J. exp. Biol. **140**, 405–420 (1988)
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PREDOMINANCE OF VAGAL BRADYCARDIA MECHANISM IN THE BRAIN STEM OF TURTLES

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Accepted 19 May 1988

Summary

Cardiovascular parameters of spontaneously breathing pond turtles (*Cyclemys flavomarginata*) anaesthetized with chloralose ($4\,mg\,100\,g^{-1}$) and urethane ($40\,mg\,100\,g^{-1}$), were examined during exploratory electrical stimulation of the brain stem. Turtles exhibited a low mean systemic arterial blood pressure (MSAP, average 25 mmHg) and slow heart rate (average 24 beats min^{-1}). Upon stimulation, pressor (sympathetic), depressor (sympathetic inhibition), bradycardia and hypotensive (vagal) responses were elicited from regions of the brain stem extending from the hypothalamus to the medulla, principally in the medial region. The pressor response appeared after a longer latency than did the bradycardia and hypotensive responses. It developed rather slowly, and rarely attained a magnitude double its resting value. In contrast, stimulation of many points in the brain stem produced marked slowing or even cessation of the heart beat, and thus resulted in an immediate fall of the blood pressure even to zero. This cardioinhibitory response depended on the integrity of the vagus nerves and was particularly marked upon stimulation in the caudal medulla, the areas of the ambiguus, solitary and dorsomotor nuclei of the vagus and the midline structures. When such an area was stimulated continuously the heart stopped beating throughout the stimulation. The longest period of cardiac arrest before the appearance of escape was 35 min. With continuous stimulation of the peripheral end of the cut vagus, the earliest escape beat occurred even later (65 min). Epinephrine given intravenously produced an increase of MSAP and force of cardiac contraction, although the slope of pressor rise was shallow. Reflex bradycardia, however, was not observed. These experiments show that a very prominent vagal bradycardia can be evoked from the turtle brain stem, which may contribute to its well-known capacity for tolerating anoxia.

Introduction

In the mammalian heart, cardiac arrest and resultant anoxia produced by vagal stimulation are transitory: cessation of heart beat lasts for only a short time before

Key words: bradycardia, vagal escape, pressor response, brain stem, hypothalamus, midbrain, medulla, turtle.

the heart resumes beating in spite of continued stimulation (vagal escape phenomenon). In cats, continuous optimal stimulation of the peripheral end of the cut vagus nerve results in escape in less than 16 s (Chai & Kuo, 1967). Escape occurs even earlier (a matter of a few seconds) on stimulation of the vagal nuclei in the medulla oblongata (Chen & Chai, 1976). In the turtle, the situation has long been known to be quite different: the heart may stop beating for more than 1 h in response to continuous vagal stimulation (Mills, 1885; Hough, 1895), and this sustained cardiac arrest has been attributed to the low concentration of catecholamines in the heart (Friedman & Bhagat, 1962).

The turtle is an amphibian reptile characterized by its slow horizontal motion, low systemic arterial pressure (SAP) and heart rate (HR) and extreme tolerance to prolonged anoxia that may last from hours to even months (Robin *et al.* 1964; Berkson, 1966; Penney, 1974; Felger *et al.* 1976; Ultsch & Jackson, 1982). The turtle, therefore, can be expected to have a unique pattern of cardiovascular control as well as special metabolic mechanisms. The low SAP and slow HR, together with the prolonged cardiac arrest on vagal stimulation, suggest that vagal mechanisms in the central nervous system of a turtle might predominate in cardiovascular integration. The presence of such mechanisms is investigated in the present study.

Materials and methods

Eighty-seven spontaneously breathing turtles (*Cyclemys flavomarginata*) of either sex, weighing between 800 and 1800 g, were used. In all cases anaesthesia was achieved by immersion in iced water for sedation followed by an injection of a mixture of chloralose ($4\,mg\,100\,g^{-1}$) and urethane ($40\,mg\,100\,g^{-1}$) through a cannula in the jugular vein, which was also used for drug administration. The vagus nerves on both sides were carefully isolated. Measurement of the systemic arterial pressure (SAP) from the right carotid artery, monitoring of the mean systemic arterial blood pressure (MSAP), partial representation of cardiac contractility (dP/dt) and heart rate (HR) have been described previously (Lin *et al.* 1987; Chai *et al.* 1988). All recordings were made on a Gould 2800S polygraph.

The head of the animal was fixed in a David–Kopf stereotaxic instrument with a modified mouthpiece. The occipital and the parietal bones were removed. Electrical stimulation was accomplished with a coaxial electrode (NEX-100, Rhodes Medical Instrument; shaft diameter 250 μm, tip diameter 100 μm). In the experiments specifically for chemical activation, stimulation was accomplished monopolarly (cathodal) with 30-gauge electrode tubing. The tubing, insulated except for 200 μm at the tip, was connected through a section of PE 10 polyethylene tube to a Hamilton syringe so that it could be used for identification of positive reactive sites by electrical stimulation followed by chemical injection at the same point. The electrode or electrode tubing was positioned perpendicular to the horizontal plane of the brain. Stimulating current was provided by a constant-current unit connected to a Grass S48 square-wave stimulator. The stimulation

parameters were 20 Hz, 5 ms and 50–400 μA, for a 20-s period unless stated otherwise. Various frequencies (10, 20, 40 and 80 Hz) and durations (0·1, 0·2, 0·5, 1·0, 2·0, 3·0, 4·0, 5·0 and 6·0 ms) were tried. It was found that a pulse train of 20 Hz and 5 ms was optimal to produce the most prominent bradycardia. Each site of stimulation was marked by iron deposited by passing a direct current of 200 μA for 15 s through the stimulating point. In the experiments specifically for chemical stimulation, the stimulation sites were identified by injecting fast green dissolved in the chemicals.

The following agents were used for chemical stimulation: sodium glutamate (Glu, 1 mol l^{-1}, pH 8·0 in 0·6% saline), DL-homocysteic acid (DLH, 500 mmol l^{-1}), kainic acid (KA, 1 μg 100 nl^{-1}), acetylcholine (ACh, 5 mmol l^{-1}), norepinephrine (NE, 1 mmol l^{-1}) and epinephrine (Epi, 1 mmol l^{-1}). Each of these agents was mixed with 0·5% fast green, and administered in a volume of 100–200 nl through the electrode tubing by manual pressure.

For testing the reflex bradycardia, Epi (2 μg 100 g^{-1}) was given intravenously. To determine the nature of the cardiovascular responses, autonomic blocking agents, atropine (0·1 mg 100 g^{-1}), propranolol (30 μg 100 g^{-1}) or phentolamine (50 μg 100 g^{-1}), was administered intravenously.

