The Effect of Chronic Anti-Hypertensive Therapy with Bendroflumethiazide on Sympathetic Drive

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Abstract: Essential hypertension (EHT) and sympathetic activation are recognized as independent cardiovascular risk factors. The effects of several chronic anti-hypertensive therapies on efferent sympathetic nerve activity have been studied previously. Thiazide diuretics are often recommended therapy, either first line, or in combination, although we know little of their effects in this regard. Therefore, this study was designed in patients with untreated EHT to quantify any effect of bendroflumethiazide (BFZ) therapy on muscle sympathetic nerve activity (MSNA).

We examined 11 EHT patients before and after 3 ± 0.5 months of oral BFZ therapy (EHT + BFZ) in comparison to 11 age, gender and body weight matched group of patients with mild EHT who were followed up over a similar period of time with no anti-hypertensive therapy (EHT-N). MSNA was quantified as the mean frequency of single units (s-MSNA) and as multiunit bursts (MSNA bursts) using the technique of microneurography. BFZ significantly (at least $P < 0.03$) increased MSNA by 6.0 ± 2.3 bursts/100 cardiac beats and s-MSNA by 9.0 ± 3.2 impulses/100 cardiac beats from 57 ± 2.3 bursts/100 cardiac beats and 70 ± 3.6 impulses/100 cardiac beats respectively. The increase of indices of sympathetic nerve activity in EHT+ BFZ group amounted to at least 9.4 ± 4.6% of baseline values; in contrast, no significant changes occurred over the same time in EHT-N group.

These findings of a significant increase in central sympathetic neural drive to the periphery with bendroflumethiazide therapy have implications for the planning of anti-hypertensive therapy if the aim is to reduce the cardiovascular risk associated with sympathetic hyperactivity.

Keywords: anti-hypertensive drugs, sympathetic nervous system, hypertension, thiazide diuretics

Introduction

Sympathetic activation is known to occur in essential hypertension (EHT) and both of these are independently associated with future cardiovascular complications and risk (Grassi, 1998a; Julius, 1998; Mancia et al. 1999; Esler, 2000). Thiazide diuretics are widely recommended for the treatment of hypertension, either alone or in combination with other chronic anti-hypertensive therapeutic agents (Chobanian et al. 2003; WHO, 2003; Williams et al. 2004). The effects of many therapies including beta-blocking agents, angiotensin converting enzyme inhibitors, angiotensin receptor blockers and calcium channel inhibitors on sympathetic activity assessed by plasma norepinepherine levels (Grassi et al. 1998c; Fu et al. 2005), its spillover rate (Rongen et al. 1998), and efferent sympathetic nerve activity (Grassi et al. 1998c; Binggeli et al. 2002; Grassi et al. 2003a; Grassi et al. 2003b; Burns et al. 2004; Fu et al. 2005), have been previously studied in uncomplicated EHT (Grassi et al. 1998c; Rongen et al. 1998; Binggeli et al. 2002; Grassi et al. 2003a; Grassi et al. 2003b; Burns et al. 2004; Fu et al. 2005). These findings assume relevance as reducing sympathetic nerve activity and its effects are known to contribute to the therapeutic regression of left ventricular hypertrophy (Burns et al. 2007). Also, regression of left ventricular hypertrophy is known to reduce the risk of subsequent development of cardiovascular disease in EHT (Verdecchia et al. 2003).

However, despite their extensive use, the consequences of thiazide therapy on directly measured central sympathetic nerve activity supplying the periphery have not been previously examined. There have been reports on the effect of hydrochlorthiazide therapy on the sympathetic drive in patients with

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EHT, though these used indirect assessments through the measurement of plasma norepinephrine levels (Fernandez et al. 1987; Giles et al. 1987; Koenig et al. 1991). The findings were inconsistent with hydrochlorthiazide therapy resulting in either an increase in plasma norepinephrine levels (Fernandez et al. 1987; Giles et al. 1987) or no change (Koenig et al. 1991). This inconsistency however may be explained by the wide variability (Grassi et al. 1997) in assessing the plasma level of catecholamines and the multitude of confounding factors (Esler et al. 1990) that can affect this measurement. Indeed, studies in patients with hypertension and other conditions have reported that plasma norepinepherine levels remain normal despite an increase in sympathetic nerve activity (Grassi et al. 1997; Grassi and Esler, 1999).

