology of presynaptic inhibition[5,10] and the chloride mediated actions of GABA[7], it seems more reasonable to suggest that GABA could be the presynaptic inhibitory transmitter, and that PAD is caused by a GABA induced chloride efflux from primary afferent terminals. This would explain the large increase in evoked PAD observed in the present experiments during low chloride superfusion.

Already one depolarizing action of GABA – on sympathetic ganglion cells – has been shown to be chloride mediated, suggesting the existence of an inwardly directed chloride pump to maintain intracellular levels at greater than equilibrium distribution[11]. If the primary afferent neurone possesses such a pump it might also be expected to have a low resting chloride permeability to reduce the pump's energy expenditure. This could explain the lack of effect of low chloride superfusion on resting afferent terminal excitability (Figures 3 and 4).

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