J. Pharm. Pharmacol. 1992, 44: 142–144
Communicated June 22, 1991

Supersensitivity of isolated atria from diabetic rats to adenosine and methacholine: modulation by pertussis toxin

XIAO-JIANG LI, *Department of Pharmacology (SM), L221, The Oregon Health Sciences University, 3181 SW Sam Jackson Park Road, Portland, OR 97201-3098, USA*

Abstract—The chronotropic response of isolated right atria obtained from rats made diabetic 14–15 weeks previously by streptozotocin, was compared with age-matched controls. Diabetic rat atria are significantly more sensitive to the negative chronotropic actions of adenosine and of methacholine. Pretreating both control and diabetic rats with 2·5 mg kg^{-1} pertussis toxin attenuated the negative chronotropic effects of methacholine and adenosine on isolated atria, although diabetic atria still displayed a significantly greater sensitivity to these agonists ($P < 0·05$–$0·001$). The negative chronotropic effects of methacholine and adenosine on both control and diabetic atria were abolished following pretreatment with higher doses of pertussis toxin (10 mg kg^{-1}). These results suggest that pertussis toxin-sensitive G proteins may be involved in the supersensitivity of diabetic hearts to methacholine and adenosine.

Alterations in autonomic control of the heart have been described in human diabetes mellitus (Duchen et al 1980) and chemically-induced diabetic animals (Chang & Lund 1986). A subsensitivity to adrenergic agonists (Atkins et al 1985) and supersensitivity to cholinergic agonists (Vadlamudi & McNeill 1983; Atkins et al 1985; Li et al 1989) have also been reported. These alterations have been associated with an increase in the frequency of cardiac pump failure in diabetic subjects (Regan et al 1977; Sanderson et al 1978). However, the mechanism whereby these abnormalities occur is still unknown. Cholinergic supersensitivity in diabetic hearts was not found to be related to an increase in the number or affinity of muscarinic receptors (Williams et al 1983; Carrier & Aronstam 1987; Kofo-Abayomi & Lucas 1987). This raises the possibility that cholinergic supersensitivity in diabetic hearts may result from an alteration beyond the receptor level. Muscarinic receptors as well as adenosine receptors in the heart couple to inhibitory guanine nucleotide binding proteins (G_i and G_o), then inhibit adenylate cyclase activity and increase K channel conductance to produce inhibitory effects on the heart (Pfaffinger et al 1985; Sorota et al 1985; Kurachi et al 1986). These inhibitory effects can be attenuated by pertussis toxin (PTX) (Endoh et al 1983, 1985; Kurachi et al 1986). To study whether diabetic heart also shows supersensitivity to the inhibitory actions of different agonists via PTX-sensitive G protein transduction pathways and whether a PTX-sensitive G protein is involved in the altered function of streptozotocin-diabetic hearts, the chronotropic responses of isolated atria to adenosine and the muscarinic agonist, methacholine, with and without PTX pretreatment, were studied.

Materials and methods

Treatment. Diabetes was induced in male Sprague-Dawley rats, 200–250 g, using a single intravenous injection of streptozotocin (55 mg kg^{-1}) into the tail vein. Age-matched control rats received vehicle alone (0·01 M citrate buffer in 0·9% NaCl) (Li et al 1989). The diabetic state was verified by plasma glucose levels in excess of 300 mg dL^{-1}, and characterized by excessive daily food and water consumption and a decline in body weight. Blood samples were obtained from the tail vein of animals and non-fasting serum glucose determined by a Kodak Ektachem OT 60 glucose analyser. All rats had free access to water and standard Purina rodent chow. Preliminary study showed that PTX caused dose-dependent attenuation of the inhibitory effects of methacholine and adenosine. PTX vehicle alone (0·01 M sodium phosphate and 0·05 M NaCl) had no effect. Two doses of PTX (2·5 or 10 mg kg^{-1}) were used to treat diabetic and age-matched control rats by single injections in the tail vein 72 h before the animals were killed. No deaths occurred when animals were treated with PTX.

