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THE EFFECTS OF DOPAMINE AND DOPAMINE AGONISTS ON THE RELEASE OF ^{3}H-GABA AND ^{3}H-5HT FROM RAT NIGRAL SLICES

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Abstract—Only high micromolar concentrations of dopamine and dopamine agonists altered spontaneous and KCl-evoked release of ^{3}H-GABA and ^{3}H-5HT from rat nigral slices *in vitro*. Apomorphine (100 μM) and dopamine (100 μM) enhanced the spontaneous release of ^{3}H-5HT but the effect of dopamine was not reversed by haloperidol (1 μM). Both apomorphine (100 μM) and dopamine (100 μM) enhanced the KCl-evoked release of ^{3}H-5HT but these effects were not reversed by haloperidol (1 μM). Apomorphine (10–250 μM) and dopamine (10–250 μM) inhibited ^{3}H-5HT uptake into nigral synaptosomal preparations in a concentration-dependent manner. Accordingly, a major portion of the apparent effect of these drugs on ^{3}H-5HT release may be due to inhibition of ^{3}H-5HT uptake. Dopamine (100 and 1000 μM), amphetamine (100 μM), apomorphine (100 μM) and 2-amino-6,7-dihydroxytetralin (ADTN; 100 μM) were without effect on the spontaneous release of ^{3}H-GABA from nigral slices. Apomorphine (100 μM) and ADTN (100 μM) reduced the KCl-evoked release of ^{3}H-GABA from substantia nigra, an effect antagonized by haloperidol (1 μM). However, amphetamine (100 μM) and dopamine (100–1000 μM) were without effect on KCl-evoked ^{3}H-GABA release. These results suggest that only high concentration of some dopamine agonists can modulate ^{3}H-5HT and ^{3}H-GABA release in substantia nigra. However, dopamine either had no effect, or its actions were not reversed by dopamine receptor blockade, so it appears unlikely that dendritic dopamine release will influence GABA and 5HT release in substantia nigra.

Dopamine is released both from the nerve terminals and dendrites of nigro-striatal dopaminergic neurones [1]. Dopamine cell bodies are located in the zona compacta of the substantia nigra, but the dendritic processes extend throughout zona compacta and pass into zona reticulata [2]. The function of dendritic dopamine release is unknown, but present evidence suggests an action on dopamine autoreceptors located on the cell bodies and dendrites of nigro-striatal neurones so as to inhibit nigral cell firing [3–5] and inhibit striatal dopamine release [6]. However, dendritic dopamine release could also modulate the release of other transmitters in substantia nigra by an action on presynaptic dopamine receptors located on the terminals of nigral afferent fibres. Two major afferent projections to the rat substantia nigra are the striato-nigral GABAergic pathway [7–9] and the raphe-nigral serotonergic pathway [10, 11]. Lesions of striatonigral fibres produce a loss of dopamine-sensitive adenylate cyclase [12, 13] and reduce specific ^{3}H-*N*,*n*-propylnorapomorphine binding [14] in substantia nigra, suggesting the presence of dopamine receptors on striato-nigral terminals. Previous *in vitro* studies have indicated that dopamine and amphetamine either stimulate the release of ^{3}H-GABA from nigral slices [15] or have no effect [16]. Dopamine and apomorphine have also been reported to inhibit the potassium chloride-evoked release of ^{3}H-GABA from nigral slices [16]. There is no direct evidence to suggest the presence of dopamine receptors on the terminals of raphe-nigral fibres. However, in perfusion experiments, dopamine agonists stimulate the release of ^{3}H-5HT from nigral slices [17]. Nigral slices are a suitable preparation from which to measure the release of ^{3}H-GABA and ^{3}H-5HT. Kainic acid induced lesions of striato-nigral fibres, followed by measurement of ^{3}H-GABA uptake in substantia nigra, have shown that at least 70% of ^{3}H-GABA accumulated by nigral slices enters striato-nigral terminals [16]. The uptake of ^{3}H-5HT into synaptosomal preparations of substantia nigra is substantially reduced following lesions of the raphe nuclei [18].

The present study was designed to characterize the effects of dopamine and dopamine agonists on the *in vitro* release of ^{3}H-GABA and ^{3}H-5HT from rat substantia nigra. We found that dopamine released from dendrites is unlikely to alter the release of GABA and 5HT from nigral afferent fibres by an action at dopamine receptors located on these terminals.

