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Long-Term β_1 -Adrenergic Blockade Restores Adrenomedullary Activity in Primary Hypertension

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Summary: In this study we examined the effects of long-term treatment of 19 patients with primary hypertension with the β_1 -adrenoceptor antagonist atenolol on norepinephrine and epinephrine kinetics, at rest and during sympathoadrenal stimulation by lower body negative pressure. Norepinephrine and epinephrine kinetics were measured by using the radioisotope-dilution technique by steady-state infusion of tritiated norepinephrine and epinephrine. The patients were studied before and at the end of 3 months of treatment with atenolol (50 or 100 mg daily). A control group of four normotensive subjects was studied before and after 3 months without any drug treatment. In this group, only arterial blood samples were collected without infusion of the tritiated catecholamines. Atenolol decreased blood pressure and heart rate, but forearm vascular resistance was not affected by atenolol. During atenolol, baseline arterial plasma epinephrine decreased from 0.23 ± 0.02 to 0.17 ± 0.01 nM ($p < 0.05$), and this was accompanied by a decrease in total body ep-

inephrine spillover from 0.50 ± 0.05 to 0.35 ± 0.04 nmol/min ($p < 0.05$). In the control group, arterial plasma epinephrine had not decreased after 3 months. In addition, the increment of arterial plasma epinephrine during lower body negative pressure at -40 mm Hg was attenuated during atenolol. Atenolol had no effect on total body and forearm norepinephrine spillover rates, either at rest or during lower body negative pressure. Clearance rates of epinephrine and norepinephrine were not significantly affected by atenolol. These results suggest that treatment of patients with primary hypertension with the β_1 -adrenoceptor blocker atenolol inhibits the adrenomedullary secretion of epinephrine, but it does not affect the biochemical indices of sympathoneural activity. It remains speculative whether this selective effect of atenolol on epinephrine secretion contributes to its hypotensive action and to its cardioprotective effects in the long term. **Key Words:** β_1 -Adrenergic—Sympathoadrenal activity—Hypertension—Catecholamines.

For a long time, β_1 -adrenoceptor blocking agents have been widely used as effective antihypertensive drugs. The mechanism through which they reduce blood pressure is still incompletely understood but is presumed to be dependent on a competitive antagonism with endogenous catecholamines at the β -adrenoceptor sites. These β -adrenoceptors are found pre- and postsynaptically in many tissues, like the heart, the brain, the adrenal medulla, and the resistance arteries. Several mechanisms have been suggested to be implicated in blood pressure reduction during β_1 -blockade: resetting of the baroreflex, central nervous system mechanisms, decreased peripheral sympathetic discharge resulting from presynaptic β -receptor inhibition, and reduction of cardiac output (1,2). Long-term treatment of patients with hypertension with β_1 -blockers might have an effect on sympathetic nervous system activity, but plasma norepinephrine (NE) levels are not suited for a reliable assessment of sympathetic

nervous system activity. Plasma NE levels during long-term treatment have been reported to be increased, unchanged, or decreased (1). Plasma catecholamine levels are determined both by their spillover into the circulation and by their clearance from the circulation. The clearance, depending on cardiac output, might be affected by β_1 -adrenoceptor blocking agents. To assess the effects of β_1 -blockade on catecholamine spillover and clearance rates, the isotope-dilution method can be used (3). Previous studies, reporting on NE kinetics during long-term β -blockade, showed decreased or unchanged NE spillover rates or decreased NE clearance rates (4,5). Epinephrine (EPI) kinetics during β_1 -blockade have never been examined in humans. In this study we investigated the effects of 3 months' treatment of patients with primary hypertension with atenolol on clearance and on sympathoneural and adrenomedullary spillovers of NE and EPI. This was carried out both at rest and during sympathoad-

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renal stimulation by lower-body negative pressure (LBNP).

