Biochemical Pharmacology, Vol. 21, pp. 635–640. Pergamon Press, 1972. Printed in Great Britain

# EFFECT OF ACRYLONITRILE ON SULPHYDRYLS AND PYRUVATE METABOLISM IN TISSUES

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(*Received* 28 *July* 1971; *accepted* 3 *September* 1971)

**Abstract**—The effects of acrylonitrile ($CH_2=CH-CN$) on tissue sulphydryls and on the pyruvate metabolism *in vivo* were investigated using guinea-pigs and rabbits. The protein and non-protein sulphydryls, especially the latter in the liver and brain, greatly decreased when the animals showed severe symptoms of poisoning after a single dose of acrylonitrile. In the blood the sulphydryls decreased at the early stage of the intoxication. Both pyruvate and lactate, above all the former in the brain, accumulated when the animals suffered acute intoxication. Sodium thiosulphate, an antidote to the cyanide which is partly liberated from acrylonitrile, did not counteract the accumulation.

IN A PREVIOUS report[1] the authors have shown that acrylonitrile reacts with sulphydryl compounds *in vitro* and that some of them could be used as well as sodium thiosulphate for reducing the acute toxicity of this material.

In the present study, the reactivity of acrylonitrile with non- and protein sulphydryls of tissues *in vitro* and *vivo*, and its effect on the pyruvate metabolism in tissues have been investigated. A possible mechanism of the toxic action of acrylonitrile is discussed.

## MATERIALS AND METHODS

*Animals.* Male guinea-pigs (400 ± 30 g) and rabbits (2500 ± 250 g) were used. They were fed on a commercial diet for more than a week before the experiments.

In vitro *experiment.* Guinea-pigs were killed by decapitation and blood, the brain and the liver were quickly removed. The blood was diluted 20 times with 0·2 M tris–HCl buffer (pH 7·4). The liver and the brain separated into the grey and white matters were homogenized in the same buffer using a teflon pestle. To the diluted blood and the 10% homogenates of the liver and brain was added acrylonitrile at the final concentration of either $2 \times 10^{-3}$ M or $2 \times 10^{-2}$ M followed by incubation for 30 min at 37°. After the incubation sulphydryl contents in the blood and in the supernatant of the homogenates, obtained by centrifugation at 3000 rev/min for 20 min, were determined as described below.

In vivo *experiment.* A 5% solution of acrylonitrile in 0·9% saline solution was injected at the dose level of 100 mg/kg ($2 \times LD_{50}$) into the guinea-pig peritoneal cavity. One hour after the administration when animals showed severe symptoms of acute poisoning such as weakness of the extremities, restlessness, tremor and occasional convulsions, blood, the brain and the liver were quickly removed. Sulphydryl contents were also determined in these tissues. In rabbits 30 mg/kg acrylonitrile was given through the auricular vein and blood samples were withdrawn from

the same vein of the other side at varied times. The sulphydryl contents were determined for the plasma, red cells and the supernatant of the deproteinized red cells.

*Determination of sulphydryls.* Amperometric titration was used according to Benesch *et al.*[2] 0·13 M tris–HCl (pH 7·4) buffer and a potential of −0·10 V versus the saturated calomel electrode were used for the diluted blood and the tissue homogenates. In these samples the values obtained are sums of the protein and non-protein sulphydryls which are called total sulphydryls in this paper and expressed as SH μmoles/ml blood or g wet tissue.

The non-protein sulphydryls were determined in the supernatants of samples after deproteinization with 20% sulfosalicylic acid using ethanol and ammonia buffer (2 M $NH_4OH$ and 0·8 M $NH_4NO_3$, pH 9·5), with a potential of −0·13 V. Values were expressed as μmoles/ml blood or g wet tissue.

The protein sulphydryls of each tissue were calculated by simply subtracting the non-protein sulphydryls from the total ones and were expressed as SH μmoles/10 mg protein. The protein contents of tissues were assayed by the method of Lowry *et al.*[3]

*Determination of pyruvic and lactic acids.* Guinea-pigs were sacrificed by decapitation at either 30 or 60 min after the intraperitoneal administration of 100 mg/kg acrylonitrile. In some animals 450 mg/kg sodium thiosulphate was given with acrylonitrile to eliminate the effect of inorganic cyanide which would have been partly liberated from acrylonitrile[1] in the body. These animals were killed at 60 min after the simultaneous administration of the two compounds. Pyruvic acid was determined according to Friedmann and Haugen[4] modified by Aldridge and Cremer[5] to increase the sensitivity, as was lactic acid according to Barker and Summerson.[6] To determine these in the brain and liver, tissues were rapidly frozen with liquid nitrogen, crushed and then transferred into tared centrifuge tubes including cold 10% TCA followed by the original procedures.

