(Ib); cannogenin and corotoxigenin; cannogenol and coroglaucigenin, etc. [6]. So far as we know, however, cardiotonic activities were determined and compared by the same bioassay only in the first pair, digitoxigenin and uzarigenin[7–9]. Principally based on these comparisons, it is generally accepted that the A/B *cis*-cardenolides are much more potent than the A/B *trans*-cardenolides[10,11], although this is not always true with the cardenolide glycosides[12]. The present results with digoxigenin (IIa) and 5α-digoxigenin (Ia) thus provide the second instance which supports the above view concerning the structure-activity relationship of cardenolide aglycones[13].

Zusammenfassung. Es wurden die kardiotonischen Wirkungen des Digoxigenins, sowie deren 5 Derivate, einschliesslich des 5α-Digoxigenins, auf das isolierte Froschherz untersucht und Vergleiche mit den entsprechenden Aglykonen der Digitoxigenin-Reihe gezogen.

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[6] T. REICHSTEIN, Naturwissenschaften *54*, 53 (1967).
[7] K. K. CHEN, A. Rev. Physiol. *7*, 677 (1945). – K. K. CHEN and F. G. HENDERSON, Archs int. Pharmacodyn. Thér. *140*, 8 (1962).
[8] K. REPKE, Proc. 2nd Int. Pharmac. Meeting (Pergamon Press, New York 1965), vol. 5, p. 65.
[9] A. L. ABELES, J. gen. Physiol. *52*, 268 (1969).
[10] CH. TAMM, Proc. 1st Int. Pharmac. Meeting (Pergamon Press, New York 1963), vol. 3, p. 11.
[11] K. H. R. REPKE and H. J. PORTIUS, Planta med. Suppl. *4*, 66 (1971).
[12] K. D. ROBERTS, EK. WEISS and T. REICHSTEIN, Helv. chim. Acta *49*, 316 (1966).
[13] This study was supported in part by a research grant No. 787012 from the Ministry of Education, Japan.
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Rifampicin and Cysteamine Protect against the Mushroom Toxin Phalloidin

Phalloidin, a cyclic heptapeptide, is quantitatively the most important representant of the rapidly acting phallotoxins isolated from the poisonous mushroom *Amanita phalloides*[1]. In an earlier series of investigations, mice could be protected against lethal doses of phalloidin by prior treatment with hepatotoxic agents such as carbon tetrachloride, sodium-cinchophen (sodium salt of 2-phenylcinchoninic acid) and thioacetanide[2,3]. Subsequent studies disclosed that marked protection against phalloidin was also provided by rifampicin and phenylbutazone[4]. The present work reports, in addition to further characteristics of the rifampicin activity, the antagonistic efficacy of cysteamine. Moreover, it was tested whether other drugs with hepatotoxic potencies and whether antidotes to the slowly acting mushroom poison α-amanitin, would also affect phalloidin toxicity.

Methods. Female NMRI mice (S. Ivanovas, 7964 Kisslegg, West-Germany), weighing 18–22 g were used throughout the experiment. Phalloidin was dissolved in distilled water and applied in a volume of 0.1 ml/10 g body weight by the i.p. route. Death after lethal doses of phalloidin occurred in general within 2–5 h after the application. As no deaths were observed later than 24 h, survival was scored at this time.

The agents to be tested were dissolved immediately before use in distilled water and applied in 0.1 or 0.2 ml/10 g body weight in single doses at the times indicated in Tables II and III. The control groups received the respective volumes of distilled water only by the corresponding route. The chemicals used included: Rifampicin ('Rimactan'), Cysteamine (Fluka AG, CH 9470 Buchs), Chlorpromazine ('Largactil'), Penicillin-G ('Specilline G'), Aureomycin (Lederle, suspended in tap water), Erythromycine ('Erythrocin'), Cytochrome C ('Cyto-Mack'), Reserpine ('Serpasil'), Phenylbutazone ('Butazolidine'), 'Synthalin A' (Decamethylenediguanidine 2 HCl) and activated charcoal (Carbo adsorbens, suspended in tap water).

