Pharmacology Biochemistry and Behavior, Vol. 44, pp. 63–69, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Alterations in Regional Brain GABA Concentration and Turnover During Pregnancy

ANDREW SMOLEN,[1] TONI NESS SMOLEN AND PAUL C. HAN

Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309-0447

Received 23 June 1992

SMOLEN, A., T. N. SMOLEN AND P. C. HAN. *Alterations in regional brain GABA concentration and turnover during pregnancy*. PHARMACOL BIOCHEM BEHAV 44(1) 63–69, 1993.—During pregnancy, mice are more susceptible to flurothyl-induced seizures than are nonpregnant control mice. The potential role of brain GABA in mediating this behavior was examined in the present study. GABA concentrations in the cerebellum, hippocampus, striatum, midbrain, and cortex from individual control, pregnant (days 17–18) and delivery-day Heterogeneous Stock mice were assayed using a fluorometric method. Turnover of GABA was assessed by inhibiting metabolism with aminooxyacetic acid and measuring GABA accumulation over the next 2 h. Steady-state GABA concentrations decreased significantly from control in all brain regions during pregnancy. Reductions in GABA concentrations were approximately 25–30% in the affected regions. At parturition, GABA concentrations in the cerebellum and cortex returned to control levels, but hippocampal, striatal, and midbrain GABA levels remained significantly depressed. All the indices of GABA turnover—first-order rate constant, half-life, initial rate of synthesis, and turnover rate (product of first-order rate constant and initial concentration)—showed a significant reduction in pregnancy, which was continued through the time of delivery in all brain regions except the hippocampus. Half-life values for GABA increased nearly fourfold in the cerebellum and cortex. These results show that there is a significant alteration in GABAergic systems during pregnancy and parturition. We suggest that the reduction in GABA turnover is a compensatory anticonvulsant mechanism to offset the inherent seizure susceptibility brought about by the reduced level of the major inhibitory neurotransmitter in the brain.

Pregnancy Seizures GABA GABA turnover Flurothyl Mice Brain Brain regions Aminooxyacetic acid

DURING pregnancy, mice are more susceptible to flurothyl-induced seizures than are nonpregnant controls (47,49,50). The cause of this increased susceptibility to seizures remains unknown, but we are studying the potential neurochemical changes that occur in pregnancy in an effort to determine the factors that modulate this behavioral change.

Our studies to date have concentrated on the indirect role of vitamin B_6 in mediating the pregnancy-associated increase in susceptibility to seizures. Normal pregnancy is associated with decreased plasma and tissue levels of the coenzyme form of vitamin B_6, pyridoxal 5′-phosphate (PLP) (5,14,23,30,45), and it has been reported that the deficit is much greater in certain pathologic pregnancies (5,23). Deficiency of PLP has been shown to cause increased susceptibility to experimentally induced seizures in mice (42) and spontaneous seizures in humans (36). We have also reported that an isozyme of aldehyde dehydrogenase, π-aldehyde dehydrogenase (π-AlDH), is induced in the liver cytosol of pregnant mice (48). π-AlDH has broad substrate specificity and is capable of metabolizing a wide variety of aliphatic and aromatic aldehydes, including one form of vitamin B_6, pyridoxal (46,49). Although inactive as a coenzyme, pyridoxal is a major transport form of vitamin B_6 in blood (1,13). It occupies a pivotal role in vitamin B_6 metabolism because it may be converted to the active coenzyme, PLP by phosphorylation (13,34), or oxidized to the major excretory product 4-pyridoxic acid by aldehyde oxidases and dehydrogenases (13,49,54).

The association between increased activity of π-AlDH, decreased vitamin B_6 nutritional status, and increased susceptibility to seizures led us to investigate the possibility that neurotransmitter systems dependent upon vitamin B_6 might be altered during pregnancy. A number of neurotransmitters, including norepinephrine, dopamine, serotonin, and GABA, require PLP-dependent enzymes for their synthesis and each has been implicated in mediating seizure activity (19,43). In a previous article, we reported that the concentration and turnover of brain norepinephrine were markedly depressed during pregnancy in the mouse but concentration and turnover of dopamine were not affected (51). Those data indicated that the noradrenergic system was altered during pregnancy and suggested that norepinephrine might be involved in the mediation of increased seizure susceptibility during pregnancy.

[1] Requests for reprints should be addressed to Dr. Andrew Smolen, Institute for Behavioral Genetics, Campus Box 447, University of Colorado, Boulder, CO 80309-0447.

In this paper, we report on our continued efforts to quantify the neurochemical changes that occur in the brain during normal pregnancy in the mouse. We found that pregnancy caused significant reductions in the concentration and turnover of brain GABA. An abstract of these data has been published (48).

METHOD

Animals

Animals used in this study were female Heterogeneous Stock (HS) mice (33), 60–100 days old. Mice were maintained on a 12 L : 12 D cycle (0700–1900 h) and allowed free access to food (Wayne Lab Blox) and water. For all experiments, controls were virgin females of the same age as experimental animals. Day 1 of pregnancy was ascertained by the observation of a vaginal plug. Mated females were separated from males (also HS) and housed in groups until the day they were used for the experiment. Three groups of mice were used: nonpregnant controls, pregnant (17–18 days), and day of delivery (usually day 20). These groups will be referred to as control (C), pregnant (P), and delivery (D) in the remainder of the text. All procedures used received prior review and approval by the University of Colorado's Institutional Animal Care and Use Committee as being consistent with PHS policies on humane care and treatment of laboratory animals.

