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Noradrenaline and isoproterenol kinetics in diabetic patients with and without autonomic neuropathy

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Summary. Noradrenaline and isoproterenol kinetics using intravenous infusion of L-^{3}H-NA and of ^{3}H-isoproterenol were investigated in eight Type 1 (insulin-dependent) diabetic patients without neuropathy and in eight Type 1 diabetic patients with autonomic neuropathy matched for age, sex and duration of diabetes. Resting plasma noradrenaline and adrenaline concentrations were reduced in patients with autonomic failure ($p < 0.05$). The metabolic clearance rate of noradrenaline was similar in both groups of patients, and the appearance rate of noradrenaline in plasma was reduced in patients with autonomic failure ($p < 0.01$). The disappearance of L-^{3}H-noradrenaline from plasma after the infusion of L-^{3}H-noradrenaline had been stopped was not different in patients with and without neuropathy. The metabolic clearance of isoproterenol was not influenced by the presence of autonomic failure and mean values were similar to the corresponding values for noradrenaline. Isoproterenol was only taken up by a non-neuronal uptake; this finding may indicate that neuronal uptake is not important for the inactivation of circulating catecholamines. Alternatively, because the non-neuronal uptake of isoproterenol is probably greater than that of noradrenaline, we cannot exclude the possibility that a small decrease in the neuronal uptake of noradrenaline was compensated for by a slightly higher non-neuronal uptake.

Key words: Diabetes mellitus, noradrenaline, isoproterenol, autonomic neuropathy, catecholamine kinetics.

Plasma noradrenaline (NA) is widely used as an index for sympathetic nervous system activity in man, and represents the net result of release and re-uptake in sympathetic nerve endings and non-neuronal removal from the plasma. In chronic autonomic failure plasma NA is usually low, but may be within normal range [1–5]. Conflicting results have been reported with regard to the metabolic clearance rate in patients with autonomic failure. Esler et al. [6] found in four patients with idiopatic autonomic failure that both the plasma NA appearance rate and the clearance rate were reduced, resulting in near normal plasma concentrations of NA. In addition, the disappearance rate of NA after the infusion was stopped was found to be lower than in normal controls [7]. Hoeldtke et al. [10] found in diabetic patients with autonomic failure that the metabolic clearance rate was unchanged. The aim of the present study was to elucidate these conflicting observations. We also investigated the metabolic clearance rate of isoproterenol to examine the extent to which circulating catecholamines are cleared by non-neuronal mechanisms [8].

Subjects and methods

Subjects

Sixteen Type 1 (insulin-dependent) diabetic patients participated in the study after informed consent. Clinical data are given in Table 1. Eight patients had signs of autonomic neuropathy judged by a decreased heart rate response to deep breathing (< 10 beats/min) and orthostatic hypotension defined as a drop in systolic blood pressure of more than 30 mmHg upon standing up.

Likewise, these patients had signs of peripheral neuropathy judged by increased vibratory perception threshold in the big toe, measured by a Biothesiometer (Biomedical Instruments Co, Ohio Neubury, USA) and absent tendon reflexes. The thresholds are expressed in volts, with a threshold value of 20 V being indicative of neuropathy. All patients had retinopathy (6 proliferative, 2 background). Six patients had proteinuria; of these, four had elevated serum creatinine.

Eight Type 1 diabetic patients without any signs of neuropathy matched for sex, age and duration of diabetes were examined as controls. Four of these had retinopathy (all background) and none had any signs of diabetic nephropathy.

Apart from diabetes and diabetic complications, the patients had no other known disease. Two of the patients with autonomic neuropa-

Table 1. Clinical data on Type 1 diabetic patients with and without autonomic neuropathy

