TOXICOLOGICAL SCIENCES **97(1)**, 75–80 (2007)
doi:10.1093/toxsci/kfm015
Advance Access publication February 8, 2007

Effect of Manganese on Luteinizing Hormone–Releasing Hormone Secretion in Adult Male Rats

Juan Pablo Prestifilippo,*[,1] Javier Fernández-Solari,* Claudia Mohn,* Andrea De Laurentiis,* Samuel M. McCann,* W. L. Dees,† and Valeria Rettori*

**Facultad de Medicina, Centro de Estudios Farmacológicos y Botánicos, CONICET, Universidad de Buenos Aires, Buenos Aires, Argentina; and †Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, Texas*

Received December 5, 2006; accepted February 5, 2007

Recently studies have demonstrated that low doses of (Mn^{+2}) in the form of manganese chloride can stimulate specific puberty-related hormones and advance signs of pubertal development in immature female and male rats. In the present study, we used an *in vitro* system to evaluate the ability of 0, 50, 250, and 500μM doses of Mn^{+2} to stimulate luteinizing hormone–releasing hormone (LHRH) secretion and to assess the hypothalamic mechanism of this action in adult male Sprague-Dawley rats. We demonstrated that Mn^{+2} at 500μM, but not the lower doses, increased LHRH release, nitric oxide (NO) synthase (NOS) activity, and the content of cyclic cGMP in the medial basal hypothalamus. Inhibition of NOS with a competitive inhibitor (N^{ω}-nitro-L-arginine methyl ester hydrochloride) prevented the Mn-induced increase in LHRH release. Additionally, methylene blue and KT5823, specific inhibitors of guanylyl cyclase and protein kinase G (PKG), respectively, also blocked the stimulatory effect of Mn^{+2} on LHRH release. These *in vitro* studies demonstrated that the hypothalamic mechanism of Mn^{+2} action in adult males is by activation of the NOS/NO system, resulting in increases in cGMP and PKG and thus the secretion of LHRH from the nerve terminals. These results indicate Mn^{+2} can cause LHRH release in adult males, and this action is discussed in relation to age, gender, as well as mechanistic and functional differences between adult and immature animals.

Key Words: **nitric oxide; cGMP; protein kinase G; medial basal hypothalamus.**

Manganese (Mn^{+2}) is an essential trace metal that is involved in the metabolism of carbohydrates, lipids, and proteins and has an important function as a cofactor for a number of enzymes (Keen, 1984). High doses of Mn^{+2} produce toxic effects causing altered developmental and reproductive functions (Grey and Laskey, 1980; Laskey *et al.*, 1982). Conversely, a Mn^{+2} deficiency causes impaired growth and reproduction in both sexes (Boyer *et al.*, 1942; Smith *et al.*, 1944), suggesting that there is a role for this metal in reproductive processes. In this regard, we recently showed that low doses of Mn^{+2}, administered chronically, resulted in increased serum levels of puberty-related hormones, such as luteinizing hormone, follicle-stimulating hormone, and estradiol (Pine *et al.*, 2005) and also advanced the time of vaginal opening in female rats (Pine *et al.*, 2005) and accelerated daily sperm production and efficiency of spermatogenesis in young males (Lee *et al.*, 2006). It was concluded that these effects in both sexes (female and male papers) were due to a hypothalamic action of the metal to facilitate the secretion of luteinizing hormone–releasing hormone (LHRH). Subsequently, we showed in immature females that the mechanism of this effect was through a nitric oxide (NO)–independent activation of the cGMP/PKG/LHRH-releasing pathway (Lee *et al.*, 2007).

There have been no studies conducted to determine whether Mn^{+2} can stimulate LHRH release in adult animals. Therefore, this study was conducted to assess the ability of Mn^{+2} to stimulate LHRH secretion in adult male rats and to discern whether the sensitivity of the stimulation and mechanism of this action at the hypothalamic level differs from immature rats of both sexes.

MATERIALS AND METHODS

Chemicals. LHRH for iodination and standards were purchased from Peninsula Laboratories, Inc., Division of Bachem (San Carlos, CA). cGMP for iodinations and standards were purchased from Sigma–Aldrich (St Louis, MO). ^{125}Iodine for iodination was purchased from New England Nuclear Life Science Product (Boston, MA). Manganese chloride ($MnCl_2$) was purchased from Anedra (San Fernando, Buenos Aires, Argentina). Hepes, DL-dithiothreitol (DTT), reduced nicotinamide adenine dinucleotide phosphate (NADPH), N^{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME), hemoglobin (Hb), and methylene blue (MB) were purchased from Sigma–Aldrich. Dowex AG 50 W-X8 200–400 mesh sodium form was obtained from Bio-Rad (Hercules, CA), and the ^{14}C-arginine monohydrochloride 360 mCi/mmol was from Amersham Pharmacia (Buckinghamshire, UK). The protein kinase G (PKG) inhibitor, KT5823, was purchased from Alomone Lab (Jerusalem, Israel).

