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Role of the vagus nerves in anaphylaxis and histamine-induced bronchoconstrictions in guinea-pigs

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Summary

1. The effects of vagotomy on the respiratory responses of guinea-pigs to anaphylactic reactions and to intravenous injections of histamine acid phosphate are described.

2. In spontaneously breathing guinea-pigs, vagotomy reduced by 50% or more the decreases in total lung conductance (bronchoconstriction) and the decreases in lung compliance, and almost abolished the rapid shallow breathing due to histamine.

3. In paralysed, artificially ventilated guinea-pigs, vagotomy reduced by more than 33% the decreases in total lung conductance, but had little effect on the changes in lung compliance due to histamine.

4. In paralysed, artificially ventilated guinea-pigs, vagotomy reduced by 75% the decrease in total lung conductance and halved the decrease in lung compliance due to anaphylaxis.

5. We conclude that a vagal reflex is mainly responsible for the rapid shallow breathing due to histamine, and partly responsible for the bronchoconstrictions due to histamine and to anaphylaxis in guinea-pigs. We suggest that "lung irritant receptors" in the bronchial epithelium are the afferent end-organs involved.

Introduction

Intravenous injections of histamine cause rapid, shallow breathing and bronchoconstriction in experimental mammals. Both these effects are due mainly to a vagal reflex in rabbits (Karczewski & Widdicombe, 1969a) and dogs (de Latona, Mata & Aviado, 1961 ; de Kock, Nadel, Zwi, Colebatch & Olsen, 1966). In man, the respiratory and bronchomotor actions of histamine are partially or completely blocked by atropine or hexamethonium, which suggests a reflex mechanism (Herxheimer, 1956 ; Bouhuys, Jonsson, Lichtneckert, Lindell, Lundgren, Lundin & Ringquist, 1960).

Reflex changes in breathing and bronchomotor tone, due to anaphylaxis, have been investigated less than the responses to histamine, but a number of studies in experimental animals point to an important vagal reflex mechanism (Auer & Lewis, 1910 ; Karczewski & Widdicombe, 1969b ; Koller, 1967a, b ; 1968).

These are probably species differences in the relative importance of reflex and "direct" reactions to histamine (for example, Karczewski & Widdicombe, 1969a). Guinea-pigs have been used far more than other species for the study of bronchial

responses to anaphylaxis and to histamine and other bronchoconstrictor drugs (see Mongar & Schild, 1962 ; Collier, 1968 ; Karczewski & Widdicombe, 1969a, b for references). The lungs of the guinea-pig differ from those of most other common experimental mammals in the structure of their terminal airways and in the abundance of smooth muscle in the pleura (Miller, 1947).

Many pharmacological studies with guinea-pigs have used pithed or very deeply anaesthetized animals in which nervously mediated changes in breathing and bronchomotor tone might be blocked (for example, Collier, Holgate, Schachter & Shorley, 1960). With lightly anaesthetized guinea-pigs anaphylaxis causes hyperpnoea (or rapid, shallow breathing) only if vagal integrity is maintained (Auer & Lewis, 1910 ; Koller, 1967a, b ; 1968). However, for the guinea-pig there seem to be no unequivocal studies on the role of vagal reflexes in the respiratory responses to histamine or the bronchomotor responses to histamine or anaphylaxis. The popularity of the guinea-pig for studies on anaphylaxis and histamine-induced bronchoconstrictions, and the almost inevitable comparisons drawn between such results and the corresponding pathological conditions in unanaesthetized man, have led to presentation of our results.

Methods

Eighteen guinea-pigs were anaesthetized with pentobarbitone sodium (Nembutal, Abbott: 30 mg/kg, intraperitoneally). Tracheal cannulae were inserted and also jugular venous catheters for injections of drugs. The animals were supine. Blood pressure was recorded in mmHg (1 mmHg$\equiv$1·333 mbar) from the left femoral artery through a polyethylene catheter by a capacitance manometer (Southern Instruments). The undamped natural frequency of the manometer and catheter system was in the range 20–28 Hz, assessed by the method of Hansen (1949). Transpulmonary pressure was measured from an air-filled polyethylene cannula tied into the lower right intrapleural space, and from a wide-bore needle inserted through the rubber-tube connexion to the tracheal cannula, using a differential capacitance manometer (Southern Instruments), range $\pm$50 cm H_2O. Tidal volume was measured from a Fleisch pneumotachograph "head", linear range 0–110 ml/s, connected to an inductance differential pressure recorder and integrator (Godart).

