ELSEVIER

European Journal of Pharmacology 387 (2000) 55–58

ejp

www.elsevier.nl/locate/ejphar

Short communication

Selective antagonism of nigral neuropeptide responses to methamphetamine by conantokin G, a naturally occurring conopeptide

Lloyd Bush [a], Tyler McCabe [b], Glen R. Hanson [a,*]

[a] *Department of Pharmacology and Toxicology, Room 201, 30 S and 2000 E, Skaggs Hall, University of Utah, Salt Lake City, UT 84112, USA*
[b] *Cognetix, Salt Lake City, UT, USA*

Received 29 July 1999; received in revised form 1 November 1999; accepted 12 November 1999

Abstract

Some conopeptides derived from cone snails act on specific subunits of the NMDA receptor and thus, exert an influence on the dopamine system. In this study, one such conopeptide, conantokin G, was administered i.c.v. in conjunction with methamphetamine, a potent central nervous system stimulant known to cause dopamine release and changes in tissue levels of neurotensin and dynorphin A in some brain structures. Both single and multiple administrations of the conantokin G preferentially attenuated the methamphetamine-induced increases in tissue levels of these neuropeptides in the substantia nigra. Conantokin G also enhanced the behavioral effects of the methamphetamine.

Keywords: Conopeptide; Conantokin G; Neurotensin; Dynorphin; Methamphetamine; NMDA receptor

1. Introduction

Cone snails (*Conus*) produce a variety of potent peptides which exert profound effects on central nervous systems. Because they appear to be highly site selective in their actions, they are being evaluated for potential research and clinical value (Olivera et al., 1990). For example, some of these conopeptides selectively interact with cholinergic receptors, while others preferentially alter the function of specific Na^+ or Ca^{2+} channels. One class of conopeptide, called conantokins, are small potent peptides (17–26 amino acids) which selectively influence the NMDA receptor (Olivera et al., 1990). We examined conantokin G, one of these conantokins, derived from *Conus geographus*. We determined the effect of conantokin G on two neuropeptides, neurotensin and dynorphin A , which have been shown in extrapyramidal structures to be regulated by an interaction between dopamine and NMDA receptors (Singh et al., 1990, 1991). Thus, we assessed the response of dynorphin and neurotensin systems in the striatum and substantia nigra after i.c.v. infusion into rats of conantokin G alone and in combination with methamphetamine. This potent central nervous system stimulant causes dopamine release (Holson et al., 1996) resulting in changes in the tissue levels of both neurotensin and dynorphin in extrapyramidal structures (Letter et al., 1987a,b; Hanson et al., 1988). This methamphetamine-induced change in these neuropeptides is blocked by the noncompetitive NMDA receptor antagonist, MK801 (dizocilpine) (Singh et al., 1990). Since conantokin G modulates NMDA receptor function, we tested the possibility that this conantokin would also influence the methamphetamine-induced changes in neurotensin and dynorphin tissue content.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (200–250 g; Simonsen Laboratories, Gilroy, CA, USA) were provided food and water ad libitum. All procedures were conducted in accordance with approved National Institutes of Health guidelines. Methamphetamine (National Institute on Drug Abuse, Rockville, MD, USA) was dissolved in saline and administered by subcutaneous (s.c.) injection. The conopeptides (Cognetix, Salt Lake City, UT, USA) were dissolved in saline and administered i.c.v.

The rats were anesthetized with chloral hydrate (500 mg/kg) and placed in a stereotaxic apparatus. Guide

* Corresponding author. Tel.: +1-801-581-3174; fax: +1-801-585-5111.
E-mail address: g.r.h@pharm.utah.edu (G.R. Hanson)