At the end of each experiment, using the iron marking technique, the animal was perfused through the jugular vein with 500 ml of 0·6% saline followed by an equal amount of 10% formalin (containing 20% sucrose and 1% potassium ferrocyanide in saline). The brain was removed and further immersed in the same fixative for 1 week. The brain was then sectioned in 30 μm slices using a cryostat. In those experiments using chemical stimulation, the brain was removed fresh and sectioned in the same way. All the sections were stained with cresyl violet.

Results

Cardiovascular responses to electrical activation

The anaesthetized turtles ($N = 87$), at room temperature and breathing spontaneously, had an average MSAP of 25·2 ± 1·2 mmHg (1 mmHg = 133·3 Pa) and HR of 23·7 ± 1·3 beats min^{-1}.

Various patterns of cardiovascular responses were obtained on systematic exploration of the whole brain stem with electrical stimulation using rectangular pulses. They included depressor responses alone (Figs 1F, 2E), depressor responses with bradycardia varying from slowing to cessation of heart beat (Figs 1D,E, 3A–C, 5A–C), pressor responses alone (Figs 1B,C, 2A) or pressor responses with tachycardia (Figs 1A, 2B). A pressor response concomitant with bradycardia was rarely seen and occurred only when the stimulation intensity was so high that it may have involved the neighbouring bradycardia mechanism through current spread. In most instances, particularly in the rostral brain stem (Fig. 1A), the pressor response was mild and developed slowly, although a marked hypertension (to a level at least double the resting value) was occasionally observed (Figs 1B,C, 2A). An increase of heart rate during the rise of pressure

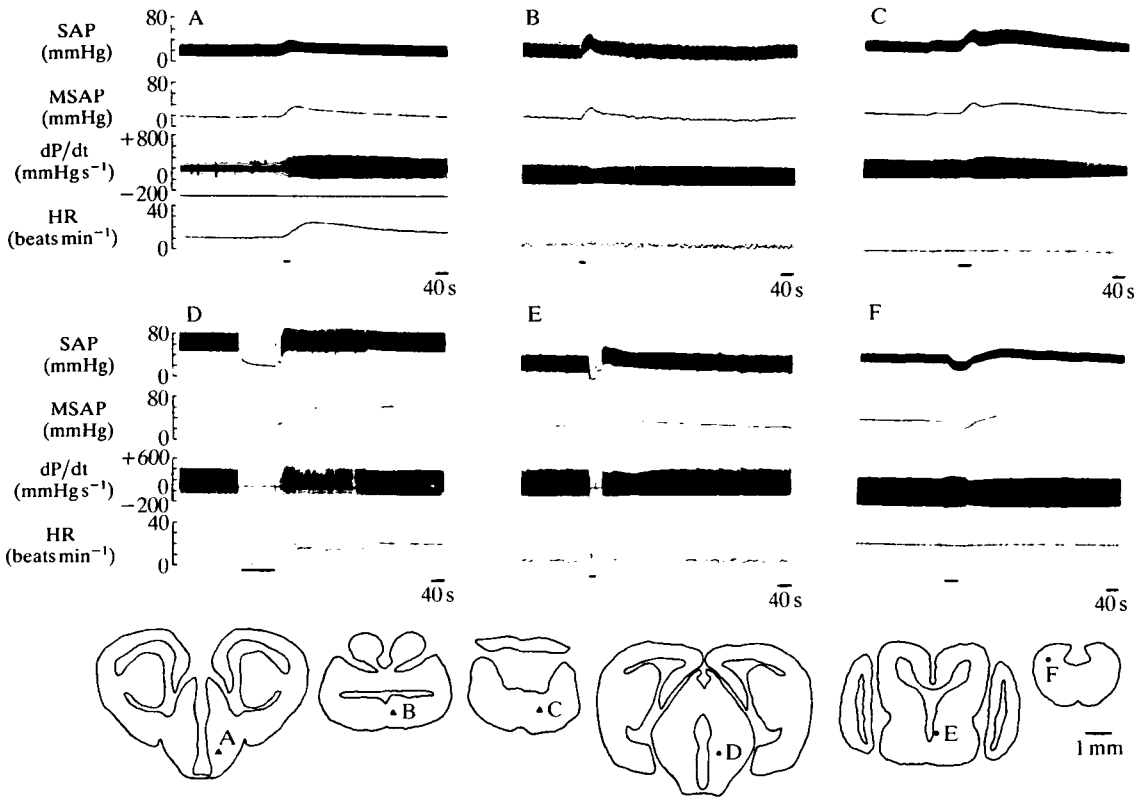


Fig. 1. General types of cardiovascular responses in brain stem stimulation. Pressor responses on stimulation of (A) the anterior hypothalamus (100 μA); (B) the midbrain (100 μA) and (C) the medulla (100 μA). Note the marked cardioacceleration in A. Depressor responses on stimulation of (D) the posterior hypothalamus (400 μA), (E) the midbrain (100 μA) and (F) the medulla (100 μA). Note the early appearance of an escape beat in the cardiac arrest produced by stimulation of the posterior hypothalamus (D), and the lack of cardiac change accompanying the depressor response (F). Sites of stimulation in this and following figures are indicated as solid dots on each diagram.

was not apparent, and cardiac contractility (dP/dt) varied from a slight increase (Fig. 1C) to a slight decrease (Figs 1B, 2A). Under electrical stimulation, therefore, the general picture was that the depressor and bradycardia responses appeared to predominate over the pressor response. In other words, the site for the former responses occupied a larger area in the brain stem and its stimulation evoked a more marked response.

