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Short communication

# Perinatal opioids reduce striatal nerve growth factor content in rat striatum

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**Abstract**

Both human and animal models indicate that perinatal methadone exposure produces a variety of short- and long-term neurobehavioral consequences, including disruption of normal development of striatal cholinergic neurons. Despite this, methadone maintenance is a standard method of managing pregnant heroin addicts, and the opioid receptor partial agonist buprenorphine is under evaluation for the same use. We now report that perinatal administration of either methadone or buprenorphine reduces the content of the neurotrophic factor nerve growth factor (NGF) in rat striatum, which may explain the behavioral deficits observed. Furthermore, although NGF content is reduced, there are no corresponding reductions in striatal NGF mRNA. © 2001 Published by Elsevier Science B.V.

*Keywords:* Buprenorphine; Methadone; NGF (nerve growth factor); Perinatal; Striatum, rat

## 1. Introduction

Findings from both humans and animal models have demonstrated that perinatal methadone exposure produces a variety of short- and long-term neurobehavioral consequences, some of which may result from disruption of the normal development of striatal cholinergic neurons (Guo et al., 1990; Robinson et al., 1993, 1996a). The acetylcholine content of neonatal rat striatum is reduced in animals that have been exposed to methadone prenatally (Guo et al., 1990). Appearance of choline acetyltransferase, the synthetic enzyme for acetylcholine, is delayed in the striatum (Robinson et al., 1993). Both choline acetyltransferase and acetylcholine reach normal levels by 3 weeks postnatally but the turnover rate of acetylcholine is significantly higher than in non-exposed animals (Robinson et al., 1991, 1993).

How exposure to methadone prenatally would result in the changes observed in the cholinergic system has not been clear, given that most cholinergic development occurs postnatally in rats (Coyle and Yamamura, 1976; McGeer et al., 1971), at a time when the concentration of methadone, given only prenatally, would be too low to be acting directly on the cholinergic neurons (Kunko et al., 1996). The changes cannot be attributed to withdrawal, because animals exposed to methadone both pre and postnatally, by fostering of pups to mothers producing methadone in the milk via implanted osmotic minipumps, show comparable changes in cholinergic phenotype (Robinson et al., 1996a,b). These results suggested that methadone must act indirectly, perhaps through some other cell type. One possibility is an effect on synthesis of a neurotrophic factor that is required for proper developmental expression of cholinergic properties in the striatum. Since nerve growth factor (NGF) is known to stimulate expression of the cholinergic phenotype in the striatum both in cultures (Ebstein et al., 1993; Hartikka and Hefti, 1988; Martinez et al., 1985) and in vivo (Johnston et al., 1987; Mobley et al., 1985; Vantini et al., 1989), it was of interest to determine whether perinatal opioid exposure reduces striatal NGF content. We now report that perinatal administration of either methadone or buprenorphine reduces the content of NGF in striatum, which may explain the behavioral deficits observed.

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## 2. Materials and methods

This study was conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Animals in Research and under protocols approved by the Animal Care and Use Committee of Virginia Commonwealth University. Rats were exposed to the μ-opioid receptor agonist methadone or to the μ-opioid receptor partial agonist buprenorphine prenatally, postnatally, or both pre and postnatally. On day 7 of gestation (detection of vaginal sperm plug = day 0), pregnant Sprague–Dawley CD rats (Harlan Sprague–Dawley, Indianapolis, IN, USA) were implanted subcutaneously, under methoxyflurane anesthesia, with 28-day osmotic minipumps (Alza, Palo Alto, CA, USA) which delivered sterile water, methadone HCl (9 mg/kg/day) or buprenorphine HCl (1.5 mg/kg/day). Within 24 h of parturition, litters were culled to 10, maintaining equal numbers of males and females when possible, and cross-fostered, resulting in the following prenatal/postnatal exposure groups: water/water, methadone/water, water/methadone, methadone/methadone, buprenorphine/water, water/buprenorphine, and buprenorphine/buprenorphine.

On postnatal day 10, pups were decapitated, and the striata dissected, rapidly frozen and pooled for analysis of NGF using a sensitive two-site enzyme-linked immunoassay (ELISA) as described (Schwartz and Mishler, 1990). For NGF mRNA analysis, pups were decapitated on either postnatal day 4 or 10, and the striata dissected, rapidly frozen and pooled for RNA extraction. RNA was prepared according to the protocol of Chomczynski and Sacchi (1987). NGF mRNA was determined using a ribonuclease protection assay protocol and RiboQuant kit purchased from PharMingen (San Diego, CA). Gels were exposed to Molecular Dynamics (Sunnyvale, CA) PhosphoImager exposure cassettes. With the use of ImageQuant software, bands for NGF mRNA were quantified relative to those for the housekeeping gene L32, and NGF mRNA is presented as units of NGF mRNA/L32 mRNA.

