Pharmacology Biochemistry & Behavior, Vol 26, pp 613–618 Copyright © Pergamon Journals Ltd , 1987 Printed in the U S A 0091-3057/87 $3 00 + 00

Alterations in Brain Catecholamines During Pregnancy

ANDREW SMOLEN,[1] TONI NESS SMOLEN AND JENNIFER L. VAN DE KAMP

Institute for Behavioral Genetics, and School of Pharmacy
Molecular and Environmental Toxicology Program, University of Colorado, Boulder, CO 80309-0447

Received 25 August 1986

SMOLEN, A , T N SMOLEN AND J L VAN DE KAMP *Alterations in brain catecholamines during pregnancy* PHARMACOL BIOCHEM BEHAV **26**(3) 613–618, 1987 —During pregnancy mice are more susceptible to flurothyl-induced seizures than are non-pregnant controls The potential role of brain catecholamines in mediating this behavior was examined in the present study The concentration and turnover of norepinephrine (NE) and dopamine (DA) were measured in hippocampus, striatum, midbrain and cortex in control, pregnant and delivery-day mice There were no significant changes from control in DA levels during pregnancy and parturition The turnover of DA was not altered during pregnancy, except for a small increase in turnover rate in the hippocampus The concentration of NE decreased during pregnancy, and rose at parturition This effect was most striking in the hippocampus The turnover of NE was markedly depressed during pregnancy, with the hippocampus again being most affected These data imply a role for NE, but not DA in the mediation of increased seizure susceptibility during pregnancy

Pregnancy Seizures Norepinephrine Dopamine Catecholamines Catecholamine turnover Flurothyl Brain Hippocampus

DURING pregnancy an isozyme of aldehyde dehydrogenase, π-aldehyde dehydrogenase (π-AlDH) is induced in the liver cytosol [29] The factors which mediate this increase in enzyme activity are not known The natural substrate for π-AlDH is not known, but it has a broad substrate specificity [29], and we have found that it is capable of metabolizing pyridoxal, a form of vitamin B_6 [30]. It is known that pregnancy is associated with decreased plasma levels of the coenzyme form of vitamin B_6, pyridoxal 5'-phosphate, PLP [4, 12, 15, 27] This occurs even in normal pregnancies, but the deficit is much greater in certain pathologic pregnancies [4, 12, 34]. Deficiency of PLP is known to be associated with increased susceptibility to experimentally induced seizures in mice [23] and spontaneous seizures in humans [19] We predicted that pregnancy would, therefore, create a predisposition to induced or spontaneous seizures, and that the risk of suffering a seizure would be greatest late in pregnancy

We investigated the influence of pregnancy on flurothyl-induced seizures, and found that pregnant mice were more susceptible to seizures than were virgin controls, thus providing our hypothesis to be correct [30]. We are currently studying the potential neurochemical changes which occur in pregnancy in an effort to determine the factors which predispose pregnant animals to increased seizure susceptibility. The apparent connection between π-AlDH, vitamin B_6, and seizures led us to investigate the role of neurotransmitter systems in mediating these increased seizures Most of the well known neurotransmitters, including norepinephrine, dopamine, serotonin and γ-amino butyric acid, require PLP-dependent enzymes for their synthesis Each has been implicated in mediating seizure activity in at least one system [10,25], but none of these are known to be specifically altered in pregnancy. In this paper we report on our studies of catecholaminergic systems in brain during pregnancy We found that dopamine was not greatly affected, but that pregnancy caused alterations in the concentration and turnover of norepinephrine.

METHOD

Animals

The animals used in this study were female Heterogeneous Stock (HS) mice [16], 60–100 days old. Mice were maintained on a 12 hr light/dark cycle (0700–1900) and were allowed free access to food (Wayne Sterilizable Lab Blox) and water.

For all experiments, controls were virgin females of the same age as the experimental animals. Day 1 of pregnancy was ascertained by observance of vaginal plug. Mated females were separated from the males (also HS) and housed in groups until the day they were used for the experiment. Three groups of mice were used: non-pregnant controls, pregnant (17 to 18 days), and day of delivery (usually day 20). These groups will be referred to as control, pregnant and delivery in the remainder of the text.

