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METHAMPHETAMINE (METH) CAUSES REACTIVE GLIOSIS IN VITRO: ATTENUATION BY THE ADP-RIBOSYLATION (ADPR) INHIBITOR, BENZAMIDE

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ABSTRACT. We examined the effects of methamphetamine (METH) in an *in vitro* model of rat fetal mesencephalic cells. METH causes loss of dopamine (DA) cells and neuronal process degeneration. In addition, the drug causes an increase in reactive gliosis as shown by the number of cells that stain for and by the intensity of staining with a glial fibrillary acidic protein (GFAP) antibody. Co-incubation of METH-treated cells with benzamide, which is a known inhibitor of ADP-ribosylation (ADPR), attenuated METH effects on both DA and glial cells. However, the effects of benzamide were somewhat more prominent on the glial cells. These results suggest that ADP-ribosylation may play a very important role in the development of reactive gliosis after the administration of neurotoxic agents.

Key Words: methamphetamine, gliosis, ADP-ribosylation, benzamide

Introduction

Methamphetamine (METH) is a drug of abuse which has deleterious effects on both the serotonin (5-HT) and dopamine (DA) systems in the mammalian brain (1,2). The changes associated with METH administration include decreases in DA and its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) (1), as well as in 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA) (1,3). Moreover, METH causes reactive gliosis in the rat brain (4,5).

Reactive gliosis is a ubiquitous response to CNS injury (6,7,8). Reactive glial cells can be differentiated from other glia by the presence of more extensive processes and increased immunostaining against glial fibrillary protein (GFAP) (7). Although this reaction was initially thought to be stereotypic, recent data have questioned that assumption (see ref. 7, for a recent review). Specifically, gliosis associated with mechanical or thermal injury is associated with disruption of the blood-brain barrier, vascular damage, as well as brain ischemia, whereas neurotoxin-induced glial reaction is not accompanied by these abnormalities (6,7,9). Moreover, while induction of ornithine decarboxylase plays a critical role in the increase GFAP staining observed after mechanical lesions in the rat (7,8), this does not seem to be the case after 1-methyl-4-phenyl-1,2,3-6-tetrahydropyridine (MPTP)-induced astrogliosis (10). As a first step towards evaluating the molecular events involved in the reactive gliosis observed after METH administration, we have developed an *in vitro* model using primary mesencephalic cells for rat fetuses.

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Methods

Cultures enriched with DA neurons were obtained by dissection of the mesencephalon of 14-day-old rat fetuses. Briefly, the cells were dissociated in a calcium and magnesium-free buffer. After a brief centrifugation, cells were resuspended in culture media (minimal eagle medium 70%, Hank's BSS 25%, Nu serum 5%, with glucose 6 g/L). Cells were then plated on poly-D-lysine coated coverslips in 24-well plates. Cultures were kept at 37 ^{o}C in a humidified (95% air/5%CO_2) incubator. Cells were grown for 7 days before treatment. On the day of treatment, cells were treated with either vehicle, METH (1.5 mM), or benzamide (250 μM) + METH (1.5 mM). This dose of METH was used because preliminary experiments had shown consistent cellular damage to DA neurons after 24 hrs of exposure to this dose (unpublished observation). Benzamide was used in these studies because it is an ADPR inhibitor (11) that can protect against cellular damage (12). Treatment lasted for 24 hours. At that time, the cells were fixed in 4% paraformadehyde and processed for tyrosine hydroxylase (TH) or GFAP staining essentially as described previously (13). After mounting the cells on microslides, the percentage of TH-positive with normal architecture and reactive GFAP-positive cells was determined by counting cells using a light microscope (Zeiss Axioscope).

Results

As shown in Fig 1A, METH (1.5 mM) caused a significant reduction of intact TH-positive cells (compare Fig 2A to 2B). Benzamide attenuated the toxic effects of METH (Fig 1A, 2C).