0014-2999/00/$ - see front matter
PII: S0014-2999(99)00806-7

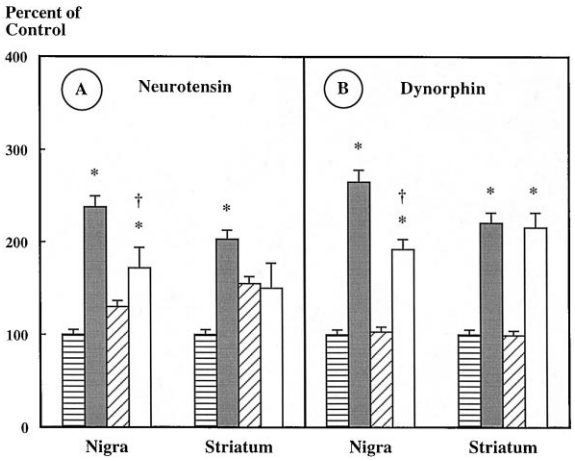

Fig. 1. Rats were injected i.c.v. with conantokin G (1 nmol/dose) 15 min prior to methamphetamine (5 mg/kg/dose, s.c.). A total of 4 injections were given at 2-h intervals and the rats were decapitated 18 h after the last dose of methamphetamine. Results are expressed as a percent of control and represent the means and standard errors of 5–8 animals. Symbols used for the groups were as follows: W saline–saline, ■ saline–methamphetamine, Z conantokin G–saline, and □ conantokin G–methamphetamine. The control values for neurotensin-like immunoreactivity (expressed as pg/mg protein) were 147 ± 17.5 in the striatum and 524 ± 60 in the substantia nigra. The control values for dynorphin-like immunoreactivity were 206 ± 24 in the striatum and 2640 ± 390 in the substantia nigra. Differences between the groups were analyzed using one-way analysis of varience followed by a Fisher protected least squares difference multiple comparisons test. Differences (* from sal.–sal. group; † from sal.–methamphetamine group) were considered significant when the probability that they were zero was less than 5%.

cannulae consisting of 20-gauge stainless steel tubing were implanted into the ventricles (0.8 mm posterior and 1.4 mm lateral to bregma and 4.2 mm ventral from the surface of the skull). Three days after the surgery the animals were injected i.c.v. with either saline or conantokin G (1 nmol in 1 μl) 15 min prior to s.c. injection with saline or methamphetamine (10 mg/kg for single dose or 5 mg/kg/dose for multiple administrations). This dose of conantokin G was selected because 1 nmol of this conantokin has biological activity in other animal models (Olivera et al., 1990) and our preliminary observation that a dose of 0.1 nmol had little effect in our treatment paradigm (data not shown). In the multiple administration protocol, a total of 4 sets of injections were given at 2-h intervals. The rats were decapitated 18 h after treatment. The brains were quickly removed and, after the removal of the striata, frozen on dry ice and stored at −80°C. The substantia nigra was later dissected from 1 mm-thick coronal slices of the frozen brains using an atlas (König and Klippel, 1963). All tissue samples were stored at −80°C until assayed for neurotensin-like immunoreactivity or dynorphin-like immunoreactivity.

In a separate experiment, to determine whether or not the effect of the conantokin G was unique or a property of a whole class of peptides, a similar conopeptide (conantokin T from *Conus tulipa*; mw 2685) was substituted for conantokin G using the multiple administration protocol.

2.2. Assays

Measurement of neurotensin-like immunoreactivity and dynorphin-like immunoreactivity was by radioimmunoassay according to the methods of Maidment et al. (1991) or Hanson et al. (1987), respectively. Sensitive and specific antibodies developed in our laboratory were used in the assays. The total protein content for each tissue was determined by the method of Bradford (1976) and neurotensin-like immunoreactivity or dynorphin-like immunoreactivity results were expressed as pg/mg protein.

2.3. Data analysis

Peptide tissue levels in Figs. 1 and 2 for neurotensin-like immunoreactivity or dynorphin-like immunoreactivity are plotted as a percentage of their respective controls. Each column in the graph represents the mean ± S.E.M. Differences between the groups were analyzed using one-way analysis of variance followed by a Fisher protected least squares difference multiple comparisons test. Differences were considered significant when the probability that they were zero was less than 5%.

3. Results

Administration of methamphetamine (either single or multiple doses) causes an elevation of neurotensin-like

