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The Blockade of Bulbospinal Inhibition by 5-Hydroxytryptamine Antagonists[1]

B. V. Clineschmidt and E. G. Anderson

Department of Pharmacology, University of Illinois College of Medicine, Chicago (USA)

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Summary. Histochemical evidence suggests that neurons in the brain stem raphé nuclei which contain serotonin (5-HT) project to the spinal cord. Stimulation of the raphé nuclei facilitated and inhibited spinal reflexes: the conduction velocity calculated for the bulbospinal inhibitory pathway was 10 m/sec. Antagonists of 5-HT were without effect on facilitation, but readily blocked raphé-evoked inhibition of the monosynaptic reflex. The antagonists also blocked the inhibition evoked from the ventral medial reticular formation. Blockade of bulbospinal inhibition was observed after LSD (0.25 mg/kg), methysergide (0.5 mg/kg), BOL (1—1.5 mg/kg) and cinanserin (4 mg/kg) but not cyproheptadine (5 mg/kg). The 5-HT antagonists were more effective when injected into the arterial supply of the spinal cord than when injected into the arterial supply of the brain stem. Thus, the antagonists appear to act at the spinal level, presumably blocking the effect of a 5-HT-releasing interneuron intercalated in the bulbospinal inhibitory pathway.

Key Words: Brain stem raphé — Bulbospinal inhibition — LSD — Methysergide — Cinanserin

Introduction

Following chronic spinal cord transection the levels of 5-hydroxytryptamine (5-HT) caudal to the section decrease dramatically (Carlsson *et al.*, 1963), but an appreciable amount (25%) persists after several weeks (Shibuya and Anderson, 1968). Histochemical examination of the spinal cord has revealed the presence of 5-HT in nerve terminals, but not in cell bodies (Carlsson *et al.*, 1964). According to Dahlstrom and Fuxe (1964, 1965), 5-HT containing cell bodies in the caudal raphé nuclei of the brain stem give rise to axons which descend the spinal cord and terminate in the dorsal, medial and ventral horns. The terminals located in the ventral horn intimately contact motoneurons (Fuxe, 1965).

The actions exerted by these descending neurons are uncertain. The microelectrophoretic administration of 5-HT to motoneurons has been reported to cause hyperpolarization (Phillis *et al.*, 1968), whereas elevation of spinal cord 5-HT levels by administration of 5-hydroxytryptophan, L-tryptophan or inhibitors of monoamine oxidase increases motoneuronal excitability — an effect readily reversed by 5-HT antagonists (Anderson and Shibuya, 1966; Anderson *et al.*, 1967; Banna and Anderson, 1968).

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In the present investigation an attempt was made to activate the serotonin containing neurons of the bulbo-spinal system through stimulating electrodes placed in the caudal raphé nuclei, while observing the effects of such stimulation on segmental reflexes. For comparison stimulating electrodes were also placed in the ventromedial reticular formation. It was anticipated that electrical stimulation of the brain stem would nonselectively activate descending systems, but that actions mediated by serotonergic neurons could be distinguished by their susceptibility to blockade with 5-HT antagonists. As reported herein, bulbospinal inhibition of the monosynaptic reflex elicited by stimulating flexor or extensor nerves was antagonized by several 5-HT antagonists. In contrast bulbospinal facilitation was unaffected. A preliminary report of these results has been published (Clineschmidt and Anderson, 1969).

Methods

Cats of either sex weighing 1.7—3.1 kg were anesthetized with ether and decerebrated between the colliculi after bilateral carotid ligation. The portion of the brain rostal to the section was removed and ether discontinued.

Brain Stem Electrode Placement

After decerebrate rigidity was well-developed, electrodes were positioned stereotaxically in the raphé nuclei just caudal to the trapezoid body (Horsley-Clarke coordinates P 7.5, L 0) and in the ventral medial reticular formation at the level of the inferior olive (P 11, L 1.5). The position of the electrode in the horizontal plane was determined by the physiological response (see Results). Concentric bipolar stainless steel electrodes with an internal tip diameter less the 0.2 mm and tip separation of 1 mm were used in most experiments. Monopolar tungsten or stainless steel electrodes tapered to a 1 μ tip diameter with 50 μ of exposed tip were also used in some experiments. Histological examination of electrode placements in inhibitory areas were made using potassium ferrocyanide staining of iron deposits resulting from passage of a direct current through the electrode.

