Significance and origin of DOPA, DOPAC, and dopamine-sulphate in plasma, tissues and cerebrospinal fluid

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1. INTRODUCTION

The sympathoadrenal system is one of the major pathways mediating physiological responses in the organism. The sympathoadrenal system plays an important role in the regulation of blood pressure, glucose, sodium and other key physiological and metabolic processes. In many disease states, the sympathoadrenal system is affected and by corrective physiological responses the sympathoadrenal system preserves homeostasis. Many therapeutic agents are either adrenergic activators or inhibitors. Therefore, measurements of the components of the sympathoadrenal system and the activity of the sympathoadrenal system have been of major interest for decades.

Levels of plasma (p-) noradrenaline (NA), the sympathetic neurotransmitter, have been used to indicate activity of the neuronal sympathoadrenal component, while adrenaline (Adr) levels indicate activity of the hormonal adrenomedullary component of the sympathoadrenal system (Christensen 1991, Goldstein 1995, Christensen & Norsk 2000).

The sympathetic nervous system is differentiated and release of NA should preferably be measured in specific organs or tissues. Forearm venous p-NA as an example is mainly derived from forearm muscle sympathetic nerves. Forearm venous p-NA mainly reflects and is furthermore a sensitive index of forearm muscle sympathetic nerve activity (Christensen 1991). Venous forearm p-NA concentrations in healthy adults in the supine resting situation were 0.06-0.52 ng/ml (0.36-3.08 nmol/l) in our studies (Eldrup et al 1989b, Eldrup et al 1994, Eldrup et al 1997), being similar to levels found by others (tables 1A and 1B). Arterial p-NA concentrations are typically lower than venous p-NA concentrations (Vendsalu et al 1960, Best & Halter 1982, Goldstein et al 1983, Hjemdahl et al 1984).

Arterial p-Adr concentration reflects and is a sensitive index of the hormonal component of the sympathoadrenal system (Christensen 1991, Goldstein 1995). P-Adr and p-NA do not necessarily change in parallel (Christensen 1991). Arterial p-Adr levels are up to approximately twice as high as venous p-Adr levels in the basal state (Best & Halter 1982, Hjemdahl et al 1984, Hjemdahl 1993). Venous p-Adr values are approximately one order of magnitude lower than p-NA levels (Table 1A and Table 1B).

NA and Adr, however, are both relatively difficult to measure in plasma with low concentrations present in the basal state (Hjemdahl 1984b).

Dopamine (DA) is an intermediate product in NA synthesis. DA was found to be an important neurotransmitter in the autonomic nervous system with its own specific effects in the kidney and gastrointestinal tract (Thorner 1975, Christensen et al 1975). Furthermore, DA is an autocrine/paracrine substance produced locally in the kidneys (Lee 1993, Goldstein 1995g). DA synthesis and actions in the kidney, however, are beyond the scope of this thesis and will not be discussed any further. Snider & Kuchel (1983) reported evidence that free DA is derived from peripheral noradrenergic nerves and from adrenal medulla. Noradrenergic neurons may co-release DA during extreme sympathetic activation (reviewed in Goldstein 1995g). Most investigators did not observe parallel changes of p-DA and p-NA during less extreme changes of sympathoadrenomedullary activity (Bell 1988 (review), Eldrup et al 1988, Hartling et al 1989, Sothmann et al 1990). Furthermore, human antecubital venous p-DA concentrations are very low and close to the detection level of the assay (tables 1A and B, Eldrup et al 1995). P-DA concentration in man is highest in adrenal venous outflow (Snider & Kuchel 1983). Although p-DA seems to be derived from sympathoadrenal nerves, p-DA concentration is not a sensitive and useful index of sympathetic activity.

The precursor of DA, NA, and Adr, the classical catecholamines, and the immediate product of the rate-limiting step in catecholamine biosynthesis is 3, 4-dihydroxyphenylalanine (DOPA). P-DOPA concentrations in humans exceed those of NA by about 10-fold and can be measured by radioenzymatic (RE) technique or by reverse-phase high performance liquid chromatography ((rp-hplc) Zürcher & Da Prada 1979, Goldstein et al 1984). Based upon the absence of an arterio-venous increase in p-DOPA concentration in sympathectomized limbs and a decrease in p-DOPA after inhibition of tyrosine hydroxylase (TH) in dogs, it was concluded that DOPA can pass across sympathetic neuronal membranes to reach the general circulation and furthermore, that p-DOPA may be related to regional rate of tyrosine hydroxylation (Goldstein et al 1987a). P-DOPA only demonstrated minimal changes during stimuli that produced significant changes in p-NA. Due to partly parallel changes of p-NA and p-DOPA, however, it was believed that p-DOPA reflect the rate of catecholamine synthesis and that p-DOPA was a simple and direct index of TH activity in vivo (Eisenhofer et al 1988, Goldstein & Eisenhofer 1988, Garty et al 1989b). It was inferred that p-DOPA levels may be an index of sympathetic activity.

DA metabolites are found in human plasma in much higher concentrations than NA, Adr and DA. Sulfoconjugated DA (dopamine-sulfate – DA-S) in humans makes up about 98% of total DA (tDA (Wang et al 1983, Snider & Kuchel 1983, Eldrup et al 1988)). DA-S concentrations in healthy human's antecubital venous plasma in the basal state are between 2 and 15 ng/ml (8.0-60.2 nmol/l, tables 1A and B). In 1979 Kuchel and co-workers found elevated plasma conjugated DA in humans with hypertension and suggested conjugated DA as a new tool for studying the role of the sympathetic nervous system in hypertension. It was later reported that ingestion of a banana increased p-DA-S (Davidson et al 1981, Dunne et al 1983, Kuchel et al 1985c). In an uncontrolled investigation, a DA-S plasma peak at 11 p.m. was found in healthy subjects eating meals at 7.30, 12.00, and 17.30, but a dietary source was excluded (Kuchel & Buu 1985) as a result of a previous finding that DA-S concentrations in 3 very obese subjects were relatively stable during 8 days of fasting (Kuchel et al 1979). Kuchel concluded that at least 12 hours of fasting was sufficient to disregard the contribution from food to p-DA-S and repeated the conclusion later without further evidence (Kuchel et al 1979, Kuchel et al 1982). In 1983 Snider & Kuchel in a review concluded: "When dietary sources are controlled, the total DA concentration can be used as an indicator of the intensity of the sympathetic response and possibly the level of training in animals and humans". Furthermore they stated: "Concentrations of total DA and of free + conjugated NE + E in plasma are a more sensitive measure of sympathetic activity than are free levels of catecholamines …". More

Table 1A. *Reference values for plasma (venous (v), arterial (a)) and cerebrospinal fluid (csf) concentrations of DOPA, NA, Adr, DOPAC, DA and DA-S in resting supine or sitting healthy human adult controls as measured by different methods and by different investigators.*

Method Reference site	Sampling (n)	DOPA ng/ml	NA ng/ml	Adr ng/ml	DOPAC ng/ml	DA ng/ml	DA-S ng/ml
Reverse phase hplc ED							
Eldrup et al 1997	v (n=7)[a]	1.58 1.18-2.51	0.16 0.14-0.28	nd	2.06 1.36-3.94	0.03 0.02-0.08	4.91 3.57-5.34
Eldrup et al 1995	v (n=21)[a]	1.40 0.80-1.88	0.36 0.12-1.24	0.03 0.00-0.12	1.64 1.02-3.46	0.02 0.00-0.13	nd
	csf (n=21)[a]	0.31 0.00-0.65	0.07 0.00-0.14	0.01 0.00-0.03	0.35 0.08-0.61	0.01 0.00-0.01	nd
Eldrup et al 1994	v (n=3-7)[a]	1.18 1.07-1.49	0.13 0.08-0.26	nd	1.58 1.30-2.23	0.02 0.00-0.03	3.57 1.95-14.16
	a (n=7)[a]	1.09 0.98-1.20	0.13 0.09-0.21	nd	1.48 1.33-2.24	nd	3.21 2.00-13.09
Eldrup et al 1989b	v (n=7)[a]	1.37 0.92-2.15	0.16 0.08-0.49	nd	nd	nd	nd
	v (n=8)[a]	1.35 0.67-1.57	0.28 0.16-0.52	nd	nd	nd	nd
Ozawa et al 1999	a (n=12)[b]	nd	0.10±0.02	nd	nd	0.04±0.01	3.37±0.50
Goldstein et al 1999	a (n=6)	~ 1.68	nd	nd	~ 1.34	~ 0.01	~ 0.5
Raskind et al 1999 Young	v (n=11)[c]	1.27±0.26	0.19±0.07	nd	1.25±0.67	0.02±0.00	nd
	csf (n=11)[c]	0.69±0.32	0.12±0.06		0.35±0.15	0.02±0.02	
Old	v (n=10)[c]	1.38±0.25	0.23±0.06		1.09±0.27	0.02±0.01	
	csf (n=10)[c]	0.86±0.33	0.12±0.04		0.49±0.27	0.01±0.01	
Yoshizumi et al 1996	v (n=36)[b]	nd	nd	nd	nd	0.05±0.00	4.80±0.90
Yamamoto et al 1996	v (n=14)[b]	1.58±0.10	0.23±0.02	nd	2.41±0.45	0.02±0.00	4.33±0.40
Ahlskog et al 1996a	v (n=15)[d]	0.87-2.43	0.13-0.50	<0.02-0.15	nd	<0.03	nd
Goldstein et al 1995	v (n=8)[b]	1.71±0.20	0.18±0.02	0.01±0.00	1.68±0.10	nd	nd
Grossman et al 1992a	v (n=10)[b]	1.27±0.08	0.35±0.05	0.04±0.01	nd	0.01±0.00	nd
Rogers et al 1991	v (n=?)[b]	nd	0.44±0.03	0.05±0.01	nd	0.04±0.01	3.10±0.42
Eisenhofer et al 1991	a (n=42)[b]	1.20±0.04	nd	nd	nd	nd	nd
Kuchel et al 1990	v (n=9-29)[b]	2.1±0.7	nd	nd	nd	nd	nd
Goldstein et al 1989b	v (n=13)[c]	1.79±0.15	0.25±0.03	nd	2.39±0.48	nd	nd
O'Hare et al 1989	av (n=8)[b]	1.54±0.16	0.21±0.05	nd	nd	nd	nd
Devalon et al 1989	v (n=17)[c]	1.57±0.10	0.38±0.06	0.04±0.01	nd	nd	nd
Boomsma et al 1988	v (n=39)[b]	2.08±0.55	nd	nd	nd	nd	nd
Goldstein et al 1987a	v (n=34)[b] a (n=34)[b]	2.29±1.12 1.73±0.75	nd	nd	nd	nd	nd
Eisenhofer et al 1986	v (n=12)[b]	3.39±0.32	0.32±0.06	0.03±0.01	0.73±0.12	nd	nd
Goldstein et al 1984	v (n=9)[e]	2.08 1.12-3.00	0.28 0.23-0.35	0.03 <0.01-0.06	1.38 0.99-3.89	0.07 <0.01-0.28	nd
Mefford et al 1981	v (n=5)[c]	nd	0.29±0.05	0.08±0.04	nd	0.03±0.00	nd
Reverse phase hplc FR							
Jeon et al 1992	? (n=10)[c]	2.29±0.37	0.54±0.05	0.13±0.05	2.67±1.34	nd	nd
Lee et al 1987	v (n=12)[c]	3.01±0.69	nd	nd	nd	nd	nd
Ion exchange hplc ED							
Hjemdahl 1993	a (n=8-12)[b]	nd	0.17±0.02	0.04±0.01	nd	0.01±0.00	nd
Hjemdahl et al 1984	v (n=12)[b] a (n=12)[b]	nd	~ 0.31±.05 0.26±0.03	0.05±0.01 0.07±0.01	nd	nd	nd

a) median with range below; b) mean±SEM; c) mean±SD; d) range; e) mean with range below; nd: not determined; ED: electrochemical detection; FR: fluorescence reaction.

than 10 years later, however, the origin and the significance of p-DA-S were considered unclear (Goldstein 1995g). Some authors have proposed that sulfoconjugated DA must be regarded as a possible physiological storage form of active free DA in plasma (Yoshizumi et al 1995).

DA is deaminated and dehydrogenated to form 3, 4-dihydroxyphenylacetic acid (DOPAC) by monoamine oxidase (MAO) and aldehyde dehydrogenase. Two isoenzymes, MAO-A and MAO-B, exist. MAO-A predominates in neural tissue, whereas both subtypes exist in non-neuronal tissue (Hovevey-Sion et al 1989, Goldstein 1995b). MAO-A and MAO-B outside the noradrenergic neurons and the central nervous system (CNS) are located primarily in the liver but activity is also found in myocardium, lung, kidney and duodenum (Saura et al 1996). Aldehyde dehydrogenase is found in

Table 1B. *Reference values for plasma (venous (v), arterial (a)) and cerebrospinal fluid (csf) concentrations of DOPA, NA, Adr, DOPAC, DA and DA-S in resting supine or sitting healthy human adult controls as measured by different methods and by different investigators.*

Method Reference site	Sampling (n)	DOPA ng/ml	NA ng/ml	Adr ng/ml	DOPAC ng/ml	DA ng/ml	DA-S ng/ml
Gas chromatography-MS							
de Jong et al 1988	v (n=11)[a]	1.77±0.39	nd	nd	nd	nd	nd
	csf (n=9)[a]	0.69±0.18	nd	nd	nd	nd	nd
Ehrhardt & Schwartz 1978	v (n=9)[a]	nd	0.20±0.12	0.06±0.04	nd	0.04±0.03	nd
PNMT-radioenzymatic							
Lake et al 1976	v (n=74)[b]	nd	0.29±0.02	nd	nd	nd	nd
COMT-radioenzymatic							
Kuchel et al 1990	v (n=9-29)[b]	nd	0.40±0.04	0.10±0.01	nd	0.05±0.01	2.49±0.7
Claustre et al 1990	v (n=7)[b]	nd	0.17±0.05	0.11±0.04	1.94±0.32	0.25±0.03	5.93±0.85
Eldrup et al 1988	v (n=7)[c]	nd	0.20 0.09-0.33	0.02 0.00-0.09	nd 0.00-0.11	0.02 1.58-11.21	3.77
Best & Halter 1982	v (n=6)[b] a (n=6)[b]	nd	0.29±0.06 0.19±0.04	0.05±0.01 0.07±0.01	nd	nd	nd
Thiede & Kehr 1981	v (n=9)[b]	2.20±0.26	0.37±0.04	0.04±0.01	4.77±0.84	0.06±0.03	nd
Wilkes et al 1981	v (n=6)[b]	nd	nd	nd	29±6	0.04±0.01	nd
Brown & Dollery 1981	v (n=8)[a]	2.06±0.48	nd	nd	nd	0.05±0.04	nd
Demassieux et al 1981	v (n=4)[a]	1.2	0.20±0.03	0.03±0.01	nd	0.02±0.01	1.06±0.16
Johnson et al 1980	v (n=10)[a]	1.82±0.61	0.20±0.05	0.04±0.01	nd	0.06±0.02	6.35±2.46
Christensen et al 1980	csf (n=18)	nd	0.01-0.18	0.01-0.09	nd	<0.02	nd
Zürcher & Da Prada 1979	v (n=5)[b] csf (n=10)[b]	1.42±0.09 0.49±0.08	nd nd	nd nd	nd nd	nd nd	nd nd
Johnson et al 1978	v (n=42)[b]	1.43±0.05	nd	nd	nd	0.02±0.02	nd
Buu & Kuchel 1977	v (n=17)[b]	nd	0.24±0.03[d]	[d]	nd	<0.02	0.74±0.17
Peuler & Johnson 1977	v (n=15)[a] csf (n=5)[a]	nd nd	0.28±0.17 0.20±0.06	0.02±0.02 0.01±0.01	nd nd	0.03±0.03 0.00±0.01	nd nd
Da Prada & Zürcher 1976	v (n=7)[b]	nd	0.20±0.02	0.05±0.01	nd	0.13±0.02	nd
Christensen 1973a	v (n=6)[a]	nd	nd	nd	nd	0.20±0.06	nd
Christensen 1972	v (n=4), mean	nd	0.21	0.05	nd	nd	nd
Engelman & Portnoy 1970	v (n=22)[a]	nd	0.20±0.08	0.05±0.03	nd	nd	nd
Fluorimetric assay							
Carruthers et al 1970	v (n=5), mean	nd	0.45	0.06	nd	nd	nd
Vendsalu A 1960	v (n=29)[b] a (n=29)[b]	nd nd	0.40±0.02 0.31±0.02	0.07±0.01 0.23±0.02	nd nd	nd nd	nd nd

a) mean±SD; b) mean±SEM; c) median with interquartile range below; d) NA+A together; nd: not determined; MS: Mass spectrometry; PNMT: Phenylethanolamine-N-methyltransferase; COMT: Catechol-O-methyltransferase.

several forms and in many organs, including brain, but is present in highest concentration in liver (Kopin 1985). Concentrations of DOPAC in healthy adult human's antecubital venous plasma are about 30-50 times that of DA, being between 1.0 ng/ml (6.0 nmol/l) and 4.0 ng/ml ((23.8 nmol/l) Eldrup et al 1995, Eldrup et al 1997, Table 1A)). Plasma DOPAC has mainly been studied by two research groups, probably due to assay problems (Holmes et al 1994, Goldstein 1995h). In rats p-DOPAC seemed to be derived from both neuronal and non-neuronal sources but the origin of the non-neuronal part was unknown (Hovevey-Sion et al 1989). Based upon existing evidence almost exclusively from animal studies, it appeared that p-DOPAC seemed to be derived at least in part from metabolism of DA in noradrenergic nerves (Goldstein 1995h). It was suggested that p-DOPAC may reflect vesicular retention and reuptake of DA similar to the MAO deaminated NA metabolite 3, 4-dihydroxyphenylglycol (DHPG; Ahlskog et al 1996a). Another suggestion was that endogenously DOPAC in plasma mainly originates from DA within the brain (Dingemanse et al 1997).

2. AIMS

The aims of this thesis are to:

- Elucidate in rats if there is a depot of DOPA in sympathetic nerves and investigate DOPA, DA and NA content in different tissues.
- Elucidate in humans if venous plasma concentrations of DOPA are indices of sympathetic nervous activity.
- Determine DOPA kinetic data in humans in the basal state and after dopa decarboxylase inhibition.
- Elucidate the effects of dopa decarboxylase inhibition on plasma concentrations of DOPA, DOPAC and DA-S in humans.
- Investigate the effects of intake of ordinary meals and 25 hour fasting on plasma concentrations of DOPA, DOPAC, and DA-S in humans.
- Investigate the effects of prolonged fasting in rats compared to sympathectomy and/or adrenalectomy on DOPA, DOPAC, DA and NA concentrations in plasma, gastrointestinal tissues and other tissues.

– Determine concentrations of DOPA, DOPAC, DA and NA in plasma and cerebrospinal fluid from controls and patients with Parkinson's disease.
– Evaluate venous p-DOPA and p-DOPAC concentrations as tumor markers in children with neuroblastoma.

Based on the author's findings and the literature, the origin of plasma content of DOPA, DOPAC and DA-S is finally elucidated.

3. MATERIAL AND METHODS

3.1. SUBJECTS

Healthy human subjects, patients with type 1 diabetes mellitus without and with neuropathy, patients investigated because of low back pain, Parkinson's disease, active or previous neuroblastoma or ganglioneuroblastoma, children with other solid tumors, and children admitted to a pediatric department for non-neoplastic diseases were investigated as described in the individual papers. Wistar rats were used in other experiments. The Regional Research Ethics Committee approved all studies individually and The Danish Animal Inspectorate approved the animal studies. In one study the approvals of the Danish National Institute of Radiation Hygiene and the Pharmaceutical Laboratory of the Danish National Board of Health were obtained as well.

3.2. EXPERIMENTAL CONDITIONS

Fasting and meals

Healthy humans fasted for at least 25 hours (h) and male Wistar rats were fasted for 4 days. Subjects and animals had free access to tap water during fasting (Eldrup et al 1997, Eldrup & Richter 2000). Ordinary meals with specified content were used (Eldrup et al 1997). The breakfast, hot meal and open sandwiches were typical Danish meals. The only exception from normal daily routine for most people was that the hot meal was served at 13.00 h and the open sandwiches meal was served at 18.00 h.

