GENDER, AGE AND DOSE EFFECTS OF NEONATALLY ADMINISTERED ASPARTATE ON THE SEXUALLY DIMORPHIC PLASMA GROWTH HORMONE PROFILES REGULATING EXPRESSION OF THE RAT SEX-DEPENDENT HEPATIC CYP ISOFORMS

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ABSTRACT:

Newborn male and female rat pups were injected with either 2 mg or 4 mg monosodium aspartate (MSA)/g body weight or diluent on alternate days for the first 9 days of life. Both doses of the amino acid had profound effects on the sexually dimorphic growth hormone secretory profiles in adulthood. There were no measurable levels of growth hormone in any of the plasma samples obtained during 8 continuous hr of serial blood collections from the adult males and females treated neonatally with 4 mg of MSA. Male rats treated with half the dose of the amino acid (i.e., 2 mg MSA/g) exhibited typical masculine profiles of growth hormone release, except that the amplitudes of the ultradian pulses were reduced to 10–20% of normal male levels. Otherwise, like normal males, the peaks occurred about every 3–4 hr and the intervening 2.5-hr troughs had undetectable levels of growth hormone. In a similar sense, females treated with 2 mg of MSA maintained their sexually dimorphic pattern of plasma growth hormone, i.e., frequent pulses of hormone followed by short-lived troughs. However, the peaks rarely exceeded 20 ng/ml and the troughs usually fell to a measurable 8 to 10 ng/ml resulting in an approximate 75% reduction in the mean plasma concentration. Growth hormone- and gender-dependent expression of CYP2C7, 2C11, 2C12, 2C13, 2A1, 2A2, and 3A2 (mRNAs, proteins, and catalytic activities) were generally unaffected by neonatal exposure to 2 mg of MSA. In contrast, the higher 4-mg dose of the amino acid completely or near completely suppressed male-specific CYP2C11, 2C13, 2A2, and 3A2 expression while inducing small increases in female-specific CYP2C12 and female-predominant CYP2A1 in the treated males. Females exposed to the 4 mg MSA dose exhibited less severe isoform changes characterized by small reductions in CYP2C12 and 2C13 levels. Whereas expression levels of most of the CYP isoforms in both sexes were lowest in the pubertal (47-day-old) rats, and occasionally higher in the adults (207-day-old) as compared with the early postpubertal (70-day-old) rats, the effects of neonatal MSA were the same at all ages studied. Since each of the CYP isoforms are regulated by different “signaling elements” in the sexually dimorphic plasma growth hormone profiles, it is possible to correlate MSA-induced alterations in CYP expression levels to specific changes in the gender-dependent growth hormone profiles.

Neonatal administration of the ubiquitous food additive monosodium glutamate (MSG)1 to rats and mice, and possibly other species, can produce a well-defined syndrome characterized by stunted body growth and obesity (1). These defects are a result of glutamate-produced lesions in the developing brain that are generally limited to growth hormone-releasing hormone (GRH) secretory neurons in the arcuate nuclei and circumventricular organs of the hypothalamus (1–3). In the absence of GRH release, the pituitary does not secrete growth hormone and the affected animals exhibit obesity and retarded growth (1, 4). MSG-induced defects, however, are not limited to abnormalities in growth patterns. We have found that the activities of hepatic drug metabolizing enzymes are dramatically depressed in adult rats neonatally exposed to MSG (4–6). Like the regulation of growth, the expression of sex-dependent2 isoforms of CYP, major components in the drug metabolizing enzyme system, is also regulated by growth hormone (7–9). CYP2C11, the predominant isoform in male rat liver comprising up to 50% of the total CYP content (10), is dependent upon the presence of the masculine episodic growth hormone profile (7–9). The elimination of this profile from the circulation, and subsequent suppression of CYP2C11, helps to explain the 80–90% decline in drug metabolizing capacity of MSG-treated male rats.

