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 ± 0.21, 10.52 ± 0.15, 9.99 ± 0.40, 9.87 ± 0.21; males, 10.29 ± 0.17, 10.36 ± 0.17, 9.47 ± 0.33, 10.35 ± 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.

IgE antibody has generally been characterized as a heat-labile homocytotropic antibody[1]; it sensitizes the tissues only of animals of the species from which it has been derived. Therefore, it is usually assayed by means of the allogeneic PCA[2-5]. However, some exceptional cases have been reported. Human IgE antibody sensitizes monkey skin for the Prausnitz-Küstner (P–K) reaction and passive cutaneous anaphylaxis (PCA), although at a much lower level of sensitivity than in humans[6]. Also, mouse IgE antibody sensitizes rat skin more strongly than mouse skin. In general, definite reproducible PCA cannot be produced by mouse IgE antibody in the dorsal skin of mouse[7], but a clear-cut reaction can be induced in rat skin[8]. This is why mouse IgE antibody has been assayed almost exclusively using rat PCA. The fact that mouse IgE antibody as well as rat IgE antibody sensitizes rat skin suggests that IgE molecules of mouse and rat are similar to each other in the Fc portion. However, no evidence has yet been offered of rat IgE antibody being able to sensitize mouse skin for PCA, most likely due to the known experimental difficulty of producing clear-cut PCA in mouse skin even with IgE antibody of mouse origin. Very recently, however, we found that mouse IgE monoclonal antibodies to benzylpenicilloyl haptens and ovalbumin mediate distinct PCA in an inbred mouse strain, DS/Shi, with a high sensitivity, comparable with that of rat PCA, and that the high sensitivity of this mouse strain is inherited by the offspring as a dominant phenotype[9]. This finding should facilitate the study of the nature of IgE antibody-mediated hypersensitivity in mice. The present study was conducted to find out whether IgE antibody of rat origin can also produce PCA in DS/Shi mice.

Materials and methods. Animals. Female Wistar/Shi rats (8–9 weeks of age; Shi: Shionogi Aburahi Laboratory, Shiga-ken, Japan) were used as antibody producers and PCA recipients. Female mice of four inbred strains, i.e., C3H/HeShi, C57BL/6JShi, DS/Shi and BALB/cCrj (Crj: Japan Charles River Inc., Atsugi, Japan), aged 7–9 weeks, were employed as PCA recipients. These animals were bred under specific pathogen-free conditions but kept under conventional conditions after the treatments.

Antigens and antibodies. Bovine serum albumin (BSA, Nakarai Chemicals, Kyoto, Japan) was used as antigen, and the antiserum to BSA of rat origin as antibody preparation. To raise anti-BSA IgE antibody, five rats were given a single i.p. injection of 1 mg of BSA with 1×10^{10} of killed *Bordetella pertussis* organisms. Two weeks later, they were bled by heart puncture and the antisera were pooled. As an IgE antibody preparation of mouse origin, ascitic fluid of (BALB/c × C57BL/6J)F_1 hybrids (abbreviated below as CBF_1 mice) containing anti-benzylpenicilloyl (BPO) IgE monoclonal antibody designated as BIE-13CE[10] was used, and BPO hapten-conjugated guinea pig serum albumin (BPO_{17}GpSA; see reference 11) was employed as the eliciting antigen homologous to BIE-13CE.

Fractionation of rat IgE antibody. A 4-ml portion of the pooled rat anti-BSA antiserum was fractionated by means of Sephadex G 200 gel filtration (column size: 3 × 80 cm; eluted with phosphate-buffered physiological saline solution (PBS, pH 7.0). The optical density of each eluate (3 ml/tube) was measured at 280 nm. When the optical density was over 2.0, eluates were diluted twofold or fourfold with PBS before the measurement. Eluates near the second and third peaks were separately pooled and concentrated three times. These two fractions were characterized by Ouchterlony's test using goat anti-rat IgG/Fc serum (Cappel, Cochranville, PA, USA), PCA and reversed PCA (RPCA) using goat anti-rat IgE/Fc serum (Cappel) and/or anti-rat IgG/Fc serum in mice and rats.

