The influence of aging on the human sympathetic nervous system and brain norepinephrine turnover

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Esler, Murray, Jacqueline Hastings, Gavin Lambert, David Kaye, Garry Jennings, and Douglas R. Seals. The influence of aging on the human sympathetic nervous system and brain norepinephrine turnover. *Am J Physiol Regulatory Integrative Comp Physiol* 282: R909–R916, 2002; 10.1152/ajpregu.00335.2001.—Investigating aging effects on the sympathetic nervous system and ascertaining underlying central nervous system (CNS) mechanisms mediating sympathetic stimulation is clinically pertinent because of the possible interconnection of cardiovascular disease development with age-dependent sympathetic nervous changes. Because of previous evidence linking human CNS neuronal noradrenergic function and sympathetic activity, we investigated the influence of aging on brain norepinephrine turnover in 22 healthy men aged 20–30 yr and 16 healthy men aged 60–75 yr by measuring the internal jugular venous overflow of norepinephrine and its lipophilic metabolites. Sympathoneural and adrenal medullary function was also studied, using plasma catecholamine isotope dilution methodology and regional central venous sampling. In the older men there was increased norepinephrine turnover in suprabulbar subcortical brain regions, 317 ± 50 ng/min compared with 107 ± 18 ng/min in younger men. A differentiated sympathetic nervous activation was also present in older men. Overall, levels of both cardiac and hepatomesenteric norepinephrine spillover were directly correlated with subcortical norepinephrine turnover. These findings suggest that in sympathetic nervous activation accompanying aging, as has previously been demonstrated with the sympathetic nervous stimulation in human hypertension and heart failure, there is an underlying sympathoexcitatory influence of noradrenergic projections to suprabulbar subcortical regions.

epinephrine; heart; kidneys; adrenal medulla

THE FUNCTION OF THE HUMAN sympathetic nervous system is altered in important ways by aging. These changes involve both the properties of the adrenergic receptors and the outflow of sympathetic neural traffic to individual organs (32). Based in large part on subcutaneous multiunit microneurographic recording from sympathetic fibers distributed in the company of motor nerves (1, 28, 37) and on the measurement of the spillover of the sympathetic neurotransmitter norepi-nephrine to plasma (14, 15, 36), there is now unequivocal evidence that progressive sympathetic activation occurs with aging. This sympathetic stimulation appears to involve the sympathetic outflow to the heart, the skeletal muscle vasculature, and the gut and liver, but to exclude the kidneys (14, 15). The nature of the underlying disturbance in central nervous system (CNS) sympathetic control, however, remains unknown.

The impetus for seeking to better understand the central nervous mechanisms by which aging causes sympathetic activation has come in part from recognition that in a variety of cardiovascular disorders, including cardiac failure, essential hypertension, and ventricular arrhythmias, for all of which incidence rises with age, the sympathetic nervous system is causally involved (11). Antiadrenergic drugs have come to occupy an important place in their treatment. Further analysis of both the effects of aging on sympathetic nervous system function and the CNS mechanisms mediating sympathetic stimulation becomes pertinent in this context of a possible interconnection of cardiovascular disease development with age-dependent changes in sympathetic nervous function.

In several clinical contexts, most notably cardiac failure and essential hypertension, we previously demonstrated the importance of projections of noradrenergic neurons to suprabulbar subcortical areas in generating the peripheral sympathetic nervous stimulation present (16, 22, 23). This contrasted with earlier experimental evidence that had documented a sympathetic suppressant rather than excitatory effect of noradrenergic neurons in the medulla oblongata mediated by the arterial baroreflex (4). In some clinical settings characterized by sympathetic nervous activation, most notably obesity, the central mechanism mediating sympathetic nervous activation also appears not to be noradrenergic. In human obesity, brain serotonin turnover is increased but norepinephrine turnover is normal (26).

The links that have been previously demonstrated between human suprabulbar subcortical human nor-

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epinephrine turnover and sympathetic activity were established by measuring CNS norepinephrine turnover, based on the overflow of norepinephrine and its lipophilic metabolites 3-methoxy-4-hydroxyphenylglycol (MHPG) and dihydroxyphenylglycol (DHPG) into the internal jugular veins (16, 22, 23) in parallel with peripheral sympathetic activity, quantified by isotope dilution measurements of whole body and regional norepinephrine spillover to the circulation. In the present paper we apply these methods to investigate the sympathoneural changes accompanying healthy aging and the possible mediating CNS mechanisms.

