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Tris reversibly inhibits secretion of albumin and α-1-antitrypsin at different sites

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We have investigated the effect of the weak base Tris on the processing and secretion of albumin and α-1 antitrypsin by hepatocytes in culture. We show that the secretion of both proteins is 90% inhibited by 30 mM Tris. The post-synthetic processing of both proteins is inhibited to the same extent. These effects are completely reversible. Cell fractionation indicates that albumin accumulates in the Golgi, whereas α-1 antitrypsin fails to leave the endoplasmic reticulum.

Tris; Albumin; α-1 Antitrypsin; Golgi; Endoplasmic reticulum; (Rat hepatocyte)

1. INTRODUCTION

A number of weakly basic amines, including Tris, chloroquine and ammonium chloride have been shown to inhibit secretion from a variety of cell types, while having little or no effect on protein synthesis. They are thought to diffuse into intracellular compartments where they accept protons and consequently accumulate and raise the pH. There is also an osmotic effect, at least in the Golgi complex that causes swelling of the cisternae and disruption of the organisation of the stacks of cisternae. It is not clear whether the inhibition of secretion is due to the combination of these effects or just to one of them. In hepatocytes, these weak bases have been shown to prevent the proteolytic processing of proalbumin to albumin, but only some, such as Tris, inhibit the maturation of the secretory glycoproteins [1–3]. The inhibition of transport is not complete, and some secretory product is released. For several proteins this has been shown to be the immature form. The implication is that both transport and processing are inhibited directly, and it is therefore not possible to use the maturation state of secretory proteins to show where in the secretory pathway the inhibition of transport is occurring.

Using hepatocytes, we show here that the inhibition of secretion of albumin and of α-1 antitrypsin (α-1 AT) and the processing of their precursors by the weak base Tris is reversible. We present evidence that Tris causes α-1 AT to be trapped in the endoplasmic reticulum (ER), whilst albumin accumulates in the Golgi complex.

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2. MATERIALS AND METHODS

These were presented in a previous paper [4] but for ease of reference we include here the significant details of the methods.

2.1. *Culture and labelling of cells*

Hepatocytes were cultured at a concentration of 10^5 cells cm^{-1} in Williams E medium supplemented with (per 100 ml of medium) 120 μg insulin, 200 μg dexamethasone, 4 mg gentamycin, 5 ml foetal calf serum and 2 mM glutamine. Cultures were washed free of medium with 3 changes of a balanced salt solution (140 mM NaCl, 7 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 1 mM Na_2HPO_4, 5 mM glucose) which contained the common amino acids at 0.1 mM with the exception of leucine and methionine and 25 mM Hepes buffer, pH 7.4. When desired the Hepes buffer was replaced by 30 mM Tris HCl, pH 7.4. The cultures were allowed to settle down in an incubator at 30°C for about 30 min. They were labelled for 5 min with 250 μCi [^{35}S]methionine (spec. act. 1 Ci/μmol) or with [^{3}H]leucine (spec. act. 190 μCi/μmol) in 1 ml for a 6 cm dish. Labelling was stopped by addition of 100 μM cycloheximide and 10 mM unlabelled amino acid. The chase was then conducted with 2 ml medium as described in the figures and tables.

2.2. *Immunoprecipitation*

Anti-albumin precipitable material was recovered from samples of the media and from cells extracted with saline containing 1% Triton X-100 with the addition of 25 μg carrier rat albumin and the appropriate amount of antibody to cause precipitation. α-1-AT was recovered by addition of rabbit anti-α-1-AT and protein-A sepharose as described in [4].

2.3. *Electrofocusing*

Albumin and proalbumin from immunoprecipitates were resolved as described by Oda et al. [5]. Isolation of Golgi fractions was as described by Fries et al. [6] except that the sucrose gradient was modified: the homogenate was made to 1.5 M with respect to sucrose, above this was 1.1 M sucrose, then 0.85 M sucrose covered with 0.25 M sucrose. The gradients were centrifuged at 50 000 rpm in a Beckman SW 50.1 rotor for 60 min. The membranes at the 0.85 M/0.25 M interface were removed and made to 1.0 ml with buffer containing 50 mM Tris-HCl, pH 7.4, 150 mM NaCl and 1% (v/v) Triton X-100 and immunoprecipitated for albumin and α-1 AT [4].