## 3. Results

Treatment with either methadone or buprenorphine, whether prenatally (methadone/water and buprenorphine/water, respectively) or postnatally (water/methadone or water/buprenorphine), or both pre and postnatally (methadone/methadone and buprenorphine/buprenorphine), led to significant 40–50% reductions in striatal NGF content, as compared to that of offspring of control dams implanted with minipumps filled with sterile water (water/water) (Fig. 1). Methadone and buprenorphine were equally effective, whether delivered prenatally, postnatally, or both.

The decrease in NGF content raised the possibility that perinatal exposure to opioids might down-regulate expression of NGF mRNA. However, no changes were detected

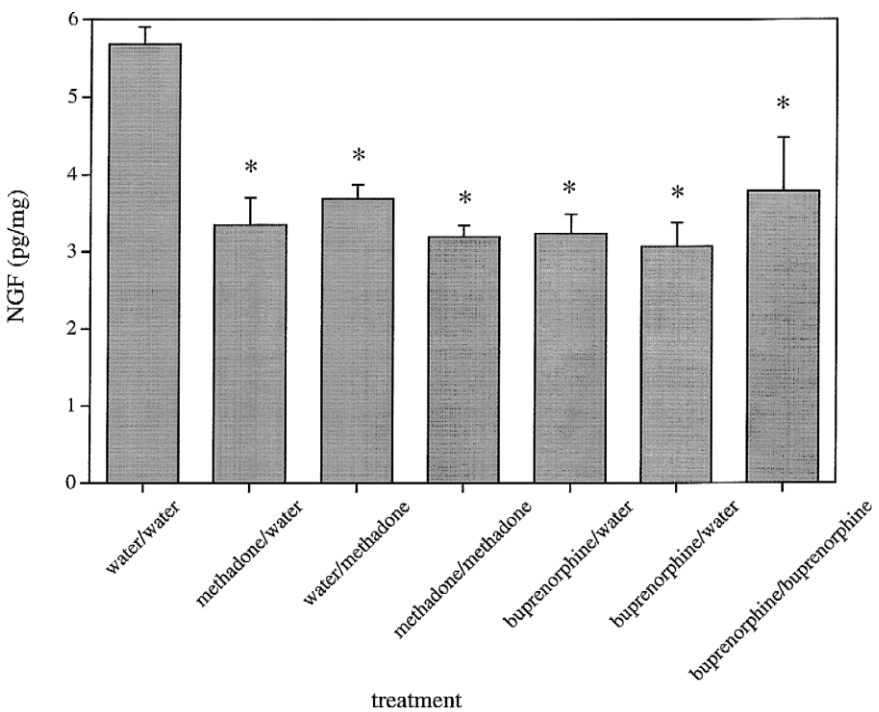


Fig. 1. NGF was measured in striatal extracts prepared from pups from the following prenatal/postnatal exposure groups (methadone—9 mg/kg/day; buprenorphine—1.5 mg/kg/day): water/water ($n = 10$); methadone/water ($n = 5$); water/methadone ($n = 5$); methadone/methadone ($n = 5$); buprenorphine/water ($n = 4$); water/buprenorphine ($n = 4$); buprenorphine/buprenorphine ($n = 4$). The data are combined from two experiments and the values are mean ± S.E.M. * The NGF content was significantly reduced ($P < 0.01$) in every group relative to the water/water control; one-way ANOVA ($F = 13.30$, $P < 0.0001$) with post-hoc Dunnett's Multiple Comparison Test.

Table 1
Effect of perinatal exposure to methadone or buprenorphine on striatal NGF mRNA content

| Treatment | Postnatal day 4 ($n$) | Postnatal day 10 ($n$) |
|---|---|---|
| Water/water | $1.79 \pm 0.16$ (6) | $5.99 \pm 0.50$ (10) |
| Methadone/water | $1.51 \pm 0.06$ (3) | $6.09 \pm 0.48$ (5) |
| Water/methadone | $1.31 \pm 0.02$ (3) | $5.43 \pm 0.62$ (7) |
| Methadone/methadone | $1.85 \pm 0.30$ (4) | $5.39 \pm 0.92$ (4) |
| Buprenorphine/water | $2.01 \pm 0.29$ (4) | $5.09 \pm 0.67$ (8) |
| Water/buprenorphine | $2.07 \pm 0.11$ (5) | $5.67 \pm 0.66$ (5) |
| Buprenorphine/buprenorphine | $1.98 \pm 0.18$ (5) | $5.11 \pm 0.60$ (9) |

RNA was extracted and analyzed by ribonuclease protection assay as described above. The data are presented as units of NGF mRNA/L32 mRNA, as mean ± S.E.M. ($n$). Analysis by two-way ANOVA revealed no significant differences between treatment groups or interactions of age with treatment but a significant increase in NGF mRNA from postnatal day 4 to postnatal day 10 ($F = 122.2$, $P < 0.001$).

in NGF mRNA in response to any of the treatments at either 4 or 10 days postnatally (Table 1).

