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RESPIRATORY MODULATION OF BARORECEPTOR AND CHEMORECEPTOR REFLEXES AFFECTING HEART RATE THROUGH THE SYMPATHETIC NERVOUS SYSTEM

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SUMMARY

1. Brief stimuli were delivered to the carotid body chemoreceptors or the carotid sinus baroreceptors at different phases of the respiratory cycle in anaesthetized dogs. Chemoreceptor stimulation was achieved by injecting small volumes (0·2–0·5 ml.) of warmed saline equilibrated with CO_2 near to the carotid bodies on both sides. Baroreceptor stimulation was achieved by injecting larger volumes (2–5 ml.) of saline equilibrated with air into the region of the carotid bifurcation on both sides, after first clamping the common carotid arteries.

2. When the vagus nerves were intact, but sympathetic nervous effects on heart rate were blocked by administration of propranolol, there was a prompt and pronounced bradycardia evoked when either baroreceptor or chemoreceptor stimuli were given in expiration, but little or no change in heart rate when they were given in inspiration.

3. When the vagus nerves were cut, but sympathetic nervous function was intact, respiratory modulation of both baroreceptor and chemoreceptor reflex effects on heart rate could still be demonstrated. The bradycardia evoked by either stimulus was more marked for stimuli given in expiration than for stimuli given in inspiration. A complementary response pattern for brief decreases in baroreceptor stimulation (carotid occlusions) was demonstrated: the tachycardia evoked by occlusions timed during inspiration was greater than that evoked by occlusions timed during expiration. All the reflex effects were mediated by the sympathetic system because they were abolished by administration of propranolol.

4. Typically, the sympathetic reflex effects were slight in comparison with the vagal reflexes evoked by either chemoreceptor or baroreceptor stimuli.

INTRODUCTION

Stimuli delivered to the carotid arterial baroreceptors or chemoreceptors evoke reflex bradycardia more effectively when they are timed to occur during the expiratory, rather than the inspiratory, phase of the respiratory cycle (Koepchen, Wagner & Lux, 1961; Haymet & McCloskey, 1974, 1975; Neil & Palmer, 1975; Kordy, Neil & Palmer, 1975). That the bradycardia is mediated principally by the vagus nerve has been shown by blocking it by vagotomy or with atropine, and by recording directly from cardiac vagal efferent nerves (Haymet & McCloskey, 1975; Neil & Palmer, 1975; Kordy *et al.* 1975; Davidson, Goldner & McCloskey, 1976).

It is known, however, that the carotid baroreceptors and chemoreceptors can also slow the heart reflexly by withdrawing cardiac sympathetic tone (e.g. Bronk, 1933; Daly & Scott, 1958; Downing, Remensynder & Mitchell, 1963). We were therefore interested to see whether or not the sympathetic effects of these reflexes can be similarly modulated during the respiratory cycle. The results we present here indicate that they can.

METHODS

Experiments were performed on twenty-one adult dogs of either sex weighing from 6 to 18 kg. All but four of the animals were anaesthetized with I.V. pentobarbitone (Nembutal, Abbott; 35 mg/kg). The remaining four received I.V. chloralose (α-chloralose, British Drug Houses; 80 mg/kg), after induction with thiopentone. In each dog the trachea was cannulated low in the neck, and nylon cannulae were inserted, with their tips pointing towards the heart, into the lingual and external carotid arteries on both sides. On each side the tips of these cannulae were positioned close to each other and in close communication with the carotid sinus. Arterial pressure was recorded through one of the lingual arteries using a Statham P23 AC transducer, and was recorded on a Grass Polygraph pen recorder. Intra-tracheal pressure, as an indicator of air-flow, was recorded through a wide (2 mm i.d.) catheter inserted into the trachea, using another Statham transducer, and was also recorded on the polygraph. In five experiments, respiratory movements were recorded by the alternative method of registering tension changes produced through an elastic band sewn to the chest wall and connected to a Grass FT03 force transducer (cf. Levy, DeGeest & Zieske, 1966). On the two remaining channels of the polygraph were recorded the electrocardiogram and the beat-by-beat heart rate (Grass 7P4 Cardiotachometer, triggered from the e.c.g.). The calibration of the cardiotachometer was checked by running simultaneous records of e.c.g. and heart rate at a fast paper speed.

