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# Role of *N*-methyl-D-aspartate (NMDA) receptors in the response of extrapyramidal neurotensin and dynorphin A systems to cocaine and GBR 12909

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Changes in the activity of dopamine (DA) receptors substantially alter the cerebral concentration of neurotensin (NT) and dynorphin A (Dyn). The administration of a dopaminergic D-1 receptor agonist, SKF 38393, increases the concentration of neurotensin-like immunoreactivity (NTLI) in the striatum of the rat, whereas the D-2 receptor agonist, LY 171555, reduces the NTLI content [1]. SKF 38393 treatment also increases the concentration of nigral dynorphin-like immunoreactivity (DLI) [2]. Interestingly, because of their ability to enhance dopaminergic activity, the DA uptake blockers, cocaine and GBR 12909 [1-(2-[bis(4-fluorphenyl)-methoxy]-ethyl-4-(3-phenyl-propyl)piperazine], as well as the DA releaser, methamphetamine, cause substantial increases in striatal and nigral levels of NTLI and DLI [3–7].

Several studies suggest an interaction between the dopaminergic and glutamatergic systems. Glutamate can induce DA release [8, 9] while DA can alter glutamate reuptake [10] and release [11, 12]. Moreover, the release of both DA and glutamate is required for the development of postischemic damage in the neostriatum [13]. Consequently, the glutamatergic system could participate with the dopaminergic system in the regulation of peptidergic systems. This is supported by the ability of MK-801 ([+]-5-methyl-10,11-dihydro-5*H*-dibenzo [*a*,*d*]-cyclohepten-5,10-imine maleate; dizocilpine), a noncompetitive antagonist at the *N*-methyl-D-aspartate (NMDA)-type glutamatergic receptor, to prevent the methamphetamine-induced increase in striatal NTLI and DLI concentrations [14, 15]. Although the mechanisms vary, administration of DA uptake blockers causes increases in levels of extrapyramidal NTLI and DLI much like methamphetamine; thus, the objective of this study was to determine the role of NMDA receptors in the cocaine- and in the GBR 12909-induced changes in the extrapyramidal neuropeptide systems.

## *Materials and Methods*

Male Sprague–Dawley rats (180–230 g) were housed six per cage in a temperature-controlled room with a 12-hr light/dark cycle and given access to food and water *ad lib*. Animals received five intraperitoneal injections of cocaine (30 mg/kg/dose; National Institute on Drug Abuse, Rockville, MD) dissolved in saline or of GBR 12909 (20 mg/kg/dose; NOVO Industri A/S, Copenhagen, Denmark) dissolved in propylene glycol at 6-hr intervals 15 min after an intraperitoneal administration of 0.9% NaCl or MK-801 (1.7 mg/kg/dose; Merck, Sharp & Dohme, Rahway, NJ) dissolved in saline. The drug doses are expressed as the free base. The animals were killed by decapitation 1 hr after the last dose, which is the time of maximum peptide response to cocaine [5]. The brains were removed rapidly and the striatum was dissected out on a cold plate. After freezing the brain on dry ice, the substantia nigras were excised with a microdissecting knife. The tissues were stored at −80°C until assayed.

The response of the NT and Dyn systems was assessed by measuring the tissue concentrations of NTLI and DLI according to previously reported techniques [3, 4] employing selective NT and Dyn antisera in a radioimmunoassay

which reliably detected 5 and 8 pg of NTLI and DLI respectively. Proteins were determined by the method of Bradford [16], and the NTLI and DLI concentrations were calculated as picograms per milligram of protein. Differences between the means were analyzed by an analysis of variance (ANOVA) test followed with a Scheffe F-test with the level of significance set at $P < 0.05$.

*Results and Discussion*

The role of NMDA receptors in the response by extrapyramidal NT and Dyn systems to cocaine treatment was examined (Fig. 1). As previously demonstrated, striatal and nigral NTLI concentrations were raised to 210% of control 1 hr after the last dose of cocaine. Cocaine treatment elevated striatal and nigral DLI levels to 324 and 567% of control respectively (Fig. 1B). Blocking the NMDA receptors with MK-801 prevented the drug-induced elevations in NTLI in both tissues while significantly attenuating the increases in DLI content. MK-801 had no effect alone on the NTLI and DLI levels from either brain structures.

