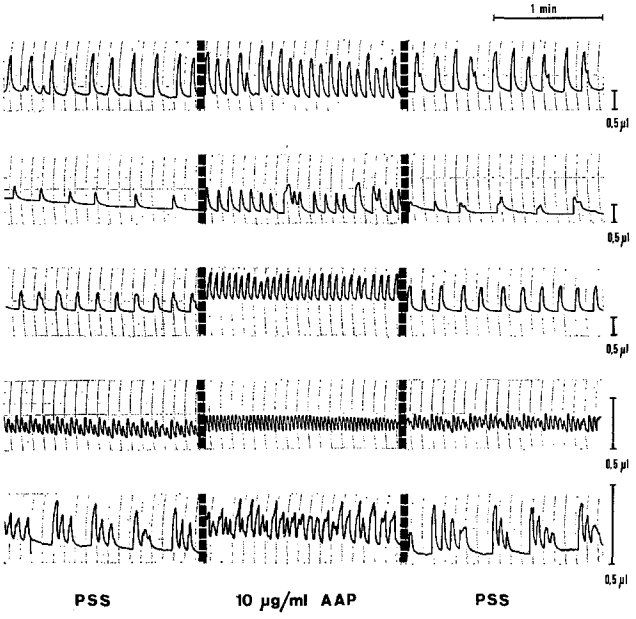
Vasotropic Activity of Arachidonic Acid Peroxide on the Isolated Murine Portal Vein and its Modification by Antiinflammatory Substances

In previous papers we have reported that arachidonic acid peroxide (AAP) elicits contractions in the isolated guinea-pig ileum and jejunum that can be counteracted by nonsteroidal, anti-inflammatory compounds displaying analgesic properties[1-4]. Recently, we demonstrated that subcutaneously administered AAP induces a paw oedema in the rat that can also be inhibited by such anti-inflammatory substances[5]. Since the microcirculation is the main target of acute inflammatory reactions, we have used the isolated, spontaneously pulsating murine portal vein[6], which shows some of the characteristics of an arteriole[7-10], as an experimental model in order to obtain some insight into the possible mode of action of these compounds in the terminal vascular bed. The following is a preliminary account of experiments in which the vasotropic activity of AAP and its modification by some substances with anti-inflammatory properties were evaluated. The method employed has been described in a previous paper[11].



Reactivity to arachidonic acid peroxide (AAP) in 5 individual vascular preparations displaying different initial activity pattern. PSS: Physiological salt solution.

When added to the bath fluid in concentrations ranging from 0.001–100 µg/ml, AAP induced a largely dose-related increase in the rate of contraction of the portal vein. The optimal concentration of AAP for test purposes was found to be 10 µg/ml, which caused an average increase of 80%. Whereas AAP regularly increased the rate of contraction, its effect on the amplitude of contraction varied. In the 70 preparations examined so far in this respect, amplitude increased in 29, diminished in another 29 and remained unaltered in 12. The 'output' of the saccular preparations of the portal vein was generally increased (57 instances), remaining unchanged in a small proportion of the vessels tested. Basal tone (base-line as shown in the Figure) was usually unaffected or slightly augmented. Although relatively drastic, short-lasting increases in tone occurred in some individual preparations, this type of reaction seems to be exceptional. When bathing solution containing AAP was replaced by physiological saline solution (PSS), the effect of AAP usually disappeared after the first change of fluid, as can be seen from the tracings below, which illustrate the reactivity of 5 individual vascular preparations displaying different initial patterns of activity. AAP can be applied repeatedly in a concentration of 10 µg/ml without inducing any signs of tachyphylaxis. As in the case of AAP-induced contraction of the guinea-pig gut, the changes induced by AAP in the isolated murine portal vein are therefore particularly suitable as a model for evaluating compounds with possible antagonistic activity.

On the strength of the available evidence, it is not possible to tell whether the action of AAP is mediated by intramural synthesis of prostaglandin, or due to the release of transmitter substances of other types. Likewise it can not be decided whether a possible direct effect results from its unsaturated fatty acid character or from its presence in peroxide form[1,2,12-17].

