Br. J. Pharmacol. (1989), **97**, 1209–1217

Effect of age and methacholine on the rate and coronary flow of isolated hearts of diabetic rats

Xiaojiang Li, [1]Ralph D. Tanz & K.S.K. Chang

Departments of Pharmacology and Anesthesiology, Oregon Health Sciences University, School of Medicine, Portland, OR (97201), U.S.A.

1 Isolated hearts perfused by the method of Langendorff from 6, 12 and 24 week streptozotocin (STZ) diabetic rats displayed a significant bradycardia following 60 min equilibration. The rate of hearts from 12-week diabetic rats (164 ± 17) displayed the greatest bradycardia compared to age-matched controls (268 ± 15; $P < 0.001$), and diabetics treated with insulin (232 ± 17; $P < 0.01$), but by 52 weeks the heart rate of the 3 groups was similar. With advancing age the effect of STZ diabetes on the rate of rat isolated perfused hearts remained unchanged but the rate of the control and diabetic + insulin groups declined.

2 Hearts from 6–52 week STZ-treated rats were found to be more sensitive to the negative chronotropic effect of methacholine, the greatest difference occurring in hearts from the 12 week animals. Atropine (10^{-7} M) did not affect the resting heart rate of age-matched controls or diabetics but blocked methacholine (2.6×10^{-6} M)-induced bradycardia in both, suggesting that the site of action of diabetic bradycardia is not the muscarinic receptors.

3 At the end of equilibration there was a significant decrease in coronary flow in hearts from 12 week diabetic animals. In spontaneously beating diabetic rat hearts administration of methacholine (2.6×10^{-6} M) produced a significantly greater decrease in coronary flow in the 12, 24 and 52 week diabetic hearts. When electrically paced (5 Hz) however, there was no difference in response to methacholine between the three groups except at 52 weeks between the age-matched control and diabetic groups. This suggests that the more pronounced reduction induced by methacholine on the coronary flow of diabetic hearts is secondary to its negative chronotropic effect.

4 In general, hearts from diabetic animals treated with insulin respond similarly to their age-matched controls in the presence and absence of methacholine.

Introduction

Coronary artery disease as well as cardiac failure are often sequelae of diabetes mellitus. Even in the absence of atherosclerosis, blood vessel disease and cardiomyopathy can occur in diabetes (Hamby *et al.*, 1974; Kannel *et al.*, 1974; Ledet, 1976; Regan *et al.*, 1977; Sanderson *et al.*, 1978). Autonomic dysfunction is considered to be one of the major secondary complications responsible for diabetic heart disease (Clarke *et al.*, 1979), but the underlying mechanism(s) remain unknown. Stuesse *et al.* (1982) reported that enhanced sensitivity to vagal stimulation was an early detectable feature of diabetic cardiac autonomic neuropathy. Bradycardia and cholinergic supersensitivity in streptozotocin-induced diabetic *in vitro* rat myocardial preparations have been reported (Foy & Lucas, 1976; Vadlamudi & McNeill, 1983; Carrier *et al.*, 1984; Carrier & Aronstam, 1987), as well as an increase in baroreflex sensitivity in conscious diabetic rats (up to 24 weeks) and then a decrease as diabetic duration progressed (48 weeks) (Chang & Lund, 1986). Thus, an alteration in parasympathetic function appears to be dependent on the duration of diabetes in the rat, but a clear picture characterizing the progression of impaired function in STZ-induced diabetes remains to be established.

[1] Author for correspondence.

Alterations in vascular sensitivity to certain agonists have been described in experimental diabetic animals (Savarese & Berkowitz, 1979; Cavaliere *et al.*, 1980; Pfaffman, 1980; Jackson & Carrier, 1983; Mueller, 1984). However, to our knowledge, no study has been performed correlating the chronotropic and coronary blood flow responses elicited by cholinoceptor agonists *in vitro* beyond diabetes of 8 weeks' duration. Moreover, 'the status of coronary flow in experimental diabetes is somewhat unclear' (Dhalla *et al.*, 1985). A number of studies utilizing streptozotocin have now been published attesting to the validity of the diabetic state it produces in animals. Accordingly, the present investigation sought to characterize some of the effects of methacholine on heart rate and coronary flow of isolated hearts from diabetic rats, 6 to 52 weeks following treatment with streptozotocin.

Methods

Male Sprague-Dawley rats (from Simonsen Laboratories, Gilroy, CA) weighing 200–250 g on arrival were randomly divided into three groups: control (C), diabetic (D) and diabetic treated with insulin (D + I). Diabetes was induced by a single injection of streptozotocin (STZ) (55 mg kg^{-1}, i.v.) in the tail vein (Rerup, 1970; Chang & Lund, 1986). Age-matched control rats received vehicle alone (0.01 M citrate buffer in saline). Diabetics treated with insulin were injected with two units of protamine zinc insulin subcutaneously daily, after streptozotocin. Blood samples were obtained just before the rats were killed and non-fasting plasma glucose determined with a Beckman glucose analyzer. The diabetic state was verified by plasma glucose levels exceeding 300 mg dl^{-1}. These animals exhibited excessive daily food and water consumption and a decline in body weight. All animals received water and standard chow *ad libitum*.

