Brit. J. Pharmacol. (1961), **17**, 224–231.

ACTION OF SOME DRUGS ON THE DORSAL ROOT POTENTIALS OF THE ISOLATED TOAD SPINAL CORD

BY

J. K. KIRALY* AND J. W. PHILLIS†

From the Department of Physiology, Australian National University, Canberra, Australia

(*Received July* 5, 1961)

A series of drugs which potentiate or antagonize transmission at cholinergic synapses have been tested on the slow depolarizing dorsal root potentials evoked by stimulation of adjacent dorsal and ventral roots. Some drugs acted specifically on the dorsal root potential evoked by ventral root stimulation. Dihydro-β-erythroidine, atropine and acetylcholine depressed this potential, whilst anticholinesterase substances in low concentrations potentiated and in higher concentrations depressed it. It is concluded that the results offer some evidence for a cholinergic link in the pathway responsible for the generation of depolarizing potentials in the dorsal root subsequent to ventral root stimulation.

The slow negative potential changes evoked in dorsal roots of the toad spinal cord by afferent volleys in adjacent dorsal roots were first described by Barron & Matthews (1938). Further investigations by Bonnet & Bremer (1938, 1952); by Dun & Feng (1944); by Eccles & Malcolm (1946); by Lloyd & McIntyre (1949) and by Göpfert (1956) have confirmed and extended these initial observations. Lloyd & McIntyre (1949) were able to distinguish in the dorsal root potentials (DRP) of the frog a series of potential changes, terminating in the prolonged negative potential described by Barron & Matthews (1938). They designated these deflections DRP I to V in order of temporal sequence and, of these, it is with DRP V that the present communication is concerned.

Dorsal root potentials set up by an antidromic volley in the segmental ventral root (VR–DRP) have also been described by Barron & Matthews (1938), Eccles & Malcolm (1948) and Lloyd & McIntyre (1949). The latter authors demonstrated that this potential, although being of lesser magnitude and having a longer latency, corresponded in general outline to the DRP V set up by stimulation of an adjacent dorsal root.

METHODS

All experiments were performed on lumbar segments of the isolated spinal cord of the Queensland Toad (*Bufo marinus*). The dissection and preparation bath have been previously described by Curtis, Phillis & Watkins (1961b). The cord was hemisected sagittally and was

* Present address: National Biological Standards Laboratory, Acton, Canberra, A.C.T., Australia.

† Present address: A.R.C. Institute of Animal Physiology, Babraham, Cambridge.

fixed in a small trough of 1.5 ml. capacity through which flowed amphibian Ringer solution of the following composition: NaCl 100 mM; KCl 2.5 mM; Na_2HPO_4 2.5 mM; NaH_2PO_4 0.45 mM; $CaCl_2$ 1.9 mM; $NaHCO_3$ 12 mM; glucose 2.8 mM. This solution had been equilibrated with 5% carbon dioxide and 95% oxygen before reaching the trough and had a *p*H 7.4. Pairs of platinum electrodes on the dorsal and ventral roots were used for both stimulating and recording. The dorsal root responses were displayed on an oscilloscope after amplification by an amplifier having a differential input and a time constant of 10 sec.

RESULTS

In accord with earlier investigations (Barron & Matthews, 1938; Eccles, 1947), the DRP was depressed by drugs which excite at peripheral cholinergic synapses. In addition it was found that these compounds also depressed the VR–DRP. Fig. 1A illustrates the potentials set up in a dorsal root by stimulation of the ventral

