

Diabetes-Induced Biochemical Changes in Central and Peripheral Catecholaminergic Systems

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Summary

A great variety of alterations have been described in the nervous system of diabetic animals. They are named as diabetic neuropathy and affect the brain, spinal cord and peripheral nerves. In diabetic animals, plasma and tissue catecholamine levels have been reported to be increased, decreased or unchanged, and these disparities have been explained by differences in the tissues selected, severity or duration of diabetes. Dopamine, norepinephrine and epinephrine from different tissues were extracted by absorption onto alumina, and measured by high performance liquid chromatography with electrochemical detection. We found that diabetes alters catecholaminergic systems in a highly specific manner. The dopamine content is reduced in the dopaminergic nigrostriatal system only. Norepinephrine is differently altered in several areas of the sympathetic nervous system. It is increased in cardiac ventricles, and decreased in stellate ganglia and the blood serum. However, it is not altered in the central nervous system. Finally, epinephrine is only altered in the adrenal gland where it is increased, and in the serum where it is reduced. Our results suggest that diabetes reduces the activity of the nigrostriatal dopaminergic system. Changes found at the sympathoadrenal level could be explained by reduced norepinephrine and epinephrine synthesis, with increased storage due to a reduced release from synaptic vesicles.

Key words

Diabetic neuropathy • Catecholamines • Synthesis • Storage • Release

Introduction

A great number of anatomical, functional and biochemical alterations have been described in the nervous system of diabetic animals (Tomlinson *et al.* 1992, Ozturk *et al.* 1996). This variety of alterations (generally named as diabetic neuropathy) affects the brain, spinal cord and peripheral nerves. They were reported many years ago as degenerative changes in the autonomic nervous system of diabetic rats, with widespread degeneration of ganglionic tissue, reduction

of axonal calibre and demyelination (Monckton and Pehowich 1980, Tomlinson and Yusof 1983, Schmidt and Pular 1986, Kniel *et al.* 1986). In the central nervous system, diabetes reduces brain weight and neocortical volume, which is associated with a reduction of the number of cortical neurons (Jakobsen *et al.* 1987). All these central and peripheral changes are consistent with decreased neuronal activity.

Biochemical changes found in diabetic neuropathy are more widespread and more controversial than the anatomical changes. When we focused on

plasma and tissue catecholamine levels, these were increased, decreased or unchanged (Fushimi *et al.* 1984, Jobidon *et al.* 1985, Hilsted 1995, Chu *et al.* 1986, Bitar *et al.* 1986). These disparities have been explained on the basis of differences in the selected tissues, severity or duration of diabetes, etc.

In the present report, we hypothesized that there is a reduction in catecholaminergic neuronal activity, with a reduction in catecholamine synthesis, associated with a difficulty to release these neurotransmitters from synaptic terminals. To test this hypothesis, we measured dopamine, norepinephrine and epinephrine levels in several discrete organs that have only catecholaminergic neuronal bodies or synaptic terminals.

Methods

Young adult Sprague-Dawley rats were divided into two groups. Animals of the control group were 10-12 weeks old and the experimental group was 6-8 weeks old at the moment of type I diabetes induction. Experiments were carried out four weeks after type I diabetes had been induced, so that all the animals were 10-12 weeks old at the moment of tissue collection. Fifteen control animals and 15 diabetics were studied. The principles of laboratory animal care were respected.

After anesthetizing the animals with an intraperitoneal injection of 6 % chloral hydrate (2 ml/kg), the jugular vein was dissected to enable a slow injection of 65 mg/kg body weight of streptozotocin. Following topical application of sulfathiazole to avoid possible infection, the wound was closed with 2-3 sutures. The animals in the control group were subjected to the same treatment, using only a citrate buffer vehicle, without streptozotocin. Experiments were performed 30 days after injection, when type I diabetes was well stabilized and streptozotocin had been totally washed away. Blood samples were taken during the surgical procedure and immediately before the experiment.

Four weeks after the streptozotocin treatment or sham operation, animals were anesthetized with chloral hydrate and perfused transcardially in order to clear plasma catecholamines from different tissues. We used saline solution plus 50 mM acid phosphate buffer (pH 5), because catecholamines are more stable in an acidic medium. After perfusion, tissues were removed, weighed and immersed in 0.5 ml of 0.4 N perchloric acid. Tissue samples were then homogenized and centrifuged at 40 000 x g for 15 min. The supernatants were stored at -80 °C until catecholamine assay.