Results were obtained on hearts from 150 rats. Six, 12, 24 and 52 weeks after induction of diabetes, hearts were excised under ether anaesthesia, arrested by plunging them into ice cold perfusion media, mounted and perfused by the method of Langendorff in a non-recirculating system at 75 cmH_2O pressure (Tanz *et al.*, 1982). The perfusion medium was a modified Krebs-Hensleit (K-H) bicarbonate solution having the following composition (mM): KCl 4.75, KH_2PO_4 1.19, $MgSO_4 \cdot 7H_2O$ 1.19, $CaCl_2 \cdot 2H_2O$ 2.54, NaCl 119, $NaHCO_3$ 25 and glucose 11.12. When equilibrated with 95% O_2 and 5% CO_2 at 37.5°C the pH was 7.35 and Po_2 598 mmHg. Three fine wire electrodes were affixed to the right and left atrial appendages and the epicardial surface of the left ventricle for the recording of heart rate, and the atrial and ventricular electrocardiogram (ECG) on a direct-writing polygraph. Coronary flow was measured and expressed as ml min^{-1} g^{-1} of wet heart weight. The first 60 min following mounting was allowed for equilibration. Hearts were then exposed to methacholine, 5.1×10^{-7}, 2.6 and 5.1×10^{-6} M for 10 min at each concentration followed by 10 min for washout with normal K-H solution to permit recovery before the next concentration. Additional experiments were performed on hearts from 12-week animals exposed to atropine (10^{-7} M) alone, methacholine (2.6×10^{-6} M) alone, and atropine followed 10 min later by methacholine. All drugs were added to the perfusate reservoir containing oxygenated medium. Since peak effects occurred 10 min after drug addition, heart rate, and coronary flow were determined at that time. The degree of the responses elicited were expressed as the percentage change from the pre-drug value so as to avoid variation between individual hearts. In order to obviate the influence of heart rate on coronary flow, hearts were also paced electrically at 5 Hz. This was accomplished by placing stimulating electrodes on the myocardium, one on the left atrium the other on the apex. Stimulating pulses were 5 ms in duration and 30–50% above threshold voltage. Hearts were then exposed to methacholine (2.6×10^{-6} M). At the conclusion of the experiment, wet weights were determined by weighing hearts dried on blotting paper.

Acetyl-β-methylcholine Cl, atropine and streptozotocin were obtained from the Sigma Chemical Co. and solutions prepared just before use; protamine zinc insulin was from Eli Lilly. Data are presented as means and the standard error of the means. Statistical analysis was by one-way analysis of variance when comparing three groups, followed by Newman-Keuls test and Student's unpaired t test when comparing two groups. A P value of <0.05 was taken as indicating statistical significance.

Results

Mortality following STZ was high with 27.2% of rats succumbing within the first 5 weeks, and a total of 42.4% within the first year. In contrast, there were no deaths in the untreated control group and a mortality of 11.8% in the diabetic + insulin group.

Animals injected with STZ had statistically significantly elevated blood glucose levels compared to the untreated controls and those treated with STZ and then insulin (Table 1). Diabetic animals killed after 6 weeks had the highest blood glucose level

Table 1 Mean blood glucose, body and heart weights after induction of diabetes in rats

Weeks	*Blood glucose* ($mg\,dl^{-1}$)	*Body wt.* (g)	*Heart wt.* (mg)†	*Heart:Body wt.*
6 Controls	96.3 (7) ±8.9	401.7 (9) ±11.2	1190 (9) ±41.0	2.96
	***	***	***	***
Diabetic	500.7 (11) ±30.0	213.8 (11) ±16.0	825 (11) ±48.3	3.95
	** ***	***	***	*
Diabetic + insulin	211.5 (7) ±32.0	396.2 (9) ±8.3	1272 (9) ±54.5	3.21
12 Controls	105.0 (8) ±17.3	429.2 (11) ±14.6	1335 (11) ±38.4	3.13
	***	***	***	***
Diabetic	491.5 (8) ±42.0	214.1 (9) ±16.4	1025 (9) ±44.7	4.88
	* ***	***	***	***
Diabetic + insulin	185.3 (6) ±39.9	444.7 (9) ±7.7	1446 (9) ±44.7	3.26
24 Controls	109.9 (11) ±14.9	512.5 (12) ±9.2	1430 (12) ±35.7	2.80
	***	***	***	***
Diabetic	410.8 (12) ±19.1	229.6 (17) ±10	1004 (17) ±41.1	4.41
	***	***	***	***
Diabetic + insulin	128.7 (13) ±16.1	525.8 (13) ±11.1	1466 (13) ±36.0	2.80
52 Controls	96.2 (6) ±16.0	580.1 (9) ±13.4	1617 (9) ±43.7	2.79
	***	***	***	***
Diabetic	347.0 (10) ±36.3	306.5 (9) ±17.7	1226 (9) ±53.6	4.04
	***	***	***	***
Diabetic + insulin	112.9 (7) ±21.5	553 (7) ±15.6	1648 (7) ±42.6	2.98

† Wet weight: number of individual observations in parentheses. Values are ± standard error of the mean.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

($500.7\,mg\,dl^{-1}$), which slowly declined with time to $347\,mg\,dl^{-1}$ at 52 weeks.