Experimental procedure. Fourteen to eighteen weeks following streptozotocin injection, rats were weighed and under ether anaesthesia their hearts excised, plunged into ice-cold Krebs-Henseleit (KH) solution, mounted and perfused with cold KH solution by the method of Langendorff (Li et al 1989). Right atria were then isolated from the ventricles and placed in organ baths containing 20 mL of modified KH bicarbonate solution having the following composition (mM): Na^+ 144, K^+ 5·94, Ca^{2+} 2·54, Mg^{2+} 1·19, Cl^{-1} 124, HCO_3^- 25, SO_2^{2-} 1·19, PO_2^{3-} 1·19 and glucose 11·12. The solution was bubbled with 95% O_2–5% CO_2 at 35·5°C. Mounted atria were placed under 1 g tension and spontaneous beating rates were recorded on a polygraph.

Cumulative concentration-response relationships were obtained for each drug. Tissues were allowed to equilibrate for 60 min in fresh KH solution which was changed 20 min before the addition of drugs. Atria were first exposed to either methacholine or adenosine from low to high concentrations. The peak effect usually occurred about 2 min following the addition of agonists and returned to base-line about 30 min following wash-out. Parallel experiments without drug treatment showed that heart rates of isolated atria were fairly stable up to 4 h. Drug was added as a solution of 20 mL containing the appropriate concentration and was placed into the 20 mL bath.

Statistical analysis. Results are expressed as the mean ± s.e. Responses to each drug are expressed as % of the control value just before the smallest dose of drug was added. The unpaired Student's *t*-test was used to compare diabetic and age-matched controls. Values of $P < 0·05$ were considered statistically significant.

Drugs. Acetyl-methacholine-Cl and adenosine were obtained from the Sigma Chemical Company; streptozotocin was obtained from the Sigma Chemical Company and dissolved in 0·01 M citrate buffer; PTX (pertussis toxin) was obtained from List Biochemical Laboratory and dissolved in 0·01 M sodium phosphate and 0·05 M NaCl (pH = 7·0). Drug solutions were prepared just before use.

Results and discussion

General features. Table 1 shows that diabetic rats, treated or untreated with PTX, had a significantly lower body weight than their age-matched controls ($P < 0·001$). Blood glucose was uniformly elevated in diabetic rats even after PTX treatment. At 60 min following equilibration, heart rate was significantly less in diabetic rats than that of age-matched controls. In a previous study we showed that these abnormalities were most apparent between 12 and 24 weeks following the administration of streptozotocin and could be corrected by insulin replacement (Li

Present address and correspondence: Department of Neuroscience, The Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205, USA.

Table 1. General features of rats 14–18 weeks after streptozotocin treatment.

Treatment	Body wt (g)	Blood glucose (mg dL^{-1})	Basal heart rate (beats min^{-1})
Control			
Age-matched	412 ± 3·3*** (8)	167 ± 12·8*** (8)	273 ± 13·1** (8)
diabetic	207 ± 17 (7)	626 ± 26 (7)	203 ± 12·6 (7)
PTX 2·5 mg kg^{-1}			
Age-matched	433 ± 21*** (7)	157 ± 9·3*** (7)	280 ± 7·7** (7)
diabetic	199 ± 14·9 (7)	656 ± 35·4 (7)	191 ± 6·0 (6)
PTX 10 mg kg^{-1}			
Age-matched	421 ± 10·2*** (3)	157 ± 23·1*** (3)	277 ± 4·1*** (3)
diabetic	244 ± 10·2 (3)	665 ± 38·1 (3)	200 ± 14·1 (3)

Data were obtained from rats 14–18 weeks following injection of streptozotocin (55 mg kg^{-1}) in the tail vein. Pertussis toxin (PTX) was injected in the tail vein 72 h before animals were killed. Body weights and blood glucose were obtained before death and heart rates were obtained from isolated atria following 60 min equilibration in the bath. Values are mean and s.e.m.; ** $P < 0.01$ and *** $P < 0.001$ compared with corresponding diabetic rats.

et al 1989). PTX did not alter the basal heart rate, as reported previously (Endoh et al 1985), and the level of blood glucose of either diabetic or age-matched control rats compared with those without PTX treatment, suggesting that the dose of PTX (2·5 and 10 mg kg^{-1}) used in this study did not alter the diabetic state.

