PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 170, 405–410 (1982)

Lidocaine in the Rat Rostral Hypothalamus: Effect on Arterial Carbon Dioxide[1] (41450)

A. F. CONNORS, JR.,[2] J. P. FARBER,[3] D. R. MCCAFFREE, R. F. HOWARD, AND M. M. STARK

Departments of Medicine and Physiology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190

Abstract. Lidocaine hydrochloride causes an increase in respiratory frequency (f) when infused into the rostral hypothalamus of the rat. The purpose of our study was to determine whether this increased f was primarily due to: (i) a direct increase in neural respiratory drive; (ii) a change in metabolism, mediated by the hypothalamus, causing increased carbon dioxide production and secondary hyperpnea; or (iii) a combination of these phenomena. Arterial carbon dioxide tension (P_aCO_2), f, and tidal volume (V_t) were measured in awake, unrestrained, tranquilized rats before and after the infusion of either lidocaine or hypertonic saline into the rostral hypothalamus. At peak increase in f, approximately 2 min after lidocaine infusion, f had increased 26.5 breaths/min (28.3%), P_aCO_2 had decreased 3.5 mm Hg (10.3%), and V_t was unchanged. There was no change in f, V_t, or P_aCO_2 2 min after saline infusion. Since an increase in carbon dioxide production typically causes increased ventilation with a stable P_aCO_2, the increased f and hypocapnia seen following infusion of the local anesthetic lidocaine are best explained by a depression of inhibitory neural discharge from the rostral hypothalamus resulting in increased discharge from the lower brainstem respiratory control centers.

The major centers responsible for respiratory control are located in the pons and medulla. However, higher structures also influence respiration. We recently demonstrated that infusion of the putative neurotransmitters norepinephrine or thyrotropin-releasing hormone into the rostral hypothalamus (RHT) of the rat, i.e., the anterior hypothalamus and preoptic area, caused an increase in respiratory frequency (1). This hyperpnea was apparently due to depression of an inhibitory neural pathway, since the injection of lidocaine into the RHT produced the same effect (1). While it was felt that the increased respiratory frequency (f) was most likely true hyperventilation, the data obtained did not exclude the possibility that the increase in breathing was a response to an increase in metabolism. The fact that the rats required tranquilization to avoid gross behavioral effects from the infusion suggested that changes in metabolism might occur even without a clear behavioral response.

The purpose of this study was to determine whether the increased f induced by lidocaine infused into the RHT was primarily mediated through: (i) a direct increase in neural respiratory drive; (ii) an increase in metabolism causing increased CO_2 production ($\dot{V}CO_2$) and a secondary increase in ventilation; or (iii) a combination of these two phenomena. A direct increase in neural respiratory drive at constant $\dot{V}CO_2$ will produce a fall in P_aCO_2 proportional to the increase in ventilation. When increasing levels of carbon dioxide are delivered to the lung due to increases in $\dot{V}CO_2$, ventilation increases but the partial pressure of carbon dioxide in arterial blood (P_aCO_2) remains stable or rises slightly (2–4). To assess the relative role of these two phenomena, we

[1] Supported by NIH Grants HL-07155 and HL-07210.

[2] Part of the work for this study was done while Dr. Connors was a Parker B. Francis Fellow in Pulmonary Research. Present address: Cleveland Metropolitan General Hospital, 3395 Scranton Rd., Cleveland, Ohio 44109. To whom correspondence should be addressed.

[3] Recipient of an NIH Research Career Development Award HL-00619.

0037-9727/82/080405-06$01.00/0

measured P_aCO_2 and ventilation before and after an infusion of lidocaine into the RHT.

Methods. Male Sprague–Dawley rats weighing 250 to 300 g were anesthetized with sodium pentobarbital (50 mg/kg), and single 23-gauge stainless steel guide tubes were chronically implanted through holes bored in the skull. The guide tubes were positioned above the RHT, 5.0 mm below the dura, 7.2 mm anterior to stereotaxic zero, and 0.75 mm left of the midline (5). The animals were allowed to recover for at least 6 days.

