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Dopaminergic Regulation of Enkephalin Release

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Abstract: The effects of dopamine receptor stimulation on enkephalin release were evaluated in vitro and in vivo by measuring the changes in the levels of [Met^5]enkephalin (YGGFM) and Tyr-Gly-Gly (YGG), a characteristic extracellular enkephalin metabolite produced under the action of enkephalinase. In rat striatal slices, D_1-receptor agonists or antagonists did not modify enkephalin release. By contrast, D_2-receptor agonists enhanced the potassium-induced release of YGGFM and YGG without affecting spontaneous release from nondepolarized slices. This response was prevented by the D_2-receptor antagonists haloperidol and RIV 2093, the latter compound being more potent, which suggested the involvement of a putative D_2-receptor subtype. Acute administration of apomorphine or selective D_2-receptor agonists, but not that of a D_1-receptor agonist, enhanced the steady-state level of YGG without affecting the YGGFM level in rat striatum. The effect was blocked selectively by D_2-receptor antagonists which, administered alone, had no effect. These observations indicate that D_2-receptor stimulation in vitro or in vivo facilitates enkephalin release from striatal neurons, but that endogenous dopamine does not exert any tonic influence upon the opioid peptide neuron activity under basal conditions. However, chronic administration of haloperidol resulted in increases in striatal YGGFM and YGG, an effect presumably reflecting a long-term adaptive process. **Key Words:** Rat—Striatum—Tyr-Gly-Gly—Dopamine D_2 receptor—Dopamine D_1 receptor—Enkephalin metabolism—Enkephalinase. **Llorens-Cortes C. et al.** Dopaminergic regulation of enkephalin release. *J. Neurochem.* **56,** 1368–1375 (1991).

The striatum contains a high density of dopamine and enkephalin axons and receptors, and complex relationships between the two neuronal systems are suggested by available experimental evidence. On one side, exogenous (Lubetzki et al., 1982), as well as endogenous, enkephalins (Wood, 1982; Llorens-Cortes and Schwartz, 1984) enhance striatal dopamine release and turnover via stimulation of receptors located on axons and/or perikarya of nigrostriatal dopaminergic neurons (Pollard et al., 1977; Gardner et al., 1980). Conversely, the activity of enkephalin neurons, which constitute a high proportion of striatal neurons intrinsic to the structure or projecting to the pallidum (Cuello, 1983), might be controlled by directly synapsing dopaminergic afferents (Kubota et al., 1986), because the dopamine D_2 receptor mRNA is expressed by these enkephalin neurons (Le Moine et al., 1990). However, the nature of the influence dopamine may exert on the activity of striatal enkephalin neurons largely remains to be clarified.

Chronic or subchronic interruption of dopaminergic transmission in rat striatum by chemical denervation (Angulo et al., 1986; Young et al., 1986; Mocchetti et al., 1987), reserpine (Mocchetti et al., 1985*a*; Zheng et al., 1988), or repeated administration of haloperidol, a predominantly D_2-receptor antagonist, enhances steady-state levels of enkephalins or proenkephalin and proenkephalin mRNA abundance (Hong et al., 1978; Sabol et al., 1983; Tang et al., 1983; Mocchetti et al., 1985*b*; Sivam et al., 1986; Morris et al., 1988). This was generally interpreted as reflecting a tonic inhibition of striatal enkephalin biosynthesis by dopamine mediated by D_2 receptors. However, chronic blockade of D_2 receptors by sulpiride, a more selective D_2-receptor antagonist than haloperidol, was found to reduce slightly striatal proenkephalin mRNA (Mocchetti et al., 1987) and a single administration of quinpirole, a selective D_2-receptor agonist (or electrical stimulation of the medial forebrain bundle), to enhance it (Bannon et al., 1989). In addition, chronic blockade of D_1 receptors was reported either to increase proenkephalin mRNA (Mocchetti et al., 1987) or to decrease it (Morris et al., 1988), whereas a single administration of a selective D_1-receptor agonist was ineffective (Bannon et al., 1989).

Hence, the direct actions of dopamine via D_1 and D_2 receptors on proenkephalin mRNA abundance cannot be considered as entirely clarified by these stud-

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ies. Furthermore, changes in mRNA levels, particularly after long-term treatments, are not easy to interpret and do not allow the direct influence of dopamine on enkephalin neuron activity or on the rate of release of the opioid peptides to be inferred. In fact, this relative lack of knowledge reflects the difficulty of assessing these parameters in peptidergic neurons by neurochemical measurements.

