EFFECT OF N-TRICYANOVINYLAMINES ON THE LEVEL OF GLUTATHIONE IN HEPATOCYTES

Dušan Podhradský[1], Helena Paulíková[1], Gabriel Toth[1] and Milan Miko[2]

[1]*Department of Biochemistry, Faculty of Science P.J. Šafárik University, Moyzesova 11, 041 01 Košice, Slovakia*
[2]*Department of Microbiology, Biochemistry and Biology Slovak Technical University, 812 37 Bratislava, Slovakia*

SUMMARY

The effects of N-substituted tricyanovinylamines (N-TCVA; $RNHC(CN)=C(CN)_2$) have been studied on rat hepatocytes and liver mitochondria. Derivatives of N-TCVA act on oxidative phosphorylation as uncouplers, and react with thiols within pH 5.0-8.5. N-Isobutyl-, N-benzyl-, and N-cyclohexyl-TCVA influence the level of GSH and GSSG in isolated hepatocytes. They can act as oxidants, but the level of GSSG increases (about 40%) only if the concentration of N-TCVA is higher than 1 μmol/l. If N-TCVA is added to a final concentration higher than 50 μmol/l a decrease of GSH and GSSG level is observed. Derivatives of N-TCVA also influence the level of GSH and GSSG in mitochondria. At 40-400 μmol/l N-TCVA in the incubation medium the level of GSSG increased and the ratio GSH/ GSSG was influenced, but the level of total SH groups did not decrease.

INTRODUCTION

Glutathione (γ-glutamylcysteinylglycine; GSH) is the major non-protein thiol in living systems. It is involved in several cellular processes, such as the synthesis of proteins, nucleic acids, prostaglandins and leukotrienes, cell division and amino acid transport /1,2/. GSH plays a critical role in detoxification reactions with xenobiotics and oxygen radicals /3/. A change of the redox status of glutathione can be achieved by using GSH oxidants, e.g. diazenes, hydroperoxides or N-substituted tricyanovinylamines (R-NH-$C(CN)=(CN)_2$; N-TCVA) /4-6/. N-TCVA derivatives were synthetized in 1957 by McKusick *et al.* /7/ and in 1987 their fungicide properties were observed /8/. In our previous work we studied the reactions of N-TCVA with thiols in aqueous solution /9/. Reduction of the C=C double bond of N-TCVA derivatives and oxidation of the thiols was observed. Derivatives of N-TCVA react with thiols as oxidizing agents at pH 5.0-8.5. N-Substituted TCVA resemble carbonylcyanide phenylhydrazone derivatives (R-NH-$N=C(CN)_2$; CCP) in their structure. Two nitrile groups of carbonyl cyanide phenylhydrazone (CCP) derivatives are highly reactive with thiols /10/ and the uncoupling effect of CCP derivatives can be reversed by the addition of thiols /11/. We found that N-TCVA stimulate the respiration of hepatocytes and mitochondria, influence membrane potential of mitochondria and reduce the level of TSH groups of the mitochondria and hepatocytes /6,12/. N-TCVA derivatives act as uncouplers on the process of oxidative phosphorylation. Because N-TCVA can react with thiols as oxidizing agents in the cell, these compounds can affect the integrity of cells. After the incubation of isolated hepatocytes with N-TCVA (at a concentration equal to 0.05-3.0 μmol/l), no change in the integrity of hepatocytes was observed /12/. This result indicated that the stimulating effect of N-TCVA on cell respiration is not due to a change in the integrity of the cytoplasmic membrane.

In the present study, we looked at the effect of N-TCVA on the level of GSH and GSSG in isolated hepatocytes and mitochondria. We attempted to compare the effect of N-TCVA on the level of glutathione with their ability to influence oxidative phosphorylation.

MATERIALS AND METHODS

Chemicals

The N-substituted tricyanovinylamines were prepared /7/ in our laboratory. These compounds were chromatographically pure and their structure was verified by IR, UV and NMR spectroscopy. Mannitol, GSH, GSSG and N-ethylmaleimide (NEM) were supplied by Calbiochem (La Jolla, USA). HEPES, carbonylcyanid-*p*-trifluormethoxy-phenylhydrazone (FCCP), albumin and 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) were obtained from Sigma Chemical Co. (St. Louis, USA). NADPH was supplied by Reanal (Budapest, Hungary) and ADP by Serva (Heidelberg, FRG). Collagenase was a product of USOL (Bohumile, CSFR). LDH-kit (lactate dehydrogenase, E.C. 1.1.1.27) and other chemicals were products of Lachema (Brno, CSFR).