	n	Age (years)	Sex (m/f)	Diabetes duration (years)	Resting blood pressure (mmHg)	Beat-to-beat variation (beats/min)	Systolic blood pressure fall on standing (mmHg)	Vibration perception thresholds (volts)	Protein-uria (mg/day)	Serum creatinine (μmol/l)	Retinopathy B = background P = proliferative N = no retinopathy	Plasma glucose during infusion (mmol/l)	HbA_{1C} (%)	Body surface (m^2)	Insulin type and dosage per day
Patients with autonomic neuropathy	1	27	M	10	190/100	3	40	35	1400	140	P	9	11.7	1.85	36 iu Monotard 8 iu Actrapid
	2	48	M	16	170/95	0	65	28	40	69	P	16	10.6	1.95	12 iu Insulatard 32 iu Actrapid
	3	49	M	18	160/90	2	38	36	32	76	B	7	8.6	1.93	18 iu Actrapid 12 iu Monotard
	4	44	M	35	150/85	3	50	22	55	115	B	8	9.4	1.80	8 iu Monotard 26 iu Actrapid
	5	42	F	24	185/100	3	45	41	24	73	B	10	9.8	2.20	28 iu Protaphane 12 iu Actrapid
	6	55	F	24	180/90	2	48	32	94	95	P	12	8.6	1.80	32 iu Insulatard 6 iu Actrapid
	7	42	M	34	160/95	3	46	42	890	251	P	11	8.8	1.80	12 iu Protaphane 24 iu Actrapid
	8	33	F	27	150/90	0	60	42	1200	300	P	8	10.8	1.52	32 iu Protaphane 8 iu Actrapid
Patients without autonomic neuropathy	1	42	M	25	140/85	22	0	10	5	76	B	11	7.1	1.80	24 iu Actrapid 16 iu Protaphane
	2	25	M	21	150/80	18	10	6	12	88	N	8	8.6	2.00	16 iu Actrapid 24 iu Protaphan
	3	39	M	28	140/85	15	15	12	60	93	B	12	8.1	1.85	28 iu Monotard 8 iu Actrapid
	4	37	M	14	125/85	19	0	7	12	104	N	8	9.4	1.45	30 iu Monotard 4 iu Actrapid
	5	43	F	23	140/80	23	5	8	6	62	B	14	9.7	1.95	20 iu Monotard 6 iu Actrapid
	6	33	M	17	135/85	21	10	6	5	88	B	9	7.4	1.74	16 iu Monotard 8 iu Actrapid
	7	30	F	24	145/90	25	5	6	7	71	N	8	8.6	1.74	14 iu Monotard 18 iu Actrapid
	8	42	F	32	135/90	21	5	7	6	75	B	10	8.0	1.75	20 iu Insulatard 8 iu Actrapid

thy were taking thiazides for a mild hypertension and the other patients took no drugs.

The study was approved by the local Ethical Committee.

Methods

Each patient participated in three experiments. All were carried out in the supine position, after 30 min of rest, in random order. All experiments were performed between 15.00 and 17.00 hours. The patients were not allowed to smoke or eat within 3 h prior the study. All patients had taken their usual dose of insulin before breakfast, but none had insulin at noon.

In the first experiment an intravenous infusion of L-^{3}H-NA (specific activity 15 Ci/mmol) was given in a dose of 0.35 μCi/min/m^2 body surface for 90 min. Before each infusion the L-^{3}H-NA was diluted with saline to a total volume of 50 ml. The infusion was given through an indwelling catheter in an antecubital vein, and venous blood samples of 8 ml were taken before the start of infusion from a catheter placed in the contralateral arm. Venous blood samples for the determination of NA kinetics were drawn after 75 and 90 min of L-^{3}H-NA infusion. Arterial blood samples of 8 ml were obtained by puncture of the femoral or radial artery at 90 min. Plasma glucose was measured immediately before the start of infusion and at 60 min. Arterial blood pressure and heart rate were measured before and 75 min after the start of infusion.

In the second study an infusion of ^{3}H-isoproterenol (specific activity 15 Ci/mmol) was given at a dose of 0.35 μCi/min/m^2 body surface for 90 min. Venous and arterial blood samples were drawn as on the first day of the examination. The two experiments were carried out at an interval of up to 5 weeks.

To investigate venous plasma NA disappearance rate, an infusion of L-^{3}H-NA (specific activity 15 Ci/mmol) was given at a dose of 0.70 μCi/min/m^2 body surface for 75 min in an antecubital vein. From a catheter placed in the contralateral arm, venous blood samples of 16 ml were obtained before the start of infusion, at 30, 60 and 75 min during the infusion and at 1, 2, 3, 5, 10, 20, and 30 min after the cessation of infusion.

Assays

All blood samples and aliquots of the infusate before and after infusion were collected in tubes with 20 μl/ml blood or infusate of a mixture of 95 mg EGTA and 60 mg reduced glutathione in 1 ml water. NA and adrenaline were measured by a single-isotope derivative assay [9]. Coefficient of variation calculated on the basis of duplicated determinations was 11% for adrenaline and 7% for NA. Tritium labelled NA and isoproterenol in plasma were determined as described earlier [11]. Haemoglobin A_{1C} was determined by thinlayer isoelectric focusing; in our laboratory normal range is 4.1-6.1%, with a between assay coefficient of variation of 4%.

Calculations

Clearance (l/min) was calculated as L-^{3}H-NA infusion dose dpm/min divided by L-^{3}H-NA plasma concentration (dpm/l).

Forearm extraction ratio

Ca - Cv/Ca where Ca and Cv are L-^{3}H-NA or (^{3}H) isoprenaline counts in arterial (a) and venous (v) blood respectively.

Appearance rate (μg/min)

Plasma concentrations of NA (μg/l) multiplied by the clearance of NA (l/min).