The developmental defects produced by early exposure to glutamate do not appear to be unique to this amino acid, but can be induced by other excitotoxic amino acids like aspartate, one of the two constituent amino acids in the artificial sweetener aspartame (1, 11). As with MSG, neonatal treatment (oral or parenteral) of monosodium aspartate (MSA) has been reported to produce hypothalamic lesions (1, 2) resulting in a permanent growth hormone deficiency and associated subnormal hepatic hexobarbital hydroxylase activity (12). However, hexobarbital hydroxylation is dependent upon the contributing activities of several CYPs (13), and it is unknown which of the growth hormone-dependent isoforms of CYP are affected by MSA. In the present study, we have examined the dose-dependent effects of neonatally administered MSA on the sexually dimorphic growth hormone secretory profiles regulating the gender-dependent expression...
of hepatic CYP isoforms in pubertal, early postpubertal, and adult male and female rats.

**Materials and Methods**

**Animals.** Animals were housed in the University of Pennsylvania Laboratory Animal Resources facility under the supervision of certified Laboratory Animal Medicine veterinarians and were treated according to a research protocol approved by the University’s Institutional Animal Care and Use Committee. At all times animals were housed on hardwood bedding in plastic cages, with water and commercial rat diet provided ad libitum. The animal quarters were air conditioned (20–23°C) and had a photoperiod of 12 hr of light, 12 hr of darkness (lights on at 0800 hr). After a 2- to 3-week acclimation period in our facilities, the animals were bred by randomly housing two adult female Sprague-Dawley rats [Crl:CD(SD)BR] with an individual adult male of the same strain. On the day of parturition all litters were mixed and microsome fractions were prepared from each individual liver. The individual livers were fractionated by electrophoresis under denaturing conditions in Rapid-hyb buffer (Amersham Corp.) using a32 P-labeled 2C11 oligonucleotide probe. The washed blots were wrapped in clear plastic and exposed to X-ray films with 2 intensifying screens at −70°C for 1–3 days. Northern blots were then successively stripped and reprobed with selective 32P-labeled 2A1, 2A2, 2C6, 2C7, 2C12, 2C13, and 3A2 oligonucleotide probes whose nucleotide sequences have been reported elsewhere (18–20). Their high degree of specificity has been demonstrated (18–20) and was further confirmed by routine competitive northern blotting analysis (21, results not presented). Evidence that RNA was equally loaded and transferred was obtained by equivalent intensity of ethidium bromide staining of 18S and 28S rRNA bands (22). Further, the rat 18S rRNA oligonucleotide probe was used as a control to verify the consistency and integrity of RNA loading (23). Quantitation of the mRNA by laser densitometry of the X-ray films was kept within the linear range as established by slot blot hybridizations and normalized to the 18S RNA signals in each lane. Their high degree of specificity has been demonstrated (18–20) and was further confirmed by routine competitive northern blotting analysis (21, results not presented).

**Testosterone Metabolism.** In vitro testosterone 2α-, 6β-, 7α- and 15α-hydroxylase, indicative of activity levels of CYP2C11, 3A2/2C12, 2A1 and 2A2 proteins, respectively, and female-specific testosterone 5α-reductase, reflective of CYP2C12 concentrations (cf. 18, 24), were assayed by our published HPLC (25) and TLC (26) methods.

**Statistics.** All data were subjected to analysis of variance, and differences were determined with r statistics and the Bonferroni procedure for multiple comparison.

**Results**

**Growth Hormone.** The established sexual dimorphisms in the ultradian patterns of plasma growth hormone were found in our control male and female rats (fig. 1). Generally in male rats growth hormone was released in pulses approximately every 3–4 hr, resulting in short-lived peaks of around 175 ng/ml, followed by approximately 2.5 hr or more of virtually undetectable trough levels (<3 ng/ml). In contrast, growth hormone was released in female rats in a more continuous pattern. Numerous low amplitude pulses of the hormone resulted in peaks of 50–75 ng/ml followed by short-lived troughs that were always measurable and rarely fell below 15–20 ng/ml.

There were no measurable levels of growth hormone in any of the plasma samples obtained during 8 continuous hr of serial blood collections from the adult males and females treated neonatally with 4 mg of MSA (fig. 1). Male rats treated with half the dose of testosterone propionate (i.e., 2 mg MSA/g) exhibited typical masculine profiles of growth hormone release, except that amplitudes of the ultradian pulses were reduced to 10–20% of normal male levels. Otherwise, like normal males, the peaks occurred about every 3.5 hr and the intervening 2.5-hr troughs had undetectable levels of growth hormone. In a similar sense, females treated with 2 mg of MSA/g exhibited normal sexual dimorphic pattern of plasma growth hormone, i.e., frequent pulses of hormone followed by short-lived troughs. However, the peaks rarely exceeded 20 ng/ml and the troughs usually fell to a measurable 8 to 10 ng/ml resulting in an approximate 75% reduction in the mean plasma concentration. Thus, while neonatal administration of the 2-mg dose of MSA did not disrupt the normal sex-dependent periodicity of growth hormone secretion in males and females, it did result in a dramatic reduction in the levels of circulating growth hormone.