Records of systemic arterial blood pressure, tidal volume and transpulmonary pressure were displayed on an oscilloscope (Tektronix 551) and photographed on 7 cm paper (with a modified Cossor camera). The photographic record was analysed after enlargement 3 to 4 times by a projector (designed and built by Dr. E. H. J. Schuster). In some experiments the variables were recorded on a Honeywell UV-31 oscillograph.

Lung conductance and compliance were measured by the "subtractor" method of Mead & Whittenberger (1953), somewhat modified (Karczewski & Widdicombe, 1969a ; Mills, Sellick & Widdicombe, 1969). This involves displaying the component of transpulmonary pressure required to overcome resistance to flow and tracheal airflow on the two axes of an oscilloscope, to give "loops", the slopes of which are proportional to total lung resistance, or its reciprocal, conductance.

Transpulmonary pressure and airflow were also recorded on a four-channel FM tape recorder (Thermionix T3000). This allowed re-evaluation of total lung resistance and lung compliance after the experiment had been completed.

Anaesthetized guinea-pigs were paralysed by intravenous injections of gallamine triethiodide (2 mg), the injections being repeated if the animals made respiratory movements. The pump frequency was 30–40 inflations/min, and the tidal volume was adjusted to maintain end-tidal CO_2% (measured with a Beckman Spinco LBI infrared absorption meter) close to the pre-paralysis value.

Thirteen guinea-pigs were first sensitized to bovine serum albumin (Armour), by administration of three intraperitoneal doses of 0·1 ml of a 20% solution at 2 day intervals. After an interval at 3–6 weeks, the animals were used for experiments, with 0·1 ml of a 20% solution of albumin as the challenging dose. Doses of histamine acid phosphate are expressed as the salt.

Results

Effects of histamine

Table 1 shows the effect of vagotomy on the responses of spontaneously breathing guinea-pigs. Table 2 shows the effect of vagotomy on the responses of paralysed, artificially ventilated guinea-pigs. Ideally, effects of the same dose of histamine before and after vagotomy should be compared. Because the sensitivity of the guinea-pigs to histamine varied considerably, however, for each animal we increased the dose before vagotomy from 1 μg (of the acid phosphate) until a dose (maximum 10 μg) was found which produced a clear decrease (about 50%) in total lung conductance (monitored on the oscilloscope). Vagotomy reduced the responses to histamine, so after vagotomy larger doses in the 1–10 μg range were often used, to test the bronchial reactivity to histamine; on average these doses produced less than a 50% decrease in conductance. Therefore Tables 1 and 2 contain two sets of averages: all responses to 5 μg histamine, which was the dose most frequently used, and all responses to all histamine injections. The results of this second analysis (all doses in the 1–10 μg range) are weighted against the chance of demonstrating a statistically significant change in the size of the response after vagotomy because, as explained, vagotomized guinea-pigs were given the large doses of histamine. Furthermore, although it would be preferable to compare responses to the same dose of histamine before and after vagotomy in the same animal, vagotomy sometimes killed the guinea-pig or stopped it breathing spontaneously (a well known effect in the guinea-pig), so that this was often impossible. (However, it was possible to arrange some of the results in comparable pairs, and these are described later in the text.)

Histamine reduced lung conductance by −42 to −46% in spontaneously breathing animals, and decreased it by −36 to −39% in paralysed animals. Vagotomy significantly reduced the effect in both groups of animals (−6% to −22% in spontaneously breathing guinea-pigs, and −25% to −23% in paralysed guinea-pigs).

In spontaneously breathing guinea-pigs histamine decreased lung compliance by −38 to −39%. This effect was significantly less after vagotomy (−7 to −15%). However, vagotomy had no such action in paralysed animals (−21 to −22% before vagotomy, −22 to 26% afterwards).

