Accelerated Resensitization of the D1 Dopamine Receptor-mediated Response in Cultured Cortical and Striatal Neurons from the Rat: Respective Role of α 1-Adrenergic and *N*-methyl-D-aspartate Receptors

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As previously shown *in vivo*, noradrenergic and glutamatergic neurons can regulate the denervation supersensitivity of D1 dopaminergic (DA) receptors in the rat prefrontal cortex and striatum respectively. Therefore, the effects of methoxamine (an α 1-adrenergic agonist) and glutamate on the resensitization of D1 DA receptors were investigated in cultured cortical and striatal neurons from the embryonic rat. In the presence of sulpiride and propranolol, DA stimulated the D1 DA receptor-mediated conversion of ^3H -adenine into ^3H -cAMP in both intact cortical and striatal cells and these responses were markedly desensitized in cells preexposed for 15 min to DA (50 μM). The complete recovery of the D1 DA response was more rapid in striatal (15 min) than in cortical (80 min) neurons. Methoxamine accelerated the resensitization of the D1 response in cortical but not in striatal neurons. The effect of the α 1-adrenergic agonist in cortical neurons was blocked by prazosin and chlorethylclonidine. In contrast, glutamate accelerated the resensitization of the D1 response in striatal but not in cortical neurons and the effect observed in striatal neurons was totally blocked by 2-amino-5-phosphonovaleric acid, an NMDA receptor antagonist. Protein kinase C was shown to be involved in the α 1-adrenergic-induced resensitization of the cortical D1 response but not in the glutamate-evoked resensitization of the striatal D1 response. Finally, for comparison, similar experiments were performed on β -adrenergic receptors using isoproterenol (1 μM) as an agonist. Methoxamine did not modify the resensitization of the β -adrenergic response in cortical neurons, but glutamate accelerated the resensitization of this response in striatal neurons.

[Key words: cortical neurons, striatal neurons, D1 DA receptors, heterologous resensitization, α 1-adrenergic receptors, NMDA receptors]

Several years ago, we provided behavioral and biochemical evidence for an interaction between the mesocortico-prefrontal dopaminergic (DA) neurons and the noradrenergic neurons originating from the locus coeruleus and innervating the cerebral

cortex. In the rat, bilateral electrolytic lesions of the mesencephalic ventral tegmental area induce a complex and permanent behavioral syndrome characterized by a locomotor hyperactivity and the incapacity of the animal to focalize its attention (Le Moal et al., 1969). Some of the behavioral deficits observed in the lesioned animals, particularly the locomotor hyperactivity, have been attributed for a large part to the selective destruction of the cortical dopaminergic (DA) innervation (Tassin et al., 1978). This locomotor hyperactivity was markedly reduced in rats with 6-hydroxydopamine (6-OHDA) lesions, which destroy not only the ascending DA neurons but also the ascending noradrenergic fibers passing near the ventral tegmental area (Tassin et al., 1982). α 1-Adrenergic receptors sensitive to the α 1 antagonist prazosin seem to play a prominent role in the permissive effect of cortical noradrenaline on the appearance of behavioral deficits in rats with ventral tegmental area electrolytic lesions. Indeed, in these lesioned animals, the peripheral injection of prazosin immediately suppressed locomotor hyperactivity (Trovero et al., 1992a). The effect of prazosin appears to be mediated at the cortical level since high affinity binding sites for prazosin were found in the prefrontal cortex, and the cortical application of prazosin abolished the increased locomotor activity evoked by amphetamine application into the nucleus accumbens (Blanc et al., 1993). In addition, the denervation supersensitivity of cortical DA-sensitive adenylate cyclase observed in rats with electrolytic lesions of the ventral tegmental area was prevented by the concomitant destruction of the cortical noradrenergic innervation (Tassin et al., 1982, 1986). Similarly, the denervation supersensitivity of the cortical DA-sensitive adenylate cyclase was not seen in rats with bilateral 6-hydroxydopamine (6-OHDA) lesions of the ventral tegmental area (Tassin et al., 1982).

Together, these behavioral and biochemical observations led us in the present study to determine whether the stimulation of α 1-adrenergic receptors could regulate the sensitivity of the D1 DA-induced formation of cAMP (D1 response) in intact cortical neurons from rat embryos in primary culture. For this purpose, we first investigated the effect of an α 1 agonist, methoxamine, on the recovery of the D1 response following a short-term DA-induced desensitization. For comparison, similar experiments were performed on cultured striatal neurons. Following this, the effects of glutamate on the rate of resensitization of the cortical and striatal D1 responses were also analyzed since the cerebral cortex is rich in glutamatergic neurons and the corticostriatal glutamatergic innervation has been shown to contribute to the regulation of the denervation supersensitivity of striatal D1 DA