Spinal Reflex Activity

Previous communications detail the methods used (Anderson and Shibuya, 1966). Reflexes were evoked by stimulating either the gastrocnemius-soleus nerves, the branches of the peroneal nerve innervating the tibialis anticus, extensor digitorum longus and the peroneus longus, or a whole dorsal root at a frequency of 18/min with a stimulus supramaximal for the monosynaptic reflex (MSR). Electrical activity of the appropriate L_7 or S_1 ventral root was amplified with a Tektronix 2A61 preamplifier and displayed on an oscilloscope (Tektronix 565 with 3A3 plug-in amplifier). The size of the MSR was quantified by summing 10 consecutive reflexes in a computer of average transients (TMC CAT 1,000), and the summed potentials were plotted on a Grass polygraph, from which the height of the MSR was measured. The amount of facilitation or inhibition produced by brain stem stimulation was determined by summing 10 consecutive MSRs, each of which was evoked at a fixed latency after a conditioning stimulus train. The temperature of the animal and that of the mineral oil bath covering the spinal cord was maintained at 37 $\pm$ 0.5° by thermister probes and temperature regulators. Blood pressure was monitored from the left common carotid artery. Animals were immobilized with intermittent doses of gallamine triethiodide, and artificially ventilated.

Drugs were injected *via* an indwelling catheter in the antecubital vein unless otherwise specified. Intra-arterial injections to the brain stem were made by ligating the internal mammary artery, costocervical arterial trunk, and the thyrocervical arterial trunk. A polyethylene cannula was then passed down the axillary artery so the tip reached the point where the vertebral artery branched from the left subclavian artery. Intra-arterial injections to the lumbar spinal cord were made *via* a polyethylene cannula which was threaded up a femoral artery into the abdominal aorta to a point just caudal to the renal arteries. All branches of the aorta below the renal arteries were ligated except the spinal arteries. The concentration of drugs for these injections was 100 μg/ml. At the end of these experiments, methylene blue was administered

intra-arterially and the results were not accepted unless the brain stem or spinal cord showed selective staining.

Doses of drugs referred to in the text are in terms of the following: LSD (d-lysergic acid diethylamide), BOL (2-bromo-LSD), ethobutamoxane HCl, methysergide bimaleate, cinanserin HCl and cyproheptadine HCl. All drugs were dissolved in 0.9% (w/v) sodium chloride solution immediately before injection.

Results

In the initial experiments, electrodes were placed stereotaxically in the vicinity of the nucleus raphé magnus and traversed in the horizontal plane through the nucleus. Both inhibitory and facilitatory effects on the spinal monosynaptic reflex (MSR) were observed following electrical stimulation. Multiple shocks were usually necessary to influence the MSR significantly, and optimal stimulus parameters for brain stem activation were a 30 msec train of 0.1 msec square wave pulses at 300 Hz. Both electrode placement and stimulus parameters determined whether facilitation or inhibition resulted from brain stem stimulation. If a MSR was evoked 2.5 msec after the end of brain stem stimulation facilitation was more frequently observed, but if 25 msec were interposed between the end of the brain stem conditioning stimulus and the test MSR, inhibition most frequently occurred. Thus, with many electrode placements both facilitation and inhibition of the MSR could be evoked from the same site in the brain stem. However, in many preparations electrode positions in the raphé and the reticular formation could be found where only facilitation or inhibition of the MSR resulted, regardless of the conditioning-test latency. These results are similar to those obtained in wide areas of the brain stem and descending fiber tracts by other workers (Sasaki *et al.*, 1962; Willis *et al.*, 1967).

Several 5-HT antagonists were tested and did not influence the facilitatory actions of raphé stimulation on the MSR. However, inhibition of the MSR produced by raphé stimulation was markedly reduced by 5-HT antagonists. In consequence, an intensive investigation was made of the effects of 5-HT antagonists on bulbospinal inhibition. In these experiments electrode positions were sought in the raphé and ventral medial reticular formation which evoked relatively pure inhibition. The sites were found by searching for electrode locations which produced maximal reduction of decerebrate rigidity in the forelimbs using a 200—300 Hz stimulus. After the animal was prepared for recording spinal reflexes, the brain stem stimulus was subsequently adjusted to produce about 50% inhibition of the MSR when the reflex was evoked 25 msec after brain stem stimulation. Current spread was not great in these experiments since movement of the brain stem electrode by 0.5—1 mm markedly altered the amount of inhibition. Histological examination of the electrode sites which produced maximal inhibition revealed that the raphé electrodes were grouped around the dorsal border of the nucleus raphé magnus, and the reticular electrodes were grouped around the ventral border of the reticularis gigantocellularis. However, significant inhibition could also be evoked when the electrodes were several millimeters from these locations. When maps of the inhibitory areas were constructed they were found to be similar to those published by Jankowska *et al.* (1968).