Brief chemoreceptor and baroreceptor stimuli were given as decribed by Haymet & McCloskey (1975). The chemoreceptor stimuli were provided by sudden retrograde injections into the external carotid arteries of small volumes (0·2–0·5 ml.) of warmed CO_2-equilibrated saline. Injections of similar small volumes of air-equilibrated saline were always without reflex effect. Baroreceptor stimuli were delivered by sudden retrograde injections of 2–5 ml. air-equilibrated saline, or of freshly drawn arterial blood, into the external carotid arteries after the common carotid arteries had first been clamped below the carotid sinus. Stimuli were usually given simultaneously on both sides.

In order to study the effects of the sympathetic nervous system on heart rate, the vagi were cut after the reflex responses with vagi intact had been observed (see Results). To ensure removal of vagal efferent effects, atropine was also given (usually 1 mg, repeated hourly). Electrical stimulation of the cardiac ends of the cut vagi was then without effect on heart rate. There then remained a slight sinus arrhythmia. This could be attributed to the waxing and waning of sympathetic tone with the respiratory cycle because in all animals it could be abolished, at the end of the experiments, by administration of propranolol (1 mg/kg). It was found that this sympathetically mediated sinus arrhythmia was most pronounced when the respiratory rate was slow, and most experiments were performed in such conditions. This was achieved by keeping the level of anaesthesia deep, and by maintaining the animal at a slightly cool temperature (35–37 °C).

RESULTS

(i) *Responses in which sympathetic effects were excluded.* Responses to brief baroreceptor and chemoreceptor stimuli were always examined first with the vagi intact. The findings of Haymet & McCloskey (1975) were confirmed: there was a prompt and pronounced bradycardia evoked when the stimuli were given in expiration, but little or no change in heart rate when they were given in inspiration. These responses were preserved in three animals in which any possible effects from sympathetic withdrawal were excluded by pretreatment with propranolol in a dose sufficient to abolish any heart rate response to administration of 10–20 μg isoprenaline (see Fig. 1). The usual dose of propranolol was 1 mg/kg. The responses were subsequently abolished by vagotomy in these animals.

(ii) *Baroreceptor responses.* After vagotomy and administration of atropine, the responses to brief bilateral baroreceptor stimuli were again tested in eighteen dogs. In ten of these, there was a slowing of at least 15 beats/min (range 15–25) whenever the baroreceptor stimuli were timed to occur in expiration, but less than 10 beats/min (range 5–10) when the stimuli were given in inspiration (for these comparisons the heart rates at corresponding points in the two respiratory cycles before stimulation were taken as controls). Typical responses of this kind are shown in Fig. 2. In the remaining animals, there was either no response to the brief baroreceptor stimuli whenever delivered (four dogs), or a small (5 beats/min) and variable response, which bore no demonstrable relation to the respiratory cycle (four dogs).

The responses to baroreceptor stimulation were typically much slower after vagotomy than when the vagi were intact. Slowing usually commenced about 1–2 sec and was most marked about 5–7 sec after the stimulus was given. Fig. 3 shows the time course of a typical response, which can be compared with the very rapid responses seen when the vagi were intact (Fig. 1).

