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SUPPRESSION OF FIRING ACTIVITY OF 5-HT NEURONS IN THE DORSAL RAPHE BY ALPHA-ADRENOCEPTOR ANTAGONISTS

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Summary—Previous pharmacological studies have suggested that the firing activity of 5-HT cells of the dorsal raphe nucleus is dependent on a tonically active, central adrenergic system. In this study, a wide variety of alpha-adrenoceptor antagonists, WB-4101 (41 ± 20 μg/kg; ED_{50} ± SD), piperoxan (0.64 ± 0.20 mg/kg), thymoxamine (0.42 ± 0.31 mg/kg) and phenoxybenzamine (3.0 mg/kg) were found to suppress firing when administered sytemically. These alpha-adrenoceptor antagonists, as well as phentolamine and dihydroergocryptine, also reduced 5-HT cell firing when applied iontophoretically. The order of potency of the drugs when applied systemically was WB-4101 ≫ piperoxan ≃ thymoxamine > phenoxybenzamine. This ranking correlates well with their activity at classical peripheral postsynaptic α-adrenoceptors. In addition, the order of potency of microiontophoretically applied adrenergic agonists (norepinephrine > phenylephrine > α-methylnorepinephrine > isoproterenol > salbutamol) in restoring 5-HT cell firing during competitive alpha-adrenoceptor blockade suggests that this receptor should be classified in the alpha-1-adrenoceptor category. Previous anatomical studies have demonstrated that the dorsal raphe receives an adrenergic input. Taken together, these findings suggest that NE terminals, present in the dorsal raphe, mediate a tonically active adrenergic influence upon which the firing of 5-HT cells depends.

Pharmacological evidence suggests that the firing activity of serotonin-containing (5-HT) cells of the dorsal raphe nucleus is dependent on a tonically active adrenergic system. Administration of either a low dose of clonidine (Svensson, Bunney and Aghajanian, 1975), which acts presynaptically to inhibit norepinephrine (NE) cell firing, or reserpine, which impairs noradrenergic transmission, suppresses 5-HT cell firing (Baraban, Wang and Aghajanian, 1978). Furthermore, amphetamine, which releases catecholamines (Glowinski and Axelrod, 1965; Stein and Wise, 1969; Carr and Moore, 1970) rapidly restores firing after either of these treatments.

Antipsychotic drugs which are thought to possess adrenergic blocking activity (Keller, Bartholini and Pletscher, 1973) reduce 5-HT cell firing when administered systemically (Gallager and Aghajanian, 1976a). An alpha-adrenoceptor is thought to mediate these effects since piperoxan, an α-antagonist, but neither sotalol, a β-adrenoceptor antagonist, nor haloperidol, a dopamine antagonist, is effective in reducing 5-HT cell firing (Gallager and Aghajanian, 1976a). In addition, clonidine, which in high doses acts centrally as a postsynaptic alpha agonist (Andén, Corrodi, Fuxe, Hökfelt, Hökfelt, Rydin and Svensson, 1970), can restore activity after the suppression of firing produced by either reserpine (Baraban *et al.*, 1978) or small doses of clonidine (Svensson *et al.*, 1975).

The dorsal raphe, as well as the adjacent central grey, receives a prominent adrenergic input as demonstrated by histofluorescence (Fuxe, 1965), biochemical (Roizen and Jacobowitz, 1976), immunocytochemical (Swanson and Hartman, 1975; Hökfelt, Fuxe, Goldstein and Johansson, 1974; Hökfelt, Johansson, Fuxe, Goldstein and Park, 1976) and ultrastructural autoradiographic techniques (Baraban and Aghajanian, 1979). Thus, the adrenergic terminals mediating the adrenergic–serotonergic interaction could be located in or near the dorsal raphe itself. This suggestion is supported by the ability of NE to reverse the reduction of firing produced by reserpine when NE is applied iontophoretically in the vicinity of 5-HT cells (Baraban *et al.*, 1978).

To determine whether alpha-adrenoceptor blockers, in general, can suppress 5-HT cell firing, several different types of alpha-adrenoceptor antagonists were tested in the present study. These agents represent a variety of chemical classes: a haloalkylamine, phenoxybenzamine (Nickerson and Nomaguchi, 1951), an ergot alkaloid, dihydroergocryptine (Rothlin, 1946), an imidazoline, phentolamine (Meier and Yonkman, 1949), two benzodioxans, WB-4101 and piperoxan (Mottram and Kapur, 1975; Bacq and Fredericq, 1934), and a thymoxyalkylamine, thymoxamine (Greeff and Schumann, 1953). The effects of these drugs on 5-HT cell firing were determined after their systemic administration. Furthermore, in the light of the possibility that alpha-adrenoceptor antagonists block the effects of NE released from adrenergic terminals located within the dorsal raphe, the effects of the local, iontophoretic application of these drugs on single unit activity of 5-HT cells in the dorsal raphe were also investigated.

Key words: dorsal raphe nucleus, alpha-adrenoceptor, WB-4101, thymoxamine, piperoxan, phentolamine.

