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Effect of tedisamil on cell communication, impulse propagation, and excitability of the failing heart

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Abstract

In the present work, the effect of tedisamil on gap junctional conductance (gj) and conduction velocity was investigated in the failing heart of cardiomyopathic hamsters (TO-2 strain). It was found that tedisamil (10^{-7} M) increased gj by $53.8 \pm 1\%$ ($n = 23$) in cell pairs isolated from 2 months old cardiomyopathic hamsters. The effect of tedisamil was suppressed by intracellular dialysis of an inhibitor of protein kinase A and also by adenosine indicating that the drug increases gj through the activation of adenylcyclase. Tedisamil also increased the conduction velocity and cardiac refractoriness of ventricular muscle from young cardiomyopathic hamsters. At an advanced stage of the disease, however, when the β-adrenoceptor, adenylcyclase signaling system is impaired, tedisamil was unable to increase gj. The present results indicate that the antiarrhythmic action of tedisamil is in part related to an increase in junctional conductance and conduction velocity.

Keywords: Tedisamil; Junctional conductance; Failing heart

1. Introduction

Tedisamil is a new Class III bradycardic agent which blocks the transient and delayed rectifier potassium currents (Dukes et al., 1990), prolongs the action potential duration and the Q–T interval in cardiac muscle (Oexle et al., 1987; Beatch et al., 1991) and has antifibrillatory properties (Adaikan et al., 1992). Indeed, tedisamil seems to inhibit reentry circuits involved in the generation of ventricular fibrillation including that produced by myocardial ischemia (see Adaikan et al., 1992).

Sotalol, another of Class III antiarrhythmic agent has a β-adrenergic blocking action and prevents dye uncoupling produced in myocardial fibers by hypoxia (Miyachi et al., 1995). Since this effect of sotalol is suppressed by an inhibitor of protein kinase A, the conclusion was that sotalol increases cyclic AMP, probably by activating adenyl cyclase, with consequent decrease of the intracellular free Ca concentration (Miyachi et al., 1995; Manoach et al., 1997).

The evidence gathered thus far strongly supports the conclusion that heart cell communication is increased by cAMP (De Mello, 1984, 1994). Indeed, the gap junctional (gj) conductance is known to be increased by isoproterenol (Burt and Spray, 1988; De Mello, 1988), and by phosphodiesterase inhibitors (De Mello, 1989) an effect abolished by intracellular dialysis of an inhibitor of protein kinase A (De Mello, 1988). Moreover, the cell-to-cell diffusion of Lucifer Yellow CH in cardiac fibers is incremented by isoproterenol (see De Mello and Van Loon, 1987). The increase in junctional communication elicited by activation of the cAMP cascade is due to phosphorylation of junctional proteins (De Mello, 1983, 1991; Saez et al., 1986).

The implication of these findings is that the activation of the sympathetic nervous system with consequent increase of cyclic AMP leads to an increase in conduction velocity thereby enhancing the electrical synchronization.

In the present work, the influence of tedisamil on cell coupling was investigated in the failing heart of cardiomyopathic hamsters which represent a good model of cardiomyopathy and heart failure in humans (see Weismand and Weinfeldt, 1987). Indeed, ventricular hypertrophy followed by progressive cardiac dilatation and death by congestive heart failure is the usual finding in these cardiomy-

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opathic animals. The relevance of the present study is related to the fact that a possible increase in electrical coupling caused by tedisamil might enhance the conduction velocity and avoid reentry and cardiac arrhythmias which are a cause of sudden death in patients with heart failure.

2. Methods

Male cardiomyopathic Syrian hamsters (2 and 11 months old) (TO-2 strain) (Biobreeders, Fitchburg, MA) and healthy male (F1B strain) control hamsters of the same age were used. Both the control and cardiomyopathic animals were kept in air-conditioned facilities at the animal house on a normal laboratory animal diet and tap water ad libitum. The animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.), and the heart was removed under deep anesthesia.

