In vivo cardiovascular reactivity and baroreflex activity in diabetic rats

Theo Van Buren a, Carina M. Kasbergen a, Willem H. Gispen a, Dick J. De Wildt a,b, *

a Department of Medical Pharmacology, Rudolf Magnus Institute for Neurosciences, Medical Faculty, Utrecht University, Universteitsweg 100, 3584 CG Utrecht, Netherlands

b National Institute of Public Health and Environment, 3720 BA Bilthoven, Netherlands

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Abstract

Objectives: Abnormalities of the cardiovascular system, e.g. impaired vasoreactivity and changes in baroreflex control of heart rate, are known to occur in experimental diabetes. It is not clear whether these cardiovascular dysfunctions are direct consequences of cardiovascular deficits and/or have autonomic neuropathy as a cause. Methods: To differentiate between cardiovascular deficits or neuronal impairment as a cause for these cardiovascular dysfunctions, we tested the effects of the ACTH analogue, Org 2766, a neurotrophic compound without cardiovascular effects, on arterial pressure, heart rate and baroreflex control of heart rate. At 15 weeks, rats were made diabetic by injection of streptozotocin, and from 0–6, 6–12 or 12–18 weeks thereafter 3 groups of rats were treated with Org 2766. These effects were evaluated during phenylephrine-induced increases, and sodium nitroprusside-induced decreases, in blood pressure, in rats that had been diabetic for various periods (2–42 weeks). Results: Throughout, both depressor response and maximal vasodilator activity in response to sodium nitroprusside were significantly (P < 0.05) reduced as compared to those of the non-diabetic controls. The pressor response of the diabetic rats to phenylephrine was only significantly (P < 0.05) reduced at 4, 6 and 12 weeks, and at 18 weeks, the diabetic rats were either hypo- or normoresponsive; Org 2766 did not restore the disturbed pressor response. From weeks 4 to 42 both maximal decrease in heart rate and sensitivity of baroreflex-mediated bradycardia in the diabetic rats were significantly less (P < 0.05) than those in the non-diabetic controls. Org 2766 restored the diminished baroreflex-mediated bradycardia of diabetic rats to non-diabetic control levels at 6 weeks, had an ameliorating effect at 12 weeks and no effect at 18 weeks. Conclusions: Time-dependent decreases in baroreflex sensitivity in diabetic rats was demonstrated and a much less steep decline of baroreflex sensitivity occurred in non-diabetic control rats. The ACTH analogue, Org 2766, when given immediately upon the induction of diabetes seem to delay the development of autonomic neuropathy, which suggests that cardiovascular factors appear to be of minor importance. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Streptozotocin-induced diabetic rat; Autonomic neuropathy; Cardiovascular reactivity; Baroreflex

1. Introduction

Cardiovascular dysfunctions are a major cause of death in patients with diabetes mellitus. Vascular pathology, e.g. atherosclerosis [1] and microangiopathy [2] could play a role in the aetiology of these dysfunctions. However, these patients are also commonly afflicted with peripheral neuropathy. Diabetic neuropathy can affect both somatic and autonomic nervous systems. Autonomic neuropathy might cause profound disturbances of cardiovascular control mechanisms (see Ref. [3] for review). However, the contribution of this autonomic neuropathy to cardiovascular disease in patients with diabetes mellitus is not yet clear.

In experimental diabetes, abnormalities of the cardiovascular system are known to occur, e.g. impaired responsiveness of the vasculature to vasoactive agents and changes in the baroreflex control of heart rate (see Ref. [4] for review). Whether these cardiovascular dysfunctions are a consequence of direct cardiovascular deficits (i.e. abnormalities of the heart and vasculature apart from neural defects) and/or of autonomic neuropathy (indirect) is still

The baroreflex control of heart rate has been found to be altered in rats with streptozotocin-induced diabetes [9–14], in diabetic BB/Wor rats [15] and in diabetic rabbits [16]. Findings regarding baroreflex-mediated bradycardia in diabetic animals are contradictory [10,13,15,16]. Likewise, the reflex tachycardia seen during decreases in arterial pressure in diabetic animals was found to be either pronounced [15] or normal [14,16]. Interestingly, Chang and Lund [12] concluded that, in diabetic rats, baroreflex activity changes as diabetes progresses. These results raise the possibility of time-dependent alterations in cardiovascular control mechanisms in experimental diabetes.

