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GABA-MEDIATED INHIBITION OF MEDULLARY VASOMOTOR NEURONES BY AREA POSTREMA STIMULATION IN RATS

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SUMMARY

1. The cardiovascular responses, together with the effects on medullary sympathoexcitatory (vasomotor) neurones of the rostral ventrolateral medulla, of area postrema stimulation have been studied *in vivo*.

2. Electrical (10 Hz) or chemical stimulation using microinjections of L-glutamate of the area postrema produced a vasodepressor response and an inhibition of the medullary sympathoexcitatory neurones in the nucleus reticularis rostroventrolateralis (RVL), while similar stimulation in the adjacent nucleus tractus solitarii (NTS) caused increases in arterial pressure.

3. Single-pulse stimulation of the area postrema revealed at least three influences on the activity of RVL vasomotor neurones, one being excitatory and two inhibitory.

4. The inhibitions evoked in the medullary vasomotor neurones on area postrema stimulation were blocked by ionophoretic application of bicuculline, a $GABA_A$ receptor antagonist, without altering the excitatory input to the same neurones. Bilateral microinjections of bicuculline into the RVL in regions where the vasomotor neurones had been identified totally eliminated the vasodepression due to area postrema stimulation.

5. These data support a role for the area postrema in cardiovascular control. It is concluded that the area postrema exerts its action on cardiovascular control in part via GABAergic inhibition of the 'vasomotor' neurones in the nucleus reticularis rostroventrolateralis.

INTRODUCTION

Considerable evidence is now available suggesting a role for the area postrema in autonomic control mechanisms. These include cardiovascular control, cerebrospinal fluid regulation, food intake, body fluid regulation and the control of vomiting (Borison, 1984; Ferguson & Marcus, 1988).

The possible involvement of this mid-line circumventricular structure in cardiovascular control is based on several pieces of evidence. First, stimulation of the area postrema produces profound cardiovascular responses although these vary in different studies from sympathoinhibitory responses (Hasser, Nelson, Haywood &

Bishop, 1987) or vasodepressor responses (Gatti, Souza, Taveira da Silva, Quest & Gillis, 1985; Ferguson & Marcus, 1988) to vasopressor responses (Barnes, Ferrario & Conomy, 1979; Mangiapane, Bruner & Fink, 1985), and from bradycardia (Gatti *et al.* 1985; Hasser *et al.* 1987; Ferguson & Marcus, 1988) to tachycardia (Barnes *et al.* 1979).

Second, the area postrema has a dense vascular supply but is devoid of a blood–brain barrier, making it readily accessible to peripherally circulating substances such as peptide hormones (Wislocki & Leduc, 1952; Joy & Lowe, 1970; Undesser, Hasser, Haywood, Johnson & Bishop, 1985; Applegate, Hasser & Bishop, 1987). In relation to this absence of a blood–brain barrier there are numerous receptors in the area postrema, including those for angiotensin II (Mendelsohn, Quirion, Saavedra, Aguilera & Catt, 1984), atrial natriuretic peptide (Bianchi, Gutkowska, Ballak, Thibault, Garcia, Genest & Cantin, 1986) and vasopressin (Applegate *et al.* 1987). Microinjection of angiotensin II into the area postrema has been shown to increase blood pressure in dogs and to decrease heart rate in rats (Casto & Phillips, 1984). The sympathoinhibitory effect of systemic vasopressin is considered to be dependent on the intact area postrema (Applegate *et al.* 1987).

Third, the area postrema may play an important role in the development and control of hypertension in rats. In normotensive and spontaneously hypertensive animals, destruction of the area postrema has been reported to result in long-term increases in blood pressure (Ylitalo, Karppanen & Passonen, 1974), markedly attenuate the development of hypertension in the spontaneously hypertensive rats (Mangiapane, Skoog, Rittenhouse, Blair & Sladek, 1989) or impair the expression of angiotensin II-induced hypertension in rats (Fink, Bruner & Mangiapane, 1987). It is, however, unclear which central neural pathways are involved in expressing these cardiovascular responses. There are, however, indications of reciprocal connections between the area postrema and the rostral ventrolateral medulla from anatomical tracing studies (Andrezik, Chan-Palay & Palay, 1981), and major efferent projections of this area to nucleus tractus solitarii (NTS), the dorsal motor nucleus of vagus, nucleus ambiguus and parabrachial nucleus (Kooy & Koda, 1983; Shapiro & Miselis, 1985). Further, the area postrema receives afferent inputs from the caudal NTS and the hypothalamic paraventricular nucleus (Kooy & Koda, 1983; Shapiro & Miselis, 1985). All these regions are believed to play important roles in control of the cardiovascular system.

Within the ventral medulla, the nucleus reticularis rostroventrolateralis (RVL) contains the cell bodies of reticulospinal neurones with sympathoexcitatory function, and direct and perhaps monosynaptic connections to the intermediolateral cell column (Ross, Ruggiero, Joh, Park & Reis, 1984; Brown & Guyenet, 1985; Sun & Guyenet, 1985, 1986*a*). These neurones are tonically active and exhibit a pattern of activity similar to the vasomotor sympathetic discharge (Brown & Guyenet, 1985; Sun & Guyenet, 1985, 1986*b*). They receive GABA-mediated baroreflex inhibition, vagal afferent inputs, and a glutamatergic hypothalamic excitatory input (Sun & Guyenet, 1985, 1986*c*, 1987). Furthermore, some evidence supports the concept that their tonic activity sustains the basal sympathetic tone, and therefore arterial blood pressure, in anaesthetized animals (Sun & Guyenet, 1985; Sun, Hackett & Guyenet, 1988*a*). The reticulospinal projection from RVL neurones consists in roughly equal

proportion of adrenergic and non-adrenergic neurones (Tucker, Saper, Ruggiero & Reis, 1987), with the latter having some properties indicative of intrinsic pacemakers (Sun *et al.* 1988*a*; Sun, Young, Hackett & Guyenet, 1988*b*, *c*).

