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Hepatic thiol and glutathione efflux under the influence of vasopressin, phenylephrine and adrenaline*

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Thiol and glutathione (GSH) efflux across the sinusoidal plasma membrane in isolated perfused rat liver was stimulated by additon of hormones such as vasopressin, phenylephrine and adrenaline, whereas glucagon or dibutyryl cyclic AMP were without effect. Phenylephrine and adrenaline effects were sensitive to prazosin and phentolamine, respectively. The increase in thiol efflux was largely accounted for by an increase in GSH efflux. Thiol efflux and the hormone effects were abolished in GSH-depleted liver. Biliary GSH efflux was diminished upon hormone addition. The newly discovered hormone-dependence of GSH release across the sinusoidal plasma membrane may explain the known loss of GSH during conditions of experimental shock (traumatic or endotoxin) and stress and peripheral inflammation.

Hepatic efflux of GSH, initially described by Bartoli & Sies (1978), substantially accounts for the turnover of the tripeptide in this organ [for reviews, see Sies (1983) and Meister & Anderson (1983)]. A system of GSH transport across hepatocyte sinusoidal plasma-membrane vesicles has been described (Inoue *et al.*, 1984). Factors that govern GSH release under physiological conditions are not well known. The present work was carried out to evaluate possible hormonal effects on release of GSH and, more globally, thiol from the isolated perfused rat liver.

Materials and methods

Haemoglobin-free rat liver perfusion

Livers of male Wistar rats (200–250g body wt.), fed on stock diet (Altromin, Lage, Germany), were perfused as described previously (Sies, 1978) without recirculation (open system) at 37°C. Additions were made by micropumps directly before the portal vein. For biliary glutathione measurements, bile was collected from a cannula into cups containing metaphosphoric acid, as described by Eberle *et al.* (1981) and Akerboom *et al.* (1982). For pretreatment of rats with phorone (Younes & Siegers, 1981), 250mg/kg body wt. was injected intraperitoneally 2h before the experiment.

Assays

Oxygen concentration, pH and Ca^{2+} in the effluent perfusate were monitored continuously with Clark-type, pH-glass and Ca^{2+}-sensitive electrodes (Sies *et al.*, 1981), respectively. Thiol in effluent perfusate was detected continuously with the 5,5′-dithiobis-(2-nitrobenzoic acid) method (Graf & Sies, 1984); GSH was measured by the 1-chloro-2,4-dinitrobenzene/GSH transferase test (Brigelius *et al.*, 1983), and GSSG was assayed as described by Akerboom *et al.* (1982).

Materials

[Lysine]Vasopressin (grade I-S, oxytocin-free), phenylephrine, adrenaline, taurocholic acid, GSH transferase and 5,5′-dithiobis-(2-nitrobenzoic acid) were obtained from Sigma (Munich, Germany). Phorone (2,6-dimethylhepta-2,5-dien-4-one) was from Fluka (Neu-Ulm, Germany), GSSG reductase and biochemicals were from Boehringer (Mannheim, Germany) and other chemicals were from Merck (Darmstadt, Germany). L-Buthionine sulphoximine was given by Professor A. Meister.

Abbreviations used: GSH, reduced glutathione; GSSG, oxidized glutathione (glutathione disulphide).

* Dedicated to Professor Dr. Karl Decker, Freiburg, on the occasion of his 60th birthday.

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Results and discussion

Thiol release upon hormone addition

The isolated perfused rat liver releases thiols and GSH at a concentration of around 5 μM at a perfusion rate of 4 ml/min per g of liver, corresponding to a release of 23 nmol/min per g wet wt. (Table 1, Fig. 1*a*). This represents the release across the sinusoidal membrane, since the perfusate leaving the liver via the caval vein is analysed. Thus biliary efflux is to be added (see below). The addition of vasopressin (12–14 nM) leads to a rapid increase in thiol release (Fig. 1*a*, Table 1), reaching a rate of 33 nmol/min per g wet wt. Phenylephrine

Table 1. *Thiol release from perfused rat liver under hormonal control*

Thiol release was measured by the 5,5'-dithiobis-(2-nitrobenzoic acid) reaction in a flow-through cuvette attached to the effluent caval cannula (see the Materials and methods section). Concentrations are converted into rates (nmol/min per g liver wet wt.) by multiplication by the perfusate flow rate (ml/min per g wet wt.) in the individual experiment. Data are given as means ± S.E.M. for the numbers of different perfusion experiments in parentheses.