Orthostatic test

An orthostatic test with measurements of catecholamines after 10 min in the upright position (Eldrup et al 1989b) was used as a stimulus with well-defined increase in muscular and other sympathetic activity due to unloading of sino-aortic and cardiopulmonary baroreceptors (Christensen 1991, Goldstein 1995, Jacobsen 1996, Vissing 1997). Venous p-NA increases after only 5 min and no further increase is normally seen after another 5 min in the upright position (Cryer et al 1974, Lake et al 1976). From the results of tracer NA kinetic studies it has been claimed that the increase in venous p-NA was largely due to decreased NA clearance during orthostasis (Linares et al 1987, Esler et al 1988, Hjemdahl 1993). The results from NA tracer kinetic studies, however, have been seriously questioned (Henriksen & Christensen 1989, Christensen & Norsk 2000). Moreover, muscle sympathetic activity increases between 30 and 90% in parallel to venous p-NA during orthostatic stress (Burke et al 1977, Rea & Wallin 1989, Baily et al 1990). Others have found that both p-DHPG and p-NA levels are indices of sympathetic activity during orthostatic stress (Howes et al 1986). The increase of muscle sympathetic activity produced by the orthostatic test and as reflected in venous p-NA concentration, however, was considered a sufficient stimulus to test the hypothesis that p-DOPA is a useful index of muscle sympathetic activity.

Tracer kinetics

Plasma DOPA kinetics were studied in healthy young men by infusion of l-^{3}H-DOPA with a specific activity of 0.6-0.7 mCi/ml at 1.7-ml/min infusion rate (Eldrup et al 1994). Tracer DOPA was infused into a peripheral vein and blood samples were collected from an antecubital vein catheter on the opposite arm and from the femoral artery. The purity of the l-^{3}H-DOPA in the infusate was between 70 % and 85% as measured by the percentage of ^{3}H-activity eluted from the hplc-system in the DOPA fraction. In plasma, l-^{3}H-DOPA purity remained unchanged during the 120-minute infusion period (infusate vs. plasma (210 min) in controls: p=0.32 (Mann-Whitney)) and almost remained unchanged up to 90 min after the infusion was discontinued (controls: p=0.05 (Friedman)). This result is superior to that seen in a tracer DOPA study in rats where only 27% of radioactivity was l-^{3}H-DOPA after 90 min infusion of radiolabelled DOPA (Grossman et al 1990). L-^{3}H-DOPA activity was followed after cessation of the infusion in order to calculate the mean residence time of tracer DOPA in plasma, a method similar to what has been calculated for tracer NA in ^{3}H-NA kinetic studies (Henriksen & Christensen 1989). Arterial p-DOPA clearance was calculated as tracer DOPA infusion rate divided by tracer DOPA arterial plasma concentration. Arterial plasma DOPA appearance rate was calculated as the product of DOPA clearance and arterial plasma DOPA concentration. The lower arm extraction fraction was calculated as the fractional arterio-venous decrease in plasma tracer DOPA concentration relative to the arterial supply of plasma tracer DOPA. Finally, lower arm plasma DOPA production was calculated as the arterio-venous increase in p-DOPA concentration across the lower arm plus the calculated fraction of arterial p-DOPA concentration extracted in the lower arm (Eldrup et al 1994). DOPA activity in peripheral vein samples was stable after 90 min infusion in the basal state and a 120 min infusion period was chosen. L-^{3}H-DOPA also appeared to be stable after 120 min infusion when infusion of l-^{3}H-DOPA was started 30 min after oral ingestion of 50 mg benserazide (Eldrup et al 1994).

Clonidine administration

Clonidine is a selective α_2-adrenoceptor agonist but clonidine also has affinity to nonadrenergic imidazoline binding sites in the central nervous system and in the adrenal medulla (reviewed in Goldstein 1995d). Clonidine decreases venous, arterialized and arterial p-NA concentrations in a dose dependent way without affecting plasma NA clearance (Hokfelt et al 1975, Bravo et al 1981, Veith et al 1984). Presynaptic α_2-adrenoceptor mediated inhibition of neuronal NA release as well as inhibition of NA synthesis is believed to be the mechanism (Veith et al 1984, Fillenz 1990, Goldstein 1995d). We chose a dose of 300 mg clonidine given orally as this dose had previously significantly decreased noradrenergic activity (Bravo et al 1981, Veith et al 1984, Goldstein et al 1987a, Eldrup et al 1988).

Benserazide administration

DOPA accumulates in rat brain after administration of an aromatic amino acid decarboxylase (AADC) inhibitor and plasma ^{14}C-DOPA increases after extracerebral dopa decarboxylase (DDC) inhibition in humans and rats (Kuruma et al 1972, Carlsson et al 1972). These observations led to the discovery that the therapeutic efficacy of DOPA in Parkinson's disease was greatly enhanced by coadministration of peripheral AADC inhibitors such as benserazide or carbidopa (Pinder et al 1976, Da Prada et al 1987). Benserazide is an about 10 times more potent inhibitor of peripheral AADC than carbidopa in rats, mice and man (Da Prada et al 1987). Equimolar doses of benserazide inhibited AADC most in man, less in rats and least in mice. Inhibition lasted for more than 8 h in rats and for more than 24 h in man when 5.1 µmol (1.5 mg)/kg benserazide was given orally. This dose caused incomplete peripheral inhibition of AADC (Da Prada et al 1984, Da Prada et al 1987). Concerns have previously been raised about ^{3}H-DOPA infusions in humans (Goldstein et al 1991a). To investigate the effects of peripheral AADC inhibition in healthy human subjects weighing 70-80 kg, a dose of only 50 mg benserazide or about 2.1-2.4 µmol/kg was chosen. In mutual agreement with the company supplying benserazide and the Pharmaceutical Laboratory of the Danish National Board of Health we choose a lower dose of benserazide than that used in Da Prada's studies. Peripheral AADC inhibition was expected to last shorter and therefore cause a lower radiation exposure than a higher dose of

benserazide. Furthermore, in a preliminary study with one patient with autonomic neuropathy administration of 50 mg benserazide caused severe and prolonged hypotension (unpublished). P-DOPA increments increased with increasing benserazide doses up to 200 mg benserazide three times daily in humans (Dingemanse et al 1997). P-DOPAC concentrations were not affected by higher benserazide doses. P-DA-S and p-NA levels were not investigated in the study. It is unknown if higher doses of benserazide would have decreased endogenous p-NA levels in healthy humans as expected and contrary to our finding (Eldrup et al 1994). Existing evidence indicates, however, that main information and conclusions from our study would have been unchanged if higher dose of benserazide had been used.

Regional and whole-body sympathectomy in rats

Unilateral sympathectomy was performed surgically in rats by removing the abdominal part of the sympathetic chain from the second lumbar to the second sacral ganglion on one side. Measurement of muscle NA content indicated that sympathectomy was successful (Eldrup et al 1989a).

Chemical sympathectomy with 6-hydroxydopamine (6-OH-DA) in doses similar to those used in our study (Eldrup & Richter 2000) has been shown in a combined morphological and biochemical study to selectively destroy noradrenergic nerve endings, while noradrenergic nerve cell bodies and adrenal glands in rats were unaffected by the treatment (Thoenen & Tranzer 1968). Tissue NA content indicated that sympathectomy was successful in our study (Eldrup & Richter 2000). We also investigated rats that had been sympathectomized after adrenal demedullectomy. Such rats may die in hypotensive shock (Goldstein 1995c). The rats in our study, however, behaved normally and gained weight until final anesthesia.

Adrenal demedullectomy in rats

Adrenal medulla on both sides was destroyed by electrocoagulation in anesthetized rats. This procedure does not affect basal adrenal glucocorticoid secretion (Richter et al 1980). Tissue content of Adr indicated that adrenal demedullectomy was successful (Eldrup & Richter 2000).

3.3. STATISTICAL METHODS

Concentrations of DOPA, DOPAC, NA, DA and Adr in tissues were given as ng/g wet weight or µg/g wet weight, while plasma or cerebrospinal fluid (csf) concentrations of DOPA, DOPAC, DA-S, NA, DA and Adr were given in ng/ml, µg/l or the SI unit nmol/l and presented as medians with interquartile ranges. Proportions were presented as percentages with the 95% confidence interval in parentheses. Results from different individuals or animals are unpaired data and differences between medians of plasma, csf or tissue concentrations were analyzed with the Mann-Whitney rank sum test/Mann-Whitney two-sample rank sum test/Mann-Whitney test (erroneously named Wilcoxon rank sum test in Eldrup et al 1989a). The Kruskal-Wallis analysis on ranks with post hoc tests by Dunn's multiple comparison procedure was used to compare more than two groups of animals. Results from the same individual or animal are paired data and differences between medians of plasma or tissue concentrations were analyzed with the Wilcoxon's matched pairs test (Wilcoxon's one-sample rank sum test; erroneously named Mann-Whitney matched pairs test in Eldrup et al 1989a). Repeated samples from the same individual were analyzed by Friedman repeated measures anova on ranks/Friedman test and in some cases post hoc tests were done by Dunn's multiple comparison procedure. Two-way analysis of ranks was used to analyze interaction from an intervention (Bradley 1968 but also described in Andersen & Holm 1984). Relationship between data was initially analyzed by linear correlation and regression analysis (Pearson's correlation coefficient r) or later and more correctly for non-normally distributed data with the Spearman rank order correlation (Spearman's correlation coefficient R_s). The chi-square test was used to compare proportions in several groups.

The power of a test, P_W, is 100% – β, β being the type 2 error. The chance of a false negative finding depends on the interaction between the strength of the signal (i.e. the true difference between groups), the amplification (i.e. the number of subjects investigated) and the amount of noise (i.e. the stochastic variation). If data fulfil the assumptions of the t-test, the relationship between type 1 and type 2 errors (2α and β), stochastic variation (the true standard deviation, SD, of the parameter measured), number of patients (N_1 and N_2 subjects that are compared), signal (the true difference, D, between the central measure of the samples in the two groups of subjects) may be described mathematically by the equation, $N_2 = N_2 = 2(t_{2\alpha} + t_\beta)^2 SD^2/D^2$, where $t_{2\alpha}$ and t_β are the significance limits of the Student t-test for the degrees of freedom that the number of subjects determine (Andersen & Holm 1984). If comparing groups of 8 subjects with a power of 80% and an error of the first kind of 5%, it can be calculated that the true difference, D, of a measure must be 1.5 times the true SD of this measure. Often, however, the true SD in the population is not known.

Statistical analyses were done with the Medstat Program vers.1-3, Astra-Gruppen A/S, Denmark, or SigmaStat Statistical Analysis System vers.1.02, Jandel Corporation. $P<0.05$ defined statistical significance.

3.4. BIOCHEMICAL MEASUREMENTS

Initially, an analysis was set up in order to measure DOPA, NA, Adr, and DA simultaneous in one sample. The method should analyze samples of plasma, cerebrospinal fluid, and tissue extracts. Later, during 1989, it became evident that the analysis could be optimized and measure DOPAC as well.

Fluorimetric techniques have occasionally been found useful to study plasma catecholamine levels (Vendsalu 1960, Lee et al 1987) and urine DOPA and DOPAC excretion (Von Studnitz et al 1963, Sourkes et al 1963). It has, however, generally been agreed that the development of radioenzymatic assays (REA) and assays based on hplc have provided better sensitivity and accuracy in this field (Hjemdahl 1984a, Kaagedal & Goldstein 1988).

A method based on rp-hplc with electrochemical detection (rp-hplc-ED) was chosen. The analyses on which this thesis is based were performed from October 1987 to October 1992.

Sample handling and storage

Plasma samples were prepared as blood was drawn into ice-chilled tubes containing 1.7 mg/ml ethylene glycol-bis (β-aminoethyl ether)-N, N, N', N'-tetraacetic acid (EGTA) and 1.1 mg/ml reduced glutathione (final concentrations). After centrifugation plasma was stored at –80°C or at –20°C until analysis.

The importance of the temperature of the blood samples until centrifugation and of the time that elapsed from blood sampling to centrifugation with subsequent separation of the plasma were investigated during our studies. Venous blood samples were obtained from 4 of the 7 men that participated in the meal study (Eldrup et al 1997). Tubes with EGTA and glutathione were stored at –20°C. After blood sampling the tubes were left for 0, 15, 30, 45, 60, 90, and 120 min either at ambient temperature (approximately 20°C) or at 0°C. Then samples were proceeded for measurements of plasma catecholamines as described below. The plasma concentration of each compound was related to the plasma concentration at 0 min. The mean percentage of between 2 and 4 measurements are shown in Table 2. Statistical analysis was not done due to the few measurements. It appears, however, that tubes should be kept ice-chilled until centrifugation which should be performed within 60 min when measuring NA and Adr, while samples apparently can be left either at room temperature or in ice up to 2 h when measuring DOPA, DOPAC and DA. These results are in accordance with those of others regarding NA, Adr and DA (Bouloux et al 1985, Boomsma et

Table 2. *Average percentages of initial concentrations of DOPA, NA, Adr, DOPAC, and DA after 1 ml blood sample was drawn into an ice-chilled tube with 1.7 mg EGTA and 1.1 mg glutathione and left at room temperature (20°C) or on ice (0°C) for 15, 30, 45, 60, 90, and 120 minutes, respectively (n=2-4 each).*

	DOPA		NA		Adr		DOPAC		DA	
	0 °C	20 °C	0 °C	20 °C	0 °C	20 °C	0 °C	20 °C	0 °C	20 °C
0’	100	100	100	100	100	100	100	100	100	100
15’	104	99	107	94	94	93	95	97	101	98
30’	102	98	94	82	107	97	94	99	103	98
45’	104	101	104	83	91	99	99	100	99	100
60’	105	104	98	74	95	84	97	99	104	93
90’	98	104	88	77	83	69	94	100	99	98
120’	100	109	88	74	103	73	96	102	105	102

al 1993, Hjemdahl 1993), but in contrast to reports concluding that samples of catecholamines in blood can be left at room temperature for up to 7 hours (Pettersson et al 1980, Brent et al 1985, Weir et al 1986, Rumley 1988). No other similar investigations of DOPA and DOPAC were found in the literature. The reasons for the different findings are not known. No reports indicate that keeping blood samples ice-chilled for 60 min are harmful to stability of Na, Adr, DA, DOPA or DOPAC. We chose to keep whole-blood samples in ice and separation of plasma by centrifugation was done within 30 min. Generally, both a chelating agent and an antioxidant are used to preserve catecholamines in many (Peuler & Johnson 1977, Johnson et al 1978, Hjemdahl 1987, Eriksson 1989), but not all methods (Eisenhofer et al 1986, Kaagedal & Goldstein 1988, Boomsma et al 1993, Holmes et al 1994). Systematic investigations of DOPA and DOPAC in this respect have not been done. The use of both glutathione and EGTA was adapted from Peuler & Johnson (1977) and was also later recommended by Hjemdahl (1993).

Plasma was separated by centrifugation at 4°C and immediately frozen at –20°C or at –80°C. We also investigated the stability of DOPA, NA, Adr, DOPAC, and DA in plasma as plasma samples from the plasma pool used in each analysis as described below were kept at –20°C and thawed after different intervals up to 26 months after blood sampling. The concentration of each compound was related to the mean concentration at the time of blood sampling being 1.22 ng/ml for DOPA, 0.18 ng/ml for NA, 0.10 ng/ml for Adr, 2.63 ng/ml for DOPAC, and 0.07 ng/ml for DA. The mean of 1-4 measurements at different months after blood sampling is shown in Fig. 1. As can be seen from this fig, DOPA, DOPAC and DA seem to be quite stable at –20°C, while NA and Adr stored at this temperature probably should be analyzed within 3 months. Eriksson (1993) reported that NA in plasma (1.01 ng/ml) stored with EGTA and glutathione were stable at –20°C up to 18 months, while heparinized or

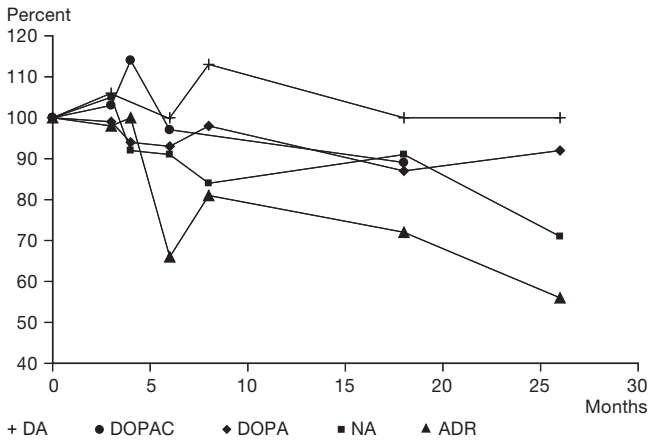

Fig. 1. *Average percentages (n=1-4) of original concentrations of DOPA, DA, DOPAC, NA and A measured after storage of samples at –20°C (Eldrup, unpublished).*

EGTA samples were only stable between 2 and 6 months. We did not perform experiments with storage of plasma at –80°C. Others have reported that heparinized samples of endogenous catecholamines are stable at –70°C to –80°C between 1 and 3-6 years after blood sampling (Goldstein 1986, Eriksson 1993, Hjemdahl 1993). Very high concentrations of DOPA are also stable at –80°C up to 4 months but unstable at –25°C and +4°C (Zürcher and Da Prada 1990). Samples in our investigations were kept at –20 °C for up to 2 months and up to 6 months at –80°C before analysis.

Tissue was homogenized in ice-chilled 0.4-0.6 M perchloric acid (PCA) containing 1.7 mg/ml EGTA and 1.1 mg/ml reduced glutathione (final concentrations) (Kehr et al 1972, Wilk 1986). Homogenates were stored at –80°C until assayed (within 3 months).

Cerebrospinal fluid was drawn into ice-chilled tubes containing 1.7 mg/ml EGTA and 1.1 mg/ml reduced glutathione (final concentrations). Samples were immediately frozen and stored at –20°C at first and then at –80°C until analyzed within 3 months. Others have used a similar procedure (Kaagedal & Goldstein 1988).

Sample preparation for assay of dopamine sulfate

DA-S was measured as tDA after addition of 0.75 unit sulfatase (arylsulfatase EC 3.1.6.1) to 1 ml plasma with subsequent incubation at 4°C for 10 min. This procedure compared to incubation at higher temperatures or for longer time periods was only of minor importance in respect to tDA measurements (Eldrup et al 1997). This procedure, however, resulted in the largest amounts of total NA and total Adr (unpublished results). Results from measurements of sulfoconjugated NA and Adr have not been included in this thesis. Based on the results described we choose the above protocol for our analyses. Sulfatase was diluted by distilled water. Alumina was added directly to the mixture of plasma and sulfatase. Others found that 95 mU sulfatase incubated with 760 µl plasma for 20 min at 37°C hydrolyzed DA-S almost completely (Yamamoto et al 1996). Some investigators have used acid hydrolysis for the determination of tDA (Tyce et al 1987). Acid hydrolysis does not separate DA-S from dopamine glucuronide but in human plasma the concentration of dopamine glucuronide is negligible and reasonable agreement has been found between DA-S measurements obtained by enzymatic hydrolysis and those obtained by acid hydrolysis though no direct comparisons have been published (Kuchel & Buu 1983, Ziegler et al 1986, Tyce et al 1987).

Sample preparation – alumina batch extraction

DOPA, DOPAC, and catecholamines were extracted from plasma, csf, and tissue homogenates with alumina (Al) as described by others (Anton & Sayre 1962, Anton & Sayre 1964, Hjemdahl 1987, Ehrenström 1988). Tris buffer containing 2% sodium EDTA was added to increase pH to 8.6. Furthermore, 30 µl of 10 mM sodium metabisulphite ($Na_2S_2O_5$) was added per µl sample. We used 25 mg activated Al for 1 ml plasma and 150 mg activated Al for 3 ml plasma and eluted with 250 µl or 2.5 ml 0.2 M PCA with 0.1 mM $Na_2S_2O_5$. The larger volume was freeze-dried and reconstituted in 500 µl HCl before 10 ml Instagel® liquid scintillation fluid was added. Recovery of ^{3}H-DOPA during Al extraction without reconstitution in HCl after freeze-drying was 31.3 (28.6-35.4)% (mean (range) of 6 samples) but increased to a mean (range) of 76.4 (70.4-78.2)% (n=6) with reconstitution in HCl as described ($p<0.01$). Slightly lower recovery (48.2-63.4%, n=2) was obtained if 75 mg Al was used instead of 150 mg Al (56,0-61,0%, n=2) when 3 ml plasma was extracted (unpublished results).

