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THE MODIFICATION OF LUMBAR MOTONEURONE EXCITABILITY BY STIMULATION OF A PUTATIVE 5-HYDROXYTRYPTAMINE PATHWAY

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1 Changes in lumbar motoneurone excitability were monitored by recording spinal reflex activity from the ventral roots of rats anaesthetized with fluothane.

2 Electrical stimulation of nucleus raphes medianus increased the amplitude of the monosynaptic reflex via a pathway having a slow conduction velocity. Stimulation elsewhere in the lower brain stem was less effective. This increase in motoneurone excitability was potentiated by the intravenous injection of L-tryptophan and reduced by intravenous injections of lysergic acid diethylamide (LSD), methysergide or Cinanserin.

3 Extracellular field potential responses to stimulation of dorsal or ventral roots were recorded with six barrelled microiontophoresis electrodes. Stimulation of nucleus raphes medianus and iontophoretic application of 5-hydroxytryptamine (5-HT) both increased the excitability of lumbar motoneurones as reflected by an increase in field potential amplitude.

4 Responses to both stimulation of raphe nuclei and iontophoretic application of 5-HT were reduced by iontophoretic application of Cinanserin and methysergide.

5 The similarities of the responses of lumbar motoneurones to applied 5-HT and activity within the raphe-spinal pathway are discussed. It is suggested that activity within the raphe-spinal pathway can increase lumbar motoneurone excitability via the release of 5-HT in the ventral horn of the spinal cord.

Introduction

It is now several years since the distribution of 5-hydroxytryptamine (5-HT) containing nerve terminals in the central nervous system has been studied using techniques of fluorescence histochemistry (Dahlström & Fuxe, 1964; Fuxe, 1965). These studies suggested that the terminals originate from cell bodies in the raphe nuclei of the lower brain stem and make intimate contact with interneurones and motoneurones of the spinal cord. Evidence from the central nervous system (CNS) generally supports the belief that 5-HT may be a neurohumoral transmitter (Chase & Murphy, 1973) but controversy exists between the view that its actions when applied to cells by microiontophoresis are depressant (Frederickson, Jordan & Phillis, 1972) and that it has both excitatory and depressant effects (Avanzino, Bradley & Wolstencroft, 1966; Roberts & Straughan, 1967; Hosli, Tebecis & Schonwetter, 1971).

The physiological significance of activity in the raphe-spinal pathways is also unclear. Studies involving the intravenous administration of precursors of 5-HT (Andén, Jukes & Lundberg, 1964; Anderson & Shibuya, 1966; Marley & Vane, 1967; Banna & Anderson, 1968) showed that the resulting increase in neuronal 5-HT levels is accompanied by an increase in amplitude of the spinal monosynaptic reflex (MSR) evoked from a dorsal root. Selective perfusion of the spinal cord and rostral sectioning of the cord indicated that these effects arose from an action at the level of the spinal cord. The effect of these drugs could have resulted from the direct excitation of motoneurones or the inhibition of inhibitory interneurones.

Studies by Ahlman, Grillner & Udo (1971) and Ellaway, Pascoe & Trott (1973) have indicated that there is an increase in gamma motoneurone discharge following the intravenous administration of 5-hydroxytryptophan (5-HTP). In addition there is an increase in the tonic stretch reflex although the underlying mechanism is uncertain.

The direct application of 5-HT onto spinal interneurones or motoneurones by microiontophoresis has usually caused either the depression of cell excitability or has been without effect

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(Curtis, Phillis & Watkins, 1961; Engberg & Ryall, 1966). Two studies, however, have noted the occurrence of infrequent excitatory responses (Weight & Salmoiraghi, 1966; Phillis, Tebecis & York, 1968). In the spinal cord therefore the direct effects of 5-HT are uncertain, the effects of intravenous precursor administration resulting from either an action upon motoneurones or interneurones.

Direct electrical stimulation of the raphe-spinal pathway has been studied by Clineschmidt & Anderson (1970). The amplitude of the monosynaptic reflex could be depressed or increased by stimulation of raphe nuclei depending upon the interval between the stimulation of raphe nuclei and stimulation of the dorsal roots. The authors found that only the depressant effects of stimulating raphe nuclei were blocked by a 5-HT antagonist. It is therefore uncertain whether activity in spinal 5-HT systems excites or inhibits motoneurones (or both); whether stimulation influences the motoneurone directly or via an interneurone; and whether the stimulation of raphe nuclei has effects which are physiologically and pharmacologically similar to those of applying 5-HT by iontophoresis. The present studies were undertaken in an attempt to answer these questions.