Addition	Thiol release (μM)	Thiol release (nmol/min per g liver wet wt.)
None (12)	4.9 ± 0.2	23 ± 1
Vasopressin, 12 nM (6)	7.1 ± 0.2	33 ± 1
Phenylephrine, 1.6 μM (3)	7.3 ± 0.4	38 ± 1
plus prazosin, 3 μM (2)	4.1	22
Glucagon, 11 nM (3)	6.0 ± 0.4	27 ± 2

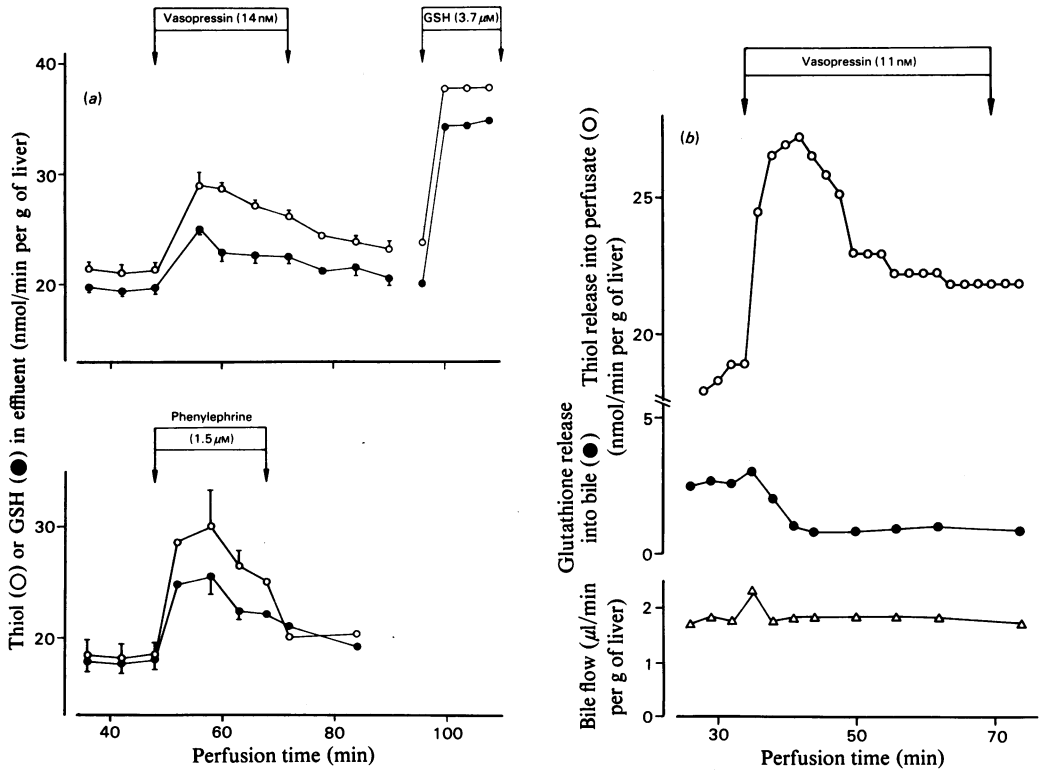

Fig. 1. *Thiol and GSH release from perfused rat liver*

(*a*) Stimulation of thiol (○) and GSH (●) release by vasopressin (top) or phenylephrine (bottom). On top right, recovery of GSH infused into the portal vein is also shown; 3.7 μM-GSH corresponds to a rate of 15 nmol/min per g wet wt. Results are means ± S.E.M. (n = 3). (*b*) Thiol efflux into perfusate (○), and glutathione (GSH plus GSSG) release into bile (●), as influenced by vasopressin. To maintain bile flow (△), taurocholate (10 μM) was present in the perfusion medium throughout the experiment.

does likewise, and this increase is abolished when prazosin is present as antagonist. Adrenaline also shows the effect, and this is phentolamine-suppressible. With adrenaline (28nM), thiol release showed more scatter than with vasopressin, and in some experiments there was a pronounced initial peak (Fig. 2) that was not found in others (results not shown). Thiol release remained at the lower plateau value for as long as the hormone was present in the perfusate, tested up to 30 min. (The scatter in the adrenaline response might be due to use of submaximal concentrations in the dose–response experiments).

Other hormones and compounds that had no significant effect on thiol release include glucagon (Table 1), dibutyryl 3′,5′-cyclic AMP (55μM), DL-β-hydroxybutyrate (5mM), L-lactate (5mM), L-buthionine sulphoximine (0.2mM), NaCN (1mM) and N_2 anoxia.

Relation to glutathione

When the liver GSH has been depleted to values less than 5% of the controls by pretreatment of the animals with phorone (see the Materials and methods section), thiol release is practically undetectable, and the vasopressin response in thiol release is abolished, but the glucose-release and O_2-uptake responses are still present (results not shown); this is presumably due to the lack of GSH that could be transported from the cells.

