J. Physiol. (1976), **259**, *pp.* 523–530
With 5 text-figures
Printed in Great Britain

RESPIRATORY MODULATION OF BARORECEPTOR AND CHEMORECEPTOR REFLEXES AFFECTING HEART RATE AND CARDIAC VAGAL EFFERENT NERVE ACTIVITY

BY N. S. DAVIDSON,* S. GOLDNER AND D. I. McCLOSKEY

From the School of Physiology and Pharmacology, University of New South Wales, Post Office Box 1, Kensington 2033, Sydney, Australia

(*Received 20 January 1976*)

SUMMARY

1. Brief stimuli were delivered to the carotid chemoreceptors or baroreceptors in dogs anaesthetized with chloralose. Chemoreceptor stimulation was achieved by rapid retrograde injections of 0·2–0·5 ml. CO_2 equilibrated saline through a cannula in the external carotid artery. Baroreceptor stimulation was achieved by forceful retrograde injection of 2–5 ml. air-equilibrated saline into the external carotid artery after first clamping the common carotid artery.

2. Prompt decreases in heart rate were elicited by brief sudden chemoreceptor or baroreceptor stimuli when these were delivered during the expiratory phase of respiration. The stimuli did not modify the control heart rate pattern when delivered in the inspiratory phase of respiration. This respiratory modulation of reflex effectiveness persisted when the animals were completely paralysed and the phase of the respiratory cycle was monitored through a phrenic electroneurogram.

3. Single cardiac vagal efferent nerve fibres were dissected from the cut central end of the right cervical vagus nerve. They were classified as cardiac efferents by their cardiac and respiratory rhythmicity, and by their increased activity in response to stimulation of a carotid sinus nerve or to mechanical elevation of the systemic arterial pressure. These efferent fibres increased their activity in response to brief chemoreceptor or baroreceptor stimuli delivered in expiration, but did not respond to stimuli delivered in inspiration. This respiratory modulation of both reflexes persisted after bilateral cervical vagotomy.

* Asthma Foundation of N.S.W., Scholar

INTRODUCTION

In 1961, Koepchen, Lux & Wagner demonstrated that the reflex slowing of the heart evoked by stimulation of the carotid sinus baroreceptors is more pronounced when the baroreceptor stimuli are timed so as to coincide with the expiratory, rather than with the inspiratory, phase of the respiratory cycle.

Haymet & McCloskey (1975) confirmed these findings, and observed that the reflex bradycardia evoked by stimulation of the carotid chemoreceptors was similarly more pronounced when the stimuli were given during expiration rather than inspiration. They showed also that the respiratory modulation of the baroreceptor and chemoreceptor reflexes is not entirely caused through the activation, during inspiration, of slowly adapting mechanoreceptors in the thorax or lungs: both reflexes remain strong during the inspiratory apnoea of the Hering–Breuer inflation reflex, and during maintained inflation in periods of post-hyperventilation apnoea. Moreover, the respiratory effects upon both reflexes persist after pulmonary denervation.

In the present study we show first, that respiratory modulation of both reflexes persists in dogs which are paralysed, and second, that the observations of Haymet & McCloskey (1975), referred to above, are borne out by the responses of single cardiac vagal efferent nerve fibres to brief baroreceptor or chemoreceptor stimuli. Part of this work has been reported in brief form (Goldner & McCloskey, 1975).

METHODS

Experiments were performed on twenty-seven adult dogs of both sexes weighing from 5 to 17 kg. The animals were anaesthetized with I.V. chloralose (α-chloralose: British Drug Houses: 80 mg/kg) after induction with thiopentone. Usually a supplemental dose of morphine (1–2 mg/kg) was given early in the period of anaesthesia before the experimental procedures. In each dog the trachea was cannulated low in the neck, and nylon cannulae were inserted, with their tips facing towards the heart, into the lingual and external carotid arteries, usually on the left side. The tips of these cannulae were positioned close to each other and in close communication with the carotid sinus. A nylon cannula was inserted into a femoral or external jugular vein for administration of anaesthetics and drugs and, in some dogs, a balloon-tip cannula was inserted through a femoral artery and advanced so that the inflatable balloon lay in the upper abdominal aorta. Rectal temperature was kept between 37 and 39 °C.