In spontaneously breathing guinea-pigs, histamine increased the frequency of breathing by +17 to +26%, and decreased tidal volume by −18 to −19%. After vagotomy the effects were much smaller and not statistically significant. Histamine

TABLE 1. *Effect of vagotomy on some respiratory and cardiovascular responses of spontaneously breathing guinea-pigs to histamine acid phosphate*

Variable	Dose of hist. (μg)	Vagi intact				Vagi cut			
		N	Control (abs.)	Change (abs.)	Change (%)	*N*	Control (abs.)	Change (abs.)	Change (%)
GL ((l./min)/cm H_2O)	5	11	0·29±0·036	−0·12±0·015**	−46·1±4·3**	3	0·34±0·085	−0·016±0·012‡	−5·7±3·9‡
	All	22	0·31±0·043	−0·14±0·018**	−42·4±4·0**	7	0·32±0·047	−0·069±0·014**†	−22·3±6·25**†
CL (ml/cm H_2O)	5	10	0·49±0·050	−0·18±0·032**	−38·4±6·7**	3	0·60±0·13	−0·047±0·020†	−7·3±1·6**†
	All	22	0·51±0·040	−0·18±0·025**	−38·7±4·6**	7	0·57±0·064	−0·081±0·021**†	−15·1±5·8**†
FBR (min^{-1})	5	10	90±8·8	+17·6±5·2**	+16·5±5·1*	3	26±1·9	+2·7±1·45†	+10·0±5·7
	All	20	84±6·8	+23·1±5·9**	+25·6±7·6**	6	29±2·8	+0·83±4·58†	+2·8±5·5
VT (ml)	5	10	3·5±0·40	−0·68±0·19**	−18·2±5·0**	3	5·1±0·41	0±0·25†	−0·7±0·5†
	All	20	3·4±0·25	−0·71±0·15**	−18·9±4·0**	6	4·9±0·46	−0·27±0·14†	−5·5±3·1†
B.P. (mmHg)	5	10	68·6±4·0	−13·4±2·7**	−21·6±2·7**	3	70·2±11·3	−15·0±10·2	−11·3±14·2
	All	19	64·0±3·2	−12·4±3·2**	−19·1±4·2**	6	68·1±5·9	−6·7±10·1	−12·4±15·7
H.R. (min^{-1})	5	9	254±6·5	−2·7±2·9	−1·2±1·3	3	264±18·3	+10±2·0*	+3·7±0·9
	All	18	250±4·6	−2·6±3·0	−1·4±1·3	6	259±11·8	+7·7±2·9	+2·8±1·1

Values are means±S.E.
** $P<0·01$, * $P<0·05$ for significance of mean changes.
‡ $P<0·01$, † $P<0·05$ for significance of mean change after vagotomy compared with that before vagotomy.
GL, Total lung conductance; CL, lung compliance; FBR, breathing frequency; VT, tidal volume; B.P., systemic arterial blood pressure; H.R., heart rate; abs., absolute.
Doses of histamine refer to the acid phosphate.

usually caused a hypotension (−19 to −28%) which recovered over about 20–60 seconds. Vagotomy reduced this hypotension in paralysed and spontaneously breathing animals (−11 to −21%) but not by a statistically significant amount. Heart rate changes, after histamine, were small and statistically insignificant and were not affected by vagotomy.

The results were also arranged in comparable pairs: that is, the response to histamine before vagotomy was compared with that to the same dose after vagotomy when other conditions (in particular the presence or absence of paralysis) were identical. Histamine decreased total lung conductance by $-0{\cdot}12 \pm 0{\cdot}0097$ (l./min)/cm H_2O ($-42{\cdot}9 \pm 5{\cdot}89\%$) before vagotomy, and by $-0{\cdot}059 \pm 0{\cdot}017$ (l./min)/cm H_2O ($-22{\cdot}2 \pm 4{\cdot}75\%$) after vagotomy ($n=9$, $P<0{\cdot}05$ for paired values). Compliance was decreased by $-0{\cdot}19 \pm 0{\cdot}035$ ml/cm H_2O ($-33{\cdot}9 \pm 5{\cdot}83\%$) before vagotomy, and by $-0{\cdot}058 \pm 0{\cdot}020$ ml/cm H_2O ($-12{\cdot}0 \pm 3{\cdot}79\%$) after vagotomy ($n=9$, $P<0{\cdot}05$ for paired values). Four of the experiments with paired values were with spontaneously breathing guinea-pigs, and in all of these the increases in breathing frequency and decreases in tidal volume due to histamine were abolished or greatly reduced by vagotomy.