Measurement of Brain GABA Concentrations

Animals were killed by focused microwave irradiation. The brain was removed, cooled, and dissected on ice into the cerebellum, hippocampus, striatum, midbrain (mostly thalamus), and cortex. All dissections were performed between 1300 and 1600 h because our previous flurothyl seizure measurements were also done during this time period (49). The tissue was weighed to the nearest milligram and homogenized in 1.0 ml ice-cold 0.5 M perchloric acid. Samples were stored at −70°C until assayed.

For assay, the samples were thawed to 4° and centrifuged for 10 min at 15,000× *g* to pellet the precipitated proteins. Extracts of cortex were diluted 1 : 1 with buffer before proceeding. Fifty microliters of a solution composed of 3.7 M KOH, 0.6 M KCl, and 0.6 M imidazole was added to 500 μl of the resulting supernatant to precipitate the perchlorate anion as the potassium salt and adjust the pH to near neutrality. Samples were centrifuged at 2,000× *g* to remove the precipitate. One hundred microliters of the resulting supernatant was used for analysis of GABA using a modification of the fluorometric method described previously (15).

To 100 μl of the neutralized supernatant was added 25 μl of reaction mixture composed of 10 mM 2-oxoglutarate, 5 mM NADP, 25 mM 2-mercaptoethanol, and 0.25 units of GABAase/ml (GABA aminotransferase/succinic semialdehyde dehydrogenase, Sigma Chemical Co., St. Louis, MO) in 400 mM Tris-HCl, pH 8.9. A set of 10 GABA standards (0, 0.3–12.5 nM GABA/100 μl) were run daily. Samples and standards were assayed in duplicate. Blanks contained no GABAase. The reaction was allowed to proceed for 2 h at room temperature. The NADPH produced was measured after enhancing the fluorescence as described by Lowry and Passonneau (29). From this point on, the samples were kept in total darkness except for those brief periods during reagent addition, which were done under dim (40 W) red light. The unreacted $NADP^+$ was destroyed by the addition of 100 μl 0.25 M potassium phosphate, pH 12. After a 1-h incubation, 100 μl of the reaction mixture was added to 1 ml $NaOH/H_2O_2$ reagent (9 vol of 6 M NaOH + 1 vol 3% H_2O_2, prepared immediately before use) and incubated at 60°C for 15 min. The tubes were cooled to room temperature (about 15 min) and the fluorescence measured in a Farrand (Valhalla, NY) Mark I spectrophotofluorometer using excitation and emission wave lengths of 340 and 460 nm, respectively.

GABA Turnover

The turnover of GABA in the five brain regions was measured in control, pregnant, and delivery day mice. GABA turnover was assessed by inhibiting the metabolism of GABA with aminooxyacetic acid (AOAA), an inhibitor of the major GABA metabolizing enzyme GABA transaminase (4-aminobutyrate : 2-oxoglutamate aminotransferase, EC 2.6.1.19, GABA-T) (2). Mice were injected with AOAA, 20 mg/kg, and the content of GABA in the five brain regions was measured 0.25, 0.5, 1, and 2 h later. Separate mice were used for each time point.

Data Analysis

Data in Fig. 1 were analyzed by one-way analysis of variance (ANOVA) followed by posthoc *t*-test using a commercial software package (CRUNCH Software Corporation, Oakland, CA). GABA turnover was calculated by linear regression analysis of natural logarithm transformations of the brain GABA values vs. time. The resulting constants (first-order rate constant, half-life, turnover rate, and initial accumulation rate) were analyzed by two-way ANOVA for nonreplicated measures as described by Spiegel (52). An α level of 0.5 was considered significant and is the only level reported.

RESULTS

The GABA content of the cerebellum, hippocampus, striatum, midbrain, and cortex in C, P, and D mice is shown in Fig. 1. There were statistically significant reductions of GABA from control levels in the cerebellum, hippocampus, and striatum of the pregnant mice. This was followed by a small, nonsignificant increase at the time of delivery.

Neurotransmitter turnover is often thought to be a better

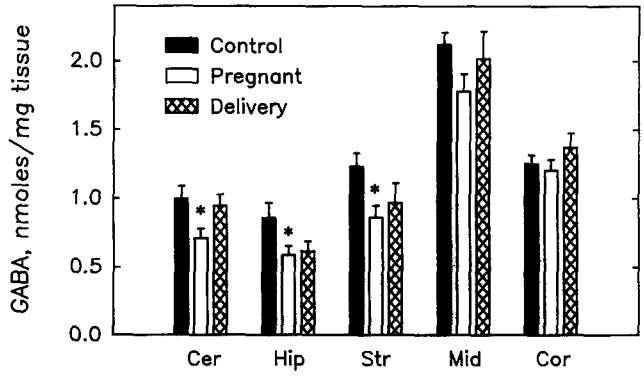

FIG. 1. GABA concentrations in brain as a function of pregnancy in the mouse. GABA was measured in the cerebellum (Cer), hippocampus (Hip), striatum (Str), midbrain (Mid), and cortex (Cor) of control mice (c), pregnant mice (p), and on the day of delivery (d). Values are the mean ± SEM of 6–15 individual animals. *Significantly different from control, $p < 0.05$.

