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Chronic GBR 12909 administration differentially alters prodynorphin gene expression compared to cocaine

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Abstract

The effect of the selective dopamine uptake inhibitor 1-[2-[*bis*(4-flourophenyl)methoxy]ethyl]-4-[3-phenylpropyl]piperazine dihydrochloride (GBR 12909) was examined on prodynorphin gene expression. GBR 12909 or vehicle was continuously infused for 7 days via osmotic minipump, or injected daily into male rats. Both continuous infusions and daily injections of GBR 12909 produced significant decreases in prodynorphin expression in the hypothalamus (37% and 31% decreases, respectively). There were no significant changes in the caudate putamen, hippocampus or nucleus accumbens. One injection of GBR 12909 had no effects on prodynorphin expression in any of the brain regions studied, suggesting that the effect in the hypothalamus is not an acute effect. As previously reported for other treatment regimens, continuous infusion of cocaine produced a 35% significant decrease in the hypothalamus, consistent with the effects of GBR 12909. In contrast to GBR 12909, however, cocaine also produced a significant increase in prodynorphin expression in the caudate putamen. Thus, chronic inhibition of dopamine uptake can regulate prodynorphin expression in the hypothalamus. In contrast, the increase in the caudate putamen following cocaine administration may not be related to the inhibition of dopamine uptake, since it was not produced by a selective dopamine uptake inhibitor. These findings suggest that regulation of prodynorphin gene expression by cocaine in the caudate putamen may be mediated by the inhibition of norepinephrine or serotonin uptake, by a combination of effects on two or three monoamine transporters, or by a mechanism unrelated to transporter inhibition. © 2001 Published by Elsevier Science B.V.

Keywords: Dynorphin; Dopamine uptake; Dopamine transport; Cocaine; GBR 12909

1. Introduction

The expression of both opioid receptors and opioid peptides is altered following chronic administration of cocaine to rats. Continuous administration of cocaine via subcutaneously implanted osmotic minipumps (Hammer, 1989; Izenwasser et al., 1996) or repeated daily injections (Unterwald et al., 1992) leads to increased μ-opioid receptor density in the nucleus accumbens. In contrast, continuous administration of cocaine produces no change in opioid receptor density in the caudate putamen (Hammer, 1989), while intermittent cocaine treatment increases μ-opioid receptors in the rostral caudate putamen (Unterwald et al., 1992). In addition, chronic cocaine administration leads to an increase in κ- but not δ-opioid receptors (Unterwald et al., 1994). Further, cocaine administration leads to increases in circulating β-endorphin levels (Forman and Estilow, 1988; Moldow and Fischman, 1987), striatal prodynorphin mRNA levels (Daunais et al., 1993; Mathieu-Kia and Besson, 1998; Romualdi et al., 1996; Spangler et al., 1993; Turchan et al., 1999) and striatonigral dynorphin content (Sivam, 1989; Smiley et al., 1990). In contrast to this, chronic cocaine administration produces a decrease in prodynorphin mRNA levels in the hypothalamus (Romualdi et al., 1996).

Since cocaine acts by inhibiting the reuptake of dopamine, norepinephrine, and serotonin, it is not known which of these actions is responsible for the changes in opioid receptors and peptides. One way to study this is to look at the effects of selective uptake inhibitors. 1-[2-[*Bis*(4-flourophenyl)methoxy]ethyl]-4-[3-phenylpropyl]piperazine dihydrochloride (GBR 12909) is a selective dopamine uptake inhibitor that is structurally different

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from cocaine. It has been shown that while GBR 12909 produces some of the same behavioral effects of cocaine, following chronic treatment there are some different behavioral and neurochemical adaptations (Izenwasser et al., 1999; Kunko et al., 1997, 1998). For example, chronic continuous administration of GBR 12909 produces a down-regulation of dopamine transporters in the caudate putamen and nucleus accumbens, whereas continuous cocaine administration does not (Kunko et al., 1997). In fact, when dopamine transporter density is measured within 48 h after treatment with cocaine, regardless of the treatment paradigm employed, there is no decrease in density (Boulay et al., 1996; Kula and Baldessarini, 1991; Wilson et al., 1996). These findings suggest that although both cocaine and GBR 12909 bind to the dopamine transporter, the consequences of binding are different. They may bind at different sites or through different mechanisms and have different binding kinetics. It is also possible that the inhibition of norepinephrine and serotonin uptake by cocaine in some way modulates its effects on the dopamine transporter.

