Acknowledgements—This work was supported by a grant from the NIH (DK 39168).

Department of Physiology and Biophysics
LSU Medical Center
Shreveport, LA 71130
U.S.A.

MATTHEW B. GRISHAM*

REFERENCES

1. van Hees PAM, Bakker HJ and van Tongeren JHM, Effect of sulfapyridine, 5-aminosalicylic acid, and placebo in patients with idiopathic procitis: A study to determine the active therapeutic moiety of sulfasalazine. *Gut* **21**: 632–635, 1980.
2. Hoult JRS and More PK, Effects of sulfasalazine and its metabolites on prostaglandin synthesis, inactivation and actions on smooth muscle. *Br J Pharmacol* **68**: 719–730, 1980.
3. Stenson WF and Lobos E, Sulfasalazine inhibits the synthesis of chemotactic lipids by neutrophils. *J Clin Invest* **69**: 494–497, 1982.
4. Allgayer H, Eisenberg J and Paumgartner G, Soybean lipoxygenase inhibition: Studies with the sulfasalazine metabolites *N*-acetyl-aminosalicylic acid, 5-aminosalicylic acid and sulfapyridine. *Eur J Clin Pharmacol* **26**: 449–451, 1984.
5. Carlin G, Djursater R, Smedegard G and Gerdin B, Effect of anti-inflammatory drugs on xanthine oxidase and xanthine oxidase induced depolymerization of hyaluronic acid. *Agents Actions* **16**: 377–384, 1985.
6. Ahnfelt-Ronne I and Nielsen OH, The antiinflammatory moiety of sulfasalazine, 5-aminosalicylic acid, is a free radical scavenger. *Agents Actions* **21**: 191–194, 1987.
7. Betts WH, Whitehouse MW, Cleland LG and Vernon-Roberts B, *In vitro* antioxidant properties of potential biotransformation products of salicylate, sulphasalazine and aminopyrine. *J Free Radic Biol Med* **1**: 273–280, 1985.
8. Aruoma OI, Wasil M, Halliwell B, Hoey BM and Butler J, The scavenging of oxidants by sulfasalazine and its metabolites. A possible contribution to the anti-inflammatory effects? *Biochem Pharmacol* **36**: 3739–3742, 1987.
9. Pryor WA, Oxy radicals and related species. Their formation, lifetimes and reactions. *Annu Rev Physiol* **48**: 657–667, 1986.
10. Gutteridge JMC, Ferrous salt promoted damage to deoxyribose and benzoate. The increase effectiveness of hydroxyl radical scavengers in the presence of EDTA. *Biochem J* **243**: 709–714, 1987.
11. Halliwell B and Gutteridge JMC, Oxygen free radicals and iron in relation to biology and medicine. Some problems and concepts. *Arch Biochem Biophys* **246**: 501–514, 1986.
12. Cohen G, The Fenton Reaction. In: *Handbook of Methods for Oxygen Radical Research* (Ed. Greenwald RA), pp. 55–64. CRC Press, Boca Raton, FL, 1985.
13. Heath RL and Tappel AL, A new sensitive method for the measurement of hydroperoxides. *Anal Biochem* **76**: 184–191, 1976.
14. Kvietys PR, Inauen W, Bacon BR and Grisham MB, Xanthine oxidase-induced injury to endothelium: Role of intracellular iron and hydroxyl radical. *Am J Physiol* **257**: H1640–H1646, 1989.
15. Rowley D, Gutteridge JMC, Blake D, Farr M and Halliwell B, Lipid peroxidation in rheumatoid arthritis: thiobarbituric acid-reactive material and catalytic iron salts in synovial fluid from rheumatoid patients. *Clin Sci* **66**: 691–695, 1984.
16. Halliwell B, Gutteridge JMC and Blake D, Metal ions and oxygen radical reactions in human inflammatory joint disease. *Philos Trans R Soc Lond [Biol]* **311**: 659–671, 1985.
17. Blake DR, Hall ND, Bacon PA, Dieppe PA, Halliwell B and Gutteridge JMC, Effects of a specific iron chelating agent on animal models of inflammation. *Ann Rheum Dis* **42**: 89–93, 1983.
18. Yoshino S, Blake DR and Bacon PA, The effects of desferrioxamine on antigen-induced inflammation in the rat air pouch. *J Pharm Pharmacol* **36**: 543–545, 1984.
19. Samuni A, Aronovitch J, Godinger D, Chevion M and Czapski G, On the cytotoxicity of vitamin C and metal ions. A site specific Fenton mechanism. *Eur J Biochem* **137**: 119–124, 1983.
20. Aruoma OI, Grootveld M and Halliwell B, The role of iron in ascorbate-dependent deoxyribose degradation. Evidence consistent with a site specific hydroxyl radical generation caused by iron ions bound to the deoxyribose molecule. *J Inorg Biochem* **29**: 289–299, 1987.
21. Aruoma OI and Halliwell B, The iron-binding and hydroxyl radical scavenging action of anti-inflammatory drugs. *Xenobiotica* **18**: 459–470, 1988.
22. Spiro G and Saltman P, Polynuclear complexes of iron and their biological implications. *Struct Bonding* **6**: 116–156, 1969.

* Address all correspondence to: Matthew B. Grisham, Ph.D., Department of Physiology and Biophysics, LSU Medical Center, 1501 Kings Highway, P.O. Box 33932, Shreveport, LA 71130-3932.