[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

Measurement of Brain Catecholamines

Animals were killed by focused microwave irradiation. The brain was removed, cooled, and dissected on ice into hippocampus, striatum, midbrain (mostly thalamus), and cortex The tissue was weighed to the nearest milligram, homogenized in 1.0 ml (2.0 ml for cortex) of 50 mM perchloric acid containing 0.1 mM sodium bisulfite Twenty μl (100 ng) of the internal standard, 3,4-dihydroxybenzylamine, was added to each sample. Samples were stored at −70°C until assayed All dissections were performed between 1300 and 1600, the same as the previous seizure tests [30]. For assay, the samples were thawed to 4°C, and centrifuged for 10 min at 10,000 × g to precipitate proteins. The resulting supernatants were transferred to tubes containing 50 mg of alumina (Woelm, acid washed and neutralized) and 1.0 ml of 3.0 M Tris-HCl buffer, pH 8.6 (adjusted at room temperature). The tubes were immediately capped and shaken by hand This was followed by an additional 10 minutes of mechanical shaking The tubes were then centrifuged 60 sec in a table top centrifuge to sediment the alumina. The supernatant was aspirated from the alumina and discarded The alumina was washed once with 1.0 ml of 6 mM Tris-HCl, pH 8 0, and twice with 2.0 ml of distilled water using brief centrifugation and aspiration as above. The catecholamines adsorbed to the washed alumina were extracted with 0.5 ml of 0 1 M perchloric acid Efficiency of extraction averaged approximately 70% as measured by external catecholamine standards.

Norepinephrine (NE) and dopamine (DA) content were measured by high pressure liquid chromatography with electrochemical detection using the method of Felice and coworkers [8]. The chromatograph consisted of a Beckman model 110 pump, Rheodyne model 7125 injector, Waters C-18 μBondapak reversed phase column (25 cm) and a Bioanalytical Systems model LC4B detector. A 50 μl sample was injected onto the column, and the chromatogram was developed isocratically using a mobile phase consisting of 60 mM citric acid, 40 mM dibasic sodium phosphate and sodium octyl sulfate (5 8 mg/l) adjusted to pH 5.5 with NaOH. Detector potential was set at +0 72 V vs. an Ag/AgCl reference electrode Standard curves (from standards frozen along with the samples) were run daily

Catecholamine Turnover

The turnover of DA in the four brain regions was measured in control, pregnant, and delivery day mice. Dopamine turnover was assessed by inhibiting the synthesis of DA with the tyrosine hydroxylase inhibitor, α-methyl-*p*-tyrosine (MPT) [3]. Mice were injected with MPT, 250 mg/kg, and the content of DA in the four brain regions was measured 0.25, 0.5, 1, 2 and 4 hours later Norepinephrine turnover was assessed by inhibiting its synthetic enzyme, dopamine-β-hydroxylase with bis-(4-methyl-l-homopiperazinylthiocarbonyl)-disulfide (FLA-63) [32]. Mice were injected with FLA-63, 40 mg/kg, and the content of NE in the four brain regions was measured over the next four hours

Data Analysis

Data were analyzed by one-way analysis of variance. Turnover studies were analyzed by linear regression analysis of natural logarithm transformations of the brain catecholamine values vs time

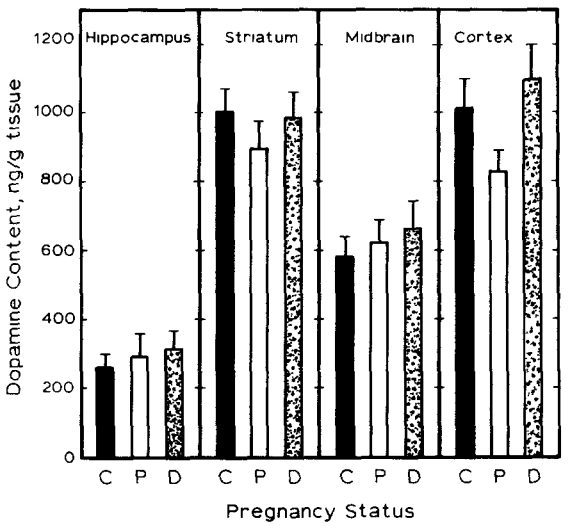

FIG 1 Dopamine concentrations in brain as a function of pregnancy in the mouse Dopamine was measured in hippocampus, striatum, midbrain and cortex of control mice (C), pregnant mice (P) and on the day of delivery (D) Values are the mean±SEM of 10–23 individual animals Values for striatum are 10 fold higher than the scale on the left-hand axis

RESULTS

The DA content of the hippocampus, striatum, midbrain and cortex in control (C), pregnant (P) and delivery (D) mice is shown in Fig 1. There were slight, statistically nonsignificant reductions of DA in striatum and cortex in the pregnant mice and a small increase in DA concentrations at the time of delivery Note that the striatal dopamine concentrations are 10 fold higher than the values given on the left-hand axis. This was done for perspective so that all four regions could be shown in the same figure.