We have found that facilitation of the monosynaptic reflex is preferentially evoked from the midline of the lower brain stem at the level of the nucleus raphes medianus. The facilitation is reduced by 5-HT antagonists and increased by potentiating agents. Microiontophoretically applied drugs have qualitatively similar effects upon both the response to raphe stimulation and the response to iontophoretic application of 5-HT.

Preliminary accounts of some of these experiments have been published elsewhere (Barasi & Roberts, 1973a, b).

Methods

Experiments were performed upon 136 Wistar rats of either sex weighing between 300-400 grams. The animals were anaesthetized with 1.5% fluothane in O_2; a tracheal cannula was inserted and a maintenance level of 0.5% fluothane administered for the rest of the experiment. Rectal temperature was maintained at $37 \pm 1^{\circ}C$ by an automatically controlled heating pad. Blood pressure was monitored via a cannula in the femoral artery, and in most experiments was approximately 100 mmHg. The femoral vein was also cannulated to allow intravenous injections.

A lumbar laminectomy was performed between L2 and L5 as soon as the animal was in the stereotaxic frame. Dorsal and ventral roots originating from segments L2-L5 were cut and laid upon silver wire electrodes. The temperature of the liquid paraffin covering the spinal cord was controlled at $37 \pm 1^{\circ}C$ via a second automatic heating device.

Stimulation of raphe nuclei

A concentric bipolar stimulating electrode was stereotaxically placed into nucleus raphes medianus at co-ordinates A.P. + 0.35; L 0.0; V −2.6 according to the atlas of Konig & Klippel (1963). These electrodes were constructed from stainless steel tubing with an outer diameter of 0.5 mm and insulated with araldite epoxy resin (Pz 985). The inner wire projected 0.5 mm. In some experiments an array of 5 bipolar steel electrodes was inserted into the lower brainstem. The electrodes were 1.0 mm apart in a line parallel to the coronal plane of the brain. The central electrode was stereotaxically positioned in the nucleus raphes medianus. In the course of the experiment, the array was systematically moved in steps of 1 mm in the vertical plane to the limits of 2 mm below and 4 mm above the target. The effectiveness of conditioning stimulation (see below) at each of 35 points in the brain stem was subsequently superimposed upon a photograph of the tracks made by these electrodes in the brain. In this way the anatomical structure associated with potentiating or depressant effects of brain stem stimulation was identified. These results were confirmed in other experiments where accidental or deliberate misplacement of a single stimulating electrode was identified by histological examination of the brain stem after the experiment.

Stimulation parameters

Optimum stimulation parameters as judged by the maximal MSR change were identified in preliminary experiments. As a result, conditioning stimulation of the raphe nuclei consisted of 10 square pulses, 0.5 ms in duration at a frequency of 100 Hz with an intensity of between 0.025 and 0.175 mA. The interval between the onset of the conditioning train and the test stimulation of the dorsal root was set at 120 milliseconds.

The excitability of motoneurones and the effects of stimulation and drugs were examined by two techniques.

Recording the monosynaptic reflex from ventral roots and intravenous administration of drugs

Dorsal and ventral roots were selected which gave a good amplitude MSR. The potentials were

displayed on an oscilloscope (Tektronix 502 A) and the reflexes were photographed directly or averaged by a computer (Biomac 1010) or recorded by a pen writing oscillograph.

Stimulation of the dorsal root (test stimulation) was often varied in intensity to allow the construction of stimulus/response amplitude curves. When a standard test stimulus was used, its duration was 0.1 ms and its intensity was always just greater than that giving a maximal response. The reflex was evoked by dorsal root stimulation every 4 seconds. Following establishment of constant MSR and polysynaptic reflex amplitudes to test stimulation of dorsal roots, the effect of conditioning stimulation of raphe nuclei was studied. The test response amplitudes were often found to be stable to within 10% over periods of 3 h or more. Usually, however, after 30 min of constant response amplitude a drug was administered intravenously. The changes in response amplitude were plotted against time. The maximum change in response amplitude is reported as a percentage of the pre drug response amplitude. The following drugs were administered dissolved in injection saline (0.9% w/v NaCl solution): lysergic acid diethylamide tartrate (Sandoz) 5-15 μg/kg in solution adjusted to give a total injection volume of 0.5 ml; 2'-(3-dimethylaminopropylthio) cinnamanilide hydrochloride (Cinanserin–Squibb), 3-4 mg/kg, total volume 0.5 ml; methysergide bimaleate (Sandoz) 0.75-1.0 mg/kg also administered in a total volume of 0.5 ml. L-tryptophan was emulsified with Tween 80 and suspended in saline; 0.5 ml was administered either intravenously or intraperitoneally in doses ranging between 80-200 mg/kg.

