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Research Article

The purpose of our study was to identify central nervous system sites involved in the respiratory depressant effect of drugs that stimulate opioid receptors. Diacetylmorphine (heroin) was administered into several cerebroventricular regions of chloralose-anesthetized cats, while monitoring pulmonary ventilation with a Fleisch pneumotachograph. Administration of heroin (17, 50, 150, and 450 micrograms) into the forebrain ventricles, which was restricted to these ventricles, resulted in no significant respiratory effects. In contrast, administration of heroin into either the fourth ventricle or the cisterna magna resulted in a significant (P less than 0.05) decrease in respiratory minute volume (VE). In the fourth ventricle this was because of a decrease in frequency (f) and in the cisterna magna, to a decrease in tidal volume (VT). Intravenous administration of heroin in the same dose-range produced a decrease in VE, which was primarily due to a decrease in f. Bilateral application of heroin (70 micrograms/side) to each of three ventral medullary surface sites (Mitchell's, Schlaefke's, and Loeschcke's areas) known to influence respiration elicited a decrease in VE only at Mitchell's area. This decrease was due to decreases in f and VT. The role of this site in the action of intravenously administered heroin was tested by topical application of naloxone to this area in animals with respiratory depression evoked by intravenous heroin. Bilateral application of naloxone […]

Central Nervous System Site of Action for the Respiratory Depressant Effect of Diacetylmorphine (Heroin) in the Cat

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ABSTRACT The purpose of our study was to identify central nervous system sites involved in the respiratory depressant effect of drugs that stimulate opioid receptors. Diacetylmorphine (heroin) was administered into several cerebroventricular regions of chloralose-anesthetized cats, while monitoring pulmonary ventilation with a Fleisch pneumotachograph. Administration of heroin (17, 50, 150, and 450 μg) into the forebrain ventricles, which was restricted to these ventricles, resulted in no significant respiratory effects. In contrast, administration of heroin into either the fourth ventricle or the cisterna magna resulted in a significant ($P < 0.05$) decrease in respiratory minute volume ($\dot{V}_E$). In the fourth ventricle this was because of a decrease in frequency (f) and in the cisterna magna, to a decrease in tidal volume (V_T). Intravenous administration of heroin in the same dose-range produced a decrease in $\dot{V}_E$, which was primarily due to a decrease in f. Bilateral application of heroin (70 μg/side) to each of three ventral medullary surface sites (Mitchell's, Schlaefke's, and Loeschcke's areas) known to influence respiration elicited a decrease in $\dot{V}_E$ only at Mitchell's area. This decrease was due to decreases in f and V_T. The role of this site in the action of intravenously administered heroin was tested by topical application of naloxone to this area in animals with respiratory depression evoked by intravenous heroin. Bilateral application of naloxone (15 μg/side) to Mitchell's area restored breathing to normal. These results lead us to suggest that the site of heroin-induced respiratory depression is a specific area (Mitchell's area) on the ventral surface of the medulla.

INTRODUCTION

The general site of action of the opioids that depress respiration is considered to be in the central nervous system (CNS)[1] (1); however, the exact location is unknown. Studies using intracerebroventricular injections of drugs that activate opioid receptors in anesthetized cats and dogs have implicated the hindbrain areas in this response (2–4). Studies using techniques to apply these drugs to more discrete sites in the hindbrain have revealed that the ventral surface of the medulla may be a prominent target area for the respiratory depression induced by these drugs (5–7). In all these studies, the mechanism for the respiratory depression has not been uniform. In some investigations, the predominant effect was a decrease in respiratory rate (7) and in others the predominant effect was a decrease in tidal volume (V_T) (6).

These effects have all been found by using locally administered drug into the brain. To our knowledge, no studies have been performed where the role of various brain sites has been evaluated in the respiratory depressant effect of systemically administered opioid drugs. The present study was initiated in an attempt to (*a*) re-evaluate the sensitivity of various brain re-

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[1] Abbreviations used in this paper: CNS, central nervous system; f, respiratory rate; $PeCO_2$, end tidal CO_2 tension; T_E, expiratory duration; T_I, inspiratory duration; T_T, total cycle duration; $\dot{V}_E$, respiratory minute volume; V_T, tidal volume

gions to the respiratory depressant effect of diacetylmorphine (heroin) and (*b*) determine which of these sites might be of importance in the respiratory depressant effect produced by systemic administration of this drug. Heroin was chosen as the drug because it is a widely abused drug and very few data exist on the respiratory depressant effect of this agent; in addition, it is more lipid soluble than morphine, and thus crosses the blood brain barrier more readily (1).

METHODS

General. Experiments were performed on 66 adult cats of both sexes weighing between 2.5 and 3.6 kg. Anesthesia was induced with 70–80 mg/kg i.v. alpha-chloralose. We cannulated a femoral artery and vein for measurement of arterial blood pressure and systemic administration of drugs, respectively. Lead II of the electrocardiogram was recorded and heart rate was determined by measurement of the R-R interval. Rectal temperature was monitored and maintained between 37.0 and 38.0°C by an infrared heating lamp.