In the present study on rate-acceleration induced by AAP, the effects of sodium salicylate, phenylbutazone, aminopyrine, indomethacin and tribenoside have been examined. Sodium salicylate in concentrations up to 10 µg/ml had no influence, whereas a concentration of 100 µg/ml reduced this effect. Phenylbutazone exerted

Substance	Dose (µg/ml)	N	% Change in frequency ($x \pm s\bar{x}$) [a]	
Sodium salicylate	100	5	-45 ± 9	
Phenylbutazone	1	4	-16 ± 6	
	10	4	-71 ± 23	
Aminopyrine	10	3	-47 ± 9	
	100	3	-45 ± 8	
	10	4		$+9 \pm 6$
	100	4		$+16 \pm 10$
Indomethacin	0.1	5	-17 ± 14	
	100	5	-20 ± 12	
Tribenoside	0.1	2		$+40 \pm 20$
	1	7		$+91 \pm 33$

[a] According to LORD.

[1] R. JAQUES, Helv. physiol. pharmac. Acta *17*, 255 (1959).
[2] R. JAQUES, Helv. physiol pharmac. Acta *23*, 156 (1965).
[3] R. JAQUES, Experientia *25*, 1059 (1969).
[4] M. RÜEGG and R. JAQUES, Experientia *28*, 1525 (1972).
[5] L. RIESTERER, H. HELFER and R. JAQUES, in preparation.
[6] H. MISLIN, Rev. Suisse Zool. *70*, 317 (1963).
[7] B. FOLKOW and E. NEIL, *Circulation* (Oxford University Press, New York, Toronto 1971), p. 272.
[8] H. HELFER and R. JAQUES, Pharmacology *5*, 23 (1971).
[9] H. HELFER and R. JAQUES, Angiologica *8*, Part I, 172 and Part II, 121 (1971).
[10] B. JOHANSSON and B. LJUNG, Acta physiol. scand. *70*, 312 (1967).
[11] H. HELFER and R. JAQUES, Pharmacology *4*, 65 (1970).
[12] P. A. BERRY, Thesis, Council for National Academic Awards, London 1966, p. 91.
[13] S. H. FERREIRA, Nature New. Biol. *240*, 200 (1972).
[14] M. HAMBERG and B. SAMUELSON, Proc. natn. Acad. Sci. *70*, 899 (1973).
[15] H. VON MEFFERT and P. REICH, Derm. Wschr. *155*, 948 (1969).
[16] S. ICHIKAWA and J. YAMADA, Am. J. Physiol. *203*, 681 (1962).
[17] S. C. SHARMA, H. MUKHTAR, S. K. SHARMA and C. R. KRISHNA MURTHY, Biochem. Pharmac. *21*, 1210 (1972).

an antagonistic action at a concentration of 10 µg/ml. Aminopyrine in concentrations of 10 and 100 µg/ml reduced the rate-accelerant action of AAP in 3 out of 7 experiments, even in those instances in which AAP displayed intrinsic, positively chronotropic properties. This antagonistic action of aminopyrine was persistent: even when the vascular preparation was rinsed several times, the rate of contraction produced by subsequent applications of AAP no longer attained its original level. In the other 4 experiments aminopyrine proved virtually inactive. Indomethacin, applied in concentrations between 0.01 and 10 µg/ml, caused practically no modification of the rate-accelerating effect of AAP, even in cases in which it displayed an intrinsic negatively chronotropic action. In contrast, tribenoside[18] in concentrations of 0.1–1 µg/ml markedly intensified the rate-accelerating effect of AAP. Furthermore, preparations that had become largely insensitive to AAP through exposure to aminopyrine reacted again to AAP after being treated with tribenoside.

It is evident from the results of the experiments described above that substances possessing anti-inflammatory properties can be distinguished on the basis of quantitative and qualitative differences in their influence on the stimulant effect of AAP on the isolated murine portal vein[17].

Zusammenfassung. An der isolierten, autonom pulsierenden Portalvene der Maus zeigt Arachidonsäureperoxid einen ohne Tachyphylaxie-Erscheinungen wiederholbaren, vasotropen Effekt, der hauptsächlich in dosisabhängiger Steigerung der Kontraktionsfrequenz besteht. Dieser Effekt kann durch anti-inflammatorisch wirksame Substanzen entweder antagonistisch (Natriumsalicylat, Phenylbutazon, Aminopyrin) oder synergistisch (Tribenoside) beeinflusst werden. Die mit Arachidonsäureperoxid stimulierte Portalvene der Maus erscheint deshalb geeignet, Antiphlogistika zu differenzieren und Hinweise auf einen eventuellen Wirkungsmodus im Gebiet der terminalen Strombahn zu liefern.