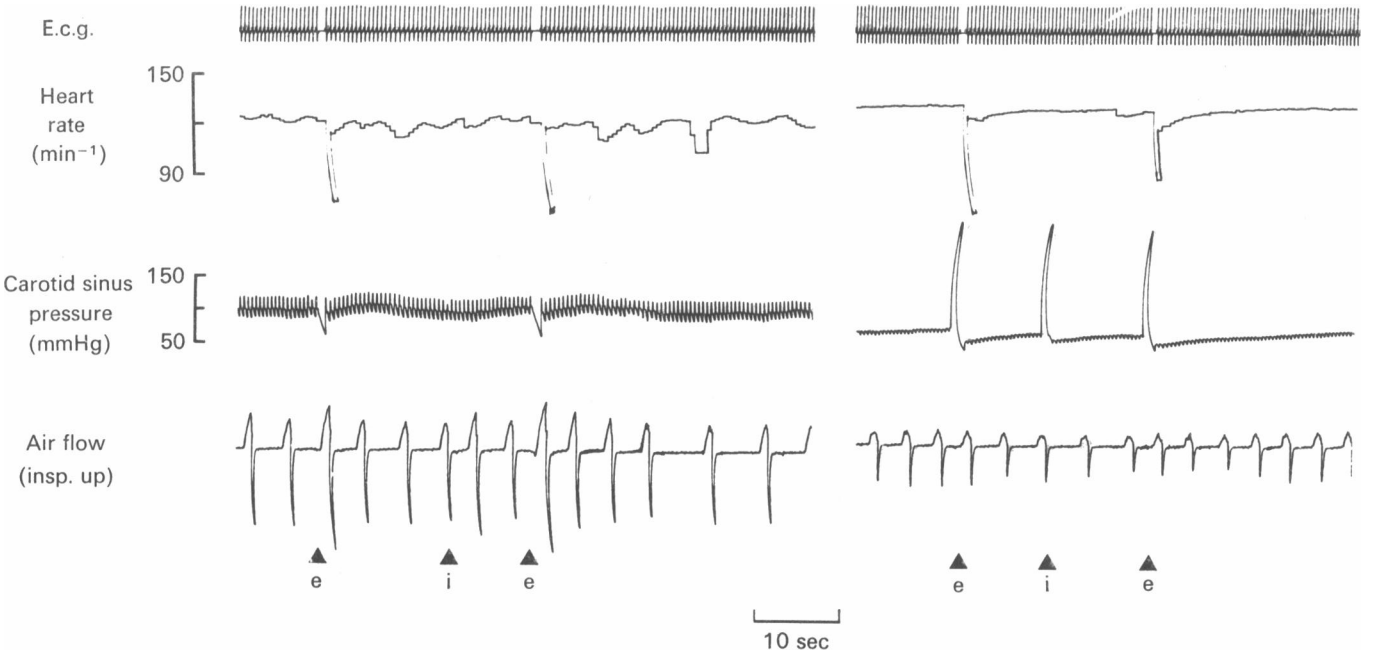

Fig. 1. Dog: chloralose and propranolol. These records show reflex effects of brief chemoreceptor and baroreceptor stimuli delivered at different points in the respiratory cycle – during expiration marked 'e', or during inspiration marked 'i'. Electrocardiogram, heart rate, carotid sinus blood pressure and tracheal air flow are shown. In the panel on the left are shown the effects of three successive chemoreceptor stimuli (injections of 0·5 ml. CO_2-equilibrated saline into the carotid bifurcation): the first and third stimuli, which were given during the expiratory phase of breathing, evoked a prompt reflex bradycardia; the second stimulus, given during inspiration, did not affect the heart rate. In the panel on the right are shown the effects of three successive baroreceptor stimuli (injections of approx. 3 ml. air-equilibrated saline into the carotid bifurcation after clamping the common carotid artery): the first and third stimuli, which were given during the expiratory phase of breathing, evoked a prompt reflex bradycardia; the second stimulus, given during inspiration, did not affect heart rate (the sensitivity of the tracheal air flow trace was altered between records).