We also examined the effects of MK-801 on the changes in extrapyramidal NTLI and DLI levels induced by the selective DA uptake blocker, GBR 12909 (Fig. 2). Like cocaine, multiple doses of GBR 12909 substantially increased striatal and nigral levels of NTLI (182 and 223% of control respectively) (Fig. 2A). GBR 12909 also increased striatal and nigral DLI levels to 695 and 436% of control respectively (Fig. 2B). MK-801 completely blocked all GBR 12909-induced increases in neuropeptide content and, as in Fig. 1, had no effect of its own on extrapyramidal NTLI and DLI levels.

This study demonstrates that the increases in NTLI and DLI content induced by treatment with cocaine or GBR 12909 can be blocked or attenuated by antagonizing the NMDA receptors with MK-801. Because MK-801 displays

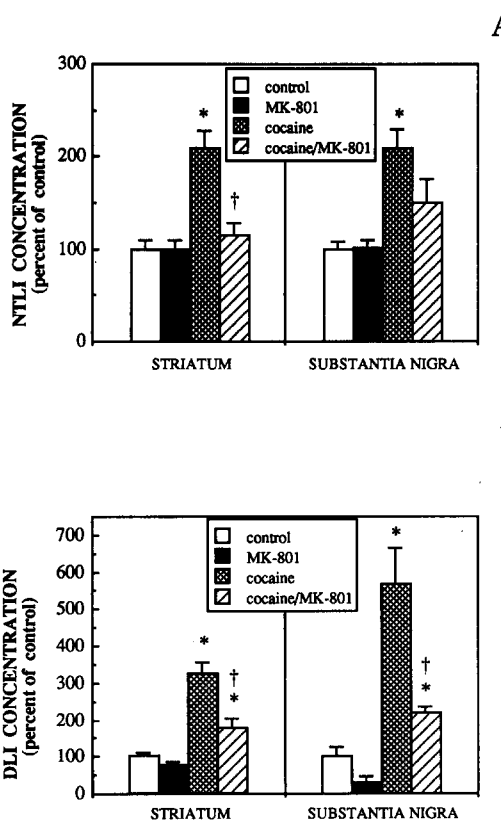


Fig. 1. Effect of MK-801 on the cocaine-induced increase in striatal and nigral NTLI (A) and DLI (B) concentrations. Rats were given five doses at 6-hr intervals of 0.9% NaCl or cocaine (30 mg/kg/dose, i.p.) 15 min after the administration of the NMDA antagonist, MK-801 (1.7 mg/kg/dose, i.p.), or 0.9% NaCl and were killed 1 hr after the last dose. Each bar represents the mean ± SEM for each treatment group, expressed as a percentage of control NTLI (A) or DLI (B) concentration (N = 3–6 per group). The average control NTLI concentrations were 87.8 ± 9.1 and 336 ± 26.8 pg/mg protein in the striatum and substantia nigra respectively. The average striatal and nigral control DLI concentrations were 98 ± 9 and 174 ± 48 pg/mg protein respectively. Key: (*) $P < 0.05$ versus control, and (†) $P < 0.05$ versus cocaine-treated group.