Nature of the cardiovascular responses

Bradycardia

The onset of the induced bradycardia was prompt. 1–4 s after the delivery of electrical stimulation, bradycardia or cardiac arrest occurred (Figs 1, 3, 5). The

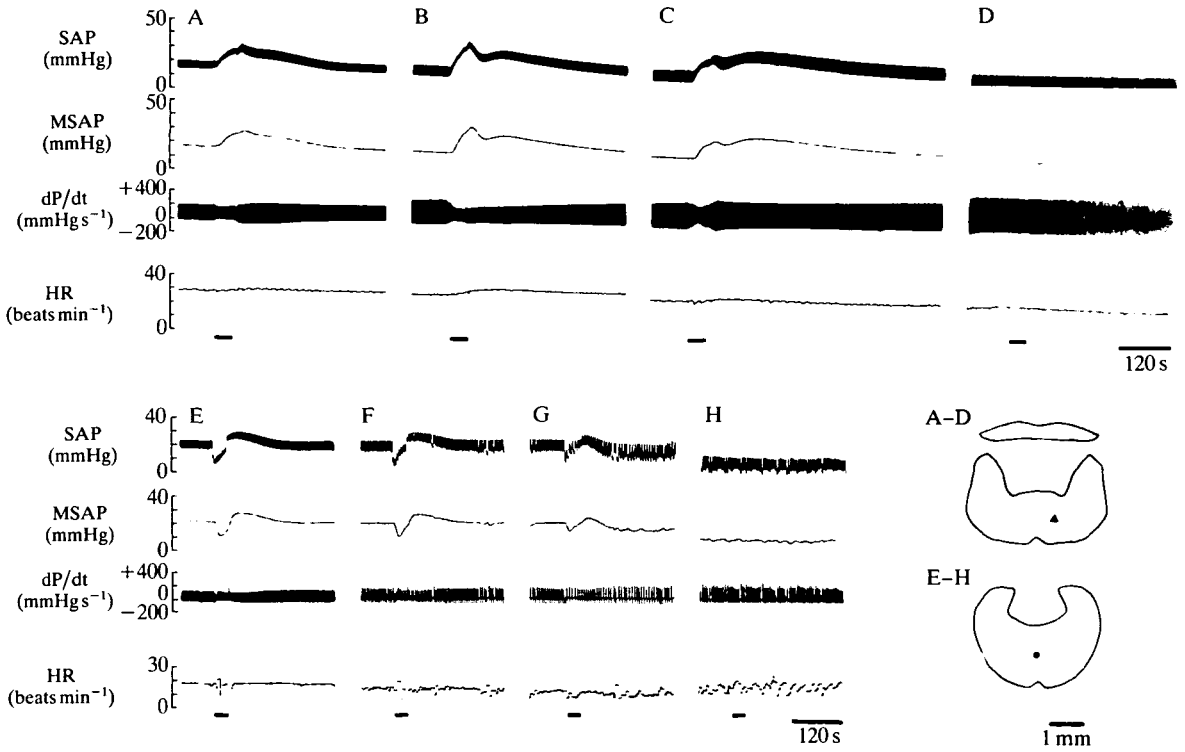


Fig. 2. Effects of sympathetic blocking agents on the pressor and depressor responses elicited from medullary stimulation. (A–D) Pressor response obtained from stimulating (50 μA) a point in the medulla of a turtle as shown in the diagram. (A) Control; (B) after bilateral vagotomy; (C) after propranolol (30 μg 100 g^{-1} i.v.); (D) after additional phentolamine (50 μg 100 g^{-1} i.v.). Note the complete abolition of the pressor response elicited from stimulation. (E–H) Depressor response elicited from stimulation (200 μA) of a point in the medulla of another turtle as shown in the lower diagram. (E) Control; (F) after bilateral vagotomy; (G) after propranolol (30 μg 100 g^{-1} i.v.); (H) after additional phentolamine (50 μg 100 g^{-1} i.v.). Note the complete elimination of the response.

bradycardia was vagal in origin, brought about mainly through a unilateral descending pathway, since vagotomy ipsilateral to the site of medullary stimulation reduced the bradycardia by 80 ± 2·4 %. Subsequent interruption of the contralateral vagus nerve then completely eliminated the bradycardia (three animals). However, initial vagotomy contralateral to the medullary stimulation decreased the bradycardia by 22 ± 3·4 %. Subsequent removal of the remaining vagus then completely eliminated the bradycardia (six animals). This lateralization was also noted in the study of vagal escape phenomena resulting from prolonged stimulation of a bradycardia point in the medulla (Fig. 3).

There was little relationship between the bradycardia and the inhibition of the sympathetic system, since intravenous administration of sympathetic blocking

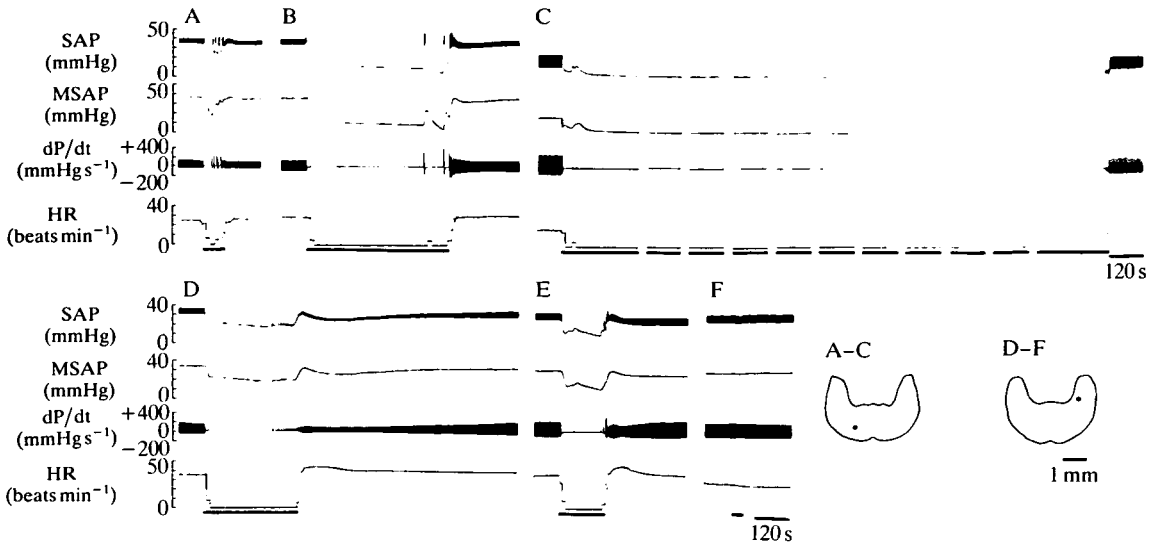


Fig. 3. Effects of sympathetic blocking agents and vagotomy on the vagal escape resulting from medullary stimulation. (A–C) Vagal bradycardia elicited from stimulating (100 μA) a point in the medulla (bottom left) of a turtle. (A) Control, escape after 20 s; (B) after propranolol (30 μg 100 g^{-1} i.v.), escape after 280 s; (C) after additional phentolamine (50 μg 100 g^{-1} i.v.), escape after 2110 s. (D–F) Vagal bradycardia elicited from stimulating (200 μA) a point in the medulla (bottom right) of another turtle. (D) Control, escape occurred at 240 s; (E) after contralateral vagotomy, escape after 140 s; (F) after section of the remaining vagus nerve, there was complete elimination of bradycardia.

agents, propranolol (30 μg 100 g^{-1}) or phentolamine (50 μg 100 g^{-1}), did not prevent the cardiac arrest even though they slightly reduced the resting MSAP (from 21·4 ± 2·9 to 20·4 ± 3 mmHg). In contrast, after treatment with either sympathetic blocker, the vagal escape from medullary stimulation was prolonged (see below).

