The effect of MSG and/or GTG treatment on selected parameters in male CFI mice

Parameter measured	Experimental group Control	MSG	GTG	MSG + GTG
Food consumption (g/week at 100–125 days of age)	41.5 ± 1.2 (6)	37.2 ± 1.2 (6)*	45.3 ± 1.0 (6)	43.4 ± 1.5 (6)
Body weight (g at 125 days of age)	35.5 ± 0.6 (21)	36.8 ± 1.1 (21)	46.8 ± 2.3 (7)	45.7 ± 2.4 (13)*
% Body lipid (% of wet wt)	10.7 ± 3.1 (6)	18.7 ± 2.7 (6)*	27.2 ± 1.3 (6)*	30.2 ± 2.8 (6)**
% Liver lipid (% of wet wt)	2.9 ± 0.9 (6)	8.6 ± 2.3 (6)*	3.2 ± 0.5 (6)	3.6 ± 0.1 (6)
Average total liver lipid (mg)	69 ± 8 (6)	189 ± 18 (6)*	87 ± 10 (6)	101 ± 6 (6)

Mean ± SEM (n). *Significantly different from controls ($p < 0.05$). **Significantly different from control and MSG groups ($p < 0.05$) but not from GTG group.

(10% w/v) at 4.0 mg/g b.wt. The remaining pups were injected with a comparable volume of saline. Animals were weaned at 25 days of age, separated by sex, and one half of the controls and one half of the MSG-treated animals were injected with GTG. The GTG was dissolved in saline (100 mg/ml) and s.c. administered at 1 mg/g of b.wt. Body weights were recorded each week. At 100 days of age 6 male animals were randomly selected from each group (controls, MSG, GTG and MSG + GTG) and placed in suspended wire-bottomed cages for a 3-week food consumption study. All animals were sacrificed at 125 days of age. 6 male animals were randomly selected from each group for total lipid analysis. Wet carcass weight was recorded, the liver removed and after drying to a constant weight the total amount of lipid in the body and liver was determined by extracting with petroleum ether. All data were subjected to statistical analysis including T-tests and ANOV.

Data for male animals are presented in the table. Food consumption data indicated a significant inhibition in the MSG treated animals with no difference between the other groups. Body weight was elevated in the GTG and MSG + GTG-treated groups, indicating that MSG-treated animals are capable of responding to GTG. The data for total body lipid in the control, MSG and GTG treated groups are similar to other published information[2,3]; however, the 30.2% value for the MSG + GTG-treated animals is not significantly different from GTG treatment alone. In the MSG-treated mice both percent and total liver lipid are elevated. These results indicate that 2 relatively separate metabolic systems leading to body lipid accumulation may be affected by the treatments. The MSG lesions favor liver lipid deposition while GTG-treatment results in greater depot lipid accumulation. In the combined MSG + GTG treatment group, the MSG does not block a subsequent response to GTG and the resulting changes appear to be the effect of the GTG rather than the MSG.

1 Debons, A.F., Krimsky, I., From, A., Siccari, E., Maayan, M.L., Fani, K., and Jimenez, F.A., J. Path. *129* (1979) 73.
2 Djazayery, A., and Miller, D.S., Proc. Nutr. Soc. *32* (1973) 30A.
3 Olney, J.W., Science *164* (1969) 719.

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Binding of levonorgestrel to monkey plasma proteins[1,2]

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Summary. ^{3}H-levonorgestrel, a protein progestational steroid, showed a high affinity saturable binding to monkey plasma. Competitive protein binding experiments suggested that the levonorgestrel binds to a protein which resembles sex hormone-binding globulin.