**CYP Proteins.** The gender-dependent expression levels of the hepatic microsomal CYP proteins were in agreement with previous reports (7–9). That is, CYP2C11, 2C13, and 3A2 were all male specific, whereas CYP2C12 was female specific and CYP2C7 was female predominant (fig. 2). Although there were some age-dependent

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**Blood Collection.** Three to four animals (4 to 5 months of age) from each treatment group, and representing all litters in the group, were implanted with a single-step guanidinium-thiocyanate method (17). RNA samples from individual livers were fractionated by electrophoresis under denaturing conditions in Rapid-hyb buffer (Amersham Corp.) using a32 P-labeled 2C11 oligonucleotide probe were performed with high stringency washings. The washed blots were wrapped in clear plastic and exposed to X-ray films with 2 intensifying screens at −70°C for 1–3 days. Northern blots were then successively stripped and reprobed with selective 32P-labeled 2A1, 2A2, 2C6, 2C7, 2C12, 2C13, and 3A2 oligonucleotide probes whose nucleotide sequences have been reported elsewhere (18–20). Their high degree of specificity has been demonstrated (18–20) and was further confirmed by routine competitive northern blotting analysis (21, results not presented). Evidence that RNA was equally loaded and transferred was obtained by equivalent intensity of ethidium bromide staining of 18S and 28S rRNA bands (22). Further, the rat 18S rRNA oligonucleotide probe was used as a control to verify the consistency and integrity of RNA loading (23). Quantitation of the mRNA by laser densitometry of the X-ray films was kept within the linear range as established by slot blot hybridizations and normalized to the 18S RNA signals in each lane. Their high degree of specificity has been demonstrated (18–20) and was further confirmed by routine competitive northern blotting analysis (21, results not presented).

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effects, in general neonatal exposure to the 2 mg/g dose of MSA resulted in either a small or no reduction in the levels of the measured CYP proteins. In contrast, expression levels of the isoforms were dramatically affected by the 4-mg dose of the amino acid. CYP2C11 and 3A2 were suppressed in male liver to the point of being undetectable, whereas CYP2C13 in males and CYP2C12 in females were reduced by 60 to 70% and CYP2C7 measured in female liver was reduced 30 to 50%. Although the 4-mg dose of MSA had a small suppressive effect on the already low concentrations of hepatic CYP2C7 levels in males, it actually induced measurable concentrations (i.e., 10% of female levels) of female-specific CYP2C12 in male rats (fig. 2).

**Testosterone Metabolites.** MSA-induced effects on the *in vitro* catalytic activities of the male-dependent testosterone hydroxylases reflected similar changes in male-specific CYP protein levels. Whereas CYP2C11-dependent testosterone 2α-hydroxylase, CYP3A2 and 2C13-dependent testosterone 6β-hydroxylase and CYP2A2-dependent testosterone 15α-hydroxylase were unaffected by neonatal exposure to 2 mg/g of MSA, the 4-mg dose produced a long lasting 80% or more reduction in the CYP-dependent enzyme activities (fig. 3). Female-dependent testosterone metabolites were much less affected by the neonatally administered MSA. Like the male-dependent CYP catalytic enzymes, the activities of female-predominant CYP2A1-dependent testosterone 7α-hydroxylase, and testosterone

![Fig. 1. Plasma levels of circulating growth hormone in adult rats neonatally exposed to MSA.](image1)

Plasma was obtained from 120- to 140-day old individual undisturbed catheterized control and neonatally monosodium aspartate (MSA)-treated rats at 15-min intervals for 8 continuous hr. Similar results were obtained from two to three additional animals in each treatment group.