Experiments indicated decreasing recovery of ^{3}H-DOPA with increasing amounts of Al, decreasing amounts of PCA, and decreasing concentrations of PCA as shown in Table 3 (unpublished). Recovery was 15-20% lower if 0.2 M HCl was used to elute catecholamines from Al. Recovery experiments in the laboratory with addition to plasma of ^{3}H-DOPA and ^{3}H-NA, demonstrated a recovery of 73.0 (70.2-74.8)% and 42.5 (41.1-44.3)%, respectively (mean (range);

Table 3. *Average recovery (range; n=1-4) of ^{3}H-DOPA after alumina extraction with different amounts of alumina and different amounts and different concentrations of perchloric acid (PCA).*

Alumina Mg	0.05M PCA 200 µl	0.1M PCA 200 µl	0.2M PCA 100 µl	0.2M PCA 200 µl	0.2M PCA 300 µl	0.2M PCA 400 µl
25	27.0% (23.9-32.3)	48.4% (47.0-50.5)	41.0%	60.1% (59.4-60.7)	69.7%	72.8%
37.5			24.0%	55.3%	64.4%	71.8%
50		19.0% (15.3-21.0)	8.4%	48.0%	60.6%	66.0%

n=10) after extraction with 25 mg Al and desorption with 250 µl 0.2 M PCA, which was the procedure finally chosen. Our experiments confirmed that ^{3}H-DA eluted easily with many acids from 25 mg Al as 71.8-75.1% recovery was obtained when 200 ml of 0.05 M PCA, 0.1 M PCA, or 0.1 M HCl were used (n=2 each, unpublished). Recovery experiments with DOPAC or Adr were not performed. Others have used an alumina extraction procedure comparable to ours (Eriksson & Persson 1982, Eisenhofer et al 1986, Hjemdahl 1987, Premel-Cabic & Allain 1988, Ehrenström 1988, Eriksson 1989).

The PCA eluate was centrifuged and 25 µl 3 M KCl was added to 200 µl of the supernatant. This mixture was passed through a 0.45 µM filter and injected into the hplc system. Eluting with phosphoric acid and also with sulphuric acid resulted in higher recoveries than those obtained with PCA (Wenk & Greenland 1980, Bouloux et al 1985). In our hands, however, these acids created a large solvent front and hampered detection of DOPA. The use of PCA, sulphuric acid or phosphoric acid with high ionic strength is necessary to desorp catecholamines from alumina but they create large solvent fronts on the chromatogram. We minimized this problem by adding KCl to the PCA eluate and thus injecting catecholamines dissolved in HCl into the hplc system. Other techniques for extracting catecholamines from body fluids could not be used, as they do not extract DOPA or DOPAC (Higa et al 1977, Smedes et al 1982, Macdonald & Lake 1985). The importance of adding an antioxidant to the acid eluting catecholamines from alumina and keeping the alumina eluates refrigerated have been emphasized by others (Hugh et al 1987, Holmes et al 1994, Candito et al 1995). Candito and coworkers (1995) observed that DOPAC only remained stable in perchloric acid extracts for 2 hours at 4°C in the dark. No signs of a systematic decrease in detector response to DOPAC were observed in our assay. An improved assay which used only 5 mg alumina per ml of plasma and eluted with 100 µl 0.04 M phosphoric acid – 0.2 M acetic acid (20:80, v/v) was published after we had completed our measurements (Holmes et al 1994).

Chromatographic and detection conditions

Equipment from Millipore Waters A/S, Denmark, was used. An automated sample processor, model 710B equipped with a cooling device that kept the samples at 4°C injected samples of 50 to 100 µl. A M510 pump with a pulse dampener delivered the aqueous mobile phase of 0.1 M sodium phosphate with (per liter) 100 mg sodium EDTA and 200 mg sodium octane sulfate and 2% acetonitrile. The buffer was adjusted to pH 3.5 with 85% phosphoric acid. This ionic strength, pH, concentration of metal ion chelating agent, concentration of ion pairing agent and concentration of organic modifier resulted in the best chromatographic conditions measuring both DOPA, NA, Adr, DOPAC, and DA. The composition was based on previous extensive studies of hplc in measurements of NA, Adr, DA, DOPA and DOPAC (Moyer & Jiang 1978, Moyer et al 1979, Mefford et al 1981, Mefford 1981, Krstulovic 1982, Goldstein et al 1984, Eisenhofer et al 1986, Hjemdahl 1987) and our own experiences, and they were in accordance with later reports (Kaagedal & Goldstein 1988, Ehrenström 1988, Bartlett 1989). The mobile phase was degassed by helium, filtered and was not recirculated during analyses. The analytical column was a prepacked stainless steel column, 150×4.6 mm (not 150×46 mm as erroneously stated in Eldrup et al 1989a and Eldrup et al 1989b), with RP C-18 hydrocarbonaceous surface 5 µm particles (Millipore Waters, Denmark). In order to optimize chromatographic conditions a C-18 Spherisorp 5 µm particle size column (Microlaboratory A/S, Aarhus, Denmark) later replaced the RP column. Waters Temperature Control System kept the column at a constant temperature of 30°C. Temperature influences retention time as has been reported by others (Krstulovic 1982, Holmes et al 1994). A M460 amperometric electrochemical detector with a glassy carbon working electrode operated at 600 mV with a working potential of 0.57-0.59 V at ambient temperature detected the separated substances. Data were analyzed by Waters 810 baseline data system. We did not succeed when we tried to separate catecholamines with solvent gradients as had been done by others (Goldstein et al 1984, Eisenhofer et al 1986). Initially the mobile phase flow rate was 0.4 to 0.6 ml/min while eluting DOPA (detection time 7.30-7.55 to 9.25-9.50 min) and NA (detection time 9.50-9.75 to 12.50-12.75 min), and 1.0 ml/min while eluting DA (detection time 27.00-27.50 to 30.25-30.75 min). This flow rate, however, partly impaired Adr detection and was therefore changed to a constant rate of 0.6-0.8 ml/min. DOPA was then detected about 5.00-5.25 min after injection, while NA, Adr, DOPAC, and DA were detected after approximately 6.50-6.75, 10.75-11.00, 12.75-13.25, and 25.00-26.00 min, respectively. The internal standard dihydroxybenzylamine (DHBA) was detected after approximately 14.25-14.75 min. Run time per sample was between 35 and 45 min depending on the body fluid assayed and the age of the column.

An assay consisted of up to 25 samples. In every assay, the first four and the last four samples were one of each aqueous standard solution of 0 pg/ml, 500/100/500/25/25 pg/ml, 1500/300/1500/75/75 pg/ml, and 3000/600/3000/150/150 pg/ml of DOPA/NA/DOPAC/Adr/DA, respectively (original concentrations). The electrochemical detector response was linear up to 10 ng/ml for all components measured. Others have found linearity up to 500 ng/ml with amperometric electrochemical detectors (Moyer et al 1979, Shum et al 1982, Ehrenström 1988, Premel-Cabic & Allain 1988). Samples with higher concentrations than 10 ng/ml were diluted or only 10 µl were injected in a second run. An assay was only accepted if the determination coefficient (r^2) of the standard curve was 0.99 for DOPA, NA, and DOPAC, and above 0.95 for Adr and DA. Moreover, two samples from a plasma pool were included in every assay. Seventy-five pg of Adr and 75 pg of DA were added to one of these samples. If one of these samples was more than 10% above or below the mean of the DOPA, NA, or DOPAC concentrations (15% for Adr and DA concentrations) found in the primary intra-assay variation analysis, the analysis of the compound in that assay was not accepted.

Calculation of sample concentrations – precision of analysis

Each compound was identified by the detection time, i.e. the time from injection to peak maximum as registered electronically by the data system. Detection times of DOPA, NA, Adr, DOPAC, DHBA, and DA in standard solutions were determined within narrow limits, below 0.15 min, in every analysis. Addition of known amounts of each compound to unknown plasma samples identified each compound during development of the analysis (Hjemdahl et al 1979, Eriksson 1989).

The baseline in all chromatograms was visually corrected if necessary, which was often the case. Baseline separation was most often obtained for NA, Adr, DOPAC, DA, and DHBA, while DOPA eluted near the basis of the solvent front of the chromatogram. Peak heights of the sample against those of the standard curves were used to calculate the concentrations in the sample. Correction was made for amount injected and the volume of the original sample before Al extraction.

The limit of detection in aqueous standards defined as two times baseline noise was 0.02 ng/ml for DOPA (decreasing from 0.05

ng/ml) and DOPAC, and 0.01 ng/ml for NA (decreasing from 0.02 ng/ml), Adr and DA. Interfering peaks in the chromatogram, "ghost peaks", occasionally made identification of Adr, DA and DOPAC peaks difficult or impossible. This problem increased with age of the column but also with age of the chromatographic system. Others have found similar (D'Eril & Rizzo 1991), higher (Lee et al 1987, Ahlskog et al 1996a) and slightly lower (Grossman et al 1992a, Yamamoto et al 1996) limits of DOPA sensitivity. Some authors present the limit of sensitivity as the lowest amount injected into the HPLC system to give a certain response of the detector and a comparison is not possible (Eisenhofer et al 1986). For NA, Adr, DOPAC, and DA our limits of detection were better or comparable to other assays, but performance of the assay regarding resting endogenous plasma concentrations of Adr and DA was not always satisfactory as also found by others (Rogers et al 1991, Ahlskog et al 1996a). Samples sometimes had to be reanalyzed several times and thus material could become sparse. This is the reason that we did not report plasma and csf DA-S concentrations in Parkinsonian patients (Eldrup et al 1995).

Methyl-dopamine (mDA, also called deoxyepinephrine) was used initially as an internal standard but after the flow rate became uniform, DHBA was used. Each sample was manually corrected for analytical recovery using the internal standard (Eldrup et al 1989a, Eldrup et al 1989b). Analytical recovery of mDA and DHBA, however, clearly varied with an intra-assay coefficient of variation (CV) of 3-4% (each n=10). This observation is in accordance with reports from others (Moyer et al 1979, Ehrenström 1988). One author found that recovery of the internal standard DHBA consistently was higher than the recovery of DOPA and DOPAC (Eisenhofer et al 1986). In a plasma sample with DOPA, NA, Adr, DOPAC, and DA mean concentrations of 1132 pg/ml, 241 pg/ml, 107 pg/ml, 2838 pg/ml, and 80 pg/ml (n=10), intra-assay CVs were 3.5%, 6.1%, 16.3%, 1.6%, and 24.7%, respectively, when corrected for analytical recovery by DHBA. Intra-assay CVs, however, were 3.9%, 6.6%, 4.6%, 3.4%, and 11.5% (n=10) when DHBA analytical recovery was not taken into account. We therefore decided that DHBA should be included in each sample as an internal standard, but no correction for DHBA recovery was made. A sample was reanalyzed, however, if the DHBA peak height in the sample was more than 10% different from the mean of DHBA peak heights of the standard samples. Others have also questioned the use of internal standards and omitted these in HPLC catecholamine analysis (Moleman & Borstrok 1985).

Intra-assay CVs were determined several times during the studies in 10 samples of pooled plasma (Eldrup et al 1989a, Eldrup et al 1995, Eldrup et al 1997). CVs were 3.9-5.4% for DOPA (at 1.1-1.3 ng/ml), 3.7-6.6% for NA (at 0.1-0.5 ng/ml), 4.6-11.4% for Adr (at 0.11-0.13 ng/ml), 3.4-4.6% for DOPAC (at 2.1-2.8 ng/ml), 9.7-11.5% for free DA (at 0.07-0.08 ng/ml), and 2.8% for tDA (at 4.6 ng/ml).

Inter-assay CVs of an identical plasma sample as analyzed in every assay were 4.5-7.6% for DOPA, 3.6-8.5% for NA, 12.5% for Adr, 5.9% for DOPAC, 14.0-16.0 % for free DA, and 5.5% for tDA (n=10-18, Eldrup et al 1989a, Eldrup et al 1995, Eldrup et al 1997). Others found similar or slightly lower intra-assay and inter-assay CVs (Goldstein et al 1981a, Davies & Molyneux 1982, Eisenhofer et al 1986, Dizdar et al 1991, Goldstein et al 1999), but most studies did not report CVs. Within-day and between-day CV decreased with increasing concentrations of NA, Adr and DA (Davies & Molyneux 1982). The precision of our assay was satisfactory and state of the art regarding DOPA, NA, DOPAC and DA-S. In contrast to these compounds, larger percentage changes in plasma concentrations at normal plasma levels of Adr and DA are necessary before they will be detected. We did not, however, draw any major conclusions from measurements of free Adr and free DA in plasma. Only measurements of free DA after conversion of tDA to free DA with sulfatase in human plasma were concluded upon. DA concentrations were higher in most rat tissues than in plasma and thus detection was easier and at higher concentrations variance was clearly smaller with the present rp-hplc-ED method.

Accuracy of analysis

The true values of NA, Adr, DA, DOPA, DOPAC or DA-S in any body fluid are not known. The use of aqueous standards may theoretically result in some inaccuracy of our assay (Moleman & Borstrok 1985). It is not, however, possible to provide plasma or csf without the compounds measured (Eriksson 1989). Standard addition as used for Adr and DA in each assay and as successfully performed for DOPA, NA and DOPAC during development of the analysis partly compensated for this source of error that are inherent to all assays measuring these compounds. Others, however, proposed the use of calibration with aqueous standards for the analysis of NA, Adr, and DA (Candito et al 1990, Candito et al 1996).

Results from healthy humans obtained with our assay and by others are shown in tables 1A and 1B. No major differences were observed.

In 81 samples the results from measurements of NA and Adr by our rp-hplc-ED method were compared with the results obtained by REA (Christensen et al 1980). The variables were not normally distributed. The Spearman rank correlation coefficient Rs was 0.91 ($p<0.005$) for NA and 0.80 ($p<0.005$) for Adr. The concentrations in the samples were in the range of 0.12-0.91 ng/ml for NA and 0-0.64 ng/ml for Adr (unpublished results). Others reported that the correlation between an rp-hplc-ED method and a REA method was 0.99 for NA and Adr (Goldstein et al 1981b). In an inter-laboratory comparison of plasma catecholamines-assays it was concluded that rp-HPLC methods were among those with the largest variability for Adr and NA (Hjemdahl 1984b). The same author obtained a Pearson correlation coefficient of above 0.99 for both Adr and NA measurements when a cation exchange HPLC assay was compared with a REA assay (Hjemdahl 1984a). If the Pearson correlation coefficient was calculated in our comparison of 81 samples determined by RP HPLC-ED and REA, r was 0.97 for Adr and 0.91 for NA.

Thus, our RP HPLC-ED assay measuring DOPA, NA, Adr, DOPAC and DA in the same sample performed well and was state of the art with the well-known limitations with respect to measurements of Adr and DA concentrations below 0.1 ng/ml. The analysis was both accurate and precise. The assay could be improved and variation decreased if duplicate measurements of each sample were performed.

4. RESULTS

4.1. RELATIONSHIP TO SYMPATHETIC ACTIVITY

DOPA

Early in the era of reliable p-DOPA measurements Eisenhofer, Goldstein, Kopin and co-workers made the assumption that p-DOPA concentrations are dependent on neurotransmitter turnover in noradrenergic neurons. This was evidenced by p-DOPA changes after pharmacological intervention supposed to change TH activity in rats, dogs and humans (Goldstein et al 1987a, Eisenhofer et al 1989, Garty et al 1989b). Absence of an arterio-venous increase in p-DOPA levels in sympathectomized limbs and a decrease in p-DOPA concentration in humans after administration of clonidine were found (Goldstein et al 1987a). Furthermore, correlations were observed between p-DOPA and NA metabolism and the rates of NA entry into plasma (Eisenhofer et al 1989). It was concluded, that p-DOPA quantification was useful as a simple and direct index of TH activity in vivo (Eisenhofer et al 1988). Others (Devalon et al 1989) made the same conclusion because p-DOPA increased after peak exercise and increased after exercise training. Boomsma et al (1988), however, in 5 hypertensive patients found no changes in p-DOPA during various sympathetic stimuli like standing, tilting, graded bicycle exercise till exhaustion and i.v. administration of tyramine, but no data were presented.

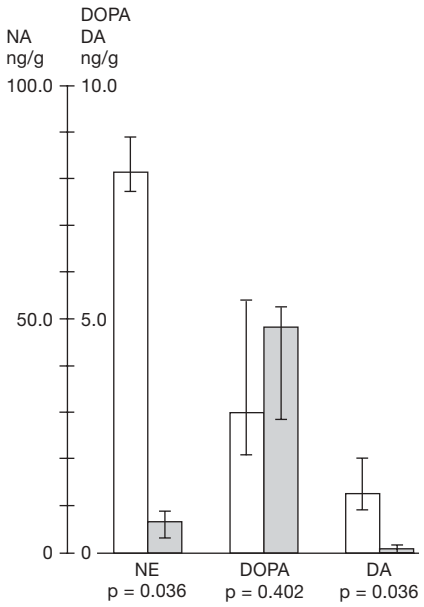

Fig. 2. *Concentrations (ng/g wet wt, medians and interquartile ranges) of NA, DOPA, and DA in rat quadriceps muscle. Open bars are values from controls and hatched bars are values from surgically sympathectomized animals. (Modified after Eldrup et al (1989a), Am J Physiol Endocrinol Metab, 256, E284-E287; with permission).*

Our investigations have seriously questioned a close relationship between sympathetic nervous activity and p-DOPA concentrations. First we have demonstrated in rats that muscle content of DOPA was unchanged after sympathectomy decreasing NA content more than 90% suggesting that there is no depot of DOPA in sympathetic nerves (discussed in more details below). Thus, DOPA is unlikely to spill over to plasma as a result of NA release (Fig. 2, Eldrup et al 1989a). Secondly, we have demonstrated that there is no relationship between forearm venous p-DOPA and muscle sympathetic activity as measured by forearm venous p-NA concentrations. P-DOPA levels were unchanged after standing up both in middle-aged healthy subjects and in diabetics with and without autonomic neuropathy (Fig. 3, Eldrup et al 1989b). Furthermore, we demonstrated

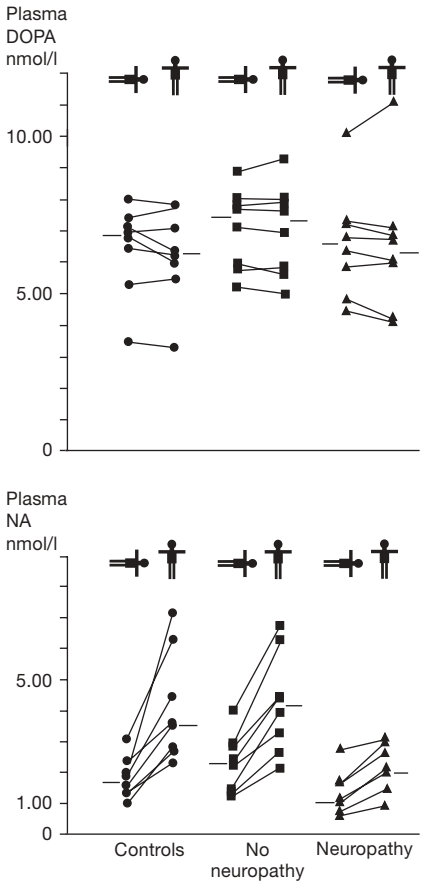

Fig 3. *Individual values of venous p-DOPA and p-NA concentrations in healthy controls (•), diabetic patients without neuropathy (■), and diabetic patients with neuropathy (▲) in supine position and after 10 min standing up. Horizontal bars indicate median values. (modified after Eldrup et al (1989b), Eur J Clin Invest, 19, 514-517; with permission).*

