DIFFERENTIAL EFFECTS OF MANGANESE AND ALCOHOL ON MAMMALIAN PUBERTAL DEVELOPMENT.

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INTRODUCTION.

The age at which the onset of puberty begins is variable and depends on a complex series of centrally mediated events resulting in an increased pulsatile pattern of luteinizing hormone releasing hormone (LHRH) secretion from the hypothalamus. This changing pattern in LHRH secretion at puberty has been associated with the removal of an inhibitory tone and/or with the developmental responsiveness to excitatory components within the hypothalamus. In recent years much progress has been made in identifying inhibitory components such as gamma aminobutyric acid and the opioid peptides (1, 2), as well as stimulatory components such as excitatory amino acids (3), leptin (4), transforming growth factor a (TGFa; 5), insulin like growth factor -1 (IGF-1; 6), KiSS-1/kisspeptin (7) and manganese (Mn; 8). Importantly, all of these substances are capable of influencing LHRH secretion at puberty. The increased release of LHRH at puberty appears to utilize an interactive participation of neural circuits and glial cells (9). Furthermore, it is well known that neuronal and glial functions can be further influenced by peripheral metabolic signals, genetic and environmental influences, as well as drugs of abuse. Thus, any substance, whether it be endogenous or exogenous, that is capable of stimulating or inhibiting prepubertal LHRH secretion could have an impact on pubertal development.

In recent years we have studied the actions of manganese chloride ($MnCl_2$), IGF-1 and alcohol (ALC) on puberty related events. This article will review our current understanding of the positive and/or negative influences of each of these substances on pubertal processes in females. Specifically, we will describe the positive action of $MnCl_2$ on LHRH release, and point out the potential beneficial and harmful effects of prepubertal exposure to low but elevated levels of this element. We will also describe the positive role of IGF-1 on puberty, and the negative action of prepubertal ALC exposure during pubertal development. Furthermore, we will discuss the actions and interactions between ALC and IGF-1 on puberty related genes, hormonal secretions and the timing of female puberty.

NEUROENDOCRINE EFFECTS OF MN ON FEMALE PUBERTAL DEVELOPMENT.

Mn is a naturally occurring essential element that is required for normal mammalian physiological functions. Both excesses and deficiencies of Mn can affect brain functions and are associated with serious health-related problems. It has been known for years that laboratory animals of both sexes that are deficient in Mn show signs of impaired

growth and reproduction, suggesting a role for this element in development and reproduction. Because Mn can cross the blood-brain barrier more efficiently in young compared with adult animals (10), and because it accumulates in the hypothalamus (11), we assessed whether Mn was involved in the neuroendocrine control of female puberty.

Initial studies were conducted to determine the effects of Mn on puberty-related hormones and the timing of female puberty (8). Specifically, we demonstrated that $MnCl_2$, delivered into the brain third ventricle, acted dose dependently to stimulate prepubertal luteinizing hormone (LH) secretion. Hypothalami incubated *in vitro* indicated this was due to a Mn-induced release of LHRH. This site of action was confirmed by *in vivo* blockade of pituitary LHRH receptors with acyline, and the lack of a direct action at the pituitary to stimulate LH release *in vitro*. Because of these acute actions, we assessed the potential short-term effects of prepubertal exposure to a low dose of this metal. Twelve-day old female rats were supplemented with $MnCl_2$ (10 mg/kg) by gastric gavage from day 12 until day 29, or in some rats, until vaginal opening (VO). In this regard, Mn accumulated in the hypothalamus and caused elevated serum levels of LH, follicle stimulating hormone and estradiol (E_2), and furthermore, advanced the age at VO (Figure 1). Collectively, these results indicate Mn can stimulate release of puberty-related hormones, and suggest it may facilitate the normal onset of puberty. Additionally, they also suggest the possibility that Mn may contribute to precocious puberty if an individual is exposed to low, but elevated levels of the element too early in development.

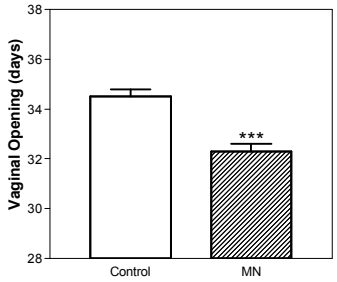

Figure 1. *Effect of short-term Mn exposure on the timing of female puberty. Mn was administered at a dose of 10 mg/kg by gavage from day 12 until vaginal opening. Note that Mn advanced the day of vaginal opening. Bars represent the mean ± SEM. ***p<0.001.*

While the intent of this report is to discuss effects of Mn in immature females, it is of importance to mention that there appears to be a gender difference between the effects of Mn in immature females and males. As opposed to the above stated effects in females, a 2.5 fold dose increase was required in males to significantly elevate gonadotropins and accelerate spermatogenesis (12); hence, suggesting that immature males are less sensitive to the hypothalamic influence of Mn than females. A possible explanation for this may be a gender metabolic variation, in that males clear Mn two times faster than females. It is important to note, however, that in both cases the oral doses used were much lower than those shown to produce neurotoxicological effects in adult rats and primates (13).