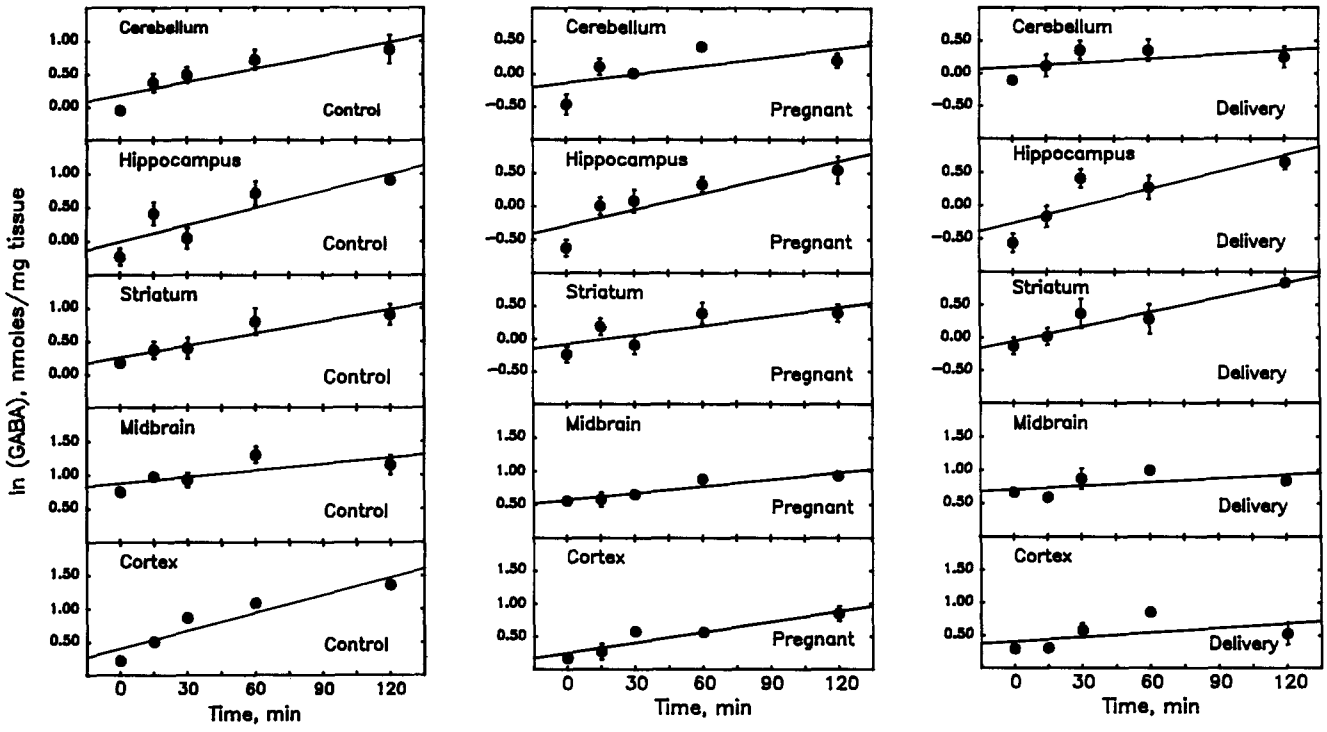

FIG. 2. GABA turnover in five brain regions as a function of pregnancy in the mouse. Turnover was estimated by inhibiting the metabolism of GABA with the GABA transaminase inhibitor aminooxyacetic acid (AOAA), and GABA content was measured at the times indicated. Each point is the mean ± SEM of 6–15 separate determinations. Where not indicated, the SEs are contained within the plotted symbol.

measure of the status of a neurotransmitter system than is the tissue content of the transmitter. Figure 2 shows the accumulation of GABA in brain regions following inhibition of GABA transaminase with AOAA. There was a general tendency for GABA turnover to be reduced during pregnancy and continue through to the day of delivery as evidenced by the shallower slopes. This trend is seen more clearly in Fig. 3, which shows the calculated indicators of GABA turnover in the five brain regions. Two-way ANOVA of the data showed significant overall effects of pregnancy on each of the turnover indices. There was a general tendency for the first-order rate constant (panel A) to decline during pregnancy, $F(2, 8) = 4.83$, $p < 0.05$, most clearly seen in the cerebellum, striatum, and cortex. GABA half-life values (panel B) showed a corresponding generalized increase, $F(2, 8) = 6.34$, $p < 0.05$. Half-life values increased nearly fourfold in the cerebellum and cortex. GABA turnover rate (the product of the first-order rate constant and extrapolated time zero value, panel C) showed a significant overall reduction during pregnancy, $F(2, 8) = 12.83$, $p < 0.05$, as did the initial GABA accumulation (synthesis) rate (panel D), $F(2, 8) = 10.75$, $p < 0.05$. Except for initial rate of GABA synthesis, hippocampal turnover values did not change with pregnancy. These changes each continued through the day of delivery, which represents the maximum change the pregnant state is able to produce in these measures of GABA turnover.

DISCUSSION

We reported previously that pregnant mice are more susceptible to flurothyl-induced seizures than are nonpregnant controls (47,49,50). The purpose of this study was to investigate the possibility that these pregnancy-associated seizures could be explained by potential alterations in GABAergic systems.

Markedly lowered GABA concentrations were found during pregnancy in the cerebellum, hippocampus, striatum, and midbrain, but cortical GABA levels remained unchanged. The reductions in GABA levels in the pregnant mice were substantial, 25–30% below levels in nonpregnant controls, suggesting a reduction of synthesis or increase in metabolism of GABA during this period. Reductions of GABA concentrations only slightly greater than these were found to occur immediately before the onset of 3-mercaptopropionic acid (3-MPA)-induced seizures (21). Thus, pregnant mice appear to have GABA levels close to those that might result in spontaneous seizures. Further evidence of altered GABAergic function during pregnancy is indicated by the highly significant decrease in GABA turnover in virtually all brain regions examined.

Although the involvement of GABAergic systems in human epilepsy and experimental seizure models is well established (35), the exact mechanisms through which GABA acts to regulate seizure susceptibility remain unknown. GABA appears to be the primary inhibitory neurotransmitter in the CNS. Its action is mediated through two major subtypes of receptors, $GABA_A$ and $GABA_B$ (17). Recent evidence suggests that the $GABA_B$ receptors may be involved with some types of seizures (22), but it is the $GABA_A$ receptors that appear to be quantitatively more important in the regulation of seizure susceptibility. Thus, blockade of $GABA_A$ receptors with a specific antagonist such as bicuculline facilitates seizures, whereas treatment with $GABA_A$-mimetic agents such as muscimol suppresses seizures (9,53).

