Bradykinin stimulates respiratory drive by activating pulmonary sympathetic afferents in the rabbit

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Soukhova, G., Y. Wang, M. Ahmed, J. F. Walker, and J. Yu. Bradykinin stimulates respiratory drive by activating pulmonary sympathetic afferents in the rabbit. J Appl Physiol 95: 241–249, 2003. First published April 4, 2003; 10.1152/japplphysiol.00582.2002.—We recently identified a vagally mediated excitatory lung reflex by injecting hypertonic saline into the lung parenchyma (Yu J, Zhang JF, and Fletcher EC. J Appl Physiol 85: 1485–1492, 1998). This reflex increased amplitude and burst rate of phrenic (inspiratory) nerve activity and suppressed external oblique abdominal (expiratory) muscle activity. In the present study, we tested the hypothesis that bradykinin may activate extravagal pathways to stimulate breathing by assessing its reflex effects on respiratory drive. Bradykinin (1 µg/kg in 0.1 ml) was injected into the lung parenchyma of anesthetized, open-chest and artificially ventilated rabbits. In most cases, bradykinin increased phrenic amplitude, phrenic burst rate, and expiratory muscle activity. However, a variety of breathing patterns resulted, ranging from hyperpnea and tachypnea to rapid shallow breathing and apnea. Bradykinin acts like hypertonic saline in producing hyperpnea and tachypnea, yet the two agents clearly differ. Bradykinin produced a higher ratio of phrenic amplitude to inspiratory time and had longer latency than hypertonic saline. Although attenuated, bradykinin-induced respiratory responses persisted after vagotomy. We conclude that bradykinin activates multiple afferent pathways in the lung; portions of its respiratory reflexes are extravagal and arise from sympathetic afferents.

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kg). The protocol used conforms to ethics requirements and has been reviewed and approved by IACUC of the University of Louisville. To assess central respiratory drive we recorded phrenic efferent activity. To evaluate respiratory pattern further, external oblique abdominal muscle (expiratory) activity and phrenic (inspiratory) activity were simultaneously examined in some rabbits. Anesthesia was established with pentobarbital sodium intravenously (30 mg/kg) during surgery. After surgery was completed, anesthesia was maintained with a mixture of α-chloralose (1%) and urethane (10%) by intravenous infusion (−1.6 ml/h). Measurements were made at least 90 min after the final dose of pentobarbital.

After cannulation of the trachea low in the neck, mechanical ventilation was established with a Harvard ventilator (model 683). A pressure transducer attached to a side arm of the tracheal tube monitored airway pressure. Tidal volume was set at 10 ml/kg body weight, and 3 cmH2O of positive-end expiratory pressure were applied. The chest was then opened by midline incision, with the opening covered with saline-soaked gauze to prevent dehydration of the underlying tissue. The femoral artery was cannulated to measure arterial blood pressure. Airway pressure, phrenic nerve activity, and expiratory muscle activity were thermorecorded (Dash IV, Astro-Med). Because the expiratory muscles are often quiet during rest, ventilation frequency was adjusted to produce active abdominal muscle activity (36).

The methods of recording phrenic nerve and external oblique abdominal muscle activities and injecting the stimulants directly into lung parenchyma appear in a previous report (36). Briefly, the electromyogram (EMG) was recorded from the right or left phrenic nerve. The phrenic nerve was separated from the surrounding tissue in the neck and transected. The central end of the nerve was desheathed and placed on a bipolar silver electrode connected to a high-impedance probe (model 7P3D integrator, Grass; time constant, 50 ms). The electromyogram (EMG) of the external oblique abdominal muscle was recorded from bipolar needle electrodes inserted into the muscle. The EMG signals were preamplified and band-pass filtered between 30 and 3,000 Hz. The processed signals were then passed through moving-time-averaged integration (model 7P3D integrator, Grass; time constant, 50 or 200 ms). Amplitudes of the ENG and EMG activity were taken as the peak height above the baseline of the moving-time-averaged signal.