Following their release from enkephalin neurons, enkephalins and the enkephalin heptapeptide, which represent together five-sixths of the opioid peptide molecules derived from the preproenkephalin gene, are inactivated in the brain by the operation of two membrane metallopeptidases, namely, aminopeptidase M (EC 3.4.11.2), which releases tyrosine (Gros et al., 1985; Giros et al., 1986*a*), and enkephalinase (EC 3.4.24.11), which releases Tyr-Gly-Gly (YGG) (Malfroy et al., 1978; Patey et al., 1981). As a result of the development of a sensitive radioimmunoassay, this tripeptide was shown to represent a characteristic extracellular metabolite closely associated with enkephalinergic neurons. Furthermore, YGG is in a highly dynamic state and its level appears to reflect the activity of enkephalinergic neurons in brain and spinal cord under a variety of circumstances (Llorens-Cortes et al., 1985*a,b*, 1986, 1990; Giros et al., 1986*b*; Houdi and Van Loon, 1990).

In the present study, we have assessed the influence of cerebral dopamine receptor stimulation on enkephalin release in vitro on depolarized slices, as well as in vivo, by measuring the changes in YGG levels.

MATERIALS AND METHODS

Animals and treatments

Male Wistar rats (180–210 g; Iffa Credo, France) were kept under artificial light (12-h cycles) with food and water ad libitum. Treatments were performed between 2 and 5 p.m. Injections were performed subcutaneously or intraperitoneally, volumes being adjusted according to body weight.

Apomorphine hydrochloride was dissolved just before use in distilled water containing 0.1% ascorbic acid; lisuride, RIV 2093, RU 24926, and SKF 38393 were dissolved in 0.05 *M* lactic acid and then the solutions were brought to neutrality with 0.1 *M* NaOH. SCH 23390 hydrochloride, quinpirole, and the injectable trade form of haloperidol were dissolved or diluted in distilled water. Control animals received only the corresponding drug vehicles.

Preparation of brain extracts for YGG and YGGFM assays

Animals were decapitated; brain regions were immediately dissected out on a cold plate and homogenized by sonication (Ultrasons, Annemasse, France) in 10–30 volumes of cold 0.4 *M* $HClO_4$, the duration of the whole procedure not exceeding 2 min. Unless otherwise stated, the dissected "striatum" comprised not only the caudate-putamen, but also the globus pallidus. After centrifugation (15,000 *g*, × min) of the homogenate, the pellet was treated with 0.4 *M* NaOH for protein determination, whereas 3 *M* KOH was added to the supernatant for perchlorate precipitation and to adjust the pH to 6.

After centrifugation, aliquots of the supernatant were applied to cartridges of bonded SiO_2 (Sep-Pak C-18; Waters Assoc.), and then YGGFM and YGG were eluted with 1.5 ml of methanol with 80% recovery, as determined with [^{3}H]YGG and [^{3}H]YGGFM. The eluates were dried under reduced pressure, the residues redissolved in distilled water, and aliquots subjected to YGG and YGGFM radioimmunoassays.

K^+-evoked release of peptides from rat striatal slices

Rats were decapitated; striata were rapidly dissected out from the brain on a cold plate and sliced bidirectionally (by a 90° rotation of the plate) at 250-μm intervals, using a McIlwain tissue chopper. The resulting slices were suspended in a modified 4 m*M* K^+ Krebs–Ringer medium of the following composition: 6.6 m*M* glucose, 25 m*M* Na-HEPES, 122 m*M* NaCl, 3 m*M* KCl, 2.6 m*M* $CaCl_2$, 0.67 m*M* $MgSO_4$, and 1.2 m*M* KH_2PO_4, pH 7.4, previously saturated with pure O_2 and maintained at 37°C. In order to obtain about 1 mg of protein/incubation, pooled tissues from 10 rats generally were used for 24 incubations. After three successive washes with fresh medium, slices were transferred to a plastic test tube containing 25 ml of fresh medium and the medium was changed three times over 45 min for complete removal of soluble peptidase activity. Slices were then suspended in about 3 ml of fresh medium and 60-μl aliquots of the suspension (corresponding to 0.5–1.5 mg of protein in the final assay) were distributed to Eppendorf plastic tubes containing 40 μl of solution (in 4 m*M* K^+ medium) of the various agents to be tested. After a 10-min preincubation at 37°C, 400 μl of fresh medium (with or without drugs), containing amounts of KCl calculated to give final concentrations of 4, 30, or 50 m*M* K^+, were added. In the presence of 30 or 50 m*M* K^+, the NaCl concentration was reduced in order to maintain isoosmolarity. After 5 min, the incubations were stopped by rapid centrifugation and 700 μl of the supernatant transferred to tubes containing 100 μl of 4 *M* $HClO_4$. Pellets were sonicated in 500 μl of 0.4 *M* NaOH and kept at 4°C overnight for protein determination. Perchlorates in the supernatant were precipitated by addition of 3 *M* KOH to pH 6. After centrifugation, the supernatants were frozen and lyophilized. The resulting dry extracts were then resuspended in 500–800 μl of water for YGG and YGGFM assays. The release of YGGFM evoked by the K^+ stimulus under the various conditions was evaluated as the difference between the mean YGGFM or YGG levels in the 4 and 50 (or 30) m*M* K^+ media and expressed per milligram of protein.