## RESULTS

*Reactivity of acrylonitrile with sulphydryls* in vitro. As shown in Table 1 both total and non-protein sulphydryls in the liver were highest among the tissues tested. After the incubation of tissues with acrylonitrile at its pharmacologically effective concentration *in vivo* their sulphydryls, above all the non-protein ones, in the liver and brain, greatly decreased in 30 min.

TABLE 1. THE EFFECT OF ACRYLONITRILE ON TISSUE SULPHYDRYLS *in vitro*

| | | | SH μmoles/ml blood or g tissue | | | | |
|---|---|---|---|---|---|---|---|
| | | | Controls | Acrylonitrile | | | |
| | | | Mean | $2 \times 10^{-3}$ M Mean | % of control | $2 \times 10^{-2}$ M Mean | % of control |
| Blood | | total SH | 14 | 12 | 83 | 12 | 83 |
| | | soluble SH | 0·79 | 0·43 | 54 | 0·59 | 75 |
| Liver | | total SH | 19 | 14 | 75 | 11 | 57 |
| | | soluble SH | 5·1 | 0·49 | 10 | 0·50 | 10 |
| Cerebrum | gray | total SH | 4·8 | 3·1 | 64 | 2·3 | 48 |
| | | soluble SH | 1·8 | 0·57 | 42 | 0·095 | 7 |
| | white | total SH | 4·4 | 2·6 | 59 | 1·8 | 41 |
| | | soluble SH | 1·5 | 0·42 | 28 | 0·15 | 10 |

Values are the means of the results of two experiments. Incubation in 0·2 M tris–HCl buffer (pH 7·4) at 37° for 30 min.

*Effect of acrylonitrile on the sulphydryl contents of tissues* in vitro. Changes of the sulphydryls in tissues after about 1 hr of the administration of 100 mg/kg acrylonitrile are shown in Table 2. The non-protein sulphydryls decreased to 15 per cent of control

TABLE 2. THE EFFECT OF ACRYLONITRILE ON THE TISSUE SULPHYDRYLS IN GUINEA-PIGS

| | | | SH μmoles/ml blood or g tissue Controls | Acrylonitrile (100 mg/kg body wt.) | % of control |
|---|---|---|---|---|---|
| Soluble SH | Blood | | 0·82 ± 0·19 (5) | 0·68 ± 0·18 (8) | 83 |
| | Liver | | 4·7 ± 1·9 (5) | 0·61 ± 0·15 (8) | 13* |
| | Cerebrum | gray | 1·3 ± 0·13 (5) | 0·66 ± 0·24 (8) | 51* |
| | | white | 1·4 ± 0·10 (5) | 0·68 ± 0·16 (8) | 49* |
| Protein SH | Blood | | 0·76 ± 0·28 (5) | 0·40 ± 0·081 (6) | 53* |
| | Liver | | 1·4 ± 0·45 (5) | 0·48 ± 0·26 (6) | 34† |
| | Cerebrum | gray | 1·1 ± 0·24 (5) | 0·50 ± 0·35 (6) | 45* |
| | | white | 0·94 ± 0·32 (5) | 0·50 ± 0·26 (6) | 53* |

Values are means ± S.D. Numbers of experiments in parentheses.
* $P < 0·05$; † $P < 0·01$. Sulphydryl contents were measured after 1 hr of the administration of acrylonitrile.

in the liver and about 50 per cent in both gray and white matter of the brain, but did not decrease in the blood. The decrease of the non-protein sulphydryls was much greater than that demonstrated with acrylamide.[7] The protein sulphydryls were decreased in the blood as well as in the liver and brain.

