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Mechanisms of nicotine actions on dorsal raphe serotoninergic neurons

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Abstract

Nicotine, locally administered into the dorsal raphe nucleus (DRN) of rat midbrain slices, increased the discharge rate of 70% of serotoninergic neurons, decreased it in 30% and induced reciprocal oscillatory increases in serotonin (5-hydroxytryptamine, 5-HT) and γ-aminobutyric acid (GABA) release. All of nicotine's stimulatory effects were maximal at 2.15 μM. Bicuculline, a $GABA_A$ receptor antagonist, increased the firing rate in 64% of serotoninergic neurons, decreased it in 36% and augmented serotonin and GABA release. Bicuculline increased nicotine's stimulatory effects on firing rate but did not reverse the inhibitory ones. *N*-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinil-cyclohexanecarboxamide (WAY-100635), a $5\text{-}HT_{1A}$ receptor antagonist, increased the firing rate of 88% of serotoninergic neurons, as well as serotonin and GABA release and reversed nicotine's inhibitory action on serotoninergic neurons. These data suggest that nicotine decreases the firing rate of one third of serotoninergic neurons through serotonin release and increases the firing rate of the remaining two thirds, due to stronger stimulatory than indirect inhibitory effects.

Keywords: Dorsal raphe nucleus; 5-HT (5-Hydroxytryptamine), serotonin; Nicotine; GABA (γ-Aminobutyric acid)

1. Introduction

The dorsal raphe nucleus contains the largest pool of serotoninergic neurons in the brain (Dahlström and Fuxe, 1964) and is an important target for drugs used in the management of depressive disorders (Blier and De Montigny, 1994). Recently, it has been shown that transdermal administration of nicotine improves mood in patients with mayor depression (Salín-Pascual and Drucker-Colín, 1998). Since some reports have demonstrated that nicotine stimulates dorsal raphe serotoninergic neurons in vitro (Li et al., 1998; Mihailescu et al., 1998) while also inducing release of serotonin (Mihailescu et al., 1998), it is likely that the antidepressive actions of nicotine are mediated by serotonin. This interpretation is strengthened by the fact that the inhibition of serotonin uptake is one of the most effective treatments of depression (Blier and De Montigny, 1998). On the other hand, it has been reported that nicotine suppresses the ponto-geniculo occipital (PGO) spikes, during rapid eye movement (REM) sleep in freely moving cats (Vazquez et al., 1996). Dorsal raphe neurons (DRN) stop firing as soon as REM sleep sets in (McGinty and Harper, 1976), thus playing a permissive role in the generation of the PGO spikes of REM sleep (Lydic et al., 1983). Nicotine prevents the shutting off of DRN neurons during REM as recently demonstrated by Guzmán-Marín et al. (2001), thereby inducing the inhibition of the PGO spikes. All of these suggest that nicotine exerts its effects by stimulating serotonin activity.

In the studies of Li et al. and Mihailescu et al., it was shown that nicotine increased the discharge rate in 60–68% of dorsal raphe serotoninergic neurons and decreased it in the remaining 32–40%. Evidence suggesting that nicotine's stimulatory effects are direct comes from studies indicating the presence of α4β2 and α7 nicotinic receptors in dorsal raphe serotoninergic neurons of rats (Bitner et al., 2000a,b). There are, on the other hand, indirect stimulatory effects of nicotine on dorsal raphe serotoninergic neurons, mediated by norepinephrine and glutamate (Li et al., 1998; Pan and Williams, 1989). The inhibitory effects of nicotine on firing rate are probably indirect, via γ-aminobutyric acid (GABA) and/or serotonin release. It has been demonstrated that

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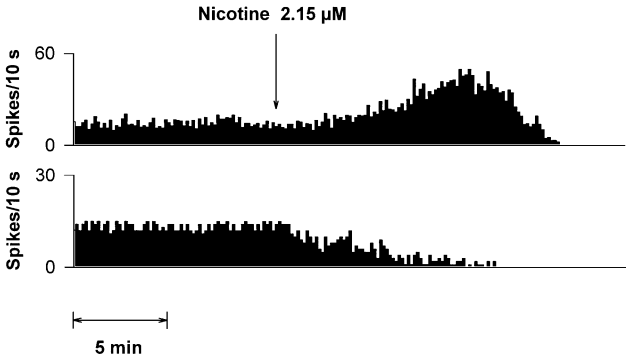