MATERIALS AND METHODS

Release of ^{3}H-5-hydroxytryptamine and ^{3}H-GABA from substantia nigra. Female Wistar rats (151–175 g; Charles River Ltd.) were killed by cervical dislocation and decapitation and the brains removed. The brain was sliced in a vertical plane just rostral

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to the substantia nigra and nigral tissue from both hemispheres was rapidly dissected from the ventral surface of the brain. The nigral tissue from individual animals was then chopped in two directions (0.2 mm × 0.2 mm) using a McIlwain Tissue Chopper (Mickle Laboratory Engineering Co. Ltd.). The resulting slices were dispersed in 1.0 ml of oxygenated Krebs buffer, pH 7.4, at 37° of the following composition: 124 mM NaCl, 1.3 mM $MgSO_4 . 7H_2O$, 3.5 mM KCl, 1.2 mM KH_2PO_4, 1 mM $CaCl_2.6H_2O$, 10 mM glucose, 25 mM $NaHCO_3$. For ^{3}H-5HT and ^{3}H-GABA release, 25 μM pargyline hydrochloride (Sigma Chemical Co.) or 10 μM aminooxyacetic acid (Sigma Chemical Co.) were included into the buffer to prevent enzymatic degradation of labelled substances. In some experiments nomifensine hydrogen maleate (1 μM; Hoechst) was included to inhibit dopamine uptake. In addition, 0.17 μM ascorbic acid (Sigma Chemical Co.) was added to inhibit the chemical oxidation of ^{3}H-5HT and dopamine. After incubation for 5 min, ^{3}H-5HT (21 Ci/mmole; Amersham International) or ^{3}H-GABA (74 Ci/mmole; Amersham International) were added to the incubates to give final concentrations of 6×10^{-7} M ^{3}H-5HT and 1×10^{-7} M ^{3}H-GABA respectively. After a further 15 min incubation the slices were dispersed randomly between 4 Perspex perfusion chambers and perfused with Krebs buffer at 37°, constantly gassed with 95% O_2/5% CO_2, generally at a rate of between 0.8 and 1.0 ml/min for 30 min. In a few experiments slices were perfused at a higher rate of between 1.6 and 2.0 ml per min. At the end of this time the perfusate was serially collected for periods of 2 min.

The effects of dopamine agonists on the spontaneous release of ^{3}H-5HT and ^{3}H-GABA. To assess the basal efflux of radioactivity, three fractions of perfusate were first collected. In the case of ^{3}H-5HT release dopamine (100 μM) or apomorphine (100 μM) were then included in the perfusing buffer for a period of 2 fractions (4 min; see Fig.1). For ^{3}H-GABA release dopamine (100 and 1000 μM), apomorphine (100 μM), ADTN (100 μM) or amphetamine (100 μM) were included in the perfusing buffer for 2 fractions (see Fig. 4). After this the tissue was perfused again by Krebs buffer alone for a further period of 4 fractions. Following collection, a 250 μl aliquot of each fraction was retained for liquid scintillation counting.

In some experiments ^{3}H-GABA release was estimated in small slices of zona reticulata micro-dissected from 0.4 mm slices of ventral mid-brain under a dissecting microscope.

The effects of dopamine agonists on the KCl-evoked ^{3}H-5HT and ^{3}H-GABA release. Following collection of one fraction of perfusate, dopamine (100 μM) or apomorphine (100 μM) in the case of ^{3}H-5HT release, or dopamine (100 and 1000 μM), apomorphine (100 μM), ADTN (100 μM) or amphetamine (100 μM) in the case of ^{3}H-GABA release were included into the perfusing Krebs buffer for a period of 4 fractions (8 min). Potassium chloride (KCl; 25 mM) was then included into the perfusing Krebs buffer, 3 fractions after commencement of collection and for a period of 2 fractions (4 min; see Fig. 2). The dopamine agonists were thus included into the perfusing Krebs buffer for 4 min prior to, and then during, the period of potassium chloride addition (Fig. 2). Following the potassium chloride addition, the tissue was perfused again by Krebs buffer alone for a further period of 4 fractions. Following collection of all the fractions, a 250 μl aliquot of each was retained for liquid scintillation counting.

In some experiments $CaCl_2.6H_2O$ was omitted from the perfusing medium and replaced osmotically by $MgCl_2.6H_2O$. In other experiments, haloperidol (1 μM; dissolved in a minimum quantity of glacial acetic acid before being made up to volume with Krebs buffer; Janssen Pharmaceutica, Belgium) was included into the Krebs buffer from 15 min prior to collection of perfusate until the end of the experiment.

