ELSEVIER

European Journal of Pharmacology 338 (1997) 7–10

ejp

Short communication

Paradoxical behavioral response to apomorphine in tenascin-gene knockout mouse

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Received 7 August 1997; revised 22 August 1997; accepted 26 August 1997

Abstract

Tenascin is a large extracellular matrix glycoprotein which is highly expressed in the developing nervous system. To examine the role of tenascin in vivo, we have produced mice in which the tenascin-gene is inactivated. These animals did not easily habituate to unfamiliar circumstances and displayed hyperlocomotion. A dopamine receptor agonist, apomorphine, reduced this hyperlocomotion dose dependently, but this phenomenon was not due to the appearance of apomorphine-induced stereotypic behavior, suggesting that tenascin-gene mutant mice have a paradoxical behavioral response to apomorphine compared to wild-type mice. © 1997 Elsevier Science B.V.

Keywords: Tenascin; Knockout mouse; Apomorphine; Dopamine receptor

1. Introduction

Tenascin is a very large glycoprotein of the extracellular matrix, and at least three different tenascin subtypes, tenascin-R, tenascin-X and tenascin-C (tenascin-C is simply designated as tenascin in this report), have been found (Erickson, 1993). It appears electronmicroscopically as a hexabrachion with six similar arms extending from a central core joined by disulfide bonds at the amino termini (Vaughan et al., 1987). Members of tenascin subtypes share a distinctive pattern of domains, such as epidermal growth factor-like units, fibronectin type III homology repeats and a fibrinogen-like domain in the carboxy terminal end. Tenascin has multifunctional roles in embryonic cellular pattern formation, cellular attachment to basement membranes and cell division. By contrast, its expression is generally limited to adult tissues, with the exception of several organs such as the thymus, small intestine and uterus. In addition, tenascin is strongly reexpressed in the stroma of malignant tumors (Chiquet-Ehrismann et al., 1986) and is transiently expressed during wound healing and brain injury (Laywell et al., 1992).

Tenascin is sequentially expressed in different regions of the developing brain and is particularly rich in certain areas of the brain, such as the frontal cortex, hippocampus and cerebellum, making it one of the most abundant extracellular matrix proteins in the central nervous system (CNS). Tenascin plays a pivotal role in neural migration, axonal outgrowth and neuron–glia cell interactions (Steindler et al., 1995). For instance, the expression of tenascin on Bergmann glia cells coincides with the migration of cerebellar granule cell neurons, indicating that tenascin guides granule cells from the external to the internal cell layer in the early stage of development of the brain (Husmann et al., 1992). Recently, we reported that tenascin-gene knockout mice, which were generated by homologous recombination (Saga et al., 1992), showed hyperactivity in an open field exploratory test. Biochemical analysis, with ion-pair high-performance liquid chromatography, showed dopamine transmission to be significantly attenuated in the striatum and hippocampus in these animals. Moreover, the intraperitoneal administration of a dopamine D2/D3 receptor agonist, LY171555 (0.5 mg/kg), completely inhibited this hyperkinesia (Fukamauchi et al., 1996). These findings suggest that tenascin may play an important role in dopamine neurotransmission related to behavior.

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In the present study, we attempted to analyze the hyperlocomotion of tenascin-gene mutant mouse in response to the dopamine receptor stimulating agent, apomorphine.

2. Materials and methods

The tenascin-gene knockout mice, in which the second exon of the tenascin-gene is replaced with the *lacZ* gene, were generated in 1992 (Saga et al., 1992) and backcrossed and maintained under specific pathogen-free conditions in the animal facility of Tsukuba Life Science Center, the Institute of Physical and Chemical Research (RIKEN) (Ibaraki, Japan). They were housed in group cages and were provided with solid food and water ad libitum under standard conditions (12 h light/12 h dark cycle). All experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals. For this study, homozygous knockout mutant mice and normal wild-type mice (age postnatal day 21–28) were individually put into empty plastic cages (16×22 cm). Spontaneous locomotor activity was assessed in the test cage every 10 min for 60 min by using Supermex (Muromachi Kikai, Tokyo, Japan). For the test on the effect of apomorphine, after 30 min acclimatization in the test cage, animals were given single intraperitoneal injections of various dosages of apomorphine hydrochloride (Sigma, St. Louis, MO, USA) or the same amount of physiological saline as a vehicle control and placed back into the individual cages as described above. Locomotor activity was recorded with Supermex for 30 min after the injection. Supermex consists of body-temperature sensors and was mounted above the cage to detect changes in heat across multiple zones of the cage through an array of Fresnel lenses. Body heat radiated by an individual animal was detected with the sensor head of the monitor, which contained paired extreme infrared ray pyroelectric detectors. In this way, the system could monitor and count all spontaneous movements. Ambulation counts were automatically totalled and scored with a personal computer.