As changes in the activity of the RVL neurones will exert influences on sympathetic tone, and neuroregulatory peptides have been shown to modify their discharge (Sun & Guyenet, 1989), the neural mechanisms controlling their activity are likely to be crucial for cardiovascular regulation. In view of the prominent roles of both the medullary vasomotor neurones of the RVL (or sympathoexcitatory neurones) and of the area postrema in cardiovascular regulation, the present study was initiated to investigate the influence of the activation of the area postrema on RVL neurones, and the neurotransmitters that are involved.

METHODS

General procedures

Male Sprague–Dawley rats (300–400 g) were anaesthetized with a mixture of urethane and sodium pentobarbitone (20 and 0·24%, respectively) given via a tail vein at doses adjusted to eliminate the paw-pinch reflex (usually 1·3–1·5 ml). Additional anaesthetic doses were given as I.V. injection of pentobarbitone to maintain a stable level of anaesthesia. A catheter was placed into a femoral vein for injection of drugs and another into the carotid artery for measurement of arterial pressure. The trachea was cannulated and the rats were mechanically ventilated with oxygen. The endotracheal CO_2 concentration was continuously monitored and the end-tidal CO_2 was regulated between 3·5 and 4·5%. Muscular paralysis was produced with gallamine (4–5 mg/kg given I.V.). The adequacy of anaesthesia under paralysis was assessed with regard to the stability of blood pressure and heart rate in the absence of stimulus-evoked responses. In the experiments in which the activity of medullary 'vasomotor' neurones was recorded, an inflatable cuff made of heat-stretched soft plastic tubing was wrapped around the descending aorta below the diaphragm and was used to gradually increase total peripheral resistance as described previously (Sun & Guyenet, 1985), thereby increasing arterial pressure and in consequence baroreceptor activity. Each animal was then placed in a stereotaxic frame.

The body temperature of the rats was maintained at $37·0 \pm 1·0$ °C throughout experiments using a feedback-controlled heating blanket.

Electrical stimulation

A concentric bipolar electrode (Clark Electromedical Instruments NE 100, tip separation 0·5 mm) was placed in the fascia surrounding the right marginal mandibular branch of the facial nerve to locate the motor nucleus of the facial nerve by means of antidromic field potential recording. This field potential was used as guidance for the location of medullary vasomotor neurones as described previously (Brown & Guyenet, 1985; Sun & Guyenet, 1985). A similar stimulating electrode was implanted in the spinal cord, following a laminectomy at the T2–T3 level. The tip of the electrode was placed on the right side with its tip 1·0 mm below the dorsolateral sulcus. A third smaller concentric bipolar metal electrode (Clark Electromedical Instruments, SNE100, tip separation 0·25 mm) was positioned at an angle of 20–30 deg caudorostral to the dorsal surface of the area postrema. To expose the area postrema, it was usually necessary to aspirate a part of the posterior cerebellum.

At the completion of each experiment, a marking lesion was made by passing direct anodal current through the stimulating electrode within the area postrema. The rat was then perfused with 0·9% saline followed by 10% formalin administered through the left ventricle of the heart. The brain was removed and 50 μm coronal sections were cut through the medulla. The anatomical locations of the stimulating sites were verified histologically.

Electrical recording and ionophoresis

Extracellular potentials were recorded with glass microelectrodes filled with 3 M-NaCl (4–8 MΩ resistance, measured DC) by means of a dorsal transcerebellar approach, amplified, counted, and

recorded on a chart recorder and on tape. Electrodes were advanced using a nanostepper microdrive. Peristimulus time histograms and ECG-triggered discharge histograms were generated with a microcomputer (CED). Response histograms were analysed to determine the firing ratio (response discharge/average resting discharge) of the medullary vasomotor neurones.

For ionophoresis, six-barrelled electrodes were used with the recording pipette protruding 25–35 μm from the other barrels. Current balancing was performed via a channel filled with 3 M-NaCl. The other channels were filled with one of the following solutions: 0·8 M-GABA (Sigma) in 160 mM-NaCl, pH 4·5; 0·2 M-L-glutamate (Sigma) in 160 mM-NaCl, pH 8·5; 0·2 M-kynurenic acid (Sigma) in 160 mM-NaCl, pH 8·5; 20 mM-bicuculline methiodide (Sigma) in water, pH 4·0. A retaining current of 10 nA was applied between periods of drug ejection.

Microinjections

Drug microinjections into the brain parenchyma were made as described previously (Sun & Guyenet, 1987). Briefly, microinjections of either L-glutamate or bicuculline were made using a 1 μl Hamilton syringe with a blunt needle glued with epoxy inside a glass micropipette, which was pulled and cut to a 10–20 μm tip (o.d.). Microinjection by applied pressure of L-glutamate into the area postrema was achieved under the guidance of a microscope and by use of a micromanipulator. For RVL microinjections, the co-ordinates of the medullary sympathoexcitatory neurones were determined bilaterally in each rat, by mapping the contour of the posterior extent of the facial motoneurones with reference to the antidromic field potential as described above. The injection was made over a period of 10 s. Histological verification of the injection sites indicated accurate placement of the pipette tips in the RVL.

Statistics

All data are expressed as means $\pm$ S.E. of the mean. The differences are considered to be significant at P values smaller than 0·05 using Student's paired t test.

