Pontile Pneumotaxic Center Regulation of Doxapram-Induced Respiratory Alterations[1,2] (37211)

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(Introduced by T. I. Koike)

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The analeptic doxapram hydrochloride (Dopram–Robins) has been described as a potent stimulator of respiratory activity (9, 10). The respiratory stimulating action of doxapram has been reported to result from a direct pharmacological action at the peripheral chemoreceptors (3, 4) and the medullary respiratory area (2, 4). Therefore, Hirsch and Wang (3) reported that injection of 0.05 mg/kg of doxapram into the carotid chemoreceptor area may induce ventilatory alterations in cats. Funderburk, Oliver, and Ward (2) found that injections of 0.20 mg/kg of doxapram iv significantly altered the electrical activity of the medullary respiratory area. Electrical activity of other portions of the central nervous system was altered only when doses of doxapram were in excess of 3.00 mg/kg iv (2).

Administration of doxapram subsequent to barbiturate anaesthesia produced alterations in ventilation primarily by elevations of tidal volume (1, 10). Following bilateral ablation of the brain stem pneumotaxic center, decerebrate cats exhibited a significant diminution of the tidal volume response to hypercapnia (8). Confirmation of this observation was obtained in decerebrate and barbiturate anaesthetized cats (6). Additionally, unilateral lesions of the pneumotaxic center were found to significantly reduce the hypercapnia-induced tidal volume response (6). Concomitant with the reduction in tidal volume response to hypercapnia following pneumotaxic center ablation, an augmented frequency response has been observed (8).

The response to hypoxic stimulation of respiration following pneumotaxic center ablation may not be identical to the respiratory response to hypercapnia. Therefore, under certain experimental conditions following pneumotaxic center ablation, the hypoxia-induced tidal volume response may be maintained while the hypercapnia-induced tidal volume response is eliminated (6). St. John has hypothesized that this difference in respiratory response to hypercapnia and hypoxia following pneumotaxic center lesioning is qualitative in nature and reflects different primary chemoreceptor sites for the hypoxic and hypercapnic gases (7).

The purpose of this study was to examine the effects of bilateral or unilateral pneumotaxic center ablation upon doxapram-induced respiratory alterations. It is believed that the studies described herein may also serve to further characterize the respiratory stimulating action of doxapram.

Methods. Experimental animals and groups. Twenty-three adult cats of both sexes of mean weight 3.0 kg were utilized in this study. Animals were divided into the following experimental groups: Group PC, having bilateral pneumotaxic center lesions; Group U, having unilateral pneumotaxic center lesions; and Group C, having control brain stem lesions.

Phase I. Control. Prelesion. All animals were anaesthetized with 35.0 mg/kg of sodium pentobarbital (Nembutal–Abbott) admini-

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stered ip. Additional increments of pentobarbital were administered as required during the experimental procedure. Endotracheal intubation and femoral vein cannulation were performed utilizing procedures previously defined (6). Pneumotachograph methods utilized for measurement of the respiratory parameters of tidal volume (V_T), frequency (f) and minute volume ($\dot{V}_E$) have also been described previously (6). Respiratory parameters were recorded with animals breathing air.

Following a period of control respiratory parameter measurement (designated "predrug" in Table I), the animals were administered the following substances in a random order: 1.00 mg/kg doxapram iv, 2.75 mg/kg doxapram iv or 2.00 cc 0.9% saline placebo iv. The respiratory responses observed for the first minute following doxapram or placebo administration (designated "postdrug" in Table I) are reported. In those animals receiving more than one doxapram administration, a temporal interval was taken between drug administrations until respiratory parameters approximately "predrug" values.

Phase II. Lesion placements. Following Phase I control testing, electrolytic bilateral or unilateral pneumotaxic center lesions or control brain stem electrolytic lesions were placed using stereotaxic procedures identical to those previously described (6). Following appropriate lesion placements, animals were retested in response to doxapram or placebo administrations in the same manner as in Phase I.

Phase III. Vagal section. The vagi of all animals were bilaterally sectioned at the level of the thyroid cartilage.

Histological evaluation of lesion placements. The electrolytic lesions in the brain stem were localized according to the histological method of Powell (5).

Statistical evaluation of data. Data obtained in this study compare the doxapram-induced respiratory responses in surgically intact animals with the responses elicited in these same animals following the placement of brain stem lesions. Statistical evaluation of data may therefore be performed by "paired" comparisons. For comparison of the absolute value of respiratory parameters, a paired t test was utilized. A Wilcoxon Matched-pairs Signed-ranks test was employed for comparison of percentage alterations of ventilation. For evaluation of certain unpaired observations, a Student's t test was used.