YGG and YGGFM radioimmunoassays

YGG was radioimmunoassayed according to Llorens-Cortes et al. (1985*a,b*). Briefly, standards and purified extracts from brain tissues or from supernatants of incubation media were treated with *p*-benzoquinone in order to derivatize YGG into its immunoreactive Schiff base. Triplicate 50-μl aliquots of the derivatized materials were mixed with the tracer consisting of ^{125}I-Tyr-Gly-Gly-benzoquinone-Tyr-Gly-Gly (20,000 dpm) and, usually, a 1:18,000 dilution of the antiserum was added to a final volume of 0.3 ml. After incubation, the bound radioactivity was precipitated by addition of polyethylene glycol and counted in a gamma spectrometer. YGG levels were calculated by comparison with the standard curve, using a logit–log transformation. The detection limit of the YGG radioimmunoassay was 5 pg/0.3 ml, and the antiserum showed negligible cross-reactivity to YGGFM and parent peptides (Llorens-Cortes et al., 1985*a,b*).

For YGGFM, assays in purified extracts were performed as described (Gros et al., 1978). Briefly, Tris-maleate buffer (0.05 *M*, pH 8.6) was used routinely to dilute the reagents. In polypropylene test tubes, standard solutions of YGGFM or samples (0.1 ml), iodinated tracers (17,000 dpm in 0.1 ml), and diluted antiserum (0.1 ml) were added successively. The mixture was stirred gently and incubated overnight at 4°C. γ-Globulin solution (0.1 ml of a 0.5% solution in Tris buffer) was added to each tube at 4°C, followed immediately by the addition of 0.5 ml of 40% polyethylene glycol. The tubes were centrifuged immediately at 2,200 *g* for 20 min at 4°C. The radioactivity of the precipitates was measured by gamma spectrometry.

Analysis of data and statistical treatment

For determination of EC_{50} and IC_{50} values, the total curves were analyzed with an iterative computer least-squares method derived from that of Parker and Waud (1971). Apparent dissociation constants (K_i values) of antagonists were calculated from their respective IC_{50} values, assuming a competitive antagonism, according to the equation: $K_i = IC_{50}/(1 + S/EC_{50})$, where S represents the agonist concentration and EC_{50} is the agonist concentration required for a half-maximal inhibitory effect (Cheng and Prusoff, 1973).

Statistical evaluation of the results was by Student's *t* test.

Drugs and reagents

Apomorphine hydrochloride was a gift from Sandoz (Basel, Switzerland); lisuride and quinpirole (LY 171555, i.e., *trans*-(−)-4*aR*-4,4*a*,5,6,7,8,8*a*,9-octahydro-5-propyl-1*H*-pyrazolo[3,4-*g*]quinoline HCl), were purchased from Lilly Laboratories (Indianapolis, IN, U.S.A.); RU 24926, i.e., *N*-*n*-propyl-di(3-hydroxylphenyl)ethylamine was from Roussel Uclaf (Romainville, France); SKF 38393, i.e., 2,3,4,5-tetrahydro-7,8-dihydroxyl-1-phenyl-1*H*-3-benzazepine, was from Smith, Kline, and French Laboratories (Philadelphia, PA, U.S.A.); RIV 2093, i.e., methyl-*N*-[(1-allyl-2-pyrrolidinyl)-methyl]-2-methoxy-4-amino-5-methylsulfamoylbenzamide, and (−)-sulpiride were generous gifts from Laboratoires Delagrange (Paris, France); haloperidol and domperidone were from Janssen (Paris, France); SCH 23390, i.e., (*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol, was from Schering Laboratories (Bloomfield, NJ, U.S.A.); bestatin was a generous gift from Laboratoires R. Bellon (Neuilly s/Seine, France); $Na^{125}I$ (sp. act. 2,000 Ci/mmol) was from Amersham (Bucks, U.K.); YGG and YGGFM were from Bachem (Bubendorf, Switzerland). Analytical grade reagents were from Prolabo (Paris, France).

