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Central Respiratory and Cardiovascular Effects in the Rat of some Putative Neurotransmitter Amino Acids

P. Wessberg[1], J. Hedner[1], T. Hedner[2], and J. Jonason[1]

Departments of Pharmacology[1] and Clinical Pharmacology[2], University of Göteborg and Sahlgren's Hospital, Box 33031, S-400 33 Göteborg, Sweden

Summary. Respiratory performance was studied in halothane anesthetized rats after intracerebroventricular (i.c.v.) injection of β-alanine, taurine or glycine (0.01–1 mg). The amino acids induced a marked decrease in both respiratory frequency (f) and tidal volume (V_T), which was immediate and longlasting. The respiratory depressant action of glycine could readily be reversed by strychnine, a glycine antagonist. Measurement of respiratory time intervals, inspiratory time (T_I), expiratory time (T_E) and total cycle duration (T_{TOT}), after administration of the putative neurotransmitter amino acids revealed that the effects on f were due to prolongation of the duration of expiration. The duration of inspiration was principally unaltered, but mean inspiratory flow (V_T/T_I) and respiratory timing (T_I/T_{TOT}) decreased. In experiments employing the occluded breath technique, $P_{0.1}$ was reduced in the same magnitude as the mean inspiratory flow (V_T/T_I). The results also showed a change in central (bulbopontine) setting for T_E, while the setting to T_I was unaltered. An inert amino acid, valine, which was administered i.c.v. in the same doses, had no effects on respiratory parameters.

Apart from the effects on basal ventilation of β-alanine, taurine and glycine, the CO_2 induced respiratory response was blunted. These three amino acids also depressed heart rate and mean arterial pressure.

Although relatively high doses were used to induce the respiratory effects, it may be hypothetized that the putative neurotransmitters β-alanine, taurine and glycine may have a physiological role in the central regulation of breathing.

Key words: Brain – Respiration – β-Alanine – Taurine – Glycine

Introduction

In the central nervous system (CNS), several amino acids normally occur in relatively high amounts (Uhr and Sneddon 1972; Guidotti et al. 1972; Martin del Rio et al. 1977). Some of them e.g. β-alanine, taurine and glycine, depress neuronal functions. According to established criteria these amino acids are generally accepted to have a neurotransmitter function within the CNS.

Among the many functions investigated after local administration of β-alanine, taurine and glycine is their ability to depress bulbar respiratory neuronal units in the brain (Denavit-Saubié and Champagnat 1975; Hösli et al. 1969). These neurons which maintain activity during either the inspiratory or expiratory phase, are more or less confined to certain areas in the brain stem (see Berger et al. 1977; Cohen 1979; Hukuhara et al. 1980; St John 1973). Although the anatomical and physiological characteristics of the respiratory units are relatively well established, less is known about their neurochemical and neuropharmacological qualities. In several recent studies, it has been shown that central respiratory control mechanisms may be influenced by several known monoamine (Armijo and Florez 1974; Lundberg et al. 1979; Mueller et al. 1980; Hedner et al. 1982), aminoacid (Hedner et al. 1981b) and peptide neurotransmitters (Florez et al. 1980; Hedner et al. 1981a, c; Moss and Friedman 1978).

Since the putative amino acid neurotransmitters β-alanine, taurine and glycine have a regional distribution in the brain with relatively high levels in the brain stem area (Uhr and sneddon 1972; Guidotti et al. 1972; Martin del Rio et al. 1977), it seemed relevant to further investigate their role in central respiratory regulation. In the present study we have investigated their actions on basal and CO_2-stimulated respiration as well as circulatory regulation in the rat after intracerebroventricular (i.c.v.) administration.

Materials and Methods

Male Sprague-Dawley rats weighing 200–300 g were used. Two–three days before experiments the rats were anesthetized with sodium pentobarbitone (40 mg/kg i.p.). The skull was exposed by a midline incision and holes were drilled bilaterally in the skull 2 mm posterior to the bregma and 2 mm lateral from the sagittal suture. Through these holes polyethylene cannulae (PP25, Portex Ltd., Hythe, Kent, England) were implanted reaching the lateral ventricles 4 mm below the surface of the skull. The cannulae were fixed to the skull by acrylic dental cement supported by metallic screws in the parietal bone on each side. The gross behaviour of the animals was normalized in all cases within 24 h after the operation.

Immediately before the experiments the rats were anesthetized with ether during the cannulation of the trachea (Venflon cannula 2.0 mm, Viggo AB, Helsingborg, Sweden), the ventral tail artery and in some cases the femoral vein (PP50, polyethylene, Portex Ltd., Hythe, Kent, England). After these operative procedures the animals were placed in a closed cylinder-formed body plethysmograph (internal diameter 80 mm, length 300 mm) and anesthesia was maintained with 0.7% halothane in O_2, continuously administered via the tracheal cannula by means of a Draeger vaporizer. By a

Send offprint requests to P. Wessberg at the above address

plastic tubing the body plethysmograph was connected to a low pressure transducer (Grass Model PT 5A) and a Grass Polygraph (Model 5 or 7). At the end of each experiment, during anesthesia, the rats were injected with 0.4 mg pancuronium bromide. After cessation of respiratory movements, a graded 2 ml syringe was connected to the external fitting of the tracheal cannula and a stepwise (0.5, 1, 1.5 and 2 ml) calibration of tidal volume was performed. Blood pressure was recorded via the arterial catheter by means of a pressure transducer (Statham P23 Db) connected to the Grass polygraph and heart rate was calculated from an electrocardiogram recorded from subcutaneously placed electrodes in the two front limbs and the right hind limb of the rat. Mean arterial pressure (MAP) was calculated from the blood pressure registrations. The internal temperature in the body plethysmograph was continuously recorded. Rectal temperature was measured by a Telethermometer (Opti-lab Instrumentation AB) and if necessary the body temperature was adjusted towards normal with a heating pad.

Tidal volume (V_T) and respiratory frequency (f) were continuously recorded by the Grass polygraph. Minute ventilation ($\dot{V}_E$) was calculated according to the formula $V_T \cdot f = \dot{V}_E$.

