Antidromic Impulses Recorded from the Dorsal Root and Peripheral Nerve of the Frog[1), 2)]

By

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(*With 5 Text-figures*)

Many investigators have observed that the antidromic impulses which originate from the spinal cord along the dorsal roots (dorsal root reflex) are elicited by an afferent volley (e.g. Matthews 1934, Barron and Matthews 1938a, b, Toennies 1938, 1939, Eccles and Malcolm 1946, Brooks and Koizumi 1956, Tregear 1958). Some of them have suggested a hypothesis to explain this dorsal root reflex, in which it is suggested that the antidromic dorsal root impulse is probably elicited by depolarization of the central terminals of the dorsal root fibres, which is recorded as the dorsal root potential (d.r.p.). On the other hand, it has also been suggested that some antidromic impulses are not generated by the d.r.p., but by the recurrent fibre or fibre interaction in the dorsal column fibres (Barron and Matthews 1935, Barron 1940, Habgood 1953). The dorsal root reflex, therefore, may contain various antidromic impulses produced by different physiological events in the spinal cord. If any of this antidromic volley is transmitted to the dorsal root through the ordinary synatpic connections in the central nervous system, and if it possesses a physiological function in controlling a peripheral effector or receptor, some of the impulses usually referred to as antidromic should be regarded as orthodromic efferent impulses in the dorsal root, which indicates a necessity for revision of the classical Bell-Magendie's law.

In the present experiments, the frog spinal cord was used in an attempt to clarify the nature of the antidromic impulse by examining its relation to the d.r.p. and its physiological significance in the peripheral function.

Material and Methods

Preparation: The spinal cord of the bullforg, *Rana catesbeiana* Shaw was used in all of the experiments. The spinal cord was separated from the medulla oblongata by a thick needle inserted from the dorsal side. The skin covering the hind-limbs was completely

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stripped away and the peripheral nervous system innervating the hind-limb muscles was severed. In addition, after the skin on the dorsal side of the body and the musculature surrounding the vertebrae were removed, a laminectomy was carefully performed and the entire length of the spinal cord was exposed. The spinal cord attached to the ventral half of the vertebrae and its peripheral nerves supplying the hind-limbs was removed and immediately placed in a plastic chamber filled with frog Ringer's fluid. Experiments were made on preparations maintained in a physiologically stationary state for about half an hour. Recording and stimulation were made on the 9th and 10th dorsal roots and on their peripheral nerves such as the sciatic, the tibial and the peroneal nerve. All of the ventral roots supplying the hind-limbs were removed in order to eliminate the influence of peripheral stimulation on the antidromic conduction of the motoneurones. Before recording from the drosal roots, the condition of the spinal cord was tested by the reflex impulse discharge recorded from a central portion of the cut ventral roots.

Stimulation and recording: A square pulse generator was used as a stimulator. A single pulse, usually 0.1 msec in duration, was applied with a pair of stimulating electrodes of the Ag-AgCl type through an isolating circuit to reduce the stimulus artifact. The dorsal roots or the peripheral nerves were lifted out of the saline bath and laid over a hooked electrode of the Ag-AgCl type for recording. A silver plate about 4 square cm was placed in the Ringer's fluid bath as an indifferent electrode. Nerve responses were recorded through a high gain, R-C coupled amplifier and photographed from an oscilloscope screen.

Solution and temperature: Frog Ringer's fluid has the following ionic composition; 120.0 mM NaCl, 2.0 mM KCl, 1.2 mM $CaCl_2$, 2.0 mM Na-phosphate, buffered to a pH of 7.2 by isotonic $NaHCO_3$. The cooled and oxygenated Ringer's solution was perfused around the isolated spinal cord and the temperature of the saline bath was maintained at 8–15°C. The room temperature during the experiments was 18–25°C.

