Whole Body Clearance of Norepinephrine

THE SIGNIFICANCE OF ARTERIAL SAMPLING AND OF SURGICAL STRESS

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ABSTRACT The whole body clearance of norepinephrine (NE) was measured in seven patients pre- and postoperatively. L[^{3}H]NE was infused intravenously for 90 min and steady-state concentrations of L[^{3}H]NE were measured at 75 and 90 min in both arterial and peripheral venous blood. Preoperatively, in the resting supine position, the clearance values based on arterial and venous sampling averaged 1.4 and 2.5 liter/min, respectively ($P < 0.02$). The difference in clearance values was due to a peripheral uptake of NE averaging 45%.

The mean plasma NE increased from 1.70 nmol/liter preoperatively to 5.20 nmol/liter postoperatively ($P < 0.02$). The plasma appearance rate of NE averaged 2.4 nmol/min before surgery and it increased to 9.5 nmol/min postoperatively ($P < 0.02$). The plasma clearance of NE averaged 1.4 and 1.6 liter/min pre- and postoperatively, respectively (not significantly different).

Our study demonstrates that the calculation of plasma NE clearance based on venous sampling results in values that are too high. Furthermore, such values may be influenced by individual variations in the peripheral uptake of NE, since we found no correlation between clearance values based on venous and arterial sampling. The increase in plasma NE postoperatively was due to an increase in the plasma appearance rate of NE because the clearance rate did not change.

INTRODUCTION

After the advent of sensitive and precise radioenzymatic assays for the determination of catecholamines in plasma there has been a renewed interest in determining the plasma appearance rate and plasma clearance of norepinephrine (NE)[1] and epinephrine. Recently, several authors using intravenous infusion of either labeled or unlabeled NE have reported a mean plasma clearance of NE of ~3 liter/min in resting supine man (1–3). However, as pointed out elsewhere (4), this is a surprisingly high value, close to cardiac output, suggesting that the extraction of NE is nearly 100% in several organs. In the studies mentioned above NE was infused intravenously in one arm and blood for the determination of steady-state plasma concentrations of unlabeled or labeled NE was collected from a vein in the opposite arm. Evidently, steady-state concentrations of NE have to be determined in arterial blood or preferably in mixed venous blood in the pulmonary artery. When venous sampling is used for measuring the steady-state concentration, the uptake of NE in peripheral tissue is disregarded and the clearance values may therefore be too high. The aim of this study was, firstly, to examine if the whole body clearance of NE differed when the steady-state concentrations were measured in arterial rather than in venous blood and, secondly, to examine the influence of surgical stress on the plasma appearance rate of NE.

METHODS

Patients

Clinical data are given in Table I. Seven female patients undergoing elective cholecystectomy participated in the study after giving informed consent. The patients had no other known disease and they took no drugs apart from oral contraceptives in two patients. The patients were examined 1–2 d before surgery and 2–4 h postoperatively. The patients were operated under standard anesthesia using halothane in

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Received for publication 2 August 1982 and in revised form 8 November 1982.

[1] *Abbreviation used in this paper*: NE, norepinephrine.

 J. Clin. Invest. © *The American Society for Clinical Investigation, Inc.* · *0021-9738/83/03/0500/06 $1.00*
Volume 71 March 1983 500–505

five patients and neurolept anesthesia in two patients. The perioperative blood loss was minimal and the postoperative period was uncomplicated in all patients. Postoperatively the patients received no medication apart from intramuscular injections of meperidine (75 mg) for pain and a slow intravenous infusion of saline. This therapy was discontinued before the experiment was started.

Procedure

The experiments were always performed between 2 and 6 p.m. The patients were studied in the supine position after at least 1 h of rest. Preoperatively the patients had fasted for 6 h and abstained from tobacco for at least 2 h preceding the study. The study was carried out 2–4 h after surgery.

A catheter was placed in a superficial vein in both forearms for blood sampling and intravenous infusion of L[^{3}H]NE. L[^{3}H]NE (3–5 Ci/mmol, sp act, New England Nuclear, Boston, MA) was prepared pharmaceutically. The radiochemical purity was measured before each experiment by chromatography and was at least 97%. The L[^{3}H]NE was stored in tartaric acid. Before the experiments the solution was diluted in saline. The infusion rate was ~0.7 μCi/min for 90 min. Aliquots of the L[^{3}H]NE infusate were collected for further analysis at the start and at the end of the infusion.