Fig. 1. Rate histograms of two dorsal raphe serotoninergic neurons simultaneously recorded. Nicotine (2.15 μM) produced reciprocal changes in discharge rate of these neurons.

nicotine increases the release of GABA in brain slices (Yang et al., 1996) and that serotoninergic neurons are subject to GABAergic inhibition during rapid eye movement sleep (Nitz and Siegel, 1997). Likewise, in rats, 25–30% of the total neuronal population of the dorsal raphe nucleus is GABAergic (Johnson, 1994). Serotoninergic neurons possess 5-HT_{1A} autoreceptors, which show strong inhibition of their firing rate (Penington et al., 1993).

The goal of this study was to characterize in a better way some mechanisms of nicotine's actions on dorsal raphe serotoninergic neurons in rat midbrain slices.

2. Materials and methods

2.1. Slice preparation

Experiments were carried out on brains of young male Wistar rats (140–160 g). After anesthesia with chloral hydrate (400 mg/kg, i.p.), the rats were decapitated and the brain was rapidly (<1 min) removed and transferred into a Petri dish, containing ice-cold Yamamoto buffer (composition in mM/l: NaCl 124, KCl 5, KH_2PO_4 1.24, $MgSO_4$, $CaCl_2$ 2.4, $NaHCO_3$ 26 and glucose 10). Coronal midbrain slices (350 μm width) were obtained using a McIlwain tissue chopper. The slices containing the dorsal raphe nucleus were transferred into a tissue slice recording chamber (Fine Science Tools) and immersed into the flowing perfusate (Yamamoto buffer: 1 ml/min, pH maintained at 7.4 by bubbling it with carbogen). Phenylephrine (12 μM, Sigma) was added to the buffer in order to induce the automatic firing of otherwise silent serotoninergic dorsal raphe neurons (VanderMaelen and Aghajanian, 1983).

2.2. Recordings and drugs

Extracellular recordings were performed 2 h after slice preparation, using multibarrel glass micropipettes (World Precision Instruments). One of the barrels was filled with NaCl 3 M and used for recording of electrical activity (impedances between 4 and 8 MΩ). Another barrel was filled with nicotine (bitartrate salt, Sigma, at 0.215, 2.15, 21.5 or 215 μM). A third barrel was used for administration of either bicuculline (Sigma, 20 μM) or *N*-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinil-cyclohexanecarboxamide (WAY-100635, RBI/Sigma, 140 nM). In other experiments, nicotine (2.15 and 21.5 μM), bicuculline (20 μM) or WAY-100635 (140 nM) were administered in perfusion, using separate reservoirs connected to the slice perfusion chamber through an electronic valve system (ValveBank8, World Precision Instruments).

Signals were amplified (500–1000 times), filtered (300–3000 Hz), visualized in a Tektronix (2213A) oscilloscope and recorded with a digital tape recorder (BioLogic). Rate histograms were obtained off-line, using a BrainWave (DataWave) system. All neurons used for recordings were found in the area of the dorsal raphe nucleus, identified in the midline under the cerebral aqueduct and above the longitudinal fasciculus.

After the baseline recording of an isolated unit, nicotine was administered using a pressure injection system (Picospritzer II, General Valve Corporation). The standard duration for local nicotine administration was 5 ms at a pressure of 20 psi, whereas for perfusion administration of drugs, 6- to 8-min administration periods were used.

The responses of dorsal raphe serotoninergic neurons to drugs were considered significant if their firing rate changed by more than 15% with respect to baseline. Periods of at

Table 1
Effects of various drugs on the firing rate of dorsal raphe serotoninergic neurons

