Subchronic Preexposure to Carbon Monoxide Modifies Heart Response to a Combined CO + Isoproterenol Challenge in Rats

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Abstract. The effect of repeated exposure to carbon monoxide (CO) on the response of middle-age rats to an acute CO exposure combined with a low dose of a sympathomimetic agent was studied. A group of 12 rats (male albino, Wistar, age 9 months) without ECG abnormalities was divided into two subgroups matched for weight, heart rate and ECG: one subgroup was exposed to 500 ppm CO for 6 h/d, 5 d/w, for 6 weeks (peak COHb 31.5%, SD 3.5); the other one (controls) was exposed to fresh air. Two or three days after the last exposure both groups underwent combined challenge with 0.025 mg/kg isoproterenol s.c. and 90 minute exposure to CO in a concentration increasing from 500 to 1500 ppm; ECG was recorded continuously. The hearts were examined morphometrically and histologically.The CO-preexposed subgroup had, as compared to controls: 1) significantly higher blood hemoglobin (by 25%), erythrocyte count (by 28%) and volume (by 6%), and hematocrit (by 33%); 2) the same peak COHb; 3) lower basic heart rate and an earlier decrease after isoproterenol, 4) significantly smaller increase in ECG abnormalities and arrhythmias after isoproterenol and during CO exposure; 5) nonsignificantly higher heart weight indexes; 6) a nonsignificantly lower score of histological abnormalities. The global score of ECG pathology during CO exposure (abnormal pattern or arrhythmia) correlated best (multiple corr. coef. >0.9) with end-exposure free (non CO bound) hemoglobin (negatively) and with mean heart rate during exposure (positively): the lower score in the preexposed subgroup was attributable primarily to the increased hemoglobin. Six- week intermittent CO exposure induced marked compensatory processes (hematological) but only a tendency to adaptational changes in the heart (by gross morphometry), and decreased the ECG response to CO+ isoproterenol challenge at the same COHb.

Key words: Carbon monoxide — Isoproterenol — Rat — ECG — Morphometry — Hematology

Introduction

Acute exposure to carbon monoxide (CO) evokes a typical series of cardiovascular

responses: the blood flow through the myocardium and the brain increases already at 5–10% of carboxyhemoglobin (COHb), then heart rate (HR) increases, arrhythmias and hypoxic changes of ECG appear; at higher COHb levels, HR and blood pressure decrease (Penney 1990; Trávníčková 1995). This typical course could be modified in animal experiments. The decrease in HR was delayed and the intensity of ECG abnormalities (response to myocardial hypoxemia) was enhanced by sympathomimetic stimulation; opposite changes were observed in sedated animals or after administration of a beta blocking agent (Trávníčková and Frantík 1994).

While the acute neurotoxicity and cardiotoxicity of CO is generally acknowledged, the evidence for chronic toxicity is controversial. In experimental studies, continuous or repeated exposure of young rats to CO has been described to provoke compensatory reactions involving polycythemia, hypervolemia and cardiomegaly (Penney 1988). The latency and the extent of changes depended on the intensity of exposure. At the same time, repeated exposure to simulated altitude hypoxia induced a similar compensatory response, but in addition clear pathological changes, both haemodynamic and biochemical (Ošťádal et al. 1981), which – if present in CO exposed animals – might be of central importance in the mechanism of chronic CO toxicity.

In preliminary experiments, to detect functional effects of subchronic CO exposure, we found no persisting changes in endurance performance or resting ECG of young rats exposed repeatedly to CO in concentrations about 500 ppm. In the present study, a modified procedure was adopted based on the experience of the laboratory with chemical cardiotoxicity testing: a subtle toxic lesion became observable only in combination with model challenges, such as aging, spontaneous pathology or momentary functional load.

Ageing, spontaneous pathology, momentary functional load and their interactions modify substantially the response of the cardiovascular system to chemical agents. The adult pattern of ECG was characteristic for rats in the resting state up to the age of 14 months, but under various challenges the number of extrasystoles and other pathological ECG findings increased with age beginning with 9 months. Most of the 2 years old rats had spontaneous arrhythmias, half of them displayed also mild ECG shape changes and pathological findings in the myocardial tissue (Osborne 1981; Kolesár 1988; Trávníčková et al. 1996).