Preparation and incubation of hepatocytes

The hepatocytes were isolated from male Wistar rats (200-400 g) fed with Larsen diet, according to Moldéus *et al.* /13/. Liver was perfused with Krebs-Henseleit bicarbonate buffer supplemented with HEPES (25.0 mmol, pH 7.4) and bubbled continuously with a gas mixture of O_2/CO_2 (19/1). Cell viability was assessed by trypan blue exclusion and lactate dehydrogenase leakage.

Incubation of hepatocytes ($1x10^6$ cells/ml) was carried out at 37°C in a rotating round flask with a gas mixture of O_2/CO_2 (19/1). The N-TCVA derivatives, FCCP and NEM, were added to the incubation medium in the form of methanolic solutions (the concentration of methanol in the incubation medium never exceeded 1%, v/v).

Determination of total SH groups

The total SH groups (TSH; TSH = protein SH groups + non-protein SH groups) were measured by Ellman's method /14/. A 1 ml sample of incubation medium ($1x10^6$ cells) was centrifuged (2 min, 500 *g*) and the sediment was suspended in 2 ml of buffer solution (0.2 mol Tris.HCl and 20 mmol EDTA, pH 8.0). To 1 ml of this suspension was added 0.1 ml 10 mmol DTNB and 15 minutes later 2 ml of ice-cold methanol. After centrifugation (10 min, 800 *g*) the absorbance at 412 nm was measured in the supernatant.

Preparation of samples for glutathione analysis

The incubation of isolated hepatocytes was finished by adding 2 ml of the incubation medium ($2x10^6$ cells) to 1 ml of 1 mol $HClO_4$ containing 1 mmol EDTA. After 10 minutes, 0.8 ml of 1.3 mol K_2HPO_4 was added, the solution was centrifuged for 10 minutes (800 *g*) and afterwards the concentration of total glutathione (extracellular + intracellular) was determined in the supernatant.

2 ml of the incubation medium were centrifuged (1 min, 500 *g*) and subsequently both the sediment and the supernatant were processed as follows: 0.2 ml 1 mol $HClO_4$ containing 1 mmol EDTA was added to 1 ml of supernatant and after 10 minutes 0.1 ml of 1.3 mol K_2HPO_4 was admixed. After centrifugation (10 min, 800 *g*) the supernatant was used for determining the extracellular glutathione.

The sediment was resuspended in 2 ml of the Krebs-Henseleit solution supplemented by HEPES (20 mmol, pH 7.4), and for deproteination, 2 ml 1 mol $HClO_4$ containing 1 mmol EDTA was added. After 10 minutes 1.6 ml of 1.3 mol K_2HPO_4 was added and the mixture was centrifuged for 10 minutes at 800 *g*. The intracellular glutathione was determined in the supernatant.

Preparation and incubation of rat liver mitochondria (RLM)

The liver mitochondria were isolated from male Wistar rats (200-400 g) fed with Larsen diet /15/. The isolation medium was composed of 0.25 mol sucrose, 10 mmol Tris.HCl and 0.5 mmol EDTA (pH 7.2).

RLM (8 mg protein) were incubated in 2 ml of incubation medium (0.25 mol sucrose, 10 mmol Tris. HCl, 0.05 mmol EDTA, pH 7.4) supplemented by 2.5 mmol K_2HPO_4 and 7.5 mmol succinate at 25°C. Methanolic solutions of N-TCVA derivatives were added to the incubation medium.

Determination of the concentration of GSH and GSSG

0.3 ml of 1 mol $HClO_4$ was added to 1 ml of incubation solution and after 10 minutes the pH value was adjusted to 7.2 by addition of 1.3 mmol K_2HPO_4. After centrifugation (10 min, 800 *g*) the supernatant was used for the determination of glutathione.

Assays

Glutathione (GSH and GSSG) was measured by the kinetic recycling assay with DTNB and glutathione reductase /16/. For the determination of GSSG, GSH was trapped with N-ethylmaleimide. Excess N-ethylmaleimide was quantitatively removed by extraction with ethyl acetate. The concentration of proteins was determined according to Lowry *et al.* /17/. All concentrations were expressed as final concentrations.

Statistical analysis of the data was carried out using Student's t-test.

RESULTS

The viability of hepatocytes

Derivatives of N-TCVA are SH reagents and can influence the viability of hepatocytes. The viability of hepatocytes was investigated by measuring extracellular LDH activity. The level of total glutathione in the extracellular space was estimated continuously.

Figure 1a shows the cell damage by 5 mmol N-isobutyl-TCVA. The viability of cells decreased to about 50% 30 min after N-TCVA addition. In our previous work we found that N-TCVA in the concentration range of 10^{-6}-10^{-5} mol/l stimulated the respiration of hepatocytes /12/. This concentration of TCVA did not influence the release of LDH and even the higher concentration of N-TCVA (50 μmol/l) after 20 min incubation caused 25% cell damage (Fig. 1b).

