Psicothema 2005. Vol. 17, nº 3, pp. 492-495
www.psicothema.com

ISSN 0214 - 9915 CODEN PSOTEG

Acute and subchronic effects of agmatine on anxiety tested in the elevated plus maze in male mice

José Francisco Navarro, Gema Luna and Francisca B. García
University of Málaga

Agmatine is an endogenous polyamine, identified in mammalian brain, which meets the main criteria to be considered a neurotransmitter. Agmatine-like immunoreactivity has been described in numerous brain regions which have long been implicated in the regulation of anxiety. However, animal preclinical studies investigating effects of agmatine on anxiety are unclear. In this study, an attempt was made to clarify the actions of agmatine (7.5-60 mg/kg, ip) on anxiety tested in the elevated plus maze mice. Moreover, the possible development of tolerance to the effects of agmatine on anxiety after its subchronic administration for 7 consecutive days was also examined. A number of classical parameters were collected (open arm duration and frequency, closed arm duration and frequency and central platform duration and frequency). Different ethological measures were also obtained (rearings, head-dipping and stretched attend posture). Results showed that agmatine did not produce any significant behavioural change in any of the parameters examined, suggesting that it might not be involved in the regulation of anxiety in mice.

Efectos de la administración aguda y subcrónica de agmatina sobre la ansiedad evaluada en el test del laberinto elevado en cruz en ratones machos. La agmatina es una poliamina endógena, identificada en el cerebro de mamíferos, que cumple los principales criterios para ser considerada un neurotransmisor. Se ha descrito inmunorreactividad a la agmatina en numerosas regiones cerebrales tradicionalmente implicadas en la regulación de la ansiedad. Sin embargo, los estudios preclínicos con animales de experimentación que han investigado los efectos de la agmatina sobre la ansiedad no son concluyentes. Este trabajo representa un intento por clarificar las acciones de la agmatina (7.5-60 mg/kg, ip) sobre la ansiedad evaluada en el laberinto elevado en cruz en ratones. Asimismo, se examinó también el posible desarrollo de tolerancia a sus efectos sobre la ansiedad tras administración subcrónica durante siete días consecutivos. Se analizaron parámetros clásicos (frequencia y duración en brazos abiertos, cerrados y en la plataforma central del laberinto, así como diferentes medidas etológicas («rearings», «head-dipping» y «stretched attent posture»). Los resultados mostraron que la agmatina no produjo ningún cambio significativo en ninguno de los parámetros examinados, sugiriendo que podría no estar involucrada en la regulación de la ansiedad en ratones.

Agmatine is an endogenous amine and ionic cation, synthesized following decarboxylation of L-arginine by arginine decarboxylase. It has been recently identified in mammals, with a wide distribution into a number of tissues including brain, stomach, intestine and aoerta (Li et al., 1994; Raash et al., 1995). Agmatine is considered as a neurotransmitter or neuromodulator in the brain, being synthesized, stored in vesicles, released from specific networks of neurons in a Ca2+ dependent manner, inactivated by energy-dependent reuptake mechanisms and degraded enzymatically (Navarro, 2002). Agmatine acts as an agonist at imidazoline and alpha-2-adrenergic receptors, also modulating NMDA receptor activity. Likewise, it has been characterized as a weak inhibitor of varios nitric oxide synthase (NOS) isoenzymes (Yang and Reis, 1999; Reis and Regunathan 1999, 2000). The presence of agmatine in neurons and astrocytes has been demonstrated by immunohistochemical examination with anti-agmatine antibody (Regunathan et al., 1995; Otake et al., 1998).

From a clinical point of view, agmatine seems to have therapeutic potential in the treatment of brain injury (Gilad et al., 1996; Gilad and Gilad, 2000), addiction to alcohol and opiates (Aricioglu-Kartal and Regunathan, 2002; Uzbay et al., 2000), epilepsy (Demehri et al., 2003; Su et al., 2004), depression (Zomkowski et al., 2002; Li et al., 2003) and chronic pain (Fairbanks et al., 2000; Yesilyurt and Uzbay, 2001). Therefore, it might be a pleiotropic molecule with many functions in mammals.