SUBJECTS AND METHODS

Subjects

Nineteen patients with primary hypertension (12 men and seven women; mean \pm SD age, 40.9 ± 5.2 years) participated in the study. All participants had a normal physical examination before entry in the study. Secondary hypertension was excluded according to standard clinical criteria, and all subjects had normal renal function. The mean \pm SD Quetelet index was 25.2 ± 2.2 kg/m². Thirteen of these 19 participants used antihypertensive medication, which was discontinued ≥ 4 weeks before the study. After stopping the antihypertensive medication, blood pressure was measured 3 times at 2-week intervals. The mean \pm SD basal systolic (SBP)/diastolic blood pressure (DBP) before treatment with atenolol was $156 \pm 16/100 \pm 8$ mm Hg, and the heart rate (HR) was 70 ± 11 beats/min. Four normotensive subjects (mean \pm SD basal SBP/DBP, $125 \pm 6/82 \pm 5$ mm Hg) served as a control group (mean \pm SD age, 45.9 ± 2.1 years). All subjects gave their written informed consent. The study protocol was approved by the Hospital Ethics Committee.

Study protocol

All patients with hypertension were studied twice; before and after treatment with atenolol for 3 months. Each patient started with atenolol at 50 mg/day, and blood pressure was recorded every 3 weeks. The dose was increased to 100 mg/day after 6 weeks unless the HR had decreased by $> 25\%$ or to < 45 beats/min. After 3 months, eight subjects were taking atenolol, 50 mg/day, and 11 subjects were taking 100 mg/day. The control group of four normotensive subjects was also studied twice with an interval of 3 months. This group was enclosed to control for a time effect, so they did not receive atenolol. However, in this group we studied only the reproducibility of the arterial plasma catecholamine levels, and we did not use the tracer infusions in this group.

On each study day, the subjects were allowed a light breakfast. All participants were required to abstain from alcohol, nicotine, and caffeinated foods and beverages for ≥ 24 h before each study day. All studies were carried out in the morning in a temperature-controlled room. During the study the subjects remained supine in a LBNP box that was used to stimulate sympathoadrenomedullary activity. After instrumentation, radiotracer infusions (see the following) were started, and the subjects rested for 30 min. During the last 3 min, baseline recordings of blood pressure, HR, and forearm blood flow were obtained. Then arterial and venous blood samples were drawn simultaneously to determine plasma concentrations of endogenous and tritiated catecholamines. Thereafter, LBNP was applied at -15 mm Hg for 15 min. Blood pressure, HR, and forearm blood-flow recordings and blood samples were collected in sequence beginning after 12 min of LBNP. A rest period of 30 min ensued, and then another 15 min of LBNP at -40 mm Hg was applied, and blood pressure, HR, and forearm blood-flow recordings and blood samples were obtained as before.

Procedures

A brachial artery was cannulated to monitor blood pressure and HR (Hewlett Packard GmbH, Böblingen, Germany) and to draw arterial blood samples. An intravenous catheter was inserted into a deep brachial vein in the ipsilateral arm to collect venous blood samples. A forearm venous catheter in the con-

tralateral arm was used for simultaneous infusion of [³H]NE and [³H]EPI. Forearm blood flow was recorded by venous occlusion strain-gauge plethysmography with air-filled cuffs (6). During the measurement of forearm blood flow and the sampling of the blood samples, the hand circulation was excluded by inflation of a wrist cuff to 100 mm Hg above systolic blood pressure (7).

Radiotracer infusion

[³H]NE (levo-[ring-2,5,6-³H]NE) and [³H]EPI (levo-[N-methyl-³H]EPI, with high specific activity, were infused intravenously to assess catecholamine kinetics. Tritiated catecholamines were obtained from Du Pont New England Nuclear, Hertogenbosch, the Netherlands), sterilized by using a micropore filter (0.22 μ m), and diluted in NaCl 0.9%, containing acetic (0.2 M) and ascorbic (1 mg/ml) acid. The vials were stored until use at -80°C for a maximum of 3 months. Sterilization, dilution, and storage took place under nitrogen. Just before the study, an aliquot of each radiotracer was diluted in normal saline.

After a bolus injection of each radiotracer at $15 \mu\text{Ci}/\text{m}^2$, both tracers were infused for 90 min at a continuous rate of $0.35 \mu\text{Ci}/\text{m}^2/\text{min}$. The weight of the two syringes containing the radiotracers was measured before and after the infusion to verify the infusion rate. Samples of the infusate were taken at the end of the infusion and stored at -80°C until assayed.