The purpose of the present study was to investigate the vasoreactivity and baroreflex control of heart rate over time in diabetic rats, when altered reflex bradycardia was found, additional experiments were done with the ACTH analogue, Org 2766, to differentiate between an autonomic neuronal or a direct cardiovascular origin of the alterations in vasoreactivity and baroreflex control of heart rate. The ACTH analogue, Org 2766, does not have a direct effect on the cardiovascular system [17] and has been shown to be effective in peripheral nerve disorders in both rats and humans [18]. In addition, Org 2766 can have neurotrophic and neuroprotective effects on the autonomic nervous system of the experimentally diabetic rat [6,7].

2. Methods

2.1. Animals and induction of experimental diabetes

Male U:WU/CPB-Wistar rats weighing approximately 295 g (age 15 weeks) were housed in Macrolon cages (two rats per cage). Diabetes mellitus was induced by a single i.v. injection of streptozotocin (Serva, 40 mg/kg). Rats with blood glucose levels higher than 15 mmol/l as measured by an Ames Glucose Test Pack [6,7] were considered diabetic. They were maintained on a 12-h light/dark cycle (light on at 7.00 h). All rats received water and standard rodent chow ad libitum. The non-diabetic control rats were given a food-restricted diet (14 g rat chow/24 h) with the calorie intake adjusted to keep their body weights similar to those of diabetic rats. All experimental protocols were approved by the Ethical Committee on the use of experimental animals of the Faculty of Medicine of Utrecht University.

2.2. Preparation and measurements

2.2.1. General

Surgery was performed on animals anaesthetized with urethane (10% dissolved in 0.9% NaCl solution) injected i.p. in a dose of 1.2 ml/100 g body weight. Urethane was used instead of barbiturates in order to ensure minimal anaesthetic interference with the cardiovascular system [6]. Body temperature was recorded with a rectal probe, and maintained at 37°C with a homeothermic blanket system (Harvard Apparatus).

2.2.2. Experiment I (time–effect relationship)

Indwelling catheters (polyethylene tubing, Intramedic) were inserted into the right femoral vein and right femoral artery to record direct blood pressure and heart rate, and to administer drugs. Arterial blood pressure and heart rate were measured by means of a Viggo-Spectramed DTX/plus transducer connected to a preamplifier/biortachometer system (University of Utrecht). All the data were processed with a Bio Signal Processing System (Instrumental services, University of Limburg, Maastricht, The Netherlands) and a Wekagraph WK-821 AR recorder. The collected data were processed with a Compaq Desktop 386/20 MHz computer and were determined once per 500 ms. The mean arterial blood pressure was calculated using the formula: MAP = (2 × diastolic blood pressure + systolic blood pressure)/3. Initial haemodynamic values were estimated after a 15-min stabilisation period. To elicit baroreflexes, phenylephrine (reflex bradycardia) at doses of 0.3–30 µg/kg and sodium nitroprusside (reflex tachycardia) at doses of 0.1–10 µg/kg were administered as bolus injections through the femoral vein. The injection volume, 100 µl, was found to have no haemodynamic effects when saline was used. Heart rate and blood pressure were allowed to return to baseline values before the next dose of a drug was administered. A resting period of 15 min was allowed between the highest dose of phenylephrine and the lowest dose of sodium nitroprusside. Heart rate and blood pressure were recorded continuously throughout the procedure.

2.2.3. Experiment II (effects of Org 2766)

At weeks 6, 12 and 18 after the induction of diabetes the rats were fitted with indwelling arterial and venous cannulae to record direct blood pressure and heart rate, and to administer phenylephrine (see Section 2.2.2). The experimental groups are detailed in ‘Outline of the study’.

2.3. Drugs

Sodium nitroprusside (E. Merck, Darmstadt, Germany), a vasodepressor drug, and l-phenylephrine hydrochloride (Sigma, St. Louis, USA), a vaspessor drug, were dis-
solved in saline and administered i.v. Org 2766, an ACTH$_{1-24}$ analogue (H−Met−(O$_2$)−Glu−His−Phe−d-Lys−Phe−OH), was a gift from Organon Int. B.V. Oss, The Netherlands. The peptide was dissolved in saline and administered s.c. at a dosage of 75 µg/kg every 48 h [6,7]. The control rats received 0.5 ml saline.