Regarding the nature of the thiol released, perfusate samples obtained from livers of untreated animals were assayed for GSH. As shown in Fig. 1(*a*), the release of thiol before hormone addition is practically fully accounted for by GSH. The increase in thiol release on addition of hormone, vasopressin (top) or phenylephrine (bottom), appears to exceed the rise in GSH release, but the predominant part (about 60–70%) of the extra thiol release is GSH. Recovery of GSH infused into the liver via the portal vein is complete (top right segment of Fig. 1*a*); this confirms that externally added GSH at physiological concentrations does not enter the liver cells (Hahn *et al.*, 1978).

Biliary response

Although GSH efflux occurs predominantly across the sinusoidal membrane, a small amount is also released across the canalicular membrane into the biliary space (Sies *et al.*, 1979; Eberle *et al.*, 1981; Akerboom *et al.*, 1982). Under conditions of

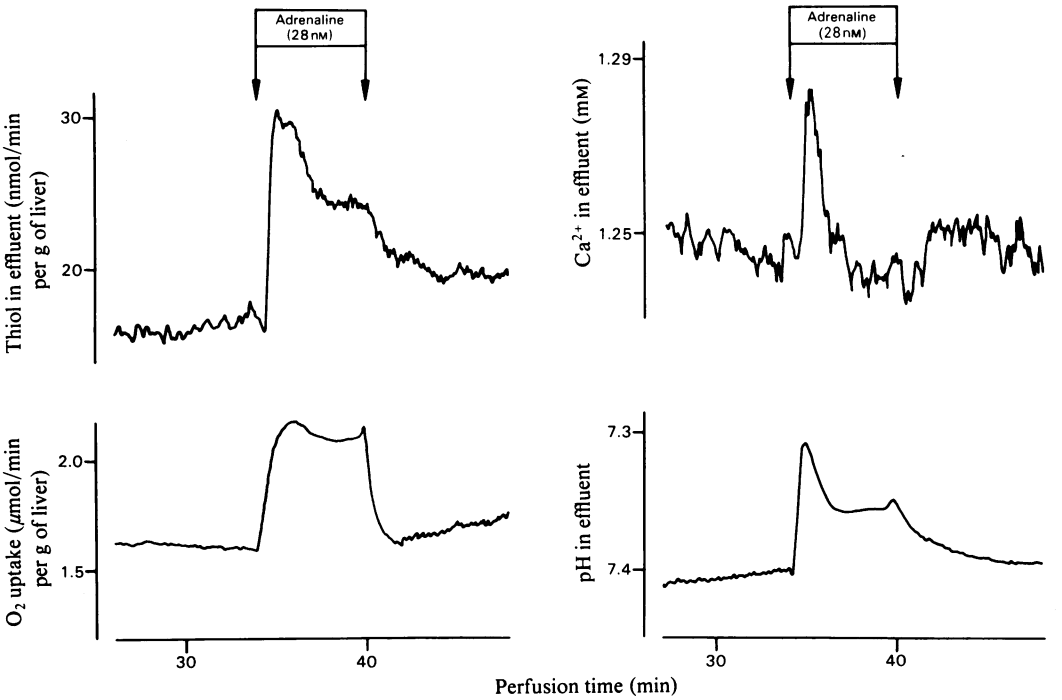

Fig. 2. *Stimulation of thiol release by adrenaline*

Thiol release was recorded continuously (top left), and O_2 uptake (bottom left) and Ca^{2+} and H^+ effluxes (right-hand side) are also shown. Thiol release in some experiments lacked the initial peak, thus reaching the plateau rate of about 7nmol of thiol/min per g wet wt. above control values [5.6$\pm$1.2 (n=7)] directly.

so-called oxidative stress, the efflux of GSSG is increased, and this occurs selectively into bile (Sies *et al.*, 1978; Sies & Akerboom, 1984). This area of redox transitions in the glutathione system is not involved here in the hormone responses, because there is no detectable change in intracellular GSSG (22 nmol/g) or extracellular GSSG contents (no biliary or sinusoidal increase) on vasopressin addition (four experiments).

As shown in Fig. 1(*b*), the increase in thiol release into the perfusate on addition of vasopressin is accompanied by a decrease in the release of glutathione into bile; release of GSH plus GSSG into bile is shown in the centre of Fig. 1(*b*). Since the predominant compound present before the hormone addition is GSH (Akerboom *et al.*, 1982), the decrease on addition of vasopressin is attributable to a decrease in biliary GSH excretion. This decrease is relatively small compared with the increase in sinusoidal efflux, so that the hormone response comprises a net increase in thiol release, not merely a change in the excretory pathway.

