Influence of age on inducibility and cholinergic modulation of arrhythmia in isolated rat right atria

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Abstract The effects of carbachol and atropine on the number of trains (NT) and on the train stimulus strength (SS) necessary to induce arrhythmia were studied in isolated right atria of infant, young, adult and mature rats submitted to electric field stimulation (66.7 Hz, 5 ms pulse-duration, 250 pulses). Carbachol (1 μ M) decreased NT from four (control) to two in all ages tested. Atropine (1 μ M) prevented tachyarrhythmia induction in tissue of all ages, even with NT equal to 12, except for mature rats (typically four trains). The SS decreases from infant to adult age [5- to 2-fold atrial threshold (AT)] and increases in mature animals (5-fold AT). Carbachol changes this result only for mature rats (5- to 2-fold AT). The SS was decreased by carbachol (1 μ M) from 5- to 3-fold AT in mature rats, but atropine did not modify SS in this age. These results indicate that inducibility and cholinergic modulation of atrial tachyarrhythmia is influenced by age.

Keywords Acetylcholine receptor · Age · Arrhythmia · Cardiac muscle · Cholinergic

Introduction

Cardiac arrhythmias significantly affect heart function, posing a risk to the patient's life if not suitably treated. In order to treat arrhythmias adequately, a knowledge of the mechanisms whereby they are generated and modulated, and of potential modifying factors such as gender or age, is important. Although the influence of these mechanisms and factors on cardiac function is not fully understood, one reasonably well known generating mechanism of arrhythmia is the reentry mechanism, which is, in turn, under the influence of the autonomic nervous system as an important modulating mechanism, whereas one important factor affecting the inducibility and the cholinergic modulation of tachyarrhythmias is the influence of age. In fact, it has been shown that the reentry mechanism can be affected greatly by alterations in action potential duration mediated by cholinergic mechanisms (Taggart et al. 2003; Olshansky 2005; Mantravadi et al. 2007), which, in turn, can be affected by aging (Kellihier and Conahan 1980; Poller et al. 1997; Brodde et al. 1998; Brodde and Michel 1999).

In this sense, some experimental studies have shown that inducibility of electrically stimulated arrhythmias can be modified by agonists of cardiac

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muscarinic acetylcholine receptors (mAChR). For example, Schuessler et al. (1992) and Godoy et al. (1999) showed that the inducibility of electrically stimulated arrhythmias was increased by acetylcholine (ACh) and carbachol and reduced by atropine in isolated atria of adult rats. Such findings suggested that mAChR could be involved in the regulation of excitability of cardiac cells, and its activation by endogenous ACh could increase reentrant circuit formation (Allessie et al. 1984; Schuessler et al. 1992; Godoy et al. 1999). Additionally, some studies have suggested that functional responses mediated by cardiac mAChR, such as positive or negative chronotropic effects (Poller et al. 1997; Kelliher and Conahan 1980) and expression of mRNA and protein corresponding to cardiac mAChR (Brodde et al. 1998; Brodde and Michel 1999; Lo et al. 2001), can be influenced by age. These findings suggest that autonomic modulation mediated by mAChR is modified in animals of different ages.

Therefore, supported the evidences that cholinergic mechanisms are involved in the genesis of atrial arrhythmias and that these mechanisms are modified by the animal's age, we have evaluated the influence of age on the inducibility and on the cholinergic modulation of atrial tachyarrhythmia. We have specifically studied the electrophysiological and pharmacological characteristics of electrically-induced tachyarrhythmia in isolated right atria of rats in different ages.

Materials and methods

Animals

This study used isolated right atria of male Wistar rats of different ages (in days): 14–16 (infant); 28–32 (young); 90–100 (adult) and 290–310 (fully mature adult; hereafter referred to as “mature”). The body and atrial weights of rats were determined using a conventional scale and an analytical scale, respectively.

