The Making of Diabetic Guinea Pigs by Streptozotocin and High Incidence of Triggered Activity in the Ventricular Muscle

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Abstract Myoplasmic Ca^{2+} metabolism is reported to be impaired in diabetic rat heart. We studied the possibility that the ventricular muscles of diabetic guinea pig are prone to develop delayed afterdepolarizations (DADs) and triggered activity (TA), because DADs and TA are believed to be a possible index of increased level of intracellular Ca^{2+} concentration. To establish an experimental diabetic model from the guinea pig, male animals were divided into four groups: 1) control group: intracardiac injection of citrate buffer; 2) IP group: intraperitoneal injection of streptozotocin (STZ, 200 mg/kg); 3) IC group: intracardiac injection of STZ; and 4) Ins-IC group: intracardiac injection of STZ after pretreatment with insulin (20 IU/kg). We found that: 1) only in the Ins-IC group was the fasting plasma glucose concentration (determined 40 days after STZ injection) significantly higher than in the control group; 2) oral glucose tolerance test performed 40 days after treatment also showed glucose intolerance in the Ins-IC group. These findings evinced the successful making of diabetic guinea pigs by an intracardiac one-shot injection of STZ during development of insulin-induced hypoglycemia. *In vitro* electrophysiological experiments were performed on ventricular papillary muscle from diabetic animals (Ins-IC group) by conventional glass microelectrode techniques. Transmembrane action potentials were elicited by pulse trains with various rates (2–5 Hz) and durations (10–30 stimuli) in the presence of ouabain ($1\ \mu\text{M}$) and various Ca^{2+} concentrations (1.8–7.2 mM). The incidence of TA in the muscles from diabetic animals was significantly higher (χ^2 -test, $p < 0.05$) than that from controls. The findings gave evidence that Ca^{2+} homeostasis in the myocardium of diabetic guinea pigs is impaired, and this may be a cause of arrhythmia.

Key words: diabetic guinea pig, streptozotocin, delayed afterdepolarization, triggered activity.

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Acute myocardial infarction is a major complication of diabetes which carries a high mortality (WATSON and WALDRON, 1987). The high incidence of mortality in patients with diabetes has been attributed to coronary atherosclerosis (PARTAMIAN and BRADLEY, 1965; RUBLER *et al.*, 1972; HAMBY *et al.*, 1974; KANNEL *et al.*, 1974). There is a growing body of evidence to suggest that diabetes mellitus induces myocardial dysfunction, including cardiomyopathy (BLUMENTHAL *et al.*, 1960; RUBLER *et al.*, 1972; FACTOR and SONNENBLICK, 1982). However, the mechanism underlying the functional disturbance of the hearts of diabetic patients is still uncertain.

A series of *in vitro* studies (FEIN *et al.*, 1980, 1981, 1983; PENPARGKUL *et al.*, 1981), using streptozotocin (STZ)-induced diabetic rat hearts, showed that the contractile force is significantly depressed. However, there is little information available regarding the electrophysiological properties of diabetic cardiac tissue. FEIN *et al.* (1983) reported that the action potential duration (APD) in diabetic ventricular muscle was longer than that in normal muscle, and was susceptible to the effects of ouabain and altered external Ca^{2+} concentration. NORDIN *et al.* (1985) reported that the ventricular muscle from diabetic rats was prone to develop delayed afterdepolarizations (DADs) and triggered activity (TA) in the presence of increased Ca^{2+} concentration, suggesting that myocardium from the diabetic rat has some abnormality in its intracellular Ca^{2+} homeostasis.

To the best of our knowledge, there is no report of electrophysiological change of the cardiac muscles from diabetic guinea pigs. In the present study, we will first show success in making a diabetic model of guinea pig, by a one-shot injection of STZ. The ventricular muscles from diabetic guinea pigs were more likely to develop DADs and TA, than the ones from normal guinea pigs. Some results have been reported in abstract form (NOBE *et al.*, 1988, 1989).