Reflex effects were evoked by direct injection of bradykinin (1 μg/kg in 0.1 ml of 0.9% NaCl) into the lung parenchyma. These effects were compared with the responses to direct injection of hypertonic saline (8.1% NaCl, 0.1 ml) (32, 37). This bradykinin concentration was chosen on the basis of our preliminary study. In most cases, this concentration could consistently produce a response; on the other hand, at doses ≤0.01 μg/kg, bradykinin usually did not produce any effect, whereas at higher doses bradykinin might cause extreme responses. Therefore, in six rabbits we tested the dose-response relationship (0.05, 0.5, 1, and 2 μg/kg) in a relatively narrow range.

Data are reported as means ± SE. Each data point for respiratory variables represents the average of three ventilatory cycles. Statistically, a Student’s paired t-test compared two groups of data from the same animals. The paired Wilcoxon test evaluated a variable’s change from its control value. Statistical significance was established as values of P < 0.05.

RESULTS

In most cases, direct injection of bradykinin into the lung elicited one or more responses (85.6% of 201 observations). There was a range of excitatory, inhibitory, and biphasic respiratory responses. Blood pressure either decreased or remained stable. The typical pattern was increased phrenic amplitude, increased phrenic burst rate, and decreased blood pressure (Figs. 1, 2, and 3). In a minority of cases (14.4%), no change in either respiration or blood pressure took place (29 of 201 observations). Data from the animals that did not respond do not appear in the group values that follow.

Effects on Phrenic Nerve Activity

Respiratory responses to bradykinin were classified as either depressor associated or non-depressor associated (73% and 27% of total trials, respectively). Depressor-associated respiratory responses were further classified as excitatory or inhibitory, with total trial occurrence rates of 55 and 18%, respectively. All non-depressor-associated bradykinin-induced phrenic nerve responses were excitatory. All hypertonic saline responses were also excitatory.

Excitatory responses. Excitatory responses to bradykinin include increased phrenic amplitude and burst rate, i.e., neural hyperpnea and tachypnea (Fig. 2). In this respect, bradykinin behaves like hypertonic saline; however, on closer inspection, the responses to bradykinin and hypertonic saline differ (Fig. 4). Bradykinin response involves a tail and narrow ENG, that is, high amplitude and short inspiratory time (Ti) (Figs. 1 and 4B). The ratio of the amplitude (in arbitrary units) and Ti (in s) of phrenic activity was 3.2 ± 0.2 before and 6.1 ± 0.6 after bradykinin. Correspondingly, the ratio was 2.9 ± 0.2 before and 3.8 ± 0.6 after hypertonic saline. The ratio of amplitude to Ti increased by 90% after injection of bradykinin (n = 10; P < 0.01), but it changed little with hypertonic saline injection (n = 10; +29%; P = 0.23). The latency of increased phrenic nerve amplitude after bradykinin was 14.8 ± 2.1 s, peaking at 35.2 ± 3.0 s. The excitatory responses in phrenic nerve activity persisted after bilateral vagotomy. Increases in both phrenic amplitude and burst rate were still significant (n = 18; P < 0.05); however, they substantially decreased (Fig. 2A).

Although the increase in phrenic amplitude in response to bradykinin was greatly attenuated after bilateral vagotomy, the time course did not change (Fig. 5). In four rabbits, respiratory responses persisted (+23.3 ± 6.0% in phrenic amplitude and +16.5 ± 3.6% in phrenic burst rate) after sectioning of the superior laryngeal nerves in addition to vagotomy.

Sympathectomy was performed in four rabbits, and the cardiopulmonary responses persisted after unilateral local avulsion of white rami in T1-T4. Complete blockade occurred but only after extensive bilateral section of sympathetic chains and white rami extending into the cervical and lumbar segments. With such extensive surgery, the animal’s condition was unstable.

