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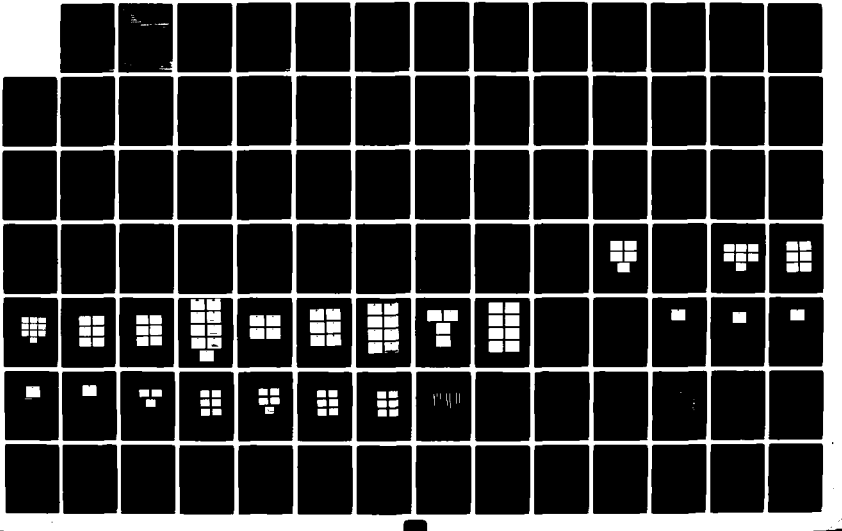

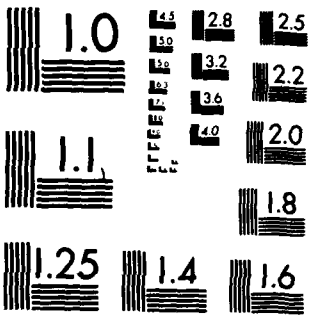

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THE ELECTROPHYSIOLOGIC MECHANISMS OF HALOGENATED ALKANE ARRHTHMOGENESIS

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Bromochlorodifluoromethane (1211) has been shown to sensitize the myocardium to the arrhythmogenic effects of adrenergic amines. Various physiologic and pharmacologic interventions were shown to modify both FC 1211 membrane effects as well as the FC 1211 sensitization process. These interventions included alterations in potassium concentration, applying stretch to Purkinje fibers, production of hypoxic conditions, alpha adrenergic effects and beta blockade of calcium mediated slow channel effects. In studies combining cyclic nucleotide measurements with electrophysiologic parameters, it was shown that

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20. Abstract (continued)

isoproterenol and FC 1211 act in an additive way to increase cyclic adenosine monophosphate levels in Purkinje fibers. Effects of FC 1211 in conscious dogs indicated that the arrhythomgenic action is due to cardiac sensitization and mediated through beta receptors.

RESEARCH OBJECTIVES

1. To provide an accurate description of the dose-related intrinsic effects of bromochlorodifluoromethane (1211) and dichlorodifluoromethane (Freon 12) on the canine cardiac action potential and the cardiac membrane properties underlying arrhythmogenesis.
2. To evaluate the extent to which various factors, including hypoxia, alteration of potassium concentrations and fiber stretch modify the cardiac action potential and membrane properties underlying arrhythmogenesis induced by bromochlorodifluoromethane and/or adrenergic amines.
3. To investigate how bromochlorodifluoromethane and adrenergic amines interact at the cardiac membrane to produce arrhythmias (cardiac sensitization).
4. To determine the role of cyclic AMP in altering the cardiac action potential and membrane properties underlying arrhythmogenesis resulting from the interaction of bromochlorodifluoromethane and adrenergic amines.
5. To determine what effects on the cardiac rhythm are due entirely to bromochlorodifluoromethane in the absence of adrenergic amine effects, i.e., do arrhtymias occur other than those from cardiac sensitization.
6. To relate the cardiac membrane effects of bromochlorodifluoromethane directly to the production of arrhythmias in the intact animal.

PROCEDURE

For each experiment, one mongrel dog weighing between 15 and 35 kg was anesthetized with an intravenous injection of sodium pentobarbital, 30 mg/kg. Rapid removal of the heart was achieved by right lateral thoracotomy, opening of the pericardium, and cutting the heart from the aorta, vena cava, and pulmonary vessel attachments at its base. The heart was placed in room-temperature carbogenated (95% O_2, 5% CO_2, Liquid Carbonic, Chicago, IL) Tyrode's solution to allow it to pump free of blood. The heart was then placed in 4°C carbogenated Tyrode's solution to slow contractions and allow excision of Purkinje fibers. The composition of the Tyrode's solution was, in millimoles per liter: NaCl, 137.0; $NaHCO_3$, 12.0; $CaCl_2$, 1.8; $MgCl_2$, 0.5; NaH_2PO_4, 1.8; KCl, 4.0; and glucose, 5.5.

Right ventricular Purkinje fibers were exposed by cutting through the pulmonary artery and down along the anterior interventricular sulcus, taking care not to disrupt larger, desirable Purkinje fibers. Chordae tendineae and valves were split to allow the opened heart to lie flat. The heart was immersed in Tyrode's solution at ten second intervals to prevent hypoxia. Purkinje fiber bundles measuring at least seven millimeters in length were cut from their distal (ventricular freewall) and proximal (interventricular septum) attachments, avoiding stretch of the fibers and leaving a myocardial pad at the distal end as a marker. Right ventricular Purkinje fiber bundles were placed in a separate jar of Tyrode's solution bubbled with carbogen by a glass dispersion tube.

Left ventricular Purkinje fibers were exposed by cutting through the aorta and left atrium, then down along the circumflex branch of the

left coronary artery to the apex of the left ventricle. Chordae tendineae and valves were split. Purkinje fiber bundles were excised and placed in a separate jar of carbogenated Tyrode's solution.

A total of three or four Purkinje fiber bundles were collected from the average heart. Fiber bundles were used in random order according to source (left or right ventricle) and treatment (control or drug-exposed fiber bundle) to average out possible changes in Purkinje fiber cyclic nucleotide and electrophysiology characteristics related to time elapsed from heart removal.

One Purkinje fiber bundle was mounted on a molded acrylic tissue holder with the proximal end (from the interventricular septum, anatomically) of the tissue overlying two platinum stimulating electrodes imbedded in the holder. The holder was secured to a Lucite tissue bath by two nylon screws. Tyrode's solution, which was controlled for temperature (37°C), pH (7.4) and pO_2 (at least 400 mmHg), was circulated by a pump from a one liter reservoir, through a glass heat exchanger and glass cuvette assembly with attachments to a Blood Gas Monitor (Radiometer, Copenhagan, Model PHM 73). From the cuvette assembly, Tyrode's solution traveled into the tissue bath, then returned to the reservoir. A thermistor immersed in the tissue bath relayed temperature readings to a digital thermistor unit (Markson Scientific, Inc., Model 90). The glass heat exchanger and water jacket surrounding the tissue bath were supplied by a water bath temperature control unit (Model 2067, Forma Scientific, Marietta, OH) set to maintain a tissue bath temperature of 37°C + 0.5°C. Tygon tubing was used to make all connections within the system. Oxygen and carbon dioxide were bubbled into the reservoir

through a glass dispersion tube and could be adjusted to desired levels by flow meters.

Microelectrodes with resistances ranging from 10 to 40 MΩ were made from precision glass capillary tubes (WP Instruments, Inc., New Haven CT, 1 μ fiber) on a horizontal magnetic electrode puller (Narishige Scientific Instrument Laboratories, Tokyo, Japan). Microelectrodes were filled with 2.5 M KCl by capillary action and a small guage needle and syringe, then coupled by an Ag-AgCl cell to an amplifier (M750, WP Instruments, Inc., New Haven CT) with high input impedence and input capacity compensation (Fig. 1). The output of the amplifier was connected to an oscilloscope (Model 5111 with one 5A14N four channel and 5B12N dual time base, Tektronix, Inc., Beaverton, OR). The first derivatives of the action potentials were obtained electronically using a differentiator linear from 0 to 1000 volts/sec. Records of transmembrane potentials and their first derivatives were calibrated by passing 100 mV and 600 V/sec signals, respectively, between the electrodes and the Ag-AgCl ground reference cell. The differentiator also amplified the action potentials and fed them to a high speed oscillograph (Model 1508 Visicorder Oscillograph, Honeywell, Denver CO) and the derivative signals to one channel of a dual trace oscilloscope ampifier (Model 5A18N, Tektronix).

The bath and microelectrodes were enclosed in a Faraday cage (Erik A. Lindgren and Associates, Chicago, IL) to minimize electrical noise.

Each microelectrode was positioned in a Purkinje fiber cell by means of a micromanipulator (Prior, England). Cell penetrations were made in the proximal, middle, and distal areas of the fiber bundle. A

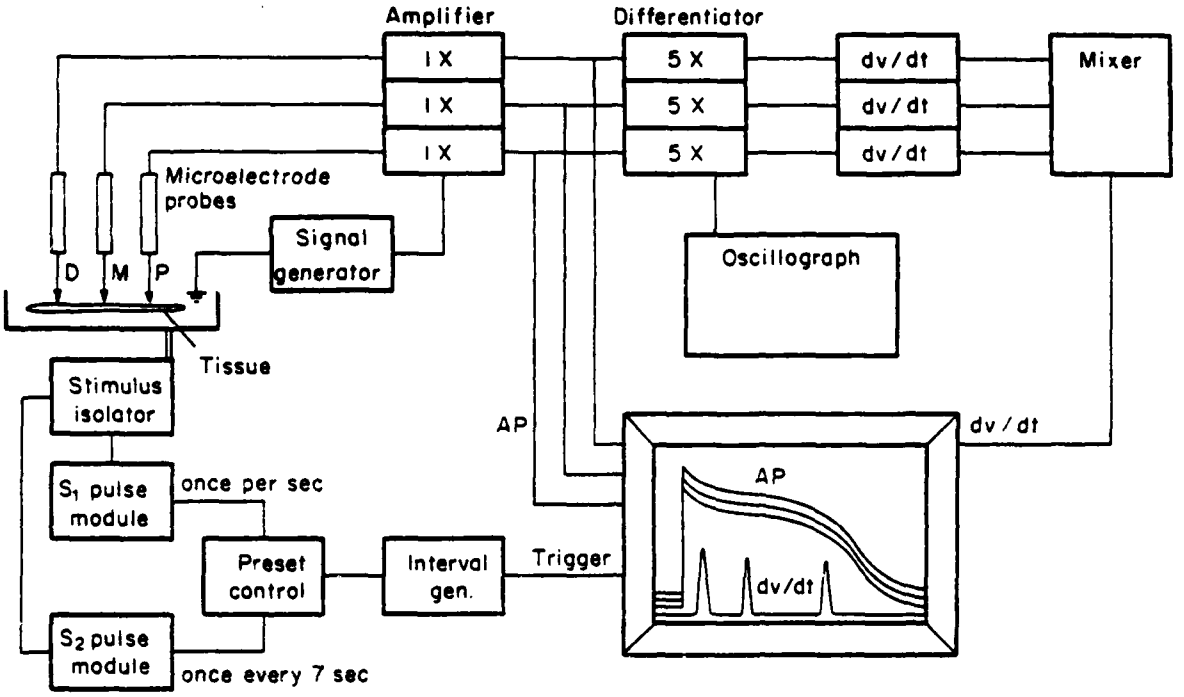


Figure I.

SCHEMATIC DIAGRAM OF ELECTRONIC EQUIPMENT

stimulus of 2 msec duration was applied to the tissue once every second. An equilibration period of 30 to 45 minutes was allowed before taking the following measurements:

1. Threshold: the minimum voltage required to stimulate the tissue. Parameters listed below were measured while the tissue was stimulated at 1.5X threshold voltage.

2. dV/dt or $\dot{V}_{max}$: the derivative of the rapid depolarization phase (or phase 0) in volts per second.

3. Effective refractory period, ERP: the time, in milliseconds, required for the cell to respond to a second stimulus, S_2, identical to S_1 applied after every seventh primary stimulus, S_1. The time delay between S_1 and S_2 was decreased by one or two msec increments until the cell no longer responded to S_2.

4. Automaticity:

a. Escape time, ET: the time from cessation of stimulus to the appearance of the first spontaneous action potential.

b. Spontaneous rate, SR: the rate in beats per minute of action potentials occurring after the Purkinje fiber had assumed its own inherent rhythm in absence of external stimulus, S_1. SR was measured from a Gould Brush recorder (Model 440, Cleveland, OH). A Visicorder oscillograph record was taken for measurement of the following (Fig. 3):

1'. Maximum Diastolic Potential, MDP: the most negative potential recorded at the beginning of phase 4.

2'. Activation Voltage, AV: the potential at the beginning of phase 0; the voltage at which the action potential is elicited.

3'. Slope of Diastolic Depolarization/Phase 4: was computed by dividing the difference between MDP and AV by the time duration of phase 4.

5. From a Visicorder record taken when the tissue was paced by a stimulus, the following parameters were measured (Fig. 2):

a. Resting Membrane Potential, RMP: the potential in millivolts just before phase 0.

b. Action Potential Amplitude, AP AMP: the height in millivolts, of phase 0, from RMP to peak.

c. Overshoot, OS: the peak positive potential, in Mv, measured from ground electrode potential to the peak potential of the action potential.

d. Action Potential Duration, APD: time, in msec, from the beginnning of the action potential (phase 0) to 50% (APD_{50}), 75% (APD_{75}), and 90% (APD_{90}) of the action potential repolarization.

After control measurements of a Purkinje fiber were taken, a treatment was administered or a second set of control measurements were taken twenty minutes later. Treatments included exposure to:

FC 1211 alone (I.C.I. America, Scofield Ave., Bridgeport, CT)

FC 1211, followed in 20 minutes by isoproterenol (Sterling-Winthrop Research Institute) 1.0×10^{-6} M or 1.0×10^{-7} M

Isoproterenol 1.0×10^{-6} M or 1.0×10^{-7} M alone

Atenolol (Stuart Pharmaceuticals, Division of I.C.I. Americas, Inc.) 1.0×10^{-5} M alone

Atenolol 1.0×10^{-5} M, followed by FC 1211

Atenolol 1.0×10^{-5} M, followed by isoproterenol 1.0×10^{-6} M

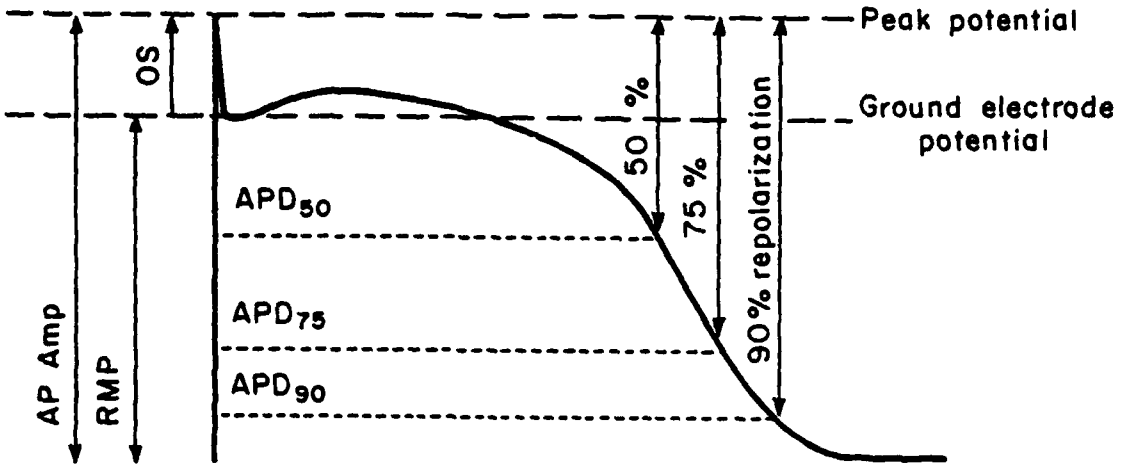


Figure 2. ACTION POTENTIAL OF A PACED PURKINJE FIBER

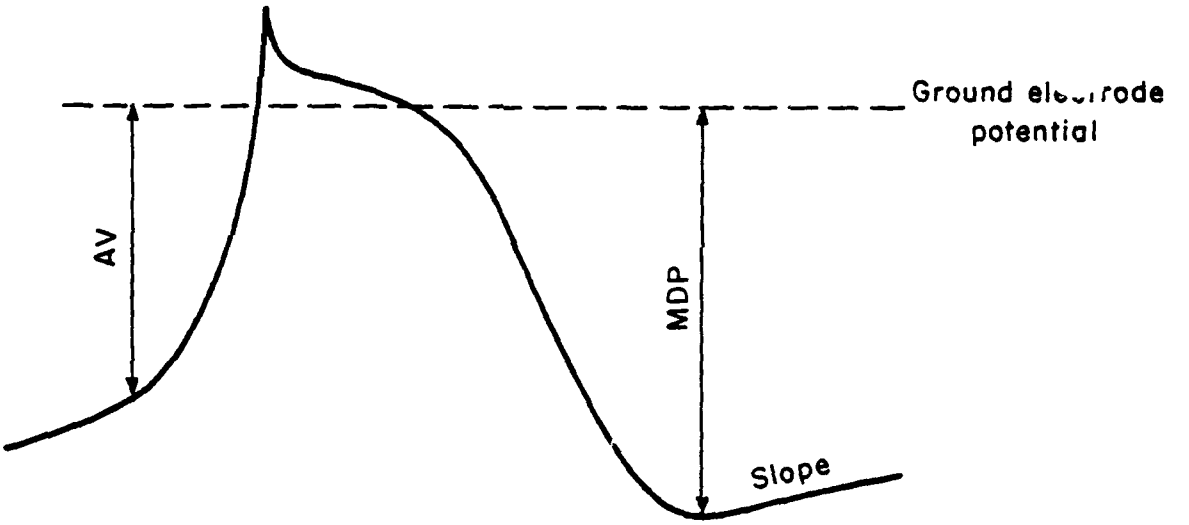


Figure 3. ACTION POTENTIAL OF AN AUTOMATIC, UNPACED PURKINJE FIBER

AP Amp = action potential amplitude in mV

RMP = resting membrane potential in mV

$APD_{50, 75, 90}$ = action potential duration at 50,70, 90% repolarization in msec

OS = overshoot of the action potential

AV = activation voltage

MDP = maximum diastolic potential

Atenolol 1.0 X 10^{-5} M, followed by FC 1211, then isoproterenol 1.0 X 10^{-6} M

While the Visicorder was run at slow paper speed, electrodes were carefully lifted out of the tissue, but left in the bath so that ground electrode potentials could be recorded. A 100 mV calibration signal was also recorded. The tissue was carefully removed from the bath, placed in 1.0 ml of cold 5% trichloroacetic acid (TCA), and homogenized using a 1.0 ml ground glass pestle and tube grinder (Kontes Scientific Glassware/Instruments, Vineland, NJ, Size AA) and a homogenizer motor (Stirrer type RZ R1-64, Caframo, Wiarton, Ontario, Canada). After homogenization, the grinder tube was rinsed with 1.0 ml of cold TCA which was then added to the first 1.0 ml. The sample was frozen until assayed for cyclic nucleotides.

To measure the concentration of FC 1211 in the Tyrode's solution bathing the tissue, two 1.0 ml samples of the solution were removed immediately after the tissue had been homogenized. The 1.0 ml samples were injected into sealed vials and then placed in a headspace sampler for analysis by gas chromatography (Model 3700, Varian Co., Sunnyvale, CA). The chromatographic peaks of FC 1211 from the bathing Tyrode's solution were compared with peaks produced by standard samples of FC 1211. The standards were made by injecting 0.0, 50.0, 100.0, 250.0, and 500.0 µl of a 8% 1211/air mixture into 1.0 ml of fresh Tyrode's solution.

Cyclic AMP and Cylic GMP Assay

Each Purkinje fiber bundle was assayed in duplicate for cAMP and cGMP using radioimmunoassay technique and for protein using the Lowry

Folin-Phenol reagent protein determination (Lowry, 1951). The following protocol (Fig. 4) based on Steiner et al. (1969) was suggested by Dr. Richard Fertel, Department of Pharmacology, Ohio State University (Steiner et al., 1969).