PCA and RPCA. PCA was produced in mice and rats. A 0.05-ml portion of the twofold serially diluted antibody preparations was injected into the dorsal skin of mice and rats, and was followed at various time intervals by i.v. challenge with 1 mg of an elicitor homologous to the antibody preparation used (0.2 ml for a mouse and 1.0 ml for a rat) including 0.5 mg (mouse) or 5 mg (rat) of Evans blue. One hour later, the animals were sacrificed and the dye leakage in the inner surface of the skin observed. Blueing with a diameter of more than 5 mm was recorded as a positive reaction. Tests were performed in duplicate using two recipients for each antibody dilution. RPCA was done in a similar way except for the use of anti-rat IgE/Fc serum or anti-rat IgG/Fc serum (0.2 ml of the 1:8 dilution for a mouse) as the elicitor in place of an antigen. In some experiments, rat antiserum was heat-inactivated at 56 °C for 30 min before use to prove that the antibody responsible for PCA and RPCA is of the IgE class.

Results. Strain difference in PCA and RPCA mediated by rat anti-BSA IgE anti-body. As shown in the table, 2- to 3-day PCA was clearly produced by rat anti-BSA serum in DS/Shi mice but not in any of the other strains tested. The antibody titer in DS/Shi mice was 1:8, which was lower than that in rats only by one grade of the twofold dilution. No individual difference was observed between the two recipients in the duplicate test. PCA in DS/Shi mice was abolished by heat-treatment of the antiserum, which strongly suggests that the antibody involved is of the IgE isotype. Similarly, 2-day RPCA was produced in DS/Shi mice but not in C3H/HeShi mice by challenging anti-rat IgE/Fc serum (table and fig. 1). In addition, RPCA was not induced in DS/Shi mice by anti-rat IgG/Fc serum.

Fractionation of the antibody responsible for PCA and RPCA in DS/Shi mice. As illustrated in figure 2, four peaks were obtained by Sephadex G 200 gel filtration of the rat antiserum. Ouchterlony's test using anti-rat IgG/Fc serum revealed that the third peak contained the IgG fraction. This fraction, heat-treated at 56 °C for 30 min, produced 1-h PCA in DS/Shi and C3H/HeShi mice but not 3-day PCA in rats, which was in accordance with the result of Ouchterlony's test. On the other hand, 3-day PCA was clearly provoked in rats by the contents of the second peak, indicating that this was the IgE fraction. This fraction produced 1-day PCA in DS/Shi mice. Also, it produced 1-day RPCA in DS/Shi mice

PCA and RPCA in the mouse and rat mediated by IgE antibodies of rat and mouse origin

Antibody preparation	Recipients	PCA titer at latent period of 2-day	3-day	5-day	7-day	9-day	RPCA *titer 2-day
Rat anti-BSA serum	DS/Shi	1:8	1:8	1:1–1:2**	Neg.	Neg.	1:16
	BALB/cCrj	Neg.***	Neg.				Neg.
	C57BL/6JShi	Neg.	Neg.				
	C3H/HeShi	Neg.	Neg.				
	Wistar/Shi rats	1:16	1:16	1:16	1:16	1:8	
Mouse anti-BPO IgE Ab	DS/Shi	1:4096	1:4096	1:4096	1:2048	1:512	
(BIE-13CE ascites)	Wistar/Shi rats	1:8192	1:8192	1:4096	1:4096	1:1024	

* Elicited by goat anti-rat IgE/Fc serum (diluted 1:8 with PBS). ** Variance depending on the recipients. *** Neg.: Negative.

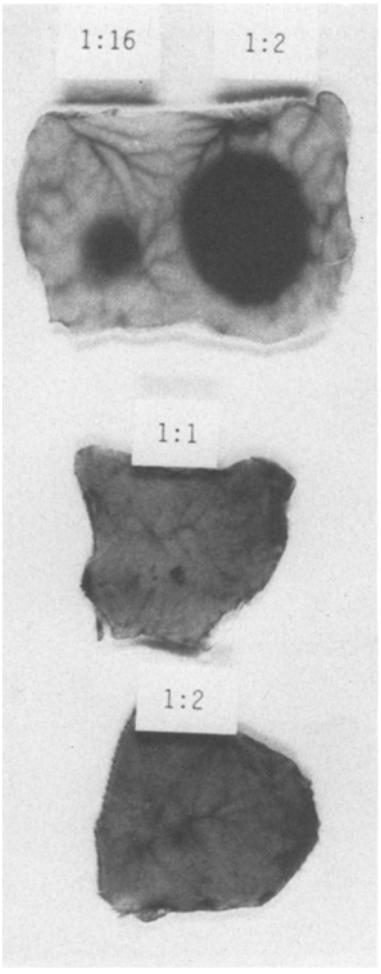

Figure 1. RPCA in DS/Shi and C3H/HeShi mice produced by rat anti-BSA serum and goat anti-rat IgE/Fc serum.