Agmatine-like immunoreactivity has been described in numerous brain regions which have long been implicated in the regulation of anxiety (Otake et al., 1998). However, results from animal studies investigating effects of agmatine on anxiety are unclear. Thus, although Lavinsky, Arteni and Netto (2003) observed a mild

Fecha recepción: 22-6-04 • Fecha aceptación: 18-10-04
Correspondencia: José Francisco Navarro
Facultad de Psicología
Universidad de Málaga
29071 Málaga (Spain)
E-mail: navahuma@uma.es

anxiolytic-like action of agmatine (20 and 40 mg/kg) in rats tested in the elevated plus maze, Uzbay and Lal (2002) did not found effects in the pentylenetetrazol (PTZ) model of anxiety (20, 40 and 60 mg/kg). In our study, an attempt was made to clarify the effect of agmatine (7.5-60 mg/kg, ip) on anxiety by examining its effects on behavior of male mice in the elevated plus-maze. Likewise, the possible development of tolerance to the effects of agmatine on anxiety after its subchronic administration for 7 consecutive days was also analyzed.

Methodology

Animals

129 albino male mice of the OF.1 strain weighing 25-30 g were used. Animals were housed in groups of five in plastic cages (24 × 13.5 × 13 cm) under standardized lighting conditions (white lights on 20:00-8:00), a constant temperature (20 ºC), and food and tap water available ad libitum, except during behavioural tests. Cage maintenance was undertaken twice weekly, but never on the day of testing. Mice were housed 7 days before the experiment.

This experiment was carried oyt in accordance with the Guiding Principles for Care and Use of Laboratory Animals approved by the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Drug administration

Nine groups of mice were used. Animals were randomly allocated to one control group (n= 14) receiving physiological saline and eight experimental groups (n= 13-15 each) receiving agmatine injections. Agmatine was diluted in physiological saline to provide appropriate doses for injections and administered acutely and subchronically (for 7 consecutive days) in four doses: 7.5, 15, 30 and 60 mg/kg. Theses doses were selected on the basis of previous behavioural studies (Uzbay and Lal, 2002; Lavinsky et al., 2003; Navarro and Luna, 2005). Drug or vehicle was injected intraperitoneally in a volume of 10 ml/kg. Tests were performed 30 min after injections.

Apparatus and behavioral test

Plus-maze test consisted of two open arms (30 x 5 cm, surrounded by a 0.25-cm-high border) and two closed arms (30 × 5 cm, surrounded by 15-cm-high walls) with the two pairs of identical platform, which emerged from a central platform (5 × 5 cm), positioned opposite each other (Lister, 1987; Manzaneque et al., 2002; Navarro and Maldonado, 2002; Navarro, Burón and Martín-López, 2002; Navarro et al., 2003). The aparatus was elevated 40 cm above the floor.

Mice were tested on the maze in a randomized order. The test was initiated by placing the mouse on the central platform of the maze, facing one of the open arms, and letting it move freely. Each session lasted 5 min, being recorded by a videocamera. All tests were carried out under dim red lighting between the second and seventh hour of the dark phase. After each test, the maze was thoroughly cleaned. Behavioural analysis was performed by a trained experimenter who was blind to treatment condition.

A number of classical parameters were collected during the session: (a) Open arm duration: the total amount of time the mouse spent in the open arms; (b) Closed arm duration: the total amount of time the mouse spent in the closed arms; (c) Central platform duration: the total amount of time the mouse spent in the central platform; (d) Open arm frequency: the frequency of mouse entry with all four paws into the open, unprotected arms; (e) Closed arm frequency: the frequency of mouse entry with all four paws into

Table 1
Median values with ranges of classical behavioural measures in the elevated plus-maze after acute and subchronic agmatine treatment (7.5, 15, 30 and 60 mg/kg, ip)

Treatment	Doses	Duration (sec)			Frequency (number of entries)		
		Open	Closed	Center	Open	Closed	Center
	Saline	39.98 (0-118)	156.88 (85-125)	121.1 (54-147)	3 (0-14)	14.5 (7-23)	17.5 (8-29)
Acute	7.5	19.94 (0-144)	138.9 (9-209)	128.25 (73-271)	2 (0-11)	13 (2-22)	16 (5-24)
	15	36.65 (0-87.5)	157.75 (45-280)	94.77 (13-255)	2 (0-12)	14 (2-23)	17 (2-32)
	30	12.10 (0-115)	176.41 (100-251)	96.01 (48-141)	1 (0-11)	16 (6-24)	20 (6-28)
	60	34.35 (0-98)	132.18 (47-192)	120 (80-193)	3 (0-19)	13 (7-20)	17 (10-32)
Subchronic	7.5	27.30 (0-116)	156.31 (83-230)	97.12 (66-199)	3 (0-10)	13.5 (6-24)	18.5 (8-25)
	15	21.98 (0-92)	173.44 (105-270)	110 (30-142)	2 (0-6)	14 (7-21)	16 (7-22)
	30	22.45 (0-77)	162.43 (110-218)	98.41 (82-150)	3 (0-13)	17 (10-26)	18.5 (11-30)
	60	23.42 (0-89)	161.69 (57-227)	106.31 (63-213)	3 (0-8)	15 (6-24)	18.5 (10-26)

the closed, protected arms, and (f) Total number of entries in the arms.