Relation to Ca^{2+}

Ca^{2+} release on the addition of hormone (cf. Fig. 2) occurs also when the cellular GSH is depleted (not shown), but the thiol release is abolished when the hormone is given under conditions of low Ca^{2+} (10 μM), shown in Fig. 3. This suggests that the release of thiol from the cell and the release of Ca^{2+} are related, but not obligatorily coupled. It was noteworthy that the omission of external Ca^{2+} has a pronounced effect of its own on thiol and GSH release, causing an increase even larger than that observed with the hormones (H. Sies & P. Graf, unpublished work).

Possible biological significance

The present work demonstrates the novel effect that GSH (and thiol) transport across the sinusoidal plasma membrane is stimulatable by hormones such as vasopressin, phenylephrine and adrenaline and is inhibitable by α_1- or α_2-receptor antagonists, allowing the conclusion that the stimulation of GSH release is hormone-receptor dependent. The experiments were carried out at hormone doses in the maximal, but not supramaximal, physiological concentration range, except for adrenaline.

Relation to GSH loss in shock or stress

It has been known for some time that glutathione concentrations decrease under conditions of stress. For example, non-protein thiol contents were found to be decreased in liver, kidney, spleen or heart in different types of shock, such as haemorrhagic, traumatic (Beck *et al.*, 1954; Jeffries, 1963; Reichard *et al.*, 1981) or endotoxin (Yamada, 1977) shock. Interestingly, GSH concentrations in rat adrenals were increased rather than decreased under stress conditions (Sedlack & Hanus, 1982). From our results, we propose that such phenomena may be explained, at least in part, by an increased transport of GSH from the organ.

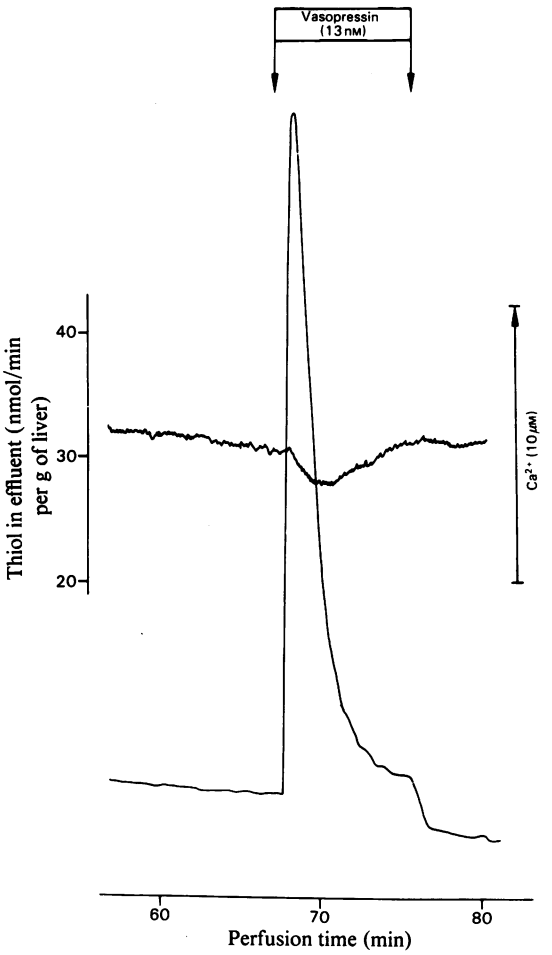

Fig. 3. *Retained calcium release and lack of thiol response upon vasopressin addition at low calcium concentration*
At 20 min of perfusion, the Ca^{2+} concentration was decreased from 1.25 mM to 10 μM. For further details see the text.

Thus the observations of positive effects of thiol compounds such as cysteine or GSH in shock therapy (Szymanski & Jeffries, 1968; Galvin & Lefer, 1978; Sumida & Yagi, 1981; Kosugi *et al.*, 1983) may find a pathophysiological explanation in serving for enhanced hepatic GSH supply for transport and utilization. Such a feedback system between liver and periphery may operate on the hormone-sensitivity of GSH transport. Another observation of interest is that the hepatic glutathione content decreases to half that of controls in

peripheral inflammation (Bragt & Bonta, 1980), and the signal(s) of hormone nature that emanate from the peripheral site of inflammation might elicit an increase in GSH transport from the liver. James *et al.* (1983) reported that adrenaline decreased hepatic GSH by about 30% at 3h after a single intraperitoneal injection into the mouse.

Relation to toxicity

As pointed out by James *et al.* (1983), the potentiation by adrenaline of the hepatotoxicity of CCl_4 (Schwetz & Plaa, 1969; Strubelt *et al.*, 1970) may be explained by the hormone-induced loss of GSH. Similarly, ethanol potentiation of CCl_4 toxicity and the lack of this effect in adrenalectomy (Lindstrom *et al.*, 1978; Strubelt, 1980) agrees with the catecholamine-dependent loss of GSH being critical.

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