Table I. Toxicity of phalloidin in NMRI mice

Dose of phalloidin (mg/kg, i.p.)	Proportion of mice surviving at 24 h	Survival(%)
1.0	6/6	100
2.0	4/6	67
3.0	4/39	10

Results. As seen from Table I, the LD_{50} of phalloidin amounted to ~2.5 mg/kg and the LD_{90} to 3 mg/kg. The drugs were tested against this latter dose. Rifampicin provided again a complete protection at both doses tested of 100 and 300 mg/kg (Table II). In addition, it was demonstrated that rifampicin is still active if applied 24 or even 48 h prior to the phalloidin. The protection afforded by phenylbutazone, after intervals of 8 or 24 h, was only marginal. Significant protection was caused by cysteamine and, to a lesser degree, by chlorpromazine. No significant activity was demonstrated by the agents entered in Table III.

Discussion. Fiume had observed that newborn rats were resistant to lethal doses of phalloidin[5]. He ascribed this phenomenon to the immaturity of drug metabolizing enzymes in newborns. Based on this and other[6] observations, it was held likely that hepatotoxic agents such as carbon tetrachloride exert their protective effect by damaging drug-metabolizing enzymes and thus preventing the transformation of phalloidin to toxic metabolites[2,3].

[1] TH. WIELAND, Science *159*, 946 (1968).
[2] G. L. FLOERSHEIM, Biochem. Pharmac. *15*, 1589 (1966).
[3] G. L. FLOERSHEIM, Helv. physiol. pharmacol. Acta *24*, 219 (1966).
[4] G. L. FLOERSHEIM, Agents Actions *2*, 142 (1971).
[5] L. FIUME, Lancet *1*, 1284 (1965).
[6] A. VON DER DECKEN, H. LÖW and T. HULTIN, Biochem. Z. *332*, 503 (1960).

Table II. Protective effects against 3 mg/kg phalloidin in NMRI mice

Treatment	Dose (mg/kg)	Application before phalloidin	Proportion of mice surviving at 24 h	Survival (%)	P
Controls	Saline	30 min	4/39	10	
Rifampicin	100 p.o.	30 min	6/6	100	< 0.001
Rifampicin	100 p.o.	8 h	4/4	100	< 0.001
Rifampicin	100 p.o.	24 h	4/4	100	< 0.001
Rifampicin	100 p.o.	48 h	1/4	25	n.s.
Rifampicin	300 p.o.	8 h	4/4	100	< 0.001
Rifampicin	300 p.o.	24 h	4/4	100	< 0.001
Rifampicin	300 p.o.	48 h	3/4	75	< 0.01
Phenylbutazone	100 s.c.	8 h	2/4	50	< 0.05
Phenylbutazone	100 s.c.	24 h	1/4	25	n.s.
Phenylbutazone	300 s.c.	8 h	1/4	25	n.s.
Phenylbutazone	300 s.c.	24 h	1/4	25	n.s.
Cysteamine	150 i.p.	30 min	8/12	67	< 0.001
Chlorpromazine	3 s.c.	30 min	5/10	50	< 0.02

n.s. = not significant. Statistical significance determined according to the χ^2 test.

Indeed, the recently reported protection in rats against carbon tetrachloride by prior carbon tetrachloride administration was also paralleled by a depression of the liver drug metabolizing system[7].

As phenylbutazone is known to affect liver enzymes[8], the protective efficacy of this agent[4] if applied at suitable intervals before the phalloidin would also tend to support the phalloidin toxification hypothesis.

With rifampicin, no investigations on its effect on drug metabolizing enzymes seem to have been published. However, recent clinical reports[9,10] on liver damage and abnormal liver function after rifampicin places this drug in the category of agents capable of causing liver injury. In the light of the new finding that rifampicin protects against phalloidin also if it is administered 48 h earlier, the alternative explanation that rifampicin blocks the phalloidin uptake by liver cells seems less likely.

It appears that the protection against phalloidin is afforded predominantly by compounds capable of causing hepatic damage. From a series of chemicals with a more patchy incidence of hepatotoxic effects such as aureomycin, erythromycin, synthalin A and chlorpromazine, only the latter affected phalloidin toxicity[11] (Table II). However, the toxification hypothesis is challenged by the failure to detect in studies with rats a metabolite of [^{3}H] desmethylphalloin[12] and by the finding that carbon tetrachloride seems to prevent the binding of phalloidin onto liver cells[13].