Pressor response

The onset of any pressor response upon brain stem stimulation was comparatively slow. 15–20 s after application of the electrical stimulation the SAP began a rise that did not reach its peak until 30–40 s. The pressure rise was sympathetic in origin, since administration of sympathetic blockers eliminated it. In six animals with a previous bilateral vagotomy, propranolol (30 μg 100 g^{-1} i.v.) was administered first, followed by phentolamine (50 μg 100 g^{-1} i.v.). On average, propranolol decreased the resting MSAP from 19·8 ± 1·8 to 7·0 ± 1·7 mmHg and diminished the tachycardia response (from 19·6 ± 2·9 to 15·0 ± 2·9 beats min^{-1}). Yet the pressure rise on medullary stimulation remained essentially unchanged (MSAP 21·0 ± 1·4 mmHg). Subsequent administration of phentolamine further lowered the resting MSAP to 10·2 ± 1·6 mmHg, and the pressor response on stimulation was completely abolished. A typical case is illustrated in Fig. 2A–D. In another

four animals the blockers were administered in the reverse order. In these animals phentolamine decreased the resting MSAP from 21·5 ± 1·6 to 18·0 ± 1·2 mmHg but both eliminated the pressure rise (21·0 ± 2·6 mmHg, no change in BP) and left the cardioacceleratory response unchanged (23·0 ± 1·8 *vs* 23·3 ± 1·9 beats min^{-1}). Further intravenous administration of 30 $\mu g\,100\,g^{-1}$ propranolol then abolished the tachycardia.

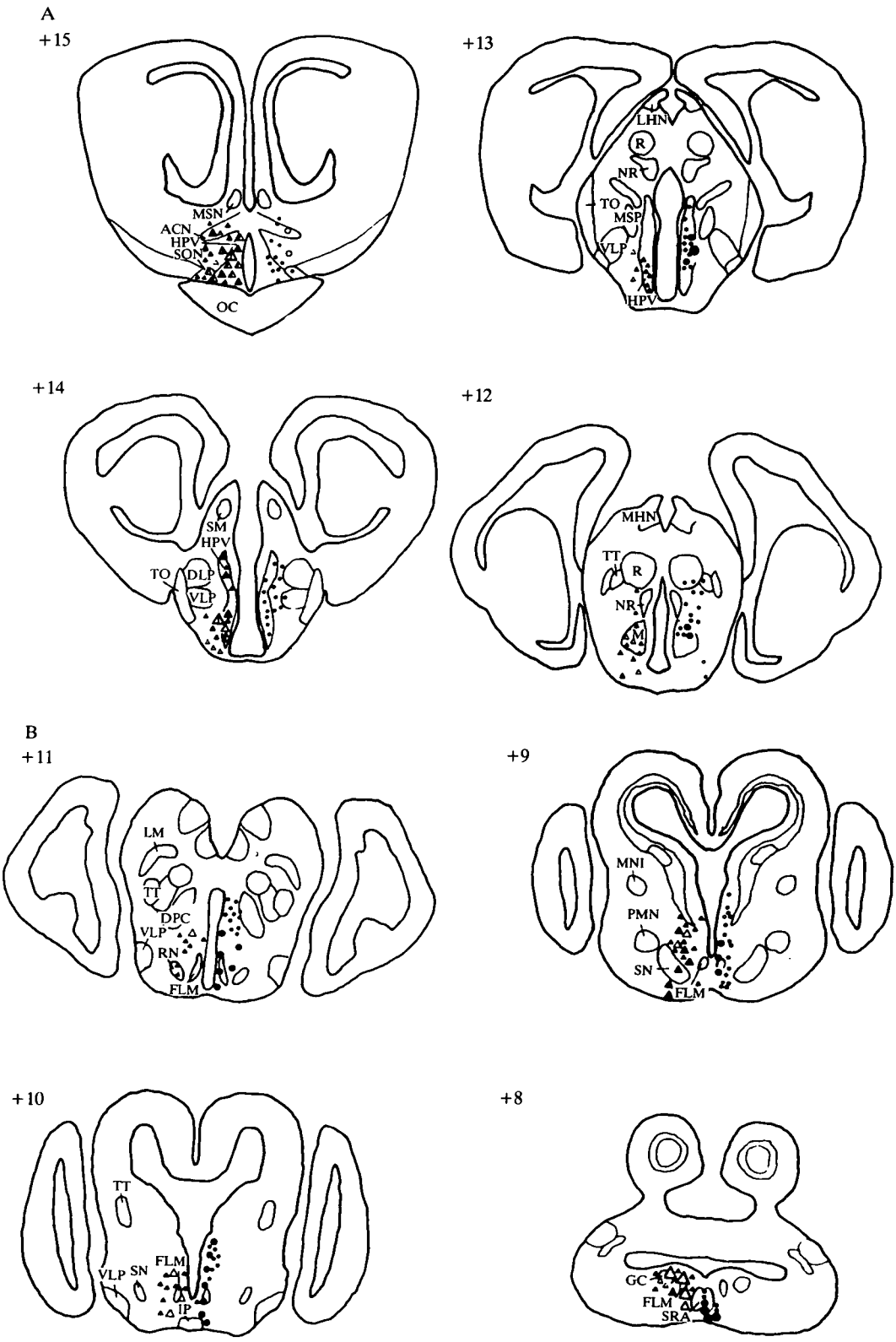
The pressure rises noted upon brain stem stimulation appeared to be independent of inhibition of vagal function. This was demonstrated by the observation that bilateral vagotomy (nine animals), even with additional administration of atropine, 100 $\mu g\,100\,g^{-1}$ i.v., did not reduce the elicited pressor response. Instead, a slight increase of the pressure rise was noted (Fig. 2A,B). Furthermore, exploration of the brain stem in these vagotomized animals revealed no significant increase in the number of the reactive sites for the pressure increase.

Depressor response

Pure depressor responses were occasionally observed, and were due to sympathetic inhibition because bilateral vagotomy did not affect them. Administration of phentolamine, 50 $\mu g\,100\,g^{-1}$ i.v., however, completely eliminated the hypotension (three animals, Fig. 2E–H).