This study was designed to determine whether bendroflumethiazide (BFZ) therapy affected central sympathetic nerve activity quantified directly by peroneal microneurography. We compared one group of patients with untreated, uncomplicated mild to moderate essential hypertension (EHT + BFZ), studied before and after 3 months BFZ therapy, with a control group of EHT patients whose pressure elevation was at levels at which the attending physician thought it unnecessary to start drug treatment and who were followed up without therapy for a similar time period (EHT-N).

Methods

Subjects

We examined 22 patients with EHT on two occasions; 11 patients before and after BFZ (EHT + BFZ), and 11 patients with no such therapy (EHT-N). All individuals had similar occupational status and were screened by history, physical and laboratory examination. None had any evidence of secondary hypertension, left ventricular hypertrophy, peripheral vascular disease, arrhythmia, neuropathy or chronic disease that may influence the autonomic nervous system. Arterial pressure was defined on the basis of the average of at least 3 sphygmomanometer readings, taken on separate occasions spanning the morning and afternoon, using a standard measurement technique (Chobian et al. 2003; WHO, 2003). The presence of hypertension was accepted if the systolic or diastolic arterial pressures were $\geq$140 mmHg or $\geq$90 mmHg respectively (Chobanian et al. 2003; WHO, 2003). The EHT-N group was made up of patients in whom the attending physician wished to defer treatment pending review, for example, because of apparently mild and intermittent levels of raised arterial pressure. The investigation was carried out with the approval of St. James's University Hospital Ethics Committee, and all subjects provided informed written consent.

Study design

The patients in both groups were matched during recruitment according to gender, age, body weight, body mass index and heart rate. Given that deferment of therapy was a clinically based decision, matching did not include arterial pressure levels. It was considered inappropriate on ethical grounds to use a placebo group with matched arterial blood pressure as a control, as this would have entailed a delay in commencement of clinically indicated oral anti-hypertensive therapy. The patients in the EHT + BFZ group were chosen on the basis of having mild-to-moderate hypertension to justify using BFZ monotherapy in doses of 2.5 mg per day, given in the morning. This dose of BFZ has been reported to reduce arterial pressure without incurring significant metabolic or hormonal changes (Harper et al. 1995; Wiggam et al. 1999; Haenni et al. 2002) which is of particular relevance to this study, as increased plasma insulin levels and insulin resistance are known to increase muscle sympathetic nerve activity (Huggett et al. 2005). Both groups of patients were examined twice, 3 months apart, using an identical protocol.

General protocol

Microneurographic and hemodynamic measurements were obtained in an identical manner during each session, as we have previously reported in detail (Greenwood et al. 1999; Greenwood et al. 2000; Burns et al. 2004). All investigations were performed under similar conditions between the hours of 09:00 and 12:00. Patients were asked to have had a light breakfast, and to empty their bladder before commencing the study. They were instructed to maintain a normal dietary intake of sodium, and to avoid products containing nicotine or caffeine for 12 hours, as well as alcohol

and strenuous exercise for 24 hours prior to investigation. During each session, the subjects were studied in the semi-supine position and when the measurements had attained a steady state for at least 30 minutes. Recordings were made in a darkened laboratory in which the temperature was constant between 22 and 24 °C. Resting blood pressure was measured from the arm, using a mercury sphygmomanometer. Changes in heart rate and arterial pressure were monitored and recorded, using a standard electrocardiogram and a Finometer device (FMS, Arnhem, The Netherlands, TPD Biomedical Instruments).

Microneurography

Post-ganglionic muscle sympathetic nerve activity (MSNA) was recorded from the right peroneal nerve, simultaneously with the other data as previously described (Greenwood et al. 1999; Greenwood et al. 2000; Burns et al. 2004). The neural signal was amplified (x50,000) and either filtered (bandwidth of 700–2000 Hz) and integrated (time constant 0.1 sec) for the purpose of generating bursts representing multiunit discharge, or left intact to examine raw action potentials. The output of action potentials and bursts from this assembly was passed to a PC-based data-acquisition system (LabView, National Instruments Corp, Austin, TX, United States), which digitised the acquired data at 12,000 samples/second (16 bits).

