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INHIBITION BY APOMORPHINE OF THE POTASSIUM-EVOKED RELEASE OF [^{3}H]-γ-AMINOBUTYRIC ACID FROM THE RAT SUBSTANTIA NIGRA *in vitro*

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1 The spontaneous and potassium-evoked release of tritium from the rat substantia nigra prelabelled with [^{3}H]-γ-aminobutyric acid [^{3}H]-GABA were assessed *in vitro* under conditions of superfusion.

2 Kainic acid lesions performed in the right caudate nucleus resulted in a 70% reduction in the ability of the homolateral nigral cells to take up and retain [^{3}H]-GABA when compared with the unlesioned side. The potassium-evoked release of [^{3}H]-GABA remained proportional to the radioactivity retained in the tissue suggesting that the nigral GABA neurones that survived kainic acid treatment were still functional.

3 The spontaneous outflow of [^{3}H]-GABA was significantly increased by exposure to different concentrations of exogenous GABA (10 to 1000 μM) when amino-oxyacetic acid was present in the incubation medium.

4 Apomorphine in concentrations ranging from 1 to 30 μM inhibited the calcium-dependent release of [^{3}H]-GABA induced by 1 min exposure to 30 mM K^+. These concentrations of apomorphine did not affect the spontaneous outflow of radioactivity. *In vivo* administration of haloperidol 0.2 mg/kg antagonized the *in vitro* inhibition by apomorphine of the K^+-evoked release of [^{3}H]-GABA.

5 The results obtained with apomorphine and haloperidol suggest the presence of presynaptic dopamine-like inhibitory receptors in gabaergic nerve terminals.

6 Dopamine in concentrations ranging up to 300 μM did not modify either the spontaneous or the K^+-evoked release of [^{3}H]-GABA from the substantia nigra. These concentrations of dopamine effectively displaced [^{3}H]-dopamine recently taken up into the substantia nigra.

7 Our results do not support the view that dendritic release of dopamine from the substantia nigra might be involved in the physiological modulation of the spontaneous or the stimulation-evoked release of GABA.

Introduction

Dopamine receptors in the rat substantia nigra have been shown to be located in the cell bodies of the dopaminergic neurone (Carlsson, 1975; Aghajanian & Bunney, 1977). However, the activity of the dopamine-sensitive adenylate cyclase identified in the substantia nigra (Phillipson & Horn, 1976), occurs on cellular elements other than the dopaminergic neurone (Kebabian & Saavedra, 1976) and is, apparently, located presynaptically on terminals of striatonigral afferents (Gale, Guidotti & Costa, 1977).

Several *in vitro* studies on the modulation of the calcium-dependent release of neurotransmitters support the view that presynaptic receptors can play a role in the interaction between neurotransmitters at the level of transmitter release (for reviews see Langer, 1977; 1980; 1981).

Facilitation by γ-aminobutyric acid (GABA) of the potassium-induced release of dopamine from the rat substantia nigra has been demonstrated in *in vitro* experiments (Starr, 1978). Conversely, dopamine can increase the spontaneous outflow of [^{3}H]-GABA from the rat substantia nigra (Reubi, Iversen & Jessell, 1977). Based on these observations it was concluded that presynaptic dopamine receptors located on gabaergic nerve endings facilitate the release of GABA in the rat substantia nigra (Reubi *et al.*, 1977).

The aim of the present experiments was to determine whether dopamine receptors are located on the gabaergic nerve terminals of the rat substantia nigra and, if so, whether these receptors are able to modulate the potassium-evoked release of GABA. We have therefore compared the effects of apomorphine

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and dopamine on the spontaneous and the potassium-evoked release of [^{3}H]-GABA in the rat substantia nigra.

Methods

Male rats of 150–200 g were killed by decapitation. The substantia nigra was dissected and each segment chopped from the lateral to medial and caudal to rostral side in two halves. The tissues were incubated at 25°C for 30 min in 2 ml of Krebs medium containing 2.3×10^{-7} M [^{3}H]-GABA (sp. act. 45 Ci/mmol, CEA, France) or [^{3}H]-dopamine 1×10^{-7} M (sp. act. 10 Ci/mmol, The Radiochemical Centre Amersham). The Krebs medium had the following composition (mM): NaCl 118, KCl 4.7, glucose 11.1, $NaHCO_3$ 25.0, $MgCl_2$ 1.2, $NaHPO_4$ 1.0, $CaCl_2$ 1.3, ascorbic acid 0.11, disodium edetate (EDTA Na_2) 0.04, amino-oxyacetic acid 0.1 and in one group of experiments, pargyline 10 μM. The medium was equilibrated with 5% CO_2 in O_2. After the incubation, the tissue was rinsed three times with 4 ml of Krebs and two halves of the substantia nigra were transferred to a superfusion chamber and superfused for the next 45 min at a rate of 0.5 ml/min maintaining the temperature at 25°C. A separate group of experiments described in more detail in the Results section was carried out at 37°C either in the presence or the absence of amino-oxyacetic acid in the Krebs medium and at a superfusion rate of 1 ml/min.

