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THE EFFECTS OF 5-HYDROXYTRYPTAMINE ANTAGONISTS ON SPINAL NEURONAL ACTIVITY[1, 2]

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ABSTRACT

BANNA, NABIL R. AND EDMUND G. ANDERSON: The effects of 5-hydroxytryptamine antagonists on spinal neuronal activity. J. Pharmacol. Exp. Therap. 162: 319-325, 1968. The effects of several 5-hydroxytryptamine (5-HT) antagonists were studied on potentials evoked in acute spinal cats by supramaximal stimulation of an L_7 dorsal root or peripheral nerve and recorded from the ipsilateral L_7 ventral root. The effects of the different 5-HT antagonists on the evoked potentials varied from marked depression to slight facilitation. Since these actions still appeared in animals depleted of endogenous 5-HT, they apparently are not associated with the antagonism of 5-HT. The 5-hydroxytryptophan (5-HTP)-induced increase in the monosynaptic spike amplitude and spontaneous motoneuronal discharge and decreased stimulus-response latency were immediately antagonized by the injection of the 5-HT antagonists, methysergide, 2-brom-*d*-lysergic acid diethylamide, *d*-lysergic acid diethylamide, cinanserin and cyproheptadine. The amount of antagonist necessary for complete reversal was directly proportional to the dose of 5-HTP. None of the 5-HT antagonists effectively antagonized the depressant actions of 5-HTP on polysynaptic and dorsal root reflexes. Pretreatment with a 5-HT antagonist also prevented the excitatory effects of 5-HTP. It is concluded that the 5-HT antagonists will effectively antagonize the excitatory, but not the depressant, effects of 5-HTP on evoked potentials in the spinal cord.

Recent studies in this laboratory (Anderson and Shibuya, 1966; Anderson *et al.*, 1967) have shown that raising the levels of 5-hydroxytryptamine (5-HT) in the cat spinal cord by the use of 5-HT precursors (5-hydroxytryptophan and tryptophan) and monoamine oxidase (MAO) inhibitors produced marked changes in spinal synaptic activity. The most notable of these changes were a pronounced increase in the monosynaptic spike amplitude and in spontaneous ventral root discharge and a decrease in stimulus-response latency. In addition, 5-hydroxytryptophan (5-HTP) produced a depression of the polysynaptic and dorsal root reflexes.

In view of the possible role of 5-HT in modifying motor outflow, it became of great interest to investigate the effects of some 5-HT antagonists on spinal synaptic activity. The following experiments were designed to study the effects of several 5-HT antagonists on spinal neuronal potentials in spinal cats which were untreated, depleted of endogenous 5-HT or pretreated with agents to elevate endogenous 5-HT.

METHODS. The methods used have been previously described in detail (Anderson *et al.*, 1967). Cats under ether anesthesia had the spinal cord transected at the C_1 level or were decerebrated at the intercollicular level, artificially respired and immobilized with gallamine triethiodide. After laminectomy, stimuli (15 times group I threshold) were applied to a whole dorsal root or peripheral nerve at a frequency of 12/min. This stimulus was supramaximal for the monosynaptic spike. Neuronal potentials were recorded from the ventral root and from an adjacent dorsal root (dorsal root reflex) or dorsal root entry zone (dorsal root potential). The lumbosacral cord was isolated from afferent inflow by severing all dorsal roots from L_5 to S_3. The mineral oil pool bathing the spinal cord and the rectal temperatures were held constant at 37°C. Carotid blood pressure was continuously

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TABLE 1

The effect of methysergide on spinal neuronal potentials evoked by stimulation of various nerves