MATERIALS AND METHODS

A. Preparation of diabetic guinea pig model. Experimental animals: All procedures met the guiding principles set by the Physiological Society of Japan on the care and use of animals in the field of physiological sciences. Male guinea pigs weighing 270–380 g were housed in self-flushing stainless steel cages in our animal facility, maintained at $24 \pm 2^\circ\text{C}$ with humidity at $60 \pm 10\%$, and with a 12-h light/dark cycle. The animals were given free access to food (RC-4, Oriental Yeast Co., Japan) and water, before and after the streptozotocin treatment (STZ, Sigma Chemicals Co., U.S.A.) except as described.

After 24 h of fasting, the animals were divided into four groups: 1) control group ($n=9$), treated with a single intracardiac injection of 0.05 M citrate buffer (1.2 ml/kg, pH 4.5), a vehicle of STZ; 2) IP group ($n=5$), treated with a single intraperitoneal injection of STZ (200 mg/kg); 3) IC group ($n=10$), treated with a single intracardiac injection of STZ (200 mg/kg); 4) Ins-IC group ($n=20$), first injected subcutaneously with 20 IU/kg of insulin (Actrapid, Novo Industry Co.,

Table 1. Body weight and concentration of plasma glucose at Days 0, 10, and 40 after injection of streptozotocin or buffer in control group, IP group, IC group, and Ins-IC group guinea pigs.

Group	Day 0			Day 10 (1st oral glucose tolerance test)				Day 40 (2nd oral glucose tolerance test)			
	Body weight (g)	Plasma glucose (mg/dl)	Body weight (g)	Plasma glucose (mg/dl)			Body weight (g)	Plasma glucose (mg/dl)			Body weight (g)
				0 h	1 h	2 h	3 h	0 h	1 h	2 h	3 h
1. Control group	322±12 (n=9)	114±2 (n=9)	386±11 (n=9)	119±3 (n=9)	331±13 (n=9)	321±29 (n=9)	161±27 (n=9)	114±6 (n=9)	291±34 (n=9)	230±48 (n=9)	149±19 (n=9)
2. IP group	323±5 (n=5)	109±3 (n=5)	327±12* (n=5)	119±3 (n=5)	371±53 (n=5)	321±44 (n=5)	194±50 (n=5)	119±3 (n=5)	321±10 (n=5)	311±39 (n=5)	169±15 (n=5)
3. IC group	334±4 (n=10)	120±2 (n=10)	330±8** (n=8)	122±8 (n=8)	412±37 (n=8)	374±33 (n=8)	274±50 (n=8)	118±7 (n=4)	300±30 (n=4)	328±18 (n=4)	290±39 (n=4)
4. Ins-IC group	310±5 (n=20)	118±2 (n=20)	308±18** (n=7)	128±5 (n=7)	354±27 (n=7)	377±37 (n=7)	248±42 (n=7)	143±6* (n=6)	361±46 (n=6)	440±17* (n=6)	318±34** (n=6)

Values expressed as mean±S.E.M.; number of surviving animals in parentheses. * $p < 0.05$ and ** $p < 0.01$, compared to controls measured on corresponding day.

Denmark). Within 1 h after this injection, hypoglycemic attack (muscle twitching and extreme weakness) occurred in all ($n=20$) of the animals. During the attack, STZ (200 mg/kg) was injected intracardially.

Just prior to injection of STZ or the vehicle, body weight and plasma glucose concentration were measured using a spring scale and a glucose analyzer (Model Fijichem 1000, Fuji Film Co., Japan), respectively. Blood samples were obtained by translumbar vena cava puncture described by WINSETT *et al.* (1985). Then either STZ or an equivalent volume of the vehicle (citrate buffer) was injected intraperitoneally or intracardially as mentioned (Day 0). All STZ solutions were prepared immediately before use. In all four groups, on the tenth (Day 10) and the fortieth (Day 40) days of treatment, body weight was measured after 24-h fasting, and the oral glucose tolerance test (OGTT) was performed. Glucose (2 g/kg as a 30% aqueous solution) was administered through a polyethylene catheter inserted to the stomach. Plasma glucose concentration (mg/dl) was determined at zero (immediately before the glucose loading), 1, 2, and 3 h after glucose loading, by the method described before.