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Figure 1. Pharmacological characterization of the dopamine-induced production of cAMP in cortical neurons. Cortical neurons from rat embryos were cultured for 6 d in serum-free medium and then incubated for 2 hr with ^3H -adenine. DA ($50 \mu\text{M}$) and antagonists (sulpiride, $5 \mu\text{M}$; propranolol, $1 \mu\text{M}$; yohimbine, $1 \mu\text{M}$; SCH 23390, $0.2 \mu\text{M}$) were then added to the medium in the presence of IBMX (1 mM) and incubated for 5 min. The ^3H -cAMP that accumulated in cells was then estimated. Neither sulpiride nor propranolol nor yohimbine significantly modified the response obtained with DA alone. Results are the mean $\pm$ SEM of values obtained from triplicate in a typical experiment. *, significantly different ($p < 0.05$, Student's t test) from basal values; **, significantly different ($p < 0.05$, Student's t test) from the DA-evoked response. Four other experiments performed with different cultures provided similar results.

receptors (Hervé et al., 1989). Finally, in order to demonstrate further the specificity of the effects observed with methoxamine and glutamate, parallel experiments were made on the resensitization of the β -adrenergic-induced formation of cAMP (β -adrenergic response), following isoproterenol-induced desensitization of β -adrenergic receptors in cortical and striatal neurons.

Materials and Methods

Primary cultures of cortical and striatal neurons

Cortices and striata were removed from 17 d old Sprague-Dawley rat embryos (Iffa Credo, Lyon, France) and mechanically dissociated in serum-free medium with a fire-narrowed Pasteur pipette. Cells were plated (5.10^5 cells/ml) on 24-well (0.5 ml/well) Nunc culture dishes previously coated successively with poly-L-ornithine ($1.5 \mu\text{g/ml}$; MW, 40,000; Sigma) and culture medium containing 10% fetal calf serum. After removing the last coating solution, cells were seeded in a culture medium composed of a 1:1 mixture of Dulbecco's modified Eagle's medium (DMEM) and Ham's F12 nutrient (GIBCO, Paris), supplemented with glucose (33 mM), glutamine (2 mM), sodium bicarbonate (3 mM), and HEPES buffer (5 mM , pH 7.4). To this culture medium was added a mixture of hormones and salt composed of insulin ($25 \mu\text{g/ml}$), transferrin ($100 \mu\text{g/ml}$), putrescine ($60 \mu\text{g/ml}$), progesterone (20 nM), and sodium selenate (30 nM) (all from Sigma). After 6 d in culture,

more than 95% of the cells were immunocytochemically defined as neurons according to El Etr et al. (1989).

^3H -cAMP assay

Cells cultured in 24 well culture dishes were incubated for 2 hr with $2\text{-}^3\text{H}$ -adenine (27 Ci/mmol , Amersham, $2 \mu\text{Ci}$ per well containing 0.5 ml of culture medium).

Desensitization procedure. DA ($50 \mu\text{M}$) was added to the culture medium 15 min before the end of ^3H -adenine incorporation, in the presence of both propranolol ($1 \mu\text{M}$) and sulpiride ($50 \mu\text{M}$) to prevent β -adrenergic and D2 DA receptor stimulation.

Resensitization procedure. After removal of the desensitization medium, neurons were washed once with 0.5 ml of Krebs' phosphate buffer (in mM: NaCl, 120; NaH_2PO_4 , 15.6; KCl, 4.8; MgSO_4 , 1.2; CaCl_2 , 1.2; glucose, 33.3; pH 7.4), and then incubated for the indicated time in 0.5 ml of the same medium containing appropriate agonists and/or antagonists.

^3H -cAMP measurement. After removal of the resensitization medium, cells were incubated for 5 min in 0.5 ml of Krebs' phosphate buffer containing DA ($50 \mu\text{M}$), sulpiride ($5 \mu\text{M}$), propranolol ($1 \mu\text{M}$), and the nonselective inhibitor of phosphodiesterases, 3-isobutyl-1-methylxanthine (IBMX; 1 mM). The incubation was stopped by replacing the medium with 0.5 ml of ice-cold trichloroacetic acid (6%). Cells were then scraped and $100 \mu\text{l}$ of medium containing 1% SDS, 50 mM Tris-HCl, 3 mM ATP, 3 mM cAMP, pH 7.4, was added to the samples. After centrifugation (10 min at $12000 \times g$), the labeled ATP and cAMP present in 0.5 ml of supernatant were separated according to the procedure described by Salomon et al. (1974). Radioactivity was estimated in 10 ml of Aquasol-2 (New England Nuclear-Du Pont) with a beta scintillation counter. Results, calculated as percentage of conversion of ^3H -ATP into ^3H -cAMP by $(^3\text{H}\text{-cAMP} \times 100)/(^3\text{H}\text{-ATP} + ^3\text{H}\text{-cAMP})$, were expressed as a percentage of the basal values obtained without agonists.

Inositol phospholipid turnover assay

Neurons were incubated for 24 hr in culture medium supplemented with $1 \mu\text{Ci/well}$ of $myo\text{-}^3\text{H}$ -inositol. Cells were then preincubated for 10 min in Krebs' phosphate buffer supplemented with LiCl (10 mM). Methoxamine ($100 \mu\text{M}$) or glutamate ($100 \mu\text{M}$) was then added for 20 min . The incubation was stopped by adding successively $200 \mu\text{l}$ of 0.1% Triton X-100/ 0.1 M NaOH, $200 \mu\text{l}$ of 0.1% Triton X-100/ 0.1 M HCl, and $500 \mu\text{l}$ of water. Cells were then scraped, and ^3H -inositol phosphates were extracted and estimated according to the procedure of El Etr et al., (1989).