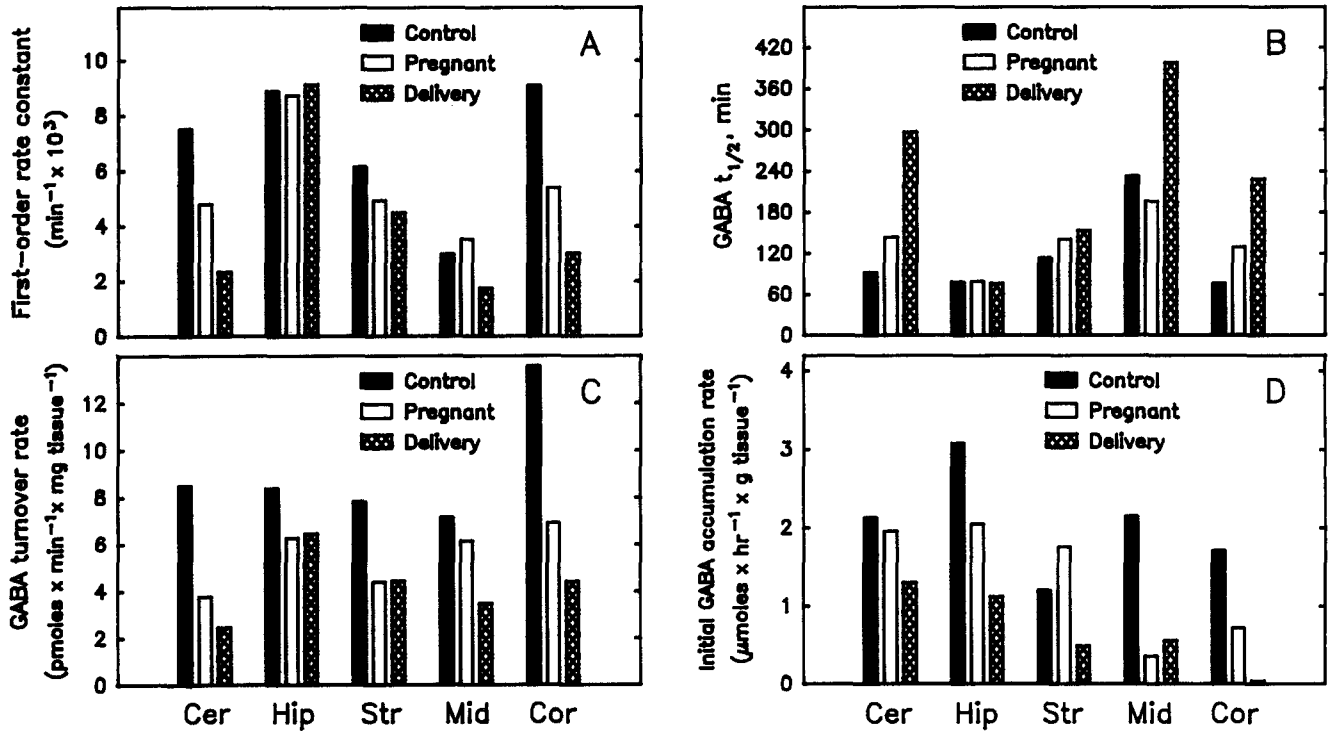

FIG. 3. GABA turnover constants in five brain regions as a function of pregnancy in the mouse. Indices of GABA turnover were calculated from the data in Fig. 2. (A). First-order rate constant. (B). Half-life of GABA. (C). GABA turnover rate (calculated as the product of the first-order rate constant and extrapolated time zero concentration). (D). Initial rate of GABA accumulation. Cer, cerebellum; Hip, hippocampus; Str, striatum; Mid, midbrain; Cor, cortex.

There have been many studies demonstrating that pharmacological manipulation of GABA levels in the CNS cause alterations in seizure susceptibility. The consensus appears to be that high levels of GABA protect from, and low levels of GABA facilitate, seizures, although there is more direct experimental evidence for the former case. Treatment of animals with GABA-T inhibitors such as AOAA or γ-vinyl GABA (GVG) protect against a variety of seizure-inducing agents. This anticonvulsant effect is associated with, and presumably mediated by, significantly increased CNS GABA levels (2,8,28). Other clinically useful anticonvulsants such as valproic acid inhibit GABA-T and cause an increase in brain GABA levels (41), although the doses required are in excess of those required for anticonvulsant activity.

The other side of the hypothesis, that low GABA levels are associated with increased seizure susceptibility, has not been as clearly demonstrated. Blockade of the GABA synthetic enzyme, glutamate decarboxylase [EC 4.1.1.15, (GAD)], with 3-MPA causes severe seizures in mice and rats that are presumed to be precipitated by decreased GABA concentrations. Karlsson et al. (21) reported a significant decrease in regional brain GABA levels in rats following a 35-mg/kg dose of 3-MPA, but Löscher (27) found brain GABA levels to be unchanged in mice at seizure-producing doses (60 mg/kg) of the drug. In the latter study, the dose of 3-MPA had to be increased threefold to demonstrate a consistent decline in GABA concentrations.

While pharmacological manipulations that decrease CNS GABA concentrations precipitate seizures and pharmacological manipulations that increase GABA concentrations or GABAergic transmission protect from seizures, to date there has been no evidence of depressed brain GABA levels in a seizure-susceptible animal. For example, Sykes and Horton (56) found no differences in GAD activity or brain GABA levels between audiogenic seizure-susceptible DBA and seizure-resistant TO mice. Our demonstration of depressed GABA levels in pregnant mice is apparently unique in that pregnant mice, which are more susceptible to seizures than nonpregnant controls, also have depressed brain GABA concentrations in the absence of any pharmacological intervention. This also implies that lowered brain GABA concentrations are a normal consequence of pregnancy in this species. It is not known if GABA concentrations decline in other species during pregnancy.

