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A CENTRALLY-MEDIATED EFFECT OF MORPHINE TO DIMINISH HEPATOCELLULAR GLUTATHIONE

STEPHEN M. ROBERTS,*†‡ NICHOLAS P. SKOULIS† and ROBERT C. JAMES†

†Division of Interdisciplinary Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR; and ‡Division of Pharmacology and Medicinal Chemistry, College of Pharmacy, University of Cincinnati Medical Centre, Cincinnati, OH, U.S.A.

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Abstract—Morphine administration has been associated with a decrease in hepatic glutathione (GSH) and an increase in the hepatotoxicity of compounds dependent upon GSH for detoxification. In this study, intraperitoneal administration of 100 mg/kg morphine in mice resulted in approximately a 25% decrease in hepatic GSH. The same magnitude of GSH depletion was also observed following intracerebroventricular (i.c.v.) injection of 100 μg of morphine, but no effect was observed when 100 μg of morphine was administered intravenously. Pretreating animals with either yohimbine (5 mg/kg, i.p.) or prazosin (5 mg/kg, i.p.) resulted in a partial blockade of i.c.v. morphine-induced change in hepatic glutathione concentrations. Adrenalectomy prior to i.c.v. morphine treatment completely prevented morphine-induced changes in hepatic GSH concentrations; however, the morphine response was restored in adrenalectomized mice supplemented with hydrocortisone (2.5 mg/kg). No effect on the ability of i.c.v. morphine to diminish GSH concentrations in the liver was observed following pretreatment with either propranolol (20 mg/kg, i.p.), atropine (1 mg/kg, i.p.), hexamethonium (15 mg/kg, s.c.), or destruction of peripheral adrenergic nerve terminals with 6-hydroxydopamine (30 mg/kg, i.p.). It is concluded that hepatocellular GSH concentrations may be diminished as a consequence of a central action of morphine. The response by liver GSH to this action does not appear to be mediated through adrenal medullary release of catecholamines or by autonomic stimulation of the liver. While corticosteroids are a necessary component of this response, their role is probably permissive. The ability of both prazosin and yohimbine to antagonize the effect of i.c.v. morphine on hepatic GSH, coupled with the apparent absence of a peripheral catecholaminergic mechanism, suggests that the adrenergic interaction with the i.c.v. morphine effect is also of central origin. Thus, the results of this study show that the central effects of morphine can result in a decrease in hepatic GSH, and that this effect is not mediated by a peripheral catecholaminergic mechanism.

Administration of morphine to mice has been shown to produce a rapid, dose-dependent decrease in hepatic glutathione (GSH) concentrations [1]. It is not clear to what extent this effect may contribute to the hepatotoxicity that has been attributed to certain opioid drugs [2, 3]. Even though GSH serves a variety of important biochemical functions, including the detoxification of reactive and toxic intermediates formed during oxidative metabolism [4], the GSH depression produced by opioids does not always correlate to the sometimes modest changes observed in those biochemical indices that have been used to monitor liver damage after the administration of even high doses of opioids [1]. An opioid-induced decrease in hepatic GSH, however, would be expected to increase the hepatotoxicity of compounds dependent upon GSH for detoxification. Consistent with this expectation, the hepatotoxicities of carbon tetrachloride, cocaine, and acetaminophen are potentiated by the coadministration of morphine [1, 5].

The effect of opioid drugs to diminish GSH in the liver may be direct and due, at least in part, to their conversion to a reactive, electrophilic metabolite which depletes GSH [6, 7]. However, the observations of Chang and Ho [8] and Needham *et al.* [3] suggest that the major portion of these opioid effects on the liver may originate in the central nervous system (CNS). They observed that intracerebroventricular (i.c.v.) injections of small doses of morphine or [D-Ala^2]-Met-enkephalinamide in mice produce abnormalities in liver function, as indicated by elevations in serum aminotransferase activities, that were of the same magnitude as those produced by much larger systemic doses. They also found that the serum aminotransferase activity elevations after morphine were blocked by the opioid receptor antagonist naloxone, and were antagonized by either adrenalectomy or hypophysectomy. These observations led us to determine if morphine-induced lowering of hepatic GSH is also centrally-mediated. If so, it was our further objective to determine whether adrenal epinephrine release might be the mechanism by which this action of morphine on the CNS produces an effect on GSH in the liver.