Results

Responses of an intact dorsal root to peripheral stimulation: Figure 1 shows the responses elicited in an intact dorsal root by a single electrical stimulation of the peripheral nerve. There was a large afferent volley followed by an irregular burst of impulses lasting about 30–50 msec, which were surmounted on the d.r.p. The pattern of these irregular impulses was not consistent, even in the same preparation. There question remains whether all irregular impulses are ordinary antidromic impulses originating from the spinal cord, or orthodromic impulses from the spinal ganglion. It is also possible that they are a mixture of both. To examine this problem, records were obtained from the peripheral portion of the cut dorsal root following peripheral stimulation. The response produced in the cut dorsal root showed a pattern of irregular impulses very similar to that in the intact dorsal root. This indicates that most of the irregular impulses were initiated by repetitive firing of the spinal ganglion cells following the arrival of the afferent volley. But this still leaves the possibility of ordinary antidromic impulses being included in this irregular pattern. The true antidromic impulses leaving the spinal cord could not be accurately analysed following peripheral stimulation in an intact dorsal root.

It was observed that impulses from the spinal ganglion, generated either spontaneously or following a peripheral volley, conducted in both centripetal and

centrifugal directions.

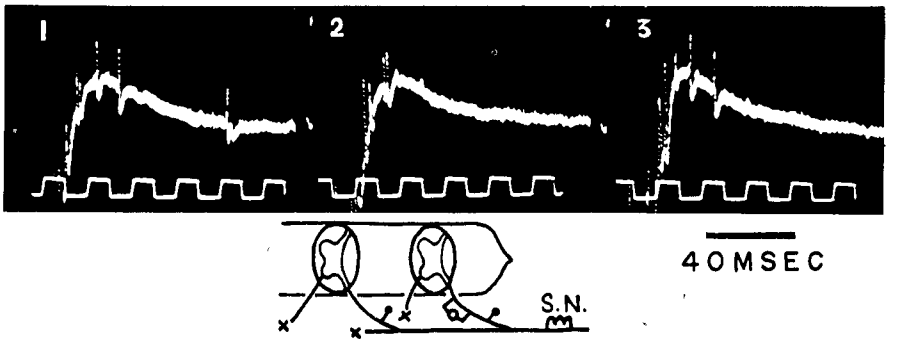

Fig. 1. Responses in the 10th dorsal root to a single stimulus to the sciatic nerve. Three records from the same preparation. Positions of the stimulating and recording electrodes are shown in the inset diagram. The stimulus artifact appears at the beginning of the sweep. × shows position of severed nerves.

Patterns of antidromic discharges: In order to eliminate discharge in the dorsal root originating from the spinal ganglion, and to examine the true components of the antidromic impulses leaving the spinal cord, the response from a

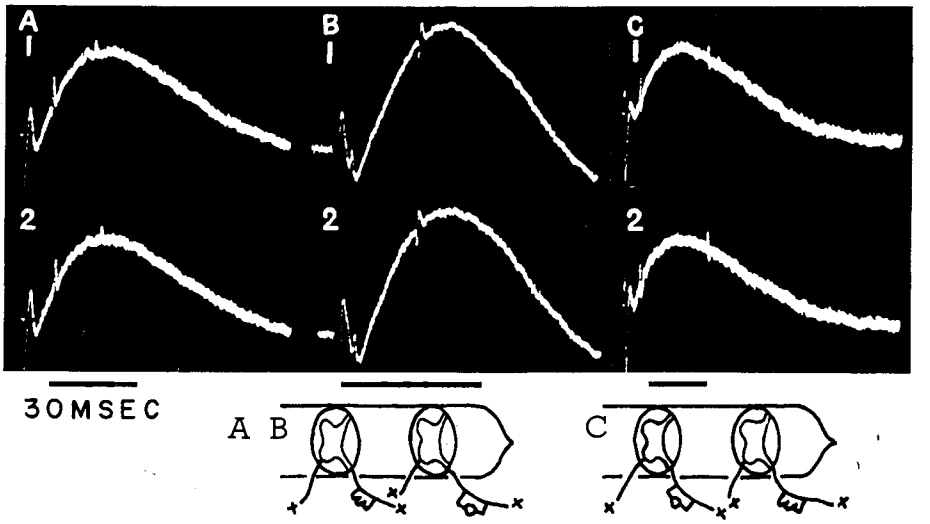

Fig. 2. Antidromic impulses in severed dorsal root produced by a single stimulus to adjacent dorsal root. Most of the impulses are surmounted on the d.r.p. Records taken from three different preparations (A, B and C). Potential deflections seen just after the start of stimulation are the catelectrotonic potential. Positions of the recording and stimulating electrodes shown in the inset diagrams.