Inspiratory time (T_I), expiratory time (T_E) and total cycle duration (T_{TOT}) were calculated from the respiratory curve at high chart speed as previously described (Hedner et al. 1980). At certain times during the experiments the mean inspiratory flow (V_T/T_I) and "respiratory timing" (T_I/T_{TOT}) were calculated. If the animal showed respiratory arrest at such a time the first representative breath following was used for calculation.

In some experiments the tracheal cannula was connected to a low pressure transducer (Grass model PT 5A) for registration of the intratracheal pressure. At certain points during the experiments the tracheal cannula was occluded at functional recidual capacity (FRC) for 2–3 breaths. The negative intratracheal pressure generated after 0.1 s ($P_{0.1}$, Lynne-Davies et al. 1971) was calculated from the registration chart after calibration of the transducer to 0 and −10 cm H_2O. Measurements of $P_{0.1}$ always refers to the first occluded breath in order to eliminate the effects of increasing chemical stimuli during the occlusion period.

During the occluded breath, expansion of the pulmonary volume is seen due to decompression of the gas mixture in the respiratory system. The maximum value of this expansion and the corresponding value of the preceding unoccluded breath (V_{max}; in an unoccluded breath V_{max} is equal to V_T) was plotted against the inverse of T_I, T_E and T_{TOT} of the same breaths. According to Grunstein et al. (1973) the slope of the function V_{max} vs $1/T_I$ is indicative of vagally mediated volume control of the length of the inspiratory phase, whereas the T_I value corresponding to $V_{max} = 0$, represents the bulbopontine setting of inspiratory time.

The effects of β-alanine, taurine and glycine on the respiratory and circulatory parameters studied were measured after an initial stabilization period of 20 min. The drugs were slowly injected bilaterally via the intracerebroventricularly placed cannulae (10 µl followed by 8 µl saline into each ventricle). Control animals were given 18 µl of saline into each lateral brain ventricle. The animals used for dose-response studies were given three to four subsequent injections into each side with 20 min intervals. In a separate experiment in order to rule out osmotic pressure effects, valine was given in the same dose range as β-alanine, taurine and glycine. In the time-effect experiments CO_2 challenges (5% CO_2 or 10% CO_2 in O_2) were performed immediately before and 30 min after drug injection. In some experiments animal given glycine i.c.v. were allowed to recover after 15 min in the plethysmograph. Twenty-four hours later, the experimental procedures were repeated and the effects of another injection of glycine i.c.v. was studied.

At the end of some experiments 0.5 µl of Evans blue dye was injected bilaterally throiugh the i.c.v. cannulae and the injection site was then located at autopsy.

At certain time intervals (before and 15 min after drug injection) 0.5 ml of arterial blood was withdrawn from the ventral tail artery. Maximum two samples per rat were taken and the withdrawn volume of blood was immediately replaced by an equivalent volume of 0.9% NaCl solution. Arterial blood pH, standard bicarbonate (SB), partial pressures of carbondioxide ($PaCO_2$) and oxygen (PaO_2) were analyzed by means of a blood gas analyzer (Instrumentation Lab. Inc., analyzer 413).

For statistical analysis, Student's t-test, one or two way analysis of variance followed by t-test, or paired t-test were used.

Drugs used: Halothane (Hoechst, Frankfurt, FRG), pancuronium bromide (Organon, Oss, The Netherlands), glycine (Sigma Chemical Co. St. Louis, MO, USA), β-alanine (Sigma Chemical Co. St. Louis, MO, USA), taurine (Sigma Chemical Co St. Louis, MO, USA), strychnine (Sigma Chemical Co, St. Louis, MO, USA).

Results

During the experiments, the internal temperature of the plethysmograph was 27.1 ± 0.6° C and the body temperature of the animals was 35.2 ± 2° C. After i.c.v. administration of the various amino acids the body temperature of the animals fell by 2.0 ± 0.4° C during the course of the experiment.

Single i.c.v. saline injections under halothane anesthesia did not influence respiratory performance during the 45-min period studied (Fig. 1 a–c). Previous control experiments have shown that repetitive i.c.v. injections (totally 108 µl) did not alter respiratory performance during a 60 min interval (data not shown). Similarly, repetitive i.c.v. administration of a hyperosmotic solution like the inert amino acid valine, in the same dose range as β-alanine, taurine and glycine did not affect central respiratory activity (Fig. 3 d). Nor did any significant effects occur after a single dose of 1 mg valine i.c.v. during the following 45 min period studied (data not shown). Compared to some other anesthetics like pentobarbitone, chloral hydrate and enflurane only minor influences on basal respiratory performance was seen after halothane (unpublished results).

Effects of 1 mg of Taurine, β-Alanine or Glycine i.c.v. on f, V_T and $\dot{V}_E$.

The effects of i.c.v. injections of the drugs used on f, V_T and $\dot{V}_E$ at various time intervals after administration are shown in Fig. 1. Within 1 min after i.c.v. injection of 1 mg of either taurine, β-alanine or glycine, f markedly decreased (Fig. 1 a). Glycine and β-alanine reduced f to approximately 75 and 50% of the initial values. After the initial fall, f maintained at this level during the rest of the 45-min observation period.