2.4. Outline of the study

2.4.1. Experiment I (time−effect relationship)

Phenylephrine and sodium nitroprusside were administered to measure alterations in cardiovascular function and baroreflex control of heart rate. Blood pressure and heart rate responses were recorded at 2, 4, 6, 12, 18, 26, 34 and 42 weeks after the induction of diabetes.

2.4.2. Experiment II (effects of Org 2766)

The effect of different treatment schedules with the ACTH$_{1-24}$ analogue, Org 2766, on cardiovascular function and reflex bradycardia was investigated at different times during the induction of diabetes. Series I: group 1, the non-diabetic control rats were given a food-restricted diet during the induction of diabetes. Series II: the second series followed the same protocol as the first series. However, the treatment of groups 2 and 3 (see above) was started 6 weeks after the streptozotocin injection, when experimental diabetic neuropathy was manifest [19,20] and was continued for 6 weeks, up to 12 weeks. Series III: the third series followed the same protocol as the second series. However, the treatment with the ACTH$_{1-24}$ analogue, Org 2766, and placebo was started 12 weeks after the induction of diabetes and was continued for 6 weeks, up to 18 weeks.

2.5. Data analysis

The data are presented as means ± s.e.m. The significance of differences in initial haemodynamic variables and maximal changes in heart rate and mean arterial pressure between two groups was assessed with Student’s t-test and differences between more than two groups were assessed by analysis of variance (ANOVA), followed by the Student–Newman–Keuls test. The significance of differences between groups for dose−response curves to vasoactive drugs was assessed by a multivariate analysis of variance.

### Table 1

General features and initial haemodynamic variables: experiment I

<table>
<thead>
<tr>
<th>Week/group (n)</th>
<th>Body weight (g)</th>
<th>Glucose (mmol/l)</th>
<th>MAP (mmHg)</th>
<th>$P_{\text{dias}}$ (mmHg)</th>
<th>$P_{\text{syst}}$ (mmHg)</th>
<th>HR (bpm)</th>
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<td>132±5</td>
<td>305±16</td>
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<td>225±10*</td>
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<tr>
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<td>331±12</td>
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<tr>
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<td>321±15</td>
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<td>Diet (8)</td>
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<tr>
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<td>83±6</td>
<td>122±6</td>
<td>283±13</td>
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<td>292±13</td>
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<td>92±4</td>
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<td>322±18</td>
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<tr>
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<td>21±1*</td>
<td>88±7*</td>
<td>73±7*</td>
<td>120±8</td>
<td>294±8</td>
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</table>

General features and initial haemodynamic values (means ± s.e.m.) for body weight, blood glucose levels (glucose), mean arterial blood pressure (MAP), diastolic pressure ($P_{\text{dias}}$), systolic pressure ($P_{\text{syst}}$) and heart rate (HR) at weeks 2, 4, 6, 12, 18, 26, 34 and 42 after the induction of diabetes: non-diabetic controls (Diet group); diabetic rats (DM group). Number of animals is given in parentheses.

*Statistically significant difference from non-diabetic controls, $P < 0.05$. 

with repeated measurements (MANOVA). The ‘ramp’ technique was used to construct baroreflex curves [21]. Use of this technique allowed the sensitivity of the baroreflex control of heart rate to be calculated by linear regression [22]. The regression coefficient (slope of the regression line) was taken as an index of baroreflex sensitivity [21] and the difference in slopes for the response to phenylephrine injection at weeks 6, 12 and 18 after the induction of diabetes: non-diabetic controls (Diet group), placebo-treated diabetic rats (DMPla group) and Org 2766-treated diabetic rats (DMOrg group). Number of animals is given in parentheses.

Statistically significant difference from non-diabetic controls, $P<0.05$.

Statistically significant difference from placebo-treated diabetic rats, $P<0.05$.

as compared to preinjection values. A $P$-value of less than 0.05 was accepted as indicating a significant difference.