We cannulated the trachea of each cat and fitted each with a Fleisch no. 0 pneumotachograph connected to a respiratory flow transducer (HP 4730A, Hewlett-Packard Co., Waltham, MA). The airflow signal obtained was integrated (HP 8815A respiratory integrator) to obtain V_T and respiratory minute volume ($\dot{V}_E$). Respiratory rate (f) and inspiratory (T_I), expiratory (T_E), and total cycle (T_T) durations were obtained from fast tracings on the flow signal. Mean inspiratory flow (V_T/T_I) and ratio of inspiratory time to total cycle time (T_I/T_T) were calculated from these values. End tidal carbon dioxide tension ($PeCO_2$) was measured with a CO_2 analyzer (Instrumentation Laboratory, Inc., Lexington, MA, model 200 CO_2 monitor). All respiratory and cardiovascular parameters were recorded continuously on a Hewlett Packard eight-channel recorder (model 7758B).

Control measurements were taken at ~5-min intervals for at least 30 min before administration of drugs in each experiment. Once a drug was administered, measurements were made at the time at which peak responses occurred and at 10-min intervals thereafter until respiratory activity had returned to a predrug level of activity or had stabilized at new levels.

Intracerebroventricular injections of drugs. For administration of drugs into the CNS, animals were mounted in a David Kopf stereotaxic apparatus. The cranium and atlanto-occipital junction were exposed by a midline incision and retraction of the cervical musculature. Three types of injections were performed.

(*a*) Perfusion of the fourth ventricle only. Perfusion of the fourth ventricle was accomplished by making injections through a spinal needle placed in the fourth ventricle at coordinates AP (antero-posterior) −8.5, HD (height-depth) −4.5, and RL (right-left) 0 (8). The needle was inserted through a burr hole in the occipital bone at a 36° angle. A short length of polyethylene tubing (PE-160) was inserted horizontally into the cisterna magna to serve as a pressure bleed.

(*b*) Intracisternal injection. Injections into the cisterna magna were performed by inserting a polyethylene tube (PE-160) into the cisterna magna and injecting drug through this tubing. A volume of cerebrospinal fluid equal to the amount of drug solution plus the volume for flushing the cannula was withdrawn and discarded before drug administration.

(*c*) Perfusion of the forebrain ventricles only. Perfusion of the forebrain (lateral and third) ventricles was accomplished by making injections through a 26-gauge stainless steel needle positioned in the left lateral ventricle at coordinates AP +11.5, HD +8.5, and RL 4.0 (8). Collection of perfusate was made through a cannula (i.e., a 10-cm length of PE-160 tubing tipped with a 2.5-mm perforated steel ball) lodged in the aqueduct of Sylvius, thereby preventing drug from reaching the fourth ventricle and the ventral surface of the brainstem. To insert the cannula, the dura and arachnoid membranes were removed, and the steel ball of the cannula was advanced through the cisterna magna, along the floor of the fourth ventricle, and into the Sylvian aqueduct. In all experiments, needle or cannula placement and drug distribution were confirmed by infusion of acridine orange dye and postmortem examination of the brain.

Ventral surface application of drugs. For application of drugs to the ventral surface of the medulla, a longitudinal midline incision was made in the neck. The trachea and esophagus were retracted; the prevertebral muscles were retracted, and the basal plate of the skull was removed along with a large portion of the bullae tympani and the ventral arch of the atlas. After the removal of the dura and subarachnoid membranes, small cottonoid pledgets (3-mm in diameter and 0.5 mm in thickness) were soaked with 5 μl of either heroin solution (i.e., 70 μg were contained in 5 μl of saline) or naloxone solution (i.e., 15 μg were contained in 5 μl of saline) and applied to the ventral surface of the medulla. Administration of both drugs in the volume of 5 μl resulted in the saturation of the cottonoid pledgets. A cottonoid wick was arranged at the rostral and caudal edges of the craniotomy to prevent excessive pooling of cerebrospinal fluid during the experiment and thereby prevent excessive dilution and spread of drug solution. Subsequent to local drug administration, acridine orange dye was applied using cottonoids to confirm proper localizaion of ventral surface application.

Intravenous injections of drugs. Cardiorespiratory effects of intravenously administered heroin were evaluated in neurally intact animals and in bilaterally vagotomized animals. For the latter purpose, both vagus nerves were isolated and sectioned at the midcervical level to eliminate chemosensitive afferent neural input originating from the cardiopulmonary regions. In one of these animals the carotid sinus nerves were also bilaterally sectioned. In additional animals, the effects of intravenously administered heroin were examined after the antagonist, naloxone, had been applied to the ventral surface of the medulla.

Drugs used and drug preparation. The following drugs were used: alpha-chloralose (Establissments Kuhlmann, Paris), heroin (Research Technical Branch, National Institute of Drug Abuse, Rockville, MD), and naloxone (Endo Laboratories, Inc., Garden City, NY). Alpha-chloralose was dissolved by heating it in distilled water. Heroin and naloxone were dissolved in 0.9% NaCl. Single injections into the CNS were made in a volume of 0.1 ml followed by a saline flush of 0.05 ml with the intracerebroventricular injections and 0.1 ml with the intracisternal injections. Intravenous injections of drugs were made in a volume of 1 ml, including flush. All injections or applications of drugs were controlled by administering the same volumes of saline containing no drug. This procedure had no significant effect on the indices used to monitor respiratory function or on arterial pressure or heart rate. The rate of injection of drug and control solution into the CNS ranged from 30 to 60 s, and the period of application of drug and control solution to the ventral

medullary surface ranged from 2 to 5 min. The pH of the solutions of heroin and naloxone ranged from 5.5 to 6.5.

