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Atypical effect of dopamine in modulating the functional inhibition of NMDA receptors of cultured retina cells

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Abstract

Cultured retina cells released accumulated [^{3}H]GABA (γ-aminobutyric acid) when stimulated by L-glutamate, *N*-methyl-D-aspartate (NMDA) and kainate. In the absence of Mg^{2+}, dopamine at 200 μM (IC_{50} 60 μM), inhibited in more than 50% the release of [^{3}H]GABA induced by L-glutamate and NMDA, but not by kainate. This effect was not blocked by the D_1-like dopamine receptor antagonist, *R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH 23390), neither by haloperidol nor spiroperidol (dopamine D_2-like receptor antagonists). The dopamine D_1-like receptor agonist *R*(+)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,diol hydrochloride (SKF 38393) at 50 μM, but not its enantiomer, also inhibited the release of [^{3}H]GABA induced by NMDA, but not by kainate; an effect that was not prevented by the antagonists mentioned above. (±)-6-Chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide (SKF 812497) had no effect. Neither 8BrcAMP (5 mM) nor forskolin (10 μM) inhibited the release of [^{3}H]GABA. Our results suggest that dopamine and (+)-SKF 38393 inhibit the glutamate and NMDA-evoked [^{3}H]GABA release through mechanisms that seem not to involve known dopaminergic receptor systems. © 1998 Elsevier Science B.V.

Keywords: Cell culture; Retina, chick; GABA (γ-aminobutyric acid) release; Dopamine; Glutamate; NMDA receptor complex

1. Introduction

Glutamate, dopamine and γ-aminobutyric acid (GABA), are widely recognized as neurotransmitters in the central nervous system, including the retina of many vertebrate species (Lam et al., 1979; Brandon, 1985; Yazulla, 1986; Massey and Redburn, 1987).

L-Glutamate is an excitatory neurotransmitter that mediates its effects through ionotropic and metabotropic receptors. The activation of the ionotropic receptors induces the release of several neuroactive substances including GABA (Miller and Slaughter, 1986; Barnstable, 1993; Hamassaki-Britto et al., 1993). A considerable number of publications have demonstrated that a substantial portion of the release of GABA induced by excitatory amino acids occurs via the activation of NMDA and non-NMDA ionotropic receptors by mechanisms independent of external calcium (Szerb, 1979; Harris and Miller, 1989; Pin and Bockaert, 1989; Dunlop et al., 1991; Duarte et al., 1993) and is present in a variety of central nervous system regions, including the retina (Yazulla and Kleinschmidt, 1983; Do Nascimento and De Mello, 1985; Schwartz, 1987; Ferreira et al., 1994).

Dopamine is the predominant biogenic amine in the retina and both, GABAergic and dopaminergic cells seem to interact in the processing of visual information at the retina level (Kramer, 1971; Araki et al., 1983; Kato et al., 1984; Dowling, 1987; Gardino et al., 1993). Until recently only two subtypes of dopamine receptors (D_1 and D_2) had been identified in the central nervous system. Both types of receptors are present in the retina (Iuvone et al., 1978; Ventura et al., 1984; Schorderet and Novak, 1990; Ventura and De Mello, 1990). With the advent of the recombinant DNA methodology, seven different dopamine receptors

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have been cloned and functionally, pharmacologically and molecularly classified as belonging to the D_1-like subclass (D_1, D_{1D}, D_{1C} and D_5) or to the subclass D_2 (D_2, D_3 and D_4) (Sibley and Monsma Jr., 1992; Demchyshyn et al., 1995). Several studies have revealed that the dopaminergic, glutamatergic and GABAergic systems interact in the processing of neural information in the CNS including the retinal tissue (Zucker and Yazulla, 1982; Yazulla, 1986). In fish retina, for instance, dopamine inhibits the release of GABA stimulated by L-glutamate, an effect that is mimicked by dibutyril cyclic AMP and forskolin and is apparently mediated by activation of dopamine D_1-like receptors present in the tissue (Yazulla and Kleinschmidt, 1982; Kato et al., 1985; O'Brien and Dowling, 1985).

In the present communication, using the release of GABA as a signal that measures the functional activation of excitatory amino acid receptors present in cultured retina cells, we show that dopamine and (+)-SKF 38393 (but not its S(−) enantiomer) inhibit glutamate evoked [^{3}H]GABA release through mechanisms that seem not to involve known dopaminergic receptor systems. In addition, we show that dopamine inhibits [^{3}H]GABA release when NMDA receptors are activated, while kainate induced [^{3}H]GABA release is not affected by dopamine.