Right atria preparation

Rat isolated right atria were prepared as previously described (Godoy et al. 1999, 2002; Godoy and Galvão 2007). Briefly, the animals were sacrificed

by cerebral concussion and immediate section of cervical blood vessels for surgical removal of the heart. The isolated heart was placed in Krebs-Henseleit solution for isolation of the right atrium. The isolated right atrium was placed in the centre of a 50 ml cylinder-shaped organ bath chamber. The chamber was filled with Krebs-Henseleit solution up to a height of 0.5 cm (solution volume of 25 ml) that was kept constant by application of solution or vacuum. The Krebs-Henseleit solution had the following composition (mM): NaCl 126.4, KCl 4.6, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 13.6, CaCl₂ 1.5, glucose 11.11, pH 7.4, saturated with 95% O₂ plus 5% CO₂, at 36.5°C.

A period of 40 min was allowed for atrial natural rhythm stabilisation before application of the electrical field stimulation (EFS) protocol. The EFS-protocol and electrogram detection of isolated atria were performed using two platinum wire electrodes and two Ag–AgCl electrodes, respectively. A third Ag–AgCl electrode was used as an electrical reference. Accordingly, these electrodes were connected to an electric stimulator (S48-Stimulator, Grass Technologies, Astro-Med, West Warwick, RI) and to an amplifier (Iso-Dam8; World Precision Instruments, Sarasota, FL). Both devices were connected to a computer-based system via an acquisition system (analog/digital converter CAD1236; acquisition software Aqdados 4; Lynx Electronics Technologies, São Paulo-SP, Brazil). The positioning of the stimulation electrode at the chamber was made in such a way that the electric field direction of a biphasic voltage pulse (5-ms rectangular pulse with capacitive coupling) could be applied in the base–apex orientation of the atrium. This was performed to minimise the electric field strength necessary for atrial stimulation (Godoy et al. 2002). All procedures were approved by The Committee of Ethical in Animal Research of University of Mogi das Cruzes (UMC).

Experimental protocol

The EFS-protocol used for induction of atrial tachyarrhythmia, based on the previous work of Godoy et al. (1999), consisted of the application of a train of 250 biphasic voltage pulses (66.7 Hz) in the perfusion chamber. The number of trains (NT) and the train stimulus strength (SS) necessary to induce tachyarrhythmia were determined in every age range as follows: a set of up to 12 trains with SS equal to 2-

fold the atrial threshold (AT) was initially applied. If, for example, tachyarrhythmia was induced in the third train of that set, we stopped the stimulation and considered that tachyarrhythmia was inducible with three trains whose SS is equal to 2-fold AT. If, on the other hand, tachyarrhythmia was not induced within that set of 12 trains, the SS was increased by one step (from 2- to 3-fold AT) and another set of up to 12 trains was applied. This procedure of increasing SS (steps of 1-fold AT) in a set of up to 12 trains was repeated until tachyarrhythmia was induced or until maximum stimulus capability was reached by the stimulator (typically 12-fold AT). A stabilisation period of 10 min was allowed between the train sets. The AT, determined before every train set, was considered to be the minimum SS of a train that would pace the atrium at a frequency 10% above the sinus normal rate.

To investigate the influence of age on the cardiac mAChR, we studied the effects of an agonist (carbachol, 1 μ M) or antagonist (atropine, 1 μ M) of these receptors on EFS-induced tachyarrhythmia in isolated right atria of infant, young, adult and mature rats. Tachyarrhythmia induction was attempted 30–40 min after addition of the drug to the perfusion solution. These drug studies were performed after a set of trains whose atrial tachyarrhythmia was induced (control condition). A washout of the preparation was performed after each drug test and control conditions were obtained before each drug perfusion.

Data analysis

The means of either SS, NT or atrial rate (AR) in the different age ranges were compared by analysis of variance for paired samples. This analysis was performed using the GraphPad InStat software (Graphpad Software, San Diego, CA). The null hypothesis (no statistical difference) was rejected considering $P > 0.05$. Data were expressed as mean $\pm$ standard error of mean (SEM).

