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Excretion and distribution of [^{14}C]rhein and [^{14}C]rhein anthrone in rat

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Abstract—After single intracaecal administration of [^{14}C]rhein (25 mg kg^{-1}) and [^{14}C]rhein anthrone (20 mg kg^{-1}) to rats, the summated recovery rates of ^{14}C after five days were in urine 37(±8·3)% and 2·8(±0·4)% and in faeces 53(±9·5)% and 95 (±10·1)%, respectively. The clearance of radioactivity from the organs and tissues was almost complete within three days, with the exception of the kidney which exhibited pronounced retention of radioactivity even after five days (< 61% of 24 h values). Extracts of faeces from animals treated with [^{14}C]rhein of [^{14}C]rhein anthrone, revealed rhein as well as other radioactive substances, which chemically did not react as 1,8-dihydroxyanthraquinones.

Sennosides, the main constituents of senna leaves and pods, are bacterially degraded to rhein and rhein anthrone in the large intestine after oral administration (Lemli & Lemmens 1980). These metabolites locally exert their laxative action both by affecting fluid absorption (Beubler & Juan 1979; Donowitz et al 1984) and by decreasing transit time (Hardcastle & Wilkins 1970; Garcia-Villar et al 1980; Wienbeck et al 1985; Leng-Peschlow 1986).

Previously, several authors have dealt with the metabolism of different monohydroxy- (Fujita et al 1961) and 1,8-dihydroxy-anthraquinones in rats and mice (Gebhardt 1936; Moss 1969; Breimer & Baars 1976; Lemmens 1978, 1979; Lemmens & Dreessen 1979; Moreau et al 1985), but all of those authors met with the same problem of low recovery, especially from faeces. However, after the administration of [^{14}C]emodin, Bachmann & Schlatter (1981) were able to recover 90% of the activity.

To account for the low recovery of anthranoids from urine and particularly faeces after oral administration of rhein (Lemmens & Dreessen 1979) and sennosides (Lemmens 1979) to rats, we synthesized [^{14}C]rhein, according to a new route to anthraquinones proposed by Dodsworth et al (1981), using carbon dioxide (Amersham, UK) as a source of ^{14}C and subsequently reduced the product to the corresponding [^{14}C]rhein anthrone.

Methods and materials

[^{14}C]Rhein anthraquinone ([^{14}C]rhein). The anthraquinone (1,8-dihydroxy,3-carboxyanthraquinone) was prepared according to De Witte & Lemli (1988) (radiochem. purity >99%, spec.act. 55·7 mCi mmol^{-1}). To reduce the specific activity, dilutions were made with unlabelled rhein, prepared from aloin according to Bellaart (1952). Before injection, a homogeneous suspension of [^{14}C]rhein was achieved by adding 0·5 mL of a NaOH solution (5 mM) to 1 mg of rhein.

[^{14}C]rhein 9-anthrone. 100 mg of radioactive rhein was boiled under reflux in 20 mL acetic acid, while 3·5 mL of a 40% (w/v) $SnCl_2$ in conc. HCl was added dropwise. After 2 h the mixture was placed under argon at 4 °C to crystallize. After 12 h the solvent was decanted and the precipitate washed with oxygen-free water until the acid was removed. The product was dried under a gentle stream of argon (Stoll et al 1950, modified).

Yield: 80%, radiochem. purity: 94%.

Before injection, [^{14}C]rhein anthrone was homogeneously suspended in a 1% tragacanth solution.

Animals and doses. Male Wistar rats (ca 200 g) were kept individually in plastic metabolism cages during the experiments, with free access to water and commercial food (Muracon-G-Star, Gent, Belgium). [^{14}C]rhein (25 mg kg^{-1}) and [^{14}C]rhein anthrone (20 mg kg^{-1}) was administered intracaecally as a suspension under ether anaesthesia, after a midventral abdominal incision was made. A sample of the suspension was taken for [^{14}C] determination and quantification.

The rate of [^{14}C] excretion was studied, urine and faeces being collected separately over 24 h for each of five days. For that purpose five animals received individually 1 μCi of [^{14}C]rhein or [^{14}C]rhein anthrone. The body distribution was analysed by giving each of nine animals 10 μCi [^{14}C]rhein or [^{14}C]rhein anthrone. Of these, three were killed after 1, 3 and 5 days. After dissection, the organs and tissues were thoroughly rinsed with 0·9% NaCl (saline) and dried on filter paper. Blood was sampled from the heart with heparinized syringes and centrifuged. For faecal metabolites, three rats were treated with 100 μCi of [^{14}C]rhein or [^{14}C]rhein anthrone and faeces pooled at −10 °C over 24 h for [^{14}C]rhein anthrone and three days for [^{14}C]rhein. Animals were killed by an overdose of chloroform.