MATERIALS AND METHODS

Morphine sulfate was obtained from Merck & Company (Rahway, NJ). Yohimbine hydrochloride, 5,5′-dithiobis(2-nitrobenzoic acid), 6-hydroxydopamine hydrobromide, and DL-propranolol were purchased from the Sigma Chemical Co. (St. Louis,

* Address all correspondence to: Dr. Stephen M. Roberts, Division of Interdisciplinary Toxicology, University of Arkansas for Medical Sciences, 4301 West Markham, Little Rock, AR 72205.

MO). Atropine sulfate was purchased from EM Science (Gibbstown, NJ); ascorbic acid, USP from the J. T. Baker Chemical Co. (Phillipsburg, NJ); and hexamethonium chloride from ICN (Cleveland, OH). Prazosin hydrochloride was a gift from Pfizer, Inc. (New York, NY). Hydrocortisone, USP (free alcohol), was purchased from the Upjohn Co. (Kalamazoo, MI).

Male ICR Swiss mice, 20–25 g, were obtained from Harlan Industries (Indianapolis, IN). Animals were housed with a 12-hr light/dark cycle on sawdust bedding with free access to food and water for at least 1 week prior to experimentation. Atropine, prazosin, propranolol, yohimbine, hexamethonium, and morphine were dissolved in saline; hydrocortisone was dissolved in corn oil; and 6-hydroxydopamine was dissolved in ice-cold 10% ascorbic acid in saline for administration. Intraperitoneal (i.p.) and intravenous (i.v.) doses were administered in a volume of 0.1 ml vehicle/10 g body weight, whereas i.c.v. doses were administered in a total volume of 5 μl of saline. Atropine, prazosin, propranolol, hexamethonium, and yohimbine were each given i.p. 15 min before treatment with morphine at doses demonstrated in the literature to produce receptor blockade (e.g. atropine [9], prazosin [10], propranolol [11], hexamethonium [12], and yohimbine [13]).

Destruction of peripheral noradrenergic nerve terminals was achieved by administering 6-hydroxydopamine, 30 mg/kg, i.p., 48 hr before morphine treatment [14]. The effectiveness of this regimen was confirmed by measurements of heart tissue catecholamine concentrations which revealed a 95% depletion of norepinephrine (data not shown).

The method of i.c.v. injection was similar to that of Lipman and Spencer [15], except that the animals were lightly anesthetized with ether prior to injection, and a small midline incision was made in the scalp. This incision facilitated visual location of cranial sutures and enhanced the uniformity of depth of penetration of the i.c.v. needle. Following i.c.v. injection, this incision was closed with a single ligature.

Total adrenalectomy or sham surgery was performed 48 hr prior to treatment with morphine in some experiments. Adrenalectomized mice were given normal saline as drinking water until the time of the experiment to minimize the potential for hyponatremia. Hydrocortisone-maintained adrenalectomized animals received doses of 2.5 mg/kg hydrocortisone just after surgery, 24 hr later, and 2 hr before the experiment.

James *et al.* [1] have shown that hepatic GSH is maximally diminished within 3 hr after i.p. administration of morphine. In the present study, animals were killed and the livers were removed 3 hr after treatment with morphine, and GSH was determined as previously described [1]. GSH concentrations are expressed as a percent of concurrently measured, saline-treated controls. All experiments were conducted at the same time of day to minimize the influence of diurnal variations in hepatic GSH.

Data were analyzed using a one-way analysis of variance (ANOVA). Differences among individual treatment groups were detected using a Student Newman–Keuls *a posteriori* test. The level of statistical significance was set at $P < 0.05$.

RESULTS

Intracerebroventricular injection of 100 μg of morphine produced a GSH depression equivalent to a systemic (intraperitoneal) dose of 100 mg/kg (Table 1). Distribution of morphine to functional sites outside the CNS after i.c.v. administration, e.g. to the liver, cannot account for this effect as i.v. administration of the same dose (100 μg) produced no effect on hepatic GSH. This result, therefore, indicates a central mechanism for the effect of morphine on hepatic GSH. The dose–response relationship for this effect was steep, and doses below 40 μg did not produce significant depression whereas doses above 100 μg produced little additional depression (Fig. 1).