Results. Phase I. Control. Prelesion. The respiratory parameters of tidal volume (V_T), frequency (f), and minute volume ($\dot{V}_E$) for the various animal groups breathing air before and after administrations of doxapram or saline are reported in Table I. Examination of the alterations of ventilation subsequent to doxapram administrations (Fig. 1) confirms previous observations that doxapram stimulates respiratory changes primarily through tidal volume elevations in barbiturate anesthetized animals. Data reported in Table I also evidence the rather large variations in respiratory response to doxapram administration.

Phase II. Lesion placements. Bilateral removal of the brain stem pneumotaxic center (Group PC) effectively eliminated the doxapram-induced tidal volume elevation (Fig. 1). Concomitant with this absence of the tidal volume response, these Group PC cats exhibited a significant elevation in the doxapram-induced frequency response. A significant reduction in the tidal volume response to doxapram administration was likewise observed following unilateral pneumotaxic center ablation (Group U) (Fig. 1). The Group U cats, however, did not display the significant elevation of the frequency response comparable to that observed in Group PC animals. Following control lesion placements (Group C), doxapram-induced respiratory changes did not differ significantly from prelesion values.

Examination of Table I reveals that a significant elevation of the respiratory tidal volume and a significant diminution of the respiratory frequency resulted concomitant with bilateral pneumotaxic center ablation. Upon unilateral pneumotaxic center lesion placement, the elevation of tidal volume was not significant; however, the frequency of respiration was significantly less in the post-lesion phase. Administrations of doxapram subsequent to bilateral or unilateral pneumotaxic center ablation resulted in tidal volumes

which were slightly in excess or not significantly different from those observed in the prelesion phase. The Group PC and Group U cats exhibited frequencies and minute volumes of respiration which were significantly less than prelesion values following doxapram administrations in the post lesion phase.

Phase III. Vagal section. Upon bilateral vagotomy, all animals having bilateral pneumotaxic center lesions (Group PC) exhibited apneustic respiration. In three of the eight Group PC animals, this inspiratory apnea persisted without expiratory interruption. After these three cats had maintained inspiration for 120.0 sec, artifical respiration was administered in an attempt to prolong survival. Upon cessation of the artificial ventilation, these Group PC cats returned to inspiratory apnea which then persisted until death. For purposes of statistical comparison, the inspiratory duration time of these three cats was considered to be 120.0 sec. The mean inspiratory duration time exhibited by the eight Group PC cats was, therefore, 70.9 sec. Respiratory activity of Group U or Group C animals in the post vagotomy phase was not characterized by any significant pause of respiration in the inspiratory position with the exception of one Group U cat. The one exceptional animal displayed apneusis with an inspiratory duration time of 50.0 sec following bilateral vagotomy. The inspiratory duration times of Group PC animals were significantly different from those of Group U and Group C cats with $p < 0.005$ (Student's t test).

The tidal volume of respiration of Group U animals averaged 46.0 ml in the post vagotomy phase while that of Group C cats was 39.1 ml. As noted above, in three of the eight Group PC animals, inspiratory apnea persisted without interruption; hence, no accurate measure of tidal volume was possible. In the remaining five Group PC animals, tidal volumes averaged 98.0 ml following bilateral vagotomy. It is of interest to note that the tidal volumes exhibited by Group PC and Group U animals following vagal section were significantly greater ($p < 0.025$, paired t test) than the maximal tidal volumes exhibited by these same animals upon doxapram administration in the post lesion phase. Tidal volumes of Group C cats in the post vagotomy phase did not differ significantly from the maximal tidal volumes exhibited upon doxapram administration in the post lesion phase.

Histological evaluation of lesion placements. In a previous report (6), identical methods to those of the present study were utilized for the placement of brain stem electrolytic lesions and for the histological localization of these lesions. In that study, the pontile pneumotaxic center, that area of the brain stem in which the placement of bilateral lesions resulted in apneusis upon vagotomy, was defined. All Group PC animals in the present study had bilateral lesions within this pneumotaxic center; likewise, all Group U cats had unilateral lesions which encroached on this area. Lesion placements in Group C animals were anterior and/or ventral to pneumotaxic center. Histological illustrations of lesion sites comparable to those of the present study may be seen in an earlier publication (6).

Discussion. Subsequent to bilateral or unilateral pneumotaxic center ablation, the tidal volume is "reset" at a higher level while the frequency is "reset" at a lower level. Potential tidal volume deviations from this "reset" level are minimized following pneumotaxic center lesions, whereas potential frequency elevations are enhanced. Therefore, doxapram stimulation of respiration following pneumotaxic center ablation results in only minor alterations of tidal volume. In contrast, the doxapram-induced respiratory frequency alterations are augmented following pneumotaxic center lesioning.