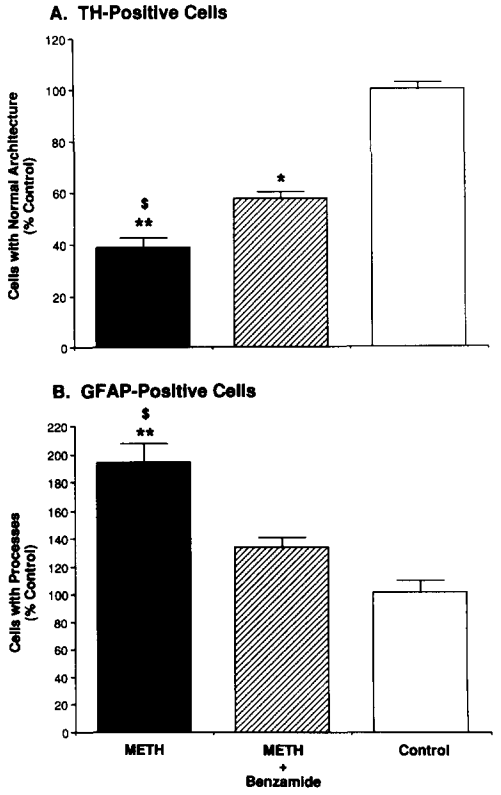

Fig. 1

Effects of METH (1.5 mM) on (A) TH-positive and (B) GFAP-positive cells *in vitro*. METH caused a marked reduction of TH-positive cells with normal processes and cell bodies but a marked increase in GFAP-positive cells with extensive processes and increased staining. Key: * = $p < .01$; ** = $p < .0001$ in comparison to control. $ = $p < .01$ in comparison to METH + Benzamide (Scheffe's test).

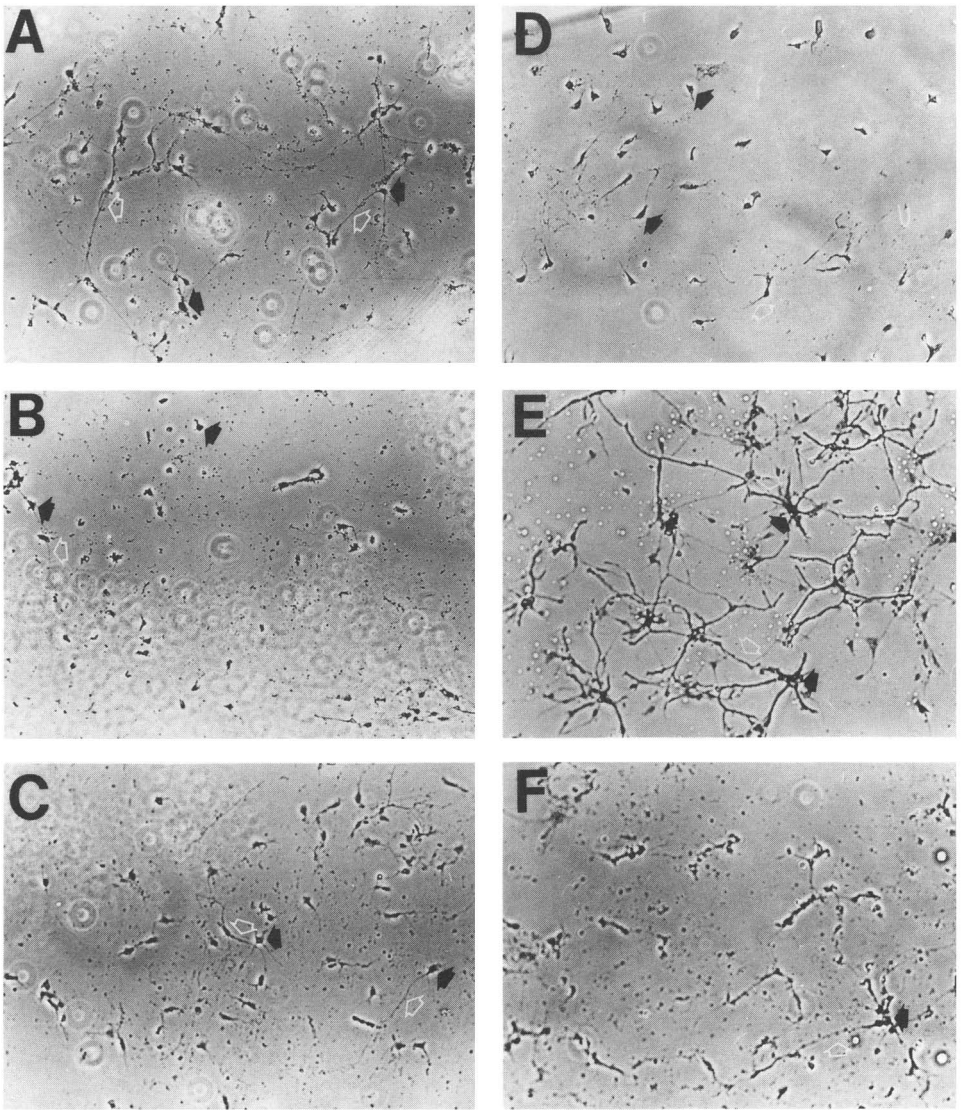

Fig. 2

Photomicrographs showing the effects of vehicle (A, D), METH (B, D), and METH + benzamide (C, F) on TH-positive (A, B, C) and GFAP-positive (D, E, F) mesencephalic cells *in vitro*. METH caused marked loss of TH-positive cells (black arrowheads) with normal architecture. Note the loss of neuritic processes (white arrowheads) in the TH-positive cells treated with METH (2B). Benzamide attenuated these changes (2C). Note the presence of more neuritic processes in the presence of METH + benzamide (compare 2B to 2C). On the other hand, METH caused a marked increase in the number of GFAP-positive cells (black arrowheads) with extensive processes (white arrowheads) (compare 2D to 2E). Note the hypertrophic response of the glial cells in the presence of methamphetamine (2E). Note the lack of multiple processes in the cells treated with METH + benzamide (compare 2E to 2F).