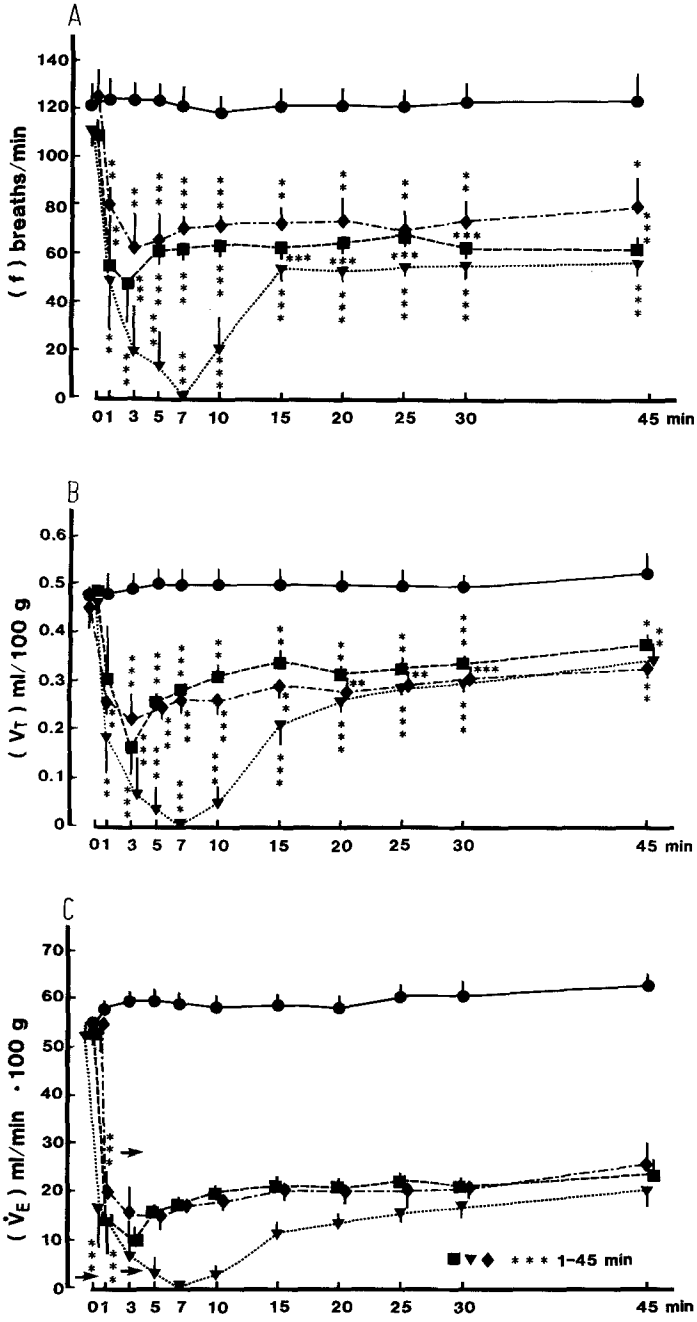

Fig. 1. Effects of a single i.c.v. injection of saline, β-alanine 1 mg, taurine 1 mg or glycine 1 mg, on respiratory frequency (**a**), tidal volume (**b**) and minute volume (**c**). Shown are means ± S.E.M. Statistical comparison versus the saline curve was performed by means of Student's *t*-test. In Fig. 3c note significance indicated with arrows; $P < 0.001$ from 1 to 45 min. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (●——●) saline ($n = 7$); (■----■) β-alanine ($n = 4$); (▼·····▼) taurine ($n = 5$); (◆-·-·◆) glycine ($n = 4$)

The effect of taurine was more pronounced than that of glycine and β-alanine. After taurine a progressive decline in *f* was noted up till 7 min after the i.c.v. injection. At this time interval all animals showed respiratory arrest. In two of the five animals apnea was noted already 1 min after injection and in three of the five animals apnea still persisted 10 min after the i.c.v. injection. No apnoic spells were seen after glycine, whereas one of the four rats injected with β-alanine remained apnoic between 1 and 3 min after the i.c.v. injection.

Table 1. 24 h interval experiments

	Time (min)	*f* (breaths/min)	V_T (ml/100 g)	V_E (ml/min · 100 g)
	Control	118 ± 10.8	0.53 ± 0.05	60 ± 2.3
Day 1	5	76 ± 16.5***	0.27 ± 0.06***	22 ± 7.2***
($n = 4$)	10	80 ± 15.3***	0.30 ± 0.03***	24 ± 5.9***
	15	82 ± 15.3**	0.30 ± 0.02**	25 ± 5.6***
	Control	138 ± 5.1	0.59 ± 0.04	81 ± 5.5**
Day 2	5	115 ± 8.6*	0.46 ± 0.02	53 ± 3.8***
($n = 4$)	10	116 ± 7.5*	0.42 ± 0.03**	49 ± 3.7***
	15	113 ± 9.0*	0.40 ± 0.03**	44 ± 5.0***

The effect of 1 mg glycine i.c.v. on some respiratory parameters. I.c.v. injections were given to the same individual animals with an interval of 24 h. Statistics by 2 way analysis of variance followed by *t*-test. Statistical significances represent comparison to the control registration of each day. Control registration of day 2 compared to control registration of day 1. Shown are the means ± S.E.M., *n* represents the number of animals. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

In the taurine-treated animals *f* reached approximately the same value as for glycine and β-alanine at the end of the apnoic period.

Similarly as for *f*, V_T decreased immediately after i.c.v. injection of the amino acids (Fig. 1 b). The decrease in V_T after taurine was more pronounced than after glycine and β-alanine. Thus, after taurine V_T decreased to zero at 7 min, when apnea was present in all animals. After the initial decrease, V_T increased slowly during the rest of the period studied, but was still significantly depressed at 45 min after i.c.v. injection.

As a consequence of the marked decreases in *f* and V_T, $\dot{V}_E$ was markedly depressed after taurine as well as after glycine and β-alanine. The decrease in $\dot{V}_E$ followed the same time sequence (Fig. 1 c). In the animals reinvestigated 24 h after a single i.c.v. injection of 1 mg glycine, $\dot{V}_E$ was significantly increased compared to the preinjections values (Table 1). A new i.c.v. injection of 1 mg glycine again resulted in respiratory depression, however slower in onset and less pronounced than after the initial injection.

The respiratory depressant effect (*f*, V_T and $\dot{V}_E$) of glycine could readily be reversed by an i.v. injection of strychnine (1 mg/kg) administered 15 min after the i.c.v. glycine injection (Fig. 2, data for *f* and V_T not shown). Administration of strychnine (1 mg/kg) before glycine injection was, due to generalized convulsions, not possible.

Dose-Response Effects of i.c.v. β-Alanine, Taurine and Glycine on f, V_T and $\dot{V}_E$

β-Alanine, taurine and glycine were given in four consecutive i.c.v. injections; 0.01, 0.1, 0.3 and 1 mg (Fig. 3 a–c). All amino acids studied induced a progressive decline of *f*, V_T and $\dot{V}_E$, in a dose-response manner. Taurine seemed to be somewhat more potent than glycine and β-alanine. It produced respiratory arrest in all animals at the highest dose level and in 2 out of 4 animals after 0.3 mg.