A tabulation of body weight, heart weight and the ratio of heart to body weight is also shown in Table 1. The body weight of diabetic rats was significantly less than both the untreated control group and those injected with STZ + insulin. This also obtained for heart weight. However, since body weight declined to a greater extent than did heart weight, the ratio of heart to body weight ($mg\,g^{-1}$) was consistently greater for the diabetic group than the other groups.

Heart rate

Actual heart rate at the end of equilibration (+60 min) was invariably less in the diabetic group than for either the untreated control or the diabetic + insulin groups, with the exception of the 52 week animals which, although less, was not significantly different (Figure 1).

When preparations were first exposed to 5.1×10^{-7} M methacholine for 10 min the decrease in heart rate from pre-drug levels was significantly greater in hearts from 12 week diabetic rats than in the untreated control and diabetic + insulin groups, but not in the 6, 24 or 52 week groups (Figure 2). However, following exposure to 2.6×10^{-6} M methacholine, the decrease in heart rate was significantly different between all diabetic and untreated control groups. This difference was also present at 12 and 52 weeks between the diabetic and the diabetic + insulin groups. Following exposure to 5.1×10^{-6} M

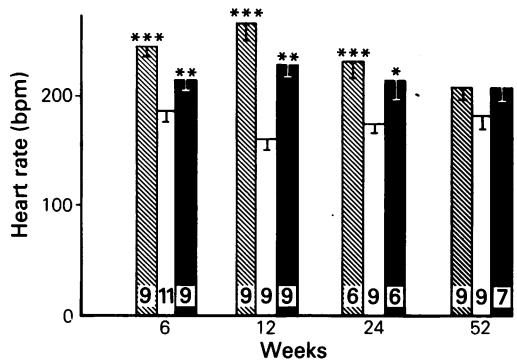

Figure 1 Mean heart rates of the three groups of rat Langendorff hearts at the end of 60 min equilibration 6, 12, 24 and 52 weeks after streptozotocin. Number of individual preparations indicated within the column. Hatched = controls; open = diabetic; solid = diabetic + insulin. Vertical bars show s.e.mean.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

there was a significantly greater decrease in the rate of hearts from the 12 and 24 week diabetic animals and their controls, but not from the 6 or 52 week animals. In the 12 week group, a significant difference in rate between hearts from the diabetic + insulin and diabetic groups was also observed. Analysis of the electrocardiograms revealed no discernible rhythmic alterations.

Effect of atropine on heart rate

In an attempt to ascertain whether increased cholinergic sensitivity of the S-A node is the basis for diabetic bradycardia, additional studies were performed in the presence of atropine (10^{-7} M). In the presence of atropine exposure to methacholine (2.6×10^{-6} M) 10 min later did not raise the rates of either control or diabetic hearts above pre-treatment levels (Table 2), though atropine very effectively blocked methacholine-induced bradycardia. This suggests that the bradycardia of isolated hearts from diabetic rats is not due to a supersensitivity of muscarinic receptors, but that the site of action of enhanced cholinergic sensitivity in diabetics lies elsewhere.

Coronary flow

Actual coronary flow (ml min^{-1} g^{-1}) at the end of equilibration (60 min) was significantly less only in

