Asymmetry in the control of cardiac performance by dorsomedial hypothalamus

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ABSTRACT:

Dorsomedial hypothalamus (DMH) plays a key role in integrating cardiovascular responses to stress. We have recently reported greater heart rate responses following disinhibition of the right side of the DMH (R-DMH) in anesthetized rats and greater suppression of stress-induced tachycardia following inhibition of the R-DMH in conscious rats (both compared to similar intervention in the left DMH), suggesting existence of right/left side asymmetry in controlling cardiac chronotropic responses by the DMH. The aim of the present study was to determine whether similar asymmetry is present for controlling cardiac contractility. In anesthetized rats, microinjections of the GABA_A antagonist bicuculline methiodide (BMI, 40pmol/100nl) into the DMH evoked increases in heart rate (HR), left ventricular pressure (LVP), myocardial contractility (LVdP/dt), arterial pressure and respiratory rate. DMH disinhibition also precipitated multiple ventricular and supraventricular ectopic beats. DMH-induced increases in HR, LVP, LVdP/dt and in the number of ectopic beats depended on the side of stimulation, with R-DMH provoking larger responses. In contrast, pressor and respiratory responses did not depend on the side of stimulation. Newly described DMH-induced inotropic responses were rate-, preload- and (largely) afterload-independent; they were mediated by sympathetic cardiac pathway as revealed by their sensitivity to beta-adrenergic blockade. We conclude that recruitment of DMH neurons causes sympathetically-mediated positive chronotropic and inotropic effects, and that there is an asymmetry, at the level of the DMH, in the potency to elicit these effects, with R-DMH>L-DMH.

KEYWORDS: cardiac contractility; dorsomedial hypothalamus; asymmetry.
Psychological stress may cause cardiovascular disturbances, including potentially fatal cardiac arrhythmias (29). The end-organ (cardiac) mechanisms precipitating stress-induced arrhythmias are relatively well understood; they comprise excessive release of noradrenaline from the cardiac sympathetic terminals (20, 22, 28). Meanwhile, the mechanistic link between mental events and activation of cardiac sympathetic neurons still remains unclear, and many details of central pathways that lead to their activation during stress are unknown. Animal experiments conducted during the two last decades provided the basis for understanding these pathways, and it is now firmly established that the dorsomedial hypothalamus (DMH) is a key region in orchestrating neurally-mediated cardiovascular responses to stress. Indeed, integrity of synaptic transmission in the DMH is essential for the expression of stress-induced tachycardic and pressor responses (15). These functional studies are well supported by neuroanatomical data: the DMH receives synaptic inputs from many cortical and subcortical areas that are activated during stress and are involved in emotional processing, including the amygdala, the medial prefrontal cortex and the insular cortex (7, 18, 34, 41). DMH descending projections, after relaying in the medullary raphe, can excite cardiomotor spinal sympathetic neurons and thus enhance cardiac sympathetic outflow [for review, see (15)]. However, with the exception of two earlier works employing electrical stimulation of the hypothalamic area (11, 21), the knowledge about the DMH involvement in the cardiac control is limited to the effects on the heart rate. Changes in heart rate however are not sufficient for drawing conclusion about autonomic influences on the myocardial performance as there are examples of dissociation between neurally-induced inotropic and chronotropic effects [eg. (25)]. Consequently, it remains entirely unknown whether DMH activation could lead to changes in myocardial contractility and/or to cardiac arrhythmias.

In 2005 Critchley at al. (8) have provided the first human brain imaging data showing a robust positive relationship between midbrain activity and potentially pro-arrhythmic
abnormalities in ventricular repolarization during psychological and physical stresses. These cardiac effects were attributed to the increase in sympathetic outflow to the ventricular myocardium, suggesting that relevant cardiac sympathetic neurons were activated concurrently with, and potentially from the midbrain. While the authors carefully avoided using more specific anatomical terms, it is most likely that the DMH was activated in this brain imaging study as it represents an integral part of the midbrain (both anatomically and functionally). Of particular interest in this work was also the finding that the brain/heart correlation was observed exclusively for the right hemisphere. Based on previous evidence for right-side cortical dominance in cardiac control (43, 44), the authors suggested that “right-lateralized shift in midbrain activity reflects dysfunction, during stress, of a system that translates cortical activity into bilateral autonomic responses in the periphery”. This human study triggered our interest in the lateralization of cardiac control in the brain, and in our recent work we attempted to determine whether this phenomena is present in experimental animals. We found that pharmacological inhibition of the right DMH (R-DMH) performed in rats prior to air-jet stress nearly completely abolished the tachycardia caused by this stress whereas inhibition of the left DMH (L-DMH) was without effect (47). In anesthetized rats, disinhibition of the R-DMH evoked more potent tachycardic responses compared to the L-DMH. In contrast, pressor responses elicited from right or left DMH did not differ (13, 46, 47). In this previous work we did not assess DMH-induced effects on myocardial contractility, and the principal aim of our current work was to determine whether such effects exist, and if so, whether there is an inter-hemispheric asymmetry in their origin. More specifically, we hypothetized that neural influences from R-DMH will elicit more potent cardiac inotrope responses compared to L-DMH. To this end, in anesthetized rats we recorded ECG and measured changes in the left ventricular pressure (LVP) and contractility (LVdP/dt) elicited by unilateral (right and left) disinhibition of the DMH with GABA\textsubscript{A} antagonist bicuculline. Our additional aims were to determine whether DMH activation
could provoke cardiac arrhythmias, and whether, if present, DMH-induced inotropic and pro-arrhythmic effects are sympathetically mediated. Part of our results have been published in abstract form (45).