Likewise, different ethological measures were also quantified: (a) Rearings: a boyd stance in which the animal sets his forepaws onto the wall of a closed arm while keeping his rear legs on the floor; (b) Streched attend posture (SAP): a body posture in which the mouse streches forward and then retracts to its original position without moving the feet, and (c): Head-dipping (HD): movement of the head over the side of the maze and down towards the floor.

Data analyses

Nonparametric Kruskal-Wallis tests were initially used to assess the variance of the behavioural measures over different treatment groups. Subsequently, if necessary, appropriate paired comparisons were performed using Mann-Whitney U tests to contrast the parameters in the different treatment groups. The analysis was carried out using nonparametric statistics since the criteria for parametric statitistics were not met by the data.

Results

Table 1 illustrates medians (with ranges) of duration and number of entries in the different areas of the maze, and Table 2 shows the medians (with ranges) of the ethological behavioural measures examined. Kruskal-Wallis and Mann.Whitney U-tests analysis showed that there were no significant differences between control and experimental groups in any of the behavioural parameters examined.

Table 2
Median values with ranges of ethological behavioural measures in the elevated plus-maze after acute and subchronic agmatine treatment (7.5, 15, 30 and 60 mg/kg, ip)

Treatment	Doses	Frequency		
		Rears	HD	SAP
	Saline	6 (1-28)	9 (5-21)	7.5 (1-19)
Acute	7.5	10 (1-28)	10 (4-16	6 (0-16)
	15	10 (0-32)	7 (2-14)	8 (1-14)
	30	12 (1-31)	10 (4-16)	7 (3-14)
	60	6 (1-24)	10 (3-18)	5 (3-15)
Subchronic	7.5	6.5 (1-25)	11 (5-21)	7 (2-20)
	15	9 (3-13)	13 (4-31)	7 (1-14)
	30	12 (4-27)	11 (5-18)	6.5 (1-15)
	60	9.5 (0-27)	9 (6-15)	7 (0-14)

Discussion

Exploratory behaviour in novel environments is the basis for many psychopharmacological tests of anxiety, including the elevated plus maze. This test is routinely used to study anxiety-related behaviours in mouse (Lister, 1987). In this situation, mice will show a pattern of behaviour characterized by open-arm avoidance. This tendency is suppressed by anxiolytics and potentiated by anxiogenic agents (Belzung and Griebel, 2001). Furthermore, the inclusion of ethological measures results in a more sensitive methodology to characterize drug effects than if only are included spatial (classical) measures (Ohl, 2003).

Overall, the results obtained in the present study indicate that agmatine, acutely or subchronically administered, does not exhibit an anxiolytic/anxiogenic activity in male mice. Thus, as Table 1 and 2 show, no significant differences were observed between experimental and control groups in any of the parameters of anxiety analyzed in the elevated plus maze. These findings are in concordance with those comunicated by Uzbay and Lal (2002), who did not find any effect of agmatine (20, 40 and 60 mg/kg, ip) on anxiety in the pentylenetetrazol (PTZ) model in Long-Evans rats. Likewise, our results are consistent with a recent study in which agmatine did not produce any significant changes on agonistic behaviour of mice. In this work, some behavioural categories that have been traditionally interpreted as an index of anxiolytic/anxiogenic-like actions (eg, social investigation, avoidance/flee and defense/submission) (Beltrán and Navarro, 2002; Brain, Kusumorini and Benton, 1991; Luna, Pedraza and Navarro, 2004; Maldonado and Navarro, 2001; Navarro and Maldonado, 2004) were not significantly affected after treatment with agmatine (Navarro and Luna, 2005). However, our findings differ from Lavinsky et al. (2003), who found that agmatine (20 and 40 mg/kg) caused in Wistar rats a mild anxiolytic-like action in the elevated plus maze. This discrepancy may represent a species difference, perhaps related to the distinct functions of agmatine in rats and mice. In fact, species differences in receptor functions have been described with other neurotransmitter systems, such as 5-HT (Griebel et al., 2000; Larm, Shen and Gundlach, 2003).