Arterial pressure was recorded from a lingual artery using a Statham P23 AC transducer. In paralysed animals, respiratory activity was recorded from a whole de-sheathed phrenic nerve through Ag-AgCl electrodes, and this phrenic neural discharge was integrated using a Grass 7P3B preamplifier. To produce paralysis I.V. D-tubocurarine (1–2 mg/kg) was given slowly until all respiratory movements ceased; the animals were then ventilated on pure oxygen using a Starling Ideal pump,

adjusted so that some phrenic neural activity remained. Observations on reflex effectiveness during paralysis were made in periods of 20–40 sec during which the respiratory pump was temporarily stopped. In these same animals records of electrocardiogram and heart rate were obtained using a Grass 5P4D preamplifier. Blood pressure, e.c.g., heart rate, and phrenic neural activity were recorded on a Grass polygraph.

Spontaneously breathing animals were used in the study of activity in cardiac vagal efferent nerves. In these animals a record of tracheal air-flow was obtained by passing a wide (2 mm i.d.) nylon tube about 5 cm into the trachea and connecting it to a Grass 5PT5A volumetric pressure transducer. Arterial pressure was obtained from a lingual artery as before. The entire pharynx and larynx were removed from just above the sternum up to the level of the hyoid bone, and the skin flaps were raised to make a pool which was then filled with liquid paraffin. The vagus nerve on the right-hand side was then cut, desheathed, and the central end was laid across a rigid, earthed, stainless-steel plate with a blackened upper surface. The nerve was then divided into filaments under the microscope with fine forceps. Neural activity in the filaments was recorded by lifting them, one by one, on to fine stainless-steel electrodes, which were connected through a Tektronix 122 preamplifier to an oscilloscope and speaker. Filaments were dissected until single cardiac efferents were recorded. Nerves were considered to be cardiac efferents if they demonstrated a cardiac rhythm, and a waning of their activity during inspiration (Jewett, 1974). Confirmation of this characterization was always obtained in one of two ways, (i) the nerve responded with a latency of 40–60 msec to single shocks applied to the central end of a carotid sinus nerve (cf. Iriuchijima & Kumada, 1964) or (ii) the nerve increased its discharge while maintaining its cardiac and respiratory rhythms, in response to a 'mechanical' elevation of blood pressure produced by inflating an aortic balloon, Records of carotid sinus blood pressure, tracheal air-flow, and cardiac vagal activity were obtained on an ultraviolet recorder (Y.E.W. Type 2901), with galvanometers giving a flat frequency response to 1000 Hz. Records obtained with this instrument have required retouching.

Brief baroreceptor and chemoreceptor stimuli were given in the way described by Haymet & McCloskey (1975). The chemoreceptor stimuli were provided by sudden retrograde injections into an external carotid artery 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 of freshly drawn arterial blood into an external carotid artery, after first clamping the common carotid artery downstream of the carotid sinus.

RESULTS

(i) *Respiratory modulation of reflexes during paralysis.* In this part of the study eight dogs were used. All showed the phenomenon described by Koepchen *et al.* (1961) and Haymet & McCloskey (1975): brief baroreceptor or chemoreceptor stimuli evoked no reflex bradycardia when delivered during inspiration, but did evoke prompt bradycardia when given in expiration. The dogs were then paralysed, as described, and artificially ventilated on O_2. During periods in which the ventilation was temporarily stopped, baroreceptor and chemoreceptor stimuli were again delivered. The activity recorded from the phrenic nerve was used to indicate the

phase of the paralysed animals' continuing respiratory cycle. Baroreceptor and chemoreceptor stimuli which evoked no reflex bradycardia when delivered in the inspiratory phase of the neural respiratory cycle, evoked prompt bradycardia when delivered in the expiratory phase of the cycle. This occurred in all dogs. Characteristic responses from one dog are shown in Fig. 1.