Negative chronotropic response to methacholine and adenosine. The concentration-response relationships of isolated, spontaneously beating right atria from diabetic and age-matched control rats to the negative chronotropic effect of methacholine and adenosine are shown in Figs 1 and 2, respectively. Atria from diabetic rats were more sensitive to the inhibitory effects of adenosine (Fig. 1A) and methacholine (Fig. 2B). Significant differences from adenosine occurred at concentrations between 10^{-5} and 10^{-4} M and to methacholine between 10^{-6} and 10^{-4} M. Although many reports have described alteration of diabetic heart response to muscarinic agonists, this study provides the first evidence for the altered response to adenosine, another agonist for receptors linked to inhibitory G proteins in the heart. Since muscarinic and adenosine receptors are coupled to the same PTX-sensitive G proteins (G_i and G_o) to produce their inhibitory effects on the myocardium (Endoh et al 1983, 1985; Kurachi et al 1986), these results suggest that in diabetic hearts an alteration other than at the sarcolemmal receptors may be responsible for supersensitivity to these two different receptor agonists. The previous studies showing that cholinergic supersensitivity in diabetic hearts could not be related to increases in the number or affinity of muscarinic receptors (Williams et al 1983; Carrier & Aronstam 1987; Kofo-Abayomi & Lucas 1987) also support this hypothesis.

PTX modulation of diabetic rat atria responses. To confirm that diabetic-induced supersensitivity to adenosine and methacholine involve a G protein-coupled transduction, PTX was used to pretreat rats since PTX has been demonstrated to attenuate or abolish the effect of muscarinic and adenosine receptor function in cardiac tissue, through ribosylation of G_i and G_o proteins (Endoh et al 1983, 1985; Kurachi et al 1986). When rats were pretreated with PTX (2·5 mg kg^{-1}), the negative chronotropic responses were attenuated in both diabetic and control atria. However, atria from diabetic rats were still more sensitive to adenosine and methacholine than those from age-matched control rats (Figs 1B, 2B). This also confirms the supersensitivity of diabetic atria to agonists even when G protein function has been partially attenuated by small doses of PTX. A larger dose of PTX (10 mg kg^{-1}), however, abolished the inhibitory effects of adenosine and methacholine on all atria, regardless of whether they were from diabetic or age-matched control rats (Figs 1C,

