Biochemical Pharmacology, Vol. 17, pp. 1501–1509. Pergamon Press. 1968. Printed in Great Britain

THE INFLUENCE OF SODIUM SALICYLATE ON THE METABOLISM OF COLLAGEN IN THE LATHYRITIC RAT

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(*Received* 25 *January* 1968; *accepted* 13 *March* 1968)

Abstract—In rats treated simultaneously with beta-aminopropionitrile (BAPN) 70 mg/kg and with 300 mg/kg sodium salicylate dose the metabolism of collagen was studied by determining the specific and total activities of ^{14}C-hydroxyproline in collagen fractions of skin and in urine after the injection of ^{14}C-proline. BAPN and sodium salicylate was administered daily for 56 days, the ^{14}C-proline was injected on 28th day of experiment. At the dose used BAPN produced in untreated animals a heavy degree of lathyritic changes with depression of collagen metabolism, and the well known defect manifested by slower conversion of extractable into insoluble collagen. Simultaneous treatment with sodium salicylate resulted in a partial return to normal of these changes, e.g. elevation of metabolic turnover of collagen with faster maturation of collagen.

EXPERIMENTAL lathyrism is the most widely investigated molecular disease of connective tissue, manifested by defects of the growth of cartilage and bones, generalised weakness of connective tissue and formation of aortal aneurysms.[1] The main effect of these changes is an increase in extractable fractions of collagen proteins in tissues, which is the result of the inhibition of covalent inter- and intramolecular cross-links by lathyrogens.[2, 3] It has recently been postulated that lathyrogens may exert their effect by inhibition of amine oxidase necessary for deamination of lysyl side chains of collagen participating in cross-link formation. The collagen molecule is intrinsically normal.[4, 5] Its biosynthesis is either increased,[6] unchanged[7, 8, 9] or it may be toxically decreased, depending on the dose and duration of administration of the lathyrogen.[10, 11]

Some substance of the group of hormones and antirheumatic drugs—especially salicylates—have a beneficial influence on changes in experimental lathyrism, which fact is manifested by a decrease of the elevated proportion of extractable collagen in tissues and normalization of histologic changes.[12–14]

We have previously shown[15] that the effect of chronic administration of sodium salicylate in doses 300 mg/kg is a speeding up of the metabolic turnover of collagen. In the present study an attempt was made to verify the influence of sodium salicylate, this time on damaged metabolism of collagen. With the dose of BAPN—70 mg/100 g, administered for a prolonged time—56 days, we intentionally brought about a high degree of lathyritic defect. Lathyritic changes were manifested not only with the great retardation of the intra- and intermolecular bonds formation, but also by a decrease of collagen biosynthesis.

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MATERIAL AND METHODS

Sixty male albinos rats of an initial weight of 80 g were divided into three groups of 20 animals each. The first, control group was fed a standard diet—Altromine + 10 g forcemeat for each animal. Physiologic solution was administered s.c. twice a day. The second—lathyritic—group was fed Altromine + 10 a forcemeat mixed with 70 mg BAPN/100 g body wt. Physiologic solution was administered to this group twice a day s.c. The third group—lathyritic—treated with sodium salicylate, was fed like the lathyritic group, 300 mg sodium salicylate/kg body wt. being administered s.c. The daily dose was divided into two portions so as to keep the salicylate level in the body as constant as possible.

On the 28th day the experiment, to each rat was administered 10 μc of uniformly labelled ^{14}C-proline/100 g body wt. The administration of BAPN and sodium salicylate was continued for a total of 28 days after administration of the isotope. Twenty four-hr urine was collected from 4 rats each time from every group at intervals of 1, 7 and 28 days after administration of the isotope. Four rats from each group—control, lathyritic and sodium salicylate treated lathyritic—were sacrificed at intervals of 0·5; 1; 3; 7 and 28 days after administration of ^{14}C-proline.