Data analysis: Data are presented as mean $\pm$ S.E.M. The difference was analyzed by Student's *t*-test (for body weight in Table 1 and Fig. 1 and plasma glucose concentration in Table 1) and by χ^2 -test (for survival rate in Fig. 1).

B. Electrophysiological studies. Preparations: For the electrophysiological study, guinea pigs of both control and Ins-IC group were used. Animals were anesthetized with ethyl ether, and the hearts were removed quickly after thoracotomy

Fig. 1. Changes in survival rate (A) and body weight (B) after streptozotocin (STZ) or vehicle injection in each group. Open circles, control group; open triangles, IP group; open squares, IC group; closed circles, Ins-IC group. Data expressed as percentage of value at Day 0 in each group. * $p < 0.05$, * $p < 0.01$, compared to values of the control group measured at the same day.

and immersed in oxygenated Tyrode's solution (see below). The ventricular papillary muscles (3–5 mm long, 0.5–1.5 mm diameter) were excised from both ventricles and mounted in a 1.5 ml muscle bath, superfused with Tyrode's solution gassed with 95% O₂ and 5% CO₂. The composition of the control Tyrode's solution was as follows (in mM): NaCl, 137; KCl, 5.4; NaHCO₃, 11.9; MgSO₄, 1.05; NaH₂PO₄, 0.42; CaCl₂, 1.8; and glucose, 10. After oxygenation the pH of the solution was 7.4, and the bath temperature was kept constant at 36.5 ± 0.5°C.

Microelectrode recordings: Glass microelectrodes filled with 3 M KCl (10–30 MΩ) were used to record transmembrane potentials. The microelectrode was coupled to the input of a capacitance neutralizing preamplifier (Model MEZ 7107, Nihon Kohden Co., Japan). The first derivative of the transmembrane potential was recorded using an electronic differentiator with linearity over a range of 0–600 V/s. Both signals were displayed on an oscilloscope (Model VC-9, Nihon Kohden) and recorded using a long recording camera (Model RLG 6101, Nihon Kohden) and a chart recorder (Model RJG 4004, Nihon Kohden).

The preparations were electrically stimulated by isolated rectangular 1 ms pulses at 1 Hz through a stimulating electrode, a pair of silver wires placed 1–2 mm from the recording electrode. Electrical stimuli of 1.5 × threshold were supplied from the stimulator (Model SEN 3201, Nihon Kohden) via an isolater.

The preparations (driven at 1 Hz) were superfused with the oxygenated Tyrode's solution (1.8 mM Ca²⁺) and equilibrated for at least 1 h before starting the experiment. After equilibration, the characteristics of action potentials between control and diabetic muscles were compared (cf. Table 2).

For induction of delayed afterdepolarization (DAD) and triggered activity (TA), the preparations were superfused with high Ca²⁺ (3.6 or 7.2 mM) Tyrode's solution containing 1 μM ouabain. Exposure to these solutions with progressively higher levels of extracellular Ca²⁺ concentration ([Ca²⁺]_o) in the presence of ouabain produced myoplasmic Ca²⁺ overload (ALLEN *et al.*, 1984; NORDIN *et al.*, 1985). The other solutes in the solution were the same as in Tyrode's solution. The preparations were superfused with the following sequence: 1) Tyrode's solution containing ouabain (1 μM) alone, 2) Tyrode's solution containing ouabain (1 μM) with Ca²⁺ concentrations increased to 3.6 mM, and 3) to 7.2 mM. The preparations were superfused with each solution for 20 min before recording. The following stimulation protocol was used to produce DAD and TA during superfusion with each solution. The preparations were first driven at the basic stimulation rate of 0.5 Hz and then at increasingly higher frequencies of 2, 2.5, 3.3, and 5 Hz, each in trains of 10, 20, and 30 pulses. After each train, the evolution of DADs and TA was checked during a rest period of 15 s. After the 15-s rest period, basic stimulation (0.5 Hz) was repeated and followed by the next test train pulses. This sequence of stimulation was repeated to cover all stimulation frequencies and all pulse numbers during a train (cf. Figs. 2 and 3).