2. Materials and methods

2.1. Materials

Hepes (*N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid]), trypsin, NMDA (*N*-methyl-D-aspartate), kainate, L-glutamate, forskolin (7β-acetoxy-1α,6β,9α-trihydroxy-8,13-epoxy-labd-14-en-11-one) and IBMX (3-isobutyl-1-methylxanthine) were obtained from Sigma Chemical Co., St. Louis, MO. Dopamine, CNQX (6-cyano-7-nitroquinoxaline-2,3-dione), NO-711 (1-(2-(((diphenilmethylene)imino)oxy) ethyl)-1,2,5,6,-tetrahydro-3-pyridine-carboxylic acid hydrochloride, haloperidol, MK 801 hydrogen maleate (5*R*,10*S*-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate), *R*(+)-SKF 38393, *S*(−)-SKF-38393 hydrochloride (1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride), (±)-SKF 81297 hydrobromide ((±)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide), *R*(+)-SCH 23390 hydrochloride (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride) and (−)-quinpirole (*trans*-(−)-4a*R*-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1H-pyrazolo[3,4-g]quinoline hydrochloride), were obtained from Research Biochemicals, Natick, MA. Spiroperidol was from Jansen Pharmaceuticals. [^{3}H]GABA (100 Ci/mmol) was from New England Nuclear. All other reagents were of analytical grade. Fertilized white Leghorn eggs were obtained from a local hatchery.

2.2. Retina cultures

Chick embryos were staged according to Hamburger and Hamilton (1951) and retina cultures prepared as described previously (Do Nascimento and De Mello, 1985). Briefly, 8-day-old chick embryo retinas were dissected, cleared from the pigmented epithelium and placed on a calcium and magnesium-free salt balanced solution. Trypsin, 0.05% (w/v) was added and incubated at 37°C for 8–10 min. The retinas were mechanically dissociated with a 5 ml pipette and seeded in 35 mm plastic dishes at a cell density equivalent to 30×10^6 cells per plate.

2.3. Uptake and release of [^{3}H]GABA

Cultures were incubated for 2 h at 37°C with 1 ml of modified Hanks balanced salt solution containing: 128 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 20 mM HEPES, 12 mM glucose and 0.5 μCi of [^{3}H]GABA (100 Ci/mmol), pH 7.4. After the incubation period, the cells were washed and bathed successively with 1 ml of the same solution in the absence or presence of test substances, for periods of 5 min. The medium was pipetted out after each incubation period, followed by immediate addition of another sample of the bathing solution and so forth. The radioactivity present in each fraction was determined by liquid scintillation. At the end of the experiment, the cells were lysed with 1 ml of water followed by 3 freeze–thaw cycles for at least 1 h and the remaining intracellular radioactivity determined. The radioactivity present in each fraction was expressed as the fractional release, which corresponded to the percentage of radioactivity released at each time point, as compared to the total radioactivity present in the cells at the beginning of each procedure. Greater than 95% of the radioactivity released was recovered as GABA after dansylation and separation by thin layer chromatography. Since most of the experiments were conducted using bathing solutions without Mg^{2+}, with added EDTA (2 mM), the integrity of the cells was accessed by visual inspection of the cultures, by trypan blue exclusion or by detecting the presence of lactate dehydrogenase (LDH) in the bathing solution after 25 min incubation using the Cyto Tox 96 kit from Promega. Greater than 98% of LDH activity remained inside the cells after the incubation procedure. No differences were observed when EDTA exposed cultures were compared to cultures incubated in Hanks solution containing 1 mM Mg^{2+} without EDTA. Also, 80 to 90% of the radioactivity corresponding to the [^{3}H]GABA incorporated remained inside the cells after the incubating periods in the absence of Mg^{2+}. Under this condition the stimulated efflux of [^{3}H]GABA always returned to the basal efflux level 10 to 15 min after ceasing the stimulation.

2.4. Cyclic AMP assay

Cyclic AMP (adenosine 3′:5′-cyclic monophospate) was assayed according to Gilman (1970). Briefly, the samples

containing cAMP were incubated in the presence of the regulatory subunit of protein kinase-A and a fixed, trace amounts of [^{3}H]cAMP in 50 mM acetate buffer, pH 4.0, at 4°C for 90 min. The reaction was stopped by the addition of 200 mM phosphate buffer at pH 6.0. The samples were filtered through Millipore filters HAWP-0045. The radioactivity bound to the protein kinase retained in the filters was measured in a liquid scintillation counter. The radioactivity retained in the filters was inverse function to the amount of cAMP present in the samples.