Location of Stimulating Electrode	Dose or Dose Range of Methysergide	Percent Change in		
		Monosynaptic spike height	Polysynaptic area	Dorsal root reflex
	mg/kg			
Dorsal root	0.005	−6 (3)[a]	0 (3)	0 (2)
	0.01	−18 (4)	−7 (3)	−5 (4)
	0.02	−20 (1)	+30 (1)	0 (1)
	0.05	−30 (4)	−2 (2)	0 (1)
	0.15	−59 (3)		−6 (2)
	0.2-0.4	−55 (3)	16 (2)	
	0.8	−59 (1)		
	1.0-2.5	−49 (4)	−18 (3)	−23 (1)
	5.0-10.0	−52 (3)	−27 (2)	−37 (1)
Gastrocnemius-soleus	0.05	−18 (1)		
	0.10	−25 (2)	0 (2)	
	0.20	−26 (2)	0 (1)	
Anterior tibialis nerve	0.20	−60 (1)	0 (1)	
Hamstrings nerve	0.20	−58 (2)	−23 (2)	
Sural nerve	0.20		+5 (2)	
	2.0		−30 (2)	

[a] Results are average values of the number of experiments given in parentheses.

monitored and all drugs were dissolved in saline and injected into the antecubital vein *via* an indwelling catheter. A minimum of 3 hr elapsed between the cessation of ether anesthesia and the start of control recordings. During the control period the response of the preparation was monitored for 1 hr. Preparations showing a variability in potential size greater than ±10% were not used.

The following 5-HT antagonists were studied: methysergide (UML 491), BOL (2-brom-*d*-lysergic acid diethylamide), LSD (*d*-lysergic acid diethylamide), cinanserin [2′-(3-dimethylaminopropylithio)cinnamanilid hydrochloride], cyproheptadine and BAS (1-benzyl,2-methyl,5-methoxy tryptamine hydrochloride).[4]

RESULTS. *5-HT antagonists and spinal neuronal activity.* Immediately after the injection of methysergide, the monosynaptic spike evoked by dorsal root or peripheral nerve stimulation was selectively depressed. This effect was maximal within 1 to 4 min of injection and was accompanied by an increased stimulus-response latency which averaged 0.1 msec at doses producing maximal depression. The degree of depression of the monosynaptic spike was dose-dependent at doses below 0.2 mg/kg (table 1). Cumulative dose-response curves were obtained in individual animals by injecting an initial dose of 5 mg/kg of methysergide, followed by additional injections at 4-min intervals which doubled the total amount of drug in the animal with each injection. Figure 1 depicts the depression of the monosynaptic spike obtained by averaging the cumulative dose-response curves from five animals. From both table 1 and figure 1 it is apparent that about 0.2 mg/kg of methysergide produced a maximal depression of the monosynaptic spike height of about 50% which was not increased further, even by doses as high as 10 mg/kg.

Approximately 1 hr was required for complete recovery of the monosynaptic spike from the depressant effect of methysergide. After full recovery from an initial dose, a second dose produced much less depression of the monosynaptic spike. This tachyphylaxis to the depressant effects of methysergide persisted for several hours. However, when submaximal doses were repeated within short time intervals, as when obtaining cumulative dose-response curves, little tachyphylaxis was observed.

Methysergide in doses below 0.2 mg/kg caused no significant effect on polysynaptic or

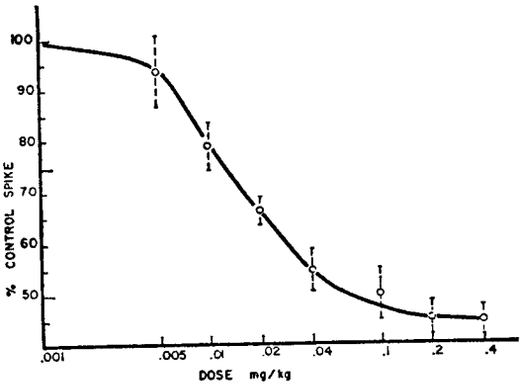

FIG. 1. A cumulative dose-response curve depicting the effects of the sequential injection of increasing doses of methysergide on the monosynaptic spike amplitude. This curve is the average of 5 separate experiments with the standard error indicated.

[4] We are indebted to Dr. John Gogerty of Sandoz Laboratories for supplies of methysergide and BOL and to Dr. Bernard Rubin of the Squibb Institute and Dr. Clement Stone of the Merck Institute for supplies of cinanserin and cyproheptadine respectively.

dorsal root reflexes. Higher doses depressed these potentials, with the longer latency polysynaptic potentials being affected more than the shorter latency potentials. The same depressant action was observed on the pure polysynaptic potentials evoked from sural nerve stimulation (table 1).

