ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 1996, 56: 129-135

Brainstem neurones and postganglionic sympathetic nerves: does correlation mean connection?

Robin M. McAllen and **Clive N. May**

Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne, Parkville, Vic. 3052, Australia

Abstract. Short-term correlations in activity have been widely used as evidence to connect brainstem units with postganglionic sympathetic nerves. These may be detected by spike-triggered averaging, cross correlation or coherence analysis. The specificity of this type of evidence has been investigated by cross-correlating the activity of identified cutaneous vasoconstrictor postganglionic fibres with that of medullary premotor neurones of like and of unlike functional type, as determined by physiological testing (preoptic warming), in anaesthetised cats. Single medullary premotor neurones of both types were recorded from the subretrofacial nucleus: they were identified by their barosensitivity and, in most cases, their spinally projecting axons. By the test criteria chosen, the correlation method gave both false-positive and false-negative results as commonly as it gave correct ones. We conclude that it is not a reliable way to determine brainstem-postganglionic connectivity.

Key words: medulla oblongata, sympathetic, vasomotor, skin, correlation, blood pressure

INTRODUCTION

Premotor neurones in the rostral ventrolateral medulla are considered to be the most important source of descending drive to sympathetic neurones, and to mediate tonic and reflex control of vasomotor activity (Calaresu and Yardley 1988, Dampney 1994). Data from several laboratories now support the notion that these premotor neurones are not homogeneous, but contain subsets with preferential or selective effects on different sympathetic outflows. Three main lines of evidence favour this view. First, localized microinjections of glutamate into this region may preferentially or exclusively excite one sympathetic outflow rather than another, depending on injection site (Lovick 1987, Dampney and McAllen 1988, Dean et al. 1992, McAllen and May 1994a). Second, the functional identity (in this case cutaneous vasoconstrictor) of certain premotor neurones has been determined by their response to a specific physiological test (inhibition by preoptic warming: McAllen and May 1994a). Third, short-term (c. 100 ms) firing correlations may be found between individual medullary neurones and various postganglionic sympathetic nerves: in some cases the correlation is selectively with one sympathetic nerve rather than another (Barman et al. 1984). These correlations may be detected either by spike-triggered averaging of nerve activity (Barman and Gebber 1981, 1985, 1987, Morrison and Gebber 1982, 1984, McCall 1988, Morrison et al. 1988, McCall and Clement 1989, Gebber 1990, Huangfu et al. 1991) or by spectral analysis, measuring standard or partial coherence (Gebber et al. 1990, Barman and Gebber 1992, Cohen et al. 1995).

When results from the first two approaches are compared, as has been done for the cutaneous vasoconstrictor pathway in the cat, they show reasonably close agreement. Both methods indicate that cutaneous vasoconstrictor premotor neurones are concentrated around the ventromedial part of the subretrofacial nucleus, and are partly intermingled with the premotor neurones of other outflows (Dampney and McAllen 1988, McAllen and May 1994b). However, the correlation approach has never been tested for specificity against any independent reference. It is therefore of interest to compare its results with those of another method. This paper describes such a test.

METHODS

The present results constitute a new analysis of data from experiments reported elsewhere (McAllen and May 1994b). The recordings were made from 14 cats, 11 anaesthetized with alpha chloralose (70 mg/kg i.v.) and 3 with an infusion of the steroid mixture "Saffan" (Pitman - Moore; 0.3-2 mg/kg/h), given after 11 mg/kg i.m. ketamine. During recording, animals were paralysed with pancuronium (2 mg i.v.), while appropriate care was taken to ensure adequate anaesthesia at all times. Full details of anaesthesia, preparation and experimental procedures are given elsewhere (McAllen and May 1994b). Briefly, the preoptic region, medulla and cervical cord were exposed from a ventral approach, and a few-fibre cutaneous vasoconstrictor (CVC) preparation was dissected from a fascicle of the left superficial peroneal nerve that supplied hairy skin. A blind sac was prepared from one carotid sinus, with the common carotid occluded by an inflatable cuff for baroreceptor tests, but otherwise left open. A thermode was placed into a site in the preoptic region that, when warmed, inhibited ongoing CVC activity. Microinjections of glutamate into the rostral ventrolateral medulla were used to map the region of the subretrofacial (SRF) nucleus that excited CVC activity. Extracellular single unit recordings were then made from that region. All units studied were inhibited by baroreceptors (carotid blind sac inflation). Most could also be antidromically activated by electrical stimulation in the spinal cord (shown by collision test). Medullary units were then classified by their response to 1-7 min. episodes of preoptic warming (McAllen and May 1994b).

