Central nervous system control of cardiorespiratory nasopharyngeal reflexes in the rabbit

SAXON WHITE, ROBERT J. McRITCHIE, AND PAUL I. KORNER
Department of Medicine, University of Sydney, and Hallstrom Institute of Cardiology,
Royal Prince Alfred Hospital, Sydney, 2050, Australia

White, Saxon, Robert J. McRitchie, and Paul I. Korner. Central nervous system control of cardiorespiratory nasopharyngeal reflexes in the rabbit. Am. J. Physiol. 228(2): 404-409. 1975.—The role of different central nervous regions in the reflex apnea, bradycardia, and mesenteric vasoconstriction evoked by nasopharyngeal stimulation with cigarette smoke was examined in unanesthetized sham-operated, thalamic, and pontine rabbits with intact and sectioned carotid sinus and aortic nerves (CS and AN). Apnea occurred in all preparations. In pontine animals with intact CS and AN, the heart rate response was reduced but not the mesenteric vasoconstriction. The role of suprabulbar and bulbospinal regions became more apparent when individual components of the input profile were examined in animals with controlled ventilation. The bradycardia and mesenteric vasoconstriction evoked by apnea without smoke, but not by smoke without apnea, were reduced in pontine animals. Prior section of the CS and AN attenuated the response in all neural preparations but to the least extent when cerebral hemispheres were intact. The data indicate that the respiratory reflex is predominantly integrated at bulbospinal sites, but the cardiovascular reflex is integrated at both bulbospinal and suprabulbar sites, or is integrated at bulbospinal and modulated from suprabulbar sites.

Central nervous cardiovascular control; central nervous respiratory control; smoke reflex; trigeminal; carotid sinus and aortic nerves; apnea.

Nasopharyngeal stimulation in the rabbit with cigarette smoke and many other vapors evokes a reflex response consisting of apnea in expiration, bradycardia, a rise in blood pressure, and widespread peripheral vasoconstriction (5, 20, 21). Trigeminal afferents and those from the arterial baroreceptors contribute to the circulatory response, and the input from the chemoreceptors influences the duration of the apnea (14). The question arises as to whether suprapontine mechanisms play a role in the “smoke” reflex as they do in other reflex disturbances (9–11, 19). No analysis of the role of the central nervous system (CNS) in the smoke reflex of the rabbit has been made so far. However, the reflex resembles the diving response of aquatic birds, in which Huxley (6) and Andersen (1) demonstrated that reflex apnea and bradycardia were unaffected by decerebration. They therefore considered the diving reflex was integrated predominantly by bulbar mechanisms.

The purpose of the present study was to examine the role of suprapontine and bulbospinal regions in the reflex response to nasal smoke inhalation in unanesthetized rabbits.

We studied the responses of spontaneously breathing sham-operated, thalamic, and pontine rabbits in which the input from the carotid sinus and aortic nerves was intact and also when these nerves were sectioned. In these same preparations we also analyzed under conditions of controlled ventilation the effects of apnea without smoke stimulation and of smoke without apnea.

METHODS

The experiments were performed with New Zealand white rabbits, weighing between 2.4 and 3.5 kg. Preliminary operations were performed 10–14 days before an experiment, with halothane anesthesia. At this operation, a Doppler ultrasonic flow transducer was placed around the superior mesenteric artery, as described previously (21). On the day of the experiment the central ear artery was cannulated under local lidocaine anesthesia. A frontoparietal craniotomy was then performed with halothane anesthesia. Sham-operated rabbits, thalamic rabbits (with cerebral hemispheres and basal ganglia removed and thalamus and hypothalamus left intact), and pontine animals (infracollicular decerebration) were prepared as described previously (11). There were four rabbits in each of these groups. In four additional animals of each group the carotid sinus and aortic nerves were cut bilaterally in the neck (7) immediately after neurosurgical procedures while the rabbits were anesthetized with halothane. Sham-operated and thalamic animals recovered normal movement and posture within 5–1 h from the end of anesthesia. The pontine animals lay on their side until .5 h before the start of the experiment, and their body temperatures were maintained with an electric blanket. Details of the postoperative management were as described previously (11).