3. Results

3.1. Effect of dopamine on the glutamate-evoked release of GABA

[^{3}H]GABA incorporated by cultured retina cells was released into the incubating medium when cultures were stimulated with 100 μM glutamate. As reported by others (Hofmann and Mockel, 1991; Duarte et al., 1993) in the presence of Mg^{2+}, glutamate evoked the release of [^{3}H]GABA, in part (30%) due to the activation of NMDA receptors and in part (greater than 50%) due to the activation of non-NMDA receptors (De Mello et al., 1993; Kubrusly et al., 1997). The amplitude of the release reached levels 4 to 5 fold higher than the basal, non-stimulated release. In the presence of Mg^{2+} dopamine did not interfere with the glutamate evoked release of GABA (Fig. 1).

One of the characteristic and diagnostic properties of the NMDA receptor is the voltage-dependent channel block by magnesium (Barnard, 1997). Therefore, when Mg^{2+} was omitted and 2 mM EDTA was added to the bathing medium containing 2 mM Ca^{2+}, the glutamate evoked [^{3}H]GABA release was increased by more than two fold. Under this condition, dopamine inhibited by more than 50% the release of [^{3}H]GABA promoted by glutamate, suggesting that dopamine was affecting the release of GABA induced by NMDA receptor activation only.

3.2. Dopamine inhibited GABA release induced by NMDA

In cultures treated with 100 μM NMDA in Hanks solution containing 2 mM calcium and 1 mM glycine, without added Mg^{2+} (nominal magnesium), a three fold increase in [^{3}H]GABA release was observed. Under this condition, dopamine added at 200 μM concentration reduced the release by 50% (basal efflux, 1.16 ± 0.12%; with 100 μM NMDA, 3.5 ± 0.1%; with 100 μM NMDA plus 200 μM dopamine, 2.5 ± 0.01% measured in triplicate cultures respectively). In order to maximize the cultures response to NMDA, the cells were bathed with a Mg^{2+} free Hanks medium containing 2 mM EDTA and 1 mM of glycine (calcium always at 2 mM). Under this condition, the stimulation of the cultures with 100 μM NMDA increased the efflux of [^{3}H]GABA by 8 to 12 fold as compared to control, non-stimulated levels. MK 801, (a non-competitive NMDA receptor antagonist) at 10 μM concentration completely blocked the release. Dopamine added at 200 μM concentration in the medium inhibited the release of [^{3}H]GABA evoked by NMDA by 55% (Fig. 2). At 50 μM dopamine inhibited [^{3}H]GABA release by 30% and at 100 μM the inhibition was 50%. At 10 μM, dopamine had no significant effect. The estimated IC_{50} for dopamine was approximately 60 μM, at least one order of magnitude higher than the EC_{50} for dopamine to activate receptor mediated functions associated with adenylyl cyclase (Kebabian and Calne, 1979; Demchyshyn et al., 1995). Moreover, norepinephrine at 200 μM reduced the release of GABA by only 4% while epinephrine reduced the release by 30% at 200 μM concentration. Serotonin had no effect (not shown).