TABLE 3. THE EFFECT OF ACRYLONITRILE ON THE TISSUE SULPHYDRYLS IN RABBITS

| | | | SH μmoles/ml blood or g tissue Controls | Acrylonitrile (100 mg/kg body wt.) | % of control |
|---|---|---|---|---|---|
| Soluble SH | Blood | | 0·79 ± 0·25 (6) | 0·55 ± 0·30 (3) | 70 |
| | Liver | | 6·5 ± 1·4 (3) | 1·2 ± 0·13 (4) | 18† |
| | Cerebrum | gray | 1·3 ± 0·20 (6) | 0·85 ± 0·086 (4) | 65* |
| | | white | 1·2 ± 0·35 (6) | 0·63 ± 0·13 (4) | 53† |
| Protein SH | Blood | | 0·58 ± 0·12 (6) | 0·47 ± 0·054 (3) | 81 |
| | Liver | | 0·80 ± 0·00 (3) | 0·83 ± 0·10 (4) | 104 |
| | Cerebrum | gray | 1·5 ± 0·11 (5) | 0·85 ± 0·086 (4) | 57† |
| | | white | 1·4 ± 0·31 (6) | 0·85 ± 0·17 (4) | 61* |

Values are the means ± S.D. Numbers of experiments in parentheses.
* $P < 0·05$; † $P < 0·01$. Sulphydryl contents were measured after 1 hr of the administration of acrylonitrile.

Table 3 shows the results obtained from the rabbits given 100 mg/kg acrylonitrile intravenously. The decrease of sulphydryls in tissues was similar to that seen in guinea-pigs.

*Time course of the changes in blood sulphydryls.* Figure 1 shows the changes of sulphydryl contents in the blood after the injection of 30 mg/kg acrylonitrile in rabbits. The protein sulphydryls in red cells and serum sulphydryls, being mostly the non-protein ones, decreased quickly and recovered within 1 hr, while the non-protein sulphydryls in red cells showed a slower decrease and recovery.

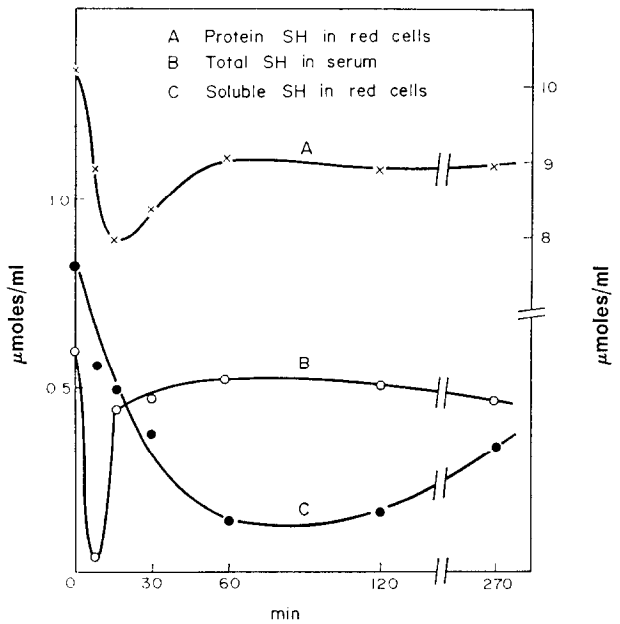


FIG. 1. The effect of acrylonitrile on the blood sulphydryls after a single dose in rabbits.

*Effect of acrylonitrile on the pyruvic acid metabolism in tissues.* From the results obtained above an experiment was designed to examine whether the pyruvate metabolism of the tissues, especially of the brain, was affected by acrylonitrile in the poisoning of which nervous symptoms were dominant.

As shown in Table 4 tissue pyruvate in the guinea-pigs treated with 100 mg/kg acrylonitrile significantly increased, especially in the brain, at 1 hr after the treatment when the animal showed severe symptoms of poisoning. The increase was also demonstrated when acrylonitrile was given with sodium thiosulphate. Tissue lactate was also increased at 1 hr after the injection, although the extent was less than that demonstrated in pyruvate. Sodium thiosulphate was also ineffective to prevent the increase of lactate.

## DISCUSSIONS

It has been known that acrylonitrile reacts with active hydrogen atoms such as those of sulphydryls through cyanoethylation, and this property has been used for studying the structure of proteins such as β-lactoalbumin.[8] In the previous report[1] the authors described how acrylonitrile easily formed stable conjugates with L-cysteine and L-glutathione *in vitro*.