Conduct of experiments and statistical methods. Experiments began 3 h after the operation. The sham-operated and thalamic animals sat quietly in their rabbit box, while the pontine animals were placed in a special holder and studied in the posture of a normally sitting rabbit. Nasopharyngeal stimulation was produced by blowing cigarette smoke through a narrow polyvinyl chloride tube. One puff of smoke was blown gently in front of the animal's nostrils and the cardiorespiratory response was observed (see below). The response was highly reproducible in a given animal (20). A given response in a particular animal was estimated from the mean of three puffs of cigarette smoke administered at 3- to 5-min intervals. For each test, measurements...
preparations in the present study were generally small (see its own control. An alternative method is to express responses as percent change with each animal formation since differences in initial resting values between changes as the absolute difference between prestimulus and values. Both methods provide similar types of in-

animals: the mean for an individual animal during sponta-
test (13). The significance of differences between re-

of arterial pressure, heart rate, mesenteric blood velocity (which in chronic preparations is directly proportional to volume flow (21)), and mesenteric “conductance” were made just before smoke stimulation and 7–12 s later when the response was at its height. The duration of apnea was also measured using a light, AC-coupled carbon-in-plastic strain gage strapped about the rabbit’s thorax.

After determination of the response to smoke stimulation in each animal during spontaneous respiration, it was given the short-acting anesthetic propanidid (Eptonol, Bayer) and intubated with a no. 14 Foregger pediatric endo-

Animal after receiving a puff of smoke and was tolerated by the latter without apparent distress.

Five minutes after this test we studied the circulatory effects of blowing a small amount of smoke into the nose while ventilation was controlled at 1 liter/min. The alternate stimuli of apnea in the absence of smoke stimulation and smoke stimulation in the absence of apnea were repeated twice more at 5-min intervals. Resting values (Table 1) were calculated for each preparation when spontaneously breathing and also during controlled ventilation. The resting value for each preparation was calculated from four animal during spontaneous respiration, it was given

Mean and standard error of the mean for an individual animal during spontaneous breathing and also during controlled ventilation. The resting value for each preparation was calculated from four

I -..- .-

FIG. 1. Effects of nasal inhalation of a single puff of cigarette smoke in a sham-operated rabbit. R = respiration rate; AP = ear artery pressure; MF = mean mesenteric flow (calculated from mean velocity \( \times \) measured diameter of vessel at postmortem); MV = mean mesenteric velocity; MC = mesenteric conductance; HR = heart rate.

RESULTS

Normal response to smoke inhalation. When a bolus of smoke was blown close to the sham-operated animal’s nose it developed apnea in expiration and remained motionless for the next 10–15 s, as described previously (21). The circula-
tory response consisted of a small rise in arterial pressure, marked bradycardia, and a fall in mesenteric vascular con-
ductance (Fig. 1). Respiration was then resumed and the circulatory variables rapidly returned to their resting value.

In the group with intact carotid sinus and aortic nerves, (buffer nerves), resting arterial pressures and heart rates differed little between sham-operated, thalamic, and pontine animals. Mesenteric blood velocity did not differ signifi-
cantly in any of the preparations with intact buffer nerves. In animals with section of the buffer nerves the arterial pressure was higher than in corresponding groups in
which the nerves were intact, while mesenteric blood velocity and conductance were lower (Table 1). For this reason most of the quantitative comparisons of conductance changes between different neurological preparations have been made within each of these two subgroups.

In the different preparations, controlled ventilation had minimal effects on resting circulatory variables and also on arterial P<sub>O<sub>2</sub></sub>, P<sub>CO<sub>2</sub></sub>, and pH. Table 2 illustrates results from the sham-operated group.

**Duration of apnea**. After inhalation of cigarette smoke the apnea time was similar in sham-operated and pontine rabbits with intact carotid sinus and aortic nerves, but was shorter in thalamic animals (P = 0.05, Table 3). In all groups with section of the buffer nerves the duration of the apnea was longer than in corresponding groups in which the nerves were intact. In animals with section of the buffer nerves there was again no difference in apnea times between sham-operated and pontine groups, but in thalamic animals apnea time was shorter (P = 0.02).

**Heart rate responses**. In the spontaneously breathing groups with intact buffer nerves, smoke inhalation evoked a fall in heart rate (Fig. 2) that was similar in sham-operated and thalamic animals but tended to be less marked in pontine rabbits both on a percent basis and in terms of the absolute number of beats, i.e., the resting heart rate of sham-operated rabbits was 264 beats/min and fell to 130 beats/min (to 49 ± 4.0 SEM, % of control), while the resting heart rate of pontine rabbits was 220 beats/min and fell to 145 beats/min (to 66 ± 1.8 SEM, % of control). Thus the absolute changes in beats per minute were 134 ± 15.0 and 75 ± 16.0, respectively. On the basis of the t test, the probability that the differences between responses on a percent basis could be accounted for by chance was slightly greater than 0.05, whereas on an absolute change basis the probability was slightly less than 0.05. Each group had a similar reflex rise in arterial pressure (Table 4). When the same animals were subsequently subjected to a brief period of apnea without smoke stimulation, the difference between sham-operated and thalamic rabbits on the one hand and pontine rabbits on the other was greater than in spontaneously breathing rabbits (P < 0.05, Fig. 2). When smoke stimulation was performed while ventilation was maintained constant the evoked bradycardia was similar in all three neural preparations (Fig. 2).