2.1. Measurements of junctional conductance

Cell pairs were obtained by enzymatic dispersion of hamster ventricle following the methods of Powell and Twist (1976) and Tanigushi et al. (1981). The animal was anesthetized and the heart was removed and immediately perfused with normal Krebs' solution containing (mM): NaCl 136.5, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, NaH_2PO_4 0.3, $NaHCO_3$ 11.9, glucose 11, HEPES 5, with pH adjusted to 7.3. After 20 min, a Ca^{2+}-free solution containing collagenase (0.4%) (Worthington Biochemical) was recirculated through the heart for 1 h. The collagenase solution was washed out with 100 ml of recovery solution containing (mM): taurine 10, oxalic acid 10, glutamic acid 70, KCl 25, KH_2PO_4 10, glucose 11 and EGTA 0.5 with pH adjusted to 7.3. All solutions were oxygenated with 100% O_2.

The ventricles were minced (1 to 2 mm thick slices) and the resulting solution was agitated gently with a Pasteur pipette. The suspension was filtered through nylon gauge and the filtrate centrifuged for 4 min at $22 \times g$, and the cell pellets were then resuspended in normal Krebs' solution. All experiments were made at 36°C.

Suction pipettes were pulled from microhematocrit tubing (Clark Electromedial Instruments) by means of a controlled puller (Narishige) and their tips were polished with a microforge (Narishige). The pipettes, which were prepared immediately before the experiment, were filled with the following solution (mM): KCl 125, NaCl 10, $MgCl_2$ 3, Na_2-ATP 5, EGTA 10 and HEPES 5, with pH adjusted to 7.3. The resistance of the filled pipettes varied from 2.5 to 3 MΩ.

2.1.1. Drugs

Tedisamil was kindly provided by Solvay Pharmaceuticals, Germany. Propranolol, the inhibitor of protein kinase A (Walsh inhibitor) and adenosine were from Sigma, St. Louis, MO.

2.1.2. Experimental procedures

All the experiments were performed in a small chamber mounted on the stage of an inverted phase-contrast microscope (Diaphot, Nikon). The junctional resistance was determined in cell pairs with the use of two separate voltage-clamp amplifiers. Gigaohm sealing was achieved in each cell and then the surface cell membrane of both cells was broken by application of a stronger suction (−30 to −65 cm H_2O) and a whole-cell clamp configuration was produced. Each pipette was connected to a separated voltage-clamp amplifier (Dagan) that made possible the control of the nonjunctional membrane potential in each cell as well as the voltage gradient across the intercellular junction.

The experimental procedure consisted of holding the membrane potential of both cells at −40 mV. Cell 1 was then pulsed to 0 mV while the membrane potential of cell 2 was maintained unchanged. A voltage was created across the junctional membrane (V_1), and a compensating current of opposite polarity recorded from pipette 2 (I_2) represents the current flowing through the gap junction. As I_2 equals V_1/rj, the junctional resistance (rj) or conductance (gj) can be easily estimated. Data acquisition and command potentials were controlled with PCLAMP software (Axon Instruments, CA).

Series resistances (Rs_1 and Rs_2) originated from the tips of the micropipettes were compensated electronically before the experiment and checked periodically during the experiment. When necessary, the gj was corrected taking into consideration the changes in series resistance. For this, the following equation (see Giaume, 1991) was used:

$$gj = \Delta_2/\Delta I_1 - (Rs_1\Delta_1 - Rs_2\Delta_2)$$

The change in patch electrode solution was made using an electrode similar to that described by Irisawa and Kokubun (1983). During these experiments, measurements of gj were made under control conditions and then the protein kinase A inhibitor was added to the pipette solution and the compound was dialysed into the cell.

Voltage and current signals were displayed simultaneously on an oscilloscope (Tektronix 5113) and chart recorder (Gould 2400).

2.2. Measurement of conduction velocity and refractoriness

Twenty-five 11-month old adult male cardiomyopathic hamsters (TO-2) (110 to 115 g) from Biobreeders, Fitchburg, MA, were anesthetized with sodium pentobarbital (50 mg/kg) intraperitoneally, and the hearts were immediately removed and immersed in oxygenated Krebs' solution (36°C). The wall of the right ventricle was dissected

and transferred to a bath, through which normal Krebs' solution flowed continuously.