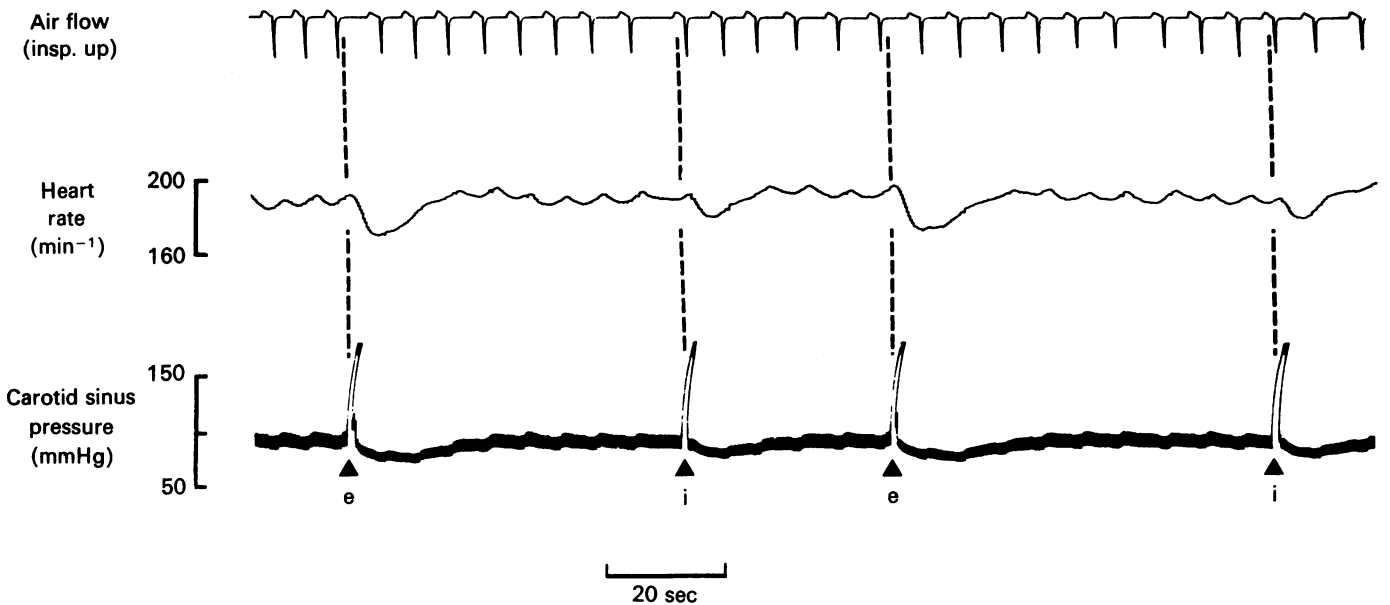

Fig. 2. Vagotomized dog, anaesthetized with pentobarbitone and given atropine. Record shows tracheal air flow, heart rate and carotid sinus blood pressure (both common carotid arteries clamped). Four brief baroreceptor stimuli were delivered, by simultaneous injections of approx. 3 ml. air-equilibrated saline into both carotid sinuses, at the markers. The first and third stimuli (marked 'e') were delivered during the expiratory phase of breathing, and slowed the heart rate more than the second and fourth stimuli (marked 'i'), which were delivered during inspiration.

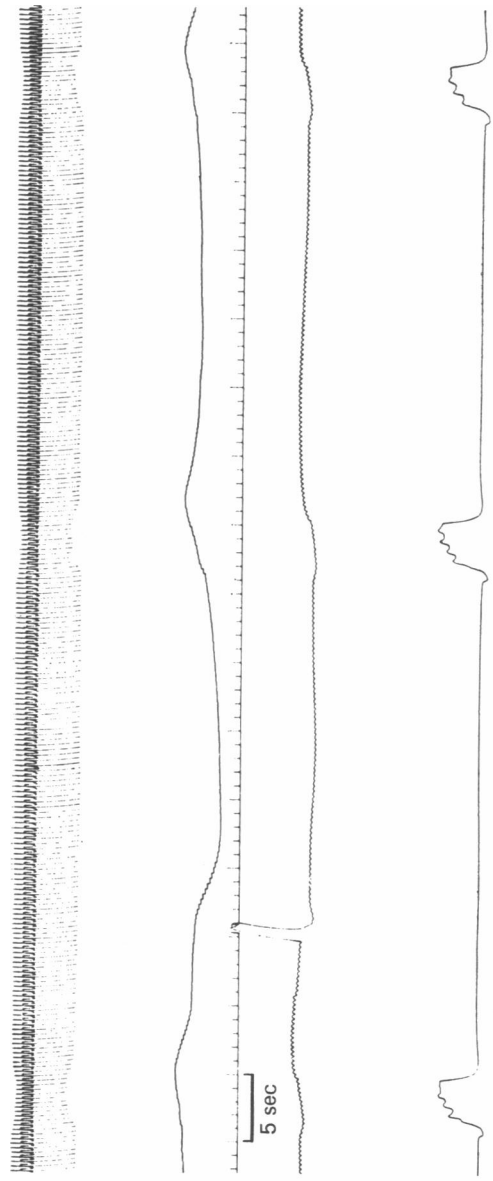

Fig. 3. Vagotomized dog, anaesthetized with pentobarbitone and given atropine. Record shows tracheal air flow, heart rate, carotid sinus blood pressure, and respiratory movements. A brief pulse of pressure in the carotid sinus, caused by the sudden injection of approx. 3 ml. air-equilibrated saline, was a baroreceptor stimulus delivered during the expiratory phase of breathing. The time course of the reflex slowing of the heart evoked by the stimulus is shown.