For all infusions the rats were tranquilized with haloperidol (2–6 mg/kg) to prevent the activity (exploratory behavior and grooming) that frequently accompanied lidocaine infusions in preliminary experiments. Such activity would make accurate determination of respiratory rate impossible and would affect CO_2 production in an unpredictable manner. Then 28-gauge stainless steel cannulae were inserted through the guide tubes to approximately the depth of the RHT, 8.0 mm below the dura. Either hypertonic saline or lidocaine hydrochloride was infused, using a Model 351 Sage pump (Sage Instruments, Cambridge, Mass.) driving a 10-μl Hamilton syringe (Hamilton Co., Reno, Nev.). A volume of 0.25 μl was infused over 30 sec in most studies; however, in some animals a volume of only 0.125 μl infused over 30 sec was found to be effective. Lidocaine was infused at a concentration of 70 mg/ml (0.249 mmo/ml) and a pH of 4.9 units. Hypertonic saline was infused at a concentration of 14.6 mg/ml (0.252 mmo/ml) and a pH of 5.6 units. Previous studies show that infusing fluids into the RHT with pH differences in this range do not cause changes in f (1). No animal was given more than one infusion per day.

Ventilation was monitored using a whole body plethysmograph. The unrestrained animals were placed in a Plexiglas box through which a 2 liter/min flow of humidified room air was maintained across very high inflow and outflow resistances (modified from Pappenheimer) (6). The tidal volume (V_t) was determined using the increase in pressure caused by the warming and humidification of each inspired breath (7). These pressure changes were measured using a Statham PM-5 transducer (Statham Instruments, Hato Rey, Puerto Rico) whose output was amplified and recorded on a Grass Model 5 polygraph (Grass Instruments, Quincy, Mass.). The box volume was much greater than the $\dot{V}CO_2$ of the rat. Consequently, the PCO_2 of the effluent gas was too low for accurate measurement and the washout of the box was slow, obscuring detection of transients of $\dot{V}CO_2$. Thus, $\dot{V}CO_2$ could not be measured. The animals were kept in the plethysmograph for about 5 min, until f was constant, before the 30-sec infusion was begun and then for an average of 8 min after the infusion, until f had returned to baseline.

In order to obtain serial samples of arterial blood, a second operation was performed on those animals with appropriately positioned injection cannulae. Cannula placement was assumed to be accurate, pending histologic confirmation, if lidocaine infusion caused an increase in f of greater than 20% (1). Each animal was anesthetized as before and a polyethylene cannula was inserted into the femoral artery according to a technique described by Jerome Dempsey (personal communication). The cannula consisted of 6 cm of PE10 tubing (Intramedic polyethylene tubing, i.d. 0.011 in., o.d. 0.024 in., Clay Adams, Parsippany, N.Y.) fused with 25 cm of PE50 tubing (Intramedic polyethylene tubing, i.d. 0.023 in., o.d. 0.038 in., Clay Adams, Parsippany, N.Y.). The PE10 tubing extended about 5 cm into the artery and the PE50 tubing was passed subcutaneously to a point between the scapulae where it was brought out through the skin. The cannula was then filled with heparinized saline (10 units/ml), plugged, and secured with adhesive tape. The animals were allowed to recover for 24 hr before further study.

After recovery from the arterial cannulation, the animals were tranquilized as before and placed in the whole body plethysmograph. The arterial cannula was flushed with heparinized saline (10 units/ml). The f and V_t were continuously monitored. The infusion of lidocaine was conducted as be-

fore, except that a 0.25-ml arterial blood sample was allowed to flow spontaneously into a heparinized glass capillary tube at three points during the experiment: (i) preinfusion, at the end of a control period of at least 5 min during which the animal had established a stable *f;* (ii) postinfusion I, at the peak increase in *f;* and (iii) postinfusion II, after the animal had returned to the baseline *f,* usually about 8 min after the infusion. All arterial blood samples were transported in melting ice. The pH, P_aCO_2, and partial pressure of arterial oxygen (P_aO_2) of the samples were measured by a blood gas analyzer (IL 813, Instrumentation Laboratories, Inc., Lexington, Mass.) within 15 min of being drawn. When possible, the studies were repeated at 1- to 2-day intervals.