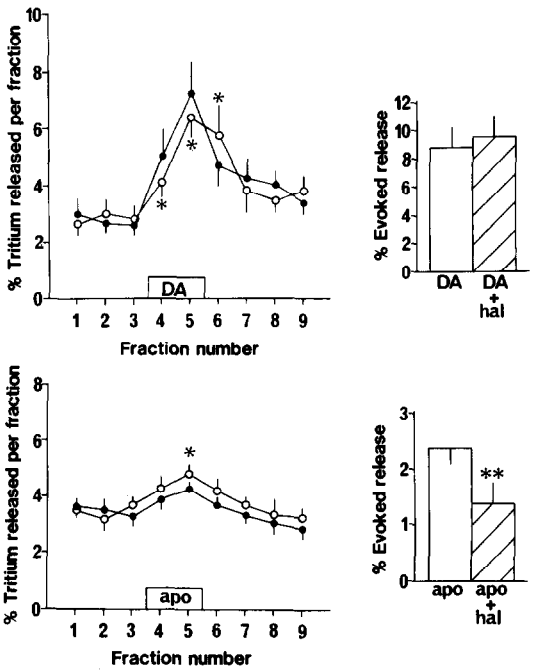

Fig. 1. The effect of dopamine (DA; 100 μM) and apomorphine (APO; 100 μM) in the presence or absence of haloperidol (hal; 1 μM) on the spontaneous release of ^{3}H-5HT from nigral slices. In each case the results are expressed both as efflux curves and as the release of ^{3}H-5HT evoked over basal levels in the 3 fractions (6 min) immediately following addition of dopamine or apomorphine. Dopamine (100 μM) or apomorphine (100 μM) were included into the perfusing buffer for a period of 4 min in the presence (—●—) or absence (—○—) of haloperidol (1 μM). Points on the efflux curves represent the mean ± 1 S.E.M. of the percent radioactivity released in each fraction. In experiments performed in the absence of haloperidol, those points on the efflux curves significantly higher than the respective basal value in fraction 3 are denoted by a single asterisk. * $P < 0.05$; Student's *t*-test. Histograms show the mean evoked release of ^{3}H-5HT ± 1 S.E.M. under each treatment, hatched bars denoting those experiments performed in the presence of haloperidol (1 μM). The evoked release of ^{3}H-5HT by apomorphine (100 μM) was lower in the presence of haloperidol (1 μM). **. $P < 0.05$; Student's *t*-test, N = 6–8 for each manipulation.

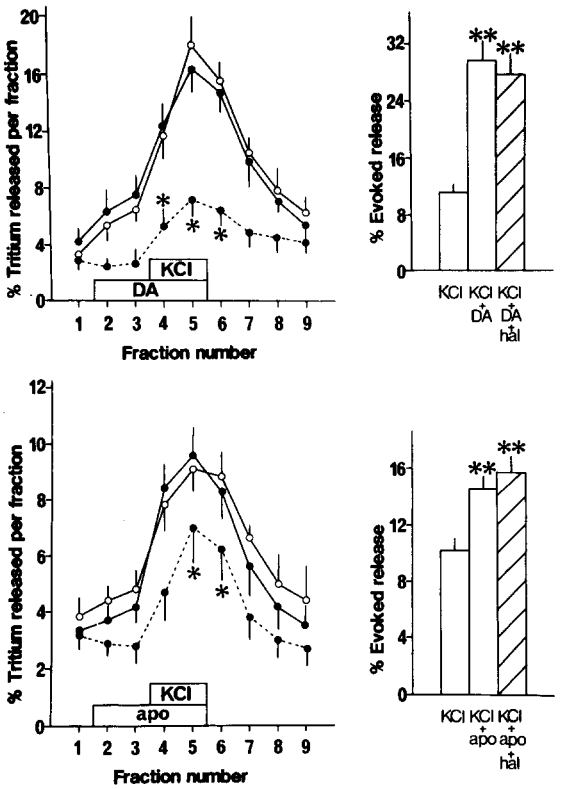

Fig. 2. The effect of dopamine (DA; 100 μM) and apomorphine (apo; 100 μM) in the presence or absence of haloperidol (hal; 1 μM) on the 25 mM KCl-evoked release of ^{3}H-5HT from nigral slices. In each case the results are expressed both as efflux curves and as the release of ^{3}H-5HT evoked in the 3 fractions (6 min) immediately following addition of KCl. 25 mM KCl was included into the perfusing buffer for a period of 4 min either alone (--●--), or with dopamine (100 μM) or apomorphine (100 μM) in the presence (—●—) or absence (—○—) of haloperidol (1 μM). Points on the efflux curves represent the mean ± 1 S.E.M. of the percent radioactivity released in each fraction. In experiments performed in the presence of 25 mM KCl alone, those points on the efflux curves significantly higher than the respective basal value in fraction 3 are denoted by a single asterisk, * $P < 0.05$; Student's *t*-test. Histograms show the mean evoked release of ^{3}H-5HT ± 1 S.E.M. under each treatment, hatched bars denoting those experiments performed in the presence of haloperidol (1 μM). Note the differing scales with dopamine and apomorphine. The evoked release of ^{3}H-5HT by 25 mM KCl in the presence of dopamine (100 μM) or apomorphine (100 μM) was significantly greater than 25 mM KCl alone, ** $P < 0.05$; Student's *t*-test. Haloperidol (1 μM) had no effect on the potentiation of the 25 mM KCl-evoked release of ^{3}H-5HT by dopamine (100 μM) or apomorphine (100 μM). N = 4–8 for each manipulation.