Our hypothesized role for epinephrine as a potential mediator of i.c.v. morphine-induced effects was initially tested by pretreating mice with adrenoreceptor blocking drugs prior to administration of i.c.v. morphine. Yohimbine, a relatively selective α_2-adrenoreceptor antagonist, and prazosin, a relatively selective α_1-adrenoreceptor antagonist, both partially antagonized the effect of i.c.v. morphine on hepatic GSH to a similar extent (Table 2). Propranolol, a relatively selective β-adrenoreceptor antagonist, had no effect on the decrease in hepatic GSH by i.c.v. morphine. Thus, the attentuation of the

Table 1. Effect of morphine on hepatic GSH

	Hepatic GSH 3 hr after treatment (% of control)	
Morphine administration	Saline	Morphine
Intraperitoneal, 100 mg/kg	100 ± 7 (7)	75 ± 6* (7)
Intracerebroventricular, 100 μg	100 ± 8 (6)	73 ± 7* (6)
Intravenous, 100 μg	100 ± 7 (7)	101 ± 7 (7)

GSH concentrations are expressed as a percent of the mean GSH concentration of concurrently measured saline-treated controls (2.80, 2.73, and 2.79 mg GSH/g liver for saline-treated mice in the intraperitoneal-, intracerebroventricular-, and intravenous-route experiments respectively). All values are expressed as mean ± SD. The numbers of animals are in parentheses.

* Significantly different from saline-treated control, $P < 0.05$.

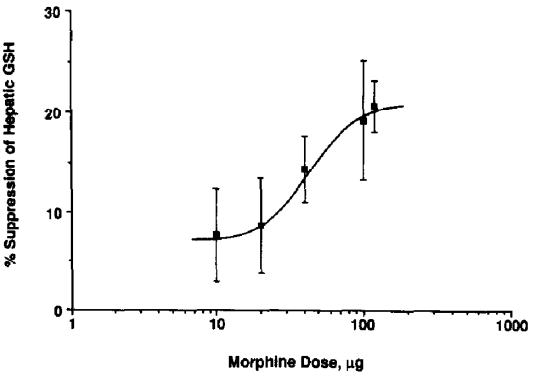

Fig. 1. Dose–response relationship for intracerebroventricularly-administered morphine on hepatic glutathione (GSH) concentration. Hepatic GSH concentrations were measured 3 hr after morphine administration. The percent suppression of hepatic GSH is as compared to mice administered an equivalent volume of saline by the same route. Results are mean ± SD, N = 6. Mean hepatic GSH concentration for saline-treated mice was 2.10 mg GSH/g liver. Percent suppression was statistically significant ($P < 0.05$) at the 40, 100, and 120 μg doses.

effect of morphine on GSH appeared to involve α-adrenoreceptors, with potential roles for both α_1- and α_2-receptors.

The α-adrenoreceptor antagonism of i.c.v. morphine on hepatic GSH need not necessarily involve epinephrine and may have occurred either centrally or peripherally. To explore further whether the i.c.v. morphine effect was mediated through adrenal epinephrine release, the influence of adrenalectomy on hepatic GSH response to morphine was examined. Adrenalectomy abolished the effect of either systemically-administered (i.p.) or centrally-administered (i.c.v.) morphine on hepatic GSH (Table 3). However, when adrenalectomized mice were maintained on hydrocortisone, morphine administered either i.p. or i.c.v. was capable of producing a diminution of hepatic GSH of the same magnitude as that induced in sham-operated controls. These results indicate that it is the adrenal cortex rather than the medulla which must be present for morphine to exert an effect on hepatic GSH, and that this effect cannot be mediated via adrenal medullary release of epinephrine.

An alternative mechanism by which catecholamines might serve as intermediaries between a central action of morphine and a hepatic GSH response is through direct sympathetic innervation. Destruction of peripheral sympathetic nerve terminals with 6-hydroxydopamine, however, failed to antagonize the ability of i.c.v. morphine to lower hepatic GSH concentrations (Table 4). Blocking tissue receptors with atropine also did not antagonize the response to i.c.v. morphine, indicating that parasympathetic stimulation is not involved. The absence of a role for autonomic stimulation was confirmed by the observation that ganglionic blockade with hexamethonium pretreatment did not diminish the effect of morphine on hepatic GSH.

