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Plasma adrenaline kinetics in Type 1 (insulin-dependent) diabetic patients with and without autonomic neuropathy

A. Dejgaard[1], J. Hilsted[2], J. H. Henriksen[3] and N. J. Christensen[4]

[1] Hvidøre Hospital, Klampenborg, [2] Department of Internal Medicine F, Glostrup Hospital, Glostrup, [3] Department of Clinical Physiology and Nuclear Medicine, Hvidovre Hospital, Hvidovre, and [4] Department of Internal Medicine and Endocrinology, Herlev Hospital, Herlev, Denmark

Summary. Plasma adrenaline kinetics (clearance, extraction across the forearm, initial plasma disappearance rate, mean sojourn time, volume of distribution) were studied in sixteen Type 1 (insulin-dependent) diabetic patients during constant i.v. infusion of tritium labelled adrenaline. In patients with ($n=8$) and without ($n=8$) neuropathy forearm venous plasma noradrenaline and adrenaline concentrations as well as plasma clearance of adrenaline based on arterial sampling (1.7 vs 2.1 l/min) were not significantly different. The initial disappearance time ($T_{1/2}$) after the infusion of the tritium labelled adrenaline had been stopped was significantly prolonged in Type 1 diabetic patients with neuropathy compared to those without (after 20 min infusion 2.7 vs 2.2 min, $p<0.02$, after 75 min infusion 3.7 vs 2.9 min, $p<0.05$). The corresponding values for the mean sojourn time of adrenaline in plasma were 6.5 vs 4.7 min ($p<0.05$) after 20 min infusion and 18 vs 10 min ($p<0.05$) after 75 min of infusion. The unchanged plasma clearance and the prolonged initial halftime and mean sojourn time of adrenaline in plasma suggest that adrenaline is distributed in a larger volume in Type 1 diabetic patients with neuropathy as compared to patients without neuropathy (estimated space of distribution 29 vs 20 l). Our results suggest that patients with diabetic neuropathy do not adjust the plasma adrenaline concentration to changes in adrenaline infusion rate as rapidly as those without neuropathy, i.e. the effect of an elevated adrenaline secretion rate may be prolonged in patients with diabetic autonomic neuropathy.

Key words: Adrenaline, autonomic neuropathy, catecholamine, kinetics, Type 1 (insulin-dependent) diabetes.

It is generally held that the sensitivity to catecholamines is increased in patients with autonomic failure [1-3]. Indeed, it has recently been shown that i.v. adrenaline (A) induced significantly greater cardiovascular and metabolic changes in diabetic patients with autonomic neuropathy than in patients without neuropathy [4]. The mechanism underlying the increased responsiveness is not known; altered kinetic properties of infused A as well as changes in β-adrenoceptor density and/or affinity could be involved. To clarify whether plasma A kinetics differ in Type 1 (insulin-dependent) diabetic patients with and without autonomic neuropathy, we have studied plasma clearance of A and the disappearance rate of A after continuous infusion of tritium labelled A (L-^{3}H-A) in these patients.

Subjects, material and methods

A total of 16 Type 1 diabetic patients were examined in the study. All patients gave informed consent and the study was approved by the local ethics committee. Clinical data of the patients are given in Table 1. Eight patients, six males and two females, showed signs of autonomic neuropathy judged by a decreased heart rate response to deep breathing (<10 beats/min) [5] and by orthostatic hypotension defined as a drop in systolic blood pressure of more than 30 mm Hg upon standing up. Peripheral neuropathy assessed by increased vibratory perception thresholds in the big toe measured by a Biothesiometer (Biomedical Instruments Co., Newbury, Ohio, USA) and absent tendon reflexes were likewise present in all these patients. At the time of the study, all these patients had retinopathy (five proliferative, three background), and five patients had albuminuria (two more than 300 mg/day, three microalbuminuria). Eight patients matched for sex, age and duration of diabetes were examined as control subjects. These patients showed no signs of autonomic or peripheral neuropathy. Three of these patients had retinopathy (all background) and all had normal levels of albuminuria (<30 mg/day). All 16 patients were treated with insulin with at least two daily injections. Besides insulin all patients were free of other medication during the study period. 3/4-labelled A (L-N-methyl-^{3}H-A, New England Nuclear, specific activity of 2.6 TBq/mmol (65 Ci/mmol)) was pharmaceutically prepared by Isotopapoteket, Copenhagen, Denmark. The preparation was stored under nitrogen and the radio-chemical purity checked by high performance liquid chromatography. L-^{3}H-A in 2 ml samples of plasma and aliquots of the infusate were extracted by alumina and eluted by 6 ml of acetic acid. The eluate was freeze-dried and subjected to liquid scintillation counting. Deaminated metabolites of L-N-methyl-^{3}H-A were determined as described earlier [6]. No radioactivity could be ascribed to deaminated catecholamines.