Microelectrode recording from motoneurones and iontophoretic application of drugs

The animals were prepared in the same way as for the previous experiment. Dorsal and ventral roots were laid upon silver wire electrodes used to elicit orthodromic and antidromic field potential responses from motoneurones. These responses were recorded with a 5 or 6 barrelled microelectrode inserted into the lumbar enlargement of the cord just lateral to the entry of the dorsal roots. The electrode followed a vertical path through the cord to a maximum depth of 2 mm, antidromic responses being encountered most frequently between 1 mm and 1.5 mm. A conditioning–test design was used as in the previous experiment to study the effect of raphe stimulation on the orthodromically or antidromically evoked response of motoneurones. Stimulation parameters were identical with those used in the previous experiment.

The methods of recording and microiontophoretic ejection of drugs from microelectrodes have been described elsewhere (Roberts & Straughan, 1967). The recording barrel of the microelectrode was filled with 3 M saline and connected via an electrometer amplifier to the oscilloscope. Ions were ejected from the remaining four (or five) barrels of the microelectrode by the passage of a metered current from a constant current generator. Between periods of ejection of the biologically active ions, their diffusion into the environment of the cells was inhibited by passage of a 25 nA current of the opposite polarity (retaining current).

The electrodes were filled by boiling at 70°C under reduced pressure in double distilled water; the water was replaced with drug or saline solution and 24 h allowed for diffusion of the solution to the electrode tips. Drug solutions used were: 5-hydroxytryptamine bimaleate (0.2 M, pH 3.0, Koch-Light. In some experiments solutions at pH 5.0 and 7.0 were made by the addition of NaOH); lysergic acid diethylamide tartrate (0.01 M, pH 4.5, Sandoz); Cinanserin hydrochloride (0.2 M, pH 3.0, Squibb); methysergide bimaleate (0.01 M, pH 3.5, Sandoz); imipramine hydrochloride (0.2 M, pH 7.0, Geigy); sodium L-glutamate (0.2 M, pH 6.5); glycine 0.2 M, pH 3.0).

Results

The effect of stimulating raphe nuclei on the spinal monosynaptic reflex

The dorsal root evoked MSR recorded from ventral roots was studied in 68 rats. In most preparations the MSR exceed 1.5 mV with a latency to onset between 0.8 and 1.2 ms and a duration of 1.5-2 ms (Figure 1a). Polysynaptic discharges (PSR) were also seen, having a lower amplitude and a longer latency. Both MSR and PSR could be recorded for 2-3 h under stable conditions, with a variation usually around 10%.

In 85% of the experiments, conditioning stimulation of the raphe nuclei increased the MSR amplitude by between 50 and 200% depending upon the experimental conditions (see below). In the remaining experiments raphe stimulation was ineffective or elicited weak or variable increases in MSR amplitude. Polysynaptic reflexes were reduced or abolished by raphe stimulation. An example of these effects is shown in Figure 1b.

To control for the effects of fluothane, 12 animals were prepared by a mid-collicular decerebration (crossing H 0.0 at AP + 1.50), and fluothane anaesthesia withdrawn. In all these

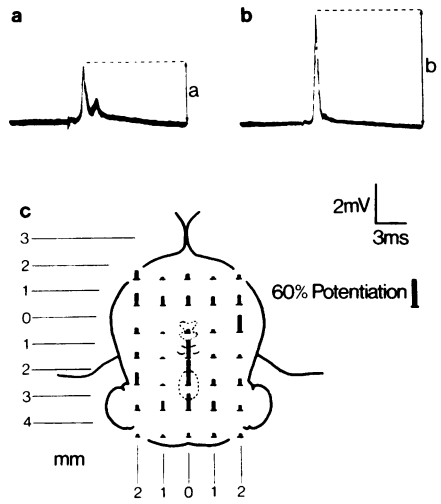

Fig. 1 Potentiation of the monosynaptic reflex (MSR) by stimulation of the lower brain stem. The upper traces are three superimposed spinal reflexes recorded from lumbar ventral roots and evoked by stimulation of the corresponding dorsal root. Column height = $((b - a)/a) \times 100$.

(a) The reflex evoked in the absence of conditioning stimulation (unconditioned). The MSR has a latency of approximately 1.0 ms and is followed by polysynaptic activity.

(b) Spinal reflex evoked 120 ms after the start of conditioning stimulation applied to the raphe nuclei. The MSR amplitude has been doubled whilst the polysynaptic responses have been abolished.

(c) The percentage increase in the MSR amplitude resulting from conditioning stimulation applied to different areas of the brain stem. A calibration bar representing 60% potentiation is shown to the right. The most marked MSR potentiation was evoked from a region corresponding to nucleus raphe medianus (the approximate limits of which are outlined by a dotted line). Stimulation of nucleus raphe dorsalis (also outlined) provided little MSR potentiation.