In the group of rabbits with section of the buffer nerves the bradycardia evoked by smoke stimulation in the spontaneously breathing animals was significantly smaller than in corresponding groups with intact nerves (Fig. 2) both on a percent basis and in terms of absolute numbers.

### Table 1. Resting circulatory data

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Heart rate, beats/min</th>
<th>Mean arterial pressure, mmHg</th>
<th>Mesenteric blood velocity, cm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>104 ± 3.2</td>
<td>245 ± 5.0</td>
<td>23 ± 3.0</td>
</tr>
<tr>
<td>Thalamic</td>
<td>110 ± 4.2</td>
<td>265 ± 6.5</td>
<td>21 ± 3.0</td>
</tr>
<tr>
<td>Pontine</td>
<td>113 ± 3.3</td>
<td>257 ± 7.4</td>
<td>22 ± 4.0</td>
</tr>
</tbody>
</table>

### Table 2. Resting P<sub>O<sub>2</sub></sub>, P<sub>CO<sub>2</sub></sub>, and pH in sham-operated animals with spontaneous breathing and controlled ventilation

<table>
<thead>
<tr>
<th>Preparation</th>
<th>P&lt;sub&gt;O&lt;sub&gt;2&lt;/sub&gt;&lt;/sub&gt;, mmHg</th>
<th>P&lt;sub&gt;CO&lt;sub&gt;2&lt;/sub&gt;&lt;/sub&gt;, mmHg</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>104 ± 4.6</td>
<td>245 ± 0.3</td>
<td>7.45</td>
</tr>
<tr>
<td>Controlled ventilation</td>
<td>106 ± 4.0</td>
<td>245 ± 0.2</td>
<td>7.45</td>
</tr>
<tr>
<td>Controlled ventilation</td>
<td>102 ± 4.2</td>
<td>245 ± 0.2</td>
<td>7.45</td>
</tr>
</tbody>
</table>

### Table 3. Duration of apnea

<table>
<thead>
<tr>
<th>Anapne Duration, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact carotid sinus and aortic nerves</td>
</tr>
<tr>
<td>Sham</td>
</tr>
<tr>
<td>Thalamic</td>
</tr>
<tr>
<td>Pontine</td>
</tr>
</tbody>
</table>

### Table 4. Arterial pressure data

<table>
<thead>
<tr>
<th>% Resting Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
</tr>
<tr>
<td>Thalamic</td>
</tr>
<tr>
<td>Pontine</td>
</tr>
</tbody>
</table>

Values are averages for 4 animals ± SEM.
CNS CONTROL OF SMOKE REFLEX IN RABBIT

FIG. 2. Upper panels, left to right: effects on heart rate (expressed as % resting control) of nasal smoke stimulation in 4 spontaneously breathing (Spont) sham-operated (Sh), thalamic (Th), and pontine (Po) rabbits, with intact carotid sinus and aortic nerves, and in the same animals with controlled ventilation during apnea without smoke stimulation (Apnea), and during smoke stimulation without apnea (Smoke). Lower panels: data from identical experiments carried out in groups of 4 similar preparations in which there was prior section of carotid sinus and aortic nerves. Bars on each response = 1 SEM.

of beats \( P < 0.05 \). After section of the carotid sinus and aortic nerves reflex bradycardia was greater in the sham-operated animals than in thalamic and pontine rabbits \( P = 0.05 \). Similarly, in the same animals apnea without smoke stimulation evoked a significantly greater fall in heart rate in sham-operated rabbits than in thalamic and pontine animals, and smoke stimulation without apnea produced significant bradycardia in all preparations, with the response greatest in the sham-operated group \( P < 0.05 \).

Mesenteric conductance changes. In spontaneously breathing rabbits with intact carotid sinus and aortic nerves the mesenteric conductance decreased considerably in all preparations (Fig. 3). The decrease was slightly smaller in pontine rabbits than in thalamic or sham-operated rabbits, but the differences were not significant. In these animals apnea without smoke stimulation did not significantly alter mesenteric conductance in the pontine group, but evoked a reduction to about 50% of control in both sham-operated and thalamic preparations. After smoke stimulation without apnea there was a substantial and similar decrease in conductance in all three preparations.

