Bradykinin causes hypotension by activating pulmonary sympathetic afferents in the rabbit

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Wang, Y., G. Soukhova, M. Proctor, J. Walker, and J. Yu. Bradykinin causes hypotension by activating pulmonary sympathetic afferents in the rabbit. J Appl Physiol 95: 233–240, 2003. First published April 4, 2003; 10.1152/japplphysiol.00584.2002.—Bradykinin (BK) activates sympathetic afferents in the heart, intestine, and kidney, and it alters hemodynamics. However, we know little about the influence of pulmonary sympathetic afferents on circulation. Activation of pulmonary afferents by directly injecting stimulants into the lung parenchyma permits examination of reflexes that originate in the lung without confounding effects from the systemic circulation. In the present study, we tested the hypothesis that pulmonary sympathetic afferents exert a significant influence on hemodynamics. We examined reflex effects of injecting BK (1 μg/kg in 0.1 ml) into the lung parenchyma on circulation in anesthetized, open-chest, artificially ventilated rabbits. BK significantly decreased mean arterial blood pressure (BP) (27 ± 3 mmHg) and heart rate (19 ± 4 beats/min). Both effects remained after bilateral vagotomy. To rule out possible direct systemic vasodilation by BK, we examined renal sympathetic nerve activity (RSNA) in response to BK injection and examined BP responses to injection of ACh (0.1 ml of 10−4 M). BK suppressed the RSNA before and after vagotomy. ACh did not change BP when injected into the lung parenchyma, but it decreased BP (31 ± 3 mmHg) when injected into the right atrium. Our data indicate that activating pulmonary sympathetic afferents reflexly suppresses hemodynamics.

FOR DECADES IT HAS BEEN RECOGNIZED that cardiovascular regulation is strongly influenced by inputs from the lung (24). These pulmonary inputs are believed to travel mainly in the vagus nerves. Mechanically activating pulmonary stretch receptors (33) or chemically activating pulmonary C fibers (4) in the vagus nerve can cause peripheral vasodilation and changes in heart rate. Activation of some pulmonary vagal afferents in the rabbit causes a slight pressor response in addition to stimulating breathing (25, 34). However, information regarding the reflex effects evoked from pulmonary sympathetic afferents is scarce and debatable (23).

Sensory information in the lung is transmitted through vagal and sympathetic nerves (32). Morphological evidence points to the existence of sympathetic afferents in the lungs (13, 18, 22). Pulmonary sympathetic afferent activity has been recorded in stellate ganglia (9) and white rami of T2–T4 (12). This afferent activity increases during lung inflation (12). Activation of pulmonary sympathetic afferents may excite (26) or inhibit breathing (11). Stimulation of cardiopulmonary sympathetic afferents can produce a depressor response in monkeys and dogs (10), and produces either depressor or pressor responses in cats (31). Similarly, it may stimulate or inhibit renal sympathetic nerve activity (RSNA) (31). In all, reflex effects from activating pulmonary sympathetic afferents are inconclusive and require more study.

The reason for the delay in research of lung sympathetic afferents likely relates to technical difficulties in separating pulmonary from cardiovascular inputs. By locally injecting stimulants directly into the lung, pulmonary reflexes may be examined without the confounding effect of activating afferents through the systemic circulation. Bradykinin is reported to produce hemodynamic changes by stimulating sympathetic afferents in the heart (19, 30), gut (17), and kidney (1). We hypothesize that bradykinin also alters circulation by activating pulmonary sympathetic afferents. In the present study, the reflex effects of activating pulmonary sympathetic afferents on hemodynamics were evaluated by directly injecting bradykinin into the lung parenchyma before and after vagotomy. There is evidence of a profound cardiopulmonary reflex that produces systemic hypotension, bradycardia, and decreased RSNA, in addition to stimulated respiratory drive (26). The observed depressor effects may derive from withdrawal of sympathetic efferent activity.

METHODS

General. Experiments were conducted on open-chest, mechanically ventilated New Zealand White rabbits (body weight, 1.9–2.2 kg). They were conducted in conformity with the APS’s Guiding Principles in the Care and Use of Animals and were approved by IACUC of the University of Louisville. Initially, the rabbits were intravenously anesthetized with pentobarbital sodium (30 mg/kg). The trachea was cannulated...
lated low in the neck, and mechanical ventilation was provided with tidal volume set at 10 ml/kg body weight and 3 cmH2O of positive-end expiratory pressure (model 683, Harvard). Airway pressure was monitored at the tracheal tube inlet. The chest was opened by midline incision, with the opening covered by saline-soaked gauze to prevent dehydration of the underlying tissue. After the surgery was completed, anesthesia was maintained with a mixture of α-chloralose (1%) and urethane (10%) by intravenous infusion (~1.6 ml/h). All experimental measurements were made at least 90 min after the final dose of pentobarbital.