RESULTS

Effects of dopaminergic agents on K^+-induced release of enkephalins from striatal slices

Enkephalin release from depolarized striatal slices was evaluated by measuring the levels of YGGFM and/or YGG, its main metabolite, particularly when the aminopeptidase M metabolic pathway is blocked by bestatin (Giros et al., 1986*b*).

When slices were incubated for 5 min in a 4 m*M* K^+ medium containing no aminopeptidase inhibitor, YGGFM and YGG levels in the medium represented 0.8 ± 0.1 and 1.3 ± 0.1 pmol/mg of tissue protein, respectively (means ± SEM of 21 values). Addition of various dopaminergic agents, i.e., lisuride, RU 24926, apomorphine, SKF 38393, haloperidol, RIV 2093, domperidone, and SCH 23390 (1 μ*M*), did not result in any significant change in these basal levels (not shown). After a 5-min incubation in the presence of 50 m*M* K^+ and in the absence of aminopeptidase inhibitor, YGGFM and YGG levels in the medium became 2.3 ± 0.1 and 4.6 ± 0.1 pmol/mg of protein, respectively, corresponding to 180% and 200% increases. These various values were not modified significantly by addition of either 1 μ*M* SKF 38393, a selective D_1-receptor agonist, or 0.1 μ*M* SCH 23390, a D_1-receptor antagonist (Table 1). However, the lack of change in the presence of these dopaminergic agents may be due to the fact that, in the absence of bestatin, an aminopeptidase inhibitor, a considerable proportion of YGGFM is degraded to products which cannot be detected. In order to exclude this possibility, the same type of experiment was performed in the presence of bestatin. The recovery of YGG in the medium of slices depolarized by 30 m*M* K^+ and incubated in the presence of bestatin was not modified by the same agents (Table 1).

In order to assess selectively D_2 receptor-mediated modulations of enkephalin release, all further studies were conducted in the presence of 0.1 μ*M* SCH 23390.

In the presence of 0.1 μ*M* lisuride, the YGG level in the medium of 50 m*M* K^+-depolarized slices was enhanced by 40%, an effect prevented by either RIV 2093 or domperidone, two selective D_2-receptor antagonists (Table 2).

In the presence of bestatin, an aminopeptidase M inhibitor, depolarization of the slices by 30 m*M* K^+ for 5 min resulted in a 300% increase in YGGFM levels (3.06 ± 0.18 instead of 0.81 ± 0.10 pmol/mg of protein in 4 m*M* K^+ medium) and a 300% increase in YGG levels (Table 2). Under these conditions, addition of 0.1 μ*M* apomorphine, RU 24926, or lisuride resulted in further elevations in YGG levels of 35%, 53%, and 39%, respectively. The effect of lisuride was prevented

TABLE 1. *Effects of dopaminergic agents on YGGFM and YGG levels in the incubation medium of K^+-depolarized striatal slices*

Conditions	YGGFM level	YGG level
50 m*M* K^+	1.2 ± 0.1	2.8 ± 0.2
+ 1 μ*M* SKF 38393	1.1 ± 0.2	2.8 ± 0.3
+ 0.1 μ*M* SCH 23390	1.4 ± 0.2	3.3 ± 0.2
30 m*M* K^+ with 0.1 m*M* bestatin		4.8 ± 0.6
+ 1 μ*M* SKF 38393		5.3 ± 0.1
+ 0.1 μ*M* SCH 23390		5.1 ± 0.4

Striatal slices were preincubated for 10 min in the presence of 1 μ*M* SKF 38393 or 0.1 μ*M* SCH 23390 before being exposed for 5 min to 50 or 30 m*M* K^+ in the absence or presence of 0.1 m*M* bestatin, respectively. Values, expressed as pmol/mg of protein, are the means ± SEM of values from four to 10 experiments done in quadruplicate.