cut dorsal root was recorded after stimulation of an adjacent dorsal root. Figure 2 gives three pairs of examples of the antidromic impules in the cut 10th (A and B) and 9th dorsal root (C) produced by stimulation of the adjacent 9th or 10th dorsal root. Most of the antidromic spike potentials were surmounted on large d.r.p. following small catelectrotonic potentials just after the beginning of stimulus (Lloyd 1952). The nature of the impulses produced differed from the irregular pattern originating in the spinal ganglion, because the latent period of the responses obtained in any one of the preparations was uniform with each stimulus as is shown in the two paired records in Fig. 2. Since there is no spinal ganglion in the transmission route between the positions of stimulation and recording, the regular impulses obtained were undoubtedly true antidromic impulses descending from the spinal cord along the dorsal root.

However, from preparation to preparation, there was considerable variation in the latent period of the antidromic discharges on the dorsal roots. And, in addition, in some preparations, no antidromic discharges could be elicited, even though the preparations were quite fresh.

In general, most of the antidromic discharges appeared in the rising phase and the peak of the d.r.p. Occasionally, some additional discharges appeared in the exponential decay phase, as is shown in Figs. 2, C and 4, No. 9.

Central reflex delay: The central delay within the spinal cord of the dorsal root reflex was calculated by subtracting the conduction time of the centripetal and centrifugal nerve from the conduction time between the stimulating and the recording position. The minimal delay was 1–2.5 msec (first small spike in Fig. 2, B) and a longer delay of 5.5–29.5 msec was obtained from impulses appearing on the rising phase and the summit of the d.r.p.

Since the observed minimal delay in the central reflex was more than 1 msec, it is inconceivable that the antidromic impulses are initiated by the recurrent fibre (Matthews 1934, Barron and Matthews 1935). The wide variation in the measured delay results from the difference in the response patterns from preparation to preparation.

Response of the peripheral nerve: Figure 3 shows the simultaneous responses recorded from the 10th dorsal root and the sciatic nerve following stimulation of the 9th dorsal root. As is shown in this figure, impulses on the dorsal root which were surmounted on the d.r.p. were antidromically conducted towards the periphery. Corresponding spikes of very small size appeared on the sciatic nerve. These spikes could not be the electrotonic spread of the antidromic impulses, because there is no sign of spread in the d.r.p. The recorded sciatic nerve responses were very small. This apparently suggests that only a few fibres in the nerve trunk are responsible for conduction of the antidromic impulses. Small impulses of the same nature were also recorded from the tibial and peroneal nerve branching away from the sciatic nerve.

The conduction velocities of the dorsal 'efferent' fibres which transmitted the

antidromic impulses from the spinal cord were calculated from the conduction time and the distance between two recording sites. At 12°C, the conduction velocity of the spikes appearing on the rising phase of the d.r.p. was 13m/sec, and that of the spike on the rounded peak, 19m/sec. These values indicate that the fibres conducting the antidromic impulses are 'A' fibres.