The initial step in the mechanism of steroid hormone action is the binding of the hormone to a specific receptor protein in the cytosol of the target cell[3]; binding to plasma proteins, however, also occurs. The binding of steroid hormones to plasma proteins is of great physiological significance because steroids are biologically inactive when associated with protein; they can be activated by dissociation to form the unbound hormone. In fact, steroid hormone binding to specific plasma proteins provides a pool of the steroid in equilibrium with unbound fraction and thus limits the availability of steroid hormone to the target tissue. Specific high affinity binding proteins in plasma have been identified for cortisol and sex steroids and the physiological effect of steroid protein interactions have been well established[4,5]. Levonorgestrel is a potent progestin widely used as a contraceptive agent in women. In the present study, we report the ^{3}H-levonorgestrel binding to plasma proteins of rhesus monkey *(Macaca mulatta).*

Material and methods. ^{3}H-levonorgestrel (D-15,16-(^{3}H) 13β ethyl-17β-hydroxy-18,19-dinor-17-pregn-4-en-20-yn-3-one; sp.act. 39 Ci/mmole) was a generous gift from Schering AG, Berlin. The unlabeled levonorgestrel was obtained from Wyeth Chemicals, USA. Other radioinert steroids were authentic standards provided by Prof. D.N. Kirk, Steroid Reference Collection, England. Plasma samples were collected from healthy adult female monkeys (b.wt

4-5 kg) of our primate colony. To remove endogenous steroids, the plasma was treated with dextran-coated charcoal suspension (0.025 g dextran and 0.25 g Norit (A) 100 ml Tris-HCl buffer pH 7.4) for 10 min and then centrifuged. The supernatant after dilution (1:1) with 50 mM Tris-HCl buffer containing 1 mM EDTA, 1 mM mercaptoethanol, and 10% glycerol, pH 7.4, was used for steroid binding assays. 100 µl of diluted plasma was incubated with ^{3}H-levonorgestrel (0-20 nM) for 2 h at 4 °C in the absence and presence of a 100-fold molar excess of unlabeled levonorgestrel. Following incubation, the protein bound and free steroids were separated by addition of 500 µl of dextran-coated charcoal suspension. The radioactivity was assessed as described earlier[6].

Results and discussion. The present study demonstrated the presence of high affinity binding protein for ^{3}H-levonorgestrel in monkey plasma (fig.). The binding is saturable with unlabeled levonorgestrel and the Scatchard analysis of the binding data suggested a dissociation constant (K_d-value) $3.2 \times 10\ M^{-9}$.

The specificity of levonorgestrel binding to plasma proteins was established by competition studies (table). Incubation in the presence of a 100-fold molar excess of various radioinert steroids revealed that dihydrotestosterone, testosterone and estradiol-17β were strong competitors, whereas cortisol and progesterone were weak competitors for levonorgestrel binding to plasma.

Further validation of the above finding was obtained by displacement studies (table) with tritiated dihydrotestosterone and ^{3}H-progesterone, which are known to bind sex hormone-binding globulin and cortisol-binding globulin, respectively. Binding studies with monkey plasma using ^{3}H-dihydrotestosterone as a tag, indicated that levonorgestrel, testosterone and estradiol-17β competed strongly for dihydrotestosterone-binding sites. In contrast, binding studies with tritiated progesterone as a tag showed that levonorgestrel was unable to suppress the progesterone-binding to plasma proteins.

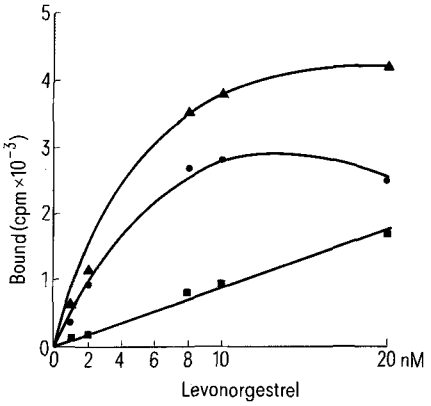
It is evident from the results reported here that levonorgestrel binds to the protein which shows affinity for dihydrotestosterone, testosterone and estradiol-17β. These steroids are known to have affinity for sex hormone-binding globulin (SHBG). This finding is in agreement with that of levonorgestrel binding to human plasma. Using polyacrylamide gel electrophoresis and dextran-coated charcoal method, the binding protein for levonorgestrel in human plasma has been demonstrated as sex hormone-binding globulin[7]. Similarly, Victor et al. have reported that sex hormone-binding globulin is the major carrier protein for levonorgestrel in plasma of women[8]. This observation has been further confirmed by Jenkins and Fotherby[9] who reported that levonorgestrel binds to sex hormone-binding globulin and albumin in human plasma.