On the other hand, motility of mice was not significantly affected after treatment with agmatine. These findings are foreseeable since a decreased motor activity has been only described with higher doses of those employed in our study (Lavinsky et al., 2003), and they are also in concordance with the recent study by Karadag et al (2003), who did not found effects on locomotor function after agmatine administration (10, 30 and 100 mg/kg, ip).

In conclusion, our results does not support the hypothesis that agmatine may play a role in modulation on anxiety in mice. Further studies using other animal models of anxiety and different mice strains are needed to confirm these findings.

References

Aricioglu-Kartal, F. and Regunathan, S. (2002). Effect of chronic morphine treatment on the biosynthesis of agmatine in rat brain and other tissues. *Life Sciences, 71,* 1.695-1.701.

Beltrán, D. and Navarro, J.F. (2002). Efectos de la administración de N-metil-ß-carbolina-3-carboxamida sobre la conducta agonística en ratones machos. *Psicothema, 14*, 205-208.

Belzung, C. and Griebel, G. (2001). Measuring normal and pathological anxiety-like behaviour in mice: a review. *Behavioral Brain Research, 125*, 141-149.

Brain, P.F., Kusumorini, N. and Benton, D. (1991). 'Anxiety' in laboratory rodents: a brief review of some recent behavioural developments. *Behavioral Processess, 25,* 70-80.

Demehri, S., Homayoun, H., Honar, H., Riazi, K., Vafaie, K., Roushanzamir, F. and Dehpour, A.R. (2003). Agmatine exerts anticonvulsant effect in mice: modulation by alpha-2 adrenoceptors and nitric oxide. *Neuropharmacology, 45*, 534-542.

Fairbanks, C.A., Schreiber, K.L., Brewer, K.L., Yu, C.G., Stone, L.S., Kitto, K.F., Nguyen, H.O., Grocholski, B.M., Shoeman, D.W., Kehl, L.J., Regunathan, S., Reis, D.J., Yezierski, R.P. and Wilcox, G.L. (2000). Agmatine reverses pain induced by inflammation, neuropathy and spinal cord injury. *Proceedings of the National Academy of Sciences USA, 97,* 10.584-10.589.

Gilad, G.M., Salame, K., Rabey, J.M. and Gilad, V.H. (1996). Agmatine treatment is neuroprotective in rodent brain injury models. *Life Sciences, 58*, 41-46.

Gilad, G.M. and Gilad, V.H. (2000). Accelerated functional recovery and neuroprotection by agmatine after spinal cord ischemia in rats. *Neuroscience Letters, 296,* 97-100.

Griebel, G., Rodgers, R.J., Perraul, G. and Sanger, D.J. (2000). The effects of compound varying in selectivity as 5-HT1A receptor antagonists in three rat models of anxiety. *Neuropharmacology, 39*, 1.848-1.857.

Karadag, H.C., Ulugol, A., Tamer, M., Ipci, Y. and Dokmeci, I. (2003). Systemic agmatine attenuates tactile allodynia in two experimental neuropathic pain models in rats. *Neuroscience Letters, 339*, 88-90.

Larm, J.A., Shen, P.J. and Gundlach, A.L. (2003). Differential galanin receptor-1 and galanin-expression by 5-HT neurons in dorsal raphe nucleus of rat and mouse: evidence for species-dependent modulation of serotonin transmission. *European Journal of Neuroscience, 17*, 481-493.

Lavinsky, D., Arteni, N.S. and Netto, C.A. (2003). Agmatine induces anxiolysis in the elevated plus maze task in adult rats. *Behavioral Brain Research, 141,* 19-24.

Li, G., Regunathan, S., Barrow, C.J., Eshraghi, J., Cooper, R. and Reis, D.J. (1994). Agmatine: an endogenous clonidine-displacing substance in the brain. *Science, 263*, 966-969.

Li, Y.F., Gong, Z.H., Cao, J.B., Wang, H.L., Luo, Z.P. and Li, J. (2003). Antidepressant-like effect of agmatine and its possible mechanism. *European Journal of Pharmacology, 469*, 81-88.

Lister, R.G. (1987). The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology, 92*, 180-185.

Luna, G., Pedraza, C. and Navarro, J.F. (2004). Efectos de la coadministración de gammahidroxibutirato (GHB) y L-741-741, un antagonista selectivo de los receptores dopaminérgicos D4, sobre la conducta agonística en ratones machos. *Psicothema, 16*, 282-287.

Maldonado, E. and Navarro, J.F. (2001). MDMA («ecstasy») exhibits an anxiogenic-like activity in social encounters between male mice. *Pharmacological Research, 44*, 27-31.