The TCA-Purkinje fiber homogenate was centrifuged for 10 min at 2000 X g. The precipitate was used for protein determination. The TCA in the supernatant was extracted three times with water-saturated ethyl ether. The bulk of the ether was aspirated and the remainder was evaporated in a hot water bath under a hood. Each tube was then adjusted to a pH of 6.5 using sodium acetate buffer. Standard samples of cAMP and cGMP ranging from 0.31 to 10,000 femtomoles per tube, blank tubes (B_0 or maximum binding tubes), and total radioactivity tubes (containing only ^{125}I-cyclic nucleotide) were set up. Standard and Purkinje samples were acetylated by adding two parts acetic anhydride and five parts triethylamine under a fume hood. ^{125}I-cyclic nucleotide in 0.25% gamma globulin and cAMP or cGMP antiserum in buffered bovine serum albumin were added to each tube of sample or standard. Tubes were incubated at 0-4°C for 6 to 60 hours. Next, the antibody-cyclic nucleotide complexes were precipitated by addition of ammonium sulfate. After a 20 minute incubation the tubes were centrifuged for 20 minutes at 2000 X g, the supernatant was decanted and tubes were counted for radioactivity in a gamma counter programmed to determine percent bound ^{125}I-cAMP or ^{125}I-cGMP. Standards were plotted on log-logistic probability graph paper with percent bound vs. femtomoles of cyclic nucleotide per tube. The percent bound values for Purkinje samples were plotted on the line drawn through the standard

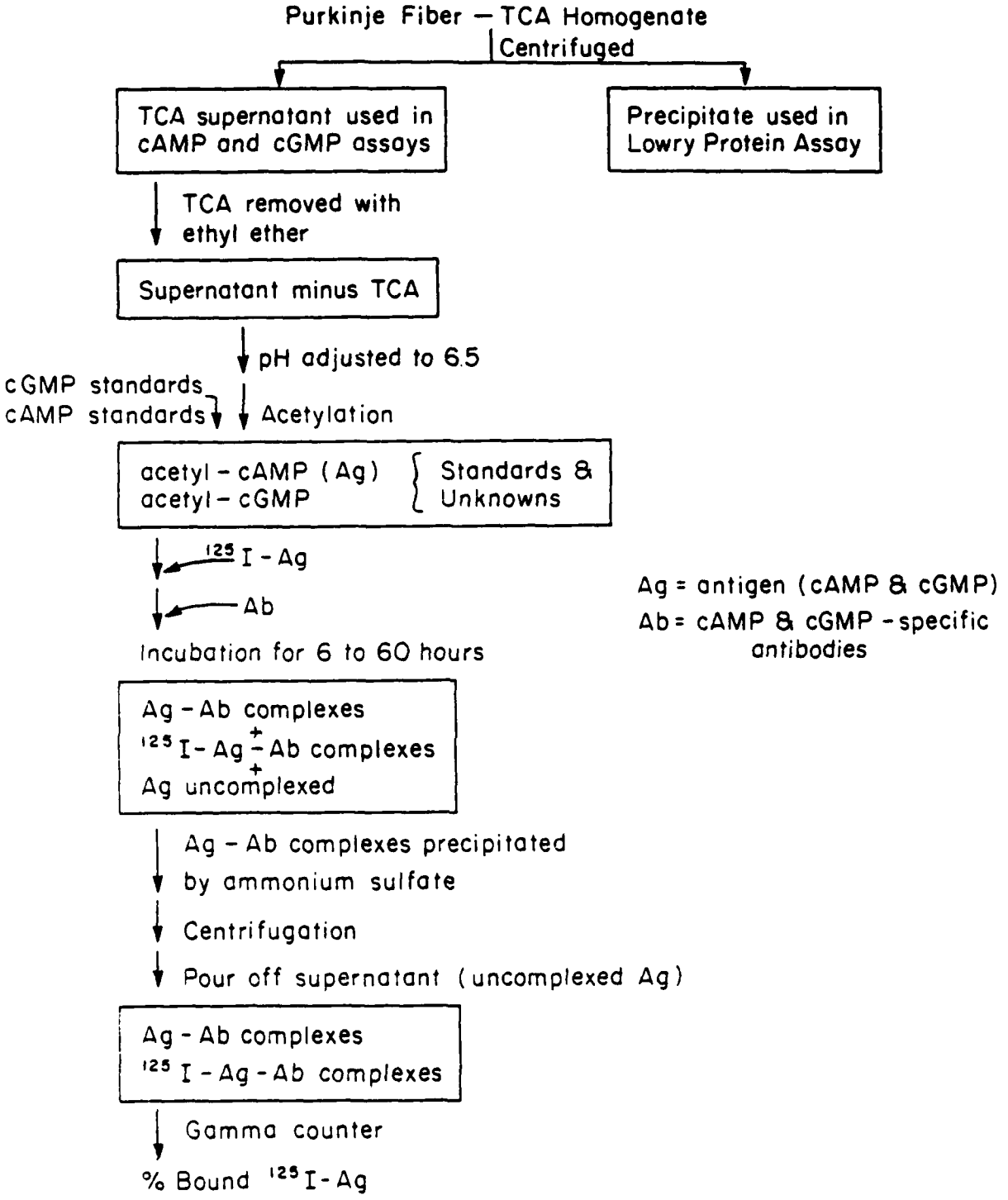


Figure 4.
FLOW DIAGRAM OF ASSAYS

values. The cyclic nucleotide concentration in Purkinje samples was then derived from the graph.

Electrophysiologic and cyclic nucleotide data were evaluated statistically using analysis of variance by the general linear models procedure, Duncan's multiple range test, and correlation coefficients. Significance was reported at the $p<0.05$ level unless otherwise noted.

Steady State Determination

One dog anesthetized with sodium pentobarbital was used for this experiment. The dog was intubated with an endotracheal tube which was attached to the FC 1211 breathing system. This system consisted of a two-way respiratory valve attached to the end of the tracheal tube. The intake side received flow through an inline 4 liter bag from an Ohio anesthetic machine. In this machine the NO_2 calibration tubes had been recalibrated by water displacement method to flows of FC 1211. It was at this machine that the mixing of FC 1211 and oxygen took place. The exhaust side of the respiratory valve was connected to a scavenging system that collected and disposed of exhaled gases (including FC 1211) through the in-house vacuum.

The dog received three different concentrations of FC 1211 (2%, 5%, 10%), one on each of three consecutive days. The mixture of FC 1211 was inhaled for 60 minutes. Blood samples for gas chromatograph analysis (discussed later) were taken at times 1, 3, 5 and 10 minutes, and additionally every 10 minutes until the end of the 60 minute exposure time. The inspiration of FC 1211 was then terminated immediately, and the dog inhaled 100% oxygen for the next 60 minutes. Again, gas

chromatograph blood samples were taken every 10 minutes. The results of the blood FC 1211 concentrations were plotted against time to determine steady state time of inhalation.

Conscious Mobile Dog Experiments

Dog Preparation (Tracheostomy Procedure)

Ten dogs, all healthy and weighing between 16 and 18 kg., were used for this procedure. Each dog was anesthetized with 1 ml of fentanyl citrate and droperidol and thiamylal sodium (4 mg/lb IV to effect). In addition, all dogs received an IM injection of 1 ml of glycopyrrolate to decrease salivary secretions during the procedure. The dogs were then prepared for surgery, shaved and scrubbed with betadine.

Each dog was situated in dorsal recumbency and an anterior midline incision 5 cm long over the second to fifth tracheal cartilage was made. The sternohyoideus and sternothyroideus muscles were freed by blunt dissection and pulled to the sides. An oval window was then cut into the trachea excising the anterior half of tracheal cartilages 3,4,5. The strap muscles were then secured using continuous 2.0 chromic gut sutures between the subcutaneous tissue and the connective tissue overlying the trachea. Finally, the skin was inverted around the oval window and sutured through the tracheal cartilage using 2.0 chromic gut interrupted sutures.

Post-surgically the area was covered with sterile gauze padding and wrapped with roller gauze. Daily injections of 400,000 units of Distrycillin containing penicillin G procaine in dihydrostreptomycin sulfate solution, and topical application of Panolog were administered

for five days after the surgery. Protective leather collars were also placed on the dog's neck to prevent any damage to the surgical area. These collars were worn continuously until the study had been completed. Tracheostomies were allowed to heal for at least three weeks before the dogs were used for an experiment.

Inhalation Experiments

Ten tracheostomized dogs trained to stand in a vinyl abdominal sling for up to two hours were used in these experiments. Dogs were positioned in such a way that their heads and legs had full mobility while their bodies were completely supported and movement was therefore restricted. All four legs were shaved free of hair and EKG plate leads were positioned. These leads recorded an EKG (lead II) on a Narco MK-IV-P pen-recorder physiograph operating at a paper speed of 5 cm/sec.

Using cetacaine as a local anesthetic, an endotracheal tube was inserted into the tracheostomy and tied into position with roller gauze. The end of the tube was connected with the intake side of the FC 1211 breathing system respirator valve as described previously.

All dogs in these experiments were pretreated with an IV injection of 15 mg/kg phenobarbital to block the convulsive effect (discussed later) of FC 1211. After a control period of breathing pure oxygen, dogs inhaled varying mixtures of FC 1211 (2%, 5%, 10% or 20%) for a period of 10 minutes. Blood samples for determination of blood FC 1211 concentration were taken at the end of this 10 minute period through a 16 ga., 20.3 cm long jugular catheter. During these experiments a constant EKG trace was recorded.

In an additional experiment, EEG tracings were recorded as well while a dog breathed a consecutive series of FC 1211 concentrations (2%, 5%, 10%, 20% and 30%). Finally, one dog breathing 20% FC 1211 was stimuated with stroboscopic lights and noise in an attempt to cause release of endogenous catecholamines. EKG and EEG tracings were recorded.

Conscious Immobile Dog Experiments

Dog Preparation

All dogs were anesthetized with Surital, and intubated with an endotracheal tube. A cut-down and catheterization procedure was performed on one femoral artery and vein using 2% carbocaine as a local anesthetic. The dogs were then placed in the abdominal sling and attached to a Honeywell VR-12 which monitored EKG's (lead II) and blood pressure. As the animal displayed signs of awakening from the anesthetic, 1 mg pancuronium bromide (nondepolarizing competitive neuromuscular blocking drug) was injected IV to immoblize them. The dogs were mechanically respired, receiving inspired air through the FC 1211 mixing system.

Sensitization Experiments

Nine immobilized dogs inhaled 100% oxygen for at least 20 minutes to establish oxygen control values. While still breathing O_2, these animals were administered several doses of isoproterenol IV in a 10 cc total volume. The dosing regimen was 0.5, 1, 3, 5 and 10 µg/kg of isoproterenol. Blood pressure and EKG's were monitored for 2 minutes after each injection. Between each injection of the dose response the animal

was allowed to return to control (at least 10 minutes). The dose response end-point was considered reached when EKG aberrations consisted of at least 3 PVC's in a three second interval or ventricular tachycardia. If no aberrant rhythms were apparent at the 10 µg/kg injection, this was considered the end-point.

The second portion of the sensitization experiments involved eighteen immobile dogs breathing varying concentrations of FC 1211 for 10 minutes (2%, 5% or 10%). While still breathing the respective FC 1211 concentration, a dose response with isoproterenol was performed. The dosing regimen began with 0.005 µg/kg and was doubled with each consecutive injection until the same desired cardiac aberration end-point was achieved. Blood samples for FC 1211 concentration determination were taken every 10 minutes.

B_1 Adrenergic Blockade

Six dogs were prepared the same as for all other immobile experiments with one exception. After obtaining oxygen control values, the dogs were administered 6 mg/kg atenolol (cardiospecific adrenergic antagonist) in a total volume of 20 cc over a two minute injection period. Ten minutes after the atenolol injection new control values were recorded and breathing of 2% FC 1211 was initiated. After 10 minutes of FC 1211 inhalation, a blood sample for the determination of FC 1211 concentration was taken, and a single injection of isoproterenol in a dose of 1 µg/kg was given IV. EKG and blood pressure tracings were recorded for two minutes post-injection.

Immediately following this two minute period, the FC 1211 mixture was increased to a concentration of 5% and was inhaled for 10 minutes. At this time blood samples were again taken and a second challenge dose of 1 µg/kg isoproterenol was administered. The monitored EKG and blood pressure parameters were similarly recorded. The inhalation concentration of FC 1211 was then increased to 10% and 20%, respectively, and a third and fourth injection of isoproterenol was administered at the end of the 10 minute inhalation period of these two FC 1211 concentrations. Identical parameters were measured at these FC 1211 concentrations as were measured at the lower concentrations.

Bromochlorodifluoromethane Determination

Blood samples from all experimental animals were analyzed for FC 1211 concentrations through a standard flame ionization gas chromatography technique. A series 3700 Aerograph gas chromatograph fitted with a HS-6 rotating head space injector was employed. The gas chromatograph operated at a detector temperature of 240°C, an injector temperature of 210°C, and a column temperature of 180°C. The glass separation column used measured 6 feet in length, with a 6 mm outer diameter and a 2 mm inner diameter. The column was prepacked with chromosorb 102, mesh size 80/100, and nitrogen at a flow of 30 ml/minute was used as the carrier gas. This system also employed a BD-40 pen recorder, run at a chart speed of 2 mm/sec.

One milliliter blood samples were taken from each dog breathing FC 1211 and immediately injected into a heparinized air-tight head space vial. Sample vials were placed in the head space injector and brought to

temperature (minimum of 10 minutes) before the sample was injected through the column. All samples were run in duplicate, and an average of the two peak heights was recorded.

A standard curve of FC 1211 was run every experiment day. This was necessary because parameters including barometric pressure and temperature influence the density of FC 1211, and thus, its corresponding gas chromatograph peak heights. A one liter standard of 8% FC 1211 mixed in a model S-1000 acrylic resin syringe was prepared and allowed to equilibrate. From this standard liter, samples of 0, 50, 100 and 250 microliters were taken and injected into head space vials over one milliliter of control dog blood. Samples were analayzed as described above, and a standard curve was calculated. Peak heights of unknown samples were then compared to the standard curve and corresponding amounts of FC 1211 in μg/ml of blood were obtained.

Analysis of Data

Comparison of mean values for sinus rate, systolic and diastolic pressure, blood FC 1211 concentrations, and threshold dose of isoproterenol was performed with a Student's t-test (Larsen, 1975). This test determines the probability that the control mean and a specific treatment mean are statistically equal.

Arrhythmogenic frequency data of the atenolol blocked experiments was compared to the frequency data of the sensitization experiments using Chi square test (Larsen, 1975). This statistical analysis employs the use of a 2 X 2 contingency table which categorizes qualitative criteria for its values. Chi square determines if an observed treatment

difference of a particular experiment could have been caused by randomization alone.

Status of Research

Range Finding Experiments

An initial set of experiments was performed to (1) determine the effective range of 1211 concentrations which produced measurable responses in the parameters in questions, and (2) determine the time of onset and duration of effects. A flow of 11 ml/min of 1211 alone produced no measurable changes from control. The threshold effect was produced by a flow of 20 ml/min while the maximum response we could measure was seen at a flow of 115 ml/min. (This was limited by the changes in $_pO_2$ and $_pH$ produced by flows above 115 ml/min). It was determined that bath concentration reached a stable maximum value of 1211 for any given flow rate at 15 minutes following the initiation of 1211 flow. Also, the changes in AP parameters were maximum by 17 or 18 minutes and remained unchanged as long as flow of 1211 was maintained. Twenty-five minutes was selected as the period of exposure at each flow. In several experiments, it was determined that the effects of 1211 were readily reversible in that the tissue characteristics returned to control values with 15-30 minutes of washout.

Nitrogen Control Experiments

To assure that the introduction of 1211 would not compromise the partial pressure of oxygen or alter the $_pH$, five experiments were performed in which nitrogen was substituted for 1211 at identical flow

rates. In these experiments, there were no significant changes ($p = 0.5$) in any parameters provided PO_2 was maintained above 200 mmHg. Likewise, $_pH$ changes, up to 7.45, had no effect on these parameters. In the experiments with 1211 at a maximum flow of 115 ml/min, PO_2 never fell below 250 mmHg and $_pH$ never exceeded 7.45.

Status of Research Effort

Dose-Response Studies

The dose-response effects of bromochlorodifluoromethane and dichlorodifluoromethane on the Purkinje action potential have been determined. The results of these experiments are presented in Tables 1 and 3 and Figures 5 and 6. The tabulated results of individual experiments have been normalized to the concentrations presented. Mean values have been compared to the control using Dunnet's test for statistical significance. Experiments for both compounds were conducted identically with one exception. The perfusate potassium concentration used with bromochlorodifluoromethane was 2.7 mM and the concentration with dichlorodifluoromethane was 4.0 mM. This difference is reflected in the resting potential (RP) and the action potential amplitude (AP).

The results of these experiments indicate that the action potential durations (APD_{50} and APD_{90}) as well as the effective refractory period (ERP) are quite sensitive to these compounds. Abbreviation of these two parameters has been shown to facilitate re-entry rhythms allowing the heart to sustain rapid trains of impulses leading to tachycardia and fibrillation. These are reported to occur in the whole animal following exposure to these compounds. In comparing the relative potency of these

two compounds, a concentration of 50 μg/ml of bromochlorodifluoromethane is 3.07 X 10^{-4} M and 40 μg/ml of dichlorodifluoromethane is 3.39 X 10^{-4} M, suggesting the former compound is more potent relative to the above mentioned parameters.

Sensitization to Adrenergic Amines

The halogenated alkanes were tested together with isoproterenol to determine the combined action on canine Purkinje electrical activity. The results are presented in Tables 2 and 4 and Figures 7 and 8. In each series of experiments, the response to three concentrations of isoproterenol was determined. Following a period of drug washout in which all parameters returned essentially to control values, either bromochlorodifluoromethane or dichlorodifluoromethane was introduced at a flow rate which alone produced minimal effects. The same three concentrations of isoproterenol were then repeated in the presence of either halogenated alkane. The results were compared statistically using the Newman-Keul's test.

As can be seen, especially with the brominated compound, the combined action on certain parameters was greater than either agent alone. The halogenated alkanes appeared to have the effect of increasing the potency of isoproterenol. An arrhythmogenic action is again suggested by the decrease produced in action potential duration (APD) and refractoriness (ERP). This can be related to the whole animal where endogenously released adrenergic amines may be potentiated by halogenated alkanes at otherwise innocuous levels.

Hypoxia Experiments

In these studies, Figure 9, the PO_2 was reduced in the presence of bromochlorodifluoromethane alone and in combination with isoproterenol. As can be seen, decreasing PO_2 to 100 mmHg produced only minor changes in the action potential configuration as well as other parameters. The addition of bromochlorodifluoromethane drastically altered the action potential configuration, decreased refractoriness and reduced upstroke velocity. Hypoxia, bromochlorodifluoromethane and isoproterenol completely eliminated phase 2 plateau of the action potential, further decreasing refractoriness. This loss of plateau is indicative of depression of the slow inward current carried primarily by Ca++. This effect may be related to the negative inotroprism in ventricle muscle reported by others for this halogenated alkane.

The combination of hypoxia and isoproterenol in the presence of bromochlorodifluoromethane is quite relevant when one considers the potential for arrhythmias in a fire emergency situation in which this agent is used as an extinguisher.

Altered Potassium Experiments

The membrane potential as well as certain other phases of the action potential are critically dependent on extracellular potassium concentrations. Therefore, experiments shown in Figure 10 were performed in which the normal potassium concentration was halved or doubled. At all potassium concentrations, isoproterenol alone reduced the action potential duration. Bromochlorodifluoromethane alone at 2.0 and 4.0 mM reduced the total duration, refractoriness and the plateau. At 8.0 mM

the effect was to reduce both amplitude and duration and on occasion to completely abolish a conducted impulse (F). This response is interesting not only from the standpoint of conduction abnormalities leading to cardiac arrhythmias but also because isoproterenol, when added under these conditions, restored conduction of the abnormal impulse (J). In this case isoproterenol effects were not potentiated but rather isoproterenol reversed the effects of bromochlorodifluoromethane. Either elimination of impulse conduction (block) or production of an abnormal, slowly conducted impulse (re-entry) may be related to rhythm disturbances.

Cyclic AMP Mediated Effects

Since beta adrenergic effects in the heart are in part mediated via the second messenger cyclic AMP, experiments were performed to assess the influence of dichlorodifluoromethane on this system (Fig. 11). Aminophylline, a phosphodiesterase inhibitor, was used to elevate levels of cyclic AMP by inhibiting its destruction. Isoproterenol alone and aminophylline alone both produce shortening of the action potential and refractoriness (B + C). Dichlorodifluoromethane enhanced this effect of aminophylline (D). When isoproterenol was added to a preparation exposed to aminophylline, the response was greater than either alone (E). Finally, combining dichlorodifluoromethane, isoproterenol and aminophylline produce the maximum response, greater than any combination of two of the above.

These results would indicate that the interaction of adrenergic amines and halogenated alkanes may be mediated via cyclic AMP. The possibility exists that the interaction could be via a direct increase in

cyclic AMP levels or an indirect action on the beta receptor-cyclic AMP system.

Slow Channel Mediated Action Potential Effects

It has been shown that certain oscillatory potentials in cardiac tissue resulting from a slow channel current may give rise to aberrant rhythms. The experiments depicted in Figure 12 provide an experimental model for determining the effects of the halogenated alkanes on slow channel action potentials. The potassium concentration is elevated to 16 mM, resulting in depolarization of the membrane (B). Additional Ca++ (2.7 mM) is then added (C). Isoproterenol alone enhances conduction of these action potentials (D) while dichlorodifluoromethane appears to further slow the conduction under these conditions(E). The combined action of isoproterenol and dichlorodifluoromethane produce an effect which appears as a summation of the individual effects.

In comparing action potentials in B and E, it appears that dichlorodifluoromethane may reduce the slow channel current since both action potentials are similar while the perfusing media contains one-half the amount of Ca++ in B than in E.

Fiber Stretch Experiments

In an effort to simulate the effects of increased blood pressure and, hence, increased afterload which produces stretch in the ventricular wall, Purkinje fibers were stretched 20% beyond their relaxed length and exposed to various concentrations of 1211 (Fig. 13). In the paced fiber, stretching alone had little effect on the action potential configuration

or other parameters. However, with the application of 1211, the rate of upstroke, overshoot and duration were all reduced, giving the Purkinje fiber action potential the appearance of a ventricular muscle action potential. In the spontaneously beating fiber, stretch can be seen to increase the intrinsic rate which is further enhanced when 1211 is again applied.

Alpha Stimulation by Phenylephrine

Realizing that alpha receptors exist in the myocardium, though relegated to a minor role in comparison to beta receptor regulation, it was important to determine how 1211 might interact with alpha stimulation (endogenously released epinephrine is both alpha and beta). In a manner similar to the sensitization experiments with the pure beta stimulant, isoproterenol, the response to various concentrations of phenylephrine was determined with and without 1211 (Fig. 14). Phenylephrine at the applied concentrations, is a pure alpha agonist; the alpha effects of epinephrine in the heart are masked by the dominant beta effects. It is apparent from these results that doses of phenylephrine in the range of 10^{-9} M to 10^{-7} M produce practically no change in action potential configuration or other characteristics. This may result from the paucity of alpha receptors in the ventricle. Phenylephrine causes a slight increase in action potential duration which is not potentiated by 1211, but rather the combined effects are additive in that the net results are a summation of shortening due to 1211 and the prolongation due to phenylephrine. This apparent lack of alpha adrenergic potentiation by 1211 is not

surprising because such action has not been reported for other sensitizing halogenated alkanes.