METHODS

1. *Single unit recording and microiontophoresis*

Male Sprague–Dawley albino rats (Charles River) 200–300 g were anesthetized with chloral hydrate, 400 mg/kg (i.p.); additional injections were given as needed. Rats were mounted in a stereotaxic instrument; a burr hole was made over the raphe area whose center was in the midline approx. 0.5 mm anterior to lambda.

Single barrel micropipettes were filled with 2M NaCl saturated with Fast Green FCF (Fisher) or Pontamine Sky Blue (Searle) dye. Electrode impedances were in the range of 3.5–5.5 MΩ when measured at 60 Hz *in vitro*. For microiontophoresis, 5-barrel or 6-barrel electrodes were used. Five-barrel micropipettes were prepared according to the technique previously described (Haigler and Aghajanian, 1974). Impedances were 3.5–4.5 MΩ in the recording barrel. Six-barrel electrodes were constructed by positioning a single-barrel recording electrode 20–30 μm ahead of a 5-barrel micropipette that had been broken back to a tip diameter of 10–20 μm and cementing them together as previously described (Wang and Aghajanian, 1977a). The drug solutions were directly injected into 3 of the 4 side barrels of the 5-barrel micropipette. Each of these contained a few strands of fiberglass to allow rapid filling of the tips by capillary action (Tasaki, Tsukahara, Ito, Wagner and Yu, 1968). One side barrel, the balance channel, was filled with 4M NaCl. Circuits for drug ejection and current balancing were similar to those described by Salmoiraghi and Weight (1967), except that solid-state components were used.

Electrode signals were passed through a high-input impedance amplifier and monitored on an oscilloscope. Integrated firing rate was recorded in consecutive 10 sec samples from the rate meter. The body temperature was kept at 36–37°C. Units displaying a characteristic slow firing rate (≃1 spike/sec), regular rhythm, and wide duration of action potential (1–2 msec) typical of histochemically and antidromically identified 5-HT cells were tested (Aghajanian and Haigler, 1974; Wang and Aghajanian, 1977b). Recording sites were marked at the end of each experiment by passing 20 μA of cathodal current through the recording barrel for 15 min, depositing a green or blue spot at the electrode tip (Thomas and Wilson, 1965). Rats were perfused with 10% formalin. For all cells described below, the location of the electrode tip was confirmed by histological examination to correspond to the location of 5-HT-containing neurons in the dorsal raphe nucleus.

2. *Intraventricular 6-hydroxydopamine (6-OHDA) injections*

Twenty minutes prior to intraventricular injections, animals were pretreated with chlorimipramine 20 mg/kg (i.p.) (Ciba) in order to decrease the possibility of 6-OHDA uptake into, and subsequent damage to, 5-HT neurons. Rats were anesthetized with chloral hydrate 400 mg/kg (i.p.) and mounted in a stereotaxic apparatus. 6-Hydroxydopamine (Regis) was prepared in a concentration of 7.5 mg/ml (free base) in normal saline solution containing 1 mg/ml ascorbic acid. Twenty microliters of this solution, containing a total of 150 μg of 6-OHDA was injected at 1 μl/min according to the technique described by Svensson *et al.* (1975). The injected animals were used for recording experiments 4–7 days later.

3. *Drugs*

The following drugs were administered intravenously: WB-4101 (2-2′,6′-dimethoxy (phenoxyethylamino) methyl benzodioxan HCl) (WB, WB Pharmaceuticals), piperoxan chlorhydrate (PIP, Rhone-Poulenc), thymoxamine HCl (TMX, Wm. Warner, Ltd), phenoxybenzamine HCl (PBZ, SK & F), dihydroergocryptine mesylate (DHK, Sandoz), phentolamine mesylate (PHE, Ciba) *d*-lysergic acid diethylamide bitartrate (LSD), *l*-amphetamine sulfate (A, Aldrich), reserpine (RES, Regis).

Solutions for microiontophoresis were as follows: WB (0.1 M) pH 5.0; PIP (0.1 M) pH 4.0; TMX (0.1 M) pH 4.0; PBZ (2.5 mg/ml) pH 3.6: DHK (3.0 mg/ml) pH 5.0; PHE (20 mg/ml) pH 5.5; serotonin creatine sulfate (0.05 M) pH 5.0 (5-HT, Regis); *l*-norepinephrine bitartrate (0.1 M) pH 4.0 (Regis, NE); monosodium glutamate (0.1 M) pH 8.0 (G. Mann Labs); Sotalol HCl (0.1 M) pH 4.0 (SOT, Regis); L-isoproterenol-D-bitartrate (0.1 M) pH 4.0 (ISO, Regis); *l*-phenylephrine HCl (0.1 M) pH 4.0 (PE, Sigma); salbutamol sulfate (0.1 M) pH 4.0 (SALB, Schering); *l*-α-methylnorepinephrine (0.1 M) pH 4.0 (α-MNE, Sterling–Winthrop). The following drugs were also used in ionically diluted solutions when iontophoresed from 5-barrel electrodes (section 8 of Results): WB-4101 (0.01 M) in 0.1 M NaCl, pH 5.0; glutamate (0.01 M) in 0.1 M NaCl, pH 8.0

4. *Data analysis*

The ED_{50} of systemically administered drugs was determined by log-probit analysis from the cumulative dose-response curves. The IT_{50} of iontophoretically applied drugs was calculated as the product of I, the current in nanoamperes, and T_{50}, the time in seconds required to obtain a 50% depression (deMontigny and Aghajanian, 1977).