The increased levels of glutathione in the extracellular space may be directly or indirectly responsible for cellular damage. The addition of 1.15 mmol N-isobutyl-TCVA decreased the intracellular glutathione (GSH+GSSG) by more than 50% after 5 min, but the level of extracellular glutathione increased only about 5% (Fig. 1c). The level of extracellular glutathione increased about 60%, but only after 60 min incubation. This increased level of glutathione is, at least partly, explained by the oxidation initiated by N-TCVA.

Derivatives of N-TCVA react with SH groups and they can not only cause depletion of glutathione but also decrease the level of total SH groups (TSH) in hepatocytes. N-Isobutyl-N-cyclohexyl- and N-benzyl-TCVA (in milimolar concentrations) decrease the level of TSH

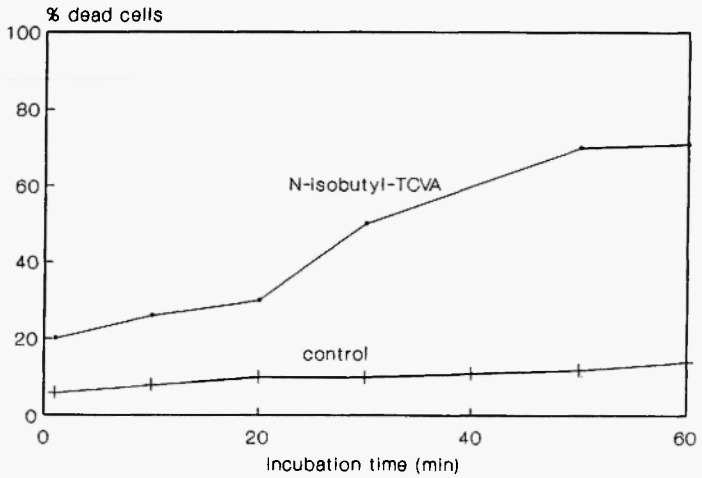


Fig. 1a: Time course of the loss of cellular viability by N-isobutyl-TCVA. Hepatocytes ($1x10^6$ cells in 1 ml incubation medium) were incubated with N-TCVA (final concentration 5 mmol/l). For controls, incubation solution was complete medium plus methanol (0.1%, v/v). Cell killing was assessed by the release of LDH into the medium. Experimental details are given in Materials and Methods. Each point represents the mean value of three determinations.

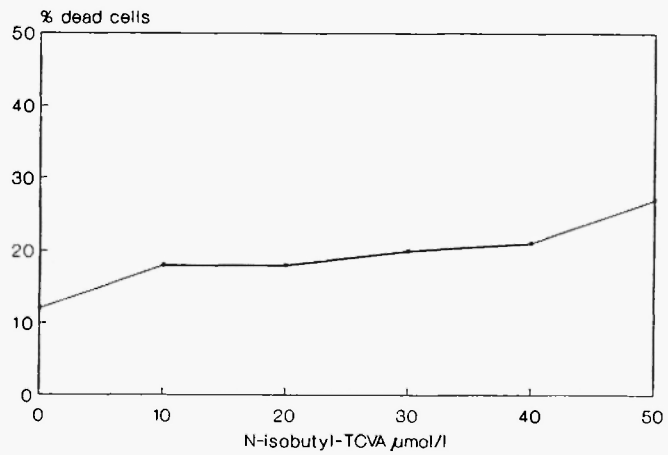


Fig. 1b: Effect of N-isobutyl-TCVA on cellular viability. Hepatocytes ($1x10^6$ cells in 1 ml incubation medium) were treated with varying concentrations of N-TCVA for 20 min. Cell killing was assessed by the release of LDH into the medium. Experimental details are given in Materials and Methods. Each point represents the mean value of three determinations.

