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EFFECTS OF NON-STEROID ANTIRHEUMATIC AGENTS ON MICROSOMAL DRUG-METABOLIZING ENZYMES OF RAT LIVER*

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Abstract—The effects of several non-steroid antirheumatic drugs on ascorbic acid excretion in urine, hexobarbital sleeping time, liver fresh weight, ascorbic acid concentration in the liver and amidopyrine *N*-demethylation by 15,000 *g* liver supernatant of the rat were investigated. The drugs tested were acetylsalicylic acid, amidopyrine, benzydamine, flufenamic acid, indomethacin, mefenamic acid, sodium salicylate and salicylamide. There are differences in effects between various agents. Of the eight drugs tested, only amidopyrine increased microsomal liver enzyme activities as phenobarbital.

Neither phenobarbital-like induction nor inhibition of phenobarbital induction are common properties of non-steroid antirheumatic drugs.

NUMEROUS chemically heterogenous drugs induce microsomal liver enzymes.[1] Among them, phenylbutazone and amidopyrine are antirheumatic drugs.[2] There seem to be no systematic investigations on induction of liver enzymes by non-steroid antirheumatic drugs. Nor is it known whether non-steroid antirheumatic drugs may inhibit enzyme induction by other drugs, for instance by phenobarbital.

Phenobarbital is a strong inducer of microsomal liver enzymes.[3] Therefore the effects of several clinically used antirheumatic drugs on various parameters of microsomal liver enzyme induction (see Ref. 4) have been investigated in the rat: (1) ascorbic acid excretion as a measure of the activity of the glucuronic acid system[5] in the liver. It is increased severalfold by treatment with phenobarbital;[6] (2) ascorbic acid concentration in the liver; it was found increased after treatment with barbital;[7] (3) liver fresh weight; it is increased by treatment with inducing drugs;[8] (4) hexobarbital sleeping time as a measure of hexobarbital biotransformation *in vivo*. It is strongly shortened by phenobarbital treatment;[3,9] (5) amidopyrine *N*-demethylation by liver supernatant. It is markedly increased by phenobarbital treatment (see Ref. 10). Steroid antirheumatic drugs were not included in the experiments reported here because of their different induction pattern.[11–13]

EXPERIMENTAL

Materials

The following drugs were used: acetylsalicylic acid, salicylamide (VEB Promassolwerk Erfurt), amidopyrine (aminophenazon DAB 7), sodium salicylate, hexobarbital

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sodium, phenobarbital sodium (all DAB 7 quality), indomethacin,* benzydamine-HCl,* flufenamic acid* and mefenamic acid.*

Animals

Male, 30–35-day-old Wistar rats (Jena) were used. At this age, enzyme induction is produced more easily than at other age groups.[10] Rats were bred in litters of six animals each and were weaned after 28 days. Standard diet (Arbeitsgemeinschaft Versuchstierzucht Berlin) and drinking water were available without limitation. Room temperature was 22–24°, air humidity > 50 per cent.

Regimen of trials and methods

Animals were randomized and divided into four groups for each experiment. One group received the antirheumatic drug, another group received phenobarbital, the third group received both antirheumatic drug and phenobarbital. The control group was treated with a solution of 1% tylose in water.

Drugs were given on 3 following days, once daily between 10 and 11 a.m. All agents were administered i.p. dissolved or suspended in a 1% tylose solution.

The daily doses of the antirheumatic drugs were 50 per cent of the acute i.p. LD_{50}, i.e. acetylsalicylic acid 250 mg/kg body weight,[14] amidopyrine 125 mg/kg,[14] benzydamine-HCl 55 mg/kg,[15] flufenamic acid 225 mg/kg†,[16] indomethacin 6 mg/kg,[17] mefenamic acid 80 mg/kg,[18] sodium salicylate 300 mg/kg‡ and salicylamide 300 mg/kg.[19] The injection volume was 2 ml/100 g.

