PHARMACOLOGICAL STUDIES ON THE MYOCARDIAL EFFECTS OF O,S-DICARBETHOXYTHIAMINE (DCET)*

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It was presumed in the previous report (1) that DCET potentiates the twitch tension and the incomplete tetanus of striated muscle by facilitating effects in the excitation-contraction coupling process. The effects of DCET on the striated muscle are similar to those of quinidine (2, 3), i.e., (i) *d*-tubocurarine-like blocking at neuro-muscular junction, (ii) stabilizing effects on the muscle membrane, (iii) potentiation of both the twitch tension and incomplete tetanus, (iv) depression of the complete tetanus tension in higher doses.

DCET also has antiveratrinic effects in the isolated diaphragm of rats and the isolated sartorius muscle of toads (1). Arora *et al.* (4) demonstrated that antiveratrinic responses are the common properties of many antiarrhythmic agents, including quinine analogues, ataxic agents, and antihistaminics.

So it is well anticipated that DCET has quinidine-like antiarrhythmic properties in the cardiac muscle. In this experiment the antiarrhythmic activities of DCET are tested and compared with those of quinidine, and some mechanisms of these antiarrhythmic activities of DCET are clarified in the isolated atrium of guinea-pigs and rabbits.

METHODS AND MATERIALS

1) Preparation of isolated atrium and papillary muscle of the guinea-pig or rabbit

Atria of guinea-pigs or rabbits were isolated under unanesthetized condition and prepared for recording the twitch tension isotonically or isometrically. Twitches of the isolated guinea-pig left atrium and rabbit papillary muscle were electrically driven by a square pulse generator (MSE 3, Nihon Koden Kogyo, Japan). Krebs-Ringer-bicarbonate solution saturated with 95% O₂ and 5% CO₂ was used at 29-30°C in all experiments except for the papillary muscle. In this preparation modified Locke solution, in which CaCl₂ and NaHCO₃ concentration were decreased to one half, was employed.

2) Determination of effective refractory period, threshold voltage, and conduction velocity

Following Dawes method (6), we compared the effects of DCET and quinidine on the effective refractory periods of isolated right atria of guinea-pigs. Effects on the three-

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threshold voltage and conduction velocity in the isolated rabbit atrium, electrically driven at the rate of 1.5-3 c/s, were conventionally compared using a cathode ray oscilloscope.

3) Induction of fibrillation in the atrium and ventricular muscle

i) *Electrical fibrillation* (7): Fibrillation of isolated rabbit atrium was caused by the application of high frequency electrical stimulation (10 c/s, 3 msec). Atrial fibrillation continued during electrical stimulation.

ii) *Aconitine fibrillation*: Aconitine fibrillation was caused both in the isolated right atrium and the right ventricular muscle of guinea-pig by the administration of aconitine in a dose of 10^{-6} . It was, however, necessary to electrically drive the ventricular muscle until flutter or fibrillation appeared in the muscle.

4) Application of microelectrode technique (8)

In order to observe the effects of DCET on the action potential of a single cardiac cell, we used the glass microelectrode filled with 3 M KCl.

Materials

O,S-Dicarbethoxythiamine hydrochloride (DCET), quinidine hydrochloride, dissolved in frog Ringer solution, and aconitine nitrate were administered into the Krebs-Ringer solution.

RESULTS

1) Effects on the spontaneous movements of isolated guinea-pig right atrium

DCET in a dose of 2×10^{-5} to 10^{-4} showed slightly positive inotropic effects following negative inotropic effects. But the frequency of atrial contraction decreased from 16 to 30% (Fig. 1). Atrial arrest following arrhythmia was observed in 5×10^{-4} of DCET. Only marked negative chronotropic effects were shown by the administration of quinidine, 2×10^{-5} . Negative chronotropic effects by DCET appeared immediately after its application and continued for about 60 minutes. On the other hand, negative chronotropic effects were gradually intensified with quinidine administration.

FIG. 1. Effects of DCET and quinidine on the spontaneous movements of the isolated right atria of guinea-pigs.

W : washing, Time scale : 10 seconds.

FIG. 2. Effects of DCET on the twitch tension of isolated left atria of guinea-pigs caused by electrical stimulation (2 c/s, 1 msec).
W : washing, Time scale : 10 seconds.