DISCUSSION

The results of these experiments clearly demonstrate that morphine can lower hepatic GSH by a centrally-mediated mechanism, and that the extent of the central effect is comparable to that induced by systemic administration. These observations parallel those of Chang and Ho [8] and Needham *et al.* [3] who found that administration of 100 μg of morphine i.c.v. produces the same liver injury as a 50 mg/kg dose administered by the i.p. route. The demonstration of a central site of action for the effect of morphine on hepatic GSH does not necessarily preclude GSH depletion by other potential mechanisms. For example, Correia *et al.* [6, 7] have proposed that decreases in GSH can occur via the formation of a reactive metabolite of morphine that is detoxified by GSH. It appears from the present studies, however, that the magnitude of GSH depression elicited by the central mechanism is adequate to account for most, if not all, of the loss of GSH observed with large systemic doses of morphine.

Morphine administration is associated with adrenal medullary release of epinephrine [16]. We have shown previously that epinephrine administered sub-

Table 2. Effects of adrenergic receptor antagonists on loss of hepatic glutathione by intracerebroventricular morphine

		Hepatic GSH 3 hr after treatment (% of control)	
	Pretreatment	Saline i.c.v.	Morphine i.c.v.
(1)	Yohimbine	100 ± 10	84 ± 15*
	Saline	100 ± 7	63 ± 12
(2)	Prazosin	93 ± 7	81 ± 13*
	Saline	100 ± 7	63 ± 12
(3)	Propranolol	98 ± 9	65 ± 11
	Saline	100 ± 7	63 ± 12

All values are mean ± SD, N = 10. The morphine dose was 100 μg i.c.v. Yohimbine (5 mg/kg, i.p.), prazosin (5 mg/kg, i.p.), and propranolol (20 mg/kg, i.p.) were administered in saline 15 min before treatment with either i.c.v. morphine or saline. GSH concentrations are expressed as percent of the mean GSH concentration in saline-pretreated/saline-treated controls (2.52 mg GSH/g liver). The results from morphine i.c.v. treatment were significantly ($P < 0.05$) different from saline i.c.v. treatment in all cases.

* GSH depression after drug pretreatment was significantly different as compared to saline pretreatment, $P < 0.05$.

Table 3. Effect of adrenalectomy on loss of hepatic GSH by intraperitoneal or intracerebroventricular morphine

Treatment	Hepatic GSH 3 hr after treatment (% of control)	
	Intraperitoneal administration*	Intracerebroventricular administration†
Sham surgery		
Saline	100 ± 7 (5)	100 ± 11 (9)
Morphine	76 ± 14‡ (5)	76 ± 10‡ (9)
Adrenalectomy		
Saline	100 ± 18 (5)	100 ± 15 (5)
Morphine	95 ± 18 (5)	102 ± 12 (5)
Adrenalectomy, HC-maintained		
Saline	100 ± 11 (5)	100 ± 11 (8)
Morphine	75 ± 15‡ (5)	78 ± 10‡ (8)

Total adrenalectomy or sham surgery was performed 48 hr prior to the experiment. Morphine doses were 100 mg/kg, i.p., or 100 μg, i.c.v. Hydrocortisone (HC), 2.5 mg/kg/day, was administered i.p. in corn oil. GSH concentrations are expressed as a percent of the mean GSH concentration for saline-treated controls (see below). All values are mean ± SD. The numbers of animals are in parentheses.

* Mean GSH concentrations for saline-treated controls were 2.36, 2.32, and 2.39 mg GSH/g liver for sham-treated, adrenalectomy, and adrenalectomy/HC-maintained experiments respectively.

† Mean GSH concentrations for saline-treated controls were 2.68, 2.57, and 2.79 mg GSH/g liver for sham-treated, adrenalectomy, and adrenalectomy/HC-maintained experiments respectively.

