Pharmacology Biochemistry & Behavior, Vol. 34, pp. 681–684. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Ventilatory Responses of Dystrophic and Control Hamsters to Naloxone

EVELYN H. SCHLENKER AND TIMOTHY J. METZ

Department of Physiology and Pharmacology
University of South Dakota School of Medicine, Vermillion, SD 57069

Received 10 April 1989

SCHLENKER, E. H. AND T. J. METZ. *Ventilatory responses of dystrophic and control hamsters to naloxone.* PHARMACOL BIOCHEM BEHAV **34**(4) 681–684, 1989.—In this study we determined if endogenous opioid peptides may contribute to the depression of ventilation seen in dystrophic hamsters. Ventilation of control and dystrophic awake hamsters was determined prior to either naloxone (1 mg/kg) or saline administration and then 5, 15 and 30 minutes postinjection. Subsequently, animals were exposed to a hypercapnic challenge (7% CO_2 in O_2). Control hamsters increased ventilation significantly ($p<0.01$) after naloxone compared to saline treatment. In contrast, dystrophic hamsters showed no difference in ventilation when they received either naloxone or saline. Both groups increased ventilation significantly ($p<0.05$) after hypercapnic challenge, whether they had received naloxone or saline. Although dystrophic hamsters can respond to a ventilatory stimulant (CO_2), naloxone did not increase ventilation, possibly indicating that endogenous opioids are not responsible for their depressed ventilation.

Control of ventilation Muscular dystrophy Endogenous opioids Responses to hypercapnia

ENDOGENOUSLY produced opioid peptides have been implicated as modulators of ventilatory control (8, 10, 11). Chadha and Sackner (1) found that the development of irregular and periodic breathing induced by progressive acute hypoxemia in 6 normal awake human subjects could be blunted or eliminated by pretreatment of the subjects with naloxone, an opioid antagonist. Neubauer and her colleagues (9) were able to reverse the respiratory depression due to carboxyhemoglobin hypoxemia in cats with naloxone, also implicating opioid peptides as contributors to the respiratory depression.

The role of endogenous opioids in the ventilatory response to acute flow-resistive loads in unanesthetized goats was investigated by Scardella and his co-workers (14). They found that loading resulted in endogenous opioid generation as assessed by measurement of immunoreactive β-endorphin levels in cisternal cerebrospinal fluid. Furthermore, they were able to reverse the depression of ventilation after loading by administration of naloxone.

The role endogenous opioids play in the control of breathing of subjects with chronic obstructive pulmonary disease (COPD) has been equivocal. Reents and Beck, Jr. (12) found that naloxone resulted in a stimulation of ventilation in patients with COPD, whereas Roca and co-workers (13), as well as Simon and her colleagues (16), found that naloxone did not stimulate ventilation. In the latter studies, subjects given naloxone showed no stimulation of ventilation after hypercapnic challenge nor loaded breathing.

In our laboratory, we have been studying an animal model of alveolar hypoventilation, the dystrophic hamster (15). This animal develops progressive dystrophy as a consequence of autosomal recessive genes. In this study we investigated the possibility that endogenous opioid peptides may contribute to the depression of ventilation of the dystrophic hamster.

METHOD

Male BIO 14.6 (dystrophic) and golden Syrian hamsters (control) were obtained from Bio-Breeders, MA and Sasco, Omaha, NE, respectively. At the time of the study the dystrophic hamsters averaged a body weight of 80 g and the control hamsters, 107 g. Before experiments were initiated, the animals were housed for 2 weeks in pairs (dystrophic-dystrophic or control-control) in stainless steel cages in our animal facilities. Light-dark schedules were 14 hr on-10 hr off. Animals received food and water ad lib.