FIG. 3. Effects of DCET and quinidine on the effective refractory period in the isolated right atria of guinea-pigs. Numerals in the figure show the frequency of stimulation in one minute and Max. Rate shows the maximal follow rate of the atrium. Note the appearance of atrial arrhythmia in the electrical stimulation which exceeds the maximal frequency.

W : washing, Time scale : 10 seconds.

TABLE 1. Effect of DCET and quinidine on the maximal follow rates of the isolated right atria of guinea-pigs.

Compound	Conc. g/ml	No. of exp.	Maximal rate/min (I)	Maximal rate/min (II)	% Decrease of maximal rate
DCET	2.5×10^{-5}	5	336	312	$7.0 \pm 1.3^*$
	5×10^{-5}	6	335	294	12.2 ± 2.8
	10^{-4}	5	355	288	19.0 ± 1.9
	2×10^{-4}	6	368	244	33.8 ± 1.3
Quinidine	1.25×10^{-6}	5	344	300	13.1 ± 1.1
	2.5×10^{-6}	4	349	300	14.5 ± 3.5
	5×10^{-6}	5	333	235	29.9 ± 3.9
	10^{-5}	4	330	227	31.8 ± 3.1

The maximal follow rates of atria were measured by applying various rates of electrical stimulation (I). (II) shows the maximal follow rates at 10 minutes after drug administration. *: Standard error.

2) *Effects on the twitch tension of isolated guinea-pig left atrium*

By the administration of DCET, 10^{-4} to 5×10^{-4} , a moderate increase of twitch amplitude was observed. These positive inotropic effects were presumed to be of a lasting nature. In 2×10^{-3} concentration of DCET, however, twitches of the left atrium were arrested after marked inhibition (Fig. 2). No effects on the twitch tension were observed by quinidine in 2×10^{-3} concentration.

3) *Effects on the twitch tension of isolated rabbit papillary muscle*

Effects of DCET on the papillary muscle were observed after the marked positive inotropic effects of ouabain and strospeside were recognized. Moderate increase of twitch tension following temporal inhibition of papillary muscle appeared in a dose of 10^{-4} of DCET. Only marked inhibition of twitch was observed with 5×10^{-4} .

4) *Prolongation of the effective refractory period of isolated guinea-pig right atrium*

Maximal follow rates of the isolated right atrium of the guinea-pig to the electrical stimulation proportionally decreased with the increasing log dose of DCET and quinidine. In addition, a parallel relationship exists in the dose-response curves of DCET and quinidine. The relative potency of DCET was only 0.034 to quinidine (Fig. 3, Table 1).

5) *Elevation of the threshold voltage and slowing of the conduction velocity in the isolated rabbit atrium*

The threshold voltage of isolated rabbit atrium was elevated a few volts by DCET, while quinidine elevated it more than 10 volts (Table 2). At the same time, we observed the effects of DCET and quinidine on the extracellular action potential of isolated rabbit atrium using the isolating box method. By the administration

TABLE 2. Effects of DCET and quinidine on the threshold voltage of isolated rabbit atria.

Compound	No. of exp.	Thr. (1)	Control	Time after drug (min)				Thr. (2)	Thr. (3)	Thr. (4)
				10	20	30				
DCET ($1.9 \sim 2.3 \times 10^{-4}$)	6	$10.2 \pm 1.9^*$ volt	2.8	2.7	2.5	2.4		12 ± 1.9 volt	10.8 ± 1.5 volt	—
				(i) A.P. (mv)						
				(ii) A.P.D. (msec)	165.6	180.0	178.9			
				(iii) T. (g)	0.66	0.70	0.71			
Quinidine ($9.3 \times 10^{-6} \sim 1.2 \times 10^{-5}$)	7	9.4 ± 0.8 volt	2.7	3.4	2.4	2.3		21.6 ± 3.3 volt	15.3 ± 1.7 volt	11.6 ± 0.9 volt
				(i) A.P. (mv)						
				(ii) A.P.D. (msec)	141.9	146.0	154.3			
				(iii) T. (g)	0.60	0.65	0.58			
			93	(iv) P. (P/min)		75	69			

In order to determine the threshold voltage, electrical stimulation (3 c/sec, 3 msec) was applied to the preparation before and after the administration of compounds.