Biochemical Pharmacology, Vol. 39, No. 12, pp. 2063–2064, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

Differences in plasma carboxylesterase activity: relevance to anticholinesterase sensitivity

(*Received* 6 *October* 1989; *accepted* 19 *December* 1989)

Russell and Overstreet [1] have discussed recently the mechanisms underlying sensitivity to the organophosphorus anticholinesterase agents. In the section on noncritical binding proteins, they pointed out that serum butyrylcholinesterase may be involved in the sex but not the strain differences in anticholinesterase sensitivity [2–4]. Carboxylesterase has also been implicated as a protein to which anticholinesterases may bind, thereby reducing the amount available to bind acetylcholinesterase and produce physiological effects [5, 6]. The present study was a pre-

liminary investigation of the possible involvement of serum carboxylesterase activity in the sex and strain differences observed previously. It was hypothesized that female rats would have higher levels of carboxylesterase activity because they are resistant to organo-phosphates; conversely, the line of rat selectively bred to be more sensitive to anticholinesterases (Flinders Sensitive Line, FSL) was predicted to have lower carboxylesterase activity than the resistant line (Flinders Resistant Line, FRL).

Materials and Methods

Animals. Eight male and eight female rats of the 34th generation of the selectively bred FSL and FRL rats, which were derived originally from Sprague–Dawley albino rats, were used. They were approximately 60 days old at the time of blood collection; the males weighed approximately 300 g and the females 200 g.

Experimental protocols. Blood was withdrawn from the tail vein using a 19-gauge needle. Whole blood was transferred to microfuge tubes, which were centrifuged at 2000 *g* for 15 min. The serum was stored at 4° until assays were conducted 3–5 days later.

The assay of carboxylesterase activity was a titrimetric assay [5], using tributyrin as the substrate. The results are expressed as nanomoles tributyrin hydrolyzed per milliliter serum per minute.

Statistical analysis. Data were analyzed by a two-way analysis of variance (ANOVA), with sex and strain as the two main factors.

Results and Discussion

The males of the FSL and FRL strains had nearly identical carboxylesterase activities (Table 1); in contrast, the FRL females had higher levels than the FSL females. Statistical analysis failed to support the latter observation, as only the sex difference reached statistical significance: $F(1, 31) = 7.24$, $P < 0.001$. Neither the strain difference nor the sex by strain interaction effect was statistically significant ($P < 0.05$).

The above results for carboxylesterase activity are similar to data for butyrylcholinesterase [2–4]: female rats have higher levels than male rats, but there are no significant differences between the strains. Therefore, it is possible that binding to carboxylesterase could be a factor in the sex differences in anticholinesterase sensitivity, but it cannot be involved in the greater sensitivity of the FSL rats. As indicated in the recent review article [1], a greater number of acetylcholine muscarinic receptors is the most likely mechanism underlying the greater sensitivity of FSL rats to anticholinesterases and other cholinergic agonists.

The present finding of a sex difference in plasma carboxylesterase activity suggests that the earlier interpretation of the involvement of butyrylcholinesterase in anticholinesterase sensitivity [2, 4] may have been premature. We know that ovariectomy reduces serum butyrylcholinesterase activity, but we do not know whether this treatment also reduces carboxylesterase activity. Other work using selective inhibitors of the two enzymes concluded that butyrylcholinesterase is not very important in detoxifying anticholinesterases, whereas carboxylesterase is [7]. In any case, the present findings suggest that sex differences in serum carboxylesterase activity could well contribute to the sex differences in anticholinesterase sensitivity.

Table 1. Carboxylesterase activity in male and female Flinders sensitive line (FSL) and Flinders resistant line (FRL) rats

Strain	Serum carboxylesterase activity (nmole/mL/min)	
	Males	Females
FSL	674 ± 59	722 ± 40
FRL	671 ± 39	900 ± 64

Analysis of these data by two-way ANOVA revealed a significant ($P < 0.001$) sex difference but no significant line or interaction effect. Values are means ± SE, $N = 8$ in each group.

In summary, female rats of both the FSL and FRL strains had higher serum carboxylesterase activities than their male counterparts, but there were no significant differences between the strains. Thus, differences in basal plasma carboxylesterase activity may contribute to the previously reported sex differences in anticholinesterase sensitivity, but not the strain differences.

School of Biological Sciences
The Flinders University of South Australia
Bedford Park, S.A. 5042
Australia; and
†*Defense Research Establishment Suffield*
Biomedical Defense Section
Ralston, Alberta T0J 2N0
Canada

DAVID H. OVERSTREET*
JOHN G. CLEMENT†
ADAM BRUZZONE
JOHN KOVALISKI
GRANT D. SCHILLER

REFERENCES

1. Russell RW and Overstreet DH, Mechanisms underlying sensitivity to anticholinesterase agents administered acutely or chronically. *Prog Neurobiol* **28**: 97–129, 1987.
2. Overstreet DH, Russell RW, Runge P, Helps SC and Prescott AM, Sex differences following manipulation of the cholinergic system by DFP and pilocarpine. *Psychopharmacology* **61**: 49–58, 1979.
3. Overstreet DH, Russell RW, Helps SC and Messenger M, Selective breeding for sensitivity to the anticholinesterase, DFP. *Psychopharmacology* **65**: 15–21, 1979.
4. Overstreet DH, Russell RW, Kerni W and Netherton RA, The influence of ovariectomy on the sex-dependent effects of the anticholinesterase, DFP. *Psychopharmacology* **74**: 393–394, 1981.
5. Clement JG, Role of aliesterase in organophosphate poisoning. *Fundam Appl Toxicol* **4**: S96–S105, 1984.
6. Cohen SD, Carboxylesterase inhibition and potentiation of soman toxicity. *Arch Toxicol* **49**: 105–106, 1981.
7. Clement JG, Importance of aliesterase as a detoxification mechanism for soman (pinacolyl methylphosphonofluoridate) in mice. *Biochem Pharmacol* **33**: 3807–3811, 1984.

* Corresponding author.