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[18] Generic name of ethyl-3,5,6-tri-*O*-benzyl-D-glucofuranoside (Glyvenol®).

[19] The technical assistance of Mr. A. Blättler is gratefully acknowledged.

Influence of Erythorbic Acid on the Vitamin C Status in Guinea-Pigs

Humans as well as primates, the guinea-pig and some Indian native animal species, are not able to form L-ascorbic acid biosynthetically due to the lack of a special enzyme[1]. They, therefore, require a sufficient supply of vitamin C in the food in order to prevent a hypovitaminosis C or even scurvy.

Due to its reducing properties, L-ascorbic acid exhibits a high antioxidant potency. Thus, it is used, for instance, in fruit processing, as a curing aid in meat processing or in beer to prevent oxidative changes.

Erythorbic acid (D-isoascorbic acid, D-araboascorbic acid) is one of the stereoisomers of L-ascorbic acid with practically no biological activity (only one-twentieth of the biological activity of L-ascorbic acid)[2]. It possesses, however, similar antioxidative properties to L-ascorbic acid and is, therefore, used in some countries as an antioxidant in food[3].

[1] J. J. Burns, in: *Metabolic Pathways*, 3rd ed., vol. 1 (Ed. D. M. Greenberg; Academic Press, New York, London 1967), p. 394.

[2] J. Fabianek and H. Herp, Proc. Soc. exp. Biol., N.Y. *125*, 462 (1967).

[3] B. Borenstein, Food Techn. *19*, 115 (1965).

Effect of feeding various doses of D-erythorbic acid on the uptake of radioactivity after a single orally administered dose of L-(1-^{14}C)ascorbic acid in male guinea-pigs

Tissue	Radioactivity (dpm × 10^{-3}/g wet tissue)				
	Amount of D-erythorbic acid fed (mg/day)				
	0	20	50	100	400
Adrenal glands	177.0 ± 20.4	158.6 ± 25.0	79.9 ± 9.9[c]	108.4 ± 25.6[c]	99.8 ± 10.4[c]
Lungs	69.0 ± 8.0	64.3 ± 9.8	52.8 ± 15.3[a]	47.5 ± 13.0[c]	34.9 ± 4.4[c]
Kidneys	30.4 ± 6.3	30.5 ± 5.3	22.4 ± 3.9[b]	21.4 ± 3.4[c]	17.2 ± 2.2[c]
Testes	21.5 ± 3.2	26.0 ± 7.2	16.5 ± 3.2[b]	18.1 ± 4.4	12.4 ± 3.2[c]
Eyes	20.5 ± 4.3	19.4 ± 1.4	13.6 ± 0.3[c]	13.6 ± 2.1[c]	11.8 ± 1.2[c]
Pancreas	42.3 ± 4.3	47.0 ± 9.8	33.6 ± 6.8[b]	32.7 ± 8.9[a]	25.3 ± 3.6[c]
Cerebrum	6.5 ± 1.0	7.4 ± 0.7	5.8 ± 1.2	5.1 ± 1.0[a]	5.2 ± 0.7[b]
Cerebellum	7.4 ± 1.1	9.3 ± 1.2	7.1 ± 0.8	6.0 ± 0.6[b]	5.7 ± 1.0[b]
Liver	55.6 ± 9.8	62.7 ± 5.8	49.5 ± 12.5	33.9 ± 6.4[c]	40.6 ± 9.4[b]
Spleen	68.8 ± 21.7	83.5 ± 19.2	62.0 ± 12.2	55.8 ± 16.9	46.1 ± 5.8[b]

The experimental details are described in the text. 6 h after dosage of the radioactivity the animals were sacrificed and the tissues were quickly removed. The concentration of radioactivity in the tissues was determined by means of a Nuclear Chicago Mark II liquid scintillation spectrometer after solubilization of parts of the tissues with 1 *N* NaOH. Instagel® (Packard) was used as counting solution. The radioactivity is expressed as disintegrations per min (dpm per g of tissue) (± S.D.).

[a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.005$; as compared with 0 mg D-erythorbic acid