Venous blood was collected before (0 min sample) the infusion started and at 30, 75, and 90 min during the infusion. Arterial samples were obtained by puncture of the femoral artery at 75 min and if possible also at 90 min.

The heart rate and the arterial blood pressure were measured at 15-min intervals during the experiments.

Assays

L[^{3}H]NE. Tritiated NE in 3-ml samples of plasma and in aliquots of the infusate was extracted by alumina (5) and eluted by 6 ml of acetic acid as described earlier (6). A small sample of the eluate was stored for determination of the endogenous catecholamine concentrations whereas the larger portion of the eluate was freeze-dried and the radioactivity counted.

Deaminated metabolites. 3 ml of plasma and aliquots of the infusates were treated by alumina extraction as described above. The eluate was saturated with NaCl, acidified, and extracted into 5 vol of ethylacetate (5). An aliquot of the organic sample was evaporated in a current of air and the radioactivity was counted.

Endogenous NE and epinephrine concentrations. These were measured in the alumina eluate by means of a single-isotope derivative assay described elsewhere (7). The alumina eluate contained ~400 cpm of L[^{3}H]NE (apart from the 0 min sample). Only 50 μl of the 6 ml of alumina eluate were used in the analysis and the contribution of L[^{3}H]NE to the ^{3}H-labeled normetanephrine derivative in the assay was therefore very small, corresponding to ~1–2 cpm (taken the recovery of the assay into account) or <1% of the total counts achieved in the assay of NE. No adjustment was therefore necessary. The infusion of L[^{3}H]NE was calculated to raise the endogenous NE concentration in arterial blood ~0.12 nmol/liter at steady state (see below).

Calculations. The clearance was calculated as follows: L[^{3}H]NE infusion dose dpm/min/L[^{3}H]NE plasma dpm/liter. Both parameters were calculated on the basis of the counts per minute in the alumina eluate.

The plasma appearance rate of NE (nanomoles per minute) was calculated by the formula: plasma NE concentration nmol/liter × clearance liter/min.

The extraction ratio was calculated as $c_a - c_v/c_a$, where c_a and c_v are L[^{3}H]NE counts in arterial and venous blood, respectively.

The blood pressure was measured indirectly by a sphygmomanometer.

Statistics

Differences between mean values were tested for statistical significance by Wilcoxon's tests for two samples and for pair differences while correlations was measured by Pearson's product-moment correlation coefficient, *r*.

RESULTS

Pertinent clinical data are given in Table I. There was no difference either preoperatively or postoperatively in plasma NE before and after infusion of the tracer (Table II). The infusion of L[^{3}H]NE was calculated to increase the 75-min values in both experiments by

TABLE I
Clinical Data

Patients	Age	Height	Weight	Heart rate		Blood pressure	
				Preoperative	Postoperative	Preoperative	Postoperative
	yr	*cm*	*kg*	*beats/min*		*mmHg*	
1	57	165	61	65	96	150/100	170/100
2	52	162	70	74	74	140/90	140/85
3	35	168	85	76	80	140/95	150/95
4	45	174	101	60	83	145/100	135/80
5	26	166	65	60	88	110/70	110/65
6	60	163	68	62	82	115/70	115/70
7	36	163	71	66	70	120/80	120/80
Mean	44	166	74	66	82	131/86	134/82
SEM	5	2	5	3	3	6/5	8/5

TABLE II
Mean Plasma NE and Epinephrine Concentrations in a Peripheral Vein (V) and the Femoral Artery (A)

	Preoperatively			Postoperatively		
	0V	75V	75A	0V	75V	75A
			min			
Mean norepinephrine (*nmol/liter*)	1.70	2.07	1.81	5.20	3.93	3.07
SEM	0.23	0.30	0.19	1.96	0.85	0.49
Mean epinephrine (*nmol/liter*)	0.49	0.55	0.62	0.82	1.14	1.50
SEM	0.15	0.11	0.10	0.23	0.37	0.49

Data were obtained in seven patients pre- and postoperatively before (0 min) and at 75 min during the infusion of L[^{3}H]NE.

~0.12 nmol/liter, a value too small to be of any significance. The mean arteriovenous difference of NE and epinephrine were negative and positive, respectively, but the differences were not significant (Table II). The mean preinfusion level of plasma NE increased from 1.70 nmol/liter preoperatively to 5.20 nmol/liter postoperatively ($P < 0.02$), whereas plasma epinephrine concentrations did not differ.

