Cortical and brain stem changes in neural activity during static handgrip and postexercise ischemia in humans


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It is known from electrophysiological studies in experimental animals that unmyelinated (group IV) and small-diameter myelinated (group III) nerve fibers in muscle respond to both contraction and metabolic products of contraction (22–24). Moreover, activity in these afferents can be maintained by preventing the release of metabolic products from the muscle interstitium. Accordingly, these afferents are known as metaboreceptors (or ergoreceptors). Selective nerve blocks revealed that these group III and IV afferents are responsible for the cardiovascular (and respiratory) responses to muscle contraction (29, 42). Furthermore, lesion studies have demonstrated that the reflex increase in sympathetically mediated vasoconstriction, the metaboreflex, requires the medulla but does not require connections to more rostral regions (21); indeed, a somatosympathetic reflex can be demonstrated in an isolated brain stem-heart preparation (38). Electrophysiological evidence has revealed that group III and IV muscle afferents project to neurons in the nucleus tractus solitarius (NTS) and that neurons from the NTS directly project to the rostral ventrolateral medulla (RVLM), the primary output nucleus for muscle vasoconstrictor drive (20, 35). However, while the importance of the medulla in the metaboreflex is clear, cardiovascular responses to exercise can occur in the absence of any input from the periphery and can even occur during imagined exercise (45, 46). Given their previously documented roles in cardiovascular control (5, 6, 34, 52, 53), the insular cortex and anterior cingulate cortex have been strongly implicated in integrating the motor command with the cardiovascular adjustments to volitional exercise. Using neuroimaging techniques [single-photon emission computed tomography (SPECT)] to assess regional cerebral blood flow in human subjects, Williamson et al. (45–47) showed activation of both the anterior cingulate and insular cortex during static or dynamic exercise. Static handgrip exercise, sustained for 3 min, followed by postexercise ischemia for 100s, was used to differentiate between changes in regional blood flow associated with motor command (contraction phase) and those associated with metaboreceptor inputs during the period of circulatory occlusion (45). These authors found a marked increase in blood flow in the sensorimotor cortex, left anterior insula cortex, and anterior cingulate cortex during the contraction phase and in the sensorimotor and right anterior insula cortex during the period of postexercise ischemia (PEI), but they did not report on changes within the brain stem.

Two recent studies, using functional MRI (fMRI) during 30-s periods of static handgrip exercise, have found significant increases in blood oxygen level-dependent (BOLD) signal intensity in the sensorimotor cortices, insular cortex, midcingulate cortex, cerebellum, and brain stem and decreases in the dorsal anterior
cingulate cortex and ventral medial prefrontal cortex that were related to the increases in HR during the contraction but did not examine changes during PEI (48, 49). The purpose of the present study was to use fMRI to examine the time course of BOLD signal intensity changes within the cerebrum, cerebellum, and brain stem during a 2-min period of static handgrip (SHG) and a 6-min period of PEI. In particular, we aimed to define areas within the human brain stem that, based on their pattern of activation, are likely to correspond to those identified in animal experiments.

MATERIALS AND METHODS

Seventeen healthy subjects (9 men and 8 women; age: 19–46 yr, body mass index: 19–29) were recruited for the study. This study was approved by the Human Research Ethics Committee of the University of New South Wales. All procedures were performed in accordance with the Declaration of Helsinki and with the understanding and written consent of the subjects. All subjects were normotensive, had no history of cardiovascular or neurological disorders, and were not taking any medications. Two experimental protocols were undertaken in the same subjects on different days: 1) the fMRI protocol and 2) the laboratory protocol.

fMRI Protocol

Subjects were supine with their head and torso inside a 3-T whole body MRI scanner (Intera, Philips Medical Systems). The head was held immobile in an eight-channel RF head coil by foam pads. A deflated occlusion cuff was placed around the upper right arm; this cuff could be quickly inflated and kept at suprasystolic pressure (280 mmHg) via an automatic static inflator outside the scanner room (Hokanson) to accomplish control cuff occlusion (CCO) and PEI. Subjects were thoroughly briefed about the timeline of the protocol, such that communication could be kept to a minimum during the protocol. Subjects wore headphones to minimize the noise experienced from the magnet and to receive short messages from outside the scanner room. Subjects could communicate via both a microphone and a pneumatic buzzer.