## 4. Discussion

These results indicate that perinatal opioid exposure reduces striatal NGF content, which may be responsible for the delayed expression of the cholinergic phenotype since NGF has been shown to stimulate the cholinergic phenotype in striatum both in culture (Ebstein et al., 1993; Hartikka and Hefti, 1988; Martinez et al., 1985) and in vivo (Johnston et al., 1987; Mobley et al., 1985; Vantini et al., 1989). There were no differences between the effects of methadone and the effects of buprenorphine, suggesting that the mechanism is mediated by μ-opioid receptors. The drugs were equally effective whether given prenatally or within the first 10 days postnatally, suggesting that the cholinergic neurons remain sensitive to NGF throughout this period or at least during a time window spanning parturition.

Which cell is the source of the NGF remains to be determined. Astrocytes cultured from striatum can produce NGF (Schwartz and Nishiyama, 1994; Wu et al., 1998), and two groups have demonstrated the presence of μ-opiate receptors on striatal astrocytes (Hauser et al., 1996; Ruzicka et al., 1995). Furthermore, chronic administration of the opioid receptor antagonist naltrexone stimulated synthesis of NGF by striatal astrocytes (Mitsuo and Schwartz, 1993). In vivo, neurons in the striatum have been shown to express NGF and/or its mRNA in the adult brain (Bizon et al., 1999; Senut et al., 1990). These neurons are located in close proximity to cholinergic interneurons, where there is the potential for trophic interactions (Chang and Kita, 1992). In addition, reactive astrocytes, which re-express many of the developmental properties of neonatal astrocytes, express NGF (reviewed in Wu et al., 1998).

What is unusual is that the effect of the perinatal opioids on NGF content was not reflected in a corresponding decrease in NGF mRNA. This suggests that the regulation must be occurring at the level of processing of the NGF precursor. Attempts to measure the precursor using an antibody reputed to detect only precursor were unsuccessful (data not shown), presumably because the level of NGF itself is very low in striatum (Bizon et al., 1999; Whittemore and Seiger, 1987), and the precursor is expected to be a small fraction of that. To date, there have been no reports on regulation of neurotrophic factor synthesis at the level of precursor processing. Other possibilities include an effect on release of NGF or on its stability: one might expect either of these to result in increased synthesis of mRNA to restore endogenous levels but no evidence was seen in support of that interpretation.

In summary, perinatal exposure to either methadone or buprenorphine substantially reduces striatal NGF content without affecting the expression of NGF mRNA. The results suggest that use of μ-opioid receptor agonists or partial agonists should be approached with care in pregnant women, as changes in NGF content may perturb the development of cholinergic neurons and thereby contribute to the behavioral changes observed in the offspring of opioid-exposed mothers. Since these two opioids remain the most viable approach to treating pregnant addicts, the mechanism behind this action should be further elucidated.

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## References

Bizon, J.L., Lauterborn, J.C., Gall, C.M., 1999. Subpopulations of striatal interneurons can be distinguished on the basis of neurotrophic factor exppression. J. Comp. Neurol., 408, 283–298.

Chang, H.T., Kita, H., 1992. Interneurons in the rat striatum: relationships between parvalbumin neurons and cholinergic neurons. Brain Res., 574, 307–311.

Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal. Biochem., 162, 156–169.

Coyle, J.T., Yamamura, H.I., 1976. Neurochemical aspects of the ontogenesis of cholinergic neurons in the rat brain. Brain Res., 118, 429–440.

Ebstein, R.P., Bennett, E.R., Sokoloff, M., Shoham, S., 1993. The effect of nerve growth factor on cholinergic cells in primary fetal striatal cultures: characterization by in situ hybridization. Dev. Brain Res., 73, 165–172.

Guo, H.Z., Enters, E.K., McDowell, K.P., Robinson, S.E., 1990. The effect of prenatal exposure to methadone on neurotransmitters in neonatal rats. Dev. Brain Res., 57, 296–298.