Effects of anaphylaxis

Table 3 shows the effect of vagotomy on some of the respiratory and cardiovascular responses of guinea-pigs to anaphylaxis. Only the results from paralysed animals are shown, to eliminate complications introduced by the gross changes in breathing (breathing usually became very erratic and often stopped completely). Since anaphylaxis could be induced only once in a given animal, the experiments were limited in number and the participation of the vagi in the responses could not be estimated from comparisons in the same animal.

In guinea-pigs with intact vagi, anaphylaxis reduced lung conductance by −20%. This compares with a reduction of −39% by 5 μg of histamine. In vagotomized guinea-pigs the decrease in conductance was −5·5%, compared with a reduction of −25% by 5 μg of histamine in vagotomized guinea-pigs.

Compliance was reduced during anaphylaxis by −17% in animals with vagi intact, and by −8% in vagotomized animals. Neither effect was statistically significant, nor was the effect of vagotomy.

Anaphylaxis always produced a greater and more prolonged hypotension than histamine. The hypotension was similar in vagotomized animals. Heart rate changes were small and insignificant.

Discussion

The effects of vagotomy on the lung conductance and dynamic compliance response to histamine and anaphylaxis in spontaneously breathing animals are difficult to interpret quantitatively for two reasons: because both conductance and compliance are directly affected by the pattern of breathing which is changed after histamine and anaphylaxis; and because vagotomy may shift the control levels of conductance and compliance on which changes are superimposed. The former difficulty has been overcome by the use of paralysed artificially ventilated animals, in which the pattern of breathing is constant. Examination of Tables 1, 2 and 3

TABLE 2. *Effect of vagotomy on some respiratory and cardiovascular responses of paralysed and artificially ventilated guinea-pigs to histamine acid phosphate*

Variable	Dose of hist. (μg)	Vagi intact				Vagi cut			
		N	Control (abs.)	Change (abs.)	Change (%)	*N*	Control (abs.)	Change (abs.)	Change (%)
GL ((l./min)/cm H_2O)	5	12	0·27±0·027	−0·095±0·012**	−39·2±5·2**	16	0·26±0·017	−0·067±0·016**†	−25·0±4·8**†
	All	16	0·28±0·026	−0·083±0·011**	−36·4±5·8**	20	0·24±0·017	−0·055±0·012**†	−22·7±4·0**†
CL (ml/cm H_2O)	5	10	0·49±0·071	−0·10±0·027**	−22·3±5·4**	15	0·55±0·059	−0·16±0·039**	−26·2±5·0**
	All	14	0·56±0·066	−0·095±0·027**	−21·4±4·6**	19	0·56±0·057	−0·13±0·029**	−22·4±4·4**
B.P. (mmHg)	5	9	76·6±9·8	−19·2±3·9**	−26·7±5·3**	15	66·8±5·9	−14·5±3·7**	−20·7±5·1**
	All	12	70·9±8·1	−20·1±3·2**	−28·4±4·5**	17	66·4±5·5	−12·3±3·6**	−17·6±4·2**
H.R. (min^{-1})	5	10	238±3·35	+3·0±1·8	+1·2±1·0	12	235±2·4	+0·8±1·5	+0·7±1·2
	All	11	238±3·04	+2·7±1·6	+1·1±0·7	13	235±2·3	+0·7±1·4	+0·7±1·0

Values are means±S.E.
** $P<0·01$, * $P<0·05$ for significance of mean changes.
† $P<0·05$ for significance of mean change after vagotomy compared with that before vagotomy.
GL, Total lung conductance; CL, lung compliance; B.P., systemic arterial blood pressure; H.R., heart rate; abs., absolute.
Doses of histamine refer to the acid phosphate.

TABLE 3. *Effect of anaphylaxis on some respiratory and circulatory variables of paralysed and artificially ventilated guinea-pigs with vagi intact and with vagi cut*

Variable	Vagi intact				Vagi cut			
	N	Control (abs.)	Change (abs.)	Change (%)	*N*	Control (abs.)	Change (abs.)	Change (%)
GL ((l./min)/cm H_2O)	6	0·24±0·039	−0·047±0·012*	−19·8±5·67*	5	0·40±0·133	−0·015±0·0064†	− 5·5±2·41*†
CL (ml/cm H_2O)	4	0·52±0·16	−0·13±0·064	−17·3±6·5	5	0·53±0·066	−0·082±0·075	− 8·4±7·22
B.P. (mmHg)	4	72·5±12·6	−41·8±13·6*	−54·2±7·56**	4	63·7±22·5	−39·1±17·8	−54·3±10·1*
H.R. (min^{-1})	3	232±10·6	+16·0±10·6	+7·3±5·0	3	240±2·41	−4·7±4·7	−2·0±1·6

