ON THE METABOLIC PATHWAY OF METHYLMETHACRYLATE

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1. Introduction

In a living organism, methacrylate is probably fully oxidized because in the urine of exposed animals no characteristic metabolites are found [1,2]. According to current metabolic schemes [3,4], we can suppose that methacrylate after combination with coenzyme A is submitted to β-oxidation and after rearrangement it joins the citric acid cycle in the form of succinyl-coenzyme A. The second possibility, however, is a simultaneous α- and β-oxidation leading to pyruvate, because weak oxidation with permanganate proceeds in a similar manner [5]. In both cases, full oxidation in citric acid cycle occurs (fig. 1).

This work attempts to prove which is the actual metabolic pathway of the methacrylate.

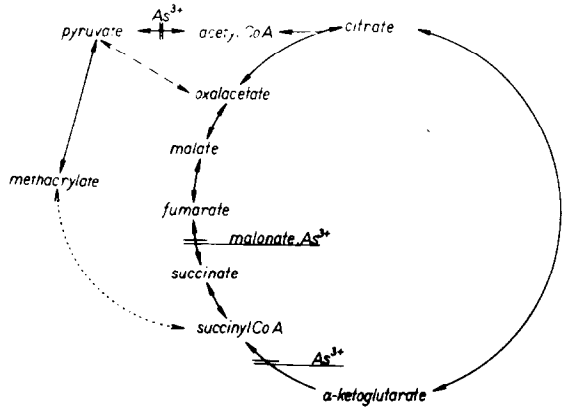


Fig. 1. Metabolic pathway of methacrylate concerning the relationship to citric acid cycle.

2. Materials and methods

For the experiments Wistar rats, male animals weighing 200–250 g were used. The rats were killed by cervical dislocation and decapitation. The livers were rapidly chilled in ice and slices 0.4–0.5 mm thick were cut by a free hand at 4°C. The slices of total area about 3 cm^2 were transferred to prepared 25 ml volumetric flasks containing 2 ml of Krebs-Ringer bicarbonate solution with inhibitor. After a definite time of preincubation under shaking in a water bath at 37°C, 5 ml of Krebs-Ringer bicarbonate solution, containing the substrate in desired concentration, are added. The flasks were tightly stoppered with ground glass stoppers and shaken in a water bath at 37°C. Total incubation periods were 30 and 60 min. Incubation medium was deproteinized according to Somogyi [6]. In the deproteinized supernatant, the methylmethacrylate was oxidized at a pH of 2–3 by means of permanganate. The resulting methylpyruvate was determined polarographically in the form of oxime in superfluous hydroxylamine at a pH of 1 [5]. Fumaric acid was determined polarographically under the same conditions, too (fig. 2).

The linkage of the methacrylate metabolism to the citric acid cycle was proved by means of inhibition with 1 mM arsenite with two-hours preincubation and with 10 mM malonate with one-hour preincubation of tissue slices in the given inhibitor.

3. Results and discussion

The comparison of the rate of metabolic transfor-

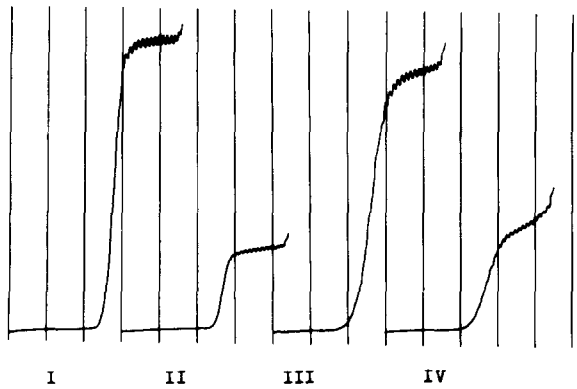


Fig. 2. Polarographic waves obtained by the determination of fumarate and methylmethacrylate. I – 7 mM fumarate, II – 7 mM fumarate after 30 min incubation with liver slices, III – 7 mM methylmethacrylate, IV – 7 mM methylmethacrylate after 30 min incubation with liver slices.

Table 1

Comparison of the rates of methylmethacrylate and fumarate metabolic transformation by rat liver slices. The incubation period was 30 min at 37°C. The original concentration of substrate was 7 mM. The values reported are the average of three determinations.

Substrate	Decrease of substrate concentration (mM)
fumarate	5.10
methylmethacrylate	4.10

Table 2

The effect of various inhibitors on the metabolic transformation of methylmethacrylate by rat liver slices. The incubation period was 60 min at 37°C. The original concentration of substrate was 7 mM. The values reported are the average of three determinations.

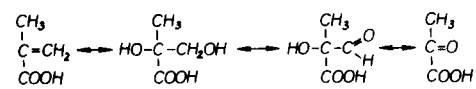
Inhibitor	Inhibitor conc. (mM)	Other additions	Decrease of substrate concentration (mM)
nil	–	–	5.60
malonate	10	–	5.30
arsenite	1	–	1.57
arsenite	1	succinate	1.57
arsenite	1	fumarate	1.94
arsenite	1	malate	1.90

mation of methylmethacrylate and fumarate by rat liver slices shows no expressive difference (table 1, fig. 2). The fumarate as a natural constituent of citric acid cycle is very quickly metabolized and hence the metabolism of methylmethacrylate proceeds very quickly too and this is the first reason for which its toxicity is very limited.

Using various inhibitors of citric acid cycle (table 2), it was stated that malonate exerts no inhibition on the methacrylate transformation. However, we should expect a braking effect on the methacrylate metabolism if the succinylcoenzyme A were supposed to be an intermediate. This metabolic way is not probable, because the expected braking effect has not been found.

The arsenite inhibition of methacrylate transformation is very effective and it can not be destroyed by means of addition of citric acid cycle constituents. Hence, we can suppose that the oxidative decarboxylation of pyruvate is a very important step in the methacrylate metabolism. The arsenite causes the citric acid cycle braking as well, due to the inhibition of the decarboxylation of α-ketoglutarate and dehydrogenation of succinate. For this reason, the addition of succinate shows no effect, but by the addition of fumarate and malate the citric acid cycle is restituted. Thus, the residual activity of the oxidation decarboxylase of pyruvic acid is restituted and a slight restitution of methacrylate metabolism is found. From these findings it follows that it is very probably the pyruvate and not the succinylcoenzyme A which is an intermediate of methacrylate metabolism (fig. 1).

The explanation of this metabolic pathway is not difficult. The hydroxylation of double bond occurs very probable at first and the resulting primary alcoholic group on the carbon end-atom is oxidized so as to form the aldehydic one. After deformylation of the resulting 2-hydroxy-2-formylbutyric acid which is running under the action of coenzyme C, pyruvic acid is formed. The following scheme tries to outline this metabolic pathway of methacrylate:



In quite a similar way goes the oxidation of methacrylate by permanganate in slightly acidic medium [5],

the hydroxylation being, however, of nonenzymatic origin here.

Thus, the low toxicity of methacrylate is biochemically well grounded, on the one hand, by the high metabolic rate of methacrylate transformation, on the other hand, by the fact that the product of the metabolism, viz. the pyruvic acid, is a compound occurring as a natural constituent of the living body.

References

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