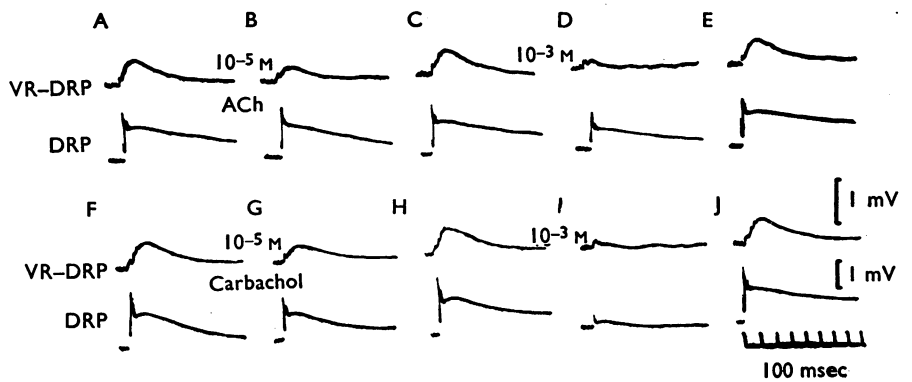

Fig. 1. VR–DRP, the potential evoked in the dorsal root (9th) by stimulation of the ventral root of the same segment. DRP, the potential evoked in dorsal root of 9th spinal segment by stimulation of dorsal root of 8th spinal segment. A, Control responses; B, after 4 min exposure to 10^{-5} M acetylcholine bromide; C, 2 min after washing; D, after 2 min exposure to 10^{-3} M acetylcholine; E, 12 min after washing. F, Control responses; G, responses after 2 min exposure to 10^{-5} M carbachol; H, recovery 8 min after washing; I, 2 min after exposure to 10^{-3} M carbachol; J, 22 min after washing. Time, 100 msec. Voltage calibration, 1 mV for VR–DRP above J, 1 mV for DRP above J.

root of the same segment (VR–DRP) and the adjacent dorsal root (DRP). A solution containing 10^{-5} M acetylcholine bromide was then passed through the bath for 4 min and the responses in Fig. 1B were recorded. The VR–DRP had been considerably reduced whereas there was very little alteration in the DRP. After washing for 4 min, the responses recovered (Fig. 1C). Two min after exposing the cord to a solution containing 10^{-3} M acetylcholine, the VR–DRP had been almost abolished and the height of the DRP had been reduced by nearly 50% (Fig. 1D). After washing, the potential took 10 min to recover (Fig. 1E).

Figs. 1F to J illustrate the effects of similar concentrations of carbachol on the same preparation. The onset of action of carbachol was slightly faster and recovery after washing slower than in the experiments with acetylcholine, but the magnitude of the depressant action of both drugs on the VR–DRP was about the same. Carbachol, however, depressed the DRP to a much greater extent than did acetylcholine. The excitant action of various amino acids (Curtis *et al.*, 1961b) on the

toad spinal cord has been found to be associated with a decrease in the magnitude of the dorsal root potential and the generation of a negative potential change in the dorsal root (Phillis, unpublished observations). As carbachol in low concentrations produced a similar increase in the excitability of the preparation, it was not unexpected that both types of dorsal root potential were depressed. When applied in low concentrations, acetylcholine was relatively ineffective in causing an increased excitability of this preparation, so that its depressant action on the VR–DRP might be a specific effect. When applied in concentrations of 10^{-3} M or more, acetylcholine depressed both the VR–DRP and DRP and increased the ventral root reflex discharge.

Koketsu (1956) has reported that the dorsal root potential set up by ventral root stimulation was increased by eserine. This was confirmed in the present investigation. Eserine and prostigmine in low concentrations caused a small increase in magnitude and prolongation in duration of the VR–DRP, but higher concentrations depressed the response. Figs. 2A and B illustrate the DRP, VR–DRP and ventral

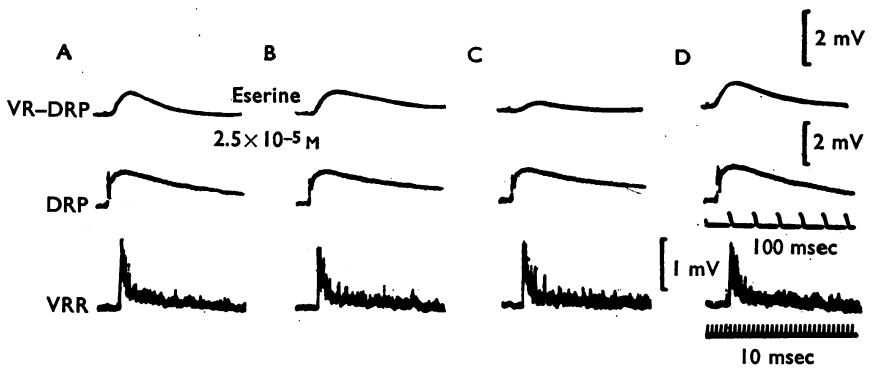

Fig. 2. Responses recorded from dorsal and ventral roots of 9th spinal segment of cord. VR–DRP and DRP are the potentials recorded from dorsal root following stimulation of corresponding ventral root and adjacent (8th) dorsal root respectively. VRR is response recorded from ventral root of segment after stimulation of 8th dorsal root. A, Control response ; B, response after exposure for 3 min to 2.5×10^{-5} M eserine salicylate ; C, response after a further 12 min of exposure to eserine solution ; D, responses after 70 min washing. Time, 100 msec for VR–DRP and DRP; 10 msec for VRR. Voltage calibration, VR–DRP, 2 mV; DRP, 2 mV; VRR, 1 mV.

