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Cardiac response to autonomic drugs in atria of diabetic mouse

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Abstract—Comparisons of chronotropic effects of sympathomimetic and parasympathomimetic agents were made in isolated atria from alloxan-induced diabetic and age-matched control mice. In atria from mice rendered diabetic for three months, the cardiac response to bethanechol was potentiated compared with that of the age-matched control atria. However, the cardiac responses of atria from alloxan-treated mice to noradrenaline, 3-isobutyl-l-methylxanthine and 1,1-dimethyl-4-phenyl piperazinium iodide were similar to those of the controls.

Cardiovascular disease is more prevalent in diabetic patients (Garcia et al 1974). Research in experimental diabetes in rats has indicated that chronotropic activity was decreased (Pfaffman 1980; Sanges et al 1980; Dowell et al 1986; Rosen et al 1986; Li et al 1989; Navaratnam & Khatter 1989), and cardiac acetylcholine was increased (Akiyama et al 1989). The cardiac sensitivity to acetylcholine was also reported to increase (Vadlamudi & McNeill 1983; Aronstam & Carrier 1989), and the number of cardiac muscarinic receptors decreased (Heyliger et al 1982; Gotzsche 1983; Atkins et al 1985; Bitar et al 1987). On the other hand, cardiac noradrenaline levels have been reported to either increase or decrease (Neubauer & Christensen 1976; Felten et al 1982; Fushimi et al 1982; Akiyama et al 1989); similarly, cardiac response to noradrenaline was reported to either increase or decrease (Foy & Lucas 1978; Ingebretsen et al 1981; Wilson et al 1982). These data suggest that diabetes affects the cardiac chronotropic activity, but that there is an inconsistency in the claims for the direction of the effect.

Rats and mice are frequently used in experimental diabetes research, but rats have been mainly used to determine effects on the cardiovascular system. The purpose of the present research was to investigate whether the response of diabetic mouse atria to parasympathomimetic drugs was changed after a 3-month alloxan-induced diabetes. The atrial response to sympathomimetic agents was also evaluated.

Materials and methods

For the induction of experimental diabetes, ICR mice, six weeks old, were injected subcutaneously with alloxan (100, 150, 200, 250, 300, 350, 400 mg kg^{-1}). Alloxan-induced hyperglycaemia was checked every day for one week by determining the concentration of glucose in blood collected from the tail vein, using the Reflolux II M glucose analyser (Boehringer Mannheim, Germany). This regimen was used to choose the most appropriate dose of alloxan.

ICR mice were killed by a blow on the head. The heart was quickly removed and transferred to a Krebs bicarbonate solution bubbled continuously with 95% O_2–5% CO_2. The Krebs bicarbonate solution had the following composition (mM) as described elsewhere (Wong 1990): NaCl (137·9), KCl (4·0), $CaCl_2$ (2·0), $MgCl_2$ (0·5), $NaHCO_3$ (12·0), KH_2PO_4 (1·0), and glucose (11·1). The right atrium was prepared for chronotropic recording in an organ bath containing the Krebs bicarbonate solution with temperature maintained at 37°C and bubbled continuously with 95% O_2–5% CO_2. The atrial response was recorded with a Grass polygraph (Model 79D) via a force displacement transducer. Drug solutions were prepared in Krebs bicarbonate solution, and the concentration effect curve of each drug was determined by a stepwise increase of the drug concentration in the bathing solution. Student's t-test was used for statistical analysis and $P < 0.05$ was considered significant. The following drugs were purchased from Sigma Co. (USA): noradrenaline bitartrate, 1,1-dimethyl-4-phenyl piperazinium iodide (DMPP), bethanechol, 3-isobutyl-1-methyl-xanthine (IBMX), alloxan.

Results

It was observed that 300 mg kg^{-1} alloxan was the optimum dose to induce increases in blood glucose in mice six weeks old. Therefore, this dose of alloxan was used to induce experimental diabetes. Changes in the chronotropic activity in response to drugs were compared between diabetic and age-matched control atria. It was shown that the parasympathomimetic agent bethanechol produced a concentration-dependent negative chronotropic effect, and a significant difference in negative chronotropic effects induced by bethanechol between diabetic and age-matched control atria was demonstrated (Fig. 1), since bethanechol could induce a greater negative chronotropic effect at 10^{-5} and 10^{-4} M in diabetic than in age-matched control atria; the ED50 values for the bethanechol response in the diabetic and control atria were $9{\cdot}4 \times 10^{-6}$ and $3{\cdot}6 \times 10^{-5}$ M, respectively. The 95% confidence interval of the difference was $6{\cdot}17 \times 10^{-6}$–$4{\cdot}7 \times 10^{-5}$ M ($P = 0{\cdot}017$).