Neutral-salt extractable collagen (NSC)—was isolated from the rat skins with 0·45 M NaCl pH 7·4, *acid-extractable collagen* (ASC) was isolated with 0·066 M Na-citrate buffer pH 3·7 and the residue after extraction was denoted *insoluble collagen* (ISC). In all extracts and with every rat, as well as in all the urine samples, the hydroxyproline content was determined according to Stegemann[16] and ^{14}C-hydroxyproline specific activity was determined by means of the Juva and Prockop method[17] in the modification of Nordwig, Kobrle and Pfab.[18] (For a detailed description of the methods, see ref. 15). Hydroxyproline content of the 24-hr urine sample was determined according to Prockop and Udenfriend[19] and sp. act. of ^{14}C-hydroxyproline was assayed by the modified method of Juva and Prockop.

The sedimentation measurements were carried out in analytical centrifuges (either the Spinco Model by Beckman Instruments, Passadena, U.S.A., or by Messrs. Fischer Wyss, Zürich, Switzerland) in 0·25 M sodium-citrate buffer at pH 3·7 (0·075 M with respect to sodium citrate). For examination of denatured collagen, the fractions of ASC from the control, lathyritic and lathyritic sodium salicylate—treated rats were denatured for 30 min at 40° before centrifugation.

RESULTS

The administration of the lathyrogenic agent BAPN in the dose of 70 mg/100 g and sodium salicylate in the dose 300 mg/kg was a long term process—56 days. The great degree of lathyrism was of course manifested in the lower weight of the lathyritic rats, yet the weight increment was constant in the course of the experiment (Table 1).

Yields of soluble and insoluble collagen in skin and hydroxyproline in urine

Table 2 gives a survey of the amount of collagen, isolated in the individual skin fractions. In lathyritic rats the proportion of NSC is very much increased and that of ISC decreased. This is proof of the high degree of lathyritic changes caused by the experimental treatment. In lathyritic rats, treated with sodium salicylate, the NSC amount is significantly lower than in lathyritic rats, although the amount of collagen

isolated from ISC is higher. Administration of sodium salicylate decreased the extractibility of collagen which had been increased by the effect of lathyrogens.

Hydroxyproline elimination in the urine is always increased in lathyritic rats (see Table 3, first column). In the group of sodium salicylate—treated lathyritic rats, hydroxyproline elimination was again normalised.

TABLE 1. INCREASE IN WEIGHT OF CONTROL RATS AND OF RATS TREATED WITH BAPN AND BAPN + SODIUM SALICYLATE

Days of the experiment	Days after ^{14}C-proline introduction	Number of the rats	Wt. of rats (g)			Increase in wt. (g)		
			Control	BAPN	BAPN + sodium sal.	Control	BAPN	BAPN + sodium sal.
0		25	80	80	80			
7		25	111	105	100	31	25	20
18		25	151	137	134	71	57	54
28	0·5	4	175	152	150	95	72	70
29	1	4	192	161	192	112	81	112
31	3	4	196	173	170	116	93	90
35	7	4	200	172	170	120	92	90
56	28	4	180	157	150	100	77	70

TABLE 2. YIELDS OF COLLAGEN FROM NEUTRAL SALT EXTRACTABLE, ACID EXTRACTABLE FRACTIONS AND INSOLUBLE COLLAGEN mg/100 mg TOTAL COLLAGEN

	NSC	ASC	ISC
Control *	3·6 ± 1·1	5·8 ± 2·7	80·1 ± 4·9
BAPN *	21·8 ± 3·7	7·6 ± 3·0	64·2 ± 10·7
BAPN + * sodium sal.	16·7 ± 2·3	7·5 ± 2·0	74·7 ± 12·5

* The mean of all rats in the group (20).
Results are presented as means together with confidence limits of the 5 per cent level of probability.

The incorporation of ^{14}C-proline into the collagen fractions of the skin

(a) *Specific activities.* It is to be seen from Table 4 that the sp. act's of NSC, ASC and ISC are substantially decreased in the lathyritic rats. The sp. act. of NSC and ASC achieves the control values on the seventh day after administration of the isotope, the ISC sp. act. is decreased throughout the duration of the experiment.

Treatment of lathyritic rats with sodium salicylate is mainly manifested in the sp. act. of ^{14}C-hydroxyproline in the NSC fraction, which is increased significantly in the five days following the adminstration of the labelled proline. The sp. act. of ASC is increased on the first and third day, and the sp. act. of ISC is increased on the third day after administration of the isotope (Table 5).