BOL in doses of 400 mg/kg rapidly depressed the monosynaptic spike height in five animals to an average of 83 ± 4% (±S.E.) of their control height. Larger doses produced little additional depression of the monosynaptic potentials, but further depressed polysynaptic activity.

LSD differed from methysergide and BOL in that doses of 300 μg/kg increased the monosynaptic spike height 54 ± 2% above their original control levels, while slightly depressing polysynaptic activity. Larger doses of LSD caused little additional increase in the monosynaptic spike amplitude, but did produce additional depression of polysynaptic potentials. These results are in close agreement with those reported by Geiger and Cervoni (1958).

Cinanserin (4 mg/kg), cyproheptadine (2 mg/kg) and BAS (7 mg/kg) were found to have minimal effects on spinal neuronal potentials immediately after injection. However, about 45 min after the injection of cinanserin a late facilitation of the monosynaptic spike was frequently observed.

The effects of methysergide and cinanserin obtained in decerebrate preparations were the same as in the acute spinal animals.

The changes in neuronal potentials caused by most of the 5-HT antagonists showed no correlation with their cardiovascular effects. The blood pressure response to methysergide in doses above 0.2 mg/kg was quite marked (10–20 mm Hg), but varied from pressor to depressor responses. However, the changes in neuronal potentials after methysergide were consistent in spite of the unpredictable blood pressure response. The cardiovascular changes following LSD, cinanserin and cyproheptadine were minimal, but the depressor response after BOL was sometimes quite marked. The rapid injection of 1 to 2 mg/kg of BOL frequently produced a precipitous fall in blood pressure in spinal cats and a subsequent depression of all spinal neuronal potentials. For this reason, the usage of BOL was restricted to slow injections of doses of 1 mg/kg or less.

5-HT antagonist in 5-HT-depleted animals. The endogenous cord levels of 5-HT were depleted by chronic cord transection and pretreatment with reserpine. Chronic cord transection has been reported by Carlsson *et al.* (1963) and Andén *et al.* (1964) to result in nearly total depletion of the 5-HT in the cord caudal to the transection. Similar results have been obtained in this laboratory (Shibuya and Anderson, 1966, unpublished observations). Chronic reserpine treatment was effected by injecting 2 mg/kg of reserpine phosphate 24 hr and 4 hr prior to reflex testing. The effects of methysergide on spinal neuronal potentials evoked from reserpine-treated and chronic transected cats were quantitatively similar to those found in acutely transected cats. Thus, the endogenous level of cord 5-HT appears to have no effect on the actions of methysergide.

Antagonism of the effects of 5-HT precursors. The injection of 5-HTP produces a number of effects on spinal neuronal potentials which correlate with rises in the cord level of 5-HT (Anderson and Shibuya, 1966). After 50 to 75 mg/kg of 5-HTP, a marked increase in the monosynaptic spike height and spontaneous ventral root discharge occurred in conjunction with a decreased stimulus-response latency. These excitatory effects were accompanied by decreases in the polysynaptic and dorsal root reflexes. All effects were maximal about 90 min after injection of 5-HTP and slowly returned toward control levels over a period of 4 to 5 hr. Hence, the effects of the 5-HT antagonists were determined 90 min after the injection of 5-HTP. In other experiments, the 5-HT antagonists were injected immediately prior to the administration of 5-HTP to determine their ability to prevent its actions.

The administration of a 5-HT antagonist during the height of 5-HTP action resulted in a dramatic reversal of all the excitatory effects of 5-HTP, but not its depressant actions. Thus, within 3 min of the injection of 0.5 to 2 mg/kg of methysergide the 5-HTP-induced increase in the monosynaptic spike height and the spontaneous motoneuronal discharge and the decrease in the stimulus-response latency were antagonized (see fig. 2). The dose of methysergide required for complete antagonism was directly proportional to the dose of 5-HTP. At the point of maximal antagonism the monosynaptic spike amplitude was brought down