In most animals multiple brain stem stimuli were necessary to detect significant inhibition of the MSR. However, occasionally, detectable inhibition was observed

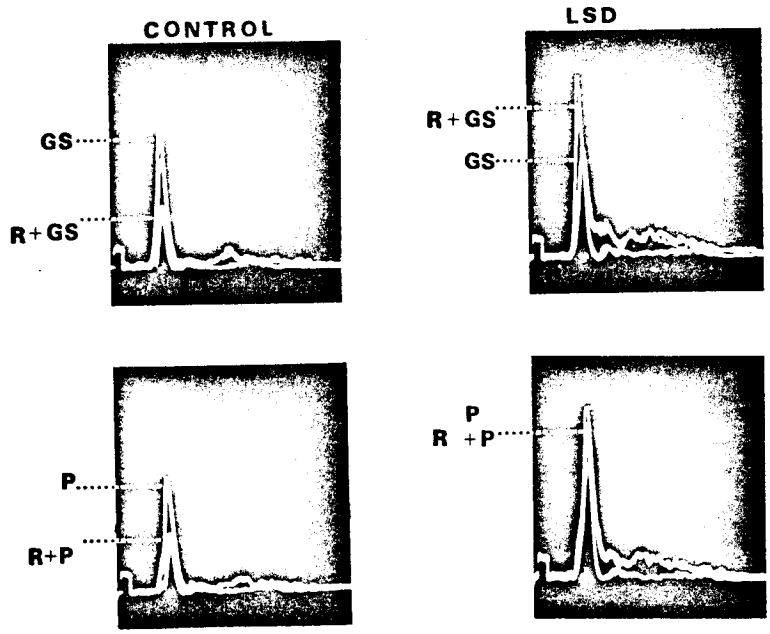

Fig. 1. *LSD-induced blockade of bulbospinal inhibition.* The column on the left shows control ventral root reflexes evoked by stimulating gastrocnemius-soleus (GS) or the peroneal (P) nerves with (R), and without, conditioning stimulation of the brain stem raphé nuclei. The reflexes in the right hand column were recorded 23 (for GS) and 30 (for P) min after the injection of 0.25 mg/kg of LSD. In each frame 3 successive reflexes with raphé conditioning (R) are superimposed on 3 successive reflexes without conditioning. A square wave calibration pulse of 0.5 mV and 0.5 msec duration precedes the reflexes

following a single pulse. In two such preparations the minimal latency required between raphé stimulation and observable inhibition of the MSR indicated a minimal conduction velocity of 10 m/sec.

LSD Blockade of Bulbospinal Inhibition

The intravenous injection of 0.25 to 0.5 mg/kg of LSD completely blocked brain stem inhibition of the MSR (Fig. 1). In some preparations LSD converted bulbospinal inhibition to facilitation (Fig. 1, top row). LSD was equally effective in blocking inhibition of the extensor and flexor MSR (Fig. 1), evoked from either the raphé or reticular formation. It did not, however, block the inhibition of polysynaptic reflexes (Fig. 1).

The effect of LSD on bulbospinal inhibition began 3—4 min after injection, was maximal in 15—30 min and lasted from 1—4 h (Fig. 2A). In most (Fig. 1, top row), but not all preparations (Fig. 2A), LSD initially depressed the size of the unconditioned MSR. Whether or not an initial depression occurred, LSD subsequently increased the size of the unconditioned MSR to well above the control level (Fig. 2A). This facilitatory effect of LSD has been reported earlier (Banna and Anderson, 1968; Geiger and Cervoni, 1958). Irrespective of changes in the unconditioned MSR, LSD always blocked bulbospinal inhibition. Within 2—4 h of the injection of LSD the inhibition produced by brain stem stimulation approximated that prior