In a previous paper, pretreatment with a synthetic catecholamine (isoproterenol) in a dose of 0.05mg/kg was selected as a model of sustained medium intensity stress characterized by an increase in heart rate by 20% over the basal level; similar increase usually accompanies behavioral arousal. HR changes during the exposure to CO were displaced to lower COHb and shifted towards lower HR values but ECG shape changes and arrhythmias were much more frequent and profound than in animals without isoproterenol (Trávníčková and Frantík 1994). The dose used does not induce histological changes in hearts of healthy rats (Benjamin et al. 1989).

On the basis of this experience, combined challenge with low dose isoproterenol and carbon monoxide in an increasing concentration, applied to 9–14 months old

rats was developed as one of standard tests for detection of subchronic or chronic cardiotoxicity (Mikisková 1988). Using this model, the effect of repeated exposure to CO on the response of middle-age rats to an acute CO exposure combined with a low dose of a sympathomimetic agent was studied in the present experiments.

Materials and Methods

Male albino rats of Wistar strain, delivered as SPF, were completely habituated to all components of the procedure, i e , to the restraining device, to insertion of subcutaneous electrodes, to subcutaneous injections, and to blood sampling ECG was recorded repeatedly in a group of 16 animals At the age of 9 months, two subgroups (6 rats each) comparable in ECG characteristics were selected from the animals with a normal ECG pattern One subgroup was then exposed to 500 ppm CO for 6 hours per day, 5 days per week, for 6 weeks The control subgroup was exposed to ambient air

CO concentration in the air was measured using infrared analyser URAS (1 ppm CO = 1 145 mg/m^3)

COHb level was determined (according to Heilmayer) after the first and the last subchronic exposure Blood for hematological analysis (hemoglobin concentration, erythrocyte count, hematocrit and mean erythrocyte volume, semiautomatic Coulter counter) was collected 24 hours after the last exposure

Two or three days after the last exposure, a single challenge exposure was performed in all animals (Trávníčková and Frantík 1994) The animals were put into a plastic holder and limb electrodes (thin silver wires) were placed (ECG leads I, II, III) After 30 minute of acute habituation, the animals received isoproterenol s c in a dose of 0 025 mg/kg and were exposed for 90 minutes to CO in concentrations increasing in steps of 500, 1000 and 1500 ppm (Fig 1) HR and ECG were continuously recorded (Bioscript RFT, cardiotachometer) The number of extrasystoles and the duration of all types of arrhythmia were evaluated from the continuous record, both separately and as a common score of arrhythmia Neither the classification system of Lown nor its modification for rats by Lessard were applicable to this purpose The ECG pattern changes (primarily flattening of T wave) were rated on a 12 point scale in 5 minute periods
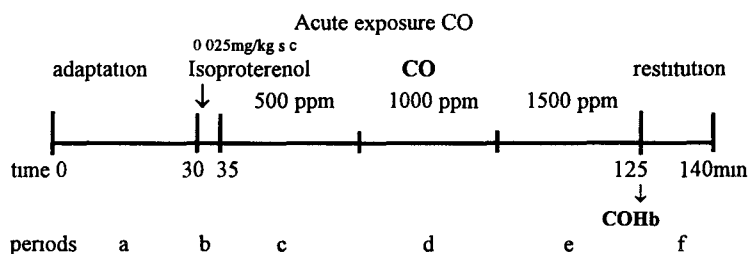
Figure 1. Time diagram of the challenge exposure

Blood for COHb determination was sampled immediately after the exposure, and ECG was recorded for further 15 minutes The animals were then killed (CO_2 anaesthesia) and the hearts were removed for morphometrical and histological examination Longitudinal sections were stained with hematoxylin and eosin

All values of preexposed and control animals were compared using Student's t-test at $p = 0.05$ or two-way analysis of variance (time course of HR and ECG findings) and multiple regression analysis (Statgraphics).

Results

The concentrations of COHb after the first and the last subchronic exposure were similar: means 32% and 31%, respectively. Mean COHb levels after the challenge exposure in preexposed and control subgroup did not differ significantly: 52.7 and 50.6, respectively ($t = 0.70$).

Both subgroups differed significantly in the heart response to the challenge exposure: the initial HR was slightly lower in preexposed rats; the isoproterenol induced increase in HR was the same in both subgroups (to 458 beats per minute on average); HR decreased earlier in the course of CO exposure in preexposed rats; hypoxic ECG changes (namely lowered or isoelectric T) and arrhythmias (ventricular extrasystoles, sinus arrhythmia, partial or complete intraventricular blocks) were significantly more frequent in control rats (Fig. 2).

All hematological values were significantly higher in preexposed rats in comparison with the control subgroup: hemoglobin by 25%, erythrocyte count by 28%, erythrocyte volume by 6%, and hematocrit by 33% (Table 1).

