Facilitation of the arterial baroreflex by the ventrolateral part of the midbrain periaqueductal grey matter in rats

Koji Inui, Sumio Murase* and Shoichiro Nosaka*

*Departments of Psychiatry and *Physiology, Mie University School of Medicine, Tsu, Mie 514, Japan*

1. The effects of stimulation of the ventrolateral part of the midbrain periaqueductal grey matter (PAG) on the arterial baroreflex were investigated in urethane–chloralose anaesthetized and artificially ventilated rats.

2. Both electrical and chemical stimulation of the ventrolateral PAG provoked hypotension, vagal bradycardia and marked facilitation of baroreflex vagal bradycardia (BVB), which was induced by stimulation of the aortic depressor nerve. The magnitude of ventrolateral PAG facilitation of BVB was $328 \pm 193\%$ ($n = 34$) for electrical stimulation and $243 \pm 224\%$ ($n = 13$) for chemical stimulation. Baroreflex hypotension was slightly augmented during either electrical or chemical stimulation of the ventrolateral PAG in vagotomized rats.

3. Stimulation of the nucleus raphe magnus (NRM) also provoked hypotension, vagal bradycardia and facilitation of BVB. The magnitude of BVB facilitation was $234 \pm 132\%$ ($n = 8$) for electrical stimulation and $328 \pm 170\%$ ($n = 7$) for chemical stimulation. After microinjection of kainic acid into the NRM region, baroreflex facilitation, as well as hypotension and vagal bradycardia, produced by ventrolateral PAG stimulation, was almost abolished.

4. In conclusion, the ventrolateral PAG, besides producing hypotension and bradycardia, facilitates arterial baroreflexes. These effects are exerted via the NRM, sharply contrasting with effects of the dorsal PAG.

The midbrain periaqueductal grey matter (PAG) is involved in a variety of functions that include defensive behaviour (Hilton & Redfern, 1986; Bandler & Carrive, 1988), vocalization (Jürgens, 1991), autonomic functions (Carrive, Bandler & Dampney, 1989), sexual behaviour (Sakuma & Pfaff, 1979) and pain control (Lovick, 1993*a*). Some recent studies have indicated, however, that the PAG is not a functionally homogeneous structure and that the dorso- and ventrolateral parts of the PAG produce different or even opposing effects. For example, stimulation of neurones in the dorsolateral part of the PAG provokes defensive behaviour that is accompanied by marked sympathoexcitation, whereas stimulation of the ventrolateral part produces immobility and sympathoinhibition (Bandler, Carrive & Zhang, 1991). Both the dorso- and ventrolateral portions of the PAG subserve antinociception, but the analgesic effects are of differing types with different descending pathways (Lovick, 1993*a*). Anatomically, it has been demonstrated that there are apparently different patterns of afferent and efferent projections to/from the dorso and ventrolateral parts of the PAG (Veening, Buma, Horst, Roeling, Luiten & Nieuwenhuys, 1991; Holstege, 1991).

Among the different or opposing effects produced by the dorso- and ventrolateral parts of the PAG, the cardiovascular changes that accompany behavioural reactions have been studied most intensively by Bandler & Carrive (1988), Carrive *et al.* (1989), Bandler *et al.* (1991) and Lovick (1992). Stimulation of the dorsolateral part of the PAG produces an increase in blood pressure, tachycardia, mesenteric vasoconstriction and vasodilation in the skeletal musculature in the hindlimbs, which represent part of a complex co-ordinated defence reaction. As a unique cardiovascular involvement, arterial baroreflexes are suppressed during dorsolateral PAG stimulation (Jones, Kirkman & Little, 1990; Nosaka, Murata, Inui & Murase, 1993). To warrant a sufficient blood supply to the dilated vessels in the skeletal musculature during defensive behaviour, the arterial baroreflex must be actively suppressed in the face of concomitant hypertension (Nosaka *et al.* 1993). In contrast to such dynamic cardiovascular changes evoked by the dorsolateral PAG, stimulation of the ventrolateral PAG produces hypotension and bradycardia (Lovick, 1992), accompanied by immobility (Bandler *et al.* 1991). Furthermore, it has been reported by Lovick (1992) that activation of neurones in the

ventrolateral PAG inhibits cardiovascular defence responses elicited by dorsal PAG stimulation. In view of these findings, it is relevant to examine whether the ventrolateral PAG affects arterial baroreflexes, a subject that has not previously been investigated.

This study was designed to determine whether and how the ventrolateral part of the PAG modulates the arterial baroreflex. Furthermore, we sought to identify the descending pathway of the modulation of baroreflexes by the ventrolateral PAG. In our study, the aortic depressor nerve (ADN) was electrically stimulated to provoke a control baroreflex. The major advantage of ADN stimulation to provoke baroreflexes in rats is the fact that the ADN in the rat contains only baroreceptor afferents (Sapru & Krieger, 1977).

METHODS

Male Wistar rats, weighing 350–400 g, were anaesthetized by intraperitoneal injection of α-chloralose (60 mg kg^{-1}) and urethane (600 mg kg^{-1}). One-sixth of the initial dose was administered at 3 h intervals to maintain anaesthesia. The trachea was cannulated and polyethylene tubes were inserted into the left femoral vein and artery for administration of drugs and measurement of blood pressure, respectively. Animals were immobilized by intravenous injection of succinylcholine (10 mg kg^{-1}) and ventilated artificially. The depth of the anaesthesia under paralysis was assessed by stability of arterial blood pressure and heart rate. β-Blockade was achieved by intravenous injection of propranolol (bolus injection of 0·4 mg kg^{-1} followed by 0·2 mg kg^{-1} at 2 h intervals). Under such conditions, changes in heart rate could be attributed to changes in vagus nerve activities. The aortic depressor nerves (ADNs) on both sides were prepared for electrical stimulation as described previously (Nosaka, Murase & Murata, 1991). Carotid sinus nerves (CSNs) on both sides were cut in each rat to eliminate any possible interactions between inputs from the ADNs and the CSNs. The ADN on a given side was electrically stimulated and responses in terms of blood pressure and heart rate were recorded. Parameters for the stimulation were 8 V and 0·5 ms duration. By adjusting the stimulation frequency, we reduced the bradycardiac response to ADN stimulation to less than one-third of the maximum response, so that both facilitatory and inhibitory effects of PAG stimulation could be examined. Once the appropriate stimulation frequency had been determined, it was held constant throughout the experiment.

Stimulation of the PAG

The PAG ipsilateral to the ADN used was stimulated either chemically or electrically. Chemical stimulation was achieved by pressure injection of DL-homocysteic acid (DLH, 6 μg in

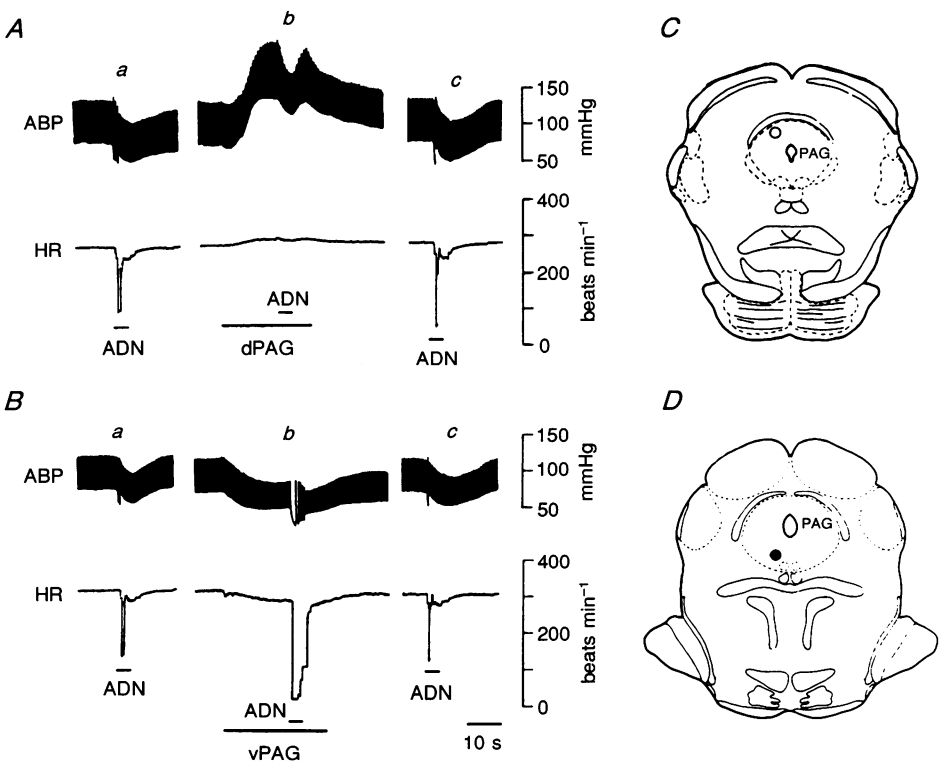

Figure 1. Effects of electrical stimulation of the dorsal PAG and the ventrolateral PAG on the arterial baroreflex

A a and *A c*, control baroreflex provoked by aortic depressor nerve (ADN) stimulation (8 V, 0·5 ms and 30 Hz); *A b*, ADN response during dorsal PAG (dPAG) stimulation (30 μA, 0·5 ms, 50 Hz). *B a* and *B c*, control baroreflex; *B b*, ADN response during ventrolateral PAG (vPAG) stimulation. *C* and *D*, stimulated sites. Periods of the respective stimulations are indicated by horizontal bars. ABP, arterial blood pressure; MBP, mean blood pressure; HR, heart rate. *A–D*, data from one and the same rat.