To verify that the lack of locomotor hyperactivity of mutant mice was not due to the influence of apomorphine-induced stereotypic behavior, we also checked the advent of stereotypic activity in each mouse. Stereotypy was scored at 5, 10, 15, 20, 25 and 30 min after treatment with apomorphine. For assessment of stereotypy, the following scoring grade was used: 0 = no stereotypy; 1 = intermittent sniffing; 2 = continuous sniffing; 3 = continuous sniffing and intermittent biting, gnawing or licking and 4 = continuous sniffing and continuous biting, gnawing or licking. Scores obtained at each time point were added to attain a total score. The mean and standard error for the group were calculated using the Mann–Whitney *U*-test. Values of $P < 0.05$ were regarded as significant.

3. Results

When the wild-type mice were placed in the test cage, they generally showed exploratory behavior at the begin-

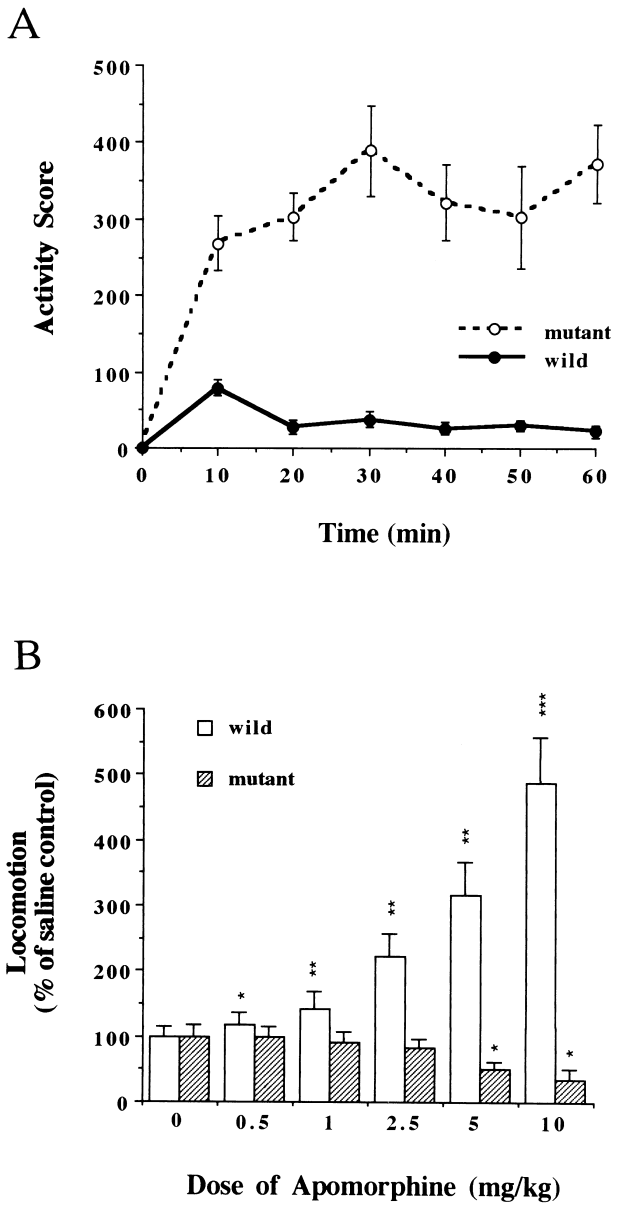

Fig. 1. (A) Mouse locomotor activity in the test cage. Locomotion was assessed for 60 min by using Supermex as described in Section 2. Each value represents cumulative scores for 10 min and the mean score with SEM of 8 observations. The scores of tenascin gene-knockout mutant mice were significantly higher than those wild-type mice from 10 to 60 min ($P < 0.001$). (B) Dose–response changes in apomorphine-induced locomotor activity. The locomotor activity was recorded for 30 min after injection and the data are the percentage values relative to those of vehicle-injected control mice. Each point represents the mean with S.E.M. obtained from 8 mice. Statistical analysis of the behavioral data was performed with the Mann–Whitney *U*-test ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared to saline challenge for each group). Injection of apomorphine (0 mg/kg) indicates saline-treated control mice.

