*J. Physiol.* (1984), **347**, *pp.* 75–83
*With 4 text-figures*
*Printed in Great Britain*



# TRIGEMINAL–BARORECEPTOR REFLEX INTERACTIONS MODULATE HUMAN CARDIAC VAGAL EFFERENT ACTIVITY

BY DWAIN L. ECKBERG*, SUJIT K. MOHANTY AND MAGDALENA RACZKOWSKA

*From the Departments of Medicine and Physiology and Biophysics, V.A. Medical Center and Medical College of Virginia, Richmond, VA 23249, U.S.A.*

(*Received 11 March 1983*)

## SUMMARY

1. Instantaneous levels of vagal cardiac inhibition reflect integrated responses of vagal motonuclei to multiple sensory neural inputs. We studied how two of these inputs, from trigeminal cutaneous receptors and carotid arterial baroreceptors interact to influence human vagal cardiac outflow.

2. Nine healthy young men voluntarily maintained breathing rates and tidal volumes within narrow limits. Carotid baroreceptors were stimulated with brief periods of moderate neck suction. Volunteers were studied prone, breathing through a snorkel, before and during face immersion in cold water, and before and after an intravenous injection of a very low dose of atropine sulphate (which increases vagal cardiac efferent activity in dogs).

3. Face immersion raised blood pressure slightly, increased heart period, and augmented baroreflex bradycardia and respiratory sinus arrhythmia significantly. Low-dose atropine together with face immersion further augmented blood pressure, heart period, baroreflex responses and sinus arrhythmia.

4. These results suggest that one input to the central nervous system (from trigeminal cutaneous receptors) which increases vagal cardiac outflow, augments vagal responses to another input (from arterial baroreceptors). Since the initial pathways of these two inputs are anatomically separate, it is likely that the influences of respiration and low doses of atropine on vagal motonuclei are exerted down-stream from the termination of primary trigeminal and baroreceptor afferent fibres.

## INTRODUCTION

Diving provokes bradycardia mediated by increased vagal cardiac motoneuronal activity (Richet, 1899). This increased rate of firing reflects an algebraic response of vagal nuclei to changing levels of several converging sensory inputs. Two of these neural inputs, from arterial baroreceptors and trigeminal cutaneous receptors, initially traverse different brain-stem pathways (Brodal, 1981). We considered that

* Medical Investigator, Veterans Administration.

the existence of this anatomical disparity might be used to try to increase understanding of central regulation of human vagal cardiac motoneurone activity. We selectively modified the afferent input from trigeminal receptors and arterial baroreceptors to determine in what ways trigeminal input modifies vagal responses to baroreceptor input; how responses to these two inputs are modified by a pharmacological intervention (intravenous low doses of atropine) which augments vagal cardiac motoneurone activity (Katona, Lipson & Dauchot, 1977; Garcia, Jordan & Spyer, 1978); and how they are modified by a third influence, spontaneous respiration.

## METHODS

We stimulated arterial baroreceptors with neck suction applied to prone healthy young men, before and during face immersion in cold water, and before and after low-dose atropine.

*Subjects.* We studied eleven healthy, normotensive men, ages 22–27 years, after they gave written consent to participate in the research. Results from two subjects were excluded; one had frequent premature atrial beats, and the other was found to have drunk alcoholic beverages excessively, shortly before the study. In two other subjects, responses to neck suction could not be measured. One developed sinus arrest with neck suction during face immersion, and the other developed premature ventricular beats at the time neck suction was applied. Thus, we report complete studies on seven volunteers and incomplete studies (comprising all interventions except neck suction) on an additional two.

*Measurements.* We used ink-writing and FM tape recorders to transcribe the electrocardiogram, beat-by-beat R–R interval, tidal volume (Respitrace Respiration Monitor), end-tidal carbon dioxide concentration (mass spectrometer) and neck chamber pressure (strain gauge pressure transducer). We measured water temperature with a thermometer and blood pressure with a sphygmomanometer cuff. A digital computer measured responses to neck suction and made frequency histograms of heart period on-line in real time, and a processing digital oscilloscope (Norland 3001) measured respiratory and cardiac intervals, tidal volumes and end-tidal carbon dioxide concentrations from FM tape play-back recordings.