Immediately after the injection the animals were placed over funnels with sieve insets. Urine was collected during 5 hr after the third injection in order to determine ascorbic acid.[7]

On the fourth day, the sleeping time after i.p. injection of 100 mg/kg hexobarbital-sodium was determined.[20] Then the animals were decapitated under ether anaesthesia. Livers were removed and weighed. After excision of suitable amounts of liver tissue for ascorbic acid determination,[7] livers were placed into ice-cold phosphate buffer and homogenized 30 min later in a Potter–Elvehjem glass homogenizer with teflon pestle. The activity of the amidopyrine *N*-demethylase was determined in 15,000 *g* liver supernatant according to the method of McMahon.[21]

One ml liver supernatant was added to 1 ml of a solution containing 0·5 μmoles NADP, 11 μmoles glucose-6-phosphate, 25 μmoles nicotinic acid amide, 25 μmoles $MgCl_2$, 45 μmoles semicarbacide and 125 μmoles phosphate buffer of pH 7·4. Reaction was started by addition of 60 μmoles amidopyrine in 1 ml aqueous solution. The final volume was 3 ml. The open flasks were incubated for 20 min at 37° in a shaking apparatus. After incubation, the formaldehyde liberated into the reaction mixture was determined according to the method of Cochin and Axelrod.[22] Two ml of double-strength Nash-reagent were added to each flask. Then the mixture was boiled in a water bath and centrifuged 10 min at 6000 *g*. The formed dye was estimated colorimetrically at 412 nm in the supernatant. Blanks contained water instead of amidopyrine. Standard flasks contained all components except amidopyrine, as well as

* For kind supply of drugs we wish to thank: Sharp & Dohme GmbH München for indomethacin Troponwerke Köln-Mülheim for benzydamine-HCl and Parke, Davis & Co. München, for flufenamic acid and mefenamic acid.

† 50 per cent of the oral LD_{50}.

‡ H. HOFFMANN, personal communication.

known amounts of formaldehyde; they were incubated in the same manner. Protein was determined by the biuret method using bovine serum albumine as standards; all the experiments have been carried out between September and March.

RESULTS

There are both activating and inhibiting effects of non-steroid antirheumatic drugs on the investigated parameters.

(1) *Ascorbic acid excretion in urine*

In all experiments (Table 1) phenobarbital increased ascorbic acid excretion significantly. Acetylsalicylic acid, amidopyrine, flufenamic acid, mefenamic acid and sodium salicylate increased ascorbic acid excretion, too. Acetylsalicylic acid, amidopyrine and mefenamic acid significantly intensified the effect of phenobarbital.

(2) *Ascorbic acid concentration in the liver*

Ascorbic acid concentration in the liver was found to be the least reliable parameter of enzyme induction. Phenobarbital significantly increased ascorbic acid concentration only in four out of eight experiments. The increase was small. In two experiments, phenobarbital had no influence, in one trial it had a lowering effect. Acetylsalicylic acid and mefenamic acid significantly lowered ascorbic acid concentration in the liver.

(3) *Liver fresh weight*

Liver fresh weight of control animals varied considerably. Phenobarbital increased liver fresh weight in three of eight trials significantly. The effects of the antirheumatic drugs were not statistically significant.

(4) *Hexobarbital sleeping time*

In all experiments (Table 2) phenobarbital reduced hexobarbital sleeping time significantly. Only amidopyrine acted like phenobarbital. Benzydamine-HCl lengthened hexobarbital sleeping time. Indomethacin depressed the phenobarbital effect.

(5) *Amidopyrine* N-*demethylation*

The inducing effect of phenobarbital could be demonstrated in all experiments (Table 3). Acetylsalicylic acid and mefenamic acid decreased, amidopyrine and benzydamine-HCl increased amidopyrine *N*-demethylation moderately. The phenobarbital effect was not influenced by any of the drugs tested.