Respiratory measurements were made in a 19.7×9.5 cm Plexiglas cylinder. One end of the cylinder was closed and contained 3 openings for measuring chamber temperature (using a Digitec HT series 5810 thermometer), O_2 and CO_2 concentrations (using Beckman M-14 O_2 and LB-2 CO_2 gas analyzers) and flow rate through the chamber (using a Gilmont rotameter). The other side of the chamber was closed with a large rubber stopper (No. 15). A port through the stopper allowed air or CO_2 to enter the chamber. Using another port, pressure changes associated with ventilation were measured by a Stathem PM 15E low pressure transducer (±2.5 cm H_2O) attached to a Grass 7D recorder. Ventilatory parameters that were measured included tidal volume (V_T), frequency of breathing (f), minute ventilation (V_E) and inspiratory and expiratory times (T_I and T_E).

Procedures

After the hamster was weighed, he was placed in the chamber through which air was constantly flushed. A preinjection reading was obtained after the hamster was acclimatized to the chamber. Subsequently, the animal received an intraperitoneal injection (0.1–0.2 ml) of either saline or 1 mg/kg naloxone (Endo Laboratories, Inc.). The hamster was then placed back into the chamber

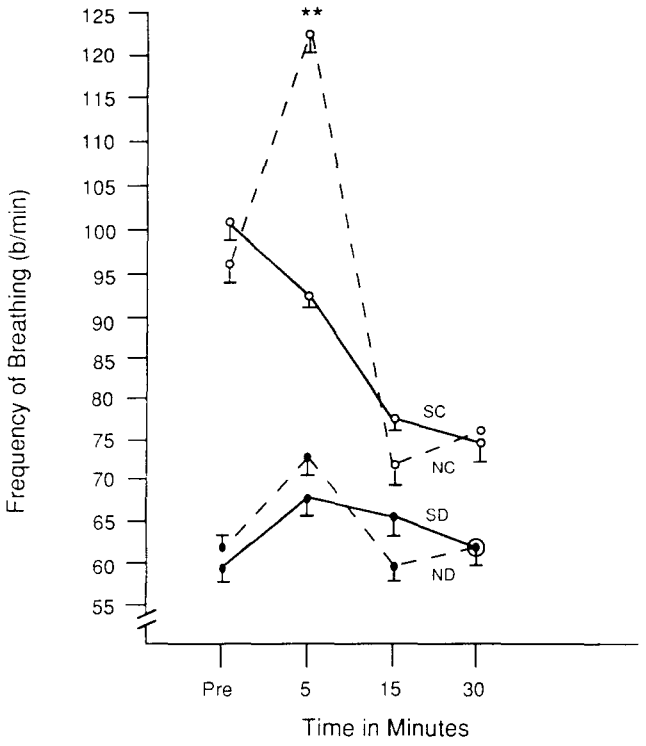

FIG. 1. Frequency of breathing of control hamsters (○) receiving saline (SC-) or naloxone (NC--) and dystrophic hamsters (●) receiving saline (SD-) or naloxone (ND--). Asterisks indicate a significant difference ($p<0.01$) between naloxone and corresponding saline levels in control hamsters. Values are mean ± SEM.

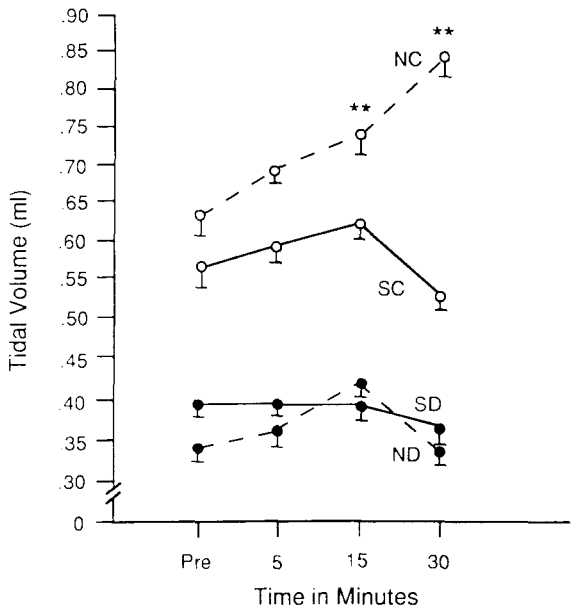

FIG. 2. Tidal volume response of control hamsters (○) receiving saline (SC-) or naloxone (NC--), and dystrophic hamsters (●) receiving saline (SD-) or naloxone (ND--). Asterisks indicate significant differences ($p<0.01$) between naloxone and corresponding saline values in control hamsters. Values are mean ± SEM.