Figure 2 shows the NE content of these four brain regions as a function of pregnancy. As was found for DA, the levels of NE also tended to rise during pregnancy, and were highest at parturition These changes were not statistically significant except for the NE values in striatum for the pregnant and delivery groups and the delivery group in hippocampus. The concentration of NE in striatum of control or pregnant mice is quite low, but the values rise dramatically at parturition.

The concentration of NE in the hippocampus, however, decreases in pregnant mice compared to nonpregnant controls This is the only brain region and the only time point where this occurs. On the day of delivery, the hippocampal NE levels increased significantly over control and pregnant mice, and at this time the seizure sensitivity returns to control [30]

Simple levels of neurotransmitters may not sufficiently describe the status of a neurotransmitter system, and it has been argued that turnover is a better measure. Figure 3 and Table 1 show the turnover of DA in the four brain regions for control, pregnant, and delivery day mice. There was a slight tendency of DA turnover to decline during pregnancy, and return to control at delivery. These changes occurred in striatum, midbrain and cortex. In the hippocampus, DA turnover rose during pregnancy

Norepinephrine turnover is shown in Fig. 4 and Table 1

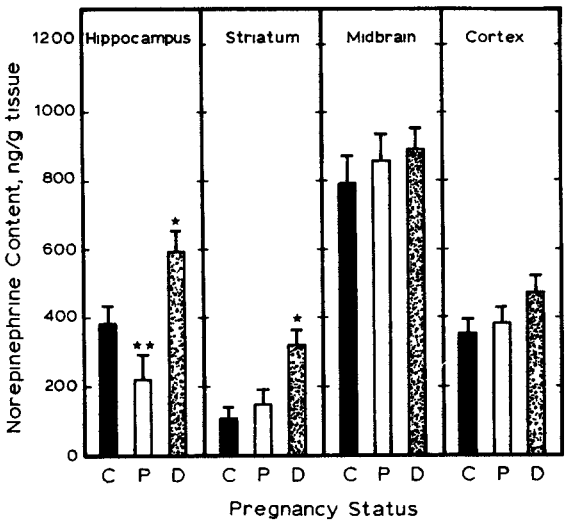

FIG 2 Norepinephrine concentrations in brain as a function of pregnancy in the mouse Norepinephrine was measured in hippocampus, striatum, midbrain and cortex of control mice (C), pregnant mice (P) and on the day of delivery (D) Values are the mean±SEM of 10–23 individual animals *Indicates significantly different from control, $p<0.05$ **Indicates significantly different from day of delivery, $p<0$ 05