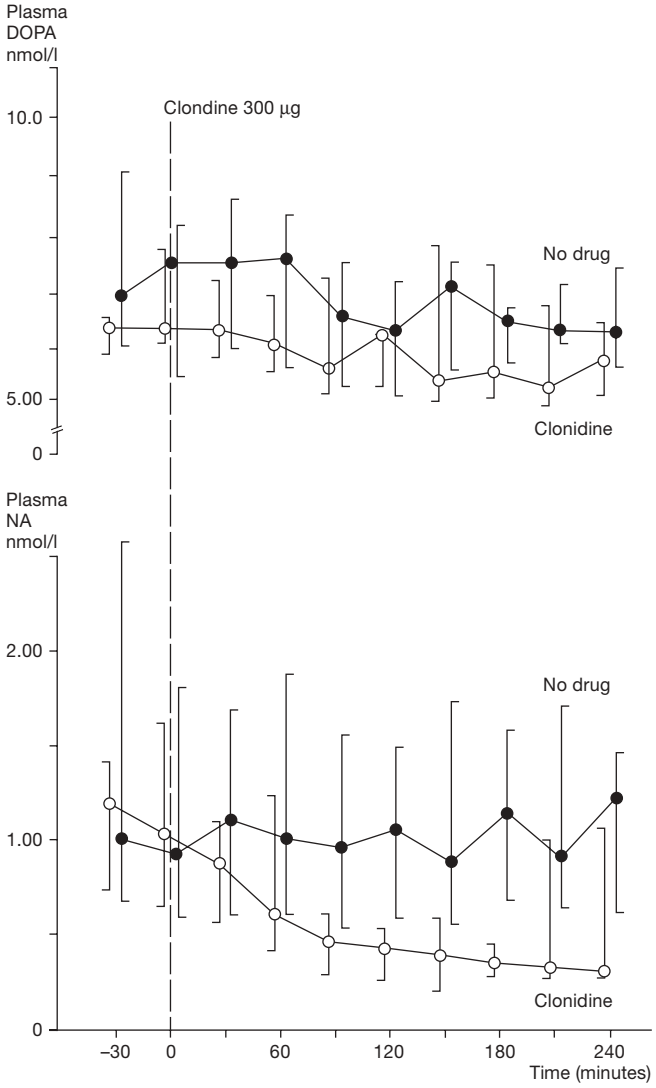

Fig. 4. *Venous p-DOPA and p-NA concentrations before and after peroral administration of 300 µg clonidine (○) or no drug (•) at t = 0 min. Values are medians (interquartile range, n = 7). (modified after Eldrup et al (1989b), Eur J Clin Invest, 19, 514-517; with permission).*

that the decrease in p-DOPA levels after clonidine administration was not related to changes in sympathetic activity caused by clonidine (Fig. 4, Eldrup et al 1989b (the p-DOPA decrease with time was analyzed with a Friedman test while the interaction between time and clonidine treatment was analyzed with a two-way analysis)). Others have confirmed that p-DOPA concentrations in healthy humans may decrease spontaneously (Goldstein et al 1992). The observation that an arterio-venous increase of p-DOPA concentrations across the forearm was absent in sympathectomized limbs (Goldstein et al 1987a) may be explained by an increase in limb blood flow. Interestingly, but unexplained, Goldstein recently found no difference in arterio-venous increase of p-DOPA between normal and sympathectomized hands or feet in patients with reflex sympathetic dystrophy (Goldstein et al 2000a). Anton (1991) also questioned that p-DOPA is a valid indicator of sympathetic activity.

In different experiments with manipulations that induced major changes of plasma NA, there were no or minimal changes of p-DOPA levels observed in humans (summarized in Table 4). After α_2–receptor blockade with yohimbine increasing p-NA more than 100%, no significant changes in venous and arterial p-DOPA levels were observed (Goldstein et al 1987a, Goldstein et al 1991b). Intravenous administration of isoprenaline (which in previous experiments increased p-NA 81%) and trimethaphan (a ganglionic blocker decreasing p-NA) did not change p-DOPA concentrations

Table 4. *The results from experiments in which changes (Δ% above baseline) in sympathetic activity measured as p-NA concentration or plasma NA spillover (SO) were related to changes of p-DOPA concentrations. A few investigations also included data for changes in p-DOPAC or p-Adr concentrations.*

Species	Stimulus	Site	NA conc Δ%	NA SO Δ%	DOPA Δ%	DOPAC Δ%	Adr Δ%	Reference
Human	Exercise	v	370		22*			*Morita et al* 1992
Human	Exercise	v	250		15			*Devalon et al* 1989
Human	Orthostasis	v	115		–7*			*Axelrod et al* 1996
Human	Orthostasis	v	113		0*			*Eldrup et al* 1989b
Human	Orthostasis	v	97		–6*	–9*		*Eisenhofer et al* 1986
Human	Isoprenaline	v	~81		–2*			*Goldstein et al* 1987a
Human	Exercise	v	45		–3*		42	*Weicker* 1988
Human	Hypoglycemia	v	32		0*	0*	2919	*Goldstein et al* 1992
Human	Water immers	v	–15		8*			*Grossman et al* 1992a
Human	Clonidine	v	–70		0*			*Eldrup et al* 1989b
Human	Insulin (euglycemia)	av	44		–17			*O'Hare et al* 1989
Human	Hypoglycemia	a	186		12*	17*	2530	*Elman et al* 2001
Human	Yohimbine	a	114		–3*			*Goldstein et a*l 1991b
Human	Exercise	a	60		2*		13	Weicker 1988
Human	Coffee	a		81	14			*Eisenhofer et al* 1991
Human	handgrib exerc.	a		59	9			*Eisenhofer et al* 1991
Human	mental stress	a		37	4			*Eisenhofer et al* 1991
Human	Desipramine	a -		17	–4			*Eisenhofer et al* 1991
Monkey	Ketamine	v	116		4*			*Goldstein et al* 1987a
Dog	Metyltyrosine	v	–12		–62			*Goldstein et al* 1987a
Dog	Metyltyrosine	v	–54		–34			Goldstein et al 1987a
Dog	Nitroprusside, 20 m	a	540		10*			*Goldstein & Eisenh.*1988
Dog	Nitroprusside, 80 m	a	340		90			*Goldstein & Eisenh.*1988
Dog	6-hydroxyDA	a	–29*		–6*	–53*		*Goldstein et al* 1991c
Dog	Reserpine	a	–70*		–20*			*Goldstein et al* 1991c
Rat	Pithed	a	2371		58			*Szemeredi et al* 1991
Rat	Hypoglycemia	a	411		39	177	5382	*Goldstein et al* 1993
Rat	Hypoglycemia	a	340		96	480	663	*Goldstein et al* 1993
Rat	Nitroprusside	a	326		96			*Garty et al* 1989b
Rat	Immobilization,60 m	a	300		51	306	2804	*Kvetnansky et al* 1992b
Rat	Hypoglycemia	a	134		0*	0*	1041	*Goldstein et al* 1993
Rat	Forskolin	a	114		34	37*		*Eisenhofer et al* 1988
Rat	Nitroprusside	a	69		11*			*Garty et al* 1989b
Rat	Hypoglycemia	a	65		20	146	580	*Goldstein et al* 1993
Rat	Reserpine,30 m	a	0*		–22	0*		*Eisenhofer et al* 1988
Rat	Desipramine	a	0*		–14	–21		*Eisenhofer et al* 1988
Rat	Phenylephrine	a	–26		0*			*Garty et al* 1989b
Rat	Chlorisondamine	a	–37		0*			*Garty et al* 1989b
Rat	6-hydroxyDA	a	–73		–42			*Grossman et al* 1992b
Rat	6-hydroxyDA	v	–82		–59			*Grossman et al* 1992b

a: arterial; v: venous; av: arterialized; *: p>0.05.

(Goldstein et al 1987a). Desipramine, inhibiting NA uptake, decreased arterial p-DOPA levels 3.6% while plasma NA spill over was decreased 17% (Eisenhofer et al 1991). During euglycemic insulin infusion arterialized p-NA concentrations increased while p-DOPA concentrations decreased (O'Hare et al 1989). Goldstein found no significant increases in venous and arterial p-DOPA concentrations during acute hypoglycemia induced by 2-deoxy-D-glucose despite 32% and 186% increase in venous and arterial p-NA levels, respectively (Goldstein et al 1992, Elman et al 2001). Hypoglycemia also significantly increased muscle sympathetic nerve activity (Frandsen et al 1989, Fagius & Berne 1989).

In dogs and rats various experiments have shown mostly minor changes in arterial and venous p-DOPA concentrations but most often in the same directions as much larger changes in p-NA concentrations. These results mainly from Eisenhofer, Goldstein, Kopin and co-workers are also summarized in Table 4.

Analysis of the data presented in table 4 indicates that there is a relationship between changes in p-NA and p-DOPA in animal experiments but not in human experiments (the estimate after isoprenaline in humans and the very high p-NA level in one rat experiment were excluded from analysis). P-DOPA seems to increase in animals provided p-NA increases 60% or more. Such large increments in sympathetic activity are likely to be associated with cardiovascular changes and possibly some decrease in the clearance of DOPA, which might explain the slight increase in p-DOPA. None of the investigations in Table 4 have evaluated if DOPA clearance was changed by the experimental conditions. Results from the investigations in table 4 are compatible with the view that DOPA is released from sympathetic nerve endings at high activity. The changes observed are, however, very small in contrast to the large increments in p-DOPA, which are observed after blockade of the decarboxylation of DOPA to DA (see below).

No close correlation between chronic changes of sympathetic nervous activity and p-DOPA levels has been demonstrated. We found no changes in basal p-DOPA levels in patients with diabetic autonomic neuropathy despite low p-NA levels (Fig 3, Eldrup et al 1989b (with Kruskal-Wallis' test, p = 0.004 for NA and p = 0.63 for DOPA)). Power of this study was adequate. Data were normally distributed and the power of finding a difference of 40% between p-DOPA in healthy individuals and p-DOPA in diabetic patients with neuropathy was 95% assuming a population SD of p-DOPA of 0.28 ng/ml (SD in controls, ref. Table 1A). Similarly, no changes of p-DOPA levels were found in multiple system atrophy (MSA, Shy-Drager syndrome) patients particularly not in the subgroup of MSA patients with low p-NA levels (Goldstein et al 1989b). In patients with pure autonomic failure (PAF), another form of neurogenic or-

thostatic hypotension, both normal and low p-DOPA levels were found despite low NA levels in all the patients (Goldstein et al 1989b, Meredith et al 1991). In another disease with orthostatic hypotension, familial dysautonomia, however, supine p-DOPA levels were 20% higher than in slightly older controls while p-NA levels were 48% higher (Axelrod et al 1996).

Furthermore, p-DOPA levels are not correlated with age-related changes in sympathetic activity. Venous p-DOPA concentrations in humans are not correlated with age (Johnson et al 1978, Eldrup et al 1995) or are decreased with age (Garty et al 1989a). Sympathetic activity, however, measured as venous p-NA concentrations increases with age (Christensen 1973b, Garty et al 1989a, Eldrup et al 1995, Jensen 1999), as does sympathetic muscle nerve activity (Wallin et al 1981).

In the heart, however, a relationship between DOPA spill over and sympathetic activity seems to exist. Goldstein found that spill over of DOPA from the heart was correlated with sympathetic activity, which may be explained by DOPA not being extracted in the heart (Goldstein et al 1991a, Eisenhofer et al 1992, Eisenhofer et al 1998, Eldrup et al 1998). P-NA as well as p-DOPA spill over across the heart are decreased in PAF patients and normal in patients with MSA (Meredith et al 1991, Goldstein et al 1997).

It may be that a proportionately larger increase in exocytotic NA release than NA synthesis in sympathetic nerves can explain a proportionately larger NA than DOPA response when increasing sympathetic activity (Goldstein et al 1995). No correlation between venous p-DOPA and venous p-NA concentrations has been found in contrast to the positive correlation that exists between venous p-NA concentrations and muscle sympathetic activity (Wallin et al 1981). Serial samples in the upright position would have been necessary to exclude that an increase in TH activity could increase p-DOPA levels after p-NA increase in orthostatic experiments. Results from such a protocol have never been published. Large increments in sympathetic activity have been related to small or insignificant increases in levels of p-DOPA in humans and major changes of sympathetic activity have been related to minor changes of arterial p-DOPA levels in rats and dogs. Taken together, existing evidence indicates that p-DOPA may be derived from sympathetic nerves as a result of TH activity, but p-DOPA is not a sensitive index of sympathetic activity or NA synthesis in the nerves.

DOPAC

Studies on p-DOPAC levels during changes of sympathetic activity in healthy humans or animals are few. P-DOPAC concentrations were unchanged during orthostasis in healthy humans while p-NA concentrations increased (Eisenhofer et al 1986, Axelrod et al 1996). After we were able to measure DOPAC in our analysis, we reanalyzed chromatograms and provided DOPAC data from the orthostatic experiments in healthy humans (Eldrup et al 1989b). These hitherto unpublished data disclosed that p-DOPAC concentrations did not change after an orthostatic manoeuvre, being 1.85 ng/ml (1.39-2.67 ng/ml, median (interquartile range), n=8) in the supine position and 1.60 ng/ml (1.33-2.05 ng/ml, n=8) 10 min after standing ($p>0.10$, Wilcoxon rank sum test). When chromatograms from our human clonidine study (Eldrup et al 1989b) were reanalyzed, DOPAC concentrations could be determined in five of seven of the healthy young men. Venous samples from the no drug and from the clonidine 300 µg experimental day disclosed no effect of clonidine on venous p-DOPAC concentrations (two-way analysis, $p>0.05$, unpublished). Again serial samples of p-DOPAC are needed to exclude that p-DOPAC reflect TH activity in orthostatic experiments but clonidine data suggest that there is no close relationship between p-NA and p-DOPAC levels in humans.

Some evidence exists that p-DOPAC concentrations are decreased in patients with chronically decreased sympathetic activity. In patients with PAF both normal and low p-DOPAC levels were found despite low NA levels (Goldstein et al 1989b, Yamamoto et al 1996). Cardiac production of DOPAC was low in PAF patients (Goldstein et al 1997). In MSA-patients p-DOPAC concentrations were both decreased and normal but cardiac DOPAC production was decreased (Goldstein et al 1989b, Goldstein et al 1997). In familial dysautonomia p-DOPAC was decreased despite increased p-NA and p-DOPA levels (Axelrod et al 1996). When chromatograms from the orthostatic experiments in diabetic patients with and without neuropathy (Eldrup et al 1989b) were reanalyzed, supine p-DOPAC concentrations were 1.84 ng/ml (1.47-2.53 ng/ml, median (interquartile range), n=8) in patients without neuropathy and 1.55 (0.93-2.79) ng/ml (n=8) in patients with neuropathy. Kruskal-Wallis one-way analysis on ranks disclosed no significant difference between supine p-DOPAC levels in healthy humans and the two groups of diabetic patients ($p=0.87$) but the power of this comparison was only 11% assuming a population SD of p-DOPAC of 0.79 ng/ml (SD in controls, ref. Table 1A). Thus, we may not appreciate a true difference of p-DOPAC concentrations between patients with decreased sympathetic activity and healthy subjects. In the upright position p-DOPAC concentrations were 1.78 ng/ml (1.45-2.53 ng/ml, median (interquartile range), n=8) in patients without neuropathy and 1.49 (0.92-2.68) ng/ml (n=8) in patients with neuropathy ($p=0.77$ when comparison was made between the two groups of diabetic patients and healthy subjects).

There is some support from animal studies that p-DOPAC originates at least in part from sympathetic nerves. DA synthesized in the axoplasma is deaminated to DOPAC if it is not taken up in vesicles and hydroxylated to NA (Goldstein 1995a). In rats DOPAC in blood was suggested to originate partly from sympathetic ganglia (Andén et al 1985). TH inhibition in dogs decreased p-DOPAC levels (Goldstein et al 1987a). Administration of reserpine, which inhibits vesicular uptake of DA and increases tyrosine hydroxylase activity, caused p-DOPAC levels to increase 10-fold after 15-16 hours in rats while p-NA levels decreased 68%. Another activator of tyrosine hydroxylase activity, forskolin, increased p-DOPAC levels 37% (but $p>0.05$%) while p-NA levels increased 114%. The NA reuptake inhibitor desipramine decreased p-DOPAC concentrations 21%, while p-NA concentrations were unchanged (Eisenhofer et al 1988). Immobilization stress increased p-DOPAC levels almost 3-fold in rat arterial plasma but p-DOPAC levels decreased during prolonged immobilization in contrast to p-NA levels that remained elevated (Kvetnansky et al 1992a). P-DOPAC levels increased in parallel with p-NA and Adr levels during and after severe hypoglycemia in rats but not in humans (Goldstein et al 1992, Goldstein et al 1993). Arterial p-DOPAC levels in rats, however, were unchanged after clonidine administration (Dong et al 1993).

Based mostly on animal studies, p-DOPAC concentration at least partly seems to be derived from sympathetic nerves. P-DOPAC may to some extent reflect TH activity, but p-DOPAC does not seem to be a sensitive index of sympathetic activity.

DA-S

Snider and Kuchel concluded in 1983 that total DA concentration is an index of sympathetic activity. Total DA are in humans almost identical to DA-S. Although investigations on this topic are not included in this thesis a short update of the relationship between plasma DA-S and sympathetic activity will be given. Several lines of evidence have accumulated that contradict the suggestion of Snider and Kuchel. In healthy humans venous p-DA-S concentrations were unchanged during orthostasis (Johnson et al 1980, Kuchel et al 1985a). No significant changes of p-DA-S concentrations were observed in healthy humans during and after bicycle exercise at between 30% and 90% maximal oxygen uptake for between 8 min and 60 min but plasma levels of NA and Adr were increased between 3-fold and more than 10-fold, respectively. (Ratge et al 1986b, Odink et al 1986, Sothmann et al 1987, Devalon et al 1989, Sothmann et al 1990, Rogers et al 1991, Ogaki et al 1995). Similarly, p-DA-S levels were not significantly changed after a steam bath, a 20 km cross-

country march, running a half-marathon or running a marathon, while p-NA and P-Adr levels increased between 1.5-fold and 10-fold (Ratge et al 1986b, Yoshizumi et al 1992, Sakai et al 1995). In one report, however, a 3.7-fold increase of venous p-DA-S was found immediately after a 10-h triathlon. Plasma free NA and Adr levels were increased 45% and 30%, respectively (Sagnol et al 1990). After a 24-h run, however, P-DA-S levels were not significantly changed while p-NA levels were increased 175% and p-Adr levels were not significantly changed (Sagnol et al 1990). Samples were collected between 10 and 11 p.m. after the triathlon. Swimming, bicycling and running activities lasted for 9.25 h of the 10 h triathlon. The finding reported by Sagnol et al could be explained by intake of food during the triathlon but no information about meal intake was given in the study and the reason for the p-DA-S increase observed remains unclear. The study does not support, however, that p-DA-S concentrations are related to sympathetic activity. Increasing or decreasing the sympathetic outflow by insulin-induced hypoglycemia, oral ingestion of clonidine, intravenous administration of nitroprusside or trimethaphan did not change p-DA-S (Eldrup et al 1988, Cuche et al 1990, Eisenhofer et al 1999). In patients with PAF p-DA-S levels were normal in contrast to p-NA and p-DHPG levels that were decreased (Yamamoto et al 1996).

Thus, reviewing the literature it may be concluded that p-DA-S concentration does not reflect sympathoadrenal activity and p-DA-S is not an index of sympathetic activity.

