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SHORT COMMUNICATIONS

THE ENDOGENOUS HEAT-STABLE GLUCOCORTICOID RECEPTOR STABILIZING FACTOR AND THE *H-2* LOCUS

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SUMMARY

It has recently been suggested that the level of the endogenous glucocorticoid receptor stabilizing factor in mouse liver is regulated by the major histocompatibility, *H-2*, complex (Katsumata *et al.* [1]). We have developed an asssay for the activity of the endogenous heat-stable factor in mouse liver and have assayed this factor in liver cytosols prepared from two pairs of two *H-2* congenic mouse strains. Our results show that the amount of the endogenous factor is the same in all four mouse strains and that it is not regulated by the *H-2* locus.

Katsumata *et al.*[1] recently reported differences in dexamethasone binding in liver cytosols prepared from mice of four congenic and recombinant strains, C57BL/10, B10.A, B10.A(2R) and B10.A(5R). These strains have almost identical genetic backgrounds and differ only in the *H-2* complex and closely linked loci. Katsumata *et al.*[1] found that plots of dexamethasone binding versus concentration of liver cytosol were linear for C57BL/10 and B10.A(5R) mice whereas similar binding plots for strains B10.A and B10.A(2R) were not. The authors suggested that the differences in binding between the strains could be due to differences in the levels of an endogenous modifier that was determined by the *H-2* complex. The small molecular weight, heat-stable transformation inhibitor reported by Cake *et al.*[2, 3] and Bailly *et al.*[4] and the heat-stable factor reported by Sando *et al.*[5–7] were suggested as possible candidates for the endogenous modifier.

We [8] have recently examined in detail the characteristics of the endogenous heat-stable factor prepared from rat liver, which both stabilizes the binding capacity of the unbound glucocorticoid receptor and inhibits transformation of the bound receptor to the DNA-binding form. The receptor stabilizing and transformation inhibiting activities coelute on both Sephadex G-10 and Dowex 1 chromatography, suggesting that the same endogenous factor is involved in both processes. This factor appears to be the same as the transformation inhibitor reported by Cake *et al.*[2, 3] and Bailly *et al.*[4]. We have developed an assay system to quantitate the receptor stabilizing and the transformation inhibiting activities of the endogenous heat-stable factor and in this study we use this system to test the proposal of Katsumata *et al.*[1] that the level of the endogenous modifier is controlled by the *H-2* complex.

Livers from 10–12 week old C57BL/10J (B background, $H\text{-}2^b$), B10.A (B background, $H\text{-}2^a$), A/J (A background, $H\text{-}2^a$) and A.BY (A background, $H\text{-}2^b$) mice were homogenized in 1.5 vol. (w/v) of 10 mM Hepes buffer, pH 7.35 and centrifuged at 27,000 *g* for 20 min. The supernatant fractions were heated at 100°C for 15 min and centrifuged at 27,000 *g* for 10 min to remove denatured protein. Various amounts of each heated mouse liver preparation were added to filtered rat liver cytosol as previously described [8] and, after incubation at 20°C for 45 min, the specific binding capacity was measured in 0.1 ml aliquots by charcoal assay. To measure the transformation inhibiting activity of the heated mouse liver preparations, various amounts of each preparation were added to steroid-bound filtered rat liver cytosol and the binding to DNA-cellulose was measured after incubation at 15°C for 60 min as we have previously described [8].

The receptor stabilizing activity in the heated preparations from the four mouse strains is shown in Fig. 1A. All the heat-stable preparations contain stabilizing activity and there is no difference in the concentration dependency curves for the four mouse strains. As shown in Part B of the Figure, the transformation inhibiting activity in the four mouse strains is similar and is also concentration dependent. It has been demonstrated [4, 8] that the heat-stable factor in rat liver cytosol inhibits transformation but it does not affect the ability of already transformed glucocorticoid–receptor complexes to bind to nuclei or to DNA-cellulose. In our previous work [8] we expressed our results in units of inhibitory activity calculated from similar concentration dependency curves. Table 1 presents the units of inhibitory activity for the four mouse strain factors calculated from the data presented in Fig. 1. There is no significant difference in the activities of the mouse liver heat-stable, glucocorticoid receptor stabilizing factor.

