function in 24 h. Clearly, a substantial interindividual susceptibility to ischemic injury exists among various hepatocytes in the intact organ.

The concentration of ATP in the ischemic lobes of the liver 1 h after reperfusion proved to be a good predictor of hepatic function 24 h later (fig. 2). A similar strong correlation between postischemic ATP levels and subsequent functional recovery has recently been demonstrated in a model of renal ischemia [8]. In the chosen model of partial hepatic ischemia approximately 50% of the total mass of the liver is subject to ischemia. Thus, non-ischemic parts of the liver contribute one half of the total demethylation capacity, and even with total loss of function in the ischemic lobes one would expect a residual demethylation of 15–20% of the dose. This fraction corresponds to the intercept of the regression line with the ordinate in figure 2 (19% dose appearing in breath as $^{14}CO_2$). The graph indicates that with concentrations of ATP in the ischemic lobes approaching zero 1 h after reperfusion there will be essentially no functional recovery, whereas with ATP levels close to the physiological concentration complete recovery will occur.

The activity of ALT in serum shortly after ischemia was poorly correlated to hepatic function 24 h later. This is not surprising considering the fact that additional ischemic injury may occur much later, as manifested by the release of OCT [9]. In contrast to ALT 1 h after ischemia the activity of ALT at the time of the aminopyrine breath test was strongly correlated with hepatic function (fig. 1). The level of ALT at 24 h is probably related to the total amount of ALT released following the ischemic insult and therefore provides a better index of the extent of hepatic damage than the activity of ALT shortly after ischemia.

In conclusion, our data show that hepatic function decreases with increasing duration of ischemia indicating marked interindividual differences in the susceptibility of hepatocytes to the effects of ischemia in vivo. The concentration of ATP in the ischemic lobes 1 h after reperfusion predicts hepatic function 24 h later. Recent data obtained in man support this conclusion [10]. Measurements of the concentration of free ATP by non-invasive means such as NMR might serve as an index of the subsequent functional capacity of transplanted livers and might be helpful in assessing the effect of agents claimed to give protection against ischemic liver injury.

Acknowledgments. This work was supported by grant Nr. 3.824.0.84 from the Swiss National Foundation for Scientific Research.

1 VanThiel, D. H., Schade, R. R., Hakala, T. R., Starzl, T. E., and Denny, D., Hepatology *4* (1984) 66S.
2 National Institutes of Health Consensus Development, Conference Statement: Liver Transplantation, June 20–23, 1983. Hepatology *4* (1984) 107S.
3 Jennische, E., and Hannson, H.-A., Acta physiol. scand. *122* (1984) 199.
4 Nordström, G., Seeman, T., and Hasselgren, P. O., Surgery *97* (1985) 679.
5 Baker, H. C., J. Path. Bact. *71* (1956) 135.
6 Lauterburg, B. H., and Bircher, J., J. Pharmac. exp. Ther. *196* (1976) 501.
7 Fredericks, W. M., Fronik, G. M., and Hesselin, J. G. M., Exp. molec. Path. *41* (1984) 119.
8 Stromski, M. E., Cooper, K., Thulin, G., Gaudio, K. M., Siegel, N. J., and Shulman, R. G., Proc. natl Acad. Sci. USA *83* (1986) 6142.
9 Frederiks, W. M., Vogels, I. M. C., and Fronik, G. M., Cell. biochem. Funct. *2* (1984) 217.
10 Lanir, A., Jenkins, R. L., Caldwell, C., Lee, R. G. L., Khettry, U., and Clouse, M. E., Hepatology *7* (1987) 1049.