Thr., threshold voltage; A.P., action potential; A.P.D., action potential duration; T., twitch tension; P., cardiac pulse. Rabbit atria were washed twice for 30 minutes between Thr. (2) and Thr. (3), Thr. (3) and Thr. (4). *:Standard error.

TABLE 3. Effects of DCET and quinidine on the conduction velocity of isolated rabbit atria.

Compound	No.	Conc.	M/sec		% Decrease
			Control	30 min after	
DCET	1	2.0×10^{-4}	0.692	0.376	45.7
	2	1.9×10^{-4}	0.832	0.582	30.0
	3	2.0×10^{-4}	0.434	0.378	12.9
	4	1.9×10^{-4}	0.465	0.419	9.9
	5	2.1×10^{-4}	0.203	0.168	17.2
(average)			0.525	0.385	$26.7 \pm 3.4^*$
Quinidine	1	1.1×10^{-5}	0.341	0.270	20.8
	2	1.0×10^{-5}	0.313	0.189	39.6
	3	1.0×10^{-5}	0.265	0.187	29.4
	4	1.0×10^{-5}	0.603	0.418	30.7
(average)			0.381	0.266	$30.2 \pm 3.8^*$

Isolated rabbit atria were electrically driven at constant rate (1.5 c/s, 3 msec). *: Standard error.

of DCET and quinidine, the height of action potential decreased and its duration was prolonged. About 30% slowing of conduction velocity in the rabbit atrium was observed by the application of DCET (2×10^{-4}) and quinidine (10^{-5}) (Table 3).

6) Antifibrillatory effects of DCET

i) Effects on the fibrillation of isolated rabbit atrium caused by electrical stimulation

Marked fibrillation of isolated rabbit atrium was returned to normal rhythm by the administration of DCET ($1.8\text{--}2.3 \times 10^{-4}$) or quinidine ($1.0\text{--}1.5 \times 10^{-5}$), even under high

FIG. 4. Effects of DCET and quinidine on the electrically induced fibrillation in the isolated rabbit atria. Electrical stimulation (10 c/s, 3 msec). Numerals in the figure show the frequency of contraction of atria in one minute. In the atrial fibrillation electrical artifacts are shown in the lower curve.

TABLE 4. Effects of DCET and quinidine on the fibrillation of the isolated rabbit atria caused by electrical stimulation.

Compound	Conc.	No. of exp.	Time after drug administration (min)						
			0	5	10	15	20	25	30
Control	—	6	272 * ± 18.2	238 ± 19.0	224 ± 10.3	197 ± 17.7	192 ± 6.2	193 ± 13.4	198 ± 11.1
DCET	1. $2.0 \sim 2.5 \times 10^{-5}$	4	280.5 * ± 21.5	255 ± 29.9	240 ± 38.9	252 ± 40.7	249 ± 31.1	253 ± 15.3	265.5 ± 25.0
	2. $9.0 \times 10^{-5} \sim 1.1 \times 10^{-4}$	7	246.9 * ± 15.2	222.9 ± 12.0	198 ± 7.6	208.3 ± 4.5	196.3 ± 8.6	190.3 ± 10.3	204 ± 22.8
	3. $1.8 \sim 2.3 \times 10^{-4}$	6	267 * ± 14.3	200 ± 9.6	170 ± 21.1	131 ± 21.0	136 ± 15.4	143 ± 20.4	142 ± 22.6
Quinidine	$1.0 \sim 1.5 \times 10^{-5}$	5	276 * ± 12.6	210 ± 11.7	184.8 ± 19.6	169.2 ± 10.5	155.2 ± 14.5	151.2 ± 10.5	136.8 ± 8.1

Numerals in the table show the number of contractions of isolated rabbit atria in one minute. Electrical stimulation (10 c/s, 3 msec) were continuously applied to the preparation in the organ bath. *: Standard error.

frequency stimulation (Fig. 4, Table 4). But atrial fibrillation continued in the presence of DCET at a concentration less than 2×10^{-4} .

ii) *Effects on the aconitine fibrillation in the isolated right atrium and the isolated right ventricular muscle of guinea-pigs*

DCET and quinidine were administered after the aconitine Ringer was washed out, because the aconitine effects were so marked. But even toxic doses of both DCET and quinidine only showed slight antiarrhythmic effects on the atrial fibrillation caused by aconitine. In ventricular fibrillation, however, both DCET ($6.3\text{--}6.7 \times 10^{-4}$) and quinidine ($2.4\text{--}2.7 \times 10^{-5}$) decreased the frequency of action potential discharge and muscle contraction.