Localization of cardiovascular responses

Fig. 4 maps all the pressor, depressor and bradycardia points identified upon electrical stimulation of the brain stem. For clarity, depressor responses with or without bradycardia are on the right and pressor responses are on the left side of the figure. All points where pressor or depressor responses were less than 15 % of control values were neglected in an attempt to avoid the effect of current spread and also to minimize exaggeration of the size of the responsive area. Pressor responses that did not exceed a 70 % increase in MSAP were obtained from the rostral half of the brain stem, i.e. the levels rostral to the caudal mesencephalon (+15 to +9; 15–9 mm anterior or rostral to the obex). These levels included the medial or ventromedial portions of the hypothalamus (+15 to +12) and rostral midbrain (+11 to +10). Pressor points were dorsomedially located at the caudal midbrain (+9 to +8). Pressor responses causing a greater than 70 % increase were observed at levels between the caudal midbrain (+8) and the rostral medulla (+4) from the ventromedial portions of the reticular formation just ventral to the medial longitudinal fascicle (FLM). At levels in the caudal medulla (+3 to obex), only a few small pressor responses were noted from points sporadically scattered in the more lateral portion of the reticular formation. Points yielding a pressor response with tachycardia were located mainly in the hypothalamus (+15, +14) and midbrain (+8) especially in its rostral area (+15). Points that gave a pressor response with bradycardia were found infrequently in the hypothalamus (+15 to +13) and midbrain (+9), and more often in the rostral medulla (+7 to +5). Depressor points were of two types. (1) Simple depressor responses (decrease of SAP without change in HR) were rarely recorded after stimulation in the rostral


A
+15
MSN
ACN
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SON
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LHN
R
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MHN
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+11
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RN
FLM
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Fig. 4A,B

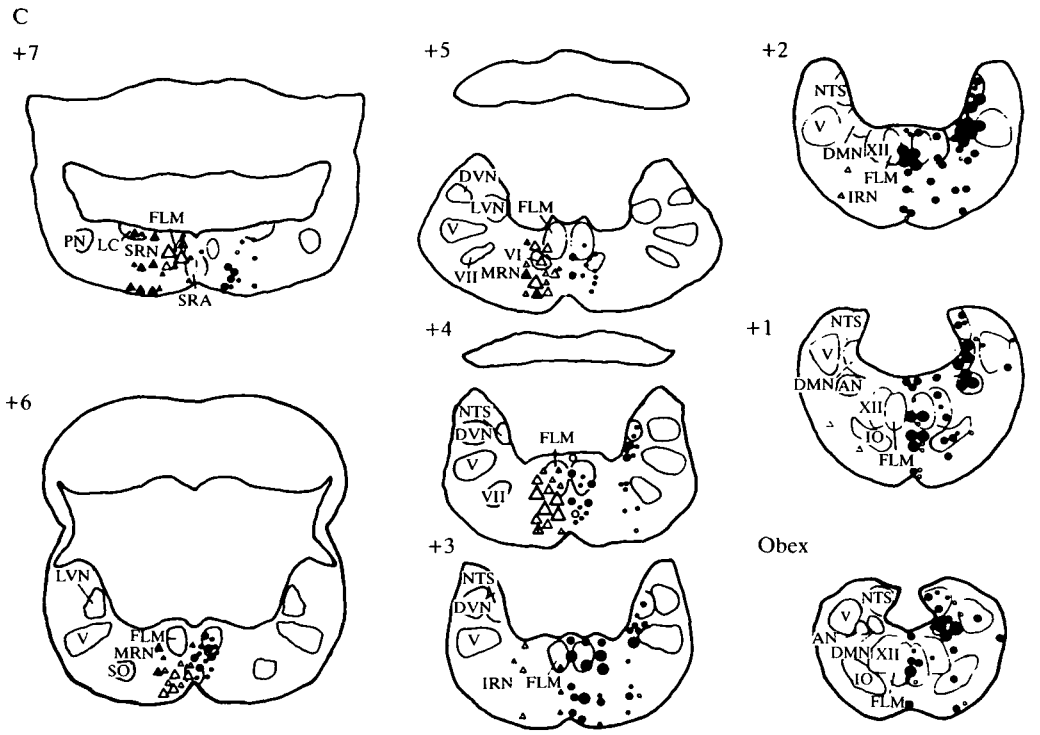

Fig. 4. Distribution of the cardiovascular responsive points in the brain stem of the turtle. For convenience all points producing an increase in arterial pressure (pressor points) are placed on the left side of each section and those producing a decrease in arterial pressure (depressor points) on the right side. (△) Pressor response with minimal or no heart rate change; (◬) pressor response with tachycardia; (▲) pressor response with bradycardia. The large, medium and small triangles indicate increases of >70 %, 69–50 % and 49–15 %, respectively. (○) Depressor response with minimal or no change in heart rate. Medium and small circles indicate decreases of 69–50 % and 49–15 % in the arterial pressure, respectively. (●) Depressor response with marked bradycardia. Because very prominent bradycardia or cardiac arrest would inevitably result in almost complete suppression of the arterial pressure, the degree of the depressor response with bradycardia could only be expressed by reference to the duration of the cardiac arrest. Large, medium and small sizes represent the length of cardiac arrest >20 s, 19–10 s and 9–5 s, respectively. The numbers at the left upper corner of each diagram represent the distance (in mm) rostral (+) to the obex. ACN, anterior commissural nucleus; AN, ambiguus nucleus; DLP, dorsal peduncle of lateral prosencephalic fascicle; DMN, dorsal motor nucleus of vagus nerve; DPC, dorsal nucleus of posterior commissure; DVN, descending vestibular nucleus; FLM, medial longitudinal fascicle; GC, griseum centrale; HPV, hypothalamic periventricular nucleus; IO, inferior olivary nucleus; IP, interpeduncular nucleus; IRN, inferior reticular nucleus; LC, locus coeruleus; LHN, lateral habenular nucleus; LM, lentiform mesencephalic nucleus; LVN, lateral vestibular nucleus; M, medial hypothalamic nucleus; MHN, medial habenular nucleus; MNI, magnocellular part of nucleus isthmus; MRN, medial reticular nucleus; MSN, medial septal nucleus; MSP, medial suprapeduncular nucleus; NTS, nucleus of tractus solitarius; NR, nucleus reuniens; OC, optic chiasma; PN, parabrachial nucleus; PMN, profound mesencephalic nucleus; R, nucleus rotundus; RN, red nucleus; SM, stria medullaris; SN, substantia nigra; SO, superior olivary nucleus; SON, supraoptic nucleus; SRA, superior raphe nucleus; SRN, superior reticular nucleus; TO, tractus opticum; TT, tectothalamic tract; V, nucleus of descending trigeminal nerve; VI, nucleus of abducent nerve; VII, nucleus of facial nerve; VLP, ventral peduncle of lateral prosencephalic fascicle; XII, nucleus of hypoglossal nerve.