The present analysis was done on simultaneous recordings of the spontaneous activity of individual SRF neurones and peripheral CVC fibres. Simultaneous records lasted 3-30 min., and included peri-

ods of preoptic warming as well as resting activity. Cross correlation histograms (cross correlograms) were constructed from the data, triggering from the SRF neurone and taking CVC unit activity as the response. This method plots the time relation of every response spike to every trigger spike. It accumulates these in terms of occurrences at each particular interval before and after the trigger spike, as a histogram with 20 ms time bins. The cutoff time was set at 4 s before and after the trigger. All records were then digitally smoothed by taking a 5 point moving average.

The amplitude of a correlation peak was measured relative to the mean of the two flanking troughs, and expressed as a percentage of the overall mean bin count. Enhanced oscillations at cardiac frequency (see below) were accepted as correlation peaks if they occurred abruptly around the appropriate time. In these cases, the mean background oscillation was subtracted from the peak before calculating the percentage. Repetitive oscillations of cardiac period that diminished gradually over the 2-4 s either side of the trigger (e.g. Fig. 2F) were attributed to a background oscillation gradually losing synchrony, and were discounted.

RESULTS

The hypothesis to be tested was that there should be a more direct relation between the firing of SRF and postganglionic neurones of the same functional class (i.e. if they responded the same way to preoptic warming) than if they were of different type.

Twenty barosensitive SRF neurones (12 with proven spinal axons) out of 77 tested were found to be significantly inhibited, in parallel with CVC activity, when the preoptic region was warmed with a thermode (Fig. 1, left). These warm-inhibited SRF neurones were inferred to be preomotor neurones driving CVC activity. In contrast, 27 barosensitive SRF neurones (20 with proven spinal axons) were significantly excited by preoptic warming, while CVC activity recorded at the same time was inhibited (Fig. 1, right). Warm-excited SRF neurones were considered unlikely to drive the CVC pathway (McAllen and May 1994b), and have been taken here as the control group. Three of these were excluded from analysis because of low spike counts. For clarity, a further 22 SRF neurones that were neither inhibited nor excited by preoptic warming, as well as 8 that gave unclear responses, were also excluded from the present analysis.

Cross correlograms were constructed from the ongoing spike discharges of the SRF neurones under study (trigger) and the postganglionic cutaneous vasoconstrictor fibres (response). Several patterns were evident among the correlograms of both the experimental and control groups. Firstly, in these baroreceptor-intact animals, the cardiac

