PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 157, 681–683 (1978)

# Effects of Carbon Monoxide on Isolated Heart Muscle (40121)

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The mechanism by which carbon monoxide (CO) affects the mammalian organism was first described by Haldane (1) and has since been confirmed by numerous other workers (2, 3). Briefly, CO binds with blood hemoglobin to form carboxyhemoglobin. This causes a functional anemia by decreasing the amount of hemoglobin available for oxygen transport and by causing the oxyhemoglobin dissociation curve to shift to the left. If the tissue hypoxia is severe enough, the resultant of these two effects can lead to a fatal event.

There are, however, several reports in the literature suggesting that CO might have a direct effect on functioning tissue in addition to its hemoglobin-mediated effects (4–7). The following experiments were undertaken to study the effects of CO on the isolated, rat right ventricle preparation contracting in a hemoglobin-free medium.

*Methods.* Male, white rats weighing 312 ± 14 g were guillotined and the hearts excised rapidly. The right ventricle was dissected away and split into two pieces. One strip was mounted on an electrode-bearing block, attached to a strain gauge transducer and immersed in an oxygenated Krebs–Ringer solution as previously described (8). The preparation was stimulated at a rate of 60 per min and resting and developed tensions were recorded. At the end of one hour the aerating gas mixture of 95% $O_2$–5% $CO_2$ was changed to either 95% $N_2$–5% $CO_2$ ($N_2$-stress) or 95% CO-5% $CO_2$ (CO-stress). After 10 min of the stress gas, oxygen was again bubbled through the medium and the contracting muscle was allowed to recover for 10 min in 95% $O_2$–5% $CO_2$ (normoxia) or 70% $O_2$–25% CO–5% $CO_2$ (CO-hypoxia).

The time in minutes required for the contracting muscle to decline to one half of its preanoxic amplitude during the $N_2$ or CO-stress was determined and expressed as $T_{50}$. The change in resting tension in grams at the end of 5 min of stress was expressed as $\Delta T_5'$. The extent of recovery after 10 minutes of normoxia or CO-hypoxia was determined by comparing the ratio of the recovery amplitude to the pre-anoxic amplitude and expressing the results as percent recovery (%R) according to the following formula (9).

$$\%R = \text{Recovery amplitude} / \text{Preanoxic amplitude} \times 100$$

The Student $t$ test was used to test the significance of differences between means (10). Tissue electrolytes were also determined on nitric acid digests by atomic absorption spectrophotometry according to the method of McGrath and Bullard (8). Briefly, one portion of the right ventricle was prepared for analysis immediately while the second portion was prepared after recovery from the $N_2$- or CO-stress. The tissues were dried overnight in an oven at 100°, digested with nitric acid and analyzed for sodium and potassium. The results were expressed as percent increase or decrease from control values.

*Results.* Developed tension decreased and resting tension increased in the isolated right ventricles during either $N_2$- or CO-stress. The time required for the developed tension to decrease to one half of its pre-stress value ($T_{50}$) was 2.5 minutes in $N_2$ and 2.3 min in CO (Table I). The difference between these values is not significant. The change in resting tension ($\Delta T$) at the end of 5 minutes of stress was 1.0 g in nitrogen and 0.9 g in CO. The difference between these values is not significant.

The percent recovery (%$R$) after 10 min of normoxia was 43.5 % in nitrogen and 63.0% in CO (Table II). Recovery was significantly greater in the CO-stressed muscles. Percent recovery was reduced when the $N_2$-stressed or the CO-stressed muscles were allowed to recover in CO-hypoxia. Under these conditions recovery was reduced to 31.6% and

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0037-9727/78/1574-0681$01.00/0

36.9% in the $N_2$ and CO-stressed muscles, respectively. The difference between these values is no longer significant.

After the $N_2$- and CO-stressed muscles had recovered in oxygen, the electrolyte content was determined and compared to pre-stress levels. With both gas stresses, sodium levels were increased and potassium levels were decreased at the end of the recovery period (Table III). Similar electrolyte changes were noted in the $N_2$- and CO-stressed muscles that recovered with CO present in the recovery mixture.

*Discussion.* We have demonstrated a direct effect of carbon monoxide on functioning heart muscle. The effect of CO on heart muscle was similar in some ways to that of nitrogen in that it caused a reduction in developed tension, an increase in resting tension and a reduction in percent recovery. However, we were surprised to observe that the effect was also different in that the CO-treated muscle appeared to be somewhat protected during the gas stress and that it recovered to a significantly greater degree when oxygen was reintroduced into the bathing medium. There is evidence in the literature that CO can be burned by living tissue. Fenn and Cobb (11) showed that the stimulatory action of CO on the respiration of frog and rat skeletal and cardiac muscle was due to oxidation of CO to $CO_2$. This work was confirmed by Schmitt and Scott (12) and Stannard (13). Clark et al (14) working with frog tissue showed that the rate of CO oxidation was greatest for heart muscle, next for skeletal muscle and negligible in skin and nerve. Clark (15) also demonstrated CO-burning in intact turtles and mice.