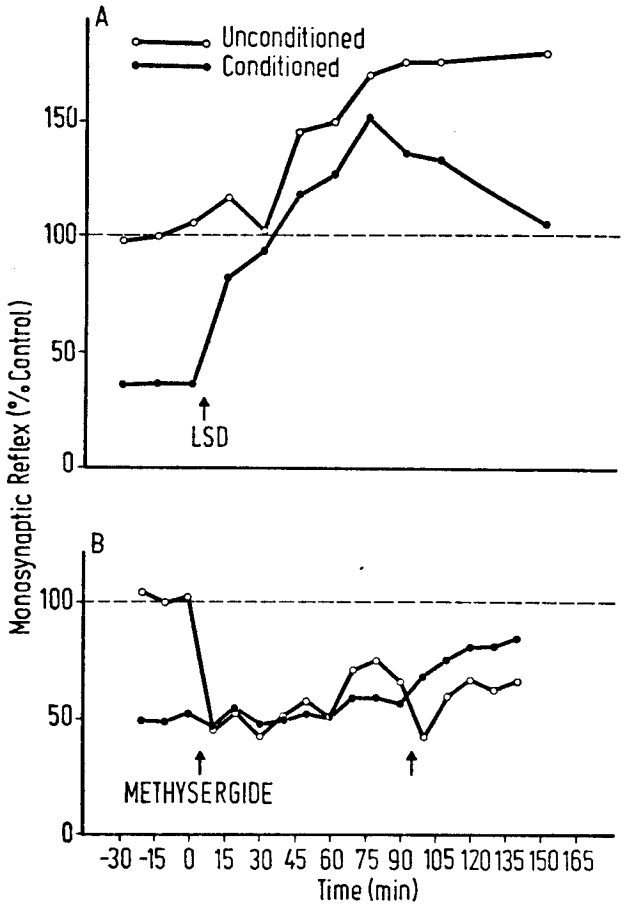

Fig. 2. *Effects of LSD and methysergide on MSR amplitude with and without brain stem conditioning.* A: The MSR was evoked by dorsal root stimulation and recorded from the L_7 ventral root. Each point represents the average of 10 consecutive MSR's without (unconditioned) or with (conditioned) previous raphé stimulation. LSD (0.25 mg/kg) was injected at arrow 5 min after zero time. B: The MSR evoked, recorded and quantified as in A, was inhibited by stimulation of the reticular formation. Methysergide (0.5 mg/kg) was injected at the arrows

to the drug, but the absolute size of both the unconditioned and the conditioned MSR was greater than the control valve (Fig. 2A).

Immediately following the injection of LSD, there was a transient fall in diastolic blood pressure of approximately 25 mm Hg. No association was observed between the time course of this fall and the block of supraspinal inhibition or changes in the MSR. Furthermore, when blood pressure was immediately restored to control levels by the injection of dextran, doxapram or methamphetamine, the effects of LSD on reflexes were not altered.

Methysergide Blockade of Bulbospinal Inhibition

Methysergide was a potent antagonist of supraspinal inhibition in 15 of 15 experiments (Fig. 2B, 3 and Table 1). Like LSD, methysergide blocked inhibition