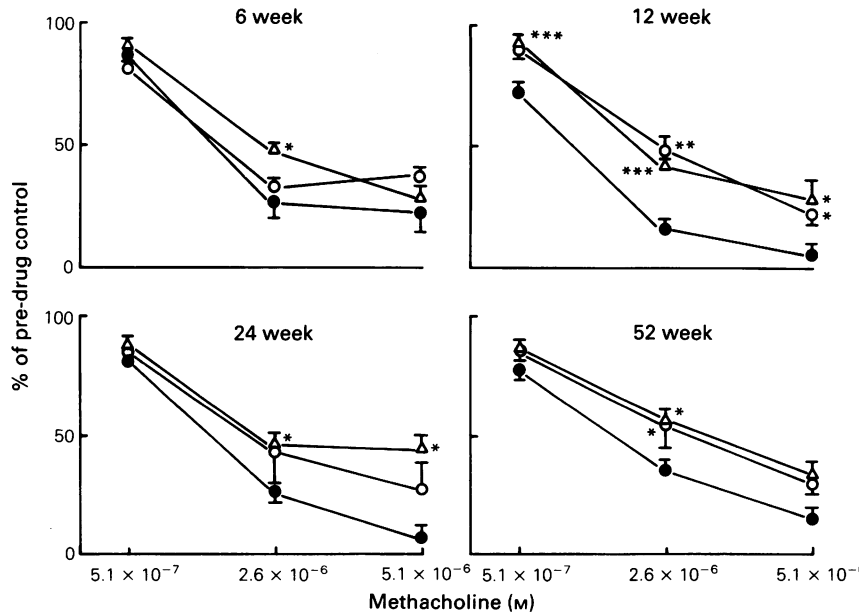

Figure 2 Effect of methacholine on the rate of spontaneously beating rat isolated hearts. The change in heart rate following methacholine was related to the pre-drug rate which was equated to 100%. Age-matched animals were killed 6, 12, 24 and 52 weeks following streptozotocin. (△) = controls; (●) = diabetic; (○) = diabetic + insulin. Vertical bars show s.e.mean.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; compared to diabetic groups.

Table 2 Effect of methacholine (2.6×10^{-6} M) and atropine (10^{-7} M) on the rate of isolated 12-week diabetic and control rat hearts

	Pre-drug	*Methacholine*	*Atropine*	*Atropine + methacholine*
Control	237.0 (15) ±8.8 ***	135.5 (9) ±7.1 ***	247.5 (5) ±10.5 ***	236.0 (7) ±7.5 ***
Diabetic	183.0 (15) ±9.7	34.4 (8) ±6.1	202.0 (5) ±12.5	196.0 (5) ±6.8

Number of individual hearts in parentheses. Values are ± standard error of the mean.
** $P < 0.01$; *** $P < 0.001$.

hearts from 12 week diabetic animals (Figure 3). Figure 4 illustrates the coronary flow observed for the three groups in response to methacholine. Regardless of age, when exposed to methacholine coronary flow was invariably lower in hearts from diabetic rats than either those treated with insulin or in hearts from control animals. Moreover, it was significantly less following exposure to 2.6×10^{-6} M methacholine in hearts from 12, 24 and 52 week animals as well as following the 5.1×10^{-6} M concentration in 52 week old animal hearts. However, when hearts were paced at 5 Hz the only statistically significant difference in coronary flow occurred at 52 weeks between control and diabetic hearts (Figure 5).

Discussion

These results demonstrate that Langendorff perfused hearts from rats made diabetic with streptozotocin

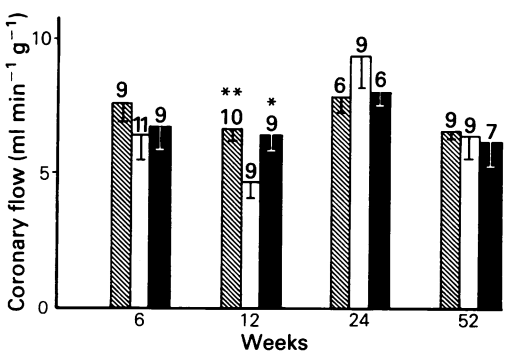

Figure 3 Mean coronary flows of spontaneously beating rat isolated hearts following 60 min equilibration 6, 12, 24 and 52 weeks after streptozotocin. Coronary flow = ml min^{-1} g^{-1} (wet wt.). Number of individual preparations indicated within the column. Hatched = controls; open = diabetic; solid = diabetic + insulin. Vertical bars show s.e.mean.
* $P < 0.05$; ** $P < 0.01$; compared to diabetic groups.

have significantly lower rates *per se* and in response to methacholine than their age-matched and insulin-treated diabetic controls. But is the defect due to streptozotocin or the diabetic state? At the end of 60 min equilibration the rate of hearts from STZ-injected animals was significantly less than the controls as well as those from diabetics treated with insulin (Figure 1). At 12 weeks the rate of diabetic hearts was lower than the rate of hearts from 6, 24 and 52 week control and insulin-treated diabetic animals. The same qualitative trend occurred at 0-time when hearts were initially mounted and perfusion begun (data not presented). By 52 weeks however, the rate of hearts from all three groups was not significantly different. These facts suggest that: (1) STZ did not destroy all the β-cells, but rather there was a partial regeneration as evidenced by the fact that the blood glucose level was declining with the passage of time (Table 1) and (2), STZ is not a direct myocardial depressant *per se*. This conclusion is in agreement with that reached by Fein *et al.* (1981) who found that papillary function returned to normal the longer insulin was given, and concluded that STZ-induced cardiomyopathy is not the result of STZ-induced cardiac toxicity. Thus, age and the duration of STZ-induced diabetes appear to be important determinants affecting chronotropic activity.