MATERIALS AND METHODS

Experimental subjects. Twenty-two men aged 20–30 yr and 16 aged 60–75 yr participated as research volunteers in the study. All were recruited from the general community by advertisement and found to be in good health. A comprehensive clinical evaluation was performed by a specialist physician in all experimental subjects, and testing included a chest X ray, hematology and multipanel serum biochemistry testing. All recruited volunteers were nonsmokers. None were obese; mean body mass index was 24.0 kg/m² in the younger men and 24.9 kg/m² in the older men. All subjects gave written informed consent for their participation in the study, which was approved by the Alfred Hospital Ethics Review Committee.

General procedure. All subjects were studied at rest in the supine position 2 h after eating a standardized light breakfast (16, 22). Tea, coffee, and alcohol were withheld for a minimum of 12 h before the study. Total body sympathetic function was assessed utilizing the principle of plasma norepinephrine isotope dilution (10). The overflow of norepinephrine and its metabolites from the brain into one or both internal jugular veins was also measured, to measure cerebral norepinephrine turnover (6, 22, 23). For this purpose blood samples for plasma catechol assay were obtained from a central venous catheter and a brachial arterial cannula. These were percutaneously inserted under local anesthesia. The central venous catheter, a 7-F coronary sinus thermodilution catheter (Webster Laboratories, type CCS-7U-90B), introduced via an antecubital venous sheath, was placed with fluoroscopic control high up in one or both internal jugular veins beyond the angle of the jaw to exclude sampling from the venous drainage of the tissues of neck and face (16, 22, 23). Jugular venous sampling was bilateral in seven younger and in five older men.

The central venous catheter was repositioned under fluoroscopic control to also allow blood sampling from the coronary sinus (25 subjects), the right hepatic vein (17 subjects), and the right renal vein (19 subjects). In each case the catheter position was verified with 2 ml radiopaque contrast medium (Omnipaque, Winthrop Pharmaceuticals). The right renal vein was used for renal sampling to avoid the contamination from adrenal venous drainage that occurs on the left (10). Arterial blood samples were obtained from the brachial artery simultaneously with venous sampling, using a percutaneously placed 21-gauge cannula. Throughout the catheter study tritiated norepinephrine was infused for the determination of plasma norepinephrine kinetics (10). Measurements of the spillover of norepinephrine to plasma from the heart, hepatomesenteric circulation, and kidneys, and from the body as a whole were used to estimate organ-specific and overall sympathetic nervous activity. The relationship that exists between the rate of sympathetic nerve firing in an organ and release of norepinephrine into its venous drainage provides the experimental justification for the use of transmitter spillover measurements in the study of sympathetic nervous function (10).

Measurement of brain norepinephrine turnover. The estimate of brain norepinephrine turnover was based on the Fick principle, utilizing measurements of venoarterial plasma concentration differences for norepinephrine and the lipophilic norepinephrine metabolites MHPG and DHPG across the brain, with arterial and internal jugular venous sampling. The associated internal jugular blood and plasma flows were measured by thermodilution, with appropriate adjustment for the hematocrit (16, 22). Cerebral norepinephrine spillover was calculated using isotope dilution in plasma, whereas for DHPG and MHPG blood flow rather than plasma flow was used in the calculations, based on results showing that DHPG and MHPG added to whole blood are rapidly and equally distributed between plasma and the red cell compartment (25).

Brain norepinephrine turnover, as calculated, represented the combined jugular venous overflow of norepinephrine, DHPG, and MHPG (16, 22, 23). This overflow of norepinephrine, DHPG, and MHPG into the cerebrovascular circulation is thought to be primarily derived from the noradrenergic neurons of the brain rather than from the sympathetic innervation of the cerebral arterial blood vessels. The evidence justifying this viewpoint is the finding that ganglionic blockade with trimethaphan does not reduce calculated cerebral norepinephrine turnover, whereas in patients with pure autonomic failure, who have sympathetic nerve degeneration, transcerebral release of norepinephrine is undiminished (24).

Cerebral venous sinus scan. Differentiating the pattern of cerebral drainage into the internal jugular veins was of crucial importance in the present study in allowing selective measurement of suprabulbar subcortical norepinephrine turnover. Using a technetium-99 cerebral venous sinus scan to delineate the pattern of venous drainage in individual subjects, one can distinguish subcortical and cortical neurotransmitter turnover (Fig. 1) (16, 22). The more common pattern is for the right internal jugular vein to have the superior sagittal sinus as its major tributary and the cerebral cortex as its predominant field of drainage; here, suprabulbar subcortical venous drainage from regions such as the hypothalamus and amygdala is into the left internal jugular vein. Venous drainage from the medulla oblongata is primarily into the veins of the spinal cord and dural venous sinuses, not the internal jugular veins (18). Sometimes the venous sinus drainage pattern is reversed, with cortical venous drainage being into the left internal jugular vein. In a minority of cases, the drainage pattern is nonlateralizing, with ready admixture of blood occurring at the confluence of the sagittal and straight sinuses.