MSNA was differentiated from skin sympathetic activity and afferent activity by previously accepted criteria (Valbo et al. 1979; Macefield et al. 1994). Single units (s-MSNA) in the raw action potential neurogram were obtained by adjusting the electrode position, whilst using fast monitor sweep, and an on-line storage oscilloscope to confirm the presence of a consistent action potential morphology, as previously described (Macefield et al. 1994; Greenwood et al. 1999; Macefield et al. 1999). Only vasoconstrictor units were accepted and examined, the criteria of acceptance being appropriate responses to spontaneous changes in arterial pressure during verification by a preliminary Valsalva maneuver and isometric handgrip exercise. During the Valsalva maneuver, sympathetic activity increased in the latter part of phase-II and/or phase-III and decreased during phase-IV (corresponding to the decrease and increase in arterial pressure). During the isometric handgrip exercise, performed using a dynamometer (MIE Medical Research Ltd, Leeds, U.K.), a delayed increase in sympathetic nerve activity was observed. In addition, simultaneous measurement of calf vascular resistance (CVR) by venous occlusion plethysmography (DE Hokanson Inc, Bellevue, WA, U.S.A.) confirmed the vasoconstrictor function of the observed neural activity.

Analysis was performed independently off-line, using dedicated software based on the LabView system (National Instruments Corp, Austin, TX, U.S.A.). An electronic discriminator window was used objectively to count s-MSNA spikes with consistent morphology and a threshold discriminator was used to count the R-waves of the electrocardiogram. The mean frequency of s-MSNA was quantified over one minute, and also over 100 cardiac cycles in order to avoid any interference caused by variation in the length of the cardiac cycle (Sundlöf and Wallin, 1977). The bursts of MSNA were identified by inspection when the signal-to-noise ratio was greater than three, and were counted and quantified in a similar manner to s-MSNA. The variability of repeated measurements of two minute segments of recordings of s-MSNA units and MSNA bursts spanning a period of 30 minutes or those of two impalements performed within 60 minutes did not exceed 10%, in terms of twice the 95% confidence intervals around individual differences relative to the mean of the repeated measurements (Greenwood et al. 1999).

Statistics

Unpaired Student *t* tests were used to assess differences between the two groups of data, and paired Student *t* tests were used to assess changes over time within the same group. The least square technique was used to assess the linear relationship between variables. Values of $P < 0.05$ were considered statistically significant. All data are presented as mean ± SEM.

Results

The details of the two groups at baseline are shown in Table 1. The two groups were matched in respect to gender, age, body weight, body mass index and heart rate. As anticipated from the study design, the EHT-N group had lower arterial pressure and

Table 1. Group characteristics and baseline data.

Patients	EHT+BFZ	EHT-N	*P* value
Number (males)	11 (7)	11 (7)	–
Age (years)	51 ± 2.7	50 ± 2.7	–
Body Weight (kg)	80 ± 4.6	80 ± 4.5	–
Body mass index (kg/m^2)	28 ± 1.1	28 ± 0.9	–
Heart rate (beats/minute)	70 ± 3.5	67 ± 2.6	–
Arterial pressure (mmHg)			
Systolic	158 ± 2.5	137 ± 8.0	<0.02
Diastolic	93 ± 2.1	86 ± 3.4	ns
Mean	114 ± 2.1	103 ± 4.8	<0.03
MSNA (bursts/minute)	41 ± 2.2	33 ± 3.1	<0.02
MSNA (bursts/100 beats)	57 ± 2.3	50 ± 4.4	ns
s-MSNA (impulses/minute)	51 ± 4.7	38 ± 3.7	<0.02
s-MSNA (impulses/100 beats)	70 ± 3.6	57 ± 5.1	<0.03

Groups shown are those assigned to bendroflumethiazide therapy (EHT + BFZ) and those who were assigned to control (EHT-N). MSNA, muscle sympathetic nerve activity. Data are mean ± SEM. *P* values refer to unpaired *t* tests; ns denotes $P \geq 0.05$.

sympathetic nerve activity indices than the EHT + BFZ group.

The changes following BFZ therapy are shown in Table 2. BFZ significantly reduced mean arterial pressure by 7.6 ± 1.2% and increased all indices of sympathetic nerve activity in the EHT + BFZ group by at least 9.4 ± 4.6%. The proportions of increases in indices of sympathetic nerve activity and decreases of indices of arterial blood pressure relative to baseline values also attained statistical significance (at least $P < 0.02$). These significant changes were not accompanied by significant alterations in body weight, body mass index or heart rate. Following BFZ therapy there were no statistically significant correlations between indices of sympathetic nerve activity or their changes relative to baseline values and those of arterial pressure.

