Effect of Caffeine on the Ventilatory Response to CO_2 in Newborn Rats

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ABSTRACT

Methylxanthines (MXs), such as theophylline (1,3-diMX) and caffeine (1,3,7-triMX), are commonly used in the treatment of recurrent apnea due to their stimulant effects on the respiratory center. In a previous study, caffeine was shown to increase tidal volume (V_T), but not respiratory rate (f) in newborn rats breathing room air. Carbon dioxide (CO_2) is known to be a powerful stimulant to breathing. The present study was designed to test the effect of various doses of caffeine on the ventilatory response to CO_2. The PowerLab computer-based physiology laboratory was used in conjunction with a body plethysmograph to study the respiratory effects of caffeine and CO_2 in 4- to 6-day-old rats. From each recording, V_T, f, minute ventilation (V_E), time of inspiration (T_i), and total time of each breath (T_{tot}) were obtained, and effective timing (T_i/T_{tot}), and mean inspiratory flow (V_T/T_i) were calculated. After an initial CO_2-response test, each rat received a s.c. injection of saline, 20, 40, or 80 mg/kg caffeine. The CO_2-response test was then repeated at three 15-min intervals after drug administration. The ventilatory response to CO_2 was pronounced in all animals before and after drug administration. Caffeine (80 mg/kg) produced a significant increase in V_E in response to CO_2, especially at 45 min after administration. There was a trend toward increasing the mean inspiratory flow response to CO_2, which did not reach statistical significance. While there was no frequency response to CO_2, there was a statistically significant change in the frequency pattern after caffeine, but there was no obvious trend. This study clearly demonstrates the usefulness of PowerLab in conducting respiratory studies in the newborn rat. Caffeine seems to be an effective drug for modifying the ventilatory response to CO_2. Further investigation is needed using higher doses in order to discern the mechanism by which caffeine modifies the CO_2-response.

INTRODUCTION

Breathing is controlled by the respiratory center in the medulla oblongata. When an infant's medulla is immature, it occasionally ceases breathing and does not respond to signals from chemoreceptors even when the carbon dioxide (CO_2) concentration in the body increases. Apnea in neonates, which may lead to sudden infant death syndrome (SIDS), is considered to be caused by the decreased ventilatory response to CO_2. Recurrent apnea is treated by methylxanthines (MXs), such as theophylline (1,3-diMX) and caffeine (1,3,7-triMX). Many studies have shown the beneficial effect of theophylline on apnea. Aranda et al. (1986) reviewed the various effects of theophylline on many organs of the newborn. It has been shown that theophylline increases minute ventilation (V_E) and tidal volume (V_T), and increases the ventilatory response to CO_2.

Although theophylline is a popular drug for treatment of apnea, caffeine is also effective. Bairam et al. (1987) found that caffeine showed an effect on respiratory rate (f) earlier than theophylline did. In addition, caffeine has fewer side effects than theophylline, such as tachycardia, arousal, and gastrointestinal intolerance. Furthermore, better stability was observed in caffeine plasma levels due to a longer half-life than theophylline and the fact that theophylline is methylated into caffeine in the liver.

Rigatto et al. (1975) studied the ventilatory response to CO_2 in infants born at 32 and 37 weeks gestational age. They observed a depression of respiration at 32 weeks of gestational age and concluded that it is due to immaturity of the respiratory center. Since the same phenomenon is observed in newborn rats, we chose to investigate the effect of caffeine on the immature breathing system of newborn rats.

McGilliard et al. (1990) reported that theophylline increases both V_T and f to increase V_E in newborn rats, whereas caffeine increases only V_T and decreases f at high doses. In other words, caffeine administration caused newborn rats to breath slowly and deeply. Based on this result, we were interested in the effect of caffeine on the ventilatory response to CO_2.

Our purpose in conducting this study was to investigate the effect of three different doses of caffeine on the respiratory pattern and ventilatory response to CO_2 of newborn rats. Our hypothesis was that the degree of ventilatory drive increases with increasing doses of caffeine. Of particular interest is the mean inspiratory flow (MIF), a measure of central respiratory drive.