Regulation of the GABA-benzodiazepine receptor complex has been implicated in mediating differential seizure sensitivity in certain seizure models. The Genetically Epilepsy-Prone Rat (GEPR) was bred for differential sensitivity to audiogenic seizures (the GEPR 3 line has mild seizures; the GEPR 9 line has severe seizures). Both lines were found to have more low-affinity GABA and benzodiazepine binding sites than a nonselected control population, and the GEPR 9 line had more of both receptors than did GEPR 3 rats (4). The GEPR were also found to have more GABAergic neurons (39), which might explain the higher binding values. In contrast, benzodiazepine binding was found to be reduced in the midbrain of the seizure-sensitive Mongolian gerbil (38) and analysis of GABA-benzodiazepine binding sites in audiogenic seizure-prone DBA mice has not yielded consistent results [summarized in (19,24)].

Changes in GABA uptake systems may also affect seizure susceptibility. Two GABA uptake inhibitors, SK&F89976-A and SK&F100330-A, were found to be potent anticonvulsants for NMDA-, pentylenetetrazol-, and picrotoxin-induced seizures (55). Because both picrotoxin and pentylenetetrazol block the GABA-benzodiazepine receptor complex, the anticonvulsant action was thought to be the result of increased GABAergic tone and subsequent increase in the threshold for seizure initiation. Conversely, Janjua and coworkers (18) reported that in the E1 mouse model of human temporal lobe epilepsy hippocampal GABA uptake was markedly decreased compared to control (ddY) mice. They concluded that decreased GABA uptake was a genetically determined predisposing factor for temporal lobe epilepsy and suggested that the clinical condition could be produced by the participation of other precipitating factors. Support for their argument comes from reports that have shown that the anticonvulsants phenytoin and phenobarbital increase GABA uptake (60), although it is not known if this effect is related to their anticonvulsant actions.

The measurement of total neurotransmitter levels measures primarily stored, not synaptic, transmitter because the majority of neurotransmitters in the tissue are found in storage vesicles. Turnover is thought to be a better indicator of the functioning of a neurotransmitter system because it measures released (synaptic) transmitter and not simply whole cell content. The present study shows that GABA turnover was markedly decreased in all areas of the brain during pregnancy except the hippocampus. Interestingly, we had shown previously that norepinephrine turnover was most affected in the hippocampus of pregnant mice (51).

There have been relatively few studies of estimates of GABA turnover rates in experimental animals. Collins (7,8) measured GABA turnover in rat brain using two different methods: monitoring the rate of GABA accumulation following inhibition of GABA-T with AOAA and monitoring the disappearance of intracisternally administered [^{3}H]GABA. Both methods yielded similar GABA turnover rates. He reported that inhibition of GABA-T did not change GABA turnover estimated by the radioactive tracer method, suggesting that GABA-T inhibitors were suitable for the estimation of GABA turnover. Previous estimates of GABA turnover in control mice measured as initial rate of GABA accumulation following inhibition of GABA-T range from 2 to 6 μM/h/g of tissue (20,32,58), which agrees well with the initial values for nonpregnant control mice we calculated in this study (Fig. 2D).

GABA turnover has not been measured in any other seizure models, but several anticonvulsants including valproic acid, diazepam, phenobarbital, and phenytoin significantly reduce GABA turnover in the mouse brain at doses equivalent to their anticonvulsant efficacy (3,6). In addition, the turnover rate of GABA in vivo is increased threefold during bicuculline-induced seizures (6). These reports suggest that seizures are associated with increased GABA turnover, while anticonvulsant activity is associated with decreased GABA turnover. It follows that an animal with increased susceptibility to seizures would be expected to have an increased rate of GABA turnover. However, we found that GABA turnover in seizure-susceptible pregnant mice was significantly lower than in seizure-resistant controls. We suggest that this apparent paradox is explained by a compensatory mechanism whereby pregnant mice, which have lowered GABA levels and are more susceptible to seizures, are maintained at homeostasis by an offsetting reduction in GABA turnover that keeps them seizure free in the absence of an external convulsive stimulus. The reduction in brain GABA turnover may then be seen as a reaction to increased seizure susceptibility, not a cause of it, much in the same way that adrenocortical steroids are elaborated in response to a variety of stressful stimuli.

It may be possible that the reduced concentration of GABA induces the synthesis of an endogenous anticonvulsant compound that acts to lower GABA turnover. There is evidence for an endogenous anticonvulsant produced in the brain following seizures that acts at opiate receptors (57), and benzodiazepines have been shown to reduce GABA turnover through interaction with the GABA-benzodiazepine receptor (3). We are currently investigating the hypothesis that the GABA turnover-reducing anticonvulsant elaborated during pregnancy is an endogenous adenosine receptor agonist, perhaps adenosine itself. Adenosine, as well as several synthetic adenosine agonists such as cyclohexyladenosine, L-phenylisopropyladenosine, and 2-chloroadenosine, are known to have anticonvulsant properties (11,12,37), while adenosine receptor antagonists such as the methylxanthines caffeine and theophylline have proconvulsant effects (10,12,37). Adenosine levels have been shown to rise dramatically following experimental seizures (25,61) and it has been hypothesized that this increase inhibits further seizure activity. Recent evidence suggests that adenosine receptors are closely linked to GABA-benzodiazepine receptors (31,40). Thus, purinergic agonists and antagonists may be capable of regulating GABAergic functioning by modulating the GABA-benzodiazepine receptor complex in brain. This may be of importance to women at risk for developing eclampsia who also consume beverages containing methylxanthines, such as coffee, tea, cocoa, and caffeinated soft drinks.