Statistical analysis. Data are presented as mean±SE. Statistical analysis was performed using the paired t test. The criterion for statistical significance was $P < 0.05$.

RESULTS

Data obtained with CNS injections. Sequential doses of 17, 50, 150, and 450 μg were administered into the fourth ventricle at intervals ranging from 15 to 35 min. These intervals were based on the time required for minute ventilation to return either to control levels or to a new stable base line after administration of a previous dose. The data, summarized in Table I, indicate that doses of 17 and 50 μg produced no significant change in respiratory and cardiovascular function. Doses of 150 and 450 μg produced a significant decrease in minute ventilation. The decrease in $\dot{V}_E$ observed with the 150-μg dose was not accompanied by any significant changes in V_T or f, although both these indices decreased slightly. The decrease in minute ventilation produced by the 450 μg dose was due to a significant decrease in respiratory frequency. The time to onset for this effect was 0.4±0.1 min, and the time to peak effect was 1.3±0.8 min. No significant decrease in tidal volume (V_T) was observed. One of the five cats studied exhibited apnea after administration of the 450 μg dose. Associated with the decrease in $\dot{V}_E$ was an increase in the $PeCO_2$; this was also significantly increased by the 50 and 150 μg doses (Table I). The changes produced by the 450-μg dose on T_I, T_E, and T_T were not statistically significant, but it appeared that lengthening of the total respiratory cycle was occurring because of prolongation of expiration. No significant changes in V_T/T_I or T_I/T_T were observed. In addition, this dose of heroin had no significant effect on heart rate or arterial blood pressure.

In four animals, heroin was injected into the cisterna magna. The reason for examining the effect of drug administered by this route was to determine whether fourth ventricular administration of drug was exerting its effect by exiting from the foramen of Lushka and acting on the ventromedullary surface. Drug injected into the cisterna magna of the cat has access to the ventromedullary surface and does not reach the fourth ventricle (9). Respiratory depression occurred as heroin decreased V_T (Table II). The decrease in V_T was observed with all of the doses used. The decrease in $\dot{V}_E$ was seen with three of the doses. This was also true for $PeCO_2$ (Table II). Doses of 17 and 150 μg also produced a significant decrease in V_T/T_I, while no significant change in T_I/T_T was observed. The time to onset for this depressant effect (i.e., decrease in V_T) after the 450 μg dose was 0.6±0.2 min and the time

TABLE I

Effect of Fourth Ventricular Administration of Heroin on Several Indices of Respiratory Activity

Group and dose of heroin	$\dot{V}_E$	V_T	f	T_I	T_E	T_T	$\frac{V_T}{T_I}$	$\frac{T_I}{T_T}$	$PeCO_2$
	ml/min	*ml*	*breaths/min*		*s*		*ml/s*		*mmHg*
Control (n = 5)	406±30	33.4±2.0	12.2±0.7	1.8±0.1	3.2±0.3	5.0±0.2	18.8±1.9	0.4±0.03	39±2.2
Saline (n = 5)	414±28	34.0±1.7	12.2±0.6	1.6±0.1	3.2±0.3	5.0±0.2	21.2±2.2	0.3±0.02	40±1.6
17 μg (n= 5)	346±32	39.2±5.0	9.4±1.3	1.8±0.1	4.3±0.5*	6.2±0.5	21.2±2.0	0.3±0.03	41±1.8
Control (n = 5)	373±35	32.4±2.9	11.6±0.7	1.7±0.1	3.2±0.3	4.9±0.3	18.0±1.0	0.3±0.03	41±2.2
50 μg (n = 5)	298±32	37.2±9.0	9.6±1.6	1.8±0.2	3.0±0.3	4.8±0.4	15.0±3.0	0.4±0.03	46±2.2*
Control (n = 5)	352±38	38.4±10.0	11.0±1.8	1.6±0.1	5.4±2.3	7.1±2.4	22.4±4.1	0.3±0.05	47±2.1
150 μg (n = 5)	294±48*	34.8±8.4	10.0±1.9	1.7±0.1	6.0±2.7	7.7±2.9	20.0±3.9	0.3±0.05	50±2.2*
Control (n = 5)	336±53	32.0±5.1	11.6±2.1	1.5±0.1	3.1±0.3	4.6±0.3	18.1±1.2	0.3±0.04	50±2.4
450 μg (n = 5)	230±52*	31.2±3.9	8.2±2.2*	1.6±0.1	5.2±1.6	6.9±1.5	18.9±1.5	0.3±0.06	57±2.6*

* $P < 0.05$ using the t test for paired data.