In experimental models diabetes-induced bradycardia has been found to occur both *in vivo* and *in vitro* (Foy & Lucas, 1976; Pfaffman, 1980; Bielefeld *et al.*, 1983; Jackson & Carrier, 1983; Chang & Lund, 1986), but the mechanism remains obscure. For example, Senges *et al.* (1980) utilizing isolated atria from rabbits treated with alloxan observed a statistically significant lower sinus rate, A-V block and inhomogeneity of atrial conduction compared to controls. Diabetic-induced bradycardia has been attributed to decreased glucose metabolism (Foy & Lucas, 1976), autonomic dysfunction (Tomlinson & Yusof, 1981), and a decrease in β-adrenoceptors

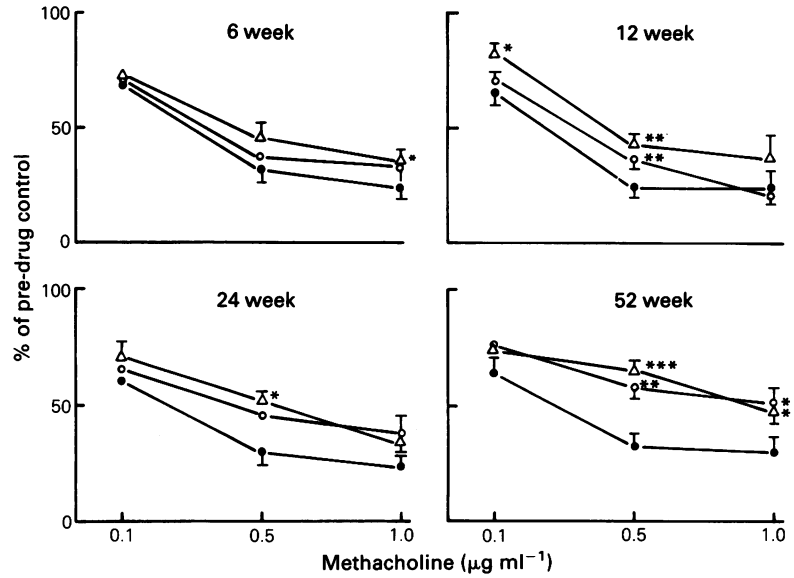

Figure 4 Effect of methacholine on the coronary flow of spontaneously beating rat isolated hearts 6, 12, 24 and 52 weeks following streptozotocin. The change in coronary flow following methacholine was related to the pre-drug flow which was equated to 100%. (△) = controls; (●) = diabetic; (○) = diabetic + insulin. Vertical bars show s.e.mean.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; compared to diabetic groups.

(Savarese & Berkowitz, 1979; Williams *et al.*, 1983; Latifpour & McNeill, 1984; Atkins *et al.*, 1985; Bitar *et al.*, 1987). Autonomic neuropathy is a clinical problem in diabetes (Wheeler & Watkins, 1973; Duchen *et al.*, 1980), as well as in experimental settings (Monckton & Pehowich, 1980; Schmidt *et al.*, 1981). But in the presence of diabetic autonomic neuropathy, tachycardia occurs apparently as a result of diminished vagal tone since hearts fail to accelerate after atropine and there is an absence of reflex bradycardia following a Valsalva manoeuvre (Wheeler & Watkins, 1973).

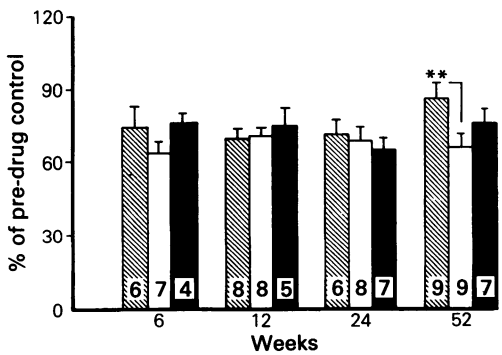

Figure 5 Effect of methacholine (2.6×10^{-6} M) on coronary flow of isolated perfused rat hearts paced at 5 Hz 6, 12, 24 and 52 weeks following streptozotocin. The change in coronary flow following methacholine was related to the pre-drug value which was equated to 100%. Number of individual preparations indicated within the column. Hatched = controls; open = diabetic; solid = diabetic + insulin. Vertical bars show s.e.mean.
** $P < 0.01$; compared to diabetic group.

The results shown in Figure 1 suggested the possibility that hearts from 12 week old animals might show the greatest disparity in response to pharmacological intervention. And indeed this proved to be the case. Figure 2 attests to the fact that methacholine-induced bradycardia produced by larger doses resulted in a significant difference between diabetic preparations and the controls as well as those treated with insulin. This disparity was barely evident at 6 weeks, became greatest at 12 weeks, and then less and less at 24 and 52 weeks. Moreover, the negative chronotropic effect elicited by 2.6 and 5.1×10^{-6} M methacholine was invariably greater in hearts from all diabetic rats, regardless of their age. This enhanced chronotropic sensitivity to a cholinoceptor agonist in diabetes has been previously reported by other investigators (Tomlinson & Yusof, 1981; Carrier *et al.*, 1984), especially in hearts from 8–10 week diabetic rats (Carrier *et al.*, 1984; Carrier & Aronstam, 1987). Vagal stimulation