Radioactivity in each fraction (250 μl aliquots) and in the tissue at the end of the experiment was measured by addition to ES299 scintillant (4 ml; Packard) and subsequent liquid scintillation spectrometry using a Packard 2425 scintillation spectrometer at an efficiency of 20–30%.

Release data are presented either as efflux curves for the total collection period, or as the amount of evoked release occurring during a 6-min period immediately following K^+ stimulation.

Each point on the efflux curves represents the mean ± S.E.M. of the amount of radioactivity released per fraction expressed as a percentage of the total radioactivity in the tissue at the time of collection. The total tissue radioactivity at the beginning of the experiment was estimated by calculating the total fractional radioactivity released and adding this to the radioactivity at the end of the experiment. Total tissue radioactivity during each collection period was determined by serially subtracting fractional radioactivity from total radioactivity.

The evoked release was calculated from values for individual collection periods and was taken as the total amount of radioactivity released during a 6-min period from the beginning of K^+ stimulation or addition of dopamine agonist, after the total amount of radioactivity released during the 6 min period immediately prior to K^+ stimulation or addition of dopamine agonist had been subtracted; this was expressed as the percentage of the total recovered radioactivity.

Analysis of the radioactivity released from nigral slices. Samples of tissue perfusate were retained for analysis of the composition of the released radioactivity.

^{3}H-5HT was separated from ^{3}H-5HIAA and other breakdown products by a modification of the method of Boireau *et al.* [19]. Briefly, 1.0 ml samples of perfusate were passed through Sephadex G10 columns (2.0 × 0.5 cm; made up in 0.5 M formic acid). Radioactive products were eluted using 6 × 1.0 ml of distilled water. Subsequently, ^{3}H-5HT was eluted using 6 × 1 ml of 1 M formic acid. Using this method, 95% of a standard of ^{3}H-5HT (0.1 μl of label diluted in 1.0 ml Krebs buffer) eluted in the acid fractions. Between 60 and 80% of the radioactivity released spontaneously from nigral slices was ^{3}H-5HT. Release of radioactivity induced by apomorphine (100 μM), dopamine (100 μM) or KCl (25 mM) consisted of between 75 and 82% ^{3}H-5HT.

^{3}H-GABA was separated from ^{3}H-glutamate, ^{3}H-glutamine and other radioactive products by a modification of the method of Gauchy *et al.* [20]. To 2.0-ml samples of perfusate were added 8.0 ml of distilled water containing 0.1% Triton-X100 and 10 ml of 0.2 M sodium acetate buffer (pH 4.5). Samples were passed through Dowex AG-X4 columns (1 × 3 cm) made up in 0.2 M sodium acetate buffer (pH 4.5). Radioactive products of ^{3}H-GABA were eluted from the column using 2 × 5.0 ml quantities of 0.02 M sodium acetate buffer (pH 4.0) containing 0.1% Triton-X100 and 1 × 2.0 ml volume of distilled water. ^{3}H-GABA then was eluted using 3 × 1.0 ml quantities of 0.4 M Tris–acetate buffer (pH 8.0). Using this method at least 90% of a standard of ^{3}H-GABA (0.1 μl of label diluted in 2.0 ml of Krebs buffer) eluted in the latter fraction whereas 95% of a standard ^{14}C-glutamate (0.1 μl of label diluted in 2.0 ml of Krebs buffer; 280 mCi/mmole; Amersham International) and 97% of a standard ^{14}C-glutamine (0.1 μl of label diluted in 2.0 ml of Krebs buffer; 40 mCi/mmole; Amersham International) were eluted with the 2 × 5.0 ml additions of 0.02 M sodium acetate buffer containing 0.1% Triton-X100.

Using this technique, between 24 and 38% of spontaneously released radioactivity was shown to be ^{3}H-GABA whereas 75–85% of radioactivity released by KCl was ^{3}H-GABA. Due to the small ^{3}H-GABA content in spontaneously released fractions of perfusate, some experiments involving the effect of dopamine on spontaneous ^{3}H-GABA release were performed in which all fractions were analysed on Dowex AG-X4 columns. Total ^{3}H-GABA remaining in the tissue at the end of the experiment was estimated by homogenizing the tissue in 2 ml 0.2 N perchloric acid followed by centrifugation at 1500 rpm. A 100 μl aliquot of supernatant was then added to 1.9 ml Krebs buffer before being analysed for ^{3}H-GABA content as described above.