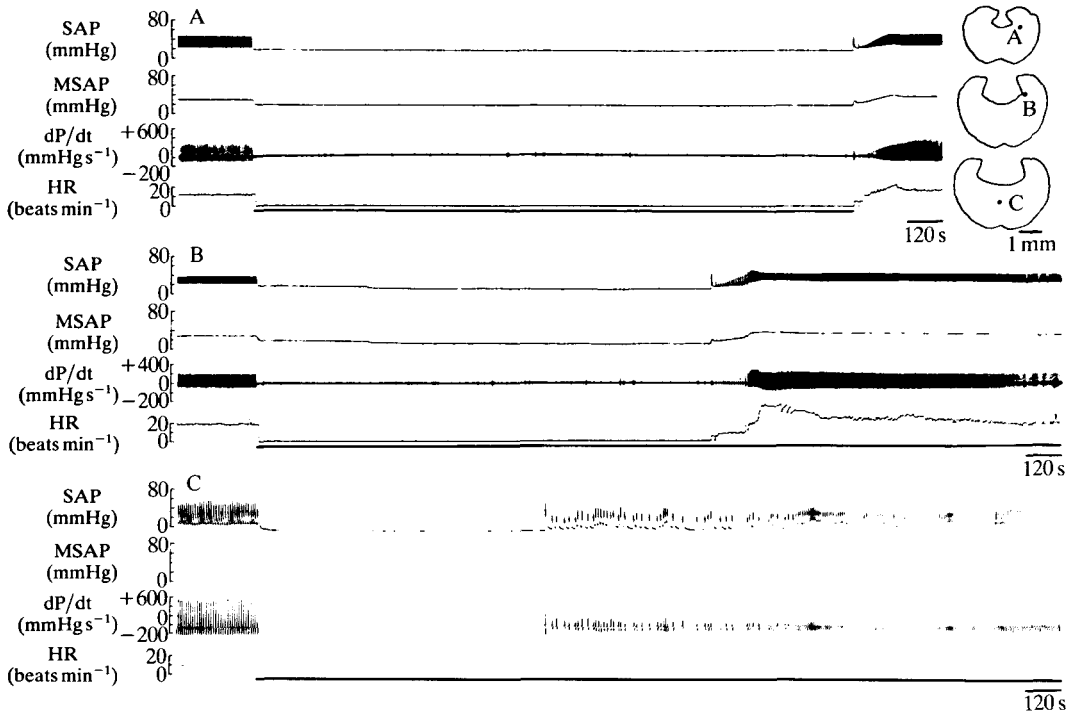

Fig. 5. Cardiac arrest produced by stimulation of various structures in the medulla. (A) Complete cardiac arrest for 34 min on stimulation of an area of the ambiguus nucleus in the medulla (200 μA); (B) for 25 min on stimulation of the dorsal motor nucleus of the vagus (200 μA), and (C) for 16 min on stimulation of the midline structure in the medulla (200 μA).

levels of the brain stem, scarcely at all after stimulation in the middle levels, but somewhat more frequently after stimulation in the caudal levels (+4 to 0). (2) Depressor responses associated with bradycardia occurred after stimulation throughout the whole length of the brain stem. The degree of the hypotensive response was proportional to the slowing of the HR, and a cardiac arrest of several seconds would inevitably result in complete suppression of SAP (Figs 1, 5). Thus, the magnitude of this depressor response was determined by the severity of the cardiac arrest rather than by the degree of the peripheral hypotensive effect. At the rostral and middle levels of the brain stem, the distribution of the depressor points was generally similar to that of the pressor points. In the caudal brain stem, pressor points were sometimes found in the area lateral to the depressor points, whereas moderate depressor responses were attained after stimulation of the area of the inferior olivary nucleus. The most distinctive feature of these results, however, was the prominent bradycardia found on stimulating the areas of the dorsal motor, solitary and ambiguus nuclei (+2 to 0), as well as the raphe area just ventral to the medial longitudinal fascicle (+2, +1).

The potent vagal bradycardia mechanism in the medulla

A predominance of vagal bradycardia in turtles was evident because stimulation of the caudal medulla immediately stopped the heart beating and thus lowered the SAP to zero with complete arrest of circulation (Figs 3A–C, 5). When stimulation was deliberately prolonged, beating resumed (escaped) after a considerable time. Six of eleven points of stimulation in the caudal medulla at a level 1 mm rostral to the obex (+1), when tested for their maximum effect, gave arrests of 25, 28, 29, 33, 34 and 35 min, whereas the other five caused arrests lasting from 8 to 12 min. The average for the 10 animals was 21 min. Of five points at the obex level tested for the same purpose, two stopped the heart for 25 and 34 min, and the rest for 5–17 min (average 15 min). These values were in contrast to the maximum arrest of 5–10 min (average 8 min) from 11 points at the +2 level, and the maximum arrest of 5–7 min (average 6 min) from six points at the +3 level. Average lengths of cardiac arrests caused by stimulation of other areas were: ambiguus, 22 min; dorsal motor of vagus, 15 min; and midline structures, 4 min. After stimulation rostral to the +3 level, cardiac arrest was not marked, less than 1 min, except for one point in the hypothalamus (+13) which caused an arrest of 2 min (Fig. 1D).

Vagal escape

For comparison, in 10 animals the peripheral end of the cut vagus nerve was stimulated to study the escape phenomenon. The responses differed insignificantly among animals. In one turtle the effect of stimulation was so marked that escape did not occur, and the animal was found dead after stimulation for 63 min. The vagal escape appeared as late after stimulation as 65 and 21 min (Fig. 6A,B) or as early as 180 s (Fig. 6C). The cardiac arrest was more prolonged after stimulation of the right vagus (30·0 ± 2·1 min) than the left vagus (13·0 ± 1·3 min).