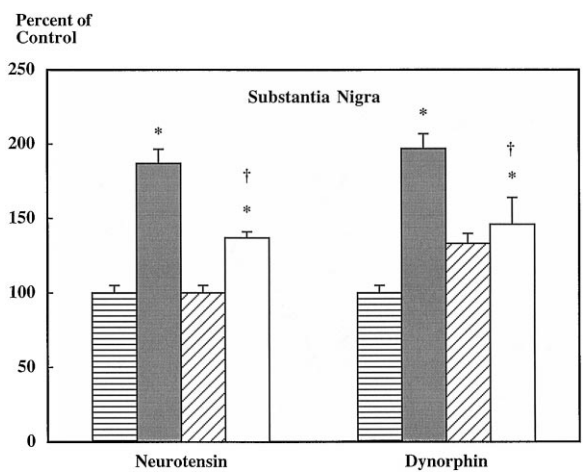

Fig. 2. Rats received a single i.c.v. injection of conantokin G (1 nmol) 15 min prior to a single administration of methamphetamine (10 mg/kg, s.c.). The rats were decapitated 18 h after the methamphetamine dose. Results are expressed as a percent of control and represent the means and standard errors of 6–8 animals. Symbols used for the groups were as follows: W saline–saline, ■ saline–methamphetamine, Z conantokin G–saline, and □ conantokin G–methamphetamine. The nigral control values (expressed as pg/mg protein) were 1165 ± 139 for neurotensin-like immunoreactivity and 3296 ± 310 for dynorphin-like immunoreactivity. Differences between the groups were analyzed using one-way analysis of varience followed by a Fisher protected least squares difference multiple comparisons test. Differences (* from sal.–sal. group; † from sal.–methamphetamine group) were considered significant when the probability that they were zero was less than 5%.

immunoreactivity and dynorphin-like immunoreactivity in both the striatum and the substantia nigra (Hanson et al., 1987; Letter et al., 1987a). In this study it was found that while multiple administrations of conantokin G by itself had no effect on peptide levels, it significantly attenuated the methamphetamine-induced increases in dynorphin-like immunoreactivity in the substantia nigra, but not the striatum (Fig. 1A). Conantokin G also partially blocked increases in nigral neurotensin-like immunoreactivity caused by methamphetamine treatment. Although not statistically significant, it appears that conantokin G may have also attenuated the response of the striatal neurotensin system to methamphetamine (Fig. 1B).

To assess whether or not the blockade of nigral peptide responses could be achieved by a single dose of conantokin G, a separate experiment was accomplished using only one administration of the drugs. The dose of methamphetamine was increased to 10 mg/kg to achieve a substantial effect on the neuropeptides with a single injection. Under these conditions, it was found that conantokin G attenuated the methamphetamine-induced nigral increases in both neurotensin-like immunoreactivity and dynorphin-like immunoreactivity (Fig. 2).

Qualitatively, in a separate experiment, it was observed that, although a single dose (1 nmol) of conantokin G slightly changed behavior by itself (minor ataxia), it enhanced the methamphetamine-induced locomotor and stereotypic activity. The onset of stereotypy was earlier and its intensity was increased in the animals receiving both conantokin G and methamphetamine when compared to those receiving only methamphetamine (5 mg/kg).

The behavioral effects of multiple administrations of conantokin G were quite pronounced. By itself this conantokin caused minimal ataxia, but in combination with the methamphetamine (4 administrations of 5 mg/kg/injection), the stimulant-induced stereotypy was greatly enhanced throughout the treatment as compared to that caused by methamphetamine alone. The progression through the various stages of motor responses was much more rapid with the final level of activity after the fourth drug treatment being more intense. The rats behaved as though they had received doses of 15 mg/kg of methamphetamine rather than 5 mg/kg of this stimulant.

To assess the selectivity of the nigral effect of conantokin G, a related conopeptide, conantokin T, was injected using the multiple administration protocol described for conantokin G. It was found that conantokin T had no effect by itself nor did it influence the methamphetamine-induced increases in nigral neurotensin-like immunoreactivity or dynorphin-like immunoreactivity (data not shown).