4.2. DOPA AND DOPAC IN MUSCLE TISSUE IN RATS

DOPA

Skeletal muscles play an important metabolic role in the exchange of amino acids with blood (Daniel 1977). After administration of DOPA to rats, skeletal muscles were the major reservoir of free DOPA (Romero et al 1973). Skeletal muscles are the largest tissue mass in non-overweight humans and rats comprising approximately 45% of body weight (Ordonez et al 1974, Daniel 1977). Muscle tissue seems to be the largest depot of endogenous DOPA at least in rats assuming homogenous DOPA concentrations in all skeletal muscles (Eldrup et al 1989a). Endogenous DOPA in rat muscle tissue does not seem to be located in sympathetic nerves as tissue concentrations of NA but not DOPA decreased after surgical sympathectomy (Fig. 2, Eldrup et al 1989a). In agreement with this finding it was evidenced that DOPA may be stored in a non-neuronal pool and released during skeletal muscle contraction (Szemeredi et al 1991). Goldstein's group found decreased DOPA content in skeletal muscle after sympathectomy with 6-OHDA indicating a neuronal localization of DOPA in muscle (Grossman et al 1992b). In contrast, in a similar experiment we found unchanged skeletal muscle DOPA content after sympathectomy with 6-OHDA, (Fig. 5, Eldrup & Richter 2000). Interestingly, Goldstein's group in the early experiments with 6-OHDA in rats found decreased DOPA content in all the tissues that were examined (Grossman et al 1992b) but in more recent and similar experiments found unchanged DOPA content after 6-OHDA in most tissues (Kawamura et al 1999). Skeletal muscle tissue DOPA content, however, was not reported in the recent investigation. Most evidence points towards a non-neuronal localization of DOPA in skeletal muscle.

Others have found that DOPA may be transferred via the blood circulation to the pancreas, liver and kidney in mice (Andén et al 1989). The exchange of DOPA between plasma and muscle has rarely been studied. Endogenous plasma insulin concentrations most probably play a role in the uptake of DOPA in muscles both in rats and in humans (Ordonez et al 1974, O'Hare et al 1989). Release of free DOPA from muscles to plasma has been shown in rats but the mechanisms of this release are unknown (Ordonez et al 1974). It is not known if humans with very low muscle mass or muscle degenerative diseases have low or normal p-DOPA concentrations. Thus, the impact of muscle DOPA depot on p-DOPA concentrations and vice versa in humans has not been clarified.

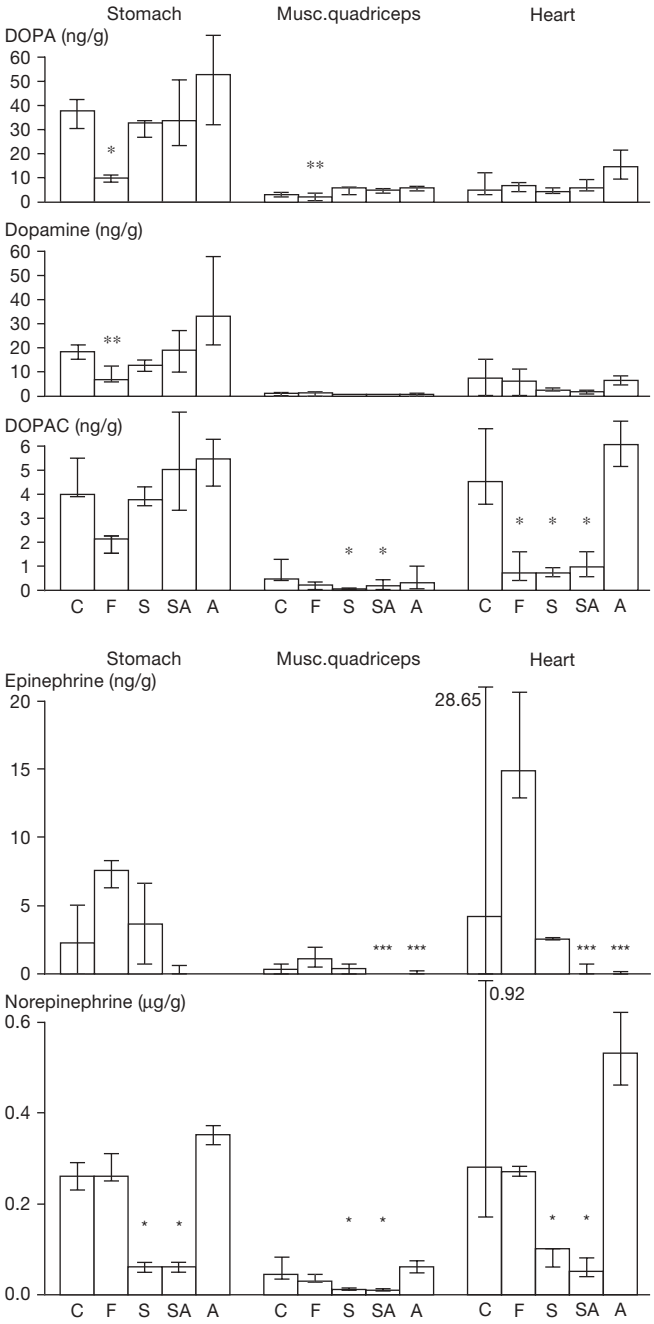

Fig. 5. *Upper panel shows median values and interquartile ranges of DOPA (top), DA (middle), and DOPAC (bottom) in stomach, quadriceps muscle and heart after different experimental procedures in rats. Lower panel in a similar way shows the values of Adr (top) and NA (bottom). C, control; F, after 4 days of fasting, S, after sympathectomy with 6-OHDA; A, after adrenal demedullectomy; SA after both sympathectomy and demedullectomy. * P < 0.05 vs. control values; **P< 0.05 vs. demedullectomy values; *** P < 0.05 vs. fasting values. (From Eldrup & Richter (2000), Am J Physiol Endocrinol Metab, 279, E815-E822; with permission).*

DOPAC

Muscle tissue content of DOPAC increases after DOPA administration (Rose et al 1994). Few, however, have studied endogenous DOPAC content in muscle tissue. Rat skeletal muscle content of DOPAC is low compared to DOPAC content in other tissues, an exception being the liver (Eldrup & Richter 2001). In contrast to DOPA, muscle DOPAC content decreased to almost undetectable values after sympathectomy with 6-OHDA in rats (Fig. 5, Eldrup & Richter 2000). Thus, based primarily on our results it may be concluded that endogenous DOPAC in skeletal muscle tissue is primarily located in sympathetic nerves.

4.3. PLASMA DOPA KINETICS WITHOUT AND WITH INHIBITION OF DOPA DECARBOXYLASE

An early estimate of p-DOPA clearance during l-DOPA administration was 0.5 l/kg/h, which equals 0.6 l/min in a 70 kg subject (Cederbaum 1987). Arterial p-DOPA clearance in cardiac patients was 0.8 l/min at p-DOPA levels 2-fold the normal range and 1.1 l/min at p-DOPA levels 10-fold the normal range. These clearances, however, were not significantly different (Goldstein et al 1991a). We were the first to determine p-DOPA clearance at endogenous p-DOPA levels. In healthy humans arterial plasma DOPA clearance was 1.0 l/min. Arterial p-DOPA appearance rate was 1.1 μg/min (Eldrup et al 1994). Venous p-DOPA clearance was 1.6 l/min in healthy humans during infusion of l-DOPA increasing p-DOPA levels 10-fold (Wolfovitz et al 1993). Tracer or compound concentrations will be lower in peripheral venous plasma than in arterial plasma if local net uptake of tracer/compound takes place. In such situations clearance estimated from venous plasma samples may be erroneously high (Christensen & Henriksen 1988). Venous sampling from extremities where DOPA is clearly extracted may have caused part of the high clearance reported by Wolfovitz et al (1993). The results of Goldstein and co-workers (1991a) taken together with those found by Wolfovitz et al (1993), however, indicate that p-DOPA clearance may also be related to p-DOPA levels. In rats arterial p-DOPA clearance in control animals was estimated to 16 ml/min or approximately 32-40 ml/kg/min (Grossman et al 1992b), which is higher than the values of 13-17 ml/kg/min found in humans weighing 60-80 kg (Eldrup et al 1994). P-DOPA clearances from other species have not been reported. The kidneys seem to account for approximately 20% of DOPA clearance if it is assumed that a local DOPA production in the kidneys contributing to DA excretion in the urine is counterbalanced by degradation of DOPA to other metabolites than DA by the kidneys (Eldrup et al 1994). No data on DOPA production in kidneys or DOPA metabolism beyond DA in kidneys have been published.

Andén proposed that DOPA could be synthesized in the sympathoadrenal system and be transferred to other organs via the blood stream (Andén et al 1989). Infusion of ^{13}C-DOPA to patients suffering from cardiomyopathy, small vessel cardiac disease and hypertension revealed a regional extraction of DOPA from plasma of 22% in the leg, 8% in the heart, 12% across the lungs and 11% between the arteries and the internal jugular vein (Goldstein et al 1991a). We measured a 24% extraction of ^{3}H-DOPA in the lower arm of healthy humans (Eldrup et al 1994). Assuming a plasma flow to the extremities in resting man of 550 ml/min it may be calculated from Eldrup et al (1994) that extremities account for removal of 207 μg DOPA per 24-hr (1.09 ng/ml × 24% × 550 ml/min × 60 min × 24 hr) or 13% of DOPA clearance.

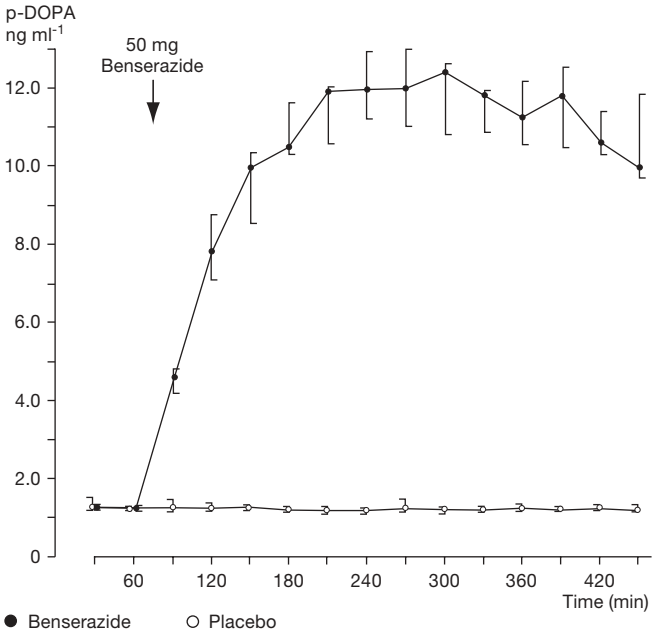

Fig. 6. *Venous p-DOPA concentrations (medians (interquartile ranges), n = 7) after oral ingestion of 50 mg benserazide (•) or placebo tablet (○) at t = 60 min. (From Eldrup et al (1994), Eur J Clin Invest, 24, 205-211; with permission).*

P-DOPA concentrations increase after inhibition of DDC with benserazide in humans and rats and after carbidopa in humans (Williams et al 1986, Da Prada et al 1987, Goldstein et al 1989a). These findings were extended when it was observed that two and a half hours after ingestion of benserazide p-DOPA appearance rate increased approximately 8-fold to 8.4 μg/min, but arterial p-DOPA clearance did not decrease significantly (Fig. 6, Eldrup et al 1994). These findings indicate that normally there is a large production and metabolism of DOPA in tissues from which DOPA only to a minor extent spill over to plasma. Tracer DOPA only mixes with these compartments to a small degree during 120 min infusion of l-^{3}H-DOPA but benserazide seems to enter these compartments immediately. When decarboxylation of DOPA to DA is blocked by benserazide, DOPA leaves the compartments and spill over to plasma (Eldrup et al 1994). Likewise, endogenous p-DOPA concentrations are elevated in the very rare children with aromatic L-amino acid decarboxylase deficiency (Hyland et al 1992, Swoboda et al 1999). The tissue in which DOPA is normally produced and metabolized to DA most probably is sympathetic nerves.

Thus, DOPA, which is synthesized in sympathetic nerves, seems to spill over to plasma provided it is not decarboxylated to DA.

4.4. P-DOPAC AND P-DA-S AFTER DA INFUSION AND AFTER DOPA DECARBOXYLASE INHIBITION

DOPAC

Endogenous p-DOPAC concentrations are decreased more after MAO-A than after MAO-B inhibition indicating that DA primarily by MAO-A is metabolized to DOPAC that is found in plasma (Hovevey-Sion et al 1989, Dingemanse et al 1996). Outside noradrenergic neurons and central nervous system the DA deaminating and dehydrogenating enzymes are located primarily in liver but MAO activity is also found in myocardium, lung, kidney and duodenum (Kopin 1985, Saura et al 1996). No other pathways from DOPA to DOPAC than via DA have been described.

P-DOPAC and p-DA-S concentrations increase when plasma free DA concentrations are elevated by DA infusion in the fasting state (Claustre et al 1990). The deaminating tissue during DA infusion has not been identified.

After partial inhibition of DDC with benserazide in fasting humans p-DOPAC concentrations were unchanged, despite major increases in p-DOPA and p-DA-S levels. Circulating free DA concentrations remained unchanged (Eldrup et al 1994). Moreover, others did not find significant changes of p-DOPAC concentrations after even higher doses of benserazide (Dingemanse et al 1997). Thus after benserazide, DA that has been derived from DOPA seems preferentially to be metabolized to DA-S and not to DOPAC. The reason may be that production of DOPAC from DOPA via DA takes place in cells accessible to benserazide and containing DDC, MAO-A and perhaps even the phenolsulfotransferase A3 isoform, while production of DA-S may take place in other cells only accessible to benserazide to a very limited degree and containing DDC and the phenolsulfotransferase A3 isoform but not MAO-A. The tissues in which these cells are located remain to be identified. Among other tissues, the role of liver in production of p-DOPAC warrant further investigation.

DA-S

Some catecholamines like isoprenaline are primarily sulfated when administered orally but O-methylated after intravenous administration (Conolly et al 1972). Plasma concentrations of DA-S, however,

invariably increase during infusion of DA in microgram doses to humans whether healthy controls, critically ill patients, patients with heart failure or during heart surgery (Ratge et al 1986a, Hashizume et al 1987, Claustre et al 1990, Ratge et al 1991, Nakaya et al 1994, Yoshizumi et al 1998). In contrast, intravenous administration of DA in dogs did not increase p-DA-S concentrations (Oka et al 1987). These observations have been explained by findings that human platelet phenolsulfotransferase can sulfoconjugate DA whereas dog platelets are relatively absent of phenolsulfotransferase (Rein et al 1981, Toth et al 1986). The importance for p-DA-S concentrations of platelet sulfotransferase in humans, however, has been a matter of debate. If platelets were responsible for the production of p-DA-S from circulating free DA by an unsaturable process and if platelets are without restrictions towards DA uptake and DA-S release one could expect a relatively constant ratio between DA-S and free DA in blood and in plasma (Eisenhofer 1999). During intravenous DA administration, however, DA-S/free DA ratio in plasma is considerably lower than before DA administration (Ratge et al 1986a, Hashizume et al 1987, Claustre et al 1990, Ratge et al 1991, Nakaya et al 1994, Yoshizumi et al 1998, Eisenhofer et al 1999). Moreover, p-DA-S concentrations are normal in patients with thrombocytopenia (Kuchel et al 1985b). Thus, platelets are probably not responsible for p-DA-S production. Another suggestion was that DA-S may be generated via DOPA sulfate (Kuchel et al 1990). No evidence, however, supporting this view has emerged. Our finding that venous p-DA-S concentrations increased after benserazide administration is in agreement with endogenous circulating DOPA being metabolized via DA to DA-S in humans in cells to which benserazide only has limited access (Eldrup et al 1994). It is not known if conversion of DOPA to DA and conversion of DA to DA-S take place in the same cells. The liver does not contain the phenolsulfotransferase A3 isoform that primarily sulfoconjugates biogenic amines including DA (Eisenhofer et al 1999). The liver is therefore probably not the site of DA conversion to DA-S. Conjugated DA concentrations in plasma increased after intravenous DA administration in humans, but conjugated DA concentration in the small intestine was even larger (Shibata et al 1987). This is consistent with the observation that human small intestine has sulfotransferase activity (Sundaram et al 1989). Thus it may be speculated that sulfoconjugation of circulating free DA or DA produced from DOPA in loco may take place in intestine. Others have based on findings that monoamine-sulfating phenolsulfotransferase is mainly found in small intestine and hardly in liver combined with data showing substantial production of DA-S by mesenteric organs, but not by the liver, suggested that sulfotransferases in the gut seem to be major determinants of p-DA-S concentrations in humans (Boulton & Eisenhofer 1998, Eisenhofer et al 1999). This view corroborates our hypothesis that p-DA-S originate from food (Eldrup et al 1988, Eldrup et al 1997) but is also compatible with the suggestion that some p-DA-S is produced from locally produced or circulating DOPA via DA (Goldstein et al 1999). Although the small intestine has been suggested, the tissue has not been finally identified in which p-DA-S is produced from DA and in which production of DA-S takes place after administration of benserazide.

4.5. RELATIONSHIP WITH FOOD

DOPA in the upper gastrointestinal tract

We found relatively high DOPA concentrations in stomach from rats that never received DOPA treatment (Fig. 5, Eldrup et al 1989a, Eldrup & Richter 2000). Stomach content of DOPA in rats decreased nearly 75% after 4 days of fasting. Lower sympathetic activity during fasting could not explain this finding, as fasting did not alter NA content in rat stomach (Fig. 5, Eldrup & Richter 2000). Surprisingly, others found decreased DOPA content in the rat stomach after chemical sympathectomy with 6-OHDA in one study but unchanged DOPA content in another very similar study (Grossman et al 1992b, Kawamura et al 1999). The reason for this discrepancy reported by the same group of investigators remains unexplained. DOPA in gastrointestinal tissues could be taken up from the blood if DOPA uptake in muscle decreased during fasting. If our finding in humans that DOPA content in meals is several folds higher than 24 h p-DOPA appearance rate (Eldrup et al 1994) also applies to rats, it seems more likely that DOPA in stomach is derived from food than from blood. TH has been found in non-neuronal cells in rat stomach and another possibility is that DOPA may be synthesized locally (Bäck et al 1995, Mezey et al 1998). At present it is unknown if the fasting state decreases TH activity in non-neuronal cells. Sympathectomy with 6-OHDA did not influence TH activity in non-neuronal cells (Mezey et al 1998). Thus, it cannot be completely excluded that a local DOPA synthesis is responsible for some of the DOPA content in rat gastrointestinal cells. It may be suggested, however, that DOPA in stomach tissue at least in part seems to be derived from food.

Impact of food intake and fasting on p-DOPA

Dihydroxyphenylalanine, DOPA, is both an amino acid and a catecholamine (Cederbaum 1987, Rose et al 1988, Waite 1991, Goldstein 1995e, Eldrup et al 1998). DOPA is, however, most often classified as a substrate in the synthesis of classical catecholamines, dopamine, noradrenaline and adrenaline. DOPA has been found in bananas (Waalkes et al 1958) and in cereals (Hoeldtke et al 1972), dietetic tuna (Williams et al 1986), bound to proteins in canned dog food (Banwart et al 1989), and in ordinary meals (Eldrup et al 1997). Amounts of DOPA in ordinary meals were 0.6-4.7 mg, which is less than 1/100-fold the amount of other amino acids but approximately 5-fold higher than the amount of DOPA endogenously produced in 24 h (Eldrup el al 1994, Eldrup et al 1997). Wheat is unlikely to be the only source of free DOPA in ordinary meals, as breakfast and open sandwiches contained an amount of DOPA inversely related to the amount of white bread. Banana, tuna or oats was not included in our ordinary meals but the exact source of DOPA in meals was not investigated (Eldrup et al 1997).

Venous p-DOPA concentrations increased slightly after a tuna test meal with a free DOPA content of 0.2-0.4 μg (Williams et al 1986). Arterialized p-DOPA levels, however, were not significantly increased in healthy and hypertensive men after a tuna test meal with an equal amount of free DOPA (Clark et al 1992). In healthy humans we found that venous p-DOPA decreased after the ordinary hot meal (3.5 mg free DOPA) and even more so after the evening open sandwiches meal (4.7 mg free DOPA, Fig. 7, Eldrup et al 1997).

In a recent investigation a tuna meal with 0.3 μg free DOPA clearly decreased venous p-DOPA in controls and hypertensive subjects although an increase was reported (Kuchel 1998). In contrast, others recently found that arterial p-DOPA increased as much as 60% after a standard meal (Goldstein et al 1999). The amount of DOPA in this standard meal, however, was not reported. From the treatment of Parkinsonian patients it is well known that oral intake of pharmacological amounts of DOPA, 100-800 mg daily or more, may increase p-DOPA concentrations and that this increase may persist for 48 h (Hare et al 1973, Eldrup et al 1995, Ahlskog et al 1996b). Fifty to seventy-five percent of orally administered DOPA is metabolized by gut decarboxylase before reaching the circulation and absorption of DOPA increases non-linearly with dose (Cederbaum 1987). It is not known, however, to what extent these results from intake of pharmacological DOPA doses apply to the intake of small amounts of DOPA that seem to be present in ordinary food.