TABLE 5. Effects of DCET on the action potential of isolated right atrium of rabbit.

	Control	Time after DCET administration (min)						Remarks	No. of exp.
		10	20	30	40	50	60		
A.P. (mv)	86.4	83.2	85.6	86.7	85.1	80.3	79.1	74.1	8
S.E.	± 1.2	± 2.3	± 1.3	± 2.5	± 2.5	± 2.4	± 1.7	± 1.5	
O.S. (mv)	18.5	14.3	15.0	16.1	15.0	14.0	15.9	11.9	8
S.E.	± 1.5	± 2.1	± 2.0	± 2.3	± 1.9	± 1.6	± 1.9	± 2.0	
R.P. (mv)	66.2	68.3	70.5	70.7	70.2	66.5	64.4	61.4	8
S.E.	± 1.3	± 2.5	± 3.1	± 2.3	± 2.2	± 2.0	± 1.8	± 1.3	
50% R. (msec)	33.0	29.3	38.5	49.1	46.7	49.7	48.7	45.1	8
S.E.	± 3.9	± 1.3	± 2.7	± 5.7	± 6.3	± 5.0	± 6.6	± 4.0	
90% R. (msec)	80.3	75.8	99.6	121.1	122.4	126.6	123.7	107.9	8
S.E.	± 9.2	± 4.5	± 3.3	± 11.0	± 11.8	± 11.9	± 8.8	± 9.3	
M.R.D. (v/sec)	121.4			79.6			55.2	40.9	6
S.E.	± 6.4			± 9.0			± 14.2	± 15.4	
T. (cm)	0.33	0.4	0.45	0.42	0.49	0.49	0.5	0.39	5
S.E.	± 0.06	± 0.04	± 0.05	± 0.08	± 0.1	± 0.08	± 0.1	± 0.1	

Right atria were electrically driven at the rate of 2 c/s with square wave pulse (3 msec). The preparations were 2-4 times washed for 30 to 70 minutes after the DCET effects. A.P., action potential; O.S., overshoot potential; R.P., resting potential; R., repolarization; M.R.D., maximal rate of depolarization; T., twitch tension.; S.E., standard error.

FIG. 5. Effects of DCET on the action potential of isolated right atrium of rabbit, electrically driven at the rate of 2 c/s with the 3 msec square wave pulse. Lower curves show the upstroke of action potentials in faster sweep of oscilloscope. Slight decrease of overshoot potential and prolongation of depolarization process as well as repolarization process were observed by the administration of DCET.

7) Effects on the action potential of isolated rabbit right atrium

Time course of control action potential in the isolated right atrium, electrically driven at the rate of 2 c/s, was carefully examined.

Both the action potential and the resting potential became rather larger until 30 minutes from the onset of electrical stimulation, but afterwards they were constant for about 1 hour. Therefore, the effects of DCET on the action potential were observed after the atrium was electrically stimulated for at least 30 minutes. DCET, in a concentration of 2×10^{-4} , markedly inhibited the maximal rate of depolarization and prolonged both the 50% and 90% repolarization time (Table 5). Sometimes notching in the upstroke of action potential was observed after DCET administration (Fig. 5).

DISCUSSION

Slight positive inotropic effects on the isolated right atria of guinea-pigs were observed by the administration of DCET. But it had some negative chronotropic effects similar to quinidine.

It is reported that thiamine propyl disulfide (TPD) has marked inotropic effects both on the isolated toad heart (9) and on the isolated atrium of guinea-pigs (10, 11). But the mechanism of positive inotropic effects by TPD is still not clear. DCET also has moderately positive inotropic effects on the isolated guinea-pig left atrium and on the rabbit papillary muscle.