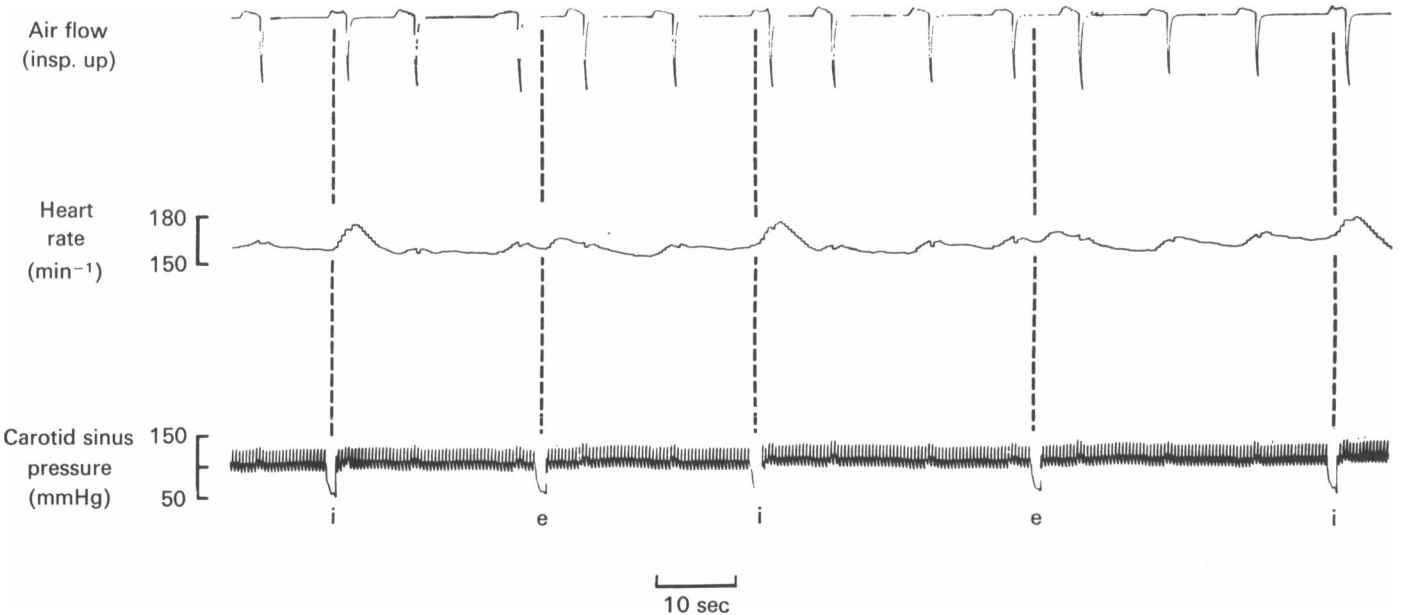

Fig. 4. Vagotomized dog, anaesthetized with pentobarbitone and given atropine. Record shows tracheal air flow, heart rate and carotid sinus blood pressure. The five falls in carotid sinus blood pressure were caused by briefly occluding both common carotid arteries. When the occlusions were timed to occur during the inspiratory phase of breathing (marked 'i') they evoked reflex increases in heart rate which were greater than those evoked by occlusions timed to occur during the expiratory phase of breathing (marked 'e').