Drugs	Conc. (μM)	Stimulatory effects				Inhibitory effects			
		Control	After drugs	Drugs/control	*n*	Control	After drug	Drug/control	*n*
Nicotine	0.215	1.5 ± 0.1	2.2 ± 0.3[a]	1.5 ± 0.1	11	1.9 ± 0.5	0.5 ± 0.2[a]	0.2 ± 0.02	4
Nicotine	2.15	1.3 ± 0.2	3.3 ± 0.5[a]	3.4 ± 0.8[b]	10	1.7 ± 0.4	0.8 ± 0.3	0.4 ± 0.06	4
Nicotine	21.5	1.3 ± 0.1	2.2 ± 0.5[a]	1.7 ± 0.4	8	1.5 ± 0.1	0.6 ± 0.1[a]	0.4 ± 0.07	6
Nicotine	215	1.6 ± 0.3	3.3 ± 0.6[a]	2.3 ± 0.3	15	1.8 ± 0.3	0.9 ± 0.4	0.4 ± 0.12	6
WAY	0.14	1.8 ± 0.3	3.7 ± 0.9[a]	2.5 ± 0.6	7	2.16	1.01	0.46	1
Bicuculline	20	1.2 ± 0.5	2.2 ± 0.7[a]	1.4 ± 0.2	7	1.6 ± 0.2	0.6 ± 0.2[a]	0.5 ± 0.1	4

Values represent neuronal firing rate (cycles/s) and are presented as means ± S.E.M.

[a] Significant increase as compared to baseline (paired t-test, $P<0.05$).

[b] Significant increase by nicotine, 2.15 μM, compared to nicotine 0.215 and 21.5 μM (one-way ANOVA on ranks followed by Dunn's test, $P<0.05$).

least 60 s were used to establish the baseline firing rate, whereas the changes induced by drugs were evaluated using periods of at least 30 s.

2.3. High performance liquid chromatography studies

GABA and serotonin levels were measured in samples collected at 4-min intervals from the vacuum line of the recording chamber. Samples were analyzed by high performance liquid chromatography with electrochemical detection. We used the method described by Donzati and Yamamoto (1995) for measuring GABA and the method of Cheng and Kuo (1995) for determining serotonin release. Both neurotransmitters were identified using external standards (fmol/5 μl) and were quantified by measuring the area under the identified peaks. Control values for neurotransmitter release were obtained from three samples of perfusate collected prior to drug administration. Drugs effects were evaluated using mean values for neurotransmitter release obtained from seven consecutive samples.

2.4. Statistics

Results are expressed as means ± standard errors of means (S.E.M.) Comparisons between groups were made using the paired *t*-test (single comparisons), one-way analysis of variance (ANOVA) followed by Dunnett's test (multiple comparisons) or one-way ANOVA on ranks followed by Dunn's test (multiple comparisons of data sets with non-normal distributions). The correlation between serotonin and GABA release was studied using the Pearson Product Moment Correlation. The differences were considered significant at $P<0.05$.

3. Results

A total of 93 cells were recorded in the area of the dorsal raphe nucleus. These cells were classified as serotoninergic according to criteria previously established (Vandermaelen and Aghajanian, 1983): slow (0.5–3 spikes/s), regular firing rate and action potentials of long duration (>2 ms).

Nicotine, locally administered into the dorsal raphe nucleus, significantly increased the discharge rate of 70% of serotoninergic neurons (44 of the 64 cells) and decreased it in the remaining 30% (Fig. 1, Table 1). The maximal increase in firing rate of dorsal raphe neurons produced by nicotine (3.3 times the control) was observed at concentrations of 2.15 μM (Table 1). This increase was significantly different from those obtained with 0.215 and 21.5 μM (one-way ANOVA on ranks, followed by Dunn's test, $P<0.05$). There were no significant differences between the decreases in firing rate induced by various concentrations of nicotine.

Serotonin and GABA levels (Table 2) increased significantly after all four nicotine concentrations tested (0.21, 2.15, 21.5 and 215 μM, paired *t*-test, $P<0.05$). The

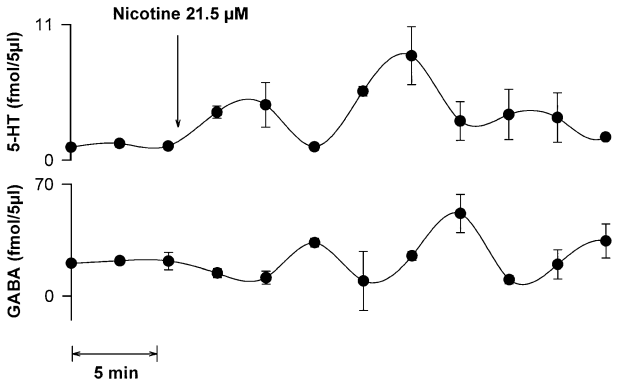

Fig. 2. Oscillatory reciprocal increases in serotonin and GABA release induced by local nicotine administration (21.4 μM) in midbrain slices. Results are presented as means ± S.E.M. ($n=6$). An inverse temporal correlation could be established between GABA and serotonin release in both experiments, using the Pearson Product Moment Correlation, $P<0.05$.

