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Carnosine prevents methamphetamine-induced gliosis but not dopamine terminal loss in rats

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Abstract

The neuroprotective effect of carnosine, an endogenous antioxidant, was examined against methamphetamine-induced neurotoxicity in rats. Carnosine pretreatment had no effect on dopamine terminal loss induced by methamphetamine (assessed by [^{3}H]1-(2-[diphenylmethoxy]ethyl)-4-[3-phenylpropyl]piperazine([^{3}H]GBR 12935) binding) but prevented microgliosis (increase in [^{3}H]1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide ([^{3}H]PK 11195) binding) in striatum. The 27-kDa heat-shock protein (HSP27) expression was used as indicator of astroglial stress. Methamphetamine treatment induced the expression of HSP27 in striatum and hippocampus, which was inhibited by carnosine, indicating a protective effect. Carnosine had no effect on methamphetamine-induced hyperthermia. Thus, carnosine prevents the microgliosis in striatum (where we did not detect loss of serotonergic terminals by [^{3}H]paroxetine binding) and the expression of HSP27 in all the areas, but fails to prevent methamphetamine-induced loss of dopamine reuptake sites. Therefore, carnosine inhibits only some of the consequences of methamphetamine neurotoxicity, where reactive oxygen species play an important role.

Keywords: Methamphetamine; [^{3}H]GBR 12935; Benzodiazepine receptor, peripheral-type; HSP27; Carnosine; [^{3}H]Paroxetine

1. Introduction

Amphetamines are indirectly acting sympathomimetic compounds that exert powerful psychomotor stimulant effects through activation of brain catecholamine-containing neurones. Chronic administration of methamphetamine also increases hydroxyl radical formation in the striatum, and endogenous dopamine and glutamate (Stephans and Yamamoto, 1994) seem to be implicated in the development of such oxidative stress, leading to neurotoxicity.

Methamphetamine-induced neurotoxicity is accompanied by structural damage in the striatum (Ricaurte et al., 1982). Any injury in the central nervous system leads to microgliosis and, as microglia contains a high amount of peripheral-type benzodiazepine receptors, their density is a suitable indirect marker of neuronal damage (Benavides et al., 1987; Stephenson et al., 1995). On the other hand, the 27-kDa heat-stock protein (HSP27) is expressed in astroglia after several kinds of brain insult (Kato et al., 1994; Plumier et al., 1996) and can be used as an indicator of astroglial stress.

Carnosine is an endogenous dipeptide (β-alanyl-L-hystidine) with chelant-antioxidant properties (Boldyrev et al., 1997). It is expressed in glial cells throughout the brain, with high concentrations in the olfactory system. Horning et al. (2000) reported a neuroprotective effect of carnosine from the toxic effects of zinc and copper in cultured neurones and Boldyrev et al. (1999) also demonstrated that carnosine protects against excitotoxic cell death via a mechanism that is more complex that its antioxidant effects.

We examined the potential neuroprotective effect of carnosine against methaphetamine-induced neurotoxicity by measuring its effect on dopaminergic terminal loss, increase of peripheral-type benzodiazepine receptor number and expression of HSP27. We also investigated its effect on methamphetamine-induced hyperthermia to assess the possibility of a thermoregulatory action.

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2. Materials and methods

The ethics committee of the Universitat de Barcelona, following the European Community guidelines, approved the protocol concerning the use of experimental animals in this study. Adult male Sprague–Dawley rats (250–300 g) were obtained from Harlan Ibérica (Spain). Methamphetamine (Sigma) was administered to the methamphetamine group (10 mg/kg, s.c.) every 2 h, for a total of four doses (equivalent to a chronic schedule). Carnosine + methamphetamine group received five doses of 20 mg/kg carnosine (total dose: 100 mg/kg, Yuneva et al., 1999), administered 24 h and 30 min before the first dose of methamphetamine and together with the following doses of methamphetamine. There were also two control groups: one was injected with saline and the other received carnosine plus saline instead of methamphetamine, following the same injection schedule. The animals were allowed to acclimatise to an environmental temperature of 28 ± 1 °C before receiving the first dose of methamphetamine and were kept under these conditions until 1 h after the last dose (Bowyer et al., 1994). Body temperature was measured through a flexible rectal probe attached to a digital thermometer (0331 Panlab), before and 1 h after the first injection of methamphetamine. Seventy-two hours after treatment (when dopamine terminal loss was maximum, Escubedo et al., 1998), rats were killed by decapitation, brains were removed and striatum and hippocampus were dissected out, frozen on dry ice and stored at − 80 °C.