Manzaneque, J.M., Brain, P.F. and Navarro, J.F. (2002). Effects of low doses of clozapine on behavior of isolated and group-housed male mice in the elevated plus-maze test. *Progress in NeuroPsychopharmacology & Biological Psychiatry, 26*, 349-355.

Navarro, J.F. (2002). Agmatina: perfil farmacológico y efectos conductuales. *Psiquiatría Biológica, 9*, 130-132.

Navarro, J.F., Burón, E. and Martín-López, M. (2002). Anxiogenic-like activity of L-655.708, a selective ligand for the benzodiazepine site of GABA-A receptors which contain the alpha-5 subunit, in the elevated plus-maze test. *Progress in NeuroPsychopharmacology & Biological Psychiatry, 26*, 1.389-1.392.

Navarro, J.F. and Maldonado, E. (2002). Acute and subchronic effects of MDMA («ecstasy») on anxiety in male mice tested in the elevated plus-maze. *Progress in NeuroPsychopharmacology & Biological Psychiatry, 26*, 1.151-1.154.

Navarro, J.F., Luna, G., García, F. and Pedraza, C. (2003). Effects of L-741,741, a selective D4 dopamine receptor antagonist, on anxiety tested in the elevated plus-maze in mice. *Methods and Findings in Experimental and Clinical Pharmacology, 25*, 45-47.

Navarro, J.F. and Maldonado, E. (2004). Effects of acute, subchronic and intermittent MDMA (ecstasy) administration on agonistic interactions between male mice. *Aggressive Behavior, 30*, 84-91.

Navarro, J.F. and Luna, G. (2005). An ethopharmacological assessment of agmatine's effects on agonistic encounters between male mice. *Aggressive Behavior, 31* (in press).

Ohl, F. (2003). Testing for anxiety. *Clinical Neuroscience Research, 3*, 233-238.

Otake, J., Ruggiero D.A., Regunathan, S., Wang, T.A., Milner, D.J. and Reis, D.J. (1998). Regional localization of agmatine in the rat brain: an immunocytochemical study. *Brain Research, 787*, 1-14.

Raash, W., Regunathan, S., Li, G. and Reis, D.J. (1995). Agmatine, the bacterial amine, is widely distributed in mammalian tissues. *Life Sciences, 56*, 2.319-2.330.

Reis, D.J. and Regunathan, S. (1999). Agmatine: an endogenous ligand at imidazoline receptors is a novel neurotransmitter. *Annals of New York Academy of Sciences, 881*, 65-80.

Reis, D.J. and Regunathan, S. (2000). Is agmatine a novel neurotransmitter in brain? *Trends in Pharmacological Sciences, 21*, 187-193.

Regunathan, S., Feinstein, W., Raasch, W. and Reis, D.J. (1995). Agmatine (decarboxylated arginine) is synthesized and stored in astrocytes. *Neuroreport, 6*, 1.897-1.900.

Su, R.B., Wei, X.L., Zheng, J.Q., Liu, Y., Lu, X.W. and Li, J. (2004). Anticonvulsive effect of agmatine in mice. *Pharmacology Biochemistry and Behavior, 77*, 345-349.

Uzbay, I.T., Yesilyurt, O., Celik, T., Ergün, H. and Usimer, A. (2000). Effects of agmatine on ethanol withdrawal syndrome in rats. *Behavioral Brain Research, 107*, 153-159.

Uzbay, I.T. and Lal, H. (2002). Effects of N-nitro-L-arginine methyl ester, 7-nitro indazole, and agmatine on pentylenetetrazol-induced discriminative stimulus in Long-Evans rats. *Progress in NeuroPsychopharmacology & Biological Psychiatry, 26*, 567-573.

Yang, X.C. and Reis, D.J. (1999). Agmatine selectively blocks the N-methyl-D-aspartate subclass of glutamate receptor channels in the hippocampal neurons. *Journal of Pharmacology and Experimental Therapeutics, 28*, 544-549.

Yesilyurt, O. and Uzbay, I.T. (2001). Agmatine potentiates the analgesic effect of morphine by an alpha-2-adrenoceptor-mediated mechanism in mice. *Neuropsychopharmacology, 25*, 98-103.

Zomkowski, A.D., Hammes, L., Lin, J., Calixto, J.B., Santos, A.R. and Rodrigues A.L. (2002). Agmatine produces antidepressant-like effects in two models of depression in mice. *Neuroreport, 13,* 387-391.