*Experimental protocol.* We studied eight of the nine subjects twice. All measurements were made with subjects lying prone, breathing through a snorkel in a quiet, warm (about 22–25 °C) room. At the beginning of an experimental session, subjects breathed at a rate of about 12/min according to an auditory signal; the tidal volume each volunteer established during this period was used throughout the remainder of the experiment. (During interventions, the investigators encouraged subjects to breathe more or less deeply as necessary, according to on-line measurements of tidal volume.)

Frequency histograms of heart period were developed over 10 min during each intervention from measurements of the intervals between threshold crossings set on the upstroke of the R wave. Blood pressures were measured three times during a period of about 3 min during the second experimental session, and were averaged. (All other measurements were made during the first session.) All measurements were made before and during face immersion and before and after intravenous atropine sulphate, 0·725 μg/kg. A recent study (Raczkowska, Eckberg & Ebert, 1983) established that this dose significantly prolongs base-line heart period. Subjects' foreheads rested on a cushion in a rectangular tray. For the face immersion portion of the protocol, ice water (maintained between 5 and 5·5 °C) was added to the tray to submerge volunteers' lower faces. Most subjects wore a nose clip during immersion.

*Arterial baroreceptors* were stimulated with 30 mmHg suction applied to a neck chamber (Eckberg, Cavanaugh, Mark & Abboud, 1975) during held expiration, beginning exactly 5 s after the end of expiratory air flow and continuing for 5 s. Twenty such stimuli were delivered and P–P interval changes were plotted as functions of the interval between the onset of neck suction and each successive P wave, as described earlier (Eckberg, 1977*b*). Results were averaged at 0·75 s intervals.

*Statistical analyses.* Statistical comparisons were made with the paired $t$ test and multivariate analysis of variance with replication (Winer, 1962). Differences were considered significant when $P$ was less than or equal to 0·05.

## RESULTS

*Baroreceptor responses.* Responses of one volunteer to baroreceptor stimulation, before and during face immersion, are depicted in Fig. 1. The baroreflex-mediated prolongation of heart period (baroreflex bradycardia) was augmented in this subject during face immersion. Volunteers did not regulate their breathing rates and depths during this portion of the experiment, and in this record, breathing interval and tidal volume were slightly greater during face immersion than before. However, these small changes are unlikely to have augmented respiratory sinus arrhythmia to the extent observed (Eckberg, 1983), and therefore, this record suggests that respiratory sinus arrhythmia also is augmented by face immersion.

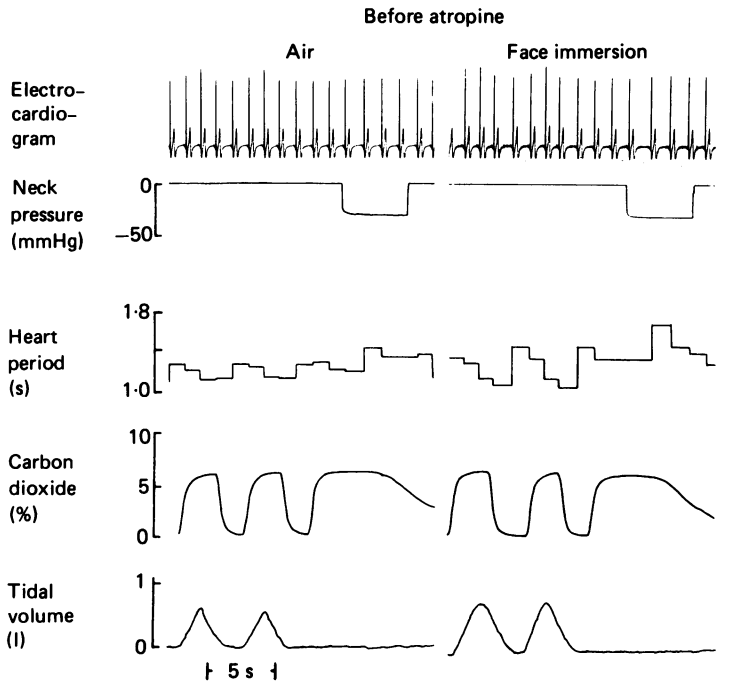


Fig. 1. Original record from one volunteer. 'Air' indicates control measurements made before face immersion (in water 5–5·5 °C). Heart period fluctuations during breathing and heart period prolongations with neck suction were greater during face immersion than before. Volunteers did not regulate their breathing rates and tidal volumes during this portion of the study. Experiment B2231.