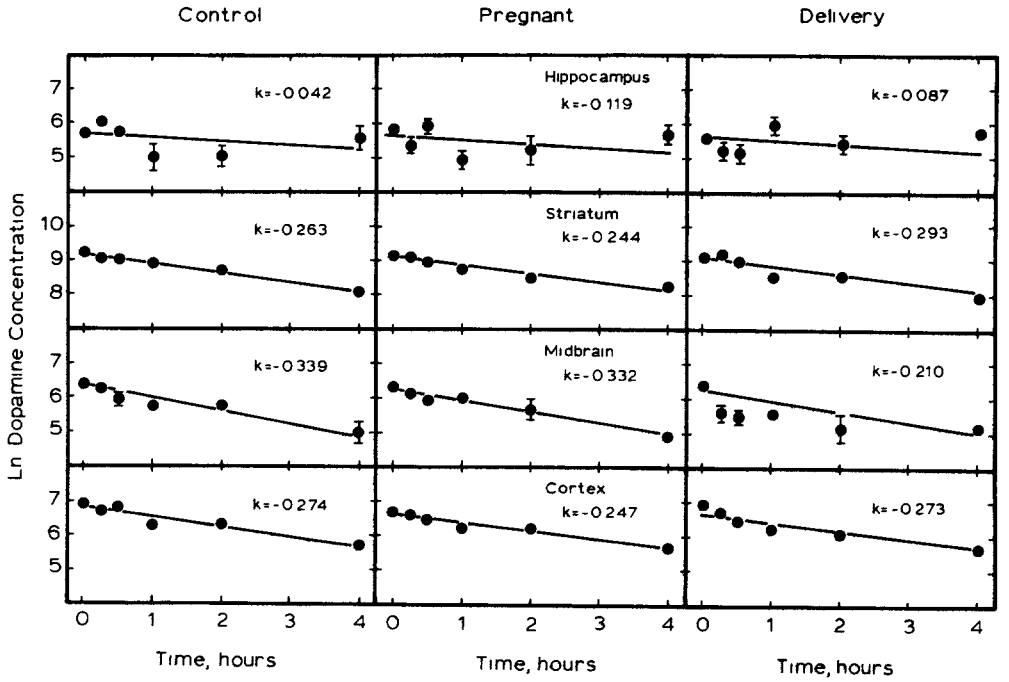

FIG 3. Dopamine turnover in four brain regions as a function of pregnancy in the mouse Turnover was estimated by inhibiting the synthesis of DA with the tyrosine hydroxylase inhibitor, MPT, and DA content was measured at the times indicated in the figure. Each point is the mean±SEM of 8–12 separate determinations. Where not indicated, the standard errors are contained within the plotted symbol. The first order rate constant, k, is given within the figure for each region

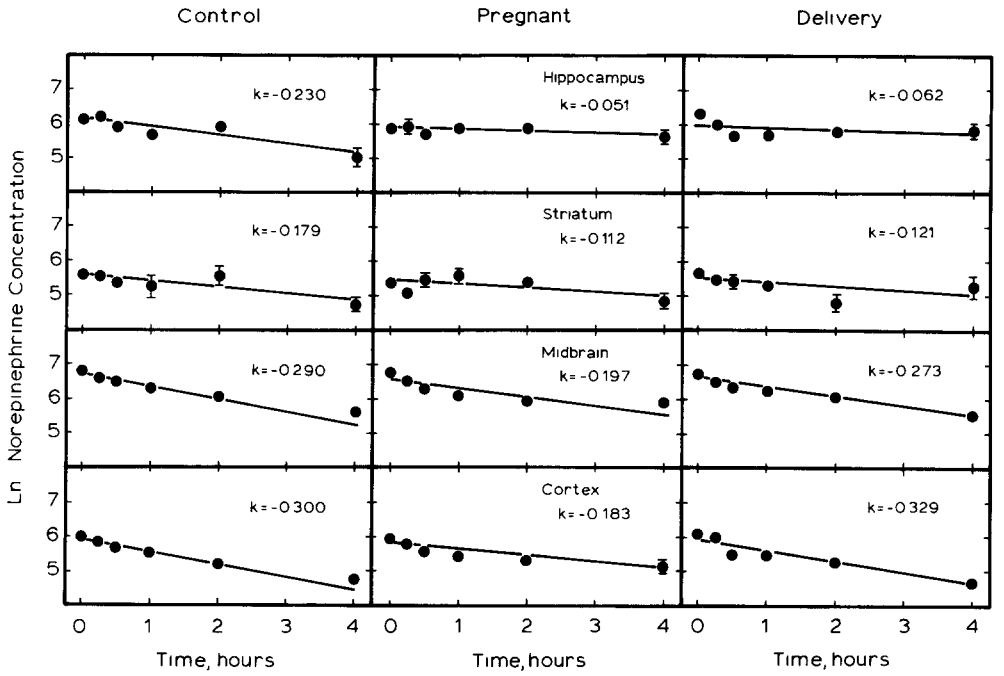

FIG 4 Norepinephrine turnover in four brain regions as a function of pregnancy in the mouse Turnover was estimated by inhibiting the synthesis of NE with the dopamine-β-hydroxylase inhibitor, FLA-63, and NE content was measured at the times indicated in the figure Each point is the mean±SEM of 8–12 individual animals Where not indicated, the standard errors are contained within the plotted symbol The first order rate constant, k, is given within the figure for each region

There was a general tendency for NE turnover to decrease during pregnancy. This was especially striking in the hippocampus in which NE turnover was very low in both pregnant mice and in mice on the day of delivery In the other regions, NE turnover returned to control levels on the day of delivery This large decline in NE turnover, especially in the hippocampus, may be involved in the increased seizure sensitivity in the pregnant mice