TABLE II

Effect of Cisterna Magna Administration of Heroin on Several Indices of Respiratory Activity

Group and dose of heroin	$\dot{V}_E$	V_T	f	T_I	T_E	T_T	$\frac{V_T}{T_I}$	$\frac{T_I}{T_T}$	$PeCO_2$
	ml/min	*ml*	*breaths/min*		*s*		*ml/s*		*mmHg*
Control (n = 4)	463±65	28.7±1.6	15.7±1.7	1.5±0.1	2.4±0.3	3.9±0.4	18.6±1.2	0.4±0.03	34±2.0
Saline (n = 4)	485±67	29.5±2.0	16.2±1.3	1.5±0.1	2.3±0.3	3.8±0.3	19.7±1.6	0.4±0.03	33±0.3
17 μg (n = 4)	337±52*	25.2±1.7*	13.0±0.4	1.7±0.0*	2.8±0.2	4.6±0.2*	14.5±1.0*	0.4±0.02	40±1.5*
Control (n = 4)	404±47	25.2±1.4	16.2±1.4	1.3±0.0	2.4±0.3	3.7±0.4	18.4±1.5	0.4±0.02	43±3.4
50 μg (n = 4)	347±23	20.7±1.7*	16.2±1.0	1.3±0.1	2.4±0.2	3.8±0.3	15.3±0.7	0.4±0.02	47±3.1*
Control (n = 4)	450±57	23.5±2.0	19.5±1.6	1.2±0.1	2.0±0.2	3.2±0.3	19.2±2.0	0.4±0.02	46±2.8
150 μg (n = 4)	375±54*	19.0±1.2*	17.7±2.0	1.3±0.1	2.0±0.2	3.4±0.3	14.3±2.1*	0.4±0.02	48±1.6
Control (n = 4)	449±20	24.0±1.8	19.0±1.2	1.2±0.1	2.0±0.3	3.2±0.2	19.6±1.7	0.3±0.04	46±3.3
450 μg (n = 4)	322±58*	17.7±3.9*	18.0±2.9	1.1±0.2	2.8±0.7	3.9±0.9	18.4±0.8	0.4±0.04	53±0.9*

* $P < 0.05$ using the t test for paired data.

to peak effect was 1.3±0.4 min. Finally, no significant changes in heart rate and arterial blood pressure occurred.

In using the above two routes of administration, heroin was excluded from the forebrain regions. To assess whether heroin might exert an effect at forebrain sites, experiments were performed in three cats in which this agent, in doses ranging from 17 to 450 μg, was injected into and restricted to the lateral and third ventricles. No respiratory or cardiovascular changes were observed.

Data obtained with intravenous injections. The above data indicate that injection of heroin into the brain by three different routes produced different respiratory responses, and the divergent results prompted an investigation of the pattern of respiratory activity that occurs with systemic administration of the drug. For this purpose, we initially examined the effect of a dose of heroin that was three times higher than the largest dose administered by the central routes, i.e., 1,350 μg. This dose was found to produce apnea in the majority of animals tested. Since the maximal respiratory depressant effect was produced by this dose, we examined the effects of the lower range of doses that were previously tested by the central routes, namely, 17, 50, 150, and 450 μg. Sequential administration produced changes in respiratory function that were indistinguishable from those produced with fourth ventricular administration. Doses of 150 and 450 μg produced a significant decrease in $\dot{V}_E$, and this decrease was due to a reduction of f, as no change in V_T was observed (Table III). The time to onset for this effect at 450 μg was 0.4±0.1 min and the time to peak effect was 1.2±0.3 min. Two of the six cats exhibited apnea after receiving the 450-μg dose. Associated with the decrease in $\dot{V}_E$ was an increase in the $PeCO_2$ (Table III). The changes produced by the 450-μg dose on inspiratory, expiratory, and total cycle durations were not statistically significant, but it appeared that the lengthening of the total respiratory cycle was occurring because of prolongation of the expiratory time. No significant changes in V_T/T_I or T_I/T_T were observed. Interestingly, 450 μg of heroin produced an alteration in arterial pressure as a significant degree of hypotension was noted. That is, arterial pressure was reduced from 128±8 to 94±7 mmHg ($P < 0.05$). No significant change in heart rate occurred after this dose or after the lower doses tested.

These data indicate that the range of doses of heroin administered centrally into the fourth ventricle produced equivalent respiratory depressant effects when given by the intravenous route. This was unexpected, in view of the earlier findings of Florez and colleagues (2), who reported that a 20-fold higher dose of morphine was needed by the intravenous route to elicit a response equivalent to that seen with fourth ventric-

TABLE III

Effect of Intravenous Administration of Heroin on Several Indices of Respiratory Activity

