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EFFECT OF ANTIPSYCHOTIC DRUGS ON THE FIRING OF DORSAL RAPHE CELLS. I. ROLE OF ADRENERGIC SYSTEM

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The activity of serotonergic (5HT) neurons in the dorsal raphe nucleus was inhibited by the i.v. administration of certain antipsychotic drugs (methiothepin, clozapine and thioridazine). However, other antipsychotic agents (chlorpromazine, haloperidol and pimozide) did not inhibit raphe cell firing. The inhibitory potency of these drugs on raphe activity correlates with reported central noradrenergic blocking efficacy. An α-adrenergic blocking agent, piperoxane, but not the β-blocking agents, propranolol and MJ 1999, inhibited raphe activity when administered systemically. All of these drugs appear to act indirectly since they (and NE) have relatively weak or variable effects when applied microiontophoretically to raphe neurons.

The depressant effects of certain antipsychotic drugs and piperoxane on 5HT neurons appears to be mediated by a central adrenergic system since (1) the depression could be reversed by the catecholamine releasing agents l- and d-amphetamine; (2) the depression could be abolished by destruction of adrenergic pathways in the CNS by chemical, mechanical, or electrothermic lesions. While a precise localization has not yet been obtained, the data suggest that these drug effects may be mediated by an adrenergic pathway ascending from the lower brainstem.

Antipsychotic drugs | Dorsal raphe nucleus | Single cell recording | Microiontophoresis
Amphetamine | Serotonin

1. Introduction

Previous studies have shown that serotonergic neurons in the dorsal raphe nucleus are inhibited by very low intravenous doses of the antipsychotic drug methiothepin (MTT) (Haigler and Aghajanian, 1974a). A number of investigators have found that brain levels of 5-hydroxyindoles are elevated following this drug (Monachon et al., 1972; Fuller and Perry, 1974; Lloyd and Bartholini, 1974; and Jacoby et al., 1975). Recently, another antipsychotic drug, clozapine (CLZ) has also been shown to increase the concentration of 5HT and 5HIAA in the brain (Bürki et al., 1975). However, alterations in 5HT metabolism or raphe cell firing are not produced by all antipsychotic drugs since systemically administered chlorpromazine (CPZ) does not affect 5-hydroxyindole levels in brain (Laverty and Sharman, 1965; Gey and Pletscher, 1961) or raphe cell firing (Aghajanian et al., 1970a). Interestingly, both MTT and CLZ have been reported to be potent central NE blocking agents while CPZ appears to have relatively weak NE-receptor blocking ability (Keller et al., 1973). Thus, the effects of these drugs on the 5HT system could correlate with central adrenolytic efficacy. There is anatomical, biochemical and physiological evidence suggesting an interaction between adrenergic and serotonergic neuronal systems within the

CNS. Fuxe (1965) demonstrated fine catecholamine (CA) terminals within the dorsal raphe nucleus. Loizou (1969) reported that some of these terminals disappeared following lesions of the n. locus coeruleus, a nucleus consisting almost exclusively of CA-containing cell bodies (Dahlström and Fuxe, 1964). Lesions of the n. locus coeruleus have been shown to produce significant increases in the 5HT metabolite 5-hydroxyindoleacetic acid (5HIAA) in the forebrain (Jouvet, 1973; Kostowski et al., 1974). An increase in serotonin (5HT) turnover has also been observed following administration of the CA-specific neurotoxin, 6-hydroxydopamine (6OHDA) (Blondaux et al., 1973), after the administration of a tyrosine hydroxylase inhibitor, α-methyl-p-tyrosine (Stein et al., 1974), or after the administration of a dopamine-β-oxidase inhibitor (Johnson et al., 1972). Morgane et al., (1974) have reported that neuronal activity in the dorsal raphe (5HT) nucleus is inhibited by stimulation of CA cell bodies in the n. locus coeruleus. In addition, Svensson et al., (1975) have shown that very small intravenous doses of the α-adrenergic agonist, clonidine, are able to inhibit most 5HT neurons in the dorsal raphe nucleus.

All of these studies suggest that CA neurons exert a regulatory influence upon 5HT neurons in the dorsal raphe nucleus, most imply that such an interaction originates in the n. locus coeruleus. However, Roizen and Jacobowitz (1976) report that the ventral norepinephrine (NE) pathway, not the locus coeruleus, may contribute most of the NE input to the raphe area. Immunohistofluorescence studies of Hökfelt et al., (1974) have demonstrated that ascending epinephrine-containing neurons within or near the ventral NE pathway also project to the vicinity of the dorsal raphe area.

In present study, we tested a series of antipsychotic and adrenolytic drugs for their effects on the rate of firing of raphe (5HT) cells in the brain. In addition, the possible mediation of such effects by various CA pathways in brain was investigated.

2. Materials and methods

2.1. Single unit recording and microiontophoresis

Male Albino rats (Charles River, 220—280 g) were used. For purposes of recording, animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic apparatus. Additional injections of chloral hydrate were given as needed throughout the experiment. In experiments where drugs were administered only by the systemic route, single barrel micropipettes (tip diameters: 1—2 μm) were used. Micropipettes were filled with 2 M NaCl saturated with Fast Green FCF (Fisher) dye. The electrodes typically had an impedance of 4—6 MΩ when measured at 60 Hz. A burr hole was made in the midline of the skull over the midbrain raphe area (approximately 0.8—1.0 mm anterior to lambda). Electrodes were lowered into the midbrain raphe region with a hydraulic microdrive system. Electrode signals were passed through a high input-impedance amplifier and monitored on an oscilloscope. With optimal electrode placement a zone of electrical silence, which corresponded to the location of the cerebral aqueduct, was encountered above the dorsal raphe area. Integrated firing rate was recorded by means of a rate meter. Body temperature was measured by a rectal probe, and was maintained between 36—37°C. Under these conditions, midbrain raphe units displayed a regular rhythm and rate of 0.5—2.5 spikes/sec which is typical for histochemically identified 5HT units (Aghajanian and Haigler, 1974). Recording sites were marked by passing a 16 μA cathodal current through the recording barrel for 10 minutes which resulted in the deposition of a discrete spot of fast green dye (Thomas and Wilson, 1965). The precise location of the electrode tip was confirmed by histological examination and compared with the previously established location of 5HT-containing neurons in the dorsal raphe nucleus. Placement of the electrodes ranged between frontal planes P 400 μm to A 650

μM of König and Klippel (1970).