DISCUSSION

We have reported previously that pregnancy results in increased susceptibility to flurothyl-induced seizures in the mouse [28, 30, 31]. The neurochemical mechanisms which mediate this effect are not yet known. The purpose of this study was to investigate the possibility that these pregnancy-associated seizures could be explained by potential alterations in catecholaminergic systems during pregnancy

The results of this study show that changes in catecholaminergic systems do indeed occur in the pregnant mouse Changes in NE and DA were found in each brain region studied, but the concentration of hippocampal NE was most affected by pregnancy. Since the hippocampus is widely recognized as a mediator of generalized seizures, this may be important in the increased susceptibility to flurothyl seizures seen in pregnancy. The reduction of NE occurs at a time of pregnancy when mice are most susceptible to flurothyl-induced seizures [30] On the day of delivery seizure suscep-

TABLE 1

CATECHOLAMINE TURNOVER IN BRAIN REGIONS

Region	Control	Pregnant	Delivery
	(ng amine/g tissue/hr)		
	Dopamine		
Hippocampus	12 1	33 6	20 0
Striatum	2436 7	2177 5	2738 9
Midbrain	170 2	177 3	138 6
Cortex	244 1	189 2	228 8
	Norepinephrine		
Hippocampus	101 4	18 7	24 7
Striatum	47 1	25 7	29 7
Midbrain	234 6	138 1	202 0
Cortex	108 9	60 4	124 0

Catecholamine turnover rates were calculated as the product of the first order rate constant (Figs 3 and 4) and the extrapolated time zero concentration of the appropriate catecholamine

tibility of mice returns to control levels [30], and the NE content of the hippocampus is significantly elevated compared to both control and pregnant animals. These data imply a positive association between NE concentrations and susceptibility to flurothyl-induced seizures in the pregnant mouse

There is ample support in the literature for the association of NE depletion with increased seizure susceptibility. Depletion of brain NE concentrations with pharmacologic agents such as 6-hydroxydopamine, reserpine, FLA-63 and disulfiram result in decreased seizure threshold, increased seizure intensity, or both [5, 10, 11, 17] Most types of induced seizures, including maximal electroshock [11,17], kindling [6] and pentylenetetrazole [6, 11, 17] are exacerbated by decreasing the concentration of NE. Depletion of NE has also been shown to increase epileptiform activity of the hippocampal slice [18]. Most of these studies, in addition to showing that NE is a modulator of seizure activity, largely rule out a role for DA in modulating these seizures. Others, however, have implicated DA in the control of some seizure types [1,2], especially audiogenic [7,10]

Scudder and coworkers [24] screened mice of six genera and three inbred strains for maximal electroshock seizure latencies and brain catecholamine content. They found that seizure latencies were strongly correlated with brain amine content—high levels were associated with long latencies. A number of seizure susceptible animal models have been described (for reviews see [10,25]), and the role of catecholamines in mediating the differential responses to seizure-inducing agents has been widely studied. The results of these studies indicate that, in general, seizure susceptible animals have lower levels of NE than do seizure resistant ones For example, DBA/2 mice, which are more susceptible to audiogenic, pentylenetetrazole, electroshock and flurothyl seizures than are C57BL/6 mice [10, 21, 28], also appear to have lower basal levels of brain NE [21,22] This view has been challenged, however [10,13] Jobe and coworkers reported that the genetically epilepsy-prone rat had lower levels of NE than did seizure resistant control rats [9] Thus, a number of studies implicate low (relative) brain NE levels with high seizure proneness Our present study is in agreement with this view

The measurement of total neurotransmitter levels measures primarily stored, not synaptic transmitters, since 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 since it measures released (synaptic) transmitter, and not simply whole cell content The present study shows that DA turnover is altered only in the hippocampus during pregnancy. In contrast, pregnancy causes a decreased rate of turnover of NE in each of four brain regions studied, and this decrease is especially marked in the hippocampus. Thus, we are in substantial agreement with a study by Jobe *et al.* [9] which showed that NE turnover was considerably lower in the genetically epilepsy-prone rat compared to seizure resistant control animals.

Our observation that brain NE content and turnover are altered during pregnancy may be useful in understanding the factors which influence development of preeclampsia and eclampsia (toxemia of pregnancy) in humans. Preeclampsia is a condition which develops late in the course of an otherwise normal pregnancy which is characterized by hypertension, proteinuria and edema. 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 [14,26].