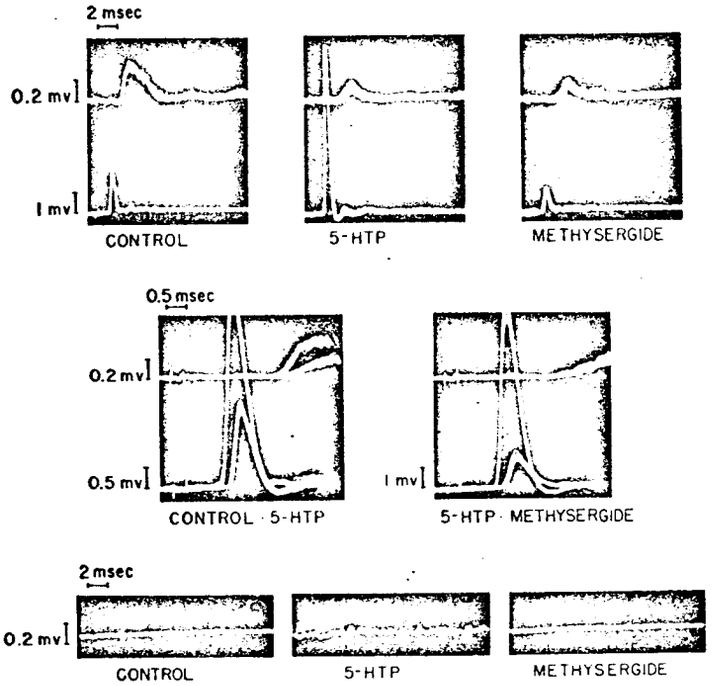

FIG. 2. A typical experiment depicting the antagonism of the effects of 5-hydroxytryptophan (5-HTP) by methysergide. Each evoked potential is a composite made by superimposing 15 consecutive oscilloscope sweeps. The lower beam is the ventral root recording and the upper beam is the dorsal root reflex. The top row depicts the effects of 75 mg/kg of 5-HTP (90 min after injection) followed by methysergide (1 mg/kg 3 min after injection) on the ventral root and dorsal root potentials. The middle row shows the latency change following 5-HTP superimposed on the control spikes (first frame) and the reversal of this latency change by 1 mg/kg of methysergide (second frame). The bottom row demonstrates the effects of 5-HTP and methysergide on spontaneous motoneuronal discharge monitored from the whole ventral root (each trace is a single oscilloscope sweep).

below, and the stimulus-response delay was increased to above, the pre-5-HTP control levels. Thus, the direct depressant effects of methysergide appeared to be imposed on top of the effects due to 5-HT antagonism.

Methysergide failed to antagonize the 5-HTP-induced depression of the polysynaptic or dorsal root reflexes. Instead, the doses of methysergide necessary to antagonize the excitatory actions of 5-HTP usually further depressed these reflexes. A partial antagonism of the 5-HTP-induced depression of the dorsal root potential was occasionally observed with doses below 1.5 mg/kg.

The antagonistic effects of methysergide toward the excitatory effects of 5-HTP declined after a period of 30 to 60 min and the facilitatory effects of 5-HTP reappeared. A second dose of methysergide again antagonized the excitatory effects of 5-HTP, but the monosynaptic spike amplitude was not depressed to below the original control level. This probably reflected the tachyphylaxis to the direct depressant effects of methysergide.

Pretreatment of cats with 2 mg/kg of methysergide just prior to the injection of 5-HTP prevented the facilitatory effects of 5-HTP from manifesting their presence for about 2 hr. However, the depressant effects of 5-HTP on the polysynaptic and dorsal root reflexes appeared as usual.

The antagonism of the effects of 5-HTP by BOL was qualitatively similar to that of methysergide. Doses of 1 mg/kg of BOL after 75 mg/kg of 5-HTP usually reversed all of the excitatory effects of 75 mg/kg of 5-HTP, returning them to near control levels. By administering doses of BOL in a stepwise manner (fig. 3), it was possible to minimize cardiovascular changes and titrate the amount of BOL necessary for complete antagonism of the excitatory effects of 5-HTP. BOL, like methysergide,

