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Mechanical and Electrical Actions of Nicotine on Hypertrophied and Failed Cat Ventricle[1] (38057)

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(Introduced by N. Kahn)

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Sustained ventricular hypertension results in compensatory myocardial hypertrophy and, if the compensation is inadequate, cardiac failure. During cardiac failure in man and also in experimentally induced ventricular failure in animals, there is a reduction in endogenous myocardial stores of catecholamine (1, 2). It has been shown that nicotine (6.2×10^{-5} *M*) increases developed force of normal cat ventricular muscle by an indirect effect, i.e., mediated through release of endogenous cardiac catecholamine (3, 4). Nicotine also has a direct effect on contractility as exposure to higher concentrations of drug increase developed force in ventricular muscles from reserpine-pretreated (4, 5) and denervated cat hearts (5). Additionally, nicotine lengthens the plateau and terminal repolarization phases of normal and catecholamine depleted cat ventricular muscle (6). In the present report, we evaluate the indirect and direct actions of the drug on the mechanical properties and single cell electrical activity of hypertrophied and failed cat ventricular muscle.

Materials and Methods. The experimental methods have been described in detail previously (4, 6–8). Briefly, right ventricular papillary muscles and trabeculae carneae were obtained from 3 groups of adult cats (1.5–2.6 kg). These 3 groups were (1) a control group consisting of cats subjected to sham operation, (2) cats with right ventricular hypertrophy (RVH) and (3) right ventricular failure (RVF). [Hypertrophy and failure resulted from chronic right ventricular pressure overload which was brought about by partial constriction of the main pulmonary artery (9).] Sham operated and RVH cats were studied 1, 3, 7, 10, 21 and >90 days after surgery. RVF cats were studied when they were considered to be in congestive heart failure. Criteria used for defining RVF were (1) markedly elevated right ventricular end-diastolic pressure (>8 mm Hg) in combination with two or more of the following physical signs: ascites, pleural effusion and hepatomegaly. The excised muscles were mounted horizontally in a lucite tissue chamber and were superfused with Tyrode's solution [for composition see (7)] saturated with a 95% oxygen and 5% carbon dioxide gas mixture. Temperature was maintained at 36 ± 0.1°. Force was measured by a Statham UC-2 transducer mounted through one wall of the tissue chamber. Muscle length was adjusted by a fine micrometer movement to obtain a

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small initial load and stimulation was initiated; muscle length was then gently set to optimal length so that each muscle developed maximum isometric active force. Active and resting force and the maximum rate of development of force are expressed per unit of cross-sectional area (7). After the muscle was obtained, the right ventricular free wall was removed, dissected free from the ventricular septum and surface fat and weighed after blotting to remove surface water. Pressure overloaded ventricles demonstrated significant increases in weight (7). Muscles were stimulated through fine bipolar silver wires at a rate of 30 per min using square wave pulses of 2–3 msec duration; stimulation voltage was set 5%–10% above threshold. Glass microelectrodes filled with 3 *M* KCl (tip resistances of 15–25 Mohms) were used to impale cells in each muscle; potentials were recorded on a Textronix oscilloscope through a preamplifier having input capacity neutralization (Bioelectric, NFI). Input capacity neutralization and the rate of rise of phase 0 of the action potential were checked on each sweep of the oscilloscope (10). Permanent records of mechanical and electrical activity were obtained with a Grass or Polaroid camera. Nicotine was added to the reservoir bottle of Tyrode's solution. Results are expressed as mean plus or minus the standard error of the mean; statistical comparisons were made using Student's *t* test (11).

Results. Nicotine (6.2×10^{-4} *M*) induces a maximum transient increase in active isometric force developed by untreated right ventricular muscles from normal cats; this transient increase in contractility results from the action of the endogenous cardiac catecholamine released by nicotine (4). Table I compares the maximum transient increase in active force provoked by exposure to 6.2×10^{-4} *M* nicotine for right ventricular muscles from sham-operated and RVH cats. There were no significant differences between the responses of sham and RVH muscles; further, the responses of both groups of muscles did not differ significantly from previously reported responses of normal muscles (4). In contrast, muscles removed from cats in overt RVF demonstrated a marked reduction in the transient increase in force. The maximum transient increase in force for 27 RVF muscles was 12 ± 3% (expressed as percent increase of control force upon exposure to 6.2×10^{-4} *M* nicotine). The RVF muscles were obtained from cats sacrificed at times when clinical and hemodynamic signs of congestive heart failure were present after pressure overloading (3–127 days). The time of study of these muscles did not correspond to the times selected for sacrifice of the RVH and sham-operated cats, hence statistical comparison between the 3 groups of muscles was not possible.

