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EFFECT OF LOWER BODY NEGATIVE PRESSURE ON HUMAN MUSCLE NERVE SYMPATHETIC ACTIVITY

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SUMMARY

1. Recordings of multi-unit sympathetic activity were made from muscle branches of the median nerve in five healthy subjects during application of lower body negative pressure (l.b.n.p.). Simultaneous recordings of arterial blood pressure were made in four subjects. The strength of the neural activity was quantitated by counting the number of pulse synchronous sympathetic bursts and their amplitudes in the mean voltage neurogram.

2. The general appearance of the sympathetic activity in pulse synchronous bursts was similar during control periods and during l.b.n.p., but during l.b.n.p. there was always an increase in the number of sympathetic bursts and usually also in the mean voltage amplitude of the bursts.

3. The probability of occurrence of a burst was correlated to different blood pressure parameters of individual heart beats and both during control periods and during l.b.n.p. there was regularly a close negative correlation to diastolic, a low correlation to systolic, an intermediate negative correlation to mean and a positive correlation to pulse pressures.

4. The changes in arterial blood pressure during l.b.n.p. were small and in most cases statistically insignificant. The observed increases in the number of sympathetic bursts during l.b.n.p. were significantly greater than what could be expected on the basis of the blood pressure changes.

5. The findings suggest that the reflex control from the arterial baroreceptors is maintained during l.b.n.p. but, since the increase of sympathetic activity during l.b.n.p. could not be explained by a change in stimulation of the arterial baroreceptors, influence from other receptor groups (presumably intrathoracic volume receptors) must also have occurred.

INTRODUCTION

As the vascular bed of skeletal muscle plays an important role for circulatory homeostasis much work has been devoted to elucidating the various reflex mechanisms controlling muscle blood flow. The nervous influence is mediated by sympathetic impulses, the outflow of which is modulated by different inhibitory reflexes. Of these the carotid sinus reflex is the most thoroughly studied but there is also evidence that in addition reflexes from intrathoracic, so called 'low pressure' receptors may affect skeletal muscle blood flow (see Paintal, 1973; Shepherd, 1973; Öberg, 1976).

Ever since Greenfield, Brown, Goei & Plassaras (1963) introduced lower body negative pressure (l.b.n.p.) as a method of studying the effects of blood volume displacement on the cardiovascular system, this technique has been widely used in studies on man (see Wolthuis, Bergman & Nicogossian, 1974). In such studies a reduction of the central blood volume caused a significant increase in forearm vascular resistance (Zoller, Mark, Abboud, Schmid & Heistad, 1972; Johnson, Rowell, Niederberger & Eisman, 1974) and since no significant changes in arterial blood pressure occurred this was interpreted as an effect elicited from the 'low pressure' receptors.

With the development of a neurophysiological technique for recording sympathetic action potentials in human peripheral nerves (Hagbarth & Vallbo, 1968) it has become possible to study the reflex effects of blood volume and arterial blood pressure changes in a more direct way. In previous recordings of muscle nerve sympathetic activity (MSA) sympathetic impulses were found to appear in response to spontaneous, temporary blood pressure reductions (Delius, Hagbarth, Hongell & Wallin, 1972; Wallin, Delius & Hagbarth, 1973; Wallin, Delius & Sundlöf, 1974; Sundlöf & Wallin 1978). This inverse relationship together with the characteristic pulse-synchronous grouping of the impulses, related to the diastoles, was taken as evidence for arterial baroreflex modulation of the sympathetic outflow.

In the present investigation multi-unit sympathetic activity in muscle nerves has been recorded during l.b.n.p. and the interest has been focused on two main questions.

(1) Does sympathetic activity change when blood is pooled in the legs?

(2) If there is a change in activity, is it elicited from arterial pressure receptors or can other receptor groups be involved?