In dogs venous p-DOPA concentrations were unchanged after feeding canned dog food with only traces of free DOPA but with 0.03-1.7 mg DOPA bound to proteins (Banwart et al 1989).

When humans fasted for 25 hours no changes of venous p-DOPA concentrations were found (Eldrup et al 1997). In rats neither 24 h nor 4 days fasting state changed arterial p-DOPA concentrations (Garty et al 1989c, Eldrup & Richter 2000).

In humans that fasted perioperatively, it was found that 128

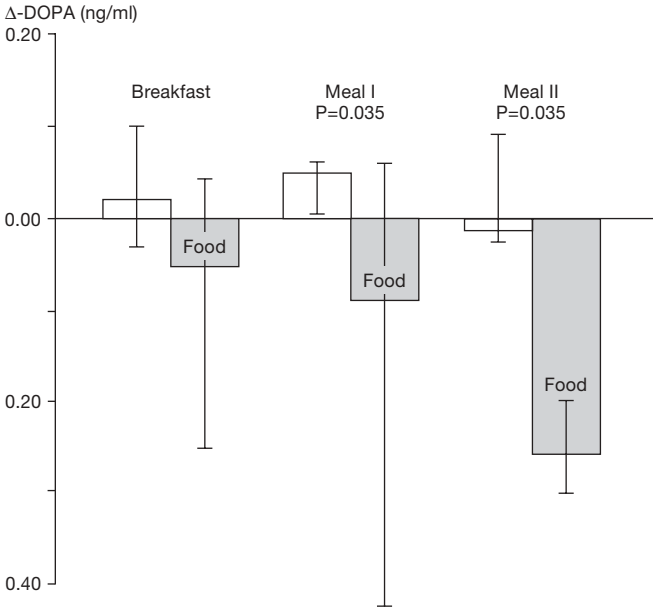

Fig. 7. *Delta values of average venous p-DOPA concentrations 3 h before and 1 h after each meal (hatched bars) or at the time of the meal on the fasting experimental day (open bars). Medians and interquartile ranges are shown (n = 7). (From Eldrup et al (1997), Clin Sci, 92, 423-430; with permission).*

ng/min DOPA was produced in mesenteric organs but an equal amount of DOPA was extracted in the liver (Eisenhofer et al 1997). In rats there was no arterio-portal venous increment in p-DOPA (Grossman et al 1992b).

O'Hare et al (1989) demonstrated that euglycemic insulin infusion decreased p-DOPA concentrations in obese and in lean men. During euglycemic hyperinsulinemia p-DOPA concentrations in rats was 19% lower but this was not statistically significant (Wolfovitz et al 1995). Plasma insulin concentrations in Wolfovitz' study, however, were 27-37% lower, which together with a possible species effect may explain the slightly different results. After 4 days of fasting we found lower DOPA content in rat skeletal muscle, which could have been caused by lower plasma insulin levels during fasting (Fig. 5, Eldrup & Richter 2000). Plasma insulin levels were not measured in any of our studies of food intake in humans and fasting in rats, but it seems possible that at least some of the different findings in studies of p-DOPA after food intake may be explained by different insulin levels. Another explanation may be that different amounts of DOPA, either as a free amino acid or bound to proteins, seem to be present in food. Several other theoretical explanations exist that post-prandial peripheral arterial and venous p-DOPA levels in humans are quite variable. DOPA spill over from neuronal tissue may vary as may possible DOPA production in non-neuronal cells in the gastrointestinal tract. DOPA uptake and release between the blood and cells in the gastrointestinal tract, extraction of DOPA across the liver, and uptake of DOPA in muscle and other tissues may also vary. These possibilities deserve further investigation.

In summary, the relationship between food intake and p-DOPA concentrations seems to be complex and not finally elucidated. P-DOPA levels after meals may at least partly depend on DOPA content of meals and may possibly also depend on circulating insulin levels. Dietary influences should be considered when interpreting changes in p-DOPA as also stated by Eisenhofer and co-workers (1999). The fact that DOPA is not only a catecholamine but also an amino acid may be more important than hitherto acknowledged in the literature.

DOPAC in the upper gastrointestinal tract

MAO activity is high in stomach as well as in small and large intestine in rats (Fogel 1990, Saura et al 1996). In fasted rats we found decreased DOPAC content in stomach and in small and large intestine, although the difference from control rats was not significant (Fig. 5, Eldrup & Richter 2000). Lower sympathetic activity during fasting could not be the reason for the apparently lower DOPAC content in the gastrointestinal tract as NA content was not decreased. Rat food content of DOPAC, DOPA, and DA was unfortunately not quantified in our study but could be sources of DOPAC in rat upper gastrointestinal tract. Another source of DOPAC in the upper gastrointestinal tract could be blood and local production from tyrosine via DOPA and DA, but these possibilities have not been elucidated. Our findings are consistent with the possibility that DOPAC at least in rat upper gastrointestinal tract is derived from food.

Impact of fasting and food intake on p-DOPAC

Fasting for 25 h decreased p-DOPAC levels 43% (Fig. 8, Eldrup et al 1997). In children hospitalized for diseases other than neuroblastoma, ganglioneuroblastoma and pheochromocytoma, p-DOPAC concentrations were decreased compared with p-DOPAC levels in

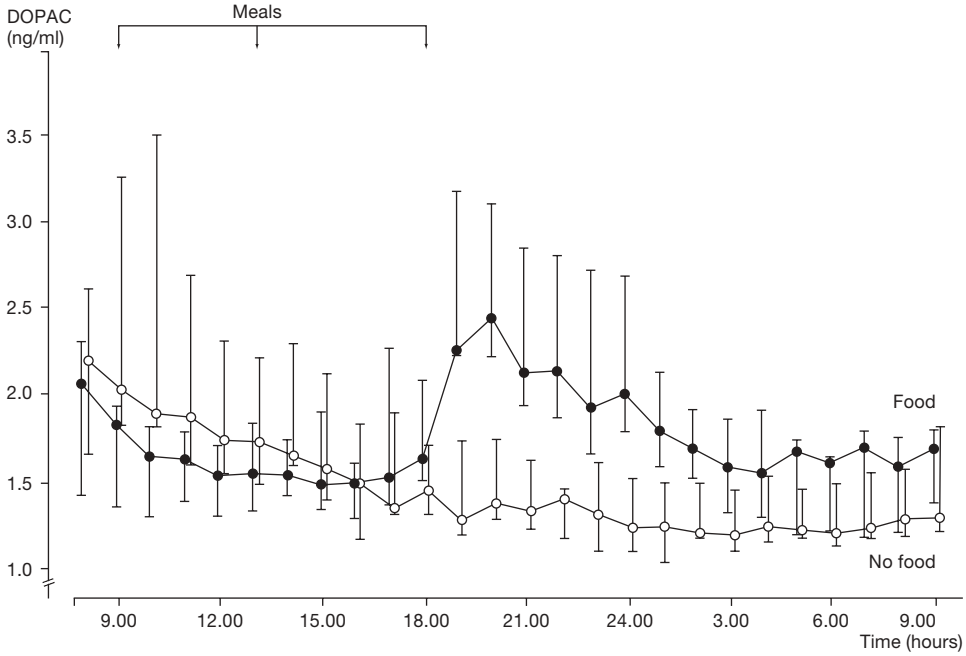

Fig. 8. *Venous p-DOPAC concentrations during the fasting (○) and the meal (•) experimental day. Medians and interquartile ranges are shown (n = 7). (From Eldrup et al (1997), Clin Sci, 92, 423-430, with permission).*

healthy children (Fig. 11, Eldrup et al 2001). Although food intake was not quantified in the neuroblastoma study, it seems most likely that reduced food intake explains this observation. In rats p-DOPAC levels decreased after 4 days fasting when compared with healthy control rats, sympathectomized and adrenal demedullectomized rats (Eldrup & Richter 2000).

We discovered that an ordinary meal increases p-DOPAC concentrations in healthy humans (Fig. 8, Eldrup et al 1997). Others have confirmed that a meal can increase p-DOPAC levels (Goldstein et al 1999). Eisenhofer reported a net production of DOPAC from mesenteric organs but a net removal of DOPAC across the liver in humans without known liver disease who fasted during upper abdominal surgery (Eisenhofer et al 1997). Although the liver may not contribute to p-DOPAC levels during surgery, the role of the liver in DOPAC production and degradation has not been completely elucidated.

P-DOPAC increased after an evening open sandwiches meal but not after breakfast or after a hot meal at lunch. Breakfast contained much more DOPAC than the evening meal indicating that DOPAC in the food is probably not the source of p-DOPAC (Eldrup et al 1997). P-DOPAC increase was not related to food content of tyrosine and phenylalanine and these amino acids are probably not the source in p-DOPAC (Eldrup et al 1997). After DOPA ingestion in >100 mg doses p-DOPAC concentrations increased 20-fold but were normalized 13 hours later (Ahlskog et al 1996b). No studies of p-DOPAC concentrations after oral DA administration have been presented. A hot meal and an open sandwiches meal each contained approximately 4 mg DOPA. The open sandwiches meal after which p-DOPAC increased contained nearly 25-fold more DA than DOPA. Our results suggest that the source of DOPAC in plasma at least partly seems to be DA originating in food. This possibility warrant further investigation.

DA-S

In 1981 Davidson and co-workers reported that ingestion of a banana increased p-DA-S. Others have corroborated this finding and ascorbic acid potentiated the increase (Dunne et al 1983, Kuchel et al 1985c). One group found no increase of p-DA-S up to 4 hours after ingestion of a morning meal low in phenylalanine and tyrosine whether tyrosine was added or not. When a large amount of DOPA was added to the meal, p-DA-S concentrations increased 100-fold (Cuche et al 1985). An evening rise in DA-S was found in healthy subjects but a dietary source was excluded (Kuchel & Buu 1985) based upon a previous finding that DA-S concentrations in 3 very obese subjects were relatively stable during 8 days of fasting (Kuchel et al 1979). It was concluded that at least 12 hours of fasting was sufficient to negate the contribution from food to p-DA-S and this conclusion was later repeated without further evidence (Kuchel et al 1979, Kuchel et al 1982). Plasma DA-S was normal or high in patients with liver-failure and before and during liver transplantation indicating that the liver is not necessary for conjugation of DA (Ratge et al 1986a, Tyce et al 1987, and later also in Gaudin et al 1990). The source of DA-S, however, was still considered a puzzle (Anton 1987). A late afternoon increase of p-DA-S was found in healthy human males after food intake but p-DA-S values were not obtained before breakfast on the day of meals. Furthermore, food intake was not standardized and the content of DOPA, DA and DA-S in the food was not quantified (Eldrup et al 1988). This observation was later acknowledged (Yamamoto et al 1996, Shannon & Robertson 1996). An uncontrolled study showed an increase in p-DA-S following a meal 2-hour post exercise (Ogaki et al 1995). In 1997 we clearly established that ordinary meals are a major source of plasma DA-S (Fig. 9, Eldrup et al 1997). Our results have been confirmed as p-DA-S increased after protein intake in healthy and hypertensive subjects (Kuchel 1998) and after a balanced meal in healthy subjects (Goldstein et al 1999). In accordance with these data and the results from patients with liver disease, mesenteric production of DA-S that was not altered by the liver has been reported (Eisenhofer et al 1997).

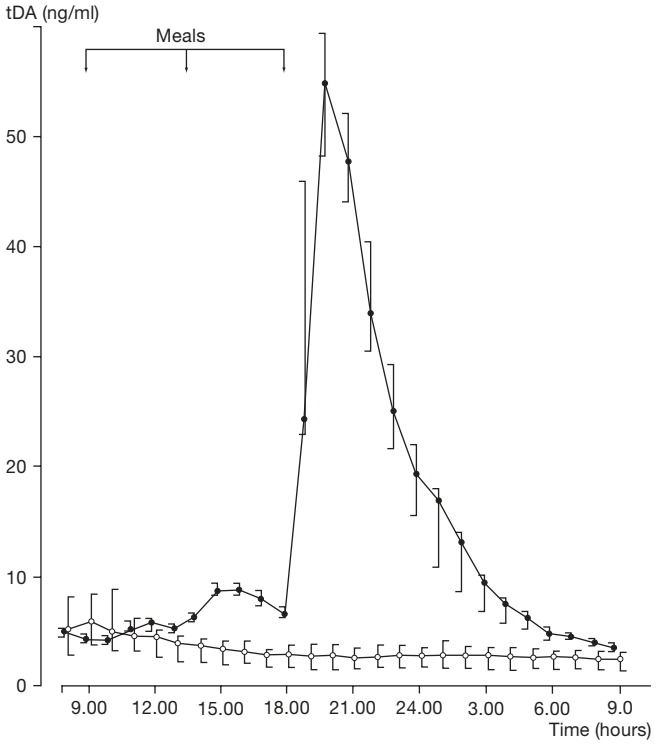

Fig. 9. *Venous plasma total DA (tDA = DA-S + free DA) concentrations during the fasting (○) and the meal (●) experimental day. Medians and interquartile ranges are shown (n = 7). (From Eldrup et al (1997), Clin Sci, 92, 423-430; with permission).*

In dogs venous p-DA-S did not increase significantly the first 5 hours after feeding (Banwart et al 1989). The same group, however, later found that conjugated DA increased in plasma and in lymph after feeding in dogs (Chen et al 1993). The reason for the different results is not known.

It is now generally agreed that p-DA-S originates predominantly from food (Eldrup et al 1997, Goldstein et al 1999). It is still not known, however, if p-DA-S comes from the food per se, comes from DA-S produced intra-luminally in the gastrointestinal tract from DOPA and DA in food, or comes from DOPA and DA in food that is metabolized to DA-S in gastrointestinal cells. Given the evidence that TH may be found in non-neuronal cells in the gastrointestinal tract (Mezey et al 1998) it could also be speculated that tyrosine or phenylalanine from food could be metabolized via DOPA and DA to DA-S by action of enzymes in the gastrointestinal tract. From food tables (20 mg tyrosine/100 g banana & 32 mg phenylalanine/100 g banana) and an estimated weight of 130 g banana pulp, tyrosine and phenylalanine content of one banana can be estimated to 26 mg and 42 mg, respectively. In ordinary meals tyrosine and phenylalanine content were 852 mg and 997 mg in breakfast, 1056 mg and 1275 mg in the hot meal, and 1209 mg and 1475 mg in open sandwiches, respectively (Eldrup et al 1997). Data from the literature give some indication about the increase in p-DA-S concentrations in relation to food content of DOPA, DA, and DA-S, respectively, as shown in Table 5. Although DA, DOPA, tyrosine and phenylalanine content in a banana is estimated, it may be summarized from Table 5 and abovementioned data that in contrast to food content of DOPA, DA and DA-S, no relationship seems to exist between tyrosine or phenylalanine intake on the one hand and increase in p-DA-S concentration on the other. DOPA, DA and DA-S content in food seem to be related to the increase in p-DA-S concentration after meals as previously concluded (Eldrup et al 1997).

The production of DA-S in the gastrointestinal tract may be delayed with respect to meals. DA-S produced in response to a meal may even be taken up in different tissues and from there released

Table 5. *Comparison of p-DA-S increase from baseline and 1 hour after oral ingestion of a measured or estimated amount of DOPA, DA, and DA-S, respectively.*

Reference	Food component ingested perorally	DOPA (mg)	DA (mg)	DA-S (mg)	DA-S increase 0-1 h (ng/ml)
Davidson et al 1981	Banana[a]	2.0[b]	13[b]		33
Kuchel et al 1985c	Banana[a]	2.0[b]	13[b]		7
Cuche et al 1985	DOPA	125			658
McCulloch et al 1987	DA		0.8		18
Hashizume et al 1987	DA		50		563
Ratge et al 1991	DA		1.22		23
Eldrup et al 1997	Breakfast	0.6	0.1	0.4	2
Eldrup et al 1997	Hot meal	3.5	0.9	0.5	4
Eldrup et al 1997	Open sandwiches	4.7	102	110	48

a) size estimated to 130 g banana pulp (*Waalkes et al* 1958); b) estimated content: 1.5 mg DOPA and 10 mg DA per 100 mg banana pulp (*Duncan et al* 1984, *Kanazawa & Sakakibara* 2000).

back to the circulation. Such a process could be passive or regulated by yet unknown mechanisms and could together with DA-S production via DA from continous DOPA synthesis and spill over to plasma explain why some p-DA-S always seems to be present even in the fasting state (Kuchel et al 1979, Eldrup et al 1997, Goldstein et al 1999) and why very high levels of DA-S are present in plasma up to several days after DA infusion (Ratge et al 1991, Nakaya et al 1994).

4.6. LEVELS OF NA, DOPA AND DOPAC IN CEREBROSPINAL FLUID AND PLASMA IN PATIENTS WITH PARKINSON'S DISEASE

Parkinson's disease (PD) is a neurodegenerative disorder of the cerebral extrapyramidal system, characterized by progressive motor dysfunction. Autonomic impairment may complicate the disease. Cerebrospinal fluid concentrations of catecholamines and their primary metabolites have been studied infrequently in PD.

NA

Cerebrospinal fluid NA concentrations are assumed to be derived from central noradrenergic neurons (Lake & Ziegler 1985, Geracioti et al 1994, Elrod et al 1997) but controversies exist. A rostrocaudal decrease of csf NA concentration is in accordance with a central origin of csf NA. Ventricular/lumbar csf ratio was 1.85 for NA in a human autopsy study (Wester et al 1990). Rostrocaudal csf NA decrease was also found in various neurological patients (Ziegler et al 1977). Others in contrast found higher NA levels in lumbar csf compared to ventricular csf in patients with normal pressure hydrocephalus (Gjerris et al 1988). Some authors have suggested that lumbar NA levels are predominantly influenced by NA release from areas caudal to the lateral ventricles (Geracioti et al 1994). Intravenous ganglion blockade in dogs decreased csf NA concentrations suggesting that csf NA was not of CNS origin (Goldstein et al 1987b). More recently, however, it was summarized that csf and plasma NA levels change in the same direction in response to most physiological and pharmacological stimuli (Goldstein 1995f). Goldstein and co-workers recently agreed that csf NA in humans probably reflects CNS NA activity though this was not considered established with certainty (Raskind et al 1999). Clearance of NA from csf has not been clarified. Variable csf NA findings may be caused by different clearance rates of NA in different parts of the central nervous system. Recently, it was claimed that csf DHPG concentration is an index of csf NA clearance (Raskind et al 1999). This has not been evidenced although plasma DHPG seem to reflect intraneuronal metabolism of NA in the peripheral sympathetic nervous system.

A significant increase of csf NA with age ($r = 0.89$, $n = 7$ and $r = 0.27$, $n = 128$) was observed in hospitalized patients who underwent a diagnostic lumbar puncture (Eide et al 1979, Ziegler et al 1980). Higher csf NA concentrations were found in 14 healthy older subjects when compared with 33 healthy young subjects (Raskind et al

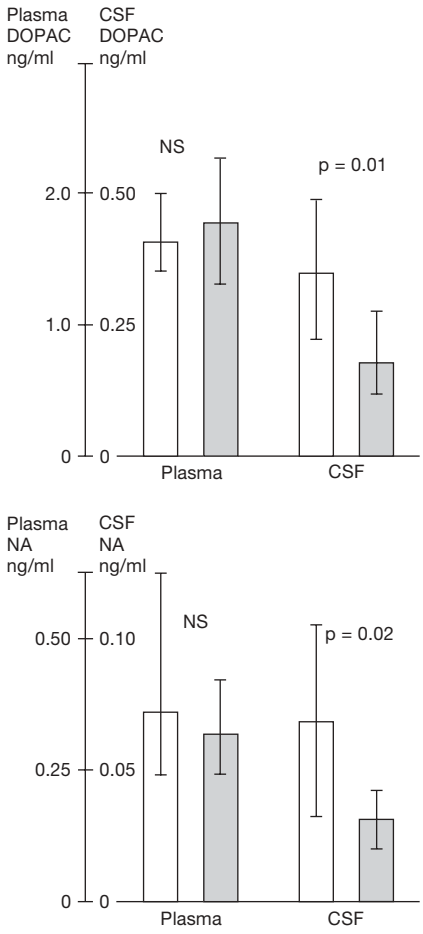

Fig. 10. *Plasma and cerebrospinal fluid (CSF) concentrations of DOPAC (upper panel) and NA (lower panel) in control patients (open bars, n=21) and Parkinsonian patients (hatched bars, n=16). Medians and interquartile ranges are shown. NS: p > 0.05. (modified after Eldrup et al (1995), Acta Nerol Scand, 92, 116-121; with permission).*

1988). In contrast, we found no relationship between age and csf concentrations of NA in 21 controls with low back pain (Eldrup et al 1995). A recent observation of similar csf NA levels in 10 young and 11 old healthy subjects corroborates our result (Raskind et al 1999). The selection of controls, performance of the lumbar puncture and other environmental variables may account for the different results. Thus, in contrast to the relationship between p-NA levels and age, the relationship between csf NA concentrations and age has not been finally clarified.