As DCET has stimulating properties on the excitation-contraction coupling process in the cardiac muscle as well as in the striated muscle, some positive inotropic effects

are presumed to be induced when the muscle membrane is depolarized by electrical stimulation. In the isolated guinea-pig right atrium, however, negative inotropic effects would appear because of the stabilizing effects of DCET. These stabilizing effects of DCET were already recognized in the striated muscle as an antiveratrinic effects.

So it is considered that DCET would have some antiarrhythmic activities as Arora *et al.* (4) stated. In fact DCET has all the common properties of antiarrhythmic agents in the cardiac muscle, i.e., (i) prolongation of effective refractory period, (ii) elevation of the threshold voltage, (iii) slowing of conduction velocity. By the administration of DCET in a dose of 2×10^{-4} , severe electrical fibrillation in rabbit atrium returned to normal rhythm. However, very slight antiarrhythmic activities were observed against aconitine fibrillation. Matsuda *et al.* (12), in studies on aconitine fibrillation, reported that aconitine fibrillation started with the oscillatory potential following the action potential which generated the conductive action potential at some critical level. These oscillatory potentials would also appear in the muscle deprived of Ca or treated with veratrine, and be antagonized with Ca or Mg. On the other hand, (i) facilitation on the pacemaker activity, (ii) conduction block in the atrium, and (iii) appearance of ectopic focus are cited as three major causes of aconitine fibrillation in the isolated rabbit atrium by Goto *et al.* (13). Anyhow ionic permeability of cardiac cell membrane is considered to be markedly stimulated in the cardiac flutter or fibrillation. According to Briggs and Holland (14), Na influx increased 4 to 5 times in the ventricular fibrillation. Arora *et al.* (4) reported the elevation of K concentration in plasma in atrial fibrillation and this high K concentration returned to normal with the termination of fibrillation after quinine administration. Atrial fibrillation by acetylcholine also depends on the K concentration in the bathing fluid (15).

Dawes (6), in studies on the effective refractory period, discovered that many spasmolytics and local anesthetics had quinidine-like properties. Using microelectrode technique, Vaughan Williams (16) suggested that antiarrhythmic effects of quinidine were derived from the marked depressive effects on the depolarization and overshoot potential in the atrium. Applying this hypothesis to the antiarrhythmic effects of pronethalol, they proved that the most important properties of antiarrhythmic agents were the depression of depolarization process (17). Maximal rate of depolarization was markedly depressed and the repolarization process in the right atrium of rabbit was slowed down by the administration of DCET.

Weidmann (18) demonstrated that the "Na theory" of Hodgkin (19) was applicable to the cardiac muscle. So these DCET effects would appear because DCET depresses not only Na permeability but also K permeability in the falling phase of action potential. As DCET was weak in elevating the threshold voltage of rabbit atrium, marked electrical fibrillation would be mainly depressed by prolongation of effective refractory period resulting from the increase of action potential duration after the administration of DCET.

We are examining whether these antiarrhythmic effects of DCET observed in the isolated atrium are also recognizable *in situ* preparations or not.

SUMMARY

1. Slight negative inotropic effects and moderate negative chronotropic effects in the isolated right atria of guinea-pigs were observed by the administration of DCET.
2. DCET, in a dose of 10^{-4} , moderately increased the twitch tension of both isolated guinea-pig left atrium and rabbit papillary muscle.
3. Effective refractory period of isolated guinea-pig right atrium was prolonged by DCET. The relative potency of DCET to quinidine was only 0.034 to 1.0.
4. Only slight elevation of threshold voltage of the isolated atrium was observed by DCET, while the conduction velocity in the rabbit atrium was slowed down about 30% by the application of DCET in 2×10^{-4} concentration.
5. Electrical fibrillation in the isolated rabbit atrium returned to normal rhythm by the administration of DCET in a dose of 2×10^{-4} . DCET, in higher concentrations, also depressed the aconitine fibrillation in the isolated guinea-pig ventricular muscle.
6. DCET depressed the upstroke of intracellular action potential and prolonged the repolarization process in the isolated rabbit atrium.
7. Antiarrhythmic activities of DCET are presumed to originate from the following causes, that DCET retards both the depolarization and the repolarization of action potential for its nonspecific inhibition to Na, K permeability and extends the effective refractory period of isolated atrium.

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