METHODS

*Animals and surgery*

Experiments were performed on adult male Hooded Wistar rats (300-350 g) bred at the animal facilities at the School of Biomedical Sciences and Pharmacy, University of Newcastle. All experimental procedures were approved by the Animal Ethics and Care Committee of the University of Newcastle, and conformed to the National Health and Medical Research Council guidelines. Experiments were conducted under combined \(\alpha\)-chloralose (60 mg/kg, i.p.) and urethane (600 mg/kg, i.p.) anesthesia. Its adequacy was verified by the absence of a withdrawal response to a nociceptive stimulation of a hindpaw. Supplemental doses of urethane were given when necessary. Following anesthesia induction, an endotracheal tube was inserted and connected to a line supplying humidified 100% oxygen. Animals were breathing spontaneously throughout the experiment. End-tidal CO\(_2\) was monitored using Normocap CO\(_2\) monitor (Datex, Helsinki, Finland). Polyethylene catheters were placed into the femoral artery and vein for recordings arterial pressure (AP) and for drug injections, respectively. Left ventricular pressure (LVP) was measured using a microtip pressure transducer (SPR-249, Millar Instruments, Houston, TX, USA) inserted into the left ventricle through the right common carotid artery. LVdP/dt (a measure of contractility) was computed online as a first derivative of LVP. Left ventricular end-diastolic pressure (LVEDP) was also calculated from LVP traces. Three-lead electrocardiogram (ECG) was recorded using subcutaneous stainless steel electrodes connected a BioAmp (ADInstruments, Sydney, Australia). Subsequently, the animals were positioned on a heating pad in a prone position, and their head was placed in a stereotaxic frame (Stoelting, IL,
USA), with the tooth bar fixed at -3.3 mm below the interaural line. A small craniotomy was made bilaterally near the Bregma level to allow later insertion of a glass micropipette into the DMH. Body temperature was monitored using rectal thermometer and maintained in the range of 37-37.5° C with a heating pad. Stable physiological state of our preparation is evidenced by stability of pressor responses (control/post-zatebradine/post-atenolol) in the course of experiment.

**Experimental design**

*Experiment 1: Effects of DMH disinhibition on cardiovascular and respiratory parameters in unpaced preparation.*

This experiment was conducted in seven rats; it comprised four steps. We initially performed the first microinjection of bicuculline methiodide (BMI; a GABA_A receptor antagonist; 40 pmol/100 nl) into the right or left side DMH, randomly chosen. After physiological variables returned to the baseline, a second injection of BMI was made into the contralateral side of the DMH. In the second step, the rats underwent i.v. administration of zatebradine (1 mg/kg), a blocker of Na pacemaker current (I_f) (10). We then repeated injections of BMI into right and left DMH. The purpose of using zatebradine was two-fold: i) to reduce, as much as possible, rate-dependent effects on cardiac contractility, and ii) to prolong vulnerable diastolic period, in anticipation that this may facilitate occurrence of ventricular arrhythmic events during DMH activation (26). In the third step we evaluated whether DMH-elicited cardiac responses were due to sympatho-excitation. For this purpose, beta-adrenergic receptor blocker atenolol was administered i.v. (2 mg/kg) prior to repeating microinjections of bicuculline into the left and right DMH. Finally, the aim of the fourth step was to determine whether a change in afterload contributed to the rise in contractility evoked by disinhibition of the DMH. For this purpose, a bolus i.v. injection of the α-adrenergic agonist phenylephrine (10 µg/kg) was performed (see below for details). In the preliminary
experiments conducted earlier, we confirmed that cardiovascular responses to repetitive intra-DMH bicuculline microinjections are reproducible providing sufficient time is given for recovery (n=3).