Plasma A and noradrenaline concentrations were determined by a single isotope radioenzymatic assay [7]. All blood samples and

Table 1. Clinical data on Type 1 (insulin-dependent) diabetic patients with or without diabetic autonomic neuropathy. Figures are mean ± SEM

	Patients with autonomic neuropathy	Patients without autonomic neuropathy
Number	8	8
Age (years)	50.3 ± 2.9	44.5 ±3.1
Sex M/F	6/2	5/3
Duration of diabetes (years)	22.9 ± 3.0	25.4 ±2.43
Beat-to-beat variation (beats/min)	3.1 ± 0.9[a]	21.4 ±1.3
Vibratory perception thresholds (volts)	41.4 ± 3.3[a]	10.1 ±1.2
Mean blood pressure (mm Hg)	113 ± 3.2[a]	94 ±2.8
Systolic blood pressure fall on standing (mm Hg)	41.5 ± 3.5[a]	3.9 ±1.2
Proteinuria (mg/day)	189 ±107[a]	7.11 ±2.5
Retinopathy		
N = none	0 N	5 N
B = background	3 B	3 B
P = proliferative	5 P	0 P
HbA_{1c} (%)	9.5 ± 0.4[a]	7.2 ±0.4
Blood glucose during infusion (mmol/l)	10.7 ± 0.9	10.5 ±0.1

[a] Significantly different from patients without neuropathy ($p<0.05$)

aliquots of the infusate for analysis of catecholamines and L-^{3}-H-A were collected in tubes containing EGTA and reduced glutathione. The samples were reanalysed and almost identical results were obtained ($r=0.97$ for a correlation between plasma A at the first and second determination. The corresponding value for noradrenaline was also 0.97).

HbA_{1c} was determined by thin-layer isoelectric focusing. Hb and serum creatinine were determined by standard techniques.

The arterial blood pressure was measured by a sphygmomanometer.

Procedure

The Type 1 diabetic patients were studied in random order. On the day of the investigation all patients had taken their usual dose of insulin before breakfast, and eaten breakfast and lunch. Two patients treated with multiple insulin injections were put on a twice daily insulin injection regime in connection with the study. All investigations were performed between 15.00 and 18.00 hours. None of the patients had smoked, eaten or injected insulin at least 3 h before the study.

An i.v. cannula was inserted into an antecubital vein in both arms and kept open with 0.9% NaCl solution. After 30 min of rest in the supine position, a blood sample for determination of endogenous circulating A and noradrenaline was taken and thereafter a continuous i.v. infusion of L-^{3}H-A was started at a dose of 0.35 μCi (1.3×10^4 Bq)·min^{-1}·m^{2-1} body surface. The first infusion period lasted for 20 min and the disappearance rate of L-^{3}H-A in plasma was followed for the next 20 min by venous blood sampling at 0, 1, 2, 3, 5, 10, and 20 min after termination of the infusion. A new infusion period was started which lasted for 75 min and, again, blood samples were collected after the infusion had stopped for determination of the disappearance rate. An arterial blood sample of 8 ml was taken by puncture of the femoral artery at the end of each infusion period. Blood glucose and blood pressure as well as heart rate were measured before and during the experiment.

Calculations

Clearance (l/min): rate of L-^{3}H-A infusion dose (dpm/min) divided by the arterial L-^{3}H-A plasma concentration (dpm/l).

Extraction ratio: (Ca-Cv)/Ca where Ca and Cv are L-^{3}H-A counts in arterial and venous plasma, respectively.

Appearance rate (ng/min): Plasma concentration of endogenous A (ng/l) multiplied by the clearance A (l/min).

Disappearance rate (ng/min): the half-life of the disappearance curve was calculated on a computer using all ln transformed values obtained during the first 5 min and expressed as $T_{1/2}$. The correlation coefficient was 0.97 or greater in 26 of the 32 experiments. In 6 experiments the values ranged between 0.90 and 0.96. Additionally, the plasma curve after ending the infusion was described by a biexponential least square iterative computer fit [8] and the disappearance rate expressed as the initial rate constant.