![Fig. 2. Long lasting effects of neonatally administered MSA on hepatic microsomal CYP proteins.](image2)

CYP isoforms were measured in livers from 47-, 70-, and 207-day-old male and female rats neonatally treated with either 2 mg or 4 mg monosodium aspartate (MSA) or diluent (0 mg MSA) on alternate days for the first 9 days of life. Each data point is a mean ± SD with a N ≥ 5 and expressed as a percentage of the mean value of the 70-day-old diluent-treated male or female group with the greater CYP concentration. *p < 0.01; compared with the diluent-treated control animals of the same age and sex, †p < 0.01; compared with the 70-day-old rats of the same sex and MSA treatment.

5α-reductase [a non-CYP-dependent enzyme whose activities generally reflect CYP2C12 levels (16, 31)] were unaltered by the 2 mg dose of MSA. Doubling the dose of MSA to 4 mg/g had no suppressive effect on the 7α-hydroxylase and 5α-reductase activities, but in most cases induced a small but significant elevation in the hepatic activities of these enzymes (fig. 3).

MSA-induced changes in testosterone metabolism could not be explained by similar changes in the activity of hepatic NADPH-cytochrome P450 reductase whose levels at all ages studied were unaltered in both male and female rats by neonatal exposure to MSA (data not presented).

Earlier, we reported an up to 100% difference in the induction levels of hepatic CYP2B1 and 2B2 between peripubertal and adult rats (32). With this observation in mind, in the present study we examined gender-dependent CYP levels in peripubertal (47 days of age), recent postpubertal (70 days of age) and mature adult (207 days of age) rats. Although CYP levels tended to be lower in the 47-day-old animals and were occasionally higher in the 207-day-old rats, the effects of MSA were the same at each age studied (fig. 2). That is, if neonatal MSA suppressed a CYP isoform at 47 days of age, the enzyme remained suppressed, and at near the same level at 70 and 207 days of age. Accordingly, we limited our present CYP mRNA mea-
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**CYP mRNAs.** The sexually dimorphic expression levels of CYP transcripts (fig. 4) reflected gender differences in CYP protein concentrations (fig. 2) and specific CYP-dependent catalytic activities (fig. 3), and were in agreement with previous observations (7, 8, 16). Hepatic mRNA concentrations showed that CYP2C11, 2C13, 2A2, and 3A2 were male-specific, CYP2C12 was female-specific, CYP2A1 and 2C7 were female-predominant, and CYP2C6 was gender-independent (fig. 4). Although neonatal exposure to 2 mg/g of MSA produced a substantially small change in the mRNA levels of some isoforms, transcript levels in the majority of CYPs were unaltered by the 2 mg dose. In contrast, exposure to the higher 4-mg dose of the amino acid virtually eliminated expression levels of CYP2C11, 2C13, 2A2, and 3A2 in males, reduced hepatic CYP2C12 mRNA by 35% in females, and slightly suppressed CYP2C7 mRNA in both sexes. Hepatic CYP2C12 and 2A1 mRNAs, however, were elevated in males. Basically, the longterm effects of neonatal MSA on CYP mRNAs were in agreement with changes in CYP protein levels and specific CYP-dependent catalytic enzyme activities.

**Drug Metabolism.** Neonatal exposure to MSA seemed to have its greatest effect on the drug metabolizing activities of pentoxysresorufin O-dealkylase and coumarin 7-hydroxylase (fig. 5). Since the major hepatic CYP isoform contributing to pentoxysresorufin O-dealkylase is CYP2C11 in male rats (33) and 2C12 in female rats (34), it is not surprising that the MSA-induced reduction in the levels of this drug metabolizing enzyme very much reflected a similar decline in CYP2C11 and 2C12 (figs. 2 and 4). Furthermore, the MSA-induced increase in hepatic coumarin 7-hydroxylase activity was in agreement with the increase in CYP2A1-dependent testosterone 7α-hydroxylase (fig. 3) and CYP2A1 mRNA (fig. 4); the major isoform contributing to coumarin 7-hydroxylase activity (35).