Experiment 2 - Effects of unilateral DMH disinhibition on cardiovascular parameters during cardiac pacing.

In this series of experiments (n=4), our objective was to completely eliminate rate-dependent effects on contractility when studying DMH-elicited cardiac effects. For this purpose, after placing tracheal tube, arterial and venous lines, LVP catheter and ECG electrodes (as described above), a midline thoracotomy was performed, and animals were artificially ventilated using Dual-Mode TOPO ventilator (Kent Scientific, Torrington, CT, USA). Two stainless-steel pacing electrodes were attached to the atria, and connected to a constant-current stimulator triggered from the PowerLab (ADInstruments, Sydney, Australia) output. The animals were placed in the stereotaxic apparatus and prepared for the injections into the DMH as described above. After stabilization of all parameters, zatebradine (1 mg/kg) was administered i.v. to decrease the heart rate to about 270 bpm. The heart was then paced at 360 bpm (the peak value achieved following DMH injection in zatebradine-treated rats without pacing). Pacing was started before the brain microinjections and continued for 15 minutes post-injection. The first microinjection of bicuculline methiodide was made in either right or left DMH, randomly selected; 30 min later the second microinjection was made into the contralateral DMH. Similar to the Experiment 1, a bolus injection of epinephrine was then administered for assessing of the afterload-dependence.

Microinjections and drugs

Microinjections of bicuculline were made into the DMH (100 nl/side) with a fine-tip (50 µm) glass micropipette. Injected volume was controlled by observing meniscus in a
calibrated glass capillary. The coordinates were 3.2 mm posterior and 0.6 mm lateral to the bregma, at a depth of 8.5 mm below the dura (27). Bicuculline methiodide, atenolol and phenylephrine were from Sigma (USA); zatebradine [1,3,4,5-tetrahydro-7,8-dimethoxy-3-[3-[2-(3,4-dimethoxyphenyl)ethyl] methylimino]propyl]-2H-3-benzazepin-2-on hydrochloride], a blocker of I_f pacemaker current (10) was a gift from Otsuka Pharmaceuticals (Tokyo, Japan).

**Data acquisition and analysis.**

All analog signals except ECG were sampled at 100 Hz; ECG was digitized at 1 kHz. Data were acquired using PowerLab 8/20 and LabChart 7.0 (ADInstruments, Sydney, Australia) and displayed online. Online computations were performed for the heart rate (from the ECG), respiratory rate (from the CO_2 signal) and LVdP/dT (by differentiating the LVP signal).

In order to control for the afterload-induced contractility changes, we compared the AP-independency contractility index computed from values obtained during DMH activation to that from values obtained during phenylephrine administration. The index was calculated according to the following equation: I = (∆dP/dt)/ (∆MAP) where ∆MAP and ∆dP/dt represent difference between the basal level and the maximal values for each variable obtained after either DMH activation or after phenylephrine.

Comparisons between responses evoked by the first and second microinjections of BMI into DMH were determined by a paired t test. For multiple comparisons, we used one-way ANOVA followed by Newman Keuls’s post hoc test. Comparisons between the ranges of the responses following left or right DMH disinhibition were performed with two-way ANOVA (factors drug and time) followed by Bonferroni’s post hoc test. Significance was taken at P<0.05. Data are expressed as mean ± SEM.
Histological verification of injection sites.

At the end of experiments rats were euthanized by an overdose of Lethabarb, and a microinjection of alcian blue dye 2% (100 nl) was made into the DMH injection sites for subsequent histological confirmation. Rats were then perfused transcardially with 50 ml of saline followed by 50 ml of phosphate-buffered paraformaldehyde (4%). The brains were removed, stored in paraformaldehyde (4%) for 24 hours and immersed in a sucrose solution (30%) for at least 48 hours. Brain slices (50 µm) were then cut in a freezing microtome. The atlas of Paxinos & Watson (27) was used as a reference for histological confirmation of the injection sites in the DMH.

RESULTS

Effects of unilateral DMH disinhibition on cardiovascular and respiratory parameters and on the ECG.