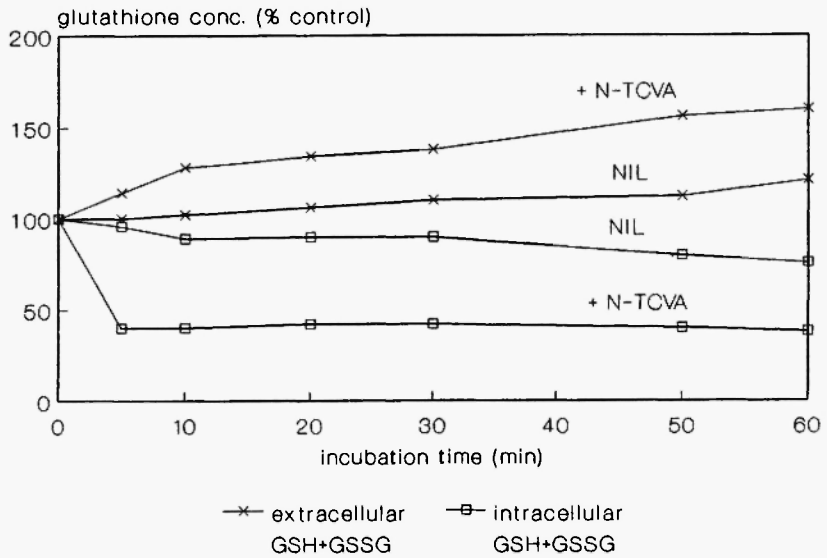


Fig 1c: The level of intracellular glutathione (GSH+GSSG) and corresponding extracellular glutathione during incubation of isolated hepatocytes ($1x10^6$ cells in 1 ml incubation medium) with N-isobutyl-TCVA (final concentration 1.15 mmol/l). Other conditions are given in Materials and Methods. The control values were (t=0 min): extracellular glutathione 4.2 ± 1.2 μmol/l; intracellular glutathione 59.5 ± 4.8 μmol/l. Each point represents the mean value of three determinations.

in isolated hepatocytes about 20-60% after 10 min incubation (Fig. 2). The reason for the different effects of N-TCVA on TSH could be their different reactivity with the thiols /9/ and their different hydrophobic properties. Derivatives of N-TCVA can react with SH groups in different locations in proteins.

The level of GSH and GSSG in hepatocytes

A loss of glutathione occurs during incubation of hepatocytes /13/. The levels of GSH and GSSG were estimated during 60 minutes incubation in controls (Fig. 3a). Extracellular GSH and GSSG content increased about 20-25% after 10 min incubation. The increase of extracellular GSSG content can be explained as a result of autoxidation of GSH, which is released from hepatocytes. The level of intracellular

GSSG did not change significantly, but the level of GSH decreased about 20%.

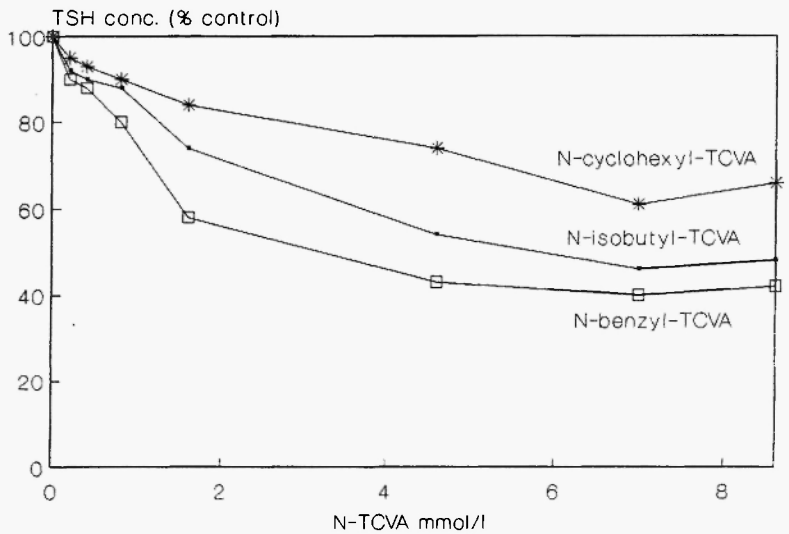


Fig. 2: Effect of N-TCVA derivatives on the TSH level in hepatocytes after 10 min incubation. The control value of TSH was 196 ± 13 µmol/l. Experimental details are given in Materials and Methods. Each point represents the mean value of three determinations.

Figure 3b and c show the time course of GSH and GSSG content in isolated hepatocytes (in the extra- and intracellular space) treated with derivatives of N-TCVA. During half an hour both a decrease in intracellular GSH and an increase in extracellular GSH was seen. The level of GSSG did not change significantly during the first 15 min of incubation. After half an hour incubation of hepatocytes with N-TCVA the level of GSSG was increased about 10%. This increase could be caused by the oxidation of extracellular GSH.

Derivatives of N-TCVA in milimolar concentrations are able to influence GSH content, but the level of GSSG did not change significantly. In this case these compounds did not act as oxidants.

The effect of N-TCVA, at a final concentration of 2-250 µmol/l, on GSH and GSSG in isolated hepatocytes was investigated. The action of the most effective derivative, N-isobutyl-TCVA, is shown in Figure 4. After 5 min incubation of isolated hepatocytes with N-TCVA at a concentration below 4 µmol/l, the content of GSH decreased and the

level of GSSG increased; N-TCVA acted as an oxidant. When the concentration of N-TCVA was higher than 50 μmol/l, the levels of both GSH and GSSG decreased.