Results

Desensitization of the D1 DA-induced formation of cAMP in cortical neurons by preexposure to DA

As illustrated in Figure 1, a 5 min exposure to DA stimulated the formation of ^3H -cAMP in cortical neurons prelabeled with ^3H -adenine ($\text{EC}_{50} = 1.5 \pm 0.2 \mu\text{M}$, mean $\pm$ SEM calculated from three independent experiments, $n = 3$). Most of the response to DA ($50 \mu\text{M}$) was blocked by the D1 antagonist SCH 23390 ($0.2 \mu\text{M}$), suggesting the involvement of D1 receptors in this process. The simultaneous addition of sulpiride ($5 \mu\text{M}$) only slightly enhanced the DA-evoked response. Moreover, the addition of propranolol ($1 \mu\text{M}$) or yohimbine ($1 \mu\text{M}$) did not alter the DA-induced formation of ^3H -cAMP, excluding the involvement of β - and/or α_2 -adrenergic receptors (Fig. 1).

A reduction in the DA-induced formation of ^3H -cAMP was observed when cortical neurons were preincubated for 15 min with DA ($50 \mu\text{M}$) in the presence of both sulpiride ($5 \mu\text{M}$) and propranolol ($1 \mu\text{M}$). In fact, a comparison of the DA dose-response curves in untreated and DA-preincubated cells revealed a marked decrease in the maximal response (-55% , $n = 10$) with only a weak but not significant reduction in the potency of DA (Fig. 2).

The DA-induced desensitization of the cortical D1 response was reversible. Indeed, a slow recovery of the initial D1 DA-

Figure 2. Dose-response curves for the D1 receptor-mediated formation of ^3H -cAMP in cortical neurons pretreated with or without dopamine. Data represent the amount of ^3H -cAMP produced (V) when neurons were incubated for 5 min with increasing concentrations (S) of DA in the presence of sulpiride ($5\text{ }\mu\text{M}$) and propranolol ($1\text{ }\mu\text{M}$), as described in Figure 1. DA-pretreated neurons were preincubated for 15 min in medium containing DA ($50\text{ }\mu\text{M}$), sulpiride ($5\text{ }\mu\text{M}$), and propranolol ($1\text{ }\mu\text{M}$). Cells were then extensively washed with Krebs' phosphate buffer before the estimation of the dopamine-induced formation of ^3H -cAMP as described above. Results are the mean $\pm$ SEM of values obtained in an experiment performed in triplicate. Curves shown are representative of data obtained in three independent experiments made with different neuronal cultures. *Inset*, Eadie's representation of the data.

induced formation of ^3H -cAMP could be observed in cortical neurons after the removal of DA, the half-maximal response being restored within 40 min and the total recovery of the full response being achieved after 80 min (Fig. 3).

Accelerated resensitization of the D1 DA-induced formation of cAMP by stimulation of $\alpha 1$ -adrenergic receptors in cortical neurons

The addition of methoxamine ($100\text{ }\mu\text{M}$), an $\alpha 1$ -adrenergic agonist, during the resensitization period of the cortical D1 response, accelerated the recovery of the D1 DA-induced formation of ^3H -cAMP (Figs. 3, 4), without modifying the fully restored D1 response (estimated after a 80 min resensitization period) (Fig. 3). $\alpha 2$ -Adrenergic receptors did not contribute to the effect of methoxamine since the $\alpha 1$ agonist-induced response was not affected by yohimbine ($1\text{ }\mu\text{M}$) (Fig. 4).

The accelerating effect of methoxamine on the resensitization of the D1 response was suppressed by prazosin ($1\text{ }\mu\text{M}$), an $\alpha 1$ -adrenergic antagonist (Fig. 4). The incubation of cortical neurons with chlorethylclonidine (CEC, $100\text{ }\mu\text{M}$, added during 30 min before methoxamine addition), a compound that has been shown to inactivate $\alpha 1\beta$ -adrenergic receptors irreversibly (Han et al., 1987), also suppressed the effect of methoxamine (Fig. 4). In the absence of methoxamine, neither prazosin nor CEC affected the resensitization of the D1 response (Fig. 4). Interestingly, in contrast to methoxamine, glutamate ($100\text{ }\mu\text{M}$), which

Figure 3. Effects of methoxamine on the resensitization of the D1 response in cortical and striatal neurons. Neurons were preincubated for 15 min with DA ($50\text{ }\mu\text{M}$) as described for Figure 2. Following washing (see Materials and Methods), cells were incubated for various times in the resensitization medium with or without methoxamine ($100\text{ }\mu\text{M}$). All incubations with methoxamine were performed in the presence of yohimbine ($1\text{ }\mu\text{M}$) to prevent a potential stimulation of $\alpha 2$ -adrenergic receptors. Values at zero time correspond to the formation of ^3H -cAMP over a 5 min period, estimated immediately after the 15 min D1-induced desensitization and washing of the cells. Values are the means $\pm$ SEM of data obtained in three experiments, each performed in triplicate. *, significantly different ($p < 0.05$, Student's t test) when compared to control values.

also stimulates phospholipase C activity in cortical neurons, did not modify the resensitization of the D1 response (Table 1).