We have chosen organs with different catecholaminergic characteristics, namely from regions that have only neuronal bodies, such as the midbrain for dopaminergic neurons, pons and stellate ganglion for noradrenergic neuronal bodies and the adrenal glands as the main source of epinephrine. Besides, we selected the striatum on the one hand and the medulla and ventricle on the other, because in these regions there are only dopaminergic and noradrenergic nerve terminals, respectively, but there are no catecholaminergic neuronal bodies. Finally, there are neither neuronal bodies nor terminals in the blood serum so that serum catecholamine levels are the net resultant of neuronal release and clearance. Serum levels can be taken as indirect evidence of catecholamine release from sympathetic nerves or the adrenal gland.

Dopamine, norepinephrine and epinephrine were extracted by absorption onto alumina and measured by high performance liquid chromatography with electrochemical detection. Catecholamines were separated by an ionic exchange column (PCAT Analytical Column, Bio-Rad Laboratories), and determined using a standard kit for serum catecholamine detection (Catec. Plasma Reagent Kit, Bio-Rad Laboratories). The model HP 1100 amperometric detector (Hewlett Packard) was used at an oxidation potential of +0.5 V.

Data are expressed as mean \pm S.E.M. Statistical significance was calculated using Student's *t*-test for paired or unpaired data, where appropriate. A two-tailed $P < 0.05$ was considered as statistically significant.

Results

Two days after streptozotocin administration, the first symptoms of type I diabetes, polyuria and polydipsia, became evident followed by polyphagia and weight loss. The body weights of four weeks diabetic rats were decreased by 15 %, from 217.6 \pm 6.2 to 185.6 \pm 8.6 g ($p < 0.005$), and plasma glucose levels were increased from 7.4 \pm 0.2 to 24.7 \pm 0.8 mmol/l ($p < 0.0001$). These results agree with those previously published (Bitar *et al.* 1986, Casis *et al.* 2000).

In peripheral tissues, dopamine levels were unchanged by diabetes in the adrenal glands, blood serum and cardiac ventricles, whereas a clear decrease was found in the stellate ganglion of diabetic rats (Fig. 1A). In the central nervous system, the dopamine content was not altered either in the medulla or pons, but it was reduced in the nigrostriatal system, both in the midbrain, where

neuronal bodies are present, and in the synaptic terminals of the striatum (Fig. 1B).

Figure 2A shows that diabetes differentially altered norepinephrine levels in the sympathetic nervous system. Norepinephrine levels were reduced in the stellate ganglion and in the blood serum, but they were increased in the cardiac ventricle. Neurohormone content was not altered by diabetes in the adrenal gland or in any region of the central nervous system (Fig. 2B).

The epinephrine content is not changed by diabetes either in the central nervous system or in the cardiac ventricle. In these regions, the epinephrine content is very low in both healthy and diabetic animals. The main differences are found in the adrenal gland, where epinephrine is synthesized and stored, and in the blood serum into which the hormone is released (Fig. 3).

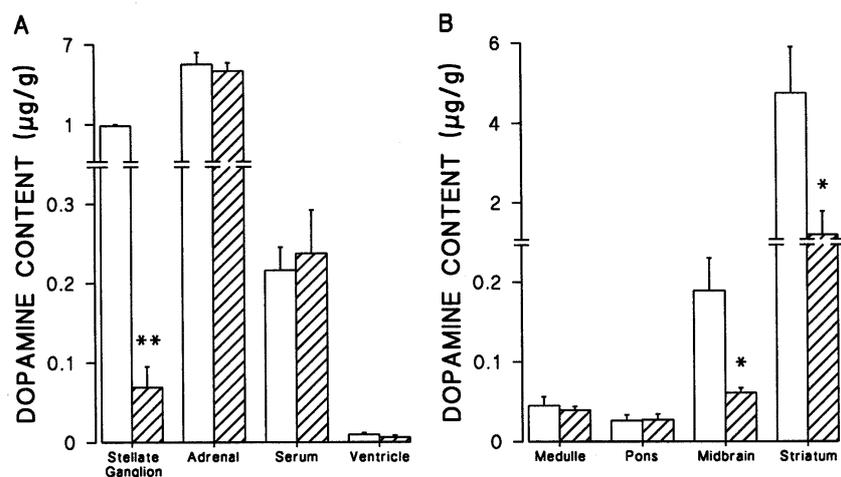


Fig. 1. Dopamine content (μg per g of tissue) in the sympathoadrenal system (A) and central nervous system (B). * $P < 0.05$, ** $P < 0.0001$.