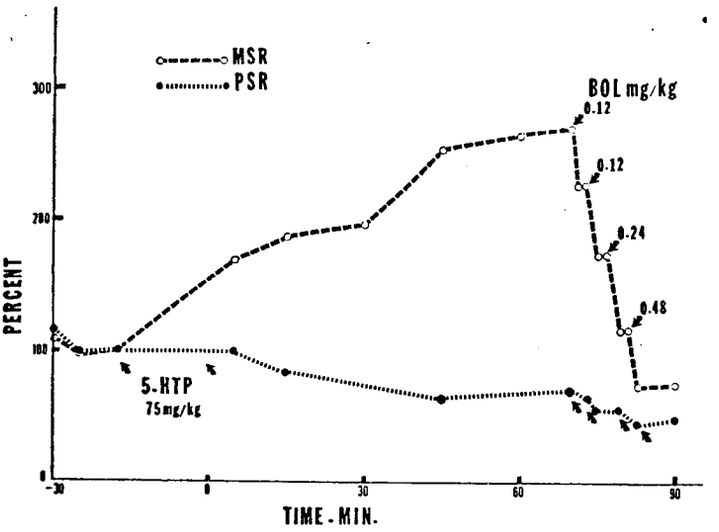

FIG. 3. A typical experiment illustrating the antagonism of the effects of 5-hydroxytryptophan by sequential injections of BOL. ○, the monosynaptic spike height; ●, the area beneanth the polysynaptic potentials.

failed to antagonize the depressant effects of 5-HTP on the polysynaptic and dorsal root reflexes. Instead, it further depressed these responses (fig. 3).

LSD, cinanserin and cyproheptadine also antagonized the facilitatory effects of 5-HTP by returning the monosynaptic spike height, stimulus-response latency and spontaneous ventral root discharge to near control levels (see fig. 4). To antagonize the effects of 50 mg/kg of 5-HTP, doses of 1.5 mg/kg of LSD and cyproheptadine and 4 mg/kg of cinanserin were required. Larger doses of 5-HTP required larger doses of these antagonists. The depressant effects of 5-HTP on the polysynaptic and dorsal root reflexes were not significantly affected by these antagonists. In an occasional experiment some antagonism of these depressant effects was observed (see LSD in fig. 4), but this was not a consistent response.

BAS in doses up to 7 mg/kg failed to show an antagonism toward the effects of 5-HTP. This was the only antagonist investigated which failed to reverse the excitatory effects of 5-HTP.

As previously reported, 100 mg/kg of *l*-tryptophan produced an increase in the monosynaptic spike height averaging 72% (Anderson and Shibuya, 1966). After pretreatment with a MAO inhibitor, 75 mg/kg of *l*-tryptophan produced excitatory effects on the spinal neuronal potentials which were quantitatively and qualitatively similar to those of 75 mg/kg of 5-HTP. The 5-HT antagonists immediately returned the excitatory effects of *l*-tryptophan to control levels.

Specificity of the 5-HT antagonists. The administration of *l*-3,4 dihydroxyphenylalanine (dopa) has been observed to alter ventral root potentials in a manner qualitatively similar to the effects of 5-HTP (Baker and Anderson, 1965). Experiments were therefore carried out to determine whether the 5-HT antagonists would show any selective antagonism toward the effects of 5-HTP or dopa. All of the 5-HT antagonists, except BAS, were found to antagonize the excitatory effects of 20 mg/kg of dopa. Both cinanserin and LSD completely antagonized dopa by returning the increased monosynaptic spike and decreased stimulus-response latency after dopa to control levels. Methysergide in doses of 1 mg/kg showed antagonism toward the excitatory effects of 20 mg/kg of dopa, but usually failed to return the potentials to their control levels, whereas the excitatory actions of 50 mg/kg of 5-HTP were returned to below control levels by this dose of methysergide. This tendency toward a selective antagonism was more clearly demonstrated in experiments in which pretreatment with 2 mg/kg of methysergide prevented the effects of up to 75 mg/kg of 5-HTP but not the