Interaction of Cyclic AMP and 1211

Cyclic adenosine monophosphate (cAMP) acts as a second messenger in response to beta adrenergic stimulation in the heart and elsewhere. That cAMP and the system that regulates it mediate cardiac membrane effects is well established. Because the interaction between 1211 and beta adrenergic stimulation to sensitize the heart may be mediated via the cAMP system, several experiments to determine this have been performed.

The effects of inhibiting phosphodiesterase, the enzyme responsible for the removal of cAMP, have been reported previously. Briefly, inhibition of this enzyme by aminophylline, while stimulating adenyl cyclase through the beta receptor with isoproterenol coupled with exposure to 1211 produced greater changes in cardiac membrane effects than did any two of these treatments alone. This suggests a common effect which may be stimulation of cAMP.

A second approach to determine cAMP relationship to cardiac sensitization was the application of dibutyryl cAMP. This compound is permeable to the cardiac membrane (the parent compound is not) and once inside the cell, it is probably converted to the monobutyryl compound and cAMP. Once inside the cell, cAMP acts on various sites, such as the endogenously stimulated release might. To test whether the effects of dibutyryl cAMP might be enhanced by 1211, experiments of the type depicted in Fig. 15 were performed. As can be seen, successively increasing amounts of dibutyryl cAMP produce changes in the action

potential configuration, including duration and refractoriness as well as enhancement of spontaneous automaticity. This is not unlike beta stimulation due to isoproterenol. 1211 augments the membranes' effects of dibutyryl cAMP, suggesting again a role for this second messenger in cardiac sensitization.

Slow Channel Calcium-Mediated Action Potential Blockade by 1211 and Atenolol

In order to examine the dose-dependent effects of 1211 on the slow channel plateau current, the tissue was depolarized by potassium and enriched with calcium (Fig. 16). Isoproterenol, by beta stimulation, caused an increased calcium current which is primarily responsible for the shortening of the action potential plateau. The slow inward calcium current was progressively diminished at increasing levels of 1211. The effect at the highest concentration was very similar to the blocking effect produced by atenolol, a beta blocker without intrinsic membrane stablilizing properties. Though the effects of 1211 and atenolol were similar, it is likely that the 1211 effect is directly on the membrane rather than at the receptor as with atenolol.

Cardiac Sensitization by 1211 in Conscious Dogs

This series of experiments was performed in order to define the conditions for cardiac sensitization to isoproterenol by 1211. The electrophysiology experiments in this study have employed isoproterenol, a beta adrenergic agonist, and the blood superfusion experiments, likewise, employ isoproterenol. Few, if any, animal studies with 1211

cardiac sensitization have been performed with isoproterenol, but rather with epinephrine or norepinephrine, alpha and beta adrenergic agonists. The use of isoproterenol in these experiments is justified because the extracardiac vasoconstriction is eliminated as well as possible alpha adrenergic cardiac effects (see phenylephrine experiments, this report). On the other hand, using epinephrine or norepinephrine more nearly reproduces the actual situation in which endogenously released catecholamines interact with 1211 in the sensitization process.

These experiments were conducted on conscious, but pancuronium-immobilized dogs. This was necessary for two reasons. First, when 1211 was administered to pentobarbital anesthetized animals, the arrhythmogenic level was excessively high when compared to previous reports with conscious animals. Second, when fully conscious, chronically tracheostomized dogs were made to breathe low levels (2%) of 1211, the initial response was a convulsive state which precluded continuation of monitoring for cardiovascular irregularities.

To conduct these conscious immobilized experiments, the animals were anesthetized briefly with sodium thiamylal and intubated with a cuffed endotracheal tube. In those experiments where blood pressure was monitored, the arterial catheter was introduced during the period of anesthesia and the site of insertion was anesthetized locally with 2% carbocaine. Before recovery from anesthesia, the dogs were administered the neuromuscular blocker, pancuronium, at a dosage of 0.05 mg/kg. Respiration and the metered delivery of 1211 was maintained by a respirator.

In the first series of experiments, dogs were given progressively larger doses of isoproterenol until a ventricular rhythm disturbance occurred (defined minimally as three abnormal beats during any three second period). In Figure 17, the results of these experiments are presented. In all animals studied, a ventricular arrhythmia, as defined above, was not seen at 1 μg/kg, but developed at a level of 3 μg/kg. After exposure to 5% 1211 for five minutes, followed by progressively larger challenging doses of isoproterenol, all animals developed an arrhythmia at or below a dosage of 0.08 μg/kg of isoproterenol. This indicates cardiac sensitization at this level of exposure and provides a basis for the subsequent blood superfusion experiments.

In the second series of experiments, the objective was to determine whether 1211, by itself, would induce substantial changes in the cardiac membrane to cause arrhythmias. Evidence for a direct, intrinsic action by this compound on the cardiac action potential has already been reported. In Figure 18, the results of these experiments are presented. Atenolol, a pure beta I blocker, was administered at the rate of 6 mg/kg. Increasing concentrations of 1211, up to 20% of the inspired air, were administered. At each concentration, a challenging dose of 1 μg/kg of isoproterenol was given. At concentrations of 1211 up to 20% in the presence of complete beta blockade, no arrhythmia developed. With a dose of isoproterenol shown above to be arrhythmogenic in the sensitized heart, the beta blockade was not overcome until the highest concentration of 1211 was given. This suggests that despite previous reports as well as substantial direct membrane effects, 1211 is not arrhythmogenic in the absence of (or blockade of) endogenous adrenergic amines.

Blood Superfusion Experiments

Blood superfusion of isolated Purkinje fibers was peformed in order to relate the cardiac membrane response to production of ventricular arrhythmias in the intact animal. A detailed description of the experimental procedure was presented in the original proposal. Briefly, Purkinje fibers from one dog's heart were mounted in the Tyrode's superfused tissue bath. A second dog was anesthetized and prepared to provide blood for superfusion of the isolated tissue. Blood pressure, left ventricular pressure and electrocardiograms were recorded from this dog and both 1211 and isoproterenol were administered directly to the animal. Following equilibration with blood superfusion and the recording of normal parameters (Fig. 19), the animal was tested with several concentrations of isoproterenol to determine an arrhythmogenic dose. Following recovery, the animal was made to breathe a mixture of 1211 (5%) in air and challenged a second time with isoproterenol. The appearance of ventricular arrhytymias occurred at a lower level of isoproterenol when combined with 1211. Decreased refractoriness and slowed conduction were noted in the Purkinje action potential. Following beta blockade with atenolol, the same challenge of isoproterenol failed to produce any rhythm disturbance and was thus, protective. Continuing experiment with this model will compare epinephrine to isoproterenol and look at other possible interventions.

Electrophysiology

The effects of FC 1211, isoproterenol and atenolol on Purkinje fiber electrophysiology and cyclic nucleotide levels were studied in 110 Purkinje fibers. Means (± S.E.M.) of control values for all parameters measured are listed in Table 5. The results of 9 treatments administered to Purkinje fiber preparations are reported in Tables 6 through 13. The treatments did not affect threshold voltage (Table 6), maximum diastolic potential (Table 10), cGMP concentrations, or cAMP:cGMP ratio (Table 11) significantly ($p<0.05$)

Escape time was increased significantly by exposing Purkinje fibers to FC 1211 or atenolol 1.0×10^{-5} M, then FC 1211. Isoproterenol at concentrations of 1.0×10^{-6} M and 1.0×10^{-7} M (Fig. 20, D and E) caused decreased escape time, as did atenolol 1.0×10^{-5} M + isoproterenol 1.0×10^{-6} M. Although the mean escape time values for the isoproterenol and atenolol-isoproterenol treatments (Table 6) were less than 30% of the control value, they were not found to be significantly different. The mean escape time value for the atenolol-FC 1211-isoproterenol treatment (81.4 sec) was much higher than the control value (38.4 sec), but lower than FC 1211 alone (123.6 sec) or atenolol + FC 1211 (103.3 sec)(Table 6). FC 1211 caused a much slower rate of spontaneous action potentials than control. Fig. 21, A, B, and C shows an increased escape time and a decreased spontaneous rate due to FC 1211, then a decrease in escape time and acceleration of spontaneous rate due to subsequent addition of isoproterenol. The atenolol-FC 1211 and atenolol-FC 1211-isoproterenol treated tissues showed little change in spontaneous rate. Isoproterenol 1.0×10^{-6} M, FC 1211-isoproterenol 1.0×10^{-6} M, FC 1211-isoproterenol

1.0 X 10^{-7} M and atenolol-isoproterenol 1.0 X 10^{-6} M treatments produced more rapid spontaneous rates (Fig. 20, D and E).

Action potential overshoot (OS) was significantly decreased by FC 1211, isoproterenol 1.0 X 10^{-6}, FC 1211-isoproterenol 1.0 X 10^{-7} M, and FC 1211-isoproterenol 1.0 X 10^{-6} M (Table 7). Resting membrane potential (RMP) generally decreased in all treatments except atenolol and atenolol-isoproterenol. Isoproterenol 1.0 X 10^{-7} M caused a decrease in RMP, however, this decrease was not statistically significant. Action potential amplitude (AP AMP) was diminished to the greatest extent by superfusion with FC 1211-isoproterenol at both concentrations. Significant differences in AP AMP occurred between the following pairs of treatments:

FC 1211 . . . . . atenolol-FC 1211

FC 1211 . . . . . FC 1211-isoproterenol 1.0 X 10^{-6} M

isoproterenol 1.0 X 10^{-6} . . . FC 1211-isoproterenol 1.0 X 10^{-6} M

FC 1211-isoproterenol 10^{-6} M . atenolol-FC 1211-isoproterenol

atenolol . . . . . atenolol-FC 1211

Action potential durations (Table 8) at 50% (APD_{50}), 75% (APD_{75}), and 90% (APD_{90}) repolarization shortened significantly during FC 1211 (Fig. 22), isoproterenol 1.0 X 10^{-6} M, FC 1211-isoproterenol 1.0 X 10^{-6} (Fig. 21), FC 1211-isoproterenol 1.0 X 10^{-7} M, atenolol-FC 1211, and atenolol-FC 1211-isoproterenol (Fig. 23). Isoproterenol did not cause significant shortening of APD when the fiber had been superfused with atenolol (Fig. 24). Difference from control in APD during the 9 treatments is shown in Fig. 25. Effective refractory period (ERP) shortened in all treatments, but not significantly in atenolol or

atenolol-isoproterenol experiments (Table 9). Shortest ERP times occurred in Purkinje fibers superfused with FC 1211-isoproterenol; this is similar to the APD result. The correlation coefficients for ERP vs. APD_{75} and ERP vs. APD_{90} are 0.90; ERP vs. APD_{50} has a r value of 0.79 ($p < 0.0001$).

The maximum rate of rise of the phase 4 upstroke (dV/dt) showed a maximum mean value during atenolol exposure and a minimum during FC 1211-isoproterenol 1.0×10^{-6} M exposure. A correlation exists between dV/dt and AP AMP: $r = 0.70$ ($p<0.0001$).

Parameters measured during spontaneous activity are shown in Table 10. Maximum diastolic potential (MDP) ranged from -81.8 mV during FC 1211 exposure to -88.0 mV during atenolol-isoproterenol exposure, however, none of the values within the range were significantly different from the others. Activation voltage (AV) was less negative during FC 1211 exposure than during control superfusion. Atenolol alone or in combina- tion with isoproterenol caused the Purkinje fiber to fire at potentials that were higher than control potentials. Phase 4 rate of rise (slope) was greatest when the tissue was exposed to FC 1211-isoproterenol (10.1 and 19.9 V/sec) and isoproterenol alone (16.0 and 12.6 V/sec). FC 1211 caused a decrease in diastolic depolarization by 3 V/sec. Atenolol alone caused flattening of diastolic depolarization and when combined with isoproterenol or FC 1211 + isoproterenol, caused a slower diastolic rate of rise than occurred with either of these treatments without atenolol. Tissues superfused with atenolol + FC 1211 had a slightly greater slope than tissues exposed to FC 1211 alone.

Measurements of ERP, OS, RMP, AP AMP, APD, dV/dt, MDP, AV and slope were taken at 3 sites on each Purkinje fiber: proximal, middle, and distal. Differences due to measurement site were significant for control measurements of OS, RMP, ADP_{50}, APD_{75}, and dV/dt (Table 12). Variation among the 9 treatment groups was not significant, therefore, in Tables 7, 8, 9, and 10, proximal, middle, and distal values were averaged together.

Differences were found between left and right ventricular Purkinje fibers during the control period (Tables 12 and 13). Escape time was about twice as long and spontaneous rate about half as fast in right ventricular fibers as left ventricular fibers. ERP, APD_{50}, APD_{75}, and APD_{90} measured from left ventricular fibers were 5-9% greater than these parameters measured from right ventricular fibers.

Cyclic Nucleotides

Table 11 shows the results of cyclic nucleotide assays. Purkinje fibers treated with FC 1211, isoproterenol 1.0×10^{-6} M, FC 1211-isoproterenol 1.0×10^{-7} M, and FC 1211-isoproterenol 1.0×10^{-6} M contained concentrations of cAMP which were significantly greater than those of the control tissues. Figure 26 depicts variation in cAMP concentration among the 9 treatment groups. Cyclic AMP concentrations in fibers exposed to atenolol alone, or in combination with isoproterenol, FC 1211, or both, were not statistically different from the control. Cyclic AMP concentrations did not correlate well with any of the electrophysiologic parameters ($r<0.5$). Cyclic GMP concentrations and cAMP:cGMP ratios during treatments showed no significant differences from control (these two parameters were not measured during atenolol experiments).

Table 14 contains a summary of the effects of treatments on electrophysiologic and cyclic nucleotide parameters.

RESULTS

Steady State Determination of FC 1211

The blood concentrations of FC 1211 related to time in an anesthetized dog are shown in Table 15. The increase in blood FC 1211 concentrations correlates directly with the increase in FC 1211 inhalation percentage. This increase represents the ability of FC 1211 to freely pass aleveolar membranes and to be transported in the peripheral blood stream. Upon withdrawal of the inspired FC 1211, blood concentrations fell quickly. The disappearance of FC 1211 from the blood also followed a concentration time dependant relationship with the 2% level requiring 20 minutes to be removed and the 10% level over 60 minutes to be completely eliminated through the lungs.

At the 2% and 5% inhalation levels, steady state was reached within 10 minutes. At the 10% inhalation level, blood concentrations of FC 1211 continued to rise throughout the 60 minute period, showing no true steady statetime. This effect has been related to the blood-tissue solubility coefficient of FC 1211. At the 10% inhalation level saturation of lipid containing tissue was not complete within 60 minutes. A true saturation-equilibrium between blood and tissue was therefore not reached during this period of inhalation.

Conscious Mobile Inhalation Experiments

In three preliminary experiments each dog inhaled one of three concentrations of FC 1211 (2%, 5% or 20%). Within 30 seconds at the 20% level, 1 minute at 5%, and 2 minutes at the 2% inhalation concentration animals experienced severe convulsive seizures. Due to the intensity of the seizures, EKG's were impossible to record. In an attempt to find an anticonvulsant agent to control these seizures, several compounds were tested. Valium at a dose of 10 mg per animal or 20 mg per animal of Rompun did not induce the necessary anticonvulsant state. However, 15 mg/kg phenobarbital was found to depress seizure activity enough to allow proper EKG recording. At this dose of phenobarbital, slight tremors were still apparent in the experimental animals, but did not interfere with recorded tracings. Therefore, all animals tested in the following experiments were pretreated with this dose (15 mg/kg) of phenobarbital.

Table 15 summarizes the results of 10 dogs breathing FC 1211 for 10 minutes. Parameters measured included sinus rate, blood FC 1211 concentration and EKG activity. At all levels of FC 1211 inhalation (2%, 5%, 10% and 20%) an increase in sinus rate was apparent. Blood concentrations of FC 1211 were also significantly different (P less than 0.01) between each of the four inhalation concentrations. Changes in EKG activity were minor except in one case. One dog breathing 5% FC 1211 for only 1 minute experienced an episode of sinus tachycardia with right bundle branch block (Figure 27). The blood level of FC 1211 in this dog at the time of the arrhythmogenic episode was 43.23 µg/ml. Excitation and tremors were apparent just prior to the onset of the arrhythmia which may have been induced due to a surge of endogenous catecholamines being

released or an increase in sympathetic tone of the heart. All other dogs experienced only minor EKG changes while breathing any concentration of FC 1211. The majority of these changes included either an increase or decrease in the amplitude of the T wave (Fig. 28). Changes in T wave amplitude are related to ischemia of the heart which may have been induced by a vascoconstrictor effect of FC 1211, or by a hypoxia effect.

EEG recordings (Fig. 29) displayed a slight decrease in amplitude after breathing concentrations of 2%, 5% and 10% FC 1211. No changes in pattern frequency was noted. At the 20% inhalation level EEG amplitude was also depressed and episodes of burst suppression were evident approximately 2 minutes after exposure to this level. It was noted that tremors in the animals followed these EEG aberrations, and occurred approximately 50 seconds apart. By the end of the 20% inhalation (10 minutes) EEG frequency and amplitude were greatly depressed with bursts of activity now occurring 90 seconds apart. At 30% inhalation of FC 1211 EEG activity remained depressed. After 4 minutes at this level, several bursts of activity were noted occurring only 15 seconds apart, followed by loss of EEG activity and death of the animal. As FC 1211 inhalation concentration increased, EKG tracings of this same animal showed a progressive increase in T and R wave amplitude.

The final experiment in these dogs was to see the effect of noise and stroboscopic lights on EEG and EKG patterns (Fig. 30 and 31) of a dog breathing 20% FC 1211 for 10 minutes. This excitation of the animal might increase release of endogenous catecholamines which would effect the normal sinus EKG patterns. In both cases (noise and lights) an increase in EEG activity was elicited showing the functional ability of

the central nervous system to be stimulated. However, changes in EKG tracings were not apparent including sinus rate. Therefore, it is not possible to conclude if endogenous catecholamine levels had been affected.

FC 1211 Sensitization

Isoproterenol induced ventricular arrhythmias in 6 of 9 dogs breathing 100% oxygen. EKG aberrations included isolated premature ventricular contractions in 5 dogs, and ventricular tachycardia in the last (Fig. 32). The threshold dose of isoproterenol producing arrhythmias was 2.66 $\pm$ 2.33 µg/kg. Three dogs tested showed no cardiac aberrant rhythms even at the end-point dose of 10 mg/kg isoproterenol. Unpublished experiments from this laboratory illustrated that if arrhythmias were not produced at 10 µg/kg isoproterenol increasing the challenge dose to as high as 40 µg/kg still would not cause the production of cardiac aberrations. This may be due to the effect of isoproterenol on blood pressure. Large doses decrease vascular tone resulting in vasodilatation. Therefore, by greatly decreasing vascular pressure they actually protect the heart from producing spontaneous ventricular arrhythmias.

Inhalation of FC 1211 at all concentrations (2%, 5% and 10%) significantly lowered (P less than 0.05) the threshold dose of isoproterenol needed to induce arrhythmias (Table 17). Threshold doses were .057 $\pm$ 053 µg/kg at the 2% level, .044 $\pm$ .038 µg/kg at 5% and .075 $\pm$ .062 µg/kg at the 10% inhalation level. Cardiac aberrations included those seen in the control experiments (isolated premature ventricular contractions and ventricular tachycardia) as well as one episode of multiple ventricular tachycardia and several cases of bigeminy (Fig. 33). The mean blood

concentration of FC 1211 at the three inhalation levels was 40.0 ± 3.80 µg/ml, 87.0 ± 8.21 µg/ml and 115.1 ± 24.4 µg/ml, respectively. Blood concentration of FC 1211 was significantly different between each of the three inhalation concentrations, while the arrhythmogenic threshold dose of isoproterenol showed no statistical difference among the three. Additionally, all three inhalation concentrations of FC 1211 increased sinus rate significantly (P less than 0.01) when compared to oxygen control. However there was no significant difference in heart rate among the three concentrations of FC 1211 when compared to each other.

Atenolol Dose

A dose of 6 mg/kg atenolol was shown to produce B_1 adrenergic blockade (Table 18). Table 18A depicts an arithmetic increase in atenolol dose given to one control dog (immobilized with pancuronium bromide). Each dose was challenged with 3 µg/kg isoproterenol (approximate mean arrhythmogenic dose of isoproterenol in control animals). An absence in sinus rate alteration upon the isoproterenol challenge was considered to demonstrate a B_1 blockade. The dose of atenolol causing this effect was between 4 mg/kg and 8 mg/kg. A second control dog was then given 6 mg/kg atenolol and challenged with the same dose of isoproterenol. Table 18B demonstrates that this dose of atenolol blocked an increase in sinus rate when the animal was challenged with isoproterenol. Therefore, this dose (6 mg/kg atenolol) produced a B_1 blockade adequate for the following experiments. In both of the atenolol dose determining experiments, decrease in vascular pressure upon the isoproterenol challenge was apparent. This was expected since atenolol

is a pure B_1 antagonist and therefore does not block the adrenergic receptors (B_2) of the vascular system from the effects of isoproterenol.

B_1 Adrenergic Receptor Blockade

Table 19 lists all measured parameters in the atenolol blocked dogs both before and after the isoproterenol challenge (1 μg/kg), and while breathing 2%, 5%, 10% and 20% FC 1211 mixtures. While inhaling any of the four FC 1211 concentrations, and before the administration of the isoproterenol challenge, no significant increase in sinus rate when compared to controls was demonstrated. However, an effect on blood pressure was apparent at all inspired levels of FC 1211. Both systolic and diastolic pressure decreased significantly (P less than 0.05) at the 2% and 5% level, and (P less than 0.01) at the 10% and 20% inhalation concentration. Blood concentrations of FC 1211 reached levels seen in other experiments (steady state determination, and FC 1211 sensitization) and were significantly increased (P less than 0.01) between each of the four inhalation levels of FC 1211.