Uptake of ^{3}H-5HT into synaptosomal preparations of substantia nigra. The uptake of ^{3}H-5HT into synaptosomal preparations of substantia nigra was determined in the presence of apomorphine (10–250 μM) and dopamine (10–250 μM). A P2 synaptosomal fraction was prepared and suspended in 20 vol. (original tissue weight) of oxygenated Krebs buffer according to the method of Carter [21]. Aliquots (100 μl) were incubated in 1.4 ml of oxygenated Krebs buffer containing apomorphine hydrochloride or dopamine hydrochloride with 1×10^{-8} M ^{3}H-5HT for 5 min at 37°. Samples were then vacuum filtered through Whatman GF/C filters and washed with 2×1.0 ml of ice-cold Krebs buffer. Blank values for each concentration of dopamine or apomorphine were obtained by incubating aliquots of the synaptosomal preparation with ^{3}H-5HT on ice at 0°. The radioactivity retained on each filter paper was determined by addition to 4 ml of scintillation fluid (ES299; Packard) and liquid scintillation counting. The protein content of the synaptosomal preparation was measured by the technique of Lowry *et al.* [22]. The results are expressed as pmoles transmitter taken up/mg protein/hr.

Unilateral 6-hydroxydopamine lesions of the medial forebrain bundle. Animals were anaesthetized using chloral hydrate (300 mg/kg, i.p.) and placed in a Kopf stereotaxic frame. Unilateral lesions of the medial forebrain bundle (MFB) (A 4.3, L 1.9, V-3.2 [23]) were made by injection of 6-hydroxydopamine hydrobromide (6-OHDA; 8 μg in 3 μl 0.9% saline containing 2 μg ascorbic acid; Sigma Chemical Co.) using a 10-μl Hamilton syringe with Luer needle (o.d. 0.33 mm; i.d. 0.18 mm) at a rate of 1 μl/min. Two weeks later the effectiveness of the lesion was examined by assessment of the contraversive circling response to apomorphine hydrochloride (0.25 mg/kg, s.c., 15 min previously). Those animals displaying contraversive rotations were retained for release experiments 6–8 days later.

Statistical analysis. Differences between ^{3}H-GABA and ^{3}H-5HT release in the presence and absence of drug, and the effect of dopamine and apomorphine on ^{3}H-5HT uptake were analysed using a two-tailed Student's *t*-test.

RESULTS

The effect of dopamine and apomorphine on the spontaneous release of ^{3}H-5HT from nigral slices

The inclusion of dopamine (100 μm) and apomorphine (100 μM) into the perfusing medium stimulated the spontaneous overflow of ^{3}H-5HT from nigral slices. Dopamine (100 μM) had a quantitatively greater effect than apomorphine (100 μM) on ^{3}H-5HT release (Fig. 1). The effect of apomorphine (100 μM) on ^{3}H-5HT release was partially reduced by inclusion of haloperidol (1 μM) into the perfusing medium whereas that of dopamine (100 μM) was not (Fig. 1). Haloperidol itself (1 μM) did not affect the spontaneous rate of ^{3}H-5HT release (control basal release of radioactivity in 6 min prior to addition of agonist = 10.5 ± 0.3% of total tissue radioactivity at that time, N = 24; basal release of radioactivity in 6 min prior to addition of agonist in presence of haloperidol = 10.7 ± 0.4% of total tissue radioactivity at that time, N = 24).

The effect of dopamine and apomorphine on KCl (25 mM)-evoked release of ^{3}H-5HT from nigral slices

The inclusion of 25 mM KCl in the perfusion medium produced a Ca^{2+}-dependent release of ^{3}H-5HT from nigral slices (evoked release of ^{3}H-5HT in 6 min following addition of 25 mM KCl in the presence of Ca^{2+} = 10.1 ± 0.8% of total tissue radioactivity at that time, N = 4; evoked release in 6 min following addition of 25 mM KCl in absence of Ca^{2+} = 0.6 ± 0.4% of total tissue radioactivity at that time, N = 4; $P < 0.05$, Student's *t*-test). The inclusion of dopamine (100 μM) and apomorphine (100 μM) into the perfusion medium enhanced the KCl-evoked (25 mM) release of ^{3}H-5HT from nigral slices. The effects of dopamine and apomorphine were unaffected by inclusion of haloperidol (1 μM) into the perfusion medium (Fig. 2).

The effect of dopamine and apomorphine on the uptake of ^{3}H-5HT into synaptosomal preparations of substantia nigra

Dopamine (10–250 μM) and apomorphine (10–250 μM) inhibited the uptake of ^{3}H-5HT into crude P2 synaptosomal preparations of substantia nigra in a concentration-dependent manner (Fig. 3). Dopamine was again more potent than apomorphine in inhibiting ^{3}H-5HT uptake (Fig. 3), IC_{50} values for dopamine and apomorphine being approximately 10 μM and 50 μM respectively (Fig. 3).

The effect of dopamine and dopamine agonists on the spontaneous release of ^{3}H-GABA from nigral slices

The inclusion of dopamine (100–1000 μM), apomorphine (100 μM), ADTN (100 μM) and amphetamine (100 μM) into the perfusion medium did not affect the spontaneous release of ^{3}H-GABA from nigral slices (Table 1).