Although each of the seven lidocaine-infused animals served as its own control (preinfusion and 8 min postinfusion values), an additional control procedure using hypertonic saline infusion was thought to be useful. Although such an infusion does not stimulate breathing (1), we wished to evaluate the possibility that infusion of fluid into the RHT might, in itself, produce alterations in arterial blood gases (presumably by changing metabolic rate). We evaluated two animals in the lidocaine-tested group for ventilatory and blood gas responses to hypertonic saline. In the other five lidocaine-tested animals, problems with the femoral cannulae precluded further evaluation. For statistical purposes, four additional animals were used to evaluate responses to saline in the RHT. Their surgical preparation and tranquilization were the same as for the previous group. Since no increase in *f* was seen postinfusion in the saline animals, the arterial blood samples were drawn arbitrarily at three points: (i) preinfusion, as above; (ii) postinfusion I, 2 min after the infusion; and (iii) postinfusion II, 7 to 8 min after the infusion. Each rat was studied twice, with at least 24 hr between studies.

Histologic confirmation of the infusion site was obtained in all animals. The rats were anesthetized, the injection cannula was placed at the same location used for all lidocaine and saline infusions, and methylene blue dye was infused (2 mg/ml, 0.25 μl over 30 sec). The animals were then sacrificed by opening the chest and perfusing the circulatory system with physiologic saline followed by 10% buffered formalin. After perfusion, the brain was cut into 75-μm sections and examined microscopically. The injection site was located relative to easily determined local landmarks (5). Tissue injury and destruction at the injection site was minimal.

Average values were used for analysis of animals receiving more than one lidocaine infusion and for analysis of all saline animals. The changes in pH, P_aCO_2, P_aO_2, *f,* and V_t in response to RHT infusions of lidocaine or hypertonic saline were analyzed using a paired Student's *t* test. The difference between the saline and lidocaine infusions were analyzed using an unpaired Student's *t* test. A *P* value of less than 0.05 was considered significant. All values are reported as mean ± standard deviation (SD).

Results. *Lidocaine infusion.* Seven animals received a total of 12 lidocaine infusions. The injection site was 7.5 ± 0.4 mm anterior to stereotaxic zero and 1.2 ± 0.3 mm to the left of midline, at a depth of 7.7 ± 0.6 mm below the dura. The responses of pH, P_aCO_2, P_aO_2, *f,* and V_t to the infusion of lidocaine into the RHT are recorded in Table I. At postinfusion I, 2.1 ± 0.5 min after the infusion, there was a significant fall in P_aCO_2 while *f* and P_aO_2 increased. The pH and V_t did not change. At postinfusion II, 8.4 ± 2.5 min after the infusion, P_aCO_2 and *f* had returned to baseline levels while P_aO_2 remained elevated.

Saline infusion. Two of the seven animals described above received infusions of hypertonic saline in addition to lidocaine. The average change of P_aCO_2 and *f* in response to both saline and lidocaine infusion for these animals is shown in Fig. 1.

For all six animals receiving hypertonic saline infusions, the injection site was 7.5 ± 0.3 mm anterior to stereotaxic zero and 0.9 ± 0.3 mm to the left of midline, at a depth of 7.6 ± 1.0 mm below the dura. The responses of pH, P_aCO_2, P_aO_2, *f,* and V_t to hypertonic saline infusion are recorded in Table I. At postinfusion I, 2.3 ± 0.4 min after the infusion, there was no change in