**Discussion**

In all species studied (9), growth hormone is secreted in sexually dimorphic ultradian profiles. In the case of the rat, males secrete growth hormone in episodic bursts every 3–4 hr. Between the peaks, growth hormone levels are extremely low or undetectable. In females peaks are of lower magnitude than in males and occur more frequently, whereas the troughs between the peaks are considerably elevated compared with those of males. It is this gender difference in the growth hormone secretory profiles that regulate the sexually dimorphic expression of nearly a dozen constitutive and inducible isoforms of hepatic CYP. That is, expression of the major female-specific CYP2C12 (as well as the non-CYP 5α-reductase) is dependent upon the feminine profile of continuous growth hormone secretion. Exposure to the masculine profile of episodic hormone release as well as the absence of the hormone from the circulation (e.g., hypophysectomy) results in the complete suppression of CYP2C12 (7, 31). Inasmuch as the highest female-specific CYP2C7 expression is also dependent upon the feminine growth hormone profile and is completely suppressed in the hypophysectomized rat. However, exposure to the masculine profile allows for expression of CYP2C7 and 25–40% of normal female levels (7, 31). Expression of the major male-specific CYP2C11 requires the episodic “on-off” masculine profile of growth hormone secretion. Whereas the feminine pattern of continuous hormone secretion blocks CYP2C11 expression, total growth hormone depletion from the circulation allows for CYP2C11 expression at 15 to 25% of intact male levels (7, 10, 16). After hypophysectomy, female-predominant CYP2A1 (1:3; M:F) concentrations decline, but remain above male levels, and are restored to intact female-like levels by continuously administered growth hormone (16, 36). Although the expression levels of CYP2C7, 2C11, 2C12, and 2A1 are greatest when exposed to their gender-dependent growth hormone profiles, other isoforms may be optimally expressed in the absence of growth hormone. Male-specific CYP2A2 and 3A2 are maximally expressed in the hypophysectomized rat, disappear when growth hormone is secreted constantly, but are only partially suppressed, relative to the high levels observed in hypophysectomized rats, under the influence of episodic growth hormone (37, 38). Male-specific CYP2C13 is optimally expressed when exposed to the masculine hormone profile or under conditions of no growth hormone, whereas the feminine growth hormone profile completely suppresses CYP2C13 (7, 16). Although there are additional examples, it becomes clear that the expression and/or suppression of each isoform of CYP is likely to be regulated by a different “signal” in the sexually dimorphic growth hormone profile. These “signals” may be recognized by the hepatocyte in the frequencies and/or durations of the pulse and interpulse periods. Or perhaps the hepatocyte can monitor the mean plasma concentration of the hormone (9).

Neonatal exposure to MSA produced a long lasting and profound reduction in the circulating levels of growth hormone that was associated, at the 4 mg dose, with a suppression of major CYP isoforms. Although CYP levels tended to be lowest in the pubertal rats, the

![Fig. 3. Long lasting effects of neonatally administered MSA on the catalytic activities of hepatic microsomal CYP isoforms.](image-url)

**Table:**

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<tr>
<th>CYP Enzyme</th>
<th>MALE</th>
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<tr>
<td>2α-Hydroxylase</td>
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effects of the amino acid were the same at all ages studied. In contrast, the dose of MSA had a dramatic effect on growth hormone secretion and CYP expression. Whereas adult male and female rats treated neonatally with 4 mg/g body weight of MSA on alternate days for the first 9 days of life had no measurable plasma growth hormone, male rats treated with half the dose of the amino acid (i.e., 2 mg MSA) exhibited typical masculine profiles of growth hormone release, except that the amplitudes of the pulses were reduced to 10 to 20% of normal male levels. Similarly, females treated with 2 mg MSA maintained their sexually dimorphic pattern of plasma growth hormone secretion, but as a result of a decline in both the pulse and interpulse amplitudes, the mean plasma concentration was reduced 75%.

