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THE EFFECTS OF CHRONIC SECTION OF DORSAL ROOTS ON THE RESPONSIVENESS OF MOTONEURONES TO 5-HYDROXYTRYPTAMINE AND A SUBSTANCE P ANALOGUE

M. H. T. ROBERTS & D. M. WRIGHT[1]

Department of Physiology, University College, P.O. Box 78, Cardiff CF1 1XL

1 Experiments were performed on rats 14–21 days after unilateral dorsal root section in order to determine if the effects on motoneurone excitability of a substance P analogue, 5-hydroxytryptamine (5-HT) and noradrenaline were altered by section of primary afferents.

2 The effects of iontophoretic applications of these agents on motoneurone excitability were quantified by measuring the change in amplitude of the short latency field potentials evoked antidromically, by ventral root stimulation.

3 Iontophoretic application of the substance P analogue (eledoisin-related peptide, ERP) always produced an increase in the amplitude of the field potential. These increases in amplitude were 25.9% larger on the sides of the cords with sectioned dorsal roots. This was not a statistically significant difference ($P > 0.05$).

4 Section of dorsal roots did not alter responses to noradrenaline.

5 Responses to 5-HT were significantly larger following section of dorsal roots. There is very little evidence for the release of 5-HT by primary afferents and denervation supersensitivity is an improbable explanation. It is possible that descending 5-HT systems directly excite motoneurones and indirectly inhibit primary afferent transmission. Dorsal root section would alter the balance between these actions of 5-HT in favour of an excitatory effect.

Introduction

Substance P has a potent depolarizing action on motoneurones (Konishi & Otsuka, 1974; Zieglgänsberger & Tulloch, 1979). Immunohistochemical evidence has shown that substance P-like immunoreactivity occurs in two systems that converge onto the spinal grey. It is contained in primary afferents with small diameter axons (Hökfelt, Johansson, Kellerth, Ljungdahl, Nilsson, Nygards & Pernow, 1977) which terminate principally in the dorsal horn and in the terminals of the medullary raphe-spinal system which also contain 5-hydroxytryptamine (5-HT) (Björklund, Emson, Gilbert & Skagerberg, 1979). Section of dorsal roots increases the responses of dorsal horn neurones to iontophoretic application of a substance P analogue but has no effect on the responses of these cells to glutamate or 5-HT (Wright & Roberts 1978). The present study examines the effect of dorsal root section on the sensitivity of ventral horn cells to a substance P analogue, noradrenaline and 5-HT.

A preliminary account of these data was communicated to the Physiological Society (Roberts & Wright, 1978).

[1]Present address: Department of Anatomy, The Medical School, University of Bristol, Bristol BS8 1TD.

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Methods

Male albino Wistar rats (300–450g) were used in these experiments.

Dorsal rhizotomy

Animals were anaesthetized with halothane (1% in O_2) administered via a face mask. An area of the dorsal surface over the lumbro-sacral region was shaved and cleaned with hibitane (ICI Ltd.). A longitudinal incision approximately 2cm in length was made along the line of the vertebral column. A limited dissection was performed to expose the dorsolateral lamina of one side of a lumbar vertebra (L4 or L5). Care was taken to ensure that the point of attachment of the dorsal long back muscles to the transverse process was not sectioned. Muellers lacrymal retractors were used to keep the bone surface exposed. A 2mm hole was drilled through the vertebral lamina with a dental burr, just dorsal to the transverse process. A microlance with a bent tip was then used to hook two or three dorsal roots of one side. These were then sectioned with a pair of iridectomy scissors. The burr hole was filled with a small piece of sterispon No. 2 (Allen & Hanburys); overlying muscle and connective tissue were sutured and the wound sprayed with terramycin aerosol spray (Pfizor).

Experiments were performed 14–21 days following dorsal rhizotomy. Halothane (0.5% in O_2) was again used as the anaesthetic. Carotid artery blood pressure, respiratory rate and ECG were monitored throughout the experiment.

Identification of sectioned dorsal roots

The lumbar spinal cord was exposed by laminectomy. Sectioned dorsal roots could be identified visually and by their inability to conduct compound action potentials.