4. Discussion

This is the first report demonstrating that a naturally occurring peptide, conantokin G, is able to block the effects of a dopamine-enhancing drug, such as methamphetamine, due to what appears to be antagonism of the glutamate NMDA receptor. This conclusion is based on: (1) the high and somewhat selective affinity of conantokin G for the NR2B subunit of the NMDA receptor, demonstrated by its ability to block NMDA-evoked current responses ($IC_{50} \sim 300$ nM) in oocytes expressing NR2B, but not NR2A, subunits (data being prepared for publication); and (2) previous reports that other NMDA receptor antagonists, such as MK801 (a noncompetitive NMDA receptor antagonist), also block methamphetamine-induced increases in the levels of neurotensin-like immunoreactivity and dynorphin-like immunoreactivity in extrapyramidal structures (Singh et al., 1990, 1991). However, important differences between the effects of MK801 and conantokin G were observed. Thus, conantokin G appeared to preferentially block the methamphetamine-induced increases in both neurotensin-like immunoreactivity and dynorphin-like immunoreactivity in the substantia nigra, which contrasts with the striatum where there was no such blockade of dynorphin-like immunoreactivity and an equivocal effect on neurotensin-like immunoreactivity. A possible explanation for the difference between conantokin G and MK801 may relate to the subunit selectivity of this conantokin. It is interesting that the NR2B subunit, for which conantokin G appears to have selective affinity, has been associated with the striatal–nigral projections (Standaert et al., 1994), possibly explaining the apparent preferential impact of conantokin G on nigral responses to methamphetamine. In contrast, MK801 has somewhat nonselective affinity for NMDA receptor subunits (Laurie and Seeburg, 1994), possibly accounting for its lack of selectivity and comparable neuropeptide effects in both the striatum and substantia nigra. The possibility that the site of action for conantokin G differs from MK801 was confirmed by our behavioral findings. We observed that the presence of conantokin G had little effect alone, but when combined, profoundly enhanced the methamphetamine-induced stereotypic motor responses. In contrast, MK801 induces substantial ataxia when administered alone and when combined, it almost completely prevents expression of methamphetamine-induced stereotypic effects (Singh et al., 1990). The potential clinical relevance of these differences between conantokin G and nonselective NMDA receptor antagonists, such as MK801, requires further study.

Acknowledgements

This study was supported by PHS grants DA04222 and DA00378.

References

Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Hanson, G.R., Merchant, K., Letter, A.A., Bush, L., Gibb, J.W., 1987. methamphetamine-induced changes in the striatal–nigral dynorphin system: role of D_1 and D_2 receptors. Eur. J. Pharmacol. 144, 245–246.

Hanson, G.R., Merchant, K., Letter, A.A., Bush, L., Gibb, J.W., 1988. Characterization of methamphetamine effects on the striatal–nigral dynorphin system. Eur. J. Pharmacol. 155, 11–18.

Holson, R.R., Bowyer, J.F., Clausing, P., Gough, B., 1996. methamphetamine-stimulated striatal dopamine release declines rapidly over time following microdialysis probe insertion. Brain Res. 739, 301–307.

König, J., Klippel, R., 1963. The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem. Williams and Wilkins, Baltimore, MD.

Laurie, D.J., Seeburg, P.H., 1994. Ligand affinities at recombinant *N*-methyl-D-aspartate receptors depend on subunit composition. Eur. J. Pharmacol. 268, 335–345.

Letter, A.A., Merchant, K., Gibb, J.W., Hanson, G.R., 1987a. Effect of methamphetamine on neurotensin concentrations in rat brain regions. J. Pharmacol. Exp. Ther. 241, 443–447.

Letter, A.A., Matsuda, L., Merchant, K., Gibb, J.W., Hanson, G.R., 1987b. Characterization of dopaminergic influence on striatal–nigral neurotensin systems. Brain Res. 422, 200–203.

Maidment, N., Siddall, B., Rudolph, V., Erdelyi, E., Evans, C., 1991. Dual determination of extracellular cholecystokinin and neurotensin fragments in rat forebrain: microdialysis combined with a sequential multiple antigen radioimmunoassay. Neuroscience 45, 81–93.

Olivera, B.M., Rivier, J., Clark, C., Ramilo, C., Corpuz, G., Abogadie, F., Mena, E., Woodward, S., Hillyard, D., Cruz, L., 1990. Diversity of *Conus* neuropeptides. Science 249, 257–263.

Singh, N., Bush, L., Gibb, J.W., Hanson, G.R., 1990. Dopamine-mediated changes in central nervous system neurotensin systems: a role for *N*-methyl-D-aspartate receptors. Eur. J. Pharmacol. 187, 337–344.

Singh, N., Midgley, L., Bush, L., Gibb, J.W., Hanson, G.R., 1991. *N*-methyl-D-aspartate receptors mediate dopamine-induced changes in extrapyramidal and limbic dynorphin systems. Brain Res. 555, 233–238.

Standaert, D.G., Testa, C.M., Young, A.B., Penney, J.B., 1994. Organization of *N*-methyl-D-aspartate receptor gene expression in the basal ganglia of the rat. J. Comp. Neurol. 343, 1–16.