After the 45 min superfusion period, 2 min samples were collected throughout the rest of the experiment. Two periods of stimulation with 30 mM K^+ for 1 min were applied 89 min (S_1) and 121 min (S_2) after the end of the incubation with the radioactive transmitter. The depolarizing medium, containing 30 mM K^+, was obtained by isomolar replacement of NaCl by KCl. At the end of the superfusion, the tissues were solubilized, and the tritium content of the tissue and superfusate samples were determined by liquid scintillation counting. The spontaneous outflow of radioactivity was calculated as fractional release, i.e. total nCi released in each sample as a fraction of the total radioactivity present in the tissue. The overflow of radioactivity elicited by potassium was calculated by subtracting the spontaneous outflow assumed to have occurred in each sample during and following exposure to potassium and expressing the results as a percentage of the total tissue radioactivity (Pelayo, Dubocovich & Langer, 1980). The spontaneous outflow in the absence of the drug was expressed as the fraction of total tissue radioactivity released spontaneously during each 2 min fraction. The effect of a drug on the spontaneous outflow of radioactivity was determined during the 12 min before the second period of stimulation with potassium. The values are expressed as the ratio between the spontaneous outflow in the presence and in the absence of the drug.

In some experimental groups indicated in Results, the rats were pretreated with haloperidol (0.2 mg/kg, i.p.) 24 h before they were killed.

Lesions of the corpus striatum were performed by microinjection of kainic acid (2 μg in 1 μl of 0.1 M Tris buffer pH: 7.2) in the right rat striatum using a cannula positioned at the following stereotaxic coordinates, A: 8.2, L: 2.6; V: 0.5 according to the atlas of Köning & Klippel (1970). The microinjections lasted 3 min.

Statistical calculations were performed using

Table 1 Effects of intrastriatal injection of kainic acid on the retention, spontaneous outflow and stimulation evoked release of [^{3}H]-GABA from the rat substantia nigra

Group of rats		n	*^{3}H-spontaneous outflow* (a) (FR × 10^{-3})	*^{3}H-stimulation-evoked release* *^{3}H-total* (nCi)(b)	*FR × 10^{-2}* (c)	*^{3}H-tissue content* (d) (nCi)
Untreated		10	1.19 ± 0.39	16.37 ± 2.29	6.46 ± 1.08	294.2 ± 49.9
Kainic acid at 6 weeks	Control	4	1.94 ± 0.38	24.89 ± 4.18	8.34 ± 1.43	278.2 ± 25.6
	Lesion	5	1.55 ± 0.26	6.68 ± 2.14*	6.00 ± 1.57	86.4 ± 7.8***
Kainic acid at 14 weeks	Control	6	1.98 ± 0.42	19.65 ± 1.65	6.22 ± 0.92	340.0 ± 42.2
	Lesion	6	2.20 ± 0.48	5.92 ± 3.01*	3.10 ± 1.27	134.3 ± 34.0**

Unilateral intrastriatal injections of kainic acid were performed 6 and 14 weeks before rats were killed. The contralateral non-lesioned substantia nigra was used as the control.

(a) fraction of the total tissue radioactivity released spontaneously during 1 min, determined 45 min after the end of the incubation with [^{3}H]-GABA; (b) nCi of tritium released above the spontaneous outflow by a 1 min exposure to 30 mM K^+; (c) fraction of the total tissue radioactivity above the spontaneous outflow released by a 1 min exposure to 30 mM K^+; (d) nCi of tritium per substantia nigra determined 45 min after the end of the incubation with [^{3}H]-GABA.

n = number of experiments. Shown are mean values ± s.e.mean.

*$P<0.008$; **$P<0.004$; ***$P<0.001$ when compared with the corresponding control group.

Mann Whitney 2 tailed test (Siegal, 1956).

The following drugs were used: apomorphine hydrochloride, dopamine, haloperidol hydrochloride, kainic acid, GABA and pargyline.