Average responses of seven subjects to baroreceptor stimulation are shown in Fig. 2. Face immersion significantly increased baroreflex bradycardia before (left panel) and after (right panel) intravenous, low-dose atropine. The average control response ('air') after atropine was greater ($P = 0{\cdot}052$, single-tailed test) than that before atropine. (In an earlier study conducted in eight supine young adults (Raczkowska *et al.* 1983), augmentation of baroreflex responses after the same dose of atropine was highly significant ($P = 0{\cdot}007$).) The average baroreflex response during immersion

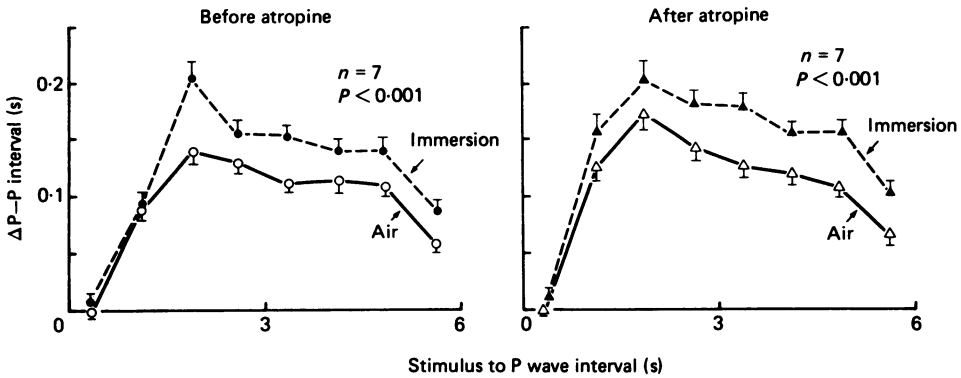


Fig. 2. Average heart period prolongations provoked by 30 mmHg neck suction applied for 5 s, in seven volunteers. Each successive P–P interval prolongation was plotted as a function of time from the onset of neck suction (time 0) until the P wave concluding each cycle. Responses were averaged at 0·75 s intervals. Face immersion significantly enhanced baroreceptor–cardiac reflex inhibition. The mean response during immersion after atropine, 0·725 $\mu$g/kg, was significantly ($P$ less than 0·001) greater than the three other mean responses. Error bars encompass one S.E. of mean.

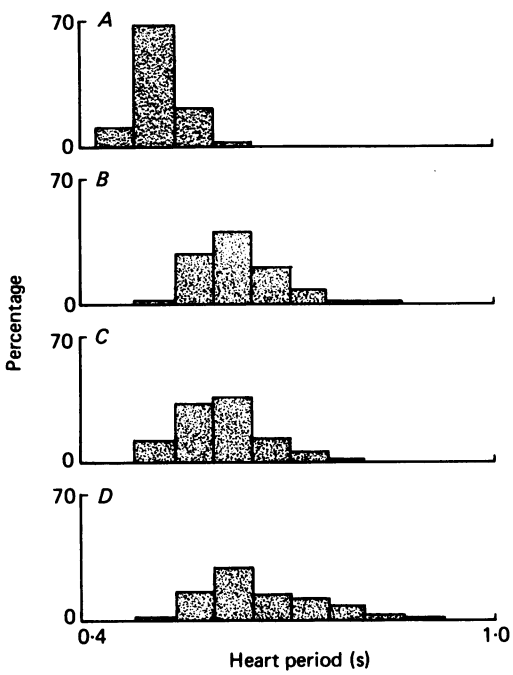


Fig. 3. Frequency histograms of heart period from another volunteer during the four interventions. Histograms were developed over 10 min periods. Experiment W2250. *A*, control/air; *B*, control/immersion; *C*, atropine/air; *D*, atropine/immersion.

after atropine was significantly greater than the control response after atropine ($P < 0{\cdot}001$) and the control and immersion responses before atropine ($P < 0{\cdot}001$).