Data analysis: The data are expressed as mean ± S.E.M. and statistical comparisons were made using Student's *t*-test for unpaired data (for Tables 1 and

5 Hz, Train 30

Fig. 2. Induction of delayed afterdepolarization (DAD) and triggered activity (TA) in the ventricular muscle from STZ-treated diabetic guinea pigs (Ins-IC group) in various levels of $[Ca^{2+}]_o$ in the absence or presence of ouabain as indicated above the top row. Top and bottom traces show records from control and diabetic papillary muscles, respectively. The preparations first stimulated at 0.5 Hz for ten beats, then stimulated at 5 Hz for 30 beats, then rested for 15 s. In each record, the first two action potentials were the last two of 10 action potentials at 0.5 Hz. Only lower portions of action potentials are displayed. Note that diabetic muscle developed DADs and TA, but controls did not. See text for details.

Ca_o 7.2 mM, Ouabain 1 μM, 5 Hz

Fig. 3. Effects of the number of train stimuli on diastolic membrane potential in ventricular papillary muscle from control and STZ-treated diabetic (Ins-IC group) guinea pigs. Preparations superfused with Tyrode's solution containing 7.2 mM $[Ca^{2+}]_o$ and 1 μM ouabain. Top and bottom traces: records from control and diabetic hearts, respectively. Only lower portions of action potentials are displayed. Number of stimuli per train increased from left to right (10, 20, and 30) as noted. Note that DADs and TA occurred only in the preparation from the diabetic animal.

Table 2. Comparison of action potential characteristics of ventricular papillary muscles from control and streptozotocin-diabetic guinea pigs.

Condition	<i>n</i>	RMP	APA	OS	$\dot{V}_{\max}$	APD ₂₅	APD ₉₀
Control	6	-91 ± 4	128 ± 4	37 ± 2	202 ± 50	104 ± 11	171 ± 8
Diabetic	5	-90 ± 5	125 ± 7	35 ± 2	215 ± 36	88 ± 16	155 ± 24

Stimulation frequency, 1 Hz, *n*, number of the preparations; RMP, resting membrane potential (mV); APA, action potential amplitude (mV); OS, overshoot (mV); $\dot{V}_{\max}$, maximum upstroke velocity of action potential (V/s); APD₂₅ and APD₉₀, action potential duration at 25 and 90% repolarization, respectively (ms).

Table 3. Frequency of occurrence of delayed afterdepolarizations and triggered electrical activity in diabetic and control guinea pig papillary muscles.

Conditions	[Ca ²⁺] _o (mM) Ouabain (μM)	Number of preparations/total number studied		
		1.8 0	1.8 1	3.6–7.2 1
Delayed afterdepolarizations and/or triggered activity				
Control		0/4 (0)	3/3 (100)	9/11 (82)
Diabetic		0/5 (0)	3/4 (75)	12/14 (86)
Triggered activity				
Control		0/4 (0)	2/3 (67)	4/11 (36)
Diabetic		0/5 (0)	2/4 (50)	11/14* (79)

* $p < 0.05$, compared with values of the control group. Percent in parenthesis.

2) and χ^2 -test (for Table 3). A value of $p < 0.05$ was considered significant.

RESULTS

A. Induction of diabetic guinea pigs

Table 1 summarizes some characteristics of four groups of experimental animals. Survival rate of the guinea pigs after injection of STZ or the vehicle (citrate buffer) for 40 days is shown in Fig. 1A, and relative changes in the body weight are shown in Fig. 1B.

Streptozotocin lethality. As shown in Fig. 1A and Table 1, there were no deaths in either the control or the IP group for 40 days. However, the survival rates in the IC and Ins-IC groups were significantly low. In the IC group, 3 of 10 guinea pigs died within 24 h after STZ injection, and 1 animal died before the 2nd oral glucose tolerance test (OGTT). In the Ins-IC group, 13 of 20 animals died within 24 h of STZ treatment, and one died before the 2nd OGTT.