RESULTS

1. *Effects of systemic administration of alpha-adrenoceptor antagonists on 5-HT cell activity*

Intravenous administration of the alpha-adrenoceptor antagonists WB-4101 ($n = 27$), piperoxan ($n = 8$) or thymoxamine ($n = 9$) rapidly and totally suppressed 5-HT cell firing. The ED_{50} for suppression of firing for these drugs was as follows: WB-4101, 41 ± 20 μg/kg (ED_{50} ± SD); piperoxan 0.64 ± 0.20 mg/kg; thymoxamine, 0.42 ± 0.31 mg/kg.

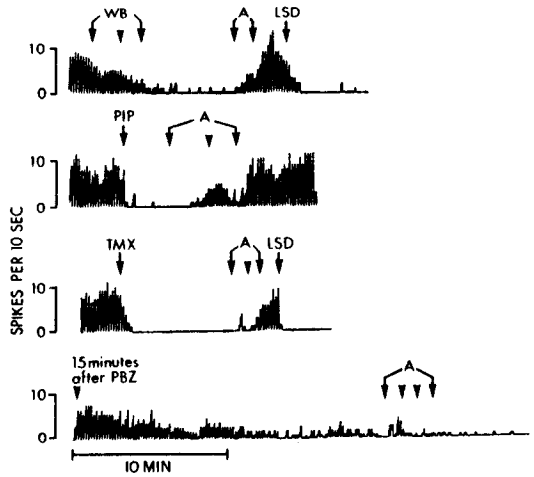

Fig. 1. Suppression of 5-HT cell firing by alpha-adrenoceptor antagonists: reversal of competitive blockade by *l*-amphetamine. Intravenous administration of WB-4101 (WB; successive doses of 30, 15, 15 μg/kg given at times indicated by arrows), piperoxan (PIP; 1 mg/kg), or thymoxamine (TMX; 1.5 mg/kg) rapidly suppressed firing. Phenoxybenzamine (PBZ; 3.0 mg/kg, i.v.) gradually suppressed firing within 30 min. *l*-Amphetamine (*l*-A; cumulative doses of 2 mg/kg, i.v.) restored activity following suppression of firing caused by the three competitive antagonists. However, *l*-A (5 mg/kg, i.v.) was ineffective in overcoming the non-equilibrium blockade produced by phenoxybenzamine. The ability of LSD (10–15 μg/kg, i.v.) to inhibit 5-HT cell firing remained unimpaired.

Phenoxybenzamine (3.0 mg/kg, $n = 6$) also suppressed firing but this occurred gradually over 30 min (Fig. 1). In animals tested after previous injections of PBZ (25 mg/kg i.p.), recovery of activity had commenced by 15 hr ($n = 4$) and gradually neared completion at 36 hr ($n = 3$). In contrast to the above drugs, large doses of dihydroergocryptine (6.0 mg/kg, i.v. $n = 4$) only partially suppressed firing (10–50%). Phentolamine, at doses up to 10.0 mg/kg (i.v.) ($n = 4$) did not suppress firing rate by more than 20%. Perhaps the weak activity of these two drugs when given by the systemic route stems from their limited ability to enter the brain (see Discussion).

2. *Effects of 6-OHDA lesions on WB-4101 suppression of 5-HT cell firing*

The destruction of adrenergic terminals by 6-OHDA has been shown to block the ability of the pre-synaptically acting drugs, reserpine and low doses of clonidine (Baraban *et al.*, 1978; Svensson *et al.*, 1975) to suppress the firing activity of 5-HT cells. Therefore, the effect of 6-OHDA pretreatment on the ability of an alpha-adrenoceptor antagonist, WB-4101, to suppress 5-HT cell firing was examined. Animals were given intraventricular injections of 6-OHDA (150 μg, free base). For the first few days, 5-HT cells fired slowly and erratically, consistent with the loss of adrenergic tone. However, in animals tested 4–7 days after injection, the normal rate and pattern of firing resumed (Svensson *et al.*, 1975), suggesting that 5-HT cells may adapt in some manner to a severely compromised adrenergic system. During this period, the ability of WB-4101 to suppress firing activity was tested. The ED_{50} for suppression of firing by WB-4101 was found to be 91 ± 45 μg/kg ($ED_{50} \pm$ SD) ($n = 9$). This value was significantly greater than that obtained in unlesioned animals, 41 ± 20 μg/kg ($P < 0.02$).