Table 2. Mean resting venous plasma concentrations of noradrenaline and adrenaline and forearm extraction ratios of noradrenaline and isoproterenol in Type 1 diabetic patients with ($n=8$) and without ($n=8$) autonomic neuropathy

	Patients with autonomic neuropathy	Patients without autonomic neuropathy
Mean noradrenaline (ng/ml)	0.168 ± 0.031	0.245 ± 0.016
Mean adrenaline (ng/ml)	0.020 ± 0.004	0.039 ± 0.005
Forearm extraction ratio of noradrenaline	0.25 ± 0.05	0.30 ± 0.06
Forearm extraction ratio of isoproterenol	0.24 ± 0.06	0.30 ± 0.07

Values are mean ± SEM

Table 3. Calculated clearances in Type 1 diabetic patients with (n = 8) and without (n = 8) autonomic neuropathy based on venous (V) and arterial (A) blood samples at 75 and 90 min during infusion with H^3 noradrenaline and H^3 isoproterenol

	Noradrenaline clearance l/min			Isoproterenol clearance l/min		
	V_{75}	V_{90}	A_{90}	V_{75}	V_{90}	A_{90}
Patients with autonomic neuropathy	1.81 (0.17)	1.76 (0.14)	1.29 (0.23)	1.69 (0.21)	1.80 (0.22)	1.33 (0.11)
Patients without autonomic neuropathy	2.29 (0.18)	2.06 (0.23)	1.38 (0.15)	2.12 (0.28)	1.70 (0.19)	1.24 (0.17)

Values are mean with ± SEM in parentheses

Statistical analysis

The Mann-Whitney rank sum test for unpaired data was used. P values less than 5% were considered statistically significant.

Results

Resting plasma concentrations of NA and adrenaline were significantly lower in Type 1 diabetic patients with autonomic neuropathy compared to patients without neuropathy ($p < 0.05$, Table 2). Plasma clearance of NA based on arterial sampling was similar in the two groups and similar to values previously observed in control subjects [11-13]. Autonomic neuropathy had no effect on the clearance of isoproterenol, which was similar to that of NA in both patient groups. (Table 3). The appearance rate of NA was significantly lower in patients with autonomic neuropathy as compared to patients without neuropathy (Fig. 1, $p < 0.05$).

The disappearance rate of L-^{3}H-NA after the infusion was stopped was not different in patients with and without autonomic neuropathy ($p > 0.1$ for all point of measurements) (Fig. 2). Finally, the arterial-venous difference of isoproterenol and of NA in the forearm was not significantly different comparing both groups of patients.

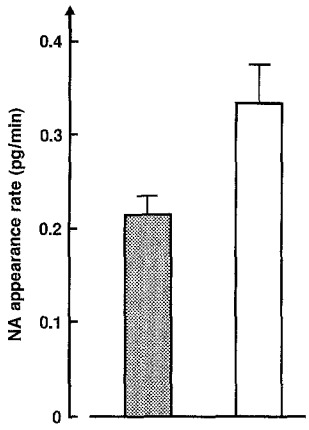

Fig. 1. Plasma appearance rate of noradrenaline (NA) in Type 1 (insulin-dependent) diabetic patients with ($n=8$) (■) and without ($n=8$) (□) autonomic neuropathy. Values are mean ± SEM. Significant difference ($p < 0.01$)

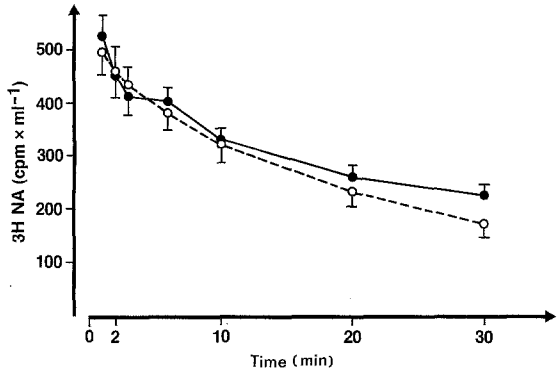

Fig. 2. Plasma disappearance rate of noradrenaline (NA) after cessation of infusion in Type 1 patients with ($n=8$) (●) and without ($n=8$) (○) autonomic neuropathy

All patients had plasma glucose values between 7 and 16 mmol/l (mean 10 mmol/l) during the infusions.

No side effect of the infusion was seen. Neither heart rate nor arterial blood pressure changed significantly during the infusion of L-^{3}H-NA or ^{3}H-isoproterenol.