When challenged with isoproterenol many changes were apparent when compared to each FC 1211 level before the isoproterenol administration. At 2% inhalation level, no change in sinus rate was noted upon the isoproterenol challenge, but both the systolic and diastolic pressure parameters decreased significantly (P less than 0.01). At all other FC 1211 inhalation concentrations (5%, 10% and 20%) increase in sinus rate and decrease in both pressure parameters were statistically significant (P less than 0.01) after the administration of the isoproterenol challenge.

The last column of Table 19 lists the aberrant changes in EKG's seen while breathing each of the four FC 1211 concentrations and following the isoproterenol challenge. At 2% inspired FC 1211 no arrhythmias occurred, and at 5% FC 1211 only one of six dogs elicited an aberrant rhythm. At the higher inhalation concentrations, the sensitization property of FC 1211 was again apparent with all but one dog developing ventricular arrhythmias at the 10% level, and three out of six at the 20% level. Arrhythmias noted here included three cases of isolated premature ventricuular contractions, four runs of multifocal ventricular ectopic beats, three episodes of bigeminy and one case of ventricular tachycardia. As table 19 displays, one dog showed a progressive change in the type of arrhythmia elicited, proceeding from bigeminy to multifocal ventricular ectopic beats and finally ventricular tachycardia.

Comparison of the arrhythmogenic effect of FC 1211 and isoproterenol during B_1 adrenergic blockade with that of the FC 1211 sensitization experiments where adrenergic blockade was not present demonstrates significant changes (Table 20). At the 2% and 5% inhalation level the arrhythmogenic sensitization property of FC 1211 was significantly blocked with the presence of the B_1 blockade (P less than 0.01 at 2% and P less than 0.05 at 5% FC 1211). Increasing the inhalation level to 10% FC 1211 in these atenolol pretreated animals caused no significant changes in the frequency of aberrant rhythms displayed as compared to the same level of the sensitization experiments. Apparently, at this higher level of FC 1211, the atenolol blockade of the B_1 receptor was overcome far enough to elicit the same response as seen in the nonblocked animals. While no sensitization experiment were performed at the 20% FC

1211 inhalation level, comparison of the arrhythmogenic frequency at this level with that seen at the 10% level (during β_1 blockade in both) shows no significant difference. This observation suggests that the inhalation of 20% FC 1211 also overcame the protection against arrhythmias produced by atenolol.

FIGURE LEGENDS

Figure 5--Control--proximal (upper) and distal (lower) action potentials; 1211 (bromochlorodifluoromethane) at flows of 20, 40, 70 and 115 ml/min producing concentrations of 50, 100, 150 and 200 µg/ml, respectively.

Figure 6--(A) Control; (B, C, D) dichlorodifluoromethane at concentrations of 20, 40, 80 µg/ml, respectively; (E) dichlorodifluoromethane washout.

Figure 7--Control--proximal (upper) and distal (lower) action potentials; 10^{-7} M isoproterenol; isoproterenol washout; bromochlorodifluoromethane alone at subthreshold concentration; combined effects of same concentrations of each agent.

Figure 8--(A) Control; (B) 10^{-7} M isoproterenol; (C) 10^{-6} M isoproterenol; (D) isoproterenol washout; (E) dichlorodifluoromethane at 40 µg/ml; (F) 10^{-7} M isoproterenol and dichlorodifluoromethane at 40 µg/ml; (G) 10^{-6} M isoproterenol and dichlorodifluoromethane at 40 µg/ml.

Figure 9--(A) Control; (B) PO_2 at 200 mmHg; (C) PO_2 at 100 mmHg; (D) PO_2 at 200 mmHg and dichlorodifluroromethane at 80 µg/ml; (E) PO_2 at 100 mmHg and dichlorodifluoromethane at 80 µg/ml; (F) PO_2 at 100 mmHg, dichlorodifluoromethane at 80 µg/ml and isoproterenol at 10-7 M.

Figure 10--(A) 2.0 mM K^+; (B) 4.0 mM K^+ (control); (C) 8.0 mM K^+; bromochlorodifluoromethane and (D) 2.0 mM K^+, (E) 4.0 mM K^+, (F) 8.0 mM K^+; 10^{-7} M isoproterenol and (G) 2.0 mM K^+, (H) 4.0 mM K^+, (I) 8.0 mM K^+ (J) 8.0 mM K^+, 10^{-7} M isoproterenol and bromochlorodifluoromethane.

Figure 11--(A) Control; (B) 10^{-6} M isoproterenol; (C) 10^{-4} M aminophylline; (D) 10^{-4} M aminophylline and dichlorodifluoromethane; (E) 10^{-4} M aminophylline and 10^{-6} M isoproterenol; (F) 10^{-4} M aminophylline and 10^{-6} M isoproterenol; (G) 10^{-4} M aminophylline, and dichlorodifluoromethane.

Figure 12--(A) Control; (B) 16.0 mM K^+ (C) 16.0 mM K^+ and 5.4 mM Ca^{++}; (D) $3X10^{-7}$ M isoproterenol; (E) dichlorodifluoromethane; (F) $3X10^{-7}$ M isoproterenol and dichlorodifluoromethane.

Figure 13--Fiber stretch (A, B) control; (C, D) 20% stretch; (E, F) 20% stretch plus 1211 at flow of 20 ml/min; (G, H) 20% stretch plus 1211 at flow of 40 ml/min; (I) 20% stretch plus 1211 at flow of 60 ml/min.

Figure 14--Phenylephrine and 1211. (A) control; (B, C, D) phenylephrine at 10^{-9} M, 10^{-8} M and 10^{-7} M; (E) control after washout, (F) 1211 at flow of 40 ml/min; (G, H, I) 1211 at flow of 40 ml/min plus phenylephrine at 10^{-9} M, 10^{-8} M and 10^{-7} M; (J) washout of 1211 and phenylephrine.

Figure 15--cAMP and 1211. (A, B) control; (C, D) $3X10^{-4}$ M dibutyryl cAMP; (E, F) $3X10^{-3}$ M dibutyryl cAMP; (G, H) 1211 at flow of 20 ml/min plus dibutyryl cAMP at $3X10^{-3}$ M; (I, J) 1211 at flow of 40 ml/min plus dibutyryl cAMP at $3X10^{-3}$ M; (K) 1211 at flow of 60 ml/min plus dibutyryl cAMP at $3X10^{-3}$ M; (L) washout.

Figure 16--Effects of 1211 on slow channel AP sequential additions. (A) Control; (B) depolarization by 16 mM K^+; (C) elevated CA^{++}, 5.4 mM; (D) 10^{-7} M isoproterenol; (E) 1211 at flow of 20 ml/min; (F) 1211 at flow of 40 ml/min; (G) 1211 at flow of 60 ml/min; (H) atenolol at 10^{-5} M.

Figure 17--Cardiac sensitization by 5% 1211. (A) control; (B) 1 µg/kg isoproterenol; (C) 3 µg/kg isoproterenol; (D) 5% of 1211; (E, F, G. H) 5% 1211 plus isoproterenol at .01, .02, .04 and .08 µg/kg. ECG is displayed in upper part of each frame and arterial BP in lower.

Figure 18--Effects of 1211 on cardiac rhythm in presence of beta blockade. (A) Control; (B, C, D, E) 1211 at 2, 5, 10 and 20% of inspired air; (F) control plus atenolol, 4 mg/kg; (G, H, I, J) atenolol and 1 µg/kg challenge of isoproterenol at 2, 5, 10 and 20% 1211 in inspired air.

Figure 19--1211 blood superfusion. Upper panel, Purkinje fiber action potential. Lower panel, ECG, left ventricular pressure, arterial pressure. (A) control in blood; (B) 2.5 min. after .8 µg/kg isoproterenol; (C) 5% 1211; (D) 2.5 min after .4 µg/kg isoproterenol in presence of 5% 1211; (E) 2.5 min after .4 µg/kg isoproterenol in presence of 5% 1211 and 4 mg/kg atenolol.

Figure 20--Purkinje fiber action potentials before and after isoproterenol 1.0×10^{-7} M.

A: Control period.
B: Isoproterenol 1.0×10^{-7} M.
C: Control action potential (longer APD) superimposed upon isoproterenol 1.0×10^{-7} M action potential (shorter APD).
D: Control recording of escape time and spontaneous action potentials: at arrow external stimulation of fiber was stopped.
E: Escape time and spontaneous action potentials after isoproterenol 1.0×10^{-7} M.

Figure 21--Purkinje fiber action potentials before and after FC 1211 + isoproterenol.

A: Control escape time and spontaneous action potentials.
B: Escape time and spontaneous action potentials during administration of FC 1211.
C: Escape time and spontaneous action potentials after isoproterenol 1.0×10^{-7} M and continued administration of FC 1211.
D: Control action potential (different Purkinje fiber from A, B, and C).
E: Action potentials during administration of FC 1211.
F: Action potentials after isoproterenol 1.0×10^{-6} M.

Figure 22--Purkinje fiber action potentials before and during FC 1211 administration.

A and B continuous: After 20 minues of FC 1211 administration during the escape time determination. Baseline oscillations grade to action potentials: an occasional observation during FC 1211 administration.

C, D, and E: Are action potentials from a different Purkinje fiber taken before and during FC 1211 administration.

C: Control.

D: Action potential after 20 minutes of FC 1211 administration.

E: Shortening of action potential due to FC 1211: the control action potential (outer edge) gradually shortens in duration while FC 1211 was administered (20 minute time lapse).

Figure 23--Right ventricular Purkinje fiber action potentials before and after atenolol 1.0×10^{-5} M + FC 1211 + isoproterenol 1.0×10^{-6} M.

A: Control.

B: Atenolol 1.0×10^{-5} M.

C: Atenolol 1.0×10^{-5} M. + FC 1211.

D: Atenolol 1.0×10^{-5} M. + FC 1211 + isoproterenol 1.0×10^{-6} M. Shortening during first 2 minutes after addition of isoproterenol 1.0×10^{-6} M.

E: Atenolol 1.0×10^{-5} M + FC 1211 + isoproterenol 1.0×10^{-6} M. Four minutes after addition of isoproterenol: action potential at its shortest duration.

F: Atenolol 1.0×10^{-5} M + FC 1211 + isoproterenol 1.0×10^{-6}. Ten minutes after addition of isoproterenol: slight lengthening of action potential duration from position in E.

Figure 24--Purkinje fiber action potentials before and after atenolol 1.0×10^{-5} M and isoproterenol.

A, B, and C: were recorded from a left ventricular Purkinje fiber.

D, E, and F: were recorded from a right ventricular Purkinje fiber.

A: Control.

B: Atenolol 1.0×10^{-5} M.

C: Atenolol 1.0×10^{-5} M + isoproterenol 1.0×10^{-7} M.

D: Control.

E: Atenolol 1.0×10^{-5} M

F: Atenolol 1.0×10^{-5} M + isoproterenol 1.0×10^{-6} M.

Figure 25--Changes from control in percent and msec units in Purkinje fiber action potential duration after treatments. The following abbreviations were used:

1211 . . . . . FC 1211

Iso 1.0×10^{-6} . . . Isoproterenol 1.0×10^{-6} M

Aten . . . . . Atenolol 1.0×10^{-5} M.

Figure 26--Changes in Purkinje fiber aAMP concentration after treatments (Δ percent and Δ pmol/mg protein), * denotes significance at $p<0.05$.

Figure 27--EKG tracings from the only dog who experienced spontaneous arrhythmias during the inhalation of FC 1211.
Strip A: Control.
Strip B: 5% FC 1211 (1 minute)-sinus tachycardia with right bundle branch block.

Figure 28--Minor EKG changes seen in phenobarbital pretreated dogs inhaling FC 1211.
Strip A: Control.
Strip B: 2% FC 1211 (10 minutes)-increased T wave amplitude.

Figure 29--EEG tracings of a dog inhaling a consecutive series of FC 1211 concentrations.
Strip A: Control.
Strip B: 2% FC 1211 (10 minutes)-decreased amplitude.
Strip C: 5% FC 1211 (10 minutes)-decreased amplitude.
Strip D: 10% FC 1211 (10 minutes)-decreased amplitude.
Strip E: 20% FC 1211 (10 minutes)-decreased amplitude and frequency with burst suppressions.
Strip F: 30% FC 1211 (10 minutes)-decreased amplitude and frequency with burst suppressions.

Figure 30--Simultaneous EKG (top) and EEG (bottom) tracings of a dog stimulated by external noise while inhaling 20% FC 1211 (10 minutes).

Figure 31--Simultaneous EKG (top) and EEG (bottom) tracings of a dog stimulated by stroboscopic lights while inhaling 20% FC 1211 (10 minutes).
Strip A: Initial stimulation.
Strip B: 15 seconds after initial stimulation.

Figure 32--Typical arrhythmias induced by isoproterenol during FC 1211 sensitization experiments.
Strip A: Isolated premature ventricular contractions.
Strip B: Ventricular tachycardia.

Figure 33--Typical arrhythmias induced by isoproterenol during FC 1211 sensitization experiments.
Strip A: Multiple ventricular tachycardia.
Strip B: Bigeminy.

PURKINJE AP - 1211 DOSE RESPONSE

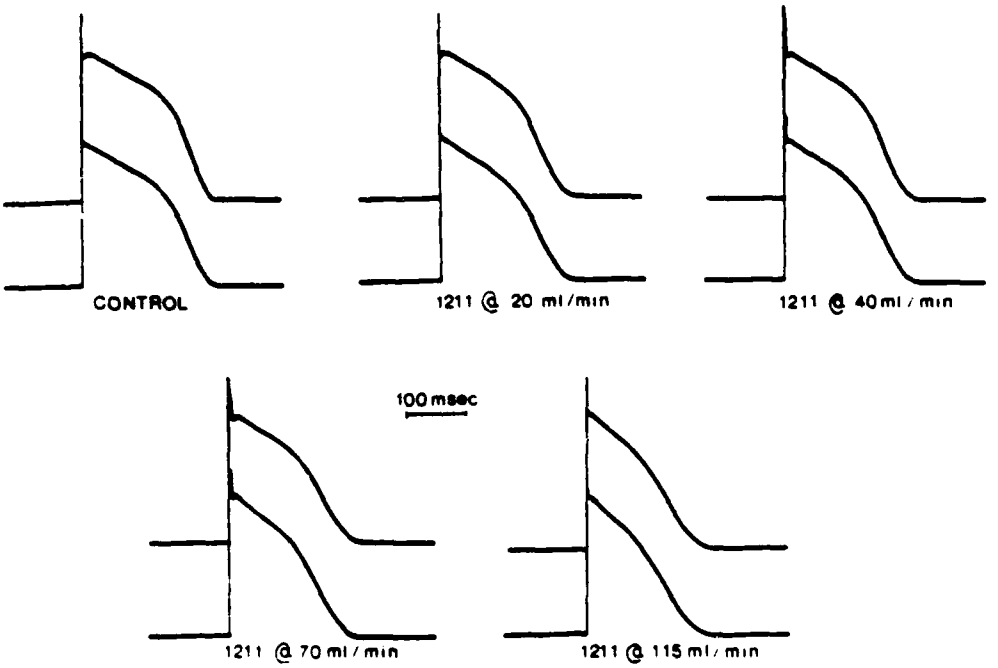


Figure 5

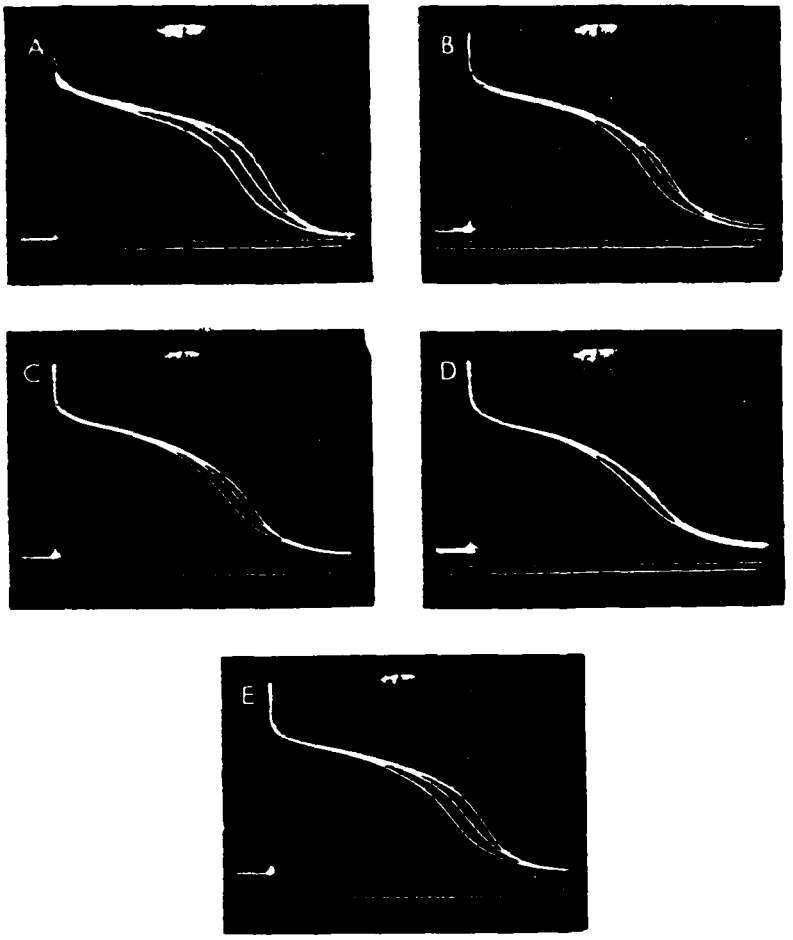


Figure 6

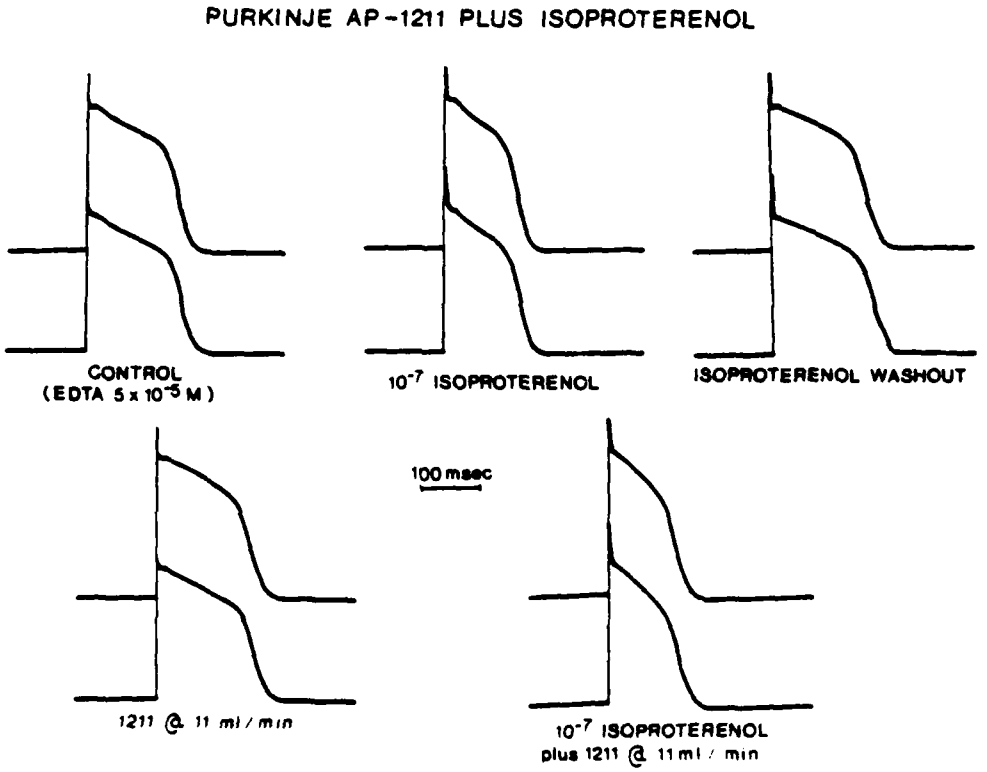
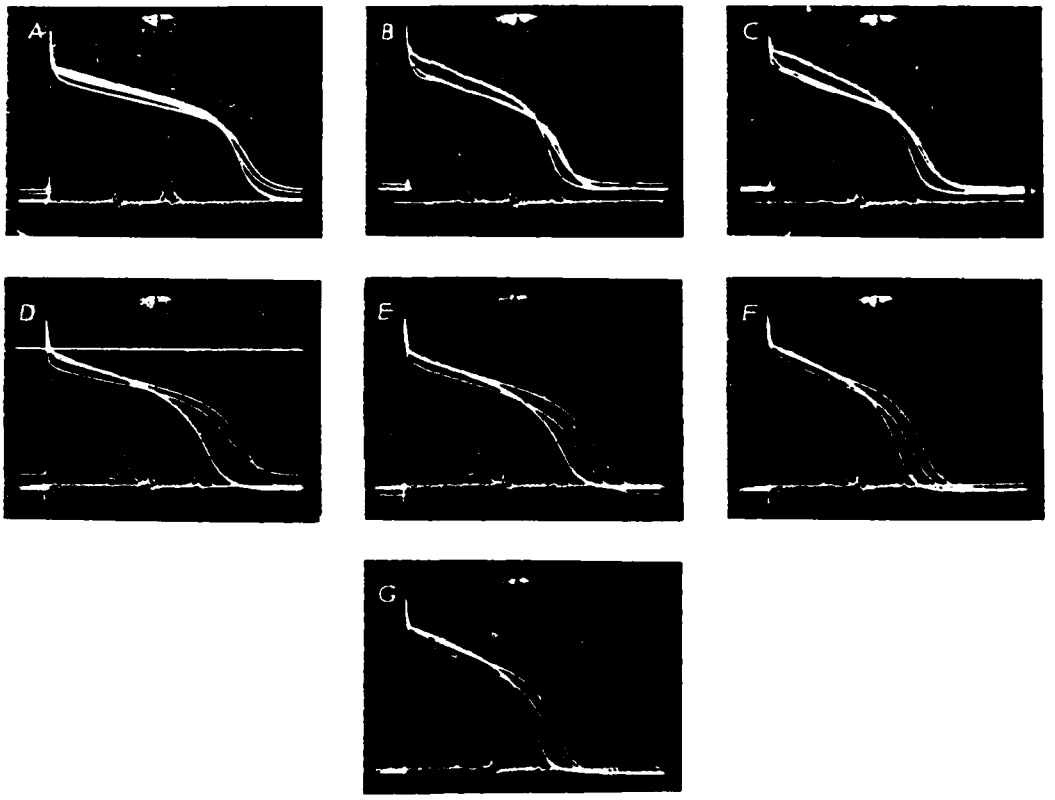