Fig. 1 is an example of a coronal brain section depicting microinjection sites in the DMH. Unilateral microinjections of bicuculline into the DMH increased heart rate, arterial blood pressure, LVP, LVdP/dt and respiratory rate. We also noted that DMH activation raised the frequency of respiratory events that we interpreted as sighs or “augmented breaths” (see Discussion). Representative traces and mean group data values for all parameters are presented in Fig. 2. Changes became noticeable within 1 min post-injection, peaked at 5-7 min and then gradually returned to the basal level within the next 25-30 min. Tachycardic and contractile (LVP and LVdP/dt) responses evoked from the right-side DMH were significantly higher compared to those evoked from the left side (Fig. 2, bar graphs). In contrast, the magnitude of pressor and tachypnoeic responses did not depend on the side of injection. There was also no difference in the incidence of sigh-like events evoked from the left and right sides. In the 2nd, 4th and 6th animals, microinjection of bicuculline was preceded by microinjection of vehicle; this did not provoke any consistent effects (data not shown).
In accord with the above-presented effects on the heart rate, DMH activation caused shortening of the ECG RR intervals, with a more potent effect provoked from right side (R: -28±9 vs. L: -15±4 msec; \( P < 0.05 \)). No further differences were observed in other ECG parameters between right and left DMH (data not shown). Importantly, DMH activation consistently provoked supraventricular and ventricular ectopic beats (Fig. 3), with significantly higher incidence for both subtypes after disinhibition of R-DMH. Such ectopic beats were never observed at baseline.

***Effects of \( I_f \) inhibition on basal cardiovascular and respiratory parameters, and on the DMH-elicited responses.***

Pharmacological blockade of the cardiac pacemaker current caused marked and sustained fall in HR (\( \Delta HR = -104\pm4 \) bpm, \( P < 0.05 \) vs. baseline), without enduring effects on other parameters. During the initial phase of this fall we observed slight transient increases in \( LVdP/dt_{peak} \) (\( \Delta LVdP/dt_{peak} = +1293\pm496 \) mmHg/s vs. baseline, \( P < 0.05 \)). This effect lasted less than 3 minutes, and at the 5th minute following injection the parameters stabilized at the levels not different from the pre-injection values (Fig. 4A).

Unilateral DMH activation performed under zatebradine blockade caused increases in MAP, HR and ventricular contractility indices (Fig. 4 B & C; mean data values shown near corresponding traces). Right-side dominance of the DMH influences on the cardiac performance was preserved after zatebradine (Fig. 4D). The drug also substantially reduced the incidence of supraventricular ectopic beats triggered by the activation of right DMH (7±2 vs. 2±1 SVEB/10 min; \( P < 0.05 \)); in contrast, the incidence of ventricular ectopics was not affected (L=3±2 vs. 2±1 and R= 9±4 vs. 6±2 VEB/10 min; before vs. after zatebradine, respectively).

The magnitude of tachypnoeic responses did not differ from pre-zatebradine condition, and was similar for both sides of injection (\( \Delta RespRate \) L= 47±8 vs. 43±9 and R=}
54±9 vs. 47±8 cycles per minute, cpm; before vs. after zatebradine, respectively). There was also no difference in the incidence of sighs evoked from the left and right sides (Δsighs = 16±2 vs. 17±2 sighs/10min, L vs. R respectively).

**Effects of atenolol on basal cardiovascular and respiratory parameters and on DMH-induced responses**

Beta-adrenergic blockade with atenolol caused falls in MAP (-6±2 mmHg), HR (-33±5 bpm), LVP (-15±4 mmHg) and LVdP/dt (-432±517 mmHg/s) (Fig. 5A). Atenolol completely blocked cardiac chronotropic responses evoked by microinjection of bicuculline into either side of the DMH (Fig. 5 B & C). However, DMH activation after atenolol still caused significant pressor responses associated with small but significant rises in the ventricular contractility (Fig. 5 B & C); there was no lateral asymmetry in eliciting these responses (Fig. 5D). Atenolol completely abolished supraventricular ectopic beats during activation of either side of the DMH; a small number of ventricular ectopics (3±1 and 6±4 VEB/10 min; L and R respectively) was however still present in 3 of 7 animals.

**Effect of afterload on ventricular contractility**

In order to determine whether DMH-elicited increases in ventricular contractility were secondary to increases in afterload (i.e. due to rises in the peripheral vascular resistance), we compared the (ΔLVdP/dt)/(ΔMAP) ratio determined during DMH activation to that determined during administration of phenylephrine. Result of this analysis is presented in Fig. 7. Both left and right DMH activation provoked pronounced increases in LVdP/dt, with similar slopes. In contrast, responses caused by phenylephrine elicited quite substantial increases in MAP, moderately affecting contractility. It is evident from this analysis that pressure rises provoked from the DMH (+15-20 mmHg) could account for only minor fraction of associated positive inotropic effect. Fig. 7 also illustrates our finding,
presented above, that activation of the R-DMH and L-DMH provoked similar pressor effect but side-dependent (R>L) inotropic effect. The values of (ΔLVdP/dt)/(ΔMAP) ratios for DMH-induced responses obtained under beta-adrenergic blockade (L=84±16 and R=79±16 s⁻¹) did not differ from the phenylephrine-induced effect (61±9 s⁻¹).