Animals. Male rats of the Sprague-Dawley strain (220–250 g) were kept in group cages in an animal room having a photoperiod of 14 h of light (0500 h–1900 h) and a room temperature of 22°C–24°C. Animals had free access to

[1] To whom correspondence should be addressed at Facultad de Medicina, Centro de Estudios Farmacológicos y Botánicos, CONICET, Universidad de Buenos Aires, Paraguay 2155, piso 16, CP 1121, Buenos Aires, Argentina. Fax: +54 11 4508 3680. E-mail: jprestifilippo@yahoo.com.ar.

© The Author 2007. Published by Oxford University Press on behalf of the Society of Toxicology. All rights reserved.
For Permissions, please email: journals.permissions@oxfordjournals.org

Downloaded from toxsci.oxfordjournals.org by guest on August 5, 2011

laboratory chow and tap water. The experimental procedures reported here were approved by the Animal Care Committee of the Center of Experimental Pharmacology and Botanicals of the National Council for Research of Argentina and carried out in accordance with the Declaration of Helsinki.

In vitro incubations. After decapitation and removal of the brain, the medial basal hypothalamus (MBH) was dissected by making frontal cuts just behind the optic chiasm, extending dorsally 1.0 mm, and a horizontal cut extended from this point caudally to just behind the pituitary stalk, where another frontal cut was made. Longitudinal cuts were made 1.0 mm lateral to the midline bilaterally. The hypothalami (7–8 for each group) were preincubated individually in glass tubes with 500 μl of Krebs-Ringer bicarbonate-buffered medium (NaH_2PO_4 1.18mM, NaCl 118.46mM, KCl 5mM, $MgCl_2$ 1.18mM, $NaHCO_3$ 24.88mM, and $CaCl_2$ 2.5mM, containing 0.1% glucose, pH 7.4) alone or with vehicle of the inhibitory drugs for control or with the inhibitory drugs such as, L-NAME, Hb, MB, and KT5823. After this preincubation (15 min), the medium was discarded and replaced with fresh medium alone or with the medium containing the substances to be tested, and the incubation continued for 30 min. The incubation was continuing for 30 min followed by removal of the medium and storage of the samples at −20°C until the respective assays were conducted. All incubations were carried out in a Dubnoff shaker (50 cycles per min; 95% O_2/5% CO_2) at 37°C.

Radioimmunoassays. LHRH in the incubation media was measured by radioimmunoassays (RIA) utilizing a highly specific LHRH antiserum kindly provided by Ayela Barnea (University of Texas Southwestern Medical Center, Dallas, TX). The sensitivity of the assay was 0.2 pg per tube, and the curve was linear up to 100 pg of LHRH. The intra-assay coefficient of variation of the LHRH RIA ranged from 7.3%, and the interassay coefficient of variation was 8.9%. All samples were measured in duplicate.

cGMP content was measured by RIA using a highly specific antiserum purchased from Sigma–Aldrich. The sensitivity of the assay was 0.24 pmol/ml per tube, and the curve was linear up to 25 pmol/ml of cGMP. Intra- and interassay coefficients of variation were 5.0% and 7.0%, respectively.

Determination of NO synthase activity. Determination of nitric oxide synthase (NOS) activity was performed by a modification (Canteros *et al.*, 1995) of the ^{14}C-citrulline method of Bredt and Snyder (Bredt and Snyder, 1989). After the incubation period, the MBHs were immediately homogenized in ice cold 0.5 ml of 20mM Hepes (pH 7.4) with addition of 1.25mM $CaCl_2$ and 1mM DTT. The reaction was started by adding 120μM NADPH and 200,000 dpm of ^{14}C-arginine (360 mCi/mmol) to the homogenates. The tubes were incubated for 15 min at 37°C in a Dubnoff metabolic shaker (50 cycles per min and 95%O_2/5%CO_2 atmosphere). At the end of this incubation period, the tubes were immediately centrifuged at 10,000 × g for 10 min at 4°C. The supernatants were immediately applied to individual columns containing 1 ml of Dowex AG 50 W-X8 200 mesh sodium form and washed with 2.0 ml of double distilled water. All collected fluid from each column was counted for ^{14}C-citrulline activity in a scintillation counter. Since NOS converts arginine into equimolar quantities of citrulline and NO, the data were expressed as pmol of NO produced per MBH per min.