In spontaneously breathing rabbits with section of the buffer nerves there was little difference in the response of sham-operated and pontine animals, but the decrease in conductance was slightly less marked in the thalamic group. Apnea without smoke stimulation was associated with a significant fall in conductance only in the sham-operated group, which was smaller than during spontaneous respiration in these animals \( P < 0.05 \). Smoke stimulation without apnea decreased mesenteric conductance in all three preparations, the reduction being somewhat smaller in the thalamic and pontine groups than in the sham-operated group.

DISCUSSION

Evaluation of differences in reflex responses of different neurological preparations. In comparing the effects of each stimulus in sham-operated, thalamic, and pontine animals we have expressed the reflex responses as a percent change from each animal's own control values. Smoke stimulation and the sudden induction of apnea are "impulse functions," and from control theory it seems reasonable to express the reflex response as a change from resting, either in percent units or as an absolute difference (15). However, care is necessary in assessing differences in responses between the neurological preparations. If there were systematic differences in their resting values, incorrect conclusions would be reached about differences in their responsiveness if the stimulus drove the variable under study to a common saturation level.

There were small differences in resting heart rate responses between the neurological preparations. In pontine animals with intact carotid sinus and aortic nerves, the resting heart rate was lower than in sham-operated and thalamic animals, but this difference was not statistically significant. There was thus a considerable overlap in the resting values of different animals in the various groups. However, the responses to the stimuli in each group were much more uniform as evidenced by the relative low SE values in Fig. 2. Further, in animals with section of the carotid sinus and aortic nerves, the resting heart rate of the pontine animals was exactly the same as that of the sham-operated group, yet the fall in heart rate was again significantly smaller, suggesting a true difference in the reflex response of that preparation. The smoke stimulus did not drive the heart rate to the same level in all preparations and in general the heart rate of the pontine animals was somewhat higher during smoke stimulation than those of sham-operated rabbits (see RESULTS). Finally, during sleep, rapid neurally mediated reductions in heart rate to about 70-80 beats/min are frequently observed in rabbits (Korner, un-
published observations), which are considerably lower than the levels of 120-150 beats/min reached after smoke stimulation in the present study. The overlap in resting heart rate between the three neurological preparations in any group and the absence of a saturation effect thus suggest that the use of percent changes is an adequate approximation for assessing differences between sham-operated, thalamic, and pontine preparations.

As regards resting mesenteric conductance, there were again systematic differences between animals with intact carotid sinus and aortic nerves on the one hand and those with section of these nerves on the other. However, within each subgroup of sham-operated, thalamic, and pontine rabbits, irrespective of their buffer nerve status, differences were slight. Thus it again appears reasonable to express the responses as a percent change from resting values and to draw conclusions from differences between sham-operated, thalamic, and pontine conductance responses for a given buffer nerve status. However, we have avoided drawing conclusions from the differences in conductance responses between preparations with intact buffer nerves and those without these nerves.

Role of bulbohypothalamic and suprabulbaria regions. Recent analysis of the response to nasal inhalation of smoke in the rabbit has shown that the apnea, bradycardia, and rise in arterial and right atrial pressure are initiated by nasopharyngeal trigeminal afferents (14). The role of olfactory afferents in these reflex effects is negligible (14). The shorter duration of apnea in thalamic animals suggests that normally cerebral structures exert a facilitatory effect on the apnea induced by trigeminal stimulation. The duration of apnea in pontine animals, however, is similar to that of sham-operated animals, suggesting in turn that thalamic/hypothalamic structures exert inhibitory effects on the reflex suppression of respiration induced at bulbohypothalamic levels, an effect normally masked by the cerebral facilitation. Trigeminal afferents could evoke the circulatory effects directly through projections to the pontomedullary reticular formation (2) and/or indirectly due to changes in the input from lung inflation receptors as a result of the reflex apnea formation (2) and/or indirectly due to changes in the input from lung inflation receptors cardiace receptors, or both and is mediated mainly through vagal afferents (3, 4, 16, 17). Previous studies have indicated that the arterial baroreceptor-heart rate reflex is dependent on suprapontine regions (8-10, 12). The present study suggests that the contribution to the reflex response of apnea without smoke stimulation made by vagal afferents on the one hand and the carotid sinus and aortic nerves on the other are both dependent on suprapontine regions.

The reflex drive of smoke stimulation without apnea in rabbits with section of the carotid sinus and aortic nerves probably arises mainly from trigeminal afferents (14). In these animals the reflex effects in part depend on intact cerebral hemispheres, since cardiac slowing is greatest in sham-operated rabbits. When the input from the baroreceptors is also intact, smoke stimulation without apnea produces greater cardiac slowing that does not differ significantly in the three neurological preparations.