in 7–9 week alloxan rats has been reported to produce a more pronounced bradycardia compared to controls (Stuesse *et al.*, 1982). Increased baroreflex sensitivity in conscious STZ diabetic rats to methacholine up to 24 weeks, with a progressive decline occurring thereafter, has also been reported (Chang & Lund, 1986). But the mechanism still remains unknown. It has been reported that acetylcholinesterase is significantly lower in right atria from 8–10 week STZ diabetic rats (Carrier & Aronstam, 1987) which could explain the presence of cholinergic supersensitivity and the resulting bradycardia. In an attempt to determine the mechanism responsible for diabetic bradycardia, the chronotropic responses elicited by methacholine in the presence and absence of atropine (Table 2) were determined. The results obtained suggest that cholinergic supersensitivity is not due to an increase in muscarinic receptor activity. Moreover cholinergic supersensitivity in diabetic hearts has been found in the presence of decreased affinity and density of muscarinic receptors (Carrier & Aronstam, 1987). This suggests that the mechanism and site of action of diabetic-induced bradycardia and chronotropic cholinergic supersensitivity lies elsewhere. That the diabetic state was the result of insulin deficiency rather than streptozotocin toxicity is attested to by the fact that the responses observed both in the controls and the insulin-treated diabetics were similar throughout.

Coronary flow at the end of 60 min equilibration was not significantly different for the three groups except in hearts from the 12 week diabetic animals (Figure 3). This correlates with the fact that 12 week diabetic hearts also displayed the greatest reduction in rate at that point in time (Figure 1). This reduction of coronary flow in response to methacholine in hearts from diabetic animals 12 weeks and older was invariably greater then in either the untreated controls or those from diabetics treated with insulin (Figure 4). In hearts from the 12, 24 and 52 week animals, the response elicited by 2.6×10^{-6} M methacholine in diabetic hearts was significantly more, as was that following exposure to 5.1×10^{-6} M in hearts from 52 week diabetics. The difference between the control and diabetic groups at 52 weeks might be due to greater cholinergic sensitivity. Yet when paced at 5 Hz, hearts from 6, 12, 24 and 52 week animals all displayed the same coronary flow in response to methacholine. However, since pacing eliminated the negative chronotropic effect of methacholine between all other groups at 6, 12, 24 and 52 weeks, we conclude that the reduction in coronary flow is secondary to the decline in heart rate in this preparation. It is well known that by autoregulatory mechanisms, myocardial oxygen consumption and demand are the major determinants of coronary blood flow (Berne, 1964). Since the rate of isolated diabetic rat hearts was lower when exposed to methacholine (Figure 2), their requirement for oxygen was also less and therefore coronary flow declined (Figure 4). Thus, hearts from 12 week diabetic rats displayed the greatest bradycardia as well as the greatest reduction in coronary flow in response to methacholine. Although muscarinic stimulation constricts coronary smooth muscle in the rat (Sakai, 1980), the pacing studies suggest that autoregulatory mechanisms predominate in determining coronary flow.

Coronary dilatation in response to cholinoceptor agonists occurs in some species (Blumenthal *et al.*, 1968), but constriction occurs *in vitro* in man, rat, pig, sheep, rabbit and cow (Smith, 1950; Nakayama *et al.*, 1978; Sakai, 1980; Ginsburg, 1983; Kalsner, 1985; Nuutinen *et al.*, 1985). In response to methacholine the coronary flow of hearts from paced STZ-treated animals was similar at all ages. But at 52 weeks control paced hearts displayed a significantly greater coronary flow when exposed to methacholine. This suggests that with advancing age hearts from normal rats become less sensitive to muscarinic agonists while those from diabetic animals retain an enhanced sensitivity, and agrees with the conclusion by Efellah *et al.* (1986) who demonstrated decreased cholinergic myocardial responsiveness in rats with advancing age. One question remaining is whether autonomic receptor density and/or affinity changes in the presence of diabetes and, if so, is there a progressive change that can be correlated with diabetic duration. Chang *et al.* (1984) have shown that 4–12 week diabetic rats have a significant decrease in choline acetyltransferase, indicative of defective parasympathetic innervation that might result in an increase in muscarinic density or affinity. But the few studies that have been reported suggest that there is a decrease in muscarinic receptor density in 12 week diabetic rat atria (Carrier *et al.*, 1984), and 6 month diabetic rat ventricular tissue (Latifpour & McNeill, 1984).

These studies suggest that of the four different diabetic groups (i.e. 6, 12, 24 and 52 weeks), isolated perfused hearts from 12 week animals exhibit the greatest sensitivity to methacholine. This sensitivity is especially evident as a negative chronotropic response and appears to be, at least in part, responsible for the accompanying fall in coronary flow. The changes in muscarinic sensitivity to methacholine in diabetic rats appears not to be due to streptozotocin toxicity *per se*. Moreover, insulin administration tends to correct the defects.