Lack of effect of methoxamine on the resensitization of the β -adrenergic-induced formation of cAMP in cortical neurons

A 5 min incubation of cortical neurons with isoproterenol ($1\text{ }\mu\text{M}$) resulted in a large formation of ^3H -cAMP (see Fig. 5 legend). This β -adrenergic response was reduced (80%) following prior exposure (15 min) of the cells to isoproterenol ($1\text{ }\mu\text{M}$) according to the procedure used for the estimation of the D1 DA-induced desensitization. The recovery of the full β -adrenergic response was more rapid than that of the D1 response since 75% of the initial response was restored 15 min after the removal of iso-

Figure 4. Effects of α_1 -adrenergic antagonists on the methoxamine-induced acceleration of the resensitization of the D1 response in cortical neurons. Experiments were performed with or without methoxamine (100 μ M, *met.*) as described in Figure 3. When used, yohimbine (*yo.*, 1 μ M) and prazosin (*prz.*, 1 μ M) were added without or with methoxamine during the 15 min resensitization period while chlorethylclonidine (*CEC*, 100 μ M) was added 15 min before the desensitization period. Values are the mean $\pm$ SEM of data obtained in triplicate from a typical experiment. Results are expressed as a percentage of the control D1 response. *, significantly different ($p < 0.05$, Student's *t* test) from the control resensitized D1 response (15 min *res.*). Three other experiments performed with different neuronal cultures led to similar results.

proterol (Fig. 5). In contrast to that observed for the D1 response, the addition of methoxamine (100 μ M) during the resensitization period did not affect the recovery of the β -adrenergic response (Fig. 6).

Accelerated resensitization by glutamate, but not by methoxamine, of the D1 DA-induced formation of cAMP in striatal neurons

Confirming previous results (Chneiweiss et al., 1990), preincubation of striatal neurons with DA (50 μ M) in the presence of

Figure 5. Resensitization of the β -adrenergic response in cortical and striatal neurons. The β -adrenergic-induced formation of 3 H-cAMP was estimated by incubating neurons with isoproterenol (1 μ M) for 5 min (β -response). Desensitization was achieved by adding isoproterenol (1 μ M) for 10 min. Following washing (see Materials and Methods), cells were incubated for various times in the resensitization medium. Values at zero time correspond to the formation of 3 H-cAMP over a 5 min period, estimated immediately after the 10 min isoproterenol-induced desensitization and washing of the cells. Results are expressed as a percentage of the control β -responses (control values). β -Responses represented 272 ± 17 and $436 \pm 23\%$ of increase in basal 3 H-cAMP formation in cortical and striatal neurons respectively, the basal 3 H-cAMP levels being 247 ± 36 and 605 ± 45 d.p.m./well. Values are the means $\pm$ SEM of data obtained in three experiments, each performed in triplicate.

sulpiride and propranolol induced a marked desensitization of the D1 response (Fig. 7). Indeed, the DA-induced formation of 3 H-cAMP was markedly reduced ($\sim 90\%$ of the maximal D1 response) following a prolonged exposure of the cells to DA. Moreover, the resensitization of the striatal D1 response was more rapid than that observed in cortical neurons since the maximal D1 DA-induced formation of 3 H-cAMP was almost completely restored (95%) only 15 min after the removal of DA (Fig. 3).

Interestingly, in contrast to cortical neurons, the addition of methoxamine (100 μ M) during the resensitization period did not modify the rate of resensitization of the D1 response in striatal neurons (Fig. 3). However, this resensitization was accelerated by glutamate (100 μ M) (Fig. 7). Indeed, the full D1 response was observed as rapidly as 5 min after the beginning of the resensitization period in the presence of the excitatory amino acid. NMDA receptors appear to be involved in the glutamate-induced resensitization of the D1 response since the effect of glutamate was completely abolished in the presence of 2-amino-5-phosphonovaleric acid (APV) (50 μ M), a competitive inhibitor of NMDA receptors (Fig. 7). In addition, the D,L- α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX; 50 μ M) was not effective (Fig. 7). Furthermore, 2-amino-3-phosphonopropionic acid (AP3; 1 mM), an antagonist of metabotropic receptors coupled to phospholipase C was also without significant effect (Fig. 7).

As observed with DA, the β -adrenergic agonist isoproterenol (1 μ M) stimulated the formation of 3 H-cAMP in striatal neurons but this effect was less pronounced than that observed in cortical neurons (see Fig. 5 legend). Following a 15 min preexposure to isoproterenol, in order to desensitize the β -adrenergic receptors on these cells, a rapid resensitization of the β -adrenergic re-

Table 1. Respective effects of methoxamine and glutamate on phospholipase C activity and on the resensitization of the D1 response in cortical neurons

	PLC activation (in % of basal activity)	Effect on D1 resensitization (in % of control resensitized response)
Methoxamine (100 μ M)	$191 \pm 7^*$	$180 \pm 14^{**}$
Glutamate (100 μ M)	$242 \pm 12^*$	97 ± 16

The production of 3 H-inositol phosphate induced by a 5 min incubation of cortical neurons in the presence of either methoxamine or glutamate is expressed as a percentage of the basal 3 H-inositol phosphate formation. Values are the mean $\pm$ SEM of data obtained in three separate experiments, each being made in triplicate. The effect of methoxamine or glutamate on the resensitization of the D1 response was expressed as a percentage of the resensitized D1 response estimated under control conditions (see Fig. 4). Values are the mean $\pm$ SEM of three independent experiments.