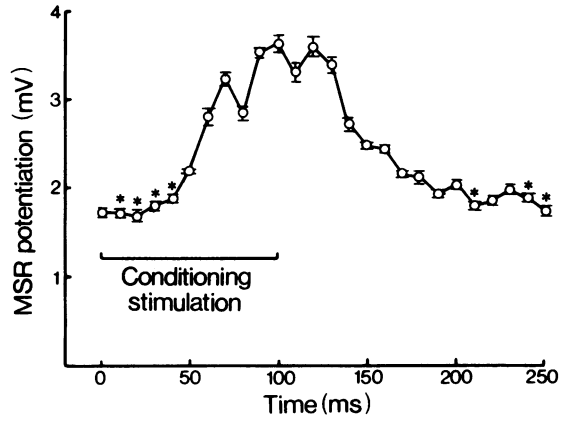

Fig. 2 The potentiation of the monosynaptic reflex (MSR) by conditioning stimulation applied at different intervals before test stimulation of the dorsal roots. Each point is the mean amplitude of 4-6 reflexes. The vertical lines indicate the standard error of the mean. Asterisks indicate a deviation from the unconditioned reflex amplitude which was not significant at the 5% level (Student's *t* test). Little potentiation was recorded during the first 40 milliseconds. Between 50 and 100 ms the degree of potentiation rapidly increased. The potentiating effects of conditioning were finally lost about 100 ms after the termination of the conditioning train.

animals conditioning stimulation increased the amplitude of the MSR.

The degree of MSR potentiation was dependent upon the intensity and frequency of conditioning stimulation. The adopted parameters of between 0.025 and 0.175 mA and 100 Hz were submaximally effective, being limited by us due to considerations of current spread within the brain stem.

The interval between the start of the 100 ms conditioning train and the test stimulation of dorsal roots was 120 milliseconds. Increasing this interval progressively reduced the degree of potentiation until at approximately 200 ms the potentiating effects were lost. Applying the test stimulus earlier than 50 ms after the start of the conditioning train also resulted in little potentiation (Figure 2).

Histological examination of the lower brain stem following each experiment indicated that the position of the conditioning electrode was an important factor influencing the degree of potentiation of the MSR. In 12 animals, we investigated this further by inserting an array of five coaxial steel stimulating electrodes stereotaxically oriented across the coronal plane of the brainstem. We applied conditioning stimulation to each electrode in turn, lowered the array 1 mm and repeated the process. Figure 1c shows the results from a typical experiment, in which a test MSR was evoked 120 ms after initiation of a 100 ms conditioning train. Although lesser degrees of potentiation were observed from other areas of the lower brain stem, the greatest potentiations were evoked from the nucleus raphes medianus. Little effect was seen following conditioning of nucleus raphes dorsalis. It should be noted that as the parameters of stimulation were constant

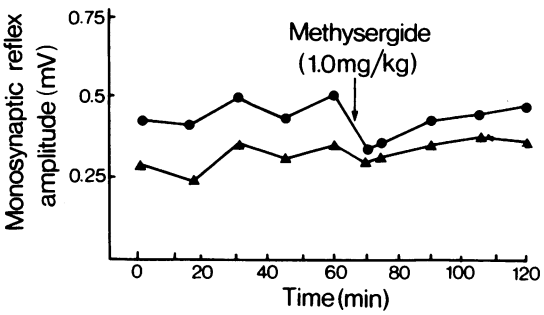

Fig. 3 Effect of methysergide (1.0 mg/kg) on the unconditioned (▲) and conditioned (●) monosynaptic reflex (MSR). Each point represents the mean of 3 consecutively evoked reflexes. Within 4 min of the intravenous injection, the effects of conditioning stimulation were markedly reduced whilst the unconditioned MSR was unaffected. The effects of conditioning reappear during the subsequent 50 minutes.

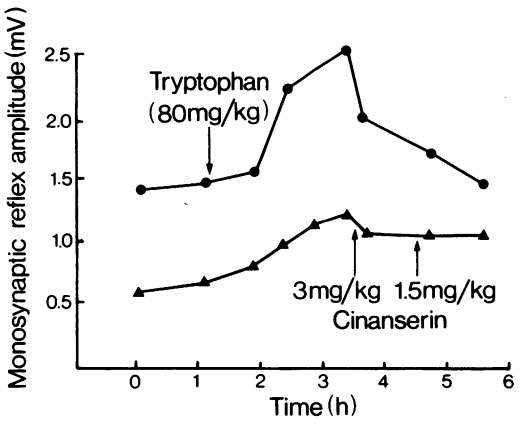

Fig. 4 The effect of intravenous L-tryptophan (80 mg/kg) and Cinanserin (3 + 1.5 mg/kg) on the unconditioned (▲) and conditioned (●) monosynaptic reflex (MSR). Each point represents the mean of 3 consecutively evoked reflexes. The unconditioned and conditioned reflexes progressively increased in amplitude during the 2 h following administration of L-tryptophan. Cinanserin reduced the potentiation of conditioning stimulation by L-tryptophan without grossly influencing the unconditioned reflex amplitude.

throughout these particular experiments, short latency responses are not illustrated by Figure 1.