The biological significance of steroid binding to plasma proteins lies in the protection of the steroid hormone from chemical and enzymatic attack, resulting in decreased metabolic clearance[10]. The contraceptive progestins such as norethynodrel[11], chlormadinone acetate[12] and levonorgestrel[13] in women have been reported to remain in the blood for longer time and this retention of progestational steroids in plasma might be due to their binding to plasma proteins. The results of the present study indicate that the levonorgestrel binding profile of monkey plasma is similar to that of human. Thus, in vitro steroid-protein interactions in monkey model may prove to be useful in research concerning human reproductive biology. Such studies may have possible application in evaluation of the repercussions of the administration of newly synthesized contraceptive agents on plasma steroid hormone binding profiles[14].

Competitive protein binding experiments with monkey plasma using labeled levonorgestrel, dihydrotestosterone, and progesterone

Competitors*	Percent inhibition of binding		
	^{3}H -DHT	^{3}H -levonorgestrel	^{3}H -progesterone
Levonorgestrel	82.0±2.2	86.3±3.1	5.0±2.0
Estradiol-17β	77.0±1.5	85.5±4.0	0
Testosterone	76.0±2.0	80.0±3.0	73.0±3.6
Dihydrotestosterone	78.0±3.2	86.0±3.0	72.5±2.6
Cortisol	27.0±4.0	26.5±2.5	90.0±2.4
Progesterone	18.0±3.0	25.5±3.1	84.0±4.0

Competition studies were performed with 4 nM of ^{3}H-steroids. *100-fold molar excess of radioinert steroids progesterone and dihydrotestosterone. Data represent triplicate analysis from 3 individual monkeys.



Binding of (^{3}H)levonorgestrel to monkey plasma. Diluted monkey plasma (1:1) was incubated with tritiated levonorgestrel (0-20 nM) in the absence and presence of 100-fold molar excess of unlabeled levonorgestrel. Protein bound and free steroid was separated by dextrancoated charcoal technique. (▲—▲) Total binding, (●—●) saturable binding and (■—■) non-saturable binding.

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3 King, R.J.B., in: Essays in biochemistry, pp. 12 and 41. Eds P.N. Campbell and R.L.A. Aldridge. Academic Press, New York 1976.
4 Westphal, U., Monograph in endocrinology, vol. 4. Springer, Berlin 1971.
5 King, R.J.B., and Mainwaring, W.I.P., Steroid-cell interactions. Butterworths, London 1974.
6 Srivastava, A.K., Stitch, S.R., and Habib, F.K., in: Endometrial cancer, p. 289. Eds M.G. Brush, R.J.B. King and R.W. Taylor. Baillier-Tindall, London 1978.
7 Srivastava, A.K., J. Steroid Biochem. *9* (1978) 1241.
8 Victor, A., Weiner, E., and Johansson, E.D.B., J. clin. Endocr. Metab. *43* (1976) 244.
9 Jenkins, N., and Fotherby, K., J. Steroid Biochem. *13* (1980) 521.
10 Westphal, U., Stroupe, S.D., and Cheng, S., Ann. N.Y. Acad. Sci. *286* (1977) 10.
11 Laumas, K.R., Murgesan, K., and Hingorani, V., Acta endocr., Copenh. *60* (1971) 355.
12 Dugwekar, U.G., Narula, R.K., and Laumas, K.R., Contraception *7* (1973) 27.
13 Uniyal, J.P., and Laumas, K.R., Biochim. biophys. Acta *427* (1976) 218.
14 Bonne, C., Mouren, M., Bouten, M.M., Philbert, D., and Raynaud, J.P., J. Steroid Biochem. *8* (1977) 16.

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