The level of GSH and GSSG in rat liver mitochondria

The role of mitochondrial glutathione is much less defined than its role in cellular metabolism (i.e. transport, catalysis and protection against toxic compounds). Its major or possibly only function appears to be a protective effect against oxidative stress. The action of N-TCVA derivatives as oxidizing agents and uncouplers of oxidative phsophorylation is striking if total cellular glutathione and mitochondrial glutathione are considered.

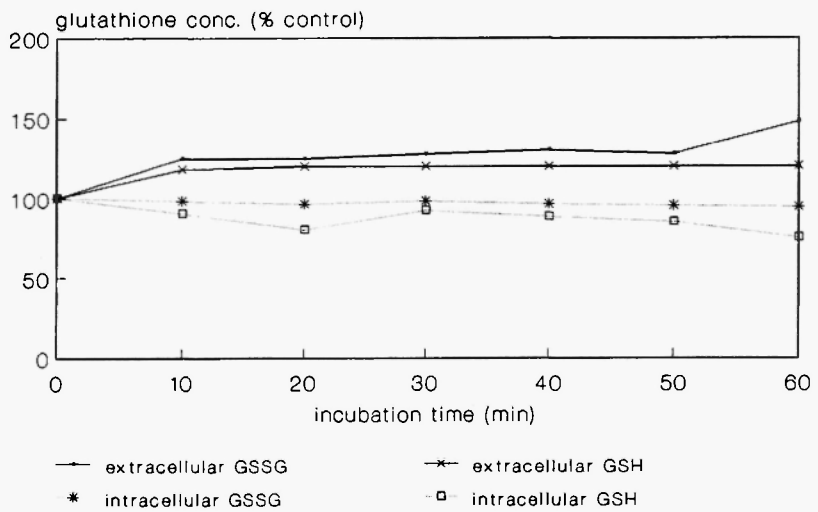
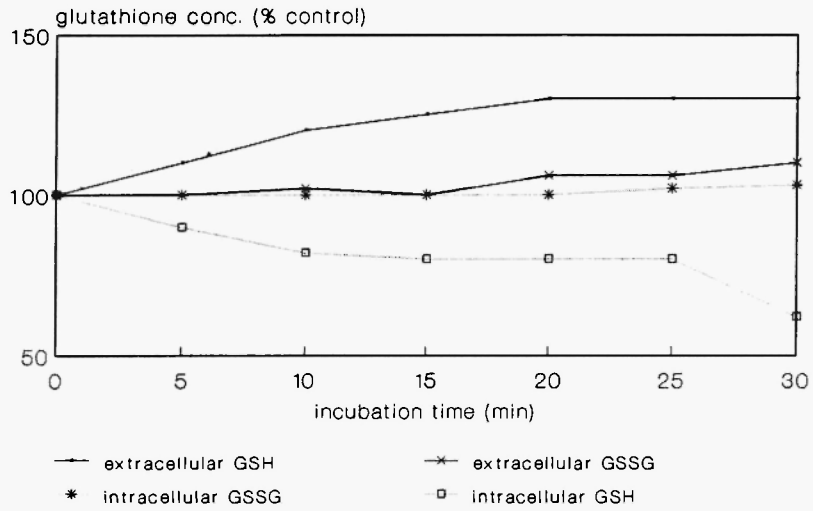


Fig. 3a: Time course of extra- and intracellular glutathione content during incubation of hepatocytes in incubation medium plus 0.5%, v/v methanol. For experimental details see Materials and Methods. The control values (t=0 min) were in μmol/l: extracellular GSH 14.3 ± 1.2, GSSG 5.2 + 0.8; intracellular GSH 66.8 ± 1.9, GSSG 1.1 ± 0.6. Each point represents the mean value of three determinations.



