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Protective effect of (+)cyanidanol-3 on the inhibition of protein synthesis and secretion after galactosamine injection

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Synthesis and secretion of serum proteins are two of the specific functions of the liver. Besides the rough endoplasmic reticulum where protein synthesis takes place, the process of secretion is localized in three subcellular structures, the smooth endoplasmic reticulum, the Golgi apparatus with its vacuoles and the plasma membrane, and involves the action of several glycosyltransferases [1]. This process is no more fully instrumental when these subcellular structures are altered. One parameter of an intact secretion process is the time between the injection of a labelled amino acid and the appearance of protein-bound radioactivity in the plasma. This time difference is normally 15 min [2, 3]. After the injection of galactosamine this time is substantially prolonged [4]. Furthermore, it is well established that the respective membranous structures are damaged [4–6]. In an earlier study it could be shown that by pretreatment of rats with the flavonoid (+)-cyanidanol-3 the plasma membrane-damaging effect of galactosamine is ameliorated [7]. In the present study synthesis and secretion of plasma proteins of (+)-cyanidanol-3 pretreated rats were investigated after injection of galactosamine.

Female Wistar rats (Invanovas; Kisslegg, F.R.G), weighing 160–180 g each, were fed on a commercial diet (Altromin, Altromin GmbH, Lage-Lippe, F.R.G.) and given water *ad lib.*

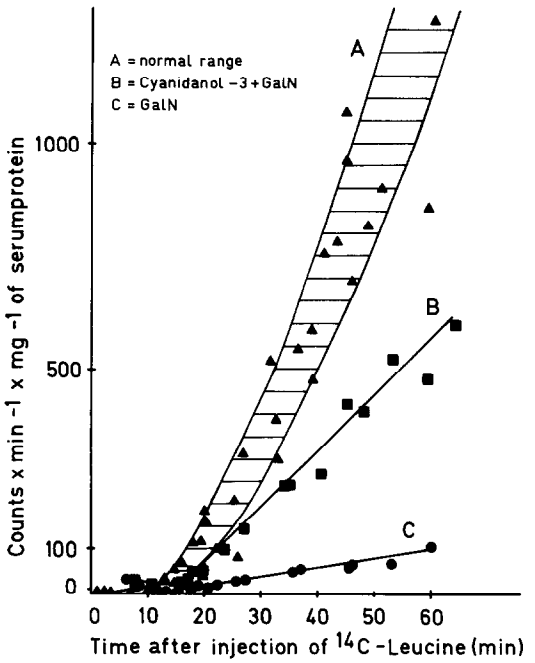

Fig. 1. Measurement of the secretion of plasma proteins. Effect of pretreatment with (+)-cyanidanol-3 with a daily injection for two days on the inhibitory effect of D-galactosamine–HCl (375 mg/kg body wt). For experimental details see the text.

D-Galactosamine–HCl (purissimum) was purchased as Hepasamin from C. Roth OHG (Karlsruhe, F.R.G.). L-[1-^{14}C]Leucine (61 mCi/mmole) was obtained from the Radiochemical Center (Amersham, U.K.). All other chemicals of analytical grade were obtained from E. Merck AG (Darmstadt, F.R.G.). (+)-Cyanidanol-3 was a kind gift of Zyma GmbH (München, F.R.G.).

The animals received at daily intervals two intraperitoneal (i.p.) injections of (+)-cyanidanol-3 as an aqueous suspension. D-Galactosamine was given 5 hr before the administration of L-[1-^{14}C]leucine.