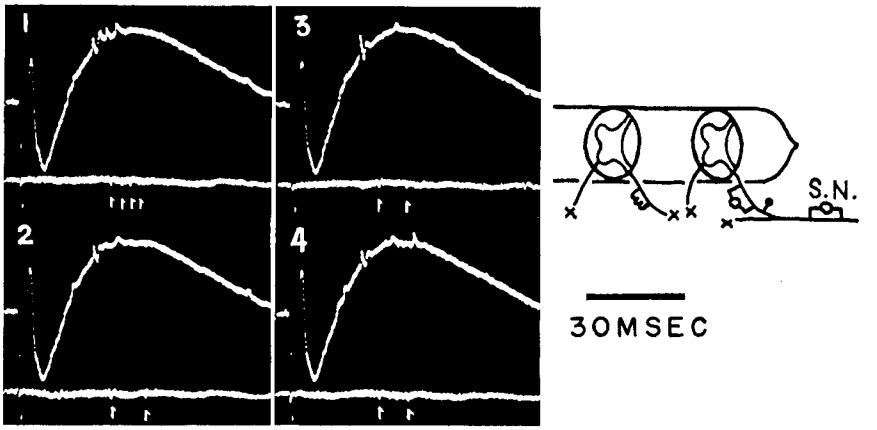

Fig. 3. Antidromic impulses simultaneously recorded from 10th dorsal root (upper traces) and sciatic nerve (lower traces) in response to a single stimulus applied to severed 9th dorsal root. Arrows under all lower traces indicate positions of small impulses. Four records of same preparation. Positions of electrodes shown in the inset diagram.

Relation between the dorsal root potential and the antidromic impulse: It has been postulated that the occurrence of antidromic dorsal root impulse was closely connected to the greatest slope of the rising phase of the d.r.p. (Eccles and Malcolm 1946, Tregear 1958). However, as may be seen in Fig. 2, antidromic impulses occurred, even when the height of the d.r.p. was small. The impulse appeared on various parts of the d.r.p., suggesting that there was no such strict requirement in the production of antidromic impulse.

In order to test the relationship between the d.r.p. and the antidromic impulse, the pattern of the antidromic impulses in response to the gradual increase in the height of the d.r.p. was examined (Fig. 4). When the stimulus intensity was relatively weak, the height of the d.r.p. was low, and the slope of its rising phase was gentle, and few impulses appeared (Fig. 4, No. 2–6). Another small impulse appeared on the decaying phase of the larger d.r.p. close to its summit, and moved closer as the stimulus strength increased (Fig. 4, No. 7, 8). After maximal stimulation was repeatedly applied, still another antidromic impulse sometimes appeared on the exponential decaying phase of the d.r.p. (Fig. 4, No. 8, 9). It should be noted that although all of the impulses on the rising phase of the d.r.p. had slight variation in the central delay, the one impulse which appeared on the decaying phase close to the summit of the d.r.p. had a central delay varying over a range of 5 msec,

depending upon the stimulus strength and the location of its appearance.

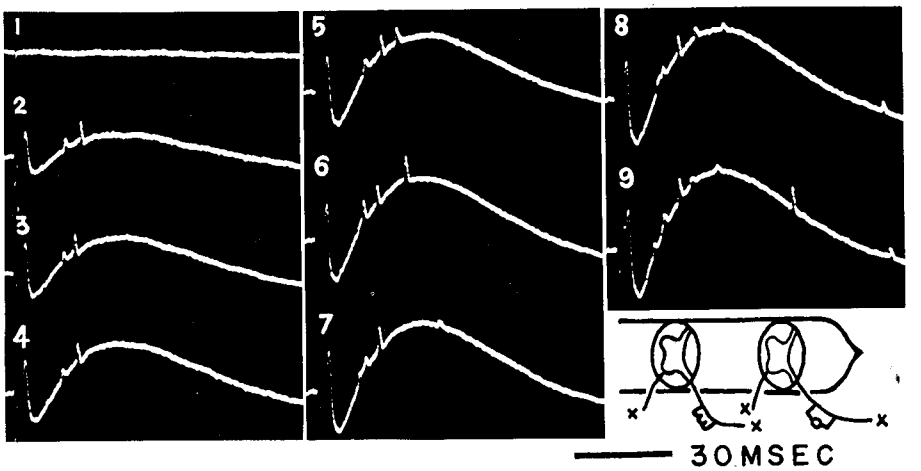

Fig. 4. Antidromic impulses in the severed 10th dorsal root produced by single stimulation of the severed 9th dorsal root. Stimulus strength gradually increases from 1 to 9. Positions of electrodes shown in the inset diagram.