A pneumatic cylindrical handgrip was positioned in the right hand and connected by nondistensible tubing to a pressure transducer (Spectramed) outside the scanner room. The pressure signal was recorded on a computer-based data acquisition and analysis system (PowerLab 4/25T and Chart 5, ADInstruments, Bella Vista, Australia), fed live to a digital projector, and displayed on a screen located inside the scanner room, which the subject could view via a noninverting mirror system. With this visual feedback and verbal encouragement, each subject made at least 3 brief (~5 s) maximum effort handgrip contractions, and the highest pressure recorded was considered the maximum voluntary contraction (MVC). This pressure was then scaled to 100%, and both a pressure reference line and the relative pressure in large digits were displayed on the feedback screen so the subjects could perform static handgrip exercise at 40% of their MVC. In a closed pressurized system, the pressure increase created by the static handgrip exercise was calibrated under a variety of conditions with known weights that pulled four cords positioned around the cuff. We determined an optimal loading volume (i.e., the largest volume that is still compatible with a good grip) and pressure [i.e., to minimize the relative volume displacement we used the highest pressure, which stayed stable during a 2-min handgrip period (100 mmHg) and used these settings in all experiments]. Despite the optimized conditions, the pneumatic device is not linear; rather, the weight-pressure curve is upward convex, corresponding to an underestimation of maximal force. In comparison, a conventional force transducer handgrip device (Stoelting) tested in the same calibration setup was completely linear over the range relevant for handgrip testing. We estimated that to perform a “true” 35% of MVC, subjects should perform 40% of MVC on the pneumatic device. We confirmed this assumption by comparing the pneumatic and the conventional devices in the same subjects in the laboratory.

Two continuous series of fMRI image volumes (gradient echo and echo-planar images using BOLD contrast) were collected. Each volume covered the entire brain (42 axial slices, repetition time: 3 s, echo time: 30 ms, flip angle: 90°, field of view: 230 mm, and raw voxel size: 1.8 × 1.8 × 3 mm thick). The first fMRI series consisted of 200 image volumes (=10 min). Baseline was recorded for 2 min, then CCO for 6 min, and finally 2 min of recovery. The purpose of this CCO protocol was to label the areas activated by unilateral sensory input from the same region but without engaging the metaboreflex. The second fMRI series consisted of 240 image volumes (=12 min). Baseline was recorded for 2 min, and subjects then performed SHG for 2 min, followed by PEI for 6 min, and finally 2 min of recovery. The purpose of continuing PEI for 6 min was to provide a long-lasting steady-state unilateral sensory input and metaboreflexly generated sympathoexcitilation without concurrent central command.

After each of the two fMRI series, the McGill pain questionnaire was read aloud, and each subject selected the words that most closely described the pain in the right arm during the cuff inflation period. Each subject also rated the maximum effort during SHG exercise and the intensity of pain and unpleasantness during CCO and PEI on a 0–10 Borg scale (2). The two functional imaging scans were separated by at least 20 min, during which a three-dimensional, T1-weighted anatomic image (voxel size: 0.4 × 0.4 × 0.9 mm) and a T2-weighted anatomic image of the brain stem (voxel size: 0.4 × 0.4 × 2.2 mm) were collected.

fMRI Analysis

Whole brain analysis. Using SPM2 (11), fMRI images were motion corrected, spatially normalized to the MNI template, and spatially smoothed (6 mm full width at half-maximum). Since global signal intensity also includes a significant portion of signal intensity changes due to “real” neural activation, and the fact that in our challenge we expected to find significant signal intensity changes of relatively long duration in widespread areas of the brain stem and cortex, a high-pass filter was not used. Significant signal intensity changes were determined during the contraction phase of the challenge only. That is, statistical time trends between the 40-volume baseline and the subsequent 40-volume contraction period only were assessed using a model of mean sympathetic nerve activity convolved with a hemodynamic delay. The mean sympathetic nerve activity model was created by averaging each individual subject’s MSNA acquired during a separate laboratory session (see below). Mean values were sampled every 3 s. Voxel-wise statistical maps were then calculated for each individual subject, indicating the fit between signal intensity and the model of mean sympathetic nerve activity (11). The resulting 17 statistical parametric maps were used in a random-effects second-level analysis to generate a statistical map representing the consistency of effects across subjects. A threshold of P < 0.05 (corrected for multiple comparisons, minimum cluster size of 10 voxels) was then applied using a random field theory (50). Significant signal intensity changes were then color coded and rendered onto an individual’s T1-weighted anatomic image set. Finally, the percent signal intensity changes relative to baseline for each significant voxel in a cluster was calculated for the baseline and contraction and ischemia phases of the challenge (i.e., volumes 1–200) and averaged to create an average cluster time trend. A cluster time trend was calculated for each subject and averaged to create a mean (±SE) group cluster time trend.