100 nl over 15 s) through a tip-blunted glass capillary pipette (tip diameter, 50 μm). Stimulated sites were confirmed histologically with Fast Green that had been dissolved in the solution of DLH. Electrical stimulation was conducted through a monopolar stainless-steel electrode (100 μm in diameter, fully insulated except for the tip) by passing cathodal square-wave current pulses (20–35 μA, 0·5 ms and 50 Hz). The sites of the stimulation were confirmed by histochemical demonstration of iron ions that had been deposited by delivery of an anodal direct current (DC, 20 μA for 25 s) at the end of each experiment.

Brain lesions

In order to localize the descending pathway of ventrolateral PAG modulation of the arterial baroreflex, lesions in the ventromedial medulla were made either electrolytically or chemically. Electrolytic lesions were made by passing a cathodal DC (1·0 mA for 60 s) through a monopolar stainless-steel electrode (150 μm in diameter). For a chemical lesion, microinjection of kainic acid (KA, 1 μg in 500 nl over 10 min) was used. The extent of the lesion was confirmed by histochemical demonstration of HRP, dissolved in KA solution, by the DAB (diaminobenzidine) method. Effects of ventrolateral PAG stimulation were compared before and after the lesions had been made.

Analysis of data

Changes in blood pressure were assessed by measurement of mean blood pressure in each experiment. The magnitudes of baroreflex vagal bradycardia (BVB) were evaluated by calculating the reduction in terms of heart beats during ADN stimulation, from measurements of the response area, the time integral of the heart rate. Although the beat to beat interval (R–R interval) has been known to provide a sensitive method for analyses of chronotrophic action of the vagus nerve on the heart, this parameter often becomes so large as to scale out the conventional recording range when the ADN is maximally stimulated or when simultaneously stimulated with the PAG, making it impossible to assess the data. Therefore, we used changes in heart rate in preference to changes of the R–R interval to evaluate the bradycardiac responses to ADN stimulation in the present study. Percentage facilitation of BVB was defined as

$$\frac{\text{actual response} - \text{control response}}{\text{control response}} \times 100.$$

Data were expressed as means ± standard deviations (S.D.) and analysed statistically by Student's t test. Pairs of data were regarded as significantly different when $P < 0{\cdot}05$.

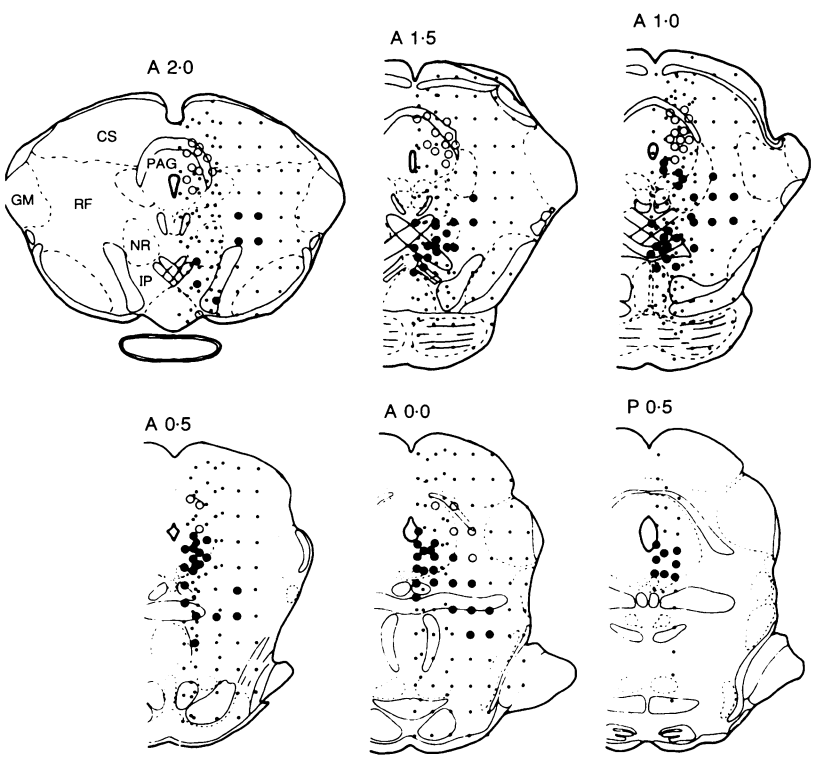

Figure 2. Midbrain sites that modulate baroreflex vagal bradycardia when electrically stimulated

The midbrain was electrically stimulated with 30 μA for 0·5 ms at 50 Hz. Sites inhibiting baroreflex vagal bradycardia (BVB) by more than 50 % and sites facilitating BVB by more than 100 % are indicated by open and filled circles, respectively. Ineffective sites are indicated by dots. CS, superior colliculus; GM, medial geniculate body; IP, interpeduncular nucleus; NR, red nucleus; PAG, periaqueductal grey matter; RF, reticular formation.

RESULTS

Effects of electrical stimulation of the ventrolateral PAG on baroreflexes

When the ventrolateral PAG was electrically stimulated, blood pressure fell (from 108·0 ± 19·2 to 88·5 ± 20·9 mmHg, $P < 0·001$, $n = 34$ rats) with a slight decrease in heart rate (from 307·4 ± 30·4 to 287·9 ± 32·2 beats min^{-1}). During ventrolateral PAG stimulation, ADN-induced baroreflex vagal bradycardia (BVB) was markedly augmented (Fig. 1*B*). The magnitude of BVB facilitation due to ventrolateral PAG stimulation ranged from 100 to 858 %, the mean value being 328·7 % above the control response. The effects of ventrolateral PAG stimulation on blood pressure, heart rate and BVB contrasted with those of dorsolateral PAG stimulation (Fig. 1*A*).

In order to localize precisely the midbrain sites that modulate BVB on stimulation, tracking studies were conducted in ten rats. The midbrain was systematically tracked and stimulated electrically (30 μA, 0·5 ms, 50 Hz) at 0·25–0·5 mm consecutive steps. In agreement with our previous report (Nosaka *et al.* 1993), stimulation of the dorsolateral PAG inhibited BVB induced by ADN stimulation. In addition, these BVB inhibitory sites were found to be located in the rostral two-thirds of the dorsolateral PAG. By contrast, stimulation of the caudal half of the ventrolateral PAG facilitated BVB (Fig. 2). Facilitation of BVB was also obtained from two other areas; the central tegmental field and the tegmentum near the decussation of the brachium conjunctivum.

Effects of chemical stimulation of the ventrolateral PAG on baroreflexes

In order to examine whether or not the facilitatory effect of the ventrolateral PAG on BVB is due to activation of neuronal cell bodies, chemical stimulation with DLH of the midbrain of seventeen rats was performed. Following administration of DLH into the ventrolateral PAG there was a fall in blood pressure (from 98·7 ± 15·4 to 86·3 ± 13·1 mmHg, $P < 0·001$, $n = 13$ rats), a decrease in heart rate (from 320·4 ± 24·5 to 310·2 ± 32·5 beats min^{-1}) and BVB was significantly facilitated (percentage facilitation, 243 ± 224 %; Fig. 3*B*). Figure 3 shows the sharply contrasting effects of chemical stimulation of the dorso- and ventrolateral PAG of a single rat, in terms of blood pressure, heart rate and BVB. Locations of sites that