The effect of dopamine and apomorphine on the spontaneous release of ^{3}H-GABA from nigral slices following 6-hydroxydopamine lesions of the medial forebrain bundle

No change in the spontaneous release of radioactivity from slices pre-incubated with ^{3}H-GABA could be detected 21 days after 6-OHDA lesions of the medial forebrain bundle (spontaneous release in 6 min prior to addition of drug from non-lesioned nigral tissue = 2.8 ± 0.4% of total tissue radioactivity at that time, N = 8; spontaneous release in 6 min

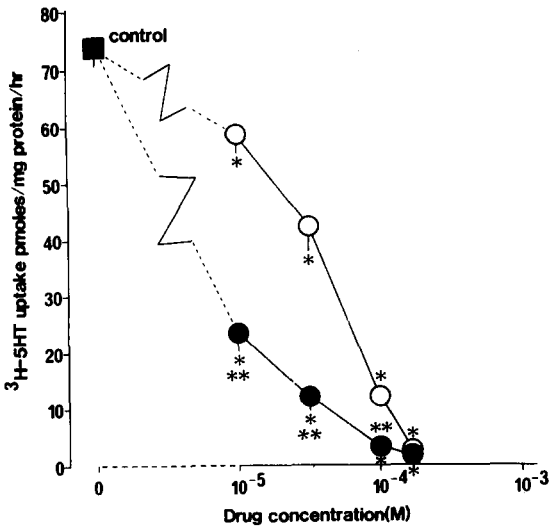

Fig. 3. The effect of dopamine (—●—) and apomorphine (—○—; both 10–250 μM) on the uptake of ^{3}H-5HT into crude P2 synaptosomal preparations of substantia nigra. Following incubation of synaptosomes in Krebs bicarbonate buffer in the presence of either dopamine or apomorphine, ^{3}H-5HT was included in the incubation medium for 5 min, after which the reaction was stopped by vacuum filtration. The experiment was performed on a single pool of nigral tissue from 12 animals. N = 6 for each point, values being ± 1 S.E.M. * $P < 0.05$ compared to control value; ** $P < 0.05$ for dopamine compared to apomorphine at the same dose, Student's *t*-test.

prior to addition of drug from 6-OHDA lesioned tissue = 3.2 ± 0.3% of total tissue radioactivity at that time, N = 8). In nigral slices from animals which had previously received a unilateral 6-OHDA lesion of the medial forebrain bundle, spontaneous ^{3}H-GABA release was unaffected by the incorporation of dopamine (100 μM) or apomorphine (100 μM) into the perfusion medium (Table 1).

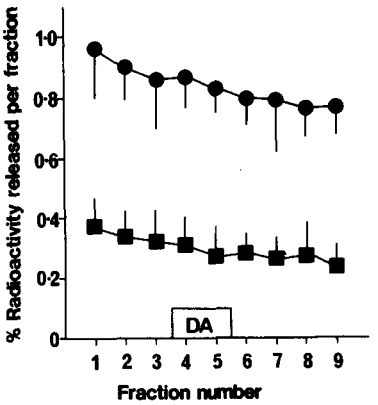

Fig. 4. The effect of dopamine (100 μM) on the spontaneous outflow of total radioactivity (—●—), or ^{3}H-GABA following separation from ^{3}H-metabolites (—■—) in nigral slices. ^{3}H-GABA was separated from other radioactive products on Dowex AG-X4 columns as described in Methods. The % ^{3}H-released per fraction represents the total radioactivity or ^{3}H-GABA released in each 2 min fraction expressed as a % of the total radioactivity or total ^{3}H-GABA remaining in the tissue at that time respectively. N = 4 for each curve, values being ± 1 S.E.M. The spontaneous release of ^{3}H-GABA was significantly lower than that of total radioactivity (^{3}H-GABA = 1.2 ± 0.2% in 6 min before addition of dopamine; total radioactivity = 2.9 ± 0.2% in 6 min before dopamine addition. $P < 0.05$, Student's *t*-test).

The effect of dopamine on the spontaneous release of ^{3}H-GABA from nigral slices: analysis of ^{3}H-GABA following separation from other radioactive compounds.

In four experiments the ^{3}H-GABA content of each fraction of perfusate was separated from other radioactive compounds using Dowex AG-X4 resin columns as described above. Under these conditions, dopamine (100 μM) also failed to influence the spontaneous release of ^{3}H-GABA from nigral slices (Fig. 4).

Table 1. The effect of dopamine and dopamine agonists on the spontaneous release of tritium from nigral slices preincubated with ^{3}H-GABA

Drug	Dose (μM)	Other treatment	Total % release of tritium ± 1 S.E.M. in 6 min following addition of drug
None	—		3.1 ± 0.1
Dopamine	100		3.1 ± 0.1
Dopamine	1000		3.1 ± 0.1
Dopamine	100	6-OHDA	3.2 ± 0.2
Dopamine	100	micro-dissected ZR	3.0 ± 0.1
Apomorphine	100		3.0 ± 0.2
Apomorphine	100	6-OHDA	2.8 ± 0.2
ADTN	100		3.0 ± 0.1
Amphetamine	100		2.9 ± 0.1

Each drug was included in the perfusing medium for a period of 4 min. In some experiments tissue was taken from the nigra ipsilateral to a 6-OHDA lesion of the medial forebrain bundle 3 weeks previously, whilst in others the zona reticulata (ZR) was used following microdissection from 0.4 mm slices of midbrain. N = 4–6 for each experiment.