root reflex response (VRR) before and after exposure of the cord to 2.5×10^{-5} M eserine salicylate. After 3 min of exposure (Fig. 2B), the VR–DRP was prolonged in duration whereas the DRP and VRR had not been altered. After 15 min of exposure, the VR–DRP had been reduced in magnitude, the DRP slightly reduced and the VRR increased slightly (Fig. 2C). After washing for 70 min, the responses recovered (Fig. 2D).

Three drugs, known to block cholinergic transmission, dihydro-β-erythroidine, hexamethonium and atropine, were tested on the magnitude of the DRP and VR–DRP. Dihydro-β-erythroidine in very low concentrations reduced the magnitude of the VR–DRP: 10 min after application of 10^{-6} M dihydro-β-erythroidine, the

control VR–DRP had been considerably reduced (Fig. 3B). After 10^{-4} M dihydro-β-erythroidine had been in the bath for a further 10 min the VR–DRP was almost abolished (Fig. 3C), but the DRP and VRR still remained virtually unaltered. Prolonged washing for 3 hr was necessary to restore the potential (Fig. 3D).

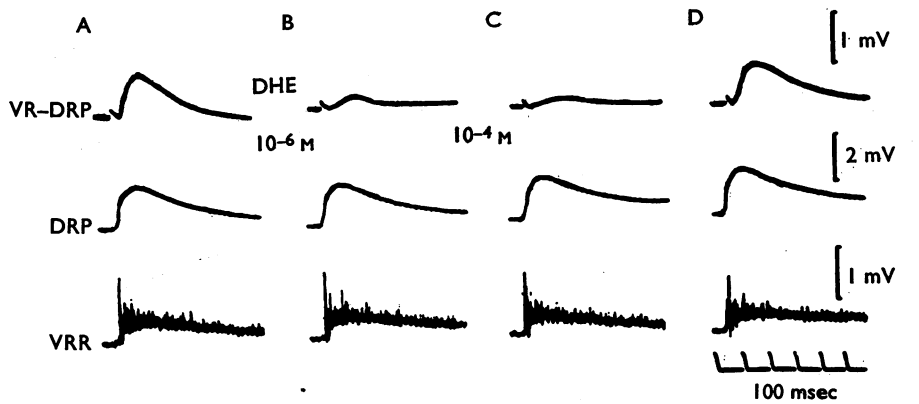

Fig. 3. Responses recorded from 9th spinal segment. See Fig. 2 for description of abbreviations. A, Control responses; B, responses after 10 min exposure to 10^{-6} M dihydro-β-erythroidine hydrobromide (DHE) ; C, after a further 10 min exposure to 10^{-4} M dihydro-β-erythroidine hydrobromide ; D, responses 3 hr after washing when VR–DRP had recovered. Time, 100 msec. Voltage calibrations, VR–DRP, 1 mV ; DRP, 2 mV ; VRR, 1 mV.

Atropine was less effective as a depressant of the VR–DRP. The minimal concentration which abolished the VR–DRP completely was 2.5×10^{-4} M (Fig. 4C), being equiactive with 10^{-5} M dihydro-β-erythroidine. Only after washing for 2 hr did the VR–DRP recover (Fig. 4D).