Stimulation of peripheral pulmonary receptors. To activate pulmonary receptors in the peripheral airway, we injected bradykinin (0.5–1 μg/kg in 0.1 ml 0.9% NaCl) directly into the lung. No specific lung lobe was selected, although the middle lobe was used for most injections. Injections were made into the lung parenchyma (5–7 mm under the surface) through a 30-gauge needle. Because the injections were into the most peripheral lung, no obvious blood vessels were encountered. Bradykinin produces tachyphylaxis in cardiac receptors (3); therefore, at least 15 min elapsed between injections. To further determine whether the observed responses were related to activation of pulmonary afferents, rather than originating from the systemic vasculature, in separate trials in six rabbits we compared the hypotensive effects of acetylcholine (0.1 ml of 10⁻⁴ M) when it was directly injected into the lung and into the right atrium. We also examined the pressor effects of phenylephrine (0.1 ml of 10⁻⁴ M) or epinephrine (0.1 ml of 1:10,000) when these agents were injected into the lung and the right atrium.

RSNA recordings. RSNA was recorded by using previously described procedures (27). In brief, a left flank incision exposed the renal sympathetic nerve. The nerve was isolated between the renal artery and vein, dissected 2 cm free from surrounding connective tissue, and then sectioned distally. The central end was placed on a bipolar electrode and covered with petroleum jelly to prevent drying. The signal was fed into an amplifier (model P511, Grass Instrument Division, Quincy, MA), with low-frequency and high-frequency cutoffs set at 30 Hz and 3 kHz, respectively. RSNA was monitored by a loudspeaker. The integrated moving-time-averaged signal was collected (model 7P3D integrator, Grass; time constant 50 ms) and thermorecorded (Dash IV, Astromed). Mean RSNA activity over time was reported by calculating the area of the integrated RSNA signal (arbitrary unit) above baseline (electrical zero). In each experiment, sympathetic activity was verified by right atrial injection of epinephrine (0.1 ml of 1:10,000) to suppress the RSNA by increasing blood pressure (Fig. 1).

Dye distribution. To assess the area in which pulmonary sensory afferents can be activated during bradykinin injection, we examined the distribution of locally injected Evans blue (mixed with saline in 0.05 ml) in four lungs of three deeply anesthetized rabbits (in 1 animal 2 lungs were used). Immediately after the injection (within 20 s), the injected lung was frozen with liquid nitrogen. After the lung was removed and sliced, the distribution of the dye was grossly examined.

Other procedures. Arterial blood pressure was measured via femoral artery cannulation to assess cardiovascular re-

![Fig. 1. Verification of renal sympathetic nerve activity (RSNA). Traces are blood pressure (BP), raw renal sympathetic nerve activity (RSNA), airway pressure (Paw), and integrated phrenic nerve activity (Phre (Integ)). Note that at the baseline (A; first 3 ventilator cycles) RSNA show clusters of the burst in phase with the ventilator cycles. B: recording of RSNA in 1 ventilator cycle with a fast paper speed. Also note that the RSNA had a cardiovascular rhythm. RSNA was suppressed as arterial BP increased after right atrial injection of epinephrine (B; 0.1 ml of 1:10,000 epinephrine, flushed with 0.4 ml normal saline). Please also note that phrenic activity is entrained with ventilator cycles. Its bursts occur during deflation.](http://jap.physiology.org/)
sponses. Phrenic efferent nerve activity was recorded from C6 (right or left) to assess central inspiratory drive. In some experiments, the electrical activity of the abdominal muscles (external oblique) was recorded to assess expiratory drive (26). These measured variables, together with airway pressure and RSNA, were recorded by a thermorecorder (Dash IV, Astro-Med). The role of sympathetic afferents in mediating cardiopulmonary responses was assessed by local injection of bradykinin before and after bilateral cervical vagotomy. In a separate sample of four rabbits, we also examined the responses after vagotomy plus section of superior laryngeal nerves.

Data analysis. Data are presented as means ± SE. A Student’s paired t-test statistically compared two groups of data from the same animals. The paired Wilcoxon test compared the percent change of a variable from its control value. A value of P < 0.05 established statistical significance.