Analytic methods

The blood samples were collected in prechilled tubes containing 0.25 M EGTA and 0.2 M glutathione in distilled water (pH, 7.4). The blood samples were placed on melting ice. Plasma was separated by refrigerated centrifugation and frozen until assayed, which occurred within 2 months after collection. The samples were analyzed for concentrations of both unlabeled and tritium-labeled NE and EPI by using high-performance liquid chromatography (HPLC) with fluorimetric detection after selective precolumn derivatization of the catecholamines with the fluorescent agent 1,2-diphenylethyl-enediamine (8). By using a Gilson fraction collector (model 201-202), connected to an automatic sample injector (Wisp 710B), we collected [³H]NE and [³H]EPI into scintillation vials, starting at the beginning of the peaks of NE and EPI in the standard mixture.

Data analysis

Forearm vascular resistance (FVR) was calculated by dividing mean arterial blood pressure (MAP) by forearm blood flow (FBF) and was expressed in arbitrary units (AU). The average of the hemodynamic data during 3 min was calculated.

The clearance rate of NE (L/min) from arterial plasma was calculated by dividing the infusion rate of [³H]NE (dpm/min) by the steady-state arterial plasma concentrations of [³H]NE (dpm/L). Total body NE spillover rate (nmol/min), the estimated rate of appearance of endogenous NE in arterial plasma, was calculated by multiplying the steady-state arterial plasma NE concentration (nM) by the clearance (L/min). Analogously, NE spillover in the forearm was estimated as:

$$\text{Forearm NE spillover (pmol/min/100 ml)} = \text{FPF} \times \text{NE}_a \times f + [\text{FPF} \times (\text{NE}_a - \text{NE}_v)] \quad (1)$$

where FPF is forearm plasma flow (ml/min/100 ml), NE_a is arterial plasma NE (nM), NE_v is venous plasma NE (nM), and f is the fractional extraction $[(^3\text{HNE}_a - ^3\text{HNE}_v)/^3\text{HNE}_a]$. The

forearm plasma flow was calculated from the forearm blood flow and hematocrit. The clearance of NE from the forearm (ml/min/100 ml) was calculated by multiplying the forearm plasma flow by the fractional extraction. The clearance of EPI from arterial plasma and the estimated rate of appearance of endogenous EPI into arterial plasma were calculated according to similar formulas.

Results are expressed as mean \pm SEM unless indicated otherwise. To test the effects of atenolol on baseline plasma kinetic variables, the Wilcoxon signed-rank test was performed. This test also was used to test for the effects of LBNP and to compare the responses to LBNP before and during atenolol. A *p* value of < 0.05 (two-tailed) was considered to be significant.

RESULTS

Hemodynamic data

After treatment with atenolol, SBP and DBP decreased significantly from $166 \pm 4/89 \pm 2$ to $146 \pm 4/75 \pm 3$ mm Hg ($p < 0.05$). HR decreased from 60 ± 3 to 49 ± 2 beats/min ($p < 0.05$). FBF and FVR were not significantly affected by atenolol (1.91 ± 0.25 and 1.72 ± 0.22 ml/min/100 ml and 81.4 ± 8.7 and 80.9 ± 9.4 AU, respectively).

LBNP at -15 mm Hg had no effects on SBP, DBP, and HR both before and after atenolol. The increase in FVR at LBNP of -15 mm Hg was not affected by atenolol. The BP responses to LBNP at -40 mm Hg were not altered, whereas the increase in HR was impaired ($+8 \pm 1$ beats/min before and $+5 \pm 1$ beats/min after atenolol; $p < 0.05$). The increase in FVR to LBNP at -40 mm Hg was similar before and after atenolol.