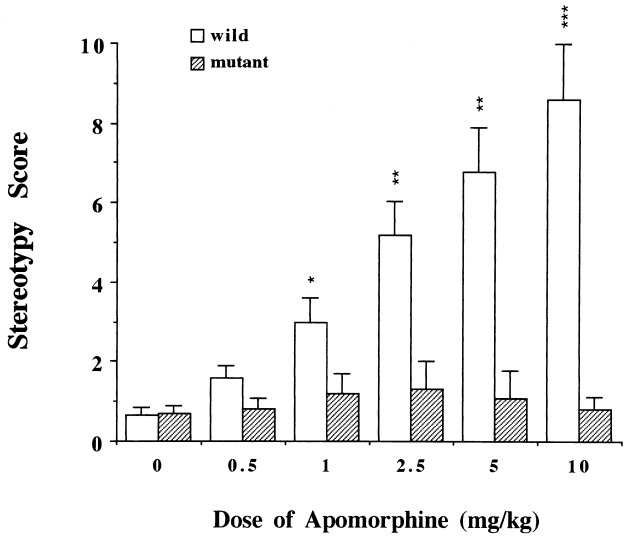

Fig. 2. Stereotypy scores after a single administration of apomorphine. Mice received various doses of apomorphine intraperitoneally and stereotypic behavior was assessed. Data presented are means ± S.E.M. of cumulated stereotypy scores of 8 mice (summation of each 5 min score for 30 min following the challenge with apomorphine, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, between groups comparison for each dose).

ning, but soon became used to the new environment and spontaneous locomotor activity was gradually reduced. However, the tenascin-mutant mice continued exploratory activity for a long time and did not easily habituate to the unfamiliar circumstances (Fig. 1A). As shown in Fig. 1B, while the normal wild-type mice demonstrated an increase in locomotor activity in a dose-dependent manner, apomorphine-induced locomotor hyperactivity was not observed in the mutant mice. Rather, there was a graduated reduction in locomotor activity with increasing doses of apomorphine.

The normal wild-type mice engaged in typical apomorphine-induced stereotypic behaviors, such as sniffing (Fig. 2). Higher levels of stereotypic behaviors, such as gnawing and licking, were not found in apomorphine-treated wild type mice. No augmentation of stereotypy was observed in the tenascin-gene mutant mice. In fact, the highest dose (10 mg/kg) of apomorphine made the mutant mice completely immobile during the first 10–20 min of the test period without producing signs of stereotypy.

4. Discussion

Mice with the disrupted tenascin gene are anatomically and histologically normal at the microscopic level. However, tenascin-gene mutant mice have a low body weight and a low pregnancy rate. In particular, hyperlocomotion has been found in homozygous tenascin-gene knockout mice. However, neither the heterozygous nor normal wild-type mice showed abnormal behavior. The present study demonstrates that apomorphine failed to produce a further increase in locomotion in tenascin-gene mutant mice but, rather, caused in a significant, dose-dependent reduction in their hyperlocomotion.