The most significant change in single cell

TABLE I. Maximum Transient Positive Inotropic Response of Sham Operated and RVH Muscles Upon Exposure to 6.2×10^{-4} *M* Nicotine.[a]

	Sham		RVH	
Day	(n)	% increase of control force	(n)	% increase of control force
1	3	206 ± 44	3	187 ± 42
3	3	149 ± 37	3	143 ± 26
7	4	100 ± 36	4	130 ± 35
10	5	116 ± 74	3	124 ± 37
21	4	119 ± 30	4	165 ± 58
>90	3	131 ± 23	6	174 ± 26
Total	22	156 ± 15	23	132 ± 20

[a] Sham—muscles from sham-operated cats. RVH—muscles from cats with right ventricular hypertrophy. *n*—number of muscles. There is no significant difference between the responses of sham and RVH muscles.

electrical activity noted during maintained exposure (>60 min) to 6.2×10^{-4} *M* nicotine of sham operated, RVH and RVF muscles was a marked lengthening of both phase 2 and total action potential duration. Sham operated muscles had normal resting potentials and action potential characteristics (7); sham muscles responded electrically to exposure to nicotine in a manner identical to that previously noted for normal muscles (6). Plateau and action potential lengthening also occurred consistently in RVH muscles with unusual action potential configurations previously reported (7) and in RVF muscles with depressed electrical characteristics (12) (Fig. 1).

Table IIA lists the changes in various mechanical and electrical properties of sham and RVH muscles brought about by 30 min of exposure to a higher concentration of nicotine (6.2×10^{-3} *M*). In general, the actions of this concentration of drug were similar in both groups of muscles. Both groups demonstrated (1) no significant change in resting potential and action potential amplitude, (2) decreased action potential rate of rise ($\dot{V}_{max}$) and (3) increased action potential duration (measured at 75% and 100% of repolarization). Further, both groups showed increases in peak active isometric force, rate of force development, time to peak force and total duration of contraction although the increase in time to peak force was not statistically significant in RVH muscles. Table IIB compares the nicotine-induced changes in the various electrical and mechanical parameters *between* the 2 groups (sham and RVH muscles). RVH muscles demonstrated significantly smaller increases in action potential duration (75% and 100% of repolarization), time to peak force and total duration of contraction during ex-

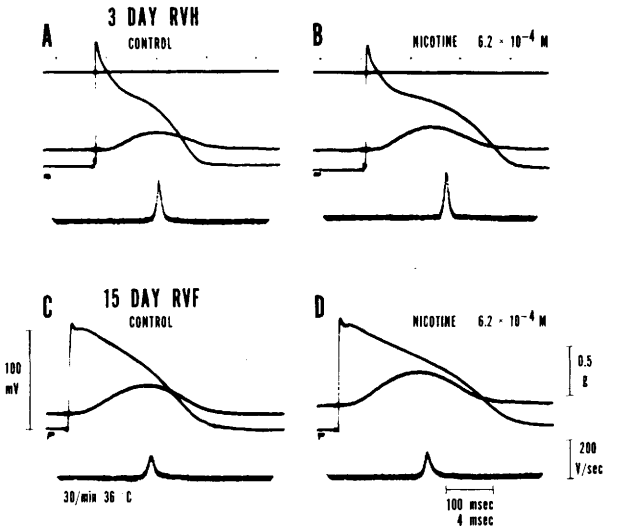

FIG. 1. Action potentials and isometric contractions recorded from an RVH muscle (pressure overloading of 3 days duration) (Panel A) and an RVF muscle (pressure overloading of 15 days duration) (Panel C). Top trace is a time and zero potential line. Second trace (from top) shows isometric contraction. Third trace shows action potential. Bottom trace shows a calibration and the rate of rise of action potential phase 0 (at faster sweep speed). Note the marked increase in duration of the action potential plateau (phase 2) and total action potential duration after 60 min of exposure to 6.2×10^{-4} *M* nicotine (panels B and D). There is a concomitant increase in active isometric force for both muscles. [The unusual action potential configuration depicted in this figure for the ventricular muscle from a cat sacrificed 3 days after partial pulmonary artery constriction (Panel A) has been described previously (7). The decreased action potential rate of rise shown for the RVF muscle (Panel C) is typical of depressed RVF muscles (12).]