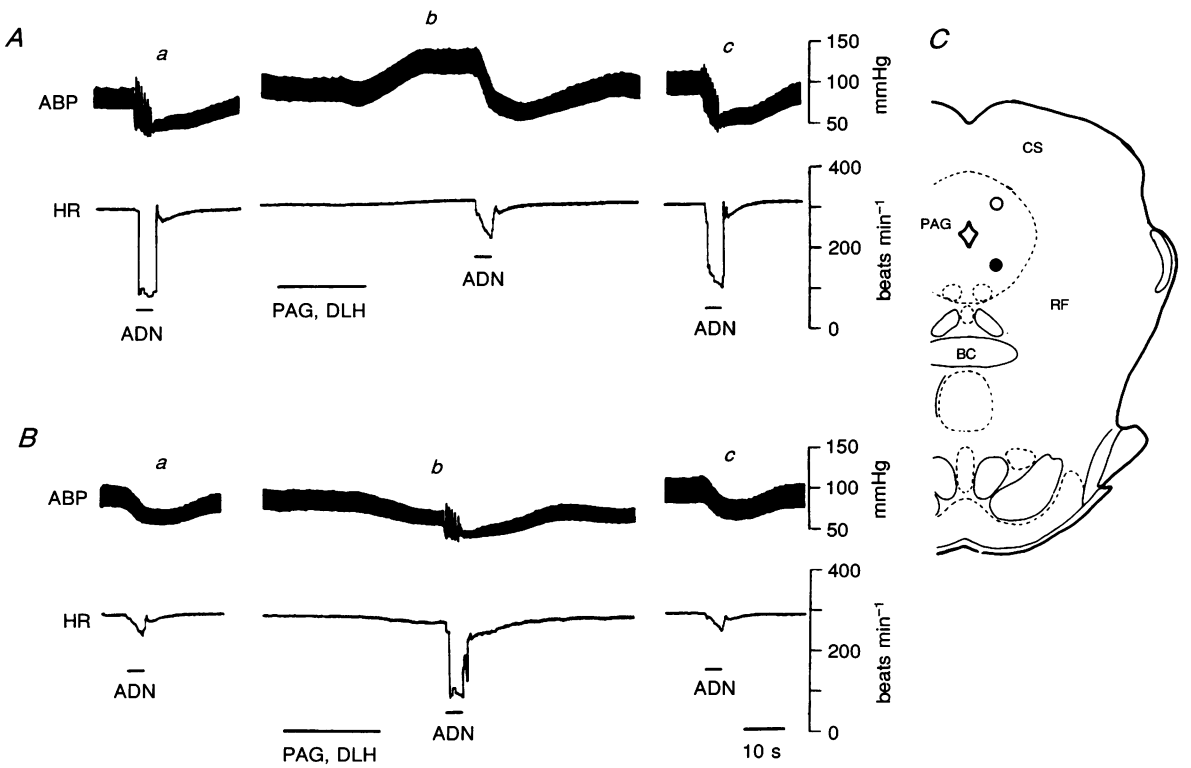

Figure 3. Effects of chemical stimulation of the dorsal and ventrolateral PAG on the baroreflex
Aa, control baroreflex provoked by aortic depressor nerve (ADN) stimulation (8 V, 0·5 ms, 50 Hz); *Ab*, ADN response following DLH microinjection (6 μg in 100 nl) into the dorsal PAG; *Ac*, control baroreflex 15 min after the injection. *Ba*, control baroreflex provoked by ADN stimulation (8 V, 0·5 ms, 25 Hz); *Bb*, ADN response following DLH microinjection (6 μg in 100 nl) into the ventrolateral PAG; *Bc*, control ADN response 15 min after the injection. *C*, sites of injection. *A–C*, data from one and the same rat. Periods of microinjection and ADN stimulation are indicated by horizontal bars.

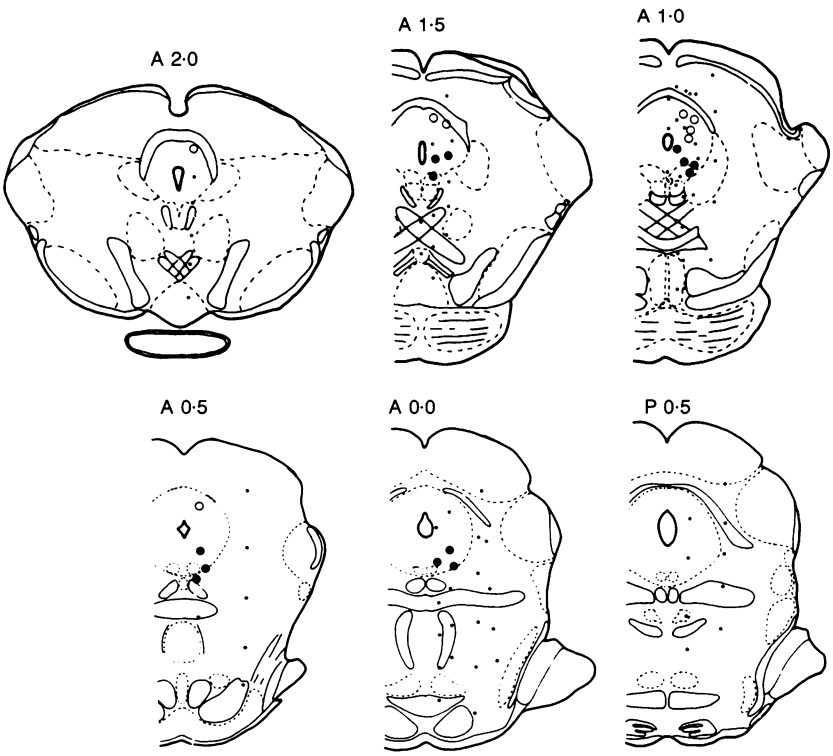

Figure 4. Midbrain sites that modulate baroreflex vagal bradycardia when chemically stimulated
The midbrain was stimulated by microinjection of DLH (6 μg in 100 nl). Sites inhibiting baroreflex vagal bradycardia (BVB) by more than 50 % and sites facilitating BVB by more than 50 % are indicated by open and filled circles, respectively. Dots denote ineffective sites.

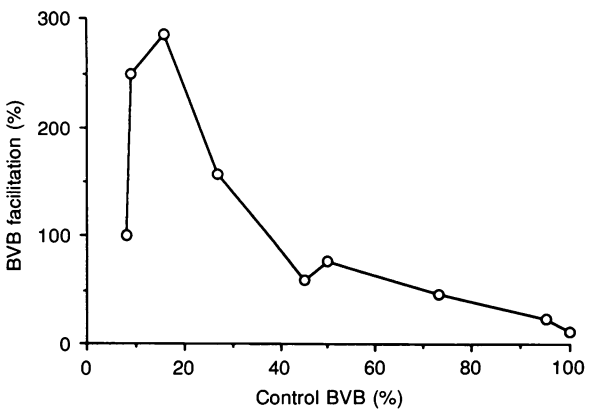

Figure 5. Relationship between the magnitude of control baroreflex vagal bradycardia and the potency of ventrolateral PAG facilitation
The magnitude of control baroreflex vagal bradycardia (BVB) is expressed as a percentage of the maximum response; the potency of ventrolateral PAG facilitation of BVB is expressed as a percentage above the control BVB.

modulated BVB on chemical stimulation are depicted in Fig. 4. Similar to the result of the tracking study described above, BVB inhibitory and facilitatory sites were shown to be located in the rostral part of the dorsolateral PAG and the caudal part of the ventrolateral PAG, respectively. Those two regions in the midbrain outside the PAG, which were shown to produce BVB facilitation upon electrical stimulation, had no effect when chemically stimulated.

Relationship between the magnitude of the control BVB and the facilitatory effect of the ventrolateral PAG

As we reported previously (Nosaka *et al.* 1993), the BVB is hardly influenced by ventrolateral PAG stimulation when a maximum ADN response is used as the control. In order to examine the relationship between the facilitatory effect of the ventrolateral PAG and the magnitude of control bradycardiac responses, the effects of ventrolateral PAG stimulation were examined on control responses of different magnitudes in four rats. For this purpose, the ADN was stimulated at 8 V with frequencies that ranged from 7 to 50 Hz. Typical data from a single rat are depicted in Fig. 5. The facilitatory effect of the ventrolateral PAG on BVB was most evident when the control BVB was reduced to about one-fifth of the maximum response. By contrast, the BVB was no longer potentiated when the maximum response was used as the control. This result indicates that there is a convergence between ADN-