RESULTS

The typical cardiopulmonary response to injecting bradykinin (0.5 – 1 µg/kg in 0.1 ml of 0.9% NaCl) into the lung parenchyma consisted of hypotension, bradycardia, and increased respiratory drive, as evidenced by increasing phrenic nerve activity and abdominal (external oblique) muscle activity (Fig. 2). Reflex effects on respiratory drive occurred in most but not all cases (26). Reflex hemodynamic effects showed a depressor response in 38 of 59 trials on 18 rabbits (64%). Only depressor responses are reported in the group data that follow. When both cardiovascular and respiratory responses were present, hypotension usually preceded the increase in respiratory drive.

Directly injecting bradykinin into the lung caused hypotension and bradycardia. The group data of changes in blood pressure and heart rate are illustrated in Fig. 3. The responses usually recovered completely within 2 min. Injection of bradykinin into the lung lowered mean arterial blood pressure by 27.1 ± 3.3 mmHg (37.7 ± 4.3%, n = 22; P < 0.005). The peak response occurred 20.9 ± 1.2 s after injection. Blood pressure also decreased in response to bradykinin after vagotomy, by 13.3 ± 3.0 mmHg from baseline (18.5 ± 4.4%, P < 0.005), although there was less depressor effect. Heart rate decreased by 19 ± 4 beats/min (n = 22; P < 0.001) at the peak response to bradykinin, which occurred 17.1 ± 1.7 s after injection. After vagotomy, heart rate showed no substantial decrease with bradykinin injection (−6.5 ± 2 beats/min), although it reached statistical significance (n = 22; P < 0.05). These cardiovascular responses persisted after section of the superior laryngeal nerves in addition to bilateral vagotomy. Blood pressure decreased by 33 ± 2.9% before and 23 ± 5.6% after the nerve sections in the four rabbits tested.

After vagotomy, no changes in cardiovascular or respiratory variables occurred with direct lung injection of acetylcholine (0.1 ml of 10⁻⁴ M), phenylephrine (0.1 ml of 10⁻³ M), or epinephrine (0.1 ml of 1:10,000) (Fig. 4, A and C). When the same dose of acetylcholine was injected into the right atrium, it produced a depressor effect (31 ± 3 mmHg), which was comparable to bradykinin (1 µg/kg) injection into the right atrium (Figs. 4 and 5). Similarly, injection of the same dose of phenylephrine (or epinephrine) into the right atrium caused significant increase in blood pressure (Fig. 4).

At baseline (resting condition), RSNA showed clear respiratory and cardiac modulation, with a cluster of bursts starting at end of the mechanical inspiration (Fig. 1, A and B). Each burst was roughly associated with the cardiac cycle (Fig. 1B). Injecting bradykinin

Fig. 2. Typical cardiopulmonary responses to local injection of bradykinin (BK; 1 µg/kg in 0.1 ml of normal saline) into the right lung parenchyma in an open-chest, artificially ventilated rabbit. Note that BK caused hypotension and bradycardia, which was accompanied by stimulation of inspiratory and expiratory activities. Traces are BP, integrated electromyogram of phrenic nerve activity [ENG (Integ)], Paw, and integrated electromyogram of abdominal (external oblique) muscle activity [EMG (Integ)]. ■, Time of injection of BK. Note that blood pressure change peaked before changes in respiratory variables.

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into the lung decreased RSNA along with the observed hypotension (Fig. 6A). As with the depressor response to bradykinin, the decrease in renal activity in response to bradykinin remained after vagotomy (Fig. 6B). In all cases where blood pressure did not decrease, RSNA did not decrease. In contrast, injection of hypertonic saline into the lung parenchyma did not cause substantial changes in blood pressure or RSNA, although it stimulated respiratory drive significantly (Figs. 7 and 8) ($P < 0.05$).

From the dye-distribution experiments, dye spread was extensive and irregular but was well within the lobe injected. The dye concentrated in a dependent area of $\sim 0.6 \text{ cm}^2$ on a slice. This concentrated area was dark blue with every bit of tissue stained. Some dye radiated from the injection point along the airways, grossly covering an area far beyond the concentrated area, up to $4 \text{ cm}^2$ on some slices. In the grossly covered area, however, some spots were unstained. Stained and unstained spots were interwoven. On rough estimation, the dye stained $<20\%$ of a lobe.

**DISCUSSION**

In anesthetized, artificially ventilated, open-chest rabbits, bradykinin activates pulmonary afferents and elicits profound cardiopulmonary reflexes in the majority of cases. These reflex effects include hypotension, bradycardia, and respiratory drive stimulation. Reflex effects on breathing pattern appear in another report (26). The reflex effects are primarily initiated through sympathetic afferent rather than vagal afferent pathways, because they persisted after bilateral vagotomy (present results) and can be blocked by bilateral section of sympathetic chains and white rami (26). The depressor effects on hemodynamics may result from withdrawing sympathetic effenter activity.