The results for CNS norepinephrine turnover reported here have been confined to subjects in whom a technically satisfactory venous sinus scan was done, because otherwise interpretation of the norepinephrine metabolite overflows from the brain is difficult (16, 22). This was achieved in the 16 men aged 60–75 yr and the 22 men aged 20–35 yr whose results form the body of this report. In these 38 men, cortical venous drainage was predominantly to the right internal jugular vein in 14 and to the left internal jugular vein in 13, a somewhat higher proportion than usual, whereas in the remaining 11 the venous drainage pattern was nonlateralizing.
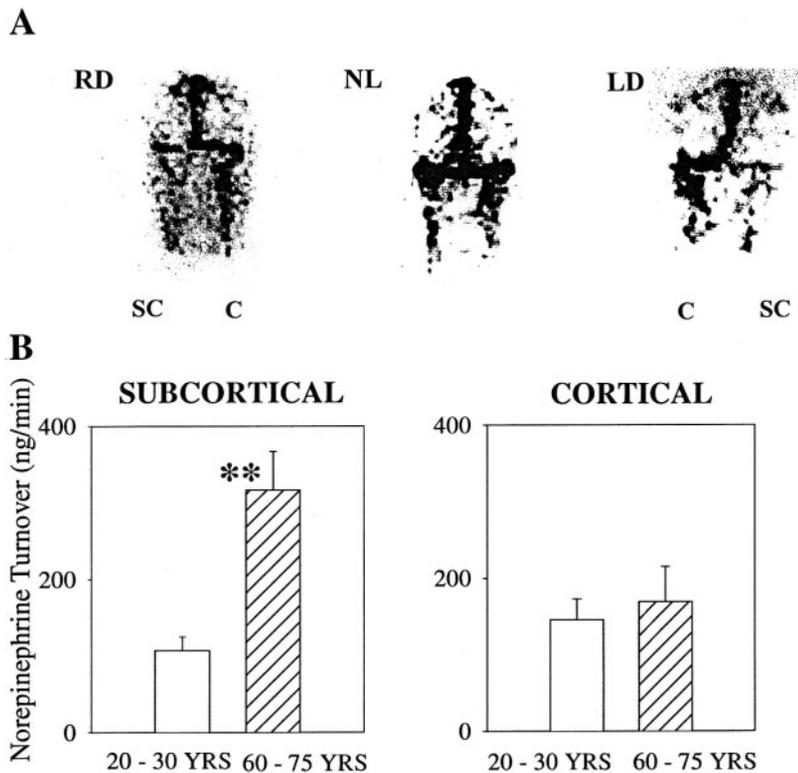
A

B

Fig. 1. *A*: patterns of cerebral venous sinus drainage demonstrated by dynamic and static single photon emission computerized tomography (SPECT) cerebral sinus technetium-99 scans representing a coronal slice through the junctions of the sagittal and transverse sinuses. Examples of internal jugular vein flow types, which allow selective sampling of cortical and subcortically derived blood, and their representatives venous sinus scans are shown. RD, right dominant (superior sagittal sinus drains to right internal jugular vein); LD, left dominant (sagittal sinus drainage to left internal jugular); NL, nonlateralizing (sagittal sinus drainage to both internal jugular veins); C, cortical drainage; SC, subcortical drainage. *B*: brain norepinephrine (NE) turnover, based on the combined overflow into the appropriate internal jugular vein of NE, dihydroxyphenylglycol (DHPG), and 3-methoxy-4-hydroxyphenylglycol (MHPG). NE turnover in subcortical areas was increased ~3-fold in older men. *$P < 0.01$

Measurement of total body norepinephrine spillover and clearance. The total body norepinephrine spillover rate and plasma norepinephrine clearance were measured using the radiotracer method developed in our laboratory (10). In brief, the method involves the continuous intravenous infusion of a tracer dose of norepinephrine (0.70 µCi/min levo-[7-^{3}H]norepinephrine, specific activity 12–20 Ci/mmol, New England Nuclear, Boston, MA) to a steady-state concentration in plasma. The total norepinephrine spillover to plasma was derived from isotope dilution, and total plasma norepinephrine clearance from the plateau plasma concentration of tracer.

Cardiac, hepatomesenteric, and renal norepinephrine spillover. The rate of norepinephrine spillover from the heart, hepatomesenteric circulation, and kidneys was calculated according to the Fick principle from regional isotope dilution and plasma flows using established methods (10)

$$\text{Organ-specific NE spillover} = [(NE_V - NE_A) + NE_A \cdot NE_{EX}]PF$$

where NE_v = plasma norepinephrine concentration in the coronary sinus or hepatic or renal vein, NE_A = arterial plasma norepinephrine concentration, NE_{EX} = the steady-state fractional extraction of plasma-tritiated norepinephrine in transit through the organ, and PF = the plasma flow (ml/min).