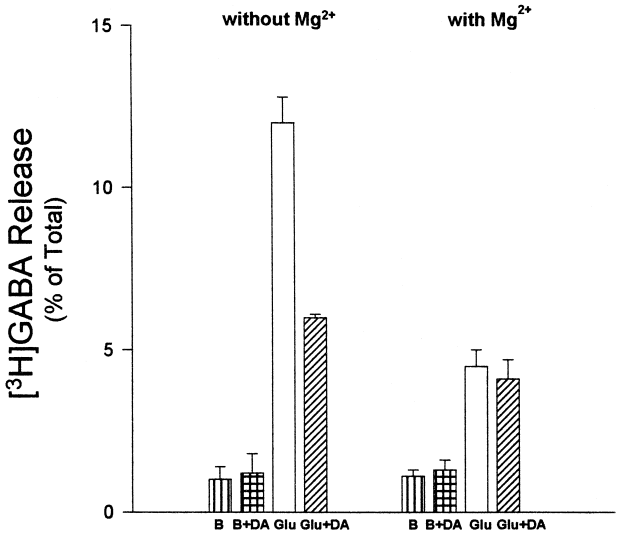

Fig. 1. Effect of dopamine on glutamate induced GABA release in the absence and in the presence of Mg^{2+}. The cultures were incubated with 0.5 μCi [^{3}H]GABA for 2.0 h and washed extensively to remove the non-incorporated radioactivity. The basal (B) efflux of GABA was measured with 5 min pulses of the bathing solution. Then a 5 min pulse with Hanks solution containing 100 μM glutamate (Glu) or glutamate plus 200 μM dopamine (glu + DA) were applied, in the absence (with 2 mM EDTA) or in the presence of 1 mM Mg^{2+}. (B + DA) is the basal efflux in the presence of 200 μM dopamine. In both cases, calcium was always added at 2 mM concentration. Note that in the presence of Mg^{2+} glutamate elicited the release of GABA that was only 40% of that released in the absence of Mg^{2+}. Dopamine inhibited in approximately 50% the GABA induced release only in the absence of Mg^{2+}. The data shown represents the mean $\pm$ S.E.M. of 3 independent cultures.

3.3. Characterization of the effect of dopamine on the NMDA mediated release of GABA

In order to characterize whether the dopamine inhibitory effect on [^{3}H]GABA release was via dopamine D_1 or D_2-like receptors, we tried to prevent the dopamine

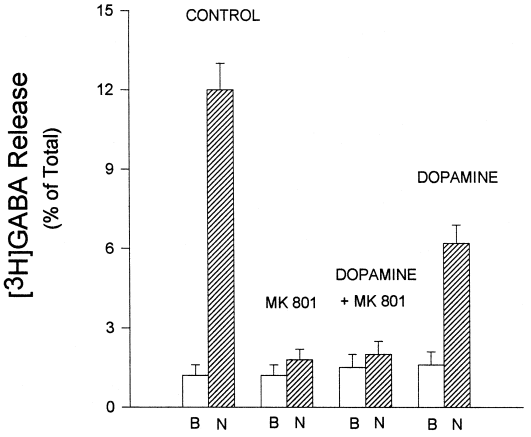

Fig. 2. Effect of MK 801 and dopamine on the NMDA mediated GABA release. B: Basal efflux of GABA measured during 5 min pulses in the absence (control) or in the presence of the pharmacological agents indicated. N: 5 min pulses with 100 μM NMDA in the absence (control) or in the presence of the pharmacological agents indicated: 10 μM MK 801, 200 μM dopamine or both. The data represents the mean $\pm$ S.E.M., or the deviation of the individual values from the mean of 2 measurements (control) or 3 independent cultures. MK 801 fully inhibited the release of GABA induced by NMDA and dopamine reduced the release by 55%. The remaining GABA release observed in cultures treated with dopamine was also inhibited by MK 801. The Hanks bathing solution used contained 2 mM Ca^{2+}, 1 mM glycine, 2 mM EDTA and no Mg^{2+}.

effect with known receptor antagonists. As shown in Table 1, SCH 23390, in concentrations as high as 10 μM, was not capable of preventing the inhibitory effect of dopamine on the release of [^{3}H]GABA induced by NMDA. Using the same culture system, SCH 23390 at 1 μM fully inhibited the cAMP accumulation promoted by 200 μM dopamine. Thus, the cAMP levels after 10 min incubation in medium containing 0.5 mM IBMX and pargyline were: basal, 147 ± 4; treated with dopamine, 614 ± 33; treated with dopamine plus SCH 23390, 161 ± 8 pmol of cAMP/mg of protein, respectively (De Mello, 1978). As for SCH 23390, haloperidol and spiroperidol, two known antagonists of the D_2-like dopaminergic receptor, were also

Table 2
Effect of dopamine D_1 and D_2-like receptor agonists on the release of GABA evoked by NMDA

Treatment	[^{3}H]GABA released (% of total)	
	basal	NMDA (100 μM)
None	1.4 ± 0.2	10.0 ± 1.0 (2)
SKF 38393 (50 μM)	1.2 ± 0.1	3.8 ± 0.4 (3)
SKF 81297 (50 μM)	1.5 ± 0.1	10.7 ± 1.0 (2)
Quinpirole (500 μM)	1.1 ± 0.1	11.1 ± 0.9 (3)

Although the dopamine D_1 receptor agonist SKF 38393 clearly inhibited the release, SKF 81297 (also D_1 agonist) had no effect. Quinpirol was also without effect. The incubation procedure was the same as described for Table 1. The values shown are the mean $\pm$ S.E.M. or the deviation of the individual values from the mean of the number of experiments indicated in parentheses. The bathing medium contained 2 mM EDTA, 1 mM glycine, 2 mM Ca^{2+} and no Mg^{2+}.

ineffective in blocking the dopamine inhibition of the NMDA mediated [^{3}H]GABA release (Table 1). The addition of SCH 23390 plus spiroperidol at 10 μM also did not prevent the inhibitory effect of dopamine. Clozapine (20 μM) was without effect (not shown).