Statistics. All data are expressed as the mean (±SEM). Comparisons between groups were performed by using a one-way ANOVA followed by the Student-Newman-Keuls multiple comparison test for unequal replicates. Student's *t* test was used when comparing two groups. Results were confirmed by at least two independent experiments. Differences with *p* values <0.05 were considered significant.

RESULTS

Effect of Mn^{+2} on the Basal Release of LHRH, NOS Activity, and the Content of cGMP from MBH

Figure 1 shows that the 50 and 250μM doses of the metal did not increase the amount of basal LHRH released from MBH, compared to control group; however, the 500μM dose of Mn^{+2} was highly effective in inducing ($p < 0.001$) the release of the peptide. To determine if NO participates in this increased secretion of LHRH produced with the 500μM dose, we measured NOS activity. Results shown in Figure 2 demonstrate that the NOS activity, as assessed by the method of conversion of ^{14}C-arginine into ^{14}C-citrulline, was increased ($p < 0.01$) by

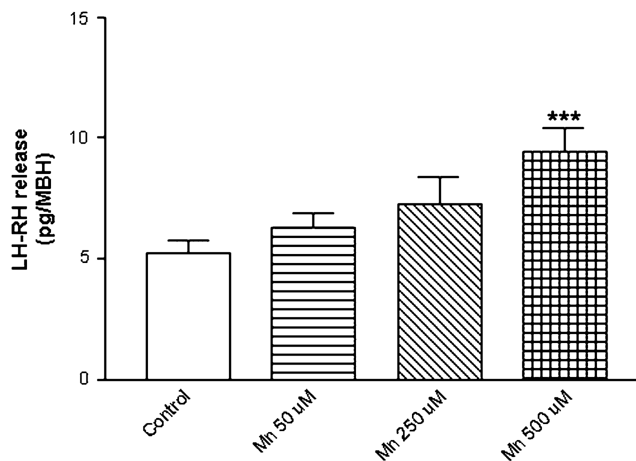

FIG. 1. Effect of different doses of Mn^{+2} on LHRH release from MBH *in vitro*. Each concentration point indicates basal LHRH levels versus Mn-stimulated levels. Tissues were incubated in Krebs-Ringer bicarbonate-buffered medium only (control) or in buffer containing 50μM, 250μM, and 500μM concentrations of Mn^{+2} for 30 min. Media were processed for determination of LHRH concentration by RIA. LHRH release into the incubation medium was increased by the 500μM dose of Mn^{+2}, but not by the 50μM and 250μM doses. Values represent mean ± SEM. The number of MBHs were 7–8 per group. ***$p < 0.001$.

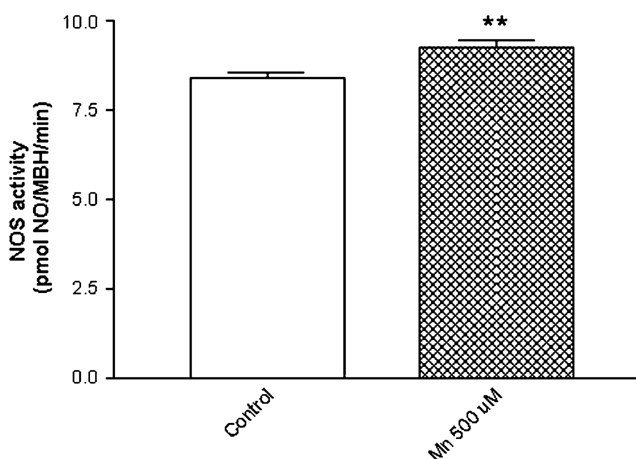

FIG. 2. The effect of incubation of MBH explants with Mn^{+2} on NOS activity. The MBHs were incubated with Mn^{+2} (500μM) as described in the "Materials and Methods" section and then NOS activity determined by the ^{14}C-arginine method. Since NOS converts arginine into equimolar quantities of citrulline and NO, the data were expressed as pmol of NO produced per MBH per min. The presence of Mn^{+2} (500μM) stimulated NOS activity in the MBH. Values represent mean ± SEM. The number of MBHs were 7–8 per group. **$p < 0.01$.

Downloaded from toxsci.oxfordjournals.org by guest on August 5, 2011

Mn^{+2} compared to the control group. Furthermore, Figure 3 shows that the content of the nucleotide cGMP, a product of guanylyl cyclase (GC) activity, was concomitantly increased ($p < 0.01$) as a result of exposure to the Mn^{+2}, an effect that was not observed in the control tissues.

Effect of NOS Inhibition on Mn-Induced cGMP Content and LHRH Release

We demonstrated that Mn^{+2} (500μM) increased the content of cGMP (Fig. 4a; $p < 0.05$) and LHRH release (Fig. 4b; $p < 0.01$) from MBH. L-NAME (1mM), an inhibitor of NOS activity, blocked both of these stimulations induced by the Mn^{2+}. L-NAME alone had no effect on the content of cGMP and LHRH release.