**Table 1.** Hematological findings in CO preexposed and control rats: means and standard deviations

	CO-preexpos. group	Control group	t
Hemoglobin (g/l)	188.3 ± 4.8	146.2 ± 11.5	8.28
Erythrocyte count ($\times 10^{12}$/l)	9.47 ± 0.24	7.43 ± 0.60	7.73
Erythrocyte volume (fl)	50.8 ± 1.16	48.0 ± 1.09	4.31
Hematocrit	0.495 ± 0.024	0.372 ± 0.041	6.57

Student's *t*-values. All differences significant ($P < 0.001$).

Morphometrical comparison has shown a slight and nonsignificant increase in almost all weight indexes in preexposed rats compared to controls (Table 2). The histological picture was similar in both subgroups: slight abnormalities were ascribed to aging. In only two animals a pathological picture was found (focal postdystrophic myocarditis with fibrotic reparation in one control rat, and focal myodystrophy with frequent scars in a preexposed rat).

Discussion

Equal COHb levels after the first and the last preexposure, and equal levels in preexposed and in control animals after the acute exposure testify against preexposure induced changes in the kinetics of CO. The modified heart rate and ECG respose
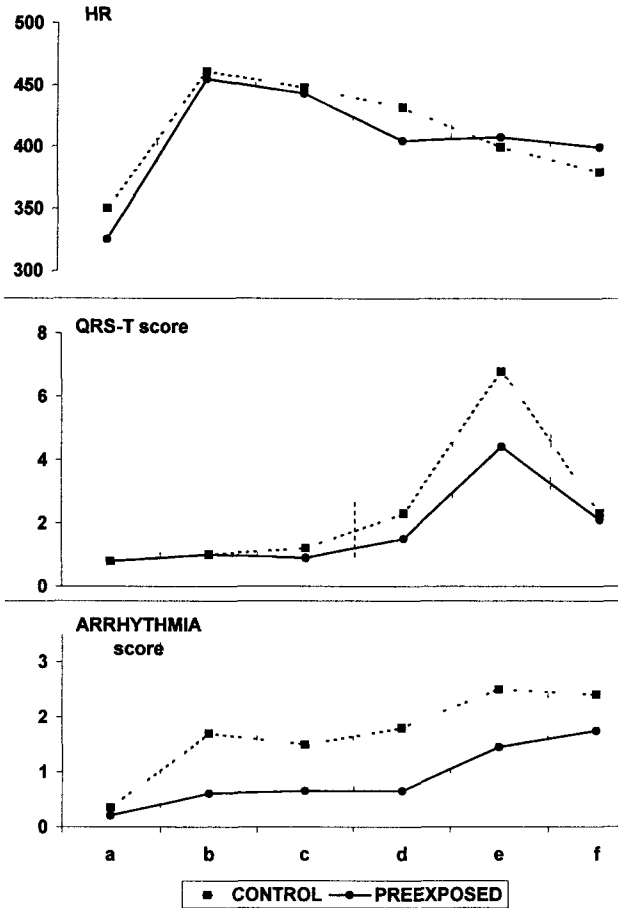
Figure 2. Heart rate (HR, beats/min), score of ECG QRS-T abnormities, and score of ARRHYTHMIA in preexposed and control rats in the course of the challenge exposure a – habituation period, b – 5 min after isoproterenol injection, c – 30 min inhalation of 500 ppm CO, d – 30 min inhalation of 1000 ppm, e – 30 min inhalation of 1500 ppm, f – after 15 min desaturation ECG changes (intensity and duration) were evaluated in 5 min periods and means were computed for periods (a) through (f) Individual period values were processed using two-way analysis of variance The F and P values for the effects of factors A (subgroups) and B (periods), and their interaction are below HR Covariate control HR $F = 28\,9$, $P < 0\,0001$ A $F = 1\,8$, $P = 0\,18$, B $F = 65\,1$, $P < 0\,0001$, AxB $F = 4\,8$, $P = 0\,0003$ Significant interaction indicates that the effect of pre-exposure changed in the course of the challenge exposure, but the difference between subgroups was not significant for any individual time point QRS-T score A $F = 5\,1$, $P = 0\,025$, B $F = 68\,6$, $P < 0\,0001$, AxB $F = 3\,6$, $P = 0\,004$ Arrhytmias A $F = 11\,7$, $P = 0\,0007$, B $F = 4\,6$, $P < 0\,0004$, AxB $F = 1\,0$, $P = 0\,4$