**3. Results**

**3.1. Experiment I: general features and initial haemodynamic values**

All rats that received streptozotocin became diabetic. The blood glucose levels remained over 15 mmol/l throughout the study (Table 1). The body weights of diabetic rats were significantly lower than those of non-diabetic controls at 2, 4 and 12 weeks of diabetes (Table 1). The initial values for haemodynamic variables are summarised in Table 2. Body weight and haemodynamic values (means $\pm$ s.e.m.) for mean arterial blood pressure (MAP), diastolic pressure ($P_{\text{diast}}$), systolic pressure ($P_{\text{syst}}$), heart rate (HR) and baroreflex sensitivity (slopes) after phenylephrine injection at weeks 6, 12 and 18 after the induction of diabetes: non-diabetic controls (Diet group), placebo-treated diabetic rats (DMPla group) and Org 2766-treated diabetic rats (DMOrg group). Number of animals is given in parentheses.

**Fig. 1**. The depressor response of sodium nitroprusside at 6 weeks after the induction of diabetes. The changes in mean arterial blood pressure (MAP) are shown as absolute increase in mmHg from the preadministration basal values. At 6 weeks, the MAP response to sodium nitroprusside administration in the diabetic rats ($\bullet$) was significantly smaller than in the non-diabetic controls (○) ($P<0.05$).

**Fig. 2**. The maximal vasodilator (hypotensive) activity of sodium nitroprusside (10 $\mu$g/kg). The changes in mean arterial blood pressure (MAP) are shown as absolute decrease in mmHg from the preadministration basal values. At all time points, the response of MAP to sodium nitroprusside in the diabetic rats ($\bullet$) was significantly smaller than that of the non-diabetic controls (○) ($P<0.05$). $^{a}P<0.05$ vs. non-diabetic control rats.
Fig. 3. The vasoconstrictor (hypertensive) activity of phenylephrine at 4 (A) and 34 weeks (B) after the induction of diabetes. The changes in mean arterial blood pressure (MAP) are shown as absolute increase in mmHg from the preadministration basal values. At 4 weeks, the MAP response to phenylephrine administration in the diabetic rats was significantly smaller than in the non-diabetic controls \( P < 0.05 \). At 34 weeks, the MAP response to phenylephrine administration was the same for both groups.

Table 1. The mean arterial blood pressure of diabetic rats was significantly lower than that of non-diabetic control rats \( P < 0.05 \) at 12, 18 and 42 weeks after the induction of diabetes. There were no significant differences in heart rate between the diabetic and non-diabetic group at any time.

3.2 Experiment II: general features and initial haemodynamic values

All rats in the three series which received streptozotocin became diabetic. The blood glucose levels of these diabetic rats remained above 15 mmol/l throughout the experimental period (data not shown). The mean body weights of the non-diabetic rats and of the diabetic rats are given in Table 2. After 6 weeks of treatment, there was no difference between the blood glucose levels and body weights of the Org 2766-treated diabetic rats and those of the placebo-treated diabetic rats. The data for the initial haemodynamic variables are summarized in Table 2. The mean arterial blood pressure of the placebo-treated and Org 2766-treated groups was not significantly different from the mean arterial pressure of the non-diabetic control group after 6, 12 and 18 weeks of diabetes. Neither systolic nor diastolic blood pressure differed significantly among the three experimental groups after 6, 12 and 18 weeks of diabetes. The heart rate was not significantly different between any of the experimental groups at any time (Table 2).