The effect of intravenously administered drugs on the spinal monosynaptic reflex

Effect of intravenous methysergide. Methysergide (0.75-1.0 mg/kg) was administered to 4 rats. The antagonist produced a small fall in the amplitude of the unconditioned MSR and markedly reduced the effects of conditioning. The average reduction of the effect of conditioning was 48% ranging from 32 to 73%, whilst the unconditioned MSR was reduced by 15% (range 8-24%). Figure 3 illustrates these changes. Within 4 min of the injection of methysergide (1.0 mg/kg) the effects of raphe stimulation were markedly reduced and recovery occurred progessively during the 50 min following the injection. Administration of the drug was not accompanied by any change in blood pressure.

Effect of intravenous Cinanserin. Intravenous Cinanserin was administered to six animals in doses between 3-4 mg/kg. In all animals the conditioned response was reduced within 10 min of the injection; the reduction being maximal after 15-30 minutes. The potentiating effects of conditioning stimulation were reduced by 73% (range 38-100%) whilst the average reduction in the unconditioned response was 11% (range 0-36%). However, increasing the dose of Cinanserin above 4 mg/kg caused profound reduction of the unconditioned MSR. The effect of Cinanserin (subsequent to administration of L-tryptophan) is shown in Figure 4.

Effect of intravenous lysergic acid diethylamide. This drug was administered to 13 rats in doses ranging between 5 and 25 μg/kg. At doses above 10 μg/kg both conditioned and unconditioned MSR amplitudes were profoundly depressed within 3 min of the injection. Recovery did not occur within 200 minutes. With doses of 10 μg/kg or less, LSD also depressed both conditioned and unconditioned MSR's in 4 animals but in 5 animals the effects of conditioning were reduced by 69% (range 46-94%) whereas the unconditioned response was depressed by only 10% (range 0-14%).

The effect of intravenous and intraperitoneal L-tryptophan. L-tryptophan was administered to 14 rats. It produced a gradual increase in both the unconditioned and the conditioned MSR amplitude. However, as can be seen from Fig. 4, the effect on the conditioned MSR was greater than on the unconditioned reflex. This change in reflex amplitude was observed between 90 and 120 min after the i.v. administration of 80-200 mg/kg of the precursor (2-3 h for the i.p. route). After about 150 min the conditioned MSR

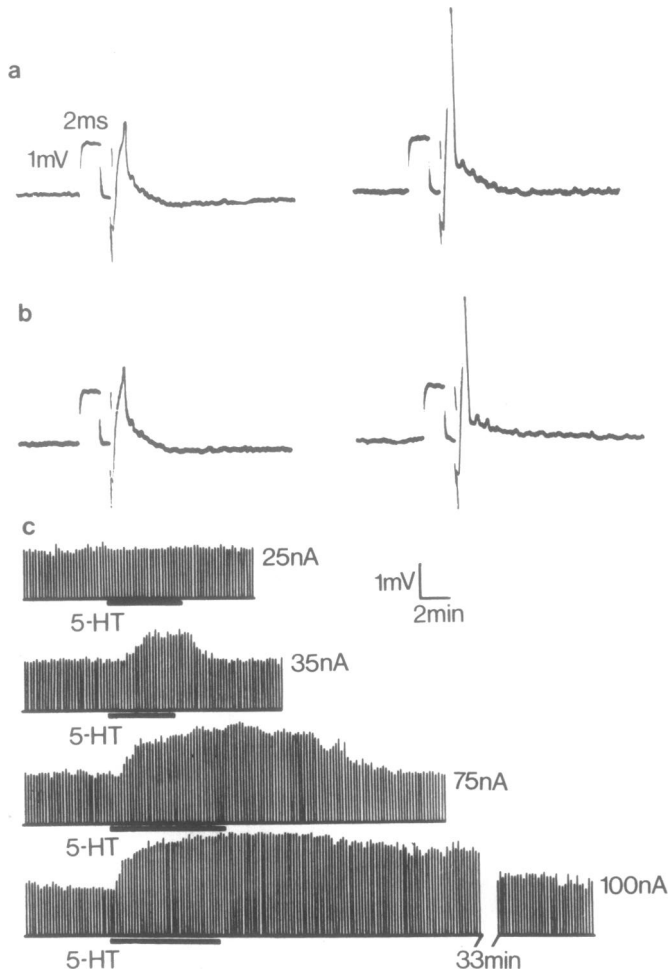

Fig. 5 The effect of raphe stimulation and iontophoretically applied 5-hydroxytryptamine (5-HT) on the antidromic field potential.