The calculated clearance values are given in Table III and the results are summarized in Fig. 1. The alumina eluates of plasma and infusate were also examined for deaminated catecholamines. Only a very small number of counts could be ascribed to deaminated products, <1% of the counts in the alumina eluate from the plasma samples. There was no difference in the L-[^{3}H]NE counts in aliquots of the infusate taken at the start and the end of the 90-min infusion period. The preinfusion sample at the postoperative examination did not contain radioactivity.

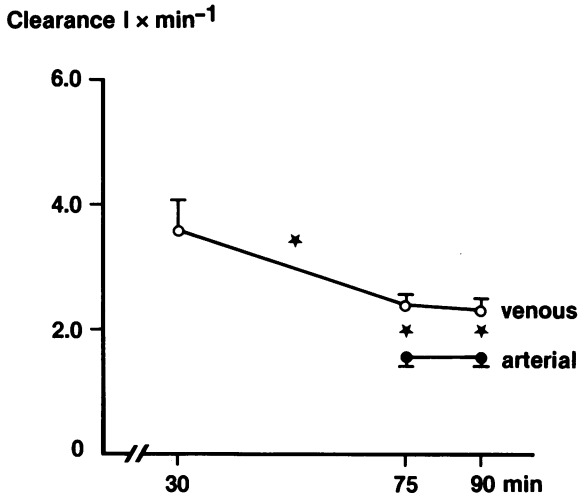

FIGURE 1 All calculated NE clearance values (liter [l] per minute) based on venous (upper curve) or arterial (lower curve) samples. Data represent mean±SEM.
* $P < 0.01$.

The clearance values calculated on venous samples after a 30-min infusion period were higher than the corresponding values calculated after 75 min (Fig. 1, $P < 0.01$), whereas there was no difference between the 75- and 90-min values either on venous or on arterial samples, respectively. Thus, steady-state concentrations were obtained after 75 min of infusion. All clearance values based on venous sampling were significantly higher than the corresponding clearance values based on arterial sampling both at 75 and 90 min (Fig. 1, $P < 0.01$). No significant correlation was obtained between venous and arterial clearance values. There was a highly significant inverse correlation between the forearm fractional extraction and the calculated whole body clearance based on arterial ($r = -0.79$, $P < 0.01$) but not on venous samples (not statistically significant).

Preoperatively the clearance values based on arterial samples at 75 min averaged 1.4 liter/min and the extraction ratio averaged 0.45. Postoperatively the corresponding values were not significantly different (Fig. 2).

Preoperatively the plasma appearance rate of NE averaged 2.4 nmol/min and it increased to 9.5 nmol/min postoperatively (Fig. 3, $P < 0.02$).

The heart rate and the arterial blood pressure did not change significantly during the infusions of L[^{3}H]NE. The mean heart rate was significantly higher postoperatively compared with the preoperative value (Table I, $P < 0.05$), whereas the mean blood pressure was the same.

DISCUSSION

The present study shows that (*a*) the plasma clearance of NE and the plasma appearance rate of NE are lower when the calculations are based on arterial sampling compared with venous sampling and (*b*) surgical stress in the immediate postoperative period increased the plasma appearance rate of NE whereas the clearance of NE was not altered.

Using arterial sampling we found a mean whole body clearance of NE of 1.4 liter/min in resting human subjects, considerably lower than the values previously reported in the literature (1–3). In these studies venous blood was used for determination of steady-state concentrations of the infused NE. The difference in the clearance values in our study compared with previous studies can be explained in the following way: When venous blood is used the steady-state concentrations

TABLE III

Calculated Clearance Values of NE and the Extraction Ratio (E) Based on Blood Samples Obtained from a Peripheral Vein (Cl_V) and the Femoral Artery (Cl_A).