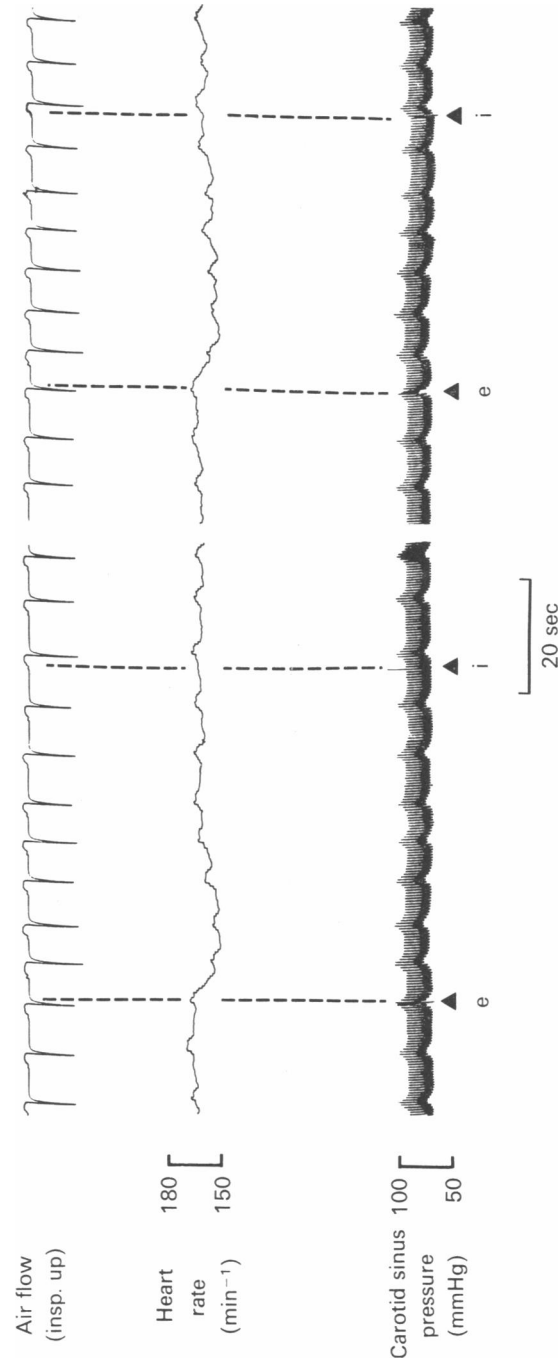

Fig. 5. Vagotomized dog, anaesthetized with pentobarbitone and given atropine. Record shows tracheal air flow, heart rate and carotid sinus blood pressure. Four brief chemoreceptor stimuli were delivered by simultaneous injections of 0·5 ml. CO_2-equilibrated saline into both carotid sinuses, at the markers. The first and third stimuli (marked 'e') were delivered during the expiratory phase of breathing, and slowed the heart more than the second and fourth stimuli (marked 'i'), which were delivered during inspiration.

In ten of the vagotomized animals the effects of decreased baroreceptor activity were investigated. Cotton loops passed around both common carotid arteries low in the neck were pulled upon to produce brief reductions in the pressure within the carotid sinus. During this manoeuvre care was taken not to pull *along* the common carotid arteries, so as to avoid stimulating stretch-sensitive baroreceptors further downstream. In six of the animals, occlusions timed to occur during inspiration caused reflex increases in heart rate of at least 15 beats/min (range 15–30), whereas occlusions occurring during the expiratory phase of the respiratory cycle caused increases of less than 6 beats/min (range 2–6). An example of this type of response is shown in Fig. 4. This pattern of response complements the pattern described above for responses to brief rises of carotid sinus pressure: it is easier to evoke a reflex bradycardia with pressure increases timed in expiration, and easier to evoke a reflex tachycardia with pressure decreases timed in inspiration (we also looked for responses to brief withdrawals of baroreceptor, and chemoreceptor, stimulation in animals with intact vagi – responses which would have involved withdrawal of vagal tone – but found no consistent effects). In the remaining animals in which brief carotid occlusions were performed, variable responses (sometimes of as much as 20 beats/min) were observed, but no relation to the respiratory cycle could be demonstrated.

All of the baroreceptor responses, whether to pulses of increased pressure or to brief carotid occlusions, were abolished following administration of propranolol (1 mg/kg).

(iii) *Chemoreceptor responses.* Brief bilaterial stimuli were delivered to the carotid arterial chemoreceptors by retrograde injections of 0·2–0·5 ml. CO_2-equilibrated saline into the external carotid arteries in the same eighteen vagotomized dogs in which the brief baroreceptor stimuli were given. In six of these animals, chemoreceptor stimuli given during expiration evoked a reflex slowing of the heart of at least 15 beats/min (range 15–20), while stimuli given during the inspiratory phase of breathing evoked reflex responses of less than 5 beats/min (range 0–5). These same animals gave similarly modulated responses when baroreceptor stimuli were applied during different phases of the respiratory cycle. Records obtained from one of these animals are shown in Fig. 5.