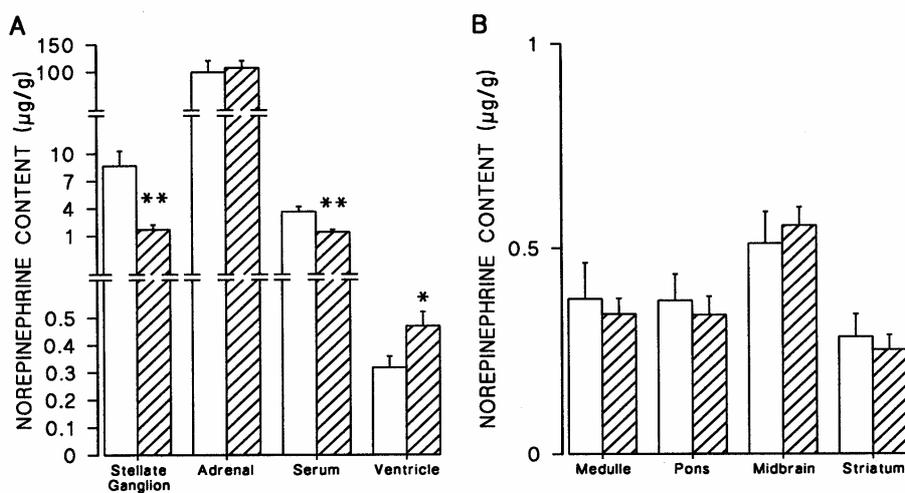


Fig. 2. Norepinephrine content (μg per g of tissue) in the sympathoadrenal system (A) and central nervous system (B). * $P < 0.05$, ** $P < 0.01$.

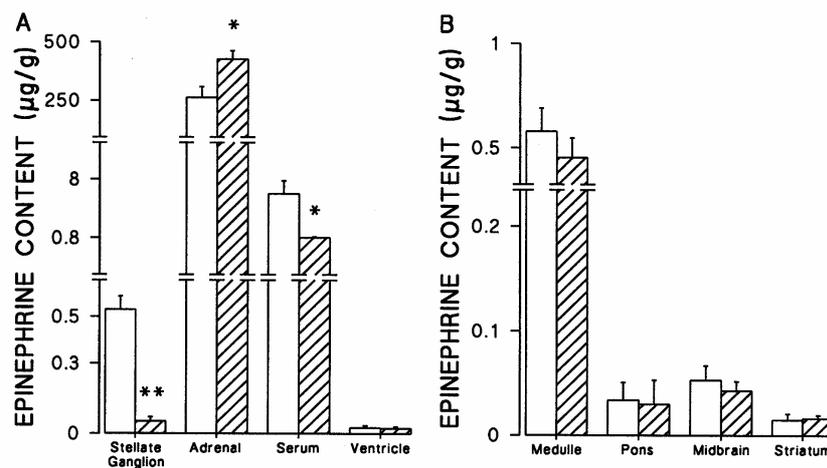


Fig. 3. Epinephrine content, in μg per g of tissue, in the sympathoadrenal system (A) and central nervous system (B). * $P < 0.01$, ** $P < 0.0001$.

Discussion

Previous reports have studied plasma and tissue catecholamine levels in diabetic animals. However, a detailed description of dopamine, norepinephrine and epinephrine in the central and peripheral regions of the autonomic nervous system in diabetic animals has not been previously performed in a systematic manner. The results of these reports were ambiguous, catecholamine levels were found to be increased, decreased or unchanged. These disparities were explained by differences in the tissues selected, severity and duration of diabetes. The present report shows that there are characteristic effects of diabetes in different tissues irrespective of the severity or duration of diabetes.

We have found that diabetes alters the catecholaminergic system in a very specific manner. The dopamine content is reduced only in the dopaminergic nigrostriatal system. Norepinephrine is altered, increased or decreased, in the sympathetic nervous system, but not in the central nervous system, and epinephrine is only altered in the adrenal gland and serum.

The present results concerning dopamine changes agree with previous works that reported reduced dopamine synthesis and turnover in several brain areas including the striatum (Trulsson and Himmel 1983, Kwok and Juorio 1986, Shimomura *et al.* 1988). This indicates that the activity of the dopaminergic system in diabetics is reduced with respect to control rats. Based on this result, one could hypothesize that this could lead to Parkinson's disease. Although there are no systematic

studies in this regard, it has recently been reported that there is a high prevalence of association between different forms of Parkinsonism and diabetes in specific communities with a high incidence of diabetes, suggesting the possibility of a cause-effect relationship or a common etiopathology (Ahlskog *et al.* 1997, Singh *et al.* 1999, Chaudhuri *et al.* 2000).