The purpose of this study was to measure the effect of chronic continuous GBR 12909 treatment on prodynorphin mRNA levels in the caudate putamen, hippocampus, nucleus accumbens, and hypothalamus.

2. Materials and methods

2.1. Chemicals

Isotopes and drugs were obtained from the following sources: cocaine hydrochloride from Sigma (St. Louis, MO); GBR 12909 from Research Biochemicals International (Natick, MA, USA).

2.2. Chronic drug treatment

Male Sprague–Dawley rats (200–250 g, Taconic, Germantown, NY, USA) were maintained on a 12/12 h light/dark cycle with unrestricted access to rat chow and water. Animals were anesthetized with halothane, and Alzet osmotic minipumps were implanted subcutaneously between the scapulae, as previously described (Izenwasser et al., 1999). Pumps contained a concentration of drug resulting in the delivery of approximately: 50 mg/kg/day of cocaine (expressed as free base); 30 mg/kg/day of GBR 12909 · 2HCl or vehicle (50% dimethyl sulfoxide/50% sterile water; used to dissolve GBR 12909) as the appropriate control. Doses were determined by average weight of each group of animals, and average pumping volume of the pumps. Alternatively, rats received 30 mg/kg/day of GBR 12909 in a single daily injection for 3 days. Because GBR 12909 has been shown to have a slow rate of association at the dopamine transporter (Pogun et al., 1991) and behavioral studies have shown that it is much less potent in vivo than would be predicted on the basis of its affinity for the dopamine transporter in vitro (Rothman et al., 1992), a behaviorally active dose of GBR 12909 was selected. This dose was higher than one that would have been chosen on the basis of in vitro binding alone.

2.3. Tissue processing

Two hours after the last daily injection, or on the 8th day of the continuous infusion, the rats were killed by decapitation, their brains rapidly removed and the hypothalamus, hippocampus, striatum and nucleus accumbens were dissected and frozen on dry ice.

Total RNA was prepared according to the method of Chomczynski and Sacchi (1987). Briefly, RNA was extracted from single tissue samples by homogenizing in a mixture of acid guanidinium thiocyanate/phenol (2 ml/100 mg tissue), adding 0.2 ml chloroform/2 ml of homogenate, and centrifuging the suspension at $12{,}000 \times g$ for 15 min at 4°C. The aqueous phase was transferred to a fresh tube, an equal volume of isopropanol was added, incubated for 15 min at 4°C and the RNA pellet was isolated by centrifugation at $12{,}000 \times g$ for 25 min at 4°C. The pellet was washed twice with 75% ethanol, dried under vacuum and then resuspended in 0.5% sodium dodecyl sulfate (SDS). Total RNA content was quantitated by measurement of absorbance at 260 nm (1 OD/ml = 40 μg RNA/ml). The ratio OD260/OD280 > 1.8 provided an estimate of the purity of the total RNA.

2.4. Probes

Blots were hybridized with the probe BgBa, the restriction enzyme *Bgl*II to *Bam*HI fragment (920 base pair) of the rat genomic DNA complementary to the prodynorphin mRNA, consisting of the 5′-translated region of the prodynorphin gene, encoding for all prodynorphin.

The cDNA fragment, inserted in the plasmid vector pUC19, was kindly supplied by Drs. O. Civelli and J. Douglass (Civelli et al., 1985). BgBa was released by restriction enzyme *Eco*RI and *Pst*I digestion, labeled by random priming methods using α-[^{32}P]dCTP to a specific activity of $7–9 \times 10^5$ cpm/ng.

A cDNA fragment recognizing β-actin mRNA (clone pHFβA-1, containing the full-length cDNA insert for human cytoplasmic β-actin) was used as internal standard to hybridize the same blots (Gunning et al., 1983).

2.5. Northern blot analysis

Total RNA from each tissue (20 μg) was electrophoresed through a 1% agarose gel containing 2.2 M