	Preoperatively							Postoperatively						
	Cl_V	Cl_V	E	Cl_A	Cl_V	E	Cl_A	Cl_V	Cl_V	E	Cl_A	Cl_V	E	Cl_A
Patients	30 min	75 min			90 min			30 min	75 min			90 min		
1	3.1	2.6	0.54	1.2	2.8	0.46	1.5	8.3	3.7	0.46	2.0	—	—	1.9
2	2.5	2.5	0.52	1.2	—	—	—	3.9	2.5	0.20	2.0	—	—	—
3	3.9	2.7	0.52	1.3	2.9	—	—	5.0	3.2	0.50	1.6	3.0	0.40	1.8
4	3.1	2.3	0.65	0.8	1.9	0.32	1.3	3.5	2.4	0.37	1.5	2.4	0.25	1.8
5	2.7	3.3	0.40	2.0	—	—	—	2.8	2.4	0.42	1.4	2.8	0.31	2.0
6	2.9	2.3	0.20	1.9	2.5	—	—	—	2.1	0.07	2.0	1.6	0.19	1.3
7	1.7	1.6	—	—	1.8	0.34	1.2	2.8	2.3	0.12	2.0	1.7	0.46	0.9

Results were obtained in seven patients pre- and postoperatively at various time intervals (minutes) during intravenous infusion of L[^{3}H]NE.

• Values were expressed in liters per minute.

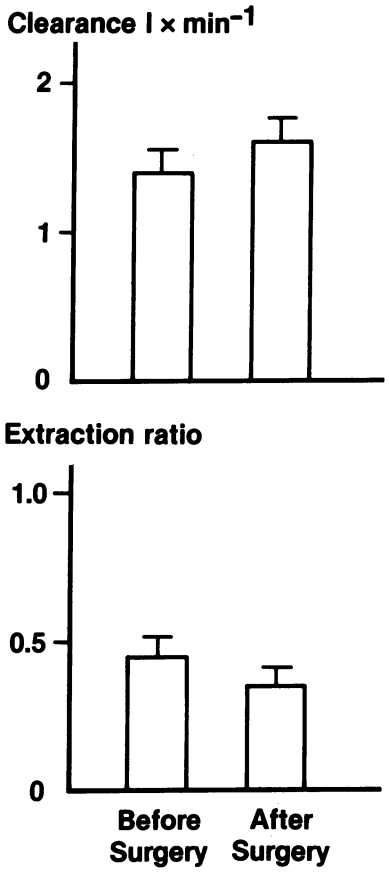

FIGURE 2 Arterial NE clearance values (liter [l] per minute) at 75 min pre- and postoperatively (upper curve). The extraction ratio pre- and postoperatively based on L[^{3}H]NE counts in arterial and venous samples at 75 min (lower curve). (In case 7 the values obtained at 90 min were used in the calculations.) Data represent mean±SEM.

of the infused NE are lower due to the peripheral uptake of NE and therefore the calculated clearance values are higher. The peripheral arteriovenous difference of endogenous plasma NE is relatively small because the uptake of NE is masked by a concomitant release of NE from the peripheral tissue. The finding that upper thoracic sympathectomy lowers the NE concentration in the forearm venous blood shows that

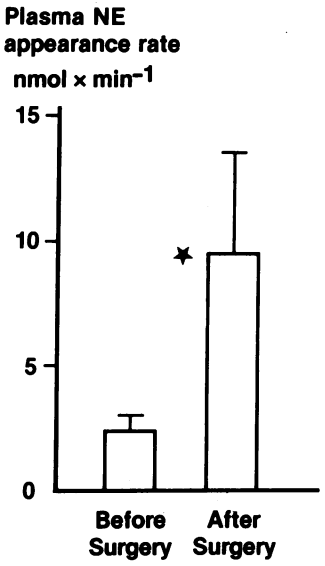

FIGURE 3 The calculated plasma appearance rate of NE (nanomoles per minute) based on the preinfusion endogenous NE concentration and arterial clearance values measured after 75 min of infusion of L[^{3}H]NE. (In case 7 the clearance values obtained at 90 min were used in the calculations.) Data represent mean±SEM.

• $P < 0.02$.

a substantial amount of NE is added to the blood in peripheral tissues (8).

It is possible that we slightly overestimated the whole body clearance of NE because small amounts of NE may be taken up by the lungs (9–12). In humans, Sole et al. (11) found aortic NE levels to be 25% lower than pulmonary artery levels, indicating that the lungs extracted at least 25% of the NE passed through them. However, in a recent study by Kjeldsen et al. (12) virtually no difference was found between plasma NE concentrations in the brachial and the pulmonary artery in a group of subjects undergoing cardiac catheterization but showing normal hemodynamics.