PURKINJE AP-1211 PLUS ISOPROTERENOL
CONTROL
(EDTA 5 x 10⁻⁵ M)
10⁻⁷ ISOPROTERENOL
ISOPROTERENOL WASHOUT
100 msec
1211 @ 11 ml / min
10⁻⁷ ISOPROTERENOL
plus 1211 @ 11 ml / min

Figure 7



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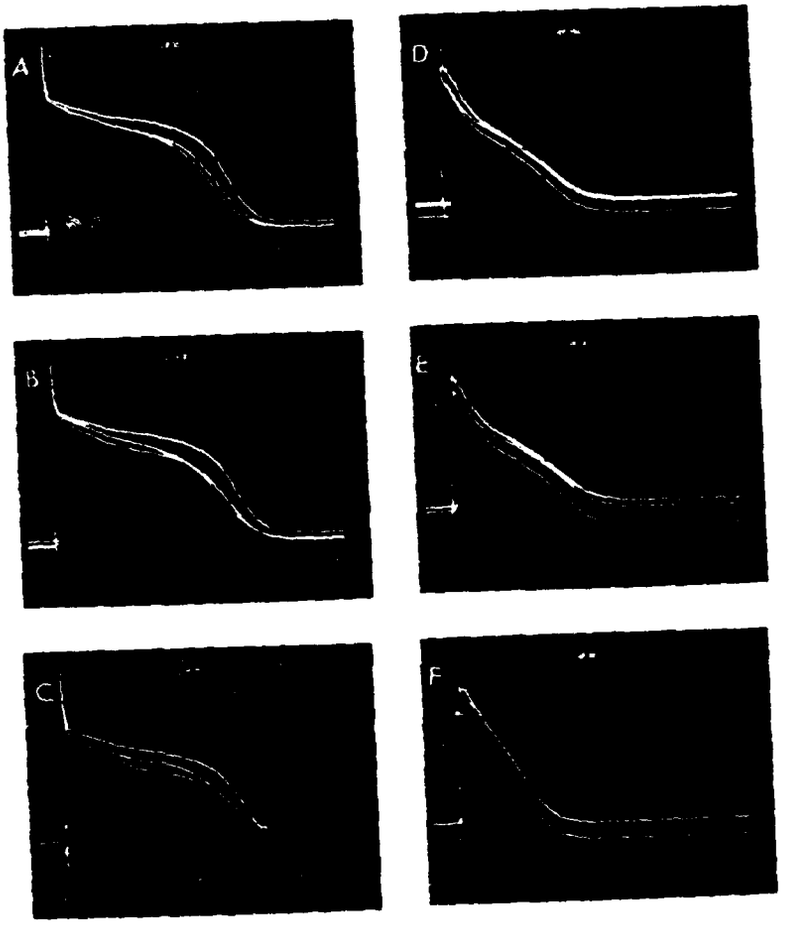
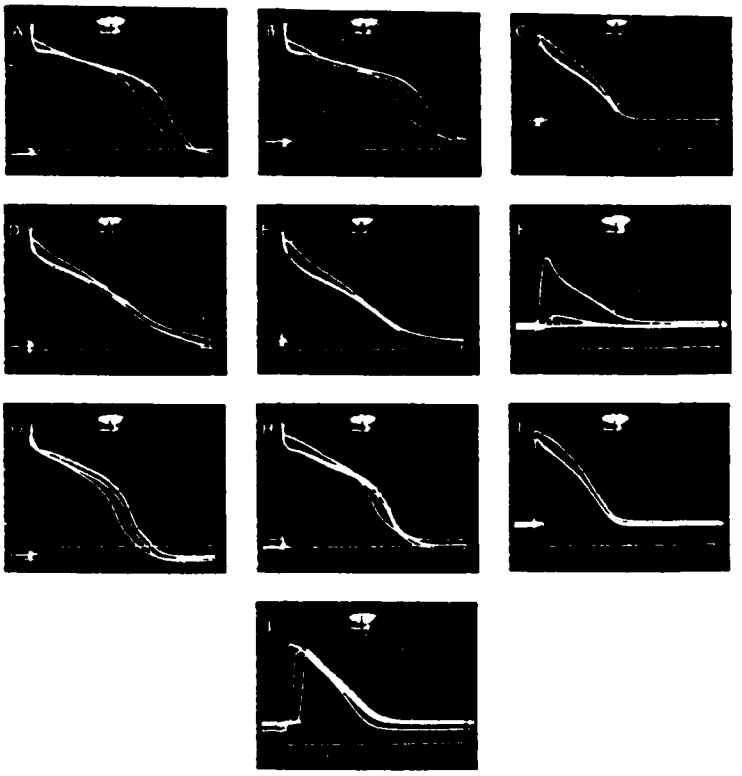


Figure 9



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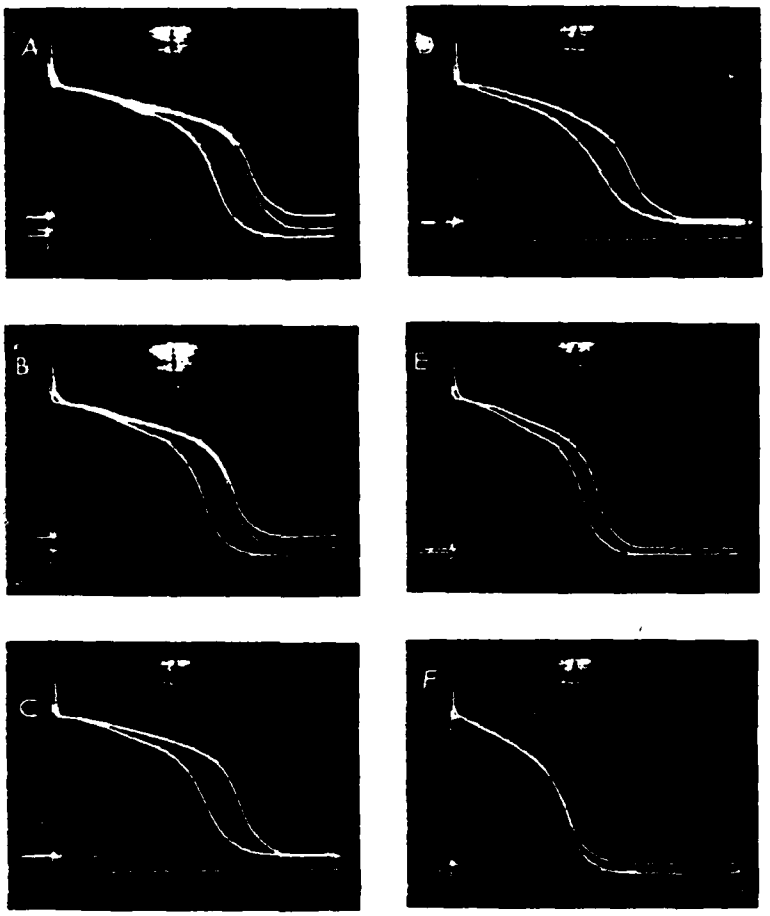
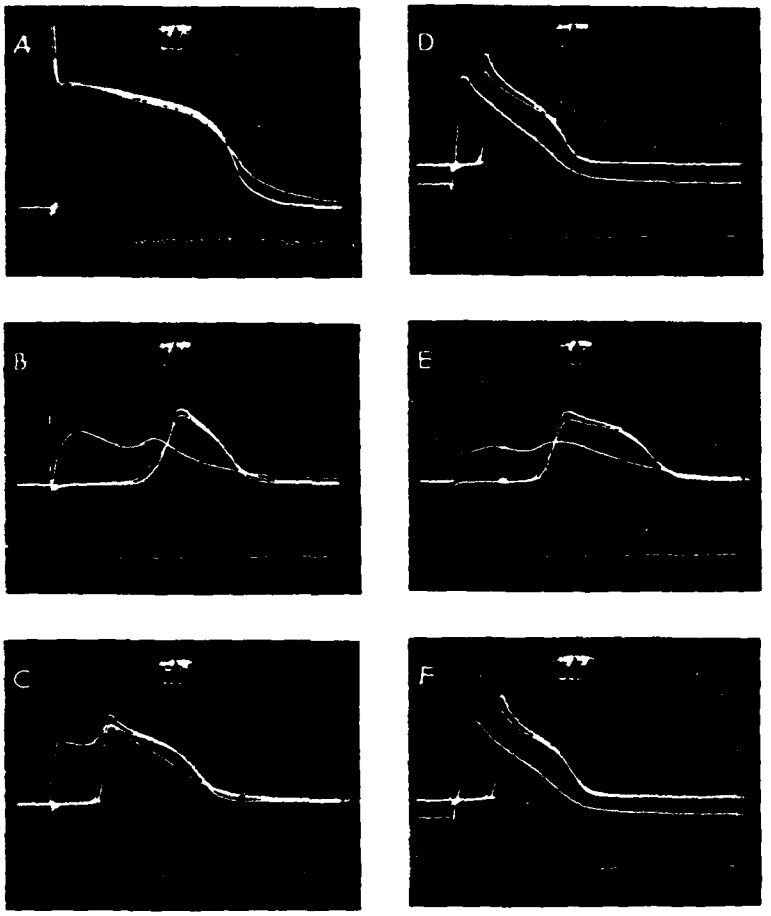


Figure 11



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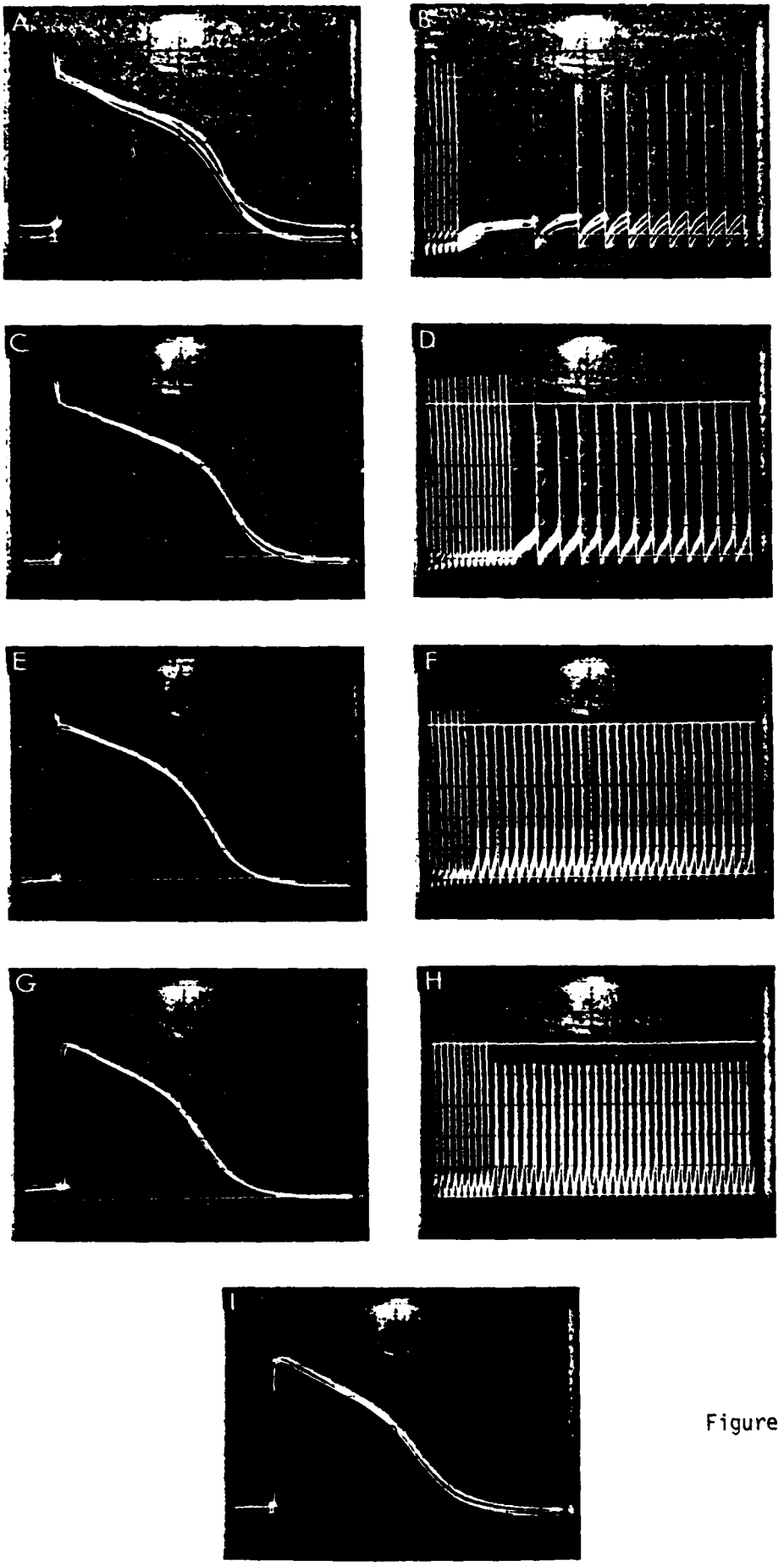


Figure 13

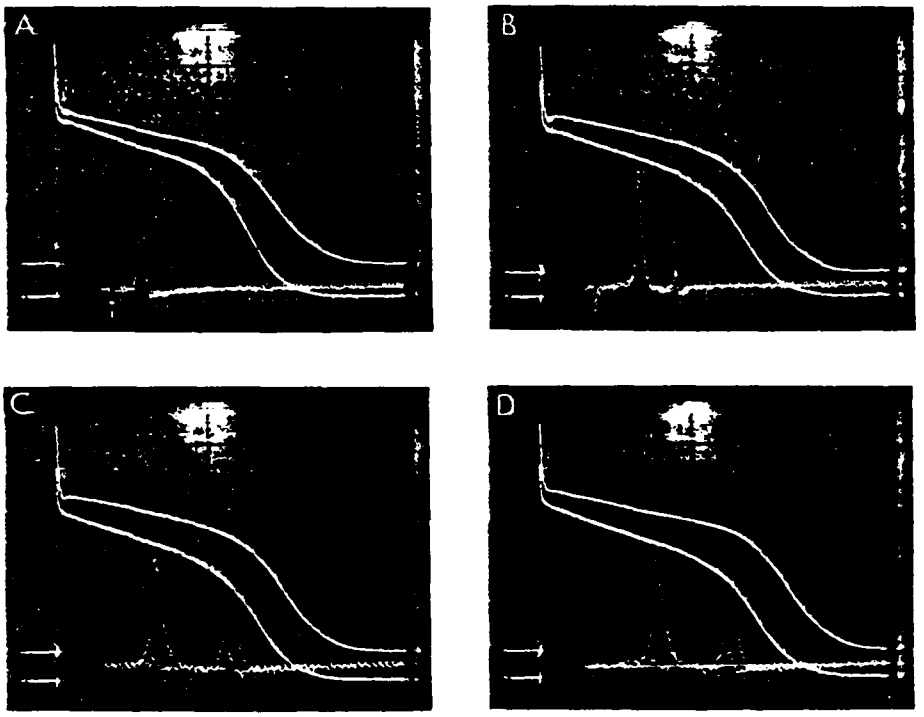


Figure 14

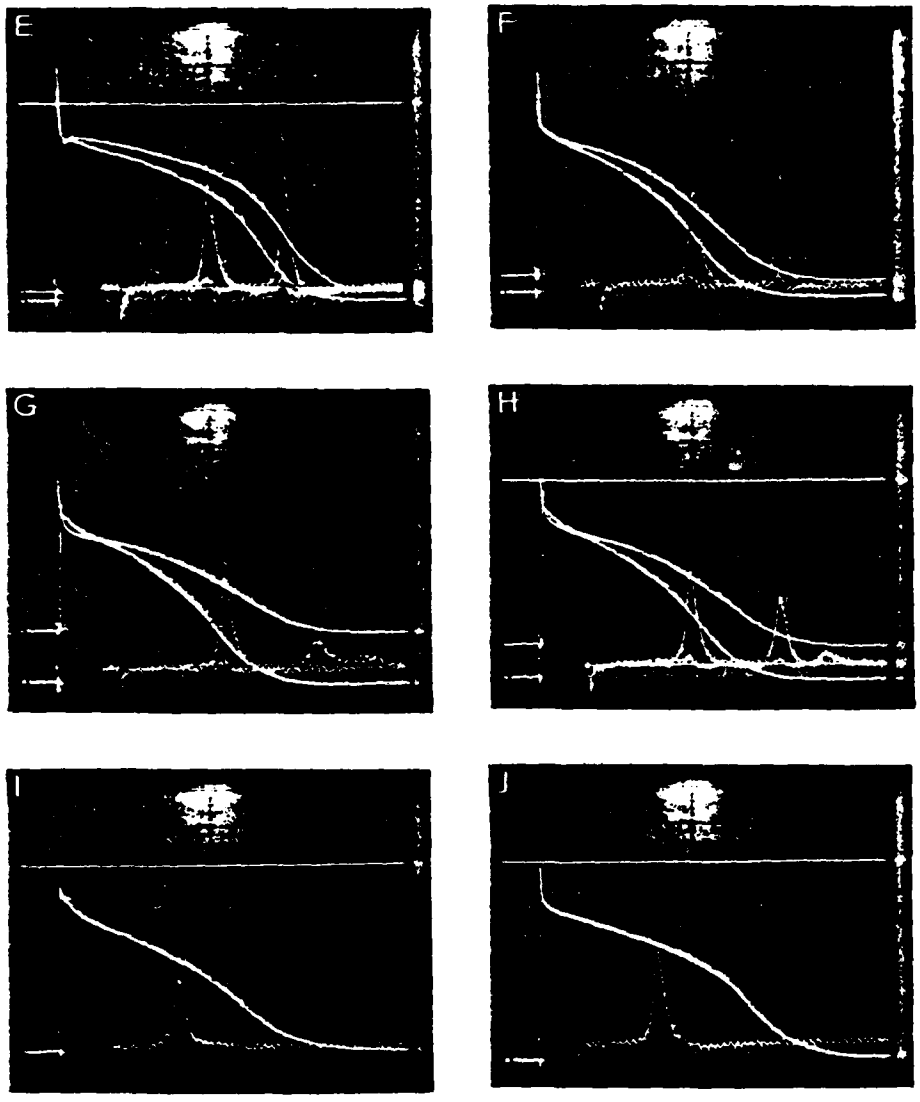


Figure 14--Continued

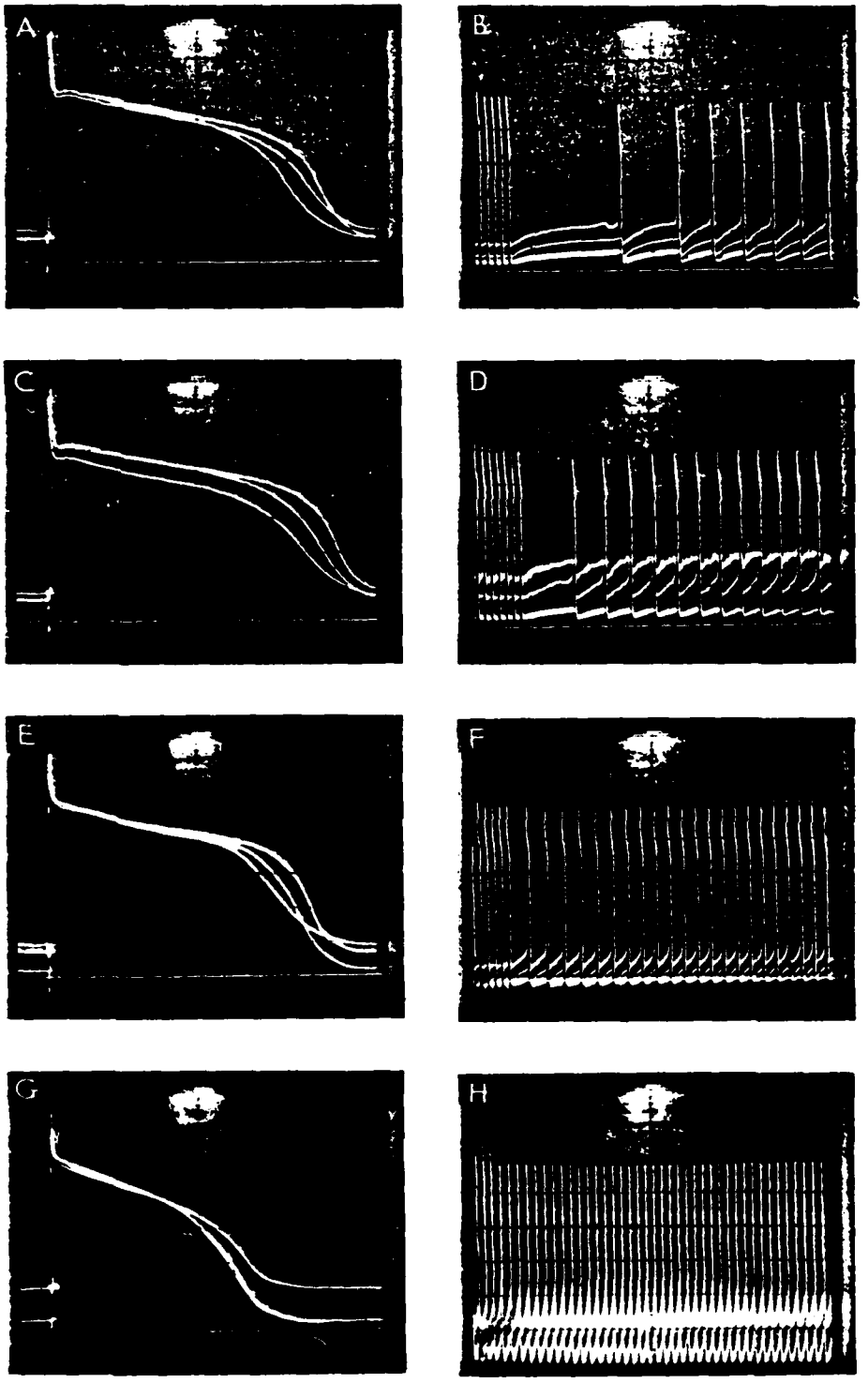
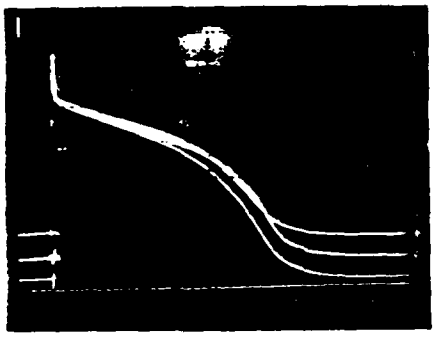
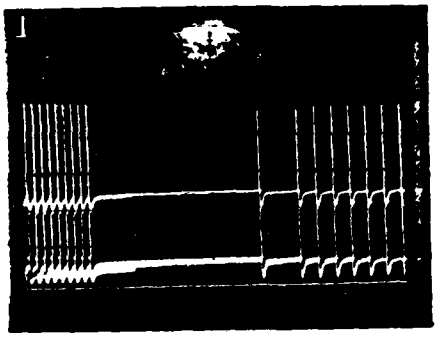
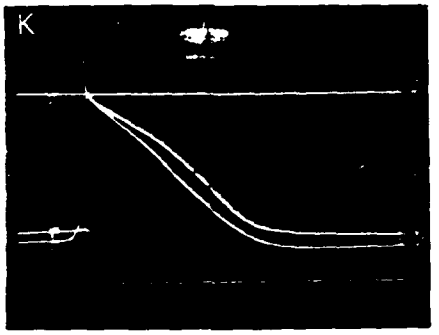
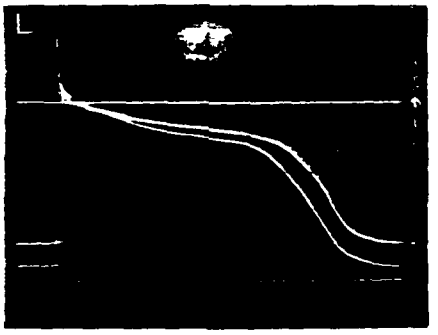