formaldehyde at 75 V using a 0.04 M morpholinopropanesulfonic acid (MOPS, pH 7.0) buffer containing 10 mM sodium acetate and 1 mM EDTA. RNA was transferred by overnight capillary blotting to nylon membranes and then air-dried, UV crosslinked and then hybridized into oven (Maniatis et al., 1982). After prehybridization for 3–6 h, blots were hybridized for 24 h at 42°C in a solution of 6 × SSC (sodium chloride/sodium citrate) (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate), 1 × Denhardt's solution (0.02% polyvinylpyrrolidone, Ficoll and BSA), 100 μg/ml denatured salmon sperm DNA, 0.1% SDS, 50% formamide, 10 mM Tris and 10% dextran sulfate, containing the probe at the concentration of $1–2 \times 10^6$ cpm/ml. Upon removal of the probe solution, blots were washed three times for 10 min at 42°C with a solution of 2 × SSC/0.1% SDS followed by three times for 10 min at 65°C with a solution of 0.1 × SSC/0.1% SDS. X-ray films (Amersham β-max) were exposed to the hybridized blot backed by an intensifying screen (Dupont Cronex) at −70°C for 3–6 days. Blots were hybridized serially twice with probes directed against prodynorphin and β-actin mRNA. For β-actin mRNA hybridization, blots were prehybridized and hybridized overnight at 65°C in a solution of 4 × SSC, 50 mM NaH_2PO_4, 5 × Denhardt's solution and 10% dextran sulfate. Blots were washed three times for 10 min at 65°C with a solution of 0.5 × SSC, 0.1% SDS on a rocker and then exposed to X-ray films at −70°C for 24 h. Total RNA from treated animals was compared to RNA from control rats. Optical densities for autoradiographic bands produced by prodynorphin and β-actin hybridization were determined using a Video Densitometer system (MDL 620). The prodynorphin mRNA/β-actin mRNA ratios of hybridization values for treated or control animals were analyzed and then expressed as percentages of controls (100%) for each experiment. Data were statistically analyzed by analysis of variance (one-way ANOVA), followed by Newman–Keuls test.

Briefly, background densities from areas of film away from the lanes were subtracted from observed values, which were obtained from non-saturated autoradiographic exposures in which standardizing lanes revealed a linear relationship between the amount of prodynorphin mRNA and hybridization signal. Two autoradiograms for each blot were scanned. Multiple exposures to film and the presence of standardizing lanes allowed us to avoid saturation of The X-ray film.

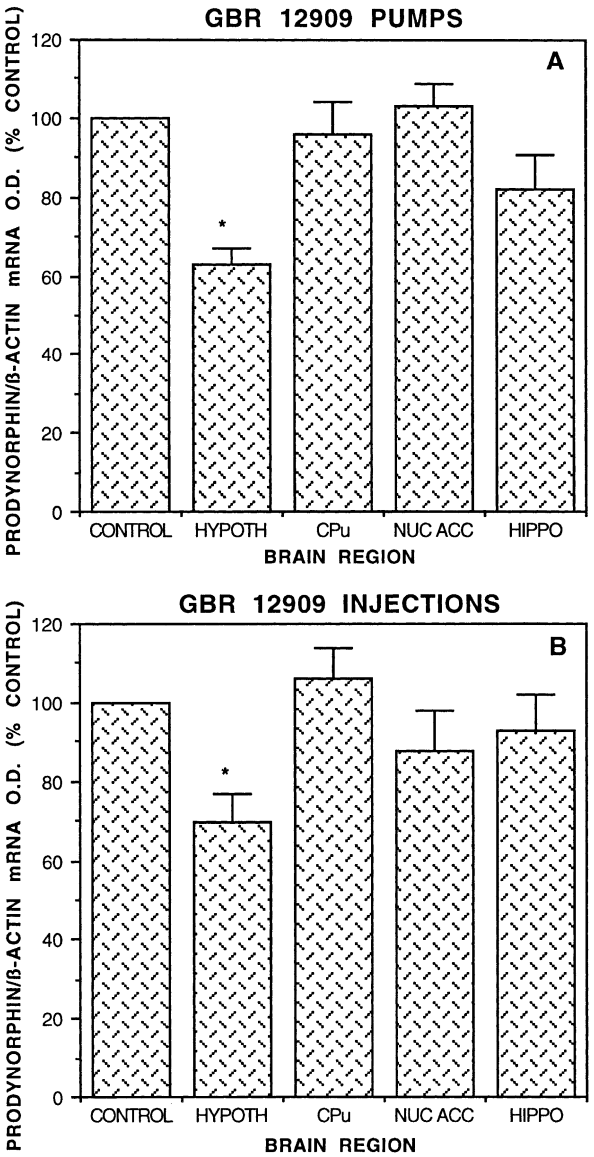

Fig. 1. Changes in prodynorphin mRNA levels in rat hypothalamus, caudate putamen, nucleus accumbens, and hippocampus after (A) continuous infusion for 7 days or (B) three daily injections of GBR 12909 (30 mg/kg/day). Values are prodynorphin/β-actin mRNA optical density ratios expressed as percentage of vehicle control (100%) for each tissue ($n = 6–12$). * Indicates a significant difference from vehicle, $P < 0.05$.