Effect of a Scavenger of NO on Mn-Induced LHRH Release

Figure 5 shows that the effect of Mn^{+2} (500μM) to stimulate ($p < 0.01$) LHRH release was blocked by the presence of Hb (40 μg/ml), scavenger of NO, in the media. Hb alone had no effect on the basal release of the peptide.

Effect of GC Inhibition on Mn-Induced cGMP Content and LHRH Release

Figure 6 shows as before that Mn^{+2} (500μM) increased both cGMP content (Fig. 6a; $p < 0.01$) and LHRH release (Fig. 6b; $p < 0.01$) into the media. This figure also shows that MB (10mM), an inhibitor of GC activity, blocked these stimulatory effects induced by the Mn^{2+}. MB alone had no effect on the content of cGMP and LHRH basal release.

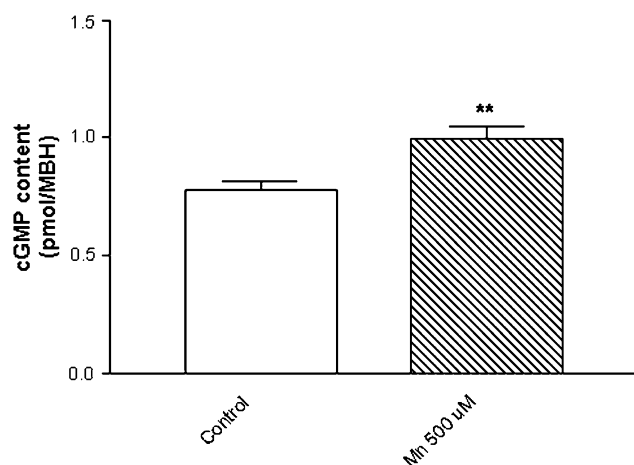

FIG. 3. The effect of Mn^{+2} on cGMP content in MBH. The nucleotide, cGMP, is a product of GC activity and furthermore transduces the signal of NO in brain. The hypothalamic fragments were incubated with Mn^{+2} (500μM) for 30 min. After the incubation period, the MBHs were immediately homogenized and cGMP content was determined by RIA using a highly specific antiserum. The presence of Mn^{+2} (500μM) significantly increased the content of cGMP in MBH. Values represent mean ± SEM. The number of MBHs were 7–8 per group. **$p < 0.01$.

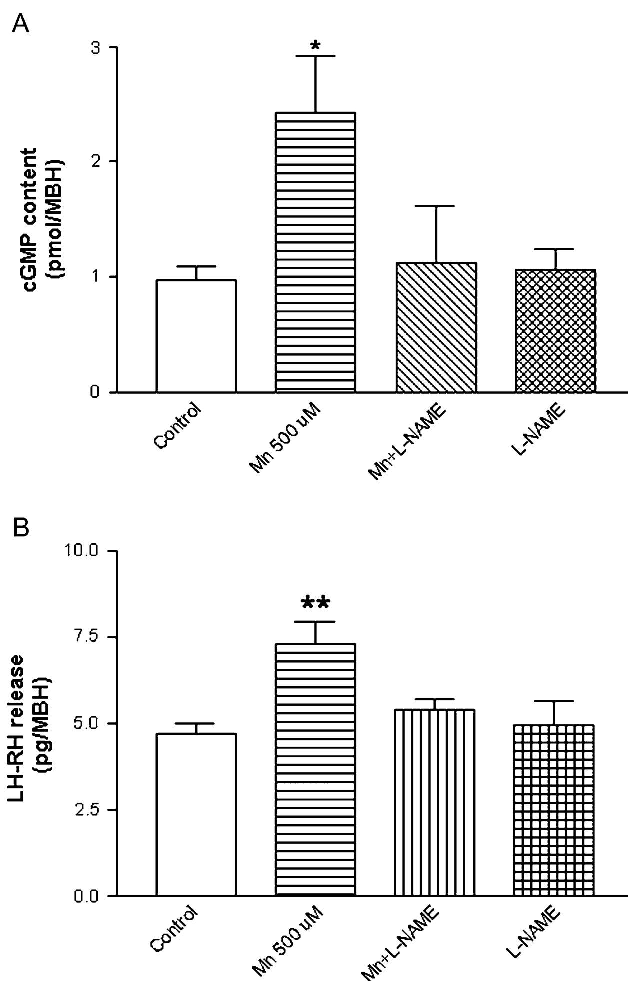

FIG. 4. The effect of Mn^{+2} in the presence of a NOS inhibitor on cGMP content and LHRH release by the MBH. The hypothalami were incubated individually in Krebs-Ringer bicarbonate buffered or with Mn^{+2} (500μM) alone or together with L-NAME 1mM for 30 min. In the groups incubated with L-NAME, the inhibitor was also present in the preincubation period. Mn^{+2} (500μM) increased the content of cGMP (a) and the release of LHRH (b). Both of these stimulatory actions were blocked by the addition of L-NAME (1mM) to the medium; L-NAME alone had no effect on cGMP content and basal LHRH release. Values represent mean ± SEM. The number of MBHs were 7–8 per group. *$p < 0.05$; **$p < 0.01$.