Table 2
Increases in serotonin and GABA release from midbrain slices following the administration of nicotine, bicuculline and WAY-100635

Drugs	Conc. (μM)	*n*	5-HT overflow (drug/baseline)	GABA overflow (drug/baseline)
Nicotine	0.215	15	1.7 ± 0.2[a]	2.6 ± 0.6[a]
Nicotine	2.15	14	7.4 ± 3.1[a,b]	5.9 ± 1.3[a,b]
Nicotine	21.5	14	2.1 ± 0.1[a]	2.72 ± 0.7[a]
Nicotine	215	14	3.8 ± 0.3[a]	4.8 ± 0.5[a]
WAY	0.14	21	1.8 ± 0.2[a]	2.9 ± 0.6[a]
Bicuculline	20	11	1.6 ± 0.2[a]	2.3 ± 0.2[a]

Values are presented as means ± S.E.M. They represent the ratio between mean values ($n=7$ for each experiment) of neurotransmitter release after drug administration and baseline values ($n=3$ for each drug).

[a] Significant increase as compared to baseline (paired t-test, $P<0.05$).

[b] Significant increase induced by nicotine, 2.15 μM, as compared with all other nicotine concentrations (one-way ANOVA followed by Dunnett's test, $P<0.05$).

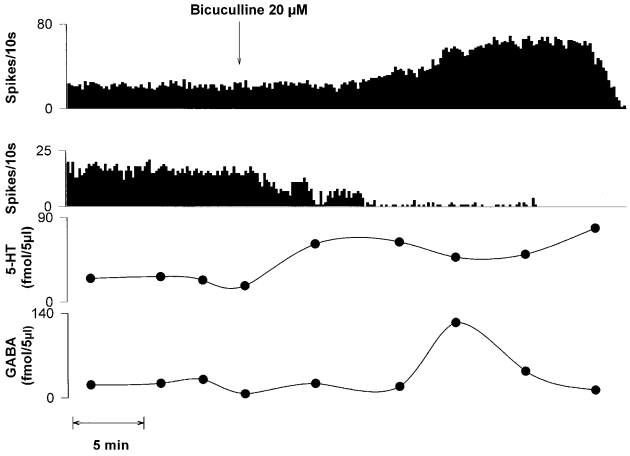

Fig. 3. Opposite effects of bicuculline on the firing rate of two dorsal raphe neurons simultaneously recorded. Bicuculline also produces a long-lasting increase in serotonin release which accompanies the changes in discharge rate of the two neurons.

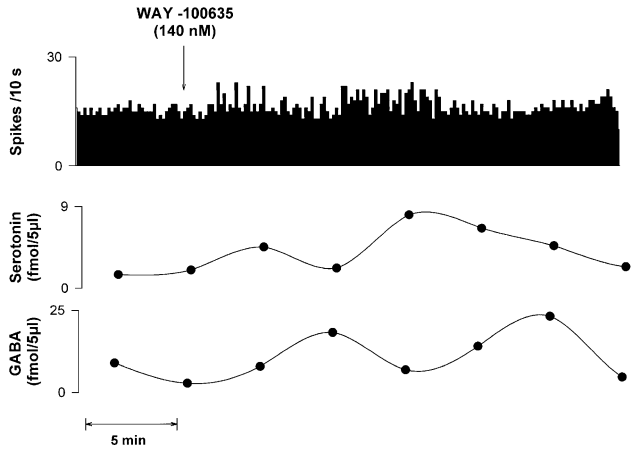

Fig. 4. Discrete increases in firing rate of a dorsal raphe neuron produced by WAY-100635 (upper trace), accompanied by oscillatory increases in serotonin and GABA release.

increases in the release of both neurotransmitters were significantly greater at 2.15 μM nicotine (7.4 times for serotonin and 5.9 times for GABA) than for the other nicotine concentrations tested (one-way ANOVA followed by the Student–Newman–Keuls test, $P<0.05$).

After nicotine, both GABA and serotonin release presented oscillations with periods of 12–16 min. The oscillations of serotonin levels were reciprocal with those of GABA (Fig. 2) and an inverse temporal correlation could be established between the releases of the two neurotransmitters after nicotine using the Pearson Product Moment Correlation ($P<0.05$).