‡ Significantly different from saline-treated controls, $P < 0.05$.

cutaneously may substantially diminish hepatic GSH concentrations *in vivo*, and that the maximal decrease induced by epinephrine is equivalent to the maximal depression caused by i.p. morphine [1, 17]. This, and the partial antagonism of morphine's effect on hepatic GSH by phentolamine, has led us to postulate earlier that epinephrine may be a mediator for at least part of the narcotic-induced depression of liver GSH levels [17]. While the present study has shown that adrenalectomy abolished the effect of morphine on hepatic GSH, the finding that hydrocortisone maintenance restored this effect indicates that the CNS effects of morphine on liver GSH are not mediated by adrenal medullary release of epinephrine as originally hypothesized. The data from these experiments also indicate that the effect was not mediated by corticosteroid release from the cortex. The saline-treated controls for the adrenalectomized mice received the same dose of hydrocortisone, and presumably had the same blood hydrocortisone concentrations, as the morphine-treated mice. Since the saline-treated controls showed no loss of hepatic GSH, it is clear that the hydrocortisone dose itself does not diminish hepatic GSH. Therefore, it appears that the corticosteroids play a necesssary role in this phenomenon by permitting the morphine-induced effect on hepatic GSH. A similar permissive role for corticosteroids

Table 4. Effects of autonomic antagonists on loss of hepatic glutathione by intracerebroventricular morphine

	Pretreatment	Hepatic GSH 3 hr after treatment (% of control)	
		Saline i.c.v.	Morphine i.c.v.
(1)	Hexamethonium	98 ± 11	74 ± 15
	Saline	100 ± 8	75 ± 15
(2)	6-Hydroxydopamine	108 ± 11	82 ± 7
	Ascorbic acid/saline	100 ± 10	82 ± 4
(3)	Atropine	100 ± 12	71 ± 16
	Saline	100 ± 8	75 ± 15

All values are mean ± SD, N = 10. Morphine dose was 100 μg i.c.v. Hexamethonium (15 mg/kg, s.c.) and atropine (1 mg/kg, i.p.) were administered in saline 15 min before treatment with either i.c.v. morphine or saline. 6-Hydroxydopamine (30 mg/kg, i.p.) was administered in cold ascorbic acid/saline 48 hr before treatment. GSH concentrations are expressed as percent of the mean GSH concentration in saline-pretreated/saline-treated controls (2.64 mg GSH/g liver for hexamethonium and atropine experiments; 2.41 mg GSH/g liver for 6-hydroxydopamine experiment). There were no significant differences ($P < 0.05$) between drug-pretreated and saline-pretreated groups.

has been observed for stress-induced decreases in hepatic GSH [18].

While stress- and morphine-induced hepatic GSH depletion share a requirement for corticosteroids, they apparently differ in the involvement of sympathetic innervation of the liver. Beck and Rieck [18] found that pretreatment with the ganglionic blocker chlorisondamine dimethochloride prevents stress-induced alterations in hepatic GSH, and concluded from this and their adrenalectomy data that the effects on GSH are due to direct sympathetic stimulation of the liver. In contrast, we found that neither ganglionic blockade with hexamethonium nor destruction of adrenergic nerve terminals with 6-hydroxydopamine had any effect on morphine-induced loss of hepatic GSH. Parasympathetic stimulation can also be ruled out from the hexamethonium and atropine experiments. The apparent absence of participation by the autonomic nervous system makes it unlikely that the CNS to liver pathway for morphine's effect is neuronal.

Adrenergic sites may be important in the hepatic response to morphine, despite the evidence against peripheral catecholamine involvement. Yohimbine, a relatively selective α_2-adrenoreceptor blocking drug, partially blocked the effects of morphine on hepatic GSH. This observation was not unexpected as yohimbine has been observed to antagonize some central opioid effects [19]. Suprisingly, prazosin, a relatively selective α_1-adrenoreceptor blocking drug, also partially blocked the effects of i.c.v. morphine. Previous studies have demonstrated that decreases in GSH in response to systemically-administered epinephrine are mediated selectively through α_2-receptors [17]. Thus, the present yohimbine and prazosin data suggest that it is centrally-located α-adrenoreceptors that have some role in the hepatic response to i.c.v. morphine.

The difficulty in finding a neuronal pathway between the CNS and the liver for the opioid effect on GSH suggests that this effect may be hormonally-mediated. While administration of morphine or opioid peptides can alter serum levels of several important hormonal substances (e.g. growth hormone, prolactin, luteinizing hormone, follicle-stimulating hormone, and thyrotropin [20]), the effects of these hormones on liver GSH concentrations are poorly understood. Currently, the best evidence for a hormonal mediator of centrally-initiated opioid-induced depression of hepatic GSH exists for vasopressin. Vasopressin is released in response to beta-endorphin in the rat [21] and has been observed to stimulate the efflux of GSH from the isolated perfused rat liver [22]. Further study will be required to define the role of vasopressin and other hormones in opioid-induced alterations of hepatic GSH concentrations.