Results

The body weights of rats used in this work were 35 ± 18 g (infant, $n = 20$), 104 ± 14 g (young, $n = 20$), 356 ± 27 g (adult, $n = 16$), 413 ± 15 g (mature, $n = 20$). The isolated right atria weights were 8 ± 1 mg (infant), $19 \pm$

4 mg (young), 46 ± 4 mg (adult) and 50 ± 10 mg (mature).

The normal AR was similar in young (4.2 ± 0.4 Hz), adult (3.5 ± 0.3 Hz) and mature (3.4 ± 0.5 Hz), but was increased in infant (7.7 ± 0.2 Hz) rats. When isolated atria were submitted to the EFS protocol in the absence of drugs, atrial tachyarrhythmia was observed at all age ranges. The duration of atrial tachyarrhythmia induced by the EFS protocol varied from 2 s to approximately 90 s at all ages. In addition, AR after the EFS protocol increased at all ages in relation to their respective normal AR, characterizing tachyarrhythmia induction with aging. This atrial tachyarrhythmia was similar in infant (24 ± 0.9 Hz), young (25 ± 0.8 Hz), adult (21 ± 0.9 Hz) and mature (19 ± 0.9 Hz) rats. A typical electrogram obtained in isolated right atria of rats of different ages submitted to the EFS protocol is shown in Fig. 1.

Figure 1 also shows the effects of an antagonist of mAChR (atropine; 1 μ M), on EFS-induced tachyarrhythmia in right atria of infant, young, adult and mature rats. Treatment of atria with atropine produced a small increase in atrial rates in young (5.5 ± 0.4 Hz), adult (4.4 ± 0.2 Hz) and mature (4.9 ± 0.3 Hz) rats, but not in infant rats (6.8 ± 0.4 Hz), compared to the absence of atropine. When atria treated with atropine were submitted to the EFS protocol, the AR was similar to that observed before EFS for infant (7.0 ± 0.6 Hz), young (5.7 ± 0.3 Hz) and adult (4.9 ± 0.4 Hz) rats, indicating no atrial tachyarrhythmia induction in these ages. In contrast, as for the mature rats, the AR in the presence of atropine was approximately 20 Hz, indicating tachyarrhythmia induction.

Figure 2 illustrates the minimum SS used for attempts of tachyarrhythmia induction in isolated atria of rats in different ages under control conditions and in the presence of carbachol (a non hydrolysable analog of ACh) or atropine in the perfusion bath.

Under control conditions, the minimum SS required to induce tachyarrhythmia changes with age, decreasing progressively from infants to adults and increasing in mature animals. The minimum SS in infant, young, adult and mature rats was 5-, 3-, 2- and 5-fold AT, respectively. The treatment of isolated atria with carbachol (1 μ M) did not change this result for infant, young and adult rats, whereas it was not possible to induce tachyarrhythmia in the presence of atropine in these same age ranges. However, for mature rats, the minimum SS necessary to induce

Fig. 1 Typical electrogram records of isolated right atria of rats of different ages submitted to an electrical field stimulation (EFS) protocol. The effect of the EFS-protocol on atrial response was studied in the absence (control, *left*) or presence of the muscarinic antagonist atropine at 1 μ M (*right*). Note that atrial tachyarrhythmia induced by EFS was totally prevented by atropine at all ages except mature rats. The normal AR (control; Hz) in infant, young, adult and mature rats was 7.0, 5.4, 5.0 and 5.0, respectively. Accordingly, atrial tachyarrhythmia rates (Hz) were 23, 19, 18 and 18. The *stimulus artifacts* indicated are due to the EFS-protocol

tachyarrhythmia, compared to control conditions, decreased in the presence of carbachol and was the same in the presence of atropine.

Figure 3 illustrates the NT used for attempts to induce tachyarrhythmia in isolated atria of rats of different ages, and after treatment with carbachol or atropine.