These results indicate that the dopamine receptors in the brains of tenascin-gene mutant mice display an aberrant function, at least, in sensitivity or response to apomorphine. Generally, the dopamine receptor antagonist itself possesses sedative effects and its dosage level is critical to assess the competitive effects of dopamine receptor agonists. Pretreatment with a low dose of the dopamine receptor antagonist, haloperidol (0.1 mg/kg), inhibited this apomorphine-induced sedative effect (unpublished observation). This finding suggests that this phenomenon is, at least partly, expressed through dopamine receptors. Undoubtedly, the dopamine system plays an important part in a wide variety of neural networks in the CNS. For instance, the nigro-striatal dopamine pathway contributes heavily to the motor system, and the meso-limbic or meso-cortical pathways are considered to be involved in motor or emotional activities. Apomorphine or methamphetamine-induced behavioral sensitization is generally accompanied by augmented central dopaminergic transmission. In particular, the striatum and hippocampus seem to be responsible for the development of behavioral sensitization during the formation of methamphetamine-induced behavioral change (Yoshikawa et al., 1991). There is general agreement that dopaminergic transmission in the striatum or limbic area, including the hippocampus, plays a key role in spontaneous behavior and in dopamine receptor simulant-induced behavioral sensitization (Creese, 1986). In this respect, it is of interest that the dopamine turnover rate was decreased in the striatum and hippocampus of tenascin-gene knockout mice (Fukamauchi et al., 1996).

Furthermore, Bloomquist et al. (1994) demonstrated that dopaminergic agents, such as bromocriptine or haloperidol, alter tenascin-gene expression at the transcription level in the neurointermediate lobe of the pituitary, supporting the interaction between dopamine receptor and tenascin. In this context, tenascin-gene mutant mice might provide clues as to the mechanisms of CNS receptors in terms of the extracellular matrix. Further detailed studies will be necessary to evaluate the characteristics of the dopamine receptor in tenascin-gene mutant mouse brain both in vivo and in vitro.

In summary, we have observed that treatment with apomorphine reduces the hyperlocomotion seen in tenascin-gene knockout mice in a dose-dependent manner without there being apomorphine-induced stereotypic behavior.

Acknowledgements

This research was supported by the Grant provided by the Ichiro Kanehara Foundation and Grant-in-Aid for Exploratory Research from the Ministry of Education, Science, Sports and Culture of Japan.

References

Bloomquist, B.T., Darlington, D.N., Mains, R.E., Eipper, B.A., 1994. RESP18, a novel endocrine secretory protein transcript and four other transcripts are regulated in parallel with pro-opiomelanocortin in melanotropes. J. Biol. Chem. 269, 9113–9122.

Chiquet-Ehrismann, R., Mackie, E.J., Pearson, C.A., Sakakura, T., 1986. Tenascin: An extracellular matrix protein involved in tissue interactions during fetal development and oncogenesis. Cell 47, 131–139.

Creese, I., 1986. Dopamine receptors. In: Conn, P.M. (Ed.), The Receptors, vol. 4. Academic Press, New York, NY, pp. 171–212.

Erickson, H.P., 1993. Tenascin-C, tenascin-R and tenascin-X: A family of talented proteins in search of functions. Curr. Opin. Cell Biol. 5, 869–876.

Fukamauchi, F., Mataga, N., Wang, Y.-J., Sato, S., Yoshiki, A., Kusakabe, M., 1996. Abnormal behavior and neurotransmissions of tenascin gene knockout mouse. Biochem. Biophys. Res. Commun. 221, 151–156.

Husmann, K., Faissner, A., Schachner, M., 1992. Tenascin promotes cerebellar granule cell migration and neurite outgrowth by different domains in the fibronectin type III repeats. J. Cell Biol. 116, 1475–1486.

Laywell, E., Dorries, U., Bartsch, U., Faissner, A., Schachner, M., Steindler, D.A., 1992. Enhanced expression of the developmentally regulated extracellular matrix molecule tenascin following adult brain injury. Proc. Natl. Acad. Sci. USA 89, 2634–2638.

Saga, Y., Yagi, T., Ikawa, Y., Sakakura, T., Aizawa, S., 1992. Mice develop normally without tenascin. Genes Dev. 6, 1821–1831.

Steindler, D.A., Settles, D., Erickson, H.P., Laywell, E.D., Yoshiki, A., Faissner, A., Kusakabe, M., 1995. Tenascin knockout mice: Barrels, boundary molecules and glial scars. J. Neurosci. 15, 1971–1983.

Vaughan, L., Huber, S., Chiquet, M., Winterhalter, K.H., 1987. A major, six-armed glycoprotein from embryonic cartilage. EMBO J. 6, 349–353.

Yoshikawa, T., Shibuya, H., Kaneno, S., Toru, M., 1991. Blockade of behavioral sensitization to methamphetamine by lesion of hippocampo-accumbal pathway. Life Sci. 48, 125–1332.