METHODS

Material. Recordings of MSA were made during twenty-nine periods of l.b.n.p. on five healthy subjects, four men and one woman, 18–27 yr of age, all of whom gave their informed consent to the investigation. The recordings were made from the right median nerve at the elbow level. Intra-arterial blood pressure was recorded simultaneously during twenty-two periods of l.b.n.p. in four subjects. In an additional fifteen subjects acceptable electrode positions for MSA recordings were searched for but not found.

Nerve electrodes, recording and display system. The nerve recordings were made with insulated tungsten micro-electrodes which were inserted manually through the intact skin into a muscle nerve fascicle and adjusted until a position was found in which sympathetic impulses could be recorded. After amplification the neural activity was fed through a RC-integrating network (time constant 0·1 sec) to obtain a mean voltage display of the nerve signals. Both the original neural record and the mean voltage neurogram were stored together with other parameters on an 8 channel FM tape recorder (Precision Instruments, PI 6200). During the experiments the neural activity was monitored continuously on a storage oscilloscope (Tektronix 549) and a loudspeaker. Details about the technique and the evidence for the sympathetic origin of the recorded impulses have been described previously (Delius *et al.* 1972; Wallin, Sundlöf & Delius, 1975; Sundlöf & Wallin, 1977).

Arterial blood pressure was monitored through a catheter in the brachial artery connected to a pressure transducer EMT 35 and electromanometer EMT 31 (Siemens-Elema Ltd, Sweden). The e.c.g. was recorded by surface chest electrodes. *Respiratory movements* were recorded by a strain gauge attached to a rubber band placed around the thorax. *The negative pressure device* consisted of a wooden box enclosing the legs and abdomen of the subject and sealed with the aid of rubber flaps around the body. Negative pressure was achieved by a standard vacuum motor and box pressure was monitored by a pressure transducer (LX 1600 A, National Semiconductor) calibrated with a mercury manometer.

Analyses. The mean voltage neurogram together with the e.c.g., arterial blood pressure and respiratory movements were displayed from the tape on an inkjet recorder (Mingograph 800, Siemens-Elema Ltd) with a paper speed of 3 or 5 mm/sec.

In order to compare the relationship between arterial blood pressure and MSA at rest and during l.b.n.p. the different pressure parameters of individual heart beats were correlated to the probability of occurrence of a sympathetic burst. For this purpose the records were divided into periods of approximately 3 min duration (range 1·5–4 min) and for each period all the pulse synchronous bursts that could be identified by inspection of the mean voltage neurogram were marked. The analogue signals of the mean voltage neurogram and the blood pressure were then converted into digital form (sampling frequency 100 Hz) and fed into a computer (Siemens 305). In previous recordings of MSA a reflex delay was demonstrated between blood pressure and neural events (Delius *et al.* 1972; Wallin *et al.* 1974, 1975; Sundlöf & Wallin, 1978). Compensation for this delay was made by the computer using a standard value of 1·1 sec for all subjects. For each heart beat the computer determined systolic, diastolic, mean and pulse pressures and marked which beats were associated with a burst. The results from each period (control or l.b.n.p.) were then summarized in so called 'threshold variability diagrams' as described by Sundlöf & Wallin (1978) (see Fig. 5 and insert in Fig. 6). As a quantitative measure of the strength of the sympathetic bursts the computer also measured the peak amplitude of the bursts in the mean voltage neurogram and the values obtained were plotted against the blood pressure.

Experimental procedure. Subjects were in a comfortable recumbent position, with the lower part of the body enclosed in a wooden box from which air could be evacuated. The nerve recording electrode was inserted into the right median nerve and the left brachial artery was catheterized for blood pressure recordings. When optimal signal-to-noise ratio for sympathetic impulses was obtained the spontaneous activity at rest was recorded during 5–10 min before l.b.n.p. was applied. The box pressure was decreased slowly (during 10–20 sec) to the desired level, where it was maintained constant for about 3 min (range 1·5–4 min). Between two periods of l.b.n.p. resting activity was recorded for 2–8 min. The periods during which the box pressure was changing were excluded from quantitative computer analysis. In each subject on the average ten control periods (range eight to twelve) and six periods (range five to seven) of l.b.n.p. of -5 to -40 mmHg were analysed.