In the experiments using chemoreceptor stimuli, marked changes in breathing were frequently evoked. It thus became difficult to compare the exaggerated sinus arrhythmia immediately following stimulation with that which preceded it. The six animals described above in which respiratory modulation of changes in heart rate was clearly shown were notably poor in their ventilatory responses to chemoreceptor stimulation. The most common problem encountered in the other animals was that

stimuli delivered during the expiratory pause evoked an immediate inspiratory effort. The tachycardia associated with such premature inspirations probably masked any immediate direct reflex effects on heart

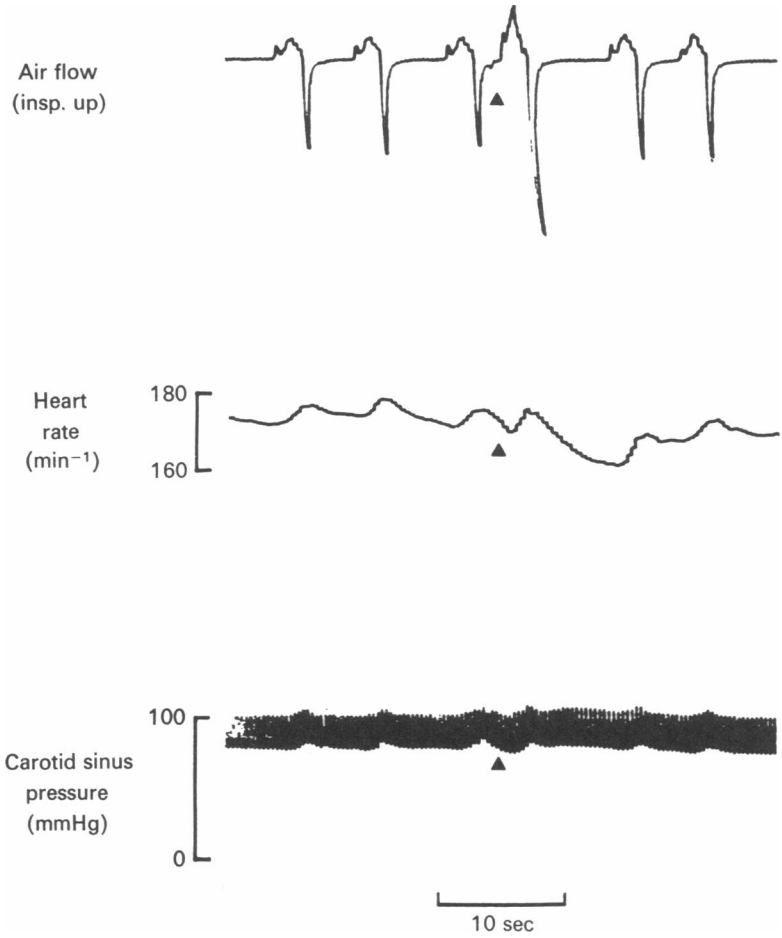

Fig. 6. Vagotomized dog, anaesthetized with pentobarbitone and given atropine. Record shows tracheal air flow, heart rate and carotid sinus blood pressure. At the marker, a brief bilateral chemoreceptor stimulus was given. The stimulus was given during the expiratory phase of breathing, but immediately evoked a large premature breath. There was a bradycardia following the evoked breath.

rate. In many animals, however, a marked bradycardia occurred in the expiratory pause following the premature inspiratory effort (see Fig. 6).

In further experiments we attempted to examine the effects of brief reductions in chemoreceptor stimulation. Retrograde injections of small volumes of O_2-equilibrated bicarbonate solution were made in animals

breathing air or hypoxic gas mixtures. Only slight and variable reflex effects on heart rate were observed in these experiments. All of the changes in heart rate evoked by arterial chemoreceptor stimulation were abolished following administration of propranolol (1 mg/kg).