Figure 15









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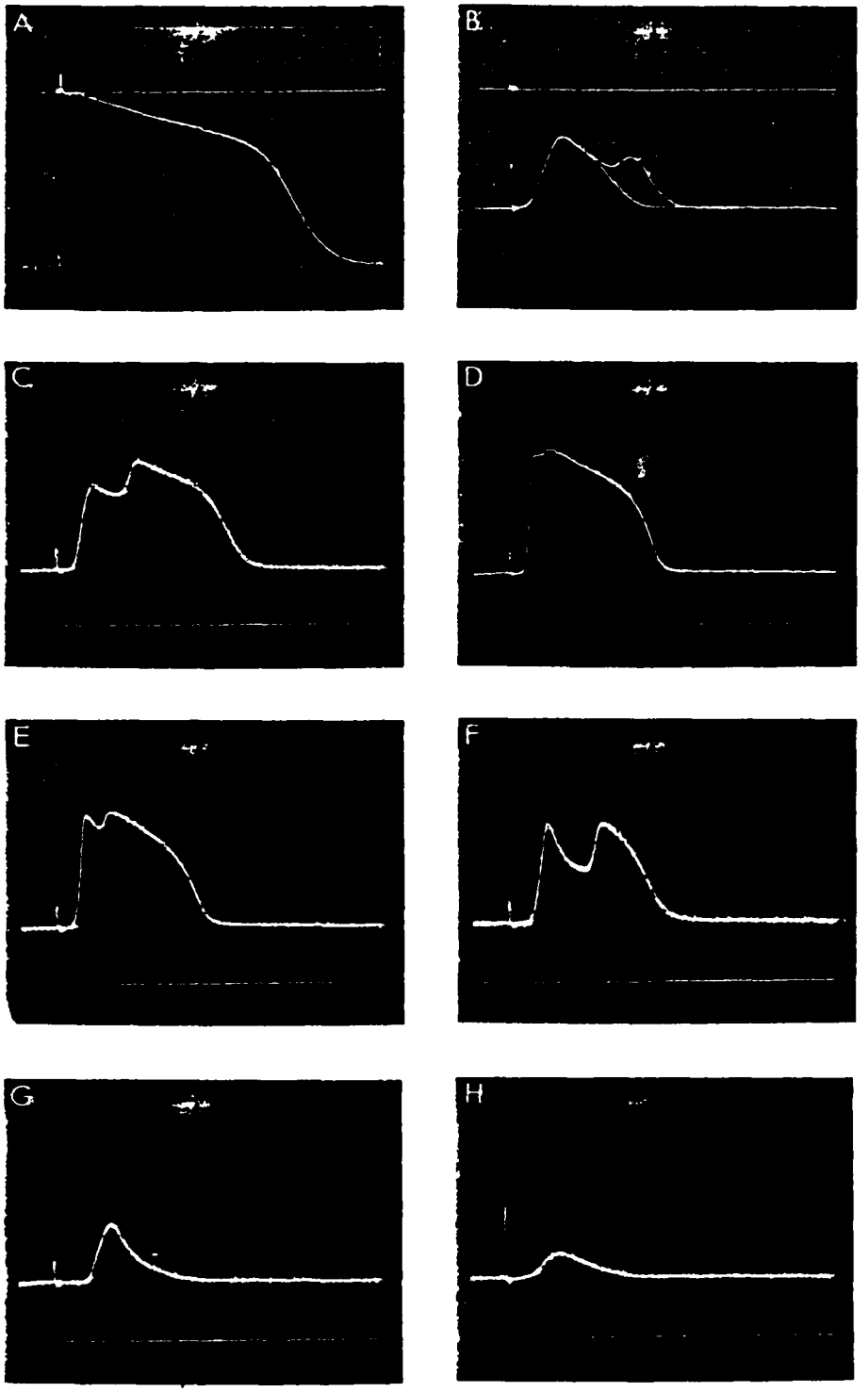
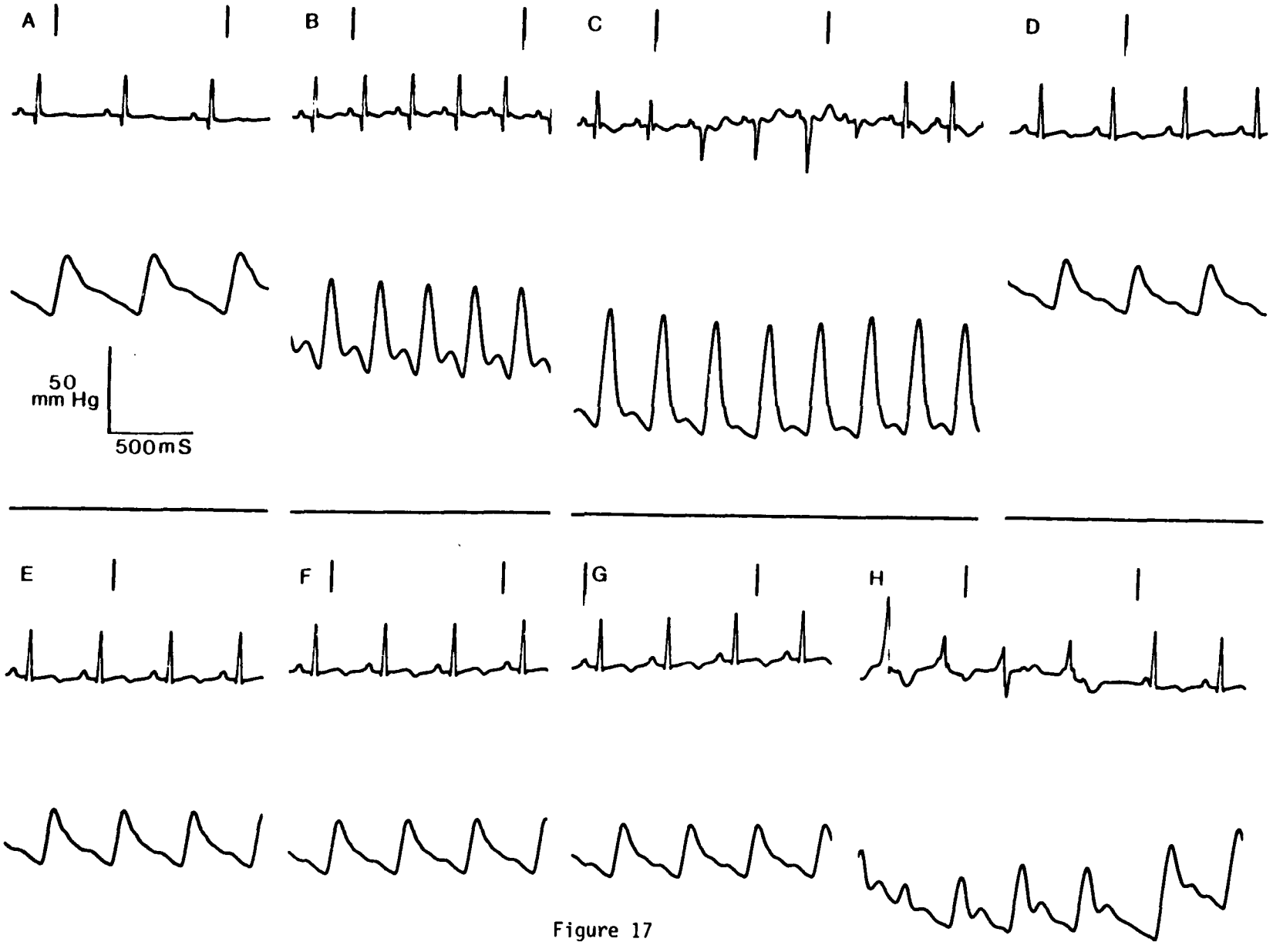


Figure 16



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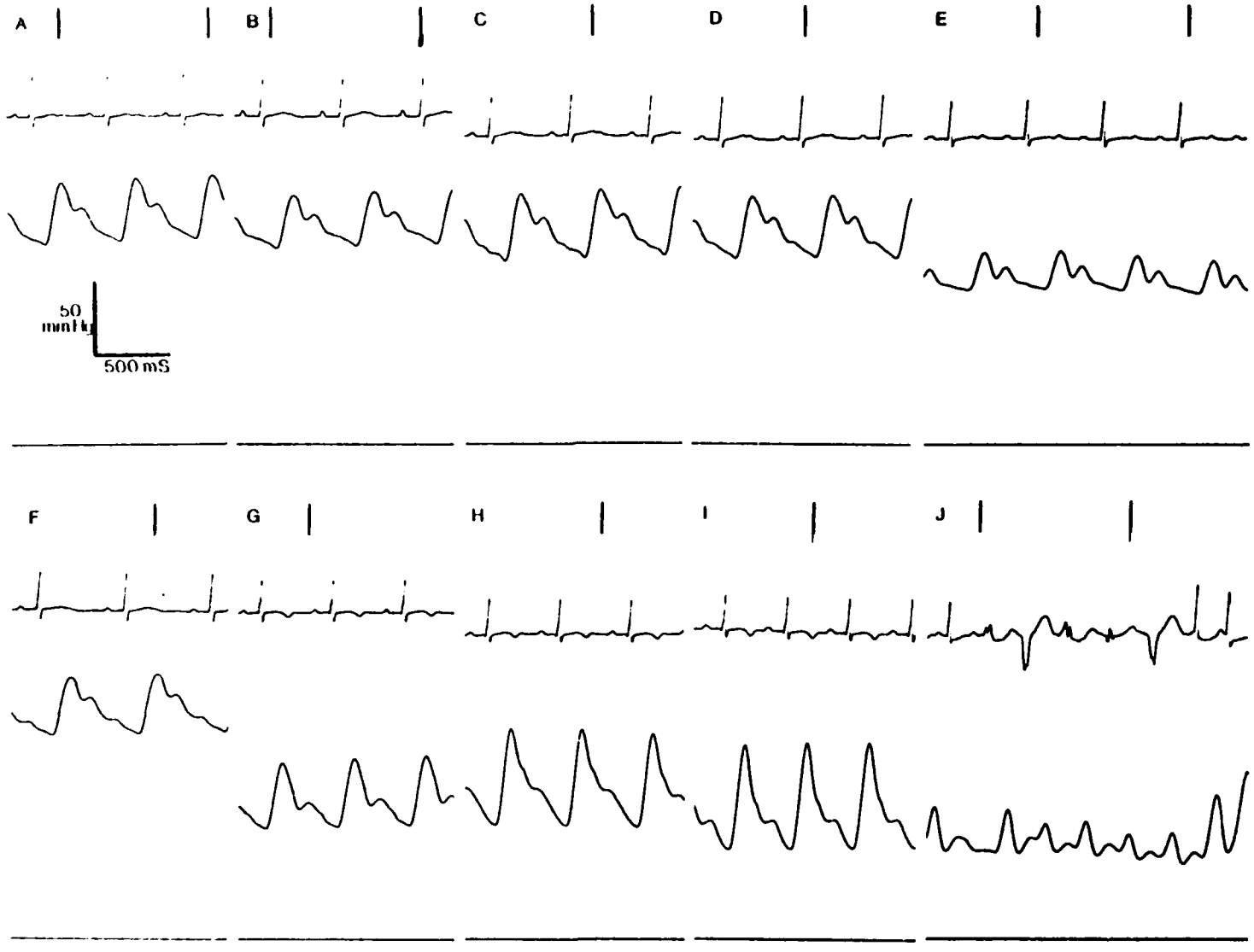
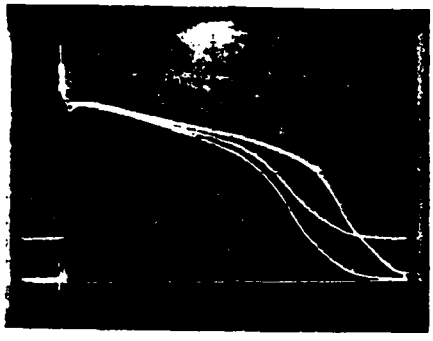


Figure 18

A



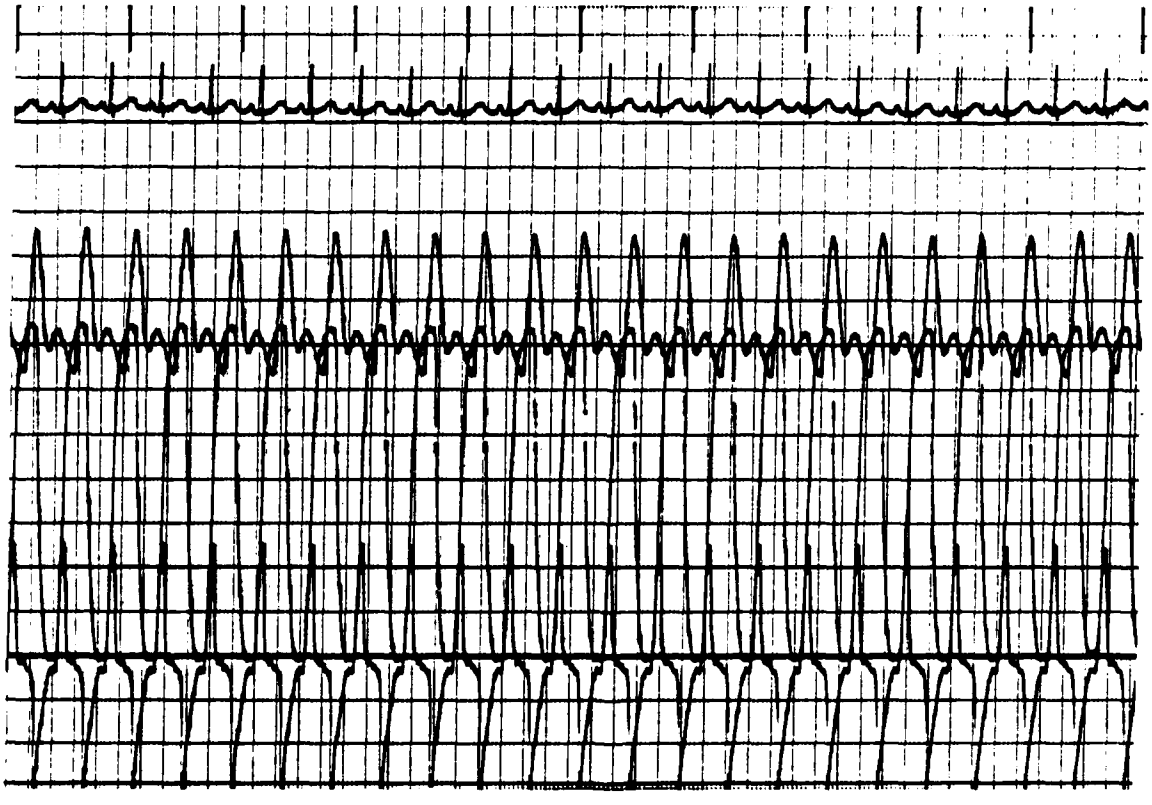
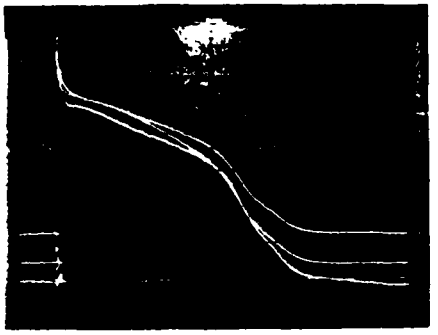


Figure 19

B



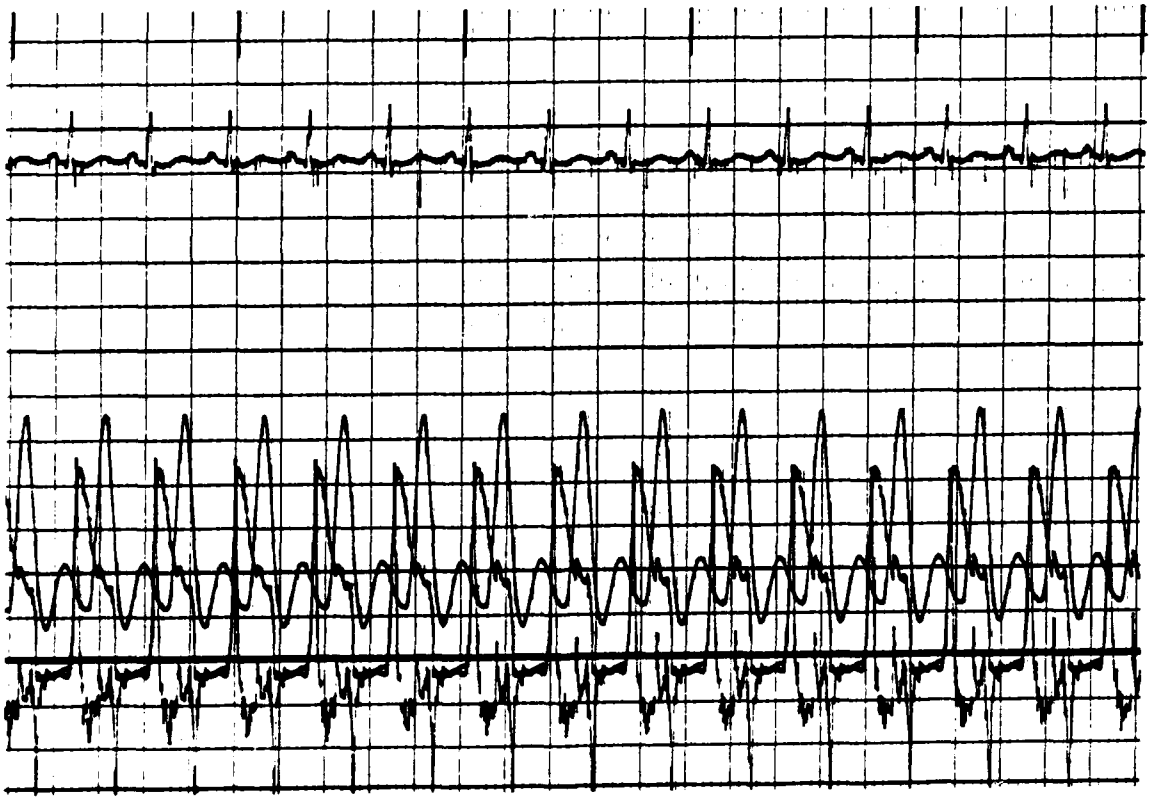


Figure 19--Continued

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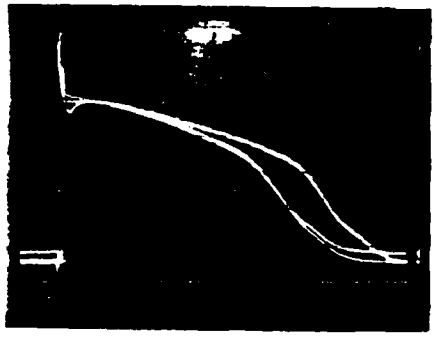