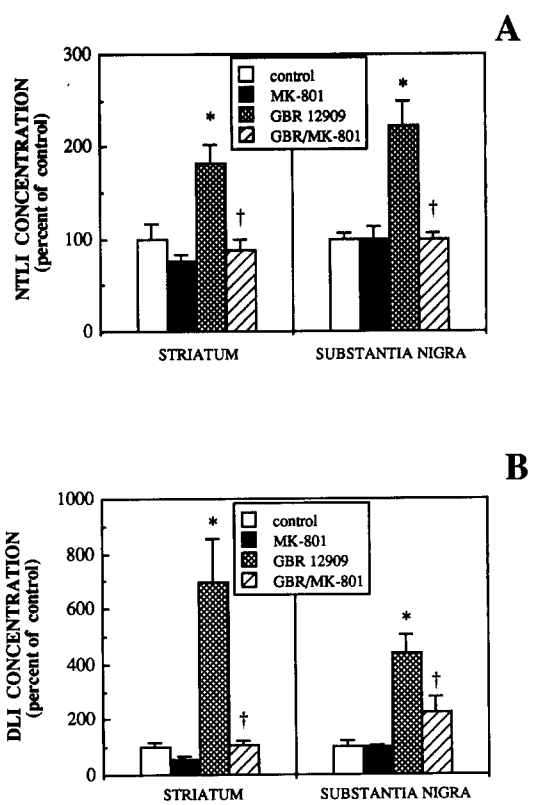


Fig. 2. Effect of MK-801 on the GBR 12909-induced increase in striatal and nigral NTLI (A) and DLI (B) concentrations. Animals were treated as described in Fig. 1 except that rats received GBR 12909 (20 mg/kg/dose, i.p.) instead of cocaine. Each bar represents the mean ± SEM for each treatment group, expressed as a percentage of control NTLI (A) or DLI (B) concentration (N = 4–6 per group). The average control NTLI concentrations were 191 ± 30 and 701 ± 41 pg/mg protein in the striatum and substantia nigra respectively. The average striatal and nigral control DLI concentrations were 110 ± 22 and 264 ± 45 pg/mg protein respectively. Key: (*) $P < 0.05$ versus control, and (†) $P < 0.05$ versus GBR 12909-treated group.

low affinity for DA receptors [17], it is not likely that this effect is due to the direct antagonism of MK-801 on DA receptors. Our results suggest that glutamaterigic systems, through NMDA receptors, participate in mediating the effects of these drugs on neuropeptide pathways.

These drug-induced increases in peptides are associated with an elevation in dopaminergic transmission. Specifically, cocaine-induced increase in striatal or nigral NTLI and DLI is attenuated or blocked by both D-1 and D-2 receptor antagonists [5–7]. Although cocaine is also a potent serotonin reuptake blocker [18], serotonin does not likely contribute to the peptide responses because serotonin uptake blockers do not cause cocaine-like effects on neuropeptides like those observed for GBR 12909 (Fig. 2) [5, 6]. Moreover, serotonin depletion fails to alter these cocaine-mediated effects [5].

The significance of the cocaine- and GBR 12909-induced elevations in NTLI and DLI concentrations is not apparent without more information about synthesis or release of these peptides. The increases in peptide content could represent an accumulation due to: (1) a decrease in peptide release, (2) a decrease in extracellular peptide metabolism, or (3) an increase in peptide synthesis. Additional studies are necessary in order to identify which of these possibilities is correct.

The ability of both DA and NMDA receptor antagonists to interfere with the cocaine-induced changes in peptides suggests an interaction between the glutamatergic and dopaminergic systems in the regulation of the peptidergic systems, although the precise nature of that interaction remains to be determined. Presynaptic regulation of DA release by glutamate through NMDA receptors has been described in the striatum [9], while others report presynaptic regulation of glutamate release by DA in the same structure [11, 12]. Also, lesions induced with the NMDA agonist, quinolinic acid, reduce the number of neostriatal D-1 receptors [19], which suggests that both receptor types are present on the same cells. It remains to establish which of these interactions between the dopaminergic and glutamatergic systems is involved in the control of NTLI and DLI levels.

This study demonstrates that MK-801, a noncompetitive NMDA receptor antagonist, blocked or attenuated the cocaine- and the GBR 12909-induced increase in striatal and nigral NTLI and DLI levels. The ability of this glutamatergic receptor antagonist to block the DA-mediated response suggests that glutamatergic systems play an essential role in the interaction between extrapyramidal peptidergic and dopaminergic pathways. Because NMDA receptors also participate in the central changes induced by methamphetamine, these results suggest that glutamatergic systems play an important role in mediating the effects of stimulants of abuse in general.

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# Thyroid hormone-independent regulation of mitochondrial glycerol-3-phosphate dehydrogenase by the peroxisome proliferator clofibric acid

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Mitochondrial glycerol-3-phosphate dehydrogenase [EC 1.1.99.5] (*sn*-glycerol-3-phosphate:acceptor 2-oxidoreductase) is regulated by thyroid hormone *in vivo* [1–5] and *in vitro* [6, 7]. Thyroid hormone treatment of rats stimulates glycerol-3-phosphate dehydrogenase activity in a number of tissues [1–3], with the largest increase occurring in the liver. This activity is also increased by treatment of animals with a number of hypolipidemic drugs [8–11] and structurally unrelated compounds such as di(2-ethylhexyl)phthalate (DEHP)* [12]. The term peroxisomal proliferator is applied to these hypolipidemic drugs [11, 13] and DEHP [14] since they increase peroxisomal β-oxidation activity.