In microiontophoretic studies, 5-barrel micropipettes were used according to techniques previously described (Haigler and Aghajanian, 1974b). The tips were broken back to 4—5 μm under microscopic control; solutions were then injected directly into the various barrels. The presence of fiberglass allowed the tips to become filled quickly by capillary action (Tasaki et al., 1968).

The central (recording) barrel was filled with a 2 M NaCl solution saturated with Fast Green dye. One side barrel, the 'balance' channel, was filled with 4 M NaCl. The other three barrels were each filled with one of the following drugs: 0.05 M serotonin creatinine sulphate, pH 4.0 (Sigma Chemical Co.); 0.1 M l-norephinephrine bitartrate, pH 4.0 (Regis Chemical Co.); 0.001 M methiothepin maleate, pH 5.0 (Hoffman-LaRoche, Inc.); 0.1 M piperoxane chlorhydrate, pH 5.0, (Rhone-Poulenc); 0.1 M thioridazine HCl, pH 5.0, (Sandoz Pharmaceuticals); 0.1 M chlorpromazine HCl, pH 5.0, (Smith, Kline & French Labs); 0.5 M MJ1999 'sotalol HCl', pH 5.0, (Mead Johnson & Co.). Impedances, measured at 60 HZ were 3—8 MΩ in the recording barrel, 10—15 MΩ in the balance channel, and 60—150 MΩ in the drug barrels.

Drugs were ejected through micropipette barrels by turning the direct current source from −8 to −12 nA of 'retaining' current to +10 to +20 nA of 'ejecting' current. Circuits for drug ejection and current balance were similar to those described by Salmoiraghi and Weight (1967), except that a solid state system was used. Recording sites were marked by ejecting fast green dye through the recording barrel as described above.

2.2. *Lesions and transections*

In some animals, acute high frequency lesions were made in the area of the n. locus coeruleus or in the reticular formation using a Grass model LM4 lesion maker (5—7 mA, 60 sec). Clay-Adams (Boston, Mass.) insect pins (size 1), insulated to within 0.8 mm of the tip, were used as electrodes. Coordinates for the lesions were: (1) locus coeruleus: ±1.1 mm, lateral to the midline, 1.0 mm posterior to the lambda and 7.0 mm ventral to skull surface; (2) reticular formation: ±0.7 mm lateral to the midline, 2.0 mm posterior to lambda, and 7.5 mm ventral to skull surface. Recording experiments in lesioned animals were performed immediately following the placement of lesions. The size and locations of lesions were verified histologically for each animal.

In another group of animals the effects of acute transections on the drug responses of dorsal raphe firing were tested using a stereotaxically positioned retractable wire knife (Sclafani and Grossmann, 1969). Transections were made at the border of the midbrain and diencephalon which separated the forebrain from the midbrain raphe recording area. In addition, transections made through the pontine reticular formation (1.6 mm to 2.1 mm posterior to lambda) separated the dorsal raphe area from connections in the lower brainstem. The completeness and location of the transections were verified histologically for each animal.

2.3. *6OHDA lesions*

In rats receiving intraventricular injections of 6-hydroxydopamine, (6OHDA, Regis) animals were pretreated 30 min prior to the intraventricular injection with 1 mg/kg chlorimipramine (CIMI) to decrease the possibility of uptake and damage of 5HT neurons by 6OHDA. An analogous pretreatment with desipramine has been shown to be useful in preventing catecholamine depletion following the neurotoxic indoleamine 5,7-dihydroxytryptamine (Björklund et al., 1975).

Following the CIMI pretreatment, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic apparatus. 200 μg (free base) of 6OHDA was injected in a volume of 20 μl of a 1 mg/ml ascorbic acid solution. Injection coordinates and technique are as reported by Svensson et al.

(1975). Lesioned animals were used for recording experiments 9—23 days later. The extent of the 6OHDA induced destruction of CA neurons was checked by histochemical fluorescence using a slight modification of the Falck-Hillarp formaldehyde condensation technique (Falck et al., 1962).

2.4. Blood pressure and heart rate measurements

Systolic blood pressure and heart rate measurements were obtained by an indirect tail-cuff method in anesthetized rats (chloral hydrate, 400 mg/kg, i.p.) using an electrosphygmomanometer (Narco Biosystems, Houston, Texas). Animals were anesthetized and maintained at 37°C. Drugs were injected i.v. in doses similar to those used in single unit recording experiments. Blood pressure and heart rate measurements were taken at 30 sec, 1 min, 3 min, 5 min, 10 min, 20 min, 30 min following drug administration.

2.5. Drugs

p-Chlorophenylalanine methyl ester HCl (PCPA, in a dose of 400 mg/kg, pretreatment 24 h prior to recording, Regis Chemical Co.) and pimozide (PIM, McNeil Labs.) were administered intraperitoneally. Other drugs, including R04-4502 ([N-(D,L-Seryl)-N-(2,3,4-trihydroxybenzyl)-hydrazine] HCl, in a dose of 800 mg/kg, pretreatment 45 min prior to recording, Hoffman LaRoche, Inc.); d-lysergic acid diethylamide bitartrate (LSD); l-amphetamine sulfate (l-A); d-amphetamine sulfate (d-A); apomorphine (APO); physostigmine salicylate, (Regis Chemical Co.); thioridazine HCl (THZ, Sandoz Pharmaceuticals); clozapine (CLZ, Sandoz Pharmaceuticals); methiothepin maleate (MTT, Hoffman-LaRoche, Inc.); haloperidol (HAL, McNeil Labs.); piperoxane chlorhydrate (PIP, Phone-Poulenc); chlorpromazine HCl (CPZ, Smith, Kline & French Labs.); and MJ 1999 ('isotalol HCl', Mead Johnson & Co.) were administered i.v. via a tail vein.