* Significantly different ($p < 0.05$, Student's *t* test) from the basal value (342 ± 14 d.p.m./well).

** Significantly different ($p < 0.05$, Student's *t* test) from the control resensitized D1 response.

Figure 6. Respective effects of methoxamine and glutamate on the resensitization of the β -adrenergic response in cortical and striatal neurons. The β -adrenergic-induced formation of ^3H -cAMP was estimated by incubating neurons with isoproterenol ($1 \mu\text{M}$) for 5 min (β -response). Desensitization and resensitization procedures were achieved as described for Figure 5. A 5 min resensitization period was chosen (5 min res.). When used, methoxamine (met., $100 \mu\text{M}$) was added during the resensitization period as described in Figure 4. Similarly, when used, glutamate (glu., $100 \mu\text{M}$) was added during the beginning of the resensitization period. Values are the mean $\pm$ SEM of data obtained in triplicate in a typical experiment. Results are expressed as a percentage of the control β -responses. Three other experiments made with different cultures led to similar results. *, significantly different ($p < 0.05$, Student's t test) from the corresponding control value (5 min res.).

sponse was observed since the initial maximal isoproterenol-induced formation of ^3H -cAMP was seen after only 10 min of the resensitization period (Fig. 5). As demonstrated for the D1 response, glutamate ($100 \mu\text{M}$) also accelerated the resensitization of the β -adrenergic response (Fig. 6).

Selective involvement of protein kinase C in the $\alpha 1$ -adrenergic accelerated resensitization of the D1 response in cortical neurons

As already indicated, $\alpha 1$ -adrenergic receptors appear to be involved in the modulation of the resensitization of the cortical D1 DA-induced formation of ^3H -cAMP. Since $\alpha 1$ -adrenergic receptors are coupled to phospholipase C (Han et al., 1987), the stimulation of these receptors may result in a subsequent activation of protein kinase C (PKC). This led us to examine the effect of phorbol myristate acetate (PMA; 10 nM), a PKC-activating agent, on the resensitization rate of the D1 response in cortical neurons.

The addition of PMA (10 nM) instead of methoxamine during the resensitization period accelerated the recovery of the D1 response in cortical neurons (Fig. 8). Interestingly, when added with DA, PMA altered neither the full DA-induced formation of ^3H -cAMP (data not shown) nor the desensitization of the D1 response (Fig. 8). Furthermore, staurosporine ($0.5 \mu\text{M}$), a non-selective inhibitor of PKC, markedly decreased the accelerating effect of methoxamine ($100 \mu\text{M}$) on the resensitization of the D1 response (Table 2).

When cells were pretreated for 40 hr with PMA (100 nM), a procedure known to downregulate the activity of PKC, no modification of the DA-induced desensitization could be observed (Table 2). The involvement of PKC in the $\alpha 1$ -adrenergic ac-

Figure 7. Accelerating effect of glutamate on the resensitization of the D1 response in striatal neurons: involvement of NMDA receptors. Desensitization with DA ($50 \mu\text{M}$, desensitized) and resensitization were performed as described for Figure 2, the D1 response (5 min formation of ^3H -cAMP in the presence of $50 \mu\text{M}$ DA) was estimated after a 5 min resensitization period (5 min res.), and glutamate (glu., $100 \mu\text{M}$) was added during the resensitization period. When used, AP3 (1 mM), DNQX ($50 \mu\text{M}$), or APV ($50 \mu\text{M}$) was added with glutamate. Data are the mean $\pm$ SEM obtained in triplicate in a typical experiment; results are expressed as a percentage of the control D1 response. *, significantly different ($p < 0.05$, Student's t test) when compared to the corresponding control value (5 min res.); **, significantly different ($p < 0.05$, Student's t test) when compared to the corresponding glutamate value (5 min res. + glu.).

celeration of the resensitization of the D1 response was further suggested by the suppression of the effect of methoxamine in PMA-pretreated cortical neurons (Table 2).

Surprisingly, in striatal neurons, the addition of PMA (10 nM) during the resensitization period did not modify the recovery of the D1 response, indicating that PKC is not involved in the accelerating effect of glutamate ($100 \mu\text{M}$) on the resensitization of the D1 response (Fig. 8).

Discussion

The prolonged exposure of cells to hormones or neurotransmitters reduces their responsiveness to further stimulation. This desensitization process has been extensively described in numerous studies dealing with receptors coupled to adenylate cyclase, particularly for the β -adrenergic receptors (for review, see Haganir and Greengard, 1990). Briefly, the process of desensitization involves a rapid and reversible uncoupling of the receptors from the α -subunits of G-proteins, followed by a slow reversible downregulation of the receptors. Two major mechanisms have been shown to be involved in the desensitization of β -adrenergic receptors: homologous desensitization, which involves a phosphorylation of the receptor by a β -adrenergic receptor-specific kinase (β -ARK), and heterologous desensitization, in which the receptor is phosphorylated by cAMP-dependent protein kinase (Clark et al., 1990; Johnson et al., 1990; for review, see Haganir and Greengard, 1990).