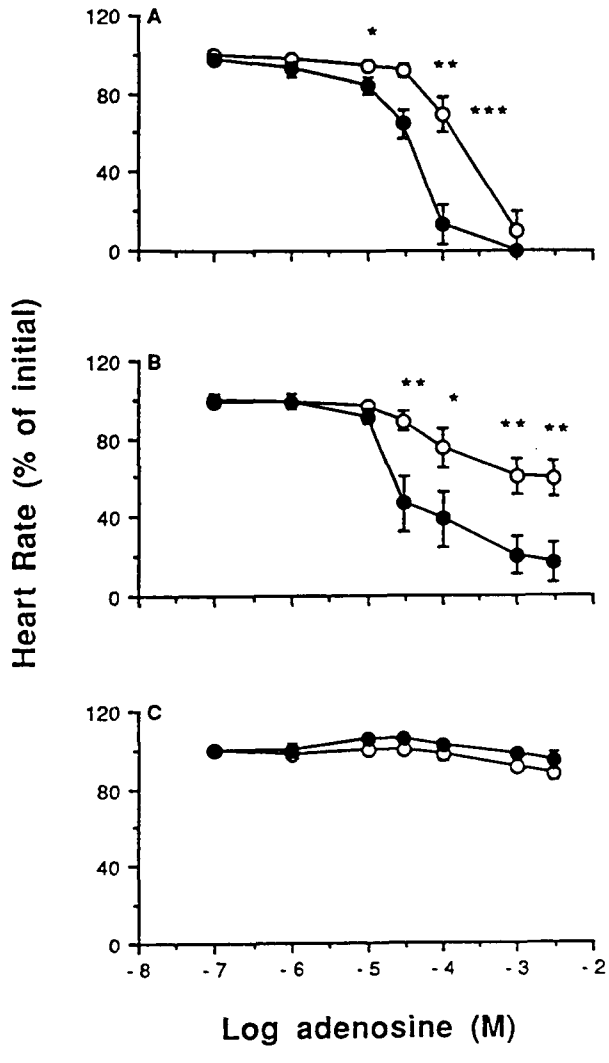

FIG. 1. The effect of adenosine on right atrial rate from age-matched control rats (○) and diabetic rats (●) in (A) the absence of PTX (n = 6–9), (B) the presence of PTX 2·5 mg kg^{-1} (n = 7–9), and (C) PTX 10 mg kg^{-1} (n = 3). Mean initial rate (beats min^{-1}): age-matched control: (A) 271 ± 11, (B) 270 ± 9·5, (C) 277 ± 4·1; diabetic: (A) 206 ± 13·6, (B) 197 ± 8·4, (C) 207 ± 17·3. Values are expressed as the % of the initial mean value. Vertical lines indicate s.e.m., * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ at corresponding concentrations.

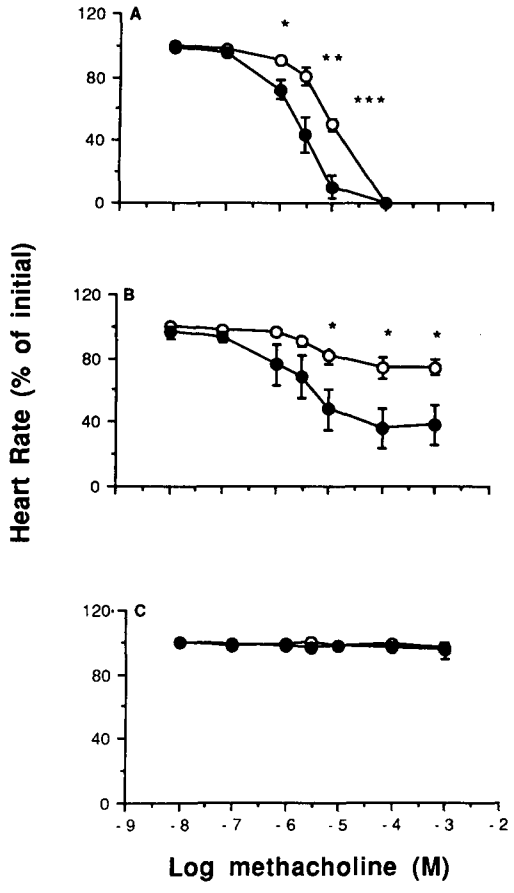

FIG. 2. The effect of methacholine on right atrial rate from age-matched control rats (○) and diabetic rats (●) in (A) the absence of PTX (n = 7-9), (B) the presence of PTX 2·5 mg kg^{-1} (n = 7-9) and (C) PTX 10 mg kg^{-1} (n = 3). Mean initial rate (beats min^{-1}): age-matched control: (A) 253 ± 8·7 (B) 262 ± 12·3 (C) 257 ± 10·8; diabetic: (A) 190 ± 16·7 (B) 191 ± 5·9 (C) 200 ± 14·1. Values are expressed as the % of the initial mean value. Vertical lines indicate s.e.m. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ at the corresponding concentrations.

2C). These results indicate that PTX-sensitive G proteins are involved in the supersensitivity of diabetic hearts to adenosine and methacholine.