TABLE 2. *Effects of dopaminergic agents on YGG levels in the incubation medium of K⁺-depolarized striatial slices*

Conditions	YGGFM level	YGG level
50 m*M* K^+	1.2 ± 0.1	3.2 ± 0.4
+ 0.1 μM lisuride	1.8 ± 0.1[a]	4.5 ± 0.4[a]
+ 0.1 μM lisuride + 0.1 μM RIV 2093	—	2.3 ± 0.2
+ 0.1 μM lisuride + 0.1 μM domperidone	—	3.3 ± 0.5
30 m*M* K^+ with 0.1 m*M* bestatin	2.0 ± 0.1	4.9 ± 0.3
+ 0.1 μM apomorphine	2.6 ± 0.2[a]	6.6 ± 0.7[a]
+ 3 μM RU 24926	2.8 ± 0.4[b]	7.5 ± 0.2[c]
+ 0.1 μM lisuride	2.6 ± 0.2[a]	6.8 ± 0.4[c]
+ 0.1 μM lisuride + 0.1 μM RIV 2093	—	4.4 ± 0.4
+ 0.1 μM lisuride + 0.3 μM haloperidol	—	4.7 ± 0.5
+ 0.1 μM lisuride + 0.1 μM sulpiride	—	4.4 ± 0.6

Rat striatal slices were preincubated for 10 min in the presence of 0.1 μM SCH 23390, a D_1-receptor antagonist, alone or together with D_2-receptor agonists in the absence or presence of D_2-receptor antagonists. Slices were then exposed for 5 min to 50 or 30 m*M* K^+ in the absence or presence of 0.1 m*M* bestatin, respectively. Values, expressed as pmol/mg protein, are the means ± SEM of values from four to 12 experiments done in quadruplicate.

[a] $p < 0.05$; [b]NS, not significant; [c] $p < 0.001$.

when slices were preincubated in the presence of the D_2-receptor antagonists RIV 2093, haloperidol, or sulpiride (Table 2).

Lisuride also enhanced significantly and in a concentration-dependent manner the recovery of YGG and the sum of YGG plus YGGFM in the medium of slices depolarized by 50 m*M* K^+ in the absence of bestatin, the maximal responses being 55% and 45%, respectively, and the corresponding EC_{50} values being 0.7 ± 0.4 n*M* and 0.6 ± 0.3 n*M* (Fig. 1 and Table 3).

A similar concentration–dependent change was observed when slices were incubated with 30 m*M* K^+ in the presence of 0.1 m*M* bestatin, the EC_{50} being 4.1 ± 2.8 n*M* and the maximal increase 40% (not shown). Under the same conditions, RU 24926, another D_2-receptor agonist, led to a maximal 54% increase with an EC_{50} of 1.7 ± 0.7 n*M* (Table 3).

The effect of 0.1 μM lisuride was inhibited progressively in the presence of RIV 2093 or haloperidol (Fig. 2) with IC_{50} values of 143 ± 73 and 11 ± 5 n*M*, respectively, leading to the apparent K_i values reported in Table 3 when a competitive inhibition was assumed.

Effect of acute treatments with dopaminergic agents on YGG and YGGFM levels in rat striatum

At 30 to 45 min after administration of apomorphine (1–5 mg/kg s.c.), YGG levels were increased in both caudate-putamen and globus pallidus (not shown). However, in view of the difficulty associated with a rapid and reproducible dissection of each area, both were dissected and analyzed together in further experiments in which the structure is referred to as "striatum."

Striatal levels of YGG were enhanced significantly in rats treated with apomorphine (26%), lisuride (37%),

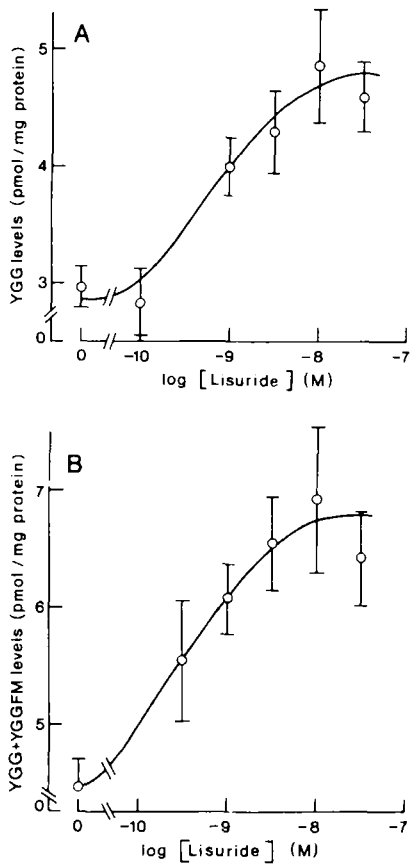

FIG. 1. Effects of lisuride on YGG and YGGFM levels in the incubation medium of K^+-depolarized slices. Striatal slices were preincubated for 10 min in the presence of 0.1 μM SCH 23390 and lisuride in increasing concentrations before exposure for 5 min to 50 m*M* K^+. **A:** Changes in YGG level. **B:** Changes in YGG + YGGFM levels. Each point represents the mean ± SEM of values from three to six experiments with quadruplicate determinations.