Effects of unilateral DMH disinhibition on cardiovascular parameters during cardiac pacing.

Changes in HR, LVP and LVdp/dt during this experimental series are exemplified in Fig. 8. In unpaced preparations, pharmacological inhibition of the pacemaker current by zatebradine evoked long-lasting bradycardia and transient contractility changes similarly to those described above. Unilateral disinhibition of the DMH during cardiac pacing caused rises in LVP and LVdp/dt; as observed in the other experiments of this study, inotropic effects elicited from R-DMH were significantly larger compared to those triggered from L-DMH (mean data values are shown near corresponding traced in Fig. 8).

Similar to the Experiment 1, in paced animals we assessed potential contribution of afterload to the DMH-induced inotropic responses, by comparing them to those provoked by a bolus injection of phenylephrine. Our finding were qualitatively similar to those in unpaced preparation, with (ΔLVdP/dt)/(ΔMAP) ratios being 195±25, 162±19 and 29±8 s⁻¹ following R-DMH activation, L-DMH activation and phenypeprine administration, respectively. The values for R-DMH and L-DMH did not differ from each other, but both were significantly different from the value for the phenylephrine (P < 0.01).

DISCUSSION

Our principal findings are: i) evidence that the DMH activation causes positive inotropic effects and alters myocardial excitability in a pro-arrhythmic manner; and ii) right-side dominance of the DMH to evoke these effects. These findings confirm and extend
previous reports showing that the right side of the hypothalamus dominates in controlling cardiac function (12, 47).

Methodological issues.

Maximal rate of LVP raise (LVdP/dt) is a well-accepted index of myocardial contractility, and we used it here to measure sympathetically-induced inotropic effects in anesthetized rats. In addition to being under autonomic control, LVdP/dt depends on several intrinsic parameters, including heart rate (Bowditch effect, treppe or frequency-dependent inotropy), preload (Frank-Starling mechanisms) and afterload (von Anrep effect) (1, 16, 42). We thus employed different approaches to exclude potential confounding effects of these influences. Firstly, we used zatebradine (a blocker of Na pacemaker current) to prevent/attenuate sympathetically-induced tachycardia. Indeed, after zatebradine administration, the maximal HR values following DMH activation were not greater than the pre-drug baseline. However, post-zatebradine DMH-induced tachycardic responses were still quite substantial, and this strategy alone was not enough to exclude the rate-dependent effects. We resolved this problem by examining the DMH-induced inotropic effect in the situation when the heart was paced at a fixed rate. Secondly, in our experiments we assessed preload by measuring LVEDP. Lack of changes of this index during DMH-induced rises in LVP and LVdP/dt suggests that contractility changes were not preload-induced. It must be acknowledged that LVEDP is an indirect estimate of the left ventricular volume and could be used as an index of preload only if the ventricular compliance remains constant. Finally, as LVdP/dt in the rat heart is strongly affected by afterload (24, 42), we have controlled for this confounder by comparing \(\Delta(LVdP/dt)/\Delta MAP\) ratio for DMH-induced responses to that provoked by administration of \(\alpha\)-adrenergic agonist phenylephrine (30). As expected, phenylephrine provoked both pressor and contractile effect, but the magnitude of this afterload-induced contractile response was substantially smaller than the DMH-induced
inotropic effect. An additional argument in favor of afterload-independence of DMH-induced inotropic effects is that they did depend on the side of activation whereas pressor effects did not.

Analysis of our data from the phenylephrine experiments clearly indicates that afterload could contribute to the DMH-induced inotropic response, but this contribution was relatively small. In rats, the relation between afterload and LVdP/dt is linear (24); this allowed to make our comparison using single dose of the phenylephrine. As can be seen in Fig. 7A, a rise of MAP by about 20 mmHg evoked by phenylephrine (a usual range of DMH-induced pressor effect) could account for not more than 25% of contractility rise evoked from the L-DMH and even less for the R-DMH-induced response. Thus, while a fraction of DMH-induced rise in cardiac contractility certainly was a consequence of increased afterload, we are confident that cardiac sympathetic nerves mediated the major part of this response. This suggestion was fully confirmed by its sensitivity to beta-blockade. Small rises in contractility that were still present post-atenolol could be most likely attributed to the above-described effect of afterload. This suggestion is supported by the fact that $\Delta$(LVdP/dt)/$\Delta$MAP ratios computed for DMH-induced responses elicited under beta-blockade were identical to those computed for responses evoked by phenylephrine.