DISCUSSION

Enzyme induction by phenylbutazone and amidopyrine is known for several years.[2] The effects resemble those of phenobarbital and partially of methylcholanthrene.[4] The present investigations on enzyme induction and inhibition by non-steroid antirheumatic agents have been stimulated by these known effects of pyrazolone derivatives.

From the tables it is evident that enzyme induction is no common property of antirheumatic drugs. Further, it is evident that non-steroid antirheumatic drugs generally do not inhibit induction by phenobarbital.

Table 1. Ascorbic acid excretion in the urine of the rat in mg/5 hr per 100 g body weight after 3 days pretreatment with antirheumatic drugs and/or phenobarbital

Control group (1 % tylose)	Treatment with phenobarbital	Tested drugs	Treatment with antirheumatic drugs alone	Combined treatment with antirheumatic drugs and phenobarbital
0·087 ± 0·012 (8)	0·522 ± 0·073 (8)*	Indomethacin	0·067 ± 0·005 (9)	0·513 ± 0·090 (9)
0·059 ± 0·007 (7)	0·261 ± 0·065 (8)*	Acetylsalicylic acid	0·156 ± 0·020 (7)*	0·743 ± 0·080 (8)†
0·053 ± 0·021 (8)	0·425 ± 0·097 (8)*	Sodium salicylate	0·257 ± 0·048 (6)*	0·583 ± 0·049 (8)
0·165 ± 0·021 (8)	0·513 ± 0·094 (7)*	Salicylamide	0·213 ± 0·051 (8)	0·635 ± 0·181 (7)
0·098 ± 0·011 (7)	0·188 ± 0·036 (7)*	Mefenamic acid	0·182 ± 0·025 (8)*	0·553 ± 0·060 (7)†
0·148 ± 0·028 (8)	0·346 ± 0·055 (7)*	Amidopyrine	0·673 ± 0·133 (8)*	1·154 ± 0·152 (7)†
0·091 ± 0·013 (8)	0·421 ± 0·077 (8)*	Flufenamic acid	0·164 ± 0·028 (6)*	0·322 ± 0·094 (6)
0·281 ± 0·058 (7)	0·603 ± 0·059 (7)*	Benzydamine-HCl	0·137 ± 0·058 (7)	0·910 ± 0·158 (7)

Means ± S.E.M.

* Significant difference ($P \leq 0.05$) to the control group.

† Significant difference ($P \leq 0.05$) to the phenobarbital group. Number of animals in brackets. The sequence of drugs corresponds to the chronological sequence of experiments.

TABLE 2. SLEEPING TIME OF RATS IN min AFTER i.p. INJECTION OF 100 mg/kg BODY WEIGHT HEXOBARBITAL SODIUM SALT

Control group (1% tylose)	Treatment with phenobarbital	Tested drugs	Treatment with antirheumatic drugs alone	Combined treatment with antirheumatic drugs and phenobarbital
45·0 ± 2·6 (5)	8·0 ± 2·5 (8)*	Indomethacin	53·0 ± 8·5 (8)	15·0 ± 2·1 (9)†
67·3 ± 5·7 (6)	17·3 ± 1·0 (7)*	Acetylsalicylic acid	81·5 ± 8·2 (8)	15·9 ± 0·7 (8)
57·0 ± 4·6 (8)	14·0 ± 1·2 (8)*	Sodium salicylate	61·1 ± 3·9 (7)	10·8 ± 0·4 (8)†
49·0 ± 2·5 (8)	11·9 ± 0·3 (7)*	Salicylamide	45·5 ± 1·8 (8)	8·9 ± 0·5 (7)†
45·4 ± 2·8 (7)	7·0 ± 1·2 (7)*	Mefenamic acid	52·3 ± 4·1 (8)	8·0 ± 0·6 (7)
46·5 ± 1·4 (8)	13·4 ± 1·3 (7)*	Amidopyrine	25·1 ± 1·4 (8)*	11·1 ± 0·3 (7)
53·6 ± 4·0 (8)	9·1 ± 2·3 (8)*	Flufenamic acid	43·0 ± 6·0 (7)	9·1 ± 1·9 (7)
35·9 ± 6·7 (7)	11·1 ± 2·1 (7)*	Benzydamine-HCl	56·0 ± 2·3 (8)*	10·1 ± 1·7 (7)

* Pretreatment and statistics see Table 1.