RESULTS

Effects of stimulation of the area postrema on arterial pressure

The effects of electrical stimulation (7–8 V, 1 ms pulses) in the area postrema were frequency dependent. At a frequency of 10 Hz and 7–8 V, stimulation elicited a decrease in arterial pressure. This decrease started 1–3 s after the onset of stimulation and lasted less than 5 s after termination of stimulation (trace *a* in Fig. 1), with a maximal average response of -22 ± 2 mmHg ($n = 10$, $P < 0{\cdot}05$). At higher frequencies (60 Hz) increases in arterial blood pressure at a similar latency (trace *b* in Fig. 1) resulted, at the same voltage of stimulation, with a maximal increase of 25 ± 4 mmHg ($n = 4$, $P < 0{\cdot}05$). The depressor responses observed at lower frequency of stimulation altered as intensities were increased. Pressor responses were usually observed at voltages > 12 V. As the electrode was advanced into the NTS, low-frequency (10 Hz), low-intensity (7–8 V) stimulation always resulted in a pressor response ($+29 \pm 3$ mmHg, $n = 8$, $P < 0{\cdot}05$). An example is shown in the trace *d* of Fig. 1.

Microinjection of 4 nmol of L-glutamate (20 nl of a 0·2 M solution in saline, pH 7·35) into the medial area postrema elicited a fall of arterial pressure (trace *c* in Fig. 1) in four rats tested, with a maximal response of -15 ± 2 mmHg on average ($n = 4$, $P < 0{\cdot}05$), while no responses were observed when similar microinjections of saline into the area postrema were performed.

Effects of single-pulse stimulation of the area postrema on the medullary vasomotor neurones

The responses of fifteen medullary vasomotor neurones recorded in the RVL to electrical stimulation of the area postrema were investigated. Fourteen of these

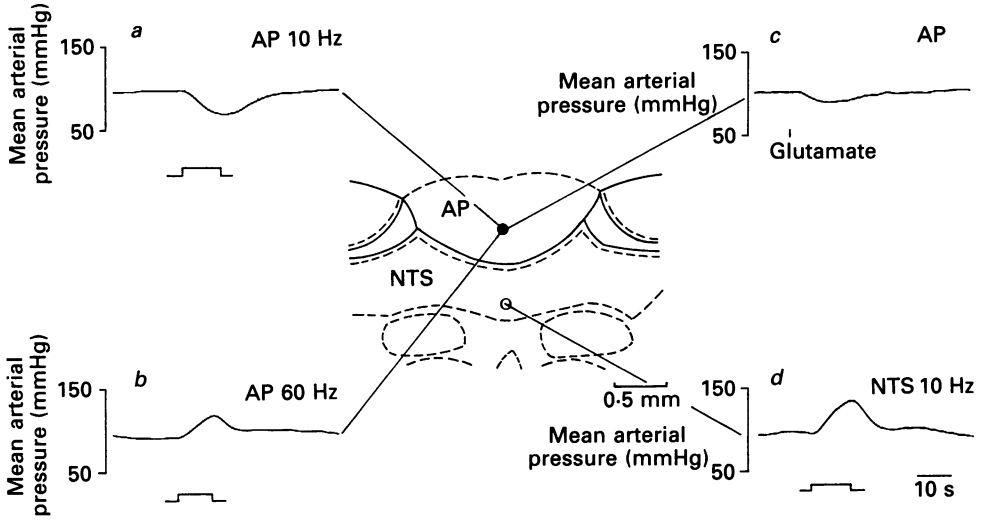

Fig. 1. Effect of area postrema stimulation on arterial pressure. Mean arterial pressure changes produced by electrical stimulation (7 V, 1 ms pulses, for 10 s as indicated below traces) in the area postrema (●: AP, 10 Hz, trace *a* and AP, 60 Hz, trace *b*) or nucleus tractus solitarii (○: NTS, 10 Hz, 7 V, trace *d*) and by microinjection of 20 nl of 0·2 M-L-glutamate into the area postrema (trace *c*).

neurones had relatively fast-conducting axons (2·0–4·7 m/s) and the other had a slowly conducting axon (0·5 m/s). These neurones were identified extracellularly according to conventional criteria (cf. Sun & Guyenet, 1985, 1986*a*). Their activity was sensitive to baroreflex inputs seen as an inhibition to increases in arterial blood pressure (Fig. 2*A*) and pulse-synchronous activity (Fig. 2*C*) under resting conditions. Their reticulospinal connections were shown with antidromic collision techniques to spinal cord stimulation (Fig. 2*B*). All these neurones were spontaneously active with a discharge rate of 21 ± 2 spikes/s ($n = 15$) under resting conditions. With the stimulus intensity to the area postrema set at the level (7–8 V) that produced a clear vasodepressor response on 10 Hz stimulation, all the neurones tested to single-pulse stimulation at 1 Hz showed an early excitatory peak followed by two periods of decreased firing as illustrated in peristimulus time histograms (see Fig. 3*A* for an example and Fig. 3*B* for the pooled data). The first peak had a latency of 16 ± 1 ms ($n = 15$), reached a maximum after 22 ± 1 ms ($n = 15$) and lasted for 12 ± 1 ms ($n = 15$). During the excitatory period, the firing ratio was $3{\cdot}34 \pm 0{\cdot}43$ ($n = 15$, $P < 0{\cdot}05$). The first inhibitory trough in the peristimulus time histogram had a latency of 31 ± 2 ms ($n = 15$), reached a minimum in firing at 41 ± 4 ms ($n = 15$) and lasted 37 ± 4 ms ($n = 15$). The averaged firing ratio during this period was $0{\cdot}29 \pm 0{\cdot}04$ ($n = 15$, $P < 0{\cdot}05$). The second inhibitory trough was distinct from the first one, and