In spontaneously breathing animals with section of the carotid sinus and aortic nerves the cardiac slowing evoked by the nasal inhalation of smoke depends not only on the effects of apnea alone but also on the direct effects of smoke stimulation, since bradycardia is evoked in thalamic and pontine rabbits as well as in sham-operated animals. With intact arterial baroreceptors the heart rate response of each preparation is still further enhanced. In the intact animal the bradycardia due to smoke inhalation thus appears to be a complex response depending on at least three sets of peripheral inputs: 1) the arterial baroreceptors; 2) the direct nasopharyngeal effects of smoke stimulation, presumably through trigeminal afferents; and 3) the effects of apnea, predominantly through various vagal afferents.

The reflex changes in mesenteric vascular conductance are probably also the result of mechanisms involving suprapontine structures. Thus in animals in which the buffer nerves have been cut the effects of apnea without smoke stimulation and the effects of smoke stimulation without apnea differ in magnitude between sham-operated, thalamic, and pontine rabbits. In these animals the effects of apnea alone rely on cerebral activity since only in sham-operated animals is there any significant fall in conductance. Smoke stimulation alone reduces conductance in all preparations but the effects are most pronounced in sham-operated animals. When the carotid sinus and aortic nerves are intact the reduction in conductance with apnea alone or with smoke stimulation without apnea is considerably more pronounced. This possibly reflects a greater degree of reflex vasoconstriction, though this conclusion is somewhat uncertain in view of the difference in resting conductance in animals with and without section of the buffer nerves (Table 1). Because of the rise in arterial pressure the arterial baroreceptor input might be expected to minimize the mesenteric vasoconstrictor response instead of accentuating it. It therefore is possible that the arterial chemoreceptor input is responsible for the enhanced constrictor response. The role of the chemoreceptors on mesenteric conductance has not previously been analyzed, but they appear to make little (if any) contribution to the bradycardia (14). An alternative explanation is that projections from the trigeminal afferents and vagal inputs could exert modulating effects on vasoconstrictor neurons also receiving arterial baroreceptor projections producing arterial baroreceptor system resetting (8,
CNS CONTROL OF SMOKE REFLEX IN RABBIT

10, 12). If the trigeminal and vagal inputs altered threshold and/or influenced gain of the baroreceptor system it would be possible to enhance the constrictor effects at elevated arterial pressures.

In the intact animal the mesenteric constrictor response of spontaneously breathing animals thus appears to depend (like the heart rate response) on the simultaneous changes in inputs from trigeminal afferents, vagal afferents, and the arterial baroreceptors (and/or chemoreceptors). No quantitative conclusions can be made regarding the relative importance of apnea alone or smoke stimulation without apnea in the mesenteric constrictor response, since the stimuli used under conditions of controlled ventilation are not likely to reproduce quantitatively the input profile evoked by the smoke stimulus in the spontaneously breathing animals.

In the present study the differences in reflex circulatory responses between sham-operated and pontine animals breathing spontaneously with all afferents intact have been relatively small. The differences between preparations become more apparent when the individual components of the afferent input profile are examined. This is consistent with Andersen's (1) conclusion that the pontomedullary breathing spontaneously with all afferents intact have been relatively small. The differences between preparations become more apparent when the individual components of the afferent input profile are examined. This is consistent with Andersen's (1) conclusion that the pontomedullary region is the major site of central nervous integration of the reflex bradycardia evoked in the somewhat analogous diving response of the duck. In contrast to the present approach Andersen did not study the individual components of the afferent input profile (i.e., the effects of apnea alone, the effects of head stimulation, or the role of the baroreceptors).

The present findings suggest that the magnitude of the interaction between the different afferent inputs differs quantitatively at different anatomical sites in the central nervous system. Alternatively, the brain sections may have interrupted facilitatory pathways descending to autonomic neurons in bulbo-sympathetic regions. In both cases the results have demonstrated the importance of suprapontine structures in the reflex response. Nevertheless, it is remarkable to what degree loss of suprapontine structures can be compensated by diencephalic or bulbo-sympathetic mechanisms, provided all relevant afferents were intact.

We gratefully acknowledge the valuable technical assistance of Mr. D. Lauff.

This study was supported by research grants from the Life Insurance Medical Research Fund of Australia and New Zealand, the National Heart Foundation of Australia, the Australian Tobacco Research Foundation, the National Health and Medical Research Council of Australia, and the Postgraduate Medical Foundation of the University of Sydney.

R. J. McRitchie is a Research Fellow of the National Heart Foundation of Australia.

The present address of S. White is: School of Medicine, The Flinders University of South Australia, Bedford Park, S. A. 5042.

Received for publication 22 August 1973.

REFERENCES