In spite of the major reduction in circulating growth hormone levels by 2 mg MSA, expressions of male-specific CYP2C11, 2C13, 2A2, and 3A2, as well as female-specific CYP2C12 and female-predominant CYP2C7 and 2A1 (mRNA, protein and catalytic activities), were generally unaffected or slightly reduced in both males and females. Although seemingly inconsistent with the role of growth hormone regulation of CYP, the results are actually in agreement with studies of CYP2C11 and 2C12 expression in incompletely growth hormone-depleted rats and hypophysectomized-growth hormone replaced rats (16, 20, 39) which demonstrated the effectiveness of “mini” amplitude pulses and subnormal concentrations of growth hormone in maintaining normal, and even supranormal levels of CYP2C11 and 2C12, respectively. In agreement with the present findings, we have reported that the critical signaling element in the masculine episodic plasma growth hormone profile that simultaneously induces CYP2C11 and suppresses CYP2C12 is the approximate 2.5 hr growth hormone-devoid interpulse periods necessarily separated by pulses of inconsequential heights (40). Similarly, the signaling element in the feminine growth hormone profile that induces CYP2C12 and represses CYP2C11 is the continuous presence of the hormone in the circulation at levels as low as 3% of normal (6, 16). Thus, it seems reasonable to conclude that the mini pulses of growth hormone secreted in the 2-mg MSA-treated male rats were sufficient to maintain normal-like male levels of CYP2C11, 2C13, 2A2, and 3A2 while suppressing female-dependent CYP2C12, 2C7, and 2A1. Moreover, the continuous secretion of subnormal concentrations of growth hormone in the 2-mg
Recently we reported that in the feminine growth hormone profile, a >97% decline in the hormone’s plasma concentration (i.e., <1 ng/ml plasma) remained highly effective in suppressing CYP2C13, 2A2, and 3A2 expression (16). Since MSA-inhibition of growth hormone secretion is probably a result of a selective block in hypothalamic release of GRH (1–3), it is possible that subassayable levels of the hormone (i.e., <3 ng/ml) continuously “leak” from a pituitary which contains substantial levels of growth hormone (41). In this regard, passive immunization with anti-rat GRH eliminates pulsatility, but does allow for a uniform growth hormone secretory profile of very low baseline concentrations (42). Thus, it is possible that the 4 mg MSA-treated male rats secrete undetectable, but sufficient levels of continuously released growth hormone to suppress male-specific CYP2C13, 2A2, and 3A2. In this regard, the above-normal expression levels (although still low in terms of female concentrations) of female-specific CYP2C12 and female-predominant CYP2A1 in the 4-mg MSA-treated males can be explained by the observation that induction of these two female-dependent isozymes is very sensitive to just nominal concentrations of continuously secreted growth hormone (16) which may likely be secreted in the 4-mg MSA rats. In contrast, it is possible that female-predominant CYP2C7 is unaffected in the 4-mg MSA-treated male rats because induction of this isoform requires higher levels of plasma growth hormone than does CYP2C12 and 2A1 (16). Accordingly, the levels of growth hormone secreted in the 4-mg MSA-treated males may be insufficient to induce CYP2C7. Similarly, low levels of continuously secreted growth hormone in 4-mg MSA-treated females as in males would explain the suppression of male-specific CYP2C11, 2C13, 2A2, and 3A2 and near female-like expression levels of CYP2C12 and 2C7 in the 4-mg MSA-treated females.

Glutamate and aspartate are both ubiquitous food additives (MSG and aspartame, respectively) whose neurotoxic properties have been reported to induce an irreversible developmental syndrome of stunted growth and obesity when administered to neonatal laboratory animals (1–3). Earlier, we had reported that neonatal exposure to high doses of MSG (4 mg/g body weight) permanently eliminates all detectable levels of plasma GH with an accompanying loss of CYP2C11, 2A2, and 3A2 in males, but surprisingly no effect on the concentration of CYP2C12 in females (4–6). A neonatal dose of 1 or 2 mg/g body weight of MSG reduced circulating GH pulse amplitudes by 70–80% without altering the general gender-dependent secretory profiles in either sex; i.e., pulsatile in males, continuous in females. In spite of these lower GH pulse heights, the gender-dependent CYP isoforms remained at normal or above-normal expression levels (6, 20, 39).

Comparing the present MSA results with the MSG findings suggests that in the broad sense both amino acids can produce similar developmental defects in growth, GH secretion, and CYP expression. Whereas 4 mg of MSG has no effect on hepatic CYP2C12 and 2C7 levels in females (5), the same dose of MSA induces a substantial decline in these isoform concentrations. Furthermore, a 50% reduction in the dose of MSG allows for expression of normal or supranormal levels of CYP2C11, 2C12, 2C7, 2A2, and 3A2 (5, 6, 20, 41), while adult rats neonatally treated with 2 mg of MSA/g body weight exhibited near or below normal levels of these isoforms. Thus, it seems reasonable to conclude that neonatal administration of MSA can induce developmental defects in the sexually dimorphic expression levels of hepatic CYP isoforms by permanently altering the “signaling elements” in the gender-dependent growth hormone secretory profiles that are similar but more severe than those induced by neonatal exposure to MSG.

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