The lack of effect of dopamine D_2-like receptor antagonists on the dopamine mediated inhibition of [^{3}H]GABA release was in agreement with the fact that the selective dopamine D_2-like receptor agonist, quinpirole, at a concentration of 500 μM produced no effect on the release of [^{3}H]GABA induced by NMDA (Table 2), therefore, making it unlikely that D_2-like receptor subclass mediated the effect of dopamine on the inhibition of [^{3}H]GABA release induced by NMDA receptor activation. However, even though SCH 23390 did not interfere with the dopamine effect, (+)-SKF 38393, a selective dopamine D_1-like receptor agonist, at 50 μM concentration, mimicked the effect of dopamine on the inhibition of the [^{3}H]GABA release evoked by NMDA (Table 2). As for dopamine, SCH 23390 did not interfere with the (+)-SKF 38393 inhibition observed (Fig. 3). On the other hand, the in-

Table 1
Lack of effect of dopamine D_1 and D_2-like receptor antagonists on the dopamine inhibition of NMDA evoked GABA release

Treatment	[^{3}H]GABA released (% of total)	
	basal	NMDA (100 μM)
None	1.9 ± 0.6	12.6 ± 1.4 (12)
Dopamine (200 μM)	1.3 ± 0.2	5.5 ± 0.6 (6)
Dopamine (200 μM) + SCH 23390 (10 μM)	1.4 ± 0.7	4.3 ± 0.7 (3)
Dopamine (200 μM) + haloperidol (100 μM)	1.3 ± 0.6	5.8 ± 0.5 (3)
Dopamine (200 μM) + spiroperidol (10 μM)	1.5 ± 0.5	3.5 ± 0.3 (3)
Dopamine (200 μM) + SCH 23390 (10 μM) + spiroperidol (10 μM)	1.3 ± 0.5	3.0 ± 0.2 (3)

Neither SCH 23390 nor haloperidol and spiroperidol were able to prevent the inhibitory effect of dopamine (DA). The basal efflux of GABA was always measured in the presence of dopamine and/or the antagonists used (5 min incubation). NMDA stimulation was also conducted for 5 min in the presence of each test compound. The values shown are the mean $\pm$ S.E.M. of the number of experiments indicated in parentheses. The bathing medium contained 2 mM EDTA, 1 mM glycine, 2 mM Ca^{2+} and no Mg^{2+}.