The data following no BFZ therapy in the EHT-N group are shown in Table 3. None of these data showed significant changes. There were no statistically significant correlations between

Table 2. Changes in the 11 patients given bendroflumethiazide therapy (EHT + BFZ).

Patients	Baseline	Follow up	Change	*P* value
Body weight (kg)	80 ± 4.6	80 ± 4.9	–0.8 ± 0.56	ns
Body mass index (kg/m^2)	28 ± 1.1	28 ± 1.1	–0.28 ± 0.21	ns
Heart rate (beats/minute)	70 ± 3.5	72 ± 3.6	1.8 ± 1.9	ns
Arterial pressure (mmHg)				
Systolic	158 ± 2.5	141 ± 3.5	–16 ± 2.7	<0.0002
Diastolic	93 ± 2.1	86 ± 2.1	–6 ± 1.3	<0.009
Mean	114 ± 2.1	105 ± 2.1	–9 ± 1.4	<0.0007
MSNA (bursts/minute)	41 ± 2.2	45 ± 3.0	4 ± 1.8	<0.04
MSNA (bursts/100 beats)	57 ± 2.3	63 ± 3.1	6 ± 2.3	<0.03
s-MSNA (impulses/minute)	51 ± 4.7	57 ± 6.1	6 ± 2.4	<0.03
s-MSNA (impulses/100 beats)	70 ± 3.6	79 ± 6.4	9 ± 3.2	<0.01

Data are mean ± SEM. *P* values refer to paired *t* tests.

Table 3. Changes in the 11 patients followed up without therapy (EHT-N).

Patients	Baseline	Follow up	Change	*P* value
Body Weight (kg)	80 ± 4.5	80 ± 4.3	–0.1 ± 0.4	ns
Body mass index (kg/m^2)	28 ± 0.9	28 ± 0.8	0.2 ± 0.18	ns
Heart rate (beats/minute)	67 ± 2.6	65 ± 3.1	–0.6 ± 1.0	ns
Arterial pressure (mmHg)				
Systolic	137 ± 8.0	137 ± 7.9	0.6 ± 0.9	ns
Diastolic	86 ± 3.4	86 ± 3.4	–0.4 ± 1.1	ns
Mean	103 ± 4.8	103 ± 4.9	0.3 ± 0.9	ns
MSNA (bursts/minute)	33 ± 3.1	33 ± 3.2	0.4 ± 0.3	ns
MSNA (bursts/100 beats)	50 ± 4.4	50 ± 4.0	0.2 ± 0.7	ns
s-MSNA (impulses/minute)	38 ± 3.7	38 ± 3.6	0.6 ± 0.5	ns
s-MSNA (impulses/100 beats)	57 ± 5.1	58 ± 4.4	0.9 ± 1.1	ns

Data are mean ± SEM. *P* values refer to paired *t* tests.

indices of sympathetic nerve activity or their alterations relative to baseline and indices of arterial pressure over the time of follow up without BFZ therapy.

By comparing absolute changes (or their proportion relative to baseline) following BFZ therapy in the EHT + BFZ group to those in the EHT-N group, only the decreases in indices of arterial pressure (at least $P < 0.004$) and increases in those of sympathetic nerve activity (at least $P < 0.03$) attained statistical significance.

Discussion

We have demonstrated for the first time that chronic bendroflumethiazide therapy augmented central sympathetic neural drive in hypertensive patients. This sympathetic augmentation was not related to the extent of the concomitant BFZ-induced reduction in arterial blood pressure.

Confounding factors known to affect sympathetic activity (age, gender, body weight, dietary intake, visceral distension, race, and diurnal variation) were avoided by the matched study design, with both groups consisting of Caucasian patients examined using the same protocol under identical laboratory conditions (Anderson et al. 1989; Fagius and Karhuvaara, 1989; Calhoun et al. 1993; Ng et al. 1993; Scherrer et al. 1994; Cox et al. 1995). In addition, the design of the investigation avoided differences in heart rate, as the latter is known to affect sympathetic nerve activity in a complex fashion (Sundlöf and Wallin, 1977; Sundlöf and Wallin, 1978).