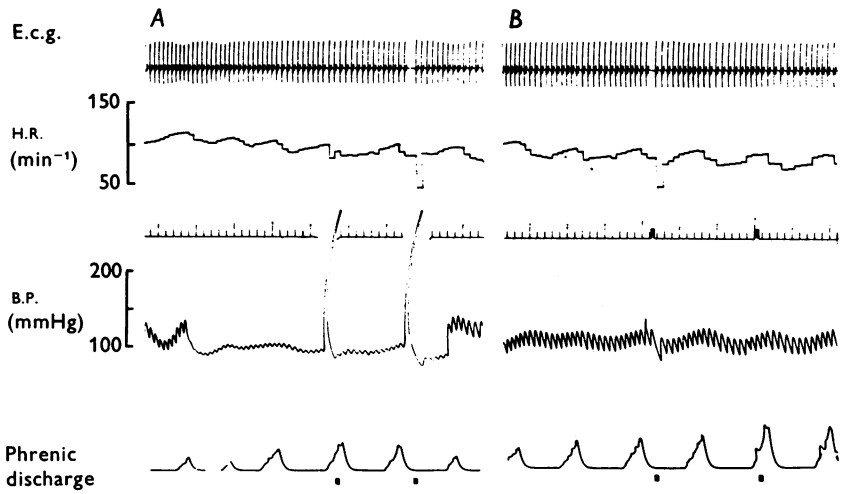

Fig. 1. This shows recordings made in a dog anaesthetized with chloralose and paralysed with D-tubocurarine. The animal had been artificially ventilated on pure O_2 until immediately before each panel of this Figure was recorded: during each recorded period the ventilator was temporarily stopped. In the period between recording the left and right panels of the Figure, artificial ventilation had been resumed for about 3 min. Records of e.c.g., heart rate, carotid sinus blood pressure, and integrated phrenic electroneurogram are shown. In the left panel, two brief baroreceptor stimuli were delivered, one during the inspiratory, and one during the expiratory phase of the respiratory cycle. Only the expiratory stimulus slowed the heart. In the right panel, two brief chemoreceptor stimuli were delivered, one during the inspiratory, and one during the expiratory phase of the respiratory cycle. Again, only the expiratory stimulus slowed the heart.

(ii) *Responses of cardiac vagal efferents to baroreceptor and chemoreceptor stimuli.* Fourteen single cardiac vagal efferent nerves from twelve dogs were studied in detail. Of these fibres (dissected from the right vagus), twelve gave their responses to stimuli given in the left carotid reflexogenic area, and two to stimuli given on the right (this grouping simply reflects the more common use of the left side for delivery of stimuli). Nine fibres responded to both baroreceptor and chemoreceptor stimuli, although commonly one form of stimulus evoked larger responses than the other.

Three fibres gave responses to baroreceptor stimuli given on the test side but not to chemoreceptor stimuli on the same side, and two fibres responded to chemoreceptor but not baroreceptor stimuli on the same side. About an equal total number of fibres, identified as cardiac efferents by our criteria, did not respond to either form of stimulus. Presumably these fibres received their afferent inputs from another source. All did, however, receive baroreceptor or chemoreceptor inputs from some source, as our identifying procedures depended upon the responses to such inputs.

All responding fibres behaved similarly. All gave bursts of activity in response to baroreceptor or chemoreceptor stimuli delivered during expiration, but were silent, or occasionally responded with just a single action potential, to similar stimuli delivered during inspiration. Typical responses are shown in Figs. 2 and 3.

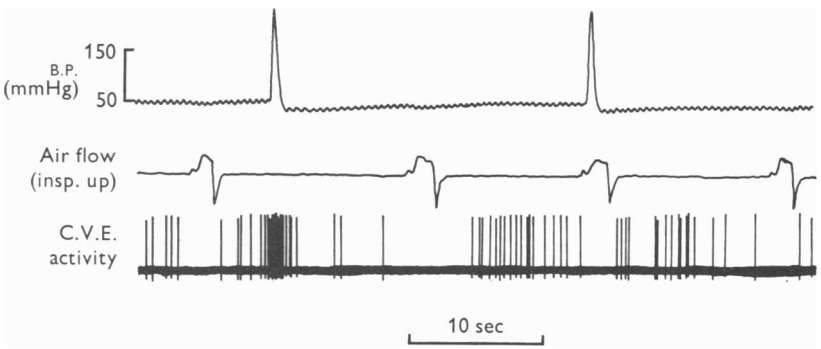

Fig. 2. Dog, chloralose and morphine. Records of carotid sinus blood pressure, respiratory air flow, and the activity of single cardiac vagal efferent nerve (C.V.E.) are shown. A burst of firing in the cardiac efferent nerve was evoked by a baroreceptor stimulus timed so as to occur in the expiratory pause. No firing was evoked when a similar stimulus was given during inspiration (the amplitude of recorded action potentials in this and other experiments was in the range 100–300 μV).

Bursts of firing were always elicited in response to baroreceptor or chemoreceptor stimuli delivered during the inspiratory apnoea of the Hering–Breuer inflation reflex (trachea occluded at normal inspiratory end-point).