Plasma epinephrine kinetics

After treatment with atenolol, the subjects had an $\sim 35\%$ lower basal arterial plasma EPI than before treatment (Table 1). This decrease in arterial plasma EPI was accompanied by a significant decrease in total body EPI spillover (Fig. 1), whereas total body clearance and forearm clearance of EPI were not altered by atenolol (Table 1). In the control group, we could not demonstrate a de-

TABLE 1. Plasma levels and kinetics of epinephrine and norepinephrine before and after treatment with atenolol

	Before atenolol	After atenolol
Epinephrine		
Arterial plasma level (nM)	0.23 ± 0.02^a	0.17 ± 0.01
Total body spillover (nmol/min)	0.50 ± 0.05^a	0.35 ± 0.04
Total body clearance (L/min)	2.20 ± 0.11	2.10 ± 0.12
Forearm clearance (ml/100 ml/min)	0.83 ± 0.09	0.69 ± 0.07
Norepinephrine		
Arterial plasma level (nM)	0.98 ± 0.10	0.92 ± 0.09
Total body spillover (nmol/min)	1.82 ± 0.16	1.75 ± 0.26
Total body clearance (L/min)	1.95 ± 0.13	1.85 ± 0.11
Forearm spillover (pmol/100 ml/min)	0.87 ± 0.09	0.82 ± 0.10
Forearm clearance (ml/100 ml/min)	0.78 ± 0.08	0.63 ± 0.06

Mean \pm SEM are given.

^a $p < 0.05$.