Effect of PKG Inhibition on Mn-Induced LHRH Release

Our results demonstrated that the ability of Mn^{+2} (500μM) to stimulate ($p < 0.001$) LHRH release was blocked by the presence of KT5823 (1μM), a specific inhibitor of PKG. The KT5823 alone had no effect on the basal LHRH release (Fig. 7).

DISCUSSION

The present results show that while Mn^{+2} at doses of 50μM and 250μM did not stimulate LHRH secretion from the basal hypothalamus of adult male Sprague-Dawley rats, the 500μM

Downloaded from toxsci.oxfordjournals.org by guest on August 5, 2011

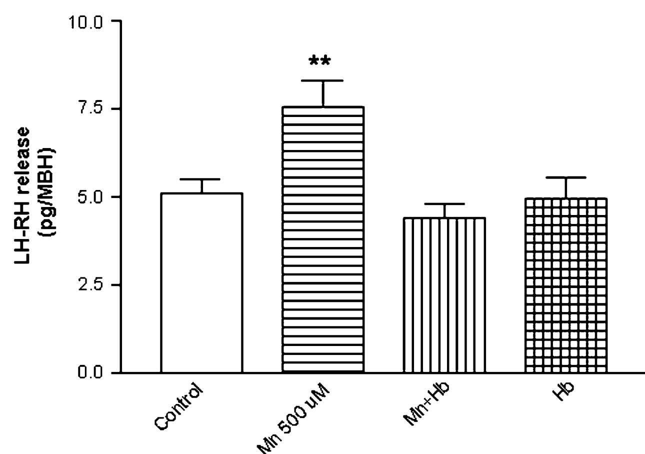

FIG. 5. The effect of Mn^{+2} in the presence of Hb, a scavenger of NO, on LHRH release from MBH. At the terminals, NO activates GC by binding to the hemegroup of the enzyme to catalyze the conversion of guanosine triphosphate to the secondary messenger, cGMP. The consequent increase in cGMP production leads to the extrusion of the LHRH secretory granules. Hb competes with the hemegroup of the enzyme and prevents the effect of NO. The ability of Mn^{+2} (500μM) to stimulate the release of LHRH was blocked by the addition of Hb (40 μg/ml) to the medium. Hb alone did not affect basal LHRH release. Values represent mean ± SEM. The number of MBHs were 7–8 per group. $^{**}p < 0.01$.