The composition of the Krebs' solution was as follows (mM): NaCl 150, KCl 5.1, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5 and glucose 11 (pH 7.3). This solution was saturated with 100% oxygen. Three M KCl glass microelectrodes used to record transmembrane potentials, were connected to a high impedance direct DC amplifier (WP Instruments, New Haven, CT). The cardiac muscle was stimulated with a fine platinum electrode by using rectangular current pulses generated by a Grass stimulator and isolation unit (Grass, Boston). The intensity of the current pulses was twice the threshold and the pulse duration was 1 ms. The stimuli were delivered to the muscle through a pair of platinum electrodes (0.3 mm in diameter) that touched the preparation at the right end. A bipolar stimulation was used and the rate of stimulation was 0.6 Hz.

After 25 to 30 min of equilibration in Krebs' solution, the membrane potential was recorded from superficial endocardial fibers located near the center of the muscle. The micropipette was kept inside the fiber during the experiments but when the impalement was not maintained the same fiber was reimpaled. If the resting potential was reduced during the reimpalement, a nearby fiber was impaled.

The conduction velocity was measured with two microelectrodes impaled at a fixed distance (about 10 mm) apart. During these measurements, the microelectrodes were maintained inside the fibers under control and experimental conditions. Since the measurements of conduction velocity were made in the ventricular wall, the values found are approximations of the real values. Measurements of the action potential duration were at 50% and 90% of action potential amplitude.

The cardiac refractoriness was investigated using strength–interval curves which were obtained by applying a second pulse of variable intensity but constant duration (1 ms) at different moments of the action potential. For each interval, the minimal current strength needed to elicit a propagated response was determined. The interval was previously selected and kept constant during the experiments, except when the increase in the duration of the action potential elicited by the drugs required a selection of a new set of intervals. The strength of the effective current was plotted against the interval in such a way that strength–interval curves were obtained for control and experimental conditions in the same fiber (about 95% of the cases) or in a nearby fiber.

The stimulus strength was recorded by amplifying the voltage drop across a 10 MΩ resistor placed between the