METHODS

Respiration was measured in 4- to 6-day-old rats using a body plethysmograph (fig. 1), which is connected to a flow transducer and the PowerLab computer-based data acquisition unit (fig. 2). Rats were randomly divided into 4 treatment groups of 8 rats each: 20 mg/kg caffeine, 40 mg/kg caffeine, 80 mg/kg caffeine, or saline controls.

Each rat was placed in the body plethysmograph and respiration was recorded while breathing room air (0 % CO_2). The CO_2 concentration was then increased in 1 % increments from 0 to 6 %. After the initial CO_2-response test, each rat received a s.c. injection of saline, 20, 40, or 80 mg/kg caffeine. The CO_2-response test was then repeated at three 15-min intervals after drug administration. Respiratory air flow and inspired CO_2 concentrations were collected by the PowerLab at a rate of 100 samples/sec. Flow data were converted by the Chart software to V_E, V_T, and f. The data were further analyzed for time of inspiration (T_i), time of expiration (T_e), total time of each breath (T_{tot}), MIF (V_T/T_i), and effective timing (T_i/T_{tot}).

The caffeine effect was expressed as % difference from pre-injection controls. We determined CO_2-response slopes by dividing average changes in respiratory values by the change in CO_2 concentrations. The data were analyzed by 3-way analysis of variance with two repeated measures (time and % CO_2), using STATPAK software. It analyzed significance of each variable (dose, time, and % CO_2) and the interactions between variables.

Figure 1. Body plethysmograph

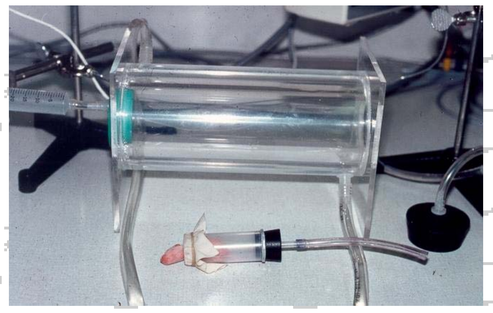


Figure 2. PowerLab

RESULTS

CO_2 response

There was a consistent and statistically significant ($p < 0.001$) increase in V_E, V_T, and MIF with increasing concentrations of CO_2 which was independent of treatment (figs. 3 and 4). Respiratory rate was unaffected by inhalation of CO_2 (fig. 5).

Effect of caffeine on respiratory pattern

In control rats breathing room air, respiratory rate declined at 30 and 45 min after the injections ($p < 0.05$). However, caffeine-treated rats did not show as much of a decrease as those with saline. V_E, V_T, MIF, and effective timing did not show any significant change with time after treatment.

Slopes of the CO_2 response

The slopes of V_T, f, and effective timing responses to CO_2 did not show any dose-related trends. The slopes of V_E and MIF responses to CO_2 increased with time at the 80 mg/kg dose of caffeine (fig. 3), whereas the slopes with 20 and 40 mg/kg of caffeine doses showed minor changes (fig. 4).

Analysis of variance

Despite a trend of increasing slope of the V_E and MIF responses to CO_2 with increasing doses of caffeine, there was no statistically significant dose or time effect. There was a significant interaction between % CO_2 and time. On the other hand, V_T, f, and effective timing showed significant interactions between dose and % CO_2 and between dose, % CO_2, and time. However, we did not find any major trend of the changes with CO_2 responses (fig. 5).

Figure 3. % change in MIF in response to CO_2 before/after caffeine (80 mg/kg)

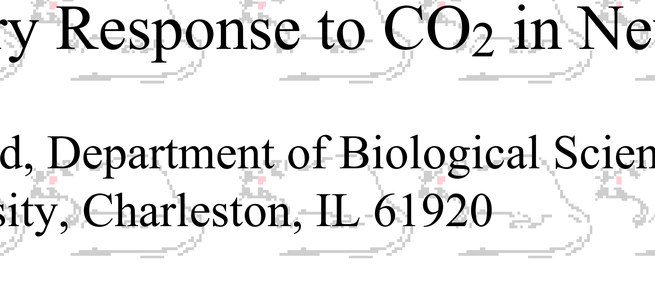


Figure 4. % change in V_E in response to CO_2 45 minutes after caffeine injection