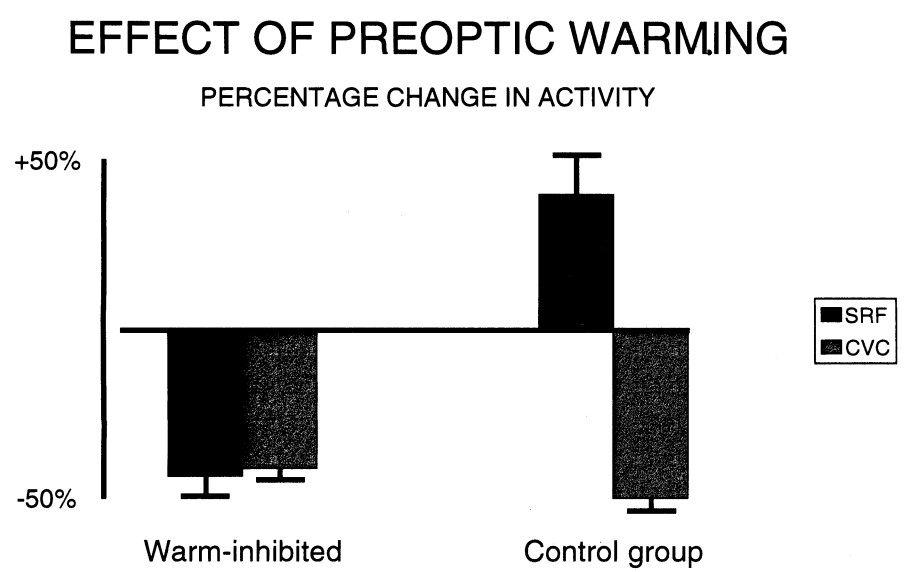

Fig. 1. Histograms of the maximum changes in neural activity in response to preoptic warming, as used to define the neural populations (data taken from the published study of McAllen and May 1994b). Values for the 20 warm-inhibited SRF neurones are shown on the left and for the 24 control (warm-excited) neurones on the right (solid bars). Accompanying values for changes in CVC activity recorded at the same time are shown as shaded bars. Error bars show SEM.

rhythmicity common to both central and peripheral units often caused periodic peaks and troughs in the cross correlogram (e.g. Fig. 2C and F). (In these experiments, perhaps surprisingly, CVC activity often showed strong cardiac rhythmicity (McAllen and May 1994b).) A common presumed respiratory rhythmicity (period 2-6 s) was also pronounced in some cases, particularly during periods of preoptic warming. This caused slower fluctuations in the cross correlogram, upon which fluctuations of cardiac period were often, but not always, superimposed (e.g. Fig. 2E and F).

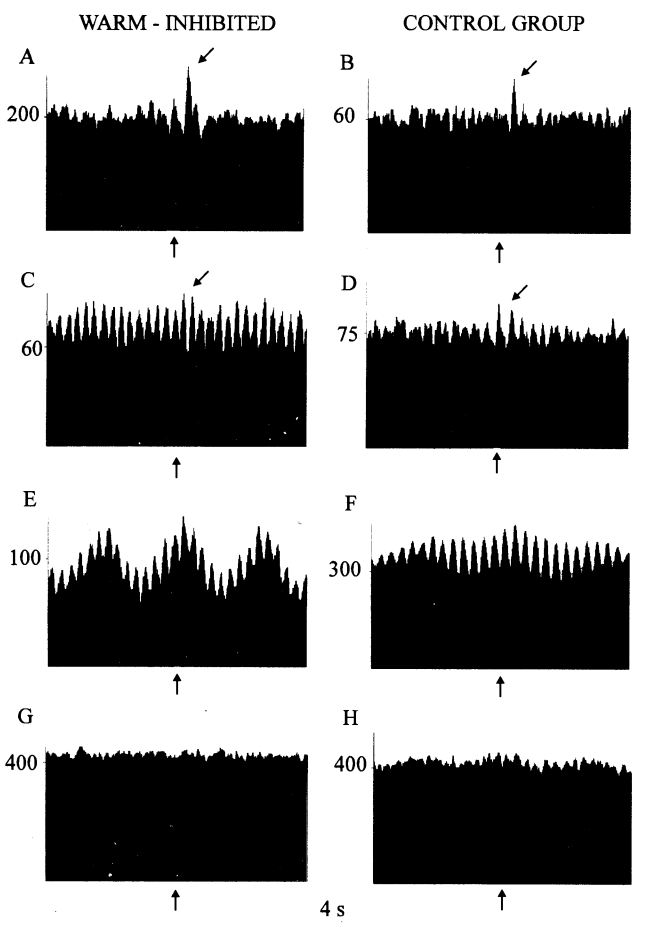

Fig. 2. Cross-correlograms of 4 warm-inhibited (A,C,E,G) and of 4 control SRF neurones (B,D,F,H) with peripheral CVC fibre activity. All show 4 s before and after the trigger (SRF neurone spike: time indicated by arrow). Oblique arrows indicate accepted correlation peaks (see text). A and B show isolated peaks; C and D show trigger-related enhanced oscillations of cardiac period; E and F show oscillations of cardiac and respiratory period, but no clear increase linked to the trigger; G and H show correlograms with no trigger-linked peak. 20 ms time bins. Numbers indicate bin counts.