Tidal volumes which would have supported a "normal" response to doxapram administrations following pneumotaxic center ablation lie well within the physiological limits. Evidence for this conclusion is provided by the observation that, in the post vagotomy phase, animals receiving no pharmacological stimulation exhibited tidal volumes greatly in excess of the maximal tidal volumes observed following doxapram administration in the post lesion phase.

The dosages of doxapram utilized in this study provide stimulation of respiratory

Table 1

Mean values (± standard deviations) of respiratory tidal volume (V_T), frequency (f) and minute volume ($\dot{V}_E$) prior to and following iv administration of 1.00 mg/kg doxapram - HCl, 2.75 mg/kg doxapram - HCl or saline placebo. Values reported are for Group PC, Group U and Group C cats prior to and following the placement of pneumotaxic center lesions or control brain stem lesions. Animals anaesthetized with pentobarbital.

GROUP (number of animals)	Phase	— 1.00 mg/kg Doxapram - HCl iv — Predrug V_T (ml)	f (min^{-1})	$\dot{V}_E$ (ml/min)	Postdrug V_T (ml)	f (min^{-1})	$\dot{V}_E$ (ml/min)
PC (8)	Prelesion	18.9 ±7.2	21.5 ±6.5	378.8 ±96.0	31.8 ±8.5	24.3 ±6.2	752.2 ±188.1
	Postlesion	48.3* ±28.2	7.4* ±5.2	297.2 ±170.8	43.8* ±12.6	11.5* ±4.3	498.2* ±230.4
U (8)	Prelesion	16.5 ±5.4	20.2 ±5.9	333.4 ±143.4	27.3 ±11.3	26.9 ±8.4	760.0 ±458.5
	Postlesion	20.7 ±7.8	11.6* ±4.4	220.3* ±60.9	26.8 ±10.3	14.3* ±4.5	371.8* ±165.8
C (7)	Prelesion	18.8 ±10.0	18.1 ±5.0	315.9 ±106.7	28.5 ±11.7	20.1 ±4.4	544.6 ±193.1
	Postlesion	18.6 ±10.3	19.7 ±7.6	344.7 ±153.2	26.5 ±11.9	22.2 ±8.9	569.6 ±272.6
		— 2.75 mg/kg Doxapram - HCl iv —					
PC	Prelesion	14.9 ±4.9	28.3 ±7.9	414.7 ±136.5	38.9 ±17.7	31.4 ±8.3	1254.1 ±667.7

(6)	Postlesion	37.5* ±21.4	9.3* ±5.8	286.4* ±108.8	43.8 ±16.7	15.1* ±3.0	672.0* ±319.4
U (8)	Prelesion	14.6 ±4.6	21.3 ±4.8	318.3 ±148.0	30.3 ±13.0	28.9 ±5.6	900.4 ±492.5
	Postlesion	18.1 ±6.2	16.3* ±4.8	276.3 ±73.6	29.6 ±11.2	19.6* ±5.0	550.5* ±169.8
C (6)	Prelesion	18.3 ±11.1	19.3 ±4.7	341.7 ±169.7	30.6 ±14.9	21.5 ±4.6	639.3 ±262.0
	Postlesion	18.4 ±10.8	19.0 ±3.6	356.9 ±224.6	31.1 ±15.6	22.0 ±4.5	699.7 ±364.9
		— saline placebo —					
PC (8)	Prelesion	17.6 ±5.7	26.4 ±7.9	441.7 ±114.0	17.1 ±5.9	25.9 ±7.6	424.9 ±115.2
	Postlesion	40.0* ±17.0	9.3* ±4.5	353.2 ±165.0	38.7* ±17.5	9.6* ±4.6	351.0 ±175.9
U (8)	Prelesion	15.5 ±4.8	26.1 ±5.9	424.2 ±211.8	14.6 ±6.2	26.0 ±6.4	403.8 ±241.5
	Postlesion	20.6* ±5.4	15.7* ±3.8	317.8* ±108.8	19.5* ±6.1	15.5* ±3.8	300.9* ±120.2
C (7)	Prelesion	18.4 ±9.8	21.2 ±4.2	369.9 ±140.0	17.3 ±9.8	20.6 ±4.5	342.5 ±154.7
	Postlesion	18.1 ±10.8	19.9 ±4.1	357.4 ±197.5	17.7 ±10.6	20.2 ±4.5	353.5 ±198.2