On the other hand, in response to sympathomimetic agents, noradrenaline-induced positive chronotropic effects in diabetic mouse atria were not significantly different from that of age-matched control atria (Fig. 2). Moreover, the positive chronotropic effects induced by IBMX and DMPP in diabetic and age-matched control atria were also similar (Fig. 3).

Discussion

In the present study, the blood glucose level in diabetic mice, three months after the subcutaneous administration of 300 mg kg^{-1} alloxan, was between 300–400 mg dL^{-1} and was much higher than the blood glucose level of 80–110 mg dL^{-1} in age-matched control mice, indicating that hyperglycaemia had been induced in the alloxan-pretreated mice. The present data showed

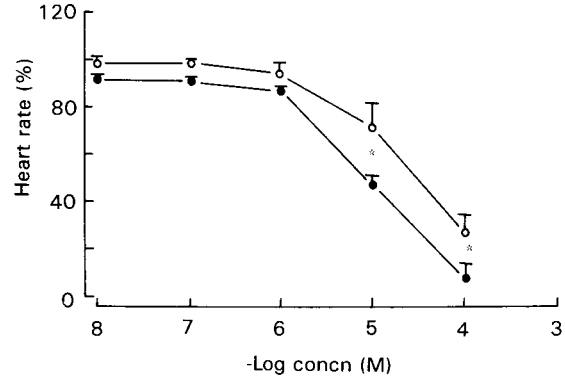

FIG. 1. Bethanechol-induced negative chronotropic activity in 3-month diabetic and age-matched control mouse atria. ○ Control atria, ● diabetic atria. Bars represent mean ± s.e.m. (n = 10 atria). *$P < 0.05$ compared with the control atria.

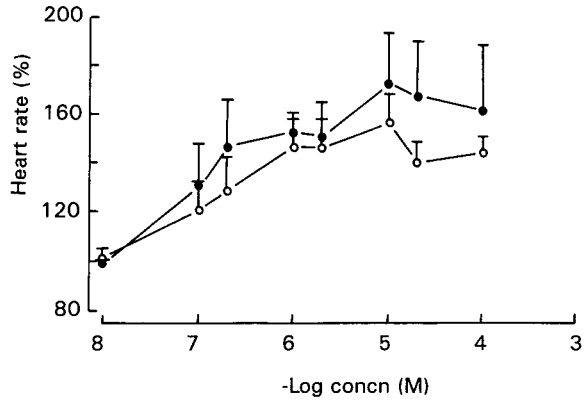

FIG. 2. Noradrenaline-induced positive chronotropic activity in 3-month diabetic and age-matched control mouse atria. ○ Control atria, ● diabetic atria. Bars represent mean ± s.e.m. (n = 10 atria).

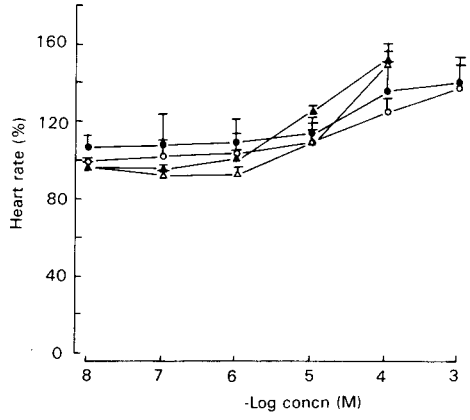

FIG. 3. IBMX-induced and DMPP-induced positive chronotropic activities in 3-month diabetic and age-matched control mouse atria. For IBMX, △ control atria, ▲ diabetic atria. For DMPP, ○ control atria, ● diabetic atria. Bars represent mean ± s.e.m. (n = 10 atria).

that noradrenaline did not induce a significant difference of positive chronotropic effect between diabetic and age-matched control atria. Since cyclic AMP is currently considered to be the second messenger following the activation by noradrenaline of adrenoceptors in the heart, the result of the IBMX experiment supported the noradrenaline data that the change of the atrial response to sympathomimetic agent in diabetic atria was not significantly different from that of the control atria, for IBMX is an inhibitor of phosphodiesterase which hydrolyses cyclic AMP. DMPP is a nicotinic agonist that induces positive chronotropic effects due to increased presynaptic release of noradrenaline (Bhagat et al 1967; Haeusler et al 1968; Westfall & Brasted 1972). The effect of DMPP was in accord with the lack of a significant difference between diabetic and age-matched control chronotropic activities in response to sympathomimetic agents. On the other hand, the bethanechol-induced negative chronotropic effect in diabetic atria was significantly different from that of age-matched control atria.

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