The anatomic location of the responsible receptors is an important issue. Identification of their location may give a clue to the mechanism(s) of afferent activation and help with understanding the physiological role of the reflex. After injection of bradykinin into the lung parenchyma, it presumably reaches the target afferent endings by both flow and diffusion. Thus the distance between the injection point and the receptor field is important for determining the peak response of the receptor. It should be helpful to know more precisely the extent of distribution of the injected bradykinin. Our results from the dye-distribution study indicate the injected solution covers only a small portion of the total lung tissue. However, injection of Evans blue dye can only indicate the general area over which the solution is distributed. It cannot indicate in which structure (alveolar wall, small airways, or microvessels) the reflex initiated. Nevertheless, this study confirms that the reflex is very powerful. Activation of a small portion of the sympathetic afferent system can produce tremendous cardiopulmonary responses.

Injection of stimulants directly into lung receptor fields has proven to be a useful method to investigate reflex effects of pulmonary afferents. One advantage is avoiding direct cardiovascular effects when a sensory nerve-ending stimulant (such as bradykinin) is administered intracardially or intravascularly, especially when cardiovascular responses are evaluated. Direct injection also avoids confounding reflex effects from activating afferents in the heart or other sites accessible through the systemic circulation. In addition, it is possible to deliver a high concentration of stimulants to pulmonary afferents without directly entering the circulation. In several respects, the use of local injection to study reflexes from pulmonary afferents is similar to topical application of stimulants in studying other organs, such as the heart (3, 20) and abdominal-visceral organs (8). The concentration of bradykinin used in the present study is comparable to that reported in the topical application to the heart [from $1 \mu\text{g/ml}$ (3, 20) to $10–100 \mu\text{g/ml}$ (30)] and to the gut [10 $\mu\text{g/ml}$ (8)].

Bradykinin is a powerful vasodilator. In dogs and rabbits, intravenous injection of bradykinin causes hypotension by decreasing peripheral vascular resistance (2, 14). In the present study, it is possible some of the bradykinin injected into the lung parenchyma was
absorbed through pulmonary capillaries and then traveled to the systemic circulation. The result could be hypotension by direct vasodilatation. We believe this is not the case, however, because local injection of another hypotensive agent (acetylcholine) as well as vasoconstrictors (phenylephrine or epinephrine) produced no changes in blood pressure. The molecular weight of these agents is comparable to that of bradykinin. They are expected to be absorbed at a similar rate. These vasoreactive agents, after being injected into the lung parenchyma, did not change hemodynamics, suggesting either absorption is minimal or so slow that the agent concentrations cannot reach the threshold to produce cardiovascular changes. In addition, bradykinin inactivates in a single passage through the pulmonary circulation (15). Any bradykinin absorbed into the pulmonary circulation will be substantially metabolized, significantly limiting access of locally injected bradykinin to the systemic circulation. For these reasons, depressor effects of bradykinin appear not to result from direct vasodilatation of the systemic circulation but rather from a reflex initiated within the lung. The RSNA results support this conclusion.

We found that injection of bradykinin into the lung decreased RSNA, suggesting that the observed hypotensive effect is a primary reflex. Were the hypotension related to direct vasodilatation, RSNA should increase, because hypotension should reflexly withdraw baroreceptor-induced tonic inhibitory effects on sympathetic activity, thereby increasing sympathetic outflow (7). In addition, bradycardia accompanied hypotension induced by locally injected bradykinin, again pointing to a reflex effect. If hypotension resulted from direct vasodilation, tachycardia mediated by the baroreflex should be observed.

Fig. 4. Effects of ACh (0.1 ml of 10^{-4}; A and B) and epinephrine (Epi; 0.1 ml of 1:10,000; C and D) on BP and heart rate. These agents were injected either into the lung parenchyma (A and C) or into the right atrium (RA; B and D). ●, Time of injection of BK. Note that both agents did not alter hemodynamics when injected into the lung but significantly affected the circulation when injected into the RA.