While the approximate 25% decrease in hepatic GSH concentration induced by morphine in this study appears modest, it is of sufficient magnitude to influence significantly the hepatotoxicity of drugs and chemicals dependent upon GSH for detoxification. A decrease in hepatic GSH of similar magnitude has been shown to substantially increase hepatic injury from acetaminophen [23], and James *et al.* [1] have found that a decrease in hepatic GSH by morphine (100 mg/kg) potentiates the hepatotoxicity of cocaine in mice. That endogenous opioids and hormones may influence hepatic GSH concentrations is a concept that has received little study to date. From these experiments and previous reports [1, 17] it is clear that the potential exists to create significant alterations in hepatic GSH through endogenous physiologic mechanisms with which a variety of drugs, chemicals, and disease processes can interact. Through these mechanisms, substances or events which are not in themselves associated with liver damage may nonetheless greatly increase the vulnerability of the liver to injury from those chemicals detoxified by GSH. Such interactions may complicate the prediction of outcome of multiple drug therapy and contribute to interindividual variations in measured toxicity or predicted risk associated with certain drugs and chemicals.

REFERENCES

1. R. C. James, D. R. Goodman and R. D. Harbison, *J. Pharmac. exp. Ther.* **221**, 708 (1982).
2. A. Thureson-Klein, Y. J. Wang and I. K. Ho, *Experientia* **34**, 773 (1978).
3. W. P. Needham, L. Shuster, G. C. Kamel and M. L. Thompson, *Toxic. appl. Pharmac.* **58**, 157 (1981).
4. A. Meister, *Science* **220**, 472 (1983).
5. J. Fontenot, Y-Y. H. Chang, H. M. Mehendale and I. K. Ho, *Pharmacologist* **20**, 270 (1978).
6. M. A. Correia, J. S. Wong and E. Soliven, *Chem. Biol. Interact.* **49**, 255 (1984).
7. M. A. Correia, G. Krowech, P. Caldera-Munoz, S. L. Yee, K. Straub and N. Castagnoli, *Chem. Biol. Interact.* **51**, 13 (1984).
8. Y-Y. H. Chang and I. K. Ho, *Biochem. Pharmac.* **28**, 1373 (1979).
9. N. Sawyer and R. Mundy, *Pharmacologist* **12**, 246 (1970).
10. N. Decker, J. D. Ehrhardt, G. Leclerc and J. Schwartz, *Naunyn-Schmiedeberg's Archs Pharmac.* **326**, 1 (1984).
11. S. L. Agarwal and D. Bose, *Br. J. Pharmac. Chemother.* **30**, 349 (1967).
12. D. R. Laurence and R. S. Stacey, *Br. J. Pharmac. Chemother.* **7**, 80 (1952).
13. D. R. Laurence and R. S. Stacey, *Br. J. Pharmac. Chemother.* **8**, 62 (1953).
14. R. M. Kostrezewa and D. M. Jacobowitz, *Pharmac. Rev.* **26**, 199 (1974).
15. J. J. Lipman and P. S. J. Spencer, *J. pharmac. Meth.* **4**, 327 (1980).
16. M. Vassalle, *Am. J. Physiol.* **200**, 530 (1961).
17. R. C. James, S. M. Roberts and R. D. Harbison, *Fund. appl. Toxic.* **3**, 303 (1983).
18. L. V. Beck and V. D. Rieck, *Proc. Soc. exp. Biol. Med.* **98**, 466 (1958).
19. M. R. Goldberg and R. Robertson, *Pharmac. Rev.* **35**, 143 (1983).
20. J. Meites, J. F. Bruni, D. A. Van Vugt and A. F. Smith, *Life Sci.* **24**, 1325 (1979).
21. R. E. Weitzman, D. A. Fisher, S. Minick, N. Ling and R. Guillemin, *Endocrinology* **101**, 1643 (1977).
22. H. Sies and P. Graf, *Biochem. J.* **226**, 545 (1985).
23. P. G. Wells, R. C. Boerth, J. A. Oates and R. D. Harbison, *Toxic. appl. Pharmac.* **54**, 197 (1980).