(a) Photographs of the field potential before (left) and after (right) application of 100 nA of 5-HT applied for 2 minutes. 5-HT more than doubled the amplitude of the field potential.

(b) The field potential before (left) and just after (right) conditioning stimulation of the raphe nuclei. Conditioning almost doubled the amplitude of the field potential.

(c) Pen writing oscillograph records of the field potential amplitude during iontophoretic application of different currents of 5-HT. 5-HT was applied during the period indicated by the bars under the records with the current shown to the right of the traces. It is apparent that the latency to onset and the maximum amplitude of the response is dependent upon the current of 5-HT.

had increased by about 100% above pre-drug levels. In parallel with these changes the PSR was progressively reduced following L-tryptophan. Control injections of Tween 80, the emulsifying agent, were without effect. In the experiment illustrated in Fig. 4 the facilitatory effects of L-tryptophan were completely blocked by the subsequent intravenous injection of Cinanserin (3 + 1.5 mg/kg). However, these doses of Cinanserin only reduced the effects of conditioning to 54% of the pre-tryptophan level.

The effect of stimulating raphe nuclei on the motoneurone field potential

Responses to ventral root stimulation recorded from the ventral horn of the lumbar spinal cord of the rat were assumed to be antidromic field

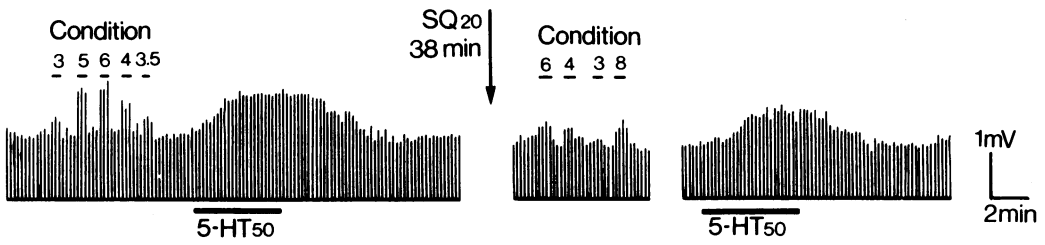

Fig. 6 The effect of iontophoretically applied Cinanserin (SQ) on the antidromic field potential response to conditioning stimulation and the iontophoretic application of 5-hydroxytryptamine (5-HT). The figures above the lines indicate the voltage applied to the conditioning electrode in the raphe nuclei. After a 38 min application of Cinanserin the response to conditioning stimulation was reduced even at higher stimulus strengths. Similarly the response to iontophoretically applied 5-HT was reduced but Cinanserin had no effect on the unconditioned field potential amplitude.

potentials from motoneurones due to their latency of 0.1-0.2 millisecond. These potentials were predominantly negative and had a duration of between 1 and 1.5 ms (Figure 5b). Recording from the same position in the cord, but stimulating a dorsal root, evoked a complex waveform. A small negative potential (superimposed upon a large positive wave) had a latency of 1-1.5 ms, suggesting it may reflect the monosynaptic depolarization of motoneurones.

The same conditioning-test design and stimulation parameters were used as in the previous study, except that test stimulation was delivered to either dorsal or ventral roots. Conditioning stimulation of the raphe nuclei increased the amplitude of the negative wave response to both dorsal and ventral roots. These changes in field potential amplitude were paralleled by changes in the amplitude of the monosynaptic reflex recorded from the ventral roots. The intensity of conditioning stimulation required to increase the amplitude of both potentials was very similar; in animals where conditioning stimulation was unsuccessful in potentiating the reflex recorded at the ventral roots, the field potentials were also unaffected; and if conditioning stimulation became less effective during any experiment both the field potentials and the reflex recorded at the ventral roots were similarly affected.

The effect of iontophoretically applied drugs on the motoneurone field potential

The effect of 5-hydroxytryptamine. The effect of iontophoretically applied 5-HT on the amplitude of the field potential response to stimulation of a ventral root is shown in Figure 5a. 5-HT has been shown to increase the amplitude of these responses in 64 recording positions in 22 animals; it reduced the response amplitude on 1 occasion only. On no occasion did 5-HT cause the spontaneous discharge of an identified motoneurone. Following 5-HT application, the negative part of the field potential increased in amplitude with no gross change in latency, amplitude of the initial positive wave, or duration of the negative part. Small currents of 5-HT potentiated the response to raphe stimulation but large 5-HT applications, resulting in large increases of the field potential, occluded the response to raphe stimulation.