Two groups reported decreased lumbar csf NA levels in PD patients (Teychenne et al 1985, Martignoni et al 1992). One investigation found no difference between PD patients and controls (Chia et al 1993) and one investigation found increased csf NA levels in PD (Turkka et al 1987). The latter result can probably be explained by methodological differences as subjects were not investigated in the supine position and perhaps the central and the peripheral sympathetic neurons were activated by physiological or psychological factors. In our study csf NA levels were lower in 6 untreated PD patients and 10 PD patients investigated at least 48 h after l-DOPA ingestion than in 21 control patients with low back pain. PD patients were without symptoms of autonomic dysfunction (Fig. 10, Eldrup et al 1995). Control patients were about 10 years younger than PD patients, 57.8 years (range 41-80 years) vs. 67.5 years (range 41-87 years). Cerebrospinal fluid NA levels at least do not decrease with age. Therefore, the age difference between cases and controls in our study is of no significance or tend to minimize the true decrease in csf NA levels in PD patients. All subjects were examined between 8 and 12 a.m. Thus, our results were not influenced by the circadian variation of csf NA levels in humans with highest values in the early afternoon and lowest values during the night (Lake & Ziegler 1985, Geracioti et al 1994). We analyzed ml 2-6 of the lumbar csf in all subjects. There are no data to suggest, that a rostrocaudal gradient of csf NA concentrations is different in PD patients compared with controls. No evidence of increased csf NA clearance in PD as com-

pared to controls has been presented. Thus, csf NA levels seem to be low in PD and low lumbar csf NA levels in PD patients are probably caused by a central noradrenergic defect. It is not possible from existing studies, however, to determine if a dysfunction in structurally intact noradrenergic neurons is also present in PD.

We only included six PD patients with disease duration of more than 5 years in our study and did not find a decrease in p-NA levels (Eldrup et al 1995) in contrast to a previous study in a larger group of long-term PD patients (Ørskov et al 1987). The well-known age related increase of plasma NA concentrations, however, was not seen in PD patients (Eldrup et al 1995). Surprisingly, other investigators later found elevated p-NA levels in untreated PD patients, but this finding was probably caused by psychological and/or physiological activation of the sympathetic nervous system in cases as discussed by the authors (Ahlskog et al 1996a). Recently, further evidence of peripheral noradrenergic defects in PD, cardiac sympathetic denervation, was found in a mixed group of PD patients (Goldstein et al 2000b).

Taken together, existing data support our finding of a noradrenergic defect in PD patients without autonomic symptoms. The defect seems to be both central and peripheral indicating a diffuse neuronal degenerative process in PD.

DOPA

Very few studies have elucidated the origin of csf DOPA. Cerebrospinal fluid DOPA may partly be derived from plasma (Pletscher et al 1967), but may also be derived from DOPA synthesized in CNS as indicated by csf DOPA concentrations being lower than p-DOPA concentrations (Tables 1A and 1B). It is not known if endogenous csf DOPA concentrations in humans are higher in ventricular csf than in lumbar csf. During l-DOPA infusion a major rostrocaudal gradient of csf DOPA with highest cisternal concentrations exists, at least in monkeys (Hammerstad et al 1990). It was recently claimed that csf DOPA provides an estimate of central catecholamine biosynthetic capacity (Raskind et al 1999), but no evidence for this concept has been published. Clearance of endogenous DOPA from csf has never been elucidated.

In one study csf DOPA concentrations in PD patients were 80% lower than in control patients (Tohgi et al 1991). In this study, however, csf DOPA levels in controls were much higher than levels found by others (Eldrup et al 1995, Raskind et al 1999). In another study csf DOPA levels in 289 PD patients with mild symptoms were almost twice as high as those found in 25 healthy controls (LeWitt 1993). LeWitt's assay clearly overestimated csf DA concentrations but csf DOPA concentrations were also high and similar results have not been found by others. We found no significant difference of csf DOPA levels in untreated PD patients compared with control patients with low back pain (Eldrup et al 1995). No later studies of endogenous csf DOPA levels in PD patients were found in the literature. Thus, most evidence indicates that endogenous csf DOPA levels are unchanged in PD.

Even 18 h after withdrawal of medication csf DOPA concentrations were 10 to 20-fold higher in l-DOPA treated PD patients than in controls (Sharpless et al 1971, Fekete et al 1984, Woodward et al 1993). Cerebrospinal fluid DOPA concentrations seem to have been normalized in our PD patients after 48 h without l-DOPA treatment as no difference was found between untreated and l-DOPA treated PD patients (Eldrup et al 1995). These results indicate that possibilities of studying csf DOPA concentrations in l-DOPA treated PD patients are limited as clinical deterioration was severe in most of our patients off l-DOPA treatment for 48 h. From investigations in monkeys Hammerstad and co-workers (1990) concluded that the lumbar csf compartment was unsuitable to investigate DOPA pharmacokinetics in the brain. DOPA in csf was not a reliable predictor of motor response in PD patients receiving l-DOPA except perhaps in the fasting state (Woodward et al 1993).

P-DOPA concentrations seem to be normal in untreated PD patients but higher in l-DOPA treated patients even 48 h after the last medication (Eldrup et al 1995, Ahlskog et al 1996b). Activity of tyrosine hydroxylase was reduced in adrenal medulla from patients with PD (Quick & Sourkes 1977, Riederer et al 1978). Our results do not support, however, that activity of tyrosine hydroxylase is generally decreased in PD patients. P-DOPA concentrations may be useful in predicting duration of the drug's beneficial motor activity in PD (Triggs et al 1996).

In summary, the origin and clearance of endogenous csf DOPA have not been clarified and it is therefore not known if csf DOPA levels mainly is the result of central TH activity. Though not fully elucidated lumbar csf DOPA concentrations in PD patients appear to be similar to csf DOPA levels in controls. Plasma but not csf DOPA levels may be of some interest in l-DOPA treated PD patients.

DOPAC

Cerebrospinal fluid DOPAC concentrations are lower than p-DOPAC concentrations (tables 1A and 1B) Early mass fragmentographic, gas chromatographic and gas chromatographic-mass spectrometric methods revealed low concentrations (<1 ng/ml) of DOPAC in lumbar csf but higher concentrations in ventricular and cisternal csf both in humans and in monkeys (Wiesel 1975, Gordon et al 1976, Wilk & Stanley 1978, Berger et al 1980). Several studies in controls, in patients with depression and neurological diseases such as Alzheimer's disease, epilepsy, migraine, ataxia, and other non-Parkinsonian movement disorders have confirmed that lumbar csf DOPAC concentrations are around or below 1 ng/ml (Stahl et al 1985, Koyama et al 1988, Devinsky et al 1992, Kaakkola et al 1993, Chia et al 1993, Eldrup et al 1995, Castillo et al 1996, Raskind et al 1999).

Cerebrospinal fluid concentrations of the final DA metabolite 3-methoxy-4-hydroxyphenylacetic acid or homovanillic acid (HVA) are much higher than csf DOPAC concentrations. HVA and DOPAC in csf were believed to be derived from DA in human brain (Wilk & Stanley 1978, Wester et al 1990, LeWitt et al 1992). Lower csf DOPAC concentrations compared with p-DOPAC concentrations and a rostrocaudal gradient with higher central csf DOPAC concentrations indicate a central origin of csf DOPAC (Eklundh et al 1996). The spinal cord is nearly devoid of dopamine and probably only contributes to lumbar csf DOPAC to a minor extent, at least in rabbits and rats (Zivin et al 1975). Cerebrospinal fluid DOPAC may also partly reflect noradrenergic transmission in the brain (Andén & Grabowska-Andén 1983, Ebinger et al 1987). Noradrenergic neurotoxin decreased brain content of NA but not csf levels of DOPAC and HVA indicating that DOPAC and HVA in rat cisternal csf derive predominantly from DA neurons in the brain (Hutson & Curzon 1986). Concerns about the use of HVA as an index of central dopaminergic cell loss have been raised (Scheinin 1985, LeWitt 1993). Most evidence, however, support that lumbar csf DOPAC at least in rat originates from brain DA neurons. The origin of csf DOPAC in humans has not been fully clarified. Clearance of endogenous csf DOPAC has not been investigated.

Few studies of csf DOPAC levels have been done in patients with PD. No difference was found when csf DOPAC levels in 25 healthy controls were compared to csf DOPAC levels in 289 non-medicated and mildly affected PD patients (LeWitt 1993). In Chinese patients no significant differences in lumbar csf DOPAC concentrations were found between 6 untreated PD patients, 37 PD patients withdrawn from l-DOPA treatment for 5 days and 26 controls, (Chia et al 1993). Lumbar csf DOPAC levels, however, significantly decreased with increased disability in 43 PD patients in Chia's study. We found decreased lumbar csf DOPAC levels in PD patients when compared to csf DOPAC levels in patients with low back pain (Fig. 10, Eldrup et al 1995). Thus, results from csf DOPAC measurements in PD patients are not uniform. LeWitt (1993) suggested that TH activity may be increased in remaining dopaminergic neurons in untreated but not in l-DOPA treated PD patients. The highly significant relationship of

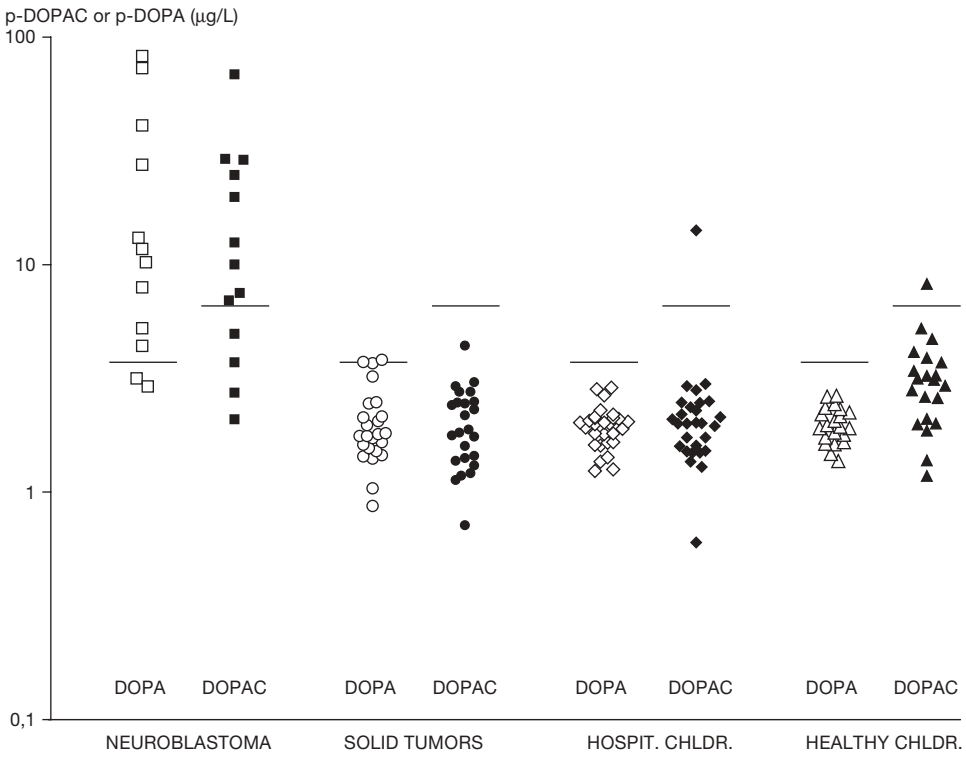

Fig. 11. *Individual values of p-DOPAC (closed symbols) and p-DOPA (open symbols) concentrations in 13 untreated newly diagnosed children with NB, 24 children with other solid tumors, 28 children hospitalised for non-neoplastic diseases and 21 healthy children. Horizontal bars indicate upper normal limits p-DOPAC: 6.58 µg/L and p-DOPA: 3.72 mg/L. (Reprinted from Eldrup et al (2001), Scand J Clin Lab Invest, 61, 479-490; by permission of Taylor & Francis AS).*

csf DOPAC levels to disability according to Hoehn & Yahr score in medicated but not in untreated PD patients in our study (Eldrup et al 1995) could be explained if TH activity is increased in mildly affected untreated PD patients and if csf DOPAC concentrations closely parallel central TH activity. The lack of relationship between csf DOPAC levels and disability score in our group of untreated PD patients, however, may also be due to the small number of subjects. Cerebrospinal fluid DOPAC clearance has not been measured in any study of PD patients but no indications as to an increased metabolism of csf DOPAC have been demonstrated in PD.

The relationship between csf DOPAC and clinical severity of PD indicates that csf DOPAC reflects brain DA metabolism to some extent. This concept is supported by the observation that patients with dominantly inherited olivopontocerebellar atrophy have decreased lumbar csf DOPAC concentrations and neuronal loss in the substantia nigra as well as low striatal DA levels (Orozco et al 1989, Kish et al 1992). Our conclusion that csf DOPAC is a reliable index of central dopaminergic activity (Eldrup et al 1995) seems to be valid. Considering clinical symptoms with severe disability that seem to be the result of nigrostriatal DA cell loss in our patients, lumbar csf DOPAC levels, however, do not seem to be a very sensitive index of PD pathology.

4.7. PLASMA DOPA AND PLASMA DOPAC AS TUMOR MARKERS IN NEUROBLASTOMA

Neuroblastoma (NB), ganglioneuroblastoma (GNB), and ganglioneuroma (GN) are derived from primordial neural crest cells, which ultimately develop into sympathetic ganglia, adrenal medulla and other tissues. NB and GNB are the most common extra cranial solid tumor in children, accounting for 7-10% of all childhood cancers (Brodeur and Castleberry 1997). Excess urinary (u-) catecholamine metabolite excretion is one of the important diagnostic criteria for neuroblastoma (Brodeur & Castleberry 1997, Sawada 1997). Furthermore, diagnosis and staging are established by histological examination of tumor tissue, biological characterization of tumor cells, meta-iodobenzyl guanidine (MIBG) scintigraphy, bone scintigraphy, computed tomography scan or magnetic resonance imaging scan (Brodeur & Castleberry 1997).

Diagnosis of neuroblastoma

Investigations of catecholamines in NB began with the study of an adrenaline secreting neuroblastoma (Mason et al 1957). Later children with NB were found to secrete excessive amounts of NA, 3-methoxy-4-hydroxymandelic acid (vanillylmandelic acid, VMA), metanephrine, normetanephrine, DOPA, DA, HVA, DOPAC, and vanillactic acid in urine (Isaacs et al 1959, Von Studnitz 1960, Greenberg & Gardner 1960, Voorhess & Gardner 1961, Voorhess & Gardner 1962, Kontras 1962, Von Studnitz et al 1963, Sourkes et al 1963, Williams & Greer 1963, Türtler & Käser 1971, Laug et al 1978).

Several studies of 40 to 60 NB/GNB patients have been published. Elevated urinary excretion was found in 80-89% (HVA), 73-89% (VMA), and 93-98% (HVA or VMA) of the patients before treatment (Gitlow et al 1973, Bond 1975, Laug et al 1978, Graham-Pole et al 1983, Tuchman et al 1987, Eldeeb et al 1988). In four studies of 288, 619, 211, and 76 NB patients, u-HVA excretion was elevated in 75-88%, u-VMA excretion was elevated in 71-84%, while either u-HVA or u-VMA excretion was elevated in 82%, 85%, 87%, and 96% of patients, respectively (LaBrosse et al 1980, Pritchard et al 1989, Berthold et al 1992, Sawada 1997). U-HVA excretion seems more frequently to be elevated than u-VMA excretion in NB patients (Williams & Greer 1963, LaBrosse et al 1980, Tuchman et al 1987). U-HVA and u-VMA excretion are not completely specific as urinary excretion of these catecholamine metabolites was increased in 10-16% of patients with the benign GN tumor (Lucas et al 1994). Consistent with previous results, we found increased u-HVA in 92% of untreated NB patients while u-VMA was only increased in 77%. In our study that did not comprise any case of GN, the specificity of normal u-HVA and u-VMA excretion in non-NB solid tumor patients was 100% and 73%, respectively (Eldrup et al 2001). Thus, u-HVA excretion and u-VMA excretion seem to be valuable in the diagnosis of NB. If random urinary samples are used, u-creatinine concentration, however, must be taken into account (Cole et al 1995, Sawada 1997).

Increased plasma or serum concentrations of DOPA (Imashuku & LaBrosse 1971, Helson et al 1980, Alvarado et al 1985, Goldstein et al 1986, Boomsma et al 1989, Jouve et al 1991, Ikeda et al 1996, Fig. 11), DA, NA (Alvarado et al 1985, Goldstein et al 1986, Eldrup

et al 2001), as well as 3-methoxy-4-hydroxyphenylglycol, HVA and VMA (Tang et al 1986, Berthold et al 1992) have been found in small series of NB patients. We extended these results and demonstrated that p-DOPAC concentrations are also elevated in NB cases compared to p-DOPAC levels in children with non-NB solid tumors and other unhealthy children (Fig. 11, Eldrup et al 2001). P-DOPAC levels were not significantly higher in NB patients compared with healthy children in our study. None of the children in our investigation was deliberately examined in the fasting state and food intake may have increased p-DOPAC levels in healthy controls.

Elevated values of p-DOPA have been found in 87-100% of untreated symptomatic NB patients from small studies (between 6 and 31 cases; Helson et al 1980, Alvarado et al 1985, Boomsma et al 1989, Jouve et al 1991, Ikeda et al 1996). We found elevated p-DOPA concentration in 85% of NB cases (Eldrup et al 2001). Lower sensitivities, 63-82% (elevated p-HVA), 57-82% (elevated p-VMA), and 71-88% (elevated p-HVA or elevated p-VMA) were found, when HVA and VMA were assayed in blood (Tang et al 1986, Gahr & Hunneman 1987, Berthold et al 1992) and even lower sensitivities of p-DA, p-NA or p-A in NB patients have been reported (Alvarado et al 1985, Eldrup et al 2001). Thus, p-DOPA seems to be superior to other single plasma measures and possibly of similar value as u-HVA and u-VMA in diagnosis of NB.

The sensitivity of an elevated value of either p-DOPA or p-DOPAC concentration was as high as 92% in 13 untreated NB patients (Eldrup et al 2001). The specificity of a normal plasma concentration of either DOPA or DOPAC was 96% among our 24 patients with a non-NB solid tumor (Eldrup et al 2001). Calculations of specificities and sensitivities involving p-DOPAC levels in NB patients in our study may be criticized. These calculations were made with the assumption that if we had included more patients in the NB group and if all children in the study had been in the same nutritional state, there would have been a significant difference between p-DOPAC levels in NB children and healthy children. Investigations of dietary and diurnal influence of p-DOPA and p-DOPAC concentrations in healthy children as well as larger studies of NB and GN patients are needed to finally elucidate the role of p-DOPA and p-DOPAC in the diagnosis of neuroblastoma. Most probably p-DOPAC concentrations should be measured in the fasting state. With these reservations, our investigation indicates that p-DOPA and p-DOPAC concentrations seem to be useful alternatives to u-HVA and u-VMA excretion in the diagnostic work-up of children with a solid tumor.