Effects on Respiratory Timing Mechanisms and Inspiratory Drive

I.c.v. administration of 1 mg of β-alanine, taurine or glycine markedly prolonged T_E and T_{TOT} while T_I was unaffected

5 min after injection (Table 2). Subsequently the respiratory timing quotient, T_I/T_{TOT}, also decreased (38–49%). There were also decreases in mean inspiratory flow, V_T/V_I, (49–56%) and $P_{0.1}$ (glycine, 58%), both reflecting inspiratory neural drive (Table 2).

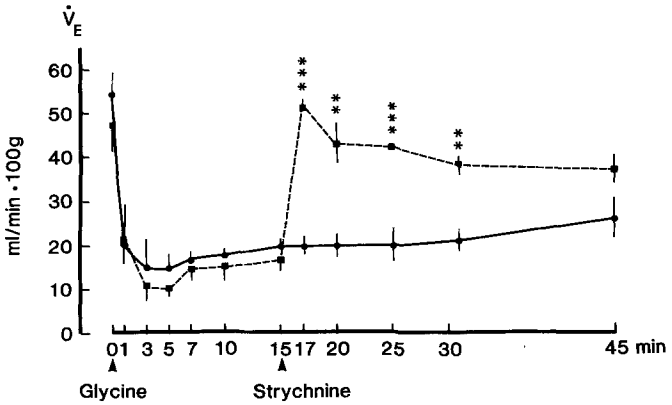

Fig. 2. Effect on minute ventilation of glycine (1 mg i.c.v.) alone or glycine (1 mg i.c.v.) followed by strychnine (1 mg/kg, i.v., 15 min). Shown are means ± S.E.M. of 4 (glycine) or 3 (glycine + strychnine) experiments. Statistics by Student's *t*-test. ** $P < 0.01$, *** $P < 0.001$. (——) Glycine 1 mg i.c.v.; (-----) glycine 1 mg i.c.v. + strychnine 1 mg/kg i.v.

Plotting the maximum volume expansion (V_{max}) in an occluded breath and the immediately preceding unoccluded breath, against the inverse of T_I, T_E and T_{TOT}, we found a decrease in the slope of the V_{max} vs $1/T_I$ function, measured as an increase in the ratio $\Delta\ T_I^{-1}/\Delta V_{max}$ from 4.76 ± 1.20 to 13.00 ± 2.41 ($P < 0.05$, $n = 5$) 5 min after glycine, 1 mg i.c.v. (Fig. 4, left panel). There was also a change in the point of intercept with the inversed time axis at $V_{max} = 0$ for the V_{max} vs $1/T_E$ function from 2.32 ± 0.62 to 1.66 ± 0.58 ($P < 0.05$, $n = 5$, Fig. 4, middle panel).

Effects of i.c.v. β-Alanine, Taurine and Glycine on CO_2-Stimulated Respiration

The addition of 5% or 10% CO_2 to the inhalation gas mixture induced a dose-related significant increase in V_T and $\dot{V}_E$ during control conditions (Fig. 5). *f* was unaltered or slightly decreased. Thirty minutes after i.c.v. injection of β-alanine, taurine and glycine the CO_2-stimulatory response on V_T and $\dot{V}_E$ was abolished.

Effects on Blood Gases

β-Alanine, taurine and glycine all induced a significant reduction in blood pH and an increase $PaCO_2$, i.e. the typical