The respiratory modulation of baroreceptor and chemoreceptor reflex effectiveness continued after the left vagus nerve was cut (the right vagus was cut in all experiments prior to recording from nerve fibres). In some animals, complete vagotomy led to a pattern of breathing characterized by inspiratory spasms lasting some seconds. Although no air-flow was occurring during large parts of these periods of inspiratory spasm, the

inspiratory block of vagal responses to baroreceptor and chemoreceptor stimuli remained throughout. Fig. 4 shows the responses of one of the fibres to chemoreceptor stimulation during the respiratory cycle after complete vagotomy.

It is possible that the inspiratory block of the reflex pathways, although remaining operative during periods of inspiratory spasm after vagotomy,

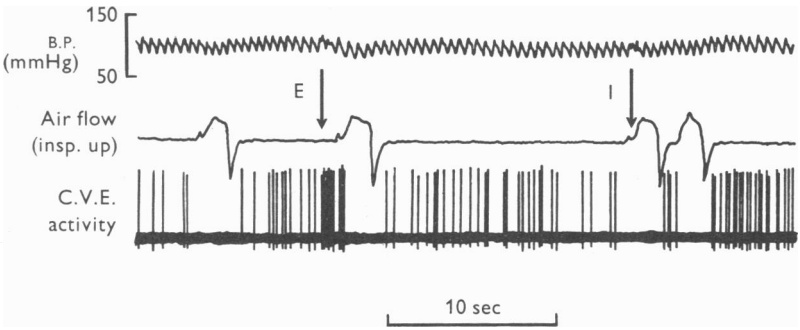

Fig. 3. Dog, chloralose and morphine. Records of carotid sinus blood pressure, respiratory air flow, and the activity of a single cardiac vagal efferent nerve (C.V.E.) are shown. A burst of firing in the cardiac efferent nerve was evoked by a chemoreceptor stimulus timed so as to occur in the expiratory pause (marked E). No firing was evoked when a similar stimulus was given during inspiration (marked I), although this stimulus was associated with the animal taking a second breath sooner than would have been expected from his previous respiratory pattern. Induced irregularities of breathing of this kind did not affect the nature of the responses observed.

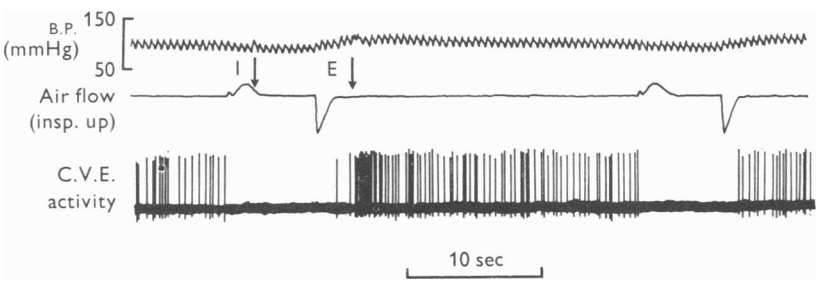

Fig. 4. Dog, chloralose and morphine, bilateral vagotomy. Records of carotid sinus blood pressure, respiratory air flow, and the activity of a single cardiac vagal efferent nerve are shown. After vagotomy this animal adopted a respiratory pattern characterized by prolonged inspiratory spasms. A burst of firing in the cardiac efferent nerve was evoked by a chemoreceptor stimulus timed so as to occur in the expiratory pause (marked E). No firing was evoked when a similar stimulus was given near the peak of inspiration (marked I).

does vary in its effectiveness. This is difficult to test using the functional, but variable, stimuli we have used here. We note, however, that when the arterial pressure is raised mechanically by aortic occlusion, the intense vagal efferent activity evoked is frequently blocked entirely at the start and finish of an inspiratory spasm, but is only reduced during the spasm (see Fig. 5). We have seen responses of this kind in six of nine fibres tested.