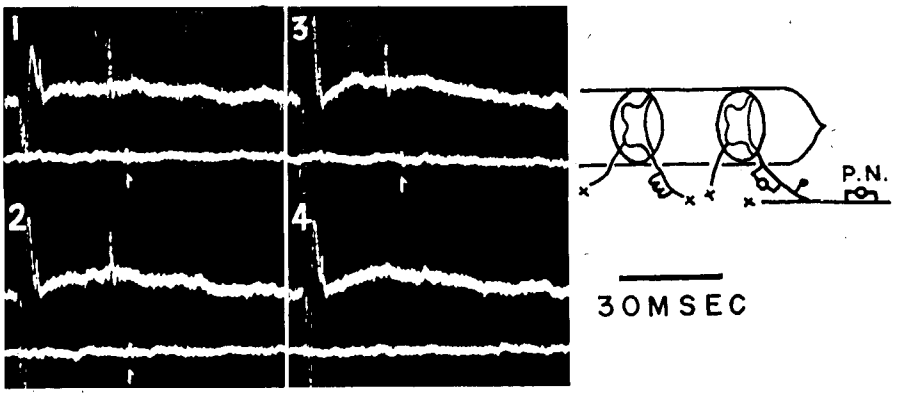

Fig. 5. Antidromic impulses in 10th drosal root (upper traces) and peroneal nerve (lower traces) produced by single stimulation of the 9th dorsal root. Stimulus intensity gradually increases from 1 to 3. All records from deteriorated preparation. Note blockage of second impulse in 4. Large potential deflections seen immediately after stimulation begins are catelectrotonic potential. Positions of electrodes shown in the inset diagram.

When the rate of stimulation was increased to 1/sec, the impulse on the summit of the d.r.p. disappeared first, while most of the impulses on the rising phase remained. Further increase of the rate of stimulation removed the dorsal root reflex. This suggests that the refractory depression of the impulse appearing on

the summit of the d.r.p. is of longer duration than that of the impulses on the rising phase. Therefore, the nature of the impulse appearing on the summit appeared to be somewhat different from that of the impulses on the rising phase.

In deteriorated preparations, no ventral root potential or resulting efferent impulse on the ventral root was produced by stimulation of the dorsal root (Sato 1962). Figure 5 illustrates the antidromic response recorded in this condition. A volley on the cut 9th dorsal root produced a few antidromic spikes on the 10th dorsal root, on which the d.r.p. appeared at a very low height. In this case, the central delay in the first and second spikes was 2 and 22 msec, respectively. These values fall in the range of the central delays in antidromic impulses on the rising phase, which were measured in the experiments shown in Figs. 2 and 4. This experimental evidence also implies that there are some differences in the mechanisms which produce impulses on the rising phase of the d.r.p. and those produced on the peak of the d.r.p.

Discussion

In some of the preparations, antidromic dorsal root impulses were not elicited, even though the preparations were quite fresh and capable of producing both d.r.p. and normal efferent impulses. There was rather wide variation in the number and correlation with the d.r.p. of the antidromic impulses produced in successful preparations. This is in striking contrast to the uniformity of responses usually observed in ordinary reflex pathways. In addition, there appeared to be no close correlation between the generation of the antidromic impulses and the magnitude of the d.r.p., with the exception of one impulse observed close to the summit. Since this impulse is the only one likely to be elicitied by the depolarizing effect of the d.r.p., the generating mechanism of the antidromic impulse suggested by Eccles and Malcolm (1946) and Tregear (1958) could only be verified with this particular impulse.