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3. RESULTS

Fig. 1 shows that 30 mM Tris inhibits the secretion of albumin, and also shows that if the medium were changed from Tris to Hepes buffering an almost complete reversal of the inhibition was seen. The secretion of α-1 AT behaved in an identical way (data not shown). During transport proalbumin, the major intracellular precursor of serum albumin, is proteolytically processed resulting in a change of p*I* from 6.1 to 5.7 [7]. The two forms can therefore be resolved by electrofocusing. In Fig. 2A, we show that the proteolytic conversion of proalbumin to albumin is grossly inhibited, and reversal of the Tris inhibition is associated with the production of the mature form of the protein and its secretion (Fig. 2B). Lane 2 shows that the small amount of material secreted in the presence of Tris was unprocessed proalbumin.

α-1 AT is a glycoprotein bearing N-linked sugars. During transport to the cell surface, these undergo a sequence of modifications changing the initially added high mannose sugars to the complex mature form which is secreted. The modification results in a marked increase in apparent molecular weight when the proteins are analyzed on SDS polyacrylamide gels. The inhibition by Tris of the production of the mature form of α-1 AT and the reversal of this effect when the buffer is changed to Hepes is shown in Fig. 3. It seems likely that Tris owes its action to the swelling of the Golgi cisternae referred to above; this in turn is probably due to discharge of their proton gradient [6]. If this were so Tris should cause the accumulation of newly labelled secretory proteins in the Golgi and this should be demonstrable by cell fractionation. Serum albumin may indeed be shown to accumulate within isolated Golgi preparations, as shown in Table I, which illustrates 4 experiments done with liver slices [4] and which also shows that α-1 AT does not appear to follow the albumin. In these experiments, the recovery of the *trans*-Golgi enzyme marker galactosyl transferase was 10%. Based on this, all the albumin would appear to have reached the Golgi. It must be noted, however, that

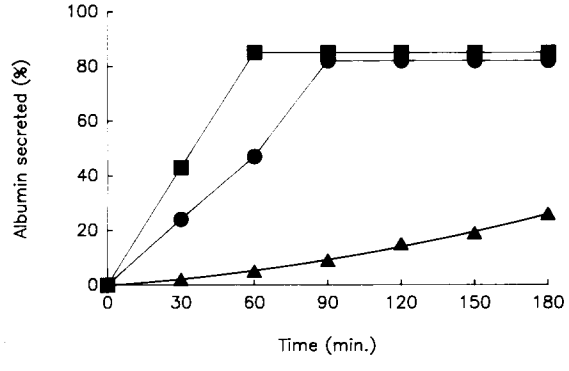

Fig. 1. The reversal of Tris-induced inhibition of secretion. The cells were incubated in Ringer buffered with 30 mM Tris-HCl, pH 7.4 for 60 min (triangles) and (circles) or with 25 mM Hepes-NaOH (squares). They were labelled with [^{3}H]leucine (50 μCi) for 10 min. The labelling was then stopped by the addition of 100 μM cycloheximide and 10 mM unlabelled leucine. At this time, the buffer in one set of samples (circles) was changed from Tris to Hepes, and the timing of the chase started. Albumin was collected from media and cells by immunoprecipitation as described in section 2. The values are expressed as percentages of the total radioactive albumin (8×10^5 dpm) in the system.