This paper reports altered GABAergic functioning in pregnant mice and is consistent with our previous reports that pregnant mice are more susceptible to flurothyl-induced seizures during pregnancy. While flurothyl has effects on several neurotransmitter systems (16), recent studies indicate an important role for GABA in the regulation of flurothyl-induced seizures. Xu and coworkers (62) reported that GVG significantly antagonized flurothyl seizures in rats, presumably by increasing CNS GABA levels. Wakamori and coworkers (59) compared the effects of flurothyl on excitatory (glutamate) and inhibitory (GABA and glycine) amino acid responses with those produced by the volatile anesthetics halothane and enflurane in cultured rat neuronal cells. The anesthetics reduced glutamate-induced excitatory responses, enhanced GABA and glycine-mediated chloride currents, and decreased the K_d of the GABA concentration-response curve. Flurothyl produced effects opposite those of the anesthetics. These investigators suggested that halothane and enflurane produced anesthesia by enhancing GABA- and glycine-mediated chloride currents, whereas flurothyl was thought to act by depressing GABA-mediated CNS inhibition.

We are studying the neurochemical changes that occur during a normal pregnancy in the mouse. In the absence of any pharmacological manipulations, pregnant mice are more susceptible to flurothyl-induced seizures than are nonpregnant controls. We found previously that noradrenergic systems are altered during pregnancy (51), and now have shown that GABAergic systems are altered as well. Our observation that brain GABA content and turnover are reduced during pregnancy may be useful in understanding the factors that influence development of preeclampsia and eclampsia (toxemia of pregnancy) in humans. Preeclampsia is a condition characterized by hypertension, proteinuria, and edema that develops

late in the course of an otherwise normal pregnancy. Eclampsia is the superimposition of generalized seizures on the preeclamptic syndrome. The incidence of preeclampsia/eclampsia has not changed over the years, its cause is unknown, treatment is symptomatic, and it remains a significant cause of perinatal morbidity and mortality worldwide (26,44). We suggested previously that the neurochemical changes that contribute to eclamptic seizures also occur in normal pregnancies, but to a lesser extent (51). By studying neurochemical changes that result during the course of a normal pregnancy, we hope to identify systems that could benefit from further study.

In this paper, we have shown that the concentration of GABA and the rate of GABA turnover is significantly reduced during pregnancy in mice. We suggest that the reduction in GABA turnover is a compensatory anticonvulsant mechanism to offset the inherent seizure susceptibility brought about by the reduced level of the major inhibitory neurotransmitter in brain.

ACKNOWLEDGEMENT

This work was supported by Grant HD21709 from the National Institute of Child Health and Human Development.