TABLE 3. *Effects of D_2-receptor agonists and antagonists on YGG levels in the incubation medium of K^+-depolarized striatal slices: analysis of concentration–response curves*

Agents	EC_{50} or K_i (n*M*)
Agonists	
Lisuride	0.7 ± 0.4
RU 24926	1.7 ± 0.7
Antagonists	
Haloperidol	5.5 ± 2.2
RIV 2093	0.6 ± 0.3

Values were obtained by analysis of the concentration–response curves shown in Figs. 1 and 2 (or derived from similar experiments with RU 24926 in the presence of 30 m*M* K^+; not shown) using a nonlinear least-square curve-fitting procedure.

RU 24926 (37%), and quinpirole (47%), whereas the D_1-receptor agonist SKF 38393 was ineffective. In the same animals, striatal YGGFM levels were not affected significantly by the administration of any of these dopaminergic agonists (Table 4). The YGG-enhancing effect of quinpirole was still observed 3 h after its administration, the tripeptide levels being 2.7 ± 0.2 pmol/mg of protein instead of 1.8 ± 0.2 pmol/mg of protein in controls (not shown). In another set of experiments, the apomorphine-induced elevation of striatal YGG was 37%, and the effect was prevented completely in animals pretreated with haloperidol or RIV 2093, whereas the D_1-receptor antagonist SCH 23390 was ineffective; YGG levels were not affected in animals receiving either RIV 2093 or haloperidol alone (Table 5).

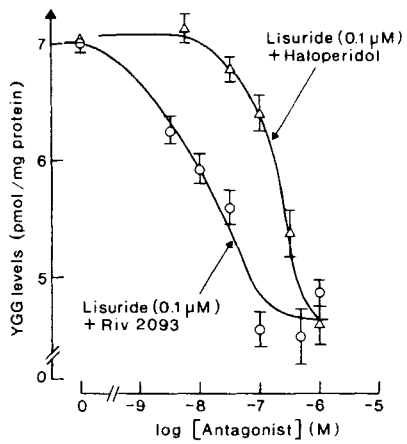

FIG. 2. Effects of haloperidol and RIV 2093 on the lisuride-induced increase of YGG levels in the incubation medium of K^+-depolarized slices. Striatal slices were preincubated for 10 min in the presence of 0.1 μ*M* SCH 23390, 0.1 μ*M* lisuride, and RIV 2093 or haloperidol, in increasing concentrations, before exposure for 5 min to 30 m*M* K^+. Preincubations and incubations were performed in the presence of 0.1 m*M* bestatin. Each point represents the mean ± SEM of data from two to four experiments with quadruplicate determinations.

TABLE 4. *Effects of acute treatments with various dopamine agonists on YGG and YGGFM levels in rat striatum*

Treatment	YGG level	YGGFM level
Vehicle	1.9 ± 0.1	35.6 ± 0.7
Apomorphine	2.4 ± 0.2[a]	33.8 ± 1.1
Lisuride	2.6 ± 0.2[b]	—
RU 24926	2.6 ± 0.1[c]	32.1 ± 3.0
Quinpirole	2.8 ± 0.1[c]	35.1 ± 1.0
SKF 38393	2.1 ± 0.1	34.3 ± 1.2

Animals were killed 30 min after injection of apomorphine (5 mg/kg s.c.) or 45 min after injection of lisuride (0.2 mg/kg i.p.), RU 24926 (5 mg/kg i.p.), quinpirole (3 mg/kg i.p.), or SKF 38393 (4 mg/kg i.p.). Values, expressed as pmol/mg of protein, are means ± SEM of 10–15 determinations.

[a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$.

The effect of the D_2-receptor agonist quinpirole on YGG levels was restricted apparently to the striatum (Table 6).

Effect of chronic treatments with haloperidol on YGG and YGGFM levels in various brain regions

Chronic administration of haloperidol (2 mg/kg/day) for 4 days induced a parallel increase in YGG and YGGFM levels in the globus pallidus (52% and 56%, respectively) 24 h after the last injection (Table 7). In the caudate-putamen, only an increase in YGGFM levels (31%) was observed. No modification in YGG or YGGFM levels was observed in the hippocampus or the cortex (Table 7).