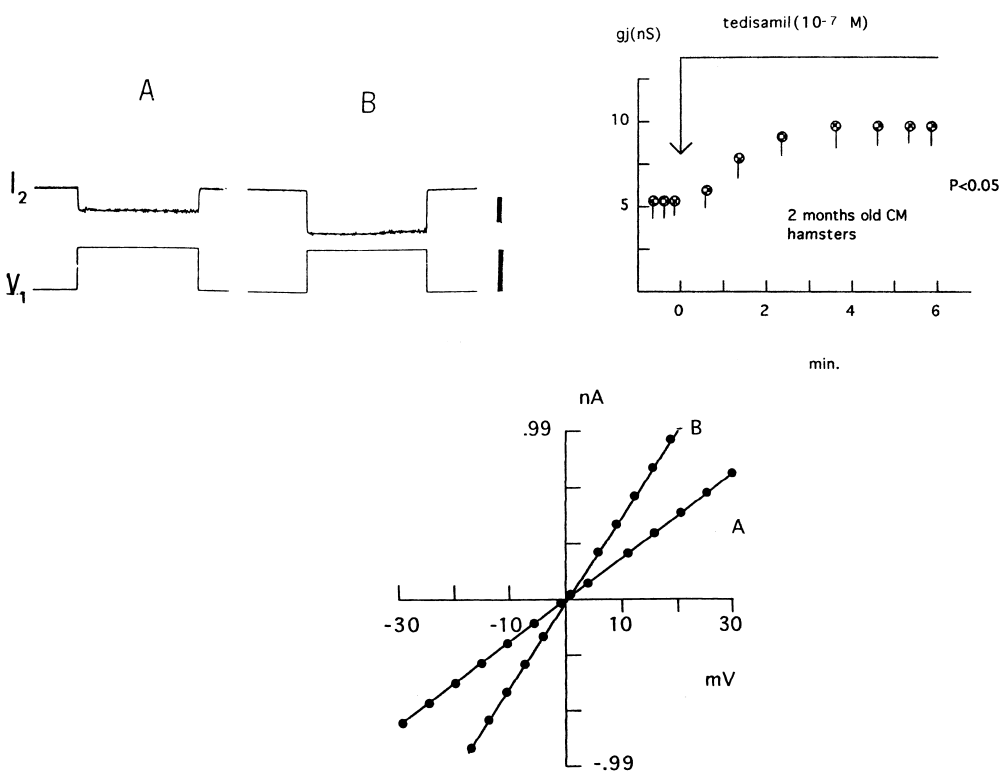

Fig. 1. Top Left: Effect of tedisamil (10^{-7} M) on gj recorded from a single cell pair isolated from a 2-month old cardiomyophatic hamster. V_1—transjunctional voltage with a calibration bar of 40 mV. I_2—junctional current with a calibration bar of 1 nA. (A) Control; (B) 4 min after the administration of tedisamil (10^{-7} M) to the bath. Top right: average change in gj elicited by tedisamil in 23 preparations. Bottom: current–voltage relationship of junctional membrane showing (A) control and (B) after 4 min of tedisamil (10^{-7} M) administration.

muscle and the ground and displaying the deflections in an oscilloscope. Voltage calibration was accomplished by injecting known voltages between the solution and the ground. Changes in membrane potential and current were displayed on an oscilloscope and photographed.

2.3. Statistical analysis

Data are presented as mean ± S.E.M. The Student's *t*-test was used to determine statistical significance, defined as $P < 0.05$.

3. Results

3.1. Effect of tedisamil on junctional conductance (gj)

To investigate the effect of tedisamil on cell communication measurements of junctional conductance (gj) were made in cell pairs isolated from the ventricle of 2 months old cardiomyopathic hamsters (TO-2). Recently, an evaluation of the electrophysiologic characteristics of 2 months old cardiomyopathic ventricle indicated that the values of junctional conductance determined in several cell pairs are distributed in two large groups: one with low gj ranging from 5 to 9 nS and the other within the range of 20–23 nS whereas in the controls the range was 40–100 nS (De Mello, unpublished; see also De Mello, 1996b). These findings are indicative that the changes in junctional conductance are already evident at this stage of the disease.

To investigate the effect of tedisamil on cell communication measurements of the junctional conductance were initially measured under control conditions and then tedisamil was added to the bath and gj was carefully monitored. As shown in Fig. 1 tedisamil (10^{-7} M) increased gj by 53.8 ± 1% ($n = 23$) ($P < 0.05$) within 4 min. Studies of the voltage–current relationship indicated that tedisamil increased gj for different values of transjunctional voltage (Fig. 1). The effect tedisamil on gj which was dose dependent (see Fig. 2) was also found in control animals of the same age. In these animals tedisamil (10^{-7} M) increased gj by 55 ± 4.6% ($n = 23$) within 4 min.

The question whether tedisamil changes gj at late stage of the disease was also investigated. Comparative studies performed in 11 months old cardiomyopathic hamsters

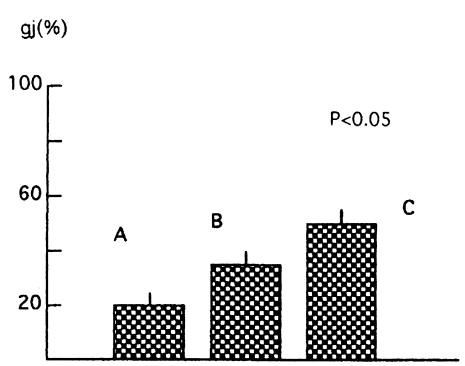

Fig. 2. Dose–effect relationship of the effect of tedisamil of junctional conductance of 2 months old cardiomyopathic hamsters. (A) 10^{-9} M, (B) 10^{-8} M, and (C) 10^{-7} M of tedisamil. Each bar is the average of eight experiments. Vertical line at each bar, S.E.M.