(b) *Total and partial activities.* The quantitative distribution of the overall amount of ^{14}C-hydroxyproline activity in the individual collagen fractions isolated from the skin, is expressed by the partial activity values. Total activity is the sum of the NSC,

ASC and ISC partial activities. All values have been calculated using 100 mg total collagen as basis.

The total and partial activity values in the group of lathyritic rats are shown in Table 6. The total activity curve in the lathyritic group has a character which is

TABLE 3. EFFECT OF BAPN AND BAPN + SODIUM SALICYLATE ON THE URINARY EXCRETION OF HYDROXYPROLINE, ON THE SPECIFIC AND TOTAL ACTIVITY OF ^{14}C-HYDROXYPROLINE AFTER THE ADMINISTRATION OF ^{14}C-PROLINE

Collection period after ^{14}C-proline	Group	Urinary hydroxyproline		
		Excretion μg/24 hr	Sp. act. cpm/μg	Total activity cpm
0–1 day (24 hr)	Control	364 (342–402)	3·56 (3·34–3·70)	1297 (1142–1447)
	BAPN	585 (512–645)	4·64 (3·20–8·20)	2659·4 (2007–4537)
	BAPN + sodium sal.	398 (332–478)	4·25 (4·00–4·50)	1519·0 (1490–1544)
6–7 day (24 hr)	Control	297 (238–388)	0·86 (0 50–1·30)	249·6 (143–356)
	BAPN	560 (425–740)	0·64 (0·70–1·00)	482·4 (408–629)
	BAPN + sodium sal.	316 (292–340)	0·58 (0·40–0·90)	303·1 (122–643)
27–28 day (24 hr)	Control	243 (218–278)	0·50 (0·30–0·57)	120·6 (93–140)
	BAPN	387 (306–425)	0·36 (0·20–0·40)	138·0 (82–193)
	BAPN + sodium sal.	262 (272–374)	0·38 (0·20–0·45)	125·6 (71–163)

TABLE 4. SPECIFIC ACTIVITIES OF THE THREE COLLAGEN FRACTIONS cpm/μg HYDROXYPROLINE

Days after ^{14}C-proline introduction	NSC		ASC		ISC	
	Control	BAPN	Control	BAPN	Control	BAPN
0·5	8·89 (8·00–10·50)	0·52 (0·20–0·81)	0·51 (0·30–0·60)	0·04 (0·037–0·044)	0·08 (0·07–0·10)	0·01 (0·008–0·018)
1	6·61 (6·45–6·88)	0·45 (0·20–0·80)	0·51 (0·39–0·69)	0·07 (0·027–0·100)	0·13 (0·10–0·15)	0·02 (0·008–0·03)
3	3·82 (3·00–4·20)	0·65 (0·43–0·90)	1·27 (0·90–1·50)	0·22 (0·090–0·300)	0·23 (0·20–0·26)	0·04 (0·02–0·07)
7	1·65 (1·40–1·70)	1·38 (0·60–2·00)	0·90 (0·40–1·20)	0·70 (0·100–0·900)	0·19 (0·17–0·20)	0·10 (0·04–0·17)
28	0·11 (0·09–0·11)	0·39 (0·20–0·72)	0·57 (0·53–0·63)	0·51 (0·410–0·600)	0·23 (0·17–0·26)	0·15 (0·14–0·16)

completely different from that of the control group. On the first 4 days after administration of the isotope the activity is very low, but on the 7th day it is on the contrary higher than in the controls, decreasing later to the control value level. The NSC partial activity takes a similar course. The very low initial values approach normal values on the third day of the experiment, rising later to a level above the control values and remaining elevated up to the end of the experiment.