and ventilation was measured 5, 15, and 30 minutes later. Then the hamster was subjected to a hypercapnic challenge consisting of 7% CO_2 in O_2. Finally, the CO_2 was flushed out of the chamber and the ventilatory response of the hamster to air was again assessed. Body temperature was measured with a BAT 12 Sensortek thermometer. A total of 32 animals were used in this study, or 8 hamsters per treatment group.

Data were analyzed using a three-way ANOVA with a least squares post-priori test using the SAS Version 5 Edition (Cary, NC, 1985). Student's paired *t*-tests were used to evaluate the ventilatory response of hamsters to CO_2.

RESULTS

The frequency of breathing of control hamsters given naloxone was significantly increased ($p<0.01$) compared to the corresponding 5-minute saline value (Fig. 1). The increased f was due to a shortening of T_I. In contrast, naloxone did not alter f of dystrophic hamsters compared to saline values.

Tidal volume of control, but not dystrophic hamsters was also increased compared to that of hamsters receiving saline at 15 and 30 minutes postinjection (Fig. 2). The minute ventilation response of control hamsters who had received naloxone was also markedly increased compared to control hamsters who had received saline at 5 and 30 minutes (Fig. 3). Unlike the control hamsters, when dystrophic hamsters were given naloxone, they showed no significant increase of f, V_T or V_E compared to those given saline.

To evaluate the question if dystrophic hamsters could respond to another respiratory stimulus, hamsters were exposed to hypercapnia. As shown in Fig. 4 and Table 1, both control and dystrophic hamsters increased V_E in response to hypercapnia. Control hamsters who had received naloxone and then were challenged with CO_2 showed a larger ventilatory response compared to CO_2 than did control hamsters receiving only saline.

DISCUSSION

This study indicates that naloxone stimulates ventilation of

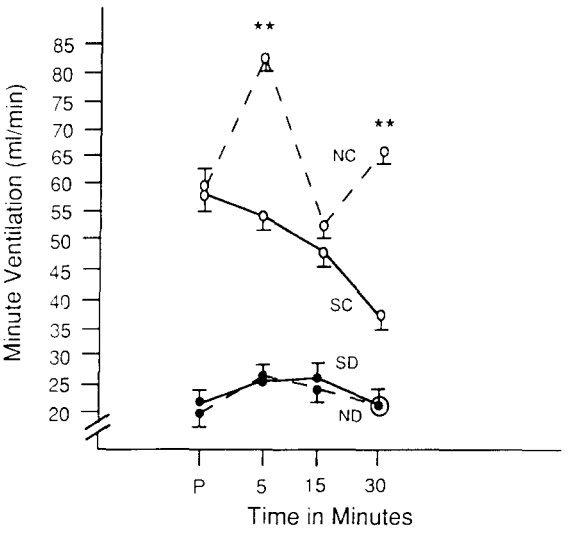

FIG. 3. Minute ventilation response of control hamsters (○) receiving saline (SC-) or naloxone (NC--) and dystrophic hamsters (●) receiving saline (SD-) or naloxone (ND--). Asterisks indicate significant differences ($p<0.01$) between naloxone and corresponding saline values.

TABLE 1

MINUTE VENTILATION (V_E, ml/min) RESPONSES OF CONTROL AND DYSTROPHIC HAMSTERS AFTER NALOXONE ADMINISTRATION TO 7% CO_2

	Control	Dystrophic
Ventilation Before CO_2	54.4 ± 4.6	22.1 ± 3.5
Response to CO_2	95.9 ± 11.2†	30.6 ± 2.5*
Ventilation After CO_2	56.5 ± 4.8	23.5 ± 1.9

Values are means ± SEM.

Superscripts indicate significant (*$p<0.05$, †$p<0.01$) of ventilation after CO_2 exposure compared to air exposure (before and after CO_2).