**DMH-induced chronotropic and inotropic responses are sympathetically-mediated.**

Our data showing right-side DMH dominance for controlling HR confirms our recent findings where we have demonstrated such dominance in both anesthetized and conscious animals (46, 47). Current data also extends these findings to cardiac contractile function, showing that myocardial contractility is increased by removal tonic GABA-ergic input on DMH neurons. Indirectly, our findings indicate that DMH neurons controlling heart rate and myocardial contractility are under tonic inhibitory influences.

We present evidence suggesting that presympathetic cardiomotor neurons controlling
HR and contractility are predominantly segregated in the R-DMH. This appears to be quite a robust observation as in none of experiments reported here did we encounter L-DMH dominance in cardiac responses. Likewise, no cases of left-side dominance were observed in our previous study (46, 47).

Substantial reduction of basal HR by zatebradine is in full accord with the view that sympathetic influences in the pacemaker cell are mediated via activation of $I_f$, a Na pacemaker current (10, 39). Zatebradine had no effect on basal contractile indices (with the exception of brief transitional periods where HR was changing) or contractile responses, in agreement with established pharmacological profile of this drug (2, 9, 40). Relatively minor effect of zatebradine on the DMH-induces tachycardic responses could be explained by the existence of sympathetically mediated but $I_f$-independent regulatory mechanism (eg. via voltage-gated Ca channels). This suggestion is in agreement with the fact that atenolol applied after zatebradine further reduced basal HR.

Beta-adrenergic blockade resulted in the reductions of basal cardiac contractile indices and HR. As in rat heart force-frequency relation is negative (24), it is likely that the magnitude of the negative inotropic effects was underestimated. Our results thus indicate that in anesthetized rats, there is a substantial basal sympathetic outflow to the ventricular myocardium. Suppression by atenolol of DMH-induced tachycardic responses confirms our previous finding that they are sympathetically mediated (15). Finally, the fact that atenolol suppressed the afterload-independent component (see below) of the DMH-induced contractile response indicates that this was also mediated via the cardiac sympathetic outflow.

Small contractile responses that persisted following beta-adrenergic blockade were no longer depended on the side of DMH stimulation, and thus were likely elicited by different mechanisms. Two arguments support the idea that these responses were the consequence of increased afterload (Anrep effect): firstly, the slope of contractility/MAP
ratio (or AP-independence index) was substantially less steep compared to the control condition but did not differ from that computed following phenylephrine test. Secondly, the time course of these contractile responses closely resembled time course of pressor changes whereas in control condition temporal differences between the two were quite obvious.

**DMH activation affects cardiac excitability.**

Activation of the DMH consistently elicited supraventricular and ventricular ectopic beats. While these cardiac arrhythmic events are not considered malignant clinically, it must be acknowledged that our study was performed in animals with normal (not predisposed) myocardium. Thus ectopic beats found in our study indicate that DMH activation resulted in observable changes in the excitability of cardiac tissues. This finding is new and may offer insight into the mechanisms generating cardiac arrhythmias during psychological stresses (26). Excessive release of noradrenaline from cardiac sympathetic terminals is a well-established peripheral mechanism of these arrhythmias (22, 23). Our results suggest that the DMH may be involved in the genesis of stress-induced arrhythmias by facilitating sympathetic outflow to the ventricular myocardium. This idea is in accord with the well-established role of the DMH in stress-induced tachycardia in rats (15). Of interest is the fact that supraventricular, but not the ventricular ectopic beats evoked from DMH were prevented by zatebradine, a blocker of the pacemaker sodium current. This suggests that supraventricular ectopics were originating in the atrial conducting system that, in contrast to the myocardium, does express sodium HCN channels responsible for $I_f$ (33). Their sensitivity to beta-adrenergic stimulation is well established, and our data suggests that excessive release of noradrenaline in atria, in addition to physiological positive chronotropic effects, may provoke arrhythmic events by activating $I_f$.

Zatebradine had no effect on the number of ventricular ectopic beats; their incidence was reduced, but not totally eliminated, by the beta-blocker atenolol. This residual ectopy
could persist either due to activation of alpha-adrenoreceptors, or due to incomplete beta-blockade.