TABLE 5. SPECIFIC ACTIVITIES OF THE THREE COLLAGEN FRACTIONS cmp/μg HYDROXYPROLINE

Days after ^{14}C-proline introduction	NSC		ASC		ISC	
	BAPN	BAPN + sodium sal.	BAPN	BAPN + sodium sal.	BAPN	BAPN + sodium sal.
0·5	0·52 (0·20–0·81)	1·19 (0·90–1·50)	0·04 (0·037–0·044)	0·05 (0·02–0·07)	0·01 (0·008–0·018)	0·01 (0·007–0·013)
1	0·45 (0·20–0·80)	1·63 (1·40–2·11)	0·07 (0·027–0·100)	0·20 (0·08–0·30)	0·02 (0·008–0·030)	0·04 (0·010–0·080)
3	0·65 (0·43–0·90)	1·36 (1·10–1·30)	0·22 (0·090–0·300)	0·40 (0·30–0·46)	0·04 (0·020–0·070)	0·11 (0·100–0·110)
7	1·38 (0·60–2·00)	1·34 (1·22–1·50)	0·70 (0·100–0·900)	0·67 (0·50–0·180)	0·10 (0·040–0·170)	0·11 (0·080–0·140)
28	0·39 (0·20–0·72)	0 (0–0)	0·51 (0·410–0·600)	0·39 (0·36–0·45)	0·15 (0·140–0·160)	0·13 (0·120–0·140)

The partial activity of ASC also is very low, attaining the control values on the seventh day only and continuing to rise later. On the other hand, the ISC partial activity is decreased throughout the experiment.

Table 7 shows a comparison of the course of total and partial activities in the lathyritic and sodium salicylate treated lathyritic group. The total activity values are increased in the sodium salicylate treated group. On the seventh day the activities of the two groups are equal, in the further course of the experiment the total activity of the treated group decreases more rapidly than that of the lathyritic group. The same course is observed with the NSC partial activity. Partial activities of ISC and ASC are increased in the treated group from the first to the 7th day after administration of the isotope, later there is no difference between the two groups.

Specific and total activities of urine hydroxyproline

Table 3. The specific and total activity of the lathyritic group is increased in comparison to the control group only during the first 24 hr after administration of ^{14}C-proline. The total activity continues to be increased on the 7th day. Differences between the lathyritic and sodium salicylate treated group are very small, only during the first 24 hr is the total ^{14}C-hydroxyproline activity lower in the treated lathyritic group than in to the non-treated lathyritic group.

Sedimentation measurements

Acid-extractable collagen (ASC), isolated from the control group of rats and denatured for 30 min at 40° gives a typical sedimentation diagramm of collagen in

TABLE 6. TOTAL AND PARTIAL ACTIVITIES cpm/100 mg COLLAGEN

Days after ^{14}C-proline introduction	Total activity		NSC		ASC		ISC	
	Control	BAPN	Control	BAPN	Control	BAPN	Control	BAPN
0·5	7157 (6240–8860)	2433 (1160–3480)	5263 (4570–5800)	1502 (797–2540)	417 (255–567)	36 (23–56)	1001 (700–1240)	85 (28–170)
1	7060 (5500–8880)	1886 (1060–3520)	4280 (3770–4790)	1653 (945–3040)	881 (565–905)	92 (39–194)	1615 (1095–1710)	147 (64–282)
3	5596 (4700–6880)	2735 (1990–3440)	2002 (1805–2330)	1919 (1624–2420)	751 (496–974)	172 (104–299)	2780 (2520–3170)	400 (232–726)
7	3126 (2170–3700)	5154 (2940–7350)	554 (447–685)	3541 (2350–4810)	450 (225–604)	435 (206–625)	2250 (2130–2580)	732 (354–1400
28	3247 (2630–3790)	3302 (2270–4310)	61 (49–83)	614 (408–820)	486 392–565)	732 (477–1060)	2543 (2120–2900)	1493 (1172–1865)

TABLE 7. TOTAL AND PARTIAL ACTIVITIES cpm/100 mg COLLAGEN

Days after ^{14}C-proline introduction	Total activity		NSC		ASC		ISC	
	BAPN	BAPN + sodium sal.	BAPN	BAPN + sodium sal.	BAPN	BAPN + sodium sal.	BAPN	BAPN + sodium sal.
0·5	2433 (1160–3480)	3040 (2000–3700)	1502 (797–2540)	2877 (1890–3540)	36 (23–56)	39 (15–54)	85 (28–170)	125 (95–171)
1	1886 (1060–3520)	4902 (3100–7350)	1653 (945–3040)	4282 (2850–6140)	92 (39–194)	385 (114–772)	147 (64–282)	407 (171–805)
3	2735 (1990–3440)	4767.. (4000–6450)	1919 (1624–2420)	3405 (2400–4930)	172 (104–299)	385 (296–502)	400 (223–726)	1111 (1075–1130)
7	5154 (2940–7350)	4946 (4150–5500)	3541 (2350–4810)	3233 (2760–3500)	435 (206–625)	705 (545–815)	732 (354–1400)	1230 (1140–1320)
28	3302 (2270–43·10)	2087 (1930–2260)	614 (408–820)	0 (0–0)	732 (477–1060)	454 (237–478)	1493 (1172–1865)	1635 (1472–1690)