Changes in body weight. Figure 1B shows that treatment with STZ,

irrespective of the route of administration, significantly suppressed the increase of body weight, whereas the body weight in the control animals increased with time.

Breeding period-dependent changes in fasting plasma glucose concentration. In all four groups, the fasting plasma glucose concentration did not change significantly for 10 days (Day 10, Table 1) after the STZ treatment. However, after 40 days of STZ treatment (Day 40, Table 1), the glucose concentration of the Ins-IC group (143 ± 6 mg/dl) was significantly higher than that of the control group (114 ± 6 mg/dl), and the glucose concentration of IP and IC groups remained unchanged.

Plasma glucose concentration during OGTT. On Day 10 and Day 40, OGTT was performed on animals of all four groups. Plasma glucose concentrations before and 1, 2, and 3 h after glucose loading are summarized in Table 1. On Day 10, there was no significant change in glucose tolerance of guinea pigs in any of the four groups. On Day 40, however, plasma glucose concentrations 2 and 3 h after glucose loading were significantly elevated in Ins-IC group, compared to those in the control group. The plasma glucose concentrations of IP group and IC group during OGTT tended to increase; however, it did not reach statistical significance.

B. DAD and TA in diabetic heart muscles

Electrophysiological properties of diabetic ventricular papillary muscle. Table 2 summarizes the characteristics of action potentials from both control and diabetic (Ins-IC group) ventricular papillary muscles, stimulated at 1 Hz. The electrical activity was recorded under superfusion with control Tyrode's solution (1.8 mM Ca^{2+}). The parameters of action potentials obtained from diabetic animals were not significantly different from those of the controls.

Induction of DADs and TA. Figure 2 (bottom trace) illustrates a representative experiment in which increasing $[\text{Ca}^{2+}]_o$ from 1.8 to 3.6 or 7.2 mM in the presence of ouabain (1 μM) produced characteristic delayed afterdepolarizations (DADs) and triggered activity (TA) when the muscles were stimulated by a 5 Hz pulse train for 6 s (30 beats). The data show that increase of concentrations of intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) enhanced the occurrence of DAD and/or TA in preparations from diabetic guinea pigs, but not in the control preparations.

Figure 3 illustrates the effect of increasing the number of stimuli (at 5 Hz) during a train on DADs recorded from control and diabetic animals superfused with Tyrode's solution containing 7.2 mM $[\text{Ca}^{2+}]_o$ and 1 μM ouabain. After 10 successive stimuli, a small DAD developed in the diabetic preparation (arrow in Fig. 3). The amplitude of DAD increased and the time course became faster with increase in the number of stimuli. In Fig. 3 (far right panel, bottom), TA was recorded (after 30 successive stimuli) in a diabetic preparation that produced another DAD. In contrast, neither DAD nor TA was produced in the particular control muscle (Fig. 3, top row).

Table 3 summarizes the frequency of occurrence of DADs and TA in three different solutions (1.8 mM Ca^{2+} -Tyrode's solution, 1.8 mM Ca^{2+} -Tyrode's solution

containing $1\text{ }\mu\text{M}$ ouabain, and $3.6\text{--}7.2\text{ mM}$ Ca^{2+} -Tyrode's solution containing $1\text{ }\mu\text{M}$ ouabain). The data show that the frequency of occurrence of DADs (which include TA not preceded by discernible DADs, because TA must be initiated by the underlying DADs) in diabetic muscles was not significantly different from that in controls, regardless of the solution used. However, the frequency of occurrence of TA in diabetic heart (11/14) in high $[\text{Ca}^{2+}]_o$ and $1\text{ }\mu\text{M}$ ouabain was significantly higher ($p < 0.05$) than that in controls (4/11). These findings imply that the probability of occurrence of DADs (local response) is much the same in control and diabetic ventricular muscles; however, the DADs are more likely to aggravate a propagated response of TA.