Results

Effects of intrastriatal injection of kainic acid on the accumulation and subsequent release of [^{3}H]-GABA from the substantia nigra

Table 1 shows that incubation of the substantia nigra with [^{3}H]-GABA for 30 min results in a high accumulation of tritium within the tissue. In order to verify that this accumulation largely results from uptake of [^{3}H]-GABA into gabaergic nerve terminals and does not involve uptake into other structures, such as glial elements, we carried out unilateral lesions in the corpus striatum by injection of kainic acid to produce a degeneration of gabaergic cell bodies of the striatum and as a consequence the degeneration of the corresponding nerve terminals in the substantia nigra.

Six weeks after the injection of kainic acid, the total radioactivity retained in the lesioned substantia nigra after incubation with [^{3}H]-GABA was significantly reduced by 70% when compared with the contralateral control substantia nigra which did not receive kainic acid (Table 1). Similar results were observed when the uptake of [^{3}H]-GABA was measured 14 weeks after kainic acid treatment (Table 1).

The fraction of tissue radioactivity released in spontaneous outflow and by depolarization with 30 mM potassium is shown in Table 1. Either 6 or 14 weeks after kainic acid injection, the spontaneous or stimulation-evoked fractional release of radioactivity were not significantly different from those in the control substantia nigra (Table 1). However, the absolute values of the overflow of radioactivity evoked by exposure to potassium measured as total tritium were significantly decreased in the substantia nigra of both groups of kainic acid treated animals when compared with the corresponding controls (Table 1).

The accumulation, spontaneous and potassium-evoked release of [^{3}H]-GABA observed in the control substantia nigra of kainic acid treated animals was similar to that obtained in untreated animals (Table 1).

Effects of dopamine agonists on the spontaneous and potassium-evoked relase of [^{3}H]-GABA

In this group of experiments, two periods of 1 min exposure to 30 mM potassium were applied. In the controls, the ratio between two consecutive periods of stimulation was lower than unity, indicating a decrease in the potassium-evoked release of [^{3}H]-GABA during the second period of stimulation (Table 2).

Apomorphine (1 to 30 μM) added to the superfusion medium 12 min before the second period of exposure to potassium reduced the stimulation-evoked release of [^{3}H]-GABA under conditions in which the spontaneous outflow of radioactivity remained unchanged (Table 2). The slope of the concentration-effect curve for this inhibitory effect of apomorphine was rather flat and even at the highest concentration (30 μM) the stimulation-evoked release of [^{3}H]-GABA was not completely inhibited (Table 2).

The effects of exogenous dopamine on the spontaneous and potassium-evoked release of [^{3}H]-GABA were studied in experiments in which monoamine oxidase activity was inhibited by 10 μM

Table 2 Effects of apomorphine on the spontaneous and K^+-evoked release of [^{3}H]-GABA from the rat substantia nigra

Apomorphine (μM)	n	*Evoked release* (a) S_2/S_1	*Spontaneous outflow* (b) Sp_2/Sp_1
0	20	0.55 ± 0.03	0.98 ± 0.03
0.01	6	0.54 ± 0.11	0.95 ± 0.03
0.1	15	0.42 ± 0.05	0.99 ± 0.07
1	15	0.36 ± 0.04*	0.99 ± 0.05
10	11	0.34 ± 0.03**	0.93 ± 0.04
30	9	0.33 ± 0.06**	0.89 ± 0.06

(a) S_1 corresponds to the % of total tissue radioactivity released by the first 1 min exposure to 30 mM K^+, and S_2 to the second release obtained 32 min later. Apomorphine in the concentrations indicated was added to the superfusion medium 12 min before S_2; (b) Sp_1 corresponds to the fraction of total tissue radioactivity released spontaneously in the absence of the drug and Sp_2 in the presence of the drug.

n = number of experiments. Shown are mean values ± s.e.mean.

*$P<0.008$; **$P<0.004$ when compared to the control group.