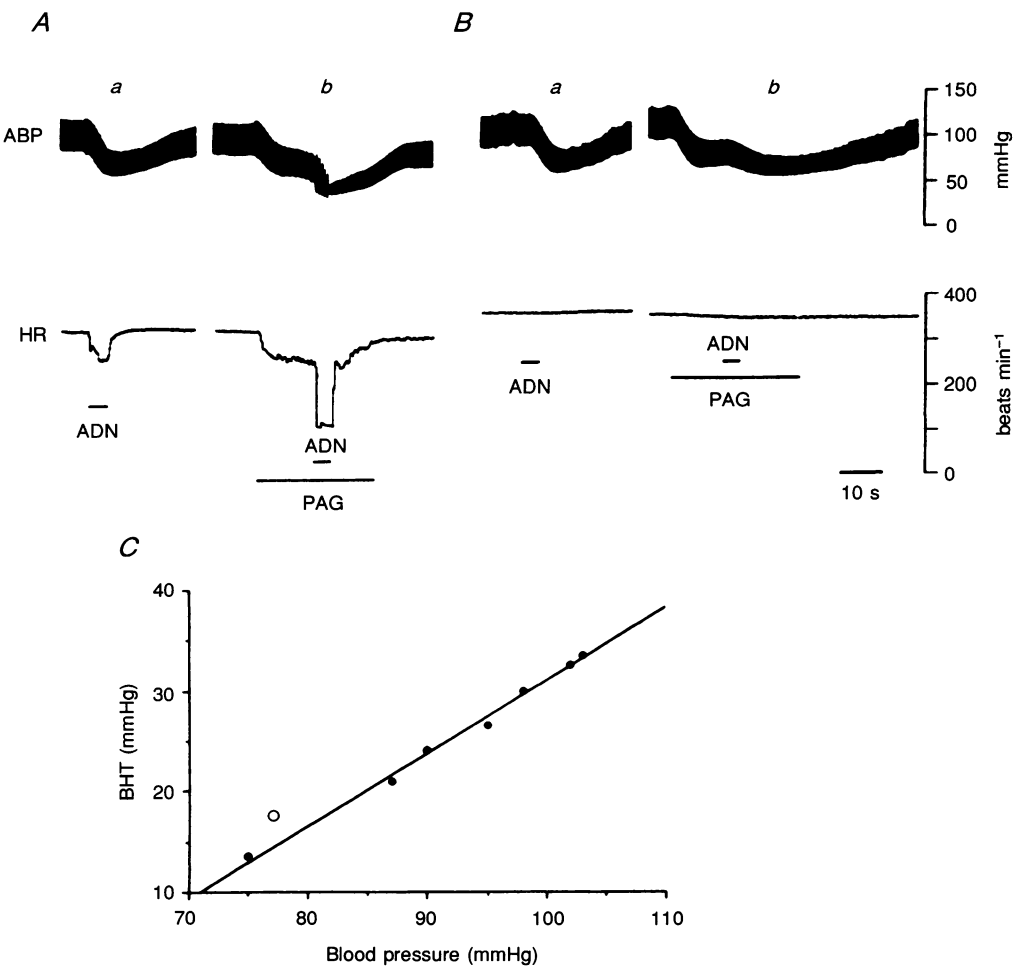

Figure 6. Effects of electrical stimulation of the ventrolateral PAG on aortic depressor nerve-induced hypotension in vagotomized rats

Aa and *Ba*, control baroreflex before and after vagotomy, respectively; panels *Ab* and *Bb*, effects of ventrolateral PAG stimulation (35 μA) on baroreflex before and after vagotomy, respectively. Periods of aortic depressor nerve (ADN) stimulation and ventrolateral PAG stimulation are indicated by horizontal bars. *C*, relationship between the magnitude of baroreflex hypotension (BHT) and the initial blood pressure after vagotomy. ○, BHT during ventrolateral PAG stimulation; ●, control BHT at different initial blood pressures.

activated and ventrolateral PAG-activated neurones somewhere in the baroreflex arc.

Effects of ventrolateral PAG stimulation on baroreflex hypotension in vagotomized rats

In order to determine whether the ventrolateral PAG modulates the vascular component of the arterial baroreflex, animals were bilaterally vagotomized so that changes in blood pressure could be attributed entirely to changes in sympathetic nerve activities. Since the baseline blood pressure was changed during PAG stimulation, baroreflex hypotension before and during PAG stimulation could not be compared simply by their magnitudes. For precise evaluation, a method that we described previously (Nosaka *et al.* 1993) was employed. In each rat, the magnitude of the decrease in blood pressure due to ADN stimulation was measured at several different initial resting blood pressure levels. The magnitude was plotted against the resting blood pressure and a straight line was obtained (Fig. 6*C*). Thus, the expected magnitude of ADN-induced reflex hypotension could be obtained from the straight line for any given blood pressure (for details of this method, see Nosaka *et al.* 1993).

In seven rats, a stimulating electrode was positioned in the ventrolateral PAG in which stimulation provoked marked facilitation of BVB. After bilateral vagotomy, stimulation at the same site produced hypotension (from 102·9 ± 11·3 to 87·1 ± 14·6 mmHg, $P < 0·05$) with a minimal change in heart rate (from 328·6 ± 22·2 to 326·9 ± 21·8 beats min^{-1}). During the stimulation, reflex hypotension was slightly but significantly facilitated (12·9 ± 9·7 % above the expected magnitude, $P < 0·05$, Fig. 6). Likewise, a slight facilitation of baroreflex hypotension was observed following DLH injection into the ventrolateral PAG (percentage facilitation, 9·0 ± 13·7 %, $n = 6$; Fig. 7). The magnitude of this facilitation of reflex hypotension by chemical stimulation of the ventrolateral PAG was not, however, statistically significant.

Descending pathway of ventrolateral PAG facilitation of BVB

The next experiments were conducted to localize the descending pathway of ventrolateral PAG effects on the cardiovascular functions. Efferent projections from the ventrolateral PAG to the ventromedial medulla including the nucleus raphe magnus (NRM) have been established anatomically (Beitz, Shepard & Wells, 1983; Fardin, Oliveras & Besson, 1984; Meller & Dennis, 1991). It seems possible that the cardiovascular effects of the ventrolateral PAG are mediated by the NRM since stimulation of this

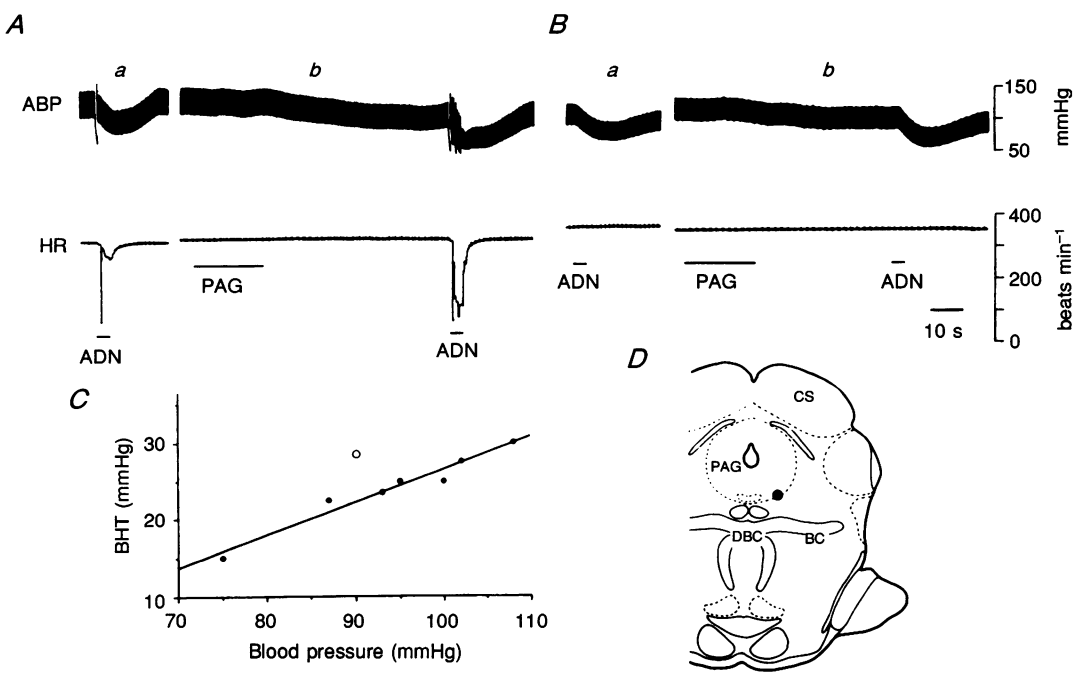

Figure 7. Effects of chemical stimulation of the ventrolateral PAG on aortic depressor nerve-induced baroreflex hypotension in vagotomized rats

Aa and *Ba*, responses to aortic depressor nerve (ADN) stimulation before and after vagotomy, respectively; *Ab* and *Bb*, ADN responses following DLH (6 μg in 100 nl) injection into the ventrolateral PAG. Periods of microinjection and ADN stimulation are indicated by horizontal bars. *C*, relationship between the magnitude of baroreflex hypotension (BHT) and the initial blood pressure level. ○, BHT following chemical stimulation of the ventrolateral PAG; ●, control BHT at different initial blood pressures. *D*, site of DLH injection.

