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Hepatic glutathione release upon decreases of extracellular calcium concentration*

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Recently, the hepatic release of GSH across the sinusoidal plasma membrane [1, 2] was found to be substantially stimulated upon the addition of hormones such as vasopressin or phenylephrine [3]. As shown in Table 1, the thiol release is about 23 nmol/min per g wet wt in the control state, and is increased to 33 or 38 nmol/min per g, respectively. Since the mechanism of action of such hormones includes calcium transients, it was of interest to examine the effects of calcium movements themselves. Therefore, the effects of the calcium ionophore, A23187 and the effects of manipulating extracellular calcium were studied; the latter involved variations in the calcium concentration as such or by the titration with EGTA.

Livers from male Wistar rats (200–500 g body wt), fed on stock diet, were perfused as previously [3]. Thiol concentration and GSH concentration as well as pH and pO_2 were followed as previously [3, 4].

Figure 1 demonstrates a significant decrease in hepatic thiol release upon the addition of A23187, a calcium ionophore. It is generally accepted that the ionophore elicits calcium transients, and in the presence of the physiological

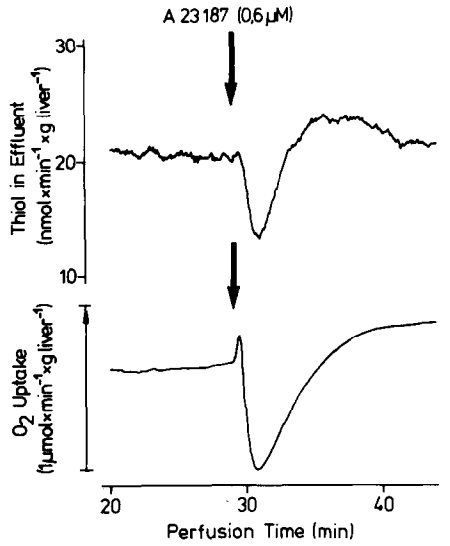

Fig. 1. A23187 induced decrease in thiol release (top) and O_2. A23187 was infused for 20 sec and corresponds to a supply of 7.4 nmol/liver. Standard perfusion condition, i.e. calcium ion concentration was 1.25 mM.

Table 1. Thiol release from perfused rat liver

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
Angiotensin II, 14 nM (3)	7.7 ± 0.4	39 ± 3
PAF, 13 nM (3)	3.6 ± 0.1	14 ± 0.3
A23187, 0.4 µM (4)	3.7 ± 0.2	14 ± 0.7
Calcium omission (4)	12.4 ± 0.5	51 ± 2
EGTA, 1.5 mM (3)	12.0 ± 0.5	54 ± 2

extracellular concentration of 1.2 mM Ca^{2+} there is an influx of Ca^{2+} across the sinusoidal plasma membrane into the cells. Thus, it appears that during the net flux of calcium into the cells there is a restriction of thiol efflux.

The reverse, e.g. an efflux of calcium from the cells, can be achieved by decreasing the calcium concentration in the extracellular space. As shown in Fig. 2, the omission of calcium in the entering perfusate is accompanied by a substantial increase in both thiol and GSH release, the rates being even higher than those obtained with vasopressin or phenylephrine (Table 1). There is no cell damage, as indicated by the lack of a significant leakage of lactate dehydrogenase in the effluent perfusate (1 mU/min per g liver) before calcium omission and until 34 min, the time point when the peak in thiol release has already occurred. The concentration dependence (Fig. 3A) shows that the thiol releasable by the omission of calcium is observed only

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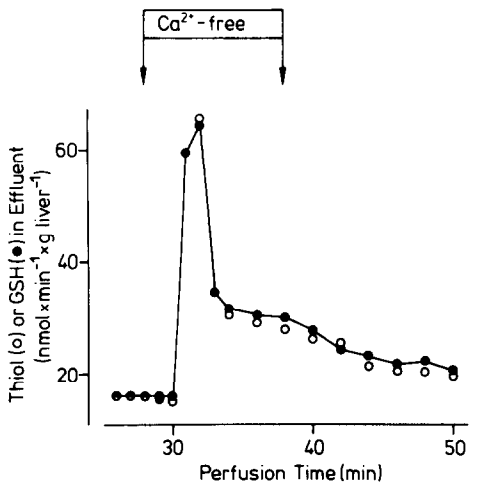

Fig. 2. Effect of calcium omission on thiol (○) and GSH (●) release into perfusate. (Note: the change in influent perfusate takes approx. 2 min, so that Ca^{2+} omission occurs at 30 min.) The figure represents one of 3 identical experiments.

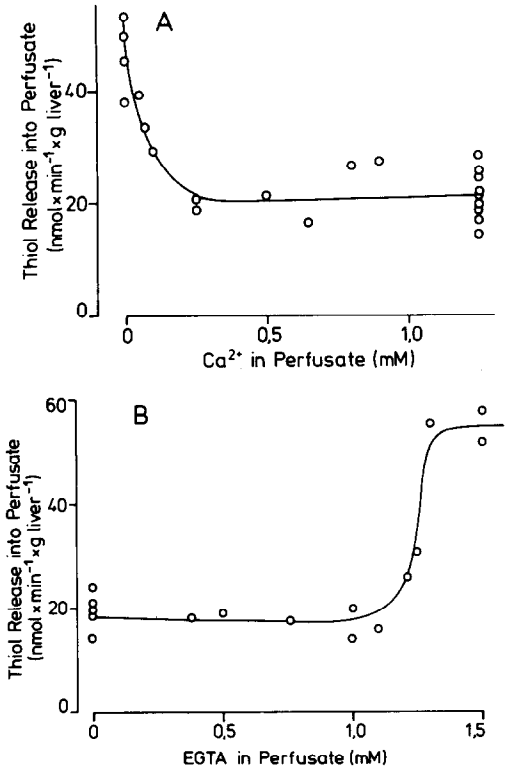

Fig. 3. Dependence of thiol release in perfused rat liver on calcium (A) and EGTA (B) concentration in influent perfusate.

at low calcium concentrations, half-maximal rates being obtained at about 30 μM extracellular calcium; the similar observation is made when, in the presence of 1.2 mM calcium ions, the chelator, EGTA, is added (Fig. 3B).

The cellular pool of calcium releasable by the addition of hormones is included in the pool released upon calcium omission. This is shown by the lack of a vasopressin response in thiol release under the condition of omitted calcium [3].

The integration of the area under the time curve in Fig. 2 reveals that the size of the releasable pool is about 300 nmol GSH/g wet wt. It will be of considerable interest to characterize the nature of this calcium-dependent cellular GSH pool. It may be speculated that this GSH pool is associated with membranes via binding to calcium. Upon the loss of membrane-bound calcium, this GSH pool would be released. The action of the hormones is known to cause a release of membrane-bound calcium; such an initial calcium mobilisation could, as a consequence, translate into an increase in GSH mobilisation. An alternative possibility regarding the nature of the releasable GSH pool is a location in the nonparenchymal cells (e.g. Kupffer cells).

In summary, there is a hepatic GSH pool of about 300 nmol/g, releasable into the plasma space upon the decrease of extracellular calcium concentration. The rate of GSH release is about 50 nmol/min per g wet wt. The hormone-releasable GSH pool is included in this pool.

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