Regarding the dose-response observation, we excluded one of the six rabbits examined from data analysis because there was a strong but irreproducible inhibitory response at the 0.5 μg/kg bradykinin dose. No detectable response occurred at higher doses in this rabbit. Breathing inhibition was also observed in one of the remaining five rabbits, manifested by a biphasic response at the 1 μg/kg dose. The lower and higher doses tested showed
only an excitatory response. All rabbits demonstrated only a marginal response at the 0.05 μg/kg dose, except one that had a significant excitatory response. Group data for peak responses at the four doses (0.05, 0.5, 1, and 2 μg/kg) were 14 ± 8, 39 ± 15, 42 ± 9, and 33 ± 11% increases in phrenic amplitude and were 37 ± 10, 56 ± 20, 72 ± 10, and 68 ± 10% increases in phrenic burst rate.

Non-depressor-associated excitatory responses showed approximately one-half the latency of depressor-associated responses (8.5 ± 1.9 s), with a small increase in phrenic amplitude (9.8 ± 3.6% at peak response). These effects were similar to the excitatory lung reflex produced by local injection of 8.1% NaCl (0.1 ml) (Fig. 6A). Hypertonic saline-induced phrenic responses are faster (Fig. 5), having latency at 3.0 ± 0.2 s and peaking at 5.5 ± 0.6 s.

**Inhibitory responses.** When bradykinin inhibited respiratory responses, these ranged from partial suppression.

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**Fig. 1.** Typical cardiopulmonary response to local injection of bradykinin (1 μg in 0.1 ml) into lung parenchyma in an anesthetized, open-chest, and artificially ventilated rabbit. Traces are phrenic electroneurogram (ENG), "integrated" ENG [ENG (Integ)], airway pressure (Paw), and arterial blood pressure (BP). Three solid squares (top) represent insertion of the needle, the start of the injection, and end of the injection, respectively. Note that bradykinin stimulated phrenic activity (which is characterized by tall and narrow burst with peak response at ~40 s and by increased burst rate) and caused hypotension.

**Fig. 2.** Percent changes in amplitude (A) and burst rate (f) of phrenic activity in response to bradykinin injection into the lung parenchyma before and after bilateral vagotomy in 2 groups of rabbits. Values are means ± SE. In A (n = 18), the same dose of bradykinin (1 μg/kg) was used before and after vagotomy. In B (n = 14), the doses of bradykinin before (1 μg/kg) and after (1–5 μg/kg) vagotomy were different. On average, the dose used after vagotomy was 2.75 times higher than that used before vagotomy for the production of a comparable response in phrenic amplitude.

**Fig. 3.** Occurrence of changes in each of the 4 variables in response to bradykinin in animals exhibiting 1 or more responses. EMG, amplitude of integrated electromyogram (abdominal muscle). These data were summarized from 58 observations in 16 vagotomized rabbits. There was no response in 14 observations (24.1%). The occurrence is calculated on the basis of the 44 observations, which had at least 1 type of response (i.e., on the basis of 75.9% responders).
sion of inspiratory activity to neural apnea (Fig. 7). Ex-
citation (increase in phrenic amplitude) followed inhibi-
tory responses at times, characterized by short latency
for onset. Phrenic nerve response pattern and time
course did not change after vagotomy. Latencies for both
excitatory and inhibitory types of inspiratory responses
appear in Fig. 8. For reporting purposes, data for partial
and complete inspiratory suppression were combined.