Coronary sinus plasma flows were derived from thermodilution-determined blood flows and the hematocrit, hepatomesenteric plasma flow was derived from the steady-state clearance and hepatic extraction of indocyanine green, and renal plasma flow was determined from the clearance and renal extraction of para-amino hippurate (10).

Measurement of the rate of epinephrine secretion. With continuous intravenous infusion of a tracer dose of epinephrine (0.5–1.0 µCi/min levo-*N*-methyl-[^{3}H]epinephrine, specific activity 55–75 Ci/mmol, New England Nuclear), the rate

of whole body epinephrine secretion was measured by isotope dilution as follows (10)

$$\text{Total Epi spillover} = \frac{[^3H]\text{Epi infusion rate (dpm/min)}}{\text{plasma Epi specific activity (dpm/pmole)}}$$

where Epi = epinephrine, dpm = disintegrations per minute of tritium-labeled epinephrine, and [^{3}H]Epi = tritium-labeled epinephrine.

Assays of catechols and MHPG. Arterial blood samples were transferred immediately to ice-chilled tubes containing an anticoagulant and antioxidant (EGTA plus glutathione). Plasma was separated by centrifugation at 4°C, and samples were subsequently stored at −70°C until assayed. Endogenous norepinephrine, epinephrine, DHPG, and MHPG plasma concentrations were measured by high performance liquid chromatography with electrochemical detection according to our previously published methods (25, 27). Fractions of the eluant leaving the electrochemical cell were collected into scintillation vials for measurement of ^{3}H-labeled norepinephrine and epinephrine by liquid scintillation spectroscopy.

Statistical methods. Data are expressed as means ± SE. Testing for normality of the data was performed with a χ^2 goodness-of-fit test and tests for skewness and kurtosis (33). Between-group comparisons were made using the Student's *t*-test for normally distributed values and the Mann-Whitney *U*-test for non-Gaussian data. The null hypothesis was rejected at $P < 0.05$.

RESULTS

Blood pressure, heart rate, and body mass index. Younger and older men had almost identical heart rates, 66 ± 3 beats/min (mean ± SE) compared with 67 ± 4 beats/min. Arterial systolic pressures were similar,

136 ± 3 mmHg in younger men and 144 ± 4 mmHg in older men (difference not statistically significant). The same applied for diastolic pressures, 76 ± 2 mmHg compared with 77 ± 3 mmHg. Mean body mass index also was similar in the two age groups, 24.0 kg/m^2 in the younger men and 24.9 kg/m^2 in the older men.

Cerebral norepinephrine turnover. CNS norepinephrine turnover in the field of an internal jugular drainage was estimated from the combined overflow of norepinephrine, MHPG, and DHPG into the internal jugular vein. Brain norepinephrine turnover in the 16 older men, based on all 21 unilateral internal jugular vein plasma samples (bilateral sampling in 5 older men), was higher than in the 22 younger men (29 unilateral jugular venous plasma samples), 231 ± 34 ng/min (mean ± SE), compared with 136 ± 19 ng/min ($P < 0.05$) (Table 1).

The information provided by the cerebral venous sinus scans indicated that this increase in brain norepinephrine turnover was confined to subcortical brain regions (Fig. 1). In the 10 older men in whom jugular venous samples were available from the subcortical brain regions, the associated brain norepinephrine turnover was 317 ± 50 ng/min, compared with 107 ± 18 ng/min in the 11 younger men in whom these jugular samples also were available ($P < 0.01$) (Table 1).

In the younger men, based on 29 unilateral internal jugular vein plasma samples, the proportionality of jugular vein overflows of norepinephrine, DHPG, and MHPG was 1.0:3.0:10.7 (with mean values for norepinephrine of 9.3 ng/min, for DHPG 27.6 ng/min, and for MHPG 99.2 ng/min). MHPG overflow contributed ~73% of the CNS turnover estimate (Table 2). In older men, based on 21 unilateral internal jugular vein plasma samples, the proportionality was similar, 1.0:4.0:9.3, with mean values for norepinephrine of 16.5 ng/min, for DHPG 65.3 ng/min, and for MHPG 153.6 ng/min, and with MHPG overflow contributing 65% of

Table 2. *Influence of aging on the relative unilateral internal JV overflow of norepinephrine, DHPG, and MHPG from the brain*

	Norepinephrine, ng/min	DHPG, ng/min	MHPG, ng/min
Age 20–30 yr	9.3 ± 2.4	27.6 ± 4.1	99.2 ± 15.5
Age 60–75 yr	16.5 ± 4.7	65.3 ± 10.8*	153.6 ± 20.9

Values are means ± SE. The proportionality of JV overflows of norepinephrine, dihydroxyphenylglycol (DHPG), and 3-methoxy-4-hydroxyphenylglycol (MHPG) from the brain in younger men, based on 29 unilateral internal JV plasma samples, was 1.0:3.0:10.7. In older men, based on 21 unilateral internal JV samples, the corresponding ratios were similar, 1.0:4.0:9.3. This finding perhaps argues against the increased overflow of norepinephrine and metabolites from the brain being due to reduced cerebral neuronal reuptake of norepinephrine, which might disproportionately increase MHPG overflow and reduce DHPG overflow (21, 25), although there is disagreement on this point (7, 8). *$P < 0.05$.