This study was supported by the G. & L. Pfeiffer Research Foundation.

References

ATKINS, F.L., DOWELL, R.T. & LOVE, S. (1985). β-Adrenergic receptors, adenylate cyclase activity, and cardiac dysfunction in the diabetic rat. *J. Cardiovasc. Pharmacol.*, **7**, 66–70.

BERNE, R.M. (1964). Regulation of coronary blood flow. *Physiol. Rev.*, **44**, 1–29.

BIELEFELD, D.R., PACE, C.S. & BOSHELL, B.R. (1983). Altered sensitivity of chronic diabetic rat heart to calcium. *Am. J. Physiol.*, **245**, E560–567.

BITAR, M.S., KOULU. M., STANLEY, I., RAPOPORT, S.I. & LINNOILA, M. (1987). Adrenal catacholamine metabolism and myocardial adrenergic receptors in streptozotocin diabetic rats. *Biochem. Pharmacol.*, **36**, 1011–1016.

BLUMENTHAL, M.R., WANG, H.H., MARKEE, S. & WANG, S.C. (1968). Effects of acetylcholine on the heart. *Am. J. Physiol.*, **214**, 1280–1287.

CARRIER, G.O., EDWARDS, A.D. & ATRONSTAM, R.S. (1984). Cholinergic supersensitivity and decreased number of muscarinic receptors in atria from short-term diabetic rats. *J. Mol. Cell Cardiol.*, **16**, 963–965.

CARRIER, G.O. & ARONSTAM, R.S. (1987). Altered muscarinic receptor properties and function in the heart in diabetes. *J. Pharmacol. Exp. Ther.*, **242**, 531–535.

CAVALIERE, T.A., TAYLOR, D.G., KERWIN, L.J. & ANTONACCO, M.J. ((1980). Cardiovascular effects of alloxan diabetes in normotensive and spontaneously hypertensive rats. *Pharmacology*, **20**, 211–223.

CHANG, K.S.K., KAELBER, W.W., JOHNSON, G.M., SUBIETA, A.R. & LUND, D.D. (1984). Changes within the autonomic system in streptozotocin induced diabetic rats. *Fed. Proc.*, **43**, 375.

CHANG, K.S.K. & LUND, D.D. (1986). Alterations in the baroreceptor reflex control of heart rate in streptozotocin diabetic rats. *J. Mol. Cell. Cardiol.*, **18**, 617–624.

CLARKE, B.F., EWING, D.J. & CAMPBELL, I.W. (1979). Diabetic autonomic neuropathy. *Diabetologia*, **17**, 195–212.

DHALLA, N.S., PIERCE, G.N., INNES, I.R. & BEAMISH, R.E. (1985). Pathogenesis of cardiac dysfunction in diabetes mellitus. *Can. J. Cardiol.*, **1**, 263–281.

DUCHEN, L.W., ANJORIN, A., WATKINS, P.J. & MACKAY, J.D. (1980). Pathology of autonomic neuropathy in diabetes mellitus. *Ann. Intern. Med.*, **92**, 301–303.

ELFELLAH, M.S., JOHNS, A. & SHEPHERD, A.M.M. (1986). Effect of age on responsiveness of isolated rat atria to carbachol and on binding characteristics of atrial muscarinic receptors. *J. Cardiovasc. Pharmacol.*, **8**, 873–877.

FEIN, F.S., STROBECK, L.E., MALHORTA, A., SCHEUER, J. & SOUNENBLICK, E.H. (1981). Reversibility of diabetic cardiomyopathy with insulin in rats. *Circ. Res.*, **49**, 1251–1261.

FOY, J.M. & LUCAS, P.D. (1976). Effect of experimental diabetes. Food deprivation and genetic obesity on the sensitivity of pithed rats to autonomic agents. *Br. J. Pharmacol.*, **57**, 229–234.

GINSBURG, R. (1983). The isolated human epicardial coronary artery. *Am. J. Cardiol.*, **52** (Suppl), 61A–66A.

HAMBY, R.I., ZONERAICH, S. & SHERMAN, L. (1974). Diabetic cardiomyopathy. *J. Am. Med. Assoc.*, **229**, 1749–1754.

JACKSON, C.V. & CARRIER, G.O. (1983). Influence of short term experimental diabetes on blood pressure and heart rate in response to norepinephrine and angiotensin II in the conscious rat. *J. Cardiovasc. Pharmacol.*, **5**, 260–265.

KALSNER, S. (1985). Cholinergic mechanisms in human coronary artery preparations: Implications of species differences. *J. Physiol.*, **358**, 509–526.

KANNEL, W.B., HJORTLAND, M. & CASTELLI, W.P. (1974). Role of diabetes in congestive heart failure. The Framingham Study. *Am. J. Cardiol.*, **34**, 29–34.