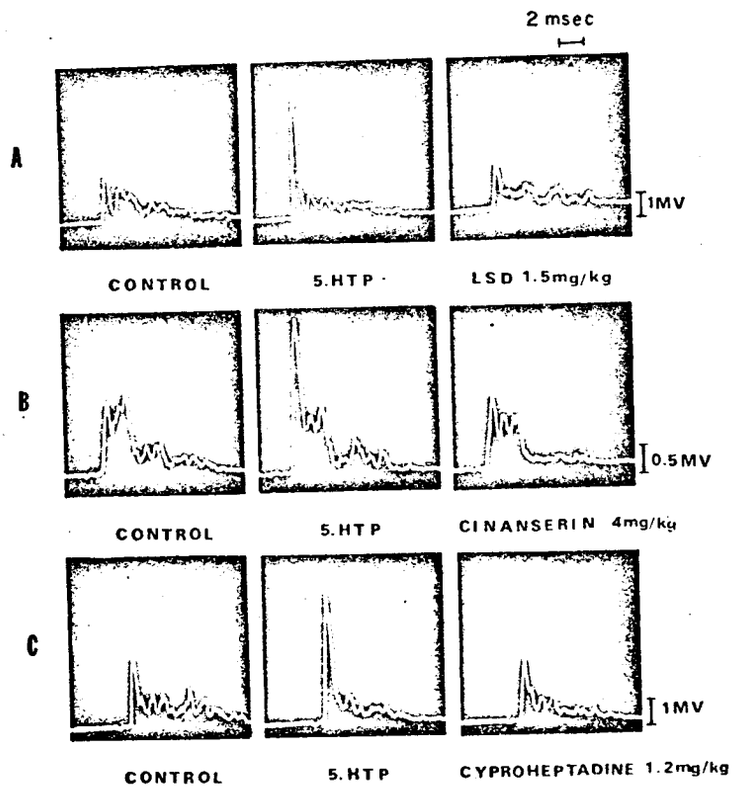

FIG. 4. Three typical experiments showing the antagonism of the effects of 5-hydroxytryptophan on ventral root potentials by LSD, cinanserin and cyproheptadine.

effects of 40 mg/kg of dopa. However, the effects of dopa were somewhat reduced. Thus, methysergide shows more antagonism toward 5-HTP than to dopa.

DISCUSSION. A careful attempt has been made in this study to separate the effects of the 5-HT antagonists on spinal neuronal potentials due to antagonism of 5-HT from those due to other actions of these compounds. In spite of the common ability of all of these compounds to antagonize 5-HT, their effects on the monosynaptic spike in acute spinal cats varied from facilitation to depression. This suggests that these actions are not due to the antagonism of endogenous 5-HT. This inference is confirmed by the experiments in which, after the endogenous 5-HT levels were depleted by chronic cord transection or pretreatment with reserpine, methysergide demonstrated the same depressant actions as seen in the normal cat.

Carlsson *et al.* (1963, 1964) have presented evidence that spinal cord 5-HT is associated with descending bulbospinal neurons in the rabbit, rat and mouse; Shibuya and Anderson (1966) have partially confirmed this in the cat. Thus, the lack of any action by the 5-HT antagonists in the acute spinal cat attributable to the antagonism of 5-HT would be expected, if the endogenous 5-HT is largely associated with descending neurons severed from their cell bodies by cord transection and thus made quiescent. These descending systems appear not to be tonically activated in the decerebrate cat since the injection of cinanserin into decerebrate animals failed to show any action on the segmental reflexes.

Methysergide's depressant action in the spinal cat, which is unassociated with 5-HT antagonism, is highly interesting because of its selectivity and potency. The potency of methysergide becomes even more impressive in view of the fact that only 13% of the amount expected in comparison to LSD has been reported to penetrate the central nervous system (Doepfner, 1962). The selectivity of

methysergide's action in depressing monosynaptic spike amplitude without affecting polysynaptic potentials was observed after flexor or extensor nerve as well as whole dorsal root stimulation. Another unusual aspect of this depressant action is the 50% limit of the block regardless of the dose. The methysergide action resembles the block of the monosynaptic reflex produced by harmaline (Anderson *et al.*, 1967), but these two compounds appear to act through different mechanisms since the combined administration of methysergide and harmaline abolished the monosynaptic spike while only partially depressing the polysynaptic activity. These observations suggest that methysergide and harmaline depress motoneuronal activation by interfering with primary afferent transmission. A further observation of interest was that the depressant action of methysergide was blocked by pretreatment with 0.3 mg/kg of LSD, but not by any of the other 5-HT antagonists.