3. Results

3.1. Effects of intravenous drugs on the firing rate of 5HT neurons

The i.v. administration of the antipsychotic agents MTT (0.12 ± 0.01 mg/kg, mean dose ±S.E., n = 26), CLZ (0.94 ± 0.18 mg/kg, n = 5), and THZ (3.9 ± 0.8 mg/kg, n = 8) were found to totally inhibit raphe cell firing (fig. 1). In contrast, 3 other antipsychotic drugs had relatively little or no effect on

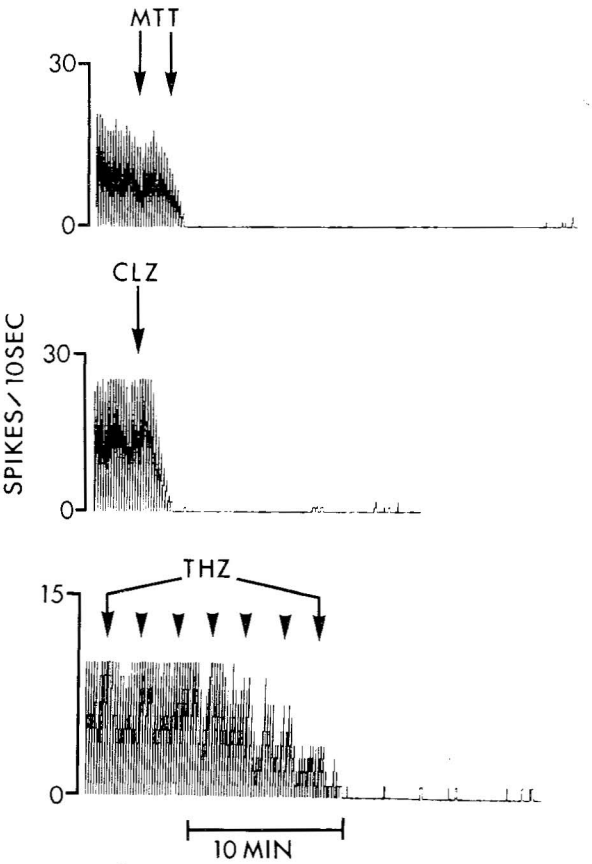

Fig. 1. Inhibition of a serotonergic cell in the dorsal raphe nucleus by the i.v. administration of methiothepin (MTT, top trace), clozapine (CLZ, middle trace) and thioridazine (THZ, bottom trace). Raphe cells were inhibited by cumulative doses of 0.1 mg/kg of MTT, 1.5 mg/kg CLZ, and 6 mg/kg of THZ, respectively.

raphe cell firing. Large doses of CPZ (17.6 ± 2.9 mg/kg, n = 5) slowed raphe firing by only 50%; in agreement with a previous study (Aghajanian et al., 1970a), the response of raphe firing to LSD was unaffected by this pretreatment. Large doses of pimozide (PIM, 30 mg/kg, i.p.) and near lethal doses of haloperidol (HAL, 9.6 ± 1.6 mg/kg, n = 4) did not inhibit raphe neuronal activity (Fig. 2).

Since the potency of these drugs to inhibit raphe neuronal activity correlates with presumed central noradrenergic blocking efficacy (Keller et al., 1973) several types of experiments were performed to investigate a possible connection between NE blockade and raphe neuronal activity. First, to determine whether the raphe effects could be classified in terms of α or β, a water-soluble α-blocking agent, piperoxane (PIP) and a β-blocking agent, propranolol (PRO) were administered. Piperoxane (7.5 ± 0.9 mg/kg, n = 4) was effective in inhibiting raphe cell firing while propranolol, which is known to readily enter the brain (Masuoka and Hansson, 1967; Laverty and Taylor, 1968), was unable to totally inhibit the firing of raphe cells even in near lethal doses (19.6 ± 0.7 mg/kg, n = 4, fig. 3). Another β-blocking agent, MJ 1999, was found to be without effects on raphe firing (14.1 ± 0.6 mg/kg, n = 3, not shown).

Next, the ability of the d- and l-isomers of amphetamine (A) to reverse the depressant effects of the antipsychotic agents and piperoxane on raphe activity was investigated. Following the inhibition of raphe units by intra-

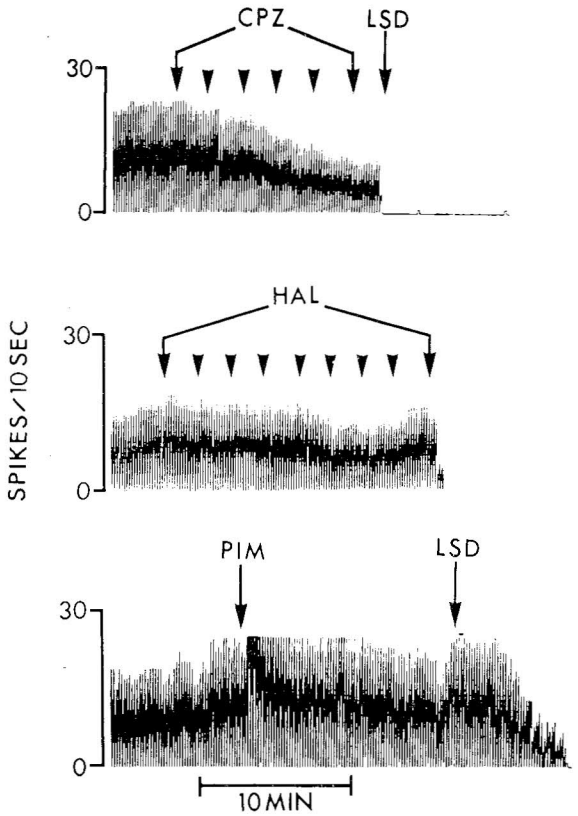

Fig. 2. Lack of effect of antipsychotic agents chlorpromazine (CPZ, top trace), haloperidol (HAL, middle trace), and pimozide (PIM, bottom trace). CPZ in a total i.v. dose of 9.4 mg/kg decreased firing by 50% while 10 μg/kg LSD totally inhibited raphe cell firing. HAL did not affect the raphe firing rate in up to a lethal dose (11.2 mg/kg, i.v.). The dorsal raphe cell was also not inhibited by PIM (30 mg/kg, i.p.), but was completely inhibited by LSD (50 μg/kg, i.p.).