A tissue homogenate preparation was used for all the assays: tissue samples were homogenised in 10 volumes of buffer (5 mM Tris–HCl, 320 mM sucrose and protease inhibitors, pH 7.4) with a Teflon-glass homogeniser. The homogenates were centrifuged at 15,000 × *g* for 30 min at 4 °C. The pellets were washed twice and the final pellets were resuspended in Tris–HCl 50 mM buffer (pH 7.4) containing protease inhibitors and stored at − 80 °C until use. Protein content was determined by the Bradford (1976) method.

The density of dopaminergic terminals was assessed by equilibrium binding of [^{3}H]1-(2-[diphenylmethoxy]ethyl)-4-[3-phenylpropyl]piperazine ([^{3}H]GBR 12935) in the striatum as described previously (Escubedo et al., 1998). Because we found no significant difference in affinity (K_D) values from control and treated rats (Escubedo et al., 1998), we used this radioligand at a single concentration of 2 nM. Similarly, serotonin uptake sites were quantified by measuring specific binding of [^{3}H]paroxetine (0.05 nM) after incubation with 150 μg of protein at 25 °C for 1.5 h in a Tris–HCl buffer containing 120 mM NaCl and 5 mM KCl to a final volume of 1.6 ml. One hundred micromoles of clomipramine (Sigma) was used to determine nonspecific binding.

Microglial activation was measured by equilibrium binding assays to peripheral-type benzodiazepine receptors using 2 nM [^{3}H]1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinolinecarboxamide ([^{3}H]PK 11195) as described previously (Escubedo et al., 1998). All radioligands were purchases from New England Nuclear, DE, USA.

Astroglial activation was determined by Western blot for HSP27. Samples (30 μg of protein) were separated by electrophoresis on 10% acrylamide gels. Subsequently, proteins were transferred to polyvinylidene fluoride sheets (Immobilon™-P, Millipore). Membranes were incubated with a primary rabbit polyclonal antibody anti-HSP27 (1:5000, SPA-801, StressGen Biotechnologies) and afterwards with a peroxidase-conjugated antirabbit IgG antibody (Amersham). Immunoreactive protein was visualised using a chemoluminescence-based detection kit according to the manufacturer's protocol (ECL kit; Amersham). Exposed photographic films were scanned and protein spots were semiquantitatively analysed using Quantiscan software (Biosoft).

Results are expressed as means ± S.E.M. of data obtained from five to eight animals. Multiple mean comparisons were done by one-way analysis of variance (ANOVA.) followed by Tukey's test. Differences between values were considered to be significant when $P < 0.05$.

3. Results

Methamphetamine induced a 35% loss of dopamine reuptake sites (920 ± 98 fmol/mg, $n = 5$ compared with control values: 1409 ± 120 fmol/mg, $n = 5$, $P < 0.05$) which was not prevented by carnosine pretreatment (921 ± 82 fmol/mg, $n = 5$) (Fig. 1).

Fig. 2A shows the peripheral-type benzodiazepine receptor levels of the striatum and hippocampus of the differently treated animals. Methamphetamine-treated animals showed a 39% increase in peripheral-type benzodiazepine receptor density in striatum (from 185 ± 16 fmol/mg, $n = 5$, to 258 ± 12 fmol/mg, $n = 8$) compared with control animals

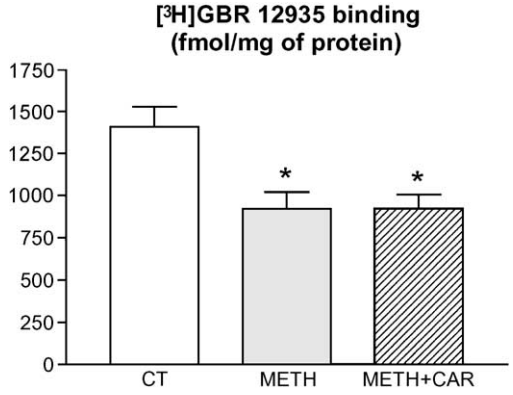

Fig. 1. Effect of treatment with methamphetamine (METH) alone or in combination with carnosine (CAR) on the density of dopamine reuptake sites assessed by [^{3}H]GBR 12935 binding density in rat striatum. Values are expressed as means ± S.E.M. of those obtained from five animals in each group (* $P < 0.05$ vs. control (CT) group).