3.3 Experiment I: vasodepressor responses and baroreflex function

Throughout, the depressor response of diabetic rats to the vasodilator, sodium nitroprusside, was significantly \( P < 0.05 \) reduced as compared to that of the non-diabetic control rats (MANOVA: week 2, \( F(1,18) = 5.48, P < 0.05 \); week 4, \( F(1,18) = 16.38, P < 0.05 \); week 6, \( F(1,14) = 25.11, P < 0.05 \); week 12, \( F(1,17) = 16.32, P < 0.05 \); week 18, \( F(1,14) = 13.14, P < 0.05 \); week 26, \( F(1,13) = 12.0, P < 0.05 \); week 34, \( F(1,13) = 8.50, P < 0.05 \); and week 42, \( F(1,14) = 6.41, P < 0.05 \). The responses to sodium nitroprusside of diabetic and non-diabetic animals at 6 weeks of diabetes are shown in Fig. 1. At all time points, the maximal vasodilator activity of sodium nitroprusside (10 \( \mu g/kg \)) in the diabetic rats was significantly less \( P < 0.05 \) than that in the non-diabetic control rats (Fig. 2). At all time points, the changes in heart rate in response to sodium nitroprusside-induced decreases in blood pressure were slight in both the diabetic and the non-diabetic rats. Even at the dose of 10 \( \mu g/kg \) sodium nitroprusside the change in heart rate was slight for both groups. The non-diabetic control rats always showed a slight tachycardia, whereas the heart rate response of the diabetic rats was variable. At all time points the blood pressure–heart rate relationship for both groups was not significantly linearly correlated \( P > 0.05 \) and, therefore, no baroreflex-mediated tachycardia could be obtained.
Baroreflex sensitivity after phenylephrine injection, as determined from baroreflex slopes (bpm/mmHg ± s.e.m.), at weeks 2, 4, 6, 12, 18, 26, 34 and 42 after the induction of diabetes: non-diabetic controls (Diet group), diabetic rats (DM group). n = number of animals.

Statistically significant difference from non-diabetic controls, P < 0.05.

The pressor response of the diabetic rats to phenylephrine was only significantly (P < 0.05) less than that of the non-diabetic control rats at 4, 6 and 12 weeks after the induction of diabetes (MANOVA: week 4, F(1,18) = 9.57, P < 0.05; week 6, F(1,15) = 6.19, P < 0.05; and week 12, F(1,16) = 18.83, P < 0.05). Typical examples of mean arterial blood pressure hyporesponsiveness (at week 4) and normoresponsiveness (at week 34) to phenylephrine in diabetic and non-diabetic control rats are shown in Fig. 3. From weeks 4 to 42 the maximal decrease in heart rate due to phenylephrine was significantly smaller (P < 0.05) in the diabetic rats than in the non-diabetic control rats (Fig. 4). The blood pressure increasing efficacy of phenylephrine 30 μg/kg was similar throughout time (age) in diabetic and non-diabetic rats (Fig. 4). The slopes for the blood pressure–heart rate relationship during phenylephrine-induced increases in blood pressure, reflecting baroreflex sensitivity, were significantly decreased (P < 0.05) compared to those for non-diabetic control rats at the various points after induction of diabetes. After 2 weeks, however, the baroreflex sensitivity was not significantly different between the two groups (Table 3).

3.4. Experiment II: vasodepressor responses and baroreflex function

At weeks 6, 12 and 18 the mean arterial blood pressure response to phenylephrine in the placebo-treated diabetic rats was significantly reduced compared to the response of the non-diabetic control rats (P < 0.05). The response of mean arterial blood pressure to phenylephrine, however, was the same in the Org 2766-treated diabetic rats and in the placebo-treated diabetic rats. At weeks 6, 12 and 18, the heart rate response to phenylephrine was significantly less in the diabetic rats than in non-diabetic control rats (P < 0.05) and Org 2766 had no influence on this response. At weeks 6, 12 and 18 the sensitivity (slope) of the baroreflex bradycardia curves elicited with phenylephrine in the placebo-treated diabetic rats was significantly less than the slope from non-diabetics (P < 0.05) (Table 2). At 6 weeks, the slope of the baroreflex bradycardia curves of the Org 2766-treated diabetic rats was similar to that of the non-diabetic control rats (Table 2 and Fig. 5). At 12 weeks, the slope of the baroreflex bradycardia curves of
the Org 2766-treated diabetic rats was increased as compared to that of the placebo-treated diabetic rats ($P < 0.05$), however, Org 2766 treatment could not completely restore this slope to the value for non-diabetic controls ($P < 0.05$) (Table 2). The blood pressure–heart rate relationship for the three groups 12 weeks after the induction of diabetes is shown in Fig. 5. At 18 weeks, the slope of the baroreflex bradycardia curves for the placebo-treated diabetic rats and Org 2766-treated diabetic rats was the same (Table 2).

### 4. Discussion

Abnormalities of the cardiovascular system, e.g. impaired responsiveness of blood pressure to vasoactive agents and changes in baroreflex control of heart rate, are known to occur in experimental diabetes. It is not clear whether these cardiovascular dysfunctions are direct consequences of cardiovascular deficits and/or have autonomic neuropathy as an indirect cause (see Ref. [4] for review).