Group and dose of heroin	$\dot{V}_E$	V_T	f	T_I	T_E	T_T	$\frac{V_T}{T_I}$	$\frac{T_I}{T_T}$	$PeCO_2$
	ml/min	*ml*	*breaths/min*		*s*		*ml/s*		*mmHg*
Control (n = 5)	453±53	32.0±2.6	14.8±2.0	1.4±0.2	3.0±0.7	4.4±0.8	23.7±7.6	0.3±0.10	37±1.7
Saline (n = 5)	400±35	29.6±7.1	14.4±2.2	1.4±0.2	3.0±0.9	4.4±1.7	22.1±4.4	0.3±0.01	36±1.2
17 μg (n = 5)	364±41	28.8±2.8	13.2±1.8	1.4±0.2	3.5±0.8	5.0±1.0*	21.2±5.5	0.3±0.01	38±2.3
Control (n = 4)	375±43	29.0±3.1	13.5±2.1	1.4±0.2	3.6±0.8	5.0±1.0	20.4±5.7	0.3±0.01	38±1.4
50 μg (n = 4)	329±31	29.3±2.4	12.8±1.7	1.5±0.2	4.2±0.7*	5.8±0.9*	21.5±5.4	0.3±0.02	39±2.0
Control (n = 5)	391±50	28.6±2.2	14.0±2.0	1.5±0.2	3.6±0.8	5.1±1.0	20.5±6.2	0.3±0.01	39±2.2
150 μg (n = 5)	267±62*	28.8±1.2	9.4±2.3	1.5±0.2	6.1±1.7	7.6±1.6	20.7±5.9	0.2±0.10	42±0.9
Control (n = 5)	335±27	30.4±2.6	11.2±0.7	1.2±0.1	3.8±0.4	5.1±0.4	24.1±5.6	0.3±0.10	43±1.5
450 μg (n = 5)	157±30*	31.6±4.1	5.2±1.0*	1.3±0.2	5.7±0.6	7.1±0.4	28.9±12.5	0.2±0.10	49±3.0*

* $P < 0.05$ using the t test for paired data.

ular injections. This was also unexpected, as drugs that are thought to exert respiratory effects in the brain exert no significant respiratory effects when the maximally effective central dose is given by the intravenous route (10, 11). This raised the possibility that centrally administered heroin might have been producing respiratory depression by leakage into the periphery. To test this point, we administered heroin intravenously to animals with all peripheral chemoreflexes denervated. This involved sectioning the vagus nerves bilaterally in three animals, and sectioning the vagus and carotid sinus nerves bilaterally in a fourth animal. These denervations removed the pulmonary J receptors and aortic and carotid chemoreceptors as potential sites where heroin could act to depress respiration.

Data obtained from the three vagotomized animals are summarized in Table IV. Doses of 17, 50, 150, and 450 μg produced respiratory depressant effects similar to those seen when these doses were given intravenously to animals with intact vagus nerves. One of the three animals developed apnea with the 450-μg dose. Hypotension was observed in the three animals given the highest dose; that is, arterial pressure was reduced from 149±5 to 114±14 mmHg ($P < 0.05$). Only the 450-μg dose was given to the debuffered-vagotomized animal. This dose resulted in apnea. These results suggested that heroin's site of action for producing respiratory depression was in the CNS and led us to study the effects of application of heroin to the ventral surface of the medulla.

Data obtained with ventral surface application. Heroin was directly applied bilaterally in a dose of 70 μg per side to three specific surface areas of the ventral medulla using cottonoids. This dose was selected in an attempt to mimic the respiratory depression observed with the intravenous dose of 450 μg. Application of heroin to Mitchell's area in nine animals produced a significant decrease in $\dot{V}_E$ (Fig. 1). This decrease was similar in magnitude to that seen with intravenous administration. As in the case of intravenous administration, application of heroin to Mitchell's area caused a significant decrease in f. In addition, application of drug to this region produced a significant decrease in V_T. Finally, local application resulted in a significant decrease in V_T/T_I. The time to onset for the depressant effects on V_T and f was ~0.6±0.2 min, and the time to peak effect was ~1.3 ± 0.2 min. There was a trend for the total cycle duration to increase, due primarily to an increase in T_E. No changes in other indices used to measure respiratory function (T_I, T_T), or in blood pressure or heart rate, occurred. In three additional animals this dose of heroin produced apnea.

Application of the same dose of heroin to Schlaefke's area produced a different respiratory response pattern. Most striking was an increase in f (Fig. 1). This response was accompanied by a decrease in V_T. As a

TABLE IV

Effect of Intravenous Administration of Heroin on Several Indices of Respiratory Activity in Vagotomized Cats

Group and dose of heroin	$\dot{V}_E$	V_T	f	T_I	T_E	T_T	$\frac{V_T}{T_I}$	$\frac{T_I}{T_T}$
	ml/min	*ml*	*breaths/min*		*s*		*ml/s*	
Control (n = 3)	538±43	38.3±3.3	14.0±1.2	1.9±0.2	2.4±0.3	4.3±0.5	19.9±1.6	0.3±0.01
Saline (n = 3)	537±38	39.0±2.6	13.6±1.2	2.0±0.1	2.4±0.4	4.4±0.4	19.4±1.0	0.4±0.03
17 μg (n = 3)	482±46	36.3±3.7	13.3±0.8	1.9±0.1	2.5±0.3	4.5±0.4	18.7±1.5	0.4±0.02
Control (n = 3)	532±44	39.0±3.2	13.6±0.3	1.8±0.1	2.4±0.0	4.3±0.1	21.8±2.0	0.4±0.01
50 μg (n = 3)	491±40*	36.0±3.0	13.0±0.3	1.9±0.2	2.8±0.1	4.7±0.1	18.7±1.2	0.4±0.06
Control (n = 3)	618±69	41.3±2.4	15.0±1.7	1.7±0.1	2.4±0.5	4.1±0.6	23.9±1.4	0.4±0.03
150 μg (n = 3)	424±20*	36.3±0.8	11.6±0.3	1.8±0.1	2.7±0.1	4.6±0.1	20.0±1.2	0.3±0.02
Control (n = 3)	644±48	46.3±4.9	14.0±0.5	1.9±0.1	3.0±0.2	4.9±0.3	26.7±5.0	0.3±0.05
450 μg (n = 3)	226±37*	40.6±2.9	5.6±1.2*	2.1±0.2	8.1±0.9	10.2±0.7	19.9±4.3	0.2±0.03