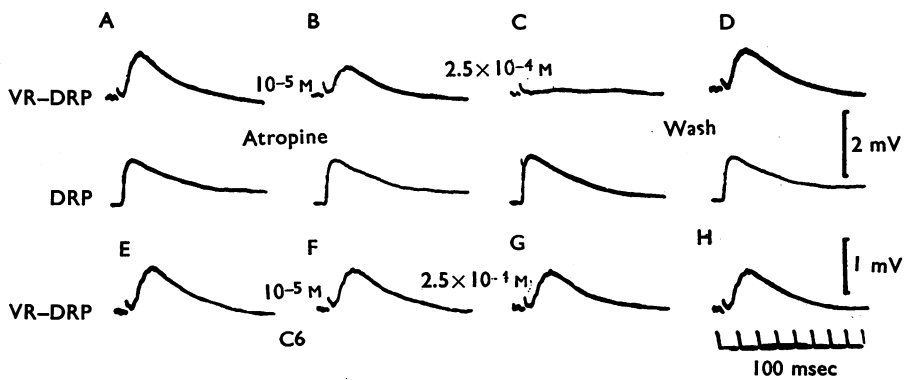

Fig. 4. Responses recorded from dorsal root of the 9th segment of spinal cord. See Fig. 2 for abbreviations. A, Control responses ; B, after 12 min exposure to 10^{-5} M atropine sulphate; C, after a further 12 min exposure to 2.5×10^{-4} M atropine sulphate ; D, 2 hr after washing. E, Control response ; F and G, after exposure for 12 min to 10^{-5} M and 2.5×10^{-4} M hexamethonium chloride (C6) respectively ; H, 1 hr after washing. Time, 100 msec. Voltage calibration, VR–DRP, 1 mV ; DRP, 2 mV.

Hexamethonium in high concentrations increased the excitability of the preparation and decreased the magnitude of both the DRP and the VR–DRP. However, the presence of 10^{-5} M and 2.5×10^{-4} M hexamethonium in the bath for periods of duration similar to those used with solutions of atropine had only a very slight depressant action on the VR–DRP (Figs. 4F and G).

The excitatory action of tubocurarine on the toad spinal cord has already been described by Eccles (1946). In most preparations in the present investigation, tubocurarine caused a reduction in the VR–DRP and the DRP, and greatly prolonged the ventral root after-discharge. After 10^{-5} M tubocurarine had been in the bath for 12 min, the responses in Fig. 5B were recorded showing that the drug

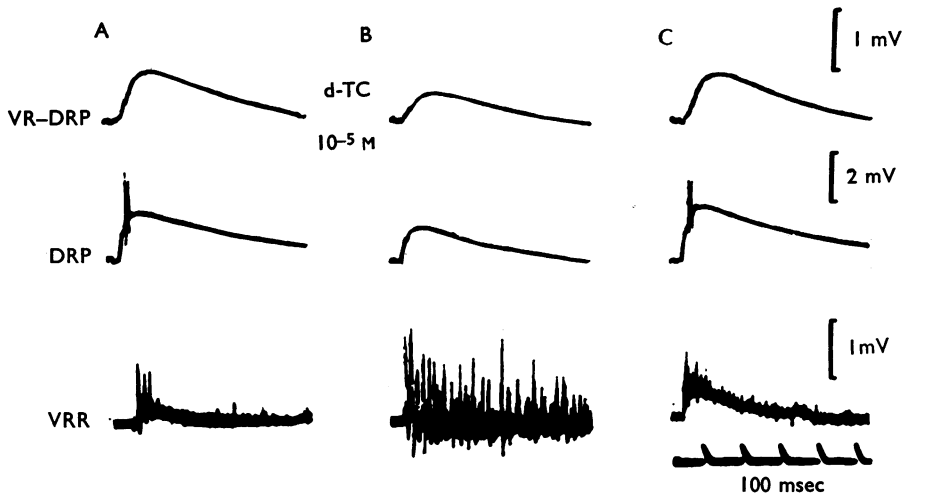

Fig. 5. Responses recorded from dorsal and ventral roots of 9th segment of spinal cord. See Fig. 2 for description of abbreviations. A, B and C, Responses before, after 12 min of exposure to 10^{-5} M tubocurarine chloride, and 1 hr after washing when the VR–DRP had fully recovered respectively. Time 100 msec. Voltage calibration, VR–DRP, 1 mV; DRP, 2 mV; VRR, 1 mV.

had a non-selective effect reducing both the VR–DRP and the DRP. The preparation was then washed and the responses recovered fully 1 hr later (Fig. 5C).

Usually the DRP was less affected than the VR–DRP, and in two preparations the VR–DRP was reduced in magnitude without a simultaneous reduction in the DRP and with very little change in the magnitude of the VRR to dorsal root stimulation. Perfusion with higher concentrations of tubocurarine in both these preparations was associated with a decrease of both the VR–DRP and the DRP and an increase in the VRR. It can be concluded, therefore, that in these cases there was evidence for a selective action on the system generating the VR–DRP.