The effect of dopamine and dopamine agonists on the KCl (25 mM)-evoked release of ^{3}H-GABA from nigral slices

The inclusion of 25 mM KCl in the perfusion medium produced a Ca^{2+}-dependent release of ^{3}H-GABA from nigral slices (evoked release of ^{3}H-GABA in 6 min following addition of 25 mM KCl in the presence of Ca^{2+} = 4.3 ± 0.4% of total tissue radioactivity at that time, N = 4; evoked release in 6 min following addition of 25 mM KCl in absence of Ca^{2+} = 0.2 ± 0.2% of total radioactivity at that time, N = 4; $P < 0.05$, Student's *t*-test). Inclusion of dopamine (100–1000 μM) or amphetamine (100 μM) into the perfusion medium failed to modify the 25 mM KCl-evoked release of ^{3}H-GABA from nigral slices (Table 2). At an increased perfusion rate (1.6–2.0 ml/min) and in the presence of nomifensine and pargyline, dopamine (1000 μM) caused a slight, but non-significant, inhibition of 25 mM KCl-evoked release of ^{3}H-GABA (Table 2). Inclusion of apomorphine (100 μM) or ADTN (100 μM) into the perfusion medium decreased the 25 mM KCl-evoked release of ^{3}H-GABA by 40% and 30% respectively (Table 2). Apomorphine and ADTN no longer reduced ^{3}H-GABA release when haloperidol (1 μM) was introduced into the perfusion medium (Table 2). Haloperidol itself (1 μM) did not alter the spontaneous (control = 3.1 ± 0.1% of total tissue radioactivity at that time in 6 min before addition of agonist, N = 15; haloperidol treated = 3.0 ± 0.1% of total tissue radioactivity at that time in 6 min before addition of agonist, N = 12) or 25 mM KCl-evoked release of ^{3}H-GABA (Table 2).

Dopamine (100 μM) remained ineffective in altering the 25 mM KCl-evoked release of ^{3}H-GABA in tissue taken from 6-OHDA lesioned nigral slices (Table 2).

DISCUSSION

The function of dendritic dopamine release in substantia nigra is unknown. One possibility we investigated in this study is that dopamine modulates 5HT or GABA release from afferent fibres terminating within nigra and on which dopamine receptors may be located.

Both apomorphine and dopamine enhanced the spontaneous release of ^{3}H-5HT from nigral slices. Previously Reubi *et al.* [17] also found that dopamine and apomorphine increased the spontaneous release of newly synthesised ^{3}H-5HT from nigral slices. However, only the effect of apomorphine was par-

Table 2. The effect of dopamine and dopamine agonists on the KCl (25 mM)-evoked release of ^{3}H-GABA from nigral slices

Drug	Dose (μM)	Other treatment	Total % release of ^{3}H ± 1 S.E.M. in 6 min following addition of drug
None	—	—	4.7 ± 0.4
Dopamine	100	—	4.9 ± 0.5
Dopamine	1000	—	4.5 ± 0.8
None	—	nom + parg	4.3 ± 0.4
Dopamine	1000	nom + parg	3.7 ± 0.3
None	—		4.6 ± 0.5
Dopamine	100	6-OHDA	4.4 ± 0.5
None	—	—	5.0 ± 0.7
Amphetamine	100	—	4.6 ± 1.0
None	—	—	4.6 ± 0.3
None	—	Haloperidol	4.2 ± 0.3
None		—	4.8 ± 0.5
Apomorphine	100	—	2.9 ± 0.5*
Apomorphine	100	Haloperidol	4.1 ± 0.5
None		—	4.4 ± 0.2
ADTN	100	—	3.1 ± 0.5*
ADTN	100	Haloperidol	4.1 ± 0.4

Dopamine (100 and 1000 μM) amphetamine (100 μM), apomorphine (100 μM) or ADTN (100 μM) were included in the perfusing medium for 4 min before, and then during, the KCl pulse (4 min). KCl (25 mM) controls were performed in parallel with each drug experiment, the effects of each drug application being compared to their respective controls. Values are ± S.E.M. for 4–10 experiments. In some experiments haloperidol (1 μM), was included into the buffer from 15 min prior to collection of perfusate until the end of the experiment. In other experiments, animals had received a 6-hydroxydopamine (6-OHDA) lesion of one medial forebrain bundle 3 weeks previously, whilst in others the slices were perfused throughout the experiment at a rate of between 1.6 and 2.0 ml/min with buffer containing nomifensine (nom; 1 μM) and pargyline (parg; 25 μM).