The relationship btween catecholamines and toxemias of pregnancy has been a subject of continuing investigation and debate. Equally good studies have reported increased NE in pregnancy [35], no change in NE [20], and decreases in NE in pregnancy [33]. These studies in humans are difficult since the tissue of interest, the brain, is obviously unavailable for study The majority of the studies, however, have indicated that pregnancy results in decreases in NE, and that in the pathological pregnancies, preeclampsia and eclampsia, this deficit is apparently much greater [33].

We are studying the neurochemical changes which occur during pregnancy in the mouse A mouse pregnancy can be considered to be a normal one, yet it is interesting that we have been able to predict, find, and partially characterize increased susceptibility to chemically induced seizures in the pregnant mouse. It is our working hypothesis that the neurochemical changes which contribute to the development of eclamptic seizures also occur in normal pregnancies, but to a lesser extent. By studying potential neurochemical changes in normal pregnancy, we hope to identify systems which could benefit from further study. While the neurotransmitter changes we have described do not mirror the behavioral changes exactly, in this paper we have largely ruled out a significant contribution of DA to the increased seizure susceptibility in pregnancy, and have presented evidence for a potential role of NE in mediating pregnancy-associated seizures

ACKNOWLEDGEMENTS

The authors wish to thank Esther Oh for technical assistance This work was supported in part by grants from the National Institute of Neurological and Communicative Disorders and Stroke, NS 20748 (A.S), the National Institute of Child Health and Human Development, HD 21709 (A S) and the National Institute on Alcohol Abuse and Alcoholism, AA 06487 and AA 06527 (T N S) Preparation of this manuscript was facilitated by a grant from the Biomedical Research Support Grant Program, RR-07013-20 awarded to the University of Colorado, Boulder, CO

REFERENCES

1 Anlezark, G M , R W Horton and B S Meldrum Dopamine agonists and audiogenic seizures the relationship between protection against seizures and changes in monoamine metabolism *Biochem Pharmacol* **27:** 2821–2828, 1978

2 Billiet, M , P Bernard, A Delaunois and A De Schaepdryver Induced changes in caudate nucleus dopamine and electroshock threshold *Arch Int Pharmacodyn* **188:** 396–400, 1970

3 Brodie, B B , E Costa, A Dlabac, N H Neff and H H Smookler Application of steady state kinetics to the estimation of the synthesis rate and turnover time of tissue catecholamines *J Pharmacol Exp Ther* **154:** 493–498, 1966

4 Brophy, M H and P K Siiteri Pyridoxal phosphate and hypertensive disorders of pregnancy *Am J Obstet Gynecol* **121:** 1075–1079, 1975

5 Chen, G , C R Ensor and B A Bohner A facultative action of reserpine on the central nervous system *Proc Soc Exp Biol Med* **86:** 507–510, 1954

6 Corcoran, M. E , H C Fibiger, J A McCaughran and J A Wada Potentiation of amygdaloid kindling and metrazole induced seizures by 6-hydroxydopamine in rats *Exp Neurol* **45:** 118–133, 1974

7 Dailey, J W and P C Jobe Effect of increments in the concentration of dopamine in the central nervous system on audiogenic seizures in DBA/2J mice *Neuropharmacology* **23:** 1019–1024, 1984

8 Felice, L J , J D Felice and P T Kissinger Determination of catecholamines in rat brain parts by reverse-phase ion-pair liquid chromatography *J Neurochem* **31:** 1461–1465, 1978

9 Jobe, P C , K. H Ko and J W Dailey Abnormalities in norepinephrine turnover rate in the central nervous system of the genetically epilepsy-prone rat *Brain Res* **290:** 357–360, 1984

10 Jobe, P C and H E Laird Neurotransmitter abnormalities as determinants of seizure susceptibility and intensity in the genetic models of epilepsy *Biochem Pharmacol* **30:** 3137–3144, 1981

11 Kilian, M and H -H Frey Central monoamines and convulsive thresholds in mice and rats *Neuropharmacology* **12:** 681–692, 1973

12 Klieger, J A , C H Altshuler, G Krakow and C Hollister Abnormal pyridoxine metabolism in toxemia of pregnancy *Ann NY Acad Sci* **166:** 288–296, 1969

13 Lints, C E , J F Willott, P Y Sze and L H Nenja Inverse relationship between whole brain monoamine levels and audiogenic seizure susceptibility in mice Failure to replicate *Pharmacol Biochem Behav* **12:** 385–388, 1980