DISCUSSION

Intracellular recording from dorsal root fibres of amphibia and mammalia (Koketsu, 1956; Eccles & Krnjevic, 1959) has shown that the dorsal root potential appears as a slow positive potential generated by a depolarization across the surface

membrane of the impaled nerve fibre. In discussing the genesis of these potentials it must be pointed out that each DRP in the present series was invariably that recorded from a root adjacent to the stimulated root and may therefore have arisen in a different fashion to the potentials generated at the pre-synaptic terminals of a stimulated root.

Available evidence indicates that dorsal root potentials are generated in the dorsal half of the spinal cord, for Pitts, McCulloch, Wall & Lettvin (1954) could only produce slow potentials in the dorsal root of cats by stimulation with a micro-electrode when the electrode was in the dorsal part of the cord, and Wall (1958) has concluded that only fibres terminating on interneurones which are concentrated in dorsal horns and intermediate nuclei appeared to be capable of generating the DRP. The ability of stimulated afferent fibres to induce potential changes in unstimulated fibres has led to the proposal that potassium ions (Barron & Matthews, 1938) or specific chemical transmitters (Fatt, 1954) released by active presynaptic fibre terminals depolarize adjacent terminals. Bonnet & Bremer (1938, 1952), Dun & Feng (1944), Eccles & Malcolm (1946), Lloyd & McIntyre (1949), Jung (1953) and Eccles & Krnjevic (1959) have suggested that interneuronal activity can contribute either by the flow of electric currents around the soma of such interneurones or by the release of transmitter from interneuronal axon terminals adjacent to primary afferent terminals. The very long duration of the DRP in amphibia suggests that sustained interneuronal activity is responsible for the later stages at least of the potential.

Koketsu (1956) concluded that the slow potential change in dorsal roots evoked by antidromic ventral root stimulation was due to the activity of motoneurones or interneurones. Its long latency (10 to 15 msec), however, apparently precludes its generation by the antidromic depolarization of the somas and dendrites of those motoneurones whose axons were stimulated. A possible route whereby interneurones in the dorsal horn of the preparation could be excited by ventral root volleys might commence along motor axon collaterals. If this be the case, then transmission at the first synapse on the pathway would be expected to be cholinergic, because other terminals of the same axons release acetylcholine at the neuromuscular junction (Dale, Feldberg & Vogt, 1936) and thus would be similar to the corresponding synapse in the cat (Eccles, Fatt & Koketsu, 1954 ; Eccles, Eccles & Fatt, 1956 ; Curtis & Eccles, 1958a and b). It was with this possible anatomical similarity in mind that this study of the pharmacology of the VR–DRP was undertaken.

The results with those drugs which depressed the VR–DRP only, and to which, therefore, may be attributed a specific action on the pathways responsible for generation of the VR–DRP, may be compared with pharmacological responses of the Renshaw cells in the cat. In low concentrations, acetylcholine, which excites Renshaw cells, specifically depressed the VR–DRP. This depressant action of acetylcholine may have been due either to a desensitization of the acetylcholine receptors (Thesleff, 1960) or to an occlusion of the response by the continuous background of excitation (Eccles, 1947 ; Curtis, Phillis & Watkins, 1961b). However, carbachol and higher concentrations of acetylcholine depressed the DRP as well as the VR–DRP and increased the general excitability of the preparation, as

evidenced by the development of depolarizing potential changes in both dorsal and ventral roots. In very low concentrations dihydro-β-erythroidine depressed the VR–DRP and was considerably more active than atropine and hexamethonium. It is also the most active of the drugs blocking cholinergic transmission on to Renshaw cells (Curtis & Eccles, 1958b ; Curtis, Phillis & Watkins, 1961a). Anticholinesterases, which prolong excitation of Renshaw cells by synaptically or iontophoretically released acetylcholine (Eccles *et al.*, 1954 ; Curtis & Eccles, 1958b), potentiated the VR–DRP when applied in very low concentrations and depressed it in higher concentrations.

While Cajal (1909) has provided histological evidence of the existence of motor axon collaterals in the amphibian spinal cord, as yet there is no physiological evidence of the existence of Renshaw-type cells (Washizu, 1960). The pharmacological findings in the present study, however, suggest that such a Renshaw-type system could be responsible for the generation of the dorsal root potential evoked by antidromic stimulation of the ventral root in amphibia.

REFERENCES

BARRON, D. H. & MATTHEWS, B. H. C. (1938). The interpretation of potential changes in the spinal cord. *J. Physiol.* (*Lond.*), **92**, 276–321.