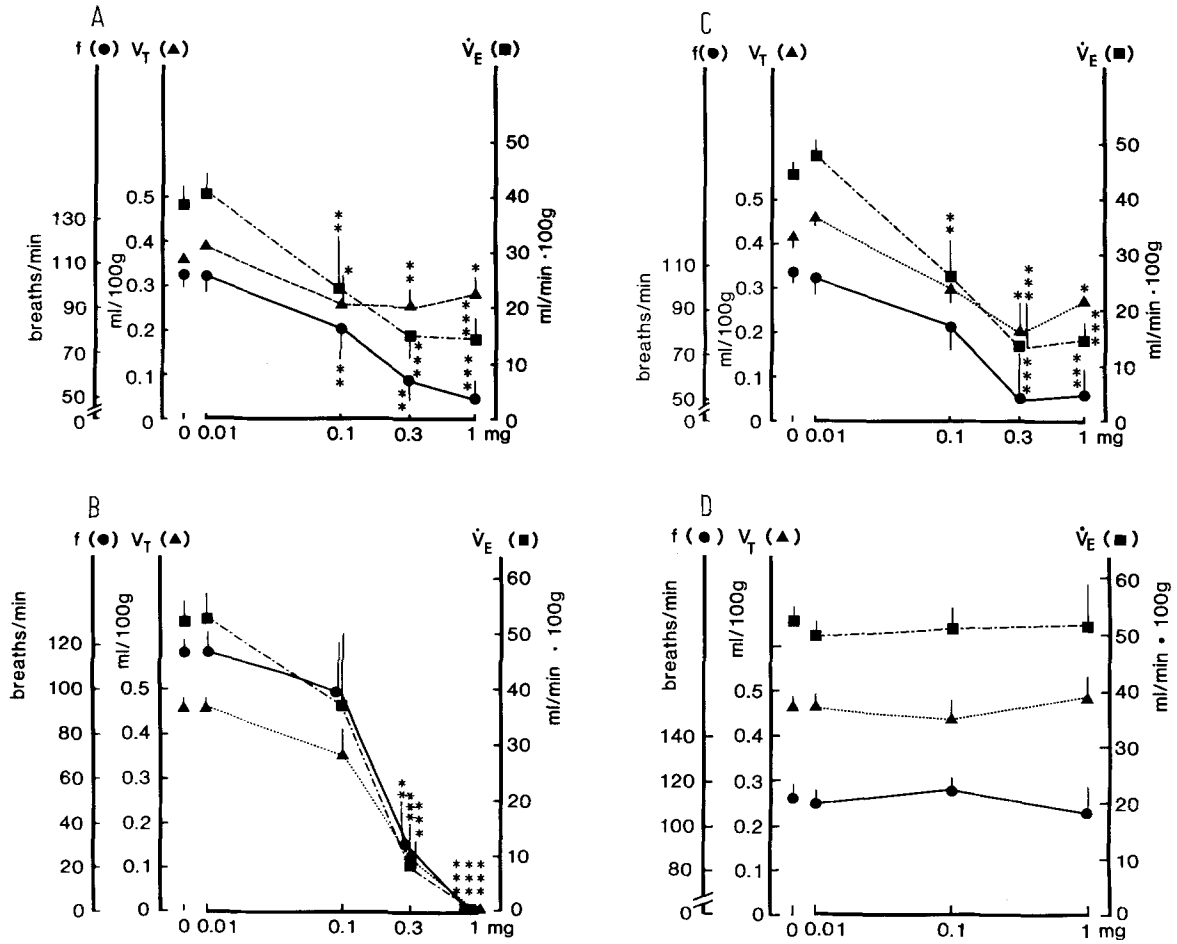

Fig. 3. Dose response relationship on respiratory frequency (*f*), tidal volume (V_T) and minute ventilation ($\dot{V}_E$) after consecutive i.c.v. injections (20 min intervals) of β-alanine (**a**), taurine (**b**), glycine (**c**) and valine (**d**). Shown are values recorded 5 min after drug administration. Means ± S.E.M. are indicated of 4 experiments (**a**, **b** and **c**) and 6 experiments (**d**). Statistics by 2 way analysis of variance followed by *t*-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 2. Time intervals and quotients of β-alanine, taurine, glycine and valine

		T_I (s)	T_E (s)	T_{TOT} (s)	V_T/T_I (ml/100g·s)	T_I/TOT	$P_{0.1}$ (cm H_2O)
Untreated		0.22 ± 0.01	0.33 ± 0.04	0.54 ± 0.04	2.26 ± 0.12	0.40 ± 0.03	
β-Alanine	1 mg	0.27 ± 0.04	0.81 ± 0.08**	1.07 ± 0.10**	1.16 ± 0.13**	0.24 ± 0.03**	
n		4	4	4	4	4	
Untreated		0.21 ± 0.01	0.32 ± 0.02	0.53 ± 0.04	2.26 ± 0.09	0.39 ± 0.01	
Taurine	1 mg	0.21 ± 0.02	0.87 ± 0.10**	1.08 ± 0.10*	1.00 ± 0.11**	0.20 ± 0.03**	
n		5	5	5	5	5	
Untreated		0.21 ± 0.01	0.33 ± 0.03	0.54 ± 0.04	2.51 ± 0.31	0.39 ± 0.01	4.0 ± 0.62
Glycine	1 mg	0.23 ± 0.02	0.94 ± 0.15**	1.17 ± 0.15**	1.10 ± 0.18*	0.24 ± 0.02**	1.7 ± 0.42*
n		7	7	7	4	4	4
Untreated		0.22 ± 0.01	0.37 ± 0.04	0.59 ± 0.05	2.05 ± 0.22	0.38 ± 0.03	
Valine	1 mg	0.20 ± 0.01	0.45 ± 0.11	0.64 ± 0.12	2.52 ± 0.17*	0.34 ± 0.03	
n		6	6	6	6	6	

Registrations were made 5 min after drug administration or in case of taurine, at first representative breath after apnea
Statistics by paired *t*-test. *n* indicates number of experiments. * $P < 0.05$, ** $P < 0.01$

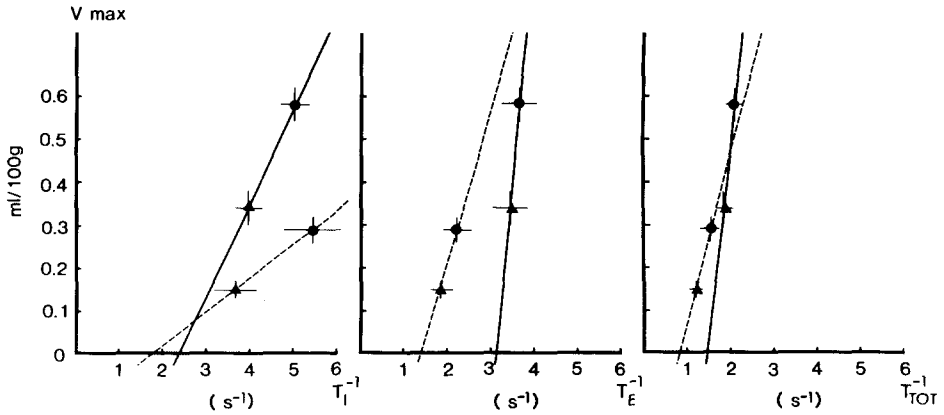

Fig. 4
Relationship between pulmonary volume expansion (in ml/100g above functional residual capacity, V_{max}) and reciprocal of T_I (*left panel*), T_E (*middle panel*) and T_{TOT} (*right panel*).
(——) Untreated animals,
(-----) animals treated with glycine (1 mg i.c.v., 5 min). (●) Control breaths, (▲) occluded breaths. Values are mean ± S.E.M. of 4 animals

findings of respiratory acidosis following decreased alveolar ventilation. β-Alanine also reduced standard bicarbonate. No significant alterations were seen in PaO_2 (Table 3).

Effects on Blood Pressure and Heart Rate

After i.c.v. administration of single 1 mg doses, all amino acids induced alterations in MAP and HR (Table 4). Both β-alanine and taurine caused marked decreases in MAP, already 1 min after injection. The hypotensive effect peristed during the 45 min registration period. Glycine was less potent, but significant decreases in MAP (approximately 25%; $P < 0.05$) were seen 3 and 5 min after drug injection. HR was significantly depressed after all amino acids. β-Alanine was more potent than taurine and glycine with a shorter onset and a longer duration.

Discussion

The putative amino acid neurotransmitters used in this study, β-alanine, taurine and glycine, all induce a marked general CNS depression when administered by the intracerebroventricular route to rats. Thus e.g. body temperature, motor behaviour and arterial blood pressure decrease after injection (Sgaragli and Pavan 1972). Regional brain analysis of the amino acids have demonstrated that high or relatively high concentrations are present in the brain stem and medulla (Uhr and Sneddon 1972; Guidotti et al. 1972; Martin del Rio et al. 1977). Electrophysiological experiments with local application of β-alanine, taurine or glycine in these areas have demonstrated a depressive or hyperpolarizing effect on neurons or neuronal activity (Hösli et al. 1969; Denavit-Saubié and Champagnat 1975). In our study i.c.v. administration of the amino acids β-alanine, taurine and glycine, caused an immediate, marked and longlasting respiratory depression, which was due to a decrease in f as well as V_T.