LATIFPOUR, J. & McNEILL, J.H. (1984). Cardiac autonomic receptors: effect of long-term experimental diabetes. *J. Pharmacol. Exp. Ther.*, **230**, 242–249.

LEDET, T. (1976). Diabetic cardiomyopathy: quantitative histologic studies of the heart from young juvenile diabetics. *Acta Path. Microbiol. Scand.*, Sect. A **84**, 421–428.

MONCKTON, G. & PEHOWICH, E. (1980). Autonomic neuropathy in the streptozotocin-diabetic rat. *Can. J. Neurol. Sci.*, **7**, 135–142.

MUELLER, S.M. (1984). Insulin treatment prevents vascular dysfunction in early juvenile alloxan-induced diabetes mellitus. *Am. J. Physiol.*, **247**, H132–138.

NAKAYAMA, K., FLECKENSTEIN, A., BYON, Y.K. & FLECKENSTEIN-GRÜN, G. (1978). Fundamental physiology of coronary smooth musculature from extramural stem arteries of pigs and rabbits. *Eur. J. Cardiol.*, **8**, 319–335.

NUUTINEN, E.M., WILSON, D.F. & ERECINSKA, M. (1985). The effect of cholinergic agonists on coronary flow rate and oxygen consumption in isolated perfused rat heart. *J. Mol. Cell Cardiol.*, **17**, 31–42.

PFAFFMAN, M.A. (1980). The effect of streptozotocin-induced diabetes and insulin-treatment on the cardiovascular system of the rat. *Res. Commun. Chem. Pathol. Pharmacol.*, **28**, 27–41.

REGAN, T.J., LYONS, M.M., AHMED, S.S., LEVINSON, G.E., OLDEWURTEL, H.A., AHMED, M.R. & HAIDER, B. (1977). Evidence for cardiomyopathy in familial diabetes mellitus. *J. Clin. Invest.*, **60**, 885–899.

RERUP, C.C. (1970). Drugs producing diabetes through damage of the insulin secreting cells. *Pharmacol. Rev.*, **22**, 485–518.

SAKAI, K. (1980). Coronary vasoconstriction by locally administered acetycholine, cabachol and bethanechol in isolated, donor-perfused, rat heart. *Br. J. Pharmacol.*, **68**, 625–632.

SANDERSON, J.E., BROWN, D.J., RIVELLESE, A. & KOHNER, E. (1978). Diabetic cardiomyopathy? An echocardiographic study of young diabetics. *Br. Med. J.*, **1**, 404–407.

SAVARESE, J.J. & BERKOWITZ, B.A. (1979). β-adrenergic receptor decrease in diabetic rat hearts. *Life Sci.*, **25**, 2075–2078.

SCHMIDT, R.E., NELSON J.S. & JOHNSON, E.M. (1981). Experimental diabetic autonomic neuropathy. *Am. J. Pathol.*, **103**, 210–225.

SENGES, J., BRACHMANN, J., PELZER, D., HASSLACHER, C., WEIHE, E. & KUBLER, W. (1980). Altered cardiac automaticity and conduction in experimental diabetes mellitus. *J. Mol. Cell. Cardiol.*, **12**, 1341–1351.

SMITH, D.J. (1950). Reactions of the isolated surviving coro-

nary artery to epinephrine, acetylcholine and histamine. *Proc. Soc. Exp. Biol. Med.*, **73**, 449–452.

STUESSE, S.L., WALLICK, D.W. & MACE, S. (1982). Vagal control of heart period in alloxan diabetic rats. *Life Sci.*, **31**, 393–398.

TANZ, R.D., RUSSELL, N.J., BANERIAN, S.P. & SHARP, V.H. (1982). Ouabain-induced tachyarrhythmias and cell damage in isolated perfused guinea-pig hearts. I: protection by propranolol. *J. Mol. Cell Cardiol.*, **14**, 655–671.

TOMLINSON, D.R. & YUSOF, A.P.M. (1981). On the structural and functional integrity of autonomic nerves in rats with long-term diabetes mellitus induced by alloxan. *Br. J. Pharmacol.*, **74**, 184P.

VADLAMUDI, R.V.S.V. & McNEILL, J.H. (1983). Effect of alloxan- and streptozotocin-induced diabetes on isolated rat heart responsiveness to carbachol. *J. Pharmacol. Exp. Ther.*, **225**, 410–415.

WHEELER, T. & WATKINS, P.J. (1973). Cardiac denervation in diabetes. *Br. Med. J.* **4**, 584–586.

WILLIAMS, R.S., SCHAIBLE, T.F., SCHEUER, J. & KENNEDY, R. (1983). Effects of experimental diabetes on adrenergic and cholinergic receptors of rat myocardium. *Diabetes*, **32**, 881–886.

(Received April 21, 1988
Revised January 5, 1989
Accepted March 17, 1989)