3. *Effect of l-amphetamine on suppression of 5-HT cell firing produced by alpha-adrenoceptor antagonists*

As the administration of a variety of alpha-adrenoceptor antagonists suppressed 5-HT cell firing, it was of interest to observe the effects of an increase in adrenergic tone produced by amphetamine on this suppression. Evidence has been accumulating that *l*- and *d*-amphetamine are nearly equipotent in affecting the function of NE neurons, while the *l*-isomer of amphetamine is much less potent than the *d*-isomer in its action on dopaminergic neurons (Svensson, 1971; Ferris, Tang and Maxwell, 1972; Harris and Baldessarini, 1973; Thornburg and Moore, 1973; Bunney, Walters, Kuhar, Roth and Aghajanian, 1975; Holmes and Rutledge, 1976). Therefore, the *l*-isomer was selected for use because of its more selective action on NE neurons. First, doses of WB-4101 or thymoxamine causing greater than 80% suppression of firing were administered. Following the administration of WB-4101, *l*-amphetamine (2.0–5.0 mg/kg, i.v.) was administered. When *l*-amphetamine was given during a period of total suppression of firing, it restored activity in only 2 out of 5 animals, suggesting that the limited catecholamine supply released by amphetamine might be unable always to overcome the high degree of competitive blockade produced by WB-4101. However, when *l*-amphetamine was given after recovery to a low level of firing, it consistently restored cell firing close to baseline rate ($n = 5$). Following thymoxamine administration, *l*-amphetamine was always able to restore firing ($n = 5$). As previously reported (Gallager and Aghajanian, 1976a), *l*-amphetamime also restored activity when administered during the suppression of firing caused by piperoxan ($n = 3$). If the NE releasing action of amphetamine does underly the observed restoration of firing activity, then amphetamine might be effective only during the suppression of 5-HT cell firing produced by competitive antagonists. Therefore, the response to *l*-amphetamine was examined when administered following phenoxybenzamine, which produces a non-equilibrium blockade (Nickerson, 1957). After phenoxybenzamine, doses of *l*-amphetamine up to 5.0 mg/kg ($n = 4$) were ineffective in restoring activity (Fig. 1).

4. *Effect of NE iontophoresis on 5-HT cell firing*

Previous studies have examined the effect of NE iontophoresis on the spontaneous firing activity of 5-HT cells (Svensson *et al.*, 1975; Gallager and Aghajanian, 1976a; Aghajanian, Haigler and Bloom,

Table 1. Response of 5-HT cells to NE iontophoresis

Current (nA)	Number of cells (Avg. rate) (spikes/10 sec)	Activated (%)	No change (%)	Inhibited (%)	Mean % response
2.5	10 (8.0)	80	20	0	152 ± 11
5	18 (9.2)	61	33	6	144 ± 13
10	8 (12.8)	25	50	25	93 ± 10
20	8 (11.2)	12	0	88	59 ± 14*

The response of 5-HT cells in the dorsal raphe to several doses of NE, iontophoretically applied from 5-barrel electrodes; NE was iontophoresed for 1 min at the currents shown. The mean baseline rate for each cell group is shown in parenthesis. Response is measured as the average rate of cell firing during the 1 min interval 30–90 sec following onset of NE iontophoresis compared to baseline rate. Activation is defined as a response of greater than 120%. Cells which did not alter their rate by more than 20% are labelled as unchanged. Inhibition is defined as a reduction in rate of greater than 20%. Mean response and standard error of mean for each group are shown in right hand column.

* Differs from both 2.5 and 5 nA groups by one-way analysis of variance ($P < 0.01$).

1972; Couch, 1970; Haigler and Aghajanian, 1973). These investigators, using 5-barrel electrodes, reported variable effects in the dorsal raphe. These observations differ from the absence of depressant responses noted when NE is iontophoresed from 2- or 6-barrel electrodes in which the drug ejection barrel is separated by 20–30 μ from the recording electrode (Baraban *et al.*, 1978). This difference between the effect of NE when iontophoresed in the vicinity of the soma with 5-barrel electrodes and when applied at a distance with 6-barrel electrodes suggests that the depressant response observed with the former may be related to high concentrations of NE deposited close to the cell body. Therefore, a more detailed investigation of NE's effects was undertaken in the present study. When low currents of NE were tested from 5-barrel electrodes, activation of 5-HT cell firing prevailed; however, at higher currents an increasing proportion of depressant responses was observed (Table 1).

5. Reversal of effects of competitive alpha-adrenoceptor blockade by NE iontophoresis

Following systemic administration of several competitive alpha-adrenoceptor blocking agents, the ability of NE iontophoresis to restore firing activity was tested. Six-barrel electrodes were used in order to avoid the direct depressant effects of NE which occur with 5-barrel electrodes. As illustrated in Figure 2, the iontophoresis of NE ($\leq$ 15 nA) reversed the diminished activity caused by the competitive alpha-adrenoceptor blocking agents, WB-4101 (7 of 8 trials), piperoxan (3 of 4 trials) and thymoxamine (5 of 5 trials). In contrast, following phenoxybenzamine, which produced a non-equilibrium blockade, NE, at currents up to 15 nA, was ineffective in restoring activity even though activation by glutamate iontophoresis could still be elicited ($n = 4$). At currents up to 15 nA, NE was also unable to reverse the inhibition produced by intravenously administered LSD (10–15 μg/kg, $n = 6$), which is thought to inhibit 5-HT cell firing by stimulating 5-HT autoreceptors (Aghajanian and Wang, 1978).