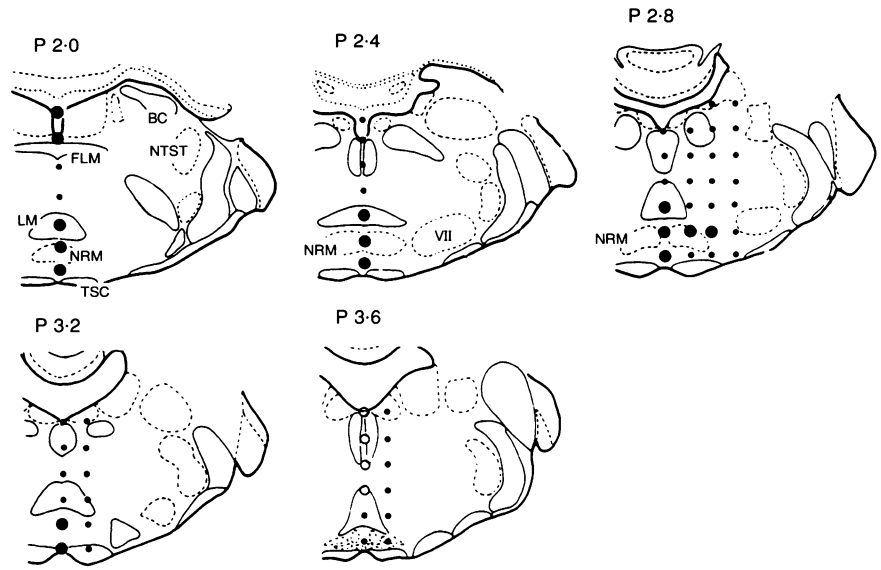

Figure 8. Sites in the ventromedial medulla and the pons that modulate baroreflex vagal bradycardia when electrically stimulated
The ventromedial medulla was electrically stimulated with 30 μA for 0·5 ms at 50 Hz. Sites that inhibited baroreflex vagal bradycardia (BVB) by more than 50 % are indicated by ○. Sites that facilitated BVB by more than 100 % are indicated by ●. Ineffective sites are indicated by dots. BC, brachium conjunctivum; FLM, medial longitudinal fasciculus; LM, medial lemniscus; NRM, nucleus raphe magnus; NTST, nucleus of the spinal tract of the trigeminal nerve; V, trigeminal nerve; VII, facial nucleus.

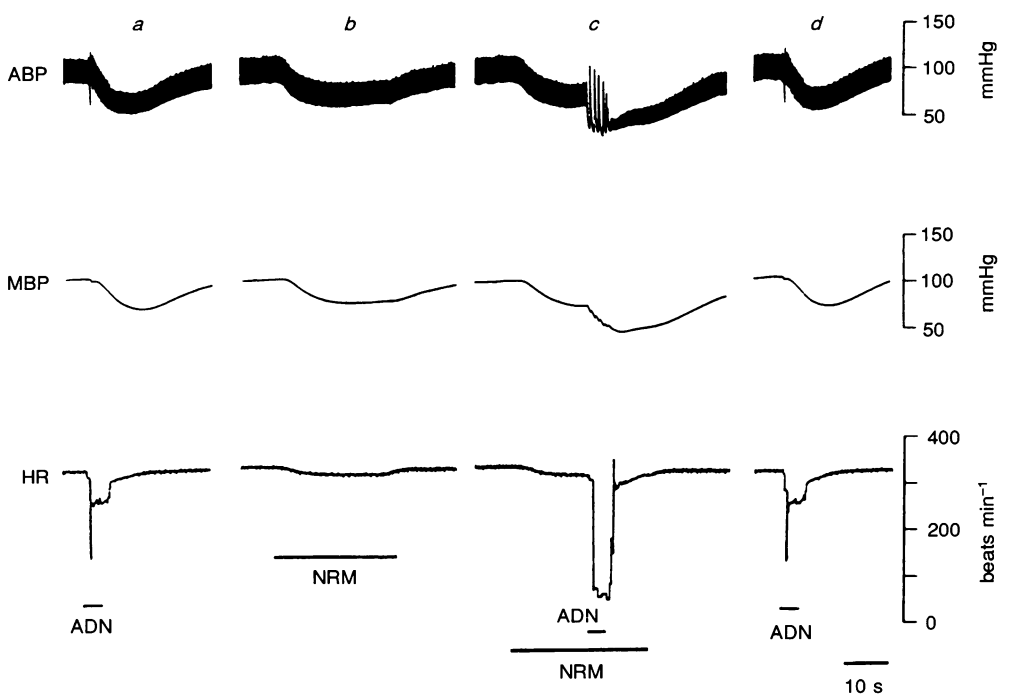

Figure 9. Effects of electrical stimulation of the nucleus raphe magnus on the arterial baroreflex
a and *d*, control baroreflexes provoked by aortic depressor nerve (ADN) stimulation (8V, 0·5 ms, 17 Hz). *b*, effects of electrical (30 μA, 0·5 ms, 50 Hz) stimulation of the nucleus raphe magnus (NRM) on blood pressure and heart rate. *c*, effects of NRM stimulation on baroreflex. Periods of ADN and NRM stimulation are indicated by horizontal bars.

nucleus has been reported to produce hypotension and bradycardia in a similar manner (Henry & Calaresu, 1974; Adair, Hamilton, Scappaticci, Helke & Gillis, 1977; Yen, Blum & Spath, 1983). To examine this hypothesis, we first conducted a tracking study in this region, with four rats. The ventromedial medulla was systematically explored with electrical stimulation (30 μA, 0·5 ms and 50 Hz) and BVB modulatory sites were mapped. Results from a single rat are shown in Fig. 8. Sites that facilitated the BVB were found in and around the NRM. When the ventral part of the NRM was electrically stimulated, blood pressure fell (from 111·7 ± 9·4 to 82·5 ± 19·9 mmHg, $P < 0·05$, $n = 8$ rats), there was a decrease in heart rate (from 309·4 ± 31·1 to 289·0 ± 37·0 beats min^{-1}) and BVB was facilitated (percentage facilitation, 234 ± 132 %, $P < 0·005$; Fig. 9). This BVB facilitation by the NRM was reproduced by selective cell body activation in this region. DLH (6 μg in 100 nl) administered into the NRM produced vagal bradycardia (from 325·7 ± 24·3 to 312·4 ± 26·1 beats min^{-1}, $n = 7$ rats) and BVB facilitation (percentage facilitation, 328 ± 170 %, $P < 0·005$; Fig. 10). Blood pressure responses to chemical stimulation of the NRM were inconsistent: a small increase in three rats, a small decrease in two and no change in two.

In spite of the inconsistent blood pressure changes, these findings indicated that there are cell bodies in the NRM that are responsible for cardiovascular effects similar to those of the ventrolateral PAG stimulation. Therefore, we examined whether or not the NRM is involved in the cardiovascular effects of ventrolateral PAG stimulation in our subsequent experiments.

The tip of the electrode was positioned in the NRM at a site at which stimulation provoked marked BVB facilitation, and an electrolytic lesion was made in this region by passing a cathodal direct current (DC) through the same electrode. The cardiovascular effects of ventrolateral PAG stimulation were compared before and after the destruction. We found that BVB facilitation by the ventrolateral PAG was almost abolished after the destruction (percentage facilitation, 350 ± 196 % before; 1·7 ± 4·0 % after, $n = 6$, Fig. 11). Moreover, the decrease in blood pressure (14·5 ± 12·5 mmHg) elicited by ventrolateral PAG stimulation was changed into a small increase (3·3 ± 6·0 mmHg) after the destruction. Likewise, the bradycardiac response to ventrolateral PAG stimulation (17·5 ± 11·1 beats min^{-1}) was almost abolished after the destruction (0·8 ± 4·9 beats min^{-1}).

Selective destruction of cell bodies in the NRM region was also effective in eliminating cardiovascular effects due to the ventrolateral PAG. Following microinjection of KA into the NRM region, BVB facilitation was reduced from 383 ± 226 % to 21 ± 35 %, $n = 9$ (Fig. 12). Depressor and bradycardiac responses to ventrolateral PAG stimulation were also almost abolished after the destruction of the cell bodies (from 24·7 ± 14·1 to −1·1 ± 7·4 mmHg and from 23·9 ± 15·0 to 4·0 ± 7·3 beats min^{-1}, respectively). Resting