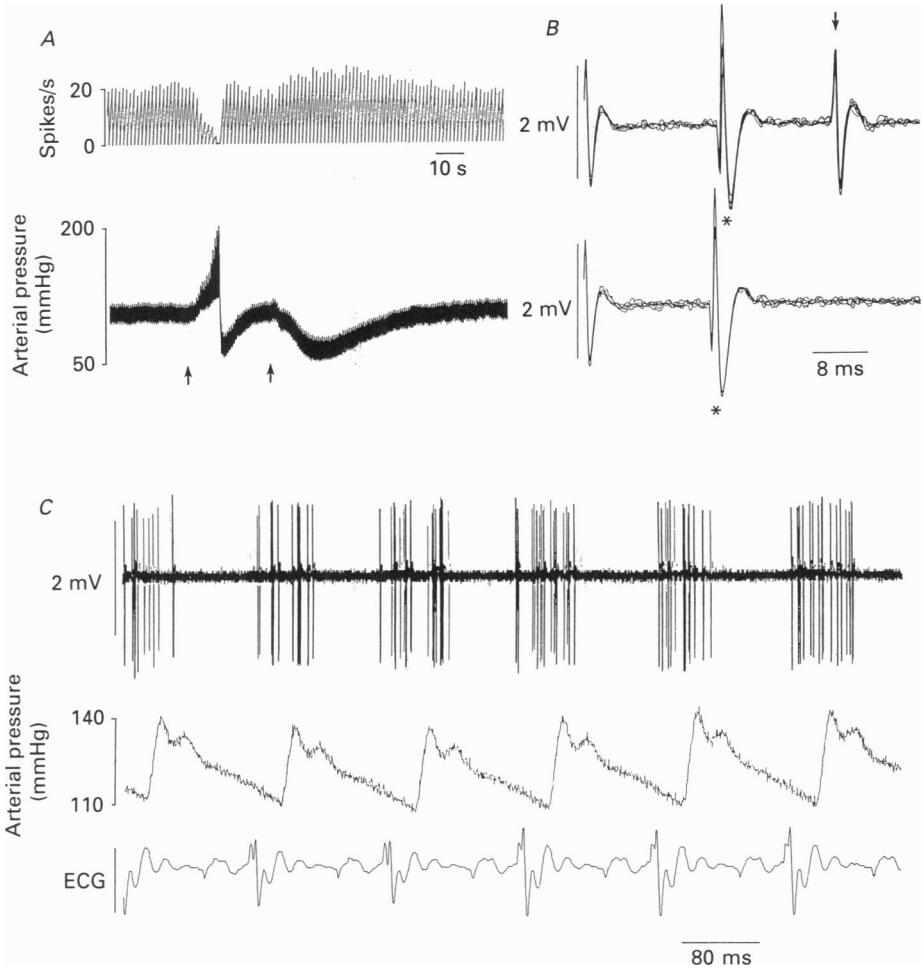

Fig. 2. Characteristics of medullary vasomotor neurones. *A*, effect of change in arterial pressure on unit activity. Neuronal activity is represented in the form of an integrated rate histogram. Arterial pressure was elevated by constriction of the descending aorta (at first arrow) and lowered by an I.V. injection of sodium nitroprusside at the second arrow. *B*, collision test used to identify medullary vasomotor units as reticulospinal neurones. Traces were triggered from spontaneously occurring spikes. Antidromic spikes (arrow in top trace) from spinal cord failed to occur (lower trace) when stimulation was delivered within critical period after trigger spikes (six superimposed traces each). *C*, pulse-synchronous discharge of the medullary vasomotor neurone. The record was triggered from the R wave of the ECG signals (lower trace). The top trace is neuronal discharge of the neurone illustrated in Fig. 1*a* and *b* and the middle trace is arterial pressure.

had a latency of 84 ± 10 ms ($n = 15$), reached a minimum 119 ± 6 ms ($n = 15$) after the stimulus, and lasted some 52 ± 7 ms ($n = 15$, firing ratio of $0{\cdot}55 \pm 0{\cdot}05$, $n = 15$, $P < 0{\cdot}05$).

The responses of five units located in the vicinity of the medullary vasomotor neurones but insensitive to brief increases of systemic arterial pressure were

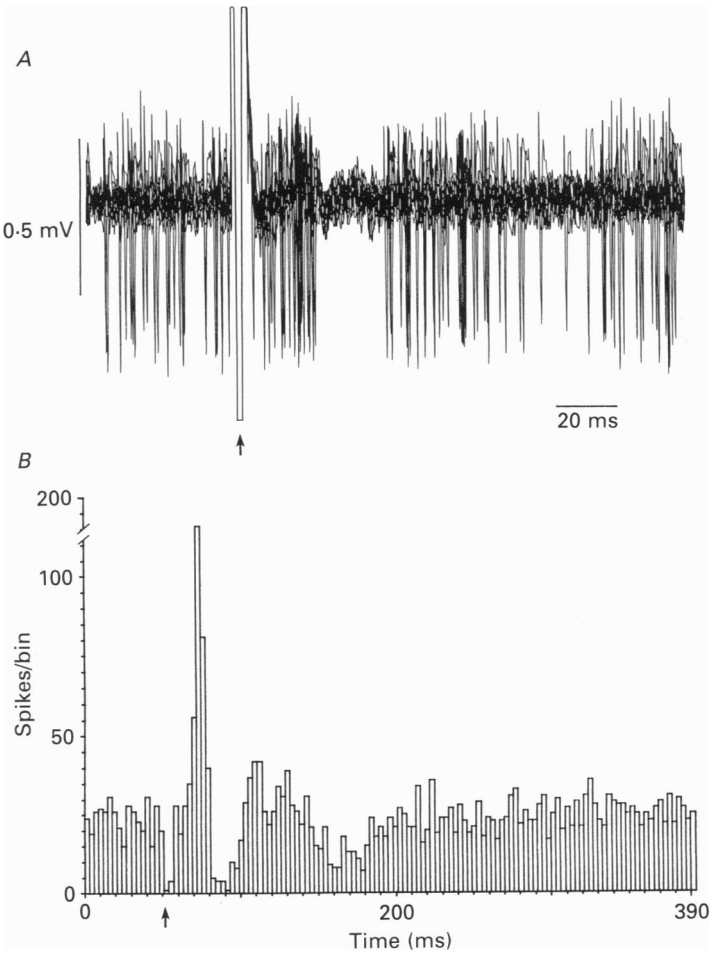

Fig. 3. Effect of single-pulse stimulation of the area postrema on the medullary vasomotor neurones. *A*, twelve single oscilloscope sweeps superimposed on neuronal responses to area postrema stimulation (8 V, 1 ms duration at 1 Hz). *B*, peristimulus time histogram of activity of a medullary vasomotor neurone during stimulation of the area postrema (8 V, 1 ms duration, 1 Hz, at arrow). 200 sweeps, 3 ms/bin. The stimulus artifact, and coincident spikes, were obliterated by processing through a window discriminator.