MECHANISM OF MN ACTION WITHIN THE PREPUBERTAL HYPOTHALAMUS.

To assess the mechanism of Mn action we used both *in vivo* and *in vitro* approaches to investigate whether the element could act via stimulation of specific pathways involved in LHRH secretion (14). Our results indicated that Mn did not operate through activation of either NMDA-receptors or IGF-1-receptors. Also, we showed that blocking nitric oxide synthase (NOS) with N-monomethyl-L-arginine (NMMA) was ineffective in blocking Mn-induced LH release *in vivo*, nor was it able to block LHRH secretion in vitro with $MnCl_2$ at doses of 50-250 µM. Importantly, the 50 and 100 µM doses did not stimulate total nitrite, a marker of NO pro-

duction, yet stimulated LHRH release. An increase in nitrite accumulation only occurred with the 250 µM dose; hence, suggesting that low doses do not induce LHRH via stimulation of NOS/NO. Conversely, we showed that a specific blocker of soluble guanylyl cyclase (sGC), ODQ, inhibited Mn-induced LHRH release dose dependently, demonstrating that the sGC is the site of action of $MnCl_2$ to facilitate LHRH release (Figure 2). Additionally, we showed that the metal stimulated release of cGMP and LHRH from the same incubates, and finally, that a protein kinase G (PKG) inhibitor blocked Mn-induced release of LHRH. Thus, these results reveal that the principal action of $MnCl_2$ is to facilitate activation of sGC, which subsequently stimulates the cGMP-PKG pathway controlling prepubertal LHRH in female rats.

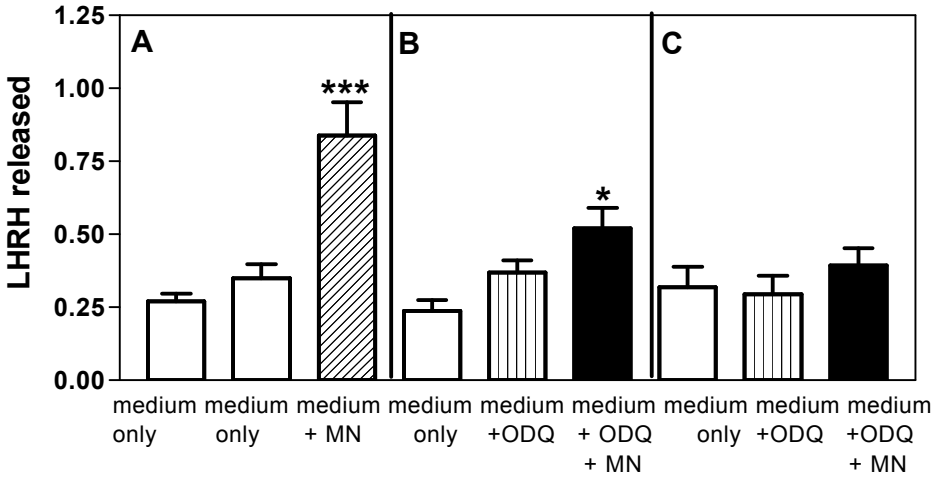

Figure 2. *Effect of inhibiting guanylyl cyclase on Mn-stimulated LHRH release in vitro. Note that $MnCl_2$ induced LHRH release in the controls (panel A), but this stimulatory effect was dose dependently blocked when 100 µM (panel B) and 250 µM (panel C) doses of 1H-[1,2,4] oxadizolo [4,3-a] quinoxalin-1 one (ODQ) were added to the medium. Bars represent mean ± SEM. ** p<0.001 and * p<0.05.*

NEUROENDOCRINE EFFECTS OF ALC ON PUBERTAL DEVELOPMENT.

Surveys by the National Institutes of Health indicate an alarming trend of ALC use and abuse occurring at earlier ages, often during adolescence and with the greatest change being in the female population. This is noteworthy because adolescence is a potentially vulnerable time of development when individuals may be more sensitive and less tolerant to the drug than adults. ALC abuse by the young is associated with several risks including altered growth, endocrine functions and development, all of which are relevant to adolescent health. Some of the early work showed ALC delayed puberty in both male and female rats and male mice. Case studies in humans are understandably limited in number and scope, but have shown that the drug can cause depressed LH in mid-pubertal boys and depressed E_2 in girls by age twelve. In recent years we have used rodents and rhesus monkeys to assess more specifically the effects and mechanisms of action of this drug during puberty, and results of some of this research are described below.