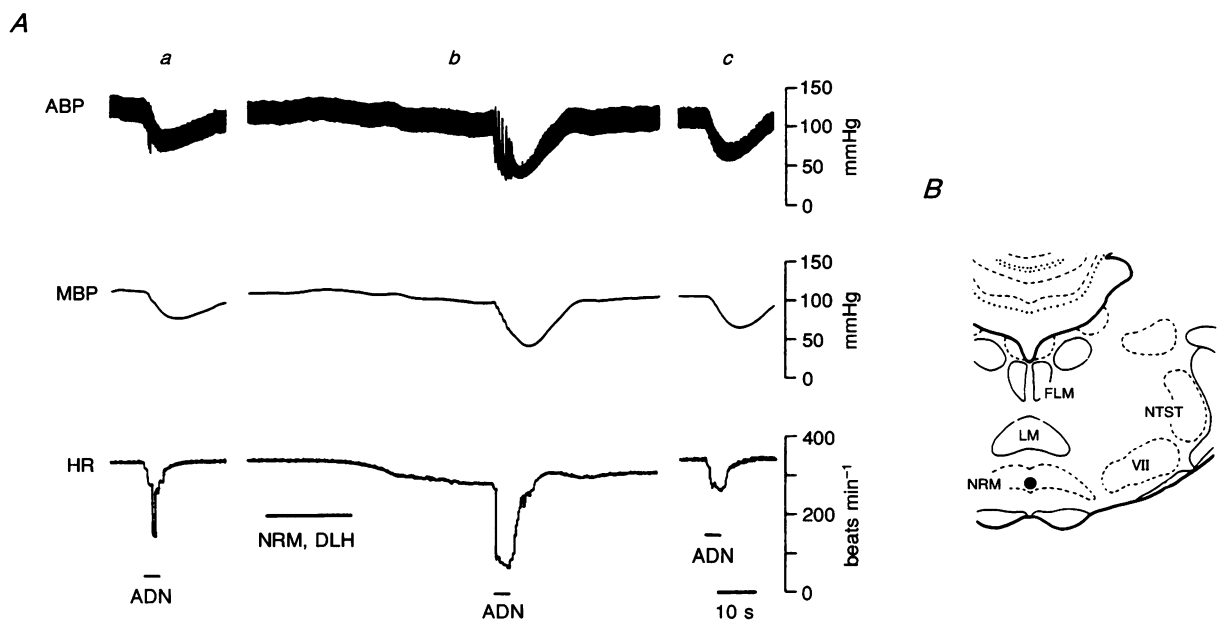

Figure 10. Effects of chemical stimulation of the nucleus raphe magnus on arterial baroreflex
A a, control responses of the aortic depressor nerve (ADN); *A b*, ADN response following DLH (6 μg in 100 nl) injection into the nucleus raphe magnus (NRM); *A c*, control ADN response 20 min after the injection. Periods of microinjection and ADN stimulation are indicated by horizontal bars. *B*, site of DLH injection. BC, brachium conjunctivum; CIF, inferior colliculus; CS, superior colliculus; FLM, medial longitudinal fasciculus; LM, medial lemniscus; PAG, midbrain periaqueductal grey matter; VII, facial nucleus.

blood pressure elevated after KA injection into the NRM region as shown in Fig. 12 (110·2 ± 15·7 before and 136·1 ± 14·1 mmHg after the injection, $P < 0.005$). Lesions that were effective in attenuating the cardiovascular effects of the ventrolateral PAG extended from the rostral part to the caudal part of the NRM, but lesions outside the NRM lateral or dorsal to it were without effect ($n = 5$).

Additionally, we examined whether the inhibitory effect of the dorsal PAG on BVB is mediated by the NRM region as well. In four rats, stimulating electrodes were positioned separately in the dorso- and ventrolateral PAG, in which stimulation provoked inhibition and facilitation of BVB, respectively. After KA injection into the NRM region, BVB facilitation due to the ventrolateral PAG was remarkably reduced (from 332 ± 184 to 25 ± 11 %), whereas BVB inhibition by the dorsal PAG was only slightly affected (from 67·5 ± 29 to 67·6 ± 23 %). Likewise, electrolytic lesions of the NRM region were almost without effect on dorsal PAG-induced BVB inhibition. The extent of the inhibition was 56·3 ± 11·9 % before, and 58·8 ± 5·4 % after the destruction ($n = 4$).

DISCUSSION

The present study confirmed the previous findings that the dorso- and ventrolateral PAG have opposing effects on blood pressure and heart rate. In addition, this study revealed for the first time that these two regions also have sharply contrasting effects on the arterial baroreflex, that is, the dorsolateral PAG inhibits the baroreflex while the ventrolateral PAG facilitates it. As we discussed previously elsewhere (Nosaka *et al.* 1993), the physiological significance of arterial baroreflex suppression during dorsal PAG activation is probably related to full-scale expression of the concomitant defence reaction. Cardiovascular components of the defence reaction include hypertension, tachycardia and redistribution of cardiac output from the viscera to the skeletal muscles, which support augmented behavioural activities of animals. If the baroreflex functions to its full extent during the defence reaction, hypertension should cause a decrease rather than an increase in cardiac output which is needed to supply the dilated vessels in the hindlimbs. Furthermore, the arterial baroreflex provokes,

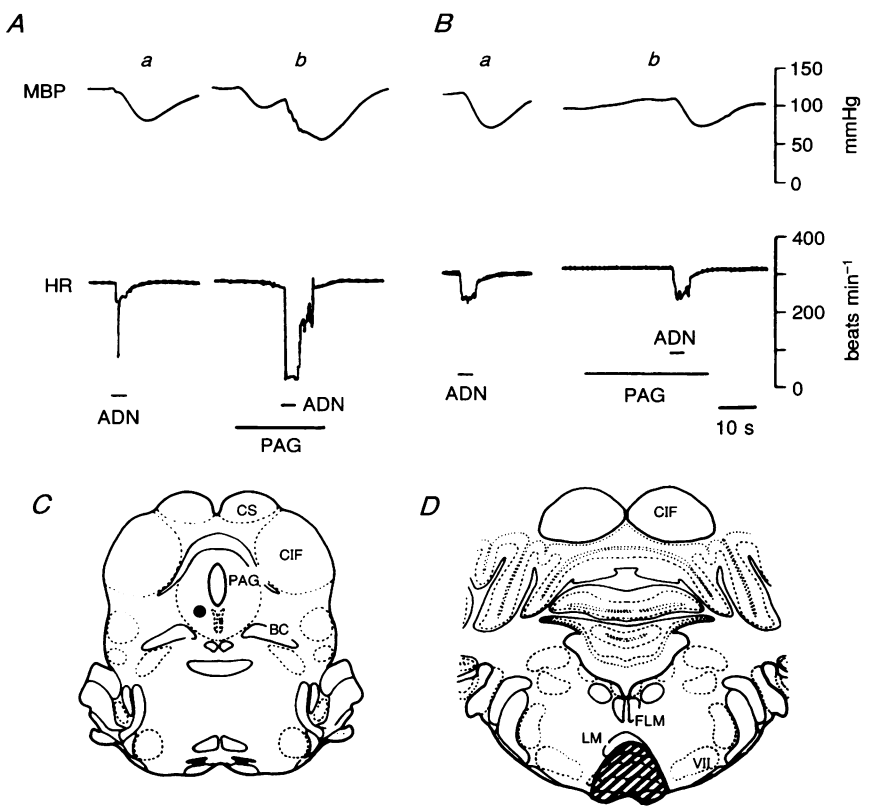

Figure 11. Effects of electrolytic destruction of the nucleus raphe magnus on ventrolateral PAG facilitation of baroreflex

Aa and *Ba*, aortic depressor nerve (ADN) responses before and after electrolytic destruction of the nucleus raphe magnus (NRM), respectively; *Ab* and *Bb*, effects of ventrolateral PAG stimulation on the baroreflex before and after the electrolytic destruction, respectively. Periods of ADN stimulation and ventrolateral PAG stimulation are indicated by horizontal bars. *C*, site of stimulation. *D*, extent of the lesion in the NRM. FLM, medial longitudinal fasciculus; LM, medial lemniscus; NTST, nucleus of the spinal tract of the trigeminal nerve; VII, facial nucleus.

in addition to the cardiovascular effects, inhibition of respiration, a decrease in muscle tone and synchronization of the EEG (Heymans & Neil, 1958), all of which may interfere with the full expression of the defence reaction. Thus, arterial baroreflex suppression is a prerequisite for the defence reaction to develop to the required extent.

In contrast to the dynamic cardiovascular changes produced by the dorsolateral PAG, ventrolateral PAG stimulation elicited hypotension, bradycardia and marked baroreflex facilitation. Although the physiological significance of the cardiovascular effects of the ventrolateral PAG has not been established, it should be emphasized that all the effects are the opposite of those associated with the cardiovascular defence reaction produced by the dorsal PAG. It is possible that the ventrolateral PAG plays a role in preventing excessive cardiovascular changes due to activation of the dorsal PAG, or in facilitating recovery from energy-consuming, ergotrophic states. Indeed, ventrolateral PAG stimulation has been demonstrated to inhibit the cardiovascular defence response provoked by the dorsal PAG (Lovick, 1992). With respect to baroreflex facilitation, many of its consequences, including sympathoinhibition, parasympathoexcitation, reduction of muscle tone and synchronization of the EEG (Heymans & Neil, 1958) are considered to be mostly advantageous in terms of recuperation after the defence reaction. Alternatively, it is possible to speculate that the ventrolateral PAG constitutes a crucial site in the forebrain mechanism reinforcing the basic medullary baroreflex function. Such a view has been proposed for the preoptic–anterior hypothalamic area (Hilton & Spyer, 1971; Spyer, 1972). It is noteworthy that anatomical connection between the preoptic–anterior hypothalamic area and the ventrolateral PAG has been delineated (Swanson, 1976).