Effects on Expiratory Muscle Activity

Most commonly, bradykinin caused abdominal mus-
cles to contract. When bradykinin inhibited phrenic ac-
tivity, usually the expiratory muscles were simulta-
nously stimulated (Fig. 7, A and B). When bradykinin
stimulated phrenic activity, it also evoked a reflex in-
crease EMG activity in the external oblique abdominal
muscle. The amplitude of the integrated EMG increased
from 2.3- to more than 11-fold above control after injec-
tion of bradykinin (0.5–1 µg/kg in 0.1 ml). A summary of
the 44 total observations is as follows: bradykinin stim-
ulated expiratory muscles in 26 observations of 15 rab-
bits (Fig. 9), expiratory muscles were inhibited in 12
observations of 7 rabbits, and there was a clear biphasic
response with initial suppression followed by excitation
in 6 observations of 5 rabbits (Fig. 6B). The latency for
inhibition on expiratory muscle, if it happened, was
shorter (4.1 ± 0.7 s) than that for excitation (20 ± 2.6 s)
(Figs. 6 and 8). The excitatory effect peaked at 32.2 ±
2.0 s (Fig. 8). Most commonly, both the tonic and phasic
activities of expiratory muscles increased (Fig. 9). Spon-
taneous activity of the expiratory muscles usually disap-
ppeared after vagotomy; therefore, excitation was the only
possible response. The latencies and peak response time
after bradykinin are illustrated for the two groups when
phrenic activity decreased (Fig. 8A) and increased (Fig.
8B). Vagotomy did not affect the time course of expiratory
muscle excitation. It is noteworthy that the inhibitory

Fig. 5. Time courses of phrenic amplitude in response to local injec-
tions of bradykinin (BK) and hypertonic saline. The curves for
bradykinin before and after vagotomy were constructed with injec-
tion doses of 1 µg/kg (vagus intact) and 1–5 µg/kg (after vagotomy),
respectively. Note the difference in time course between the re-
sponses to hypertonic saline and bradykinin injections. Values are
means from 10 rabbits.
Effects on expiratory muscle with short latency induced by bradykinin resemble those induced by hypertonic saline (Fig. 6, A and B).

DISCUSSION

Profound reflex effects occur when bradykinin activates pulmonary afferents. Bradykinin generally stimulates respiratory drive by increasing phrenic nerve amplitude and burst rate, as well as by increasing expiratory muscle activity. Yet we saw a variety of respiratory responses.

The variation in respiratory responses to bradykinin (excitation, inhibition, and biphasic) under the same experimental conditions (injection site and concentration) is perplexing. The final output of activating several afferent

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Fig. 6. Differences in expiratory responses to hypertonic saline (A) and bradykinin (B). EMG (Integ), “integrated” EMG. Note that both hypertonic saline (8.1% NaCl, 0.1 ml) and bradykinin (2 μg in 0.1 ml) stimulated inspiration and inhibited expiration. In the case of bradykinin, however, expiratory muscle was activated after 40 s, which was preceded by a slight decrease in BP. Three solid squares (top) represent insertion of the needle, the start of the injection, and end of the injection, respectively.
pathways depends on the central interaction of inputs. Coactivating multiple afferent pathways may be responsible. The injected solution covers a significant portion of a lobe (34), reaching all levels of the airway. Thus the extent of stimulant distribution within a lobe is unlikely to be a major factor in the variation. The effective dose of bradykinin reaching different types of afferent endings within the covered area may play a role; however, this issue is complicated. The dose-response study indicated that the responsible afferents operate in a relatively narrow range, making it difficult to differentiate responses by correlating them with a particular bradykinin dose.

The observed reflex effects are mainly extravagal because they persist after vagotomy alone and with simultaneous section of the superior laryngeal nerves (see RESULTS and Wang et al. (34)). Afferents from the vagus...
were presented in A; stimulatory responses were not altered by vagotomy. 

B: excitatory in A was excitatory. On the other hand, ENG was inhibitory in A and B. ENG, latency to ENG response in phrenic; PEMG, time to peak EMG response; PENG, time to peak ENG response. In A and B, ENG was excitatory. On the other hand, ENG was inhibitory in A but excitatory in B. Because there is no peak for apnea, no data (PENG) were presented in A. Note that time courses for both inhibitory and stimulatory responses were not altered by vagotomy.