6. Effects of iontophoresis of alpha-adrenoceptor antagonists on 5-HT cell activity

Adrenergic terminals have been demonstrated within the dorsal raphe. Thus, the suppressant effects produced by the systemic administration of alpha-adrenoceptor antagonists could result from the blockade of NE released from these terminals. Therefore

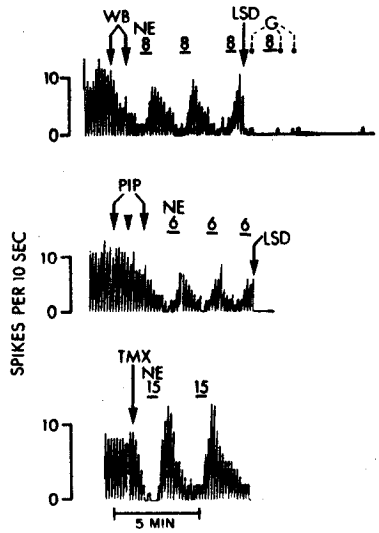

Fig. 2. Reversal by NE iontophoresis of suppression of 5-HT cell firing produced by systemic administration of alpha-adrenoceptor antagonists. Intravenous administration of the competitive antagonist WB-4101 (two doses of 25 μg/kg), piperoxan (3 doses of 0.4 mg/kg) or thymoxamine (0.8 mg/kg) caused a reduction of firing rate. Iontophoresis of NE in the vicinity of 5-HT cells promptly restored activity. As shown in the top panel, the inhibitory action of LSD (10 μg/kg) was unaffected by NE iontophoresis. Pulses of glutamate (G) elicited spikes demonstrating the continued presence of the 5-HT cell in the recording field.

Table 2. Response of 5-HT neurons in the dorsal raphe to iontophoretically applied adrenergic antagonists

Drug	Current (nA)	Duration (min)	IT_{50}	Number of cells Suppressed	No change	NE reversal
WB-4101	0.5–10	1–2	0.240 (0.210–0.278)*	42	—	14/15
PIP	10–30	2–3	0.883 (0.664–1.17)	13	—	4/4
TMX	20–35	2–4	2.05 (1.56–2.68)	8	—	4/4
PBZ	25	2–3	2.46 (2.07–2.92)	7	—	6/6
PHE	25	2–3	1.29 (0.88–1.87)	9	—	6/6
DHK	10–25	1–3	1.79 (1.50–2.13)	11	—	2/6
SOT	25	3–10		—	6	

The effect of alpha-adrenoceptor antagonists was tested from 6-barrel electrodes. The range of current and duration parameters used to obtain total suppression of activity for at least 30 sec are listed. The IT_{50} is calculated as the product of the time (sec) and current (nA) which produced 50% suppression of firing. The geometric mean is listed because it simplifies subsequent statistical analysis (Fleming, Westfall, DeLaLande and Jellett, 1972). The values in parentheses are the geometric mean ± SEM. The β-adrenoceptor antagonist sotalol did not suppress firing rate by more than 20%. Reversal of suppression by NE is defined as restoration of firing rate to greater than one-half of baseline rate. An increase in firing rate was never observed for any of the drugs tested.

* Differs from other groups by one-way analysis of varinace ($P < 0.01$).

the effects of alpha-adrenoceptor antagonists were examined when they were iontophoretically applied in the vicinity of 5-HT cells. Iontophoresis of alpha-antagonists, but not sotalol, a β-adrenoceptor antagonist (Lish, Weikel and Dungan, 1965), gradually, totally, and reversibly suppressed 5-HT cell firing (Table 2). Iontophoretic application of NE from 6-barrel electrodes effectively restored 5-HT cell firing during a period of suppression produced by alpha-antagonists (Fig. 3). Since 5-HT cells have been found to be inhibited by 5-HT (Aghajanian *et al.*, 1972), it was of interest to determine whether these agents might be stimulating 5-HT autoreceptors and thereby reducing 5-HT cell firing. However, in contrast to the ability of NE to reverse the effects of alpha-adrenoceptor antagonists, NE (10–15 nA) did not diminish the inhibition produced by 5-HT iontophoresis ($n = 6$). This observation supports the contention that alpha-adrenoceptor antagonists act independently of the 5-HT autoreceptor. Of note was the observation that the reversal of dihydroergocryptine by NE was less complete than that produced with other alpha-adrenoceptor antagonists tested. It is possible that dihydroergocryptine may share agonist activity at the 5-HT autoreceptor with another ergot alkaloid, LSD.