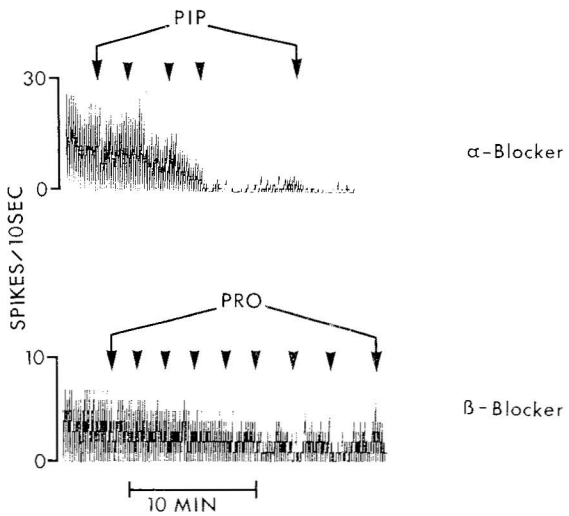

Fig. 3. Comparison of the response of a dorsal raphe cell to the systemic administration of an α-adrenergic blocking drug piperoxane (PIP) and a β-adrenergic blocking drug, propranalol (PRO). PIP totally inhibited raphe cell firing at a cumulative dose of 6 mg/kg (top trace) while PRO was not able to totally inhibit the cell until a lethal dose (20 mg/kg) was administered (bottom trace).

venous MTT and CLZ (fig. 4) or other agents (THZ and piperoxane — not shown), l- and d-amphetamine were approximately equipotent in reversing the depressant effects. A 50% reversal of MTT-induced depression of raphe firing was achieved with an average dose of 1.50 ± 1.08 mg/kg of d-A (n = 6) and 1.58 ± 1.02 mg/kg of l-A (n = 6).

The intraventricular injection of 6OHDA (9—23 days prior to recording) produced a resistance to the depressant effects of MTT (and other antipsychotic agents) on raphe firing in some animals (fig. 5 — top trace). However, in approximately one-half of animals given intraventricular 6OHDA (12 out of 25) no alteration in the response of their raphe cells to MTT was observed (fig. 5 — bottom trace). In these non-resistant animals, the reversal of raphe firing by l- and d-A, normally observed in control animals, was abolished by 6OHDA pretreatment. Histochemical fluorescence studies showed that 'resistant' rats all showed extensive destruction of CA neurons throughout the brain including terminals in the ven-

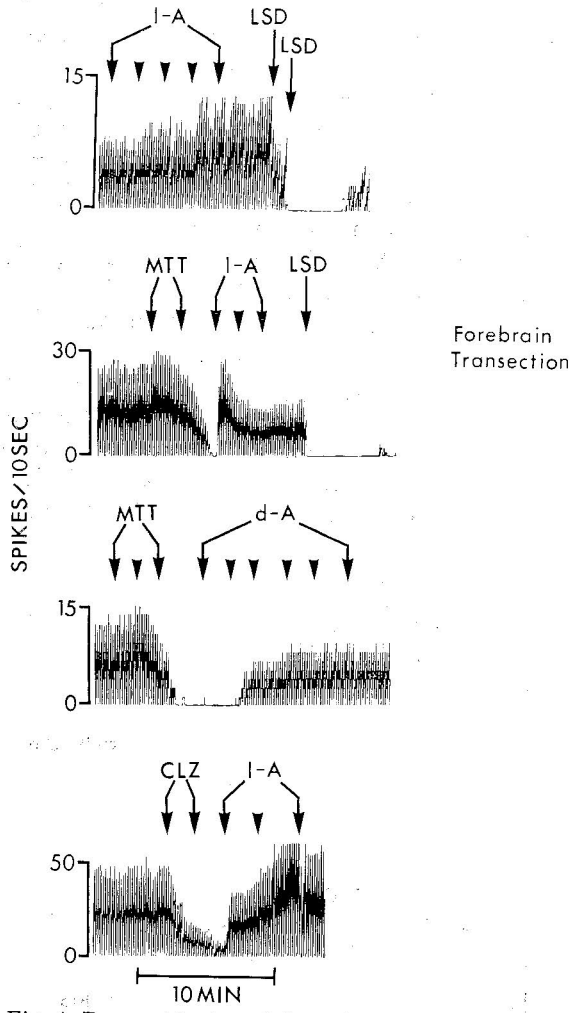

Fig. 4. Reversal by l- and d-amphetamine (l-A, d-A) of the inhibition of firing of neurons in the dorsal raphe nucleus by MTT. l-Amphetamine (total i.v. dose of 3 mg/kg) increased the firing rate of a raphe cell by approximately 30% (top trace). The cell was subsequently inhibited by LSD, 20 μg/kg, i.v. Following the inhibition of raphe cells by MTT (0.15 mg/kg, i.v.) both l-A (l-A, 2.5 mg/kg, second trace) and d-A (d-A, 3 mg/kg, third trace) reversed the inhibition of the cells. The inhibition of raphe firing produced by clozapine (CLZ, 0.8 mg/kg, i.v., bottom trace) was also reversed by l-amphetamine (l-A, 2.5 mg/ml, i.v.).