The present study reveals that the baroreflex facilitation, as well as the hypotension and bradycardia, produced by ventrolateral PAG stimulation is mediated by

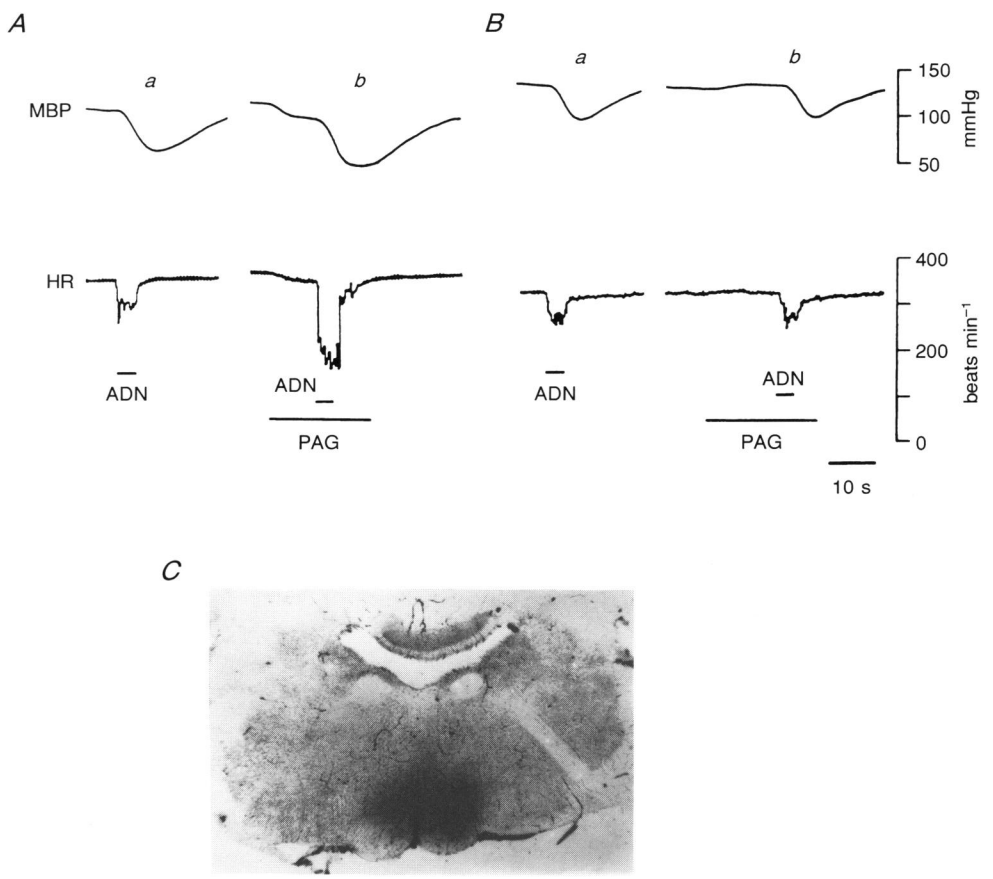

Figure 12. Effects of selective destruction of neurones in the nucleus raphe magnus on ventrolateral PAG facilitation of baroreflex

Aa and *Ba*, control responses of aortic depressor nerve (ADN) stimulation before and after an injection of kainic acid (KA, 1 μg in 500 nl) into the nucleus raphe magnus (NRM) region, respectively; *Ab* and *Bb*, ADN responses during ventrolateral PAG stimulation before and after KA injection, respectively. Periods of ADN and ventrolateral PAG stimulations are indicated by horizontal bars. *C*, a micrograph showing site of KA injection confirmed by histochemical demonstration of HRP.

the NRM. There are some results in the literature that are compatible with ours. Projections from the ventrolateral PAG to the NRM in rats have been established anatomically (Gallager & Pert, 1978; Beitz *et al.* 1983; Fardin *et al.* 1984). Shah & Dostrovsky (1980) showed that neurones in the PAG are excited antidromically by NRM stimulation, a result that indicates that there is a direct projection from the PAG to the NRM. A non-NMDA, excitatory amino acid mechanism involved in synaptic transmission from the ventrolateral PAG to the NRM has also been demonstrated (Wiklund *et al.* 1988). Furthermore, the NRM has been reported to be involved in analgesic effects elicited by the ventrolateral PAG (Prieto, Cannon & Liebeskind, 1983). Our finding that activation of neurones in the NRM provoked baroreflex facilitation and vagal bradycardia is also in harmony with the hypothesis that the NRM is a relay station of the ventrolateral PAG in terms of cardiovascular regulation.

Stimulation of the NRM produces hypotension, bradycardia (Henry & Calaresu, 1974; Yen *et al.* 1983), baroreflex facilitation (our observation), inhibition of the respiratory system (Lalley, 1986) and a decrease in muscle tone (Lai & Siegel, 1990). All these effects are mimicked by ventrolateral PAG stimulation, while being the opposite of the defence reaction elicited by the dorsolateal PAG. According to a very recent observation, activation of neurones in the medullary raphe nucleus inhibits activities of neurones in the PAG in rats (Lovick, 1993*b*). These findings may lead to the speculation that the ventrolateral PAG inhibits the defence reaction due to the dorsal PAG (Lovick, 1992), at least in part by way of a long-loop pathway that involves the NRM. It may not be too extreme to suggest that the ventrolateral PAG can exert its cardiovascular influence by suppressing a potentially interfering action of the dorsal PAG via this circuitry.

Although the target site(s) of ventrolateral PAG facilitation of the arterial baroreflex remains unclear from our study, interesting suggestions regarding this issue have been made in the recent literature. In cats, injection of serotonin receptor agonists into the nucleus ambiguus (NA) region has been demonstrated to elicit vagal bradycardia (Izzo, Jordan & Ramage, 1988). Furthermore, evidence was recently reported that cardiac vagal preganglionic neurones receive synaptic inputs from serotonin neurones in rats (Izzo, Deuchars & Spyer, 1993). With respect to sympathetic activities, serotonin receptor agonists applied in the rostral ventrolateral medulla (RVLM) produce hypotension (Mandal, Zhong, Kellar & Gillis, 1990). Since the NRM is known to contain abundant serotonin neurones and both the RVLM and the NA region are involved in arterial baroreflexes, it is possible that the ventrolateral PAG exerts its cardiovascular effects, including baroreflex facilitation, in the RVLM and the NA region via the serotonin neurones in the NRM. Although the nucleus tractus solitarii (NTS) is also a potential candidate for mediation of the ventrolateral PAG effects on the cardiovascular system, our results imply that, baroreflex facilitation occurs at higher-order structures in the baroreflex arc than the NTS, the site of primary terminations of baroreceptor afferents. If this baroreflex facilitation occurs at the NTS, it would be reasonable to expect that both the sympathetic and parasympathetic components of the baroreflex would be equally affected. In fact, however, our results showed that the ventrolateral PAG strongly facilitates the cardiac component of the baroreflex, while the vascular component is only slightly affected.

There are some conditions under which arterial baroreflexes do not function to their full extent. For example, arterial baroreflexes are suppressed during stimulation of the hypothalamus (Gebber & Snyder, 1970; Humphreys, Joels & McAllen, 1971; Coote, Hilton & Perez-Gonzalez, 1979; Nosaka, Nakase & Murata, 1989), during noxious somatosensory activation (Kumada, Nogami & Sagawa, 1975; Nosaka & Murata, 1989) and during viscerosensory receptor activation (Nosaka *et al.* 1991). By contrast, however, facilitatory modulation of this reflex has been less frequently described. The present study provides evidence that there is a centrally mediated, powerful facilitation of the arterial baroreflex. More importantly, the results of the present study indicate that the PAG is an important structure that subserves baroreflex modulation, exerting both inhibitory and facilitatory effects on the arterial baroreflex, in particular on its cardiac component.

REFERENCES

Adair, J. R., Hamilton, B. L., Scappaticci, K. A., Helke, C. J. & Gillis, R. A. (1977). Cardiovascular response to electrical stimulation of the medullary raphe area of the cat. *Brain Research* **128**, 141–145.

Bandler, R. & Carrive, P. (1988). Integrated defence reaction elicited by excitatory amino acid microinjection in the midbrain periaqueductal grey region of the unrestrained cat. *Brain Research* **439**, 95–106.

Bandler, R., Carrive, P. & Zhang, S. P. (1991). Integration of somatic and autonomic reactions within the midbrain periaqueductal grey: viscerotopic, somatotopic and functional organization. *Progress in Brain Research* **87**, 269–305.