Brain stem-only analysis. Given that the brain stem is often not accurately spatially normalized using whole brain normalization procedures, a separate brain stem analysis was performed. After realignment, the brain stem and cerebellum were extracted from each subject’s fMRI image sets. Using one subject’s image set as a template, the brain stem/cerebellum images from all 17 subjects...
were spatially normalized. One subject’s brain stem images were removed due to inaccurate normalization. The remaining subjects’ (n = 16) images were then spatially smoothed (5 mm full width at half-maximum), and significant signal intensity changes were determined using an identical model as that used for the whole brain analysis (random effects, corrected P < 0.01). Significant signal intensity changes were then color coded and overlaid onto a mean T2-weighted anatomic series and cluster time trends were calculated as described above.

**Laboratory Protocol**

All 17 subjects were also studied supine in our neurophysiology laboratory on separate study days. HR was determined by the continuous recording of ECG from Ag-AgCl chest electrodes, respiration by a strain-gauge band around the chest (Pneumotrace, UFU, Morro Bay, CA), and blood pressure by oscillometric sphygmomanometry (Colin CBM7000, Colin); mean arterial pressure (MAP) was calculated as diastolic pressure + 1/3 of pulse pressure. In 13 subjects, we obtained direct continuous recordings of MSNA via tungsten microelectrodes inserted percutaneously into the common peroneal nerve at the fibular head (ISO-80, WPI, bandpass: 0.3–3.0 kHz). This signal was root mean square processed (moving average over 100 ms), and standard burst analyses were performed. All physiological data were stored on computer via a data acquisition and analysis system (PowerLab16SP and Chart 5, ADInstruments). MSNA was sampled at 10 kHz, ECG at 2 kHz, and blood pressure and respiration at 400 Hz.

All subjects first performed exactly the same protocol as in the MRI scanner using the same cuff inflation and pneumatic device for SHG (40% of MVC). A subset of the subjects (n = 13) subsequently performed a 2-min SHG using the conventional device (35% of MVC) to compare the time course and level of sympathoexcitation and perceived effort as well as pain and discomfort to pneumatic SHG (40% of MVC). All analysis of MSNA was performed by one microneurographer (M. Sander) without knowledge of the FMRI data. MSNA is expressed both as burst frequency and total activity (burst frequency × voltage). Sympathetic and hemodynamic data are expressed as means ± SE, and statistical analysis of within-group differences were assessed by one- and two-way ANOVA for repeated measures (protocol-time and protocol-time and handgrip-type, respectively). Post hoc analysis was performed by Dunnett’s procedure for one-way ANOVA and by Tukey’s procedure for two-way ANOVA. The level of significance was set at P < 0.05 and adjusted by the Bonferroni method as appropriate.

**RESULTS**

**Physiology**

As previously demonstrated, 2 min of submaximal static handgrip exercise caused a progressive increase in MSNA and arterial pressure. These increases were sustained in the absence of motor command during a 6-min period of PEI, which was induced by inflation of a cuff around the upper arm, immediately before the cessation of the exercise. Conversely, during the CCO, performed while the subject was relaxed, there were no increases in MSNA or arterial pressure. Experimental records from one subject are shown in Fig. 1; mean data from all 17 subjects are shown graphically in Fig. 2. It can be seen that HR increased at the commencement of the exercise but returned to resting levels at the end of the contraction phase, whereas MSNA and arterial pressure remained elevated throughout the period of PEI. There were no differences in the cardiovascular responses to static handgrip and PEI performed with the pneumatic device or conventional device.