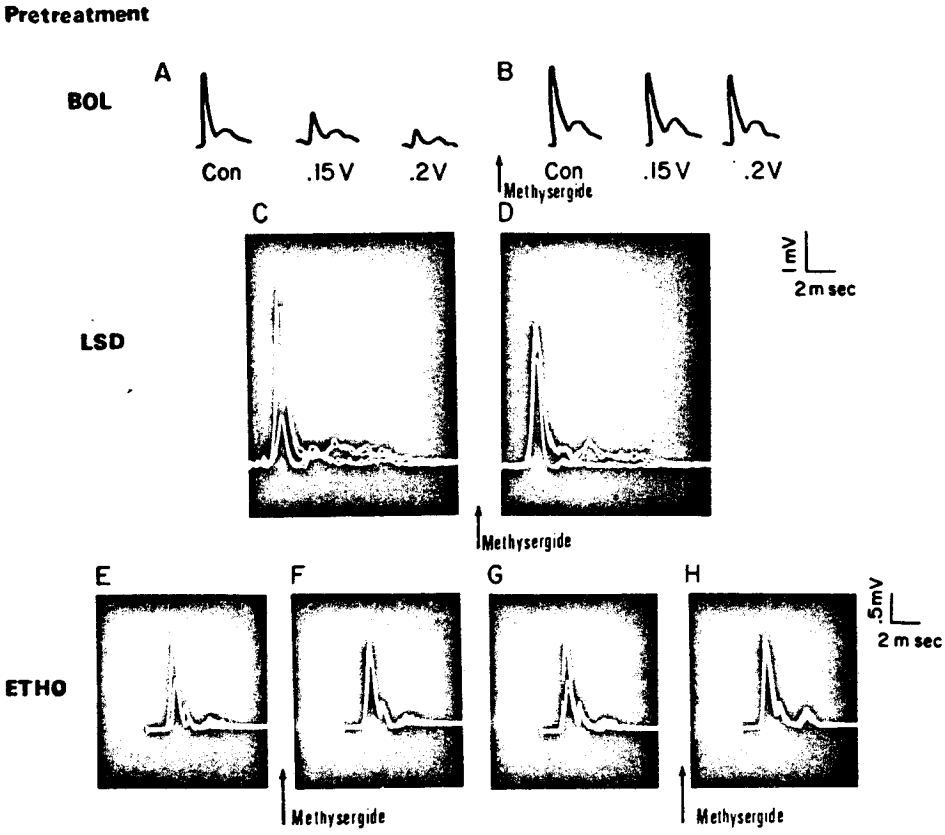

Fig. 3. *Dissociation of the methysergide-induced antagonism of supraspinal inhibition from the depression of the unconditioned MSR.* A—B, C—D, and E—H are three separate experiments. A—B: Reflex activity evoked by stimulating the L_7 dorsal root and recorded from L_7 ventral root. Control (Con), the average height of ten spinal reflexes as printed out from the CAT 1,000. The values .15 V and .2 V are the voltages of the conditioning stimulus to the reticular formation. Thirty minutes before A the animal received 2 mg/kg of BOL. After recovery from the immediate effects of BOL, methysergide (0.5 mg/kg) was injected prior to the records of B. C—D: Spinal reflexes evoked by stimulating gastrocnemius-soleus nerve. Three successive reflexes with raphé conditioning (small spikes) are superimposed on three successive reflexes without conditioning. Fifty-five minutes before C, 0.25 mg/kg of LSD was administered. Following recovery of descending inhibition, methysergide (1 mg/kg) was injected prior to the records of D.

E—H: Spinal reflexes initiated by stimulating the L_7 dorsal root and recorded from the corresponding ventral root. Three successive reflexes with raphé conditioning are superimposed on three successive reflexes without conditioning. Animal was pretreated 30 min before E with 1 mg/kg ethobutamoxane. Two mg/kg of methysergide were injected between E and F causing complete blockade of descending inhibition. Two hours and 30 min elapsed between F and G. A further dose of methysergide (2 mg/kg) was administered between G and H

evoked from the raphé and the reticular formation of both flexor and extensor MSR's. Figure 2B shows the time course of its action. Methysergide sometimes converted supraspinal inhibition to facilitation (2nd dose in Fig. 2B). However, in addition to reducing reticular inhibition, methysergide decreased the unconditioned

MSR to one-half its control size (Fig. 2B). The concomitant decrease in the unconditioned MSR and the blockade of bulbospinal inhibition might suggest that blockage of inhibition is secondary to the depression of the MSR. However, by using previous observations that pretreatment with LSD, BOL or ethobutamoxane desensitizes the MSR to the depressant action of methysergide (Banna and Anderson, 1968; Baker and Anderson, 1970), it was possible to test for a relationship between the depressant action of methysergide on the MSR and the blockage of bulbospinal inhibition. The results of three of these experiments are shown in Fig. 3. In the top tracings of Fig. 3 the animal was pretreated with 2 mg/kg of BOL, and the traces labeled A were recorded 30 min after the supraspinal inhibition had recovered. The subsequent injection of 0.5 mg/kg of methysergide completely blocked reticular inhibition without depressing the unconditioned MSR (Fig. 3B). In the experiment depicted in the second row, frame C was taken 55 min after the injection of 0.25 mg/kg of LSD, at which time supraspinal inhibition had completely recovered from the LSD-induced blockade. The subsequent injection of methysergide (1 mg/kg) again blocked supraspinal inhibition with only slight depression of the unconditioned MSR. The animal used in the experiment shown in the bottom row was pretreated with 1 mg/kg of ethobutamoxane 30 min before frame E was taken. Ethobutamoxane, like methysergide, depressed the unconditioned MSR to about 50% of its control size, but did not block supraspinal inhibition. Frame E was taken after recovery from the depressant effects of ethobutamoxane. The injection of 2 mg/kg of methysergide promptly blocked supraspinal inhibition without depressing the MSR (Frame F). Frames G and H demonstrate recovery from methysergide, and indicate that tolerance to a single dose of methysergide does not occur. These experiments clearly demonstrate that the ability of methysergide to block bulbospinal inhibition is unrelated to its depressant action on the MSR.

BOL, Cinanserin and Cyproheptadine on Bulbospinal Inhibition

Other 5-HT antagonists were examined in addition to LSD and methysergide (Table 1). A compound was characterized as reducing inhibition only if it antagonized bulbospinal inhibition as completely as methysergide and LSD in Figs. 1—3. BOL (1—1.5 mg/kg) completely antagonized supraspinal inhibition in 5 out

Table 1. *Effect of serotonin antagonists on supraspinal inhibition*

Serotonin antagonist	Dose range mg/kg i. v.	Total No. animals	No. showing reduced inhibitition	Comments[1]
LSD	0.25—0.5	5	5	Transient ↓ control MSR (4/5) followed by prolonged ↑ ↓ blood pressure (BP)
Methysergide	0.5—1	15	15	Prolonged ↓ control MSR ⇄ BP
Cinanserin	4—16	11	8	⇄ control MSR, ⇄ BP
BOL	1—1.5	5	5	Transient disinhibition Transient ↓ control MSR ↓ BP
Cyproheptadine	5	4	0	—

1 ↓ = decrease; ↑ = increase; ⇄ = no change.

of 5 experiments, but the antagonism lasted only 10—15 min. Such antagonism of inhibition was accompanied by slight depression of the unconditioned MSR. Higher doses of BOL were not investigated because they produced marked hypotension.