However, the pattern sought as evidence of connectivity was a peak, or an enhanced oscillation in CVC firing probability, at a plausible latency (up to c. 500 ms) after the trigger spike. These were apparent in 9/20 cross correlograms triggered by warm--inhibited SRF neurones and also in 8/24 of the control group (triggered by warm-excited SRF neurones). Examples are shown in Fig. 2A–D; Fig. 2E–H show correlograms without trigger-related peaks. Abruptly enhanced oscillations of cardiac period accounted for 6 of the 9 cases in the warm-inhibited group and 5 of the 8 in the control group (e.g. Fig. 2C and D, the remainder were *de novo* peaks (e.g. Fig. 2A and B). Oscillations with a cardiac periodicity were present but showed no clear enhancement around trigger time in the cross correlograms of a further 5 warm-inhibited neurones and 8 of the control group (e.g. Fig. 2E and F).

The mean amplitude of the correlated peak, or excess oscillation, in the 9 positive correlograms triggered by warm-inhibited SRF neurones was 18.9% of background. It was 16.5% for the 8 control correlograms showing peaks.

DISCUSSION

When postganglionic whole nerve activity is recorded with wide bandpass (extending down to 1 Hz, as used by Barman, Gebber and others), slow waves represent envelopes of fibre activity (Gebber 1990). Brainstem spike-triggered averages of that activity are thus homologous with the brainstem spike-triggered histograms (cross correlograms) of postganglionic fibre activity studied here. Counting spikes in this way has two advantages, however, and one disadvantage. First, whole nerve recordings can detect only activity that occurs in bursts, reflecting the simultaneous activation of hundreds or thousands of fibres; but the spikes in a few-fibre preparation monitor activity at all times, including that between bursts. Second, spike counts are quantitative, giving histograms with a meaningful zero point; one can therefore assess the strength of a peak

relative to ongoing nerve activity. The disadvantage of few-fibre counting is that statistical fluctuations in bin count may make histograms less sensitive than averages for detecting correlation peaks.

The issue of this paper is whether a peak in a spike-triggered average (or histogram) of postganglionic nerve activity gives a reliable guide to connectivity. The present analysis takes advantage of an independent physiological test, the response to preoptic warming (in addition to barosensitivity), to classify SRF premotor neurones by function. By these criteria (as well as location) warm-inhibited SRF neurones may be presumed to belong to a CVC pathway (although conceivably they might belong to a different subset than the postganglionic fibres recorded). They were all inhibited by baroreceptors, so were presumably sympathoexcitatory in type. These neurones therefore ought to show activity temporally correlated with postganglionic CVC fibres (i.e. a cross correlogram peak). Warm-excited SRF neurones may be expected to belong to non-cutaneous pathways (again, sympathoexcitatory). Warm-excited SRF neurones ought not to show activity temporally correlated with that of CVC fibres, if correlation is to be a reliable guide to connectivity.

The first finding of this analysis is that appropriate, convincing peaks in the cross correlogram were present in only around half the cases where central (trigger) and peripheral (response) neurones were of the same functional class. The remainder constitute "false negatives" according to this test. Of course the failure to detect peaks in the "false negative" group could have been due to inadequate sensitivity in the cross correlation technique; but if so, those peaks must have been rather small. There is also the possibility that small trigger-related peaks could have been masked by cardiac-period oscillations in 5 cases where that feature was prominent. (And this could apply equally to the 8 such correlograms in the control group). It should be noted that for the present purpose we demanded that a true correlation of CVC activity with the trigger spike *per se*, should show as an appropriately-timed peak in excess of the ongoing oscillation. The latter was assessed from sections of the correlogram away from the trigger, and attributed to the barosensitivity common to both SRF and postganglionic neurones.