DISCUSSION

The experiments we have described here show that the effectiveness of carotid baroreceptor and chemoreceptor stimuli in evoking reflex changes in heart rate through the sympathetic nervous system depends on the phase of the respiratory cycle in which the stimuli are given. The sympathetic efferent components of these reflexes are thus modulated by the respiratory cycle in a manner similar to the vagal components (Koepchen *et al.* 1961; Haymet & McCloskey, 1975). We have demonstrated the sympathetic modulation in the absence of vagal effects, and the vagal modulation in the absence of sympathetic effects. The responses mediated by withdrawal of sympathetic tone are slower and smaller than vagally mediated reflex changes in the same direction, and we found them considerably more difficult to demonstrate than the vagal effects.

There have been reports that it is difficult to demonstrate a sympathetically based sinus arrhythmia following vagotomy or the administration of atropine (e.g. Anrep, Pascual & Rossler, 1936). Most of our animals showed the phenomenon, probably because our efforts to slow the respiratory rate meant that there was time for the rather sluggish sympathetic effects to develop fully with each breath. The same slow respiratory rate probably also gave time for the sympathetic reflex effects to become apparent. If this is so, the responses we have described here are likely to be much less marked in animals with a more normal respiratory rate. Certainly, we had difficulty in demonstrating responses in faster breathing animals, and often saw them only after the respiratory rate had been slowed by moderate cooling or by deepening the level of anaesthesia.

Respiratory effects on reflexes involving the sympathetic nervous system have been described previously. Seller, Langhorst, Richter & Koepchen (1968) showed that a more pronounced vasodilatation in the vascular bed of the gracilis muscle was evoked by electrical stimulation of the carotid sinus nerve applied in the expiratory phase of breathing, than by similar stimuli given in inspiration. It has also been found that electrical stimuli given to the carotid sinus nerve during the expiratory phase of breathing gives a stronger inhibition of abdominal, cervical and lumbar sympathetic neural activity than stimuli given during inspiration (Seller *et al.* 1968; Richter, Keck & Seller, 1970). Difficulty in interpreting these results arises, however, because both baroreceptor and chemoreceptor

afferents would have been excited by electrical stimulation of the sinus nerve, and these are known to have opposite effects on sympathetic vascular tone: chemoreceptor stimulation causes sympathetic vasoconstriction (Daly & Scott, 1963), and baroreceptor stimulation withdraws sympathetic tone (Koizumi, Seller, Kaufman & Brooks, 1971). Seller *et al.* (1968) argued that the chosen intensities of electrical stimulation applied to the sinus nerves in their experiments were such as to stimulate baroreceptor afferents alone, without exciting chemoreceptor afferents. This is supported by their finding little evidence of respiratory responses to their stimuli. Nevertheless, it is known that baroreceptor and chemoreceptor afferents are represented in both the myelinated and the unmyelinated fibre groups of the sinus nerve (Fidone & Sato, 1969), and complete selectivity in any form of electrical stimulation would appear impossible. Indeed, in other studies (e.g. Black & Torrance, 1971; Eldridge, 1972), electrical stimulation of the sinus nerve has been employed specifically to study the effects of excitation of the chemoreceptor fibres within it. If Seller *et al.* (1968) are correct in assuming that their electrical stimuli involved mainly baroreceptor afferents, then their results are consistent with those we have presented here: if they stimulated mainly chemoreceptors, then their results and ours are at variance. By studying the sympathetic control of heart rate, however, we have chosen to examine a system in which stimulation of either chemoreceptors or of baroreceptors will evoke a similar response, namely sympathetic withdrawal (e.g. Bronk, 1933; Daly & Scott, 1958). It is worth noting that we also performed another long series of experiments (more than twenty dogs) in which we looked for changes in the resistance of the vascular bed of the isolated gracilis muscle in response to specific functional baroreceptor and chemoreceptor stimuli of the type used here: we found only small and variable responses which bore no obvious relationship to the phase of the respiratory cycle in which they were given.

The respiratory modulation of cardiac sympathetic activity which we have described here was clearly not imposed by phasic afferent traffic travelling along the vagi, because the vagi were cut in our experiments. Nevertheless, it remains possible that the modulation could be altered, perhaps augmented, by such afferent inputs in intact animals. An augmentation of the effect might indeed by expected from the work of Daly & Scott (1958), who showed that the activation of intrapulmonary receptors by inflating the lungs could accelerate the heart by both vagal and sympathetic reflex mechanisms.

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