Table 2. Morphometrical and histological findings in CO preexposed and control rats means and standard deviations

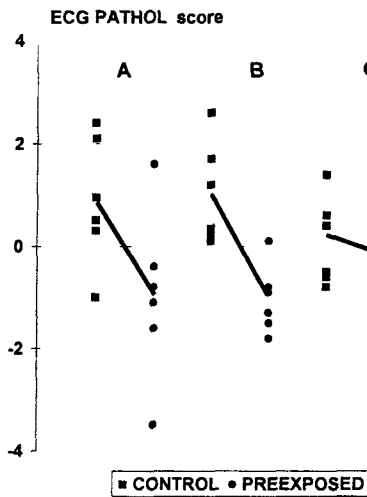|	CO-preexpos group	Control group	t
Body weight – BW (g)	360 5 ± 15 8	355 7 ± 11 3	0 61
Heart weight – HW (g)	1 079 ± 0 120	1 039 ± 0 100	0 63
R and L atria weight (g)	0 144 ± 0 047	0 126 ± 0 026	0 82
R ventricle free wall (g)	0 163 ± 0 037	0 166 ± 0 036	0 14
L ventricle free wall + S (g)	0 702 ± 0 113	0 684 ± 0 070	0 33
Index HW/BW	0 300 ± 0 027	0 292 ± 0 014	0 64
Histological score	0 92 ± 1 11	2 00 ± 1 41	1 47
Histological rank	5 25 ± 3 45	7 75 ± 3 46	1 25

Student's t-values, S – interventricular septum, R – right, L – left, score of histological positivity (0   4) and mean rank (1   12) All weights are weights of wet tissue No statistically significant differences

observed several days after the last preexposure can be thus ascribed to cumulated general and/or local effects of the preceding intermittent CO exposure

The 6-week intermittent exposure of middle aged rats to 500 ppm led to a clear-cut increase in the oxygen transport capacity of the blood and to a significant increase in the resistance to a combined CO+isoproterenol challenge, as assessed by ECG: the global score of ECG pathological changes was significantly lower in preexposed rats than in controls (Fig. 3A) Statistical elimination of the variance of mean heart rate during the challenge exposure further enhanced the difference in the global scores (Fig. 3B), whereas statistical elimination of variance of the amount of the end-exposure active hemoglobin eliminated the difference almost completely (Fig. 3C). A comparison of models A, C and D shows that the effect of preexposure on the acute-exposure-induced ECG pathology could be almost completely explained by differences in the amount of available hemoglobin.

The increase in hemoglobin was comparable to adaptation to hypoxic conditions (gradual intermittent adaptation to 7000 m, 8h/d, 24 exposures) in 2.5 months old rats (Ošťádal et al. 1981) but – unlike adaptation to altitude hypoxia – morphological signs of heart hypertrophy were negligible in CO preexposed rats The histological findings corresponded to the age of animals in both subgroups. The absence of CO related changes does not rule out subcellular response to the subchronic exposure: the threshold exposure for electron microscopic changes in the heart of rabbits was 4 hours in as low concentrations as 100–180 ppm CO (Thomsen and Kjeldsen 1974).

A complex adaptational response has been repeatedly described in young rats after repeated or long-lasting exposure to CO in concentrations similar to those used in our experiment. Young Sprague-Dawley rats (weight 175 g) displayed hematological changes and signs of heart hypertrophy after 5 weeks of exposure to 450 ppm (6 h/day, 5 days per week) (McGrath et al. 1979). A more expressive increase in the weight of the heart, morphological and hematological changes developed in male

Figure 3. Global score of ECG abnormities (sum of logarithms of the scores of QRS-T wave abnormities and arrhythmia) as a function of subgroup 0 - control rats, 1 – preexposed rats The Figure illustrates subgroup component effects in analyses (A, B and C) of multiple regression of the global score on various combinations of four independent variables SUBGRoup membership, COHb concentration at the end of the challenge exposure, mean Heart Rate during the challenge exposure, and concentration of ACtive Hemoglobin, i e , hemoglobin in blood $\times$ (1 – COHb) Model fitting results (t-values and P-values for regressors and multiple correlation coefficient r) for   Analysis A $r = 0\,63$ SUBGR $t = -2\,75$, $P = 0\,022$, COHb $t = 1\,84$, $P = 0\,098$ Analysis B $r = 0\,82$ SUBGR $t = -3\,775$, $P = 0\,006$, COHb $t = 3\,18$, $P = 0\,013$, HR $t = 2\,99$, $P = 0\,017$ Analysis C $r = 0\,88$ SUBGR $t = 0\,45$, $P = 0\,66$, AC HG $t = -4\,32$, $P = 0\,003$, HR $t = 4\,03$, $P = 0\,004$ Analysis D $r = 0\,90$ AC HG $t = -6\,04$, $P = 0\,0002$, HR $t = 4\,25$, $P = 0\,002$ A comparison of models C and D shows that the effect of preexposure on acute exposure induced ECG pathology could be almost completely explained by differences in available hemoglobin and heart rate at the challenge exposure