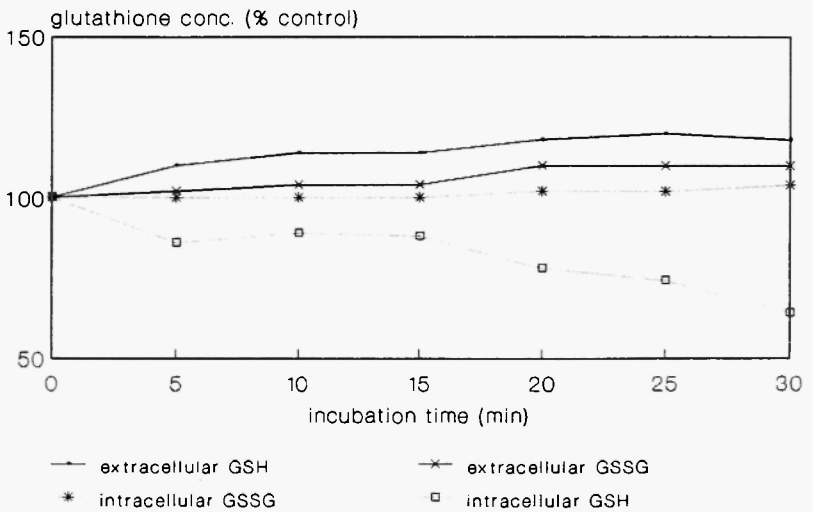


Fig. 3b,c: Time dependent effects of N-cyclohexyl- (**b**) and N-benzyl-TCVA (**c**) (final concentration 1.18 mmol/l) on the levels of GSH and GSSG in extra- and intracellular space. For experimental details see Materials and Methods. The control values (t=0 min) were in µmol/l: extracellular GSH 7.8 ± 1.3, GSSG 4.2 ± 0.8; intracellular GSH 63.1 ± 1.6, GSSG 1.8 ± 0.7. Each point represents the mean value of three determinations.

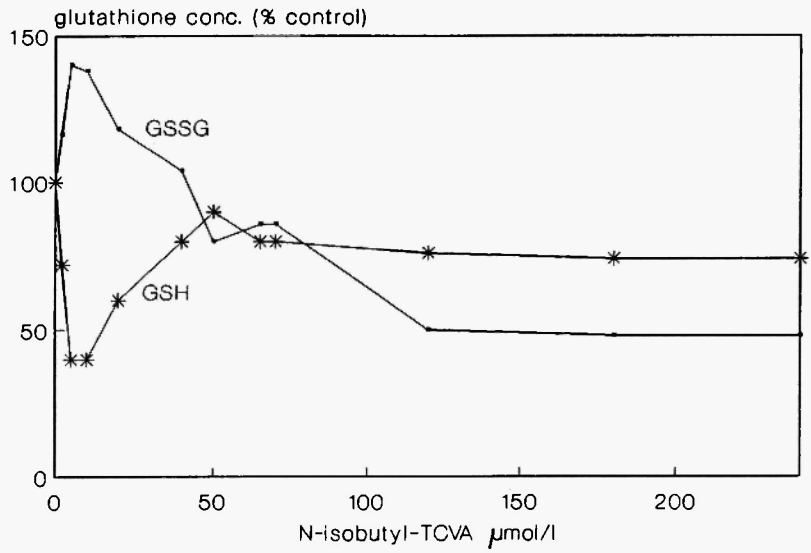


Fig. 4: Concentration dependent effect of N-isobutyl-TCVA on the GSH and GSSG contents in hepatocytes after 5 min incubation. For experimental details see Materials and Methods. The control values of total cellular glutathione (t=0 min) were in µmol/l: GSH 68.7 ± 1.9; GSSG 3.2 + 0.8. Each point represents the mean value of three determinations.

The effect of N-TCVA on the level of SH groups of rat liver mitochondria was studied earlier; we observed changes of glutathione content after the action of N-TCVA in concentrations higher than the concentration of TSH in RLM /6/. The specific action of the most effective derivative, N-cyclohexyl-TCVA, on the level of GSH and GSSG in RLM is shown in Figure 5. In spite of the protection by succinate against oxidant, N-cyclohexyl-TCVA induced glutathione depletion; at a concentration of N-TCVA below 400 µmol/l, the level of GSSG increased and the level of GSH decreased. Maximal effect was achieved at a concentration of N-TCVA of 40 µmol/l, when the level of GSSG increased by approximately 180%.

As seen from Tables 1 and 2, the effect of N-TCVA on the glutathione level depends on the ratio of TSH/N-TCVA concentration

TABLE 1

The effect of N-isobutyl-TCVA (final concentration) on GSH/GSSG ratio and the rate of respiration[a] (STIM [%] = stimulation of respiration) in hepatocytes

N-TCVA	GSH	GSSG	TSH/N-TCVA	GSH/GSSG	STIM[a]
[μmol/l]	[nmol/1x10^{6}cells]				[%]
-	68.70	3.23	-	21.26	100
10	27.48	4.41	19.6	6.23	205
20	40.11	3.70	9.8	10.84	-
30	-	-	-	-	155
50	41.22	2.50	2.5	16.48	127
120	49.41	1.61	1.6	30.68	111
240	50.69	1.50	0.8	33.79	-
560	35.66	3.04	0.3	11.73	-

TSH 196.13 nmol/1x10^6 cells; [a]the values are from /12/.