* $P < 0.05$ using the t test for paired data.

result of these contrasting responses, there was no significant change in $\dot{V}_E$. The time to onset for these changes was ~1.1±0.4 min, and the time to peak effect was 4.3±0.8 min. No change in other indices used to measure respiratory function (T_I, T_E, T_T), or in blood pressure or heart rate, occurred.

Application of this dose of heroin to Loeschcke's area failed to evoke any significant respiratory or cardiovascular changes (Fig. 1).

Effects of ventral surface application of naloxone on respiratory depression produced by intravenous administration of heroin: treatment studies. The purpose of these experiments was to test the hypothesis that intravenously administered heroin was depressing respiration by interacting with opioid receptors on the ventral surface of the medulla. This was done by first administering 450 μg of heroin intravenously and allowing a maximal depressant effect on respiration to occur; subsequent to this, naloxone was applied bilaterally to one of the three areas on the ventral medullary surface. In seven animals, intravenously administered heroin produced a significant decrease in $\dot{V}_E$ due to a decrease in f (Table V). This depressant effect was completely reversed by topical naloxone applied bilaterally in doses of 15 μg to Mitchell's area. Naloxone produced complete reversal of heroin response within 1.5±0.3 min. A representative experiment showing this antagonism appears as Fig. 2.

In the studies with naloxone on Schlaefke's area, prior administration of heroin produced a significant decrease in $\dot{V}_E$; in this case, however, the decrease was due to a reduction not only in f, but in V_T (Table V). Naloxone application to Schlaefke's area reversed all the respiratory depressant effects produced by heroin. Complete reversal was observed, on the average, 2.8±0.4 min after naloxone application. This time was significantly longer than that noted above in the studies with Mitchell's area.

Application of naloxone on Loeschcke's area had no effect on the respiratory depression (i.e., decrease in $\dot{V}_E$ produced by a reduction of f) produced by intravenously administered heroin (Table V).

Effect of ventral surface application of naloxone on the respiratory depression produced by intravenously administered heroin: pretreatment studies. In these experiments, naloxone was first applied to Mitchell's (four experiments) or Schlaefke's (two experiments) areas (15 μg to each side) before the administration of heroin intravenously (450 μg). In each case, pretreatment with naloxone blocked the usual respiratory depressant effects of intravenous heroin. For example, in the case of Mitchell's area, control values for $\dot{V}_E$, V_T, and f were 424±64 ml/min, 27±0.8 ml, and 15.5±2.1 breaths/min, respectively. The corresponding values after naloxone pretreatment were 417±63 ml/min, 27±0.8 ml, and 15.2±2 breaths/min. After the subsequent administration of heroin, values for $\dot{V}_E$, V_T, and f were 384±44 ml/min, 27±1, and

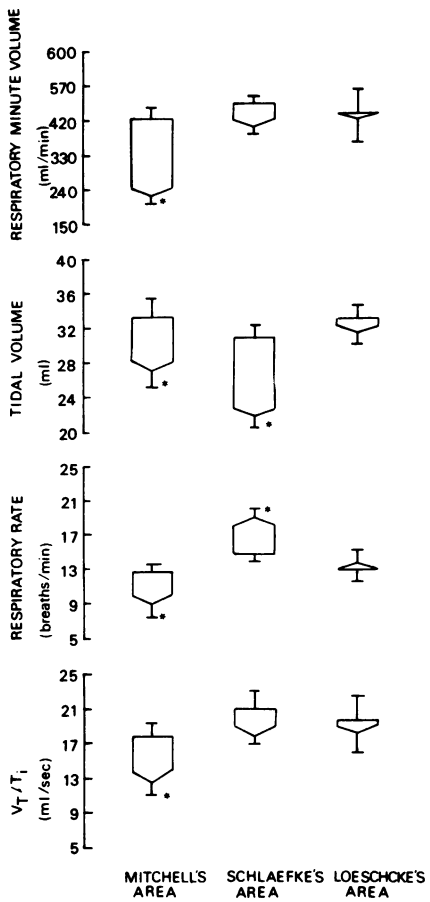

FIGURE 1 Changes in $\dot{V}_E$, V_T, f, V_T/T_I produced by bilateral application of 70 μg of heroin to each side of either Mitchell's area (n = 9 animals), Schlaefke's area (n = 7 animals), or Loeschcke's area (n = 5 animals) on the ventral surface of the medulla. Columns depict the base-line levels (flat end) and the direction and magnitude of responses (pointed end) after administration of heroin. The vertical bars on the columns indicate the standard errors. The asterisks indicate significant changes (using the paired t test) in respiratory response.