Fig. 1. Exemplary recordings of handgrip force and microneurographic raw filtered and integrated muscle sympathetic nerve activity (MSNA) in the same subject during 2 min of baseline (BL), 2 min of static handgrip (SHG), and the first 2 min of postexercise ischemia (PEI) (A) as well as during 3 min of BL and the first 3 min of control cuff occlusion (CCO) (B; no handgrip force recording).
cortex; this was apparent on both sides (Table 1). The con-
siderable increase was observed in the secondary somatosensory
of the period of ischemia. Although not shown in Fig. 3, a
forearm and hand and the initiation of paresthesia after the end
perhaps not surprising given the rush of warm blood into the
increase persisted during the short recovery period, which is
an exercise, continued throughout the period of PEI (Fig. 3). This
phase that, after a transient depression at the conclusion of the
a gradual increase in signal intensity during the contraction
increase, in retrospect, a recovery period of 2 min (40 volumes) was
resolved during the recovery period, but we acknowledge
mild and not painful; there were no differ-
tralateral insula and ipsilateral parietal association cortex
showed identical changes during the maneuver: a rapid
crease at the beginning of the contraction phase, a slower linear
crease during the static phase that was similar to that seen in
primary motor cortex, a small depression at the end of the
exercise, and a steady level of increased signal intensity
(~2.5%) during the 6-min period of PEI. Moreover, there were
signs of a reversal during the period of recovery. Decreases in
signal intensity, which commenced during the contraction
phase as a progressive fall and were sustained during PEI at an
essentially constant level, were apparent in the midcingulate
cortex (approximately −1.5%) and perigenual cingulate cortex
(approximately −2%), as shown in Fig. 3. It is clear that the
decrease in the perigenual cingulate cortex starts to reverse
during the recovery period.

Figure 4 shows mean data for the brain stem and cerebellum. Increases in signal intensity occurred in two discrete sites
within the medulla: a progressive increase during the contraction
phase, a slight dip at the end, followed by a sustained
increase in signal intensity (~1.5%) during PEI, was observed
in both the dorsomedial medulla and dorsolateral medulla.
During the recovery phase, there was a second increase in
signal intensity within the dorsomedial medulla. The deep
cerebellar nuclei showed a pattern similar to that of the primary
motor cortex: after the contraction, the signal did not fully
return to baseline but remained slightly elevated. The increase
in signal intensity within the cerebellar cortex was limited to
the contraction phase; it returned to baseline levels at the
conclusion of the contraction.

None of these changes occurred during CCO given at rest,
but slight increases in signal intensity occurred in a discrete
region of the cerebellar cortex and ipsilateral primary somato-
sensory cortex, with bilateral decreases in signal intensity
being seen in the amygdala. Although these changes were
maintained for the entire 6-min cuff inflation period, they were
small in magnitude (~0.5%) and probably of little physiologi-
cal relevance.

Given that there was intersubject variability in the magni-
tude of the signal changes in the brain and the amplitude of the
pressor response to PEI, we attempted to examine covariation
by performing linear correlation analyses for each area in
which significant changes in signal intensity were found. These
data are shown in Fig. 5. While the correlations were weak,
which may be explained by the fact that the physiology and
fMRI were not recorded in the same sessions, there was
nevertheless a significant positive relationship between signal
intensity in the dorsolateral medulla and the increase in blood
pressure. A similar trend was apparent for the dorsomedial
medulla and contralateral insula, although only the latter
reached statistical significance.

fMRI

Whole brain group analyses (n = 17) of the changes in
BOLD signal intensity over time are shown in Fig. 3. Spatial
coordinates (in MNI space) of areas showing significant in-
creases and decreases are shown in Table 1.

Significant increases in signal intensity (~1.5%) occurred
over the contralateral (left) primary motor cortex during static
handgrip exercise: BOLD signal intensity increased rapidly at
the start of the contraction and then continued to increase
linearly at a lower rate during the static phase of the contrac-
tion, in which handgrip pressure was held constant at 40% of
MVC. When the subject was instructed to relax, and the cuff
was inflated around the upper arm, signal intensity returned
toward baseline levels, although there was a slight insignificant
increase after ~2 min. It can be seen that this increase tended
to resolve during the recovery period, but we acknowledge
that, in retrospect, a recovery period of 2 min (40 volumes) was
not adequate to allow complete recovery.