7. *Ability of adrenergic agonists to restore 5-HT cell activity during alpha-adrenoceptor blockade*

The ability of adrenergic agonists to reverse the suppressant effects of iontophoretically applied WB-4101 was tested. Each agonist was compared with NE on the same cell. The response to each drug was defined as the highest average firing rate observed

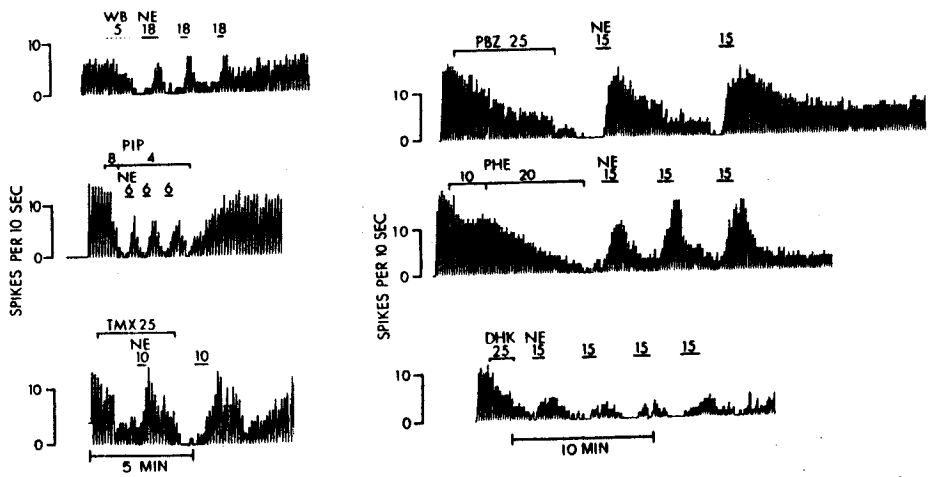

Fig. 3. Suppression of 5-HT cell firing by iontophoresis of alpha-adrenoceptor antagonists. Iontophoretic application of piperoxan, thymoxamine, WB-4101, phenoxybenzamine, phentolamine (PHE), and dihydroergocryptine (DHK) produced complete suppression of 5-HT cell firing. Iontophoretic application of NE surmounted this competitive blockade. However, NE was less effective following iontophoresis of dihydroergocryptine than after the application of the other alpha-adrenergic antagonists tested.

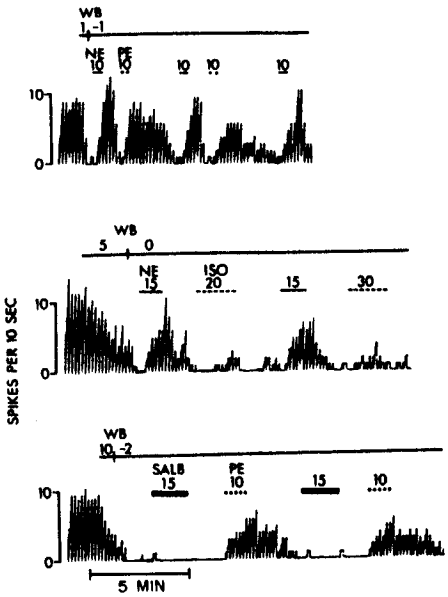

Fig. 4. Reversal of suppression of 5-HT cell firing by adrenergic agonists. Iontophoresis of WB-4101 totally inhibited 5-HT cell firing. A nearly constant degree of blockade was maintained by the use of low retaining currents. Phenylephrine (PE) was almost as potent as NE in restoring activity (top panel) whereas isoproterenol (ISO) was only weakly active (middle panel). Furthermore, salbutamol (SALB) was totally ineffective in restoring activity (bottom record).

during three consecutive 10 sec intervals. Phenylephrine (PE), a selective α-agonist (Furchgott, 1972) was nearly equipotent to NE when applied at the same current and duration. The ratio of the PE response to that of NE was 0.85 ± 0.16 (mean ± SEM, $n = 6$). Under these conditions, phenylephrine's action lasted more than twice as long as that of NE, possibly due to less efficient inactivation mechanisms (Trendelenburg, 1972; Tye, Patil and Lapidus, 1967). α-Methylnorepinephrine was less than one-fourth as potent as NE when tested at the same current and duration ($n = 5$). Isoproterenol, a strong β-agonist which possesses activity at alpha-adrenergic receptors at high doses (Trendelenburg, 1974; Bevan, Bradshaw and Szabadi, 1977) produced only a weak activation even when administered for twice as long at twice the current used for NE ($n = 5$). Salbutamol, another selective β-agonist (Cullum, Farmer, Jack and Levy, 1969; Brittain, Jack and Ritchie, 1970) was unable to produce activation under the same conditions used for isoproterenol ($n = 7$) (Fig. 4).