Antidromically evoked motoneurone field potentials

Antidromically evoked motoneurone field potentials were used as a measure of motoneurone excitability. Ventral roots were sectioned and the central ends placed over bipolar silver stimulating electrodes. Square wave pulses of 0.05 ms were delivered every 3 s to these electrodes by an isolated stimulator controlled by a digitimer (Devices Ltd.). Multibarrelled microelectrodes were used to record extracellular motoneurone field potentials. These fields were usually composed of an initial positive, negative and late positive component. Quantification of drug effects involved the measurement of the alteration in the amplitude of the negative component, thought to represent depolarization of the soma-dendritic membrane (see Figure 1). The negativity of this component varied with depth, being maximal between 1000–1500 μm below the cord surface. A peak detector unit was used to measure the amplitude of the field potential which was recorded by a polygraph. Each upward deflection of the pen gave the amplitude of the field potential. Drug effects were quantified by measuring the maximum drug-induced increase in field potential amplitude directly from the polygraph record. This was divided by the mean pre-drug amplitude of the field potential and expressed as peak % potentiation.

The other parameters measured were: the duration of the increase in field potential amplitude and the latency of the increase measured from the beginning of the drug application to the onset of the response.

Drugs

Drugs were applied iontophoretically from multibarrelled glass micro-electrodes. Two barrels were filled with 3 M NaCl; one was used for recording, the other to control and test for artifacts due to the passage of iontophoretic current (Roberts & Straughan, 1967). The other barrels contained the substance P analogue, eledoisin related peptide (ERP, 1.0 mM in 0.1 M NaCl, pH 5.0, Sigma) 5-hydroxytryptamine bimaleinate (5-HT, 0.2 M, pH 4.0, Koch-Light) and noradrenaline hydrochloride (NA, 0.2 M, pH 3.5, Koch-Light).

In a smaller preliminary series of experiments substance P (1.0 mM in 0.1 M NaCl, pH 5.0, Beckman) was applied.

Experimental design

A preliminary study was conducted to determine suitable drug 'doses'. It was necessary to apply ejecting and retaining currents with constant intensity and duration since variation in the interaction of these currents can have profound consequences on the size and time course of the neuronal response (see for example, Bradshaw, Szabadi & Roberts, 1973). A timing circuit (Bevan & Bradshaw, 1973), allowing for programmed application of ejecting and retaining currents in a regular cycle, was used to achieve this. Following examination of the preliminary study it was decided to apply ERP with a current of 100 nA for 60 s, repeated every 180 s. 5-HT and NA were always applied with a current of 100 nA for 60 s repeated every 240 s. The response to a first application of any drug was always ignored. Only one study was made per track.

The protocol used was as follows: the microelectrode was vertically advanced into the spinal cord from a position close to the entry zone of the sectioned dorsal roots. Penetrations were made alternately on the sides with sectioned and non-sectioned dorsal roots. The intensity of ventral root stimulation was adjusted to be just suprathreshold. Polygraph records were taken of the amplitude of the antidromically evoked motoneurone field potential and then drugs were applied iontophoretically.

Results

Iontophoretic applications of ERP, 5-HT, NA and substance P caused increases in the amplitude of the motoneurone field potentials evoked by ventral root stimulation. Typical polygraph records are shown in Figure 1. In this series of experiments it was found that on three occasions when substance P and ERP were applied to the same neurone, the compounds had qualitatively similar effects (for example see Figure 1d). This finding supports the suggestion made previously (Wright & Roberts, 1978) that ERP may be used as an inexpensive model for substance P action in the central nervous system. Figure 1c compares the effects of 5-HT, NA and ERP. It can be seen that although all three drugs produced similar increases in field potential amplitude there were differences in the duration of these effects. Those to ERP occurred with the shortest latency to onset and were of short duration, whereas those to the amines were often considerably longer; 5-HT effects were