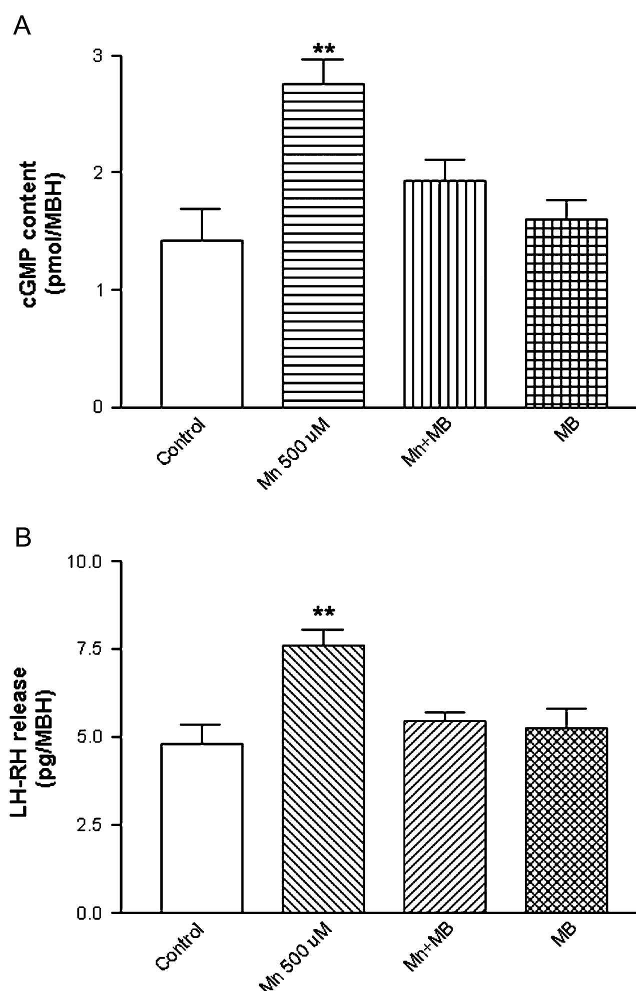

FIG. 6. The effect of Mn^{+2} in the presence of MB, a GC inhibitor, on cGMP content and LHRH release from MBH. The MBHs were preincubated in presence of MB 10mM 15 min before replacement with fresh medium or medium containing the Mn^{+2} 500μM plus MB. Mn^{+2} (500μM) increase the content of cGMP (a) and the release of LHRH (b). Both of these stimulatory actions were blocked by the addition of MB (10mM) to the medium. MB alone had no effect on cGMP content and LHRH basal release. Values represent mean ± SEM. The number of MBHs were 7–8 per group. $^{**}p < 0.01$.

dose was effective in increasing this secretion. This action to increase LHRH release was due to the ability of Mn^{+2} to stimulate NOS activity, resulting in increased production of NO and, hence, increased cGMP, PKG, and ultimately, increased LHRH release. These results are the first to demonstrate the effects of Mn^{+2} on LHRH release in adult animals; however, this stimulatory action of the metal on LHRH release in immature rats of both sexes has been reported previously (Lee *et al.*, 2006; Pine *et al.*, 2005). By comparing the results of these studies, with the results from the present study, we can now point out differences with regard to sex and age regarding sensitivity to the metal, but also specific differences in the mechanism of action.

Mn^{+2} was shown to stimulate LHRH secretion from the basal hypothalamus in immature female rats at a dose of 50μM (Pine *et al.*, 2005), whereas the dose required to stimulate release of the peptide in immature males was 250μM (Lee *et al.*, 2006). As shown in the present study, a 500μM dose was needed for LHRH stimulation in the adult males. While the adult males do appear in this regard to be less sensitive than immature males and females, it should be noted that there were modest differences in methodologies between laboratories. The present study used a shorter preincubation period that could have resulted in a greater *in vitro* basal secretion. Also, the size of the MBH in adult males is larger than those of immature animals and penetration of the metal could have been somewhat less. Taking into account these considerations, the combined results of the studies to date still suggest the potential for both a sex and age difference in sensitivity to Mn^{+2} with regard to stimulation of LHRH secretion. In that regard, the immature females were 5 and 10 times more sensitive to the metal than immature and mature males, respectively. The immature males were two times more sensitive than the mature males. These hormonal comparisons are the first and lend important support to other reports showing age and gender differences with regard to Mn^{+2} exposure (Flechter, 1999; Mena, 1974). Importantly, it has been suggested that infants and children are more sensitive to this substance than adults (Greger, 1999). Also, gender differences have been observed with male rats clearing the metal two times faster than female rats (Zheng *et al.*, 2000).

The fact that there is a NO involvement in the Mn^{+2}-stimulated release of LHRH is not surprising since NOS is

Downloaded from toxsci.oxfordjournals.org by guest on August 5, 2011

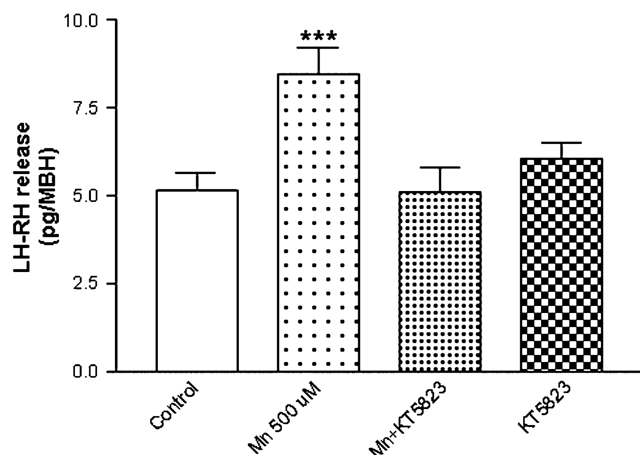

FIG. 7. The effect of cGMP-dependent protein kinase inhibition on Mn-induced LHRH release from MBH. cGMP produces the activation of a cGMP-dependent protein kinase (PKG), and the NO/cGMP/PKG pathway may control different neuronal functions. The MBHs were preincubated in presence of KT5823 1μM for 15 min before replacement with fresh medium or medium containing the Mn^{+2} 500μM plus KT5823. The ability of Mn^{+2} (500μM) to stimulate LHRH release was blocked by the addition of KT5823 (1μM) to the medium. KT5823 alone had no effect on basal release of LHRH. Values represent mean ± SEM. The number of MBHs were 7–8 per group. ***$p < 0.001$.