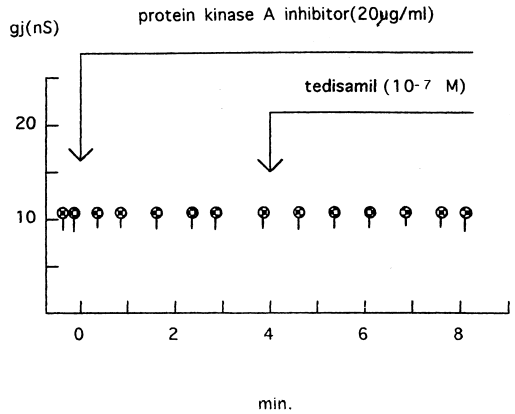

Fig. 3. Suppression of the effect of tedisamil (10^{-7} M) on gj of 2 months old cardiomyopathic hamsters caused by previous intracellular administration of an inhibitor of cyclic AMP dependent protein kinase (20 μg/ml). (A) Control in the absence of the protein kinase inhibitor; (B) 4 min after intracellular dialysis of the PKA inhibitor tedisamil was added to the bath. Vertical line S.E.M. Each point is the average of 23 experiments.

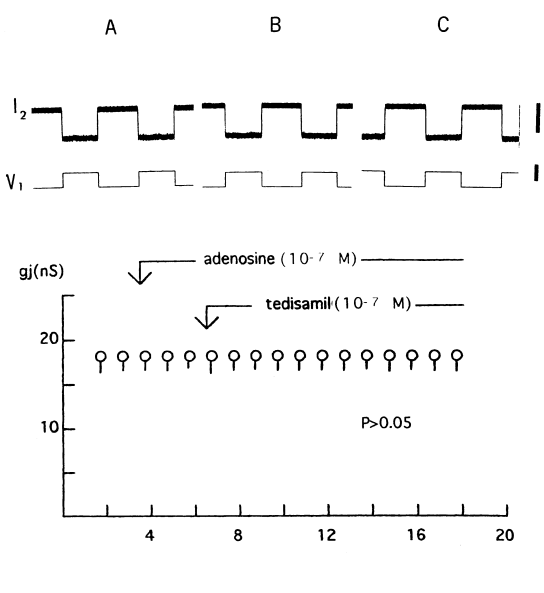

Fig. 4. Top: Lack of action of tedisamil (10^{-7} M) on gj of single cell pain isolated from 2 months old cardiomyopathic hamster previously exposed to adenosine (10^{-7} M) for 4 min. (A) Control; (B) 4 min after administration of adenosine to the bath; (C) 4 min after administration of tedisamil (10^{-7} M) to the medium containing adenosine. I_2 and V_1: same as in Fig. 1. Calibration bar for I_2: 0.2 A and for V_1: 40 mV. Bottom: suppression of the effect of tedisamil (10^{-7} M) of gj of 2 months old cardiomyopathic hamsters elicited by previous exposure of the cells to adenosine (10^{-7} M). Each point is the average of seven experiments. Vertical line at each point S.E.M.

Table 1
Conduction velocity (cm/s) measured in ventricular muscle of 2 months old cardiomyopathic hamsters

Young CM hamsters without the drug	Increment in conduction velocity caused by tedisamil		
	10^{-7} M	10^{-6} M	10^{-5} M
49.3 ± 1.4 ($n = 8$)	32.4 ± 2.2[a] ($n = 10$)	41.3 ± 2.4[b] ($n = 7$)	49.8 ± 3.1[c] ($n = 6$)

[a,b,c] $P < 0.05$.

indicated that tedisamil (10^{-7} M) was unable to increase gj as seen in young cardiomyopathic animals. On the contrary, in some cell pairs a decline in gj was found (not shown).