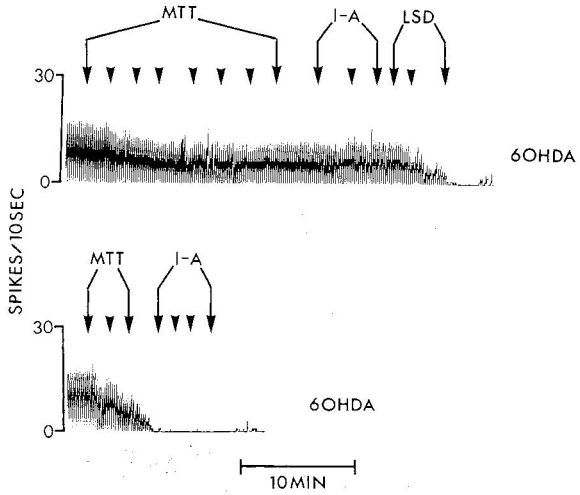

Fig. 5. Variable effects of i.v. methiothepin (MTT) on the firing rate of neurons in the dorsal raphe nucleus in rats pretreated for 12 days with 6-hydroxydopamine (6OHDA) intraventricularly (200 μg). MTT was injected via a tail vein in a cumulative dose of 0.5 mg/kg with no effect (top trace). In addition, l-amphetamine (l-A, 1.5 mg/kg) also had no effect while LSD (30 μg/kg) completely inhibited the firing of the cell. In a second 6OHDA pretreated rat (bottom trace) MTT (0.15 mg/kg, i.v.) totally inhibited raphe cell firing but l-amphetamine (l-A, 3 mg/kg, i.v.) was unable to reverse this depression.

trolateral central gray area of the midbrain and the ventral tegmental area, cell bodies in the n. locus coeruleus and fibers in both dorsal and ventral NE pathways. 'Non-resistant' animals usually showed lesser degrees of CA depletion.

In addition to 60HDA pretreatment, electrothermic lesions of the locus coeruleus were made bilaterally (n = 5). Neither the depressant effects of MTT on raphe cell firing nor the ability of amphetamine to reverse MTT-induced depression of raphe firing was affected by complete bilateral locus lesions (fig. 6).

In an effort to localize the regional site of action of the antipsychotic drugs, transections separating the raphe area from either the forebrain or lower brainstem area were performed. Following a transection which was made at the border of the midbrain and diencephalon (fig. 4 — second trace), the response of raphe unit activity to MTT was tested. In spite of the elimination of all direct forebrain influences on the recording area, the effects of MTT and l-A on raphe firing were the same as in control animals (n = 5). However, most animals (10 out of 14) with pontine transections at the level of the locus coeruleus and motor n. V, showed resistance to MTT-induced depression. These rats did not show

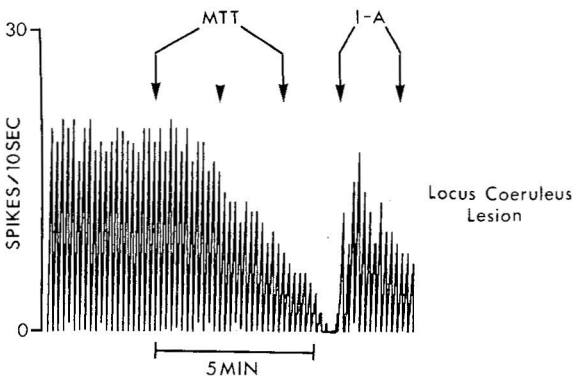

Fig. 6. Lack of effect of bilateral lesions of the n. locus coeruleus to alter the inhibitory response of methiothepin (MTT, 0.15 mg/kg, i.v.) or the reversal by l-amphetamine (l-A, 1.5 mg/kg, i.v.) of dorsal raphe cell activity.

an alteration in the responsiveness of their raphe cells to LSD, suggesting that systemically administered drugs were reaching the raphe area in spite of the transection. More discrete electrothermic lesions were attempted in the transection area in an effort to define a nucleus or pathway in this interaction. Animals with electrothermic lesions involving the n. reticularis pontis caudalis (according to Valverde, 1962) were found to be resistant to the MTT depression of raphe firing (fig. 7). Lesions of more anterior reticular areas (e.g., n. reticularis pontis oralis) or the ventral tegmental nucleus were ineffective in producing resistance to MTT.

3.2. Microiontophoretic studies

The iontophoretic application of both NE and MTT were without marked effects in the firing rate of raphe cells (fig. 8) in contrast to low doses of systemically administered MTT. However, when 5HT was applied iontophoretically, it produced a rapid inhibition in all raphe units tested. In addition, the iontophoretic application of the other antipsychotic agents and an α and a β-blocker produced either no effect or a depression of raphe firing which did not correlate with effects observed after the systemic administration of these drugs (table 1).

3.3. Interaction of antipsychotics with other drugs

Additional data indicates that the depression in raphe firing caused by antipsychotic agents (CLZ and MTT) could not be reversed by the systemic administration of physostigmine, an acetylcholinesterase inhibitor (0.24 ± 0.02 mg/kg, n = 4). In addition, apomorphine (a dopamine agonist) was found to be ineffective in reversing the effects of MTT on raphe firing (2.2 ± 0.1 mg/kg, n = 3). After the i.v. administration of apomorphine, l-A (2.0 mg/kg) was still found to be effective in reversing the depression of raphe firing caused by MTT.