Face immersion increased average ($\pm$S.E. of mean) systolic and diastolic pressures insignificantly prior to atropine (from $122 \pm 3$ to $128 \pm 3$ mmHg, $P = 0{\cdot}08$, and from $76 \pm 2$ to $79 \pm 1$ mmHg, $P = 0{\cdot}12$) and significantly after atropine (from $123 \pm 2$ to $130 \pm 2$ mmHg, $P = 0{\cdot}004$ and from $77 \pm 2$ to $83 \pm 2$ mmHg, $P = 0{\cdot}004$).

*Respiratory sinus arrhythmia.* Fig. 3 depicts frequency histograms for another volunteer during the four interventions. Face immersion (panel *B*) shifted the frequency histogram to the right (slowed the heart rate) and widened the range of intervals. Low-dose atropine without face immersion (panel *C*) provoked similar changes, and the combination of low-dose atropine and face immersion (panel *D*) provoked the largest rightward shift of the frequency histograms and dispersion of heart periods.

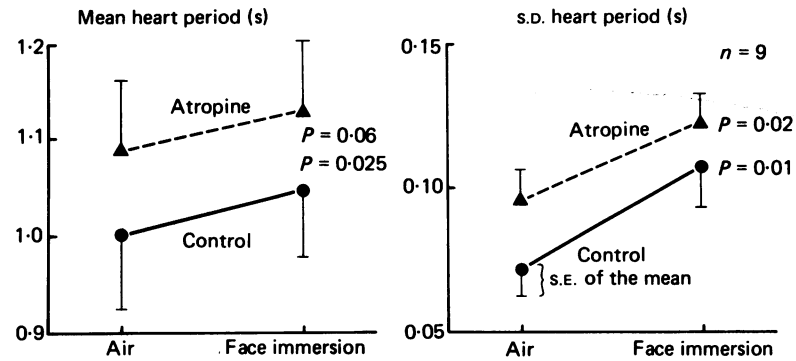


Fig. 4. Mean heart period and standard deviations of heart period (an index of the level of vagal cardiac outflow) in nine volunteers. *P* values were derived from paired *t* comparisons before and during face immersion.

Average mean heart periods and standard deviations of heart period in all nine subjects during the four interventions are shown in Fig. 4. Low-dose atropine significantly ($P < 0{\cdot}004$) increased mean heart period before and during face immersion (left panel). Low-dose atropine also produced an upward shift of standard deviation of heart period, which we used as an index of the level of cardiac vagal efferent activity (Katona & Jih, 1975; Eckberg, 1983); however, this change was significant before ($P = 0{\cdot}05$) but not during immersion ($P = 0{\cdot}08$). The increases of mean heart period and standard deviation of heart period provoked by face immersion were comparable ($P > 0{\cdot}40$) before and after atropine. Immersion provoked little change of the average minimum, and tended to prolong the average maximum heart period during a breath. Respiratory frequency, tidal volume and end-tidal carbon dioxide concentrations were comparable ($P > 0{\cdot}05$) during all interventions.

## DISCUSSION

In this study, we modified two inputs to the central nervous system (from trigeminal cutaneous receptors and carotid arterial baroreceptors), measured one

output (vagally mediated changes of heart period) and drew inferences regarding central vagal cardiac control mechanisms. The principal new findings are that trigeminal cutaneous receptor stimulation significantly increases vagal, baroreflex bradycardia and respiratory sinus arrhythmia, and that low-dose atropine similarly enhances vagal cardiac inhibition produced by trigeminal cutaneous receptor and baroreceptor stimulation.

*Diving bradycardia.* In mammals, diving bradycardia results from stimulation of receptors of the face and nares, innervated by the trigeminal nerve (Dykes, 1974; Drummond & Jones, 1979; Khurana, Watabiki, Hebel, Toro & Nelson, 1980). In contrast, in dabbling ducks, the relatively slow onset of bradycardia in forced dives is due to chemoreceptor stimulation (Jones & Purves, 1970). Cessation of ventilation (Lin, Matsuura & Whittow, 1972; Bamford & Jones, 1976; Angell-James, Elsner & Daly, 1981) and altered labyrinthine or cervical proprioceptor activity (Paton, 1913) may also contribute to diving bradycardia in both birds and mammals. In the present study, only face immersion contributed to bradycardia because ventilation, breathing rate, tidal volume, and body position were comparable before and during face immersion.