Variations at the *H-2* locus have been associated with susceptibility to glucocorticoid-induced cleft palate [9, 10]. Goldman *et al.*[11] previously suggested that the level of glucocorticoid receptor is also regulated by the *H-2* complex. We subsequently showed [12], however, that specific glucocorticoid binding activity is not mediated by the *H-2* locus, and Francke and Gehring [13] have recently demonstrated that the structural gene for the glucocorticoid receptor is located on chromosome 18, not with the *H-2* locus on chromosome 17. Katsumata *et al.* [1] have further examined the relationship of glucocorticoid binding to the *H-2* complex and proposed that the level of an endogenous modifier may be regulated by the *H-2* haplotype. The results presented herein show that the difference in dexamethasone binding observed by Katsumata *et al.* [1] cannot be explained by different levels of the endogenous heat-stable factor and that the level of the endogenous factor is not regulated by the *H-2* complex.

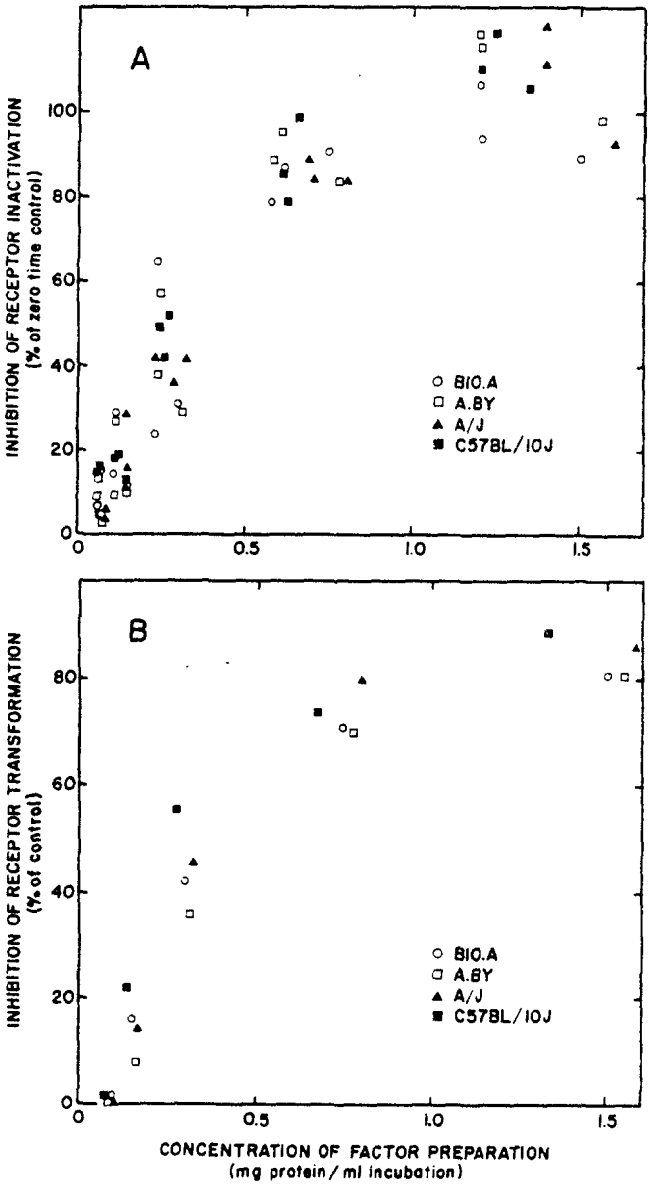

Fig. 1. Effect of heated mouse liver supernatants on glucocorticoid receptor inactivation and transformation. A, various amounts of heated mouse liver preparations were incubated with filtered rat liver cytosol at 20°C for 45 min and the specific binding capacity was assayed as previously described [8]. B, heated mouse liver supernatants were incubated with filtered rat liver cytosol, prebound with [^{3}H]-triamcinolone acetonide, at 15°C and the binding to DNA-cellulose was measured after 60 min as previously described [8]. Three different preparations for each strain are presented in part A and 1 preparation from each strain is presented in part B. ○, B10.A; □, A.BY; ▲, A/J; ■, C57BL/10J.