present in the MBH in juxtaposition to LHRH terminals and has been shown to regulate cGMP and LHRH in adult male and female rats (Bhat *et al.*, 1996; Rettori *et al.*, 1993). The Mn^{+2} dose at which this occurs, however, points to differences that can be drawn between results noted previously in immature females (Lee *et al.*, 2007). In this regard, we showed in the females that Mn^{+2} stimulated release of LHRH with a dose as low as 50μM and that this action was not altered by the presence of a NOS inhibitor. Importantly, in the immature females, we showed that low doses of Mn^{+2} acted downstream to NOS/NO by activating GC directly (Lee *et al.*, 2007). While Mn^{+2} can stimulate LHRH release at a lower dose in immature males compared to adult males, studies have not been conducted to discern whether NO plays a role in the immature males. Regardless of this particular mechanism, the gender difference is striking and notable, since a markedly lower dose of Mn^{+2} will induce LHRH release in immature females compared to either immature or adult males.

Mn^{+2} is a natural element readily available to the hypothalamus, thus further demonstrating the potential for the Mn^{+2} influence on LHRH secretion. This metal is able to enter the brain through the cerebral vasculature and the spinal fluid. The mechanism by which Mn^{+2} crosses the blood-brain barrier is not yet well understood, but involves binding of the metal to transport systems such as transferrin (Aschner and Aschner, 1990, 2000). Also, as Mn^{+2} levels rise in blood, the influx into the spinal fluid rises and entry across the choroid plexus becomes more important (Murphy *et al.*, 1991). Importantly, Mn^{+2} accumulates in the hypothalamus (Deskin *et al.*, 1980; Pine *et al.*, 2005) and is known to be taken up by both neurons and glial cells (Tholey *et al.*, 1990) and, hence, suggesting a potential role in neuronal/glial communications within the developing hypothalamus.

The effect of Mn^{+2} to stimulate LHRH with regard to dose and gender is important and worthy of additional discussion with regard to potential reproductive functions. The fact that immature animals appear more sensitive to the stimulatory effects of Mn^{+2} on LHRH release than adult animals suggests that the principal facilitative action on reproduction is perhaps most important leading up to and during pubertal maturation. Several lines of evidence support this. Because of the dose of Mn^{+2} required in the present study to induce LHRH release, adult males exhibit a higher level of resistance to the stimulatory effect of Mn^{+2}. It should be pointed out that it is possible that long-term exposure to the Mn^{+2} in males could be detrimental and they require higher concentrations of the metal, since an Mn^{+2} deficiency causes impaired growth and reproduction in both sexes (Boyer *et al.*, 1942; Smith *et al.*, 1944), suggesting that there is a role for this metal in reproductive processes. In contrast, the lower dose effect of Mn^{+2} to stimulate LHRH secretion in immature animals (Lee *et al.*, 2006; Pine *et al.*, 2005), especially in females, may be important with regard to normal pubertal development. Interestingly, however, should Mn^{+2} exposure occur too early in life, this apparent facilitatory or beneficial effect of Mn^{+2} could be harmful, since it can stimulate LHRH release and contribute to precocious pubertal development in both females and males, with females being twice as sensitive as males (Lee *et al.*, 2006; Pine *et al.*, 2005). Importantly, central precocious puberty is a much greater problem in girls with over 65% being idiopathic, compared to less than 10% in boys.

Thus, although modest differences in incubation techniques have been used between laboratories, overall the results suggest there may be dose, age, and gender differences to Mn^{2+} with immature females being more sensitive than immature males and immature males being more sensitive than adult males. In summary, our results are the first to show that Mn^{+2} is capable of inducing LHRH secretion in adult male rats. Using an *in vitro* system, we have discerned that the mechanism of this action is through activation of the hypothalamic NOS/NO system, hence, resulting in stimulation of the cGMP/PKG/LHRH-releasing pathway. Overall, these results suggest that while Mn^{+2} may facilitate LHRH secretion in adult animals, there are important age and gender differences regarding sensitivity to Mn^{+2} compared to immature animals of both sexes.

ACKNOWLEDGMENTS

This work was supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas (Grant CONICET PIP 6149), Agencia Nacional de Promoción Científica y Tecnológica (Grant BID 1201 OC-AR, PICT 03/14264), and the National Institutes of Health (ES013143 and ES09106).