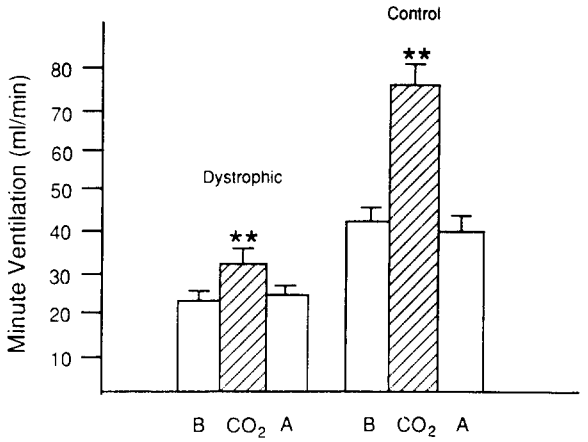

FIG. 4. Minute ventilation responses of dystrophic and control hamsters before (B), during CO_2 (CO_2) and after CO_2 (A) exposure. Asterisks indicate significant ($p<0.01$) increases of minute ventilation during CO_2 exposure compared to ventilation before and after exposure.

control, but not of dystrophic hamsters. In addition, stimulation of ventilation of dystrophic hamsters, as well as of control hamsters, occurred with hypercapnic challenge indicating that dystrophic hamsters are capable of responding to another "stimulant." A number of possibilities, which at this point are speculations, may suggest themselves for the differential responses of the two groups.

The dystrophic hamster, like the human COPD patient, is exposed to a chronic stress situation. Prolonged intermittent foot shock stress has been shown to decrease Met- and Leu-enkephalin levels 40 and 50% in the brains of rats (7). Recently, Stuckey and his co-workers (17) found that binding of (^{3}H)DAGO, a selective μ-opiate receptor agonist, was decreased in rat brains after inescapable shock. The decrease in binding was the result of a decrease in the number of μ-receptors and not a change in affinity.

Another possible mechanism responsible for the differential results we observed in this study is that of genetic variability of opioid levels, receptor numbers or activity. In a recent review, Frischknecht and co-workers (2) showed that a genetically determined dissociation of opioid effects on obesity, locomotor activity, pain inhibition, and high risk for drug addiction exist in different genetic animal models. Evaluating brain opioid levels, receptor numbers and affinities of dystrophic and control hamsters of various ages and correlating those with ventilation may allow us to evaluate both the 1st and the 2nd possibility. Ikeda and co-workers (4) have already shown that the brains of golden Syrian hamsters contain high levels of various enkephalins. In fact, they found higher levels of these chemicals in the brains of hamsters than in guinea pig or rat brains. To evaluate the functional capacities of these systems, various opioid agonists (acting on different receptor types) were administered to normal and dystrophic hamsters at various ages and the magnitude of the ventilatory depression determined.

Another factor that may influence endogenous opioid levels is increased corticosterone and adrenocorticotropic hormone levels (ACTH). Chronic stress resulting in prolonged release of ACTH and corticosterone has been shown to decrease β-endorphin levels (5). Although we did not measure levels of these hormones in control and dystrophic hamsters, there is evidence that adrenal cortical function is not normal in the latter animals (6). In fact, with progressive disease, water retention in dystrophic hamsters occurs (3,6).

In summary, this study indicates that ventilation of dystrophics is not stimulated by naloxone administration. One possibility to consider is that in the dystrophic hamster the respiratory system, because of its weakened state, is pushed to its limit and endogenous opioid inhibition of ventilation itself is inhibited. This would be analogous to the situation of chronic stress as seen in patients with chronic obstructive lung disease. In the normal hamster, in contrast, ventilation may be inhibited by endogenous opioids as part of a dynamic regulatory system (including other neurotransmittors), thus naloxone administration unmasks this component of the regulatory system.

ACKNOWLEDGEMENTS

This research was supported by a General Research Grant from The University of South Dakota. We would like to thank Shellie Delaney for typing the manuscript.