REFERENCES

1. Anderson, B. B. Red cell metabolism of vitamin B_6. In: Tryfiates, G. P., ed. Vitamin B_6 metabolism and role in growth. Westport, CT: Food and Nutrition Press; 1981:53–83.
2. Bernasconi, R.; Maitre, L.; Martin, P.; Raschdorf, F. The use of inhibitors of GABA-transaminase for the determination of GABA turnover in mouse brain regions: An evaluation of aminooxyacetic acid and gabaculine. J. Neurochem. 38:57–66; 1982.
3. Bernasconi, R.; Schmutz, M.; Martin, P.; Hauser, K. GABA hypothesis for the mechanism of action of antiepileptic drugs: Its usefulness and limitations. In: Fariello, R. G.; Morselli, P. L.; Lloyd, K. G.; Quesney, L. F.; Engel, J., Jr., eds. Neurotransmitters, seizures and epilepsy II. New York: Raven Press; 1984:95–107.
4. Booker, J. G.; Dailey, J. W.; Jobe, P. C.; Lane, J. D. Cerebral cortical GABA and benzodiazepine binding sites in genetically seizure prone rats. Life Sci. 39:799–806; 1986.
5. Brophy, M. H.; Siiteri, P. K. Pyridoxal phosphate and hypertensive disorders of pregnancy. Am. J. Obstet. Gynecol. 121:1075–1079; 1975.
6. Chapman, A. G. Effect of anticonvulsant drugs on brain amino acid metabolism and GABA turnover. In: Fariello, R. G.; Morselli, P. L.; Lloyd, K. G.; Quesney, L. F.; Engel, J., Jr., eds. Neurotransmitters, seizures and epilepsy II. New York: Raven Press; 1984:167–178.
7. Collins, G. G. S. GABA-2-oxoglutarate transaminase, glutamate decarboxylase and the half-life of GABA in different areas of rat brain. Biochem. Pharmacol. 21:2849–2858; 1972.
8. Collins, G. G. S. Effect of aminooxyacetic acid, thiosemicarbazide and haloperidol on the metabolism and half-lives of glutamate and GABA in rat brain. Biochem. Pharmacol. 22:101–111; 1973.
9. Depaulis, A.; Snead, O. C., III; Marescaux, C.; Vergnes, M. Suppressive effects of intranigral injection of muscimol in three models of generalized non-convulsive epilepsy induced by chemical agents. Brain Res. 498:64–72; 1989.
10. Dragunow, M. Purinergic mechanisms in epilepsy. Prog. Neurobiol. 31:85–108; 1988.
11. Dunwiddie, T. V. Endogenously released adenosine regulates excitability in the in vitro hippocampus. Epilepsia 21:541–548; 1980.
12. Dunwiddie, T. V.; Worth, T. Sedative and anticonvulsant effects of adenosine analogs in mouse and rat. J. Pharmacol. Exp. Ther. 220:70–76; 1982.
13. Ebadi, M. Catabolic pathways of pyridoxal phosphate and derivatives. In: Dolphin, D.; Poulson, R.; Avramovic, O., eds. Vitamin B_6 pyridoxal phosphate. Chemical, biochemical and medical aspects. Part B. New York: John Wiley & Sons; 1986:449–476.
14. Furth-Walker, D.; Leibman, D.; Smolen, A. Changes in pyridoxal phosphate and pyridoxamine phosphate in blood, liver and brain in the pregnant mouse. J. Nutr. 119:750–756; 1989.
15. George, F. R.; O'Conner, M. F.; De Fries, J. C.; Collins, A. C. Components of the GABA system in mice selectively bred for differences in open-field activity. Brain Res. 200:85–92; 1980.
16. Greer, C. A.; Alpern, H. P. Mediation of myoclonic seizures by dopamine and clonic seizures by acetylcholine and GABA. Life Sci. 21:385–392; 1977.
17. Hill, D. R.; Bowery, N. G. [^{3}H]Baclofen and [^{3}H]GABA bind to bicuculline-insensitive GABA-B sites in rat brain. Nature 290: 149–152; 1981.
18. Janjua, N. A.; Mori, A.; Hiramatsu, M. Gamma-aminobutyric acid uptake is decreased in the hippocampus in a genetic model of human temporal lobe epilepsy. Epilepsy Res. 8:71–74; 1991.
19. Jobe, P. C.; Laird, H. E. Neurotransmitter abnormalities as determinants of seizure susceptibility and intensity in the genetic models of epilepsy. Biochem. Pharmacol. 30:3137–3144; 1981.
20. Jung, M.; Lippert, B.; Metcalf, B. W.; Schechter, P. J.; Bohlen, P.; Sjoerdsma, A. The effect of 4-amino hex-5-yonic acid (γ-acetylenic GABA) a catalytic inhibitor of GABA transaminase on brain GABA metabolism in vivo. J. Neurochem. 28:717–724; 1977.
21. Karlsson, A.; Fonnum, F.; Malthe-Sorenssen, D.; Storm-Mathisen, J. Effect of the convulsant agent 3-mercaptopropionic acid on the levels of GABA, other amino acids and glutamate decarboxylase in different regions of the rat brain. Biochem. Pharmacol. 23:3053–3061; 1974.
22. Karlsson, G.; Schmutz, M.; Kolb, C.; Bittiger, H.; Olpe, H.-R. $GABA_B$ receptors and experimental models of epilepsy. In: Bowery, N. G.; Bittiger, H.; Olpe, H.-R., eds. $GABA_B$ receptors in mammalian function. New York: John Wiley & Sons; 1990:349–365.
23. Klieger, J. A.; Altschuler, C. H.; Krakow, G.; Hollister, C. Abnormal pyridoxine metabolism in toxemia of pregnancy. Ann. NY Acad. Sci. 166:288–296; 1969.
24. Laird, H. E., II; Dailey, J. W.; Jobe, P. C. Neurotransmitter abnormalities in genetically epileptic rodents. Fed. Proc. 43:2505–2509; 1984.
25. Lewin, E.; Bleck, V. Electroshock seizures in mice: Effect on brain adenosine and its metabolites. Epilepsia 22:577–581; 1981.
26. Lopez-Llera, M. Complicated eclampsia. Fifteen year's experience in a referral medical center. Am. J. Obstet. Gynecol. 142: 28–35; 1982.
27. Löscher, W. 3-Mercaptopropionic acid: Convulsant properties, effects on enzymes of the γ-aminobutyrate system in mouse brain and antagonism by certain anticonvulsant drugs, aminooxyacetic acid and gabaculine. Biochem. Pharmacol. 28:1397–1407; 1979.
28. Löscher, W.; Frey, H.-H. Aminooxyacetic acid: Correlation between biochemical effects, anticonvulsant action and toxicity in mice. Biochem. Pharmacol. 27:103–108; 1978.
29. Lowry, O. H.; Passonneau, J. A flexible system of enzymatic analysis. New York: Academic Press; 1972:8–17.
30. Lumeng, L.; Cleary, R. E.; Wagner, R.; Ye, P.-L.; Li, T.-K. Adequacy of vitamin B_6 supplementation during pregnancy: A prospective study. Am. J. Clin. Nutr. 29:1376–1383; 1976.
31. Marangos, P. J.; Paul, S. M.; Goodwin, F. K.; Syzpin, P.; Skolnick, P. Purinergic inhibition of diazepam binding to rat brain (in vitro). Life Sci. 24:851–858; 1979.
32. Matsui, Y.; Duguchi, T. Effects of gabaculine, a new potent inhibitor of gamma-aminobutyrate transaminase, on the brain gamma-aminobutyrate content and convulsions in mice. Life Sci. 20:1291–1296; 1977.