There are conflicting data for blood pressure in experimental diabetes. Hypotension [12], hypertension [23] and normotension [12] have been reported. We recently found that relatively short-term diabetic rats were normotensive [6,7,20,24]. In the present study, we found both hypotension and normotension in the relatively short-term diabetic rats. Our experimental approach for measuring blood pressure having been the same for both the earlier and the present experiments, we cannot explain these differences. Differences in rat strain, duration of diabetes, anesthesia or techniques for measuring blood pressure could explain the conflicting data [25].

The vasodilator, sodium nitroprusside, elicited in our anaesthetized control rats, a depressor response comparable to what had been measured in conscious rats [15]. Despite the clear depressor response, only minimal reflex tachycardia was elicited. The absence of a consistent, clear, reflex tachycardia in the present study might be ascribed to the urethane anaesthesia. Stornetta et al. [26] found that urethane attenuated the tachycardiac response to a decrease in blood pressure and had only a slight effect on the bradycardiac response to an increase in blood pressure when compared to the cardiac response in conscious rats. It seems that urethane anaesthesia in rats decreases sympathetic stimulation by sodium nitroprusside. The variable heart rate response to sodium nitroprusside in diabetic rats might be a consequence of the depressed vasoreactivity. Also, the underlying diabetic process could have interfered with the baroreflex arc (see below). We (unpublished observations) and others have found a diminished vasodilator response to vasodilator agents with different modes of action in diabetic rats [27,28]. There thus appears to be a general loss of vasodilator reserve, which we found to be present as early as after 2 weeks of diabetes and to persist up to 42 weeks. However, other authors have found that the endothelium-independent relaxation to nitrovasodilators is unaffected by diabetes especially in in vitro studies [29,30]. The method used in in vitro studies provides a defined endogenous postsynaptic stimulus, whereas the response to exogenously applied vasoactive agents in in vivo studies may be complicated by factors other than those directly determining vasoreactivity.

Phenylephrine, as `vasopressor', elicited a pressor response in the diabetic rats which was decreased during the relatively early stages, but returned toward its control levels after long-continued exposure to diabetes, indicating a change in vasoreactivity. Alterations in vasoreactivity to phenylephrine may thus depend upon the length of time the animals have been exposed to the diabetes. The hyporesponsiveness to phenylephrine was evidence of post-synaptic defects, confirming other findings of decreased sympathoadrenergic responsiveness [10,20]. There is, however, no general agreement as to the effect of diabetes on vasoreactivity, and little is known of the effects of long-term streptozotocin-induced diabetes on blood pressure responsiveness to sympathoadrenergic stimuli (see Ref. [8] for review). As the ACTH$_{1-9}$ analogue, Org 2766, had no effect on the hyporesponsiveness of the pressor response in diabetic rats, and has beneficial effect on the presynaptic autonomic nerve fibres [6,7,24], but no direct cardiovascular effects [17], the decreased vasoreactivity to phenylephrine appears to have a direct vascular origin.

In our anaesthetized control rats, the increases in mean arterial blood pressure evoked by i.v. phenylephrine were associated with a distinct reflex bradycardiac which was in the same range as that measured in conscious rats [15]. Our data and those of others [21,22] have shown that the blood pressure–heart rate relationship is linear when increases in mean arterial blood pressure up to 65 mmHg are evoked by phenylephrine. The sensitivity of the blood pressure–heart rate relationship was only decreased at 2 weeks of diabetes, while the decrease in sensitivity of the baroreflex-mediated bradycardia in the diabetic rats appeared at all measurements made subsequently. The baroreflex control of heart rate has been found to be altered in streptozotocin-induced diabetic rats [9–14], in diabetic BB/Wor rats [15] and in diabetic rabbits [16]. Some of these results for baroreflex activity must, however, be interpreted with caution [9,11] because the pressor agents used may have had direct cardiac effects which interfered with the reflex bradycardia. There are conflicting results for baroreflex-mediated bradycardia. An increase [10,13,14], a decrease [16] and no alteration [13,15] have been reported. Only one study, so far, has measured baroreflex control of heart rate in response to pressor stimuli in long-term experimental diabetes [12]. The authors reported an increase in baroreflex control of heart-rate sensitivity during the early stages of diabetes, which returned to its control levels and ultimately after long-term exposure to diabetes, a decrease in sensitivity was measured. We could not confirm these time-dependent alterations in baroreflex control of heart.
rate. Alterations of the integrated neural control of cardiac function might involve both the cardiac parasympathetic and sympathetic systems [31–33]. The impairment of the baroreflex-mediated bradycardia might be due to decreased sensitivity of baroreceptor afferents [34], to impaired activation of central parasympathetic pathways [14,35], or to impaired vagal efferent neural control of the heart rate [12,16]. McDowell et al. [36] found that a defect in the activation of central parasympathetic pathways could be responsible for the impairment of the baroreflex control of heart rate in diabetic rabbits. Similar experiments with induced-diabetic rats could help identify the exact site of impairment of the baroreflex arc. Our present results do not allow identification of the site of impairment of the baroreflex arc.