Radiochemical techniques. Urine (1 g) and fractions of faecal extracts were counted directly in 20 mL Lumagel (Lumac). Slices of TLC plates (silica gel F-254/Al, Merck) were eluted with 10 mL methanol–water (50:50 v/v) and the eluates counted in 10 mL Lumagel. Faeces and tissues were homogenized (Ultra-Turrax) in a sixfold and a fourfold weight of water, respectively. Fractions of homogenized faeces or tissues (1 g) and samples of plasma (0·1 g) were analysed for their ^{14}C content by combustion (Biological Material Oxidizer OX-100 L, Harvey Instruments) and subsequent trapping of released $^{14}CO_2$ in Carbomax (Lumac).

After 12 h of light and temperature equilibration, radioactive samples were counted in a β-counter (Berthold BF 5003A). Instand (Lumac) was used as an internal standard to correct for quenching effects.

Extraction of metabolites from faeces. After homogenization, faeces were extracted with methanol for 27 h in a Soxhlet apparatus, followed by an extraction with boiling water for 1 h. After cooling, the aqueous extract was centrifuged. Extracts were analysed by TLC (silica gel F-254/Al Merck), using ethylacetate–methanol–water (77:13:10 v/v) as the solvent system. After development, TLC plates were sprayed with 5% KOH in a mixture of methanol and water (1:1) to reveal anthraquinone derivatives. Radioactive spots were localized by a thin layer scanner (II LB 2723, Berthold) and autoradiographical techniques (Curix RP-1, Agfa-Gevaert). 1 mL of the different

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Table 1. Recovered radioactivity in rat organs and tissues 1, 3 and 5 days after administration of [^{14}C]rhein or [^{14}C]rhein anthrone. Values are expressed as μg rhein equivalent or μg rhein anthrone equivalent per gram wet tissue ($\mu g\ g^{-1}$).

Organ/tissue	[^{14}C]rhein[a]			[^{14}C]rhein anthrone[b]		
Liver	1·35	0·29	0·14	1·01 ± 0·78	0·50 ± 0·17	0·27 ± 0·03
Small intestine	0·81	0·10	0·03	1·26 ± 0·89	0·47 ± 0·58	0·23 ± 0·12
Caecum	1·60	0·15	0·07	1·47 ± 0·34	0·82 ± 0·73	0·48 ± 0·24
Colon	1·40	0·50	0·19	1·94 ± 1·05	0·53 ± 0·23	0·30 ± 0·15
Stomach	1·11	0·15	0·04	1·05 ± 0·96	0·31 ± 0·16	0·35 ± 0·28
Thymus	1·69	0·32	0·06	1·39 ± 0·67	0·74 ± 0·86	0·28 ± 0·33
Kidney	5·38	4·60	3·43	8·61 ± 0·63	8·01 ± 0·52	5·28 ± 0·93
Spleen	3·93	0·58	0·12	0·81 ± 0·26	0·35 ± 0·10	0·34 ± 0·19
Lung	3·27	0·41	0·05	0·70 ± 0·64	0·45 ± 0·33	0·15 ± 0·16
Striated muscle	1·24	0·17	0·11	1·07 ± 0·39	0·53 ± 0·38	0·02 ± 0·00
Brain	1·63	0·21	0·10	0·76 ± 0·14	0·34 ± 0·21	0·03 ± 0·01
Mesenterium	0·69	0·10	0·09	0·39 ± 0·17	0·30 ± 0·14	0·06 ± 0·01
Adipose tissue	1·09	0·22	0·08	1·17 ± 0·62	0·26 ± 0·19	0·04 ± 0·03
Pancreas	N.D.	N.D.	N.D.	0·55 ± 0·49	0·18 ± 0·01	0·05 ± 0·01
Plasma	0·50	0·20	0·20	0·16 ± 0·13	0·07 ± 0·02	0·08 ± 0·03
	day 1	day 3	day 5	day 1	day 3	day 5

N.D., Not Determined.
[a] Values represent one analysis as the different organs and tissues of the rats (n = 3, for each day) were pooled and processed together.
[b] Values represent the mean ± s.d. (n = 3, for each day).

aqueous or methanolic extracts was dissolved in 10 mL of 0·2 M sodium acetate buffer pH 5·0, followed by an extraction of rhein with chloroform. After removal of the remainder of the organic solvent under reduced pressure, the aqueous layer was incubated at 37 °C for 10 h with 1 mL of a solution of 20 mg β-glucuronidase/5 mL buffer (β-D-glucuronide-glucuronosohydrolase, EC 3.2.1.31, from bovine liver, Sigma) or 20 mg sulphatase/5 mL buffer (arylsulphate sulfohydrolase, EC 3.1.6.1., from limpets, Sigma). The extraction of enzymic cleavage products was performed with chloroform.