Alternatively it could be argued that the lack of correlation in the "false negative" cases was because those SRF neurones belonged to a different subset of CVC pathways (e.g. supplying hairless skin) than the postganglionic fibres recorded (which supplied hairy skin). However, most of the SRF neurones in question showed moderate or strong pulse rhythmicity - a feature reported to be absent from pathways to hairless skin (Jänig and Kümmel 1981), but present at the time in the CVC activity to hairy skin recorded in these experiments (McAllen and May 1994b).

The main finding of this study, however, is that equivalent correlation peaks are frequently found in cases where the central and peripheral neurone are of unlike functional class. These constitute "false positives" according to our test, and are difficult to explain away.

Finally, we found that when they were present in these experiments, correlation peaks were often quite weak in relation to background activity.

The present analysis uses cross correlation as others have used spike-triggered averaging, studying effects lasting tens or hundreds of milliseconds to follow pathways over at least 2 synapses. This must be distinguished clearly from the use of cross correlation to prove monosynaptic connections, which requires single units and millisecond precision (Kirkwood 1979, McAllen et al. 1994).

It is worth considering the possible mechanisms whereby brainstem spike-triggered peaks of post--ganglionic nerve activity could arise. The first would be that a single spike of that single central neurone actuality causes a measurable burst in postganglionic nerve activity. The inherent amplification of such a pathway would need to be huge, and it seems rather unlikely; but that could be tested. The more likely alternative is that the central neurone, and whatever generates postganglionic nerve bursts (perhaps a cohort of neighbouring - or other - brainstem neurones), take their time cue from a

common source: they "dance to the same tune". There is therefore no *a priori* reason to suppose that the central neurone in question is in a pathway to - or helps drive - that sympathetic nerve. Correlation on this timescale gives information about their inputs not their outputs. This could explain why the activity of central neurones of both like and of unlike functional type may be correlated with peripheral CVC activity (this study). By the same token, if a single brainstem neurone shows activity correlated with one, two or three sympathetic nerves (Barman et al. 1984, Cohen et al. 1995) this does not prove that it drives any of them.

In conclusion, this study provides the first independent test of spike-triggered correlation as a means to show specificity in medulla-to-postganglionic neurone connections. Taking physiological criteria as the standard, the correlation method apparently shows both false-negative and false-positive results as commonly as it shows correct ones. The same provisos appear likely to apply to detecting brainstem - postganglionic links by coherence analysis in the frequency domain (Barman and Gebber 1992, Cohen et al. 1995).

While correlation on this timescale may be unable to distinguish brainstem neurones whose output is destined for one particular sympathetic outflow rather than another, it remains possible that it could distinguish neurones of sympathetic pathways in general (although the present data suggest some would be missed) from those in non-sympathetic pathways. But particularly in the light of the present findings, it would seem prudent - indeed urgent - to test that assumption before using such methods to ascribe a "sympathetic" function to central neurones.

ACKNOWLEDGEMENTS

We are grateful to David Trevaks and Andrew McCubbin for excellent technical help. This work was supported by grants from the National Heart Foundation and from the National Health and Medical Research Council of Australia (Institute Block Grant).

REFERENCES

Barman S.M., Gebber G.L. (1981) Brain stem neuronal types with activity patterns related to sympathetic nerve discharge. Am. J. Physiol. 240: R335-R347.

Barman S.M., Gebber G.L. (1985) Axonal projection patterns of ventrolateral medullospinal sympathoexcitatory neurons. J. Neurophysiol. 53: 1551-1566.

Barman S.M., Gebber G.L. (1987) Lateral tegmental field neurons of cat medulla: a source of basal activity of ventrolateral medullospinal sympathoexcitatory neurons. J. Neurophysiol. 57: 1410-1424.

Barman S.M., Gebber G.L. (1992) Rostral ventrolateral medullary and caudal medullary raphe neurons with activity correlated to the 10-Hz rhythm in sympathetic nerve discharge. J. Neurophysiol. 67: 1535-1547.