It is obvious that the respiratory effects seen after the putative amino acid neurotransmitters not were due to the high molar concentrations of the infusates used as the inert amino acid valine in the corresponding dose range did not affect respiration at all! The respiratory effects were longlasting but were not due to neurotoxic effects as the animals had recovered 24 h after a single glycine injection. As a matter of fact, $\dot{V}_E$ was increased compared to initial controls. The mechanism behind this effect is not known but may be due to several factors, e.g. the initial longlasting acidosis or alterations at the neuronal or receptor level. A new i.c.v. injection of glycine again caused respiratory depression, but the animals were desensitized compared to the initial response. The reversibility of the glycine induced respiratory depression by strychnine, a recognized glycine antagonist, clearly show

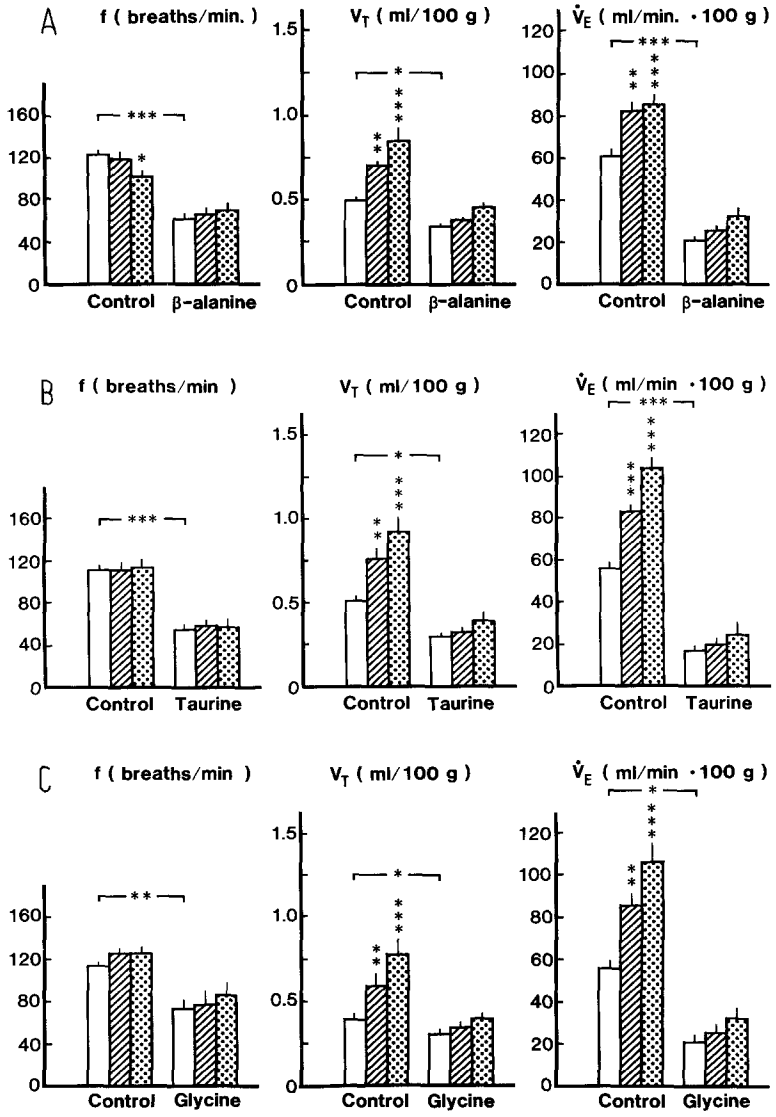

Fig. 5
Effects of CO_2 on respiratory frequency (f), tidal volume (V_T) and minute volume ($\dot{V}_E$), before and 30 min after i.c.v. administration of 1 mg of either β-alanine (**a**), taurine (**b**) or glycine (**c**). Shown are means ± S.E.M. of 4 experiments (**a, c**) or 5 experiments (**b**). Statistics were carried out by means of 1 way analysis of variance followed by t-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (□) 100% O_2; (▨) 95% O_2, 5% CO_2; (▩) 90% O_2, 10% CO_2

Table 3. Effects of saline, β-alanine, taurine and glycine administered i.c.v. on blood gases, pH and standard bicarbonate (SB) in anesthetized rats

	$PaCO_2$ (kPa)	PaO_2 (kPa)	pH	S.B. (mmol/l)
Control	6.0±2.25	29.0±7.90	7.35±0.02	29.6±3.44
Saline	6.6±0.51	23.0±8.74	7.33±0.02	25.1±2.40
$n = 3$				
Control	7.8±0.68	18.2±3.13	7.23±0.02	21.8±0.70
β-Alanine	20.0±3.84*	19.8±3.71	6.91±0.05**	18.2±1.30*
$n = 4$				
Control	5.3±0.37	32.3±7.88	7.35±0.02	22.1±0.34
Taurine	22.9±4.54*	28.1±4.51	6.86±0.02***	18.3±3.44
$n = 4$				
Control	6.4±0.65	25.9±7.41	7.36±0.02	25.3±0.82
Glycine	20.7±2.38**	26.7±5.45	6.99±0.04***	25.3±1.35
$n = 4$				

Partial pressures of carbon dioxide ($PaCO_2$), oxygen (PaO_2), pH and standard bicarbonate (S.B.) in arterial blood
The amino acids were given in a single 1 mg i.c.v. injection. Shown are means ± S.E.M. of control values and values obtained 15 min after drug injection. Statistics by paired t-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

that the effects were specific and occurring at the receptor level. The effects of strychnine were shortlasting, which was not surprising, since strychnine appears to have a very short halflife in plasma (Wang and Ward 1977).

The ventilatory depression caused by β-alanine, taurine and glycine were seen in the same dose range as the respiratory effects earlier reported for GABA (Hedner et al. 1981a). The action of all three amino acids on respiration was dose-dependent and the highest dose resulted in a marked inhibition of pulmonary ventilation. Furthermore, at this dose (1 mg), both mean inspiratory flow (V_T/T_I) and "respiratory timing" (T_I/T_{TOT}) were decreased, indicating that neural inspiratory drive as well as the rhythmicity of the frequency generator were decreased. As changes in V_T/T_I may not only be due to changes in inspiratory neural drive, but also to peripheral factors, such as airway flow resistence and compliance, we have also studied $P_{0.1}$ which is independent of peripheral influence (Lynne-Davies et al. 1971). $P_{0.1}$ decreased in the same magnitude as V_T/T_I showing that only central mechanisms were involved.