Hartikka, J., Hefti, F., 1988. Comparison of nerve growth factor's effects

on development of septum, striatum, and nucleus basalis cholinergic neurons in vitro. J. Neurosci. Res., 21, 352–364.

Hauser, K.F., Stiene-Martin, A., Mattson, M.P., Elde, R.P., Ryan, S.E., Godleske, C.C., 1996. μ-Opioid receptor-induced $Ca^{2+}$ mobilization and astroglial development: Morphine inhibits DNA synthesis and stimulates cellular hypertrophy through a $Ca^{2+}$-dependent mechanism. Brain Res., 270, 191–203.

Johnston, M.V., Rutkowski, J.L., Wainer, B.H., Long, J.B., Mobley, W.C., 1987. NGF effects on developing forebrain cholinergic neurons are regionally specific. Neurochem. Res., 12, 985–994.

Kunko, P.M., Smith, J.A., Wallace, M.J., Maher, J.R., Saady, J.J., Robinson, S.E., 1996. Perinatal methadone exposure produces physical dependence and altered behavioral development in the rat. J. Pharmacol. Exp. Ther., 277, 1344–1351.

Martinez, H.J., Dreyfus, C.F., Jonakait, G.M., Black, I.B., 1985. Nerve growth factor promotes cholinergic development in brain striatal cultures. Proc. Natl. Acad. Sci. U. S. A., 82, 7777–7781.

McGeer, E.G., Fibiger, H.C., Wickson, V., 1971. Differential development of caudate enzymes in the neonatal rat. Brain Res., 32, 433–440.

Mitsuo, K., Schwartz, J.P., 1993. Chronic treatment of newborn rats with naltrexone alters astrocyte production of nerve growth factor. J. Mol. Neurosci., 4, 21–28.

Mobley, W.C., Rutkowski, J.L., Tennekoon, G.I., Buchanan, K., Johnston, M.V., 1985. Choline acetyltransferase activity in striatum of neonatal rats increased by nerve growth factor. Science, 229, 284–287.

Robinson, S.E., Guo, H., Enters, E.K., McDowell, K.P., Pascua, J.R., 1991. Prenatal exposure to methadone affects central cholinergic neuronal activity in the weanling rat. Dev. Brain Res., 64, 183–188.

Robinson, S.E., Guo, H., Spencer, R.F., 1993. Prenatal exposure to methadone delays the development of striatal cholinergic neurons. Dev. Brain Res., 76, 239–248.

Robinson, S.E., Guo, H., Maher, J.R., McDowell, K.P., Kunko, P.M., 1996a. Postnatal methadone exposure does not prevent prenatal methadone-induced changes in striatal cholinergic neurons. Dev. Brain Res., 95, 118–121.

Robinson, S.E., Mo, Q., Guo, H., Maher, J.R., Wallace, M.J., Kunko, P.M., 1996b. Perinatal exposure to methadone affects central cholinergic activity in the weanling rat. Drug Alcohol Depend., 41, 116–126.

Ruzicka, B.B., Fox, C.A., Thompson, R.C., Meng, F., Watson, S.J., Akil, H., 1995. Primary astroglial cultures derived from several rat brain regions differentially express mu, delta and kappa opioid receptor mRNA. Mol. Brain Res., 34, 209–220.

Schwartz, J.P., Mishler, K., 1990. Beta-adrenergic receptor regulation, through cyclic AMP, of nerve growth factor expression in rat cortical and cerebellar astrocytes. Cell. Mol. Neurobiol., 10, 447–457.

Schwartz, J.P., Nishiyama, N., 1994. Neurotrophic factor gene expression in astrocytes during development and following injury. Brain Res. Bull., 35, 403–407.

Senut, M.-C., Lamour, Y., Lee, J., Brachet, P., Dicou, E., 1990. Neuronal localization of the nerve growth factor precursor-like immunoreactivity in the rat brain. Int. J. Dev. Neurosci., 8, 65–80.

Vantini, G., Schiavo, N., Di Martino, A., Polato, P., Triban, C., Callegaro, G., Toffano, G., Leon, A., 1989. Evidence for a physiological role of nerve growth factor in the central nervous system of neonatal rats. Neuron, 3, 267–273.

Whittemore, S.R., Seiger, Å., 1987. The expression, localization and functional significance of beta-nerve growth factor in the central nervous system. Brain Res. Rev., 12, 439–464.

Wu, V.W., Nishiyama, N., Schwartz, J.P., 1998. A culture model of reactive astrocytes: increased nerve growth factor synthesis and reexpression of cytokine responsiveness. J. Neurochem., 71, 749–756.