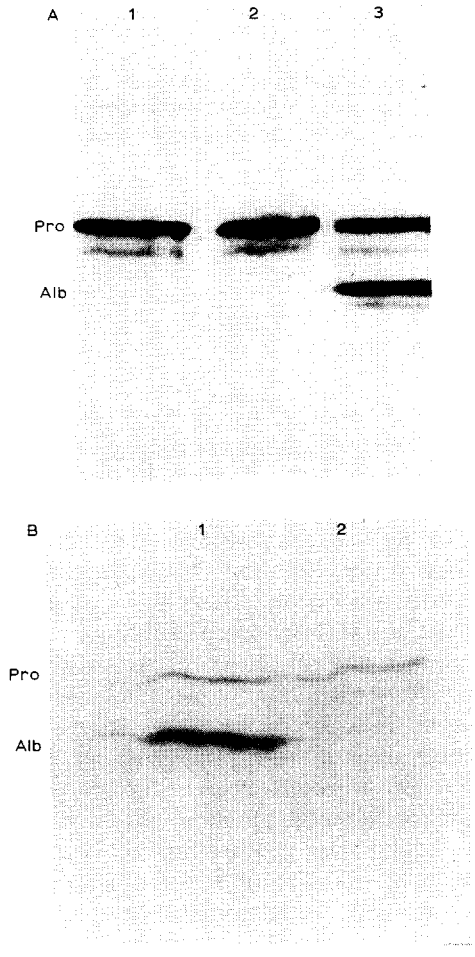

Fig. 2. The inhibition of processing of proalbumin (Pro) by Tris and its reversal. In (A), the cells were labelled with 100 μCi [^{35}S] methionine for 5 min in Ringer buffered with 25 mM Hepes, pH 7.4. Labelling was stopped by addition of unlabelled methionine (10 mM) and cycloheximide (100 μM). One sample was harvested immediately (lane 1), the others post-incubated for 40 min in the same medium (lane 3) or in medium buffered with 30 mM Tris, pH 7.4 (lane 2). Proteins were extracted from the cells into buffer containing 1% v/v Triton X-100, 50 mM Tris HCl, pH 7.4, 2 mM EDTA, and 150 mM NaCl. Albumin (Alb) was immunoprecipitated, the samples were electrofocused [6] and the gel autoradiographed. In (B), we show the material secreted from the cells with and without the removal of the Tris. The cells were pulse-labelled as above but in Tris-buffered Ringer. After 60 min further incubation, one set was changed to Hepes-buffered Ringer (lane 1) and incubation continued for 40 min and albumin immunoprecipitated from the medium and processed as described above. The second set was left in Tris (lane 2), and shows that the small amount of secreted material was unprocessed proalbumin.

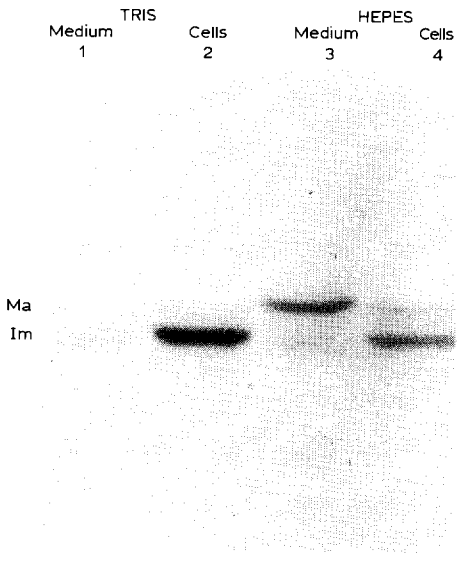

Fig. 3. The effect of Tris on the secretion and processing of α-1-AT and its reversal. Cells were pulse-labelled and then incubated for 60 min in Tris-buffered Ringer as described in Fig. 2. They were then incubated for a further 40 min in either Tris (lanes 1 and 2) or Hepes (lanes 3 and 4) buffered Ringer. Media and cells were collected and α-1-AT immunoprecipitated. Samples were analyzed on SDS-polyacrylamide gels and autoradiographed. Lanes 1 and 3, medium, lanes 2 and 4, cell extracts. Ma, mature (M_r 51 kDa); Im, immature (M_r 46 kDa).

it is an assumption that reflects a uniform recovery of the Golgi complex as a whole. We repeated this experiment with hepatocytes in culture. As shown in Table II, this experiment with its entirely different methods confirms that unlike albumin, α-1 AT does not accumulate in the Golgi complex. As removal of the Tris block results not only in secretion of the protein but also in its processing from the immature to the normal mature form, it is clear that the proteins have not passed through the Golgi but have been arrested at some point before.