For the determination of the kinetics of serum protein secretion the method of Schreiber *et al.* has been followed [2, 3]. The anesthetized rats (40 mg pentobarbital/kg body wt) received 50 μCi L-[1-^{14}C]leucine/kg body wt via the vena cava. Blood samples (0.15 ml each) were withdrawn from this vessel at timed intervals. The radioactivity of labelled proteins was determined by the method of Mans and Novelli [8]. The time between the injection of L-[1-^{14}C]leucine and the appearance of labelled proteins in the serum is called 'secretion time'. Protein was determined by the biuret method with bovine serum albumin as a standard [9]. For the determination of protein-bound radioactivity the livers were removed under pentothal anesthesia. About 1–2 g of the right liver lobe were suspended in an ice-cold Tris–KCl–$MgCl_2$ buffer as described [2] and homogenized using a motor-driven Potter-Elvejhem type homogenizer for 1 min. After centrifugation at 750 *g* for 15 min using a SS34 type rotor from Sorvall, the supernatant fraction was used for the measurement of protein-bound radioactivity [8] and protein [9].

Table 1 shows that the administration of D-galactosamine leads to an inhibition of the incorporation of labelled L-leucine into the proteins of liver and also of the serum, confirmatory to previous findings [4]. After pretreatment of the rats with (+)-cyanidanol-3 the incorporation of L-leucine is still decreased but not to the same extent as without (+)-cyanidanol-3. By lowering the dose from 250 to 66 mg (+)-cyanidanol-3 kg body wt, its beneficial effect is greatly reduced. It should be noticed that the beneficial effect of this flavonoid is more marked on the secreted proteins. It may be assumed that (+)-cyanidanol-3 exerts its protective action on membranous structures involved in the secretion process of plasma proteins. This becomes evident also from measurements of the secretion times with the result that the prolongation after galactosamine administration could not be observed in the (+)-cyanidanol-3 pretreated rats. However, the specific activity in plasma proteins is always decreased as also shown in Table 1. These findings suggest that (+)-cyanidanol-3 shows its action mainly at the level of membranous structures and not to the same extent on the protein synthesis of the liver cell. This assumption correlates with our previous investigations which have shown that galactosamine-induced alterations of the plasma membrane [6] are significantly ameliorated when (+)-cyanidanol-3-pretreated animals were injected with this amino sugar [7].

The effectiveness of (+)-cyanidanol-3 towards the endoplasmic reticulum of the liver *in vitro* could also be shown by Danni *et al.* [10]. Also lysosomal membranes have been shown to be more resistant to liver damaging substances if the animals have been pretreated with the flavonoid [11].

Table 1. Dose dependency of the effect of pretreatment with (+)-cyanidanol-3*

Treatment	(+)-Cyanidanol-3 (mg/kg)	−	+	−	66	125	250
	Galactosamine–HCl (375 mg/kg)	−	−	+	+	+	+
Organ	Serum	2430 ± 90	2610 ± 110	660 ± 50	1980 ± 210	2850 ± 430	3110 ± 410
	Liver	669 ± 213	710 ± 95	336 ± 49	386 ± 85	422 ± 102	402 ± 900

* The animals were injected i.p. with (+)-cyanidanol-3 at 8:00 a.m. and 7:00 p.m. for two days. At the third day the rats received galactosamine at 8:00 a.m. and [^{14}C]leucine 5 hr later. One hour after the injection of the label the serum and liver was removed under pentothal anesthesia. For further experimental detail see text. Values are given as c.p.m./mg protein.

From these findings it seems to be likely that (+)-cyanidanol-3 is capable of interacting with the metabolism of membranous structures of the liver. This may be done by stimulation of the biosynthesis or inhibition of the degradation of essential membrane constituents.

In summary, by the injection of D-galactosamine–HCl (375 mg/kg body wt) synthesis and secretion of proteins by the rat liver are inhibited. Pretreatment of the animal with (+)-cyanidanol (250 mg/kg body wt) for two days reduces this action of D-galactosamine. The beneficial effect seems to be mainly directed on the secretory function of the liver and is suggestive of an endomembrane-directed action of this flavonoid.

Biochemisches Institut der Albert-Ludwigs-Universität,
D-7800 *Freiburg i. Br.,*
Federal Republic of Germany.

W. REUTTER
B. HASSELS

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