Beitz, A. J., Shepard, R. D. & Wells, W. E. (1983). The periaqueductal gray–raphe magnus projection contains somatostatin, neurotensin and serotonin but not cholecystokinin. *Brain Research* **261**, 132–137.

Carrive, P., Bandler, R. & Dampney, R. A. L. (1989). Somatic and autonomic integration in the midbrain of the unanesthetized decerebrate cat: a distinctive pattern evoked by excitation of neurones in the subtentorial portion of the midbrain periaqueductal grey. *Brain Research* **483**, 251–258.

Coote, J. H., Hilton, S. M. & Perez-Gonzalez, J. F. (1979). Inhibition of the baroreceptor reflex on stimulation in the brain stem defence centre. *Journal of Physiology* **228**, 549–560.

Fardin, V., Oliveras, J. L. & Besson, J. M. (1984). Projections from the periaqueductal gray matter to the B3 cellular area (nucleus raphe magnus and nucleus reticularis paragigantocellularis) as revealed by the retrograde transport of horseradish peroxidase in the rat. *Journal of Comparative Neurology* **223**, 483–500.

GALLAGER, D. W. & PERT, A. (1978). Afferents to brain stem nuclei (brain stem raphe, nucleus reticularis pontis caudalis and nucleus gigantocellularis) in the rat as demonstrated by microiontophoretically applied horseradish peroxidase. *Brain Research* **144**, 257–275.

GEBBER, G. L. & SNYDER, D. W. (1970). Hypothalamic control of baroreceptor reflexes. *American Journal of Physiology* **218**, 124–131.

HENRY, J. L. & CALARESU, F. R. (1974). Excitatory and inhibitory inputs from medullary nuclei projecting to spinal cardioacceleratory neurons in the cat. *Experimental Brain Research* **20**, 485–504.

HEYMANS, C. & NEIL, E. (1958). Baroreceptor reflexes other than circulatory. In *Reflexogenic Areas of the Cardiovascular System*, pp. 95–100. Little, Brown & Co., Boston.

HILTON, S. M. & REDFERN, W. S. (1986). A search for brain stem cell groups integrating the defence reaction in the rat. *Journal of Physiology* **378**, 213–228.

HILTON, S. M. & SPYER, K. M. (1971). Participation of the anterior hypothalamus in the baroreceptor reflex. *Journal of Physiology* **218**, 271–293.

HOLSTEGE, G. (1991). Descending pathways from the periaqueductal gray and adjacent areas. In *The Midbrain Periaqueductal Gray Matter: Functional, Anatomical and Neurochemical Organization*, ed. DEPAULIS, A. & BANDLER, R., pp. 239–265. Plenum Press, New York.

HUMPHREYS, P. W., JOELS, N. & MCALLEN, R. M. (1971). Modification of the reflex response to stimulation of carotid sinus baroreceptors during and following stimulation of the hypothalamic defence area in the cat. *Journal of Physiology* **216**, 461–482.

IZZO, P. N., DEUCHARS, J. & SPYER, K. M. (1993). Localization of cardiac vagal preganglionic motoneurones in the rat: immunocytochemical evidence of synaptic inputs containing 5-hydroxytryptamine. *Journal of Comparative Neurology* **327**, 572–583.

IZZO, P. N., JORDAN, D. & RAMAGE, A. G. (1988). Anatomical and pharmacological evidence supporting the involvement of serotonin in the central control of cardiac vagal motoneurones in the anaesthetized cat. *Journal of Physiology* **406**, 19*P*.

JONES, R. O., KIRKMAN, E. & LITTLE, R. A. (1990). The involvement of the midbrain periaqueductal grey in the cardiovascular response to injury in the conscious and anaesthetized rat. *Experimental Physiology* **75**, 483–495.

JÜRGENS, U. (1991). Neurochemical study of PAG control of vocal behavior. In *The Midbrain Periaqueductal Gray Matter: Functional, Anatomical and Neurochemical Organization*, ed. DEPAULIS, A. & BANDLER, R., pp. 11–21. Plenum Press, New York.

KUMADA, M., NOGAMI, K. & SAGAWA, K. (1975). Modulation of carotid sinus baroreceptor reflex by sciatic nerve stimulation. *American Journal of Physiology* **228**, 1535–1541.

LAI, Y. Y. & SIEGEL, J. M. (1990). Cardiovascular and muscle tone changes produced by microinjection of cholinergic and glutamatergic agonists in dorsolateral pons and medial medulla. *Brain Research* **514**, 27–36.

LALLEY, P. M. (1986). Responses of phrenic motoneurones of the cat to stimulation of medullary raphe nuclei. *Journal of Physiology* **380**, 349–371.

LOVICK, T. A. (1992). Inhibitory modulation of the cardiovascular defence response by the ventrolateral periaqueductal grey matter in rats. *Experimental Brain Research* **89**, 133–139.

LOVICK, T. A. (1993*a*). Integrated activity of cardiovascular and pain regulatory systems: role in adaptive behavioural responses. *Progress in Neurobiology* **40**, 631–644.

LOVICK, T. A. (1993*b*). Serotonergic influence from nucleus raphe obscurus on neurones in the periaqueductal grey matter in the rat. *Brain Research* **606**, 92–98.

MANDAL, A. K., ZHONG, P., KELLAR, K. J. & GILLIS R. A. (1990). Ventrolateral medulla: an important site of action for the hypotensive effect of drugs that activate serotonin-1A receptors. *Journal of Cardiovascular Pharmacology* **15**, S49–60.

MELLER, S. T. & DENNIS, B. J. (1991). Efferent projections of the periaqueductal gray in the rabbit. *Neuroscience* **40**, 191–216.

NOSAKA, S., MURASE, S. & MURATA, K. (1991). Arterial baroreflex inhibition by gastric distension in rats: mediation by splanchnic afferents. *American Journal of Physiology* **260**, R985–994.

NOSAKA, S. & MURATA, K. (1989). Somatosensory inhibition of vagal baroreflex bradycardia: afferent nervous mechanisms. *American Journal of Physiology* **257**, R829–838.

NOSAKA, S., MURATA, K., INUI, K. & MURASE, S. (1993). Arterial baroreflex inhibition by midbrain periaqueductal grey in anaesthetized rats. *Pflügers Archiv* **424**, 266–275.

NOSAKA, S., NAKASE, N. & MURATA, K. (1989). Somatosensory and hypothalamic inhibitions of baroreflex vagal bradycardia in rats. *Pflügers Archiv* **413**, 656–666.

PRIETO, G. J., CANNON, J. T. & LIEBESKIND, J. C. (1983). N. raphe magnus lesions disrupt stimulation-produced analgesia from ventral but not dorsal midbrain areas in the rat. *Brain Research* **261**, 53–57.

SAKUMA, Y. & PFAFF, D. W. (1979). Facilitation of female reproductive behavior from mesencephalic central gray in the rat. *American Journal of Physiology* **237**, R278–284.

SAPRU, H. N. & KRIEGER, A. J. (1977). Carotid and aortic chemoreceptor function in the rat. *Journal of Applied Physiology* **42**, 344–348.

SHAH, Y. & DOSTROVSKY, J. O. (1980). Electrophysiological evidence for a projection of the periaqueductal gray matter to nucleus raphe magnus in cat and rat. *Brain Research* **193**, 534–538.

SPYER, K. M. (1972). Baroreceptor sensitive neurones in the anterior hypothalamus of the cat. *Journal of Physiology* **224**, 245–257.

SWANSON, L. W. (1976). An autoradiographic study of the efferent connections of the preoptic region in the rat. *Journal of Comparative Neurology* **167**, 227–256.

VEENING, J., BUMA, P., HORST, G. J. T., ROELING, T. A. P., LUITEN, P. G. M. & NIEUWENHUYS, R. (1991). Hypothalamic projections to the PAG in the rat: topographical, immuno-electronmicroscopical and functional aspects. In *The Midbrain Periaqueductal Gray Matter: Functional, Anatomical and Neurochemical Organization*, ed. DEPAULIS, A. & BANDLER, R., pp. 387–415. Plenum Press, New York.

WIKLUND, L., BEHZADI, G. & KALÉN, P., HEADLEY, P. M., NICOLOPOULOS, L. S., PARSONS, C. G. & WEST, D. C. (1988). Autoradiographic and electrophysiological evidence for excitatory amino acid transmission in the periaqueductal gray projection to nucleus raphe magnus in the rat. *Neuroscience Letters* **93**, 158–163.

YEN, C. T., BLUM, P. S. & SPATH, J. A. (1983). Control of cardiovascular function by electrical stimulation within the medullary raphe region of the cat. *Experimental Neurology* **79**, 666–679.

Received 22 July 1993; accepted 15 October 1994.