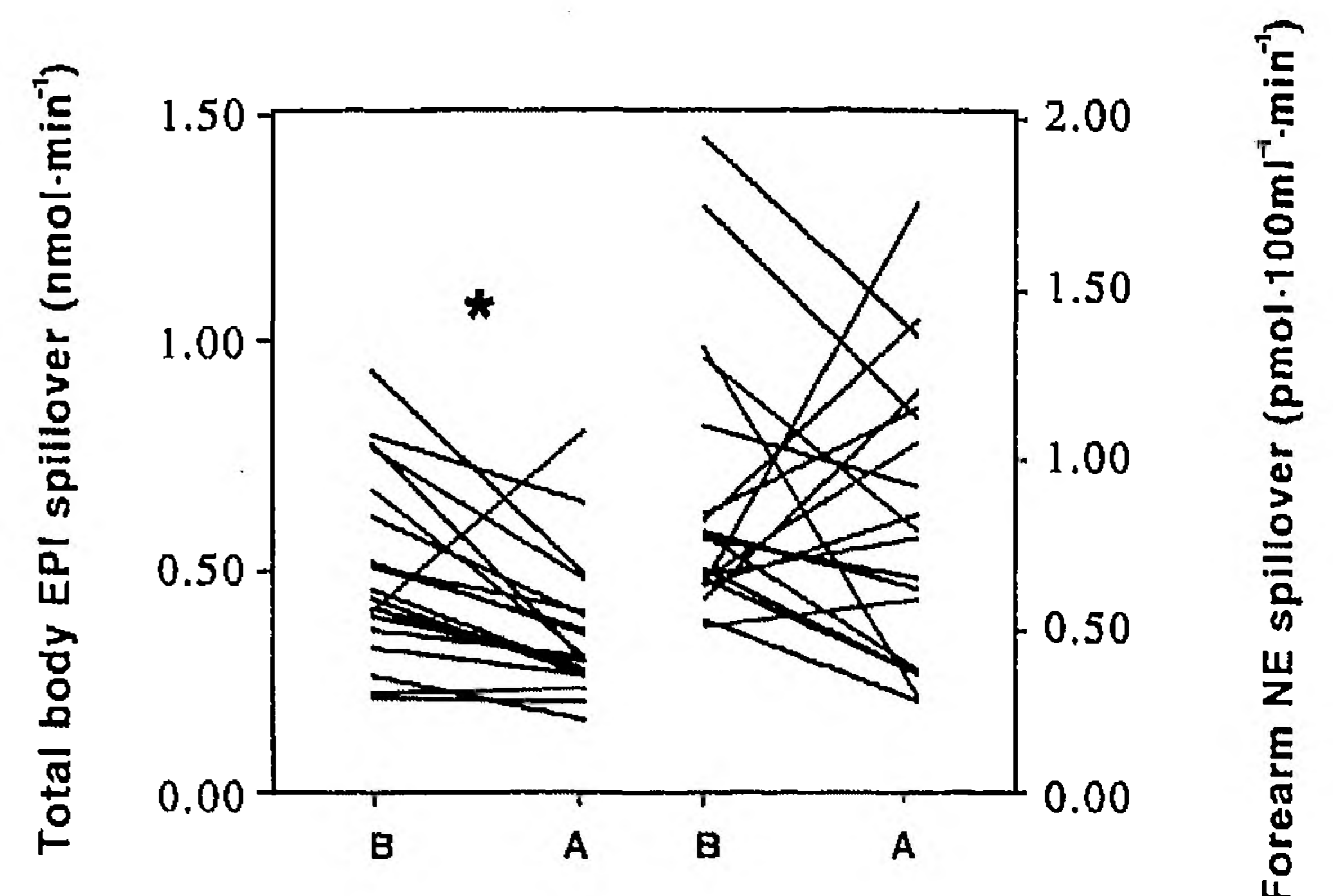


FIG. 1. Individual values of total body epinephrine (EPI) spillover and forearm norepinephrine (NE) spillover before (B) and after (A) 3 months' treatment with atenolol. $^*p < 0.05$.

crease in basal arterial plasma EPI when the subjects were restudied after 3 months: 0.29 ± 0.07 and 0.28 ± 0.06 nM, respectively.

The responses of total body EPI spillover and clearance and of forearm EPI clearance to LBNP at -15 mm Hg EPI were not affected by atenolol. However, atenolol did attenuate the increase in total body EPI spillover to LBNP at -40 mm Hg ($+0.77 \pm 0.26$ nmol/min before and 0.21 ± 0.04 nmol/min after atenolol; $p < 0.05$; Fig. 2), whereas the responses of total body and forearm clearance rates were not affected by atenolol. The attenuated response in total body EPI spillover to LBNP at -40 mm Hg after atenolol was reflected by an attenuated increase in arterial plasma EPI concentration to LBNP at -40 mm Hg after atenolol, although the difference was not significant ($+0.52 \pm 0.14$ nM before and $+0.23 \pm 0.03$ nM after atenolol; $p = 0.11$).