The NT necessary to induce tachyarrhythmia under control conditions was approximately four at all ages. In the presence of carbachol, NT decreased to

approximately two at all ages, whereas in the presence of atropine it was not possible to induce tachyarrhythmia even with the application of 12 trains. In contrast, with atria of mature rats, tachyarrhythmia induction was again possible with approximately four trains. This result shows that atropine prevented atrial tachyarrhythmia induction at all rat age ranges except in mature rats, whereas carbachol facilitated atrial tachyarrhythmia induction at all ages.

Fig. 2 Minimum stimulus strength (SS) used for attempts at tachyarrhythmia induction in isolated rat atria of different ages. Three experimental conditions are shown in each age range: absence (control) or presence of carbachol or atropine (1 μ M) in the perfusion bath. There is no data dispersion (8 or 10 atria per age range). + Data where tachyarrhythmia was not inducible

Considering all age ranges, AR after the EFS-protocol varied from 19 to 27 Hz under control conditions and in the presence of carbachol (Fig. 4a), indicating induction of typical tachyarrhythmia. This result indicates that carbachol did not change the tachyarrhythmia rate at all ages. In contrast, in the presence of atropine (Fig. 4b), AR after the EFS-protocol varied from 4 to 7 Hz (normal rate), except for mature rats, which exhibited an AR of 15 Hz (tachyarrhythmia rate).

Fig. 3 Effect of 1 μ M carbachol (a) or atropine (b) on the number of trains (NT) used in the EFS-protocol for tachyarrhythmia induction in rat isolated atria of different ages (8 or 10 atria per age range). + Data where tachyarrhythmia was not

Discussion

The present work provides experimental evidence showing that inducibility and the modulatory role of cardiac mAChR on rat atrial tachyarrhythmia are influenced by the age of the animal. The stimulation protocol for EFS-induced atrial tachyarrhythmia used in the present work was based on a method previously proposed by Godoy et al. (1999). The results obtained with this method are qualitatively similar to those obtained by other authors who have studied atrial flutter/fibrillation *in vivo* and *in vitro* (Alessie et al. 1984; Euler and Scanlon 1987; Schuessler et al. 1992; Inoue et al. 1994; Watanabe et al. 1996). However, those authors used direct stimulation whereas we have used EFS to minimise the SS applied to the cardiac tissue (Godoy et al. 2002). Additionally, in the present study, seeking a more quantitative methodological approach, we related SS to arrhythmia inducibility. By using a similar approach, Kirchhof et al. (1998, 2003) showed the antiarrhythmic and proarrhythmic effects on ventricular fibrillation of amiodarone, procainamide and propafenone. Therefore, the use of SS seems to be suitable for the evaluation of arrhythmia inducibility in diverse experimental conditions. Finally, independent of the stimulation method, these *in vivo* and *in vitro* studies seem to support the idea that the atrial tachyarrhythmia observed in the present work is probably related to reentrant circuit formation provoked by electrical stimulation of

inducible. Note that atropine blocked atrial tachyarrhythmia induction in all rat age ranges, except in mature rats. a, b, c, d Data statistically different from the respective control condition

Fig. 4 Effect of 1 μ M carbachol (a) or atropine (b) on the atrial rate (AR) after an EFS-protocol for tachyarrhythmia induction attempts in rat isolated atria of different ages (8 or 10 atria per

age range). + Data where tachyarrhythmia was not inducible (normal AR). a, b, c, d Data statistically different from the respective control condition

the atrial tissue, and, importantly, might also be mediated by cholinergic mechanisms.

Taking into account that the aforementioned studies were performed only with adult animals, the influence of age was not fully evaluated. In the present work, we obtained results that suggest that mAChR are also involved in atrial tachyarrhythmia in infant, young and mature rats, although the electrophysiological and pharmacological characteristics of this tachyarrhythmia are different in mature rats. For example, we observed that the minimum SS necessary for atrial tachyarrhythmia induction progressively decreased from infants to adults, and increased from adult to mature rats (see Fig. 2), suggesting that the arrhythmogenic mechanism(s) in atria might alter during the animal's development. Such alterations could be, at least in part, due to age-related structural and/or functional changes in cardiac tissue, such as alterations in collagen content and cholinergic mechanisms during the animal's development (Dhein et al. 2001; Caulfield and Birdsall 1998; Brodde et al. 1998; Brodde and Michel 1999).