Fig. 8. Latencies of inspiratory and expiratory responses to local injection of bradykinin before and after vagotomy. A: inspiratory inhibition type of response. B: inspiratory stimulation type of response. Values are means ± SE. LEMG, latency to EMG response; LENG, latency to ENG response in phrenic; PEMG, time to peak EMG response; PENG, time to peak ENG response. In A and B, ENG was excitatory. On the other hand, ENG was inhibitory in A but excitatory in B. Because there is no peak for apnea, no data (PENG) were presented in A. Note that time courses for both inhibitory and stimulatory responses were not altered by vagotomy.

nerves, the superior and recurrent laryngeal nerves, and sympathetic nerves each supply the lung and airways (11). The superior and recurrent laryngeal nerves mainly supply the trachea and upper airways, whereas the vagus nerves supply the trachea, upper airways, and the periphery. Our findings show that reflex responses to bradykinin mainly result from activating extraganglionic afferents running along sympathetic nerves. Supporting this are results showing reflex blockade by sectioning the sympathetic afferent pathway, although the required surgery was extensive. Perhaps sympathetic afferents, like their counterpart sympathetic efferents, have a diffuse central projection. This contention is supported by the report that a single sympathetic afferent sensory unit may supply different organs (2). Extensive sympathetic sectioning poses an interpretation problem, and it is probably the reason no studies have looked at the sympathetic nervous system’s role in control of breathing.

Bradykinin-evoked responses clearly differ from those initiated by hypertonic saline or hydrogen peroxide through pulmonary vagal afferents (32, 37). These differences can be summarized in four respects: 1) time course, 2) patterns of ENG, 3) expiratory muscle activity, and 4) hemodynamics.

In previous studies, our laboratory demonstrated that activating pulmonary vagal afferents with locally injected hypertonic saline (37) or hydrogen peroxide (32) produces an excitatory lung reflex. The excitatory lung reflex results in increased phrenic nerve amplitude and burst rate. Hypertonic saline, like other vagally mediated stimulants such as capsaicin or phenyl diguanide, produces responses with short latency (8). Although bradykinin also increases phrenic nerve amplitude and burst rate, its response latency (14 s) is longer than that for hypertonic saline (within 5 s). The time to reach peak response in the ENG is also longer for bradykinin (~40 s) than hypertonic saline (within 10 s) (32, 37) (also see Fig. 5). The differences in latency and peak response suggest separate mechanisms for phrenic activation by bradykinin and hypertonic saline. Alternatively, the differences in latencies to the onset and time to the peak response can be explained by the same afferent pathway but operating through two distinct sensing processes. Bradykinin produces its effects through at least two types of receptors, B1 and B2: the latter are found in a variety of sensory nerve endings, including nociceptive neurons, visceral afferent fibers, sympathetic nerves, and pulmonary vagal afferents (5, 13, 15, 27, 33). If hypertonic saline activates sensory endings by directly depolarizing them, whereas bradykinin binds to receptors before initiating a cascade process to depolarize them (5, 17), this may explain the difference in time course. However, differences in response pattern (see below) weigh against this explanation. It is more likely that separate afferent pathways were activated.

The latency and time to reach peak response (14 and 40 s) observed when pulmonary sympathetic afferents are activated with bradykinin agree well with those of activation of other systems. Examples include renal sympathetic afferents (11 and 26 s) (1), spermatic afferents (15.5 and 60 s) (22), and abdominal visceral afferents (17 to 19 s for latency) (24). Typically, bradykinin-induced phrenic bursts showed a tall and narrow pattern (Figs. 1, 4, and 9). The increase in phrenic amplitude caused by local injection of bradykinin was much greater than local injection of hypertonic saline (Fig. 5). The ratio of phrenic amplitude to Ti almost doubled in the response to bradykinin, but it did not change in response to hypertonic saline. These differences support the contention that these two agents stimulate respiration through different pathways.