In the contralateral primary somatosensory cortex, there was
a gradual increase in signal intensity during the contraction
phase that, after a transient depression at the conclusion of the
exercise, continued throughout the period of PEI (Fig. 3). This
increase persisted during the short recovery period, which is
perhaps not surprising given the rush of warm blood into the
forearm and hand and the initiation of paresthesia after the end
of the period of ischemia. Although not shown in Fig. 3, a
similar increase was observed in the secondary somatosensory
cortex; this was apparent on both sides (Table 1). The con-

Psychophysics

There were no statistically significant differences in the
rating of perceived effort during the contraction performed in
the scanner or laboratory (5.9 ± 0.7 vs. 6.9 ± 0.5), nor was the
rating of perceived pain or discomfort during PEI different (5.7 ±
0.6 vs. 6.8 ± 0.5). When subjects were asked to rate the
discomfort caused during the 6 min of CCO at rest, it was
considered to be mild and not painful; there were no differ-

Fig. 2. Summary data (means ± SE) for heart rate (HR), mean arterial pressure
(MAP), and MSNA during BL (2 min), SHG (2 min), PEI (6 min), and
recovery (Rec; 2 min) (○) as well as during BL (4 min), CCO (6 min), and Rec
(2 min) (□). *P < 0.05 for BL vs. time point.
ences in ratings between the scanner and laboratory (2.1 ± 0.4 vs. 2.5 ± 0.4; not significant).

**DISCUSSION**

As noted in the Introduction, both central command and reflex inputs from the contracting muscles contribute to the cardiovascular responses to exercise, whereas reflex inputs are exclusively responsible when the metabolites accumulated during the exercise cannot escape during PEI. The aim of this study was to further our understanding of how these cardiovascular responses are brought about and to differentiate between those changes that can be attributed to central command (motor effort) and those that can be attributed to reflex inputs (the metaboreflex). Using single-trial fMRI in awake human subjects, we documented the changes in the correlates of neuronal activity within the brain during a static contraction and a subsequent period of prolonged PEI. In addition to demonstrating widespread yet discrete increases in signal intensity in the contralateral primary and secondary sensorimotor cortices, insula, and cerebellum and decreases in the anterior and midcingulate cortex, we show, for the first time, robust bilateral increases in two clusters within the brain stem, areas that we believe correspond to the human equivalents of the RVLM and NTS.

**Cortical and Cerebellar Changes During Static Exercise and PEI**

As expected, signal intensity in an area of the contralateral primary motor cortex corresponding to the forearm increased...
rapidly at the start of the contraction and continued to increase linearly at a slower rate as the muscles started to fatigue. This fits with the recruitment of additional motor units in the forearm flexor muscles, required to maintain a constant force during a sustained submaximal contraction, and is consistent with a previous fMRI study (26). This increase in signal intensity returned to baseline levels at the conclusion of the exercise but then increased slightly for the duration of the PEI; the same was true for the deep cerebellar nuclei, but for the cerebellar cortex the increases were limited to the contraction phase (see Fig. 4). We also observed increases in activity in a lateral area of the primary sensorimotor cortex, corresponding to the somatotopic representation of the hand, an increase that presumably reflects an increase in sensory input. This may also account for the small increase in signal intensity within the primary motor cortex and deep cerebellar nuclei during PEI. Although force was held constant, such that afferent input from mechanoreceptors in the hand would be relatively constant, the progressive increase in signal intensity may reflect an increase in nociceptor activation during the contraction. Subjects were not specifically asked whether they were feeling pain during the 2-min contraction and were only asked to rate their effort; nevertheless, there is no doubt that there would be accumulation of metabolites during an isometric contraction and that this would activate metaboreceptors within the intrinsic muscles of the hand. Moreover, this increase in signal intensity continued to develop during the period of PEI, essentially paralleling the development of pain.

The entire contralateral insular cortex and ipsilateral (right) parietal association cortex also showed an increase in signal intensity that paralleled that observed in the contralateral primary motor cortex during the contraction phase. Unlike the motor cortex, however, this activity remained elevated throughout the PEI. Indeed, the largest and most significant increase in signal intensity during PEI encompassed almost the entire left (contralateral) insula, but there was no increase ipsilaterally. Interestingly, using SPECT, Williamson et al. (45) found activation of the insula on both sides during 2 min of PEI. Furthermore, these authors suggested that subpopulations of the insular cortex on both sides were activated during central command or PEI (45). Bilateral activation of the anterior insular cortex has also been observed during the cold-pressor response (15) and Valsalva maneuver (19) and during a maximal inspiratory breath hold (26), which, like the metaboreflex, also cause sustained increases in MSNA. During muscle pain, induced by an injection of hypertonic saline into a muscle in the forearm or leg, we recently demonstrated a robust activation of the contralateral posterior insula, the time course of which matched that of the development of pain (16, 17, 27). Given that the 6-min period of PEI is considered unpleasant (subjects want the cuff inflation to be released) and that the anterior insula is believed to encode this emotion (10), it is reasonable to conclude that the activation of both divisions of the contralateral insula may reflect both the negative affect (unpleasantness) and overt pain associated with PEI. If, on the other hand, the insula is involved in mediating the autonomic responses, then, based on animal studies showing connections between the insula and lateral posterior hypothalamus (3), we would expect to have seen activation of the hypothalamus and, via direct and indirect projections to the spinal cord, sympathetic excitation. However, while we (26) had previously seen activation of the lateral hypothalamus (bilaterally) during a maximal inspiratory breath hold, we saw no such activation in the present study. Moreover, it has also previously been shown that the lateral hypothalamus is not activated by the Valsalva maneuver (18). Accordingly, we strongly doubt that the insula is responsible for the sustained increase in MSNA associated with the metaboreflex; rather, as noted above, we believe it is engaged by the increased sensory input during PEI and is registering unpleasantness and pain.