[7] G. Ugazio, R. R. Koch and R. O. Recknagel, Exp. molec. Path. *16*, 281 (1972).
[8] A. K. Cho, B. J. Hodshon and B. B. Brodie, Biochem. Pharmac. *19*, 1817 (1970).
[9] P. J. Scheuer, J. A. Summerfield and S. Sherlock, Lancet *1*, 421 (1974).
[10] A. Austerhoff, U. Kindler, P. Knop and H. J. Knieriem, Dt. med. Wschr. *94*, 1182 (1974).
[11] O. Peczenik, I. L. Fisher and M. Librius, Toxicon *3*, 41 (1965).
[12] H. Puchinger and Th. Wieland, Eur. J. Biochem. *11*, 1 (1969).
[13] R. Kroker and M. Frimmer, Naunyn-Schmiedebergs Arch. Pharmak. *282*, 109 (1974).

Table III. Lethality of NMRI mice after 3 mg/kg phalloidin and various treatments

Treatment	Dose (mg/kg)	Application before phalloidin	Proportion of mice surviving at 24 h	Survival (%)
Controls	Saline	30 min	4/39	10
Cytochrome C	15 i.p.	30 min	1/6	17
Cytochrome C	45 i.p.	30 min	1/6	17
Cytochrome C	150 i.p.	30 min	0/6	—
Cytochrome C	300 i.p.	30 min	0/6	—
Penicillin-G	100 i.p.	30 min	0/6	—
Penicillin-G	1000 i.p.	30 min	0/6	—
Penicillin-G	100 i.p.	3 h	0/6	—
Penicillin-G	1000 i.p.	3 h	2/12	17
Aureomycin	1000 p.o.	30 min	1/12	8
Erythromycin	75 s.c.	30 min	0/5	—
Reserpine	1 s.c.	100 min	0/4	—
Synthalin A	7.5 s.c.	100 min	2/6	33
Synthalin A	7.5 s.c.	3 h	0/6	—
Activated Charcoal	2000 p.o.	15 min	1/12	8

Another aim of our study was to see whether agents capable of curing α-amanitin poisoning[4,14,15] are also effective against phalloidin. Out of this category neither penicillin nor cytochrome C protected against phalloidin. So antagonism to amatoxins does not extend to phallotoxins. This is in line with the contention that the two classes of poisonous principles in *Amanita phalloides* act by different mechanisms. On the other hand, some agents protecting against phalloidin such as carbon tetrachloride are not completely devoid of activity against α-amanitin[3]. No effect was seen with activated charcoal, tested in the light of the report indicating a hepatoenteric circulation of *Amanita* poisons[16].

A substantial protection against phalloidin was provided by cysteamine (β-mercaptoethylamine). This agent was effective in the treatment of experimental[17,18] and clinical[19] paracetamol poisoning, perhaps by scavenging the active toxic paracetamol metabolite thought to combine with vital liver-cell macromolecules and held responsible for the ensuing liver injury. Although cysteamine might inhibit the formation of a toxic phalloidin metabolite, it is known to prevent the interaction of drug metabolites with liver proteins. This opens a new vista on the mechanisms by which phalloidin toxicity may be antagonized. In current studies, agents antagonizing the single toxins phalloidin and α-amanitin are tested against total extracts of *Amanita phalloides*.

Zusammenfassung. Der Schutzeffekt von Rifampicin gegen eine Letaldosis des Pilzgiftes Phalloidin wurde bestätigt und weiter charakterisiert. Auch Cysteamin erwies sich als wirksam. Keinen Schutz gegen Phalloidin boten Stoffe, welche das Pilzgift α-Amanitin antagonisieren. Die hepatotoxische Wirkungskomponente der meisten Phalloidin-Antagonisten wird erörtert.

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[14] G. L. FLOERSHEIM, Nature New Biol. *236*, 115 (1972).
[15] G. L. FLOERSHEIM, Science *177*, 808 (1972).
[16] U. FAUSER and H. FAULSTICH, Dt. med. Wschr. *98*, 2259 (1973).
[17] J. R. MITCHELL, D. J. JOLLOW, W. Z. POTTER, J. R. GILLETTE and B. B. BRODIE, J. Pharmacol. exp. Ther. *187*, 211 (1973).
[18] J. R. MITCHELL, D. J. JOLLOW, J. R. GILLETTE and B. B. BRODIE, Drug Metab. Dispos. *1*, 418 (1973).
[19] L. F. PRESCOTT, R. W. NEWTON, C. P. SWAINSON, N. WRIGHT, A. R. W. FORREST and H. MATTHEW, Lancet *1*, 588 (1974).