examined. These units exhibited discharge patterns with a respiratory rhythm, that is they fired in phase with lung inflation. The respiratory modulation of discharge was not due to a respiratory movement of the brain since these units discharged with a similar frequency and pattern when the ventilation was stopped for one or two cycles (not shown). They displayed a flat peristimulus time histogram to single-pulse stimulation of the area postrema (Fig. 4).

Lack of effects of kynurenic acid on the area postrema inputs to the medullary vasomotor neurones

Whether the excitatory input onto medullary vasomotor neurones evoked by the area postrema stimulation is mediated by a glutamate-like substance was

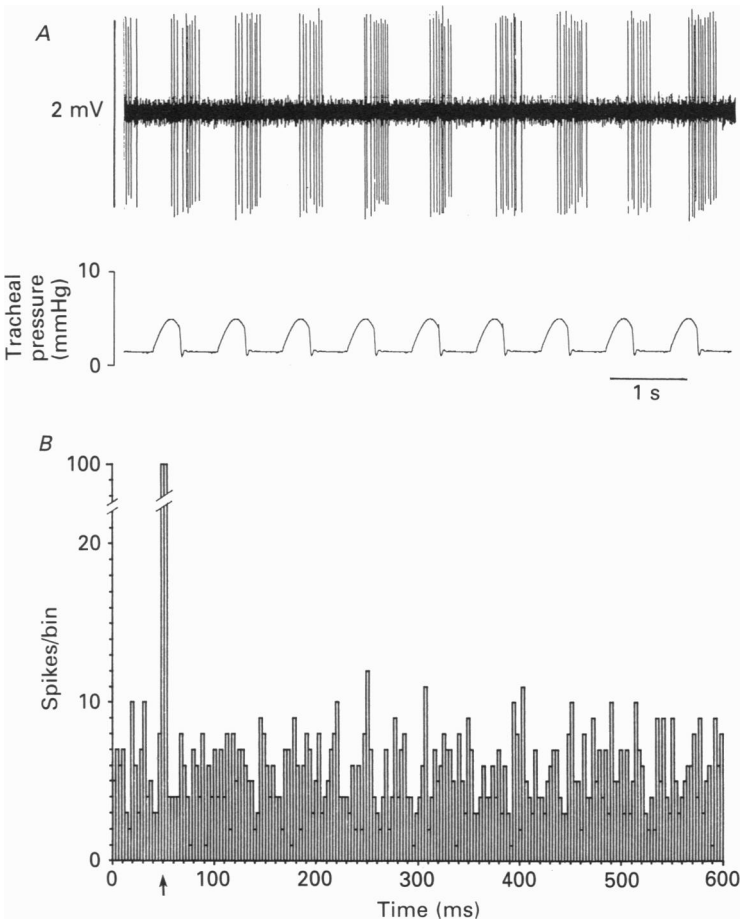

Fig. 4. Lack of effect of area postrema stimulation on medullary unit with respiratory rhythm. *A*, a single sweep of the activity (top trace) or a unit with respiratory rhythm. *B*, peristimulus time histogram of the unit shown in *A* during 1 Hz stimulation of 8 V (1 ms pulses at arrow). Bin size 3 ms, 100 sweeps.

determined. Ionophoretic application of the glutamate receptor antagonist, kynurenic acid (100 nA, 2 min), blocked the responses to ionophoretically applied glutamate but did not change the firing rate of the seven medullary vasomotor neurones tested. The peristimulus time histograms to the single-pulse stimulation of the area postrema in these neurones were not significantly altered by the application of kynurenic acid (Fig. 5). Before the application of the drug, the firing ratios of the three responses were $3{\cdot}61 \pm 0{\cdot}77$ ($n = 7$), $0{\cdot}21 \pm 0{\cdot}44$ ($n = 7$) and $0{\cdot}45 \pm 0{\cdot}05$ ($n = 7$),