TABLE I. EFFECT OF ROSTRAL HYPOTHALAMIC INFUSION OF LIDOCAINE OR SALINE ON ARTERIAL BLOOD GASES AND VENTILATION

	Preinfusion	Postinfusion[a] I	Postinfusion[a] II
Lidocaine (n = 7)			
pH	7.47 ± 0.04[b]	7.49 ± 0.04	7.46 ± 0.03
P_aCO_2 (mm Hg)	33.9 ± 3.2	30.4 ± 4.2*	34.2 ± 3.1**
P_aO_2 (mm Hg)	98.9 ± 3.9	105.8 ± 5.9*	104.8 ± 9.8*
f (breaths/min)	93.7 ± 7.1	120.2 ± 13.0*	96.3 ± 9.2**
V_t (ml)	2.5 ± 0.5	2.6 ± 0.6	2.5 ± 0.6
Saline (n = 6)			
pH	7.46 ± 0.02	7.46 ± 0.02	7.45 ± 0.03
P_aCO_2 (mm Hg)	36.0 ± 0.8	37.0 ± 1.4	37.6 ± 0.6*
P_aO_2 (mm Hg)	104.1 ± 7.0	102.6 ± 6.0	101.9 ± 7.2*
f (breaths/min)	87.4 ± 7.3	88.4 ± 8.7	88.3 ± 7.7
V_t (ml)	2.8 ± 0.4	2.8 ± 0.3	2.7 ± 0.4

Note. Abbreviations: P_aCO_2, partial pressure of arterial carbon dioxide; P_aO_2, partial pressure of arterial oxygen; f, respiratory frequency; V_t, tidal volume; MAP, mean arterial blood pressure.

[a] In the lidocaine group the postinfusion I measurements were obtained at the peak of the f response (2.1 ± 0.5 min postinfusion), and the postinfusion II measurements were obtained after the animal had returned to baseline f (8.4 ± 2.5 min postinfusion). In the saline group there was no change in f. Thus, arbitrarily, the postinfusion I measurements were made at 2.3 ± 0.4 min postinfusion and the postinfusion II measurements were made at 7.6 ± 0.9 min postinfusion.

[b] Mean ± SD.

* Significantly different than preinfusion values, $P < 0.05$.

** Significantly different than values obtained at postinfusion I, $P < 0.05$.

any variable. At postinfusion II, 7.6 ± 0.9 min after the infusion, the P_aCO_2 had increased a small amount with a corresponding fall in P_aO_2. The pH, f, and V_t did not change.

Lidocaine vs hypertonic saline. The two groups behaved differently with respect to P_aCO_2 and f after lidocaine infusion. The lidocaine group had a significantly lower P_aCO_2 at both postinfusion I and postinfusion II; the f was higher in this group at postinfusion I (see Fig. 2). The percentage change in P_aCO_2 at postinfusion I was related to the percentage change in f in a linear fashion (see Fig. 3).

Discussion. This study demonstrates a fall in arterial PCO_2 coincident with the rise in respiratory frequency which occurs about 2 min after infusion of lidocaine hydrochloride into the RHT of the rat. Infusion of hypertonic saline at the same location resulted in no change in f or P_aCO_2 at the same postinfusion interval. The rapid fall in P_aCO_2 following the lidocaine infusion strongly suggests that a direct increase in neural respiratory drive has occurred.

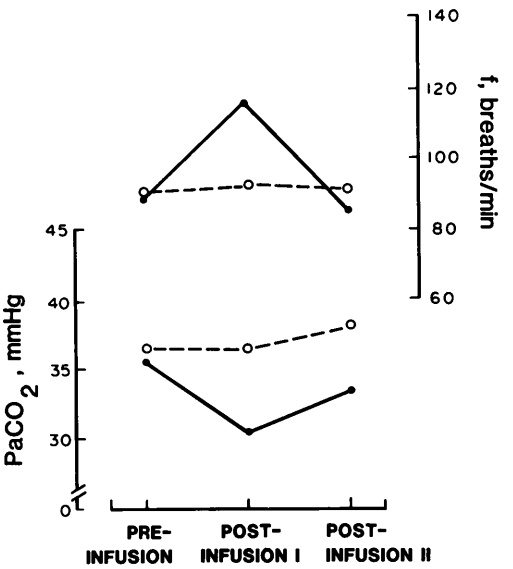

FIG. 1. The change in arterial carbon dioxide (P_aCO_2) and respiratory frequency (f) are shown for two rats that received both lidocaine (●) and saline (○) infusions.