Identification of Norcocaine as a Metabolite of [^{3}H]-Cocaine in Rat Brain

Cocaine is a powerful central nervous system stimulant of relatively short duration, low margin of safety, high systemic toxicity (LD_{50} i.v. in rats 17.5 mg/kg) and little recognized medical use. Its intense euphoriant action leads to a very high degree of psychic dependence[1,2] and a profound and dangerous type of drug abuse. Physical dependence and tolerance to its euphoria and toxic effects has, however, not been reported to develop. In spite of much work[3–14], the dispositional and metabolic profile of cocaine in the central nervous system remains unknown. This study deals with these parameters and demonstrates that: a) cocaine disappeared fairly rapidly from the rat brain after s.c. and i.v. injections; b) in addition to the formation of benzoylecgonine, benzoylnorecgonine and ecgonine as metabolites, N-demethylation occurs rapidly in the brain to form norcocaine, a lipophilic compound with convulsant properties similar to those of cocaine.

Materials and methods. Male Wistar rats (120–150 g) were injected s.c. with 20 mg kg^{-1} (free base) dose or i.v. with 8 mg kg^{-1} (free base) dose of [^{3}H] cocaine prepared as described previously[15] (radiochemical purity 98%,

[1] A. L. TATUM and M. H. SEEVERS, J. Pharmac. exp. Ther. *36*, 401 (1929).
[2] G. A. DENEAU, T. YANAGITA and M. H. SEEVERS, Psychopharmacologia *16*, 30 (1969).
[3] H. A. OELKERS and W. RAETZ, Arch. exp. Path. Pharmak. *170*, 246 (1933).
[4] H. A. OELKERS and E. VINCKE, Arch. exp. Path. Pharmak. *179*, 341 (1935).
[5] A. R. MCINTYRE, J. Pharmac. exp. Ther. *57*, 133 (1936).
[6] L. A. WOODS, F. G. MCMAHON and M. H. SEEVERS, J. Pharmac. exp. Ther. *101*, 200 (1951).
[7] R. V. ORTIZ, Annls Fac. Quim. Farm. (Chile) *18*, 15 (1966).
[8] G. WERNER, Planta med. *9*, 293 (1961).
[9] J. M. VAN ROSSUM, J. B. VAN DER SCHOOT and J. A. TH. M. HURKMANS, Experientia *18*, 229 (1962).
[10] F. MONTESINOS, Bull. Narcot. *17*, 11 (1965).
[11] H. O. J. COLLIER, Nature, Lond. *220*, 1327 (1968).
[12] F. FISH and W. D. C. WILSON, J. Pharm. Pharmac. *21*, 135 S (1969).
[13] H. LANGEKER and K. LEWITT, Arch. exp. Path. Pharmak. *190*, 492 (1938).
[14] R. C. HATCH and R. FISCHER, Pharmac. Res. Commun. *4*, 383 (1972).
[15] P. K. NAYAK, A. L. MISRA, M. N. PATEL and S. J. MULE', Radiochem. radioanalyt. Lett. *16*, 167 (1974).

Uptake of [^{3}H] cocaine in brain and plasma of male Wistar rats after a single 8 mg kg^{-1} (free base) i.v. or 20 mg kg^{-1} (free base) s.c. injection

	0.25 h	0.5 h	1 h	2 h	4 h	6 h	12 h	24 h	Half-life (h)
Brain (i.v.)	7269 ± 177	2738 ± 634	924 ± 131	212 ± 48	8 ± 3	0	—	—	0.4
Plasma (i.v.)	612 ± 62	296 ± 90	111 ± 14	26 ± 8	0	—	—	—	0.3
B/P[a] (i.v.)	11.9	9.3	8.3	8.1	—	—	—	—	
Brain (s.c.)	—	2237 ± 282	2320 ± 205	2963 ± 195	3439 ± 362	485 ± 194	4 ± 1	0	4.8
Plasma (s.c.)	—	249 ± 51	378 ± 31	448 ± 74	494 ± 59	78 ± 35	2 ± 1	0	5.0
B/P[a] (s.c.)	—	9.0	6.1	6.6	6.9	6.2	2	—	

Data represent the mean value ± S.E.M. (ng/g of tissue or ml of fluid) of 3 animals for i.v. and 5 animals for s.c. group at each time period.
[a] B/P represents the ratio of mean brain to plasma concentrations.