ultracentrifugation measurements: the components α and β are present in approximately equal ratios (Fig. 1(a)). It is known that in ASC isolated from the lathyritic group of rats the β-component is decreased as a consequence of a defect in the formation of cross-links due to the lathyrogenic factor—BAPN (Fig. 1(b)). In the group of lathyric rats, treated with sodium salicylate, ASC contains a higher amount of β-component in comparison with lathyritic ASC (Fig. 1(c)).

DISCUSSION

The influence of lathyrogens on the rate of collagen synthesis depends on their dosage and duration of administration. Radio-isotope studies have shown in lathyric *chick embryo* a normal rate of synthesis,[7-9] some reports have suggested that collagen synthesis may be retarded[10, 11] or accelerated.[20] *In young rats*, an accelerated collagen synthesis has been found after a dosage of 0·2% BAPN in food administered for 14 days,[6] a normal rate (with a slight unsignificant depression) after the intake of 0·4% BAPN in the diet for seven days.[7] High concentrations of BAPN have been found to depress collagen synthesis also in cartilage tissue cultures.[21]

In the present experiment the dosage used was 70 mg BAPN/100 g body wt. and this dosage was administered daily for 28 days before the injection of ^{14}C-proline and for another 28 days, after. These experimental conditions led to a high degree of lathyrism.

The total activity of ^{14}C-hydroxyproline in the skin of lathyritic rats shows an appreciable reduction in comparison with the control group up to the 5th day after ^{14}C-proline administration. It follows from this fact, that in latyrism caused under the above conditions collagen biosynthesis is slowed down. This is confirmed also by the great reduction of the partial NSC activity during the first days after the administration of the isotope. However, in lathyritic rats the total activity in the skin, as well as the partial NSC activity increases slowly. Both values are significantly higher on the 7th day than in the control group. This fact was also observed by Salmi and Kulonen.[11] These authors concluded that lathyrism causes probably an inhibition in the utilization of the amino acids, so that the labelled constituents remain longer in the amino acid pool and cause an apparently increased incorporation at later stages.

The inhibition of the conversion into the less extractable forms of collagen manifested itself by an appreciable depression of the partial activity of ASC and especially ISC in the course of the whole experiment.

In the urine there is an enhanced specific as well as total activity of ^{14}C-hydroxyproline during the first 24 hr after administration of the isotope, which indicates an increased degradation of NSC in lathyritic rats. It is of interest to compare the specific NSC activity and the specific hydroxyproline activity in urine in the first 24 hr after administration of the labelled proline. The sp. act. of NSC is nine times lower in lathyritic rats compared to the value which would be expected from the sp. act. in the urine, in the lathyritic group treated with sodium salicylate it is five times lower (The comparison is calculated for the situation in control rats). In both cases, however, the sp. act. of NSC is incomparably lower than the sp. act. in the urine. Therefore the source of labelled hydroxyproline in urine in the first 24 hr must be probably the newly synthetized tropocollagen.