Downloaded from toxsci.oxfordjournals.org by guest on August 5, 2011

REFERENCES

Aschner, M., and Aschner, J. L. (1990). Manganese transport across the blood brain barrier: Relationship to ion homeostasis. *Brain Res. Bull.* **24,** 857–860.

Aschner, M., and Aschner, J. L. (2000). Brain transport and emerging research needs. *Environ. Health Perspect.* **108,** 429–432.

Bhat, G., Mahesh, V. R., Aguon, K., and Brann, D. W. (1996). Evidence that brain nitric oxide synthase is the major nitric oxide synthase isoform in the hypothalamus of the adult female rat and that nitric oxide potently regulates hypothalamic cGMP levels. *Neuroendocrinology* **64,** 93–102.

Boyer, P. H., Shaw, J. H., and Phillips, P. H. (1942). Studies on manganese deficiency in the rat. *J. Biol. Chem.* **143,** 417–425.

Bredt, D. S., and Snyder, S. H. (1989). Nitric oxide mediates glutamate-linked enhancement of cGMP levels in the cerebellum. *Proc. Natl. Acad. Sci. USA* **86,** 9030–9033.

Canteros, G., Rettori, V., Franchi, A., Genaro, A., Cebral, E., Faletti, A., Gimeno, M., and McCann, S. M. (1995). Ethanol inhibits luteinizing hormone-releasing hormone (LHRH) secretion by blocking the response of LHRH neuronal terminals to nitric oxide. *Proc. Natl. Acad. Sci. USA* **92,** 3416–3420.

Deskin, R., Bursain, S. J., and Edens, F. W. (1980). Neurochemical alterations induced by manganese chloride in neonatal rats. *Neurotoxicology* **2,** 65–73.

Fechter, L. D. (1999). Distribution of manganese in development. *Neurotoxicology* **20,** 197–201.

Greger, J. L. (1999). Nutrition vs toxicology of manganese in humans: Evaluation of potential biomarkers. *Neurotoxicology* **20,** 205–212.

Grey, L. E., and Laskey, J. W. (1980). Multivariate análisis of the effects of manganese on the reproductive physiology and behavior of the male house mouse. *J. Toxicol. Environ. Health* **6,** 861–867.

Keen, C. L., Lonnerdal, B., and Hurley, L. S. (1984). Manganese. In *Biochemistry of the essential ultratrace elements* (E. Frieden, Ed.), pp. 89–132. Plenum Press, New York.

Laskey, J. W., Rehnberg, J. F., and Hein, J. F. (1982). Effects of chronic manganese exposure on selected reproductive parameters. *J. Toxicol. Environ. Health* **9,** 677–687.

Lee, B., Hiney, J. K., Pine, M. D., Srivastava, V. K., and Dees, W. L. (2007). Manganese stimulates LHRH secretion in prepubertal female rats: Hypothalamic site and mechanism of action. *J. Physiol.* **578,** 765–772.

Lee, B., Pine, M., Johnson, L., Rettori, V., Hiney, J. K., and Dees, W. L. (2006). Manganese acts centrally to activate reproductive hormone secretion and pubertal development in male rats. *Reprod. Toxicol.* **22,** 580–585.

Mena, I. (1974). The role of manganese in human disease. *Ann. Clin. Lab. Sci.* **4,** 487–491.

Murphy, V. A., Wadhawami, K. C., Smith, O. R., and Rapoport, S. I. (1991). Saturable transport of manganese across the rat blood brain barrier. *J. Neurochem.* **57,** 948–954.

Pine, M., Lee, B., Dearth, R., Hiney, J. K., and Dees, W. L. (2005). Manganese acts centrally to stimulate luteinizing hormone secretion: A potential influence on female pubertal development. *Toxicol. Sci.* **85,** 880–885.

Rettori, V., Belova, N., Dees, W. L., Nyberg, C. L., Gimeno, M., and McCann, S. M. (1993). Role of nitric oxide in the control of luteinizing hormone releasing hormone release in vivo and in vitro. *Proc. Natl. Acad. Sci. USA* **90,** 10130–10134.

Smith, S. E., Medlicott, M., and Ellis, G. H. (1944). Manganese deficiency in the rabbit. *Arch. Biochem. Biophys.* **4,** 281–289.

Tholey, G., Megias-Megias, L., Wedler, F. C., and Ledig, M. (1990). Modulation of Mn accumulation in cultured rat neuronal and astroglial cells. *Neurochem. Res.* **15,** 751–754.

Zheng, W., Kim, H., and Zhao, Q. (2000). Comparative toxicokinetics of manganese chloride and methylcyclopentadienyl manganese tricarbonyl (MMT) in Sprague-Dawley rats. *Toxicol. Sci.* **54,** 295–301.

Downloaded from toxsci.oxfordjournals.org by guest on August 5, 2011