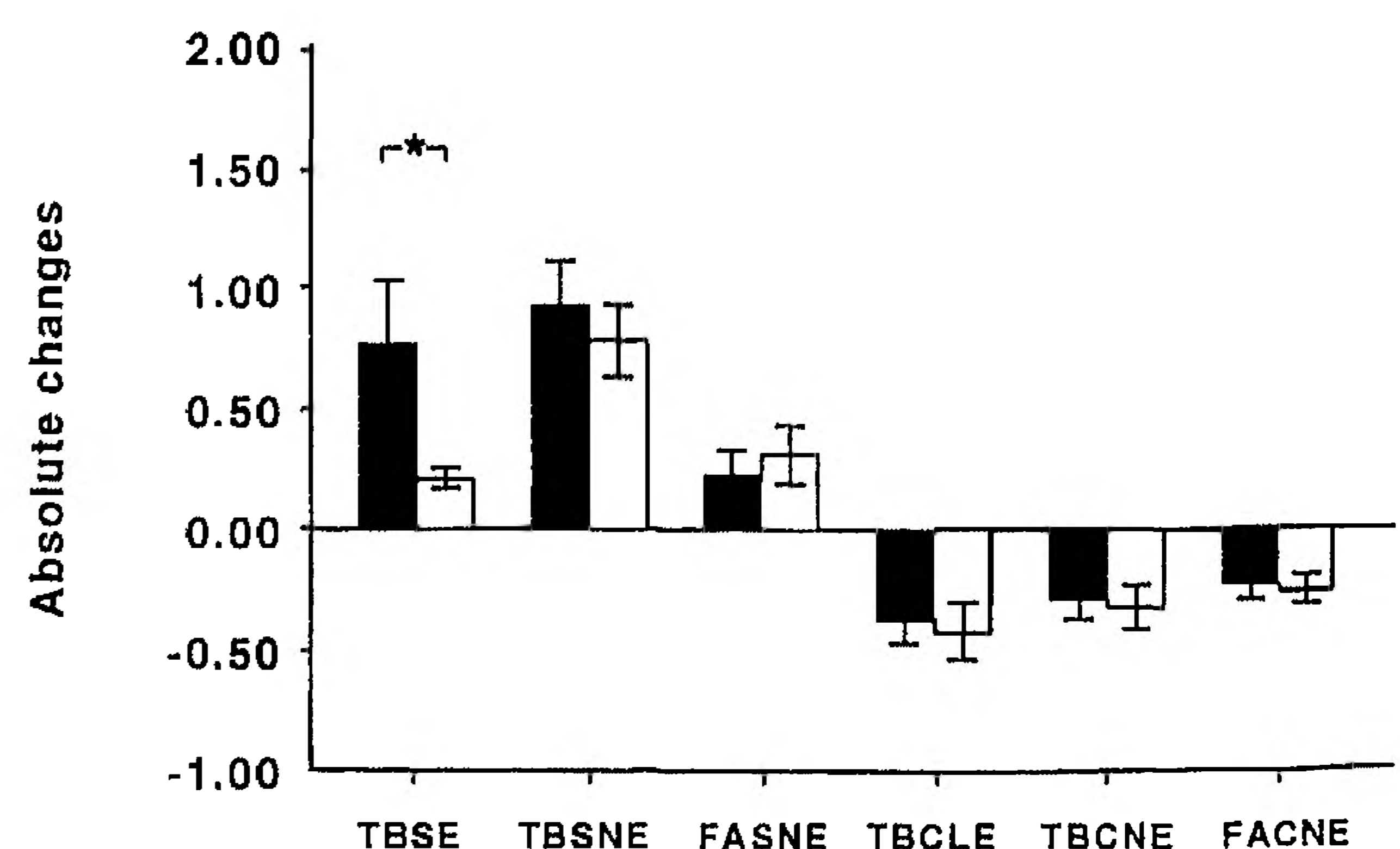


FIG. 2. The responses of total body spillover of epinephrine (TBSE, nmol/min) and norepinephrine (TBSNE, nmol/min), forearm spillover of norepinephrine (FASNE, pmol/100 ml/min), total body clearance of epinephrine (TBCLE, L/min), and norepinephrine (TBCNE, L/min) and forearm clearance of norepinephrine (FACNE, ml/100 ml forearm volume/min) to lower-body negative pressure at -40 mm Hg before (black bars) and after (open bars) long-term treatment with atenolol. Mean \pm SEM are given.

Plasma norepinephrine kinetics

Baseline arterial plasma NE, total body NE spillover, and total body NE clearance were not affected by treatment with atenolol. Forearm NE spillover (Fig. 1) and forearm NE clearance also were not changed by atenolol (Table 1). The responses of forearm NE spillover and total body NE spillover, as well as forearm clearance and total body clearance of NE to LBNP at -15 mm Hg and at -40 mm Hg, were not altered by atenolol.

DISCUSSION

The principal finding of our study is that long-term treatment with the β_1 -adrenoceptor-blocking agent atenolol decreases total body EPI spillover, whereas it does not affect total body NE spillover. The plasma clearance rates of both NE and EPI are not decreased by atenolol. Because the total body EPI spillover reflects the release of EPI from the adrenal medulla, these data imply that chronic β_1 -adrenergic blockade attenuates adrenomedullary EPI release but does not affect sympathetic NE spillover. In addition, EPI and NE spillover and clearance during sympathoadrenal stimulation by LBNP at -15 mm Hg are not affected by atenolol, whereas the adrenomedullary secretion of EPI during LBNP at -40 mm Hg is attenuated. Long-term administration of atenolol does reduce baseline as well as stimulated release of EPI from the adrenal medulla in patients with primary hypertension.