TABLE 2

The effect of N-cyclohexyl-TCVA on GSH/GSSG ratio and the rate of respiration[a] (STIM [%] = stimulation of respiration) in mitochondria

N-TCVA	GSH	GSSG	TSH/N-TCVA	GSH/GSSG	STIM[a]
[μmol/l]	[nmol/mg prot.]				[%]
-	7.21	0.67	-	10.74	100
10	6.60	1.27	20.39	5.19	-
20	6.24	1.20	10.01	5.17	400
30	-	-	-	-	500
40	5.80	1.07	5.00	5.48	526
60	5.76	0.73	3.30	7.81	350
200	3.25	0.50	1.00	6.50	-
420	1.19	0.47	0.47	2.50	-

TSH 200.39 nmol/mg protein; [a]the values are from /6/.

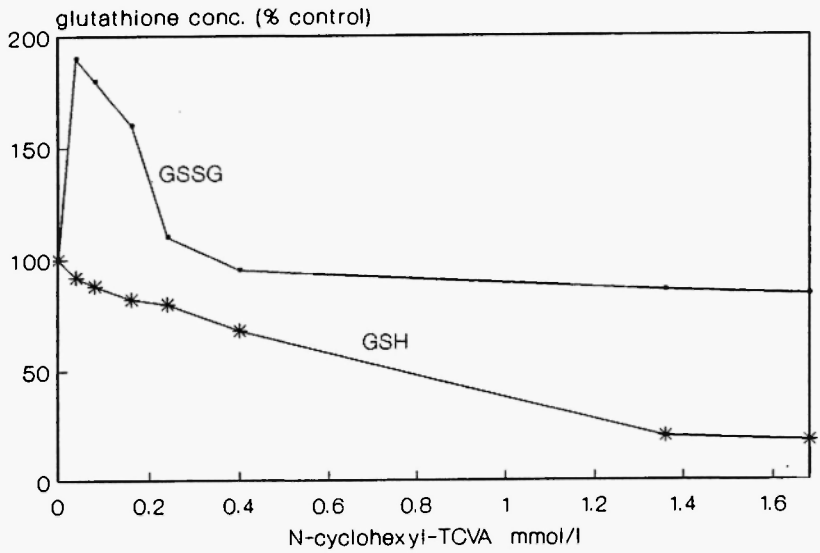


Fig. 5: Concentration dependent effect of N-cyclohexyl-TCVA on the GSH and GSSG content in rat liver mitochondria after 5 min incubation. For experimental details see Materials and Methods. The control values (t=0 min, the content of methanol was 0.1%, v/v, in the incubation medium) were in μmol/l: GSH 28.8 ± 1.2; GSSG 2.4 ± 0.8. Each point represents the mean value of three determinations.

in the incubation medium. N-TCVA derivatives at a concentration of 10^{-6} mol/l stimulate the respiration of hepatocytes and mitochondria, depolarize their membranes and cause the oxidation of GSH in mitochondria and hepatocytes. An increase of GSSG content was observed only if the ratio TSH/N-TCVA was approximately 10 or higher.

DISCUSSION

N-Tricyanovinylamines can react with thiols within pH 5.0-8.5 /9/. Results of studying these reactions indicate that N-TCVA derivatives are oxidants for thiols. Some of the N-TCVA derivatives are able to penetrate through biological membranes /6,12/. It was expected that these compounds would influence the intracellular content of glutathione.

We have previously studied the effects of N-TCVA on the respiration of hepatocytes and rat liver mitochondria /6,12/. It was found that these compounds resemble uncouplers of oxidative phosphorylation. The content of SH groups of mitochondria decreased in the presence of uncouplers /4,6,18,19/. Derivatives of N-TCVA, like other uncouplers, are able to decrease the level of TSH in RLM and hepatocytes, but only at a concentration of N-TCVA higher than the concentration of TSH (Fig. 2).

N-TCVA derivatives are probably able to modify the SH groups of proteins and to influence the integrity of membranes and/or activity of the SH-enzymes /6/. It was expected that N-TCVA would damage the isolated hepatocytes. The viability of hepatocytes was decreased only at a high concentration of N-TCVA (>1 mmol/l). N-TCVA derivatives are able to decrease the activity of SH-enzymes. For example, the activity of succinate dehydrogenase is reduced in the presence of N-TCVA at a concentration of $1x10^{-5}$ mol/l /6/ and the activity of glutathione reductase is reduced at a concentration of N-TCVA higher than $1x10^{-6}$ mol/l (results not shown). It was expected that N-TCVA at concentrations higher than $1x10^{-6}$ mol/l (glutathione reductase is inhibited) would increase the level of GSSG. The effect of N-TCVA derivatives on the GSH and GSSG levels in isolated hepatocytes or RLM depends on the concentration ratio of TSH and N-TCVA in the incubation medium (Tables 1 and 2). The level of GSSG in hepatocytes and mitochondria increased (or did not change) only when glutathione reductase was inactivated or when the concentration of N-TCVA was lower than the concentration of the total SH groups.