Table 3 Effects of exogenous dopamine on the release of [^{3}H]-GABA and [^{3}H]-dopamine ([^{3}H]-DA) from the rat substantia nigra

	[^{3}H]-GABA release		[^{3}H]-DA release
	Evoked release (a)	*Spontaneous outflow* (b)	*Spontaneous outflow* (c)
Dopamine (μM)	S_2/S_1	Sp_2/Sp_1	Sp_2/Sp_1
0	0.55 ± 0.03 (20)	0.95 ± 0.05 (20)	0.96 ± 0.02 (7)
3	—	—	1.20 ± 0.09 (4)*
30	0.58 ± 0.10 (8)	1.00 ± 0.05 (8)	4.62 ± 1.49 (3)*
100	0.44 ± 0.09 (7)	0.85 ± 0.09 (7)	5.33 ± 1.72 (3)*
300	0.55 ± 0.12 (7)	0.78 ± 0.10 (7)	—
1000	0.32 ± 0.04 (7)**	0.85 ± 0.02 (7)	—

(a) S_1 corresponds to the % of total tissue radioactivity released by the first 1 min exposure to 30 mM K^+, and S_2 to the second release obtained 32 min later. Dopamine in the concentrations indicated was added to the superfusion medium 12 min before S_2; (b) Sp_1 corresponds to the fraction of total tissue radioactivity released spontaneously during 12 min in the absence of dopamine and Sp_2 during 12 min of exposure to dopamine. Pargyline 10 μM was present throughout the experiment. The columns (a) and (b) correspond to experiments in which the substantia nigra was prelabelled with [^{3}H]-GABA.

(c) Sp_1 corresponds to the fraction of total tissue radioactivity released spontaneously during 12 min in the absence of dopamine and Sp_2 to the fraction of total tissue radioactivity released during 12 min following a 2 min exposure to different concentrations of dopamine. Pargyline 10 μM was present throughout the experiment. In this group of experiments the substantia nigra was prelabelled with [^{3}H]-dopamine.

Numbers in parentheses correspond to the number of experiments per group. Shown are mean values ± s.e.mean.

*$P<0.008$; **$P<0.004$ when compared to the control group.

pargyline. Dopamine in concentrations up to 300 μM added before the second period of potassium stimulation failed to modify the stimulation-evoked release of [^{3}H]-GABA. Nevertheless, in the presence of 1 mM dopamine a significant inhibition of release was obtained (Table 3). During exposure to concentrations of dopamine ranging from 30 μM to 1 mM, no significant changes in the spontaneous outflow of [^{3}H]-GABA were observed (Table 3).

The lack of activity of dopamine in modifying spontaneous or stimulation-evoked [^{3}H]-GABA release was not due to the fact that exogenous dopamine was not reaching the terminals in a sufficient concentration. In separate experiments in which the substantia nigra was prelabelled with [^{3}H]-dopamine, exposure to exogenous dopamine (3 to 100 μM) increased in a concentration-dependent manner the outflow of radioactivity by displacing [^{3}H]-dopamine from its storage sites in dendrites (Table 3).

It was also considered of interest to determine the influence of low temperature (25°C) and of inhibition of transaminase activity on our studies on release of [^{3}H]-GABA. Accordingly, an additional series of experiments was carried out at 37°C with and without amino-oxyacetic acid (AOAA) in the medium.

As shown in Table 4, in the presence of AOAA (100 μM) the retention of radioactivity in the tissue

Table 4 Influence of inhibition of transaminase activity with amino-oxyacetic acid (AOAA) on the spontaneous outflow and tissue retention of [^{3}H]-GABA in the rat substantia nigra

		^{3}H-spontaneous outflow		^{3}H-tissue
	n	*nCi/min* (a)	$FR \times 10^{-3}$ (b)	(nCi/nigra)
Control	14	0.80 ± 0.04	12.75 ± 0.15	62.9 ± 5.6
AOAA 100 μM	12	0.34 ± 0.03*	1.05 ± 0.03*	324.4 ± 10.6*

The experimental conditions are those described in Methods with the following modifications: the tissue was incubated with [^{3}H]-GABA for 20 min and was followed by a superfusion at a rate of 1 ml/min. The temperature was maintained at 37°C and the medium contained ascorbic acid 0.17 mM and EDTA 0.026 mM as well as AOAA 100 μM when indicated.

(a) nCi of tritium and (b) fraction of tissue radioactivity released spontaneously per min determined 85 min after the end of the incubation with [^{3}H]-GABA. (c) nCi of tritium retained per substantia nigra determined 85 min after the end of the incubation with [^{3}H]-GABA.

*$P<0.002$ when compared with control groups.

was significantly enhanced and there was a concomitant reduction in the spontaneous outflow of radioactivity both in absolute values and as fractional release when transaminase activity was inhibited.