The major question is how tedisamil enhances gj in young cardiomyopathic animals. A reasonable hypothesis is that the compound activates the cAMP cascade with consequent phosphorylation of junctional proteins and increase in gj. To investigate this possibility we started by blocking the β-adrenoceptors with propranolol (10^{-6} M) and then studying the effect of tedisamil (10^{-7} M) on gj under these conditions. The results from six cell pairs indicated that propranolol did not alter the effect of tedisamil on gj (not shown).

To investigate further the role of cAMP cascade on the effect of tedisamil gj was measured and then an inhibitor of protein kinase A (20 μg/ml) (Walsh inhibitor) was added to the pipette solution and the compound was dialysed into the cell. After 5 min of intracellular administration of the kinase inhibitor tedisamil (10^{-7} M) was added to the bath and gj was measured. As seen in Fig. 3 the inhibitor by itself did not change gj significantly but the effect of tedisamil on gj was suppressed by the inhibitor.

In other experiments adenosine (10^{-7} M), an inhibitor of adenylcyclase (Limbird, 1988), was added to the bath and gj was measured in cell pairs isolated from 2 months old cardiomyopathic hamsters. After 4 min of adenosine administration tedisamil (10^{-7} M) was added to the extracellular fluid containing adenosine and gj was monitored again. Measurements of gj made therefore and after adenosine administration shown that adenosine by itself did not alter gj but it abolished the effect of tedisamil on gj (Fig. 4).

Table 2
Effect of tedisamil (10^{-5} M) on action potential duration of ventricular fibers from 2 months old CM hamsters

Action potential duration (ms)			
50% Repolarization		90% Repolarization	
Control	Tedisamil	Control	Tedisamil
33.8 ± 2.7 ($n = 13$) $P < 0.05$	40.5 ± 2.6 ($n = 13$)	78 ± 1.9 ($n = 13$) $P < 0.05$	92 ± 2.2 ($n = 13$)

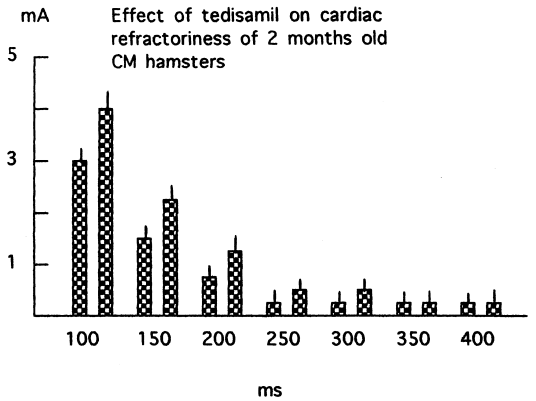

Fig. 5. Effect of tedisamil (10^{-7} M) on cardiac refractoriness of ventricular muscles of 2 months old cardiomyopathic hamsters. For each interval (in ms) the left bar is the control and the right bar represents the effect of tedisamil. Each bar is the average of 10 experiments. Vertical line at each bar, S.E.M.

3.2. Effect of tedisamil on conduction velocity and refractoriness of the failing heart

Electrophysiological studies performed on intact and isolated ventricular muscle of 2 months old cardiomyopathic hamsters showed that tedisamil at the concentration range of 10^{-7} to 10^{-5} M increased the conduction velocity by 32.4 ± 2.2 cm/s ($n = 10$) and 49.8 ± 3.1 cm/s ($n = 6$), respectively, after 10 min of tedisamil administration (see Table 1). The action potential duration measured at 50% and 90% of action potential repolarization was also significantly increased by tedisamil (10^{-5} M) as shown in Table 2 confirming other studies made on adult rats (Beatch et al., 1991).