The effects of 5-HTP have been postulated to result from 5-HT as evidenced by its slow onset of action and the marked rise in cord 5-HT levels after 5-HTP injection (Anderson and Shibuya, 1966). The ability of the 5-HT antagonists to reverse rapidly the 5-HTP-induced increase in the monosynaptic spike amplitude, increase in spontaneous motoneuronal discharge and decrease in stimulus-response latency lends further support to this conclusion. The direct depressant effects of methysergide and BOL are superimposed on the effects due to the antagonism of 5-HT, since they returned the monosynaptic spike to amplitudes below the pre-5-HTP control level. Consequently, whenever these compounds were used, care had to be taken to account for their direct depressant effects as well as their effects due to antagonism of 5-HT. No ready explanation is available for the failure of BAS to show any antagonism to 5-HTP.

The failure of any of the 5-HT antagonists to reverse effectively the depressant effects of 5-HTP on the polysynaptic and dorsal root reflexes or to prevent this depression on pretreatment is of particular interest. This suggests that the excitatory and depressant effects of 5-HTP on spinal synaptic activity are independent actions and that the respective receptors activated by 5-HT are pharmacologically distinct. It is tempting at this point to postulate that the descending serotonergic neurons contain two functionally distinct systems, one producing increased motoneuronal excitability and the other functioning to depress interneuronal systems. According to this hypothesis 5-HTP functions by increasing the 5-HT levels in all serotonergic neurons with consequent 5-HT overflow and activation of the excitatory and depressant receptor systems. The administration of a 5-HT antagonist blocks the receptors involved in the excitatory actions but allows the depressant actions of 5-HT to remain.

The ability of the 5-HT antagonists to antagonize the excitatory actions of dopa requires caution in attributing all of the actions of the antagonists to reactions with 5-HT receptors. This picture is further complicated by the marked similarities between the effects of 5-HTP and dopa on spinal neuronal potentials. Methysergide demonstrated some selectivity in more effectively antagonizing 5-HTP than dopa, but its direct depressant actions unfortunately complicate the interpretation of its actions as an antagonist.

REFERENCES

ANDÉN, N. E., HAGGENDAL, J., MAGNUSSON, T. AND ROSENGREN, E.: The time course of the disappearance of noradrenaline and 5-HT in the spinal cord after transection. Acta Physiol. Scand. **62**: 115–118, 1964.

ANDERSON, E. G., BAKER, R. G. AND BANNA, N. R.: The effects of monoamine oxidase inhibitors on spinal synaptic activity. J. Pharmacol. Exp. Therap. **153**: 405–415, 1967.

ANDERSON, E. G. AND SHIBUYA, T.: The effects of 5-hydroxytryptophan and *l*-tryptophan on spinal synaptic activity. J. Pharmacol. Exp. Therap. **153**: 352–360, 1966.

BAKER, R. G. AND ANDERSON, E. G.: The effect of *l*-3,4, dihydroxyphenylalanine on spinal synaptic activity. Pharmacologist **7**: 142, 1965.

CARLSSON, A., FALCK, B., FUXE, K. AND HILLARP, N.: Cellular localization of monoamines in the spinal cord. Acta Physiol. Scand. **60**: 112–119, 1964.

CARLSSON, A., MAGNUSSON, T. AND ROSENGREN, E.: 5-Hydroxytryptamine in spinal cord, normally and after transection. Experientia **19**: 359, 1963.

DOEPFNER, W.: Biochemical observation on LSD-25 and Deseril. Experientia **18**: 256–257, 1962.

GEIGER, L. E. AND CERVONI, P.: Effect of d-lysergic acid diethylamide on spinal reflexes. Proc. Soc. Exp. Biol. Med. **99**: 179–181, 1958.

SHIBUYA, T. AND ANDERSON, E. G.: A comparison of 5-hydroxytryptophan, l-tryptophan and pargyline in acute and chronic spinal cats. Abstracts of the Third International Pharmacological Congress, p. 91, 1966.