Figure 1 shows that both apomorphine (0.1–30 μM) and dopamine (30–1000 μM) failed to modify the spontaneous outflow of [^{3}H]-GABA in the absence or in the presence of the transaminase inhibitor, AOAA 100 μM. In the presence of AOAA 100 μM exposure to exogenous GABA (1–1000 μM) produced a marked increase in the spontaneous outflow of tritium (threshold effect at 10 μM GABA). At 1000 μM GABA the increase in the spontaneous outflow of radioactivity represented a value of approximately 15 times the basal outflow (Figure 1). In the absence of AOAA, the increase in the spontaneous outflow of radioactivity was not observed until concentrations of 1000 μM GABA were employed.

Blockade by haloperidol of the inhibitory effect of apomorphine on the stimulation-evoked release of [^{3}H]-GABA

These experiments were carried out to investigate whether the inhibitory effect of apomorphine on the potassium-evoked release of [^{3}H]-GABA was mediated through the stimulation of dopamine receptors. Rats received a single dose of 0.2 mg/kg (i.p.) of haloperidol 24 h before testing the *in vitro* effects of apomorphine on the potassium-evoked release of [^{3}H]-GABA. The release of [^{3}H]-GABA during the first period of potassium stimulation was not modified by the *in vivo* administration of haloperidol (see Figure 2, legend), and the ratio between the two periods of potassium stimulation was similar to that obtained in control experiments with untreated rats (Figure 2). The inhibition by apomorphine of the potassium-evoked release of [^{3}H]-GABA was prevented by pretreatment with this dose of haloperidol.

Similar results were obtained when haloperidol was used *in vitro* to antagonize the effects of apomorphine. Haloperidol .1 μM did not modify the potassium-evoked release of [^{3}H]-GABA but antagonized the inhibition by apomorphine 10 μM (Figure 3).

Discussion

The substantia nigra is known to be the brain region with the highest density of gabaergic innervation (Okada, Nitsch-Hassler, Kim, Bak & Hassler, 1971). The gabaergic cell bodies which originate in the globus pallidus and the corpus striatum innervate the substantia nigra (Hattori, McGeer, Fibiger & McGeer, 1973; Dray & Straughan, 1976; Nagy, Carter & Fibiger, 1978).

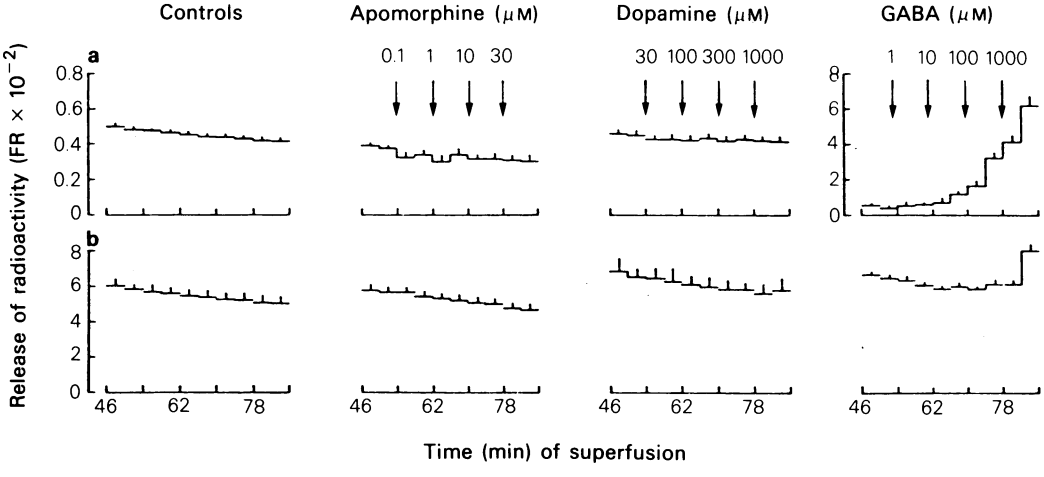

Figure 1 Influence of transaminase inhibition with amino-oxyacetic acid (AOAA) on the effects of dopamine, apomorphine and γ-aminobutyric acid (GABA) on the spontaneous outflow of [^{3}H]-GABA from the rat substantia nigra. (a) Experiments carried out in the presence of AOAA 100 μM and (b) in the absence of the transaminase inhibitor in the Krebs medium. The experimental conditions are those described in Methods with the following modifications: incubation of the tissue was for 20 min and was followed by a superfusion at a rate of 1 ml/min. The temperature was maintained at 37°C and the medium contained ascorbic acid 0.17 mM and EDTA 0.026 mM as well as AOAA 100 μM when indicated. Ordinates: spontaneous outflow of tritium expressed as fraction of the total tissue radioactivity released in a 4 min collection period. Abscissae: time of superfusion in min. The arrows indicate 8 min exposure to apomorphine, dopamine or GABA in the μM concentration indicated.