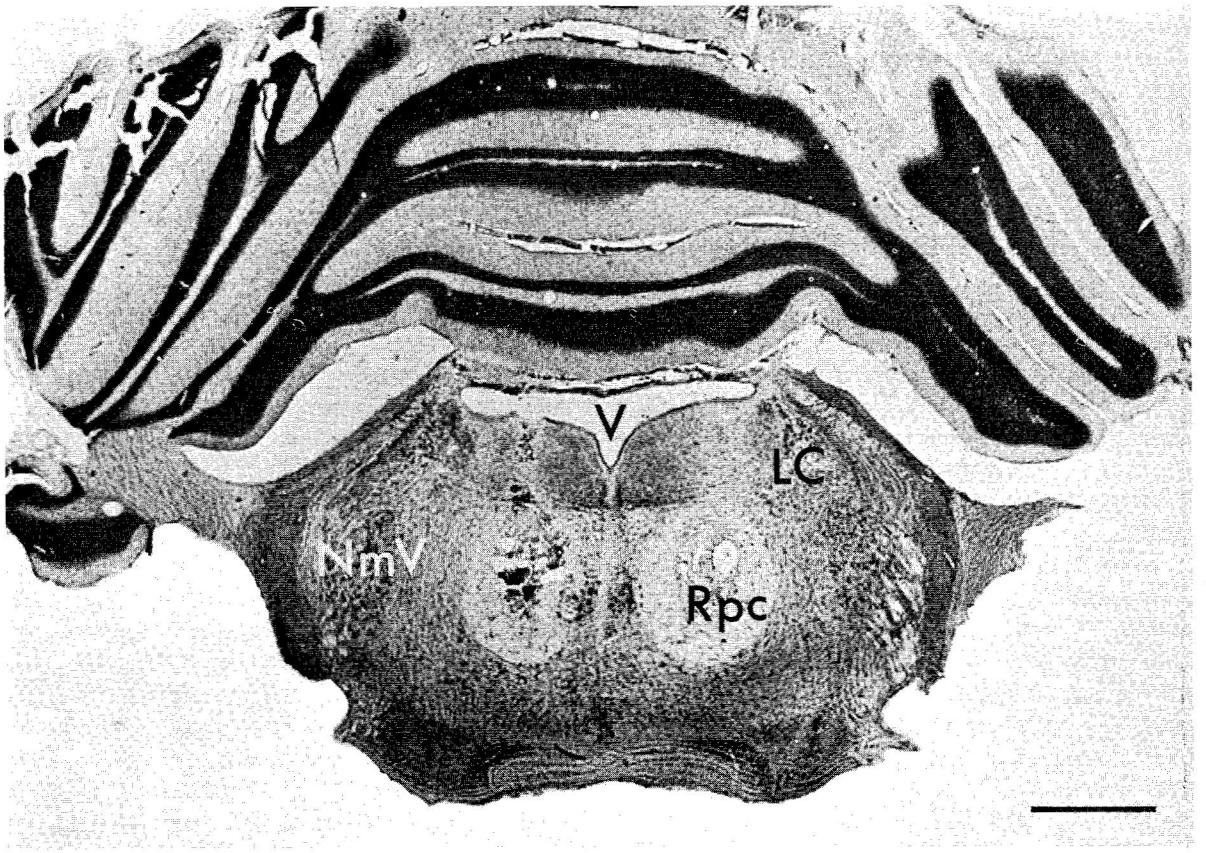

Fig. 7. Frontal plane section (50 μm) through the pontine reticular formation showing effective lesions of the n. reticularis pontis caudalis (Rpc). Raphe cells in animals with lesions of this region were resistant to the inhibitory effects of antipsychotic and adrenolytic drugs. Other anatomical landmarks on this section are the motor nucleus of the fifth nerve (NmV), the fourth ventricle (V), and the locus coeruleus (LC). Cresyl violet stain. Scale: 1 mm.

TABLE 1

Response of 5HT neurons in dorsal raphe to iontophoretically and systematically applied antipsychotic and adrenolytic drugs.

Microiontophoresis substances (nA) [1]	Effects after i.v. administration	Effects after iontophoretic application (no. of cells)			No. rats
		Excited	Inhibited	No change	
5HT (10 nA)	—	0	22—(90—100%)	0	7
PIP (10 nA)	Inhibited	0	14—(50—100%)	1	4
MTT (20 nA)	Inhibited	0	3—(30%)	6	3
THZ (20 nA)	Inhibited	0	0	3	1
MJ [2] (16 nA)	No change	0	4—(30%)	3	2
NE (16 nA)	—	0	1—(30%)	5	2
CPZ (20 nA)	No change	0	3—(50—70%)	0	1

[1] HAL, PIM, and CLZ could not be tested iontophoretically due to their insolubility in H_2O.
[2] Since Hoffer et al. (1971) have reported that propranolol produced local anesthetic effects on Purkinje cells while another β-blocker MJ 1999 did not, only MJ 1999 was tested for its iontophoretic effects on dorsal raphe cell firing.

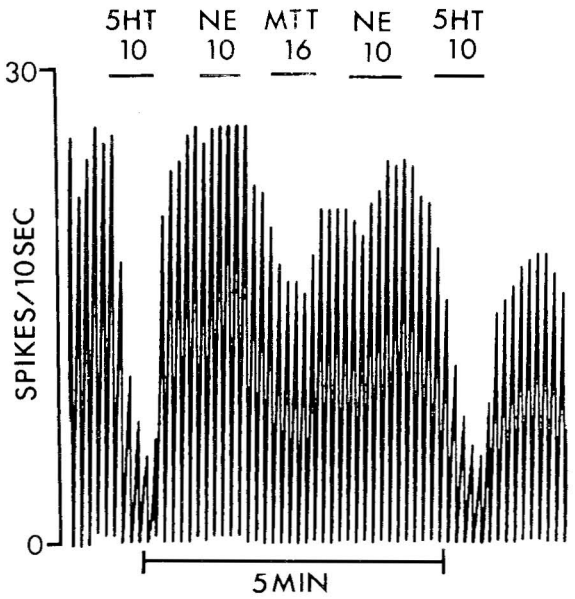

Fig. 8. The response of a dorsal raphe neuron to the microiontophoretic application 5HT, norepinephrine (NE) and methiothepin (MTT). The numbers above bars indicate ejection currents in nA. All ejections lasted 60 sec, indicated by the length of the horizontal bars. The dorsal raphe cell was inhibited by 5HT (10 nA), slightly inhibited by MTT (16nA) and not affected by NE (10 nA).

In order to determine whether the antipsychotics and α-blocking agents might affect raphe firing by altering synthesis or metabolism of 5HT, animals were pretreated with parachlorophenylalanine, a tryptophan hydroxylase inhibitor (300 mg/kg, i.p., 24 h prior to testing) or with R04-4602, an l-amino acid decarboxylase inhibitor (800 mg/kg, i.v., 45 min prior to testing). Both pretreatments failed to alter the inhibitory response of raphe cells to MTT (n = 5).