Discussion

Our results indicate that the reduced levels of plasma NA in Type 1 diabetic patients with autonomic failure is due to a decreased plasma appearance rate and not due to changes in metabolic clearance rate. These findings are in accordance with observations by Hoeldtke et al. [10], and in contrast to the findings reported by Esler et al. [6]. In both the above mentioned studies, steady state concentration of L-^{3}H-NA was obtained from venous sampling. Because of peripheral uptake of NA, which may be influenced by individual variations, clearance determination must be based on arterial rather than venous blood samples [11]. In accordance with this, we found a tendency for lower clearance values at 75 min when calculations were based on venous rather than on arterial blood sampling, but this difference was not significant (Table 3). It should be emphasized that the NA disappearance rate was also similar in diabetic patients with and without autonomic neuropathy, in contrast to the findings in patients with primary autonomic failure. Finally, the arterial-venous difference of isoproterenol and of NA in the forearm was not significantly different between the two groups of patients and in fact similar in the individual group.

Some limitations of the methods of measuring NA kinetics must be emphasized. When venous infusion of L-^{3}H-NA is employed, steady state concentrations of L-^{3}H-NA should preferably be sampled in the mixed venous blood in the pulmonary artery. Arterial sampling may be used, however, because recent studies have shown that there is a relatively small extraction of NA in the pulmonary artery (approximately 7%) and no release of NA. The calculation of the clearance rate of NA using arterial sampling may therefore be overestimated by approximately 7% [14].

Another problem with the measurement of NA clearance is that one cannot be sure that the specific activity of NA in plasma is the same as at the break-down sites. Thus, the calculated clearance of NA may probably best be viewed as a relative index of the clearance of NA.

Interestingly, the clearance of isoproterenol and NA was the same despite the fact isoproterenol is only taken up by a non-neuronal uptake (methylation) [8]. These findings may indicate that neuronal uptake is not important for the inactivation of circulating catecholamines. It is more likely, however, that extraneuronal uptake of isoproterenol is greater than that of NA [15, 16]. We cannot exclude the possibility that a relatively small decrease in neuronal uptake of NA in patients with diabetic autonomic neuropathy was outweighed by a slightly higher non-neuronal uptake. Finally, it should be noted that, in our calculation, we did not take into account the possible influence of the capillary barrier on the clearance rate.

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References

1. Kontos HA, Richardson DW, Norwell JE (1975) Norepinephrine depletion in idiopatic orthostatic hypotension. Ann Int Med 82: 336-341
2. Christensen NJ (1972) Plasma catecholamines in long-term diabetics with and without neuropathy and in hypophysectomized subjects. J Clin Invest 51: 779-787
3. Hilsted J (1984) Testing for autonomic neuropathy. Ann Clin Res 16: 128-135
4. Bannister R (1983) Autonomic failure. Oxford University Press, 200-210

5. Cryer PE, Silverberg AB, Santiago JV, Shah SD (1978) Plasma catecholamines in diabetes: The syndromes of hypoadrenergic and hyperadrenergic autonomic neuropathy. Am J Med 64: 407–416
6. Esler M, Jackman G, Bobik A, Kelleher G, Jennings P, Shews LH, Korner P (1979) Determination of norepinephrine apparent release rate and clearance in humans. Life Sci 25: 1461–1470
7. Esler M, Jackmann G, Leonard P, Shews H, Bobik, Korner P (1981) Effect of norepinephrine uptake blockers on norepinephrine kinetics. Clin Pharm Ther 29: 12–20
8. Iversen LL (1967) Uptake and storage of noradrenaline in sympathetic nerves. Cambridge, p 120
9. Christensen NJ, Vestergård P, Sørensen T, Rafaelsen OJ (1980) Cerebrospinal fluid adrenaline and noradrenaline in depressed patients. Acta Psychiatr Scand 61: 178–182
10. Hoeldtke RD, Cilmi KM (1984) Norepinephrine secretion and production in diabetic autonomic neuropathy. J Clin Endocrinol Metab 59: 246–252
11. Hilsted J, Christensen NJ, Madsbad S (1983) Whole body clearance of norepinephrine. J Clin Invest 71: 500–505
12. Hilsted J, Christensen NJ, Larsen S (1985) Norepinephrine kinetics during insulin-induced hypoglycemia. Metabolism 34: 300–302
13. Hilsted J, Christensen NJ, Larsen S (1985) Plasma clearance of noradrenaline does not change with age in normal subjects. Clin Physiol 5: 443–446
14. Henriksen JH, Christensen NJ, Ring-Larsen H (1986) Pulmonary extraction of circulating noradrenaline in men. Eur J Clin Invest (in press)
15. Goldstein DS, Zimlichman R, Stull R, Folio J, Levinson PD, Keiser HR, Kopin JF (1985) Measurement of regional neuronal removel of norepinephrine in man. J Clin Invest 76: 15–21
16. Grohmann N, Trendelenburg U (1985) The handling of five catecholamines by the extraneuronal O-methylating system of the rat heart. Naunyn-Schmiedeberg's Archiv Pharmacol 329: 264–270

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