Shown are mean values of 4–12 experiments per group; vertical lines show s.e.mean.

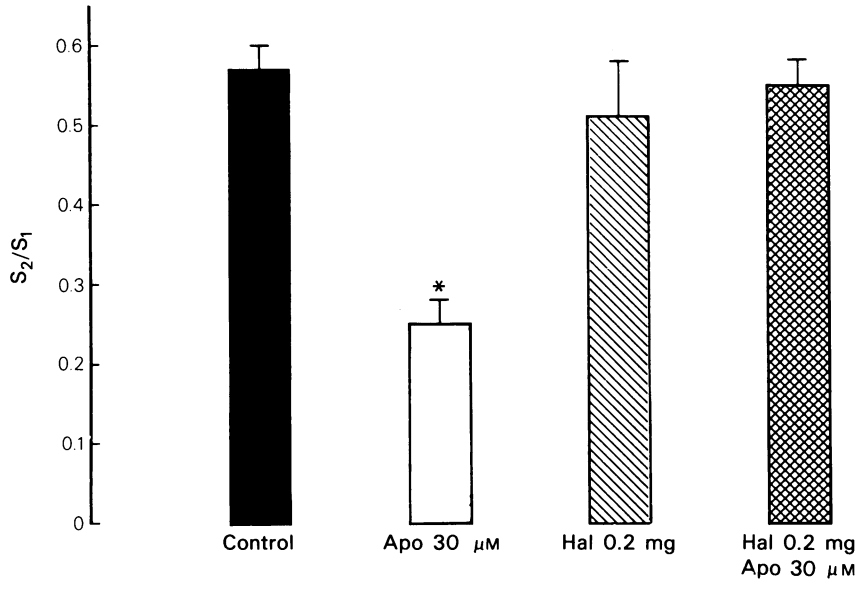

Figure 2 Effects of pretreatment with haloperidol on the inhibition by apomorphine of the potassium evoked release of [^{3}H]-γ-aminobutyric acid ([^{3}H]-GABA) in the rat substantia nigra. Rats received a single dose of 0.2 mg/kg (i.p.) of haloperidol 24 h before they were killed. Ordinate scale: ratio of fractional release of [^{3}H]-GABA (S_2/S_1); S_1 corresponds to the first 1 min exposure to 30 mM K^+ and S_2 to the second, obtained 32 min later. The % of total tissue radioactivity released by the first period of K^+ stimulation (S_1) was 5.03 ± 0.56 ($n = 20$) in control rats and 5.51 ± 0.63 ($n = 9$) in those pretreated with haloperidol. Solid column: controls (untreated rats); open column: apomorphine (Apo) 30 μM added to the superfusion medium 12 min before S_2 in the untreated rats; hatched column: control rats pretreated with haloperidol (Hal) 0.2 mg/kg; cross hatched column,: apomorphine 30 μM added to the superfusion medium 12 min before S_2 in rats pretreated with haloperidol 0.2 mg/kg. The values shown are the means of 4–20 experiments per group; vertical lines show s.e.mean.
*$P < 0.001$ when compared to the control in the untreated group and to apomorphine in rats pretreated with haloperidol.

The 70% decrease in the uptake and retention of [^{3}H]-GABA observed in the substantia nigra after 6 weeks of unilateral injections of kainic acid in the striatum reflects the degree of degeneration of the gabaergic nerve terminals. These results support the view that the uptake and retention of [^{3}H]-GABA in the rat substantia nigra takes place predominantly in gabaergic nerve endings from striato-nigral afferents. The fact that the spontaneous as well as the stimulation-evoked release of [^{3}H]-GABA represents the same fraction of total tissue radioactivity in both control and kainic acid treated tissues, indicates that the remaining gabaergic nerve terminals after kainic acid treatment are still functional.

The failure of intrastriatal injections of kainic acid to produce a total disappearance of gabaergic nerve terminals has also been reported by measuring the loss of gabaergic nerve terminals using other experimental approaches. A reduction of 49% in GAD activity, 42% in [^{3}H]-GABA synthesis (Kemel, Gauchy, Glowinski & Besson, 1979) and 44% in GABA levels (Waddington & Cross, 1978) was found after intrastriatal injections of kainic acid. Therefore, it appears that kainic acid lesions do not destroy all the gabaergic nerve terminals. A more pronounced reduction in GABA levels (Gale *et al.*, 1977), GAD activity, and [^{3}H]-GABA synthesis (Kemel *et al.*, 1979) was observed in the substantia nigra after hemitransection when compared with the results obtained with kainic acid.