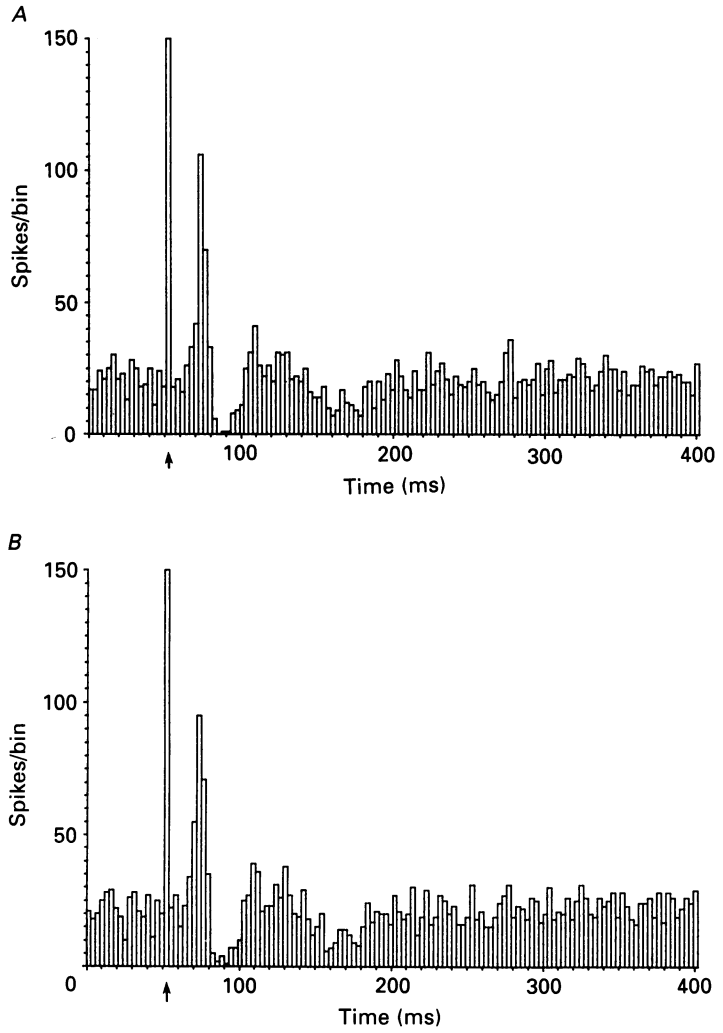

Fig. 5. Lack of effect of kynurenate on responses of medullary vasomotor neurones to single-pulse activation of the area postrema. *A*, peristimulus time histogram of medullary vasomotor neurones during stimulation of the area postrema (8 V, 1 ms duration, 1 Hz, at arrow). 150 sweeps, 3 ms/bin. *B*, peristimulus time histogram of the same neurones shown in *A* during stimulation of the area postrema (8 V, 1 ms duration, 1 Hz, at arrow) and ionophoretic application of kynurenic acid (100 nA). 150 sweeps, 3 ms/bin.

respectively; during the application of kynurenic acid the firing ratios were $3{\cdot}56 \pm 0{\cdot}67$ ($n = 7$), $0{\cdot}23 \pm 0{\cdot}04$ ($n = 7$) and $0{\cdot}42 \pm 0{\cdot}06$ ($n = 7$), respectively. These values were not significantly different from those observed in the absence of kynurenate ($P > 0{\cdot}05$ on paired t tests).

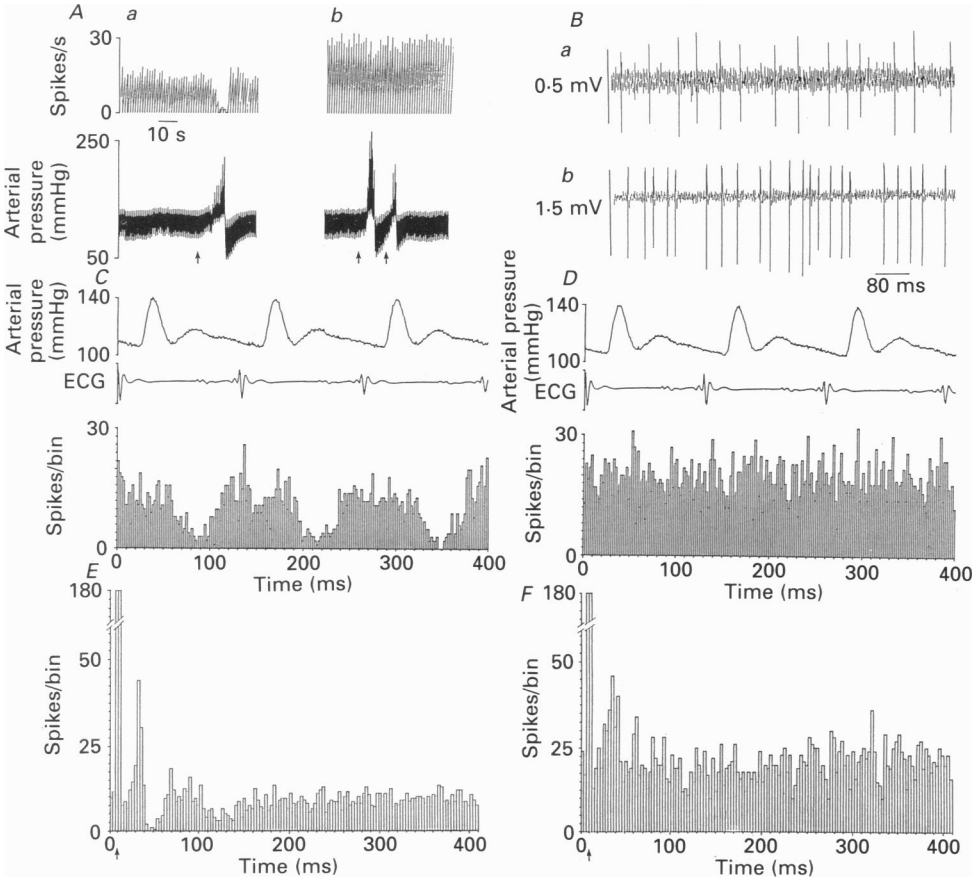

Fig. 6. Effects of bicuculline methiodide on activity of medullary vasomotor neurones. *A*, pressure sensitivity (aortic constriction initiated at arrows) of a medullary vasomotor neurone before (*a*) and during (*b*) ionophoretic application of bicuculline methiodide (100 nA). *B*, single sweep of the neuronal discharge before (*a*) and during (*b*) ionophoretic application of bicuculline methiodide (100 nA). Note that no bursting-like discharge was observed. *C* and *D*, ECG-triggered time histogram (bottom traces) of the neuronal discharge before (*C*) and during (*D*) ionophoretic application of bicuculline methiodide (100 nA). The activity of the neurone was recorded at the same arterial pressure (top trace). Note that bicuculline eliminated the pulse modulation of the neuronal activity. 300 sweeps each, 2 ms/bin. *E* and *F*, peristimulus time histogram of activity of the medullary vasomotor neurone during single-pulse stimulation of the area postrema (8 V, 1 ms) before (*E*) and during (*F*) the ionophoretic application (100 nA) of bicuculline methiodide. 180 sweeps each, 3 ms/bin.