Several mechanisms should be discussed to explain the differentiated response of the sympathetic nervous system during long-term treatment with atenolol, leading to diminished EPI release from the adrenal medulla with a concomitant unchanged NE spillover. First, diminished adrenomedullary EPI release might be a direct effect of blockade of facilitatory β_1 -adrenoceptors on the adrenomedullary chromaffin cell membranes, as was shown in rat adrenal medulla cells *in vitro* (9). In contrast, the presynaptic β -adrenoceptors at the sympathetic nerve endings appear to be of the β_2 -subtype, and therefore the NE spillover is not expected to decrease during atenolol administration (2). Second, a decreased circulating EPI concentration might result from a decreased sympathetic impulse nerve traffic to the adrenal medulla. This, however, would not be consistent with the unchanged NE spillover, unless atenolol would affect exclusively the sympathetic outflow to the adrenal medulla. Finally, the decreased arterial plasma EPI levels and the decreased EPI release from the adrenal medulla could be a time effect or could be just the expression of an attenuated defense reaction to the stress of the experimental procedure. However, in a small control group, plasma EPI levels were not reduced at a second arterial cannulation after 3 months. The ideal study setup with a randomized crossover design with placebo and atenolol would require, however, withholding antihypertensive therapy to patients with hypertension for at least another 3 months. Although this is not a randomized crossover study, the unchanged plasma EPI levels after 3 months in

the control group argue against an aspecific effect, although this can not be excluded definitely on the basis of this study.

Data on catecholamine spillover and clearance rates during long-term β_1 -adrenergic blockade in human hypertension are not available from previous studies. EPI kinetics during β -blockade in humans has never been studied. NE kinetics were studied after short-term administration of β_1 -selective (5) and nonselective β -blockers (4) or long-term administration of nonselective β -blockers (10). These data are hard to compare with our data on long-term β_1 -blockade, because short-term administration of β -blocking agents or the use of nonselective β -blockade shows important differences with regard to sympathetic nervous activity, organ blood flow, or β -receptor occupation, thus influencing catecholamine kinetics.

Short-term administration of a selective or nonselective β -blocker causes a reduction of cardiac output and a baroreflex-mediated increase in total peripheral resistance, whereas peripheral resistance generally returns to the pretreatment level during chronic β -blockade (1). The increased intraneuronally measured muscle sympathetic activity after a bolus injection with a β_1 -blocker is restored during long-term β_1 -blockade (11,12). Moreover, baroreflex sensitivity is increased after long-term β -blocker treatment but not after short-term administration (13,14). In addition to baroreceptor-mediated effects on sympathetic outflow, catecholamine kinetics are influenced at the synaptic level by local blood flow, because catecholamine clearance is blood-flow dependent (5). A nonselective β -blocker has different sites of actions from those of a β_1 -selective blocker, which may affect catecholamine kinetics as well. NE release from the sympathetic nerve terminals is modulated by presynaptic β_2 -adrenoceptors, which can be blocked by a nonselective blocker but probably not by β_1 -selective blockers. NE clearance is more β_2 -adrenoceptor dependent than β_1 -adrenoceptor dependent, because in rats, NE clearance is decreased by propranolol but not by atenolol (15). In a study with intraarterial β -blocker infusions, thus preventing systemic hemodynamic effects, both metoprolol and propranolol decreased NE spillover (5). There are, however, no inhibitory β_1 -adrenoceptors on sympathetic nerve terminals. Alternatively it remains possible that the high local dose of metoprolol that was used was not β_1 -selective.

The EPI-spillover response to LBNP at -40 mm Hg was attenuated, although the NE-spillover response was not changed. The neurohumoral responses to sympathoadrenal stimulation during β_1 -adrenoceptor blockade have never been assessed. Our data on NE kinetics confirm and extend earlier observations using microneurography that demonstrated that responses of sympathetic nerve traffic to graded LBNP were not attenuated by propranolol (16).

In conclusion, the results of this study suggest that treatment of patients with primary hypertension with the β_1 -adrenoceptor blocker atenolol inhibits the basal and stimulated adrenomedullary secretion of EPI, whereas it

does not affect the biochemical indices of sympathoneural activity. It remains speculative whether this selective effect of atenolol on epinephrine secretion contributes to its hypotensive action and to its cardioprotective effects in the long term.

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