The K^+-evoked release of [^{3}H]-GABA from the rat substantia nigra has been shown to be entirely calcium-dependent and to be reduced by both muscimol and exogenous GABA (Kamal, Arbilla & Langer, 1979). Furthermore, the inhibition by muscimol was antagonized by picrotoxin (Arbilla, Kamal & Langer, 1979). These results led the authors to postulate the presence of presynaptic inhibitory GABA autoreceptors on the gabaergic nerve endings of the substantia nigra.

The present results obtained after kainic acid injection further support the neuronal origin of [^{3}H]-GABA released under the experimental conditions employed by Arbilla *et al.* (1979). In addition, the

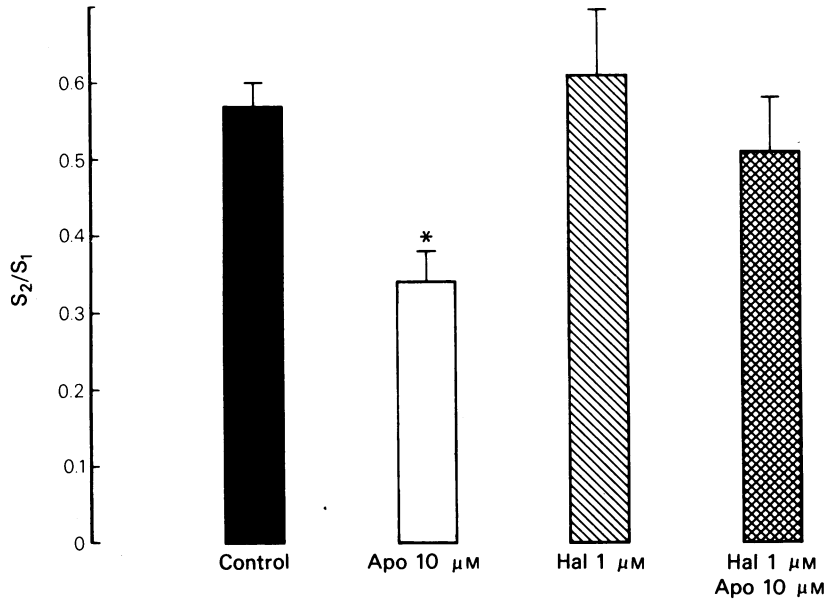

Figure 3 *In vitro* effects of haloperidol on the inhibition by apomorphine of the potassium-evoked release of [^{3}H]-γ-aminobutyric acid ([^{3}H]-GABA) from the rat substantia nigra. Ordinate scale: ratio of fractional release of [^{3}H]-GABA (S_2/S_1); S_1 corresponds to the first 1 min exposure to 30 mM K^+ and S_2 to the second, obtained 32 min later. Solid column: control; open column: apomorphine (Apo) 10 μM was added to the superfusion medium 12 min before S_2; hatched column: haloperidol (Hal) 1 μM was added to the superfusion medium 20 min before S_2; cross-hatched column: haloperidol 1 μM was added 20 min and apomorphine 10 μM 12 min before S_2. The values shown are the means of 5–20 experiments per group. Vertical lines show s.e.mean.
*$P<0.001$ when compared to the control group.

data presented show that when transaminase activity is inhibited by the addition of AOAA to the medium there is a higher retention of [^{3}H]-GABA in the tissue and concomitantly there is a slower rate of loss of radioactivity during the superfusion. These results indicate that inhibition of transaminase activity provides a useful experimental model for studies on spontaneous and stimulation-evoked [^{3}H]-GABA release. Further support for this view is provided by the fact that exogenous GABA was able to induce homo-exchange of [^{3}H]-GABA only when transaminase activity was inhibited with AOAA.

The inhibition of the potassium-evoked release of [^{3}H]-GABA obtained with apomorphine and the antagonism by haloperidol of the effect of apomorphine would suggest the presence of presynaptic inhibitory dopamine receptors located on the gabaergic nerve endings of the substantia nigra. However, since inhibition of the evoked-release of [^{3}H]-GABA was observed for apomorphine but not for dopamine, further studies with other dopamine agonists are necessary to characterize fully the receptor that mediates the effects of apomorphine.