8. *Blockade of NE activation of 5-HT cells by alpha-adrenoceptor antagonists*

Systemic administration of reserpine has been found to suppress 5-HT cell firing (Aghajanian and Haigler, 1972). Following cessation of activity, NE iontophoresis restored 5-HT cell firing (Baraban *et al.*, 1978). Since spontaneous 5-HT cell firing may be dependent on NE released from terminals present within the dorsal raphe, the effect of α-adrenoceptor antagonists was examined on 5-HT cell activity which is clearly dependent on NE applied iontophoretically in the vicinity of 5-HT cells. Reserpine (5 mg/kg, i.v.) was administered causing a suppression of 5-HT cell firing activity. Then, 5-HT cells were located by intermittent iontophoresis of glutamate from 5-barrel electrodes. This procedure caused a transient train of spikes. Iontophoresis of NE (< 10 nA for 1 min) produced a resumption of firing activity in all 5-HT cells located in this manner ($n = 15$). Low doses of WB-4101 (1–5 nA for 1–5 min, $n = 5$) and phentolamine (1–2 nA for 1–3 min, $n = 6$) blocked the activation of 5-HT cell firing by NE by more than 50%, but did not alter the ability of glutamate to excite these cells (Fig. 5).

DISCUSSION

The results of this study support the hypothesis that the firing activity of 5-HT neurons in the dorsal raphe is dependent on the tonic activation of alpha-adrenoceptors. The present authors have shown that a chemically diverse group of alpha-adrenoceptor antagonists suppressed the firing of 5-HT cells in the dorsal raphe when administered either systemically, or iontophoretically in the vicinity of 5-HT cells. Previous anatomical studies have demonstrated that the dorsal raphe receives an adrenergic input. Together, these observations suggest that NE terminals present within the dorsal raphe could mediate a tonically active adrenergic influence on 5-HT cells located there.

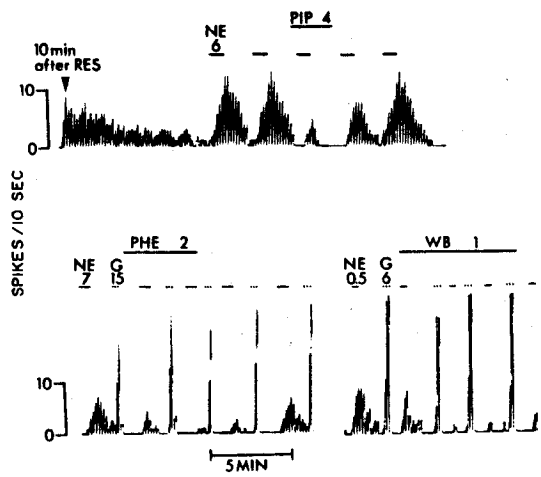

Fig. 5. Blockade of NE activation of 5-HT cell firing by alpha-adrenoceptor antagonists. Following suppression of 5-HT cell firing by reserpine (RES; 5 mg/kg, i.v.) iontophoresis of NE restored firing. The alpha-adrenoceptor antagonist piperoxan (PIP, 4 nA) blocked NE activation of 5-HT cell firing (top panel). Blockade of NE activation following reserpine (5 mg/kg, i.v., not shown) is also demonstrated for the alpha-antagonists phentolamine (PHE, 2 nA) and WB-4101 (WB, 1 nA) (bottom panel). Furthermore, the excitation produced by glutamate (G) was unaltered by these drugs, demonstrating the specificity of their blockade.

The potency of systemically applied drugs in suppressing 5-HT cell firing is WB-4101 > piperoxan ≃ thymoxamine. This ranking correlates well with other measures of alpha-adrenoceptor blocking activity. In the vas deferens, WB-4101 is much more potent than thymoxamine (Mottram and Kapur, 1975), and piperoxan and thymoxamine are of roughly similar potency at post-synaptic sites in the periphery (Drew, 1976). Upon iontophoretic application of these drugs in the dorsal raphe, WB-4101 retained a much greater potency than either piperoxan or thymoxamine. Interestingly, systemically applied dihydroergocryptine and phentolamine failed to elicit significant changes in firing at doses which cause significant peripheral alpha-receptor blockade. However, the dramatic increase in effects displayed by phentolamine and dihydroergocryptine, when they were applied iontophoretically, suggest that they possess activity at this central site, but penetrate the brain poorly. This interpretation is supported by the behavioral studies of Rothlin (1946) and the biochemical studies of Andén and Strömböm (1974).

The possibility that the effects of systemically administered agents stem from peripheral alpha-adrenergic blockade is unlikely, because (1) when these agents were administered iontophoretically they had similar effects, and (2) phentolamine at doses which produce alpha-adrenoceptor blockade peripherally but apparently penetrate the brain poorly was devoid of effects on 5-HT cell firing.

The delayed onset of action of phenoxybenzamine and prolonged effect in the dorsal raphe following systemic administration is consistent with a similar pattern observed in the periphery (Nickerson, 1962). The reduction in 5-HT cell firing produced was not reversible by either amphetamine or NE as would be expected for a nonequilibrium blockade (Nickerson, 1957). However, following short term iontophoretic application the inhibition produced was of a reversible nature, as reported by Bevan *et al.* (1977) in another area.