The role of sympathetic function in the vagal escape from medullary stimulation was studied. In six animals, propranolol (30 μg 100 g^{-1} i.v.) was administered first, followed by phentolamine (50 μg 100 g^{-1} i.v.). Propranolol prolonged the escape by 230 s (80 ± 26 s *vs* 310 ± 50 s). Subsequent phentolamine treatment further prolonged the escape (84 ± 26 s *vs* 638 ± 55 s). In one of these six animals the escape prolongation after blockers was very marked, increasing from 20 s to 400 s after propranolol and to 2110 s after the addition of phentolamine (Fig. 3A–C). In another animal, in which the order of administration of blockers was reversed, phentolamine first prolonged the escape by 180 s (from 80 s to 260 s) and then propranolol further prolonged the escape by 480 s (80 s *vs* 560 s).

Chemical stimulations

The points capable of producing significant depressor or pressor responses under electrical stimulation were injected with sodium glutamate (1·0 mol l^{-1}, 200 nl) through the same electrode tubing. In general, the response to glutamate was not marked. Although some points responded in the same way as they did to electrical stimulation, the response never reached the same magnitude. DLH

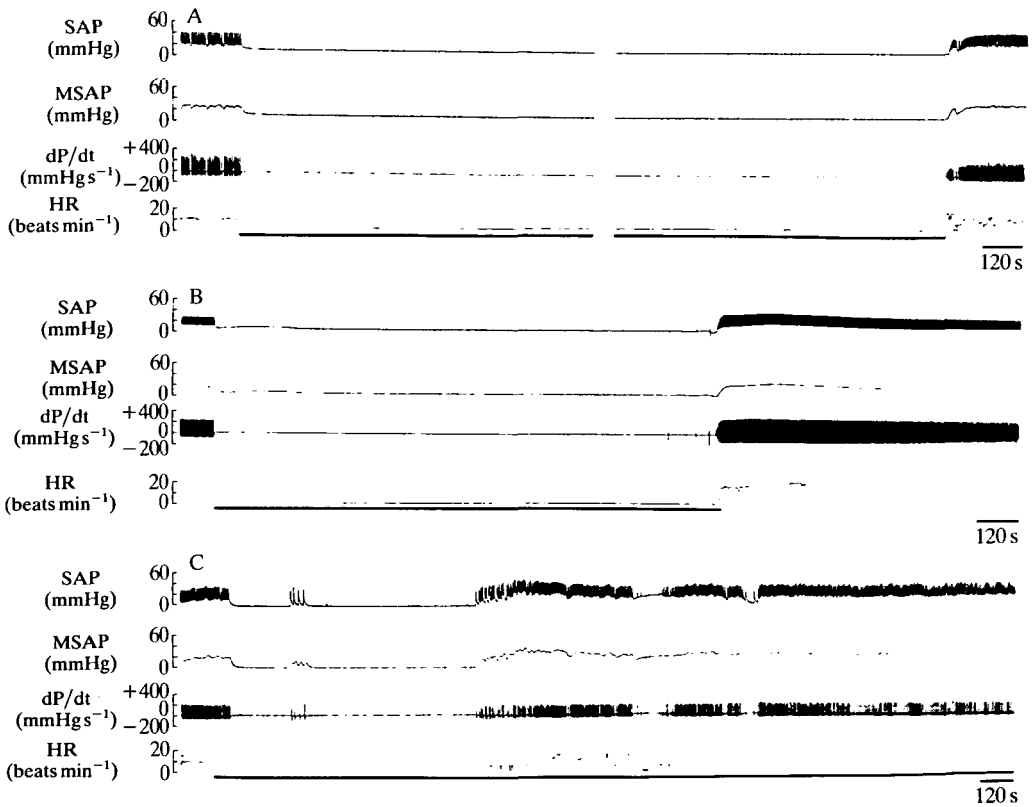

Fig. 6. Length of time before vagal escape during continuous stimulation of the peripheral vagus nerve. (A) Right vagus, 200 μA; (B) left vagus, 200 μA; (C) left vagus, 200 μA. Three different turtles were used. Note the different delays before vagal escape (A 65 min, B 21 min, C 3 min).

(500 mmol l^{-1}, 200 nl) and KA (1 μg 100 nl^{-1}, 200 nl) were later tried at some of these points, but they were also ineffective. This was also true for microinjection of NE (1 mmol l^{-1}, 200 nl), Epi (1 mmol l^{-1}, 200 nl) and ACh (5 mmol l^{-1}, 200 nl).

Effects of intravenous epinephrine

In six turtles, intravenous doses of epinephrine of 1, 2, 3, 8, 12 or 16 μg 100 g^{-1} were tested. Each dose produced a pressor response, but the response was most apparent at 2 μg 100 g^{-1}. Higher doses produced less-marked responses.

The pressor response was slow to develop, with a latency of 50–90 s. Although the rate of pressure rise was slow, its duration was long. It required 120 s to reach a maximum of 40 mmHg above the resting value and took 30–45 min to return to the pre-injection level. The pressure rise was sometimes accompanied by a slight increase in heart rate (by a maximum of 2 beats min^{-1}) and cardiac contractility (dP/dt), but never by a bradycardia of baroreflex origin. Fig. 7 shows a typical example of the response. When epinephrine injection was repeated after an

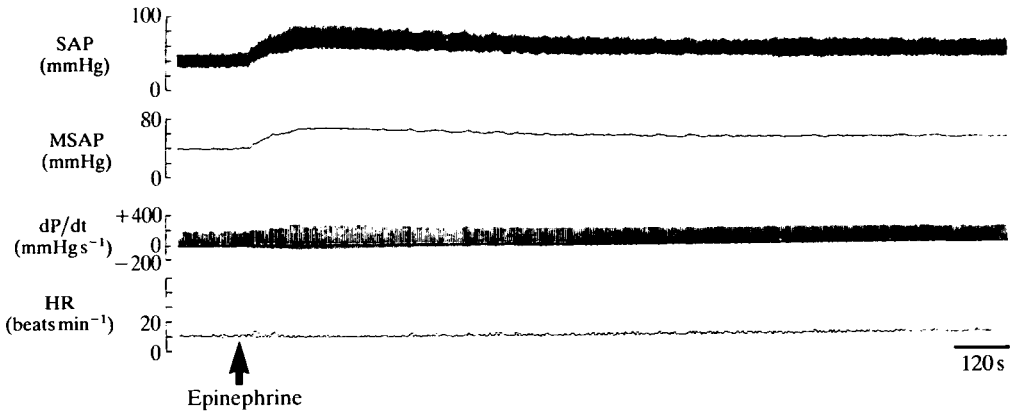

Fig. 7. Effect of intravenous injection of epinephrine, 2 μg 100 g^{-1}. Note the absence of reflex bradycardia during pressor responses.

interval of less than 15 min, the magnitudes of the pressure rise, cardiac acceleration and augmentation of contractility decreased (tachyphylaxis), a decrease that was more apparent in the latter two components.