It is possible that the clearance values of NE based on venous sampling may be used as a relative index of the whole body clearance of NE, although we found no significant correlations between the calculated venous and arterial clearances of NE. The latter conclusion is based on a relatively small number of observations. It may well be that significant correlations would be found if conditions resulting in a greater diversity of clearance values were studied. However, the finding of a negative correlation between forearm fractional extraction and clearance values at 75 min based on arterial but not on venous sampling may be due to the influence of forearm hemodynamics on clearance values based on venous sampling. It is likely that the whole body clearance of NE based on arterial sampling correlates positively to the cardiac output, whereas the forearm fractional extraction is likely to decrease with increasing regional blood flow (10). The main advantage of using arterial sampling compared with venous sampling for the determination of the whole body clearance of NE is that the clearance values are not influenced by changes in the regional fractional extraction. The main disadvantage is that the determination requires arterial puncture or catheterization.

When radioactive labeled NE is used for the determination of NE clearance it is an important assumption that there is no recycling of [^{3}H]NE i.e., [^{3}H]NE taken up into nerve terminals is not released along with endogenous NE, or that recirculation of the tracer by release from nerve terminals after uptake is negligible in comparison with the rate of infusion of tracer. This is probably so since clearance values based on venous sampling are approximately the same after intravenous infusion of unlabeled NE or labeled NE. In the study by Esler et al. (3) clearance values are identical to those reported by Silverberg et al. (1) provided that in the latter study the endogenous NE release ceased during the infusion, which is most likely. In our study clearance values based on venous sampling were only slightly lower than those reported in these two studies. Furthermore, as shown by Esler et al. (3) and confirmed in our study when steady-state concentrations of infused [^{3}H]NE have been obtained, values do not decrease further with time. Finally, calculations based on results obtained in in vitro studies suggest that recycling is unlikely to be a significant problem. A substantial part of [^{3}H]NE taken up by a neuronal mechanism is deaminated intraneuronally whereas a smaller part is reused for release (13, 14). Furthermore, the fractional turnover rate of NE in several tissues is ~0.06/h ($T_{1/2}$ is ~12 h) (15). Therefore, only a minor fraction of the NE accumulated in the organs is likely to be released during the experimental period together with endogenous NE (~6% of the [^{3}H]NE taken up in the vesicles). Finally, only a fraction of the [^{3}H]NE released from the nerve terminals will reappear in plasma as [^{3}H]NE.

Interestingly, Best and Halter (16) have recently shown that whole body clearance values of epinephrine calculated from arterial measurements were 50% lower than the clearance rates calculated from venous measurements. The authors concluded that arterial rather than venous measurements should be used to estimate catecholamine kinetics in vivo.

Sympathetic nervous activity and release of NE vary from one tissue to another. Accordingly the plasma appearance rate and the fractional extraction of NE should preferably be determined in individual organs by selective venous blood sampling. In the skin and muscles sympathetic nervous activity may be determined by recordings of impulses in the sympathetic nerves (17). However, because the release of NE may be modified by presynaptic receptors, it is conceivable that the ratio between number of bursts in the sympathetic nerves and amounts of NE released from the nerve terminals may not be identical during all conditions.

The plasma NE concentration was considerably increased in the immediate postoperative period compared with preoperative values. Plasma NE and the plasma appearance rate of NE increased to approximately the same extent because the clearance values were unchanged by surgery. The heart rate increased significantly whereas the arterial blood pressure was the same before and after surgery. It is paradoxical that the arterial blood pressure remained unchanged postoperatively despite the considerable rise in the plasma appearance rate of NE. A reduced blood volume is unlikely to be the sole explanation of the increased plasma NE appearance rate postoperatively because the blood loss during the operation was minimal and replaced by intravenous infusion of saline. The increase in the plasma appearance rate of NE postoperatively may to a large extent be mediated by afferent stimuli from the damaged tissue (18). However, this mechanism fails to explain the lack of an

appropriate rise in blood pressure in response to the increased plasma NE appearance rate.

ACKNOWLEDGMENTS

The authors thank Dr. Benny Adelhøj and Dr. Palle Neumann, Department of Anaesthesiology, Kommunehospitalet, Copenhagen, for their technical assistance.

This study was supported by the Danish Medical Research Council grants 12-3165 and 512-20320, the Danish Hospital Foundation for Medical Research; Region of Copenhagen, The Faroe Islands and Greenland grant (5/81); and P. Carl Petersen's Foundation.

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