Figure 8. Effect of phorbol myristate acetate (PMA) on the desensitization and on the resensitization of the D1 response in cortical and striatal neurons. Experiments with cortical and striatal neurons were performed as described for Figures 4 and 7, respectively. PMA (10 nM) was added during the desensitization (des. + PMA) or the resensitization period (res. + PMA). *, significantly different ($p < 0.05$, Student's t test) from the resensitized D1 response (res.). Values are the mean $\pm$ SEM of data obtained in triplicate in a typical experiment. Results are expressed as a percentage of the control D1 response. Three other experiments made with different cultures led to similar results.

Desensitization of D1 DA receptors in cultured cortical and striatal neurons

In contrast to β -adrenergic receptors, relatively little is known on the biochemical mechanisms involved in the regulation of D1 DA receptor sensitivity. Nevertheless, studies performed on cell cultures have demonstrated that desensitization of D1 DA receptors occurs following sustained exposure of neurons to DA (Balmforth et al., 1990; Barton and Sibley, 1990; Chneiweiss et al., 1990). A similar homologous desensitization has been observed in several *in vivo* studies in which striatal DA release was enhanced by amphetamine treatment (Barnett and Kuczenski, 1986; Robert-Lewis et al., 1986; Roseboom and Gnegy, 1989). In agreement with these observations, we demonstrated in the present study, that a 15 min preincubation with DA of either cortical or striatal neurons from the embryonic rat in primary culture resulted in a reversible decrease in the responsiveness of the D1 DA receptors to further DA stimulation, as estimated by the 5 min formation of ^3H -cAMP in intact cells. This effect was indeed mediated by D1 DA receptors since the experiments were performed in the presence of sulpiride and propranolol to eliminate any possible interaction of DA with both D2 DA and β -adrenergic receptors. In addition, the DA-induced desensitization was suppressed in the presence of the D1 antagonist SCH 23390.

The rapid and complete recovery of the D1 response, which was faster in striatal than in cortical neurons, strongly suggests that this desensitization involved the uncoupling of D1 DA receptors from the G-protein linked to adenylate cyclase rather than a loss of receptors from neuronal membranes. In fact, longer periods of exposure to DA are required for the occurrence of a downregulation of D1 DA receptors (Chneiweiss et al., 1990; Bates et al., 1991). This rapid and reversible desensitization

Table 2. Effect of methoxamine on the resensitization of the D1 response in cortical neurons following long-term pretreatment with PMA

	Des.	Res.	Res. + met.	Res. + met. + stauro.
Control				
cortical neurons	21.6 $\pm$ 4	47.7 $\pm$ 5	88.6 $\pm$ 7*	42 $\pm$ 9**
PMA-pretreated				
cortical neurons	27.2 $\pm$ 7	37.2 $\pm$ 5	45.4 $\pm$ 11**	—

Cortical neurons were pretreated for 48 hr with PMA 100 nM; then, following extensive washing, the D1 desensitization (des.) and the resensitization (res.) of the D1 response in the presence (res. + met.) or absence of methoxamine were induced and estimated as described for Figures 3 and 4, respectively. Staurosporine (0.5 μM) was added with methoxamine during the resensitization period only in control cortical neurons. Results are expressed as a percentage of the control D1 response. Values represent the mean $\pm$ SEM of data obtained in triplicate in a typical experiment. Three other experiments made with different neuronal cultures led to similar results.

* Significantly different ($p < 0.05$, Student's t test) from the control resensitized D1 response (res.).

** Significantly different ($p < 0.05$, Student's t test) from the corresponding value of the resensitized D1 response in the presence of methoxamine (res. + met.) in control cortical neurons.

process may result from the phosphorylation of the D1 DA receptors by a specific protein kinase as shown for β -adrenergic receptors (Balmforth et al., 1990).

Role of heterologous transmitters in the *in vitro* resensitization of the D1 response in cortical and striatal neurons

As already mentioned, little is known of the biochemical events underlying the resensitization of D1 DA receptor-mediated responses. Our study indicates that transmitters (or agonists) other than DA can play a prominent role in the regulation of the resensitization rate of the D1 DA-evoked formation of cAMP. Indeed, both methoxamine (through $\alpha 1$ -adrenergic receptors) and glutamate (through NMDA receptors) can accelerate this resensitization process. However, these regulations are structure-specific since the $\alpha 1$ -adrenergic acceleration of the resensitization of the D1 response was observed in cortical but not in striatal neurons while the reverse was found with glutamate. Finally, since there is no evidence for synaptic contacts in 6-d-old neurons in primary culture (El Etr et al., 1989), a population of cortical neurons must possess both D1 DA and $\alpha 1$ -adrenergic receptors while both D1 DA and glutamatergic receptors should be colocalized on a population of striatal neurons. This is in agreement with electrophysiological studies indicating that some cortical cells in the prefrontal cortex are sensitive to both DA and noradrenaline (Bunney and Aghajanian, 1976) and that a population of striatonigral neurons possess both D1 DA and glutamatergic receptors (Cepeda et al., 1992).