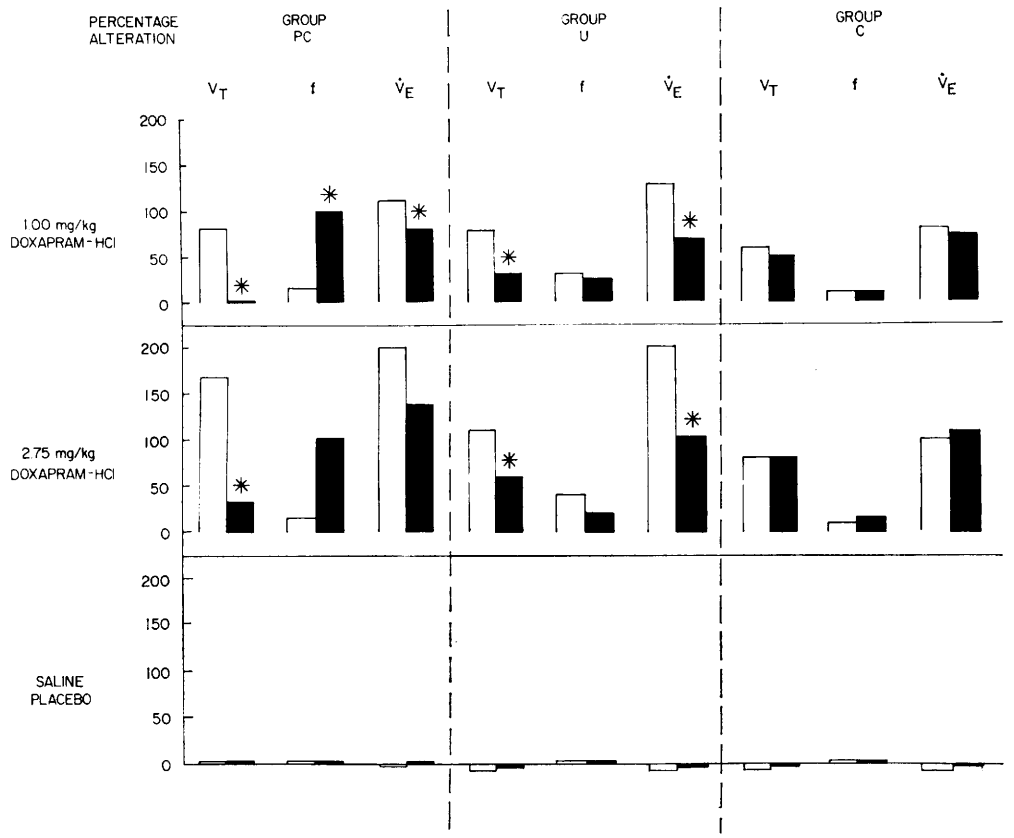

FIG. 1. Percentage alterations of respiratory tidal volume (V_T), frequency (f) and minute volume ($\dot{V}_E$) upon iv administration of 1.00 mg/kg doxapram–HCl, 2.75 mg/kg doxapram–HCl or 2.00 cc 0.9% saline placebo. Values reported are for Group PC animals prior to and following the placement of bilateral pneumotaxic center lesions, Group U animals prior to and following the placement of unilateral pneumotaxic center lesions, and Group C animals prior to and following the placement of control brain stem lesions. All animals were anaesthetized with pentobarbital. Open bars signify prelesion values; filled bars signify post lesion values * = $p < 0.025$ compaired to prelesion value (Wilcoxon test).

activity through both peripheral chemoreceptor (3) and medullary respiratory area (2) activation. Results of this study suggest that doxapram-induced stimulatory influences arising from one or both of these areas are integrated by the pontile pneumotaxic center.

The data reported in this study do not distinguish between the components of respiratory activity induced by peripheral chemoreceptor and medullary respiratory area stimulation by doxapram. Moreover, these data do not provide direct information as to the mechanism of action of doxapram in producing the respiratory stimulation. These data do indicate, however, that the respiratory stimulating action of doxapram is integrated by the same neural structure which serves to regulate the respiratory responses elicited by the neural chemical stimulation of carbon dioxide.

Summary. Doxapram–HCl effects ventilatory alterations in pentobarbital anaesthetized cats primarily by tidal volume elevations. Bilateral or unilateral ablation of the brain stem pneumotaxic center significantly reduces these doxapram-induced tidal volume elevations. Following bilateral pneumotaxic center lesions, the frequency responses elicited by

doxapram administrations are significantly elevated. Apneusis is obtained in animals having bilateral pneumotaxic center lesions upon bilateral vagal section. It is concluded that the pontile pneumotaxic center plays a fundamental role in the regulation of doxapram-induced respiratory alterations.

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