CNS turnover. The relative patterns of metabolite overflow did not differ significantly, which perhaps argues against the mechanism of increased CNS norepinephrine in the elderly being reduction in neuronal norepinephrine reuptake. There is some evidence (21, 25), although it is disputed (7, 8), that reduction in brain neuronal reuptake of norepinephrine in the elderly would be expected to reduce DHPG jugular venous overflow and increase MHPG overflow.

Whole body sympathetic activity. The mean concentration of norepinephrine in arterial plasma was 49.7% higher in the older than in the younger men, attributable to 27.5% lower clearance of norepinephrine from plasma ($P < 0.05$) and a 7.2% higher rate of whole body spillover of norepinephrine to plasma (difference not statistically significant) (Table 3). Measures of whole body sympathetic activity were unrelated to arterial blood pressures and heart rates overall for both groups combined.

Table 1. *Effect of aging on brain norepinephrine turnover: influence of jugular venous drainage emanating from different brain areas*

	Unilateral JV (Combined), ng/min	Subcortical Drainage, ng/min	Cortical Drainage, ng/min	Nonlateralizing Drainage, ng/min
Men aged 20–30 yr	136 ± 19	107 ± 18	146 ± 27	165 ± 58
Men aged 60–75 yr	231 ± 34*	317 ± 50†	169 ± 46	142 ± 22

Mean values ± SE. Patterns of cerebral venous sinus drainage were demonstrated by dynamic and static SPECT cerebral sinus technetium-99 scans (Fig. 1). Sampling from the internal jugular vein, commonly the right, which received drainage of the superior sagittal sinus and carried the bulk of cortical venous return, was used to quantify cortical norepinephrine turnover. Conversely, sampling from the internal jugular vein, commonly the left, which did not receive superior sagittal sinus drainage, was used to quantify subcortical norepinephrine turnover. In some men there was nonlateralizing sinus flow, with symmetrical drainage of the superior sagittal sinus into the right and left internals jugular veins, which thus carried blood derived from both cortical and subcortical areas. JV, Jugular vein. *$P < 0.05$, †$P < 0.01$.

Table 3. *Effects of aging on sympathetic nervous activity and the adrenal medulla*

	Age 20–30 yr	Age 60–75 yr
Plasma concentration, pg/ml		
Norepinephrine	193 ± 13	289 ± 21*
Epinephrine	84 ± 9	61 ± 6
Plasma clearance, l/min		
Norepinephrine	2.84 ± 0.17	2.06 ± 0.09†
Epinephrine	2.87 ± 0.15	1.98 ± 0.11†
Total norepinephrine spillover, ng/min	552 ± 51	592 ± 168
Epinephrine secretion rate, ng/min	248 ± 31	112 ± 6†
Norepinephrine spillover, ng/min		
Cardiac	18 ± 2	28 ± 3*
Renal	91 ± 17	130 ± 14
Hepatomesenteric	29 ± 9	63 ± 13*

Mean values ± SE. The influences of aging observed here closely matches that described in our earlier reports (13–15): elevated plasma norepinephrine concentration, reduced plasma catecholamine clearances, a lowered epinephrine secretion rate, increased spillover of norepinephrine from the heart and into the hepatomesenteric circulation. The whole body and renal norepinephrine spillover rates were somewhat higher in the older subjects, but the differences were not statistically significant. *$P < 0.05$, †$P < 0.01$.

Epinephrine secretion rate. The mean plasma concentration of epinephrine was 27.3% lower in the older than the younger men (difference not statistically significant), consequent on a lower epinephrine secretion rate, 112 ± 6 ng/min (mean $\pm$ SE) vs. 248 ± 31 ng/min ($P < 0.01$), partially cancelled out by a lower plasma epinephrine plasma clearance, 1.98 ± 0.11 l/min compared with 2.87 ± 0.15 l/min ($P < 0.01$).