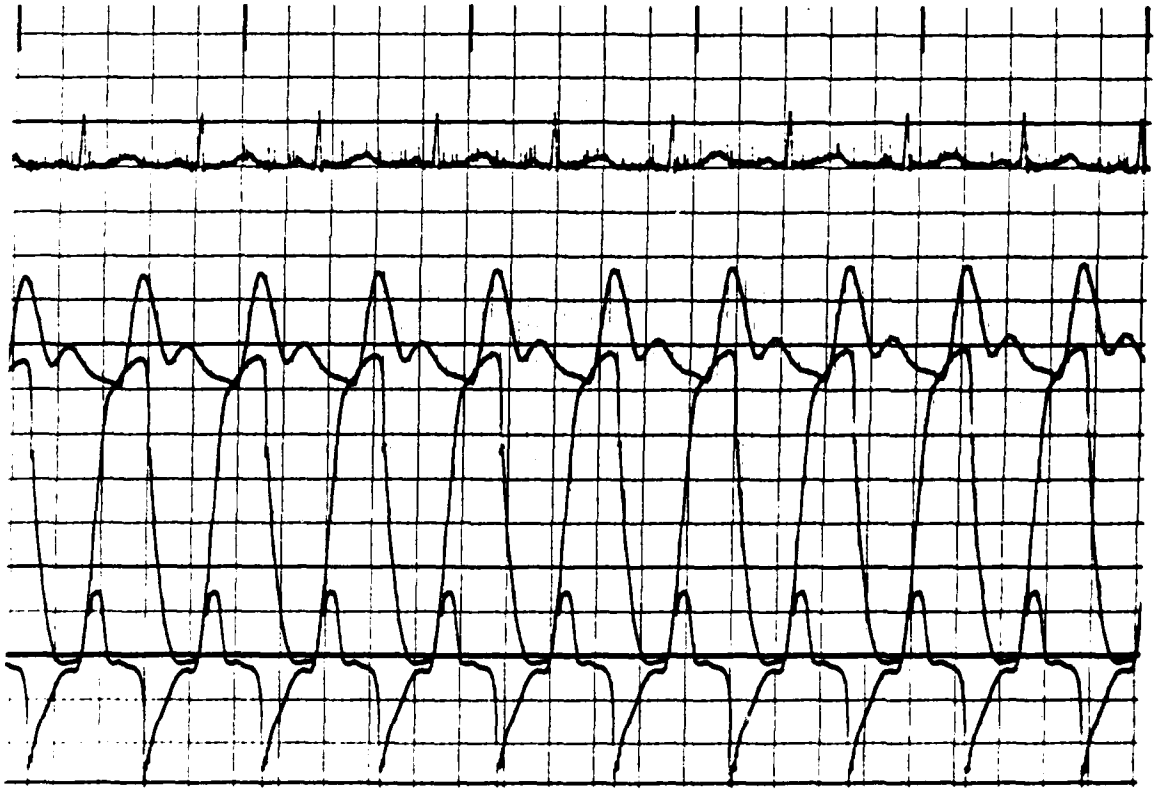

Figure 19--Continued

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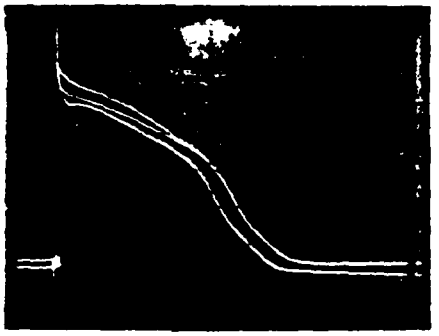


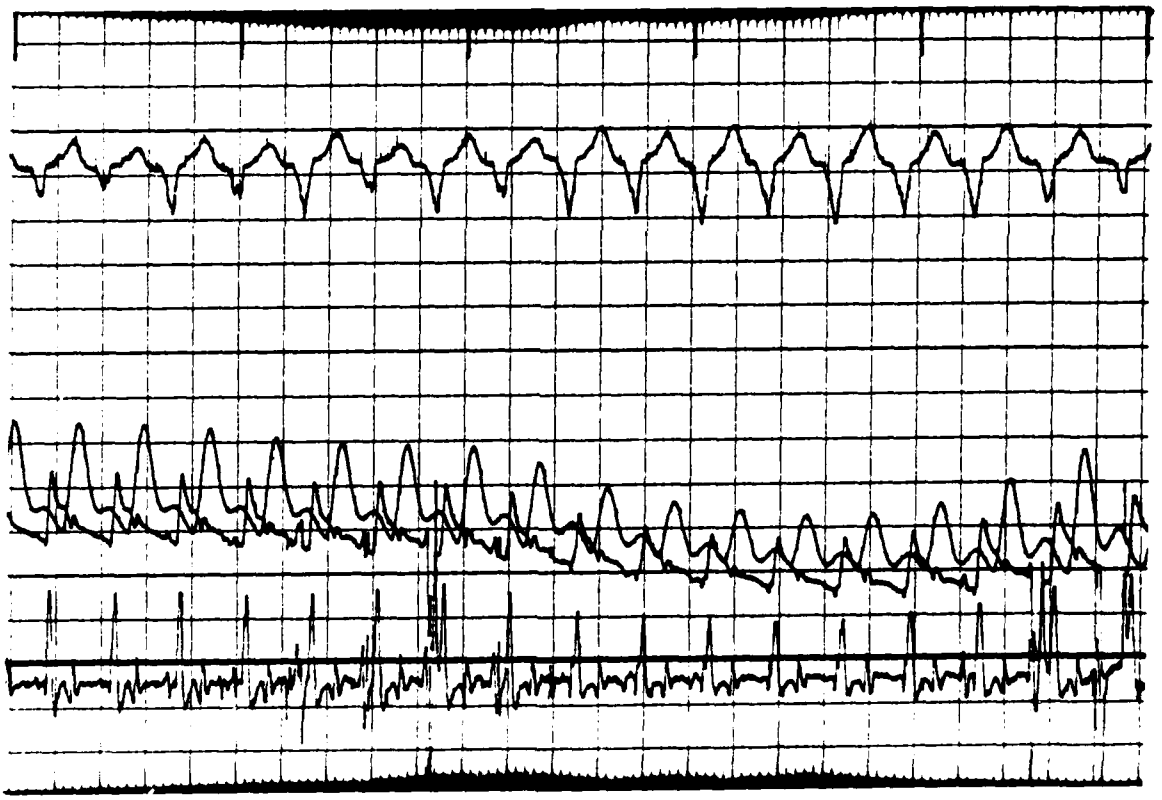
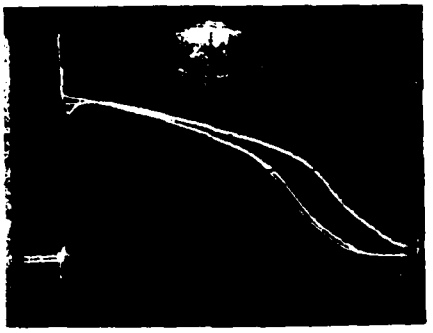
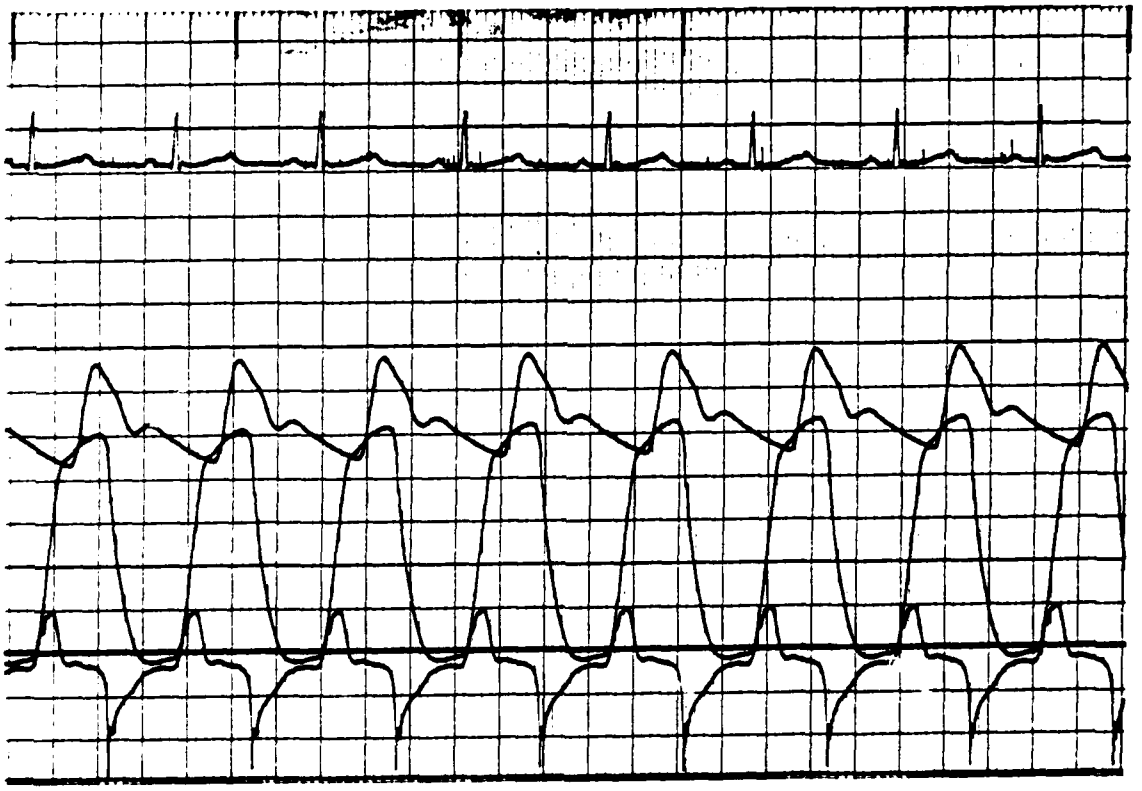


Figure 19--Continued

E





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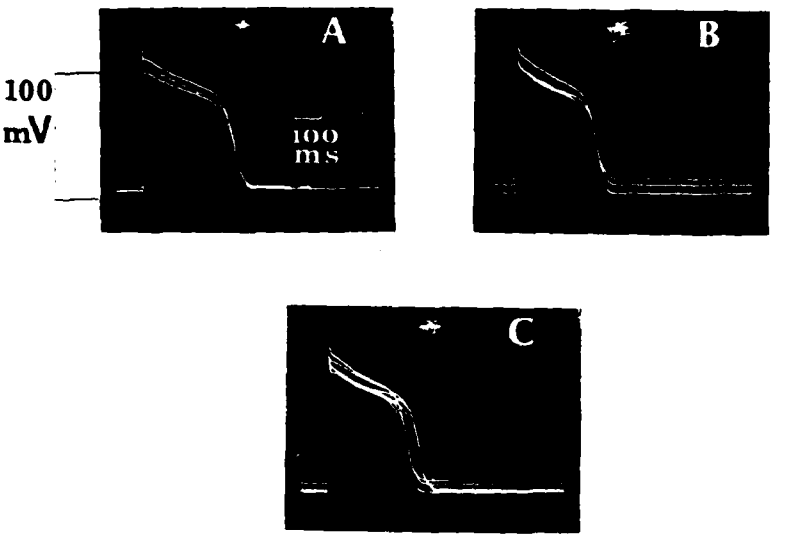

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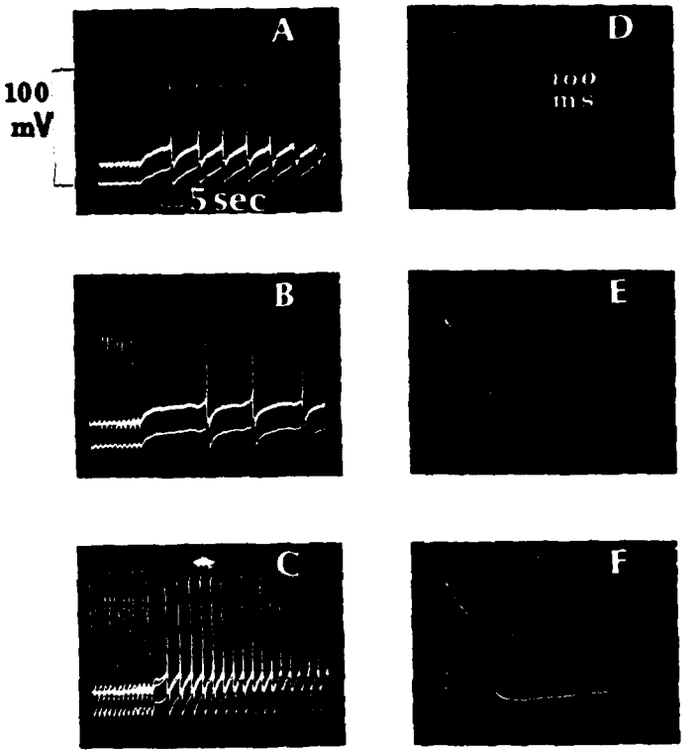


Figure 21

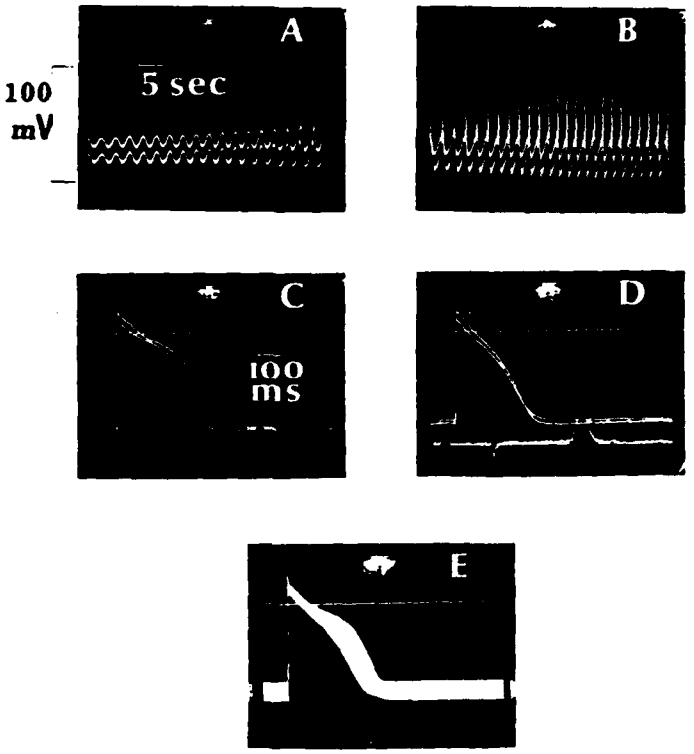


Figure 22

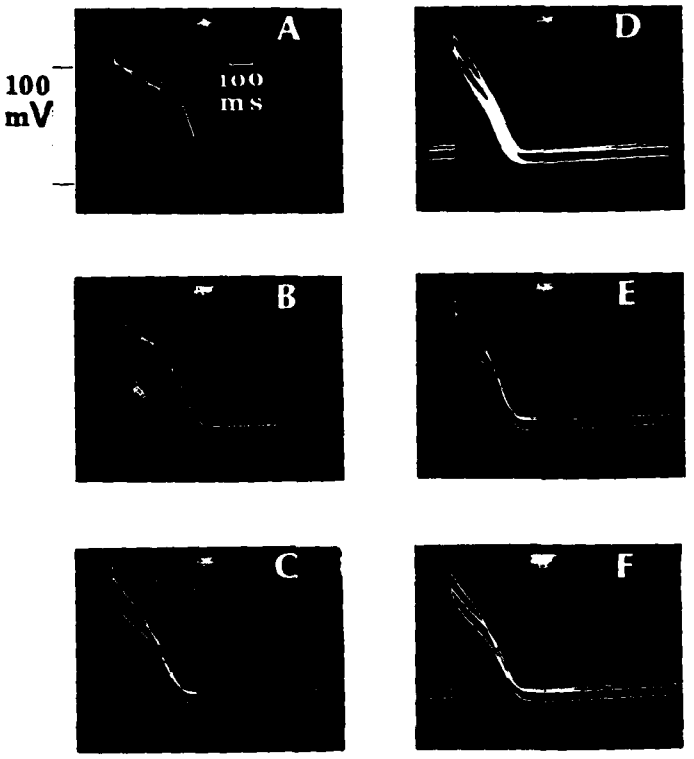


Figure 23

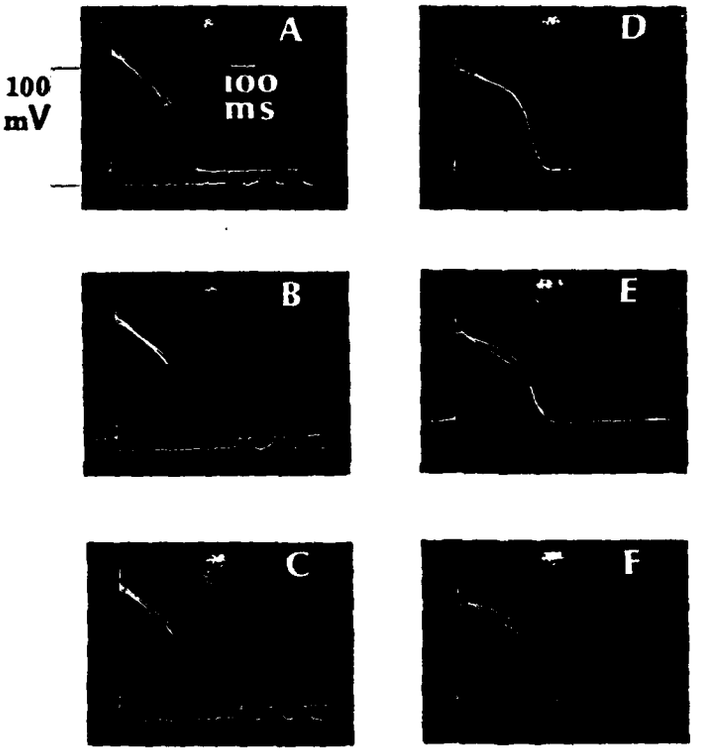


Figure 24

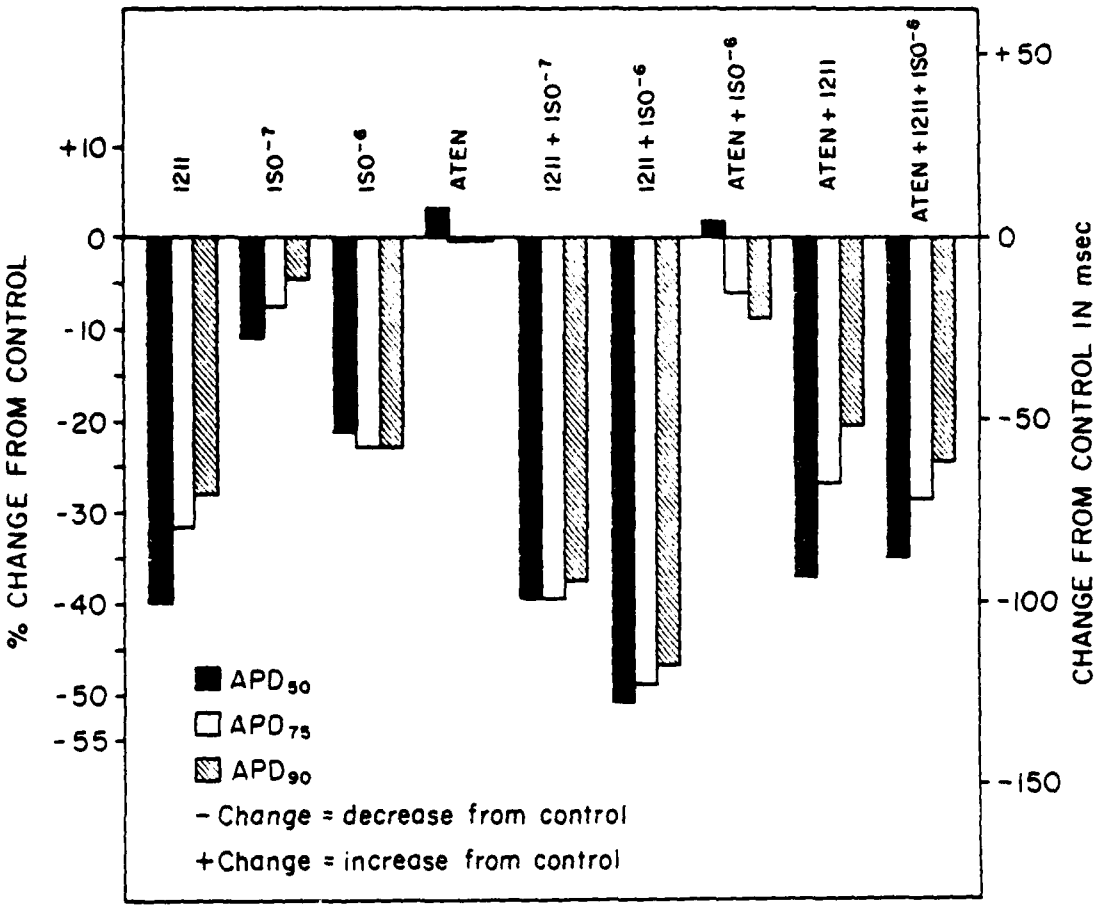

1211
ISO⁻⁷
ISO⁻⁶
ATEN
1211 + ISO⁻⁷
1211 + ISO⁻⁶
ATEN + ISO⁻⁶
ATEN + 1211
ATEN + 1211 + ISO⁻⁶
+10
0
-10
-20
-30
-40
-50
-55
% CHANGE FROM CONTROL
+50
0
-50
-100
-150
CHANGE FROM CONTROL IN msec
APD50
APD75
APD90
- Change = decrease from control
+ Change = increase from control