14 Lopez-Llera, M Complicated eclampsia Fifteen year's experience in a referral medical center *Am J Obstet Gynecol* **142:** 28–35, 1982

15 Lumeng, L , R E Cleary, R. Wagner, P -L Ye and T -K Li Adequacy of vitamin B_6 supplementation during pregnancy A prospective study *Am J Clin Nutr* **29:** 1376–1383, 1976

16 McClearn, G E , J R Wilson and W Meredith The use of isogenic and heterogenic mouse stocks in behavioral research In *Contributions to Behavior-Genetic Analysis The Mouse as a Prototype,* edited by G Lindzey and D D Thiessen New York Appleton-Century-Crofts, 1970, pp 2–22

17 Mason, S T and M E Corcoran Catecholamines and convulsions *Brain Res* **170:** 497–507, 1979

18 Mody, I , P Leung and J J Miller Role of norepinephrine in seizurelike activity of hippocampal pyramidal cells maintained in vitro alteration by 6-hydroxydopamine lesions of norepinephrine-containing systems *Can J Physiol Pharmacol* **61:** 841–846, 1983

19 Molony, C J and A H Parmalee Convulsions in young infants as a result of pyridoxine (vitamin B_6) deficiency *J Am Med Assoc* **154:** 405–406, 1954

20 Pedersen, E B , A B Rasmussen, N J Christensen, P Johannesen, J G Lauritsen, S Kristensen and M Wohlert Plasma noradrenaline and adrenaline in preeclampsia, essential hypertension in pregnancy and normotensive pregnant control subjects *Acta Endocrinol* **99:** 594–600, 1982

21 Schlesinger, K , W O Boggan and B J Griek Pharmacogenetic correlates of pentylenetetrazol and electroconvulsive seizure thresholds in mice *Psychopharmacologia* **13:** 181–188, 1968

22 Schlesinger, K and B J Griek The genetics and biochemistry of audiogenic seizures In *Contributions to Behavior-Genetic Analysis The Mouse as a Prototype,* edited by G Lindzey and D D Thiessen New York Appleton-Century-Crofts, 1970, pp 219–257

23 Schlesinger, K and R A Schreiber Interaction of drugs and pyridoxine deficiency on central nervous system excitability *Ann NY Acad Sci* **166:** 281–287, 1969

24 Scudder, C L , A G Karczmar, G M Everett, J E Gibson and M Rifkin Brain catecholamines and serotonin levels in various strains and genera of mice and a possible interpretation for the correlations of amine levels with electroshock latency and behavior *Int J Neuropharmacol* **5:** 343–351, 1966

25 Seyfried, T N and G H Glaser A review of mouse mutants as genetic models of epilepsy *Epilepsia* **26:** 143–150, 1985

26 Sibai, B M , J H McCubbin, G D Anderson, J Lipshitz and P V Dilts, Jr Eclampsia I Observations from 67 recent cases *Obstet Gynecol* **58:** 609–613, 1981

27 Sloger, M S and R D Reynolds 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

28 Smolen, A and T N Smolen Genetic influence on increased seizure susceptibility in pregnancy *Life Sci* **39:** 1525–1530, 1986

29 Smolen, A , D R Petersen and A C Collins Liver cytosolic aldehyde dehydrogenase activity in the pregnant mouse *Dev Pharmacol Ther* **3:** 31–49, 1981

30 Smolen, A , T N Smolen and A C Collins Seizure susceptibility of the pregnant mouse *Pharmacol Biochem Behav* **17:** 91–97, 1982

31 Smolen, A , T N Smolen and J L van de Kamp The effect of naloxone administration on pregnancy-associated seizures *Life Sci* **38:** 1899–1905, 1986

32 Svensson, T H and B Waldeck On the significance of central noradrenaline for motor activity Experiments with a new dopamine-β-hydroxylase inhibitor *Eur J Pharmacol* **7:** 278–282, 1969

33 Tunbridge, R D G and P Donnai Plasma noradrenaline in normal pregnancy and in hypertension of late pregnancy *Br J Obstet Gynecol* **88:** 105–108, 1981

34 Wachstein, M and L W Graffeo Influence of vitamin B_6 on the incidence of pre-eclampsia *Obstet Gynecol* **8:** 177–180, 1956

35 Zuspan, F P Adrenal gland and sympathetic nervous system response in eclampsia *Am J Obstet Gynecol* **114:** 304–311, 1972