The molecular mechanism for the supersensitivity of diabetic heart to these different agonists is not clear. An alteration in G protein-coupled transduction or effectors rather than receptor alone is likely to occur in diabetic hearts. Whether the change in function or level of G proteins occurs in diabetic hearts remains to be studied. The direct effect of diabetes on effectors such as the potassium channel may also need to be considered. The conductance of the potassium channels is important in the regulation of the pacemaker potential. This is also suggested by the observation that bradycardia is present in diabetic rats and PTX did not affect the bradycardia whereas it attenuated or abolished the stimulation by adenosine and methacholine. The previous studies showed that malnutrition and change in body weight or heart weight were not the causes of the altered function of diabetic heart (Foy & Lucas 1976; Penpargkul et al 1980). Our previous study demonstrated that insulin could reverse bradycardia and cholinergic supersensitivity of streptozotocin diabetic rat hearts (Li et al 1989). It appears that diabetic-induced changes in the PTX-sensitive G protein-coupled transduction pathway in cardiac tissue may be part of the mechanism underlying the supersensitivity to adenosine and methacholine. This hypothesis is also supported by the observation of an increase in PTX-labelled G proteins (Nishio et al 1988) and the greater sensitivity of muscarinic agonist binding to regulate GTP (Aronstam & Carrier 1989) in hearts of diabetic rats.

The author wishes to thank Dr R. D. Tanz for his guidance and suggestions, Dr K. S. K. Chang for supplying the animals used in this study, and G. & L. Pfeiffer Medical Research Foundation for financial support.

References

Aronstam, R. S., Carrier, G. O. (1989) Insulin prevention of altered muscarinic receptor-G protein coupling in diabetic rat atria. Diabetes 38: 1611-1616

Atkins, F. L., Dowell, R. T., Love, S. (1985) Beta-adrenergic receptors, adenylate cyclase activity, and cardiac dysfunction in the diabetic rat. J. Cardiovasc. Pharmacol. 7: 66-70

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

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

Duchen, L. W., Anjorin, A., Wartkins, P. J., Mackay, J. D. (1980) Pathology of autonomic neuropathy in diabetes mellitus. Ann. Intern. Med. 92: 301-303

Endoh, M., Maruyama, M., Taira, N. (1983) Modification by islet-activating protein of direct and indirect inhibitory actions of adenosine on rat atrial contraction in relation to cyclic nucleotide metabolism. J. Cardiovasc. Pharmacol. 5: 131-142

Endoh, M., Maruyama, M., Iijima, T. (1985) Attenuation of muscarinic cholinergic inhibition by islet-activation protein in the heart. Am. J. Physiol. 249: H309-H320

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

Kofo-Abayomi, A., Lucas, P. D. (1987) Muscarinic receptor density is reduced in diabetic rat atria, an effect prevented by the aldose reductase inhibitor, Statil. J. Pharmacol. 39: 1019-1021

Kurachi, Y., Nakajima, T., Sugimoto, T. (1986) On the mechanism of activation of muscarinic K channels by adenosine in isolated atrial cells: involvement of GTP binding proteins. Pflugers. Arch. 407: 264-274

Li, X. J., Tanz, R. D., Chang, K. S. S. (1989) Effect of age and methacholine on the rate and coronary flow of isolated diabetic hearts. Br. J. Pharmacol. 97: 1209-1217

Nishio, Y., Kashiwagi, A., Kida, T., Kodama, M., Abe, N., Saeki, Y., Shigeta, Y. (1988) Deficiency of cardiac beta-adrenergic receptor in streptozotocin-induced diabetic rats. Diabetes 37: 1181-1187

Penpargkul, S., Schaible, T., Yipintosoi, T., Scheuer, J. (1980) The effect of diabetes on performance and metabolism of rat hearts. Circ. Res. 47: 911-921

Pfaffinger, P. J., Martin, J. M., Hunter, D. D., Nathason, N. M., Hille, B. (1985) GTP binding proteins couple cardiac muscarinic receptors to a K channel. Nature 317: 536-538

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

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

Sorota, S., Tsuji, Y., Tajima, T., Pappano, A. J. (1985) Pertussis toxin treatment block hyperpolarization by muscarinic agonists in chick atrium. Circ. Res. 57: 748-758

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

Williams, R. S., Schiable, T. F., Scheuer, J., Kennedy, R. (1983) Effects of experimental diabetes on adrenergic and cholinergic receptors of rat myocardium. Diabetes 32: 881-886