3.4. Effects of drugs on blood pressure and heart rate

In other experiments, the possibility that the effects of these drugs on raphe unit activity could be related to changes in blood pressure or heart rate was tested. At 5 min following the i.v. administration of drugs in doses which have been shown to totally inhibit raphe firing, MTT and PIP slightly increased, THZ slightly decreased, and CLZ did not change systolic blood pressure as measured by an indirect tail-cuff method in anesthetized rats (table 2). Drugs which did not effect raphe cell firing also were found to alter systolic blood pressure a variable extent (table 2).

TABLE 2

The effect of the i.v. administration of antipsychotic and adrenolytic drugs on dorsal raphe cell firing, systolic blood pressure and heart rate.

Drug treatment (dose, mg/kg)	Effects of systemic drug on raphe firing	Change from predrug control, 5 min following i.v. administration of drug (mean ± S.E.)	
		Bp (mm Hg)	HR (beats/min)
Saline	No change	0 ± 1 [1]	0 ± 10 [2]
CPZ (7)	No change	−15 ± 4.5	−124 ± 56
PIP (9)	Inhibition	+8.3 ± 3.3	−53 ± 23
HAL (3)	No change	−4.7 ± 1.4	−116 ± 26
CLZ (2)	Inhibition	0 ± 0	−28 ± 4
MJ 1999 (4)	No change	−0.7 ± 0.7	−50 ± 27
THZ (8)	Inhibition	−3.3 ± 2	−112 ± 14
MTT (0.3)	Inhibition	+4.6 ± 4	−32 ± 20

[1] Mean systolic blood pressure measured by indirect tail cuff method (77 ± 2 mm Hg, n = 24, in choral hydrate anesthetized rats).
[2] Mean heart rate (406 ± 12 beats/min, n = 24, in chloral hydrate anesthetized rats).

All of the drugs studied, regardless of their effects on raphe firing, decreased heart rate at 5 min following their intravenous administration (table 2).

4. Discussion

These studies show that the systemic administration of a series of antipsychotic agents (MTT, CLZ, and THZ) inhibit the firing of 5HT neurons in the dorsal raphe nucleus. In contrast, three other antipsychotic agents (CPZ, HAL and PIM) did not affect raphe cell firing. The potency of these drugs to inhibit raphe neuronal activity correlates with presumed central noradrenergic blocking efficacy as reported by Keller et al., (1973). The α-blocking agent, piperoxane, but not the β-blocking agents, propranalol and MJ 1999, also inhibited dorsal raphe activity when administered systemically.

In order to test the hypothesis that raphe cell firing is affected by impairment of noradrenergic neuronal transmission, CA neurons within the CNS were destroyed by the intraventricular administration of 60HDA (Ungerstedt, 1971a). In some animals following this pretreatment, the firing of raphe neurons was no longer affected by the systemic administration of MTT (and other antipsychotic agents). This data suggests that the inhibitory effects of these drugs in the dorsal raphe is mediated by the CA system. However, the lack of resistance to the depressant effects of these drugs on dorsal raphe activity in approximately one-half of the animals receiving 60HDA could not be easily explained. Histochemical fluorescence studies suggested that 'resistant' rats all had extensive CA depletion including the ventrolateral central gray area adjacent to the dorsal raphe, the ventral tegmental area, n. locus coeruleus, dorsal and ventral NE pathways while most 'non-resistant' animals had lesser degrees of CA depletion.

If the depressant effects of the antipsychotic agents and piperoxane on raphe cell firing is due to impairment of CA transmission, then an increased release of CA by amphetamine (A) (Glowinski et al., 1966; Carr and Moore, 1970; Tilson and Sparber, 1972) should be able to reverse these inhibitory effects. Both the l- and d-isomers of A were found to be equally potent in reversing the depressant effect of MTT on raphe neurons. Recently, evidence has been accumulating to support the view that l- and d-A are equipotent in affecting the function of NE neurons while the d-isomer of A is much more potent than the l-isomer in its effects on dopamine (DA) neurons (Svensson, 1971; Ferris et al., 1972; Bunney and Aghajanian, 1973; Harris and Baldessarini, 1973; Thornburg and Moore, 1973; Bunney et al., 1975). Thus, since both l- and d-A were equally effective in reversing the MTT-induced inhibition of 5HT neurons, the data are consistent with a NE-mediated effect. To further test this view, a complete transection of the forebrain was made which eliminated all known DA systems which might have an influence on the dorsal raphe (recording) area (Ungerstedt, 1971b). In spite of the transection, the inhibitory effects of MTT on raphe cell firing and the ability of l-A to reverse the depressant effects remained intact. In addition, apomorphine (a DA agonist) was administered and found to be unable to reverse the depression of raphe firing caused by MTT. To further support this lack of correlation with the DA system, while MTT has been reported to have potent DA antagonistic properties (Monachon et al., 1972), two other antipsychotic drugs which affect dorsal raphe firing (THZ, Stawarz et al., 1975; CLZ, Bürki et al., 1974) have been observed to have weak or no DA receptor blocking abilities. Thus, these data indicate that the DA system is not involved in the depressant effects of the antipsychotic and α-adrenergic drugs on dorsal raphe cell firing.

Recently, it has been reported that CLZ and THZ have very prominent anticholinergic properties while CPZ, HAL and PIM are much less active in blocking cholinergic receptors (Snyder et al., 1974; Miller and Hiley, 1974). However, several investigators have reported

the failure of CLZ or THZ to exhibit anticholinergic properties in tests in which anticholinergic compounds (atropine, benztropine) have been shown to block the physostigmine-induced increase in brain ACh (Sethy and Van Woert, 1974a,b) or the centrally mediated sinistrotorsion in the guinea pig elicited by physostigmine (Bartholini et al., 1972). In the present study, the depression of raphe firing caused by CLZ also could not be reversed by physostigmine, suggesting that the possible anticholinergic properties of CLZ are not critical for the effects of this drug on raphe firing.