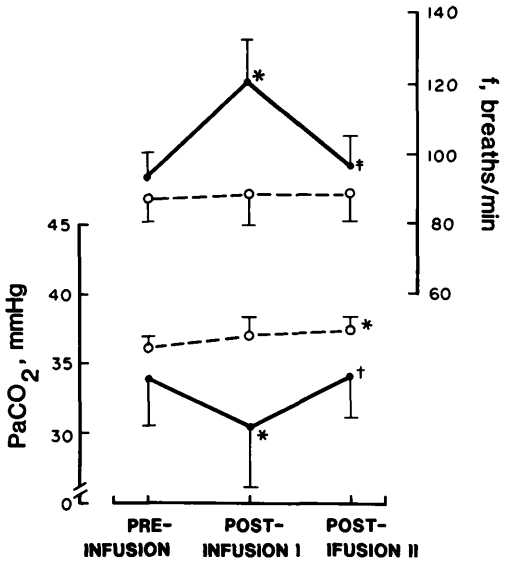

FIG. 2. The arterial carbon dioxide tension (P_aCO_2) and respiratory frequency (f) are shown for the lidocaine-infused animals (●) and the saline-infused animals (○) at three points: preinfusion, postinfusion I, and postinfusion II (see Table I). With respect to P_aCO_2, the two groups behaved differently at postinfusion I ($P < 0.01$) and at postinfusion II ($P < 0.05$). With respect to f, the two groups behaved differently only at postinfusion I ($P < 0.01$). *Different than the preinfusion values ($P < 0.01$); †different than the postinfusion I value ($P < 0.05$); ‡different than the postinfusion I value ($P < 0.01$).

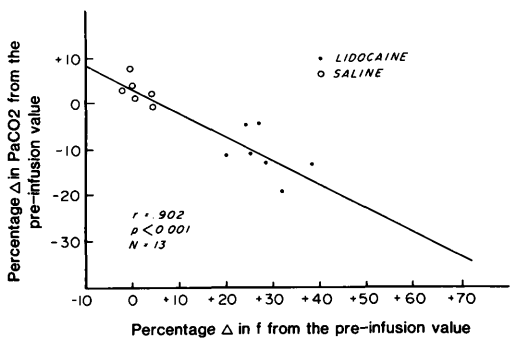

FIG. 3. The average change in arterial carbon dioxide tension (P_aCO_2) and respiratory frequency (f) from the preinfusion value to the postinfusion I value is plotted for each lidocaine-infused animal (●) and each saline-infused animal (○). A line determined by least-squares linear regression analysis is drawn through the points.

The injection sites used in these animals were the same as those reported to be associated with increases in f after infusion of norepinephrine, thyrotropin-releasing hormone (TRH), or lidocaine (1). Norepinephrine and TRH inhibit cell discharge in the RHT (8). Lidocaine also depresses neural discharge in the central nervous system, apparently preferentially affecting inhibitory fibers (9) and synapses (10). These findings suggest that the increase in f seen in this study was due to decreased discharge from inhibitory neurons in the RHT.

This proposed depression of inhibitory discharge in the RHT could increase f in one of three ways: (i) alteration of the metabolic status of the rat increasing $\dot{V}CO_2$ and, secondarily, f; (ii) true hyperventilation due to a direct increase in neural respiratory drive resulting from the release of lower respiratory centers from the inhibitory influence of the RHT; or (iii) a combination of these phenomena. If the change in f was due to increased $\dot{V}CO_2$, the P_aCO_2 at peak f should have been about the same as (2, 3) or slightly higher than (4) the preinfusion value. Instead we found a significant fall in P_aCO_2 at peak f, approximately 2 min postinfusion.

It takes 45 min to reach a new steady state for P_aCO_2 and body CO_2 stores after any change in ventilation (11). At any point following an increase in ventilation, 16% of those CO_2 stores which remain to be removed are exhaled each minute (11). At 2.1 min only 30.2% of the potential change in CO_2 stores will have been realized. Since at this point the P_aCO_2 was observed to have fallen 3.5 mm Hg from baseline, one can predict that it would have fallen 11.6 mm Hg had the increase in ventilation been maintained until a new equilibrium was reached. In that case, PCO_2 would have fallen 34.2% while minute ventilation ($f \times V_t$) rose 33.4%. The measured change in ventilation fully accounts for the extrapolated change in PCO_2. Thus, while we do not measure $\dot{V}CO_2$ and can not exclude the possibility that it increased, the demonstrated fall in P_aCO_2 suggests that the predominant effect of lidocaine infused into the RHT was a direct increase in neural respiratory drive which was clearly dispropor-

tionate to any possible response secondary to a change in metabolism.