The results of the present study suggest that the release of NE from adrenergic terminals present within the dorsal raphe maintains 5-HT cell firing activity. However, iontophoresis of NE in the vicinity of these 5-HT neurons has been previously reported to produce variable effects on their firing rate. At first glance, these findings appear to be inconsistent with the suggestion that the adrenergic input to the dorsal raphe activates 5-HT cells. However, the results of this study reveal that at low currents, NE exerts predominantly an activating influence on their spontaneous firing activity. In addition, NE can reverse the suppression of 5-HT cell firing produced by reserpine. Several lines of evidence indicate that the activation of 5-HT cells by NE is mediated by alpha-adrenoceptors: (1) NE reverses the suppression produced by alpha-adrenoceptor antagonists when they are administered either systemically or iontophoretically; (2) phenylephrine, another alpha-adrenoceptor agonist, shares the ability of NE to reverse the suppression produced by an alpha-adrenoceptor antagonist, WB-4101; (3) alpha-adrenoceptor antagonists block the activation of 5-HT cells by NE following the administration of reserpine. This demonstration that alpha-adrenoceptors mediate the activation by NE further strengthens the hypothesis that the adrenergic system exerts an activating influence on 5-HT cells in the dorsal raphe. It is unclear whether the depressant effect observed at higher levels of NE has any physiological significance.

Since the 5-HT cells of the dorsal raphe respond to alpha-adrenoceptor blockade, both locally and systemically, they provide a potentially useful means of evaluating physiologically the central alpha-adrenoceptor antagonist properties of drugs. The pharmacology of this receptor resembles that of the classical post-synaptic α-adrenergic receptor found in the periphery. Alpha-adrenergic receptors located in the locus coeruleus have been classified in the alpha$_2$-category (Cedarbaum and Aghajanian, 1977) proposed by Langer (1974) and Starke, Endo and Taube (1975). In the locus coeruleus, α-MNE is more potent than NE, while PE is only weakly active. In contrast, in the dorsal raphe the present authors have observed a reversal of this rank ordering: PE is nearly equipotent to NE, while α-MNE is only weakly active. This suggests that this receptor may be of the alpha-1 type. In addition, WB-4101 and thymoxamine exhibit preferential blockade of α_1-receptors (Kapur and Mottram, 1978; Drew, 1977; Marshall, Nasmyth, Nicholl and Shepperson, 1978).

If 5-HT cell firing is dependent on adrenergic tone, then destruction of the adrenergic input to the dorsal raphe by 6-OHDA should and does suppress firing. However, the ability of 5-HT cells to adapt to the chronic loss of adrenergic tone and resume an apparently normal pattern of firing remains unexplained. The inability of drugs which act pre-synaptically i.e., reserpine and low doses of clonidine, to suppress firing following 6-OHDA pretreatment (Baraban *et al.*, 1978; Svensson *et al.*, 1975), suggests that 5-HT cell firing has become independent of adrenergic tone. However, the partially retained ability of alpha-adrenoceptor antagonists which act postsynaptically to suppress firing activity favors the notion that some adrenergic component still contributes to maintaining 5-HT cell firing. Further study of the manner in which 5-HT cells adapt to the chronic loss of adrenergic tone is needed to explain these pharmacological responses.

There are several possible neuronal circuits through which alpha-adrenoceptor antagonists might act to suppress 5-HT cell firing. A tonically active excitatory adrenergic input could terminate directly on 5-HT cells. The ability of NE to produce excitation at alpha-adrenoceptors has been reported in the cortex by Bevan *et al.* (1977). In this case, the antagonists would act by interrupting a tonically active, excitatory adrenergic pathway. However, the ability of the

GABA receptor antagonist, picrotoxin to restore 5-HT cell activity following a reduction of adrenergic tone suggests an indirect mechanism of action (Gallager and Aghajanian, 1976b). Alpha-adrenoceptor antagonists could suppress 5-HT cell firing by blocking the tonic inhibition by NE of local GABA interneurons. A similar action of NE on interneurons has been proposed to mediate the indirect effects of NE on principal neurons in both the ventral horn of the spinal cord (Jordan, McCrea, Steeves and Menzies, 1977) and the lateral geniculate nucleus (Nakai and Takaori, 1974). In the dorsal raphe, the presence of very high levels of glutamate decarboxylase (Tappaz, Brownstein and Palkovitz, 1976) within intrinsic GABA neurons (Belin, Aguera, Tappaz, McRae-Deguerce, Bobillier and Pujol, 1979; McGeer, Scherer-Singler and Singh, 1979) is consistent with this model. Indeed, non-serotonergic interneurons which have a firing pattern reciprocal to that of neighboring 5-HT cells have been found which could receive the adrenergic input (Aghajanian, Wang and Baraban, 1978). Further anatomical and physiological investigations aimed at defining the synaptic microcircuitry of the dorsal raphe will be necessary to elucidate the mechanisms by which alpha-adrenoceptor antagonists suppress the firing of 5-HT neurons in the dorsal raphe.

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