The contribution of arterial baroreceptors to diving or to 'simulated diving' bradycardia is controversial. Andersen & Blix (1974) found that diving raises blood pressure, and they concluded that diving bradycardia results from increased levels of afferent baroreceptor traffic. Although this mechanism may contribute to diving bradycardia, it is not a sufficient explanation, since chronic baroreceptor denervation has no significant effect on diving bradycardia (Jones, 1973; Lillo & Jones, 1982). It has been unclear whether diving decreases or increases baroreflex responses. Recent inferential evidence suggests that diving *reduces* baroreflex responsiveness in ducks (Millard, 1980). This conclusion contrasts with earlier findings that diving *increases* baroreflex responsiveness in seals (Angell-James, Daly & Elsner, 1978). The latter study showed that the regression of pulse interval on blood pressure after phenylephrine injections in anaesthetized seals was steeper during simulated diving than before. However, in some trials, simulated diving also raised arterial pressure, and the authors could not determine if simulated diving increased baroreflex gain or merely shifted the sigmoidal blood pressure–heart period relation (Koch, 1931) onto a steeper segment.

Our results from seven unanaesthetized volunteers show that face immersion significantly ($P < 0{\cdot}001$) *augments* baroreflex bradycardia. It is unlikely that this change is due merely to higher blood pressures since before atropine, immersion led to only small increases of blood pressure (the average change was from 122/76 to 128/79 mmHg). An earlier study showed that healthy young subjects operate in the threshold region of their blood pressure–heart period relationships (Eckberg, 1980). On the basis of this study, which also was conducted with healthy young adult volunteers, a small elevation of blood pressure would be expected to shift pressure further into the 'linear' portion of the blood pressure–heart period relation and should not augment baroreflex bradycardia responses substantially.

*Interactions between baroreceptors and trigeminal receptors on cardiac vagal motoneurones.* Since the initial trigeminal and baroreflex pathways are different (Brodal, 1981), it seems unlikely that trigeminal receptor stimulation directly influences

activity of primary baroreceptor neurones. (However, we cannot exclude an influence of trigeminal stimulation on efferent sympathetic traffic to the carotid sinus (Felder, Heesch & Thames, 1983).) If this inference is correct, the interaction we observed occurs 'down-stream' from primary baroreceptor terminations.

We think our data point toward a specific, rather than a non-specific interaction. Increases of vagal cardiac inhibition provoked by low levels of neck suction do not augment sinus node responses to additional neck suction (Eckberg, 1977*a*). Moreover, reductions of vagal cardiac inhibition provoked by hypoxic chemoreceptor stimulation do not alter responses to baroreceptor stimulation (Eckberg, Bastow & Scruby, 1982). Thus, augmented baroreflex responsiveness would not be expected merely on the basis of trigeminally mediated increases of the level of base-line vagal cardiac activity. However, the trigeminal–baroreflex interaction we have documented may not be unique. A similar interaction may occur when cardiac receptors and arterial baroreceptors are stimulated simultaneously: patients with acute inferior myocardial infarctions appear to be unusually susceptible to sinus node inhibition provoked by carotid massage (Martens, Nikl, Beck & Hochrein, 1981).

*Diving bradycardia and respiratory sinus arrhythmia.* We found that respiratory sinus arrhythmia was significantly greater during face immersion than before. A similar observation was made in an anaesthetized seal by Angell-James *et al.* (1981); our findings extend this earlier work by showing that the effect they reported from a single animal occurs consistently and significantly in a group of conscious human subjects. Gandevia, McCloskey & Potter (1978) showed that bradycardia during simulated diving can be reversed by inspiratory efforts made against a closed glottis. Our results complement and extend this finding by showing that inspiration reverses bradycardia *during normal breathing* as well, such that the shortest heart periods during the breathing cycle are comparable, but the longest heart periods are longer during than before immersion.

Other evidence suggests that moderate fluctuations of inputs over a variety of afferent pathways influence respiratory sinus arrhythmia. Respiratory sinus arrhythmia is augmented by increased arterial pressure (Schneyer, 1935) and inferior myocardial infarction (Chung & Morgan, 1969) and reduced by hypoxic chemoreceptor stimulation (Eckberg *et al.* 1982). It is conceivable that respiration influences these disparate reflex pathways individually before they converge upon cardiac vagal motonuclei. However, a more economical interpretation is that respiratory modulation occurs at or near cardiac vagal motonuclei. This speculation is supported by more direct evidence: activity recorded from primary baroreceptor afferent fibres does not display respiratory periodicity (Jordan & Spyer, 1979), and neural activity with respiratory periodicity can be recorded in the vicinity of vagal cardiac motonuclei (McAllen & Spyer, 1978).