The mean sojourn time (min): the sojourn time is the average time the tracer remains (sojourns) in the region. It is determined according to the non-compartmental kinetic theory [9] as the area underneath the plasma tracer curve after termination of the tracer infusion (extrapolated to time infinity) divided by its height at time of ceasing infusion. The estimated volume of distribution of L-^{3}H-A (l) was assessed as mean sojourn time (min) multiplied by the clearance (l/min).

Mean arterial pressure (mm Hg): $\frac{1}{3}$ (systolic - diastolic) + diastolic pressure.

Statistical analysis

Wilcoxon's tests for 2 samples or pair differences were used when appropriate for testing statistical significance. The relationship between independent variables was determined according to the least squares method. $p<0.05$ was considered significant.

Results

Resting values of endogenous noradrenline and A did not differ significantly in the two groups of Type 1 diabetic patients (Table 2). A significant positive correlation was found between circulating noradrenaline and serum creatinine ($r=0.55$, $p<0.05$) (Table 2). There was no correlation between plasma A and serum creatinine (Table 2).

Data on plasma A kinetics are given in Table 3. The plasma clearance of L-^{3}H-A was not significantly different in Type 1 diabetic patients with and without autonomic neuropathy ($p=0.07$). The calculated clearance rate decreased in both groups from the 20 to the 75 min period ($p<0.02$ and 0.01 in Type 1 diabetic patients with and without neuropathy, respectively). The clearance values obtained at 20 and 75 min were positively correlated ($r=0.75$, $p<0.001$).

The extraction ratio of A across the forearm was not significantly different in the two Type 1 diabetic patient groups (Table 3). There was a negative correlation between the extraction ratio and the plasma clearance of A ($r=-0.61$, $p<0.05$). The plasma appearance rate of

A was not significantly different in Type 1 diabetic patients with and without autonomic neuropathy ($p=0.07$), (Table 3).

There was a significant difference in the disappearance rate between Type 1 diabetic patients with and without neuropathy (Table 3, 20 min period, $p<0.02$; 75 min period, $p<0.05$). $T_{1/2}$ increased from the 20 to the 75 min period, but the difference was only significant in Type 1 diabetic patients with neuropathy ($p<0.01$). Likewise, the mean sojourn time of L-^{3}H-A increased significantly from 20 to 75 min in both groups ($p<0.05$). The mean sojourn time was significantly longer in Type 1 diabetic patients with neuropathy (20 min period: 6.5 min; 75 min period: 18.0 min) as compared to those without (20 min period: 4.7 min, $p<0.05$; 75 min period: 10 min, $p<0.05$). The estimated volume of distribution was greater in the former group (29 vs 20 l), but the difference was not statistically significant. Type 1 diabetic patients with autonomic neuropathy had significantly higher HbA_{1c} values than Type 1 diabetic patients without neuropathy (Table 1, $p<0.01$). Blood glucose concentration did not differ between the two groups, and in none of the patients was the blood glucose below 5 mmol/l during the infusion. Blood pressure and heart rate did not change during infusion in any subject, and the Hb concentration was not different in Type 1 diabetic patients with and without autonomic neuropathy.

Table 2. Plasma noradrenaline, adrenaline, serum creatinine, and Hb in Type 1 (insulin-dependent) diabetic patients with and without autonomic neuropathy

		P-noradrenaline ng/ml	P-adrenaline ng/ml	Se-creatinine mmol/l	Hb mmol/l
Patients with autonomic neuropathy	1	0.24	0.01	81	8.9
	2	0.24	0.01	93	10.0
	3	0.44	0.03	330	9.3
	4	0.09	0.01	138	10.3
	5	0.13	0.01	80	9.5
	6	0.43	0.04	160	9.4
	7	0.40	0.01	136	9.5
	8	0.24	0.04	62	9.7
	mean ± SEM	0.28 ± 0.05	0.02 ± 0.01	135 ± 29	9.6 ± 0.15
Patients without autonomic neuropathy	1	0.20	0.05	83	9.9
	2	0.21	0.02	60	8.4
	3	0.33	0.04	72	9.2
	4	0.27	0.09	90	10.0
	5	0.19	0	65	9.3
	6	0.32	0	58	8.9
	7	0.27	0.04	81	9.3
	8	0.16	0.05	88	9.4
	mean ± SEM	0.24 ± 0.02	0.04 ± 0.01	74 ± 4	9.3 ± 0.18