The response to different currents ejecting 5-HT are shown in Figure 5c. Currents of up to 100 nA from another barrel containing NaCl had no effect during this study. It can be seen that increasing the 5-HT currents decreased the latency of the drug response and increased the plateau response amplitude. The dependence of these effects upon the pH of the solution from which 5-HT was ejected was examined in 6 electrode positions in 4 animals. 5-HT ejected from solutions at pH 3.0, 5.0 and 7.0 all caused increases in the amplitude of the field potentials, although the response latency was much greater at pH 7.0.

The effect of antagonists of 5-hydroxytryptamine. The potentiating effects of raphe stimulation and iontophoretic application of 5-HT were both reduced following iontophoretic application of Cinanserin (Figure 6). Cinanserin reduced the effect of conditioning stimulation on 9 occasions (82% of possible) and the response to 5-HT on 5 occasions (83% of possible) without grossly altering the unconditioned field potential amplitude. Currents between 50 and 100 nA were applied for 5 to 30 minutes. Similar currents of methysergide selectively affected the response to raphe stimulation or iontophoretically applied 5-HT on 3 occasions but currents of 25-50 nA of LSD always depressed the unconditioned field

potential amplitude. Considerable difficulty was experienced in identifying the narrow range of currents and times of application which influenced only the effects of conditioning and 5-HT. Within this range it was unlikely that the antagonists were exerting a directly depressant effect upon the excitability of motoneurones since no change was seen in the amplitude of the unconditioned response to test stimulation of either dorsal or ventral roots.

The effect of glutamate and glycine. Glutamate was applied by iontophoresis at 13 recording positions in 6 animals, with stepped increases in the current of application until an effect was observed. On only 6 occasions from a total of 28 applications of glutamate were there any indications of an increase in the field potential. Unequivocal reduction in the amplitude of the field potential was recorded on 17 occasions. The facilitatory effects of glutamate were always followed by profound reductions of the potential amplitude. These effects required very high currents of glutamate (100-200 nA) for long periods (2-4 minutes). These observations were made by applying initially small currents of application which were gradually increased until an effect was observed. In some studies a small increase in the ejecting current caused a progressive reduction of the field potential which was eventually abolished without any further increase of the ejecting current.

Iontophoretic currents of glycine depressed the field potential amplitude on 12 occasions in 16 attempts. Clear responses were seen to currents as low as 20 nA; increasing the current increased the degree of depression of the field potential. On no occasion did the field potential increase in amplitude.

Discussion

The increase in amplitude of the spinal MSR following stimulation of the raphe nuclei suggests that this stimulation increases the excitability of spinal motoneurones. This may result from a direct excitation of motoneurones, facilitation of interneuronal activity or the depression of inhibitory influences on the motoneurone. The polysynaptic pathways along which the longer latency responses to dorsal root stimulation travel, were depressed by raphe stimulation, however. This suggests that the effects of conditioning stimulation did not result from a general facilitation of interneuronal activity nor a widespread depression of inhibition within the cord.

The effect on the spinal MSR of stimulating nucleus raphes medianus, has also been reported by Clineschmidt & Anderson (1970). They observed both depression and facilitation of the reflex by hind-brain stimulation in the cat, and specifically examined the effect of antagonists of 5-HT on the depression of the MSR. LSD in a dose of 250-500 μg/kg was necessary to influence the inhibition and direct effects of the antagonists upon the unconditioned MSR were noticeable in most although not all experiments. The conduction velocity of the inhibitory pathway was 10 m/second. The differences between our findings may be ascribed to differences in our methods. Experimenting with cats, Clineschmidt & Anderson used 300 Hz conditioning trains which we found to be much less facilitatory in rats and a 20 times greater dose of intravenous LSD was required to influence the much faster conducting pathway studied by them. Clineschmidt & Anderson did not study the effects of precursors upon the responses to raphe stimulation. Earlier work by Anderson & Shibuya (1966), Marley & Vane (1967), and Banna & Anderson (1968) showed, as does the present paper, that intravenous precursors of 5-HT facilitate the spinal MSR and increase motoneurone excitability. These reports cannot be easily reconciled with the proposal of Clineschmidt & Anderson (1970) that activity in a spinal pathway releasing 5-HT may result in depression of motoneurone activity. Our present observation that the iontophoretic application of 5-HT results in an increase in amplitude of the MSR and antidromic motoneurone field potential recorded with microelectrodes further suggests that this amine may cause an increase in motoneurone excitability. Dahlström & Fuxe (1965) observed that the 5-HT containing terminals in the cord originated from fine (1-2 μm) unmyelinated axons descending from raphe nuclei. These axons are very similar to C fibres which have been shown to have a conduction velocity of 0.5-2.5 m/s (Iggo, 1958). The rate of conduction in the raphe-spinal pathway studied in the present experiments can be calculated from the data presented in Figure 2. No significant facilitation of the MSR occurred before 50 ms after the start of the conditioning train. As the pathway does not involve forebrain structures (mid collicular section does not change the effect of conditioning), it has a length of approximately 12 cm. The maximum conduction velocity for the pathway is therefore 2.4 m/s but the average velocity may well be less. This is within the range expected for the fibres known to contain 5-HT. The only other calculation of conduction velocity in unmyelinated fibres of 1-2 μm which may contain 5-HT was made by Couch (1970). The velocity of 0.6-0.8 m/s was calculated for the