Cardiac sympathetic activity. The spillover of norepinephrine from the heart to plasma was increased in the older men, 28 ± 3 ng/min, compared with 18 ± 2 ng/min in the younger men ($P < 0.05$) (Table 3, Fig. 2). This difference was not a consequence of hemodynamic influences on transmitter washout to the circulation, as coronary sinus plasma flows were similar, but in addition to increased cardiac sympathetic nerve firing, diminished neuronal reuptake of norepinephrine may have contributed. The extraction of tritiated norepinephrine from plasma during transit through the heart, which is predominantly into cardiac sympathetic nerves (8, 10), was lower in the older men: mean 72%, range 42–87%, compared with mean 83%, range 60–92% ($P < 0.05$, Mann-Whitney U-test). Cardiac norepinephrine spillover was unrelated to resting heart rate overall for both groups combined.

HEPATOMESENTERIC NE SPILLOVER

CARDIAC NE SPILLOVER

RENAL NE SPILLOVER
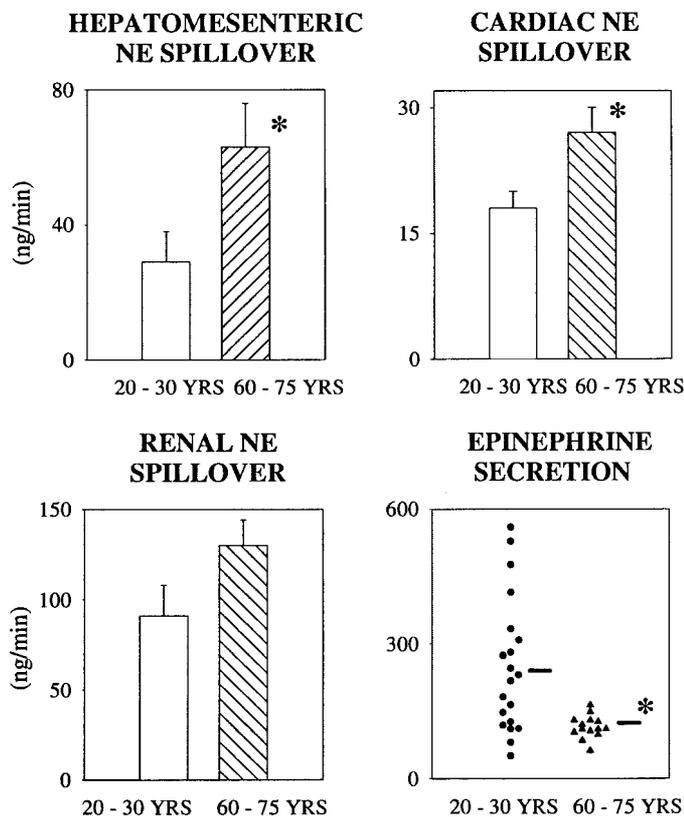
EPINEPHRINE SECRETION

Fig. 2. The influence of aging on adrenaline secretion rate and regional sympathetic nervous activity in the hepatomesenteric circulation, the heart, and the kidneys, based on the organ-specific spillover of NE to plasma is shown. In older men, the rate of NE spillover from the heart and into the hepatomesenteric circulation was increased. NE spillover from the kidneys was marginally higher in older men, but not significantly so. Conversely, aging acted to lower epinephrine secretion. *$P < 0.05$.

Table 4. *Effects of aging on hepatomesenteric norepinephrine plasma kinetics*

	Age 20–30 yr	Age 60–75 yr
Plasma norepinephrine concentration, pg/ml		
Arterial	197 ± 15	$280 \pm 24*$
Hepatic vein	52 ± 7	$98 \pm 9*$
Transhepatic plasma tritiated norepinephrine extraction	0.96 ± 0.01	0.96 ± 0.02
Hepatic plasma flow, ml/min	653 ± 97	711 ± 71
Hepatomesenteric norepinephrine spillover, ng/min	29 ± 9	$63 \pm 13*$

Mean values $\pm$ SE. Hepatomesenteric norepinephrine spillover was significantly higher in the older men, providing evidence of increased sympathetic tone in this vascular district. For hepatomesenteric norepinephrine spillover measurements, however, there is a limitation of the kinetic methodology as applied to a portal circulation, since hepatic artery and portal vein plasma flows are unknown and there is no plasma sampling downstream from the intestines in the portal vein. Accordingly, it is not possible to ascertain whether increased "hepatomesenteric" norepinephrine spillover derives from the gut, from the liver, or from both (2, 3). Hepatomesenteric extraction of tritiated norepinephrine was identical in younger and older men, so that as far as can be ascertained, the higher spillover of norepinephrine into the hepatic vein was not attributable to lesser extraction of norepinephrine by the liver. *$P < 0.05$.