DISCUSSION

The present study establishes that stimulation of dopamine D_2 receptors enhances enkephalin release in rat striatum in vitro, as well as in vivo.

Whereas D_1-receptor stimulation was ineffective, the

TABLE 5. *Effects of acute treatments with apomorphine and/or dopamine antagonists on rat striatal YGG levels*

Treatment	YGG level (pmol/mg of protein)
Vehicle	1.9 ± 0.1
Apomorphine	2.6 ± 0.2[a]
Apomorphine + haloperidol	1.8 ± 0.2
Apomorphine + SCH 23390	2.6 ± 0.3[a]
Apomorphine + RIV 2093	1.7 ± 0.3
RIV 2093	2.0 ± 0.1
Haloperidol	1.6 ± 0.2

Apomorphine was injected at the dose of 5 mg/kg i.p. 30 min before killing. Haloperidol (1 mg/kg i.p.) or RIV 2093 (50 mg/kg i.p.) was injected 60 min or 105 min, respectively, prior to apomorphine. SCH 23390 (0.5 mg/kg i.p.) was injected simultaneously with apomorphine. Values are means ± SEM of five to 10 determinations.

[a] $p < 0.05$.

TABLE 6. *Effects of an acute treatment with quinpirole on YGG and YGGFM levels in discrete rat brain regions*

Treatment	Striatum	Olfactory system	Frontal cortex	Hippocampus
YGG levels (pmol/mg of protein)				
Vehicle	2.1 ± 0.1	0.54 ± 0.1	0.19 ± 0.1	0.31 ± 0.1
Quinpirole	2.7 ± 0.3[a]	0.52 ± 0.1	0.20 ± 0.1	0.29 ± 0.1
YGGFM levels (pmol/mg of protein)				
Vehicle	35.8 ± 0.7	15.7 ± 1.1	4.9 ± 0.8	2.3 ± 0.1
Quinpirole	35.1 ± 0.6	17.5 ± 1.8	4.6 ± 0.7	2.0 ± 0.1

Animals were killed 45 min after injection of quinpirole (3 mg/kg i.p.), and brain regions were rapidly dissected out (the olfactory system corresponds to olfactory bulbs and tubercles) and sonicated in 20 volumes of cold 0.4 *M* $HClO_4$. Homogenates were centrifuged; supernatants were adjusted to pH 6.0, purified, and radioimmunoassayed. Values are means ± SEM of 10 determinations.

[a] $p < 0.01$.

action of selective D_2-receptor agonists on K^+-depolarized striatal slices was evidenced by significant increases in YGGFM and YGG recovery in the incubation medium. Depolarization-induced enhancement of YGG levels in the medium in the presence of bestatin, an aminopeptidase M inhibitor which drives most of the opioid peptides released from enkephalinergic neurons into the enkephalinase-dependent metabolic pathway, represents a most sensitive index of the release of these peptides (Giros et al., 1986*a*). Under these circumstances, agents such as apomorphine, a nonspecific dopaminergic agonist, or lisuride and RU 24926, two selective D_2-receptor agonists, maximally enhanced YGG levels by about 50% and the action of lisuride was blocked completely by various D_2-receptor antagonists. The lack of effect of the antagonists, when used alone, indicates that the amount of endogenous dopamine released by the depolarizing stimulus was not sufficient to activate the relevant D_2 receptors. The fact that the dopamine agonists did not modify significantly enkephalin release from nondepolarized slices suggests that they may have a purely facilitatory role or, alternatively, an indirect effect via release of another neurotransmitter in the preparation. Nevertheless, the recent demonstration that striatal enkephalin neurons presumably express the D_2 receptor at their surface (Le Moine et al., 1990) would be consistent with a direct facilitatory action. It is not clear whether the modulatory effect mediated by D_2 receptors occurs at the level of the caudate-putamen or the pallidum, because both structures were present in our "striatal" slices. However, a modulation of enkephalin release via stimulation of presynaptic D_2 receptors located on enkephalin axons in the pallidum would be consistent with the observations that this area receives a massive enkephalinergic input from the striatum (Cuello, 1983) and contains a relatively large density of D_2 receptors as detected by autoradiography (Altar et al., 1985; Bouthenet et al., 1987).