Sprague-Dawley rats (weight 275g) after a continuous exposure to 500 ppm for 42 days (Davidson and Penney 1988) The ratio of heart to body weight, heart weight and hematocrit increased after only a 30-day continuous exposure to 500 ppm in 60 days old rats (Penney et al 1994) Polycythemia was detectable in rats already after 3–5 days of a continuous exposure at CO concentrations higher than 100 ppm (Davidson and Penney 1988) This time corresponds also to the onset of adaptational changes at acclimatization to heights over 3000 m But in dogs a similar exposure did not induce any hematological adaptation (DeBias et al 1972) Continuous exposure seems thus considerably more efficient than intermittent exposure, modelling occupational settings

Conclusions

Subchronic CO exposure induced marked compensatory processes (detectable he-

matologically) but only a tendency to changes in the heart of rats (by gross morphometry). On the functional level, the preexposure antagonized significantly the ECG response to acute CO exposure.

References

Benjamin I. J., Jalil J. E., Tan L. B., Cho N. K., Weber K. T., Clark W. A. (1989): Isoproterenol-induce a myocardial fibrosis in relation to myocyte necrosis. Circ. Res. **65**, 657—670

Davidson S. B., Penney D. G. (1988): Time course of blood volume change with carbon monoxide inhalation and its contribution to the overall cardiovascular response. Arch. Toxicol. **61**, 306—314

DeBias D. A., Birkhead N. C., Banerjee C. M., Kazal L. A., Holburn R. R., Greene C. H., Harrer W. V., Rosenfeld L. M., Menduke H., Williams N., Friedman M. H. F. (1972): The effects of chronic exposure to carbon monoxide on the cardiovascular and hematologic systems in dogs with experimental myocardial infarction. Int. Arch. Arbeitsmed. **29**, 253—267

Kolesár J. (1988): Changes in the cardiovascular system due to aging. Brat. Lek. Listy **89**, 44—58 (in Slovak)

McGrath J. J., Chen K. C., Vostal J. J. (1979): Adaptive reaction to carbon monoxide-induce hypoxia. Toxicol. Appl. Pharmacol. **48**, A56

Mikisková H. (1988): Acute effect of carbon monoxide in the ECG picture. Pracov. Lék. **40**, 435—445 (in Czech)

Osborne B. E. (1981): The electrocardiogram of the rat. In: The Rat Electrocardiogram in Pharmacology and Toxicology (Eds. Budden R. et al.) pp. 15—27, Pergamon Press, Oxford

Ošťádal B., Widimský J., Ubranová D., Procházka J., Pelouch V. (1981): Verapamil in rats exposed to intermittent altitude hypoxia . Čas. Lék. čes. **120**, 1345—1349 (in Czech)

Penney D. G. (1988): A review: Hemodynamic response to carbon monoxide. Environ. Health. Perspect. **77**, 121—130

Penney D. G. (1990): Acute carbon monoxide poisoning: animal models: A review. Toxicology **62**, 123—160

Penney D. G., Giraldo A. A., Egmond Van E. M. (1994): Coronary vessel alterations following chronic carbon monoxide exposure in the adult rat. J. Appl. Toxicol. **14**, 47—54

Thomsen H. K., Kjeldsen K. (1974): Threshold limit for carbon monoxide-induced myocardial damage. Arch. Environ. Health **29**, 73—78

Trávníčková Z. (1995): Cardiotoxicity of carbon monoxide in dependence on circulatory load. Prakt. Lék. **75**, 406—408 (in Czech)

Trávníčková Z., Frantík E. (1994): Neurobehavioral and autonomic cofactors of toxicity: cardiotoxicity of carbon monoxide. Homeostasis **35**, 277—284

Trávníčková Z., Frantík E., Blažek K. (1996): Age and functional state as intervening variables in experimental cardiology. Proceedings: International Interdisciplinary Toxicological Conference, Piešťany Slovakia, October 1996