Barman S.M., Gebber G.L., Calaresu F.R. (1984) Differential control of sympathetic nerve discharge by the brain stem. Am. J. Physiol. 247: R513-R519.

Calaresu F.R., Yardley C.P. (1988) Medullary basal sympathetic tone. Ann. Rev. Physiol. 50: 511-524.

Cohen M.I., Yu Q., Huang W-X. (1995) Preferential correlations of a medullary neuron's activity to different sympathetic outflows as revealed by partial coherence analysis. J. Neurophysiol. 74: 474-478.

Dampney R.A.L. (1994) Functional organization of central pathways regulating the cardiovascular system. Physiol. Revs. 74: 323-364.

Dampney R.A.L., McAllen R.M. (1988) Differential control of sympathetic fibres supplying hindlimb skin and muscle by subretrofacial neurones in the cat. J. Physiol. 395: 41-56.

Dean C., Seagard J.L., Hopp F.A., Kampine J.P. (1992) Differential control of sympathetic activity to kidney and skeletal muscle by ventral medullary neurons. J. Auton. Nerv. Syst. 37: 1-10.

Gebber G.L. (1990) Central determinants of sympathetic nerve discharge. In: Central regulation of autonomic functions (Eds. A.D. Loewy and K.M. Spyer). Oxford University Press, Oxford, p. 126-144.

Gebber G.L., Barman S.M., Kocsis B. (1990) Coherence of medullary unit activity and sympathetic discharge. Am. J. Physiol. 259: R561-R571.

Huangfu D., Koshiya N., Guyenet P.G. (1991) A5 noradrenergic unit activity and sympathetic nerve discharge in rats. Am. J. Physiol. 261: R290-R297.

Jänig W., Kümmel H. (1981) Organization of the sympathetic innervation supplying the hairless skin of the cat's paw. J. Auton. Nerv. Syst. 3: 215-230.

Kirkwood P.A. (1979) On the use and interpretation of cross-correlation measurements in the mammalian central nervous system. J. Neurosci. Meth. 1: 107-132.

Lovick T.A. (1987) Differential control of cardiac and vasomotor activity by neurones in nucleus paragigantocellularis lateralis in the cat. J. Physiol. 389: 23-36.

McAllen R.M., Häbler H-J, Michaelis M., Peters O., Jänig W. (1994) Monosynaptic excitation of preganglionic vasomotor neurons by subretrofacial neurons of the rostral ventrolateral medulla. Brain Res. 634: 227-234.

McAllen R.M., May C.N. (1994a) Differential drives from rostral ventrolateral medullary neurons to three identified sympathetic outflows. Am. J. Physiol. 267: R935-R944.

McAllen R.M., May C.N. (1994b) Effects of preoptic warming on subretrofacial and cutaneous vasoconstrictor neurons in anaesthetized cats. J. Physiol. 481: 719-730.

McCall R.B. (1988) GABA-mediated inhibition of sympathoexcitatory neurons by midline medullary stimulation. Am. J. Physiol. 255: R605-R615.

McCall R.B., Clement M.E. (1989) Identification of serotonergic and sympathetic neurons in medullary raphé nuclei. Brain Res. 477:172-182.

Morrison S.F., Gebber G.L. (1982) Classification of raphe neurons with cardiac related activity. Am. J. Physiol. 243: R49-R59.

Morrison S.F., Gebber G.L. (1984) Raphe neurons with sympathetic-related activity: baroreceptor responses and spinal connections. Am. J. Physiol. 246: R338-R348.

Morrison S.F., Milner T.A., Reis D.J. (1988) Reticulospinal vasomotor neurons of the rat rostral ventrolateral medulla: relationship to sympathetic nerve activity and the C1 adrenergic cell group. J. Neurosci. 8: 1286-1301.

Paper presented at the 2nd International Congress of the Polish Neuroscience Society; Session: Dynamics of interactions between circulatory and respiratory neuronal control systems