Several neuroanatomical studies in rats have demonstrated neural pathways from the RHT to regions of the medulla and pons which are thought to be involved in control of respiration. Efferents of the lateral hypothalamus, which is richly interconnected with the RHT, extend to the parabrachial nucleus (the pneumotaxic area) and the locus ceruleus (12). Efferent fibers of the preoptic area also extend to the locus ceruleus (13). Direct efferents from the anterior hypothalamus and preoptic area extend caudally to areas of the brainstem in the general vicinity of the pons and medulla: the raphe nucleus, the ventral tegmental area, and the reticular formation (13, 14). In addition, Redgate (15) found that instillation of phenobarbital into the hypothalamus of the cat altered inspiratory area excitability in the medulla. Thus, it is reasonable to think that a depression of inhibitory discharge in the RHT might release neurons in the lower brainstem respiratory control centers and cause an increase in respiratory frequency.

In summary, we have demonstrated a fall in arterial PCO_2 occurring simultaneously with an increase in respiratory frequency approximately 2 min after the infusion of lidocaine hydrochloride into the rostral hypothalamus. These findings can be explained by a depression of inhibitory neural pathways from the rostral hypothalamus allowing increased output from brainstem respiratory control centers resulting in increased breathing frequency.

1. Farber JP, Connors AF, Gisolfi CV, McCaffree DR, Smith RM. Effects on breathing of putative neurotransmitters in the rostral hypothalamus of the rat. Brain Res Bull 6:13–17, 1981.
2. Yamamoto WS, Edwards MW. Homeostasis of carbon dioxide during intravenous infusion of carbon dioxide. J Appl Physiol 15:807–818, 1960.
3. Asmussen E. Muscle exercise. In: Fenn WO, Rahn H, eds. Handbook of Physiology. Washington DC, Amer Physiol Soc, Sect 3, Vol II, 1965.
4. Lewis SM. Awake baboon's ventilatory response to venous and inhaled CO_2 loading. J Appl Physiol 39:417–422, 1975.
5. Pellegrino LJ, Pellegrino JS, Cushman AJ. A Stereotaxic Atlas of the Rat Brain. New York, Plenum, 1979.
6. Pappenheimer JR. Sleep and respiration of rats during hypoxia. J Physiol 266:191–207, 1977.
7. Bartlett D, Tenney SM. Control of breathing in experimental anemia. Resp Physiol 10:384–395, 1970.
8. Winokur A, Beckman AL. Effects of thryotropin releasing hormone, norepinephrine and acetylcholine on the activity of neurons in the hypothalamus, septum and cerebral cortex of the rat. Brain Res 105:205–209, 1978.
9. Warnick JE, Kee RD, Yim GKW. The effects of lidocaine on inhibition in the cerebral cortex. Anesthesiology 34:327–332, 1971.
10. Tanaka K, Yamasaki M. Blocking of cortical inhibitory synapses by intravenous lidocaine. Nature (London) 209:207–208, 1966.
11. Farhi LE, Rahn H. Gas stores of the body and the unsteady state. J Appl Physiol 7:472–484, 1955.
12. Saper CB, Swanson LW, Cowan WM. An autoradiographic study of the efferent connections of the lateral hypothalamic area in the rat. J Comp Neurol 183:689–706, 1979.
13. Swanson LW. An autoradiographic study of the efferent connection of the preoptic region in the rat. J Comp Neurol 167:227–256, 1976.
14. Conrad LCA, Pfaff DW. Efferents from medial basal forebrain and hypothalamus in the rat. I. An autoradiographic study of the medial preoptic area. J Comp Neurol 169:185–220, 1976.
15. Redgate ES. Inspiratory area excitability and the hypothalamus. Amer J Physiol 198:1299–1303, 1960.

Received January 21, 1982. P.S.E.B.M. 1982, Vol. 170.