The same METH dose caused significant increases in GFAP-stained cells bearing multiple processes (Fig 1B). In addition, these cells showed qualitative evidence for increased GFAP staining (Fig 2D-2F). Post-hoc analyses shows benzamide-induced attenuation of the effects of METH on the glial cell population (Fig. 1B, also compare Fig. 2E to Fig 2F).

Discussion

The present increase in METH-induced GFAP-staining *in vitro* is consistent with that reported by other investigators in rat brain following peripheral administration of the drug (4). The present results show that, in addition, to causing deleterious effects of TH-positive cells, METH can cause marked reactive gliosis *in vitro*. Moreover, co-administration of benzamide, which blocks ADP-ribosylation (11), provides partial protection against the METH-induced toxic effects on TH-positive cells. Interestingly, benzamide also reversed METH-associated effects on glial cells. This suggests that activation of the ADP-ribosylation pathway may participate in METH-induced cellular injury as well as in the induction of reactive and hypertrophic glial cells after cellular damage. This interpretation is consistent with previous data showing that ADP-ribosylation is involved in cell proliferation and differentiation (14,15,16) as well as in cell death secondary to some toxins (12). The possibility that the glial changes that occur in the presence of METH might be due to alterations in epitope recognition which is then restored by benzamide needs also to be considered. However, the idea that ADP-ribosylation is involved in METH-induced gliosis is more consistent with the observation that inhibitors of ADPR can cause a decrease in the proliferation of astroblasts and of tumors of glial origin (reviewed in ref. 17). Thus, the observation that benzamide was somewhat more potent in inhibiting reactive gliosis in contrast to its effects on DA cell survival suggests that the two processes may be somewhat dissociated. Further experiments will be needed to clarify this point. In any case, the present data document the possibility of using an *in vitro* model to identify cellular and molecular mechanisms involved in METH-induced gliosis. This *in vitro* approach should allow for more specific manipulations of the various steps involved in that process.

References

1. J.L. CADET, P. SHENG, S. ALI, R. ROTHMAN, E. CARLSON, and C. EPSTEIN, Journal of Neurochemistry 62 380-383 (1994).
2. C.J. SCHMIDT, J.K. RITTER, P.K. SONSALLA, G.R. HANSSON, and J.W. GIBB, J. Pharmacol. Exp. Ther. 233 539-544 (1985).
3. G.A. RICAURTE, C.R. SCHUSTER, and L.S. SEIDEN, Brain Res. 193 151-163 (1980).
4. A. HESS, C. DESIDERIO, and W.G. MCAULIFFE, J. Neurocytol 19 338-342 (1989).
5. C. PU, and C.V. VORHEES, Developmental Brain Res. 72 325-328 (1993).
6. L.F. ENG, P.J. REIER, and J.D. HOULE, Prog. Brain Res. 71 439-455 (1987)
7. W.T. NORTON, D.A. AQUINO, I. HOZUMI, F.C. CHIU and C.F. BROSMAN, Neurochem. Res. 17 877-885 (1992).
8. I. ZINI, M. ZOLI, R. GRIMALDI, E.M. PICH, A. FUXE, and L.F. AGNATI, Neuroscience Letters 120 13-16 (1990).
9. J.P. O'CALLAGHAN, D.B. MILLER, and J.F. REINHARD, Jr. Brain Res. 521 73-80 (1990).
10. J.P. O'CALLAGHAN, and F.J. SEIDLER, Neuroscience Letters 148 105-108 (1992).
11. G.S. JOHNSON, Biochem. Int. 2 611-617 (1981).
12. I.U. SCHRAUFSTATTER, P.A. HYSLOP, D.B. HINSHAW, R.G. SPRAGG, L.A. SKLAR, and C.G. COCHRANE, Proc Natl. Acad. Sic. USA 83 4908-4912 (1986).
13. C. CERRUTI, M.-J. DRIAN, J.-M. KAMENKA, and A. PRIVAT, Brain Research 555 51-57, (1991).
14. R. EXLEY, J. GORDON, and M.J. CLEMENS, Proc. Natl. Acad. Sci. USA 84 6467-6470 (1987).
15. M.E. ITTEL, J. JONGSTRA-BILEN, C. ROCHETTE-EGLY, and P. MANDEL, (1983) Biochem. Biophys. Res. Commun. 116 428-434 (1983).
16. S. SHALL, Biochem. Soc. Trans. 17 317-322 (1989).
17. F.R. ALTHAUS and C. RICHTER, *ADP-ribosylation of proteins*. Spring-Verlag, Berlin Heidelberg, New York, London, Paris, Tokyo, (1987).