In the present study, the ACTH$_{1–9}$ analogue, Org 2766, was able to restore the diminished baroreflex-mediated bradycardia of diabetic rats to the non-diabetic control levels at 6 weeks, had an ameliorating effect at 12 weeks and no effect at 18 weeks after the induction of diabetes. These (ameliorating) effects can only be ascribed to neuroprotective effects [18] and not to haemodynamic actions of Org 2766 which this ACTH$_{1–9}$ analogue lacks in rats [17]. Van der Zee et al. [5] found that the responsiveness to noradrenaline within the cardiovascular system was decreased in diabetes mellitus, and can be restored by Org 2766. In addition, Org 2766 had no effect on postsynaptic defects in diabetic rats [7], and, as far we know, there is no evidence for any effect of Org 2766 on catecholamine release, synthesis or metabolism. It seems that Org 2766 exerts its protective and beneficial action on presynaptic (para)sympathetic nerve fibres in the streptozotocin-induced diabetic rat. It is suggested that exogenous melanocortins mimic or amplify a naturally occurring ACTH-like peptide signal in the repair process that is part of the regeneration repertoire [18]. The site of action of Org 2766 in the baroreceptor heart-rate reflex arc could be at the afferent, central or at the efferent level of the reflex pathway (see above). The partial character of the restoration by Org 2766 of the baroreflex-mediated bradycardia might imply that non-neuronal, i.e. direct cardiovascular, alterations could also play a role in the impairment of the baroreflex pathway seen in experimental diabetes [37,38]. Litwin et al. [37] found an abnormal cardiac function in diabetic rats. They mentioned that the alterations in ventricular relaxation and passive-elastic properties in the heart of diabetic rats might lead to loss of normal baroreflexes. On the other hand, (micro)angiopathy could lead to injury to the microvascular endothelium, and thus causes adaptive microvascular sclerosis, contributing to a loss of autoregulatory capacity [38]. Other non-neuronal explanations for the altered baroreflex-mediated bradycardia seen in streptozotocin-induced diabetic rats might be glycoprotein-induced ventricular stiffness [39] and alterations in sinoatrial nodal electrical activity [40], the latter possibly being due to changes in calcium metabolism [41].

In conclusion, time-dependent decreases in baroreflex sensitivity in diabetic rats was demonstrated and a much less steep decline of baroreflex sensitivity occurred in aging non-diabetic control rats. Furthermore, it can be concluded that cardiovascular factors do not contribute to the diminished baroreflex sensitivity in diabetic rats as the blood pressure increasing of phenylephrine seems to be quite similar throughout time in these rats. In fact, the reflectory heart rate responses are consistently decreased. Therefore, the vasoconstrictor effect of phenylephrine is well conserved, but the baroreflex pathway is impaired. The ACTH$_{1–9}$ analogue, Org 2766, when given immediately upon the induction of diabetes seems to delay the development of autonomic neuropathy, which also suggests that cardiovascular factors appear to be of minor importance. The fact, that the vasodilatory responses to sodium nitroprusside are decreased does not affect this conclusion as the effector pathway of reflex responses to blood pressure decreases are mainly sympathoneuronal, while the reflex responses to blood pressure increases are mainly parasympathoneuronally mediated.

References