Identification of rhein. Extracts of faeces from animals treated with unlabelled rhein or rhein anthrone were subjected to preparative TLC (layer thickness 0·5 mm, silica gel, Merck; solvent: ethylacetate–methanol–water (77:13:10 v/v)). After development, the yellow spot at R_F 0·33 was eluted with methanol from the plates and subjected to mass spectrometry (HP 5995 A gas chromatograph/mass spectrometer). The resulting spectrum was compared with that of rhein.

Results

Urinary and faecal recovery rate. The recovery of radioactivity in urine amounted to 37(±8·3)% for [^{14}C]rhein and 2·8(±0·4)% for [^{14}C]rhein anthrone after five days (n = 5). With [^{14}C]rhein 32(±7·3)% of the injected ^{14}C activity was excreted during the first 24 h; on the subsequent 4 days only 4·7% was recovered.

The total recovery in faeces rose to 53(±9·5)% for [^{14}C]-rhein after five days, the second day being the period of major excretion of radioactivity (28·5%). For [^{14}C]rhein anthrone the recovery was 95(±10·1)%, the first day being the phase of major excretion (87%). Fig. 1 shows urinary and faecal cumulative excretion of ^{14}C activity for [^{14}C]rhein and [^{14}C]rhein anthrone as a percentage of the given dose (n = 5).

Fig. 2 shows the sum of urinary and faecal cumulative excretion which attained 90(±3·6)% and 97·5(±10·0)%, respectively, for [^{14}C]rhein and [^{14}C]rhein anthrone after five days (n = 5).

Distribution. Table 1 presents the values for recovered radioactivity expressed in terms of μg rhein equivalents or rhein anthrone equivalents per gram wet tissue ($\mu g\ g^{-1}$). After administration of both compounds, almost all organs and tissues showed a

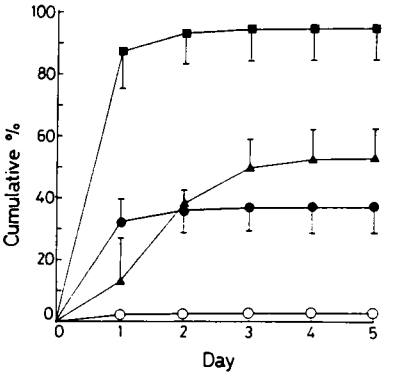

FIG. 1. Cumulative excretion of radioactivity after intracaecal administration of [^{14}C]rhein in faeces (▲) and urine (●), or [^{14}C]rhein anthrone in faeces (■) and urine (○). Mean values (±s.d.) are shown (n = 5).

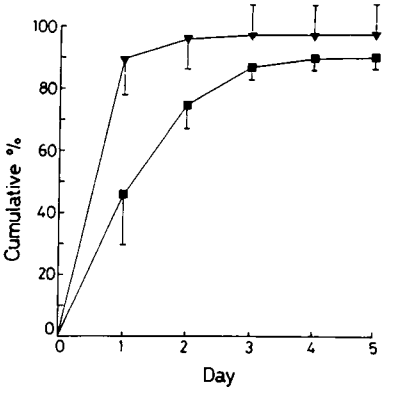

FIG. 2. Sum of urinary and faecal cumulative excretion of radioactivity after intracaecal administration of [^{14}C]rhein (■) or [^{14}C]rhein anthrone (▼). Mean values (±s.d.) are shown (n = 5).

significant clearance of ^{14}C activity within three days. One day after administration of radioactive rhein, only kidney- (5·38 $\mu g\ g^{-1}$), spleen- (3·93 $\mu g\ g^{-1}$) and lung tissue (3·27 $\mu g\ g^{-1}$) exhibited a high initial level. For lung and spleen a substantial (85%) decrease in ^{14}C activity was observed after three days, while for the kidney the loss was much smaller (36%). The kidneys of rats

treated with [^{14}C]rhein anthrone had even more pronounced radioactive levels after one day (8·61 μg g^{-1}). For both compounds no increase in activity in any organ or tissue was found after 24 h.