3. Results

Both continuous infusions (Fig. 1A) and daily injections (Fig. 1B) of GBR 12909 produced significant decreases in prodynorphin expression in the hypothalamus (37% and 31% decreases, respectively). There were no significant changes in the caudate putamen, hippocampus or nucleus accumbens. Two hours after a single injection of GBR 12909, prodynorphin expression in all studied brain regions did not differ from control levels, suggesting that the effect of GBR 12909 treatments on prodynorphin expression in the hypothalamus is not an acute effect (data not shown).

Continuous infusion of cocaine produced a significant decrease in prodynorphin mRNA in the hypothalamus (22% decrease), consistent with the effects of GBR 12909 (Fig. 2). In contrast to GBR 12909, however, cocaine also produced a significant increase in prodynorphin expression in the caudate putamen (35.6% increase). Thus, chronic

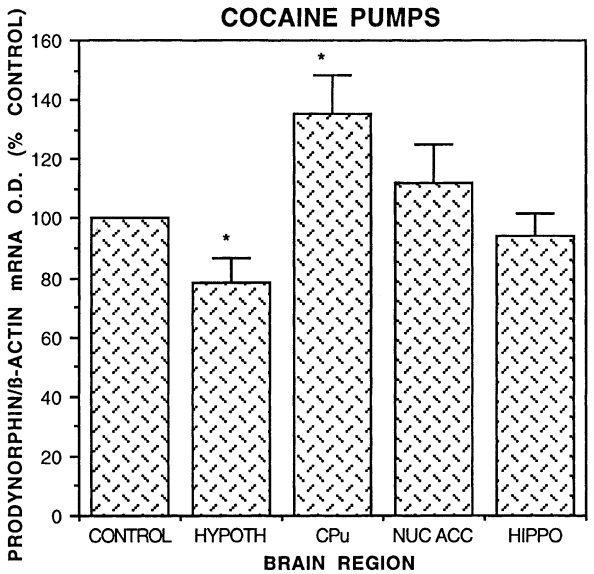

Fig. 2. Changes in prodynorphin mRNA levels in rat hypothalamus, caudate putamen, nucleus accumbens, and hippocampus after vehicle (control) or chronic continuous cocaine administration. Values are expressed as percentage of controls (100%) for each tissue. * Indicates a significant difference from vehicle, $P < 0.05$.

cocaine regulates prodynorphin expression in both the hypothalamus and the caudate putamen, albeit in opposite directions.

4. Discussion

Prodynorphin mRNA was decreased significantly in the hypothalamus and increased in the caudate putamen following treatment with continuous infusion of cocaine. This finding is consistent with reports from this, and other laboratories that cocaine, administered under a number of different regimens, increases prodynorphin mRNA in the caudate putamen (Daunais et al., 1993; Mathieu-Kia and Besson, 1998; Romualdi et al., 1996; Spangler et al., 1993; Turchan et al., 1998). In addition, this finding is consistent with our previous report showing that 7 days of intracerebroventricularly administered cocaine decreased prodynorphin mRNA in the hypothalamus (Romualdi et al., 1996).

Chronic treatment with GBR 12909 produced significant alterations in prodynorphin expression only in the hypothalamus. This was true following both continuous infusions and daily injections of GBR 12909. Thus, it does appear that the regulation of prodynorphin in the hypothalamus is mediated by dopamine, since it was produced both by cocaine and by a selective dopamine uptake inhibitor.