($P < 0.05$), indicating microglial activation. When carnosine was given before methamphetamine, this increase was prevented (210 ± 12 fmol/mg, $n = 8$) ($P < 0.05$ vs. methamphetamine group), indicating a protective effect.

In the hippocampus, no significant differences in peripheral-type benzodiazepine receptor levels were found between the different treatment groups. Carnosine alone had no effect in peripheral-type benzodiazepine receptor levels (data not shown).

Methamphetamine treatment induced a significant hyperthermia in rats that was potentiated by high ambient temperature (40.5 ± 0.4 °C, methamphetamine group, vs. 37.3 ± 0.1 °C, control group, measured 1 h after the first dose of methamphetamine). A direct effect of carnosine on body temperature is ruled out because this compound did not reduce the methamphetamine-induced hyperthermia (40.3 ± 0.4 °C for carnosine + methamphetamine group). Carnosine alone had no effect on body temperature. The severe hyperthermia and cardiovascular effects induced by methamphetamine are signs of an acute toxicity that leads to lethality. In our experiments, the methamphetamine-treated group showed a lethality of 30% that was absent in the carnosine + methamphetamine treated group. Higher doses of carnosine were also tested but not reduction of mortality were then obtained.

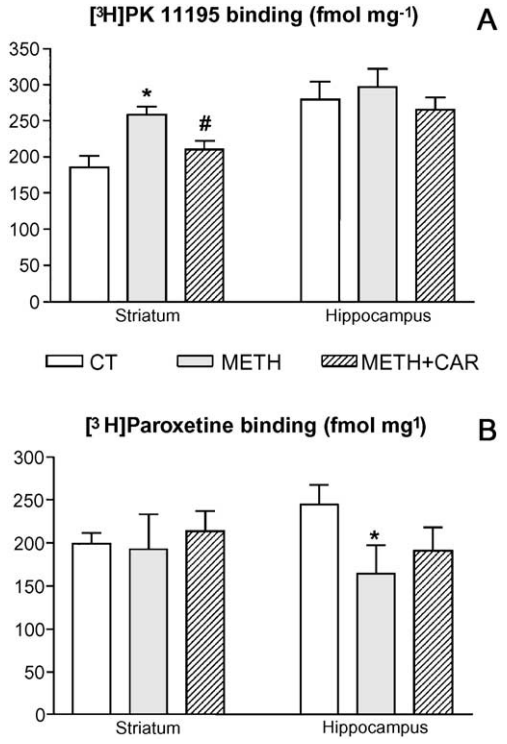

Fig. 2. Effect of the treatment with methamphetamine (METH) and pretreatment with carnosine (CAR) on the densities of PBR (panel A) and serotonin uptake sites (panel B). [^{3}H]PK 11195 (2 nM) and [^{3}H]paroxetine (0.05 nM) binding assays were performed to homogenate preparations of cortex, striatum and hippocampus of individual rats as described in the text. Values are expressed as means ± S.E.M. of those obtained from four to eight animals (* $P < 0.05$ vs. control (CT) group; #$P > 0.05$ vs. methamphetamine (METH) group).