*DMH-induced respiratory effects.*

In contrast to human studies, where close association between respiratory indices and affective states is firmly established, studies in conscious animals are mainly limited to homeostatic (rhythm-generating and chemoreflex-related) ponto-medullary mechanisms. We have recently presented the first documented evidence that in rats, psychological stressors cause rise in the respiratory rate associated with elevated incidence of sighs (augmented breaths) (19). DMH-induced tachypnoea observed in the current and several previous studies (17, 35) could well be the principal mechanism underlying these stress-related respiratory effects. We also present preliminary evidence that DMH activation could lead to an increase of sighing frequency. We acknowledge that we did not measure tidal volume (an essential condition for identifying sigh); we made this serendipitous finding when reviewing the end-tidal CO₂ signal that was used for computing respiratory rate. We believe that discrete respiratory events that we observed could be classified as sighs based on the following: i) their fairly regular occurrence; ii) a delay of the “sigh”-related peak of CO₂ signal (compared to pre-sigh regular respiratory rate) was likely due to the fact that sigh-related inspiration normally occurs at the end of a previous inspiratory phase, so that the expiratory phase is delayed; and iii) post-sigh apnea that is a distinctive feature of sighs (19).

*Neural pathways mediating DMH-induced effects.*

Beta-adrenergic blockade completely prevented the tachycardia evoked from the DMH, indicating that it was sympathetically mediated. This is in full accord with a number of studies in conscious and anesthetized animals where stress- and/or DMH-induced tachycardia could be prevented by beta-blockade or inhibition of the medullary raphe that
relays descending projections from the DMH to the spinal cardiac sympathetic neurons (5, 14, 31, 32). Beta-blockade also dramatically reduced DMH-elicited inotropic responses, suggesting that they are also largely mediated by sympathetic pathways. To the best of our knowledge, this is the first description of functional cardiac inotropic responses induced from the supra-medullary level, and it is currently unknown whether signals generated in the DMH for controlling heart rate and myocardial contractility share the same descending pathway (5, 14). In discussing this possibility, we would like to consider two medullary regions: the rostral ventro-lateral medulla (RVLM) and the medullary raphe/parapyramidal area. In rats, chemical activation of either of these areas causes sympathetically mediated tachycardia and rise in cardiac contractility (3, 4, 6, 30). Furthermore, anatomical tracing studies have demonstrated that both regions receive direct projections from the DMH (14, 37), and that both regions, in turn, project to the heart, including to the ventricular myocardium (36, 38, 48). Current consensus regarding medullary control of HR is that the RVLM mediates homeostatic (baroreflex) control and that DMH-RVLM projection carries signals for vascular but nor cardiac presympathetic neurons (14). In contrast, the raphe region is involved in cardiac responses to environmental stressors (see above). Whether presymapththetic pathways for the control of cardiac inotropic function are arranged in a similar way remains an open question. Lack of changes in cardiac sympathetic activity, HR or contractile responses to microinjection of GABA into the raphe area indicates that raphe-spinal cardiomotor neurons are not tonically active (5, 30), at least in anesthetized animals. For elaborated discussion of functional specialization of neurons controlling cardiac function (see 30).

Perspectives and significance.

One intriguing question that now remains open is how right-side dominance for controlling both HR and contractility at the midbrain level gets converted into preferentially
left-side control of contractility and preferentially right-side control of HR by cardiac sympathetic nerves. In addition, it would be most interesting to test whether DMH stimulation can trigger malignant ventricular tachyarrhythmias in animals with predisposed myocardium (eg. ischemic or post-infarction). In conclusion, activation of R-DMH provokes larger positive inotropic and chronotropic effects compared to L-DMH. In conjunction with earlier cortical data, this may mean that there exists right-hemispheric dominance in controlling cardiac sympatho-excitatory effects. Our findings are relevant for identifying mechanisms of stress-induced cardiac diseases.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.
REFERENCES


FIGURE LEGENDS:

Figure 1. Photograph of a coronal section of the rats brain documenting DMH microinjection sites (thick arrows). Abbreviations: DMH, dorsomedial hypothalamus; VMH, ventromedial hypothalamus; mt, mammillo-thalamic tract; ot, optical tract; f, fornix; III, third ventricle.

Figure 2. Cardiovascular and respiratory responses elicited by microinjection of BMI (40pmol/100nL) into right (R-) and left (L-) DMH. Left and middle columns show representative traces from a single animal; mean group data for the baseline values and maximal response values are presented below and above each trace, respectively (# - P < 0.05 vs. baseline). The inset in the left bottom panel shows respiratory event classified as a sigh. Bar graphs in the right column represent delta changes for each variable for responses elicited from the R-DMH (black bars) and the L-DMH (white bars). * - P < 0.05 L- vs. R-DMH.

Figure 3. A – Representative tracing highlighting ventricular and supraventricular ectopic beats precipitated by activation of dorsomedial hypothalamus and their effects on cardiac dynamics and arterial pressure. B – Number of ectopic beats occurring during 10 minutes period following activation of right (R-) and left (L-) DMH activation. * - P < 0.05 R- vs. L-DMH.