*Influence of low-dose atropine on trigeminal–cardiac reflex responses.* Garcia *et al.* (1978) found that iontophoretically applied, minute doses of atropine sulphate augment firing of vagal cardiac motonuclei, and Katona *et al.* (1977) found that intravenous, low doses of atropine also augment vagal cardiac efferent activity. Recently, low-dose atropine has been shown to augment average heart period and standard deviation of heart period (Raczkowska *et al.* 1983). The present results extend these earlier findings by showing that low-dose atropine also increases vagal

responses to trigeminal stimulation. Again, an economical interpretation of these results is that the *central* effect of low-dose atropine is exerted down-stream from primary baroreceptor or trigeminal afferent fibre terminations. (The references cited above indicate that low-dose atropine exerts an important central effect; however, we have not excluded an additional peripheral site of action.)

In conclusion, our results provide several new or refined insights into autonomic modulation of human vagal cardiac motoneurone activity. They provide quantitative evidence that one input which increases vagal cardiac outflow (trigeminal stimulation), augments vagal responses to another (arterial baroreceptor stimulation) and they provide inferential evidence that the influence of respiration and low-dose atropine on vagal cardiac motonuclei may be exerted down-stream from the terminations of primary autonomic afferent fibres.

We thank Janice M. Sprenkle and John J. Schelhorn for their technical assistance; Laura L. Hearne and E. Jean Ryland for their secretarial help; Vernon M. Chinchilli for his help with statistical analyses; and David R. Jones, Gary E. Musgrave and Marc D. Thames for their critical reviews of this manuscript. This research was supported by grants from the Veterans Administration and National Institutes of Health (HL22296, HL22070 and HL22546).

## REFERENCES

ANDERSEN, H. T. & BLIX, A. S. (1974). Pharmacological exposure of components in the autonomic control of the diving reflex. *Acta physiol. scand.* **90**, 381–386.

ANGELL-JAMES, J. E., DALY, M. DEB. & ELSNER, R. (1978). Arterial baroreceptor reflexes in the seal and their modification during experimental dives. *Am. J. Physiol.* **234**, H730–739.

ANGELL-JAMES, J. E., ELSNER, R. & DALY, M. DEB. (1981). Lung inflation: effects on heart rate, respiration, and vagal afferent activity in seals. *Am. J. Physiol.* **240**, H190–198.

BAMFORD, O. S. & JONES, D. R. (1976). Respiratory and cardiovascular interactions in ducks: the effect of lung denervation on the initiation of and recovery from some cardiovascular responses to submergence. *J. Physiol.* **259**, 575–596.

BRODAL, A. (1981). *Neurological Anatomy*, 3rd edn. Oxford: Oxford University Press.

CHUNG, E. K. & MORGAN, E. J. (1969). Unusually marked sinus arrhythmia as a complication of acute diaphragmatic myocardial infarction. *Jap. Heart J.* **10**, 363–368.

DRUMMOND, P. C. & JONES, D. R. (1979). The initiation and maintenance of bradycardia in a diving mammal, the muskrat, *Ondatra zibethica*. *J. Physiol.* **290**, 253–271.

DYKES, R. W. (1974). Factors related to the dive reflex in harbor seals: sensory contributions from the trigeminal region. *Can. J. Physiol. Pharmac.* **52**, 259–265.

ECKBERG, D. L. (1977*a*). Baroreflex inhibition of the human sinus node: importance of stimulus intensity, duration, and rate of pressure change. *J. Physiol.* **269**, 561–577.

ECKBERG, D. L. (1977*b*). Adaptation of the human carotid baroreceptor–cardiac reflex. *J. Physiol.* **269**, 579–589.

ECKBERG, D. L. (1980). Nonlinearities of the human carotid baroreceptor-cardiac reflex. *Circulation Res.* **47**, 208–216.

ECKBERG, D. L. (1983). Human sinus arrhythmia as an index of vagal cardiac outflow. *J. appl. Physiol.* **54**, 961–966.