If these results can be applied to our present study, it is possible that our CO-stressed muscles were able to burn a small amount of CO under the conditions of the experiment and that this somehow ameliorated its anoxic effects. It is not known what the mechanism underlying this protective effect is or how it might act to maintain tissue integrity so that tissue recovery following the CO-stress was more complete. However, if tissue integrity was better maintained during CO-stress, the tissue would be less damaged and when oxygen was again made available capable of a greater degree of recovery. That tissue integrity was affected by both gas stresses is shown by the electrolyte changes that occurred. The sodium content of both the $N_2$- and CO-stressed muscle increased, while the potassium content decreased. The results for sodium and potassium are similar to those reported by others (16, 17) and seen by us in earlier studies (18). However, it is not possible from the techniques used in this study to

TABLE I. TIME TO 50% CONTRACTION AMPLITUDE ($T_{50}$) AND CHANGE IN RESTING TENSION AT 5 MIN ($\Delta T_5'$) DURING (A) NITROGEN OR (B) CARBON MONOXIDE-INDUCED ANOXIA.

| Treatment | *n* | $T_{50}$ (min) | $\Delta T_5'$ (+g) |
|---|---|---|---|
| A 95% $N_2$-5% $CO_2$ | (12) | 2.5 ±.2 | +1.0 ±.1 |
| B 95% CO-5% $CO_2$ | (12) | 2.3[a] ±.2 | +.9[a] ±.2 |

[a] = N.S. Values are means ± SE. Student *t* test used to test significance.

TABLE II. PERCENT RECOVERY (%R) IN OXYGEN OR CO-HYPOXIA AFTER (A) NITROGEN OR (B) CARBON MONOXIDE-INDUCED ANOXIA.

| | Oxygen | | CO-hypoxia | |
|---|---|---|---|---|
| Treatment | *N* | *%R* | *n* | *%R* |
| A 95% $N_2$–5% $CO_2$ | (12) | 43.5 ±4.0 | (14) | 31.6 ±5.8 |
| B 95% CO–5% $CO_2$ | (12) | 63.0* ±4.3 | (14) | 36.9[a] ±7.5 |

[a] = N.S. Values are means ± SE. Student *t* test used to test significance. Number of hearts per group is given in parenthesis.

* $P < 0.05$.

TABLE III. TISSUE ELECTROLYTE CHANGES AFTER RECOVERY IN OXYGEN OR CO-HYPOXIA AFTER (A) NITROGEN OR (B) CARBON MONOXIDE-INDUCED ANOXIA.

| | n | Na | n | K |
|---|---|---|---|---|
| I. Recovery in oxygen | | | | |
| A 95% $N_2$–5% $CO_2$ | (10) | +23% | (11) | −18% |
| B 95% CO–5% $CO_2$ | (11) | +21% | (12) | −17% |
| II. Recovery in CO | | | | |
| A 95% $N_2$–5% $CO_2$ | (11) | +19% | (12) | −24% |
| B 95% CO–5% $CO_2$ | (10) | +16% | (12) | −24% |

determine if cellular disruption was more intense in the $N_2$-stressed muscle.

Both the $N_2$-stressed and CO-stressed muscle recovered somewhat more erratically when recovery occurred in the oxygen-CO mixture. First, the extent of recovery was reduced in both cases so there was no longer any difference in *%R*. Second, the range of *%R* was always greater when recovery occurred in the CO mixture, and with both stresses there were two experiments in which the preparations failed to recover completely. The variability of *%R* was always less and there was always some degree of recovery when recovery occurred in oxygen.

Several workers have shown that CO stimulates respiration and Breckenridge (19) demonstrated that carbon monoxide oxidation is catalyzed by cytochrome oxidase in heart muscle fraction. Thus, the depressed recovery of function in our CO-hypoxia experiments may be due to the increased $O_2$ demand placed on the muscle during recovery when the available oxygen supply was marginal. It is possible that during recovery in oxygen, more oxygen was available for restitution of the cell and normal metabolic activity, while during recovery in CO available $O_2$ was divided between these two activities and a CO-stimulated component. Thus, function as expressed by *%R* would be reduced when CO was present in the recovery mixture.

*Summary.* The direct effects of carbon monoxide and nitrogen-induced anoxia on the isolated isometrically-contracting right ventricle of the rat were assessed and compared. Both gas stresses caused a decrease in developed tension, an increase in resting tension and a reduction in percent recovery when the preparations were reoxygenated. There was no significant difference in the decline in developed tension or the increase in resting tension between the two groups. Recovery after 10 min of reoxygenation was significantly greater in the CO-stressed muscles. Recovery from either anoxia was severely depressed when CO was present during the recovery phase.

Tissue sodium levels were elevated while potassium levels were depressed by both stresses.

These results indicate that CO may have a direct effect on the heart in addition to its well known hemoglobin mediated effect.

This work was supported in part by Illinois Heart Association Grant-In-Aid No. 74-3. The authors gratefully acknowledge the technical assistance of Ms. Sharon Murnane.

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Received September 1, 1977. P.S.E.B.M. 1978, Vol. 157.