Table 3. Amidopyrine *N*-demethylation by 15,000 *g* supernatant of rat livers in μmoles formaldehyde. min^{-1}. g $protein^{-1}$

Control group (1% tylose)	Treatment with phenobarbital	Tested drugs	Treatment with antirheumatic drugs alone	Combined treatment with antirheumatic drugs and phenobarbital
0·88 ± 0·14 (5)	2·98 ± 0·40 (6)*	Indomethacin	0·75 ± 0·05 (6)	2·57 ± 0·28 (6)
0·55 ± 0·08 (6)	2·06 ± 0·28 (6)*	Acetylsalicylic acid	0·27 ± 0·06 (6)*	1·66 ± 0·11 (6)
1·19 ± 0·11 (6)	4·99 ± 0·41 (5)*	Sodium salicylate	0·93 ± 0·11 (6)	4·36 ± 0·14 (6)
1·39 ± 0·15 (6)	4·56 ± 0·41 (6)*	Salicylamide	1·06 ± 0·18 (6)	4·59 ± 0·25 (6)
2·59 ± 0·13 (6)	6·28 ± 0·32 (6)*	Mefenamic acid	2·09 ± 0·16 (6)*	5·99 ± 0·25 (6)
2·11 ± 0·02 (6)	4·35 ± 0·18 (6)*	Amidopyrine	2·63 ± 0·16 (6)*	4·46 ± 0·16 (6)
1·30 ± 0·07 (6)	3·15 ± 0·22 (6)*	Flufenamic acid	1·09 ± 0·14 (6)	3·54 ± 0·23 (6)
0·65 ± 0·04 (6)	1·54 ± 0·09 (6)*	Benzydamine-HCl	0·77 ± 0·04 (6)*	1·66 ± 0·07 (6)

* Pretreatment and statistics see Table 1.

There is no adequate explanation for the considerable variability of the control values. All experiments have been performed at the same time of day. Animals were always randomized. Seasonal fluctuations could be excluded.

There are different patterns of action of the various antirheumatic drugs. Only amidopyrine proved to be a phenobarbital-like inducer of microsomal liver enzymes. The observed effects of amidopyrine agree with published dates (see Ref. 23). Enzyme induction and suppression of enzyme synthesis by the same drug do not exclude one another.[24] Whitehouse[25] emphasized the uncoupling actions of anti-inflammatory agents. According to his theory antirheumatic drugs ought to be inhibitors of enzyme induction. If the ATP level in the cells decreases, protein *de novo* synthesis by phenobarbital induction[26] should be inhibited. Ethionin acts as an inhibitor of enzyme induction by decreasing the ATP-level.[27]

The suppression of antibody formation induced[28] by antigens might be an important property of antirheumatic drugs considering possible immunological etiology of rheumatic diseases. Non-steroid antirheumatic drugs inhibit protein synthesis in lymphocytes.[29] In the present investigations, however, no inhibition of phenobarbital induction by antirheumatic drugs could be shown.

Recently, Houck *et al.*[30] demonstrated induction of collagenolytic activity in the skin of rats by indomethacin and oxyphenbutazone. *De novo* synthesis of enzyme protein occurs in fibroblasts.[31] Acetylsalicylic acid, indomethacin, phenylbutazone and flufenamic acid increase the activity of histidine decarboxylase in the rat stomach *in vivo*.[32] In both cases, the mechanism of action resembles that of glucocorticoids.

Further investigations are in progress to elucidate whether non-steroid antirheumatic drugs influence the activity of soluble enzymes of the rat liver or interfere with enzyme induction by hydrocortisone.

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