Discussion

The present experiments show that in the brain stem of turtles vagal representation is predominant over sympathetic representation in terms of a larger area of distribution in the brain and more intense responses that varied from marked slowing to cessation of heart beat. Furthermore, the latency for producing this vagal response was shorter, less than 3 s, compared with that of the sympathetic pressor response, 10–20 s. If stimulation was given continuously in the caudal medulla, the bradycardia could last as long as 35 min before the appearance of an escape beat. Even after stimulation of the hypothalamus the cardiac arrest could last for 2 min.

This type of prolonged cardiac arrest resulting from stimulation of the brain stem is not seen in mammals. It is consistent with the finding that the turtle has a great capacity for tolerating anoxia and undergoing anaerobic metabolism. For instance: even in atmospheric air, turtles breathe irregularly and show long periods of apnoea (Boyer, 1963); turtles (*Chrysemys picta*) have been found to tolerate anoxia (breathing of air without O_2) for an average of 1104 min (20 h) and stagnant anoxia (arrest of circulation) for 72 min (Belkin, 1968); at 3 °C, turtles can submerge for 6 months in an anoxic condition (Ultsch & Jackson, 1982); at 16–18 °C they can survive an anaerobic dive for up to 2 weeks (Robin *et al.* 1964); breathing 100 % N_2, turtles (*Pseudemys scripta*) have survived for at least 48 h; and *Chrysemys* can tolerate 93 h in oxygen-free water (Folk, 1974).

The marine green turtle can bury itself in mud on the ocean floor for 1–3 months (Felger *et al.* 1976). This great tolerance of anoxia, with survival of the brain tissue,

has been attributed to a remarkable ability to use anaerobic glycolysis as an energy source (Belkin, 1961; Millen *et al.* 1964; Sick *et al.* 1982; Caligiuri & Robin, 1985). In contrast, failure of anaerobic glycolysis to maintain adequate levels of ATP and creatine phosphate limits anaerobic metabolism in freshwater turtles (*Pseudemys scripta elegans*) (Clark & Miller, 1973). The increased glycolytic capacity is related to a high activity of various glycolytic enzymes (Robin *et al.* 1979). Other mechanisms are also involved: (1) reduced energy requirement during prolonged anoxia in diving (Robin *et al.* 1981); (2) increased resistance of the brain to acidosis as severe as pH 6·20 (Caligiuri & Robin, 1985); (3) cardiovascular adjustment including bradycardia and redistribution of cardiac output such that blood from the systemic venous circulation (tissue) bypasses the lung and enters the aorta directly (Millen *et al.* 1964; White & Roos, 1966); (4) removal of plasma CO_2, probably through the body surface, during apnoea diving (Jackson & Silverblatt, 1974). It should be noted that cardiac arrest is a further extension of bradycardia. It entirely stops the circulation and hence curtails the energy expenditure of the whole body. Thus, in a turtle's brain stem the highly developed vagal mechanism responsible for cardiac inhibition is an important component of the cardiovascular adjustment that prolongs tolerance to anoxia. It is necessary, however, to find out whether a turtle heart stops beating completely or continues beating at an extremely slow rate during prolonged diving.

A pressor response effected through activation of the sympathetic system was also observed in these experiments. However, because of its long latency, slow rise and low magnitude, this hypertension appeared to be less significant than the bradycardia. Furthermore, during stimulation a concomitant increase of heart rate and cardiac contractility, very common in cats and dogs, was seldom seen in the turtles. Thus, the sympathetic mechanism in the brain stem of turtles seems principally to involve vasomotor action. It is interesting to note, however, that despite the low sympathetic function in this species, a mechanism for sympathetic inhibition does exist in the medulla.

Whether turtles are provided with reflex baroreceptor mechanisms like those of mammals has been a subject of interest for years. Some authors have supported the existence of this reflex (Millard & Moalli, 1980; Smits & Kozubowski, 1985) and others have not (Stephens *et al.* 1983). In experiments utilizing probing and mechanical occlusion of the vessels, including the region of the bulbus cordi proximal to the common pulmonary artery, an increased pressure in these vessels evoked an immediate but transient increase in synchronized traffic in the vagal efferents to the heart (Faraci *et al.* 1982). A similar baroreceptor mechanism found in the pulmonary cutaneous artery of an amphibian (the toad) serves to protect the vasculature of the lung from excessive hydrostatic pressure (West & Van Vliet, 1983).

Because the turtle lives in an aquatic habitat, usually maintaining a horizontal posture, its gravitational stress is minimal. Besides, turtles move slowly, they are extremely tolerant of anoxic conditions, and their defence mechanism seems to be escape rather than attack. It is understandable, therefore, that an active

baroreceptor mechanism for rapid cardiovascular adjustment may not be critical. In the present study, in which systemic injection of epinephrine induced an active increase in blood pressure, the heart rate remained unchanged, without the reflex bradycardia commonly seen in mammals after systemic administration of epinephrine. This observation is consistent with the absence of an active baroreceptor reflex mechanism in turtles. It should be noted, however, that for some reason, despite the use of a large dose of epinephrine (up to 16 μg 100 g^{-1} i.v.), the rate of pressure rise after administration of this pressor agent was slow in turtles. Whether this slow rise is responsible for the failure to induce a reflex bradycardia and thus hinders the detection of baroreceptors in turtle remains to be determined.

In the present study, microinjection of transmitter substances, acetylcholine, norepinephrine and epinephrine, and excitatory amino acids into the responsive regions of the brain stem failed to produce responses comparable to those evoked by electrical stimulation. This apparent difference deserves further study. Recently, Reiner (1987) reported a wide distribution of enkephalin peptides in the turtle's brain. It is interesting to note that peptide location in the brain stem shows a certain similarity to the bradycardia areas described in the present study.

In summary, the findings of the present experiments indicate the presence in the turtle brain stem of a very powerful vagal component responsible for bradycardia which may override coincident sympathetic activity. Its function may partially account for the turtle's powerful capacity for tolerating anoxia.

The authors thank R. H. Lin, Y. F. Lin, A. M. Y. Lin and C. L. Na for their most valuable assistance in these experiments, and L. L. Chen and G. T. Chen for preparation of the manuscript and illustrations. Special thanks are due to Professor John R. Brobeck of University of Pennsylvania for advice and reading the manuscript. Thanks are due to Drs Paul N. Yu and Shu Chien for their support and encouragement. This study was supported in part by the Institute of Biomedical Sciences, Academia Sinica and the National Science Council, ROC nos 755327-070 and 770412-B001.

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