projection from nucleus paragiganto-cellularis lateralis to the raphe nuclei.

Study of the precise region in the brain stem from which potentiation of the MSR could be elicited revealed that the greatest effects were evoked from nucleus raphe medianus. This is in agreement with the degeneration studies performed by Brodal, Taber & Walberg (1960) who observed that fibres from the more caudal raphe nuclei descend in the cord whilst those from anterior raphe nuclei course rostrally. Our observation that potentiation was not elicited from nucleus raphes dorsalis at stimulation intensities effective for nucleus raphes medianus would seem to suggest further that this nucleus may not project to the lumbar cord. It should be remembered that the present study was conducted with a fixed interval of 120 ms between onset of the 100 ms conditioning train and the test stimulus applied to dorsal roots. At this interval very little inhibition was evident from anywhere in the brain stem of the type reported by Magoun & Rhines (1946).

We suggest that the increased excitability of motoneurones results from stimulation in the region of 5-HT containing cell bodies and that the conduction velocity of the pathway is similar to that predicted for the 5-HT pathway. In an attempt to demonstrate the involvement of a 5-HT releasing synapse we administered L-tryptophan intravenously, and antagonists of 5-HT intravenously and iontophoretically. L-tryptophan increased and antagonists reduced the effectiveness of raphe stimulation. Both types of drug affected the amplitude of the unconditioned response to root stimulation but careful selection of the dose restricted the effects to the conditioned response.

The potentiation of the unconditioned MSR by precursors of 5-HT has been reported previously (Andén *et al.*, 1964; Anderson & Shibuya, 1966; Banna & Anderson, 1968). These authors suggested that precursors increased the spontaneous release of 5-HT. In view of the reports of a resting release of 5-HT from unstimulated spinal cords *in vitro* (Andén, Carlsson, Hillarp & Magnusson, 1964) an increased spontaneous release of 5-HT following precursor administration may account for the increase in unconditioned MSR in the present experiments. L-tryptophan reduced the PSR concomitantly with the increase in MSR amplitude, thus seeming to have effects similar to raphe stimulation. This reduction in PSR following precursor administration was also reported by Banna & Anderson (1968). We cannot confirm their observations fully, however, as we did not use 5-HTP because there is evidence that the immediate precursor is abnormally metabolized (Moir & Eccleston, 1968).

The effect of antagonists in reducing the unconditioned MSR can either be explained in terms of antagonism of spontaneous release of 5-HT or in terms of a direct depressant effect of the drug. Although it was possible to influence the effects of conditioning selectively, further discrimination between these alternatives was not possible. Banna & Anderson (1968) report that 5-HT antagonists depress the amplitude of spinal reflexes in cats following chronic spinal section and in cats depleted of central 5-HT by reserpine. This would suggest that the direct depression of reflex amplitude does not result from the antagonism of spontaneously released 5-HT. However, these authors also reported a profound reversal by antagonists of the facilitatory effects of 5-HTP with doses of antagonist which had very little effect on reflex amplitude in animals not pretreated with precursors. In the present study involving L-tryptophan and Cinanserin, very similar observations were made.

The effectiveness of iontophoretically applied 5-HT antagonists in blocking the response to raphe stimulation and the response of motoneurones to iontophoretically applied 5-HT suggest that the ventral horn of the lumbar spinal cord contains receptors to 5-HT; that 5-HT increases the excitability of motoneurones and that stimulation of raphe nuclei similarly increases motoneurone excitability. The probability of similar or identical receptors being involved in the response to both 5-HT and the stimulation of raphe nuclei is considerably increased by the observation that both responses were similarly affected by the antagonists.

Our conclusions support the suggestions of Fuxe (1965) following the observation of microfluorescent 5-HT terminals innervating both motoneurones and interneurones in the spinal cord, that 5-HT may function as a neurotransmitter in the raphe-spinal pathway. The results extend and provide a firm basis for the interpretation of the observations of Marley & Vane (1967), Anderson & Shibuya (1966) and Banna & Anderson (1968) who studied the effect of precursors, antagonists and some possible 5-HT antagonists on spinal reflexes.

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