0014-4754/88/050455-03$1.50 + 0.20/0


## Maternal stress alters monoamine metabolites in fetal and neonatal rat brain

L. R. Herrenkohl[a], U. Ribary, M. Schlumpf and W. Lichtensteiger

[a]*Department of Psychology, Temple University, Philadelphia (Pennsylvania 19122, USA), and Pharmakologisches Institut, University of Zürich, CH-8006 (Switzerland)*
*Received 5 October 1987; accepted 15 January 1988*

*Summary.* Heat-restraint stress given rats during the last week of gestation significantly altered dopaminergic dihydroxyphenylacetic acid and homovanillic acid (DOPAC and HVA) and noradrenergic 3-methoxy-4-hydroxy-phenyl-ethylene glycol (MOPEG) forebrain-hypothalamic monoamine (MA) metabolites in female offspring. On gestational day 21, HVA and MOPEG were significantly higher and lower, and on postnatal day 1 all were higher. There were virtually no differences in brain MA concentrations in males. Thus MA metabolic concentrations differ in fetal-neonatal forebrain-hypothalamus as a function of sex differences and maternal stress.
*Key words.* Maternal stress; monoamine metabolites; perinatal rat brain.

Neuroendocrine-CA systems typically undergo a period of functional organization in parallel with brain development. In the rat, the critical period corresponds to late fetal and early postnatal life. The process is most obvious in the case of the gonadal axis where it results in a number of sexually dimorphic brain functions [1, 2]. Neuroendocrine-neurotransmitter systems are particularly sensitive to environmental influences during this phase of organization. Support for this idea comes from observations that external factors such as alcohol, barbiturates, nicotine and now prenatal stress interfere with the development of the rat gonadal-CA axis [3–8]. With respect to prenatal stress, Moyer, Herrenkohl and Jacobowitz [9] have suggested that maternal stress may modify the neuroanatomical and biochemical organization of the brains of both males and females and turn the direction of male fetal brain development toward that of the female sex. They combined the microdissection procedure of Palkovits for removing individual brain nuclei with sensitive radioisotopic enzymatic assays for norepinephrine (NE) and dopamine (DA). In pregnant rats, Moyer et al. [9] discovered that stress during pregnancy reduced steady-state levels of NE in brain regions associated with gonadotropic secretion. The major noradrenergic pathway that underwent change during stress was the ventral ascending bundle (i.e., the medial preoptic nucleus, anterior hypothalamus, and median forebrain bundle). They also reported that the locations of DA decreased as a function of prepartal stress. Moyer et al. [10] also examined the effects of prenatal stress on CA concentra-

tions in the brains of the male and female offspring as adults. The major pattern of brain change in male offspring was similar to that in stressed mothers. The major system that underwent change involved NE, the major direction of change was a decrease, and the major brain regions that underwent change were those associated with gonadotropic secretion from the anterior pituitary gland and with the regulation of sexual behavior.

Among other findings[10] was the observation that prenatal stress markedly affected CA concentrations in female offspring. Prenatal stress increased the steady-state concentration of DA in the hypothalamic arcuate nucleus of stressed female offspring by 153%! Because marked alterations in arcuate DA have been associated with abnormalities in the release of gonadotropic hormones from the anterior pituitary gland, Herrenkohl[4, 11] predicted and observed that prenatal stress would produce reproductive dysfunctions in female offspring[4 - 11]. Other experimenters found that maternal stress changes testicular (Leydig cell) and brain steroid aromatase activity in rat fetuses in patterns similar to those alterations in fetal testosterone[7, 12, 13]. It has also been reported that maternal stress differentially alters pituitary, gonadal, and adrenal function in adult rats and mice[14 - 17]. Given the effects of prenatal stress on adult offspring pituitary-CA concentrations[10, 14 - 17] and on fetal gonadal function[7, 12, 13], the question arises whether prenatal stress may directly alter CA activity in the fetal neonatal stage. The present experiment employs high power liquid chromatography with electronic detection (HPLC-ED) procedures adapted by Ribary, Lichtensteiger and Schlumpf to study ontogenetic patterns of forebrain-hypothalamic monoaminergic (MA) metabolites in fetal and neonatal brain[18] as well as the consequences on these MA metabolites of prenatal exposure to nicotine[19].