Values are means±S.E.
** $P<0·01$, * $P<0·05$ for significance of mean changes.
† $P<0·05$ for significance of mean change after vagotomy compared with that before vagotomy.
GL, Total lung conductance; CL, lung compliance; B.P., systemic arterial blood pressure; H.R., heart rate; abs., absolute.

suggests that, although vagotomy affects control values of conductance and compliance, the changes are not great enough to invalidate conclusions on the role of the vagus nerves. The experiments on paralysed animals give better information about primary vagal reflex changes in lung mechanics; those on spontaneously breathing guinea-pigs, although complicated by secondary effects, both indicate more faithfully what might happen to lung mechanics in the intact animal and also give information on the role of the vagus nerves in breathing as well as in bronchomotor tone. This point can be illustrated by the decreases in lung compliance due to histamine. These were greatly reduced by vagotomy in spontaneously breathing animals, but vagotomy had no such effect in paralysed animals. Thus it appears that histamine has a non-vagal effect on lung compliance, presumably by closure of terminal airways, but that in spontaneously breathing guinea-pigs this effect is distorted by the changes in dynamic lung compliance secondary to the alterations in breathing.

In rabbits, dogs and man (see **Introduction** for references) histamine causes bronchoconstriction and stimulation of breathing which depend partly, or mainly, on integrity of conduction in the vagus nerves. For the guinea-pig, the vagal reflex role has not previously been assessed for responses to histamine, and for anaphylaxis only for the respiratory (Koller, 1967a, b; 1968) and not the bronchomotor effects.

In the rabbit, both histamine, by intravenous injection or by inhaled aerosol, and anaphylaxis stimulate "lung irritant receptors" in the bronchial epithelium which are thought to cause vagal reflex hyperpnoea and bronchoconstriction (Mills *et al.*, 1969, 1970). It is reasonable to suppose that such receptors exist in other mammalian species, including the guinea-pig, and that they mediate the results described in this paper. Koller (1967a, b; 1968) has shown that vagal afferent fibres in the guinea-pig are stimulated during pulmonary anaphylaxis; although he refers to them as "deflation receptors", they are stimulated by inflation of the lungs and their properties indicate that they are probably identical to the "lung irritant receptors" studied in the rabbit (Homberger, 1968; Mills *et al.*, 1969). These end-organs have terminals extending between the epithelial cells of the bronchial mucosa, are stimulated by intra-luminal chemical and mechanical irritants and by traction exerted on the bronchial wall, and cause reflex hyperpnoea and bronchoconstriction, and possibly also dyspnoea in man (Mills *et al.*, 1969, 1970; Sellick & Widdicombe, 1969).

Although the changes in breathing due to histamine are almost entirely vagal reflex in guinea-pigs (Koller, 1967a, b; 1968; this paper) and rabbits (Karczewski & Widdicombe, 1969a), the increase in total lung resistance—bronchoconstriction—is partly reflex and partly a direct action for histamine and anaphylaxis in both guinea-pigs and rabbits (Karczewski & Widdicombe, 1969a, b; this paper). Presumably the direct chemical action on airway smooth muscle is sufficient to stimulate epithelial "irritant receptors" which superimpose their respiratory and bronchomotor actions on the primary local response in the lungs (Mills *et al.*, 1969).

The guinea-pig has been the most popular mammalian species for studying the changes in lung mechanics and breathing due to anaphylaxis and bronchomotor drugs, and most authors have assumed that any bronchomotor changes are due to direct (rather than reflex) actions on airway smooth muscle. Our results show that the guinea-pig resembles other experimental mammals in that vagal reflexes

are of considerable importance in these responses. They are consistent with the evidence that in man histamine-induced respiratory and bronchomotor effects depend appreciably on vagal reflexes, and can be ameliorated by therapy that blocks conduction in these pathways (Petit, 1970 ; Bouhuys *et al.*, 1960 ; Herxheimer, 1956).

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