TABLE 4. THE EFFECT OF ACRYLONITRILE ON THE TISSUE PYRUVATE AND LACTATE IN GUINEA-PIGS

| | μmoles/10 ml blood or 10 g tissue | | | | | |
|---|---|---|---|---|---|---|
| | Blood | | Cerebrum | | Liver | |
| | Pyruvate | Lactate | Pyruvate | Lactate | Pyruvate | Lactate |
| Controls | | | | | | |
| 0·9% saline, 60 min (8) | 3·26 ± 0·89 | 47·1 ± 11·2 | 0·456 ± 0·100 | 49·0 ± 14·0 | 0·547 ± 0·062 | 15·3 ± 4·2 |
| Acrylonitrile | | | | | | |
| 100 mg/kg, 30 min (3) | 3·90 ± 0·79 | 55·3 ± 18·0 | 0·803 ± 0·173 | 65·8 ± 10·2 | 0·566 ± 0·030 | 27·5 ± 6·5 |
| % of control | 120 | 117 | 176 | 134 | 103 | 180 |
| Acrylonitrile | | | | | | |
| 100 mg/kg, 60 min (6) | 6·98 ± 2·09 | 129 ± 24·0 | 2·34 ± 0·54 | 93·9 ± 19·8 | 0·743 ± 0·089 | 41·8 ± 9·9 |
| % of control | 214* | 275* | 514* | 191* | 135* | 273* |
| Acrylonitrile | | | | | | |
| 100 mg/kg + Sodium thiosuphate (5) | 5·24 ± 1·04 | 131 ± 38 | 1·76 ± 0·32 | 99·3 ± 19·5 | 0·774 ± 0·074 | 47·2 ± 5·4 |
| 450 mg/kg, 60 min | | | | | | |
| % of control | 161* | 279* | 430* | 203* | 141* | 308* |

Values are the means ± S.D. Numbers of experiments in parentheses. Tissue pyruvate and lactate were measured at either 30 or 60 min after the administration of acrylonitrile. Controls were given 0·9% saline only. * $P < 0·01$.

In this study an investigation was made on the reactivity of acrylonitrile with tissue sulphydryls *in vivo* using guinea-pigs and rabbits. The biological action of acrylonitrile has been considered to be due to both inorganic cyanide liberated from it and to its molecule itself, and its effect on the nervous tissues seems to be important. Hashimoto and Kanai[1] have already shown that acrylonitrile selectively inhibited the $K^+$-stimulated respiration of the brain cortex, and that it strongly blocked the sciatic nerve conduction. In this paper it has been shown that the protein and non-protein sulphydryls, especially the latter one in the liver and brain, greatly decreased when animals suffered an acute intoxication after a single dose of acrylonitrile. In blood it became clear that the sulphydryls decreased at the early stage of the intoxication. The decrease of the brain sulphydryls, especially of the protein ones, is of considerable interest, since it has been shown by Unger and Romano[9,10] and Freundl[11] that protein sulphydryls in the brain would be closely related to nervous activity. On the other hand Hashimoto and Kanai[1] found that some sulphydryl compounds reduced the toxicity of acrylonitrile, probably due to a protection of tissue sulphydryls from acrylonitrile.

It is known that sulphydryl blocking agents such as trivalent arsenic[12] inhibit the pyruvate oxidation in tissues, above all in the brain, and cause an accumulation of pyruvic acid in the tissue. In this study acrylonitrile was also found to affect the pyruvate oxidation in tissues, above all in the brain, although it is not known yet whether the disturbance of pyruvate oxidation is due to the action of acrylonitrile on the pyruvate oxidase system as in the case of arsenites. The excess level of pyruvate in the brain might have brought about cerebral disfunctions.

The accumulation of lactate being of less extent than that of pyruvate seems to be the result of the disturbance of the pyruvate oxidation. It is known that little pyruvate accumulates under the conditions primarily permitting the formation of lactate.[13,14] The fact that sodium thiosulphate given with acrylonitrile did not prevent the accumulation of both pyruvate and lactate suggests that the pyruvate oxidation system might be sensitive to acrylonitrile, the action of which is probably due to the reactivity with sulphydryls.

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