*Materials and methods.* 23 primiparous pregnant Sprague-Dawley rats weighing 250 g were mated at Zivic-Miller (Allison Park, Pennsylvania) and sent to our laboratory one week before stressing. Upon arrival, they were housed individually in 24 × 32 × 16 cm fiberglass observation cages with San-i-cel bedding under a standard 12-h light/dark cycle beginning at 08.00 h, maintained on Purina chow and water ad libitum. On gestational day (GD) 14 (GD 1 being day of mating), 13 randomly-selected females were subjected to heat-restraint stress by being placed individually in a 18 × 8 cm semicircular plexiglass restraining cage under four incandescent lights which produced a surface illumination of 4280 lm/m$^2$ (400 ft-cd) and a surface temperature of 34 °C[4]. Three 45-min stress periods beginning at 10.00 h were alternated with 45-min rest periods in the home cage. Females readily adapted to placement in the plexiglass cage, offering little or no resistance. After a few trials, they rapidly crawled inside and sat passively within it. Ten control females remained unhandled in the home cage.

On GD 21, after one stress period at 07.00 h, fetuses were removed from mothers under anesthesia between 08.00 and 10.00 h. The forebrain was dissected and cut coronally between the bifurcation of the internal carotid artery and posterior communicating artery and the superior cerebellar artery. The piece included the hypothalamus. In the postnatal period, rats were similarly decapitated but within 1–4 h of birth (PN1). In both cases, olfactory bulbs were removed. Forebrains were assayed individually. Sex determination was based on outer genitalia and anogenital distance. Brain tissue was homogenized, centrifuged and analyzed by newly-developed HPLC-EC methodologies[18, 19].

*Results and discussion.* All but one of the six analyses of variance (ANOVAS) derived for the MA metabolites were significant. On GD 21, F-values for DOPAC, HVA and MOPEG were 1.19, 3.51 and 6.03 respectively (df's = 3,33; 3,36; 3,38). For HVA and MOPEG, p's were $<0.05$ and $<0.005$: only the differences in concentrations of DOPAC were not significant. On PN1, F-values for DOPAC, HVA and MOPEG were all highly significant (F's = 6.85, 4.58 and 3.39:df's = 3,37; 3,36; 3,36; 3,36; p's $<0.005$, 0.05, 0.05). In the case of significant F's, Tukey's test for all possible paired comparisons was conducted to reveal the p-values in the table.

The table gives mean values of MA metabolites ± standard errors in prenatally stressed and control fetuses on GD 21 and in prenatally-stressed and control neonates on PN1. The table also shows that concentrations of HVA and MOPEG were significantly higher and lower respectively on GD 21 in fetal brains of prenatally-stressed female offspring compared with nonstressed controls (p's $<0.005$, 0.05). In females, DOPAC concentrations did not differ significantly on GD 21. None of the three MA metabolites differed significantly in males on GD 21. Within 1–4 h after birth however, in the absence of heat-restraint stress, mean concentrations of DOPAC, HVA and MOPEG were all significantly higher in prenatally-stressed females compared to nonstressed controls (p's $<0.005$, 0.05, 0.05). Of the three MA metabolites, only HVA differed significantly in males on PN1[10]. It should be mentioned that regardless of treatment all MA metabolic concentrations were higher on PN1 than on GD 21. In addi-

Mean values of monoaminergic metabolites in prenatally stressed and nonstressed fetal and neonatal rats on gestational day 21 or postnatal day 1 (± SE, expressed in ng/mg protein)

| Offspring | Catecholamine metabolites | | | | | |
|---|---|---|---|---|---|---|
| | DOPAC (Dopamine) | | HVA | | MOPEG (Norepinephrine) | |
| | N | | N | | N | |
| Fetal females | | | | | | |
| Prenatally-stressed | 10 | 0.38 + 0.02 | 9 | 1.39 ± 0.05 | 10 | 0.31 ± 0.01 |
| Nonstressed | 10 | 0.35 ± 0.02 | 10 | 1.20 ± 0.06** | 10 | 0.34 ± 0.01* |
| Fetal males | | | | | | |
| Prenatally-stressed | 9 | 0.42 ± 0.04 | 11 | 1.22 ± 0.07 | 12 | 0.30 ± 0.01 |
| Nonstressed | 7 | 0.38 ± 0.03 | 9 | 1.22 ± 0.03 | 9 | 0.28 ± 0.01 |
| Neonatal females | | | | | | |
| Prenatally-stressed | 10 | 0.71 ± 0.04 | 9 | 1.50 ± 0.06 | 9 | 0.64 ± 0.03 |
| Nonstressed | 10 | 0.51 ± 0.03** | 10 | 1.31 ± 0.05* | 10 | 0.55 ± 0.02* |
| Neonatal males | | | | | | |
| Prenatally-stressed | 10 | 0.57 ± 0.03 | 10 | 1.24 ± 0.06 | 10 | 0.53 ± 0.03 |
| Nonstressed | 10 | 0.55 ± 0.03 | 10 | 1.44 ± 0.07* | 10 | 0.53 ± 0.02 |