RESULTS

Effect of l.b.n.p. on MSA

The general character of the MSA was similar during control periods and during l.b.n.p.: the sympathetic impulses were grouped in pulse synchronous bursts which often occurred in short irregular sequences interposed with periods of more or less total neural silence. In a given individual the incidence of sympathetic bursts showed fairly small random variations from one 3 min control period to the next, but between individuals there were clear differences, the mean burst incidence ranging from 39 to 62 bursts/100 heart beats (cf. Sundlöf & Wallin, 1977*a*). The occurrence of sympathetic bursts always increased during l.b.n.p. As a rule the changes in MSA occurred in parallel with the slow application and elimination of l.b.n.p. and when the desired pressure levels were reached no further changes in nerve activity occurred. In some cases, however, following a sudden release of suction, the bursts disappeared more or less completely for 5–10 sec before activity returned. An example of the increase in MSA during l.b.n.p is shown in Fig 1, and Fig. 2 summarizes the changes in occurrence of sympathetic bursts in all l.b.n.p. periods from all subjects (expressed as number of bursts/min to take account of inter-individual differences in heart rate). As illustrated in Fig. 2 the change of m.s.a. varied considerably at the same level of l.b.n.p. but there was a tendency for greater changes at higher levels of l.b.n.p. ($r = 0{\cdot}53$, slope of regression line significantly different from 0, $P < 0{\cdot}01$).

Usually not only the number of bursts but also the strength of the bursts

(i.e. their amplitudes in the mean voltage neurogram) increased during l.b.n.p. Fig. 3 illustrates that mean burst amplitude during l.b.n.p. was greater than that of the preceding control period in twenty-three and smaller in only six instances (significant increase, $P < 0{\cdot}001$, Wilcoxson's signed rank sum test).

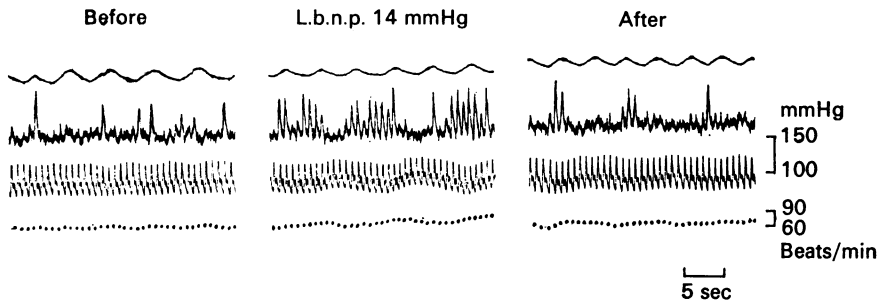

Fig. 1. Example of experimental records showing increase in number of sympathetic bursts during l.b.n.p. Traces are from above: respiratory movements (inspiration upwards), mean voltage MSA (time constant 0·1 sec), blood pressure, instantaneous heart rate.

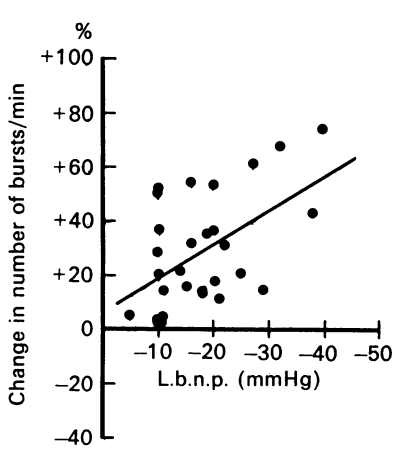

Fig. 2

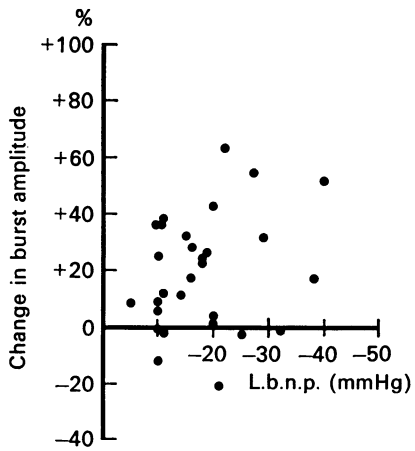

Fig. 3

Fig. 2. Changes in MSA during l.b.n.p. in all subjects. Each point represents one period of l.b.n.p.