One day after administration of [^{14}C]rhein and especially [^{14}C]rhein anthrone, all organs and tissues showed a clear increase in [^{14}C] levels in comparison with their plasma concentrations.

Metabolites. Table 2 shows the total amount of radioactivity recovered, as rhein and as NIF (non-identified fractions), after extraction of faeces by methanol in a Soxhlet apparatus for 27 h and boiling water for 1 h. Results are given as percent of the total

Table 2. The total amount of recovered, as rhein and NIF, and not extracted radioactivity, from faeces after extraction by methanol in a Soxhlet apparatus for 27 h and boiling water for 1 h. Faeces were pooled for 24 h from 3 rats which received [^{14}C]rhein anthrone, and for 3 days from 3 rats which received [^{14}C]rhein. Results are given as percent of the total faecal radioactivity.

	Faeces from rats treated with	
	[^{14}C]rhein recovery %	[^{14}C]rhein anthrone recovery %
Total Recovery (methanol)	25·5	23·7
—as rhein	14·6	15·9
—as NIF	10·9	7·8
Total Recovery (water)	15·0	12·3
—as rhein	4·0	1·2
—as NIF	11·0	11·1
Not extracted	59·5	64·0

NIF, Non-Identified Fractions.

[^{14}C] content of faeces pooled from three rats treated with [^{14}C]rhein or [^{14}C]rhein anthrone.

The radioactivity extracted from the faeces of rats treated with [^{14}C]rhein or [^{14}C]rhein anthrone with methanol during 27 h, amounted to 25·5% and 23·7%, respectively. Thin layer chromatograms of those extracts revealed a major radioactive yellow spot (R_F 0·33), which developed a red colour after the plates were sprayed with an alkaline solution. In non-radioactive experiments, this compound was identified as rhein by mass spectrometry.

Other radioactive spots were detected, but none of them developed a colour after alkaline treatment. These metabolites remained unidentified (NIF: non identified fraction). The procedure with boiling water released another 15% and 12·3% in the case of [^{14}C]rhein or [^{14}C]rhein anthrone, respectively.

After enzymic treatment of all faecal extracts, no significant amounts of radioactivity were extracted by chloroform, and cleavage products were not detected.

Discussion

Subsequent to intracaecal administration of [^{14}C]rhein, the radioactivity proved to be almost entirely excreted in urine and faeces over five days. Thus the discrepancy between this and the earlier study of Lemmens & Dreessen (1979) might be explained in terms of the different analytical procedures: in particular the recovery of ^{14}C vs chemical methods based on spectrodensitometry after extraction. Although the recovery in urine by chemical methods (ca 17%) tends to underestimate the actual urinary ^{14}C excretion, attention must be paid to the difference between ^{14}C faecal excretion and that recorded using chemically-detected anthranoids after extraction of faeces (ca 1·7%).

This distinct difference can be explained partly by the fact that even after a long extraction, the recovery of metabolites from faeces is incomplete and inadequate. This is probably due to the low solubility of those compounds and/or strong adsorption between faecal constituents (e.g. cellulose, lignin) and the metabolites. On the other hand, rhein is degraded to compounds which are no longer chemically detectable by the analytical procedure for 1,8-dihydroxyanthraquinones, i.e. the development of a red colour in an alkaline solution, followed by a spectrophotometric or spectrodensitometric measurement (Thomson 1971; Lemmens 1979). Moreau et al (1985) have demonstrated a bacterial degradation of rhein after incubation in-vitro with mice intestinal microflora. This confirms the results of Bachmann & Schlatter (1981), who demonstrated radioactive non-anthraquinone fractions in substantial quantities in faecal extracts.

The rapid oxidation of anthrone to anthraquinone in boiling methanol or water, accounts for the recovery of [^{14}C]rhein in the faecal extracts of rats treated with [^{14}C]rhein anthrone. Rhein anthrone in those extracts could no longer be detected, although the molecule was present in freshly produced faeces. The ^{14}C activity of injected rhein anthrone is excreted more effectively over a five day period than is [^{14}C]rhein, although the route is different. Indeed, with anthrone almost all the ^{14}C activity is excreted in the faeces compared with that in urine. To explain this, the much firmer and faster laxative effect of rhein anthrone (20 mg kg^{-1}) compared with rhein (25 mg kg^{-1}) should be taken into consideration; this gives the anthrone less time and opportunity to be absorbed in its passage through the colon. Also the high chemical reactivity of the anthrone could prevent the ^{14}C activity being absorbed due to linkage with high molecular weight compounds present in faeces or self-decomposition into a non absorbable material.