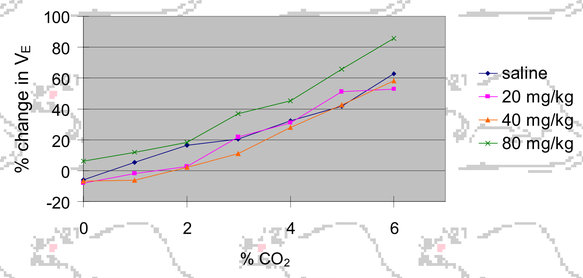
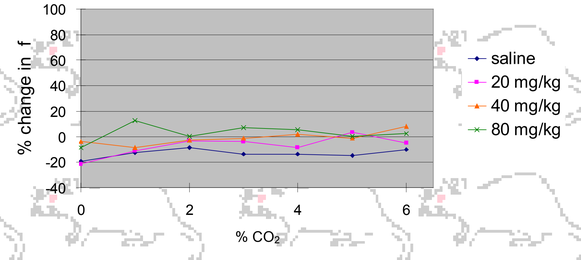


Figure 5. % change in f in response to CO_2 45 minutes after caffeine injection



DISCUSSION

These studies confirmed past observations that newborn rats show a strong ventilatory response to CO_2. The lack of frequency response to CO_2 (fig. 5) suggests that respiratory control in newborn rats resembles that of premature infants (Rigatto et al., 1975). Milic-Emili et al. (1976) and McGilliard et al. (1990) reported that the V_T and f are controlled independently. Thus, V_E is the product of interaction of two factors, V_T and f, which can independently respond to the environment.

We found that caffeine in the doses tested did not affect V_E, effective timing, nor MIF in rats breathing room air (0% CO_2). However, rats receiving 80 mg/kg of caffeine showed a trend of increased CO_2 response of V_E and MIF, although only the CO_2 x time interaction was significant. Therefore, caffeine might affect central respiratory drive during exposure to CO_2. Romagnoli (1992) suggested that caffeine stimulates central nervous system by increasing chemoreceptor responsiveness to sense CO_2.

Respiratory rate declined with time in control rats breathing room air. We assume that this was due to habituation. The decrease in f was absent when caffeine was administered. The result shows that caffeine affects respiratory rate in newborn rats although to a very asmall degree. The 3-way analysis of variance of respiratory rate showed significant interactions of dose, time, and % CO_2. This is difficult to interpret since we did not find any major trends in the f responses to CO_2 (fig. 5).

McGilliard et al. (1990) have reported that theophylline increases both V_T and f, thus increasing V_E, whereas caffeine increases V_T and decreases f at high doses. They concluded that the respiratory pattern of newborn rats administered 160 mg/kg caffeine was deep and slow. However, in our result, the newborn rats administered 80 mg/kg of caffeine showed the respiratory pattern that was faster than those administered saline.

Our 3-way analysis of variance did not show significant dose-related V_E and MIF responses to CO_2. However, there were obvious trends of increasing slope of the CO_2 response with 80 mg/kg of caffeine doses. Therefore, further investigations with higher doses are likely to show more dramatic effects of caffeine on respiratory control.

CONCLUSIONS

- **Carbon dioxide inhalation increased ventilatory volumes in all rats, regardless of treatment. However, respiratory rate did not increase in response to inhalation of CO_2.**
- **Although there was no significant effect of caffeine on the minute ventilation and inspiratory flow responses to CO_2, there was a trend toward increasing slopes of the CO_2–response curves with the 80 mg/kg dose of caffeine.**
- **Respiratory rate, tidal volume, and effective timing showed significant interactions with dose, time, and % CO_2, however, there were no trends.**
- **It is likely that caffeine stimulates the ventilatory response to CO_2, but higher doses than those tested are required to demonstrate this.**

REFERENCES

Aranda, J.V. et al., 1986. J. Allergy Clin. Immunol. 78: 773-780.

Bairam, A. et al., 1987. J. Pediatr. 110: 636-639.

McGilliard, K.L. et al., 1990. In: Modulation of Respiratory Pattern: Peripheral and Central Mechanisms, pg. 79.

Milic-Emili, J. and Grunstein, M.M., 1976. Chest 71: 131-133.

Rigatto, H. et al., 1975. Pediatrics 55: 614-620.

Romagnoli, C. et al., 1992. Ther. Drug Monit. 14: 14-19.