Figure 4. Representative tracings showing effects of zatebradine on the baseline hemodynamic parameters (column A) and changes in these parameters elicited by dysinhibition of the R-DMH (column B) and L-DMH (column C) under zatebradine blockade. The numbers near the traces are the mean group values for the pre- and post-drug conditions; # - P< 0.05 vs. baseline. Bar graphs in panel D represent delta changes for each
variable for responses elicited from the R-DMH (black bars) and the L-DMH (white bars). * - P < 0.05 L- vs. R-DMH.

Figure 5. Representative tracings showing effects of atenolol on the baseline hemodynamic parameters (column A) and changes in these parameters elicited by dysinhibition of the R-DMH (column B) and L-DMH (column C) under beta-adrenoreceptor blockade. The numbers near the traces are the mean group values for the pre- and post-drug conditions; # - P < 0.05 vs. baseline. Bar graphs in panel D represent delta changes for each variable for responses elicited from the R-DMH (black bars) and the L-DMH (white bars). * - P < 0.05 L- vs. R-DMH.

Figure 6. Representative tracings showing changes in HR, MAP, LVP and LVDp/dtpeak caused by right and left DMH activation under cardiac adrenergic blockade. Panels A and B show changes caused by microinjection of BMI into R- and L-DMH sides, respectively. Mean group data for the baseline values and maximal response values are presented near each trace; # - P < 0.05 vs. baseline. Bar graphs in panel C represent delta changes for each variable for responses elicited from the R-DMH (black bars) and the L-DMH (white bars).

Figure 7. Comparison of afterload-dependency of contractility for responses elicited from the DMH and after bolus i.v. administration of phenylephrine. A – mean group data; for each pair of points, the left one corresponds to the baseline values (±SEM) of MAP and contractility, and the right one – to maximal elicited values. Note that the slope of the line for afterload-dependency following phenylephrine was substantially less steep compared to DMH-induced responses. Bar graph in B shows these values (determined as [(Δ dP/dtpeak) / (Δ MAP)])
Figure 8. Illustration of an experiment conducted with cardiac Panel A - Representative tracings showing changes in HR, MAP, LVP and LVDp/dtpeak caused by i.v. administration of zatebradine, cardiac pacing and injection of BMI into the DMH unilaterally. Bar graphs in panel C represent delta changes for each variable for responses elicited from the R-DMH (black bars) and the L-DMH (white bars). *P<0.05 R- vs, L-DMH.
**A** Zatebradine

- **HR** (bpm):
  - Baseline: 386±10
  - Final: 271±11#

- **MAP** (mmHg):
  - Baseline: 105±7
  - Final: 100±6

- **LVdP/dt peak** (mmHg/s):
  - Baseline: 10203±712
  - Final: 9380±768

**B** R-DMH

- **HR** (bpm):
  - Baseline: 237±7
  - Final: 354±16#

- **MAP** (mmHg):
  - Baseline: 95±8
  - Final: 119±10#

- **LVdP/dt peak** (mmHg/s):
  - Baseline: 7748±408
  - Final: 14493±842#

**C** L-DMH

- **HR** (bpm):
  - Baseline: 271±9
  - Final: 333±17#

- **MAP** (mmHg):
  - Baseline: 98±8
  - Final: 119±8#

- **LVdP/dt peak** (mmHg/s):
  - Baseline: 8130±738
  - Final: 11273±755#

**D**

- **Δ Heart rate (bpm)**
  - Baseline: 119±4
  - Final: 147±5#

- **Δ MAP (mmHg)**
  - Baseline: 128±2
  - Final: 141±4#

- **Δ LVdP/dt peak (mmHg/s)**
  - Baseline: 237±7
  - Final: 354±16#

- **Δ LVP (mmHg)**
  - Baseline: 271±9
  - Final: 333±17#
A

Zatebradine 0.3 mg/kg

Pacing

BMI into unilateral DMH

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B

\( \Delta LVP_{\text{peak}} \) (mmHg)

\[ 0 \quad 10 \quad 20 \quad 30 \]

\( \Delta LVdP/dt_{\text{peak}} \) (mmHg/s)

\[ 0 \quad 5000 \quad 10000 \quad 15000 \]

R - DMH

L - DMH

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**Time (min)**

0 4 8 12 16 20 24 28 32 36

**HR (bpm)**

0 100 200 300

**MAP (mmHg)**

0 80 160

**LVP (mmHg)**

0 100 200

**LVdP/dt peak (mmHg/s)**

0 5000 10000

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**Notes**

- HR: Heart Rate
- MAP: Mean Arterial Pressure
- LVP: Left Ventricular Pressure
- LVdP/dt: Left Ventricular Pressure
- BMI: Body Mass Index
- DMH: Diaphragmatic Hemiparesis