Hepatomesenteric sympathetic activity. Hepatomesenteric norepinephrine spillover was higher in the older men, 63 ± 13 ng/min compared with 29 ± 9 ng/min (Tables 3 and 4), providing evidence of increased sympathetic tone in this vascular district. For hepatomesenteric norepinephrine spillover measurements, however, there is a limitation of the kinetic methodology as applied to a portal circulation. Because hepatic artery and portal vein plasma flows are unknown and there is no plasma sampling downstream from the intestines, in the portal vein, it is not possible to ascertain whether increased "hepatomesenteric" norepinephrine spillover derives from the gut, from the liver, or from both (2, 3). Hepatomesenteric extraction of tritiated norepinephrine was identical in younger and older men (Table 4), so that as far as can be ascertained, the higher spillover of norepinephrine into the hepatic vein is not attributable to lesser extraction of norepinephrine by the liver.

Renal sympathetic activity. The renal norepinephrine plasma kinetics measurements provided some evidence against the development of generalized sympathetic activation with aging. Norepinephrine spillover from the kidneys, although ~40% higher in older men, did not differ significantly from that in younger men (Table 3).

Relationship of brain norepinephrine turnover to regional sympathetic tone and epinephrine secretion. The central neurotransmitter mechanisms of sympathetic nervous control, and any distortion with aging, may not be identical in all sympathetic outflows. We tested whether a relationship existed between suprabulbar subcortical CNS norepinephrine turnover and the neural outflow in individual regions, specifically the heart, hepatomesenteric circulation, kidneys, and adrenal medulla.

For all experimental subjects combined, a direct relationship of suprabulbar subcortical norepinephrine turnover to cardiac sympathetic activity was present; for subcortical norepinephrine turnover vs. cardiac norepinephrine spillover, $r = 0.64$, $P < 0.05$. For hepatomesenteric norepinephrine spillover and suprabulbar subcortical norepinephrine turnover, a direct relationship also existed: $r = 0.57$, $P < 0.05$. Renal norepinephrine spillover and subcortical norepinephrine turnover were unrelated. For epinephrine secretion, a nonsignificant negative relationship to subcortical brain norepinephrine turnover existed: $r = -0.38$, $P > 0.05$).

DISCUSSION

In several clinical contexts, most notably cardiac failure and essential hypertension, we previously demonstrated the probable importance of projections of noradrenergic neurons to the forebrain in generating the peripheral sympathetic nervous stimulation present (16, 22, 24). This contrasts with the earlier experimental evidence of a baroreflex-linked sympathetic suppressant effect of norepinephrine in the medulla (4). In the present study we found the sympathetic activity accompanying aging to be associated with increased suprabulbar subcortical turnover of norepinephrine. In healthy young men also, even within the rather narrow normal range of sympathetic activity present, there is a close positive relationship between norepinephrine turnover in suprabulbar subcortical brain areas and peripheral sympathetic activity (24).

That these relationships exist was established by measuring CNS norepinephrine turnover, based on the overflow of norepinephrine and its lipophilic metabolites MHPG and DHPG into the internal jugular veins. In pilot studies (unpublished observations), it was found that CNS norepinephrine turnover shows sexual dimorphism in humans. The combined overflow of norepinephrine, MHPG, and DHPG into the internal jugular veins was substantially higher in young women than in young men, and this higher brain norepinephrine turnover in women was unrelated to the level of sympathetic nervous activity present, which was no higher in women. The present study investigating the influence of aging on brain norepinephrine turnover was confined to men because of this confounding influence of gender on brain norepinephrine turnover.

This increased brain norepinephrine turnover noted in the elderly could possibly have originated from defective neuronal reuptake of norepinephrine, which would facilitate noradrenergic neurotransmission. In sympathetic nerves there is an age-related impairment in norepinephrine transporter action (14, 15). A reduction in brain neuronal reuptake of norepinephrine in the elderly, effected by reducing intraneuronal metabolism of norepinephrine and facilitating extraneuronal metabolism, might perhaps be expected to reduce DHPG jugular overflow into the internal jugular veins and increase MHPG overflow (21, 25), although there

is disagreement on this point (7, 8). Our findings, however, argue against the importance of a central defect in neuronal norepinephrine reuptake. In the younger men the proportionality between jugular vein overflows of norepinephrine, DHPG, and MHPG was similar to that in older men, and the contribution of MHPG overflow to the estimated CNS norepinephrine turnover also was similar, 73% and 62%.

Our additional finding here, of a selective activation of the sympathetic nervous system with aging, involving the outflows to the heart and hepatomesenteric circulation accompanied by reduced adrenal medullary secretion of epinephrine, are in agreement with our previous studies (13–15). Prior clinical microneurographic measurements also document increased nerve traffic with aging in the postganglionic sympathetic fibers distributed to the skeletal muscle vasculature (1, 28, 32, 37). As in the earlier reports, we note no significant increase in the spillover of norepinephrine from the kidneys, suggesting that the renal sympathetic outflow is not activated with aging. In the present study, however, the number of observations made on renal norepinephrine spillover was insufficient for us to definitively demonstrate that renal sympathetic tone was normal in older men.