14±1 breaths/min, respectively. None of these values after drug administration was significantly different from control values. Similar results were obtained in the experiments performed with naloxone pretreatment at Schlaefke's area followed by intravenous heroin.

DISCUSSION

The purpose of our study was to determine the CNS site of action for the respiratory depressant effect of heroin. Our initial attempt involved administering heroin into the forebrain area and two hindbrain areas. Injection into the lateral ventricle with restriction of the drug to the forebrain region failed to produce respiratory depression. These results exclude areas such as the cerebral cortex, hypothalamus, and limbic system as potential sites for the respiratory depressant effects of drugs that activate opioid receptors. Comparable findings were reported by Florez and colleagues (2) who found no respiratory depressant effect of morphine when injected into the forebrain ventricle.

Injection into the fourth ventricle did produce respiratory depression manifested as a reduction in $\dot{V}_E$; this was due to a decrease in f in the absence of a change in V_T. Similarly, injection into the cisterna magna produced a decrease in $\dot{V}_E$. These findings confirm earlier data of Florez and colleagues (2) indicating that morphine injected into either the fourth ventricle or subarachnoid space causes respiratory depression.

Intravenous administration of heroin produced respiratory depression similar to that seen upon hindbrain administration. Although a decrease in $\dot{V}_E$ was consistently associated with a decrease in f, in one series of experiments, namely those involving reversal of depression with naloxone, f and V_T were both found to decrease. This effect with systemically administered heroin was due to a CNS site of action, as denervation of all known peripheral reflexogenic areas failed to influence the response.

Possible hindbrain sites where heroin could be acting

TABLE V

Effects of Ventral Surface Application of Naloxone on Respiratory Depression Produced by Intravenous Administration of Heroin

Group and dose of heroin	$\dot{V}_E$	V_T	f	MBP	HR
	ml/min	*ml*	*breaths/min*	*mmHg*	*beats/min*
Control (n = 7)	362±43	31.2±6.6	11.7±2.3	142±8.9	228±16
Heroin (n = 7)	181±33*	34.5±7.3	5.1±1.6*	120±7.8*	193±13
Naloxone on Mitchell's area (n = 7)	374±60	32.0±5.7	11.5±3.7	133±11	210±13
Control (n = 6)	471±61	29.1±4.2	16.5±6.2	163±6.4	186±11
Heroin (n = 6)	189±33*	24.3±5.5*	8.5±4.8*	126±13*	157±12
Naloxone on Schlaefke's area (n = 6)	467±40	26.5±3.8	17.1±4.8	155±6.9	212±15
Control (n = 4)	460±48	26.5±2.6	17.7±1.9	168±7.3	195±16
Heroin (n = 4)	211±33*	28.7±2.2	8.0±1.7*	138±12	199±16
Naloxone on Loeschcke's area (n = 4)	259±12	30.5±2.6	8.7±1.1	138±12	201±17

* $P < 0.05$ using the t test for paired data. *HR*, heart rate; *MBP*, mean blood pressure.

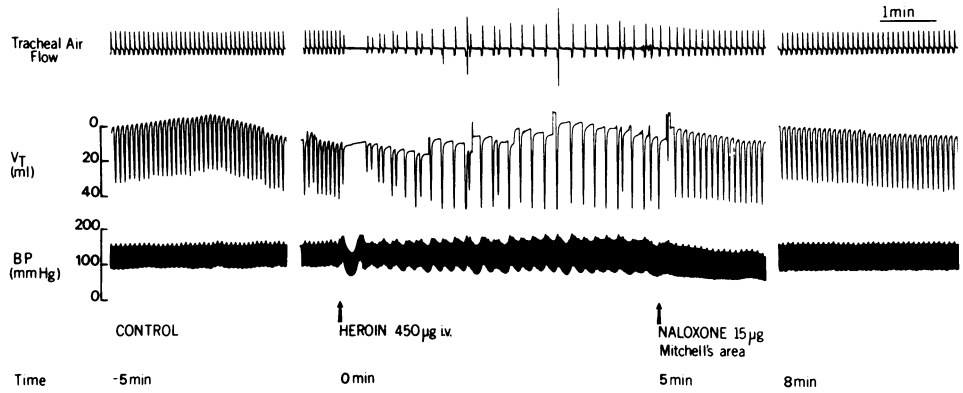

FIGURE 2 Representative experiment showing the effects of intravenous administration of heroin on tracheal airflow, V_T, and arterial blood pressure (*BP*). The tracings on the left side of the figure were obtained before heroin administration control. The middle set of tracings show the respiratory depressant effects of heroin (as indicated by the first arrow) and the reversal of this effect 5 min later with topical application of naloxone to Mitchell's area (as indicated by the second arrow). The third set of tracings on the right side of the figure were obtained 3 min after the tracings in the middle panel were taken.

are the medullary respiratory "centers" (nucleus tractus solitarius and nucleus retroambigualis (12) and ventral surface of the medulla. These medullary centers could be reached by drug placed into the fourth ventricle. Nevertheless, it is doubtful that heroin acts at these sites. Changing the activity of these neurons by either electric stimulation or lesions appears to only affect V_T and not respiratory frequency (13). Since intravenous heroin appears to have a major effect on frequency, the site of action must be elsewhere, most likely on the ventral surface of the medulla.