In 8 of 11 experiments, cinanserin (4—8 mg/kg) antagonized bulbospinal inhibition without affecting the unconditioned MSR. In some experiments, cinanserin converted bulbospinal inhibition to facilitation. However, virtually no effect of cinanserin was observed in the 3 animals even though total doses of 16 mg/kg were accumulated. Cyproheptadine (5 mg/kg) failed to block bulbospinal inhibition.

In some instances, the blockade of supraspinal inhibition by the 5-HT antagonists could be surmounted by increasing the strength of brain stem stimulation. However, in other experiments, increasing stimulus strength produced either no effect or facilitation of the subsequently evoked MSR. The latter presumably occurred from the location of the electrode in inhibitory areas near facilitatory pathways which were recruited by increases in current.

Blockade of Inhibition of Decerebrate Rigidity

In view of the effectiveness of the 5-HT antagonists in blocking bulbospinal inhibition of reflexes, we attempted to see if this action could be observed at the behavioral level as a diminution of the inhibition of decerebrate rigidity which occurs during brain stem stimulation. In 3 cats with marked decerebrate rigidity, electrodes were placed in the brain stem at sites where clear inhibition of the extensor rigidity resulted from a 200 Hz stimulus train. In all 3 animals the injection of 4 mg/kg of cinanserin markedly decreased the inhibition or "melting" of decerebrate rigidity produced by brain stem stimulation. When the dose was increased to 8 mg/kg no diminution of decerebrate rigidity could be observed following brain stem stimulation. Significant recovery was observed from this blocking action of cinanserin within 30 min. No obvious change was detected in the degree of decerebrate rigidity following cinanserin.

Site of Action of LSD and Methysergide

The results obtained thus far with the 5-HT antagonists could have been due to an action of these compounds in the brain stem or in the spinal cord. To differentiate between these two possible sites of action, LSD and methysergide were administered to these two areas by close intra-arterial injection. The injection of LSD (100 μg) or methysergide (200 μg) into the left vertebral artery failed to affect bulbospinal inhibition. When 100 μg of LSD or 200 μg of methysergide was slowly (1—2 min) injected into the arterial supply to the lumbar cord, bulbospinal inhibition was immediately reduced or blocked. Figure 4 (top row) presents results obtained with injections of methysergide into the abdominal aorta. The first injection of a small dose of methysergide (100 μg) typically decreased the unconditioned MSR without appreciably reducing the percentage inhibition. The unconditioned MSR rapidly recovered from the depressant action of methysergide (13 min) and the preparation was desensitized to methysergide in that the subsequent injection of 200 μg produced only a slight depression of the unconditioned MSR (last frame, top row, Fig. 4). However, this dose of methysergide completely blocked bulbospinal inhibition. The bottom row of Fig. 4 shows the effects of LSD injected intra-arterially. The first injection of 100 μg significantly depressed the unconditioned