In rats, short-term prepubertal ALC exposure caused delayed VO; an action associated with suppressed levels of serum growth hormone (GH) and LH (15, 16), with GH being affected first. In rhesus monkeys, these and other puberty-related hormones were also depressed by chronic ALC exposure, and the drug delayed the development of a re-

gular pattern of menstruation (17). Additionally, we have gathered compelling evidence, using both rats and rhesus monkeys, indicating that these inhibitory effects are due to actions of ALC within the hypothalamus and not due to pituitary impairments (18-21).

INFLUENCE OF IGF-1 ON PUBERTAL PROCESSES.

The observation that ALC caused prepubertal levels of serum GH to be depressed before LH, led to the question as to whether something in the GH-somatotrophic axis could be involved in activating the reproductive system at puberty. IGF-1 synthesis is influenced by GH, and the circulating levels of IGF-1 are known to increase during puberty in both primates and rodents. With this in mind we set out to determine the ability of IGF-1 to induce LHRH secretion at puberty. Our initial study showed that the peptide dose dependently stimulated LHRH release *in vitro* and was ten fold more potent than IGF-2 or insulin (6). Because IGF-1 stimulated prepubertal LHRH release, we hypothesized the peptide may be a metabolic signal capable of linking somatic development to the LHRH releasing system at puberty.

We have shown in a series of experiments that IGF-1 of peripheral origin acts centrally to stimulate LHRH release and advances female puberty (22). Expression of the IGF-1 gene did not change in the hypothalamus at puberty; however, the gene expression by the liver was markedly increased as puberty approached. This increase was followed by increases in serum IGF-1, IGF-1 receptor mRNA in the median eminence, and elevations in serum gonadotropins and estradiol. It was also shown that IGF-1 administered into the brain third ventricle induced LH release, and that this effect could be blocked by prior immunoneutralization of LHRH actions (Figure 3), confirming the hypothalamic action. Finally, we showed that the central administration of IGF-1 to immature female rats advanced the timing of their puberty.

EFFECTS OF ALC ON IGF-1 SYNTHESIS AT PUBERTY.

Because IGF-1 appears to be involved in pubertal processes and because ALC delays female puberty, we investigated, using prepubertal female rats, whether chronic exposure to the drug could affect expression of genes encoding IGF-1 in brain and liver, as well as the expression of the IGF-1 receptor in the median eminence of the hypothalamus (23). Results indicated that ALC did not affect IGF-1 or IGF-1 receptor in the brain.

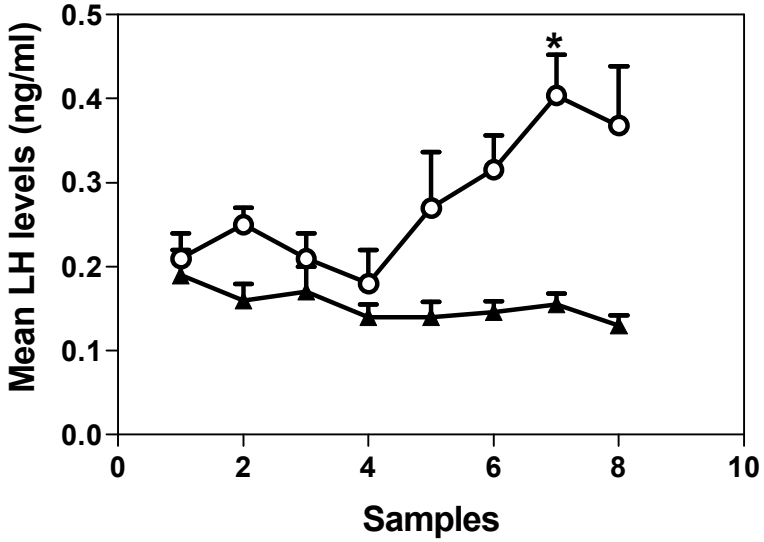

Figure 3. *IGF-1 induces LH by acting at the hypothalamic level. LH secretory profiles from immature rats are shown before (samples 1and 2) and after (samples 5-8) receiving third ventricular injections of either NRS plus IGF-1 (circle) or anti-LHRH serum plus IGF-1 (triangle). Note that the IGF-1 induced release of LH was blocked by the anti-LHRH serum.*