* $p<0.05$; ** $p<0.005$.

tion to the significant paired comparisons shown in the table, the following differences between means were also significant: GD 21-MOPEG (nonstressed fetal females vs nonstressed fetal males), $p < 0.005$; PN1-DOPAC (prenatally-stressed neonatal females vs stressed neonatal males, $p < 0.005$). PN1-MOPEG (prenatally-stressed neonatal females vs prenatally-stressed neonatal males, $p < 0.005$).

Mean values of forebrain protein content were also obtained for prenatally stressed and nonstressed fetal and neonatal rats on GD 21 and PN1 (females, means $\pm$ SE's = $9.58 \pm 0.21$, $10.52 \pm 0.15$, $9.99 \pm 0.40$, $9.87 \pm 0.21$; males, $10.29 \pm 0.17$, $10.36 \pm 0.17$, $9.47 \pm 0.33$, $10.35 \pm 0.27$). ANOVAS for GD 21 and PN1 were both nonsignificant (F's = 1.37, 1.62, df's = 3,42; 3,36), suggesting that the chemical milieu in which MA metabolites were assayed (ng/mg protein) did not contribute to the MA changes in the brain.

Thus the findings are clear that heat-restraint stress delivered to the pregnant dam during the last third of gestation significantly elevated both DA and NE forebrain-hypothalamic metabolites in female offspring on the last day of in utero life and even more so on the first day of birth, in the absence of experimental stress (table). Male offspring were relatively unaffected. There appears therefore to be sex differences in responsiveness of fetal and neonatal brain MA metabolites to maternal stress. To our knowledge this is the first demonstration that prenatal stress alters MA metabolites in fetal and neonatal brain. Acute effects of maternal restraint stress have been observed on the last day of gestation on plasma levels of NE and DA in pregnant rats and their fetuses as well as the CA content of fetal adrenals [20], but not on fetal brains. The functional and behavioral significance of prenatal-stress-induced alterations in forebrain-hypothalamic MA metabolites remains to be discovered as does the mechanism of prenatal stress. To date it has been well-established that prenatal stress feminizes and/or demasculinizes the sexual behavior of male offspring as adults [21–28]. In female offspring as adults, prenatal stress produces a syndrome characterized by markedly diminished reproductive capabilities (spontaneous abortions, vaginal hemorrhaging during pregnancy, stillbirths, neonatal mortality or low birthweight young) [4, 11, 14, 29]. Past and current research is examining the role of prenatal-stress-induced alterations in androgenic steroids as the basis of the alterations in adult behavior and reproductive function.

As mentioned previously, critical periods involving the fetal CNS have been detected for a variety of neural, hormonal and behavioral events, as for example the testosterone surge that occurs in normal fetal males on GD 18 or 19 [7]. As with the 'premature' testosterone surge in the stressed fetal male [7], prenatal stress may create a desynchrony between the state of development of the CNS and its neurotransmitter environment. Neurotransmitters 'release' hypothalamic-hypophyseal hormones that respond to and regulate gonadal, adrenal and other endocrine axes. Should there be 'deficits' in MA processes as a function of maternal stress, effects on gonadal activity, CA nerve cell development, and sex differences in fetal behavior might be expected to occur. Another question that remains open is the timing and mechanism of the onset of maternal stress effects on the developing fetal gonadal-central CA system.