Fig. 3. Changes in mean burst amplitudes during l.b.n.p. in all subjects. Each point represents one period of l.b.n.p.

Effect of l.b.n.p. on arterial blood pressure

The observed changes of arterial blood pressure during l.b.n.p. were small. In most instances there was a slight increase in diastolic blood pressure, whereas systolic, mean and pulse pressures usually decreased. The changes were never statistically significant for diastolic and mean pressures but for systolic and pulse pressures significant decreases ($P < 0{\cdot}05$) during l.b.n.p. occurred in two and thirteen instances, respectively. Fig. 4 summarizes the differences in mean values for diastolic, mean,

systolic and pulse pressures between control periods and succeeding periods of l.b.n.p., for pressures of less than 20 mmHg (open rectangles) and for pressures of 20 mmHg and more (filled rectangles).

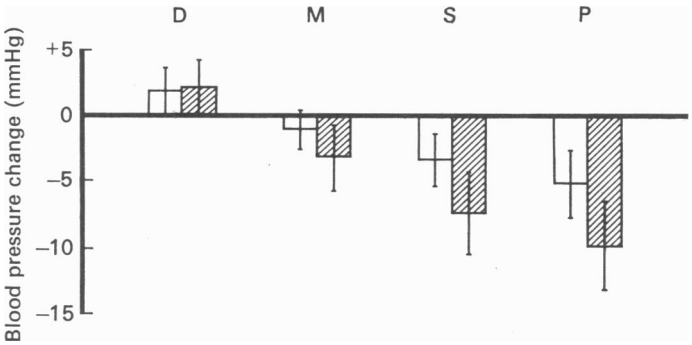

Fig. 4. Differences in mean values for diastolic (D), mean (M), systolic (S) and pulse pressures (P) between control periods and succeeding periods of l.b.n.p. Open rectangles: l.b.n.p. < 20 mmHg; filled rectangles: l.b.n.p. ⩾ 20 mmHg. Vertical bars denote ± 1 S.D.

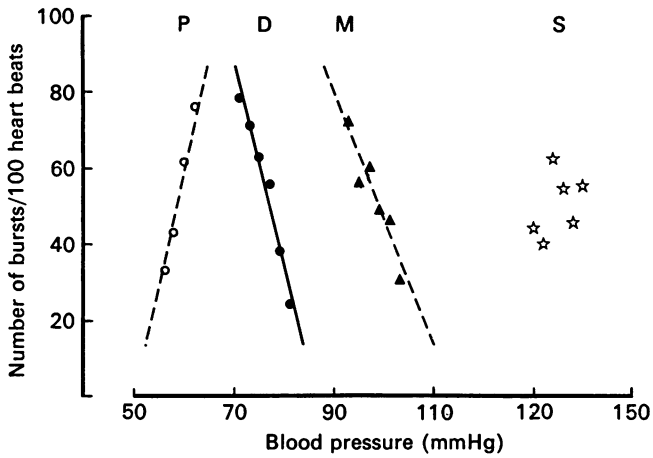

Fig. 5. The occurrence of sympathetic bursts in relation to pulse pressure (P), diastolic (D), mean (M) and systolic (S) pressures. Analyses made from one 3 min control period. Correlation coefficients for linear regression: +0·87, −0·97, −0·91 and +0·05 respectively.

The relationship between changes of MSA and blood pressure during l.b.n.p.