Bradykinin increases expiratory muscle activity, and hypertonic saline decreases it. The present study confirmed that activation of pulmonary vagal afferents suppresses abdominal muscle activity with short latency, reaching the peak at 7 s after injection (36). In contrast, bradykinin increases abdominal muscle activity with long latency, and the effect remains after vagotomy. This suggests that afferents for reflex expiratory muscle activity are located in sympathetic nerves. The expiratory muscle response to bradykinin (sympathetic activation) and to hypertonic saline (vagal activation) provides a tool to differentiate whether a reflex originates from a sympathetic or from a parasympathetic pathway. For example, in some cases bradykinin produced a biphasic response in expiratory muscle activity, i.e., immediate inhibition followed by stimulation (Fig. 6B). The initial inhibitory effect can be identified as vagally mediated
and the delayed excitatory effect as sympathetically mediated. Increased expiratory activity together with increased inspiratory activity evoked by bradykinin shapes the breathing pattern in a characteristic way, that is, with tall and narrow phrenic bursts and increased phrenic burst rate. This type of neural discharge produces a fast and deep breathing pattern (sharp cycles), which is like gasping. Because systemic hypotension may reflexly stimulate respiration, it is possible that increased respiratory activity is due to the withdrawal of a baroreflex (16). However, it is unlikely because respiratory stimulation sometimes preceded hypotension and at times occurred without changes in blood pressure.

Bradykinin evokes a variety of responses, including reflex effects that are opposite in direction (stimulation or inhibition of inspiration), which persist after vagotomy. The coexistence of stimulation and inhibition of inspiratory activity in a particular effector implies that the responses follow different pathways. This is illustrated in Fig. 7B, where local injection of bradykinin in a vagotomized rabbit caused a cessation of phrenic activity (inhibition). When phrenic activity resumes, the characteristic tall and narrow pattern (stimulation) appears. This is consistent with coactivating two extravagal (sympathetic) afferent systems. There are reports that electrical stimulation of cardiopulmonary sympathetic afferents may stimulate or inhibit inspiration (21). It is believed that stimulation of Aδ-fiber sympathetic afferent inhibits phrenic activity (21), whereas stimulation of C-fiber sympathetic afferent enhances phrenic activity. Bradykinin activates not only pulmonary sympathetic afferents in the lung but also vagal afferents, including C fibers and rapidly adapting receptors (RARs) (4, 8, 20), thereby modifying the respiratory pattern. It is still debated whether RARs are activated by bradykinin directly (18) or indirectly (4, 20). The fact that the respiratory responses attenuated after vagotomy suggests a role played by the vagus nerves. That bradykinin stimulates pulmonary vagal afferents is also supported by our results where expiratory muscle exhibited a biphasic response to bradykinin, i.e., inhibition followed by excitation (Fig. 6B). The initial suppression of expiratory muscle activity indicates activation of a vagal afferent system.

It is generally accepted that the influence of pulmonary sympathetic afferents on control of breathing, if any, is small (9, 26, 31, 36). In contrast, the present results show profound reflex effects by activation of pulmonary sympathetic afferents. There are several possible explanations. Sympathetic sensory nerves may have a higher activation threshold to stimulants such as bradykinin, i.e., responding only to high concentration. Conventionally, pulmonary sympathetic afferents are stimulated by injection of a stimulant into the pulmonary circulation. This may not provide effective sympathetic activation because blood significantly dilutes stimulant entering the circulation. On the other hand, the local injection method...
may deliver a sufficient concentration of bradykinin to the receptor field to activate sensory endings. In a way, local injection is similar to the topical application of stimulants to the surface of the other organs (34). Another reason sympathetic afferent activation in the lung may be unappreciated is that it is conventional to compare reflex effects of vagal afferents before and after vagotomy. Any remaining reflex effects are attributed to sympathetic afferents. It is possible two afferent systems (vagal and sympathetic) interact centrally and synergistically. Vagotomy alters breathing pattern. Therefore, it alters the excitability of the respiratory center. If, after vagotomy, the threshold to initiate respiratory response is high and the sensitivity to respiratory stimulants is low, the response to activating the sympathetic afferent pathway should lessen. This occurred in the present study, and it may account for why the existing literature raises questions as to the importance of sympathetic afferent system in the regulation of breathing.

In conclusion, bradykinin activates multiple afferent systems in the lung to evoke cardiopulmonary reflexes through vagal and sympathetic afferent pathways. Activation of pulmonary sympathetic afferents produces profound reflex affects that include stimulation of inspiratory and expiratory activity and sometimes apnea.

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