An interesting observation relates to the pharmacology of the receptors mediating the modulation of enkephalin release in vitro. Whereas they can be classified as D_2 receptors in a broad sense, i.e., non-D_1 receptors (Kebabian and Calne, 1979), the apparent affinity of RIV 2093, a discriminant benzamide derivative (Sokoloff et al., 1984, 1985), was significantly higher than that of haloperidol. On typical D_2 receptors, expressed by pituitary mammotrophs and striatal neurons, as well as on the newly characterized D_3 receptor expressed mainly in limbic areas, a reverse order of potency of the two compounds is found (Schwartz et al., 1984; Sokoloff et al., 1990). By contrast, RIV 2093

TABLE 7. *Effects of a chronic treatment with haloperidol on YGG and YGGFM levels in discrete regions of the rat brain*

Treatment	Globus pallidus	Caudate-putamen	Hippocampus	Frontal cortex
YGG levels (pmol/mg of protein)				
Control	2.7 ± 0.2	1.8 ± 0.1	0.34 ± 0.06	0.34 ± 0.01
Haloperidol	4.1 ± 0.3[a]	1.7 ± 0.1	0.22 ± 0.03	0.37 ± 0.05
YGGFM levels (pmol/mg of protein)				
Control	41 ± 2.0	16 ± 0.8	—	—
Haloperidol	64 ± 3.0[a]	21 ± 0.8[a]	—	—

Animals received one injection of haloperidol (2 mg/kg i.p.) every day for 4 days. They were killed 24 h after the last injection, and main regions were rapidly dissected out. Values are means ± SEM of six to 21 determinations.

[a] $p < 0.001$.

was found to be more potent than haloperidol on a putative subtype of D_2 receptors (termed D_4 sites) (Schwartz et al., 1984), suggesting that the latter might be involved, but this remains to be confirmed by its molecular identification.

That D_2 receptors also modulate enkephalin release in vivo was shown by the significant increase in striatal YGG levels elicited by apomorphine and prevented by two D_2-receptor antagonists, i.e., haloperidol or RIV 2093, but not by the D_1-receptor antagonist SCH 23390. Furthermore, this conclusion is strengthened by the observation that treatments with various D_2-receptor agonists induce similar changes which, in the case of quinpirole, result in a long-lasting effect, still significant after 3 h. The stimulation of enkephalin release appears region-selective, because it occurs in the striatum, but not in other dopaminergic areas. Again, as in vitro, no modification of enkephalin release was observed via D_1-receptor stimulation. These observations are consistent with those of Bannon et al. (1989), who detected enhanced levels of preproenkephalin mRNA in rat striatum 3 h after administration of quinpirole or metamphetamine, a direct and an indirect dopamine D_2-receptor agonist, respectively, as well as after medial forebrain stimulation, but not following D_1-receptor stimulation. Hence, the enhanced preproenkephalin gene expression is likely to reflect a rapid compensatory adaptation of the neuron biosynthetic machinery to the stimulation of the neurotransmitter release. Conversely, inhibition of striatal enkephalin release elicited by γ-aminobutyric $acid_A$ receptor stimulation was followed, after a short lag period of a few hours, by a reduced gene expression (Llorens-Cortes et al., 1990). Similar adaptive changes in biosynthesis following modified release rates are well substantiated in the case of aminergic neurons but, from the above observations, appear to occur as well in peptidergic neurons. This supports the initial assumption that YGG levels represent a sensitive and reliable index of primary changes in enkephalin neuron activity (Llorens-Cortes et al., 1986).

The unmodified YGG levels (present study) and the YGGFM and proenkephalin mRNA levels (Tang et al., 1983; Hong et al., 1985) following acute administration of D_2-receptor antagonists suggest a lack of "tonic" control of striatal enkephalin neurons by endogenous dopamine acting via D_2 receptors. By contrast, chronic blockade of D_2 receptors is followed by enhanced steady-state levels of YGGFM (Hong et al., 1978; Tang et al., 1983) and YGG (Houdi and Van Loon, 1990; present observations) accompanied by enhanced preproenkephalin mRNA abundance (Sabol et al., 1983; Tang et al., 1983; Mocchetti et al., 1985*b*; Angulo et al., 1986; Romano et al., 1987). These progressive accelerations in striatal enkephalin biosynthesis and release, which result from sustained decreases in dopaminergic transmission, might reflect a long-term adaptive process related or not to the primary action that we have presently evidenced.

In conclusion, both in vitro and in vivo studies indicate that stimulation of dopamine D_2 receptors activates enkephalin release from striatal neurons, a finding consistent with the hypothesis that excessive opioid activity may be important in the pathophysiology of some movement disorders, such as tardive dyskinesia, progressive supranuclear palsy, and a subgroup of Tourette's patients (Sandyk, 1986).

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