Some derivatives of N-TCVA stimulated the respiration of hepatocytes and RLM and influenced their membrane potential /6,12/. The abolition of the N-TCVA effects on respiration by thiol was observed /6/. The effects of N-TCVA on oxidative phosphorylation were compared with the effects of N-TCVA on the levels of GSH and GSSG. N-TCVA derivatives stimulated the respiration of hepatocytes at a concentration of 10^{-8}-10^{-6} mol/l and the respiration of

mitochondria at a concentration of 10^{-7}-10^{-5} mol/l /6,12/, but a concentration of N-TCVA below 10^{-6} mol/l did not influence the content of GSH and GSSG in hepatocytes and RLM. At this concentration the activity of glutathione reductase is not changed and this enzyme can regenerate GSH from GSSG.

It is remarkable that derivatives of N-TCVA in micromolar concentrations decrease the membrane potential /6/. This property of N-TCVA does not cause the release of glutathione from hepatocytes, but it is important to note that in the presence of N-TCVA the level of total cellular glutathione decreased together with decrease of membrane potential. The observed release of GSH and GSSG from hepatocytes at the higher concentrations of N-TCVA derivatives is a result of cell damage.

REFERENCES

1. Kosower NS, Kosower EM. The glutathione status of cells. Int Rev Cytol 1978; 54: 109-160.
2. Kaplowitz N, Aw TY, Ookhtens N. The regulation of hepatic glutathione. Ann Rev Pharmacol Toxicol 1985; 25: 715-744.
3. Reed DJ. Glutathione: Toxicological implications. Ann Rev Pharmacol Toxicol 1990; 30: 603-631.
4. Jocelyn PC. Glutathione and the mitochondrial reduction of some diazenes. Biochem Pharmacol 1980; 29: 331-333.
5. Olafsdottir K, Reed DJ. Retention of oxidized glutathione by isolated rat liver mitochondria during hydroperoxide treatment. Biochim Biophys Acta 1988; 964: 377-382.
6. Paulikova H, Podhradsky D, Antalik M. Effect of N-tricyanovinylamines on oxidative phosphorylation and level of SH-groups in rat liver mitochondria. Gen Physiol Biophys 1991; 10: 383-394.
7. McKusick BC, Heckert RE, Cairns TL, Coffman DD, Mower HF. Cyanocarbon chemistry VI. Tricyanovinylamines. J Am Chem Soc 1958; 80: 2806-2815.
8. Kreuzberger A, Daus S. Herbicides. 8. Nuclear-chlorinated and -brominated anilinoethylenetricarbonitriles. Arch Pharm 1987; 320: 93-95.
9. Podhradsky D, Paulikova H, Imrich J. Reactions of N-tricyanovinylamines with thiols in aqueous solutions. Collect Czech Chem Commun 1990; 35: 1630-1634.
10. Drobnica L, Sturdik E. The reaction of carbonyl cyanide phenylhydrazones with thiols. Biochim Biophys Acta 1979; 585: 462-476
11. Toninello A, Siliprandi N. Restoration of membrane potential in mitochondria deenergized with carbonyl cyanide p-trifluoromethoxyphenyl-hydrazone (FCCP). Biochim Biophys Acta 1982; 682: 289-292.

12. Paulikova H, Podhradsky D. Effect of N-tricyanovinylamines on respiration and ATP/ADP ratio in isolated hepatocytes. Biologia 1991; 46: 315-321.
13. Moldeus P, Högberg J, Orrenius S. Isolation and use of liver cells. Methods Enzymol 1978; 52: 60-71.
14. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and non-protein sulfhydryl groups in tissue with Ellmans reagent. Anal Biochem 1968; 25: 192-205.
15. Gazzoti P, Malstrom K, Crompton M. Preparation and assay of animal mitochondria and submitochondrial vesicles. In: Carafoli E, Semenza G, eds, Membrane Biochemistry. Berlin: Springer Verlag, 1979; 66-76.
16. Eyer P, Podhradsky D. Evaluation of the micromethod for determination of glutathione using enzymatic cycling and Ellmans reagent. Anal Biochem 1986; 153: 57-66.
17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurements with the Folin phenol reagent. J Biol Chem 1951; 193: 265-275.
18. Fuchs J, Friesleben HJ, Mainka L, Zimmer G. Reactivity of mitochondrial sulfhydryl groups toward dithionitrobenzoic acid and bromobimanes under oligomycin-inhibited and uncoupling conditions. Arch Biochem Biophys 1988; 266: 89-97.
19. Antalik M, Sturdik E, Sulo P, Properova A, Mihalovova H, Podhradsky D, Dzurilla M. Uncoupling effect of protonophoric and nonprotonophoric analogs of carbonyl cyanide phenylhydrazone on mitochondrial oxidative phosphorylation. Gen Physiol Biophys 1988; 7: 517-528.