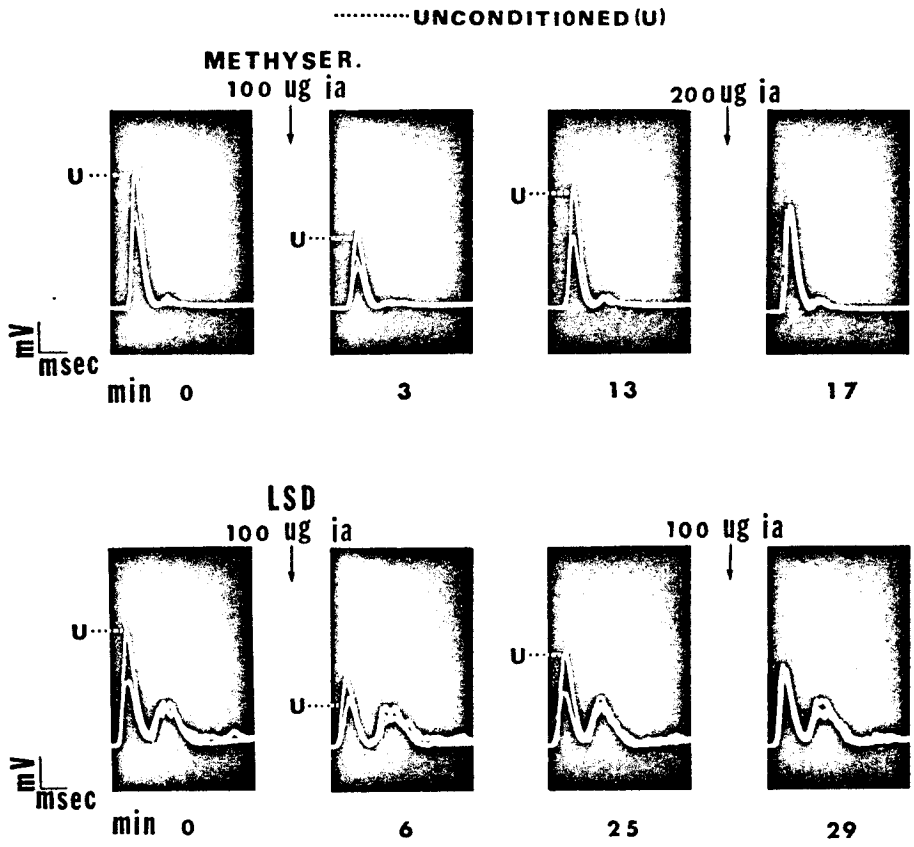

Fig. 4. *Antagonism of supraspinal inhibition by methysergide and LSD administered intra-arterially to the spinal cord.* The top frames show spinal reflexes evoked by stimulating the L_7 dorsal root and recorded from the corresponding ventral root. Three sweeps with conditioning (reticular formation) are superimposed on three sweeps without previous brain stem stimulation (U). Methysergide was injected into the abdominal aorta, as indicated by the arrows. The bottom row of frames demonstrate reflexes evoked and recorded from an L_7 dorsal and ventral root, respectively. The reticular formation was stimulated: three sweeps with conditioning are superimposed on three unconditioned reflexes. LSD was injected into the abdominal aorta as indicated by the arrows. Note that the first dose of LSD converted bulbospinal inhibition to facilitation

MSR and converted bulbo-spinal inhibition to facilitation. Following recovery from the first dose, a second injection of LSD (100 μg) blocked bulbospinal inhibition with less depressant effect on the unconditioned MSR. The injection of 100 μg of LSD or 200 μg of methysergide i.v. was without effect on bulbospinal inhibition. Thus, the above results indicate that the site of action of LSD and methysergide in antagonizing bulbospinal inhibition is most probably in the spinal cord.

Discussion

The supraspinal inhibitory system blocked by serotonin antagonists appears to be the bulbospinal system originally described by Magoun and Rhines (1946). The extent of the inhibitory area, the inhibition of both flexor and extensor motoneuro-

nal activity, and inhibition of both contralateral and ipsilateral reflexes agrees with the work of Magoun and Rhines (1946) and the findings of others studying the same inhibitory system (Jankowska *et al.*, 1968; Sasaki *et al.*, 1962). Although bulbospinal inhibition of spinal reflexes is generally assumed to emanate from the ventral medial reticular formation, anatomical studies support a participatory role for the raphé nuclei in some physiological actions of the brain stem currently attributed solely to the reticular formation (Taber *et al.*, 1960). The reduction of the effects of both raphé and reticular stimulation by 5-HT antagonists support this conclusion.

The nature of the experimental preparation prevented an analysis of dose-response relationships for quantitatively determining the potency of the various 5-HT blocking agents with regard to antagonizing bulbospinal inhibition. Nevertheless, after intravenous injection the following order of potency was evident: LSD = methysergide > BOL > cinanserin. The relative potency of BOL is less certain because of the transient nature of its antagonism toward descending inhibition and the marked hypotension following doses above 1 mg/kg. Cyproheptadine apparently failed to antagonize bulbospinal inhibition, but doses exceeding 5 mg/kg were not investigated.

The outstanding pharmacological property common to LSD, methysergide, BOL and cinanserin is antagonism of 5-HT, which suggests the blocking action of these compounds on bulbospinal inhibition most likely results from antagonism of 5 HT.

It is possible that the disappearance of bulbospinal inhibition after 5-HT antagonists could result from a blockade of inhibition or an increase in brain stem facilitation. The latter possibility is considered unlikely in that in animals which exhibited only facilitation, despite repeated attempts to position the brain stem electrode in inhibitory areas, the injection of a 5-HT antagonist did not increase the bulbospinal facilitation. Thus, the action of the 5-HT antagonists is attributed to blockade of bulbospinal inhibition. The conversion of inhibition to facilitation which occurred in some experiments following the injection of a 5-HT antagonist is probably due to electrode placements in areas contaminated with facilitatory pathways whose effects were unmasked by the blockade of inhibition.

Accepting the hypothesis that the 5-HT antagonists block bulbospinal inhibition by interfering with the function of 5-HT-releasing neurons, several possible sites of action are suggested. Figure 5 presents three of the simplest possibilities.