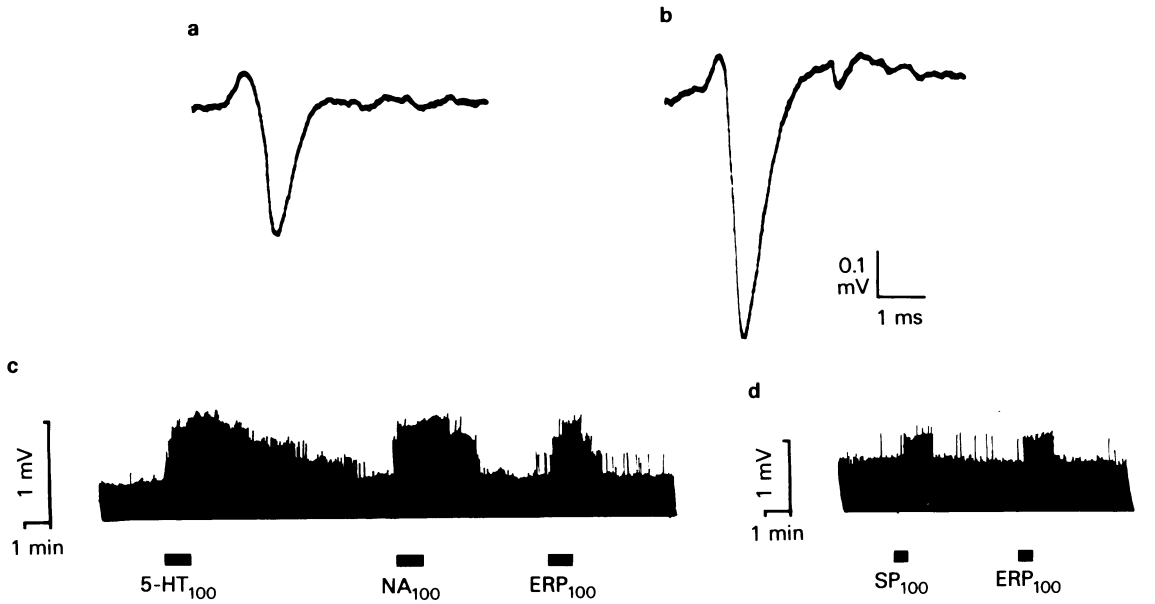

Figure 1 Effects of iontophoretically applied drugs on motoneurone field potentials. (a) Recording from the ventral horn of a motoneurone field potential antidromically evoked by stimulation of a ventral root. (b) The negative component of the field potential increased in amplitude during iontophoretic application of 100 nA 5-hydroxytryptamine (5-HT). (c) Polygraph record of the changes in amplitude of the field potential during iontophoretic applications of 5-HT, noradrenaline (NA) and eledoisin-related-peptide (ERP). The amplitude of the field potential is represented by each upward deflection of the polygraph pen. An increase in the height of this line reflects an increase in amplitude of the field potential. It can be seen that although the increases in amplitude appear similar when these drugs were applied with iontophoretic currents of equal intensity and duration, the duration of the effects were not. (d) A typical record demonstrating that similar iontophoretic currents of substance P (SP) and ERP resulted in similar increases in the amplitude of the field potential.

the most persistent.

5-HT increased the amplitude of 35 field potentials and NA had a similar effect on 20 field potentials; 17 of the potentials affected by 5-HT were also affected by ERP (48.6%) and 15 of the potentials affected by NA were affected by ERP (75%). In the side of the spinal cords with sectioned dorsal roots these percentages were not grossly different: ERP affected 48.5% of the 33 field potentials affected by 5-HT and ERP affected 55.6% of the 9 field potentials affected by NA.

Table 1 compares the effects of ERP on motoneurone field potentials recorded in the sides of rat spinal cords with with sectioned and intact dorsal

Table 1 Comparison of the effects of iontophoretic application of eledoisin-related peptide (ERP) on the amplitude of the negative component of the motoneurone field potential in the sides of rat spinal cords with sectioned and non-sectioned dorsal roots

	Sides with non-sectioned dorsal roots	*Sides with sectioned dorsal roots*	*Difference*
Peak % potentiation	79.7 ± 13.9	105.6 ± 22.8	+25.9
Latency to effect (s)	19.0 ± 2.5	16.4 ± 2.5	−2.6
Duration of effect (s)	101.7 ± 5.7	96.4 ± 6.0	−5.3
n	23	17	

Values are: mean ± s.e. mean. See Methods for details of measurement.

roots. From this table it can be seen that similar iontophoretic applications of ERP had greater effects on the motoneurone field potential when dorsal roots had been sectioned. The difference was found to be 25.9%, but neither the variance ratio test nor Student's t test showed this difference to be significant. Furthermore, there was no difference in the duration of the effect of ERP in intact and deafferented sides.