However, the drug caused a marked decrease in IGF-1 gene expression in the liver (Figure 4a), an event associated with decreased serum levels of IGF-1 and LH (Figure 4b and c, respectively). We also observed similar effects with regard to IGF-1 and LH in immature female rhesus monkeys following chronic ALC exposure (17). Since we already showed that serum GH is suppressed by ALC, we used transgenic animals with GH levels held constant by the promoter, to assess any direct action of ALC within the liver. This study showed that ALC can alter IGF-1 synthesis directly within the hepatocyte in a GH-independent manner (24). Taken together, these results are important since, as stated above, IGF-1 of peripheral origin is capable of acting at the hypothalamic level to stimulate the LHRH releasing system at puberty. Thus, ALC not only acts within the hypothalamus to decrease LHRH release, but it can also act peripherally to suppress IGF-1 synthesis and thus, the amount of peptide available to the prepubertal hypothalamus.

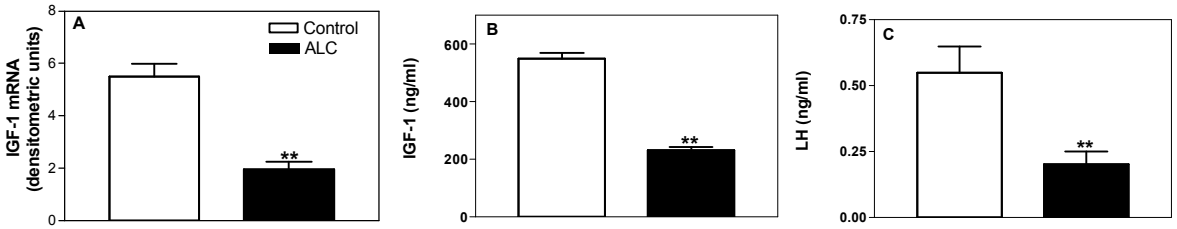

Figure 4*. ALC alters hepatic IGF-1 gene expression and serum levels of IGF-1 and LH. Bars represent mean ± SEM. **p<0.01*

ACTIONS AND INTERACTIONS OF ALC ON HYPOTHALAMIC PUBERTY-RELATED GENES.

LHRH release requires the integrative participation of glial and neuronal circuits. Specific members of the POU and Nkx gene families have been shown following activation, to transactivate genes in the prepubertal LHRH secretory pathway. Expression of both Oct-2 (which can activate the TGFa gene) and thyroid transcription factor (TTF-1) become elevated in the hypothalamus before puberty. These genes are depressed following chronic ALC exposure (25). Importantly, we have reported that IGF-1 prematurely induces expression of the Oct -2 gene; an effect blocked by ALC (26). TTF-1, however, does not appear to be regulated by IGF-1. Recently, the KiSS-1 gene, responsible for production of kisspeptins, has been shown to increase in the hypothalamus at puberty and play an important role in pubertal LHRH/LH release (7). We have shown preliminary evidence that ALC can suppress prepubertal KiSS-1 gene expression (27). Currently, we are investigating whether IGF-1 is an upstream regulator of this important puberty related gene, and whether ALC affects this action.

CONCLUSIONS.

There are both positive and negative influences on the timing of puberty. Both of these influences can be derived from endogenous and exogenous substances. Endogenous substances such as specific genes and hormones, can be either inhibitory or facilitory on pubertal development. Exogenous substances such as environmental factors and drugs of abuse can also alter pubertal events. In the context of this article, we have

shown how Mn and ALC exert opposite influences on LHRH secretion and the onset of puberty.

Mn can induce prepubertal LHRH secretion and facilitate pubertal development. The site of this action is within the hypothalamus and through a direct activation of sGC, which in turn stimulates the cGMP/PKG pathway. Because of this hypothalamic action, it is possible that this element may be an environmental factor capable of working in concert with other signals to influence the timing of normal puberty. Furthermore, we suggest that if an individual is exposed to low but elevated levels of Mn too early in life it may contribute to precocious puberty.

Conversely, ALC causes depressed prepubertal LHRH secretion and delays pubertal development. Furthermore, we have shown that IGF-1 of peripheral origin can influence LHRH secretion and the timing of puberty, and that ALC alters liver IGF-1 synthesis, resulting in decreased circulating levels of the peptide available to the hypothalamus at puberty. We have also described the ability of ALC to suppress the expression of genes associated with puberty.

Continuing to identify specific puberty-related genes and their products, as well as other neurotransmitters associated with neuronal and glial signaling will be of critical importance in determining the factor(s) controlling the onset of puberty. Determining the actions of ALC on these genes, and its effects on IGF-1 induced gene expressions will not only help us gain a better understanding of pubertal development, but will also be important in helping us to discern the mechanisms by which this drug detrimentally alters the pubertal process.

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