Both MTT (Monachon et al., 1972; Fuller and Perry, 1974; Lloyd and Bartholini, 1974; and Jacoby et al., 1975) and CLZ (Bürki et al., 1974) appear to affect the metabolism of 5HT. Compounds which decrease 5HT metabolism or increase 5HT synthesis have previously been shown to depress the firing of raphe neurons (Aghajanian et al., 1970b). The ability of these compounds to affect raphe firing is dependent upon ongoing 5HT synthesis (Aghajanian et al., 1970b; Gallager and Aghajanian, 1976). It is possible that antipsychotic and adrenergic blocking drugs also affect raphe firing by altering 5HT synthesis and metabolism. However, such a mechanism appears unlikely since pretreating animals with compounds which inhibit the synthesis of 5HT (parachlorophenylalanine, Koe and Weissman, 1966 and R04-4602, Carlsson and Lindqvist, 1970) did not alter the inhibitory response of their raphe cells to MTT.

Since it has recently been suggested that the raphe complex may be influenced by non-neuronal changes (Scheibel et al., 1975), we investigated the possibility that the effects of the antipsychotic drugs and α-blocking agents on raphe unit activity could be related to changes in blood pressure or heart rate. In agreement with previous observations (Foote et al., 1969) no correlations could be found between alterations in blood pressure or heart rate and effects on dorsal raphe neuronal activity.

These data suggest that the depression of raphe firing produced by antipsychotic drugs and piperoxane are primarily due to an interaction between the noradrenergic and serotonergic neuronal systems. However, the inhibitory effects of these drugs on 5HT neurons appears to be indirect since the direct application of these agents (Haigler and Aghajanian, 1974a) and NE (also observed by Couch, 1970; Aghajanian et al., 1972; Svensson et al., 1975) via microinontophoresis did not produce a uniform inhibitory response. In agreement with previous studies (Aghajanian et al., 1972; Bramwell and Gonye, 1973; Haigler and Aghajanian, 1973; Gallager and Aghajanian, 1976) these same dorsal raphe cells were found to be inhibited rapidly and uniformly by the microiontophoretic application of 5HT.

In an effort to localize anatomically where the adrenergic and serotonergic systems may interact, a variety of chemical, mechanical and electrothermic lesions were made. Several investigators have suggested that the interaction between the noradrenergic and serotonergic systems originates in the n. locus coeruleus (Loizou, 1969; Morgane et al., 1974; Svensson et al., 1975). However, neither the depressant effects of the antipsychotic agents nor the ability of A to reverse the inhibition of raphe cell firing was affected by complete bilateral electrothermic lesions of the locus coeruleus. In a recent study Roizen and Jacobowitz (1976) suggested that the ventral NE pathway may contribute most of the CA input to the raphe area, while the locus coeruleus may exert a lesser influence. In our studies, a complete transection at the level of the pontine reticular formation, separating the lower brainstem from the raphe (recording) area, did abolish the depressant effects on raphe firing of the antipsychotic agents and PIP. Since rats could not survive more posterior transections, discrete electrothermic lesions were attempted in the transection area in an effort to define a nucleus or pathway involved in this interaction. Animals with bilateral lesions of the medial reticular formation, specifically the n. reticularis pontis caudalis

(Valverde, 1962) which includes the 'ventral NE pathway' described by Ungerstedt (1971b) were found to be resistant to MTT and other drugs. Unfortunately, it was not possible to bilaterally lesion the cell bodies of A1, A2 and A5 reported to contribute CA fibers to this pathway due to difficulties in keeping the animals alive. Therefore, the cellular origin of this interaction between CA and 5HT systems cannot be precisely defined at present.

Recently, an axon bundle from lower brain cell bodies A1 and A2 ascending in the medial reticular formation (ventral NE pathway) has been found to contain epinephrine (Hökfelt et al., 1974). In addition, Bolme et al., (1974) have suggested that piperoxane may be a specific antagonist and clonidine a specific agonist at epinephrine-mediated synapses within the CNS. Since both of these drugs have been shown to affect dorsal raphe firing (present study and Svensson et al., 1975), it is possible that the interaction between the adrenergic and serotonergic neuronal systems may be mediated at least partially by epinephrine.

Several groups (Fuxe et al., 1970; Nybäck and Sedvall, 1970; Keller et al., 1973) have suggested that the potency of antipsychotic and α-adrenergic blocking agents in enhancing NE turnover may correlate with the sedative properties of these drugs. MTT (Jalfre et al., 1974), THZ (Fuxe et al., 1970; Bürki et al., 1975) and CLZ (Stille et al., 1971; DeMaio, 1972) as well as an α-adrenergic blocking agent, phenoxybenzamine (Boissier et al., 1967; Fuxe et al., 1970; Andén and Strömbom, 1974), have been reported to have strongly sedating actions in various animal species and man while HAL (Stille et al., 1973, Keller et al., 1973), PIM (Janssen et al., 1968; Sugerman, 1971) and a β-adrenergic agent, propranolol (Andén and Strömbom, 1974) show little, if any, sedative effects. Since it appears from the present study that drugs reported to have significant NE effects also effect 5HT neuronal activity, it is tempting to speculate that the sedative actions are due to an interaction between noradrenergic and serotonergic systems within the CNS. However, PIP does not show sedative effects (Fuxe et al., 1974) suggesting that such correlations may not have general validity.

Because drugs which do not effect serotonergic (raphe) neurons (HAL, PIM, CPZ) are at least as effective antipsychotic agents as ones which do inhibit 5HT neuronal activity (THZ, CLZ) (Angst et al., 1971; Ekblom and Haggstrom, 1974; Berzewski et al., 1969; DeMaio, 1972), it is concluded that effects on central serotonergic activity is probably not relevant to the antischizophrenic activity of these drugs.

In conclusion, these studies show that certain antipsychotic agents (MTT, CLZ, THZ) and an α-adrenergic blocking agent (PIP) inhibit the firing of 5HT neurons in the dorsal raphe nucleus. These drug effects appear to be indirect since when applied microiontophoretically to 5HT neurons, weak and/or variable responses were obtained. Various pharmacological and lesion experiments suggest that the inhibition of raphe firing is secondary to the action of these drugs on the central adrenergic system. While a precise localization for this adrenergic input has not yet been obtained, the data suggest that such an input may originate from adrenergic pathways ascending from the lower brain stem.

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