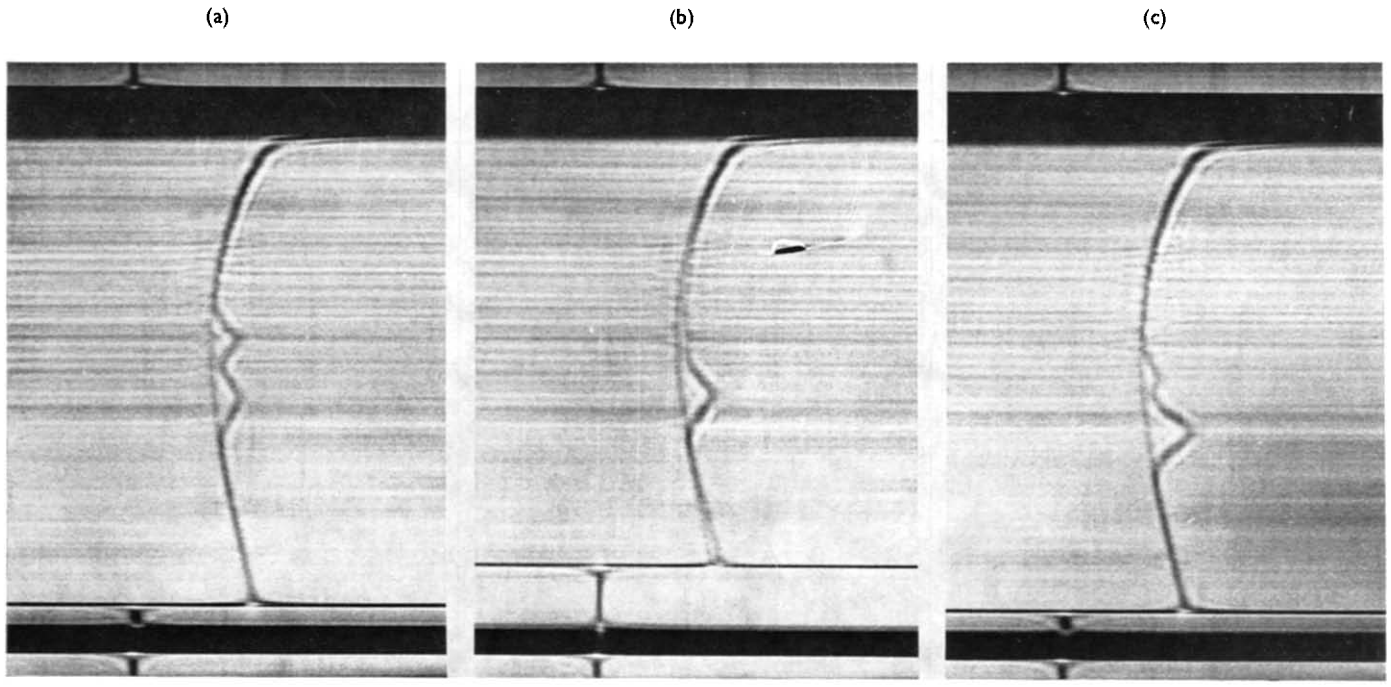

FIG. 1. Sedimentation diagram obtained from denatured acid-extractable collagen (ASC) from rat skin (in 0·25 M citrate buffer pH 3·7). Temperature 37° at 59780 rpm Phase angle 60°. Concentration c = 0·3 g/100 ml. (a) ASC—control group; (b) ASC—lathyritic group—treated with BAPN; (c) ASC—lathyritic group—treated with BAPN and sodium salicylate.

During investigations on the influence of sodium salicylate on collagen metabolism it was found that the administration of sodium salicylate in doses of 300 mg/kg for 56 days induced an increased rate of metabolic turnover of collagen.

It follows from the results obtained after the administration of sodium salicylate to lathyric rats that an identical effect can also be observed. The total activity of ^{14}C-hydroxyproline as well as the partial activity of NSC is enhanced in treated lathyric rats during the first days after the administration of the isotope, when compared with untreated animals. Specific and total activities of ASC and ISC between first and 7th day after isotope administration increase in treated lathyritic animals. The quantitative amount of insoluble collagen is higher than that in the lathyritic controls. In agreement with these findings the quantitative amount of collagen in the NSC fraction of treated lathyritic rats decreases. This was accompanied by reduced excretion of hydroxyproline and reduced total activity in the urine during the first 24 hr after isotope administration in treated lathyric rats.

It can be stated in conclusion that the collagen metabolism supporting effect of salicylates which was documented in the experiment on healthy rats manifested itself identically also in the experiment with highly lathyritic rats used as a model for the reduced collagen metabolism. This effect of salicylates might be found useful in supporting reparative and healing processes in connective tissue, especially in the case of degenerative diseases of joints.

Acknowledgement—This work was supported by a grant from Max-Planck Gesellschaft zur Förderung der Wissenschaften.

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