Discussion

The main finding of the present study was that the plasma disappearance rate of A was significantly reduced ($p<0.05$) and the mean sojourn time of A was significantly increased ($p<0.05$) in Type 1 diabetic patients with autonomic neuropathy, as compared with Type 1 diabetic patients without neuropathy. This suggests that the action of an enhanced A secretion is prolonged in the former group, a finding which may at least partially explain recent experimental results of increased β-adrenergic sensitivity in patients with diabetic autonomic neuropathy [4]. The clinical relevance of these findings is obvious, since an enhanced A secretion rate is encountered in everyday situations in diabetic patients (e.g. during exercise and hypoglycaemia) [10].

The plasma ^{3}H-A disappearance rate was significantly reduced both after a comparatively short (20 min) ($p<0.02$) infusion period and after a longer (75 min) ($p<0.05$) infusion, and mean sojourn time was significantly increased ($p<0.05$) in the Type 1 diabetic patients with autonomic neuropathy as compared to their control counterparts. These abnormalities were more pronounced than the slight decrease in plasma clearance. The main difference between the calculated disappearance rate and the clearance is that the former is also dependent on the volume of distribution. The unchanged plasma clearance and the prolonged half life and mean sojourn time indicate that the volume of distribution of L-^{3}H-A was larger in the Type 1 diabetic patients with neuropathy as compared to their control counterparts. Thus, during continuous ^{3}H-A infusion, the calculated clearance did not differ significantly be-

Table 3. Plasma adrenaline kinetics in Type 1 (insulin-dependent) diabetic patients with and without neuropathy. ^{3}H-labelled adrenaline was infused for 20 and 75 min, respectively. Results are mean values ± SEM

	With autonomic neuropathy (min)		Without autonomic neuropathy (min)	
	20	75	20	75
Plasma clearance (1 min^{-1})	2.10 ± 0.19	1.70 ± 0.10	2.52 ± 0.14	2.06 ± 0.12
Plasma appearance rate (ng/min)	39 ± 8	32 ± 7	99 ± 30	81 ± 28
Initial plasma disappearance ($T_{1/2}$, min)	2.66 ± 0.13[b]	3.73 ± 0.27[a]	2.15 ± 0.10	2.89 ± 0.24
Initial slope (K, min^{-1})	0.34 ± 0.03[c]	0.33 ± 0.02[c]	0.58 ± 0.05	0.51 ± 0.06
Mean sojourn time (min)	6.5 ± 0.6[a]	18 ± 2.8[a]	4.7 ± 0.5	10 ± 1.6
Estimated volume of distribution [1]	11 ± 1.1	29 ± 3.9	10 ± 1.1	20 ± 3.4
Extraction across the forearm	0.51 ± 0.04	0.43 ± 0.05	0.33 ± 0.06	0.32 ± 0.07

Significantly different values in patients without neuropathy. [a] = $p<0.05$; [b] = $p<0.02$; [c] = $p<0.001$

tween the groups, whereas with rapid changes in the level of ^{3}H-A after termination of the infusion, an abnormality appeared. Our results may, therefore, indicate that the Type 1 diabetic patients with autonomic neuropathy adjust their plasma A concentration somewhat slower in response to changes in the A infusion rate than Type 1 diabetic patients without neuropathy. This suggests that the action of an enhanced A secretion is prolonged in Type 1 diabetic patients with autonomic neuropathy, a finding which may explain why A mediated responses (e.g. glycogenolysis and lipolysis) tended to be preserved in spite of low A responses to various challenges (e.g. exercise and hypoglycaemia) in these patients [10].

In the present study there was no significant difference between endogenous plasma noradrenaline and A in Type 1 diabetic patients with and without neuropathy. Decreased or unchanged levels of plasma catecholamines have previously been reported in patients with diabetic autonomic neuropathy [11–13]. In the present study plasma noradrenaline correlated with serum creatinine concentrations, whereas no such correlation was found for plasma A and serum creatinine. This may indicate, that plasma noradrenaline is influenced by factors other than neuropathy, i.e. by incipient renal insufficiency.

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Dr. A. Dejgaard
Hvidøre Hospital
Emiliekildevej 1
DK-2930 Klampenborg
Denmark