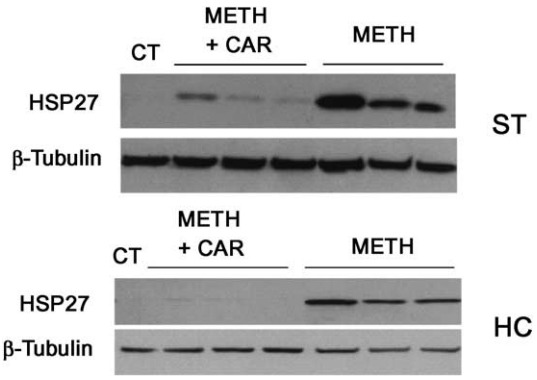

Fig. 3. Representative Western blots showing HSP27 expression in striatum (ST) and hippocampus (HC) of the same rats after treatment with methamphetamine (METH), alone or preceded by carnosine (CAR). Pretreatment with CAR prevented HSP27 expression induced by METH treatment in all these areas. Western blots were performed in duplicate, with samples originating from at least five animals of each treatment group. β-tubulin expression was used as a gel load control.

In addition, methamphetamine treatment induced the expression of HSP27 in striatum and hippocampus (Fig. 3), whereas tissues from control animals did not express this protein. In the animals pretreated with carnosine, HSP27 expression was inhibited by 60% in the striatum and by 80% in hippocampus.

In order to determine if gliosis could be consequence of a neurotoxic effect of methamphetamine on serotonergic nerve terminals, we measured [^{3}H]paroxetine binding in the same cerebral areas (Fig. 2B). No decrease in [^{3}H]paroxetine binding was observed in striatum (199 ± 12 vs. 192 ± 41 fmol mg^{-1}) whereas a 33% reduction was obtained from hippocampus (244 ± 23 vs. 164 ± 33 fmol mg^{-1}, $P < 0.05$) (values corresponding to control vs. methamphetamine group, respectively) that was not prevented by carnosine (190 ± 28 fmol mg^{-1}, $P > 0.05$ vs. methamphetamine group) ($n = 4$ for the three groups).

4. Discussion

Overall, the present study provides additional evidence that methamphetamine originates a dopaminergic neuronal lesion in striatum reflected by a significant decrease in [^{3}H]GBR 12935 binding. This damage could not be prevented by pretreatment with carnosine.

Also, there is an increase in the density of peripheral-type benzodiazepine receptors as the result of microgliosis in striatum and a generalised expression of astroglial HSP27 in striatum and hippocampus. In a previous paper (Escubedo et al., 1998), we have demonstrated that there exists a close relation between loss of dopaminergic terminals and micro-

gliosis. Here, we rule out that this microglial activation could be due to a loss of serotonergic terminals. Although there exists extensive evidence of serotonin depletion after methamphetamine treatment (LaVoie and Hastings, 1999), we did not find any decrease in the density of serotonin uptake sites in striatum, which would be a more direct indicator of terminal damage. These results are in agreement with previous reports (Haughey et al., 2000) which only detected such an effect at higher doses of methamphetamine (Kovachich et al., 1989). Moreover, it is important to note that carnosine prevents microgliosis in striatum, where there is no decrease in [^{3}H]paroxetine binding.

Carnosine, a hydrophilic antioxidant, prevented the microgliosis in striatum and the expression of HSP27 in studied areas. HSPs are induced by several noxious stimuli such as heat shock, hypoxia and reactive oxygen species. The widespread expression of this protein observed in the present study could be a response to the general hyperthermia induced by methamphetamine. Omar and Pappolla (1993) suggested a common mechanism by which various forms of injury, such as hyperthermia, cause HSP inductions, that is, via oxidative stress or increased production of oxygen free radicals. This hypothesis could explain why carnosine, as antioxidant, inhibited HSP27 expression in studied brain areas, without having effect on body temperature. In addition, carnosine reduced the mortality induced by methamphetamine. From our studies, we cannot point to a concrete effect as responsible for this increase in survival. It is known that carnosine, apart from its antioxidant properties, inhibits the nonenzymatic glycosylation (Boldyrev et al., 1999) that originates protein complexes which are increased after cerebral oxidative stress, diabetes and Alzheimer's disease. This property could play a role in the prevention of mortality.

Finally, although carnosine does not prevent the loss of dopaminergic terminals in striatum induced by methamphetamine treatment, it can prevent the gliosis associated with neuronal damage. This indicates that carnosine inhibits only some of the consequences of methamphetamine neurotoxicity, and points to a beneficial effect of this dipeptide on neurological disorders where reactive oxygen species play an important role, which deserves further studies.

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