It should also be noted that the inhibitory effect of apomorphine on the release of [^{3}H]-GABA required high concentrations of the drug, and that the concentration-effect curve to apomorphine was rather flat. The inhibitory effects of apomorphine on the electrically-evoked release of [^{3}H]-dopamine from the rabbit caudate nucleus are obtained in the nanomolar range and the concentration-effect curve is quite steep (Starke, Reimann, Zumstein & Hertting, 1978; Kamal, Arbilla & Langer, 1981). These differences in potency of apomorphine in inhibiting the stimulation-evoked release of neurotransmitter could be linked to differences in the dopamine receptor localization or in animal species. There is still some controversy about presynaptic dopamine receptors modulating the release of dopamine from the rat striatum (Farnebo & Hamberger, 1971; Seeman & Lee, 1975; Dismukes & Mulder, 1977; Arbilla, Briley, Dubocovich & Langer, 1978) while their presence in the rabbit (Starke *et al.*, 1978; Langer, Arbilla & Kamal, 1980; Kamal *et al.*, 1981) and cat caudate nucleus (Lehmann, Arbilla & Langer, 1981) is undoubtedly confirmed, suggesting the possibility of a species difference with respect to presynaptic inhibitory dopamine receptors. In support of the view that species differences could be important, it is known that the rat lacks presynaptic inhibitory

dopamine receptors in noradrenergic nerves in the heart (Lefèvre-Borg & Cavero, 1980; Cavero, Lefèvre-Borg & Gomeni, unpublished observations) while these presynaptic receptors are present in the heart of other species such as the dog, cat and rabbit (Long, Heintz, Cannon & Kim, 1975; Fuder & Muscholl, 1978; Langer & Dubocovich, 1979; Lokhandwala & Jandyala, 1979).

Under our experimental conditions exogenous dopamine did not mimic the effects of apomorphine on the K^+-evoked release of [^{3}H]-GABA. This observation may indicate that these receptors differ from the classical dopamine receptors. Such a situation resembles the differences in potency found between apomorphine and dopamine for the inhibition of the stimulation-evoked release of [^{3}H]-GABA from the rat nucleus accumbens (Beart, Kuppers & Louis, 1979) and in the rat ventral tegmentum (Beart & McDonald, 1980). The failure of dopamine to modify the potassium-evoked release of [^{3}H]-GABA is in agreement with similar results obtained by Reubi *et al.* (1977). On the other hand, our findings are at variance with these authors, because under the different experimental conditions tested, we did not find increases in the spontaneous outflow of [^{3}H]-GABA by exposure to exogenous dopamine as reported by Reubi *et al.* (1977). Yet, under our experimental conditions exogenous dopamine effectively reached its site of action because it displaced [^{3}H]-dopamine retained in dendrites of the substantia nigra. In addition, we did not observe changes in the spontaneous outflow of [^{3}H]-GABA during exposure to apomorphine (1 to 30 μM).

In the rat substantia nigra prelabelled with [^{3}H]-dopamine, a 1 min exposure to 30 mM K^+ releases approximately 1% of the total tissue radioactivity (Arbilla & Langer, 1980). Thus, the release of dopamine and GABA occur simultaneously when the substantia nigra is exposed to *in vitro* depolarizing stimuli. Yet, an interaction between endogenously released dopamine and the K^+-evoked release of [^{3}H]-GABA was excluded in experiments in which the release of [^{3}H]-GABA was studied in rats pretreated with reserpine (5 mg/kg i.p.) 24 h before the experiment. The release of [^{3}H]-GABA elicited by exposure to potassium was similar in reserpine-treated (i.e. dopamine depleted) and in control rats (Kamal, Arbilla & Langer, unpublished observations). Consequently, it seems unlikely that endogenously released dopamine could play a physiological role controlling the release of GABA in the substantia nigra.

The inhibition by apomorphine of the evoked release of [^{3}H]-GABA observed under our *in vitro* conditions has been reported *in vivo* using the push-pull cannula superfusion technique (Van der Heyden, Venema & Korf, 1980). These authors also found an inhibitory effect of low concentrations of dopamine on the release of [^{3}H]-GABA evoked by potassium stimulation. It is possible that the inhibitory action of dopamine on [^{3}H]-GABA release *in vivo* is mediated in part by the striatonigral pathway whereas under our *in vitro* conditions this means of regulation is not available.

Our results suggest that in the rat substantia nigra, the dendritic release of dopamine does not modulate the spontaneous or the stimulation-evoked release of GABA. Although the results obtained with apomorphine and haloperidol are consistent with the presence of presynaptic dopamine-like inhibitory receptors on gabaergic nerve terminals it appears that these receptors are not acted upon by endogenous dopamine to modify the release of GABA.

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