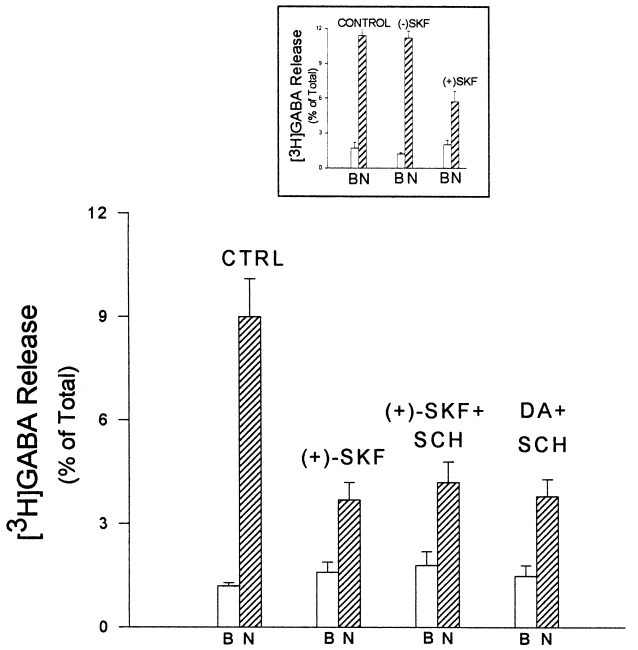

Fig. 3. Lack of effect of SCH 23390 on dopamine or SKF 38393 mediated inhibition of GABA release induced by NMDA. The incubation procedure was the same as described in the legend of Fig. 1. The basal efflux of [^{3}H]GABA during 5 min pulses was measured in the presence of the pharmacological agents indicated. In the control group no drug was added. Then a 5 min pulse with 100 μM NMDA was applied (N) in the absence (control) or in the presence of the drugs indicated. The data represents the mean $\pm$ S.E.M. of the values obtained in at least 4 independent cultures. 200 μM dopamine and 50 μM SKF 38392 inhibited in approximately 60% the release of GABA induced by 100 μM NMDA even in the presence of 1 μM SCH 23390. B: basal efflux in the presence of drugs and absence of NMDA. The modified Hanks solution used was the same as that described in the legend of Fig. 2. The inset shows the stereoselectivity of SKF 38393 inhibition of GABA release. B: basal efflux of [^{3}H]GABA during 5 min pulses in the absence (control) or in the presence of 50 μM (−)-SKF 38393 or (+)-SKF 38393. N: 5 min pulses with 100 μM NMDA in the absence or in the presence of the SKF enantiomers. The data shown represent the mean $\pm$ S.E.M. of at least 4 independent cultures. The modified Hanks bathing solution used was the same as that described in the legend to Fig. 2.

hibitory effect of (+)-SKF 38393 on the induced release of [^{3}H]GABA was stereospecific, since the less active enantiomer (−)-SKF 38393 had no effect (Fig. 3, inset).

Since retinal dopamine D_1-like receptor activation promotes the increase of the intracellular cAMP concentration (Brown and Makman, 1972; De Mello, 1978; Van Buskirk and Dowling, 1981; Ventura and De Mello, 1990), agents that are able to activate the intracellular accumulation of cyclic AMP, or analogs of cAMP capable of permeating the plasma membrane would be expected to mimic the inhibitory effect of dopamine on the NMDA-mediated [^{3}H]GABA release, if these effects were mediated by dopamine D_1-like receptors (Daly, 1975; O'Brien and Dowling, 1985). However, neither forskolin (10 μM), which in our system stimulates the accumulation of cAMP by more than 20-fold (not shown), nor 8BrcAMP (5 mM)

Table 3
Lack of effect of forskolin and 8Br-cyclic AMP in inhibiting the release of GABA induced by NMDA

Treatment	[^{3}H]GABA released (% of total)	
	basal	NMDA (100 μM)
None	1.7 $\pm$ 0.6	8.8 $\pm$ 0.8 (3)
Forskolin (10 μM)	1.5 $\pm$ 0.2	8.2 $\pm$ 1.3 (3)
8Br cAMP (5 mM)	1.5 $\pm$ 0.4	8.4 $\pm$ 1.3 (3)

The cultures were preincubated for 5 min with forskolin or 8Br cAMP before the 5 min pulses were applied to measure the basal and the stimulated levels of GABA release. Both, the basal and the stimulated GABA efflux were measured in the presence of each test substance. The values shown are the mean $\pm$ S.E.M. of experiments run in triplicate cultures. The composition of the bathing medium was the same as indicated in the legend to Fig. 2.

prevented the release of [^{3}H]GABA induced by NMDA (Table 3).

3.4. Lack of dopamine effect on the release of GABA induced by kainate

Kainate (100 μM) also elicited a 9–12 fold increase in [^{3}H]GABA release by the activation of non-NMDA receptors, an effect that was completely abolished by 100 μM CNQX (Table 4). However, the kainate effect was not additive with the NMDA-mediated release, suggesting that both agonists mediated the release of GABA from the same pool. Even though NMDA-induced release of [^{3}H]GABA was consistently inhibited by dopamine and (+)-SKF 38393, the release induced by kainate was not reduced by (+)-SKF 38393 even at 100 μM concentration (Fig. 4). In addition, the release of [^{3}H]GABA promoted by NMDA and kainate was inhibited in more than 80% by 50 μM of the non-competitive GABA carrier blocker NO-711, indicating that in both cases most of the release

Table 4
Non-additive effect of NMDA and kainate on the release of GABA

Treatment	[^{3}H]GABA released (% of total)
None	1.57 $\pm$ 0.01 (3)
NMDA (100 μM)	13.79 $\pm$ 1.27 (2)
Kainate (100 μM)	13.00 $\pm$ 1.20 (3)
NMDA (100 μM) + NO-711 (50 μM)	2.70 $\pm$ 0.06 (2)
Kainate (100 μM) + NO-711 (50 μM)	3.39 $\pm$ 0.25 (3)
NMDA (100 μM) + kainate (100 μM)	15.92 $\pm$ 0.55 (2)
Kainate (100 μM) + CNQX (100 μM)	1.25 $\pm$ 0.08 (2)
Kainate (100 μM) + MK 801 (10 μM)	13.55 $\pm$ 0.09 (2)

The [^{3}H]GABA released upon stimulation with either NMDA or kainate were not additive when the cells were simultaneously stimulated with both excitatory amino acid agonists. The values shown are the mean $\pm$ S.E.M., or the deviation of individual values from the mean of the number of experiments indicated in parentheses. The experiments were conducted with bathing medium containing 2 mM EDTA, 1 mM Glycine, 2 mM Ca^{2+} and no Mg^{2+}. Both the basal and the stimulated release were measured during 5 min incubation with each test substance.