Antagonism by bicuculline of the area postrema inputs to the medullary vasomotor neurones

Ionophoretic application of bicuculline (100 nA), a $GABA_A$ receptor antagonist, for 5 min produced an increase in the basal firing rate of the medullary vasomotor neurones ($+85 \pm 32\%$, $n = 7$, $P < 0{\cdot}05$), an elimination of both the baroreflex inhibition of the neuronal activity elicited by raising arterial blood pressure (Fig.

6*A*, compare traces *a* and *b*) and their pulse-synchronous discharge (Fig. 6*C* and *D*), in agreement with previous observations (Sun & Guyenet, 1985). During the application of bicuculline, the neurones did not exhibit a bursting-like firing pattern (Fig. 6*B*), whilst it produced a total elimination of the first period of inhibition (100 %, $n = 7$, $P < 0{\cdot}05$) and an almost total elimination of the second trough in the

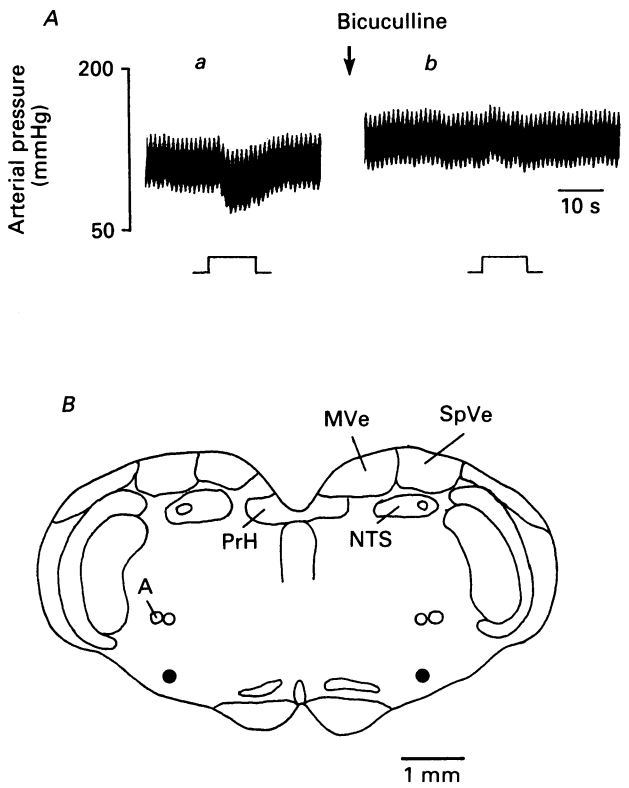

Fig. 7. Effect of bicuculline microinjections into nucleus reticularis rostroventrolateralis on the vasodepressor response of the area postrema stimulation. *A*, arterial pressure response to the area postrema stimulation (at bar, 8 V, 10 Hz, 1 ms pulses) before (*a*) and after (*b*) bilateral microinjections of bicuculline methiodide. *B*, bicuculline methiodide injection sites. Bilateral injections of 100 pmol bicuculline methiodide at sites represented by ● increased arterial pressure and eliminated the depressor responses of the area postrema stimulation, while the same amount of bilateral microinjections of bicuculline methiodide 1 mm dorsal was without such effects (○). A, nucleus ambiguus; MVe, medial vestibular nucleus; SpVe, superior vestibular nucleus; PrH, parahypoglossal nucleus; NTS, nucleus tractus solitarii.

peristimulus time histograms (92 ± 6 %, $n = 7$, $P < 0{\cdot}05$). These effects of bicuculline are illustrated in Fig. 6*E* and *F*. At the same time, the early excitation was not affected significantly (with an increase in firing ratio of $0{\cdot}01 \pm 0{\cdot}17$, $n = 7$, during the application of bicuculline, $P > 0{\cdot}05$). In an earlier study (Sun & Guyenet, 1985) the actions of bicuculline on baroreceptor inhibition were shown to be specifically related to its action on $GABA_A$ receptors (see Fig. 6*D*), as raising firing to an equivalent level

with glutamate failed to affect baroreceptor inhibitory actions. In two additional units, we have since confirmed that raising the firing level to that produced by ionophoresis of bicuculline with glutamate fails to alter the inhibition evoked from the area postrema. By analogy, as bicuculline antagonized baroreceptor-evoked inhibitions in the present study, we propose a specific role of $GABA_A$ receptors in the inhibitions evoked from the area postrema on these same neurones.

The importance of this GABAergic inhibition of the RVL vasomotor neurones on area postrema stimulation was further illustrated by making microinjections of bicuculline into the RVL whilst observing vasomotor responses. Bilateral microinjections of 100 pmol (50 nl of a 2 mM solution) of bicuculline methiodide into the RVL produced an increase in arterial pressure ($+28 \pm 7$ mmHg, $n = 5$, $P < 0{\cdot}05$, Fig. 7*A a* and *b*). This dose of bicuculline methiodide was used because it was previously demonstrated to effectively block the GABAergic inhibition of these neurones (Sun & Guyenet, 1987). The fall in arterial blood pressure accompanying low-frequency stimulation of the area postrema was either totally eliminated or converted into a vasopressor response ($+11 \pm 5$ mmHg, $n = 5$, $P < 0{\cdot}05$). The effective injection sites in the RVL are shown in Fig. 7*B* by the filled circles. Bilateral microinjections of identical amounts of bicuculline methiodide 1 mm dorsal to the location where the medullary vasomotor neurones had been recorded neither altered arterial pressure nor affected the depressor effects of area postrema stimulation.

DISCUSSION

The present study has shown that both chemical and electrical activation of the area postrema in the rat can elicit cardiovascular responses that are mediated at least partially via 'vasomotor' neurones of the RVL. We have confirmed the observations of Ferguson & Markus (1988) in the rat that low-frequency, low-intensity stimulation within the area postrema evokes a depressor response whilst high-frequency and/or high-intensity stimulation at the same site evokes a rise of blood pressure.