Table 1. Relationship of inhibitory units for mouse liver glucocorticoid receptor stabilising factor to *H-2* and genetic background

Background		*H-2* haplotype		
		H-2^a		*H-2*^{b}
A	A/J	5.9 ± 1.4 (5.6)	A.BY	5.5 ± 0.67 (4.9)
B	B10.A	5.8 ± 1.3 (6.2)	C57BL/10J	5.8 ± 0.45 (7.7)

One unit of inhibitory activity is defined as the amount of heat-stable factor preparation required to inhibit receptor inactivation or transformation by 50% in a 0.5 ml incubation containing 0.25 ml of filtered cytosol and 0.25 ml of factor preparation. The amount of factor preparation required to inhibit inactivation or transformation by 50% was calculated from the individual experiments shown in the scatter plots of Fig. 1. The numbers in the Table represent mean factor inhibitory units for receptor inactivation ± standard error for each of the four strains. Three animals were used to determine each mean. The factor inhibitory units for receptor transformation using one animal for each of the four strains is given in parentheses.

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REFERENCES

1. Katsumata M., Baker M. K. and Goldman A. J.: An H-2 linked difference in the binding of dexamethasone to murine hepatic cytosol receptor. *Biochim. biophys. Acta* **676** (1981) 245–256.
2. Cake M. H., Goidl J. A., Parchman L. G. and Litwack G.: Involvement of a low molecular weight component(s) in the mechanism of action of the glucocorticoid receptor. *Biochem. biophys. Res. Commun.* **71** (1976) 45–52.
3. Goidl J. A., Cake M. H., Dolan K. P., Parchman L. G. and Litwack G.: Activation of the rat liver glucocorticoid–receptor complex. *Biochemistry* **16** (1977) 2125–2130.
4. Bailly A., Sallas N. and Milgrom E.: A low molecular weight inhibitor of steroid receptor activation. *J. biol. Chem.* **252** (1977) 858–863.
5. Sando J. J., Nielsen C. J. and Pratt W. B.: Reactivation of thymocyte glucocorticoid receptors in a cell-free system. *J. biol. Chem.* **252** (1977) 7579–7582.
6. Sando J. J., LaForest A. C. and Pratt W. B.: ATP-dependent activation of L cell glucocorticoid receptors to the steroid binding form. *J. biol. Chem.* **254** (1979) 4772–4778.
7. Sando J. J., Hammond N. D., Stratford C. A. and Pratt W. B.: Activation of thymocyte glucocorticoid receptors to the steroid binding form. *J. biol. Chem.* **254** (1979) 4779–4789.
8. Leach K. L., Grippo J. F., Housley P. R., Dahmer M. K., Salive M. E. and Pratt W. B.: Characteristics of an endogenous glucocorticoid receptor stabilizing factor. *J. biol. Chem.*, in press.
9. Bonner J. J. and Slavkin H. C.: Cleft palate susceptibility linked to histocompatibility-2 (*H-2*) in the mouse. *Immunogenetics* **2** (1975) 213–218.
10. Erickson R. P., Butley M. S. and Sing C. F.: *H-2* and non-*H-2* determined strain variation in palatal shelf and tongue adenosine 3′:5′ cyclic monophosphate. A possible role in the etiology of steroid-induced cleft palate. *J. Immunogenet.* **6** (1979) 253–262.
11. Goldman A. S., Katsumata M., Yaffe S. J. and Gasser D. L.: Palatal cytosol cortisol-binding protein associated with cleft palate susceptibility and H-2 genotype. *Nature* **265** (1977) 643–645.
12. Butley M. S., Erickson R. P. and Pratt W. B.: Hepatic glucocorticoid receptors and the H-2 locus. *Nature* **275** (1978) 136–138.
13. Francke U. and Gehring U.: Chromosome assignment of a murine glucocorticoid receptor gene (Gr 1-1) using intraspecies somatic cell hybrids. *Cell* **22** (1980) 657–664.