REFERENCES

1. Chadha, T. S.; Birch, S.; Sackner, S. A. Periodic breathing triggered by hypoxia in normal awake adults—modification by naloxone. Chest 88:16–23; 1985.
2. Frischknecht, H. R.; Siegfried, B.; Waser, P. G. Opioid and behavior: genetic aspects. Experientia 44:473–481; 1988.
3. Galla, J. H.; Schneider, G.; Kotchen, T. A.; Hayslett, J. P. Renin and aldosterone in cardiomyopathic hamster in congestive heart failure. Endocrinology 101:389–395; 1977.
4. Ikeda, Y.; Nakao, K.; Yoshimasa, T.; Yanaihara, N.; Numa, S.; Imura, H. Existence of MET-enkephalin-Arg^6-Gly^7-Leu^8 with MET-enkephalin, LEU-enkephalin and MET-enkephalin-Arg^6-PHE^7 in the brain of guinea pig, rat and golden hamster. Biochem. Biophys. Res. Commun. 107:656–662; 1982.
5. Kanyicska, B.; Stark, E.; Horváth, Gy.; Simmonyi, A.; Fekete, M. I. K. Long term ACTH induced diminished responsiveness of prolactin secretion to morphine. Life Sci. 32:55–63; 1983.
6. Lossnitzer, K.; Bajusz, E. Water and electrolyte alterations during the life course of the BIO 14.6 Syrian golden hamster. A disease model of a hereditary cardiomyopathy. J. Mol. Cell. Cardiol. 6:163–177; 1974.
7. McGiven, R. F.; Mousa, S.; Couri, D.; Berntson, G. G. Prolonged intermittent foot shock stress decreases MET and LEU enkephalin levels in brain with concomitant decreases in pain threshold. Life Sci. 33:47–54; 1983.
8. Meuller, R. A.; Lundberg, D. B. A.; Breese, G. R.; Hedner, J.; Hedner, T.; Jonason, J. The neuropharmacology of respiratory control. Pharmacol. Rev. 34:255–285; 1982.
9. Neubauer, J. A.; Posner, M. A.; Santiago, T. V.; Edelman, N. H. Naloxone reduces ventilatory depression of brain hypoxia. J. Appl.

Physiol. 63:699–706; 1987.
10. Overholt, J. L.; Mitra, J.; VanLunteven, E.; Prabhakar, N. R.; Cherniack, N. S. Naloxone enhances the response to hypercapnia of spinal and cranial respiratory nerves. Respir. Physiol. 74:299–310; 1988.
11. Pazos, A.; Flórez, J. Interaction of naloxone with μ- and δ-opioid agonists on the respiration of rats. Eur. J. Pharmacol. 87:307–314; 1983.
12. Reents, S. B.; Beck, C. A., Jr. Naloxone and naltrexone—application in COPD. Chest 92:217–219; 1988.
13. Roca, J.; Montserrat, J. M.; Rodriguéz Roisin, R.; Guitart, R.; Torres, A.; Agusti, A. G. N.; Wagner, P. D. Gas exchange response to naloxone in chronic obstructive pulmonary disease with hypercapnic respiratory failure. Bull. Eur. Physiopathol. Resp. 23:249–254; 1987.
14. Scardella, A. T.; Parisi, R. A.; Phair, D. K.; Santiago, T. V.; Edelman, N. H. The role of endogenous opioids in ventilatory response to acute flow-resistive loads. Am. Rev. Respir. Dis. 133: 26–31; 1986.
15. Schlenker, E. H. An evaluation of ventilation in dystrophic Syrian hamsters. J. Appl. Physiol. 56:914–921; 1984.
16. Simon, P. M.; Pope, A.; Lahine, K.; Steinbrook, R. A.; Schwartzstein, R. M.; Weiss, J. W.; Fencl, V.; Weinberger, S. E. Naloxone does not alter response or resistive loading in chronic obstructive pulmonary disease. Am. Rev. Respir. Dis. 139:134–138; 1989.
17. Stuckey, J.; Marra, S.; Minor, T.; Insel, T. R. Changes in mu opiate receptors following inescapable shock. Brain Res. 476:167–169; 1989.