BONNET, V. & BREMER, F. (1938). Étude des potentiels électriques de la moelle épinière faisant suite chez la grenouille spinale à une ou deux volées d'influx centripètes. *C.R. Soc. Biol., Paris*, **127**, 806–812.

BONNET, V. & BREMER, F. (1952). Les potentiels synaptiques et la transmission nerveuse centrale. *Arch. int. Physiol.*, **60**, 33–93.

CAJAL, R. Y. (1909). *Histologie du système nerveux de l'homme et des vertèbres*, Vol. I, p. 363. Paris: Maloine.

CURTIS, D. R. & ECCLES, R. M. (1958a). The excitation of Renshaw cells by pharmacological agents applied electrophoretically. *J. Physiol.* (*Lond.*), **141**, 435–445.

CURTIS, D. R. & ECCLES, R. M. (1958b). The effect of diffusional barriers upon the pharmacology of cells within the central nervous system. *J. Physiol.* (*Lond.*), **141**, 446–463.

CURTIS, D. R., PHILLIS, J. W. & WATKINS, J. C. (1961a). Cholinergic and non-cholinergic transmission in the mammalian spinal cord. *J. Physiol.* (*Lond.*), in course of publication.

CURTIS, D. R., PHILLIS, J. W. & WATKINS, J. C. (1961b). Actions of amino-acids on the isolated hemisected spinal cord of the toad. *Brit. J. Pharmacol.*, **16**, 262–283.

DALE, H. H., FELDBERG, W. & VOGT, M. (1936). Release of acetylcholine at voluntary motor nerve endings. *J. Physiol.* (*Lond.*), **86**, 353–380.

DUN, F. T. & FENG, T. P. (1944). A note on the two components of the dorsal root potential. *J. Neurophysiol.*, **7**, 327–329.

ECCLES, J. C. (1946). Synaptic potentials of motoneurones. *J. Neurophysiol.*, **9**, 87–120.

ECCLES, J. C. (1947). Acetylcholine and synaptic transmission in the spinal cord. *J. Neurophysiol.*, **10**, 197–204.

ECCLES, J. C., ECCLES, R. M. & FATT, P. (1956). Pharmacological investigations on a central synapse operated by acetylcholine. *J. Physiol.* (*Lond.*), **131**, 154–169.

ECCLES, J. C., FATT, P. & KOKETSU, K. (1954). Cholinergic and inhibitory synapses in a pathway from motor-axon collaterals to motoneurones. *J. Physiol.* (*Lond.*) **126**, 524–562.

ECCLES, J. C. & KRNJEVIC, K. (1959). Potential changes recorded inside primary afferent fibres within the spinal cord. *J. Physiol.* (*Lond.*), **149**, 250–273.

ECCLES, J. C. & MALCOLM, J. L. (1946). Dorsal root potentials of the spinal cord. *J. Neurophysiol.*, **9**, 139–160.

FATT, P. (1954). Biophysics of junctional transmission. *Physiol. Rev.*, **34**, 674–710.

GÖPFERT, H. F. (1956). Slow potentials in the dorsal parts of the isolated spinal cord and their relation to dorsal root potentials. *J. Physiol.* (*Lond.*), **133**, 433–445.

JUNG, R. (1953). *The Spinal Cord*, Ciba Foundation Symposium, p. 130. London : Churchill.

KOKETSU, K. (1956). Intracellular potential changes of primary afferent nerve fibres in spinal cord of cats. *Amer. J. Physiol.*, **184**, 338–344.

LLOYD, D. P. C. & MCINTYRE, A. K. (1949). On the origins of dorsal root potentials. *J. gen. Physiol.*, **32**, 409–433.

PITTS, W. H., MCCULLOCH, W. S., WALL, P. D. & LETTVIN, J. Y. (1954). Maps derived by bipolar stimulation of the spinal cord. *Fed. Proc.*, **13**, 112.

THESLEFF, S. (1960). Effects of motor innervation on the chemical sensitivity of skeletal muscle. *Physiol. Rev.*, **40**, 734–752.

WALL, P. D. (1958). Excitability changes in afferent fibre terminations and their relation to slow potentials. *J. Physiol.* (*Lond.*), **142**, 1–21.

WASHIZU, Y. (1960). Single spinal motoneurones excitable from two different antidromic pathways. *Jap. J. Physiol.*, **10**, 121–131.