ECKBERG, D. L., BASTOW, H. & SCRUBY, A. E. (1982). Modulation of human sinus node function by systemic hypoxia. *J. appl. Physiol.* **52**, 570–577.

ECKBERG, D. L., CAVANAUGH, M. S., MARK, A. L. & ABBOUD, F. M. (1975). A simplified neck suction device for activation of carotid baroreceptors. *J. Lab. clin. Med.* **85**, 167–173.

FELDER, R. B., HEESCH, C. M. & THAMES, M. D. (1983). Reflex modulation of carotid sinus baroreceptor activity in the dog. *Am. J. Physiol.* **244**, H437–443.

GANDEVIA, S. C., MCCLOSKEY, D. I. & POTTER, E. K. (1978). Reflex bradycardia occurring in response to diving, nasopharyngeal stimulation and ocular pressure, and its modification by respiration and swallowing. *J. Physiol.* **276**, 383–394.

GARCIA, M., JORDAN, D. & SPYER, K. M. (1978). Studies on the properties of cardiac vagal neurones. *Neurosci. Lett.* (abstract) suppl. **1**, S16.

JONES, D. R. (1973). Systemic arterial baroreceptors in ducks and the consequences of their denervation on some cardiovascular responses to diving. *J. Physiol.* **234**, 499–518.

JONES, D. R. & PURVES, M. J. (1970). The carotid body in the duck and the consequences of its denervation upon the cardiac responses to immersion. *J. Physiol.* **211**, 279–294.

JORDAN, D. & SPYER, K. M. (1979). Studies on the excitability of sinus nerve afferent terminals. *J. Physiol.* **297**, 123–134.

KATONA, P. G. & JIH, F. (1975). Respiratory sinus arrhythmia: noninvasive measure of parasympathetic cardiac control. *J. appl. Physiol.* **39**, 801–805.

KATONA, P. G., LIPSON, D. & DAUCHOT, P. J. (1977). Opposing central and peripheral effects of atropine on parasympathetic cardiac control. *Am. J. Physiol.* **232**, H146–151.

KHURANA, R. K., WATABIKI, S., HEBEL, J. R., TORO, R. & NELSON, E. (1980). Cold face test in the assessment of trigeminal-brainstem-vagal function in humans. *Ann. Neurol.* **7**, 144–149.

KOCH, E. (1931). Die reflektorische Selbststeuerung des Kreislaufes. In *Ergebnisse der Kreislaufforschung*, ed. Kisch, B., pp. 211–215. Dresden: Steinkopf.

LILLO, R. S. & JONES, D. R. (1982). Control of diving responses by carotid bodies and baroreceptors in ducks. *Am. J. Physiol.* **242**, R105–108.

LIN, Y.-C., MATSUURA, D. T. & WHITTOW, G. C. (1972). Respiratory variation of heart rate in the California sea lion. *Am. J. Physiol.* **222**, 260–264.

MARTENS, U., NIKL, CH., BECK, O. A. & HOCHREIN, H. (1981). Über das Vorkommen eines hyperaktiven Karotissinus bei akutem Myokardinfarkt. *Z. Kardiol.* **70**, 28–31.

MCALLEN, R. M. & SPYER, K. M. (1978). The baroreceptor input to cardiac vagal motoneurones. *J. Physiol.* **282**, 365–374.

MILLARD, R. W. (1980). Depressed baroreceptor-cardiac reflex sensitivity during simulated diving in ducks. *Comp. Biochem. Physiol.* **65**A, 247–249.

PATON, D. N. (1913). The relative influence of the labyrinthine and cervical elements in the production of postural apnoea in the duck. *Q. Jl exp. Physiol.* **6**, 197–207.

RACZKOWSKA, M., ECKBERG, D. L. & EBERT, T. J. (1983). Muscarinic cholinergic receptors modulate vagal cardiac responses in man. *J. auton. nerv. Syst.* **7**, 271–278.

RICHET, C. (1899). De la résistance des canards à l'asphyxie. *J. Physiol. Path. gén.* **1**, 641–650.

SCHNEYER, K. (1935). Der pressorezeptorische herzreflex. *Z. Kreislaufforsch.* **7**, 217–226.

WINER, B. J. (1962). *Statistical Principles in Experimental Design*, 2nd edn. New York: McGraw-Hill.