* $P < 0.05$ for values of evoked release in presence of drug significantly lower than respective KCl controls, by Student's *t*-test.

tially antagonised by dopamine receptor blockade, suggesting that the effect of dopamine was not mediated by dopamine receptors. Indeed, both dopamine and apomorphine inhibited ^{3}H-5HT uptake (or displaced accumulated ^{3}H-5HT) into nigral synaptosomes in concentrations used in the release studies, suggesting that much of the observed effects of these substances on release may be due to inhibition of the re-uptake of spontaneously released 5HT. In agreement with the idea of the involvement of mechanisms other than dopamine receptors in mediating the effects of dopamine agonists on ^{3}H-5HT release from nigral slices, dopamine and apomorphine markedly potentiated the potassium-evoked release of ^{3}H-5HT, but in neither case were these effects diminished by haloperidol.

The finding that dopamine and apomorphine enhanced the spontaneous release of ^{3}H-5HT from nigral slices are at variance with *in vivo* studies in the cat where intranigral application of dopamine (10^{-7} M) inhibited nigral ^{3}H-5HT release [24] but the mechanism of this effect was not investigated.

The present results demonstrate that dopamine and apomorphine enhance ^{3}H-5HT release from nigral slices. However the effects of dopamine at least are apparently not mediated by an action at presynaptic dopamine receptors located on 5HT terminals, if these receptors are antagonised by haloperidol. Certainly the concentration of haloperidol employed (1 μM) was sufficient to antagonize the effects of apomorphine and ADTN on the potassium-evoked release of ^{3}H-GABA, whilst it has previously been shown that in slices of rat hypothalamus, low doses of haloperidol antagonise the stimulation of ^{3}H-5HT release by high doses of dopamine and apomorphine [25].

Striato-nigral GABA containing neurones represent the largest afferent input to substantia nigra [7]. Since kainic acid-induced degeneration of striato-nigral fibres produces a loss of the dopamine-sensitive adenylate cyclase [12, 13], and a loss of specific ^{3}H-*N*,*n*-propylnorapomorphine binding [14] in substantia nigra, dopamine receptors would appear to exist on these terminals so forming a potential site at which dopamine released from dendrites might act. However, dopamine, amphetamine, apomorphine and ADTN were all ineffective in altering the spontaneous release of ^{3}H-GABA from nigral slices. This was true even for dopamine using microdissected slices of zona reticulata or following prior 6-hydroxydopamine lesions of nigro-striatal dopaminergic neurones, a manipulation that we might expect to increase the sensitivity of those nigral dopamine receptors not located on dopamine neurones. Previous reports have been conflicting. Reubi and colleagues [15] found that dopamine (5–500 μM) stimulated the spontaneous release of ^{3}H-GABA from nigral slices, whereas Arbilla *et al.* [16] found that dopamine (30–1000 μM) did not. The reason for this discrepancy is unclear. Recently it has been reported that the ability of GABA to release dopamine from striatal homogenates depends upon the buffer perfusion speed [26]. However, this is unlikely to account for the difference in dopamine's ability to modify nigral GABA release since although the perfusion speed employed in the present study (0.8–1.0 ml/min) was higher than in the previous studies (0.5 ml/min) [15, 16], the present findings were similar to those of the latter authors.

Incorporation of apomorphine and ADTN into the perfusing medium inhibited the potassium-evoked release of ^{3}H-GABA from nigral slices, effects which were inhibited by haloperidol. This is in further agreement with Arbilla *et al.* [16] who showed that apomorphine inhibited the potassium-evoked release of ^{3}H-GABA from nigral slices. This suggests the presence of inhibitory dopamine receptors located on the terminals of strio-nigral GABA containing neurones. However, the incorporation of dopamine or amphetamine (which releases dendritic dopamine; [27]), did not reduce potassium-evoked ^{3}H-GABA release. Why synthetic dopamine agonists and not dopamine should inhibit potassium-evoked ^{3}H-GABA release is not clear. In *in vivo* studies in the rat, the intranigral application of dopamine and apomorphine were found to have opposite effects on local GABA release, depending on the dose applied [28]. The results of our study suggest that at least under *in vitro* conditions, those dopamine receptors located on GABA containing neuronal terminals may not be an important site for the action of dopamine released from dendritic processes.

In summary, as has been found by others [15–17], only high concentrations of dopamine or dopamine agonists can alter release of ^{3}H-5HT and ^{3}H-GABA from nigral slices. In striatal slices the inhibition by apomorphine of the depolarization-evoked release of ^{3}H-acetylcholine has been observed at nanomolar concentrations of agonist [29]. These results do not support the contention that dendritically released dopamine is likely to alter 5HT or GABA release from nigral afferent fibres *in vivo*.

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