33. McClearn, G. E.; Wilson, J. R.; Meredith, W. The use of isogenic and heterogenic mouse stocks in behavioral research. In: Lindzey, G.; Thiessen, D. D. Contributions to behavior-genetic analysis: The mouse as a prototype. New York: Appleton-Century-Crofts; 1970:3-22.
34. McCormick, D. B.; Gregory, M. E.; Snell, E. E. Pyridoxal phosphokinases. I. Assay, distribution, purification, and properties. J. Biol. Chem. 236:2076-2084; 1961.
35. Meldrum, B. S. Epilepsy and γ-aminobutyric acid mediated inhibition. Int. Rev. Neurobiol. 17:1-36; 1975.
36. Molony, C. J.; Parmalee, A. H. Convulsions in young infants as a result of pyridoxine (vitamin B_6) deficiency. JAMA 154:405-406; 1954.
37. Murray, T. F.; Sylvester, D.; Schultz, C. S.; Szot, P. Purinergic modulation of the seizure threshold for pentylenetetrazol in the rat. Neuropharmacology 24:761-766; 1985.
38. Olsen, R. W.; Wamsley, J. W.; Lee, R.; Lomax, P. Alterations in the benzodiazepine/GABA receptor-chloride ion channel complex in the seizure-sensitive Mongolian gerbil. In: Fariello, R. G.; Morselli, P. L.; Lloyd, K. G.; Quesney, L. F.; Engel, J., Jr., eds. Neurotransmitters, seizures and epilepsy II. New York: Raven Press; 1984:201-213.
39. Roberts, R. C.; Ribak, C. E. Anatomical changes of the GABAergic system in the inferior colliculus of the genetically epilepsy prone rat. Life Sci. 39:789-798; 1986.
40. Roca, D. J.; Schiller, G. D.; Farb, D. H. Chronic caffeine or theophylline exposure reduces γ-aminobutyric acid/benzodiazepine receptor site interactions. Mol. Pharmacol. 30:481-485; 1988.
41. Schechter, P. J.; Tranier, Y.; Grove, J. Effect of *n*-diproplyacetate on amino acid concentrations in mouse brain: Correlations with anticonvulsant activity. J. Neurochem. 31:1325-1327; 1978.
42. Schlesinger, K.; Schreiber, R. A. Interaction of drugs and pyridoxine deficiency on central nervous system excitability. Ann. NY Acad. Sci. 166:281-287; 1969.
43. Seyfried, T. N.; Glaser, G. H. A review of mouse mutants as genetic models of epilepsy. Epilepsia 26:143-150; 1985.
44. Sibai, B. M.; McCubbin, J. H.; Anderson, G. D.; Lipshitz, J.; Dilts, P. V., Jr. Eclampsia I. Observations from 67 recent cases. Obstet. Gynecol. 58:609-613; 1981.
45. Sloger, M. S.; Reynolds, R. D. Effects of pregnancy and lactation on pyridoxal 5′-phosphate in plasma, blood, and liver of rats fed three levels of vitamin B_6. J. Nutr. 110:1517-1524; 1980.
46. Smolen, A.; Petersen, D. R.; Collins, A. C. Liver cytosolic aldehyde dehydrogenase activity in the pregnant mouse. Devel. Pharmacol. Ther. 3:31-49; 1981.
47. Smolen, A.; Smolen, T. N. Genetic influence on increased seizure susceptibility in pregnancy. Life Sci. 39:1525-1530; 1986.
48. Smolen, A.; Smolen, T. N. Concentration and turnover of brain gamma-aminobutyric acid (GABA) during pregnancy. FASEB J. 6:A2029; 1992.
49. Smolen, A.; Smolen, T. N.; Collins, A. C. Seizure susceptibility of the pregnant mouse. Pharmacol. Biochem. Behav. 17:91-97; 1982.
50. Smolen, A.; Smolen, T. N.; van de Kamp, J. L. The effect of naloxone administration on pregnancy-associated seizures. Life Sci. 38: 1899-1905; 1986.
51. Smolen, A.; Smolen, T. N.; van de Kamp, J. L. Alterations in brain catecholamines during pregnancy. Pharmacol. Biochem. Behav. 26:613-618; 1987.
52. Speigel, M. R. Schaum's outline of theory and practice of statistics. 2nd ed. New York: McGraw-Hill; 1988:345-347.
53. Sperber, E. F.; Wurpel, J. N. D.; Zhao, D. Y.; Moshe, S. L. Evidence for the involvement of nigral $GABA_A$ receptors in seizures of adult rats. Brain Res. 480:378-382; 1989.
54. Stanulović, M.; Chaykin, S. Aldehyde oxidase: Catalysis of the oxidation of N^1-methylnicotinamide and pyridoxal. Arch. Biochem. Biophys. 145:27-34; 1971.
55. Swinyard, E. A.; White, H. S.; Wolf, H. H.; Bondinell, W. E. Anticonvulsant profiles of the potent and orally active GABA uptake inhibitors SK&F 89976-A and SK&F 100330-A and four prototype antiepileptic drugs in mice and rats. Epilepsia 32:569-577; 1991.
56. Sykes, C. C.; Horton, R. W. Cerebral glutamic acid decarboxylase activity and γ-aminobutyric acid concentrations in mice susceptible or resistant to audiogenic seizures. J. Neurochem. 39:1489-1491, 1982.
57. Tortella, F. C.; Long, J. B. Endogenous anticonvulsant substance in rat cerebrospinal fluid after a generalized seizure. Science 228: 1106-1108; 1985.
58. Van Gelder, N. M. The effect of amino-oxyacetic acid on the metabolism of γ-aminobutyric acid in brain. Biochem. Pharmacol. 15:533-539; 1966.
59. Wakamori, M.; Ikemoto, Y.; Akaike, N. Effects of two volatile anesthetics and a volatile convulsant on the excitatory and inhibitory amino acid responses in dissociated CNS neurons of the rat. J. Neurophysiol. 66:2014-2021; 1991.
60. Weinberger, J.; Nicklas, W. J.; Berl, S. Mechanism of action of anticonvulsants. Neurology 26:162-166; 1976.
61. Winn, H. R.; Welsh, J. E.; Brynor, C.; Rubio, R.; Berne, R. M. Brain adenosine production during the initial 60 seconds of bicuculline seizures in rats. Acta Neurol. Scand. 60 (suppl. 72):536-537; 1979.
62. Xu, S. G.; Garant, D. S.; Sperber, E. F.; Moshé, S. L. Effects of substantia nigra γ-vinyl-GABA infusions on flurothyl seizures in adult rats. Brain Res. 566:108-114; 1991.