We are confident that these effects produced by stimulating the area postrema at low intensity are not the result of activation of the adjacent NTS. This inference rests on the observation that with the use of concentric bipolar electrodes the possibility of current spread was minimized and that effective stimulation within the immediately adjacent regions of the NTS (that is essentially medial areas of the nucleus at obex level) with equivalent parameters of stimulation always evoked a pressor response. It should be noted that it is possible to evoke depressor responses from other sites in the NTS on electrical stimulation (see Paton & Spyer, 1990, amongst others). Further, microinjections of the excitatory amino acid glutamate into the area postrema also elicited a depressor response, indicating that the effects of electrical stimulation were at least partially due to the activation of cell bodies rather than fibres of passage. This effective concentration of glutamate is not believed to have non-specific effects beyond producing depolarization block. However, the pressor response evoked by electrical stimulation within the area postrema at low frequency and high intensity (> 12 V) is likely to be due to current spread into the medial areas of the NTS.

Electrical stimulation of the area postrema evoked both excitatory and inhibitory actions on RVL neurones. These influences on 'vasomotor' neurones are likely to be

responsible for the patterns of cardiovascular response that were evoked: the excitatory component of the response generating the pressor response, and the inhibitory influence being responsible for the depressor response. These actions of area postrema activation have been revealed for the first time in this study yet the pathways mediating them remain to be elucidated. There is evidence for a projection from the area to or through the RVL (Shapiro & Miselis, 1985). On the other hand, the possibility cannot be excluded that the inhibitory synaptic pathways involve a relay in other structures, e.g. the NTS or the caudal medullary depressor area. The pattern of response of individual RVL neurones to stimulation was an early short-lasting excitatory effect followed by two later periods of more prolonged inhibition. Hence with low-frequency stimulation the inhibitory action would predominate but at increasing frequencies of stimulation the more powerful but short-lasting excitatory peak would be expected to overcome the persistent inhibitory troughs to generate an overall increase in discharge at high frequencies. Similar effects of cerebellar stimulation on the activity of RVL neurones in the rabbit have been reported (Silva-Carvahlo, Paton, Sun & Spyer, 1990).

A major observation was that both the depressor response to area postrema stimulation and the inhibitory influences on individual RVL neurones were blocked by the application of the $GABA_A$ receptor antagonist bicuculline in the RVL. In regard to the cardiovascular response microinjections of bicuculline were only effective if restricted to the RVL. The action of ionophoretically applied bicuculline not only eliminated the inhibitions evoked from the area postrema but also blocked the inhibitory action of baroreceptor stimulation on RVL neurones and obliterated their normal pulse-related discharge as reported previously (Sun & Guyenet, 1985).

These data are suggestive of the presence of GABAergic terminals making direct contact with RVL vasomotor neurones. The location of the GABAergic neurones that mediate this inhibitory control of RVL activity remains to be determined. One possibility is that it is mediated by the glutamic acid decarboxylase (GAD)-containing neurones of the area postrema (Ruggiero, Meeley, Anwar & Reis, 1985) that have been described in the rat. There is, however, no evidence that they project to the RVL. Alternatively, GABAergic neurones located in the ventrolateral medulla might be the target for efferent projections from the area postrema. GAD-containing neurones have been localized immunocytochemically throughout the ventrolateral medulla including regions in close proximity to RVL neurones in the rat (see Ruggiero *et al.* 1985). More caudally placed GAD-containing neurones might also be involved and these have been implicated in the baroreceptor control of RVL activity (Guyenet, Sun & Brown, 1987). Whichever GABAergic neurones are involved, the pathway from the area postrema onto them and its action at the level of the RVL must represent a potential mechanism whereby circulating substances might act to influence vasomotor activity (Applegate *et al.* 1987). This follows logically from the fact that the area postrema is the most caudally placed circumventricular organ that has a diminished blood–brain barrier and so will allow access to blood-borne peptides.

One role of the area postrema in cardiovascular regulation may be related to the interaction of circulating vasopressin with reflex function. Vasopressin's action in augmenting reflex inhibitory effects such as those accompanying baroreceptor stimulation is abolished by prior lesioning of the area postrema (Applegate *et al.*

1987), suggesting that it acts via the area postrema to alter baroreflex control of sympathetic nerve activity. The electrophysiological data presented in this study suggest that this might occur via a GABAergic inhibition of the medullary 'vasomotor' neurones. It remains to be determined whether the neuronal elements activated in this study include those that are sensitive to vasopressin.

Data obtained with regard to the excitatory effects of area postrema stimulation do not favour the possibility that a glutamatergic input is responsible for the early excitation of the medullary vasomotor neurones evoked on singled-pulse stimulation. Ionophoretic application of kynurenic acid failed to block the increase in firing of RVL neurones. These observations do not preclude the possibility that glutamatergic contacts on distal dendrites are involved since these might not have been antagonized by the ionophoretic delivery of kynurenic acid. Alternatively a non-glutaminergic synapse may be involved. Interestingly, the negative observation with ionophoresis of kynurenic acid (pH 8·5) and other similarly negative observations (Sun & Guyenet, 1985) with strychnine (pH 4·0) when delivered with ionophoretic currents of up to 100 nA, mitigates against any of these observations depending on the pH of the ejected agonists or antagonists.

In summary, the present study provides evidence that RVL neurones are involved in mediating both vasodepressor and vasopressor responses to stimulation of the area postrema. The inhibitory effects on these medullary vasomotor neurones are mediated by a GABAergic synapse. This may represent a mechanism by which the area postrema senses the level of circulating vasoactive substances and participates in resetting the level of activity of medullary vasomotor neurones and hence sympathetic tone in response to changes in the titre of these substances.

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