Reduction of ventricular M₂ muscarinic receptors in cardiomyopathic hamster (CHF 147) at the necrotic stage of the myopathy

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Abstract. We have previously demonstrated that isolated ventricular myocytes from cardiomyopathic hamsters (CHF 147) during the necrotic stage (70–100 days) exhibit an attenuated contractile response to muscarinic stimulation. In the present study we have investigated whether this dysfunction may be related to a change in the density (or affinity) of cardiac muscarinic receptors. Thus, we have characterized and quantified the binding of the muscarinic antagonist [³H]-N-methyl scopolamine (NMS) to M₂ muscarinic receptors in cardiac micropunches and in suspensions of isolated intact cardiomyocytes obtained from cardiomyopathic (CHF 147) and Golden Syrian hamsters. The hamsters were either 70–100 days old, when the cardiomyopathy had reached the cytolytic and necrotic stage or 30 days old, i.e. before the onset of the cardiomyopathy. In both preparations (micropunches and dissociated cardiomyocytes) the specific binding of [³H]-NMS was stereospecific, reversible, saturable, of high affinity and linearly dependent upon increasing amounts of tissue and cells. The binding site also possessed the drug specificity typical of an M₂ muscarinic receptor. Saturation binding analysis revealed that the hearts of the older CHF 147 hamsters contain significantly fewer M₂ muscarinic receptors than the control Golden Syrian hamsters while the affinity (Kd) was not altered. This reduction of M₂ receptor number was not observed in CHF 147 hamsters at 30 days. Further, we found no differences in β-adrenergic or in α₁-adrenergic binding in the two strains of hamster at either age. Thus, our results indicate that the parasympathetic regulation of cardiac function in CHF 147 hamsters may be compromised by a decreased number of muscarinic receptors at the necrotic stage of the cardiomyopathy.

Key words: Cardiomyopathic hamster (CHF 147) — M₂-muscarinic receptors — Cardiomyocytes — [³H]-N-methyl scopolamine — Micropunches

Introduction

Major efforts in the study of heart failure in humans and experimental animals have so far focused mainly on alterations in sympathetic nervous system activity and in particular on the β-adrenergic receptor [3, 11, 22]. There is little doubt that the β-adrenergic receptor — guanine nucleotide-binding protein-adenyl cyclase system is compromised by heart disease and its accompanying increase in sympathetic activity [3, 11]. Much less studied is the parasympathetic system in cardiac pathophysiology [7], although defective cholinergic regulation has been observed in patients with heart disease [8] and in a canine model of heart failure [24].

The cardiomyopathic (CM) Syrian hamster has proved to be a valuable experimental preparation for the study of heart disease. This animal demonstrates several of the pathological changes associated with human idiopathic cardiomyopathy. For example, ventricular cellular necrosis, fibrosis, hypertrophy, dilation and congestive heart failure are distinct features of the hamster disease [10, 14–16].

In a recent study we have demonstrated that isolated ventricular cardiomyocytes, obtained from cardiomyopathic hamsters (CHF 147) at the necrotic stage of cardiomyopathy (80–110 days), have an impaired contractile response not only to β-adrenergic stimulation but also to carbachol in the presence of isoprenaline [14]. Thus, the carbachol concentration eliciting a half-maximum negative inotropic effect was significantly greater (280%) in cardiomyopathic myocytes than in control myocytes obtained from Golden Syrian (GS) hamsters. This observation suggests that muscarinic receptors in the diseased
hearts may be incorrectly coupled to the contractile machinery, or that there are fewer receptors available, or both. Evidence for the former possibility has been reported by Chidichimo et al. [6]. However, this group was unable to detect a change in the number of muscarinic receptors (quantified with [3H]-N-methyl scopolamine; [H]-NMS) in heart tissue from hamsters in the late stages of heart failure. Similarly, in very young hamsters (30 days), Wagner et al. [26] also have found no difference between muscarinic binding (measured with [H]-quinuclidinyl benzilate; [H]-QNB) in young cardiomyopathic and control hamsters. In human cardiomyopathic tissue Bühm et al. found no changes in [H]-QNB binding [2].

Our observations with carbachol [14] led us to investigate whether the density of muscarinic receptors is altered during the necrotic stage of the cardiomyopathy. Using new binding assays for muscarinic receptors we found a significant reduction in M2 muscarinic receptors in CM heart at the necrotic stage (approximately 100 days). Until now neurotransmitter binding sites have been usually quantified in broken cell preparations (homogenates) or autoradiographically in thin tissue sections. These techniques have provided invaluable data unobtainable in any other way. However, for some years now we have advocated that the study of receptor-related physiological events should ideally be carried out in intact tissue, conveniently in the form of tissue slices [27–30] or in suspensions of functionally active ventricular myocytes [13]. With the judicious use of hydrophilic ligands we have shown that it is possible to quantify cell-surface receptors on intact cells contained within tissue slices [25, 27–30]. Recently we have modified the slice assay to incorporate micropunches obtained from tissue slices [31].