Fig. 5. Comparison of the changes in blood pressure in response to BK (A) and ACh (B) injected into the lung and into RA in vagotomized rabbits. Values are means ± SE for 6 rabbits. The dose and volume for each agent used for the 2 different routes were the same. Note that BK and ACh produced comparable depressor effects when injected into the RA; however, BK but not ACh produced depressor effects when injected into the lung parenchyma. Significant difference between peak response (hatched bars) and their control (open bars): *P < 0.05.
Because bradycardia almost disappeared after bilateral vagotomy, it is uncertain whether slowed heart rate initiated by activating vagal afferents or sympathetic afferents. If it was due to activation of sympathetic afferents, then the efferent arm of the reflex may be in the vagus nerve. Afferents from the airway can traverse to the central nervous system, bypassing the main vagal trunk. They course through the recurrent and pararecurrent laryngeal branches of the vagus, then along the cervical vagal plexus, and join up with the superior laryngeal nerve to the central nervous system through their cell bodies in the nodose ganglion. For example, pulmonary stretch receptor activities were recorded in the superior laryngeal nerves (29). To ensure that this afferent pathway did not constitute the major central projection for the reflex under investigation, we assessed cardiopulmonary responses to local injection of bradykinin in four rabbits with both cervical vagotomy plus superior laryngeal nerve section. We found that the reflex effects still existed and were comparable with or without additional section of superior laryngeal nerves. This suggests that the major afferent signals for the reflex after vagotomy are mediated through sympathetic afferent pathways.

In summary, it is reasonable to conclude that activation of extravagal afferents in the lung (pulmonary sympathetic afferents) can produce reflex hypotension by withdrawal of sympathetic tone. Our results are consistent with the report that chemical activation of cardiopulmonary afferents by serotonin or phenyl biguanide suppresses RSNA (28). In contrast, local injection with hypertonic saline evokes the excitatory lung reflex while producing no clear change in blood pressure (25, 34) and RSNA (Figs. 7 and 8). The present finding is in line with the fact that blood pressure does not change or may increase slightly after evoking the excitatory lung reflex (25, 34). The difference in the hemodynamic response to hypertonic saline and bradykinin lends further support to our conclusion that these two agents activate different afferent pathways.

In anesthetized monkeys and dogs, electrical stimulation of Aδ-fiber sympathetic afferents in the stellate ganglia, the stellate cardiac nerve or anterior ansae subclavia causes a depressor response, whereas stimulation of C-fiber sympathetic afferents yields apressor effect (10). Changes in blood pressure can be used to assess overall sympathetic efferent activity in the systemic circulation. Bradykinin is known to activate vagal afferents of Aδ- and C fibers (5, 6); this may account in part for the reduction in hemodynamic responses after bilateral vagotomy in our study. In addition, bradykinin also activates visceral sympathetic afferents (16). If the rabbit is the same as the dog, the depressor response to bradykinin observed in the

Fig. 6. RSNA in response to local injection of BK (1 µg/kg in 0.1 ml) into the lung parenchyma before (A) and after (B) bilateral cervical vagotomy. ■ Time of injection of BK. Note that depressor effect was associated with suppression of RSNA. Both reflex effects still existed after the vagotomy. In this particular case, the magnitude of suppression did not change significantly after vagotomy.
present study might be due to activation of Aδ-fibers. It has been reported that electrical stimulation of thoracic sympathetic afferents at low frequency (3 Hz) decreases RSNA, whereas high frequency (30 Hz) increases activity (21, 31). In dogs, activating cardiac sympathetic afferents with bradykinin causes pressor effects and increased RSNA (30). In the present study, activating pulmonary sympathetic afferents causes depressor effects with decreased RSNA. The difference in the reflex effects on blood pressure and RSNA could be explained by activating different afferents in origin (cardiac vs. pulmonary). If this statement is true, it explains the phenomenon that electrical stimulation of cardiopulmonary sympathetic afferents can cause either pressor or depressor effects (11, 21, 31). However, the difference in blood pressure responses could be also due to species differences. Cats, dogs, and monkeys differ in their response to activation of cardiac sympathetic afferents by bradykinin (20). Activation of cardiac sympathetic afferents with bradykinin decreases blood pressure, heart rate, and RSNA in the rabbit (20). Our studies do not answer this question and further work is needed to prove or disprove this statement. Nevertheless, the present study provides more information on reflex effects evoked by activating pulmonary sympathetic afferents. These findings may explain why the depressor effect is greater when bradykinin is administered intravenously than intra-arterially (14). Intravenous administration bradykinin may activate pulmonary afferents, causing a reflex depressor effect. Because pulmonary C fibers are relatively insensitive to bradykinin (6), the difference could be due to activation of pulmonary sympathetic afferents.

Fig. 7. RSNA in response to local injection of hypertonic saline (8.1% NaCl, 0.1 ml) into the lung parenchyma. ■, Time of injection of BK. Note that hypertonic saline did not alter BP and RSNA substantially, although inspiratory activity [Phre (Integ)] increased significantly.
In conclusion, our study provides evidence that activating pulmonary sympathetic afferents can produce profound cardiopulmonary reflexes, which consist of hypotension, bradycardia, and stimulation of breathing.

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