In contrast, the increase in the caudate putamen following cocaine administration may not be related to the inhibition of dopamine uptake, since it was not produced by the selective dopamine uptake inhibitor GBR 12909. Of course, this study was done at a single time point and it is possible that GBR 12909 would have an effect at a different time point or under different conditions. An earlier study showed that a single injection of GBR 12909 increased prodynorphin mRNA in the dorsolateral, but not the ventromedial caudate, when measured 2 h after the drug administration (Hurd and Herkenham, 1992). However, when measured days later, or after a second injection, there were no changes in prodynorphin mRNA in response to GBR 12909. These findings differ from the present results showing no effect of a single injection of GBR 12909. A big difference between these two studies is that in the present study, the entire caudate was measured, and no change was observed. In the previous study, Hurd and Herkenham (1992) used in situ hybridization to demonstrate that a single injection of GBR12909 increased prodynorphin mRNA levels in only one quadrant of the caudate nucleus. This effect may not have been observed in the present study since mRNA levels were assayed quantitatively in extracts of the entire caudate nucleus. Thus, we cannot exclude small discretely localized changes in prodynorphin expression after a single dose of GBR12909. However, our study and Hurd and Herkenham (1992) are in agreement that there is no sustained change in prodynorphin mRNA levels in caudate–putamen after chronic GBR 12909 treatments. The absence of an effect of GBR 12909 is unlikely to result from a failure of the drug to gain access to the caudate–putamen since Kunko et al. (1997) reported a down-regulation of the dopamine transporters in caudate–putamen, using the same treatment protocol as that used in the present study. These observations cast doubt on the role of inhibition of dopamine uptake in the cocaine-induced increase in prodynorphin mRNA expression in caudate in these studies. It has been reported that the dopamine D_1 receptor antagonist *R*-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1 *H*-3-benzazepine-7-ol (SCH 23390) blocks the increase in prodynorphin mRNA in the caudate putamen by repeated cocaine injections (Daunais and McGinty, 1996; Spangler et al., 1996). This does not necessarily preclude a role for either norepinephrine or serotonin, or both in this effect. It could be that dopamine receptors are somehow activated by continuous inhibition of either norepinephrine or serotonin uptake, in addition to dopamine, and that this activation is blocked by a D_1 receptor antagonist. It is still possible that a serotonin or norepinephrine receptor antagonist might also block this effect. In addition, SCH 23390, while highly selective for dopamine D_1 over D_2 receptors, also binds with fairly high affinity to both $5HT_1$ and $5HT_2$ receptors (Bischoff et al., 1986; Hicks et al., 1984; Nicklaus et al., 1988; Ohlstein and Berkowitz, 1985). In light of this, the blockade of increase prodynorphin mRNA by SCH 23390 may not be entirely due to its actions at dopamine receptors. Thus, it remains possible that increased prodynorphin mRNA levels in caudate after chronic cocaine treatments result from inhibition of norepinephrine or serotonin uptake, from a combination of effects on two

or three monoamine transporters, or through a mechanism unrelated to transporter inhibition.

There are limited studies showing the effects of serotonin activation on prodynorphin mRNA, and even less on the role of norepinephrine. It has been shown that prodynorphin mRNA is increased by a $5HT_{1A}$ receptor agonist, and that the induction of prodynorphin after noxious stimulation is greatly decreased following depletion of serotonin (Lucas et al., 1993). A single injection of the selective serotonin uptake inhibitor 8-methyl-2beta-propanoyl-3beta-(4-(1-methylethyl)phenyl)-8-azabicy clo[3.2.1] (WF-31) increased prodynorphin mRNA in rat caudate putamen, while fluoxetine, another selective serotonin uptake inhibitor had no effect (Daunais et al., 1997). It is interesting to note that while selective for the serotonin over the dopamine transporter, WF-31 does bind to the dopamine transporter. Thus, it is possible that the effects of this compound are mediated by dopaminergic or by both dopaminergic and serotonergic mechanisms. It is also important to note that in this experiment, only a single injection of each drug was administered.

The present findings are consistent with other studies showing that the chronic effects of cocaine and GBR 12909 differ. For example, the patterns of locomotor activity are differentially altered during continuous infusion of these two drugs (Izenwasser et al., 1999). One caveat is that, while GBR 12909, an aryl 1,4-dialkylpiperazine derivative, binds with high affinity and selectivity to the dopamine transporter, and is a potent inhibitor of dopamine reuptake (Andersen, 1989; Heikkila and Manzino, 1984; van der Zee et al., 1980), it requires a significantly higher proportion of transporter occupancy to produce levels of behavior comparable to those produced by cocaine (Rothman et al., 1992). This may be due to the slow onset of action of GBR 12909, relative to cocaine, at the dopamine transporter (Pogun et al., 1991). Thus, the differential kinetic properties between cocaine and GBR 12909 might contribute to differences in neuroadaptive responses to these drugs. However, under conditions of continuous pump infusions of the drugs, it is likely that issues related to differences in drug distribution would be minimized due to the continual presence of the drugs.

In conclusion, it appears that there are differential adaptations in prodynorphin gene expression in response to cocaine and to the selective dopamine uptake inhibitor GBR 12909 under these conditions, and at the time point tested. These findings suggest that systems other than dopaminergic are likely to be involved in the adaptations that occur in some brain regions during chronic cocaine administration.

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