Acceleration of the resensitization of the D1 response in cortical neurons by the $\alpha 1$ -adrenergic agonist methoxamine

Several observations demonstrated that the stimulation of $\alpha 1$ -adrenergic receptors accelerates the resensitization of D1 DA receptors coupled to adenylate cyclase in cortical neurons. Methoxamine accelerated the recovery of the full D1 response and this was even observed in the presence of yohimbine, which prevents the possible stimulation of $\alpha 2$ -adrenergic receptors. Moreover, the effect of methoxamine was prevented by both prazosin, a nonselective $\alpha 1$ -adrenergic antagonist, and CEC, which is known to inactivate $\alpha 1\text{b}$ -adrenoceptors irreversibly (Han et al., 1987).

The specificity of the stimulatory effect of methoxamine on the resensitization of the D1 response was demonstrated in several ways. (1) The addition of methoxamine did not affect the full DA-induced formation of ^3H -cAMP (before the desensitization or after the resensitization) or the DA-induced desensitization. (2) As shown by experiments made with isoproterenol, methoxamine did not affect the resensitization of the β -adrenergic-induced formation of ^3H -cAMP in cortical neurons. (3) As previously indicated, methoxamine did not modify the rate of resensitization of the striatal D1 response.

Further studies will be necessary in order to elucidate the molecular mechanism(s) involved in the stimulatory effect of methoxamine on the D1 DA receptor resensitization process. Nevertheless, our results already provide some preliminary indications. A possible modulation of phosphodiesterase activity, as suggested by Beavo and Reifsnnyder (1990), can be excluded since all our experiments were performed in the presence of the non selective phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine. As already indicated, $\alpha 1\text{b}$ -adrenergic receptors appear to be involved in the stimulatory effect of methoxamine. These receptors are coupled to phospholipase C, leading to the formation of diacylglycerol and inositol phosphates. Diacylglycerol and/or calcium mobilized from intracellular stores by inositol phosphates can stimulate PKC and therefore could potentially phosphorylate a protein involved in the accelerated resensitization of the D1 response. The stimulatory effect of methoxamine persisted in both the absence of extracellular calcium or the presence of a calcium chelating agent (data not shown), suggesting that intracellular calcium could be eventually implicated in this process. Experiments performed with the phorbol ester PMA, which mimics the effect of diacylglycerol, support the involvement of PKC. Indeed, the direct activation of PKC by PMA reproduced the effect of methoxamine. Moreover, the prolonged (40 hr) preexposure of cortical neurons to PMA, a procedure that desensitizes PKC (Hepler et al., 1988), prevented the accelerating effect of methoxamine on the resensitization of the D1 response in cortical neurons. Providing further evidence for the involvement of distinct regulatory mechanisms in the desensitization and resensitization processes, the inactivation of PKC induced by long term pretreatment of cortical neurons with PMA did not affect the desensitization of D1 DA receptors. Experiments are in progress to identify the protein that is phosphorylated by PKC following exposure to methoxamine and is responsible for the accelerated recovery of the full D1 DA response in cortical neurons.

Acceleration by glutamate of the resensitization of the D1 response in striatal neurons

Through cAMP formation and protein kinase A activation, the stimulation of D1 DA receptors in the striatum leads via cAMP to the phosphorylation of DARPP 32, an inhibitor of phosphatase I (Hemmings et al., 1984). As elegantly shown, by acting on NMDA receptors, glutamate activates calcineurin, which dephosphorylates DARPP 32 and thus counteracts some of the effects of DA (Halpain et al., 1990). Interestingly, our study demonstrates a new regulatory process of DA transmission by glutamate. Indeed, glutamate was found to accelerate the resensitization of the D1 DA-induced formation of ^3H -cAMP in cultured striatal neurons.

NMDA receptors appear to be involved in the stimulatory effect of glutamate on the resensitization rate of striatal D1 DA receptors. Indeed, the glutamate-induced facilitation of this re-

sensitization process was totally prevented by APV, a competitive antagonist of NMDA receptors, while DNQX, an AMPA antagonist, was without effect. In addition to ionotropic receptors, striatal neurons in primary culture possess metabotropic glutamatergic receptors coupled to phospholipase C (Sladeczek et al., 1985). However, these receptors do not seem to be implicated in the stimulatory effect of glutamate. Indeed, the phorbol ester PMA did not accelerate the rate of recovery of the D1 response in striatal neurons, suggesting that PKC is not involved in this process. Confirming this finding, AP3, an antagonist of metabotropic receptors coupled to phospholipase C did not significantly modify the stimulatory effect of glutamate. Recent studies from our laboratory have demonstrated that a G_{off} protein is present in striatonigral neurons possessing D1 DA receptors (Hervé et al., 1993) and a specific adenylate cyclase sensitive to DA has recently been identified in the striatum (Glatt and Snyder, 1993). These observations could eventually explain why distinct intracellular biochemical events are involved in the regulation of the recovery of the full D1 DA response in cortical and striatal neurons.