According to a recent review MIBG scintigraphy has a sensitivity of 70-100 % and a specificity of 82-100% in studies including more than 20 NB patients (Shulkin & Shapiro 1998). Ganglioneuroma is one of the tumors that may cause a false positive MIBG scintigraphy (Clerico et al 1991). Most studies of the value of MIBG scintigraphy compared to other imaging procedures in the diagnostic work up of suspected neuroblastoma have been retrospective (Schmiegelow et al 1989, Abrahamsen et al 1995, Andrich et al 1996, Leung et al 1997, Perel et al 1999). One study compared u-VMA excretion and MIBG scintigraphy in 71 NB children and 25 children with other neoplastic or non-neoplastic diseases. Sensitivity and specificity of u-VMA excretion in these children were 93% and 68%, respectively. In 31 of the NB patients sensitivity of MIBG was 95%, while specificity of MIBG was 88% (Schmiegelow et al 1989). Thus, MIBG scintigraphy seems to be of similar value to p-DOPA/p-DOPAC levels and u-HVA/u-VMA excretion in the diagnosis of NB. MIBG scintigraphy has an advantage compared to biochemical measures being most helpful in staging of NB patients (Mitjavila 2002). Scintigraphy, however, requires sedation of children, is more expensive, and exposes children to irradiation. Urine sampling in small children may be troublesome and age-related normal values should be corrected for creatinine concentrations. Therefore, plasma samples seem to be preferable in primary diagnostic work up in children suspected of NB. Prospective comparisons of p-DOPA/p-DOPAC, u-HVA/u-VMA, and MIBG scintigraphy in high number of patients, however, are warranted.

Relation to prognosis

VMA and HVA excretion in urine in untreated NB have (Gitlow et al 1973, Laug et al 1978) and have not (Liebner & Rosenthal 1973, LaBrosse et al 1980, Graham-Pole et al 1983, Eldrup et al 2001) been correlated to prognosis in neuroblastoma. Ratio of urinary HVA/VMA excretion has been claimed to be a classical prognostic factor (Favrot et al 1996). Ratio between HVA and VMA urinary excretion were related to prognosis in stage IV disease with increased HVA relative to VMA being unfavorable (Laug et al 1978, LaBrosse et al 1980). In a recent study, however, low (<1) and high (>2) HVA/VMA urinary excretion ratios were related to a low survival rate (Nishi et al 1998). In other studies percentage of patients with increased urinary excretion of VMA but also of HVA as well as percentage of patients with increased serum HVA or VMA increased with increasing stage of disease. No correlation, however, between survival time and urinary excretion of catecholamine metabolites have been found (Laug et al 1978, Gahr & Hunneman 1987, Pritchard et al 1989, Berthold et al 1992, Eldrup et al 2001). When ratio of serum levels of HVA and VMA was examined, a correlation to prognosis was found only in localized but not in disseminated neuroblastoma (Berthold et al 1992).

Increased p-DOPA correlated with patient's age above 1 year, tumor stage (III and IV only) and DNA ploidy in screening-detected and non-screening-detected neuroblastoma (Ikeda et al 1996). This finding could indicate that higher p-DOPA levels are associated with unfavorable characteristics of NB. We found a significant relationship between p-DOPA and age at diagnosis in non-screening-detected neuroblastoma (Eldrup et al 2001). A larger tumor size in older neuroblastoma children is one possible explanation of our findings. We did not, however, find any relationship between p-DOPA or p-DOPAC and survival or Childrens Cancer Study Group stage in our material.

In conclusion, no definite relationship between plasma or urinary DOPA and catecholamine metabolites on the one hand and prognosis of any stage of NB disease on the other has been demonstrated.

Disease monitoring

Both VMA and HVA excretion in urine have been used to follow and guide therapy in individual patients (Liebner & Rosenthal 1973, Gitlow et al 1973, Bond 1975, Eldeeb et al 1988). Sometimes elevation of urinary catecholamine metabolite excretion preceded clinical symptoms when relapse occurred and remained elevated in fatal cases. Long-term survivors of NB with increased urinary HVA and VMA excretion, however, have been found (Liebner & Rosenthal 1973, Horn & Blatt 1992, Eldrup et al 2001). Several cases of ganglioneuroma with increased HVA and VMA secretion have also been reported (Clerico et al 1991, Lucas et al 1994). U-HVA and u-VMA excretion were used as tumor markers in a recent study of mass screening detected NB cases. Imaging procedures, however, were used in addition to catecholamine metabolite excretion, when it was determined if children suspected of NB should undergo surgery or not (Nishihira et al 2000). U-DA excretion and p-DOPA concentrations have also been utilized as tumor markers in follow-up of NB patients (Helson et al 1980, Alvarado et al 1985, Boomsma et al 1989, O'Meara et al 1994, Ikeda et al 1996). High levels of p-DOPA, however, have been reported in long-term survivors of NB (Alvarado et al 1985, Eldrup et al 2001). P-DOPA levels and u-HVA excretion from individual NB patients in our study are shown in Fig. 12. The figure shows that only in very few children serial measurements of p-DOPA and u-HVA were obtained until the time of death or for at least 2 years after diagnosis. Fig. 12 indicates that an early normalization of these markers does not in itself suggest a favorable outcome. P-DOPA remained elevated in some but not all NB patients with fatal outcome. Other investigators have probably had the same

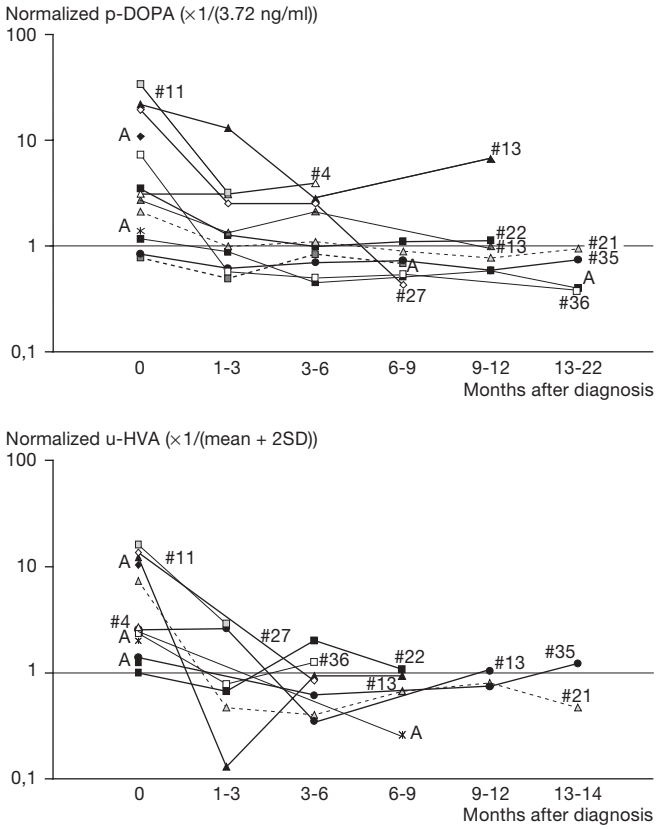

Fig. 12. *Individual values of normalized p-DOPA concentrations (upper panel) and u-HVA excretion (lower panel) in NB children (n=13) before, during and after treatment. Series are labelled: "A" denotes children who were alive more than 7 yrs after diagnosis; "# and number" denotes children who died after as many months after diagnosis as indicated by the number. (Unpublished, data from Eldrup et al 2001).*

difficulties as we had collecting serial blood samples in these small children with very serious disease as no series have been published with tumor markers sampled at regular interval until long-time survival (> 2 years) or fatal outcome. P-DOPA and p-DOPAC concentrations and urinary excretion of HVA and VMA may be of value in the follow-up of NB patients, but their significance in this respect has not been finally clarified.

Recently, it was suggested that ^{123}I-MIBG scintigraphy is better than u-VMA, u-HVA and serum neuron specific enolase for detecting recurrent NB. Seven of 8 children without clinical symptoms had true positive MIBG scintigraphy at primary recurrence of NB, while only 1 of 6 children without symptoms had elevated biological markers. Three of 5 children with normal biological markers and no clinical symptoms disclosed true positive MIBG scintigraphy at recurrence of NB (Okuyama et al 2002). Larger studies including p-DOPA and p-DOPAC determinations are warranted before it can be decided if serial MIBG scintigraphy may be superior to biochemical measures in the follow-up of NB patients. Also, ethical questions regarding irradiation dose as well as determination of appropriate interval between follow-up examinations remain to be answered and determined. Clearly, however, urine or plasma biochemical measures cannot be used as the sole determinant regarding further treatment in an individual patient with neuroblastoma or ganglioneuroblastoma. In addition, no data have been presented that indicate a better outcome, when tumor markers or repeated MIBG scintigraphy are used to detect recurrence of NB.

5. ORIGIN OF DOPA, DOPAC, AND DA-S IN PLASMA

DOPA

DOPA is synthesized by TH, the rate-limiting enzyme in synthesis of DOPA and the classical catecholamines DA, NA and Adr (Nagatsu et al 1964, Udenfriend 1966, Masserano et al 1988). TH is extensively present in vertebrates but has also been demonstrated in arthropods (for refs see Laxmyr 1985, Owen & Bouquillon 1992). TH is essential for survival, at least in mice (Kobayashi et al 1995). It has been demonstrated in dogs and rats that plasma DOPA concentrations are dependent on TH activity as TH inhibition with α-methyl-para-tyrosine decreases p-DOPA levels in the basal state (Goldstein et al 1987a, Kvetnansky et al 1992a). P-DOPA levels increased concurrent with supposed massive TH activation by immobilization stress and severe hypoglycemia (Kvetnansky et al 1992a, Goldstein et al 1993). P-DOPA seemed to increase in animals but not in humans provided p-NA increased 60% or more (Table 4). P-DOPA increased significantly without DOPA clearance apparently being decreased when DDC was partly inhibited by benserazide (Fig. 6, Eldrup et al 1994). Thus, DOPA that is normally decarboxylated to DA spill over to plasma after benserazide. TH is predominantly located in noradrenergic neurons and adrenal medulla but a non-neuronal location has recently been demonstrated (Masserano et al 1988, Bäck et al 1995, Mezey et al 1996, Mezey et al 1998). How much of p-DOPA that is derived from neuronally and non-neuronally TH is unknown. The synthesis of DOPA by the TH pathway probably is most abundant in neuronal tissue which includes adrenal glands. Adrenalectomy in rats, however, did not change p-DOPA concentrations (Hansell et al 1996, Eldrup & Richter 2000) indicating that adrenal glands are not a major source of p-DOPA. P-DOPA decreased or increased after food intake, probably depending on DOPA content of meals but possibly also depending on circulating insulin levels (Williams et al 1986, O'Hare et al 1989, Eldrup et al 1997, Goldstein et al 1999). Thus, noradrenergic neurons and to some extent food seem to be sources of p-DOPA.

Other pathways of DOPA synthesis have been demonstrated. DOPA may be synthesized from tyrosine by tyrosinase in melanogenesis (Pomerantz 1966). Catecholamine synthesis in mice seems to be mediated by tyrosinase in the absence of tyrosine hydroxylase (Rios et al 1999). Plasma DOPA concentrations, however, were similar in albino subjects and in Caucasian and black healthy subjects (Garty et al 1989a). In seven healthy females aged 20-40 years we found no seasonal variation in p-DOPA levels when measured every 3-4 months (May, August, November and March) during one year (Eldrup et al, unpublished results). Production of DOPA from m-tyrosine has been demonstrated in rats (Hollunger & Person 1974). Furthermore, administration of 3, 4-dihydroxyphenylpyruvic acid increased p-DOPA levels in rats (Lindén 1980). When either m-tyrosine or 3, 4-dihydroxyphenylpyruvic acid were administered orally to the author in mg doses in preliminary experiments, no changes of p-DOPA concentrations were observed (unpublished results). Thus, melanogenesis does not seem to contribute significantly to p-DOPA levels in humans and no other pathways than TH mediated DOPA synthesis and DOPA content in food have been demonstrated to be important for p-DOPA levels in humans.

In conclusion, DOPA is an amino acid and p-DOPA after meals may at least partly depend on DOPA content of meals. DOPA may be taken up from plasma and is probably released back to the circulation from depots in different tissues such as skeletal muscle and the gastrointestinal tract. Insulin may decrease p-DOPA levels but the mechanisms behind exchange of DOPA between blood and tissues have not been clarified. DOPA is synthesized from tyrosine by tyrosine hydroxylase in sympathoadrenal nerves but probably also in non-neuronal tissue in the gastrointestinal tract. DOPA is normally decarboxylated to DA, but DOPA that is not decarboxylated may spill over to plasma. P-DOPA concentration, however, is not a sensitive index of sympathetic activity or NA synthesis in nerves.

DOPAC

Studies in humans with autonomic neuropathy such as PAF and MSA have suggested that p-DOPAC is of neuronal origin (Yamamoto et al 1996, Goldstein et al 1997). These studies are supported by studies in dogs showing decreased p-DOPAC levels after

TH inhibition (Goldstein et al 1987a) and studies in rats showing increased p-DOPAC levels after immobilization (Kvetnansky et al 1992a). Chemical sympathectomy in rats decreased DOPAC content in skeletal muscle and heart (Eldrup & Richter 2000). After inhibition of vesicular reuptake of DA by reserpine, p-DOPAC and TH activity increased in rats (Eisenhofer et al 1988). When results from humans and rats are summarized, DOPAC most likely spill over from sympathetic neurons to plasma as a result of TH activity. P-DOPAC, however, is not a sensitive index of TH activity or sympathetic activity.

Another source of p-DOPAC has been demonstrated. An evening open sandwiches meal with high DA content increased p-DOPAC concentrations in humans (Eldrup et al 1997). Fasting in humans decreased p-DOPAC levels (Eldrup et al 1997). Prolonged fasting in rats decreased DOPAC content in plasma, heart and stomach (not significantly in stomach, Eldrup & Richter 2000).

During i.v. infusion of DA, some DA was deaminated and p-DOPAC levels in humans increased (Claustre et al 1990). It is not known, however, to what extent endogenous free p-DA may be deaminated to DOPAC in some tissue and returned to plasma.

Plasma DOPAC levels are higher than csf DOPAC levels (Table 1A) and debrisoquin, a MAO inhibitor that does not enter the brain, produces large decreases in p-DOPAC levels (Hovevey-Sion et al 1989). These results indicate that p-DOPAC is not of CNS origin.

In conclusion, evidence from mainly rats but also from humans indicates that p-DOPAC seems to be of dual origin, partly derived from neuronal tissue and partly derived from some food, possibly DA content of food.

DA-S

From the literature there is overwhelming evidence that p-DA-S is not of sympathetic nervous origin (Kuchel et al 1985a, Ratge et al 1986b, Eldrup et al 1988, Cuche et al 1990, Eisenhofer et al 1990). Circulating free DA may be sulfoconjugated after DA infusion (Claustre et al 1990). It has been suggested by others, but not proven, that DA-S production from free p-DA takes place in the small intestine (Shibata et al 1987, Sundaram et al 1989, Eisenhofer et al 1999). P-DA-S levels increase after DDC inhibition with benserazide (Eldrup et al 1994). A continous p-DA-S production from p-DOPA via DA in the fasting state seems possible (Goldstein et al 1999) but warrant confirmation in experimental studies. The origin of p-DA-S, however, seems predominantly to be food content of DOPA, DA and DA-S (Eldrup et al 1997) though a contribution from endogenously synthesized DA in the gastrointestinal tract cannot be excluded.

6. SUMMARY AND PERSPECTIVES

In summary, results from investigations reviewed in this thesis are:

- No depot of DOPA seems to exist in sympathetic nerves in rats.
- Muscle tissue seems to be the largest depot of endogenous DOPA in rats.
- P-DOPA seems to be derived mainly from sympathetic nerves as a result of TH activity but p-DOPA concentration is not a sensitive index of sympathetic activity or NA synthesis in human nerves.
- Endogenous human arterial p-DOPA clearance is 1.0 l/min and approximately 20% of this clearance is accounted for by renal excretion of DA in urine.
- Human arterial p-DOPA clearance is not significantly changed after partial inhibition of DDC which increases p-DOPA concentration approximately 10-fold.
- DOPA seems normally to be decarboxylated to DA but DOPA that is not decarboxylated may spill over to plasma.
- P-DA-S but not p-DOPAC levels increase after DDC inhibition in humans.
- Human p-DOPA concentrations decrease, increase or are unchanged after food intake and seem to depend at least partly on DOPA content of the meal.
- P-DOPA is unchanged during 25-h fasting in humans.
- Food with high DA content increases and fasting decreases p-DOPAC concentrations in humans.
- Human p-DA-S originates in the food presumably reflecting food content of DOPA, DA, and DA-S.
- Fasting for 25 h decreases but does not eliminate p-DA-S concentrations in humans.
- Food may be the source of stomach DOPA content in rats.
- Prolonged fasting but not sympathectomy decreases p-DOPAC concentrations in rats.
- DOPAC in rat skeletal muscle tissue seems primarily to be located in sympathetic nerves.
- Cerebrospinal fluid DOPAC and csf NA concentrations seem to be decreased in PD patients and in addition to the dopaminergic defect point towards noradrenergic dysfunction in PD.
- Cerebrospinal fluid and p-DOPA concentrations seem to be unchanged in untreated PD patients.
- P-DOPAC and p-DOPA seem to be alternatives to urine excretion of HVA and VMA as tumor markers in NB (but warrant confirmation in larger studies).
- P-DOPAC and p-DOPA concentrations do not seem to be general markers of prognosis in NB.

The present review also raised questions and highlighted areas that deserve further research:

- How much of p-DOPA is derived from the non-neuronally TH pathway?
- What is the influence of muscle DOPA depot on p-DOPA concentrations?
- What is the significance of insulin on DOPA exchange between plasma and other tissues?
- What is the relationship between food DA content and p-DOPAC concentrations?
- In which tissue is DOPAC and DA-S produced from circulating free DA?
- Is p-DOPA important for p-DA-S levels after prolonged fasting?
- Is endogenously synthesized DA in the gastrointestinal tract important for p-DA-S and p-DOPAC levels?
- Further characterization of noradrenergic dysfunction in PD.
- Prospective comparisons of p-DOPA/p-DOPAC with u-HVA/u-VMA and MIBG scintigraphy in children with solid tumors.

ABBREVIATIONS

Adr	adrenaline (= epinephrine)
Al	alumina
AADC	aromatic amino acid decarboxylase
Ci	Curie
CNS	central nervous system
Csf	cerebrospinal fluid
CV	coefficient of variation
DA	dopamine
DA-S	dopamine sulfate
DDC	dopa decarboxylase.
DHBA	dihydroxybenzylamine
DHPG	3,4-dihydroxyphenylglycol
DOPA	3,4-dihydroxyphenylalanine
DOPAC	3,4-dihydroxyphenylacetic acid
ED	electrochemical detection
EGTA	ethylene glycol-bis(b-aminoethyl ether)-N,N,N',N'-tetraacetic acid
g	gram
GN	ganglioneuroma
GNB	ganglioneuroblastoma
h	hours
HCl	hydrochloric acid
hplc	high performance liquid chromatography
HVA	homovanillic acid

l	litre
MAO	monoamine oxidase
mDA	methyl dopamine (= deoxyepinephrine)
MIBG	meta-iodo-benzylguanidine
min	minutes
ml	millilitre
mm	millimeter
mmol	millimol
MSA	multiple system atrophy (= Shy-Drager syndrome)
µ	micro
n	number
NA	noradrenaline (= norepinephrine)
$Na_2S_2O_5$	sodium metabisulphite
NB	neuroblastoma
ng	nanogram
nmol	nanomol
6-OH-DA	6-hydroxydopamine
p	plasma
PAF	pure autonomic failure
PCA	perchloric acid
PD	Parkinson's disease
pg	picogram
RE	radioenzymatic
REA	radioenzymatic assay
rp	reverse-phase
rp-hplc-ED	reverse-phase high performance liquid chromatography with electrochemical detection
SD	standard deviation
SEM	standard error of mean
tDA	total dopamine
TH	tyrosine hydroxylase
u	urine
VMA	vanillylmandelic acid

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