Limitations to our investigation arose from the ethical constraints of conducting a placebo-controlled study in patients with hypertension. Thus, the EHT + BFZ group had greater arterial pressure and sympathetic activity indices than EHT-N group at baseline. However, the design of the investigation included analyzing not only the absolute changes in both groups but also proportions of change relative to baseline values. The longitudinal follow-up of the present study has shown that the absolute and proportional changes of MSNA relative to baseline were different between the two groups. An increase in sympathetic nerve activity and a decrease in arterial pressure occurred only in the EHT + BFZ group. These significant changes were obtained in the context of the sympathetic nerve activity in the EHT-N group not exceeding the variability between repeated measurements shown previously in our laboratory (Greenwood et al. 1999).

Potential mechanisms underlying the BFZ induced increase in sympathetic nerve activity could include concomitant changes in central or reflex control of sympathetic drive and in metabolic or hormonal profiles such as changes in insulin levels and the renin-angiotensin system. It is not feasible to examine isolated changes in, or interaction between these factors in humans. A standard low dose of BFZ typically used to treat hypertension was used and this would not be expected to

cause metabolic or hormonal changes. The inevitable reduction in arterial pressure in the EHT-BFZ group integral to the design of our investigation would not be expected by itself to explain the increase in sympathetic nerve activity. In hypertensive patients it is well established that arterial pressure levels are neither linearly related to sympathetic nerve activity (Greenwood et al. 1999), nor do they significantly affect the gain of baroreceptor reflex control of this activity (Grassi et al. 1998). Consistent with this, and as confirmed in the present investigation, are reports showing that the blood pressure lowering effect of anti-hypertensive agents is not always associated with an increase in muscle sympathetic nerve activity in patients with EHT (Grassi et al. 1998c; Grassi et al. 2003b; Binggeli et al. 2002; Struck et al. 2002; Burns et al. 2004). Such lack of association has also been reported in obese hypertensive patients with metabolic abnormalities (Grassi et al. 2003a). In our study we found no relationship between the magnitude of decrease in arterial pressure and increase in sympathetic nerve activity, or proportionate change relative to baseline.

Our results may have implications regarding the choice of anti-hypertensive therapy in patients with essential hypertension, particularly when used in combination regimens. In hypertensive patients it has already been found that muscle sympathetic nerve activity was not affected by angiotensin converting enzyme inhibitors (Grassi et al. 1998c), was reduced (Struck et al. 2002), or remained unchanged by angiotensin receptor blockers (Struck et al. 2002; Grassi et al. 2003a) or beta blocking agents (Wallin et al. 1984; Burns et al. 2004) and was increased (Struck et al. 2002; Grassi et al. 2003b), or unchanged by calcium channel inhibitors (Binggeli et al. 2002; Grassi et al. 2003b). During the present investigation only three months of therapy were used and therefore other studies are necessary to establish whether or not such sympathetic activation persist over longer periods of treatment. However, our findings do add new information on thiazide diuretic therapy indicating that it can increase sympathetic nerve activity. It is of interest that the use of the angiotensin receptor blocker losartan in combination with hydrochlorothiazide, was also found to increase sympathetic nerve activity (Fu et al. 2005), indicating that adding thiazide therapy to other anti-hypertensive agents may augment sympathetic neural drive. It is clear however, that whilst all of these therapeutic agents have been shown to reduce levels of raised arterial pressure, their effect on sympathetic nerve activity is far from uniform. Within pharmacological classes of chronic hypertensive therapy it is unclear if there are differences on sympathetic activity.

Conclusion

Essential hypertension is known to be a state of chronic sympathetic activation, and both are independently associated with future cardiovascular complications such as left ventricular hypertrophy. Furthermore, reducing the level of sympathetic activation and its effects is known to contribute to the therapeutic regression of left ventricular hypertrophy. We have found that antihypertensive monotherapy with a thiazide diuretic to lower arterial pressure augmented the already existing sympathetic nerve activation by at least 9%. Given that thiazide diuretics are widely recommended for the treatment of hypertension, either alone or in combination with other chronic anti-hypertensive therapeutic agents, our findings have potential relevance with regard to strategies for blood pressure lowering. Given the additional burden of cardiovascular risk complicit with sympathetic activation in hypertension, antihypertensive strategies may need to be re-assessed with a view to minimizing this risk. As to the clinical significance of our findings this clearly needs further investigation including any potential difference between pharmacological classes of chronic anti-hypertensive therapy.

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Conflicts of Interest

None.

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