The $GABA_A$ receptor antagonist, bicuculline (20 μM), was administered to 11 serotoninergic neurons from the dorsal raphe nucleus, using pressure injections (5 ms, 20 psi). Bicuculline significantly increased the discharge rate in 64% of dorsal raphe serotoninergic neurons and decreased it in the remaining 36%. The increase in firing rate of dorsal raphe neurons produced by bicuculline was gradual, similarly to the one described by Sakai and Crochet (2001) for in vivo experiments and was accompanied by long-lasting significant increases in serotonin release (Fig. 3, Table 2). A transient increase in GABA release was also observed after bicuculline (Fig. 3).

The $5HT_{1A}$ receptor antagonist, WAY-100635 (140 nM), significantly increased the firing rate of seven out of eight dorsal raphe serotoninergic neurons by 62% and augmented serotonin and GABA release (Fig. 4, Tables 1 and 2).

In the experiments with perfusion, administration of nicotine followed by administration of bicuculline ($n=5$), the $GABA_A$ receptor antagonist augmented both stimulatory and inhibitory effects of nicotine on dorsal raphe neuron firing rate (Fig. 4). In this same type of experiments, WAY-100635 ($n=5$) reverted the inhibitory responses of dorsal raphe neurons to nicotine and augmented the stimulatory ones (Fig. 5).

4. Discussion

In the present study, we showed that nicotine exerts opposite effects on the firing rate of two subgroups of serotoninergic neurons from the dorsal raphe nucleus. The first subgroup (two thirds of dorsal raphe serotoninergic neurons), responded to nicotine with an increase in firing rate, whereas the neurons from second subgroup (one third of dorsal raphe serotoninergic neurons) were inhibited by nicotine through serotonin release. The maximal increases in GABA and serotonin release were observed at a relatively low concentration of nicotine (2.15 μM).

Nicotine's stimulatory effect on discharge rate observed in 70% of serotoninergic neurons may be direct, since the presence of both α4 and α7 subunit-containing nicotinic

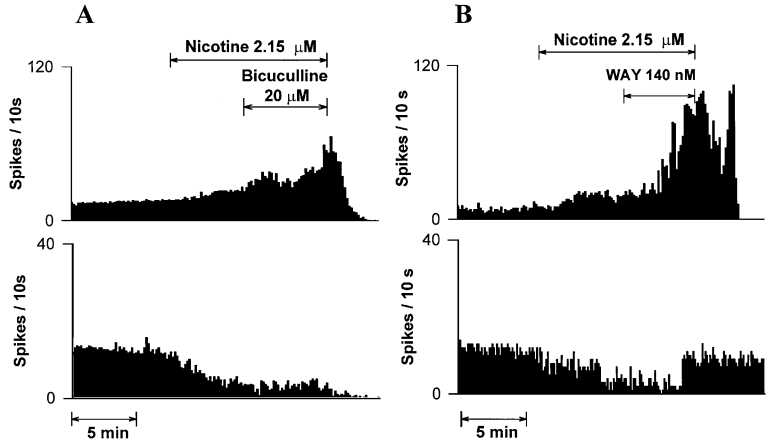

Fig. 5. Reversal of nicotine's inhibitory effects on the firing rate of a dorsal raphe serotoninergic neuron by the 5-HT_{1A} receptor blocker, WAY-100635. Both bicuculline and WAY-100635, administered in perfusion, increased the stimulatory effects of nicotine on dorsal raphe neurons (A and B, top graphs). WAY-100635 reversed nicotine's inhibitory effects on a dorsal raphe neuron firing rate (right bottom graph), an effect not observed after bicuculline (left bottom graph).