5-HT also had a greater effect on the amplitude of the field potential in the sides of the spinal cords with sectioned dorsal roots than in those with intact dorsal roots. This difference was greater than that obtained with ERP. From Table 2 it can be seen that 5-HT caused a mean peak % potentiation of $70.1 \pm 6.0\%$ in the sides with intact dorsal roots and $112.5 \pm 14.9\%$ in the sides with sectioned dorsal roots. This constituted a 42.4% increase in the effect of 5-HT in the deafferented sides which was highly significant (see Table 2). Figure 2 shows typical polygraph records of the effects of 5-HT on the amplitude of field potentials in the sides of spinal cord with sectioned and non-sectioned dorsal roots.

NA had similar effects on the motoneurone field potential on both sides of the spinal cord (see Table 3). This suggests that the increased effect of 5-HT in the deafferented sides was not a non-specific change in motoneurone responsiveness.

Discussion

This study has shown that the amplitude of motoneurone field potentials is increased by the iontophoretic application of 5-HT, NA or ERP. It has been demonstrated previously that an increase in field potential amplitude reflects an increase in the

Table 2 Comparison of the effects of iontophoretic application of 5-hydroxytryptamine (5-HT) on the amplitude of the negative component of the motoneurone field potential in the sides of the rat spinal cords with sectioned and non-sectioned dorsal roots

	Sides with non-sectioned dorsal roots	*Sides with sectioned dorsal roots*	*Difference*
Peak % potentiation	70.1 ± 6.0	112.5 ± 14.9	$+42.4^{a,b}$
Latency to effect (s)	28.6 ± 2.8	22.6 ± 2.3	-6.0
Duration of effect (s)	176.0 ± 8.7	201.6 ± 9.1	$+25.6^{c}$
n	37	38	

Values are: mean ± s.e. mean. See Methods for details of measurements
[a]F-test, $P<0.001$; [b]t test, $P<0.02$; [c]t test, $P<0.05$.

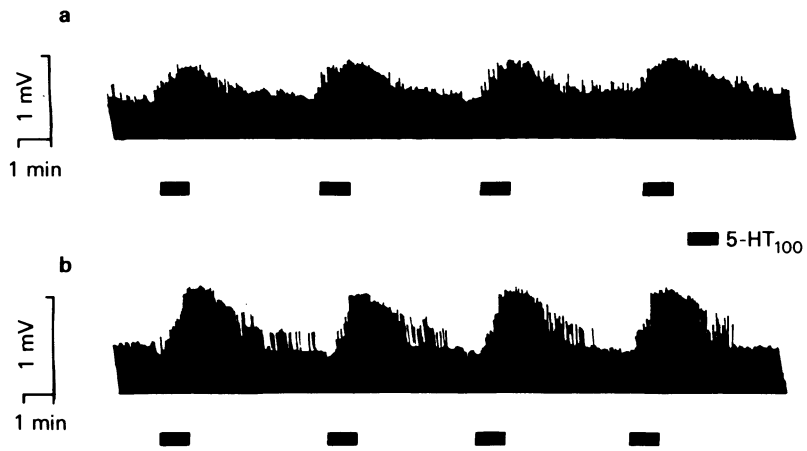

Figure 2 A typical polygraph record of the effects of 5-hydroxytryptamine (5-HT) on the amplitude of field potentials in the sides of the rat spinal cord with non-sectioned (a) and sectioned (b) dorsal roots. It can be seen that the same iontophoretic application of 5-HT (100 nA, 60 s) caused significantly greater increase in amplitude in the deafferented sides.