A well-defined clearance of radioactivity was observed for plasma and almost all tissues and organs from [^{14}C]rhein- and [^{14}C]rhein anthrone-treated rats. No accumulation could be found in mesenterium or fat tissue later than one day after administration of ^{14}C compounds, which is not in agreement with Bachmann & Schlatter (1981). Probably this is explained in terms of the more hydrophilic properties of rhein and the corresponding anthrone. This result also contradicts the hypothesis that anthranoids might possess a specific organotropy for mesenterium tissue, as Bachmann & Schlatter (1981) found with emodin.

It is clear that the kidney has the ability to store rhein or rhein derivatives: ^{14}C activity is maintained at high levels even when urinary excretion is complete, and this is especially the case for [^{14}C]rhein anthrone where only a small fraction of the given dose is excreted in urine. These data are in accordance with those of Bachmann & Schlatter (1981), who reported a high level of radioactivity in the kidney after five days. Combined autoradiographical and pathohistological studies should give more information on the question surrounding renal impairment after prolonged laxative abuse, caused perhaps by a direct action of anthranoids on kidney tissue, especially when present in their anthrone state.

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Antihistaminic and anticholinergic activities of mequitazine in comparison with clemizole

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Abstract—The antihistamine and anticholinergic properties of mequitazine have been investigated and compared with those of clemizole. Both mequitazine and clemizole antagonized the effect of histamine in guinea-pig ileum competitively, the pA_2 values calculated by Schild plot were 9.95 ± 0.44 for mequitazine and 10.54 ± 0.44 for clemizole. Mequitazine at 10^{-7} M produced a parallel shift of the dose-response curve to acetylcholine in the rat duodenum, clemizole and the lower doses of mequitazine failed to modify the effect of acetylcholine. The potency of mequitazine and clemizole as H_1-histamine blockers is similar, but only mequitazine at highest concentration used showed anticholinergic activity.

There has been a resurgence of interest in H_1-antihistamines and their clinical effects. The new antihistamine drugs such as astemizole, terfenadine or mequitazine have in common the property of crossing the blood-brain barrier with difficulty, and thus they do not cause sedation in usual dosages (Brandon 1985). Blockade of H_1-receptors is of particular therapeutic value in the treatment of several allergic symptoms. Studies to establish the clinical efficacy of these drugs have frequently been carried out, but in contrast, little attention is paid to in-vitro studies on the action of the new antihistamine drugs on the smooth muscle, and usually the information concerning their pharmacological characteristics is not available.

In this communication we describe the effect of mequitazine, a phenothiazine derivative (Fujimura et al 1981; Kanba & Richelson 1984), on the contraction induced by histamine and acetylcholine in the intestinal smooth muscle of the guinea-pig and rat in-vitro. The results are compared with those obtained with clemizole, a well-known anti-H_1 agent, in the same preparations.

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Materials and methods

Adult guinea-pigs, 350–450 g, and Wistar rats, 200–250 g, were used. Segments of ileum of the guinea-pig and of duodenum of the rat were mounted in a 20 mL organ bath containing a Tyrode solution at 37 °C of the following composition (M): NaCl 136, KCl 2·7, $CaCl_2$ 1·4, $MgSO_4$ 0·04, KH_2PO_4 0·4, $NaHCO_3$ 11·9 and glucose 5·6, aerated with 5% CO_2 in O_2. Changes in length were recorded by means of an isotonic transducer (Ugo Basile 7004) and a Ugo Basile (model Gemini 7070) recorder.

The preparations were allowed to equilibrate under a load of 1 g for at least 30 min before any drug was added. Concentration response curves to histamine (ileum) or acetylcholine (duodenum) in the absence or presence of antihistamine drugs were obtained by adding the agonist drugs cumulatively. Antagonists were incubated for 15 min.

Contractile responses to agonists were expressed as a percentage of the maximum response obtained. Effective concentration 50% (EC50) was calculated graphically from a plot of log concentrations vs percentages of the maximum response (Emax) produced by each agonist in individual experiments.

The calculation of the pA_2 values was according to Arunlakshana & Schild (1959) as described previously (Aguilar et al 1986). All data are shown as mean ± standard error of the mean. Statistical analysis of the data was carried out using Student's *t*-test at a 5% significance level.

The drugs used were acetylcholine hydrochloride and histamine dihydrochloride (Sigma Co.); clemizole hydrochloride (Schering España, S.A.) and mequitazine hydrochloride (Rhône Poulenc Farma S.A.E.).

Results

Histamine (6.5×10^{-8} to 1.08×10^{-3} M) induced concentration