Sundlöf & Wallin (1978) recently showed that if different blood pressure parameters of individual heart beats during a 3 min rest period were correlated to the probability of occurrence of a sympathetic burst (by means of 'threshold variability diagrams') there was regularly a close negative correlation to diastolic pressure, a low correlation to systolic and an intermediate correlation to mean blood pressure. They also found a positive correlation to pulse pressure. In the present work the same type of correlations were made and the results were similar to those of Sundlöf and Wallin both for control periods and for periods of l.b.n.p. Fig. 5 shows examples of the resulting threshold variability diagrams from one 3 min control period for pulse,

diastolic, mean and systolic pressures. Mean values for correlation coefficients of the regression lines calculated for a total of thirty-eight control periods were −0·85 for diastolic, −0·51 for systolic, −0·80 for mean and +0·87 for pulse pressure. The same calculations for twenty-two periods of l.b.n.p. gave similar relationships

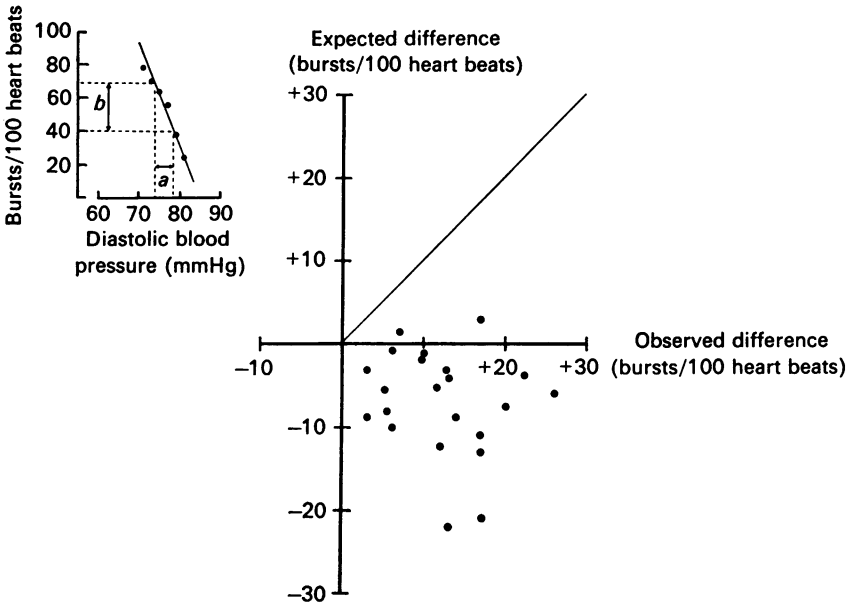

Fig. 6. Expected *vs.* observed differences in burst incidence between control periods and periods of l.b.n.p. Line at 45° denotes line of identity. Threshold variability diagram shown in upper left corner illustrates method of calculating expected difference in burst incidence (*b*) from observed difference in mean diastolic pressure (*a*) between control periods and periods of l.b.n.p.

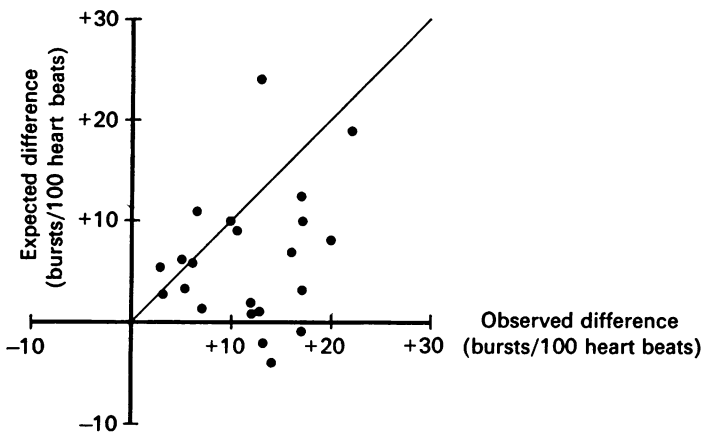

Fig. 7. Expected *vs.* observed differences in burst incidence between control periods and periods of l.b.n.p. Method of calculating expected differences in burst incidence analogous to that illustrated in Fig. 6 but based on threshold variability diagram for mean blood pressure and observed differences in mean blood pressure. Line at 45° denotes line of identity.