A different explanation appears to be necessary for the occurrence of other antidromic impulses. Among the few possible mechanisms, local fibre interaction may account for the generation of some of the antidromic impulses, especially on the basis of the observed central delay. Renshaw and Therman (1941) on the spinal cord, and Granit and Skoglund (1944) on mammalian nerve, reported a delay of between 0.1 and 0.3 msec attributable to local fibre interactions. On the other hand, Arvanitaki (1940) found the delay of less than 5 msec on non-myelinated crustacean fibres, and on myelinated mammalian fibres, Rosenblueth (1941) found a delay of between 1.0–2.5 msec. The central delay of 1–2 msec observed in the first few antidromic impulses on the rising phase of the d.r.p., is readily explainable by this mechanism, however, as most of the remaining antidromic impulses on the rising phase of the d.r.p. had central delays of more than 5 msec, they are unlikely to be initiated directly by fibre interactions.

The following process may be postulated as the generation mechanism of

these impulses: An area of local fibre interaction produced by the afferent volley, is hyperexcitable, and the same area is further excited by the d.r.p., and eventually, antidromic impulses are initiated. This hypothesis is also supported by the fact that, in the deteriorated preparations, antidromic impulses were observed on low d.r.p. with gentle slope, suggesting that a large d.r.p. is not necessary for the firing of these impulses.

The very small size of the observed antidromic impulses indicates that the number of fibres in the nerve trunk which contribute to the conduction of antidromic impulses is very small. If the size of the action potential recorded from the nerve trunk is roughly in proportion to the number of the excited fibres, the number of the fibres concerned in conduction of antidromic impulses might be calculated. Gasser and Erlanger (1927) reported that there are about 1,500 fibres in the 10th dorsal root of the bullfrog. Comparison of the height of the synchronized afferent volley in the 10th dorsal root to that of the antidromic impulses indicates that a maximum of about 70 fibres (4% of all of the fibres in the 10th dorsal root) contribute to the conduction of antidromic impulses. If those antidromic impulses have any physiological meaning, alteration of the excitability of the receptor during its refractory or supernormal period may be expected to be a possible function of the impulses. However, antidromic impulses can only be elicited with a large afferent volley set up in the dorsal root by strong stimulation. This is by no means to be expected in normal physiological activity. Although, in the present experiments spontaneous activity originating in the spinal ganglion was sometimes observed, it is difficult to presume that this activity has any physiological significance in relation to the peripheral function.

Barron (1940) and Renshaw and Therman (1941) suggested that fibre interaction was merely an artifact induced by the exposure of the spinal cord, and since, in the present experiments, only a few antidromic impulses were initiated, despite exposure of the spinal cord, it appears quite unlikely that the dorsal root reflex occurs in normal intact animals.

Kotsuka and Naito (1962) demonstrated in the bullfrog the existence of efferent autonomic nerves having a vasodilatory effect. The nerves originate in the spinal cord and pass through the dorsal root. The possibility of recording the activity of these nerves was completely eliminated in the present experiments by careful isolation of the dorsal root from the sympathetic chain. It was also eliminated by experimental determination of 'A' fibres conducting antidromic impulses.

It may, therefore, be concluded that the antidromic impulse has no physiological significance in peripheral control. This conclusion is also supported by the fact that the antidromic impulse is not of ordinary efferent nature. In other words, the classical Bell-Magendie's law still holds, in respect to the somatic nervous system of the dorsal root.

Summary

1. Irregular impulses recorded from an intact dorsal root following an afferent volley were found to be composed of (1) antidromic impulses from the spinal cord, and (2) orthodromic impulses from the spinal ganglion.

2. Most of the antidromic impulses appeared on the rising phase and at the summit of the d.r.p., with a central reflex delay of 1–29.5 msec. The impulse pattern varied among preparations.

3. Fibres conducting the antidromic impulses were determined to be of the 'A' type, composing about 4% of the fibres of the 10th dorsal root.

4. The possible mechanisms of antidromic impulses and their physiological significance in peripheral control were discussed. It was concluded that Bell-Magendie's law still holds despite the existence of antidromic impulses along the dorsal root.

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