As shown in Fig. 3, large decreases in activity were apparent in the midcingulate cortex and perigenual anterior cingulate cortex (periACC), the time course of which mirrored the changes seen in the primary somatosensory cortex. These changes at first glance might be related to the HR increase during exercise, since activity in the anterior cingulate cortex has been linked to parasympathetic activation. Thus, a decrease in anterior cingulate cortex activity could contribute to the withdrawal of parasympathetic tone at the onset of exercise, as observed by Wong et al. (48, 49). However, the continued large decrease in activity seen during PEI cannot explain the HR response, which returned to resting levels during PEI. The significance of these decreases in activity for autonomic regulation is unclear, although it is worth pointing out that a sustained decrease in the periACC has also been observed during muscle pain induced by an intramuscular injection of hypertonic saline (16, 27); this suggests that the fall in periACC activity may be related to the negative affect: both experimental muscle pain and PEI generate negative emotions, and a fall in signal intensity in the periACC has been observed in depression (9, 31).

### Brain Stem Changes During Static Exercise and PEI

Regional analysis of the brain stem revealed two distinct regions of increased signal intensity, in the dorsomedial medulla and lateral medulla, during the exercise and PEI. As

<table>
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<th>x</th>
<th>y</th>
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<tr>
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M1, primary motor cortex; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; PAC, parietal association cortex; periACC, perigenual anterior cingulate cortex; MCC, midcingulate cortex.
shown in Fig. 4, both areas increased progressively during the contraction phase, reaching a peak increase of ~2%. At the conclusion of the exercise, there was a slight decrease but then a steady-state activation during PEI. Importantly, these changes paralleled the increase in MSNA and blood pressure. We suggest that, based on our previous identification of the human correlate of the RVLM (26), the region within the lateral medulla that showed a robust increase during static handgrip and PEI is the same structure, i.e., the RVLM. From electrophysiological recordings in experimental animals, it is known that the RVLM is the primary output nucleus for muscle, splanchnic, and renal sympathetic outflow (7), so it is reasonable to expect an increase in signal intensity in this region. There was a significant positive linear relationship between the increase in blood pressure and the increase in signal intensity in the lateral medulla (RVLM); the same was true for the dorsomedial medulla, although this failed to reach statistical significance.

It is also reasonable to expect that the dorsomedial medulla, which we believe corresponds to the NTS, would show a progressive increase in signal intensity during exercise and a sustained increase during PEI: it is known that group III–IV muscle afferents (metaboreceptors) project to NTS (20, 38) and that central drive also converges in the NTS (8). However, it is clear that as blood pressure increases during the metaboreflex, there will be an increased activation of the NTS from arterial baroreceptors, which are known to also project to the NTS: this, in itself, may contribute to the sustained increase in signal intensity during PEI. We are unable to differentiate to which extent muscle and baroreceptor afferents are responsible for the increase in signal intensity within this region of medulla, although in the absence of other excitatory inputs an increase in baroreceptor input would be expected to cause a decrease in signal intensity in the RVLM (19). However, it is difficult to explain the second increase in signal intensity within the dorsomedial medulla during the recovery period in terms of an increase in blood pressure (which returned to control levels at the conclusion of PEI). Given that baroreceptive neurons have been identified in the posterior insula (52, 53), the increase in signal intensity in this region may also be related to the increase in blood pressure.