TABLE II. Changes in Transmembrane Potentials and Isometric Contraction After 30 Minutes Exposure to $6.2 \times 10^{-3} M$ Nicotine.[a]

	A				B
	Sham operated muscles [8]		RVH muscles [8]		Sham vs RVH
Parameter	Change in parameter (control to nicotine treated)	Probability P^a	Change in parameter (control to nicotine treated)	Probability P^a	Probability P^b
RP (mV)	1.5 ± 0.8	N.S.	−0.3 ± 0.8	N.S.	N.S.
AP (mV)	0.25 ± 0.8	N.S.	−0.8 ± 1.0	N.S.	N.S.
V max (V/sec)	−24 ± 7	<0.025	−14 ± 7	<0.01	N.S.
Phase 2 Duration (msec)	31 ± 6	<0.005	26 ± 6	<0.005	N.S.
APD (75%) (msec)	70 ± 8	<0.001	31 ± 4	<0.001	<0.001
APD (100%) (msec)	111 ± 8	<0.001	44 ± 6	<0.001	<0.001
Po (g/mm^2)	0.24 ± 0.07	<0.01	0.11 ± 0.04	<0.025	N.S.
dP/dt (g/mm^2/sec)	0.96 ± 0.26	<0.01	1.24 ± 0.23	<0.001	N.S.
TTP (msec)	22 ± 4.1	<0.005	5.8 ± 3.9	N.S.	<0.025
TT (msec)	38 ± 12.2	<0.025	7.5 ± 2.0	<0.01	<0.05

[a] Shows the changes in the various parameters for muscles from Sham-operated cats and cats with right ventricular hypertrophy (RVH) after 30 min exposure to $6.2 \times 10^{-3} M$ Nicotine. B—*Compares* the changes between Sham and RVH muscles. p^a—Is the probability of a significant difference using Student's t test for paired observations. p^b—Is the probability of a significant difference using Student's t test for independent observations. [ ]—Number of Muscles. ( )—Unit. N.S.—Not significant. RP—Resting potential. AP—action potential amplitude. Vmax—maximum rate of rise of action potential upstroke. APD—action potential duration (at 75% and 100% of repolarization). Po—peak active isometric force. dP/dt—maximum rate of development of force. TTP—time to peak force. TT—total time of isometric contraction.

posure to $6.2 \times 10^{-3} M$ nicotine than did the sham operated muscles.

Muscles from RVF hearts also showed increases in peak active force upon exposure to $6.2 \times 10^{-3} M$ nicotine. For 4 such muscles (from animals sacrificed 7 and 10 days after surgery) the control mean peak active force was 0.53 ± 0.11 g/mm^2; after 30 min of exposure to $6.2 \times 10^{-3} M$ nicotine, mean peak active force was increased to 0.67 ± 0.06 g/mm^2. The mean increase in force (0.14 ± 0.04 g/mm^2) was statistically significant ($P < 0.05$). For these 4 muscles, there was no initial transient increase in force; upon exposure to $6.2 \times 10^{-3} M$ nicotine, force increased and was maintained throughout exposure to the drug.

Discussion. Significant decreases in the levels of endogenous stores of ventricular catecholamine have been demonstrated for cats and dogs with chronic experimentally produced ventricular failure (2, 13). Muscles from our RVF cats showed little contractile response to a concentration of nicotine which elicits a maximal indirect (catecholamine mediated) positive inotropic response in normal cat ventricle (4). The reduced contractile effect of nicotine observed in the present study is similar to the reduced contractile response noted during exposure to tyramine of papillary muscles from dogs in experimentally induced ven-

tricular failure (13).

We previously speculated that nicotine acts to lengthen the action potential plateau in cat ventricular muscles via a release of sarcolemmal calcium associated with a possible alteration in sarcolemmal potassium and calcium conductances as sarcolemmal calcium is depleted (6). Possibly, other mechanisms may be responsible for the effects of nicotine which we have observed. Recently, nicotine has been shown to have little effect on calcium exchange in rabbit ventricle (14) while it has significant effects on both potassium and sodium conductances in squid giant axon (15). The ionic basis or other mechanisms responsible for the changes in ventricular action potential characteristics during experimentally produced ventricular pressure overload in cats are also not well understood at this time (7, 12). However, the lengthening of the action potential plateau during exposure to nicotine of normal, sham-operated, RVH and RVF muscles suggests that the membrane properties affected by the drug are probably unaltered during the development of hypertrophy and failure.

Summary. Nicotine increases peak active isometric force in sham operated and RVH ventricular muscles by indirect (catecholamine-mediated) and direct effects. In such muscles, action potential plateau and action potential duration eventually lengthen during exposure to nicotine (6.2×10^{-4} *M* and 6.2×10^{-3} *M*). Muscles from cats in overt RVF showed significantly smaller transient positive inotropic responses during exposure to nicotine than sham and RVH muscles. RVF muscles demonstrated an increased maintained peak active force during exposure to 6.2×10^{-3} *M* nicotine.

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