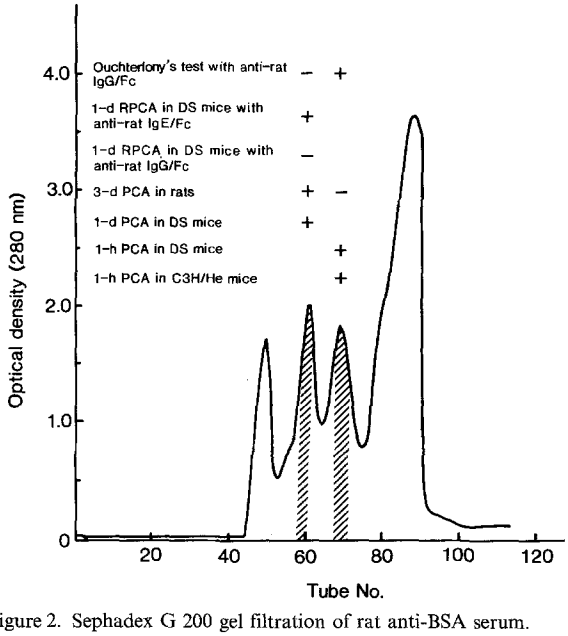

Figure 2. Sephadex G 200 gel filtration of rat anti-BSA serum.

when combined with anti-rat IgE/Fc serum, but not when combined with anti-rat IgG/Fc serum.

Duration of passive skin sensitization by rat IgE antibody. The duration of the sensitization of the skin by rat IgE antibody was compared with that by mouse IgE antibody in DS/Shi mice. The comparison was also done between rat PCA and DS/Shi mouse PCA mediated by rat IgE antibody. As shown in the table, the IgE antibody titer of the rat anti-BSA serum in DS/Shi mice did not change within 3 days after the intradermal injection. However, it decreased rapidly thereafter, PCA being negative after 7 days. In contrast, sensitization lasted longer in rat skin, the antibody titer remaining unchanged within 7 days, and decreasing only by one grade of the twofold serial dilution after 2 more days. In addition, anti-BPO IgE monoclonal antibody titer of CBF_1 mouse ascites did not change in DS/Shi mice within 5 days, although it decreased gradually after that. These results suggest that sensitization by rat IgE antibody in DS/Shi mice is alleviated sooner than sensitization by allogeneic IgE antibodies in rats and mice.

Discussion. It has been suggested that IgE molecules of rats and mice are similar in their Fc portions. This would explain why mouse IgE antibody as well as rat IgE antibody sensitized rat skin [8, 12], and why passive sensitization by mouse IgE antibody can be prevented by unrelated mouse and rat IgE molecules [13, 14]. However, information is poor concerning reciprocal passive sensitization of mouse tissues with rat IgE antibody. According to Prouvost-Danon et al. [15], rat IgE antibody sensitizes mouse mast cells for degranulation, but the results are variable and no correlation can be obtained between the PCA titer and mast cell-sensitizing activity. The present study is the first indication that rat IgE antibody produces skin sensitization for PCA and RPCA in the mouse; 1- to 3-day PCA and RPCA were clearly observed in DS/Shi mice, an inbred strain established from a closed colony of dd mice [16], but not in the other three mouse strains tested. Rat antibody of the IgG class can also mediate PCA in mice after a 1-h latent period, but the antibody responsible for 1- to 3-day PCA and RPCA was clearly distinguishable from IgG antibody in several respects such as heat lability, molecular size and lack of reactivity with anti-rat IgG/Fc antibody in Ouchterlony's test. Moreover, sensitivity to 1- to 3-day PCA by rat antiserum was markedly different depending on the strain of the recipients, while that to 1-h PCA was not. This is in accordance with our previous study dealing with the strain difference in sensitivity to PCA mediated by allogeneic IgE and IgG_1 homocytotropic antibodies [9]. The IgE antibody titer of rat antiserum obtained in DS/Shi mice was close to that in rats, which indicates that the difference if any in sensitivity between rat PCA and DS/Shi mouse PCA is small. However, it should be noticed that the duration of sensitization with rat IgE antibody was shorter in DS/Shi mouse skin than the long-lasting sensitization of the rat skin: maximal sensitization in DS/Shi mouse skin by rat IgE antibody lasted only for 3 days, while that by mouse IgE antibody lasted for 5–7 days. Thus, rat IgE antibody bound as a foreign protein to mast cells of DS/Shi mice may be metabolized relatively quickly.