From the *in vivo* results, it was inferred that peroxisomal proliferators increase hepatic glycerol-3-phosphate dehydrogenase activity through thyroid hormone based on the known thyroid hormone-dependent regulation and the observation that the stimulation with either clofibrate [9] or DEHP [12] was attenuated in hypothyroidism. Interpretation of *in vivo* data is hazardous, however, since assessment as to whether hepatic effects are primary or secondary to actions of thyroid hormone (or its lack) on other tissues is problematic. Primary cultures of hepatocytes have been used to show that clofibric acid (the active metabolite formed upon absorption of clofibrate [15]) stimulates glycerol-3-phosphate dehydrogenase activity [16], but the culture medium employed was supplemented with fetal bovine serum which contains thyroid hormone so that rigorous testing of a thyroid hormone requirement was not undertaken.

We have previously used a fully defined medium with primary cultures of rat hepatocytes to show that thyroid hormone is not required for stimulation of peroxisomal β-oxidation activity by clofibric acid [17] and now have applied this system to the study of the role of thyroid hormone in the stimulation of mitochondrial glycerol-3-phosphate dehydrogenase by clofibric acid and the relationship of this activity to that of peroxisomal β-oxidation.

## *Materials and Methods*

Biochemical reagents including collagenase (Sigma Type IV) were obtained as previously described [17]. Hepatocytes from adult (200–300 g) male Fischer F344 rats (Charles River, Wilmington, MA) were isolated and cultured as reported previously [17] with some modifications. Primaria-coated culture dishes (Baxter Scientific, Romulus, MI) were used. The attachment medium consisted of the basal medium, described below, with the following changes. Dimethyl sulfoxide was omitted, 10% (v/v) fetal bovine serum was added, and antibiotic levels were 100 units/mL penicillin G, 100 μg/mL streptomycin, and 50 μg/mL gentamicin. After 1 hr in 7 mL of attachment medium, nonadhering cells and medium were removed, and culturing was continued using 10 mL of basal medium.

The basal medium consisted of L-15 (modified) with L-glutamine supplemented as follows: 75 units/mL of penicillin G, 75 μg/mL of streptomycin, 30 μg/mL of gentamicin, 1 μM insulin, 1 μM dexamethasone, 10 mM glucose, 1 mM succinate, 2 mM L-carnitine, 0.4% (v/v) dimethyl sulfoxide, and 25 mM Hepes (pH 7.4). A sample of cells was collected after attachment and designated as Time 0. Unless noted otherwise, clofibric acid was added immediately following attachment using dilutions of a 250 mM clofibric acid stock solution in 500 mM sodium carbonate. 3,5,3′-Triiodothyronine ($T_3$) additions were made using a 1 mM stock solution of the sodium salt in 0.5 mM NaOH. Control media contained equivalent volumes of vehicle. All media were replaced every 24 hr. The harvesting of cells and isolation of an organellar pellet are described elsewhere [17].

Glycerol-3-phosphate dehydrogenase activity was assayed in organellar fractions as described previously [17]. Data are expressed as the ratio of glycerol-3-phosphate dehydrogenase : citrate synthase rates (GPD : CS). The ratio was used since organellar specific activity of citrate synthase declined with time in culture whereas homogenate citrate synthase specific activities were constant, indicating that the proportion of non-mitochondrial protein in the organellar fraction increased with time in culture. Citrate synthase and fatty acyl-CoA oxidase (the rate-limiting enzyme for peroxisomal fatty acid β-oxidation [18]) activities and protein were determined as described previously [17].

## *Results and Discussion*

Addition of clofibric acid increased mitochondrial glycerol-3-phosphate dehydrogenase activity in a time-dependent manner in the absence of exogenous thyroid hormone and serum (Fig. 1). The time course paralleled that for the peroxisomal marker, fatty acyl-CoA oxidase, with both activities reaching their maximum after 144 hr of exposure.

Although thyroid hormone was not added, the possibility

* Abbreviations: DEHP, di(2-ethylhexyl)phthalate; EGTA, ethylenebis(oxyethylenenitrilo)tetraacetic acid; GPD:CS, ratio of glycerol-3-phosphate dehydrogenase activity to citrate synthase activity; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; Tris, tris(hydroxymethyl)aminoethane; and $T_3$, 3,5,3′-triiodothyronine.