Because it is not known whether tedisamil increases the refractory period in the failing heart the influence of tedisamil on cardiac refractoriness was investigated. For this, strength–interval relationship was obtained from young cardiomyopathic hamsters ventricular muscle before and after administration of the drug to the bath. As shown in Fig. 5 (see De Mello et al., 1997) tedisamil (10^{-7} M) displaced the curve to the right indicating an increase in refractoriness. Indeed, stronger stimuli were required to elicit propagated responses at different intervals in muscles exposed to tedisamil ($P < 0.05$) (Fig. 5). Similar results were obtained with normal controls exposed to tedisamil (10^{-7} M). In these experiments, the minimal current needed to elicit a propagated response was increased by $31 \pm 3.5\%$ for the intervals of 100 ms and by $28.6 \pm 2.8\%$ for the interval of 150 ms whereas for smaller intervals the required increment in current was of smaller magnitude (not shown).

4. Discussion

The present results indicate that tedisamil enhances cell communication and impulse propagation in the failing heart at an early stage of the disease. Indeed, the conduc-

tion velocity was appreciably increased in the right ventricular wall of young cardiomyopathic hamsters, an effect that is in part explained by the increment in junctional conductance.

Since propranolol did not influence the effect of tedisamil on junctional conductance it is possible to conclude that the mechanism by which tedisamil increases gj is related to the activation of cAMP cascade at a step beyond the β-adrenoceptors. This notion is supported by the following findings: (a) an inhibitor of protein kinase A dialysed into cell abolished the effect of tedisamil; (b) adenosine—an inhibitor of adenylcyclase, by itself had no effect on gj but suppressed the effect of tedisamil; (c) tedisamil did not increase gj in cardiomyopathic animals at a late stage of the disease when it is known there is a defect of the β-adrenoceptor-G protein-adenylcyclase signaling system (Feldmann et al., 1990). Indeed, evidence has been provided that at late stage of heart failure forskolin is not able to increase gj as usually seen in the controls of same age (De Mello, 1996a).

It is important to add that despite the apparent activation of adenylcyclase tedisamil did not induce arrhythmias in normal controls or in young cardiomyopathic hamsters. Indeed, in experiments with isolated cell pairs or with isolated ventricle exposed to tedisamil no evidence of spontaneous rhythmicity was found. This finding confirms previous observations which indicated that tedisamil is not arrhythmogenic in normal rat heart (Beatch et al., 1991).

In conclusion, the present results indicate that tedisamil increases cell coupling in the failing heart at early stage of the disease when the β-adrenoceptor-G protein-adenylcyclase signaling systems is still preserved. The effect of tedisamil on cell communication leads to an increment in conduction velocity thereby preventing the establishment of reentry circuits and the consequent generation of cardiac arrhythmias. These effects of tedisamil as well as the increase in cardiac refractoriness elicited by the drug might protect the failing heart against arrhythmias.

Considering that sudden death of patients with congestive heart failure is commonly related to the generation of malignant ventricular arrhythmias the use of tedisamil might represent an important additament to the therapeutic scheme.

References

Adaikan, G., Beatch, G.N., Lee, T.L., Ratnam, S.S., Walker, M.J.A., 1992. Antiarrhythmic actions of tedisamil: studies in rats and primates. Cardiovasc. Drug Ther. 6, 345–352.

Beatch, G.N., Abraham, S., MacLeod, B.A., Yoshida, N.R., Walker, M.J.A., 1991. Antiarrhythmic properties of tedisamil (KC 8857), a putative transient outward K^+ current blocker. Br. J. Pharmacol. 102, 13–18.

Burt, J., Spray, D.C., 1988. Inotropic agents modulate gap junctional conductance between cardiac myocytes. Am. J. Physiol. 254, H1206–H1213.

De Mello, W.C., 1983. The roles of cAMP and Ca on the modulation of junctional conductance: an integrated hypothesis. Cell Biol. Int. Rep. 7, 1033–1040.

De Mello, W.C., 1984. Effect of intracellular injection of cAMP on the electrical coupling of mammalian heart cells. Biochem. Biophys. Res. Commun. 119, 1001–1007.

De Mello, W.C., 1988. Increase in junctional conductance caused by isoproterenol in heart cell pairs is suppressed by cAMP-dependent protein kinase inhibitor. Biochem. Biophys. Res. Commun. 154, 509–514.