Thus, we examined the effects of direct application of heroin to the ventral medulla. The site where respiratory responses most closely resembled those produced by intravenous administration of the opioid was Mitchell's area. At this site, decreases in both f and V_T occurred. Indeed, this was the only site where heroin application resulted in a decrease in $\dot{V}_E$. At Schlaefke's area there was an increase in f and a decrease in V_T, the net effect of which was no change in $\dot{V}_E$. No effects were observed when heroin was applied to Loeschcke's area.

Consistent with an action of heroin on Mitchell's area is the result obtained with naloxone. When applied locally to Mitchell's area, naloxone restored respiratory depression produced by intravenous heroin to normal. Similar results occurred with naloxone as a pretreatment.

We also observed that the respiratory depression produced by intravenous heroin could be either prevented or counteracted by application of naloxone to Schlaefke's area. We attribute this activity of naloxone at this site to diffusion to Mitchell's area, since the time required to observe full reversal of the intravenous heroin effect was significantly longer with application to Schlaefke's area as compared with Mitchell's area. In addition, heroin exerted no significant respiratory depressant effect when applied directly to Schlaefke's area.

Even though heroin appears to act at Mitchell's area, the drug given intravenously appears to reach the site primarily by the blood stream rather than the cerebrospinal fluid. Evidence for this is that heroin given intravenously has a shorter time to onset of action than does the drug administered directly to Mitchell's area. This finding was also reported by Florez and co-workers for morphine (2). Consistent with this is that it appears that less drug is required for an effect by the intravenous route if one takes into consideration the respective dilution factors of this route vs. local application; that is, 450 μg of heroin will be diluted by the systemic route of administration, making it impossible that the total amount of 140 μg, as administered by local application, would ever be achieved through the bloodstream. Indeed, with intracerebroventricular injections, a dose of 450 μg produced responses equivalent to a dose of 450 μg i.v., and the dilution factor would be ~30-fold higher for the intravenous route. However, it is not unexpected that it would take a significant time period for drug applied locally to reach Mitchell's area as these cells do not appear to be on the surface (14) but are mostly located between 100 and 1,000 μm below the surface (15). Furthermore, the necessity of using a relatively high dose of heroin on the ventral surface (i.e., 70 μg per site) may be due to the high lipid solubility of heroin and, consequently, its rapid diffusion to other areas before it has had a chance to exert an effect. Additionally, heroin's high lipid solubility and rapid ability to penetrate the blood-brain barrier could also result

in the circulatory removal of drug before it has had a chance to diffuse to the depth of opioid receptors, presumably to chemosensitive sites below the ventral surface.

The present results support the recent findings of Hurle and colleagues (7) indicating that Mitchell's area is an important site for the depressant effects of opioid drugs. They demonstrated that morphine placed on Mitchell's area produced decreases in both f and V_T, with the decrease in V_T being the most striking effect. Similar, but less pronounced effects were observed at Schlaefke's area. We observed that heroin produced the same effects as morphine when applied to Mitchell's area. However, heroin placed at Schlaefke's area did not reduce f; instead, there was a significant increase in this index. Heroin, as in the case of morphine in the study of Hurle et al. (7), did produce a decrease in V_T when localized to both ventral surface areas. Our results with heroin at Schlaefke's area are similar to those obtained by Pokorski and collegues (6) with fentanyl. They observed a decrease in V_T and an increase in f. In addition, they reported no respiratory depressant effect of fentanyl when placed on Loeschcke's area. This was also the case with heroin in the present study.

Although Mitchell's area appears to be the site of action of heroin to depress respiration, our data suggest that no "tonic opioid system" is present in this brain region. Evidence for this was our finding that naloxone placed locally at this site had no effect on f or V_T. These results confirm the findings of Hurle et al. (7) who found no significant change in respiratory activity when naloxone was locally applied to Mitchell's area. Hence, while there is no evidence for the presence of an endogenous opioid system at Mitchell's area, there are clearly opioid receptors present at this site that can influence breathing.

A major difference between our study and those of other investigators examining the effects of opioid drugs at the ventral surface of the medulla (5–7) is that we sought to determine the role of these brain sites in the response to systemically administered opioids. Indeed, no information is available on this point. Our findings with local application of naloxone, used either as a treatment or a pretreatment in animals given intravenous heroin, indicate that Mitchell's area is an important site for the respiratory depressant effects of these drugs.

Our results with systemically administered heroin in cats are consistent with respiratory effects described for intravenous heroin in humans. Tress and El-Sobky (16), reported that intravenous heroin produces decreases both in f and V_T. These effects were associated with hypotension, an effect also noted in the present study. Hence, the similar pattern of responses in both species suggests that Mitchell's area may be of importance in the mediation of the respiratory depressant effect of heroin in man.

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