The minimum SS necessary for atrial tachyarrhythmia induction could be influenced by the atrial mass required to induce tachyarrhythmia and/or the anatomical structure of the atrial tissue and cholinergic mechanisms. In this work, we observed that atrial mass increased with age from infant (8 mg), young (19 mg), adult (46 mg) to mature (50 mg) rats. Although the atrial mass of mature rats was similar to that of adult rats, the minimum SS necessary for

tachyarrhythmia induction was higher in mature than in adult rats. These results indicate that other non-electrophysiological factors, including an increase in collagen synthesis and reduced mAChR expression (Brodde et al. 1998; Brodde and Michel 1999; Lo et al. 2001), could be involved in the arrhythmia induction process.

In the present work we also observed that EFS-induced atrial tachyarrhythmia was facilitated or inhibited by micromolar concentrations of agonist (carbachol) or antagonist (atropine) of mAChR in infant, young and adult, but not in mature, rats (Figs. 2–4). The treatment with carbachol—a non hydrolysable analog of ACh—did not significantly change EFS-induced atrial tachyarrhythmia in infant, young and adult rats, whereas it was not possible to induce tachyarrhythmia in the presence of atropine at the same age ranges. However, in mature rats, the minimum SS necessary to induce tachyarrhythmia, compared to control condition, decreased in the presence of carbachol and it was the same in the presence of atropine. Therefore, an mAChR antagonist (atropine) prevented atrial tachyarrhythmia induction at all rat age ranges except in mature rats, whereas carbachol facilitated atrial tachyarrhythmia induction at all ages.

The blockade of cardiac mAChR by atropine increased the NT necessary for attempts at tachyarrhythmia induction, but not the minimum SS necessary for tachyarrhythmia induction, at all ages studied except in mature rats. Atropine was also able to prevent the

induction of tachyarrhythmia and to reduce AR (atrial frequency) after tachyarrhythmia at all ages studied except in mature rats. These results suggest that stimulation of mAChR facilitates induction of atrial tachyarrhythmia in infant, young and adult rats. The increment of reentrant circuit formation is a plausible mechanism that would, at least in part, be related to this facilitation. However, regardless of the mechanism of this arrhythmogenic facilitation, the results indicate that it is altered in mature rats.

Some previous results could partially explain the age-related decrease of carbachol and atropine effects observed in the present work. For example, Chevalier et al. (1991), Dhein et al. (2001) and Brodde et al. (1998) showed that the density of cardiac mAChR in rats and humans decrease with age. In addition, Lo et al. (2001) and Brodde and Leineweber (2004) showed that expression of mRNA encoding mAChR in rat and human hearts was lower in older than in younger subjects. Therefore, it seems reasonable to conclude that the decreased effects of carbachol and atropine on EFS-induced atrial tachyarrhythmia in mature rats observed in the present work could be related to a reduction of function and/or number of cardiac mAChR with the animal's age.

In summary, the results obtained in the present work indicate that electrically induced atrial tachyarrhythmia is influenced by age-related changes in cholinergic mechanisms. These changes could be related to a reduction in the function and/or number of mAChR, as well as a reduction in endogenous ACh release (Oberhauser et al. 2001). In humans, these age-related alterations of cholinergic mechanisms could dramatically interfere with the diagnosis and treatment of cardiac dysfunction, e.g. interfering with the dobutamine-stress echocardiography used to evaluate autonomic function in transplanted hearts, and affecting treatment of sinus bradycardia in patients with excessive vagal tonus. This work therefore raises a word of caution, and suggests that the suitability of a given treatment for cardiac dysfunction involving interventions affecting cholinergic mechanisms could differ with patient age.

Conclusion

In conclusion, results obtained with isolated rat atria suggest that the electrical inducibility and the cholin-

ergic modulation of atrial tachyarrhythmia are greatly influenced by the animal's age.

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