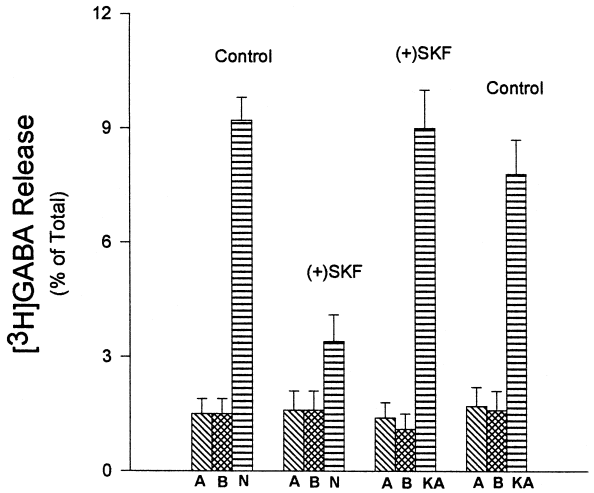

Fig. 4. Effect of (+)-SKF 38393 on the release of GABA elicited by NMDA and kainate. In the control group, A and B indicate the basal efflux of GABA in the absence of any added agent. In the (+)-SKF 38393 treated groups A indicates the basal efflux of [^{3}H]GABA in the absence of the drug, and B, the basal efflux of GABA in the presence of 100 μM (+)-SKF 38393. N−5 min pulses with 100 μM NMDA in the presence or absence of SKF 38393. KA: 5 min pulses with 100 μM kainate in the presence or in the absence of the dopaminergic agonist. (+)-SKF 38393 reduced the NMDA induced GABA release in approximately 60% but had no effect on the kainate induced GABA release. The data shown are the mean ± S.E.M. of 3 to 4 independent cultures. The modified Hanks bathing solution used was the same as that described in the legend to Fig. 2.

was mediated by the functional reversal of the GABA transporter (Table 4).

4. Discussion

In the present study we show that dopamine inhibits the release of [^{3}H]GABA induced by excitatory amino acids when the release is evoked by the activation of NMDA but not kainate receptors. In a previous publication we have shown that NMDA increases the release of [^{3}H]GABA only when Mg^{2+} ions are omitted from the bathing medium (Reis et al., 1995). In fact, dopamine only inhibits the release of [^{3}H]GABA evoked by glutamate in the absence of Mg^{2+}, suggesting that the dopamine effect occurs when [^{3}H]GABA release is induced by the activation of NMDA receptors. The fact that dopamine has no significant effect on the GABA release when dispersed cultured cells are stimulated by glutamate in the presence of Mg^{2+} is not entirely understood, and remains to be determined. However, using retina cell aggregate culture in which the cells are reorganized histotypically (Linser and Moscona, 1979), the glutamate induced release of GABA, in the presence of Mg^{2+}, is inhibited in more than 50% by dopamine (Do Nascimento et al., 1996). Therefore, the role of dopamine in controlling the dynamics of the NMDA receptor system may have important physiological significance in the organized nervous tissue.

Typical antagonists of the dopamine D_1 and D_2-like receptors, even at high concentrations, were not able to prevent the inhibitory effect of dopamine on the release of [^{3}H]GABA induced either by glutamate or NMDA, suggesting that the dopamine effect was not mediated by known dopamine receptors. Also, attempts to inhibit GABA release induced by excitatory amino acids by the dopamine D_2 receptor agonist quinpirole were unsuccessful, reinforcing the idea that D_2-like receptors were not involved. Although (+)-SKF 38393 was able to inhibit the release of [^{3}H]GABA promoted by activation of NMDA receptors in a way identical to the inhibition caused by dopamine, SCH 23390 did not prevent this effect, even at high concentrations. This dopamine D_1-like receptor antagonist was highly effective in inhibiting dopamine-induced cAMP production under the same conditions used for the study of GABA release. The possibility of a nonspecific effect of (+)-SKF 38393 was ruled out by the fact that its inactive isomer was ineffective in inhibiting the NMDA-mediated [^{3}H]GABA release. Even though the stereoselective effect of (+)-SKF 38393 suggested an interaction with dopamine D_1-like receptors, another agonist of this receptor subclass, SKF 81297, was without effect (Table 3). In addition, forskolin and 8BrcAMP did not interfere with the activated release of [^{3}H]GABA, showing that the classical adenylyl-cyclase pathway was not involved in the effects of dopamine and (+)-SKF 38393.