In previous studies by Sgaragli and Pavan (1972), a marked respiratory depression, sometimes leading to apnoic spells, were reported after intracisternal administration of

Table 4. The effects of i.c.v. administered saline, glycine (1 mg), β-alanine (1 mg) or taurine (1 mg) on mean arterial blood pressure and heart rate in anesthetized rats

			Control	1 min	3 min	5 min	15 min	30 min	45 min
Saline	$n=6$	MAP	104± 1.9	103± 2.4	107± 2.1	107± 2.5	103± 3.6	98± 4.3	110± 7.5
	$n=7$	HR	381±10.4	385± 7.7	385± 8.0	395±10.9	377±10.3	369±17.0	391±19.4
β-Alanine	$n=3$	MAP	98± 0.3	65±12.6**	50± 9.0***	53± 7.2***	70± 3.8***	75± 3.5*	81± 5.5*
	$n=4$	HR	359±16.3	306±12.4**	268± 8.7***	252± 0***	250± 7.9***	237±17.2***	246±20.9***
Taurine	$n=5$	MAP	104± 5.8	68± 6.0***	45± 9.6***	48±11.1***	88±10.8	86± 7.1	85± 5.6*
		HR	420±27.2	356±25.4	298±43.6*	291±24.2**	343±40.9	319±41.5	340±32.6
Glycine	$n=4$	MAP	114± 4.7	95± 4.1	81±11.7*	85± 7.9*	97± 4.2	103± 3.1	102± 4.1
		HR	405± 7.9	374± 2.9	343±18.9*	341±17.2*	332±18.2*	333±16.0	323±22.5

Values represent mean ± S.E.M. n represents the number of animals in each group. Statistical comparison was performed between the saline control and the drug treated animals at the corresponding time intervals by means of Student's t-test
* $P<0.05$, ** $P<0.01$, *** $P<0.001$

taurine and glycine. This is in agreement with our findings, where periods of transient apnea were seen shortly after and up to 10 min after i.c.v. injection of β-alanine and taurine. As for the central route of administration, peripheral injections of β-alanine and taurine, may also induce transient apnea in the rat (Holzer and Hagmüller 1979).

Apart from the effects on basal respiration, β-alanine, taurine and glycine also abolished the ventilatory response, seen after 5% and 10% CO_2 which further demonstrates that these amino acids depress ventilation due to central mechanisms, as the CO_2 sensing and responding mechanism is considered to be of central origin, and located in the reticular formation near the ventral surface of the medulla (see Berger et al. 1977).

After the respiratory depressive amino acids there were significant increases in arterial CO_2 tensions and a lowering of arterial blood pH. These changes are the typical findings of acidosis following primary respiratory depression.

In order to further investigate the central site and mechanisms behind the respiratory depressant actions of β-alanine, taurine and glycine, we applied the occluded breath technique described by Lynne-Davies et al. (1971) and Goldberg and Milic-Emili (1977). These data show that the central bulbopontine setting for T_I was not changed as the intercept at $V_{max} = 0$ was not altered by i.c.v. glycine injection. However, glycine treatment changed the sensitivity of the inspiratory offswitch mechanism to vagal afferent impulses since the slope of the V_{max} vs $1/T_I$ function was altered.

Furthermore there was a decrease in $1/T_E$ at $V_{max} = 0$ indicating that the central bulbopontine setting for T_E was prolonged.

In microiontophoretic studies, depressant actions have been found on neurons in the brain stem after glycine and taurine (Denavit-Saubié and Champagnat 1975; Hösli et al. 1969). In the study by Denavit-Saubié and Champagnat (1975) they found that non-respiratory neurons were more sensitive to the depressant amino acids than the respiratory network. However, when comparing inspiratory and expiratory units, the eaxpiratory neurons were more readily depressed than the inspiratory ones. The results presented in our study are in agreement with those earlier presented by Denavit-Saubié and Champagnat (1975) and Hösli et al. (1969). However, other outhors (Hagmüller and Holzer 1978; Holzer and Hagmüller 1979) have concluded that the respiratory depressant actions of the putative neurotransmitter amino acids were elicited from more rostral areas, in studies using peripheral injections. In their studies it was put forward that respiratory effects by the amino acids were mediated by areas in the brain supplied by the internal carotid arteries and not from areas (such as e.g. the respiratory brain stem area) supplied by the vertebral arteries.

MAP and HR were rapidly and markedly decreased after i.c.v. injections of the amino acids. β-Alanine and taurine were more potent than glycine in inducing hypotension and bradycardia. These circulatory effects were also seen after intracisternal injections (Sgaragli and Pavan 1972). The time courses for the hypotensive effects and bradycardia were relatively similar to those seen for the respiratory effects. However, the effects on respiration were more marked, slightly earlier in onset and had a longer duration than the circulatory depression seen after i.c.v. administration of the amino acids. It is therefore not likely that the respiratory changes are the result of a generalized circulatory depression. In previous studies investigating cardiovascular changes after i.c.v. taurine and glycine administration, hypotension and bradycardia could be elicited at lower doses (Bousquet et al. 1981). Furthermore, the cardiovascular actions of glycine could be antagonized by strychnine, a recognized glycine antagonist (Bousquet et al. 1981; Curtis and Johnston 1974). Moreover, the cardiovascular response elicited by i.c.v. taurine could also be reversed by strychnine, indicating that this amino acid may act via glycine receptors (Bousquet et al. 1981; Haas and Hösli 1973). However, the circulatory and respiratory results obtained by taurine do not formally exclude the action on specific taurine receptors as proposed by Frederickson et al. (1978). The studies on specific effects of taurine, as well as β-alanine are complex, as readily available, selective, competetive antagonists for these putative neurotransmitters are lacking.