Table I

The accumulation of labelled albumin (RSA) and α-1-antitrypsin (α-1-AT) in Golgi fractions from liver slices incubated in Tris-or Hepes-buffered Ringer

Buffer (25 mM)	Time (min)	% Secreted		% In isolated Golgi fractions	
		Albumin	α-1-AT	Albumin	α-1-AT
Tris	45	2	0.7	7.3	1.1
Hepes		11	2.5	5.0	2.1
Tris	90	15	2.0	10.5	1.6
Hepes		79	10.1	0.8	2.0

Liver slices were incubated in Ringer buffer at pH 7.4 with 25 mM Tris or Hepes. They were labelled for 10 min with 300 μCi [^{3}H]leucine and the labelling was then stopped as described in Fig. 1. At the times shown, the slices were homogenised and Golgi fractions isolated as described in section 2. The recovery of the Golgi marker enzyme galactosyl transferase was 10%, with a relative enrichment of 50 fold. The proteins were recovered by immunoprecipitation. The percentage figures shown were calculated from the total of the radioactive protein secreted and remaining in the cells. This was 48×10^5 dpm for albumin, and 1.25×10^5 dpm for the α-1-antitrypsin.

4. DISCUSSION

The best documented inhibitor of protein transport is monensin, which causes newly synthesised plasma membrane and secretory proteins to accumulate in the Golgi complex [8,9], and prevents the processing of both proalbumin and α-1-AT by cultured rat hepatocytes [10]. Unfortunately, we have found that the effects of monensin are not readily reversed in these cells (data not shown). In contrast, the effects of Tris, which also causes albumin to accumulate in the Golgi complex, are readily reversible by simply washing the cells with buffered saline. This is of considerable interest for it means that transport of albumin from the Golgi to the plasma membrane can be isolated from the rest of the secretory pathway for investigation of its functional requirements. It is of course quite possible that the action of other weak bases which appear to exert their effects by the same mechanism, could also be reversed in the same way.

Whilst we have shown that Tris causes proalbumin to accumulate in the Golgi complex, this is not the case for α-1-AT. For both proteins it is clear that they do not leave the secretory pathway, nor are they irreversibly altered as removal of the Tris permits their secretion. α-1-AT apparently fails to reach the Golgi, but we cannot formally eliminate the possibility that it reaches a *cis*-Golgi compartment which we selectively lose during our isolation of the Golgi which is monitored using a *trans*-Golgi marker enzyme. It seems more likely that the α-1-AT is retained in the ER or a compartment between the ER and the Golgi such as the 'salvage compartment' that has been postulated to mediate the return of resident ER proteins escaping into the

Table II

The effect of Tris on the relative enrichment in the Golgi of albumin and α-1-antitrypsin

Experiment	Radioactivity in	Albumin	α-1-Antitrypsin
1	Homogenate (H)	24.3	15.1
	Golgi (G)	9.3	2.0
	G/H	0.38	0.13
2	Homogenate	33.7	17.7
	Golgi	4.9	0.8
	G/H	0.15	0.05

The experiments were done with hepatocytes in culture. The cells were labelled for 10 min with [^{35}S]methionine. Labelling was stopped by the addition of 100 μM cycloheximide and 10 mM unlabelled leucine, and incubation continued for a further 90 min. The cells were then homogenised and fractionated as described in section 2. Antibody precipitates were prepared from the fractions and run on SDS-polyacrylamide gels. After fluorography, the autoradiograms were scanned and the areas of the peaks determined. The figures represent peak areas and are expressed in arbitrary units.

secretory pathway [11]. The mechanism underlying the difference between α-1-AT and albumin in their response to Tris remains a matter for speculation. Normally, albumin is transported from the ER to the Golgi complex at twice the rate of α-1-AT [4,6]. This finding led to the suggestion that some sort of receptor(s) holds the protein in the ER, perhaps until it has been correctly folded. It is possible that Tris is exerting its effect by increasing this interaction and is an amplification of an effect visible under 'physiological' conditions.

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