Table 3 Comparison of the effects of iontophoretic application of noradrenaline (NA) on the amplitude of the negative component of the motoneurone field potential in the sides of rat spinal cords with sectioned and non-sectioned dorsal roots

	Sides with non-sectioned dorsal roots	*Sides with sectioned dorsal roots*	*Difference*
Peak % potentiation	74.3 ± 10.0	68.5 ± 13.3	−5.8
Latency to effect (s)	19.8 ± 3.0	13.7 ± 2.7	−6.1
Duration of effect (s)	218.8 ± 12.0	189.6 ± 17.8	−29.2
n	20	10	

Values are: mean ± s.e. mean. See Methods for details of measurement

excitability of motoneurones (Barasi, Parry & Roberts, 1976; Parry & Roberts, 1980). Konishi & Otsuka (1974) and Zieglänsberger & Tulloch (1979) have reported that substance P depolarizes motoneurones. As ERP is a potent and relatively specific agonist of substance P receptors (see Wright & Roberts, 1978), it is likely that ERP increases the excitability of motoneurones by an action on substance P receptors.

Partial deafferentation of the spinal cord by cutting dorsal roots on one side led to a significant increase in the size of responses to 5-HT; a non-significant increase in responses to ERP and no change in responses to NA. These results contrast with those made in the dorsal horn where section of dorsal roots increased the sensitivity of interneurones to ERP but did not alter responses to 5-HT or glutamate (Wright & Roberts, 1978).

It is well known that partial deafferentation of the spinal cord leads to hyperactivity of spinal neurones (Loeser & Ward, 1967; Macon, 1979), and it may be predicted that section of dorsal roots would cause a general alteration in the responsiveness of cells to excitatory drugs. However, it is unlikely that such a general increase in excitability caused the increased responsiveness to 5-HT as responses to NA were not affected by dorsal root section and responses to ERP were not significantly larger. Although several authors found that the section of afferent pathways in the CNS failed to cause a specific supersensitivity (Krnjević, Reiffenstein & Silver, 1970; Macon, 1979) some recent reports show that selective increases in sensitivity to a known neurotransmitter can occur (Wald, 1976; Haas, Wolf, Palacios, Garbarg, Barbin & Schwartz, 1978; Wright & Roberts, 1978; McCall & Aghajanian, 1979). Nevertheless there seems to be little reason to suggest that section of dorsal roots cuts axons which contain 5-HT and that cells in the ventral horn become supersensitive to 5-HT as a result. 5-HT is not known to exist in dorsal root axons and interneurones containing 5-HT have not been detected (Steinbusch, Verhofstad & Joosten, 1978). Although Lacković (1978) gave a preliminary report that 5-HT may exist in primary afferents and Shibuya & Anderson (1968) showed that 25% of 5-HT in the spinal cord survived total spinal transection, these reports have not yet been confirmed or explained.

It is believed that all or most 5-HT in the spinal cord is contained by axons and terminals of the raphe-spinal system (Dahlstrom & Fuxe, 1965). Electrophysiological experiments have shown that some interneurones (Biscoe, Curtis & Ryall, 1966; Randić & Yu, 1976; Belcher, Ryall & Schaffner, 1978) and motoneurones (Barasi & Roberts, 1974) are excited by 5-HT and Belcher *et al.* (1978) have also suggested that 5-HT has a presynaptic inhibitory action on some primary afferents. It seems possible that the section and degeneration of dorsal roots removed the presynaptic inhibitory influence of iontophoretically applied 5-HT and, as a result, the postsynaptic excitatory effects of 5-HT appeared much larger.

The non-significant increase in the size of responses to the substance P analogue, ERP, suggests that no supersensitivity of motoneurones to ERP results from section of dorsal roots. This might be in keeping with the view that either motoneurones do not receive an innervation by fibres from the dorsal roots which contain substance P, or that the innervation is too sparse to influence the responsiveness of motoneurones significantly.

Some neurones in the medullary raphe-spinal system contain both 5-HT and substance P (Chan-Palay, Jonsson & Palay, 1978; Hökfelt, Ljungdahl, Steinbusch, Verhofstad, Nilsson, Brodin, Pernow, & Goldstein, 1978; Singer, Sperk, Placheta & Leeman, 1978). It is not possible to speculate on the interaction between 5-HT and substance P on the pre or postsynaptic membranes of these cells but the results of the present study may indicate that such an interaction exists and that section of afferent fibres containing substance P alters the sensitivity of cells to 5-HT.

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