Methodological Considerations

Unlike many fMRI studies that used repeated-trials methodology [including our own (26)], which ameliorates much of the variability in signal intensity and allows analysis of the data...
according to the classic boxcar approach, in the present study we used a single-trial approach. We acknowledge that repeating trials obtained from single subjects could greatly strengthen our results. However, our single-trial approach allowed us to sample continuously from baseline through to contraction, PEI, and recovery. We have successfully used this single-trial approach in the study of acute pain (lasting 8–10 min) induced by an intramuscular or a subcutaneous injection of hypertonic saline (16, 17, 27). In these studies and the present study we have not just reported “blobs” of increased and decreased BOLD signal intensity; we have shown the time trends of the changes in signal intensity. These time trends revealed that some areas showed a progressive increase in signal intensity during the contraction that was sustained during the period of PEI; other areas showed a progressive decrease in signal intensity, likewise sustained during the ischemic phase. It is hard to envisage these divergent responses, which were consistent across subjects in each of the responding areas, as being largely influenced by large, systematic, low-frequency drifts in the baseline signal during the continuous sampling of BOLD signal intensity.

Fig. 5. Correlational analyses of changes in fMRI signal intensity in eight different regions versus increases in ΔMAP during the last 30 s of static handgrip. Data were obtained on 2 different study days.
Indeed, although it was initially proposed that low-frequency drifts in BOLD signals were due to scanner instabilities (40), more recent work has shown that the magnitude of these low-frequency drifts varies between gray and white matter (51). Yan et al. (51) suggested that frequency drifts within gray matter actually result from changes in neural activity but attributed any drift in white matter to systematic or thermal noise. These data support our approach to not apply a low-frequency filter to our fMRI dataset. Unfortunately, our experimental paradigm did not allow sufficient recovery time (only 2 min) to document a complete return of signal intensity to baseline levels, although there were signs that this recovery had commenced. Nevertheless, BOLD signal intensity had returned to essentially resting levels in the primary motor cortex and in the cerebellum after the contraction phase, further arguing against low-frequency drifts having a significant effect on regional signal intensity during the period of PEI.

Perhaps one of the greatest limitations is that we are comparing changes in BOLD signal intensity with cardiovascular parameters recorded on a different day, albeit in the same subjects. This was necessary: we could not record the MSNA while scanning the brain, relying on the assumption that the cardiovascular responses to the handgrip contraction and PEI would be similar in the same individuals when tested on different days. Since the completion of this study, we have now succeeded, for the first time, in recording spontaneous MSNA while performing fMRI of the brain (28). Application of this approach to specific maneuvers (such as the current maneuver) promises to further increase our understanding of how the human brain controls blood pressure, but unless we have a means of recording continuous blood pressure (noninvasively) while scanning the brain we will not know the magnitude of any change in blood pressure and will not be able to correlate any change in signal intensity with the change in blood pressure.

Conclusions

Our fMRI data have shown that certain areas of the brain exhibit an increase in activity, as judged by regional increases in BOLD signal intensity, whereas others show a decrease, during static handgrip exercise and PEI. It is well accepted that the increase in HR at the beginning of exercise is mediated by central command, returning as it does to baseline levels during PEI. It is the underlying neural processes during the subsequent sympathoexcitation that are poorly understood. Studies (8, 32, 43) in experimental animals have found direct projections from the sensorimotor cortex to the NTS and RVLM, so central command could cause an increase in MSNA. However, one would expect that if this were to occur the increase in MSNA would commence at the beginning of the contraction phase. In fact, there is no increase at the beginning (48, 49) but rather a progressive increase during the course of the contraction. While central command may contribute to this increase, it is clear that metaboreceptor activity will increase progressively during the contraction phase and remain essentially constant during PEI. Based on the present data, we conclude that the metaboreflex is mediated via the medulla: projection of group III/IV muscle afferents to the NTS, excitation of neurons in the RVLM, increase in muscle sympathetic outflow, and hence increase in blood pressure. Furthermore, we believe that the increase in activity in the contralateral insula and the decrease in perigenual anterior cingulate cortex, rather than causing cardiovascular adjustments of exercise, are related more to the unpleasantness and pain subjects experience during PEI, although the posterior insula may also be activated by increase in baroreceptor input during the resultant increase in blood pressure.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

REFERENCES

15. Harper RM, Macey PM, Henderson LA, Woo MA, Macey KE, Fryssinger RC, Alger JR, Nguyen KP, Yan-Go FL. fMRI responses to...