In our work, the epinephrine and norepinephrine content is not altered in the central nervous system of diabetic rats. The increments or decrements are specifically confined to the sympathoadrenal system. Less than 10 % of norepinephrine is synthesized in the neuronal body. However, at this level there is only synthesis but no storage of the neurotransmitter which is stored at the synaptic terminals. Thus, the altered norepinephrine content in the stellate ganglion, where the neuronal bodies but no noradrenergic synaptic terminals are located, indicates that diabetes affects neurotransmitter synthesis only. The norepinephrine content is reduced in the stellate ganglion, suggesting a diminution of sympathetic neuronal activity. Sympathetic nerves coming from the stellate ganglion innervate the cardiac muscle so that the norepinephrine content in the ventricle reflects neurotransmitter storage at synaptic terminals. Facing reduced norepinephrine synthesis, the increased norepinephrine content in the synaptic vesicles could imply a difficulty in neurotransmitter release. This possibility is further supported by a reduction of serum norepinephrine, which can be taken as an indirect indicator of neurotransmitter release, because there are neither neuronal bodies nor terminals in blood serum.

These results agree with those previously published reporting reduced norepinephrine release from nerve terminals (Tomlinson and Yusof 1983, Yoshida *et al.* 1985). Our results further support the proposed hypothesis of an increase in norepinephrine storage due to reduced release (Tomlinson *et al.* 1992).

A reduction of the norepinephrine content in the stellate ganglion has previously been published by us (Gallego *et al.* 2000). On the other hand, a number of authors have reported that norepinephrine in cardiac ventricle is either unchanged (Fushimi *et al.* 1984, Jobidon *et al.* 1985, Kaul and Grewal 1985) or elevated (Paulson and Light 1981, Lucas and Quirbi 1989, Kurata *et al.* 1997). These discrepancies seem to depend on the severity and duration of diabetes, because no changes were reported after 2 weeks of streptozotocin treatment, or when a low dose (less than 50 mg/kg) was used. Besides, norepinephrine release from nerve terminals has also been reported to be increased (Lucas and Quirbi 1989), unchanged (Houwing *et al.* 1997), or reduced (Yoshida *et al.* 1985, Hart *et al.* 1988, Sato *et al.* 1989, Gando *et al.* 1993, Wilke and Hilarid 1994, Bitar *et al.* 1999). These discrepancies can also be related to the severity and duration of diabetes. Increases or no changes in norepinephrine release are found with low doses or short-term duration streptozotocin treatment. More than 40 mg/kg of streptozotocin or more than 30 days of diabetes duration are associated with a reduction of norepinephrine release. All the above reports, together with our own work, suggest that the alterations in catecholamine metabolism are dependent on the severity and duration of diabetes. Thus, short-term diabetes is accompanied with increase or no change in

catecholamine content or release, but reduced release and increased storage of catecholamines are found in long-term diabetes (Gotzsche 1983, Gallego and Casis 2001).

The epinephrine content is increased by diabetes in the adrenal gland, these results also agree with the hypothesis of reduced epinephrine release, which enhances hormone storage. Furthermore, reduced blood epinephrine levels further support a reduction in hormonal release. This reduced epinephrine release has been related to a decreased adrenomedullary response to cholinergic stimulation by presynaptic nerves (Hart *et al.* 1988).

However, the etiology of diabetic neuropathy has not yet been clarified. It has been related to excessive generation of sorbitol by aldolase reductase due to maintained hyperglycemia, altered metabolism of phosphoinositides and reduced Na/K-ATPase activity (Greene *et al.* 1987, Tomlinson *et al.* 1992). However, the clinical efficacy of aldolase reductase inhibitors has been less than expected. Recently, diabetic neuropathy has been related to a diminished production of trophic factors by peripheral tissues (Hellweg and Hartung 1990, Fernyhough *et al.* 1995, Anand *et al.* 1996).

In summary, our results indicate that diabetes reduces the activity of the nigrostriatal dopaminergic system. A reduction of norepinephrine and epinephrine synthesis, with increased storage probably due to a reduced release from terminal vesicles are also suggested.

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