Dopamine could exert its effect via a direct inhibition of the GABA carrier responsible for the release. However, dopamine does not interfere with the uptake of [^{3}H]GABA (not shown). This is the case when the inhibition of GABA release is caused by specific carrier blockers (Levi and Raiteri, 1993; Pin and Bockaert, 1989). In addition, kainate induced the release of [^{3}H]GABA apparently from the same pool that was mobilized by NMDA receptor activation. However, the release of [^{3}H]GABA induced by kainate (which is also carrier mediated, see Table 4) was not affected by (+)-SKF 38393 and dopamine. Therefore, the possibility that dopamine interferes with the release by acting on the carrier system seems to be ruled out.

Another possibility that has to be considered is that dopamine could be mediating its effects via an alternative configuration of the dopamine receptor, displaying pharmacological characteristics different from the ones known for the classical receptor systems. This possibility is consistent with reports that show, in rat brain, that dopamine activates phosphoinositide metabolism, via the activation of D1-like receptors with pharmacological characteristics that differs from the ones associated with classical D1 dopamine receptors coupled to adenylyl cyclase (Undie and Friedman, 1990; Undie and Friedman, 1992; Undie et al., 1994). Although the data presented in this communication cannot rule out the participation of this novel type of D1-like effects, the pharmacology reported for the dopamine mediated inositol phosphate accumulation display subtle differences when compared to the effects of

dopamine receptor agonists upon the release of [^{3}H]GABA reported above. Thus, while SKF 81297 has a higher potency than SKF 38393 in promoting inositol phosphate accumulation in rat brain (Undie et al., 1994), in our system SKF 38393 is highly effective in inhibiting [^{3}H]GABA release while SKF 81297 has no effect. Also, in the case of phosphoinositide turnover, SCH 23390 at 10 μM already promoted a significant 25% inhibition of the accumulation of the inositol derivatives induced by 500 μM dopamine (Undie and Friedman, 1992). In the case presented in this report, at 10 μM, SCH 23390 had no effect. Attempts to raise the concentration of this antagonist up to 1000 μM were not conclusive because, in our system, that high concentration of SCH 23390 seemed to be toxic to the cells, with visible damage of the cultures (not shown).

Several lines of evidence indicate that neurotransmitter receptor channels can be influenced by neuroactive substances. For instance, haloperidol, a known dopaminergic ligand has been suggested to interact with glycine sites of the NMDA receptor complex in hippocampal neurons (Fletcher and Mac Donald, 1993). In horizontal cells of the fish retina, dopamine seems to alter the kinetics of excitatory amino acid gated channels with characteristics of a dopamine D_2-like receptor mediated event. In this case, dopamine seems to modulate the kainate receptor channel present in fish horizontal cells (Knapp et al., 1990). Dopamine also modulates GABA elicited currents through GABAc receptors of fish horizontal cells. This effect, on the other hand, seems to be mediated by dopamine D_1-like receptors (Schmidt et al., 1994). Therefore, based on our data and on the evidence in the literature mentioned above, we also have to consider the possibility that dopamine could act directly in or near the NMDA receptor complex, changing the functional dynamics of the NMDA channel. Using patch-clamp electrophysiological measurements in cultured single cells from the chick retina and rat thalamic and substantia nigra neurons, it has been shown that ionic currents elicited by NMDA but not kainate receptor activation are inhibited in approximately 50% by dopamine and (+)-SKF 38393, with the pharmacological characteristics described for the NMDA-mediated GABA release reported in the present communication (Castro et al., 1995).

The association of dopaminergic inputs with dendritic spines enriched in NMDA receptors in different areas of the brain (Kornhuber and Kornhuber, 1983; Freund et al., 1984; Johnson and Jeng, 1991), makes the possibilities considered above attractive with respect to alternative mechanisms of control by dopamine, of the normal and altered physiology of glutamatergic circuitry.

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