The first diagram presents the hypothesis which initiated this study: the brain stem electrodes excite 5-HT neurons, the axons of which descend the spinal cord to directly inhibit the motoneuron. However, this explanation can be excluded on the basis of conduction velocity. A minimal conduction velocity of 10 m/sec was observed for the bulbospinal inhibitory pathway, which contains an unknown number of synapses. If several synapses are present in the pathway the actual conduction velocity of the descending axon would be much faster than 10 m/sec. However, the descending 5-HT containing neurons in the spinal cord have unmyelinated axons of 2—4 μm in diameter (Dahlstrom and Fuxe, 1965) which would conduct far slower than 10 m/sec.

This leaves the last two diagrams of Fig. 5 as alternative explanations. To differentiate between these remaining alternatives, 5-HT antagonists were in-

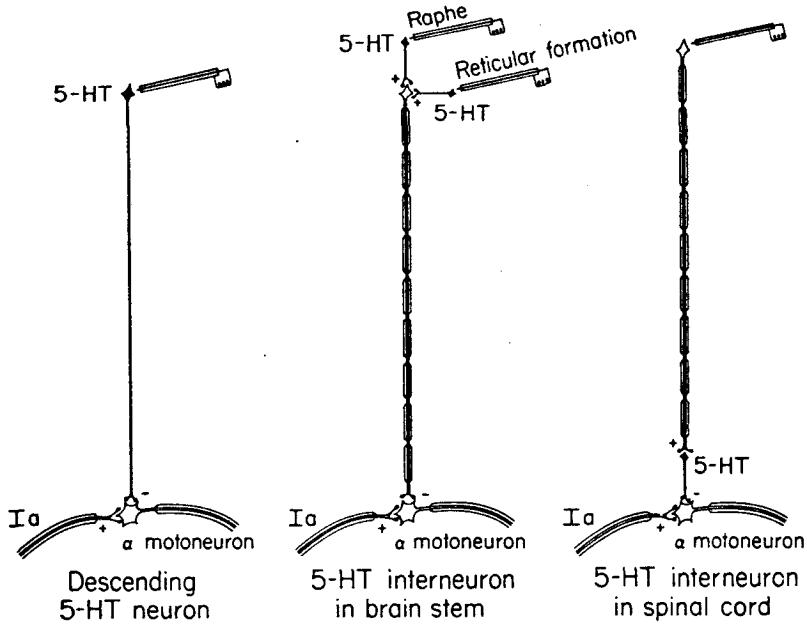

Fig. 5. *Diagram of three hypothetical neuronal arrangement which could explain the block of bulbospinal inhibition by 5-HT antagonists.* The solid filled neurons represent 5-HT-containing neurons. The + and — symbols designate excitatory and inhibitory synapses respectively

jected into the arterial supply to the brain stem or spinal cord. Serotonin antagonists injected into the vertebral artery were no more effective than when injected intravenously. However, the dose of LSD or methysergide required to block bulbospinal inhibition when injected into the abdominal aorta was less than one fifth the dose required by the intravenous route, when compared on the basis of animal weight. A greater differential between the intra-arterial and intravenous dose had been anticipated. However, the efficiency of the intra-arterial route was probably impaired by slow penetration of drug into the brain, since it was noted that rapid injections were less effective than slow ones. In any case injections of LSD and methysergide into the arterial supply of the spinal cord were clearly more effective than those administered intravenously.

These data suggest that the third diagram in Fig. 5 is most acceptable. This postulates the existence of a 5-HT containing interneuron in the lumbar spinal cord. Histochemical studies have failed to demonstrate such neurons (Carlsson *et al.*, 1964; Dahlstrom and Fuxe, 1964), and have suggested that all 5-HT in the cord is associated with descending neurons. However, chemical assay of 5-HT in the spinal cord following chronic mid-thoracic transection has shown that more than 0.3 μg/g of 5-HT remains in the lumbosacral cord 3 weeks after transection (Shibuya and Anderson, 1968). Since the sensitivity of the histochemical method for the detection of 5-HT is admittedly low (Jonsson *et al.*, 1969), it is possible that such interneurons have escaped detection.

The data from this study do not show whether the postulated 5-HT interneuron is the ultimate interneuron in the pathway with 5-HT functioning as an inhibitory

transmitter (as drawn in the 3rd diagram of Fig. 5), or whether the 5-HT interneuron is intercalated in the inhibitory pathway as an excitatory interneuron. The recent work of Phillis *et al.* (1969) showing that microelectrophoretically administered 5-HT hyperpolarizes motoneurons is compatable with the third diagram of Fig. 5.

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Dr. Edmund G. Anderson
Department of Pharmacology
University of Illinois College of Medicine
901 South Wolcott Avenue
Chicago, Illinois 60612 (USA)