Some specificity was also found in the stimulatory effect of glutamate on the resensitization of the striatal D1 DA receptors. Indeed, glutamate affected neither the full DA response nor the DA-induced desensitization. Moreover, glutamate did not affect the resensitization rate of the cortical D1 DA receptors although there is evidence, particularly in the prefrontal cortex, that there are cortical cells that are sensitive to both DA and glutamate (Pralong and Jones, 1993). However, glutamate also accelerated the resensitization of β -adrenergic receptors coupled to adenylate cyclase in striatal neurons, indicating that the glutamatergic and β -adrenergic receptors involved are colocalized on the same population of striatal neurons. These neurons could also possess D1 receptors since we have previously shown using additivity experiments that D1 DA and β -adrenergic receptors are in part colocalized in a population of striatal neurons (Chneiweiss et al., 1985).

Relevance of the stimulatory effects of methoxamine and glutamate on the resensitization processes of cortical and striatal D1 receptors to in vivo findings

Although the questions raised in the present study were suggested by *in vivo* findings, the interpretation of *in vivo* events in adult rats based on data obtained from cultured embryonic neurons can be criticized. For instance, the long term development of the denervation supersensitivity of the cortical or striatal D1 DA receptors may involve additional or different mechanisms than those implicated in the rapid resensitization processes described in the present study. In fact, it has been reported in several binding studies that following denervation, changes in the sensitivity of receptors coupled to adenylate cyclase are in most cases very likely to be associated with the appearance of new receptors on the postsynaptic neuronal membrane (Sporn et al., 1976). As already discussed, this is certainly not the case in our *in vitro* experimental paradigm. However, the amplitude of the full DA responses in cortical and striatal embryonic neurons, when compared to those observed in tissues of normal adult rats, suggests that D1 DA receptors in neuronal cultures are likely to be in an hypersensitive state. Although not strictly comparable, the hypersensitive D1 DA receptors in cultured neurons and in adult rats with denervation of DA neurons may thus have some common properties.

According to our *in vivo* data, the presence of the cortical

noradrenergic innervation plays a prominent permissive role in the appearance of the denervation supersensitivity of the prefrontocortical D1 DA receptors (Tassin et al., 1986). Moreover, $\alpha 1$ -adrenergic receptors sensitive to prazosin were shown to be involved in this heterologous receptor regulation (Trovero et al., 1992b). This is in agreement with our present results. Indeed, by acting on receptors colocalized on identical cortical neurons, methoxamine was found to accelerate the resensitization of the D1 response by stimulating $\alpha 1$ -adrenergic receptors sensitive to prazosin. However, as indicated by autoradiographic studies performed on slices of the adult rat brain, D1 binding sites labeled with the D1 DA antagonist ligand, ^3H -SCH 23390, are mainly located in deep layers of the prefrontal cortex (V and VI) while high-affinity ^3H -prazosin binding sites are mainly concentrated in layer III (Trovero et al., 1992a). Nevertheless, as suggested by our *in vitro* results, this does not exclude the possibility that $\alpha 1$ -adrenergic receptors sensitive to prazosin are located with the D1 DA receptors on a population of cortical cells. In fact, these $\alpha 1$ -adrenergic receptors could be partly distributed on apical dendrites of pyramidal neurons present in deep layers.

It is well established that the 6-OHDA-induced denervation of the nigrostriatal DA neurons leads to the development of the denervation supersensitivity of the striatal D1 DA receptors coupled to adenylate cyclase. Changes in signals conveyed by the corticostriatal glutamatergic neurons induced by bilateral alteration of catecholaminergic transmission in the prefrontal cortex prevent this denervation supersensitivity of striatal D1 DA receptors (Hervé et al., 1989). Moreover, D1 DA agonists have been shown to activate cFos expression in striatal neurons and these effects were found to be blocked by D1 antagonists (Robertson et al., 1989). The activation of adenylate cyclase is involved in this phenomenon since a positive regulation of cFos by cAMP has been demonstrated and it was also shown that the cFos promoter contains one or more cAMP response elements (Morgan and Curran, 1991). Interestingly, the D1 DA receptor-mediated cFos activation can be blocked by pretreatment with the NMDA glutamatergic antagonist MK801 (Snyder-Keller, 1991; Paul et al., 1992). Furthermore, in rats with unilateral 6-OHDA-induced lesion of the nigrostriatal DA neurons, both the marked D1-induced activation of cFos in the ipsilateral striatum and the contralateral turning induced by the mixed D1 and D2 agonist apomorphine can be prevented by transection of corticostriatal afferents (Cenci and Björklund, 1993). Therefore, the full expression of the D1 receptor-mediated response depends on an intact glutamatergic corticostriatal input. Our *in vitro* results demonstrating that glutamate increases the rate of resensitization of D1 DA receptors by acting through NMDA receptors, can be compared with *in vivo* results. They provide the cellular basis for the modulation of DA transmission by glutamate through the effect of the excitatory amino acid on the sensitivity of D1 receptors coupled to adenylate cyclase in striatal target cells.

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