De Mello, W.C., 1989. Effect of isoproterenol and 3-isobutyl-1-methylxanthine on junctional conductance in heart cell pairs. Biochem. Biophys. Acta 1012, 291–298.

De Mello, W.C., 1991. Further studies on the influence of cAMP-dependent protein kinase on Junctional conductance in isolated heart cell pairs. J. Mol. Cell. Cardiol. 23, 371–379.

De Mello, W.C., 1994. Gap junctional communication in excitable tissues: the heart as a paradigm. Prog. Biophys. Mol. Biol. 61, 1–35.

De Mello, W.C., 1996a. Impaired regulation of cell communication by β-adrenergic receptor activation in the failing heart. Hypertension 27, 265–268.

De Mello, W.C., 1996b. Renin–angiotensin system and cell communication in the failing heart. Hypertension 27, 1267–1272.

De Mello, W.C., Van Loon, P., 1987. Further studies on the influence of cyclic nucleotides on junctional permeability in heart. J. Mol. Cell. Cardiol. 19, 763–771.

De Mello, W.C., Cherry, R.C., Manivannan, S.J., 1997. Electrophysiologic and morphologic abnormalities in the failing heart: effect of enalapril on the electrical properties. J. Cardiac Fail. 3, 53–61.

Dukes, Z.D., Cleeman, L., Morad, M., 1990. Tedisamil blocks the transient and delayed K^+ currents in mammalian cardiac and glial cells. J. Pharmacol. Exp. Ther. 254, 560–569.

Feldmann, A.M., Rowena, G.T., Kessler, P.D., Weisman, H., Schulman, S.P., Blumenthal, R.S., Jackson, K.D.G., Van Dop, C., 1990. Diminished β-adrenergic receptor responsiveness and cardiac dilation in heart of my apathic syrian hamsters are associated with functional abnormality of G stimulations protein. Circulation 81, 1341–1352.

Giaume, C., 1991. Application of the patch-clamp technique to the study of junctional conductance. In: Peracchia, C. (Ed.), Biophysics of Gap Junction Channel. CRC Press, Boca Raton, FL, pp. 175–188.

Irisawa, H., Kokubun, S., 1983. Modulation by intracellular ATP and cyclic AMP of the slow inward current in isolated single ventricular cells of the guinea-pig. J. Physiol. (London) 338, 321–337.

Limbird, L.L., 1988. Receptors linked to inhibition of adenylate cyclase: additional signaling mechanisms. FASEB J. 2, 2686–2695.

Manoach, M., Uchiyama, H., Miyachi, E., Thormahlen, D., 1997. Tedisamil enhances intercellular coupling: a new explanation of its antiarrhythmic defibrillating effects. Exp. Cardiol. 2, 47–50.

Miyachi, E., Manoach, M., Uchiyama, H., Watanabe, Y., 1995. Is cAMP involved in the defibrillating effect of sotalol?. Life Sci. 57, PL393–399.

Oexle, B., Weirich, J., Antoni, H., 1987. Electrophysiology profile of KC 8857: a new bradycardic agent. J. Mol. Cell. Cardiol. 19, S65, Suppl. III.

Powell, T., Twist, T., 1976. A rapid technique for the isolation and purification of adult cardiac muscle cells having respiratory control and a tolerance to calcium. Biochem. Biophys. Res. Commun. 72, 327–333.

Saez, J.C., Spray, D.C., Nairn, A.C., Herzberg, E., Greengard, P., Bennett, M.V.L., 1986. cAMP increases junctional conductance and stimulates phosphorylation of the 27-kDa principal gap junction polypeptide. Proc. Natl. Acad. Sci. USA 83, 2473–2477.

Tanigushi, Y., Kokubun, S., Noma, A., Irisawa, H., 1981. Spontaneously active cells isolated from the sinoatrial and atrioventricular node of the rabbit heart. Jpn. J. Physiol. 31, 547–558.

Weismand, H.F., Weinfeldt, M.L., 1987. Toward an understanding of the molecular basis of cardiomyopathies. Am. Cell. Cardiol. 10, 1135–1138.