1 Naftolin, F., Science *211* (1981) 1263.
2 McEwen, B., Science *211* (1981) 1303.
3 Ward, I. L., Science *175* (1972) 82.
4 Herrenkohl, L. R., Science *206* (1979) 1097.
5 Gupta, C., Sonawane, B. R., Yaffe, S. J., and Shapiro, B. H., Science *208* (1980) 508.
6 Taylor, A. N., Branch, B. J., Lieu, S. H., and Kokka, N., Pharmac. Biochem. Behav. *16* (1982) 585.
7 Ward, I. L., and Weisz, J., Science *207* (1980) 328.
8 Lichtensteiger, W., and Schlumpf, M. (Eds), Drugs and Hormones in Brain Development. Karger, Basel 1983.
9 Moyer, J. A., Herrenkohl, L. R., and Jacobowitz, D. M., Brain Res. *121* (1977) 385.
10 Moyer, J. A., Herrenkohl, L. R., and Jacobowitz, D. M., Brain Res. *144* (1978) 173.
11 Herrenkohl, L. R., and Politch, J. A., Experientia *34* (1978) 1240.
12 Weisz, J., Brown, B. L., and Ward, I. L., Neuroendocrinology *35* (1982), 374.
13 Orth, J. M., Weisz, J., Ward, O. B., and Ward, I. L., Biol. Reprod. *38* (1983) 1287.
14 Herrenkohl, L. R., and Scott, S., Experientia *40* (1984) 101.
15 Politch, J. A., and Herrenkohl, L. R., Physiol. Behav. *32* (1984) 95.
16 Politch, J. A., and Herrenkohl, L. R., Physiol. Bahav. *32* (1984) 135.
17 Politch, J. A., and Herrenkohl, L. R., Physiol. Behav. *32* (1984) 447.
18 Ribary, U., Schlumpf, M., and Lichtensteiger, W., Neuropharmacology *25* (1986) 981.
19 Ribary, U., Schlumpf, M., and Lichtensteiger, W., Neurosci. Lett. Suppl. *18* (1984) 201.
20 Rohde, W. T., Ohkawa, K., Dobaski, K., Arai, S., Okinaga, S., and Dörner, G., Exp. clin. Endocr. *82* (1983) 268.
21 Ward, I. L., Science *175* (1972) 82.
22 Ward, I. L., J. comp. physiol. Psychol. *91* (1977) 465.
23 Herrenkohl, L. R., and Whitney, J. B., Physiol. Behav. *17* (1976) 1019.
24 Whitney, J. B., and Herrenkohl, L. R., Physiol. Behav. *19* (1977) 167.
25 Chapman, R. H., and Stern, J. M., J. comp. physiol. Psychol. *92* (1978) 1024.
26 Dählof, L. G., Härd, E., and Larsson, K., Anim. Behav. *25* (1978) 958.
27 Dunlap, J. L., Zadina, J. E., and Gougis, G., Physiol. Behav. *21* (1978) 873.
28 Götz, F., and Dörner, G., Endokrinologie *76* (1980) 115.
29 Herrenkohl, L. R., and Gala, R. R., Experientia *35* (1979) 702.

0014-4754/88/050457-03$1.50 + 0.20/0


## Production of passive cutaneous anaphylaxis (PCA) and reversed PCA by rat IgE antibody in the mouse

M. Harada, M. Nagata, and M. Takeuchi

*Shionogi Research Laboratories, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553 (Japan)*
*Received 27 August 1987; accepted 22 December 1987*

*Summary.* Although IgE antibody is generally characterized as a homocytotropic antibody, it has been well known for some time that mouse IgE antibody causes potent sensitization of rat skin for PCA. The present study clearly shows the reciprocal cross-sensitization of mouse skin with rat IgE molecules. PCA and RPCA were produced by rat IgE antibody in an inbred mouse strain, DS/Shi, though not in C3H/HeShi, C57BL/6JShi and BALB/cCrj strains. Sensitization of DS/Shi mouse skin for PCA with rat IgE antibody was comparable in sensitivity with that of rat skin, but lasted only for a short term in comparison with the long persistence in rat skin.
*Key words.* PCA; reversed PCA; rat IgE antibody; DS/Shi mouse.