(r_{mean} = −0·85 for diastolic, −0·51 for systolic, −0·78 for mean and +0·66 for pulse pressure).

To test whether the observed small blood pressure changes during l.b.n.p. could explain the changes in MSA, a comparison was made between each period of l.b.n.p. and the preceding control period with respect to sympathetic burst incidence and blood pressure. By entering the observed blood pressure differences in the threshold variability diagram for the control period (*a* in the insert in the upper left corner of Fig. 6), an expected difference (*b*) in burst incidence was calculated and 'expected' and 'observed' differences in burst incidence were then plotted against each other. As shown for diastolic blood pressure in Fig. 6 the observed differences in burst incidence were always much greater than the expected (in most cases even of opposite sign), implying that l.b.n.p. caused increases in MSA which could not be explained by changes in diastolic pressure. Although the occurrence of sympathetic bursts probably is determined mainly by the diastolic pressure variations (cf. Sundlöf & Wallin, 1978) corresponding tests were performed for all pressure parameters and none of them could explain the increase in MSA. For systolic pressure there was regularly a poor or no correlation with changes in MSA (cf. Fig. 5) and both for pulse and mean pressures the observed changes in MSA were significantly greater than the expected. For mean pressure, the results of the analysis are shown in Fig. 7 ($P < 0·01$, Student's *t* test).

DISCUSSION

Peripheral pooling of blood by means of lower body negative pressure is a well-documented technique for making blood volume displacement studies, and previous haemodynamic findings (see Wolthuis *et al.* 1974) have suggested that a reduction of the central blood volume increases the sympathetic outflow to the vascular bed of skeletal muscle. The increase in MSA during l.b.n.p. seen in the present study provides direct confirmation of these previous findings. Since both the number of sympathetic bursts and their amplitude increased and since the general pattern of the changes in activity associated with l.b.n.p. were reproducible the increase in MSA cannot be explained as an artifact due to a change in the position of the recording electrode. The recordings were made in the median nerve only and consequently the present experiments do not reveal whether the observed changes of activity during l.b.n.p. were representative for other muscle nerves as well. However, Sundlöf & Wallin (1977*a*) recently showed that in resting subjects sympathetic neurones in different muscle nerves are subjected to a fairly homogenous central drive and it seems unlikely that circumstances would be different during l.b.n.p.

In accordance with previous haemodynamic findings (Zoller *et al.* 1972; Johnson *et al.* 1974), there was a tendency for a greater increase in nerve activity with higher levels of l.b.n.p. The wide scatter between the experimental points seen in Fig. 2 is not surprising since a given level of l.b.n.p. may cause different changes in central blood volume (i.e. different degrees of receptor stimulation) on different occasions and in different subjects. Irrespective of whether the increase in MSA was great or small, however, it appeared to be constant during the whole 3 min period of l.b.n.p. and consequently the results give no indication that the responsible reflex mechanism adapted during this period of time.

Underlying reflex mechanisms

The general character of the sympathetic outflow was similar during control periods and during l.b.n.p. and in both situations the transient variations in strength of the nerve activity appeared to be determined mainly by diastolic blood pressure fluctuations (cf. Sundlöf & Wallin, 1978). Consequently the present results indicate that arterial baroreflex modulation of the sympathetic outflow is maintained during l.b.n.p. However, since l.b.n.p. caused an increase of MSA which could not be explained by a change in arterial baroreceptor stimulation, a reflex influence from other receptors must have occurred. The location of these receptors is not indicated by the present experiments but it seems likely that intrathoracic 'low pressure' (volume) receptors are involved (Zoller *et al.* 1972; Johnson *et al.* 1974; Abboud, Mark, Heistad, Eckberg & Schmid, 1975; Bevegård, Castenfors, Lindblad & Tranesjö, 1977).

Part of the results have been reported at the VIIth European Congress of Cardiology, Amsterdam, Abstract book I, p. 159 (1976).

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