1 Ito, K., Clin. Immun. *16* (1984) 724 (in Japanese).
2 Binaghi, R. A., and Benaceraff, B., J. Immun. *92* (1964) 920.
3 Hogarth-Scott, R. S., Intern. Archs Allerg. appl. Immun. *32* (1967) 201.
4 Mota, I., and Perini, A., Life Sci. *9* (1970) 923.
5 Patterson, R., J. Allergy *31* (1960) 351.
6 Ishizaka, K., Ishizaka, T., and Arbesman, C. E., J. Allergy clin. Immun. *39* (1967) 254.
7 Inagaki, N., Goto, S., Nagai, H., and Koda, A., Intern. Archs Allerg. appl. Immun. *74* (1984) 91.
8 Mota, I., and Wong, D., Life Sci. *8* (1969) 813.

9 Harada, M., Ikeda, R., and Fukushima, H., Ann. Allerg. *55* (1985) 267.
10 Fukushima, H., Misaki, R., Takeuchi, M., Niinomi, Y., and Harada, M., Clin. exp. Immun. *68* (1987) 427.
11 Harada, M., Takeuchi, M., and Watanabe, A., Chemotherapy *35*, Suppl. 1 (1987) 435 (Tokyo).
12 Prouvost-Danon, A., Binaghi, R., Rochas, S., and Boussac-Arou, Y., Immunology *23* (1972) 481.
13 De Souza, C. M., Luiz, C., Souberbrown, M., and Vaz, N. M., J. Immun. *112* (1974) 1369.
14 Lehler, S. B., Immunology *32* (1977) 507.
15 Prouvost-Danon, A., Wyczolkowska, J., Binaghi, R., and Abadie, A., Immunology *29* (1975) 151.
16 Japan Experimental Animals Research Association, Exp. Anim. *17* (1967) 27.

0014-4754/88/050459-04$1.50 + 0.20/0

Announcements

Italy

Vth International Symposium on Bioluminescence and Chemiluminescence

Florence/Bologna, 25–29 September 1988

For information, please contact the Organizing Secretariat, O.I.C., Via G. Modena 19, I-50121 Florence/Italy.

Instructions to Authors

Experientia is a monthly journal for life sciences devoted to publishing articles which are interdisciplinary in character and which are of general scientific interest. Considered for publication will be hitherto unpublished papers that fall within one of three categories:

Reviews (one-man and multi-author reviews)
Mini-reviews (1–2 printed pages)
Short Communications (1–2 printed pages)

Papers reporting on work that is preliminary in nature, or wherein animal experiments have been conducted without the appropriate anesthesia, will not be accepted.
Manuscripts (including all tables and figures) must be submitted in *triplicate* and must be in *English*. *Title pages* should bear the author's name and address (placed directly below the title), a brief *abstract* (of approximately 50 words for short communications) mentioning new results only, and a listing of *key words*. *Footnotes* must be avoided. *Tables*, and then *figures*, are to follow the body of the text and should be marked with self-explanatory captions and be identified with the author's name. All *data* should be expressed in units conforming to the Système International (SI). *Drawings* are to be on heavy bond paper and marked clearly in black. *Photographs* should be supplied as glossy positive prints. Please note that we use two different systems for citing references. 1. For *Review Articles*, references should be arranged alphabetically and be numbered. Within the text, literature should be referred to by number and, where indicated, by author. The references should contain full journal article titles and the first as well as the last page of the article cited. 2. For *Short Communications* and *Mini-reviews*, an abbreviated bibliography is requested and references should be listed chronologically. Please consult a current issue of Experientia or inquire at the editorial office for details on form.

Authors are requested to specify into which discipline their papers fall:

1. Anatomy, Physiology
2. Biochemistry and Biophysics
   Metabolism
   Neurobiology
   Pharmacology
3. Endocrinology
4. Cellular Biology
   Molecular Biology
   Immunology
5. Genetics, Developmental Biology
6. Ethology, Ecology
   Natural Product Chemistry

All incoming manuscripts are acknowledged immediately. Authors will be notified of the editorial board's publishing decision once their manuscripts have been evaluated by a minimum of two field experts. Fifty reprints of papers accepted for publication will be sent to authors free of charge; additional reprints may be ordered.
Manuscripts and all communications to the editors should be addressed to:

Experientia
Birkhäuser Verlag
P.O. Box 133
CH-4010 Basel/Switzerland
Tel. 061 73 53 00