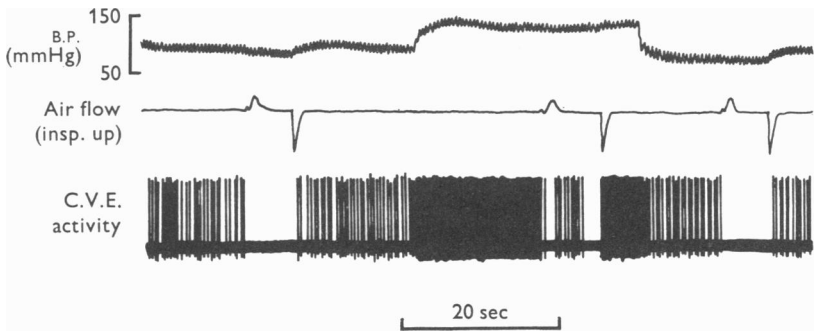

Fig. 5. Dog, chloralose and morphine, bilateral vagotomy. Records of carotid sinus blood pressure, respiratory air flow, and the activity of a single cardiac vagal efferent nerve are shown. After vagotomy, this animal adopted a respiratory pattern characterized by prolonged inspiratory spasms. At a normal blood pressure these spasms were associated with complete abolition of discharge in the cardiac efferent nerve. When the arterial pressure was raised mechanically by the inflation of a balloon in the aorta, the discharge of the cardiac efferent nerve was intensified. At an elevated blood pressure the pattern of discharge during the respiratory cycle was altered. During an inspiratory spasm there was complete abolition of discharge in the cardiac efferent nerve only at the start and finish of the spasm: some firing of the nerve occurred in the middle of the inspiratory period.

DISCUSSION

This study confirms and extends the work of Koepchen *et al.* (1961) and Haymet & McCloskey (1975). The experiments on paralysed animals indicate that the cycling of central neural respiratory mechanisms is sufficient to impose a modulation upon the effectiveness of both baroreceptor and chemoreceptor reflexes on heart rate. It remains possible that this centrally imposed modulation can be altered, perhaps augmented, by phasic afferent inputs in the spontaneously breathing animal.

The studies in which activity in cardiac vagal efferent nerves was recorded provide direct confirmation of the principal findings of Koepchen *et al.* (1961) and Haymet & McCloskey (1975) in experiments in which heart rate was recorded. Similar results for baroreceptor reflex effects were recently reported in brief form by Neil & Palmer (1975). The criteria

we used for identifying vagal efferents as cardiac are not new (cf. Jewett, 1964; Iriuchijima & Kumada, 1964), and indeed, their use is further vindicated here by the reproducibility of Haymet & McCloskey's findings in the fibres so identified. Our sample of fibres is not large enough for us to gauge accurately the extent of convergence of chemoreceptor and baroreceptor inputs on to vagal efferents. Some of our efferents were very responsive to one or other input while showing only small responses, or no responses at all, to the other. Certainly our findings suggest that inputs from one reflexogenic zone may fail to influence vagal efferents which receive inputs from some other zone. Moreover, it should be noted that there may be efferents to the heart which we did not identify or test because they did not receive the baroreceptor or chemoreceptor inputs by which we identified cardiac efferents.

This work was supported by grants from the National Heart Foundation of Australia and the Australian Research Grants Committee. Miss Jaclyn Symonds provided expert technical assistance. The Department of Medical Illustration at the University of New South Wales prepared the Figures.

REFERENCES

GOLDNER, S. & MCCLOSKEY, D. I. (1975). Central respiratory modulation of baro- and chemo- receptor reflexes on heart rate. *Proc. Aust. Physiol. Pharmacol. Soc.* **6**, 60–61*A*.

HAYMET, B. T. & MCCLOSKEY, D. I. (1975). Baroreceptor and chemoreceptor influences on heart rate during the respiratory cycle in the dog. *J. Physiol.* **245**, 699–712.

IRIUCHIJIMA, J. & KUMADA, M. (1964). Activity of single vagal fibres efferent to the heart. *Jap. J. Physiol.* **14**, 479–487.

JEWETT, D. L. (1964). Activity of single efferent fibres in the cervical vagus nerve of the dog with special reference to possible cardioinhibitory fibres. *J. Physiol.* **175**, 321–357.

KOEPCHEN, H. P., LUX, H. D. & WAGNER, P. H. (1961). Untersuchungen über Zeitbedarf und Zentrale Verarbeitung des pressoreceptorischen Herzreflexes. *Pflügers Arch. ges. Physiol.* **273**, 413–430.

NEIL, E. & PALMER, J. F. (1975). Effects of spontaneous respiration on the latency of reflex cardiac chronotropic responses to baroreceptor stimulation. *J. Physiol.* **247**, 16*P*.