In conclusion, this study shows that the putative amino acid neurotransmitters, β-alanine, taurine and glycine, have potent depressive effects on respiratory activity when administered by the intracerebroventricular route. The doses used to elicit the effects on respiration are relatively high, but in agreement with the dose range needed to induce other physiological effects on e.g. motor behaviour (Mena Gomez et al. 1978; Garcia de Yebenes et al. 1978), temperature regulation (Sgaragli and Pavan 1972), and cardiovascular parameters (Bousquet et al. 1981; Sgaragli and Pavan 1972).

Furthermore, the effects on respiration appears to be elicited from the brain stem region due to decreased inspiratory neural drive, prolonged bulbopontine setting for T_E and increased inspiratory offswitch sensitivity to vagal afferent impulses.

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References

Armijo JA, Florez J (1974) The influence of increased brain 5-hydroxytryptamine upon the respiratory activity of cats. Neuropharmacology 13:977–986

Berger AJ, Mitchell RA, Severinghaus JW (1977) Regulation of respiration. New Eng J Med 297:92–97, 138–143, 194–201

Bousquet P, Feldman J, Bloch R, Schwarz J (1981) Central cardiovascular effects of taurine: Comparison with homotaurine and muscimol. J Pharmacol Exp Ther 219:213–218

Cohen MI (1979) Neurogenesis of respiratory rhythm in the mammal. Physiol Rev 59:2205–2273

Curtis DR, Johnston CAR (1974) Amino-acid transmitters in the mammalian central nervous system. Ergeb Physiol Biol Chem Exp Pharmakol 69:97–188

Denavit-Saubié M, Champagnat J (1975) The effect of some depressing amino acids on bulbar respiratory and non-respiratory neurons. Brain Res 97:356–361

Florez J, Medavilla A, Pazos A (1980) Respiratory effects of β-endorphin, D-ala^2-metenkephalinamide and metenkephaline injected into the lateral ventricle and the pontomedullary subarachnoid space. Brain Res 199:197–206

Frederickson RCA, Neuss M, Morrorati SL, McBride WJ (1978) A comparison of the inhibitory effects of taurine and GABA on identified Purkinje cells and other neurons in the cerebellar cortex of the rat. Brain Res 145:117–126

Garcia de Yebenes Prous J, Carlsson A, Mena Gomez MA (1978) The effect of taurine on motor behaviour, body temperature and monoamine metabolism in rat brain. Naunyn-Schmiedeberg's Arch Pharmacol 304:95–99

Goldberg MS, Milic-Emili J (1977) Effect of pentobarbitone sodium on respiratory control in newborn rabbits. J Appl Physiol 42:845–851

Grunstein MM, Younes M, Milic-Emili J (1973) Control of tidal volume and respiratory frequency in anesthetized cats. J Appl Physiol 35:463–467

Guidotti A, Badiani G, Pepeu CG (1972) Taurine distribution in cat brain. J Neurochem 19:431–435

Haas HL, Hösli L (1973) The depression of brain stem neurons by taurine and its interaction with strychnine and bicuculline. Brain Res 52:399–402

Hagmüller K, Holzer P (1978) On the site of action of intravascular GABA on the respiration of the rat. Zool Jb Physiol 82:505–514

Hedner J, Jonason J, Lundberg D (1980) Respiratory effects of gamma-hydroxybutyric acid in anaesthetized rats. J Neural Transm 49:179–186

Hedner J, Hedner T, Jonason J, Lundberg D (1981a) Centrally respiratory stimulant effect by TRH in the rat. Neurosci Lett 25:317–320

Hedner J, Hedner T, Jonason J, Lundberg D (1981b) GABA-ergic mechanisms in central respiratory control in the anaesthetized rat. Naunyn-Schmiedeberg's Arch Pharmacol 317:315–320

Hedner T, Hedner J, Jonason J, Lundberg D (1981c) Evidence suggesting a role for substance P in central respiratory regulation in the rat. Acta Physiol Scand 112:487–489

Hedner J, Hedner T, Jonason J, Lundberg (1982) Evidence for a dopamine interaction with the central respiratory control system in the rat. Eur J Pharmacol 81:603–615

Holzer P, Hagmüller K (1979) Transient apnea after systemic injection of GABA in the rat. Naunyn-Schmiedeberg's Arch Pharmacol 308:55–60

Hukuhara JrT, Goto K, Takano K, Hattammaru Y (1980) Localization and functional organization of bulbar and pontine respiratory neurons in the rabbit. In: Hutas I, Debreczeni LA (eds) Advances in physiological sciences, vol 10, Respiration. Pergamon Press and Akadémiai Kiadó, Budapest, p 579

Hösli K, Tebecis AK, Filias N (1969) Effects of glycine, β-alanine and GABA and their interaction with strychnine, on brain stem neurones, Brain Res 16:293–295

Lundberg D, Breese GR, Mueller RA (1979) Dopaminergic interaction with the respiratory control system in the rat. Eur J Pharmacol 54:153–159

Lynne-Davies P, Couture J, Pengelly LD, Milic-Emili J (1971) Immediate ventilatory response to added inspiratory elastic loads in cats. J Appl Physioiol 30:814–819

Martin del Rio R, Orensanz Munoz LM, de Feudis FV (1977) Contents of β-alanine and α-amino-butyric acid in regions of rat CNS. Exp Brain Res 27:225–227

Mena Gomez MA, Carlsson A, Garcia de Yebenes J (1978) The effect of β-alanine on motor behaviour, body temperature and cerebral monoamine metabolism in the rat. J Neural Transm 43:1–9

Moss IR, Friedman E (1978) β-Endorphin effects on respiratory regulation. Life Sci 23:1271–1276

Mueller RA, Lundberg D, Breese GR (1980) Evidence that respiratory depression by serotonin agonists may be exerted in the central nervous system. Pharmacol Biochem Behav 13:247–255

Sgaragli G, Pavan F (1972) Effects of aminoacid compounds injected into cerebrospinal fluid spaces, on colonic temperature, arterial blood pressure and behaviour of the rat. Neuropharmacology 11:45–46

St John WM (1973) Characterization of tidal volume regulation function of the pneumotaxic center. Respir Physiol 18:64–79

Uhr ML, Sneddon MK (1972) The regional distribution of D-glycerate dehydrogenase and 3-phosphoglycerate dehydrogenase in the cat central nervous system. Correlation with glycine levels. J Neurochem 19:1495–1500

Wang SC, Ward JW (1977) Analeptics. Pharmacol Ther B 3:123–165

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