acetylcholine receptors in these neurons has recently been demonstrated (Bitner et al., 2000a,b). However, the results obtained by Li et al. (1998) suggested that nicotine stimulates dorsal raphe neurons by presynaptic release of noradrenaline inside the dorsal raphe nucleus. Previous studies (Sprouse and Aghajanian, 1987) have shown that noradrenaline depolarizes dorsal raphe neurons by acting on postsynaptic α_1 receptors. This indirect stimulatory action of nicotine seems unlikely in our experiments, since the α_1 adrenergic receptors of serotoninergic neurons were near to maximally stimulated by the presence of phenylephrine (12 μM) in the perfusate. Glutamate mediation of nicotine's stimulatory effects on dorsal raphe neurons was expectable because nanomolar concentrations of nicotine induce glutamate release (McGehee et al., 1995) and electrical stimulation of the dorsal raphe nucleus induces glutamate-dependent excitatory postsynaptic potentials in dorsal raphe serotoninergic neurons (Pan and Williams, 1989). However, this possibility was discarded in the study of Li et al. (1998) who used pharmacological methods. It is important to mention that dorsal raphe serotoninergic neurons stimulated by nicotine underwent inhibitory influences from serotonin and GABA, since both WAY-100635 and bicuculline further increased the discharge rate of these neurons when administered after nicotine.

Nicotine's inhibitory effects on the discharge rate of 30% of dorsal raphe serotoninergic neurons are indirect, via release of inhibitory neurotransmitters. Our data indicate that nicotine, at all concentrations tested, significantly increased the mean levels of both serotonin and GABA. It was shown that serotonin decreases the discharge rate of serotoninergic neurons by stimulating their somatodendritic 5-HT_{1A} autoreceptors (Penington et al., 1993) and that GABA exerts a similar inhibitory action through somatic $GABA_A$ receptors (Levine and Jacobs, 1992). According to our data, the inhibition of dorsal raphe neuron firing rate induced by nicotine appears to be dependent on serotonin and not on GABA, since it was reversed by WAY-100635 but not by bicuculline. A serotonin-dependent inhibition of dorsal raphe neurons by nicotine was also described by Engberg et al. (2000), using in vivo experiments. The site of serotonin release inside the dorsal raphe nucleus may be the somatodendritic region of serotoninergic neurons or serotoninergic terminals present in the nucleus. Li et al. (1998) indicated that nicotine-induced serotonin release inside the dorsal raphe nucleus is insensitive to TTX and low extracellular calcium, characteristics strikingly similar to those of the somatodendritic release of serotonin described by Adell et al. (1993). In contrast, Engberg et al. (2000) found that the iontophoretic administration of nicotine inside the dorsal raphe nucleus failed to inhibit serotoninergic neurons, which suggests an indirect inhibitory effect of systemic nicotine, involving afferent pathways. Interestingly, in our experiments, bicuculline decreased the firing rate of 36% of dorsal raphe neurons, a percentage very close to the one observed for nicotine. On the other hand, both nicotine and bicuculline increased serotonin release. It is tempting to assume that the neurons inhibited by nicotine and bicuculline belong to the same population, insensitive to nicotine's stimulatory actions but sensitive to serotonin's inhibitory effects.

In the present study, the time course of neurotransmitter release obtained from individual experiments revealed that GABA and serotonin overflows produced by nicotine were oscillatory and reciprocal. This suggests that nicotine stimulated the release of both neurotransmitters, which afterwards exerted reciprocal inhibitory actions. The facts that the $GABA_A$ receptor antagonist, bicuculline, increased serotonin release and that the 5-HT_{1A} receptor antagonist, WAY-100635, increased GABA release support this view. Likewise, recent studies have shown that: (a) GABA inhibits serotonin release through activation of both $GABA_A$ and $GABA_B$ receptors of serotoninergic neurons (Bagdy et al., 2000); (b) serotonin inhibits GABA release through inhibitory mechanisms dependent on both somatic 5-HT_{1A} and presynaptic 5-HT_{1B} receptors (Abellán et al., 2000; Bagdy et al., 2000).

Besides reciprocal inhibition, both GABA and serotonin exerted autoinhibitory effects. Thus, bicuculline increased GABA release whereas WAY-100635 augmented serotonin release. These data are in agreement with results of previous studies indicating that a part of the negative feedback control of serotonin release depends on activation of 5-HT_{1A} receptors (Bagdy et al., 2000; Tao et al., 1996) and that GABA release is limited by presynaptic $GABA_A$ autoreceptors (Ennis and Minchin, 1993; Bagdy et al., 2000).

In summary, the results of this study indicate the presence of two subpopulations of dorsal raphe serotoninergic neurons with opposite firing rate responses to both nicotine and bicuculline. Our experiments also revealed that nicotine induces oscillatory and reciprocal overflows of serotonin and GABA from rat midbrain slices.

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