In the present paper we describe the characterization and quantification of [H]-NMS binding to an M2 muscarinic receptor in 1-mm-diameter micropunches cut from 350-μm slices of CM and control GS hamster left ventricle. These data are compared to those obtained from identical assays on suspensions of enzymatically dissociated intact ventricular cardiomyocytes in which the functional responses were determined previously [14]. Both techniques yielded similar results, i.e. the CM heart from animals aged 70–100 days contains fewer M2 muscarinic receptors compared with hearts from control GS hamsters. This difference was not observed in micropunches from younger (30 days) hamsters. Additional experiments on two other receptor types i.e. α1-adrenergic; (using [H]-prazosin binding) and β-adrenergic (using [H]-CGP-12177 binding) revealed that CM hearts are not different from GS hearts at either age. Thus, the CM heart shows a selective reduction in M2 receptors at the necrotic stage of the disease.

Materials and methods

**Animals.** Male and female CM hamsters of the CHF 147 strain (25–30 days old or 70–100 days old) and age-matched control GS hamsters were obtained from Canadian Hybrid Farms, Hall's Harbour, Centerville, Nova Scotia, Canada. Hamsters were kept in the Animal Care Centre for at least 7 days before use and maintained under constant temperature (21°C) and lighting conditions (lights on 7 am–7 pm) with free access to food and water. It was visually observed that all the older (70–100 days) CM hamsters used in these studies had calcified lesions in their hearts, as reported previously [14].

**Materials.** [H]-NMS as hydrochloride spec. act. 83 Ci/m mole, [H]-prazosin (spec. act. 80 Ci/m mole) and [H]-CGP-12177 (spec. act. 38 Ci/m mole) were obtained from Amersham Canada (Toronto). (-)-Nicotine, carbachol, atropine, prazosin and (±)-timolol were from Sigma (St. Louis, Mo., USA); pirenzepine, (+)- and (-)-scopolamine hydrobromide were from Research Biochemicals (Natick, Mass., USA). The muscarinic M2 antagonists AF-DX 384 and AF-DX 116 were generous gifts from Boehringer Ingelheim (Canada). Collagenase was from Boehringer Mannheim (Montreal, Canada) and trypsin from Sigma.

**Preparation of micropunches.** Hamsters were transferred to the laboratory 1–2 h before use and sacrificed by decapitation. The heart was quickly removed and placed in ice-cold Dulbecco's phosphate buffered saline (DPBS) (Gibco) for 5–10 min. The tissue was then placed on an inverted petri dish (on ice) and the atria and the base of the heart excised with a razor blade. A longitudinal cut removed the septum and right ventricle. The left ventricle was further trimmed approximately 4 mm towards the apex producing a block of tissue for slicing.

Slices (350 μm) were made using the Campden Vibroslice model 752/M (World Precision Instruments, New Haven, Conn., USA). The tissue block was blotted dry and fixed to the chuck with cyanoacrylate tissue adhesive such that the natural curvature of the ventricle was retained. Settings of maximum amplitude and a slow rate of advance produced slices of uniform thickness. Each slice contained epicardium, myocardium and endocardium. Slices were put into petri dishes containing DPBS (on ice) and a cushion of 4–5 mm thick of Sylgard elastomer (Sylgard 184 silicone elastomer kit, Dow Corning). Slices were then viewed under a dissecting microscope and punches from myocardium were obtained using a 1-mm punch (Fine Science Tools, Vancouver, B.C., Canada). Single punches were placed in separate wells of Linbro 24-well tissue culture plates each containing 0.5 ml DPBS. For the α1- and β-adrenergic assays, 2 mm punches were used.

**Micropunch binding assay.** Muscarinic receptor binding was determined using [H]-NMS. Non-specific binding (two wells) was determined in the presence of AF-DX-384 (10−6 M) and was subtracted from total binding (six wells) to give specific binding. Labelled and unlabelled drugs were added as 20-μl aliquots to give a final assay volume of 540 μl. After incubation at 30°C for 90 min (see below for characterization) the culture plate was placed on ice and 50-μl aliquots of buffer were removed for the determination of the free (equilibrium) concentration of [H]-NMS. The remaining buffer was pipetted out and the wells washed twice for 5 min with 0.5 ml cold DPBS using a Jet-Pipet (Cole Parmer, Chicago, Ill., USA). The wells were finally drained and punches removed by touching with a small square (approximately 2 mm) of glass fibre filter paper (Whatman GF/B). Both were placed in a vial containing scintillation fluid (1.8 ml; EcoLite ICN, Irvine, Calif., USA). The vials were thoroughly mixed and then allowed to sit overnight before counting in an LKB 1218 Rackbeta liquid scintillation counter (efficiency 37%).