The lowered epinephrine secretion rate with aging, accompanied by sympathetic nervous system activation, emphasizes that the two elements of the "sympathoadrenal medullary system" do not always act in concert, so that a mismatching of sympathetic activity and epinephrine secretion rate can occur. Two other examples, of several, are the increased epinephrine secretion accompanying sympathetic inhibition that is present in both fasting and vasovagal syncope (19). Whether any functional disability is conveyed by reduced epinephrine secretion in the elderly is not clear. Epinephrine secretion with mental stress and with aerobic and isometric exercise are also reduced in the elderly (13), but it is not known if this contributes to observed reductions in vigilance and exercise capacity accompanying aging.

The functional significance of the sympathetic nervous activation with aging is also problematic, although potentially of importance. The impetus for continuing interest in this topic has come in part from the recognition that in a range of cardiovascular disorders, including essential hypertension, cardiac failure, and ventricular arrhythmias, for all of which incidence rises markedly with age, sympathetic nervous system pathophysiology may be an important causal component (11).

A statistically significant direct relationship existed between subcortical norepinephrine turnover and sympathetic tone in the cardiac and hepatomesenteric sympathetic outflows. Sympathoexcitatory projections from regions such as the locus ceruleus and A5 region of the brain stem to the hypothalamus and amygdala are important in the central regulation of sympathetic outflow (20, 35), and caudal projections from the hypothalamus are known to be of importance in the sympathetic regulation of the hepatic circulation (34). Our

results here in humans, in which with aging neuronal norepinephrine turnover was increased in a field of brain subcortical venous drainage that excludes the medulla oblongata (18), are in agreement with experimental studies in animals, which indicate that the baroreflex-related noradrenergic bulbar inhibition of sympathetic outflow, although important, can be overridden by stimulatory suprabulbar noradrenergic mechanisms, of which rostral projections to the hypothalamus are among the most important (20, 35). Independent observations made in parallel to the present study provide further evidence that the sympathetic activation of human aging is not generated by the arterial baroreflex (5).

In summary, we document increased turnover of norepinephrine in subcortical areas of the brain, in company with activation of the sympathetic outflows to the heart and hepatomesenteric circulation, with human aging. The findings suggest that noradrenergic neuronal projections to the suprabulbar subcortical areas produce the sympathetic nervous activation characteristic of healthy aging, a mechanism that also appears to underlie the sympathetic activation present in heart failure and essential hypertension.

Limitations

Although differentiating the pattern of cerebral drainage into the internal jugular veins was of crucial importance in the present study in allowing selective measurement of suprabulbar subcortical norepinephrine turnover, our methodology does not have the topographic precision to allow study of specific brain nuclei or their projections. In the future, brain imaging techniques may possibly acquire the specificity and precision to quantify highly regionalized monoamine neurotransmitter turnover in this way, but at present this capacity does not exist. Magnetic resonance imaging can be applied to measure regional blood flow and oxygen utilization but cannot give specific information on neuronal monoamine release. Positron emission tomography (PET) using 18-F dopamine can be applied to the study of norepinephrine turnover in sympathetic nerves (17), but no suitable PET agents for studying norepinephrine turnover in the human brain are available. The deficiency of a lack of topographical detail with our technique for measuring brain monoamine turnover to some extent was overcome in the present study by ascertaining the separate origins of cerebral drainage into the right and left internal jugular veins, to allow selective measurement of suprabulbar subcortical and cortical norepinephrine turnover (6, 22).

Perspectives

Activation of the sympathetic nervous system is one of the cardinal pathophysiological features of essential hypertension (29–31). Until now it has been surmised that stimulation of sympathetic nervous outflows to organs involved in blood pressure regulation with aging is a causal mechanism of hypertension in the elderly. The present study provides evidence, however, that this scenario is improbable on two counts.

First, hypertension in the elderly, in fact, is typically not accompanied by sympathetic nervous system activation above a level seen in elderly people with normal blood pressure (9). Increased resting sympathetic tone is much more typical of hypertension in young and middle-aged adults (1, 25, 29–31).

Second, the pattern of sympathetic nervous and adrenal medullary changes in hypertension and aging differ markedly. The sympathetic nervous activation present in essential hypertension involves the renal sympathetic nerves, which appears to be crucial in hypertension pathogenesis (6, 12), spares the hepatomesenteric circulation, and is accompanied by normal adrenal medullary secretion of adrenaline (25, 29–31). None of these apply to aging, in which epinephrine secretion is reduced and the sympathetic outflow to the kidneys appears to be normal, whereas that to the hepatomesenteric circulation is increased (15, 19, 36).

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