The amplitude and coupling interval (time from onset of the last driven action potential to the peak of the following DAD) of DADs in the diabetic myocardium were not significantly different from those in the controls. For instance, in $3.6\text{--}7.2\text{ mM}$ $[\text{Ca}^{2+}]_o$ in the presence of $1\text{ }\mu\text{M}$ ouabain, the amplitude and coupling interval of DADs induced by a train of 20 stimuli (at 3.3 Hz) were $3.0 \pm 1.1\text{ mV}$ and $148 \pm 70\text{ ms}$ in the controls ($n = 5$), and $2.6 \pm 0.6\text{ mV}$ and $168 \pm 34\text{ ms}$ in the diabetics ($n = 7$).

DISCUSSION

Induction of diabetic guinea pigs. Injection of STZ to the animals generates free radicals in the pancreatic islet beta cells, damages the islet cell DNA, and produces diabetes mellitus (OKAMOTO, 1981).

The severity and time course of diabetes induced by STZ vary markedly among species (KUSHNER *et al.*, 1969; DOI, 1975). In rat, mouse, and dog, intravenous injection of $50\text{--}65\text{ mg/kg}$ STZ readily produced hyperglycemia within 48 h (JUNOD *et al.*, 1967; BROSKY and LOGOTHETOPOULOS, 1969; GANS and CATER, 1971; DOI, 1972; GANDA *et al.*, 1976), whereas in rabbit, cat, and guinea pig even larger doses ($150\text{--}300\text{ mg/kg}$) of STZ failed to induce hyperglycemia within 48 h (LAZARUS and SHAPIRO, 1972; DOI, 1975). In addition, STZ-induced lethality was remarkably higher in the rabbit, cat, and guinea pig (about 80% after 6 days) than in the rat, mouse, and dog (about 20% after 30 days) (DOI, 1975; LAZARUS and SHAPIRO, 1972; KLEIN *et al.*, 1980; SCHLOSSER *et al.*, 1984).

In the present study, on Day 40, we successfully produced severe hyperglycemia in the guinea pigs with intracardiac STZ injection during insulin-induced hypoglycemia (Ins-IC group, Table 1). Hyperglycemia within 7 days after intravenous STZ injection has been reported in the same animal species by other investigators (BROSKY and LOGOTHETOPOULOS, 1969). On the other hand, KUSHNER *et al.* (1969) and SCHLOSSER *et al.* (1984) reported no change in plasma glucose concentration 2 days after intracardiac STZ injection (150 mg/kg), or 2 or 7 days after intraperitoneal STZ injection (130 mg/kg), respectively. The reason for these apparent discrepancies in inducing hyperglycemia in guinea pigs is not clear; however, the different routes and dosages of STZ administration might explain the apparent discrepancies.

Although SCHLOSSER *et al.* (1984) failed to produce hyperglycemic guinea pigs 2 days after STZ injection, they reported depression of the growth rate, impaired, but insulin-reversible, tolerance of glucose loading, and pancreatic islet lesions in the STZ-treated animals. In the present study, we observed similar changes in glucose tolerance and depression of growth rate, especially in the Ins-IC group. This procedure for inducing diabetes was introduced for the first time by BROSKY and LOGOTHETOPOULOS (1969), who showed that the insulin-induced hypoglycemia enhanced the cytotoxic effect of STZ on the beta cells.

DADs and TA in diabetic myocardium. Characteristics of the action potentials of papillary muscles obtained from STZ-treated guinea pigs (Ins-IC group) were not significantly different from those of normal animals (Table 2). This is the first documentation of the transmembrane potential of heart muscle from diabetic guinea pigs. In the diabetic rat ventricular muscles, the APD was reported to be remarkably prolonged, compared to that of the normal rat (FEIN *et al.*, 1983; NORDIN *et al.*, 1985). Recently, we confirmed that the total APD of ventricular muscle from STZ-induced diabetic rat is significantly longer than that of normal rat (APD₉₀ at 1 Hz: normal rat, 81.1 ± 3.7 ms ($n=21$); diabetic rat, 101.9 ± 4.7 ms ($n=22$), $p < 0.05$) (NOBE *et al.*, 1989). In ventricular myocytes from diabetic rats, however, no consistent change of action potential contour was recognized (HORACKOVA and MURPHY, 1988), although some cells had markedly retarded repolarization. The reason for the different response of APD to diabetes in guinea pigs and rats is not obvious but may be related to a different ionic basis for the development of action potentials.

The TA activated by DADs may be implicated in ventricular tachyarrhythmias (FERRIER *et al.*, 1973; WIT and CRANFIELD, 1977). TA and DADs are potentiated by increased $[Ca^{2+}]_i$. This could be attained by increasing $[Ca^{2+}]_o$ or decreasing extracellular Na^+ concentration, or the inhibition of the Na^+-K^+ pump, due to either lowered extracellular K^+ concentration or application of digitalis (HASHIMOTO and MOE, 1973; ROSEN *et al.*, 1973; KASS *et al.*, 1978; ALLEN *et al.*, 1984; NORDIN *et al.*, 1985; HIRAOKA, 1987). Increased $[Ca^{2+}]_i$ results in an oscillatory release of Ca^{2+} from internal stores and hence DADs or TA (see for review, JANUARY and FOZZARD, 1988). More direct evidence that increase in $[Ca^{2+}]_i$ is essential for induction of TA and DADs came from studies using intracellular injection of Ca^{2+} or Ca^{2+} -chelator (ethyleneglycol-bis(beta-aminoethyl ether)-N,N'-tetraacetic acid; EGTA). DADs were induced by injection of Ca^{2+} , but were abolished by an injection of EGTA (KASS and TSIEN, 1982; MATSUDA *et al.*, 1982). Thus, there is abundant evidence indicating that increase in $[Ca^{2+}]_i$ is important for the genesis of DADs and TA.

In the present study, we adopted the procedure of ALLEN *et al.* (1984) to increase $[Ca^{2+}]_i$ and thus produce DADs and TA. They found that increasing $[Ca^{2+}]_o$ from 2 to 8 mM in the presence of strophanthidin enhanced the signals of aequorin luminescence, an indicator for $[Ca^{2+}]_i$, in ferret ventricular muscles. It is therefore reasonable to consider that $[Ca^{2+}]_i$ in similarly exposed guinea pig myocardium is also elevated.

Evidence is now accumulating to show that long-lasting hyperglycemia and/or hypoinsulinemia in diabetes is related to the disturbance of intracellular Ca^{2+} homeostasis. For instance, the contractile force from chronically diabetic animals is significantly depressed and has slower development and relaxation rates of active force with elevated resting tension (PENPARGKUL *et al.*, 1980; FEIN *et al.*, 1980, 1981), compared to normal myocardium. Also, Ca^{2+} uptake by sarcoplasmic reticulum and the rate of Ca^{2+} binding to the sarcolemma are depressed in hearts from diabetic animals (PENPARGKUL *et al.*, 1981; PIERCE *et al.*, 1983; HORACKOVA and MURPHY, 1988). It has also been reported that chronic diabetes decreases the $\text{Na}^+ - \text{K}^+$ pump concentration in ventricular muscle, skeletal muscle, and peripheral nerves (KJELDSSEN *et al.*, 1987). This agrees with the findings that the activity of $\text{Na}^+ - \text{K}^+$ ATPase is depressed in diabetic cardiac muscle (IMANAGA *et al.*, 1981).

In conclusion, we confirmed the method of BROSKY and LOGOTHETOPOULOS (1969) for inducing diabetic guinea pigs, and demonstrated, for the first time, that ventricular myocardium from the diabetic guinea pig is more prone than the normal one to develop TA under Ca^{2+} overload (Figs. 2 and 3, Table 3). In contrast, the myocardium from normal guinea pig seemed very resistant to develop DADs and TA, thereby suggesting that the myoplasmic Ca^{2+} overload sufficient to induce the TA may hardly occur in normal myocardium. These findings are consistent with a pioneering study using ventricular muscle from diabetic rats (NORDIN *et al.*, 1985), which lends support to the idea that the evolution of impaired Ca^{2+} homeostasis in diabetic hearts is not species-limited, but is rather an ubiquitous phenomenon among different species. Thus the mechanism of ventricular arrhythmias encountered in diabetic patients may, at least in part, be explained by abnormality in Ca^{2+} homeostasis or the development of triggered activity.

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