Figure 25

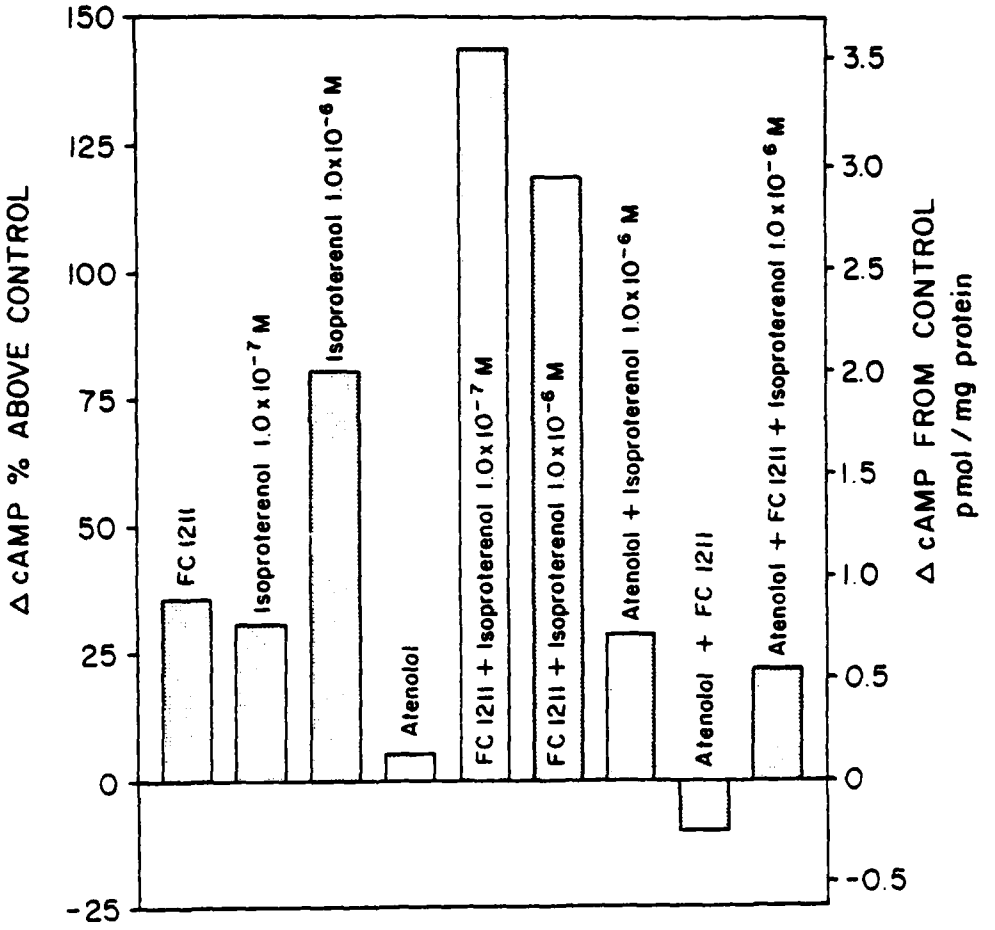

Δ cAMP % ABOVE CONTROL
150
125
100
75
50
25
0
-25
FC 1211
Isoproterenol 1.0x10⁻⁷ M
Isoproterenol 1.0x10⁻⁶ M
Atenolol
FC 1211 + Isoproterenol 1.0x10⁻⁷ M
FC 1211 + Isoproterenol 1.0x10⁻⁶ M
Atenolol + Isoproterenol 1.0x10⁻⁶ M
Atenolol + FC 1211
Atenolol + FC 1211 + Isoproterenol 1.0x10⁻⁶ M
Δ cAMP FROM CONTROL
p mol / mg protein
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5

Figure 26

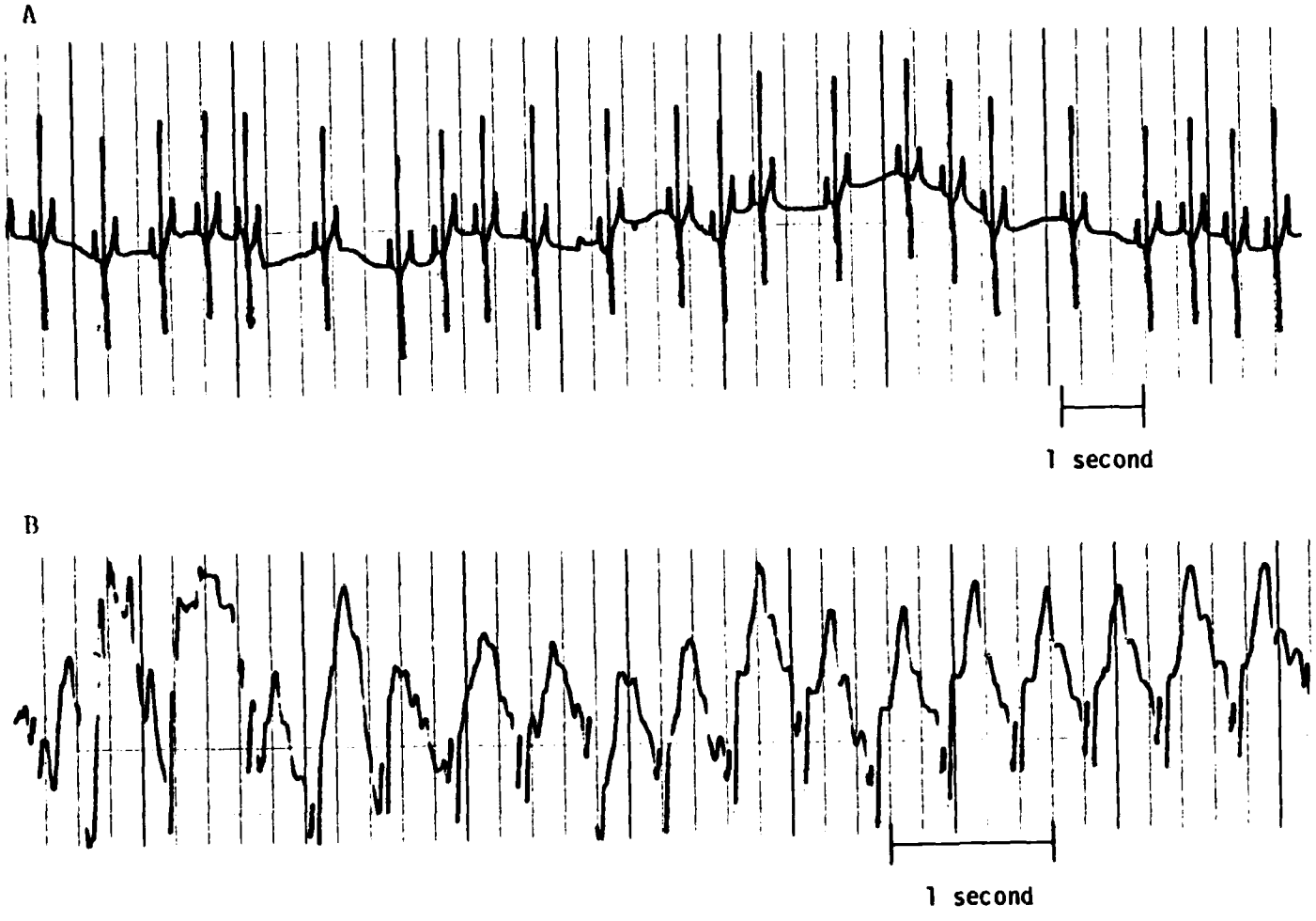
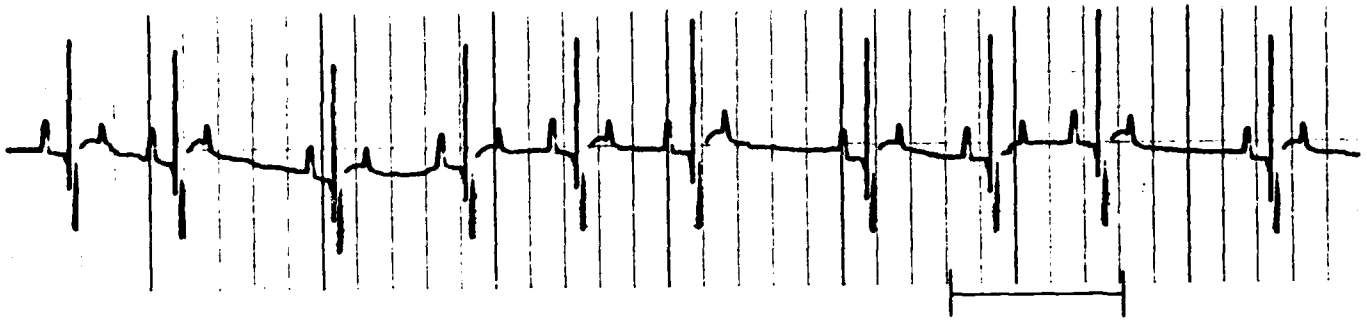


Figure 27

A



B

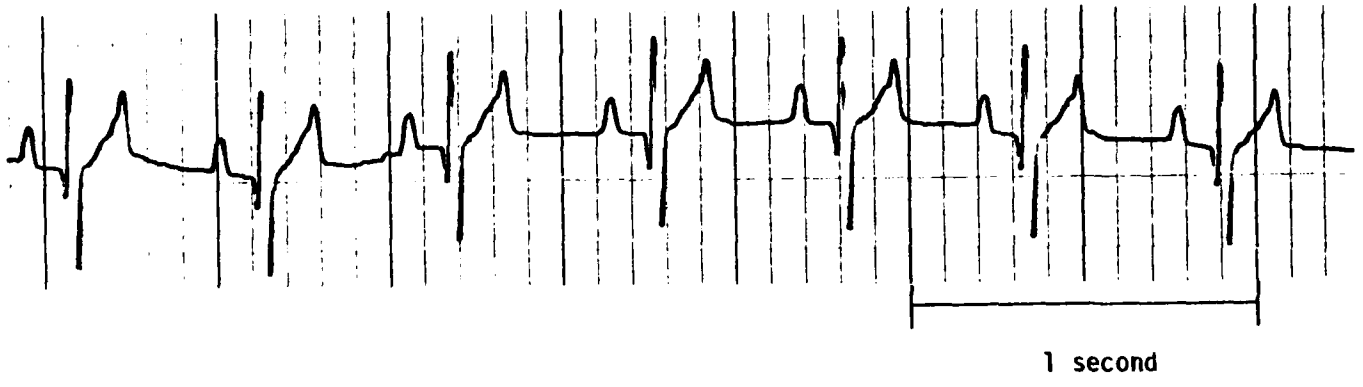


Figure 28

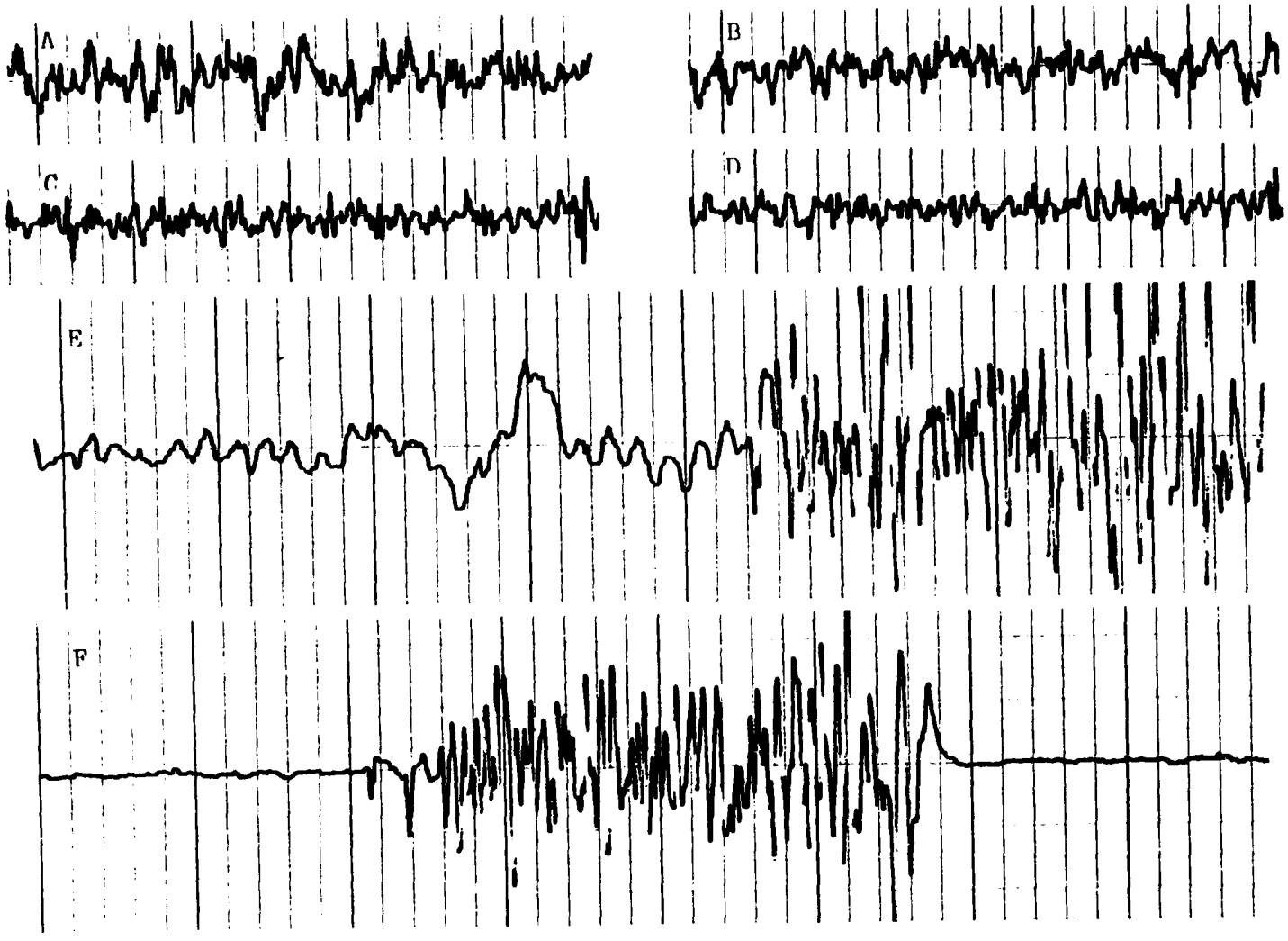


Figure 29

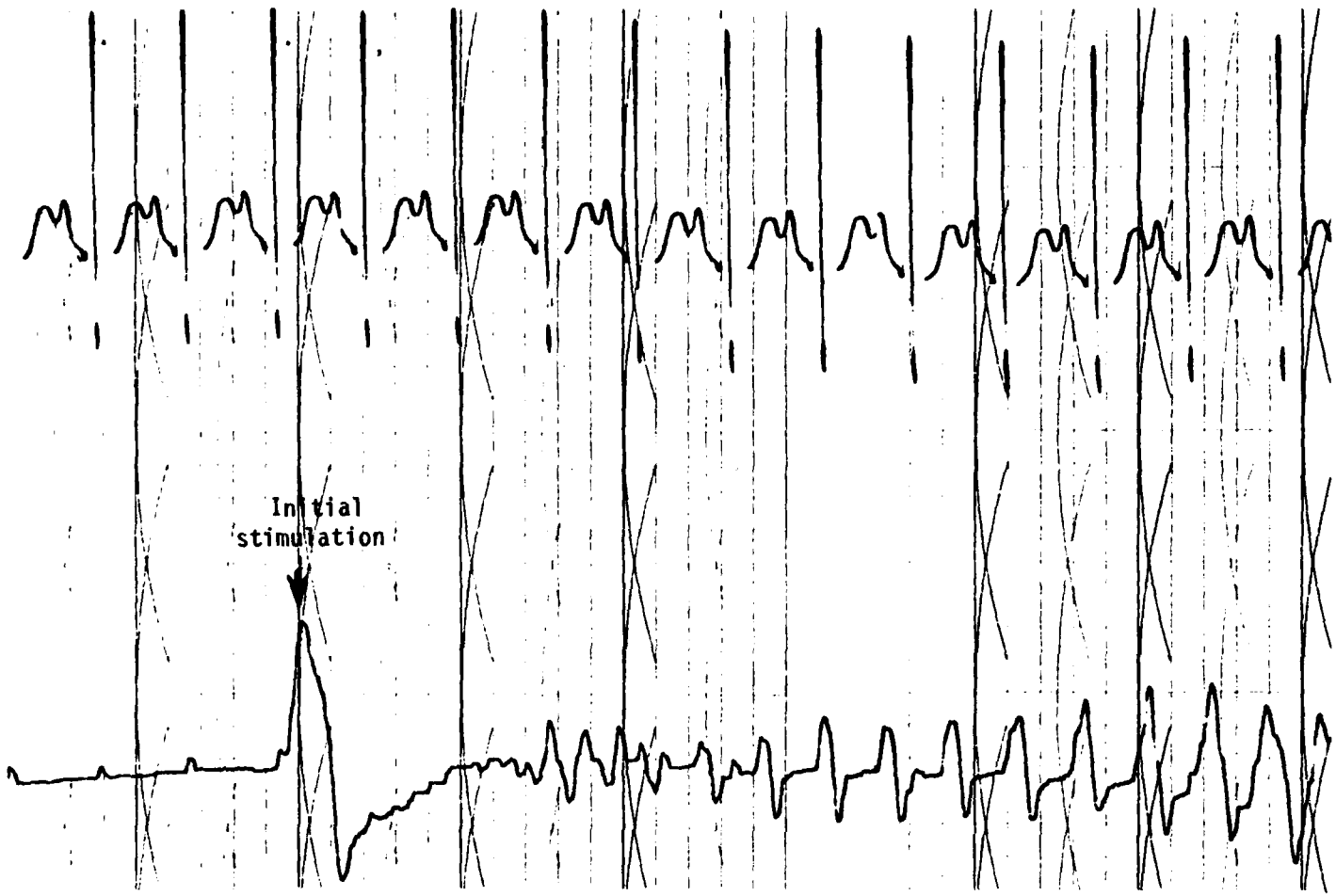


Figure 30

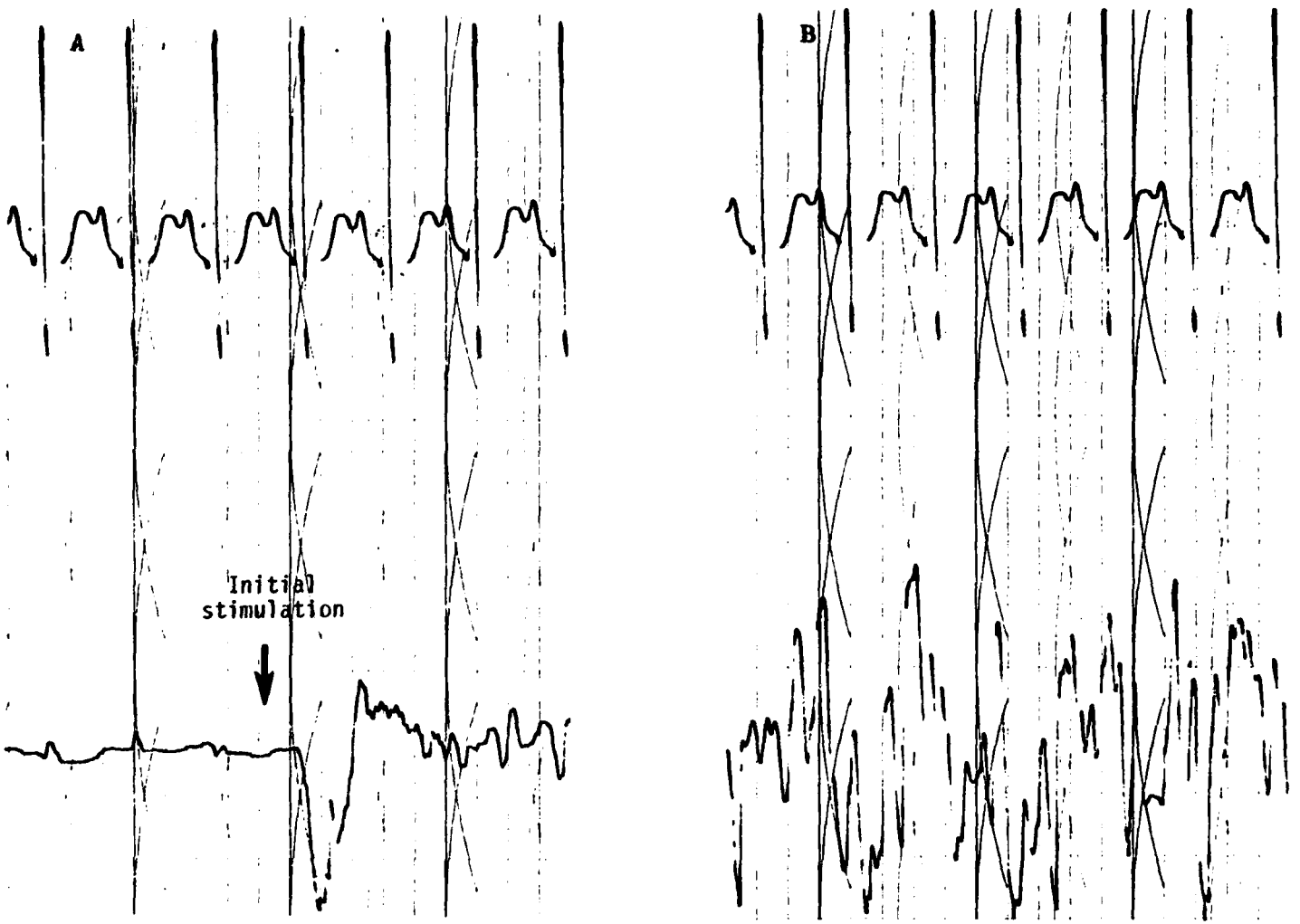


Figure 31

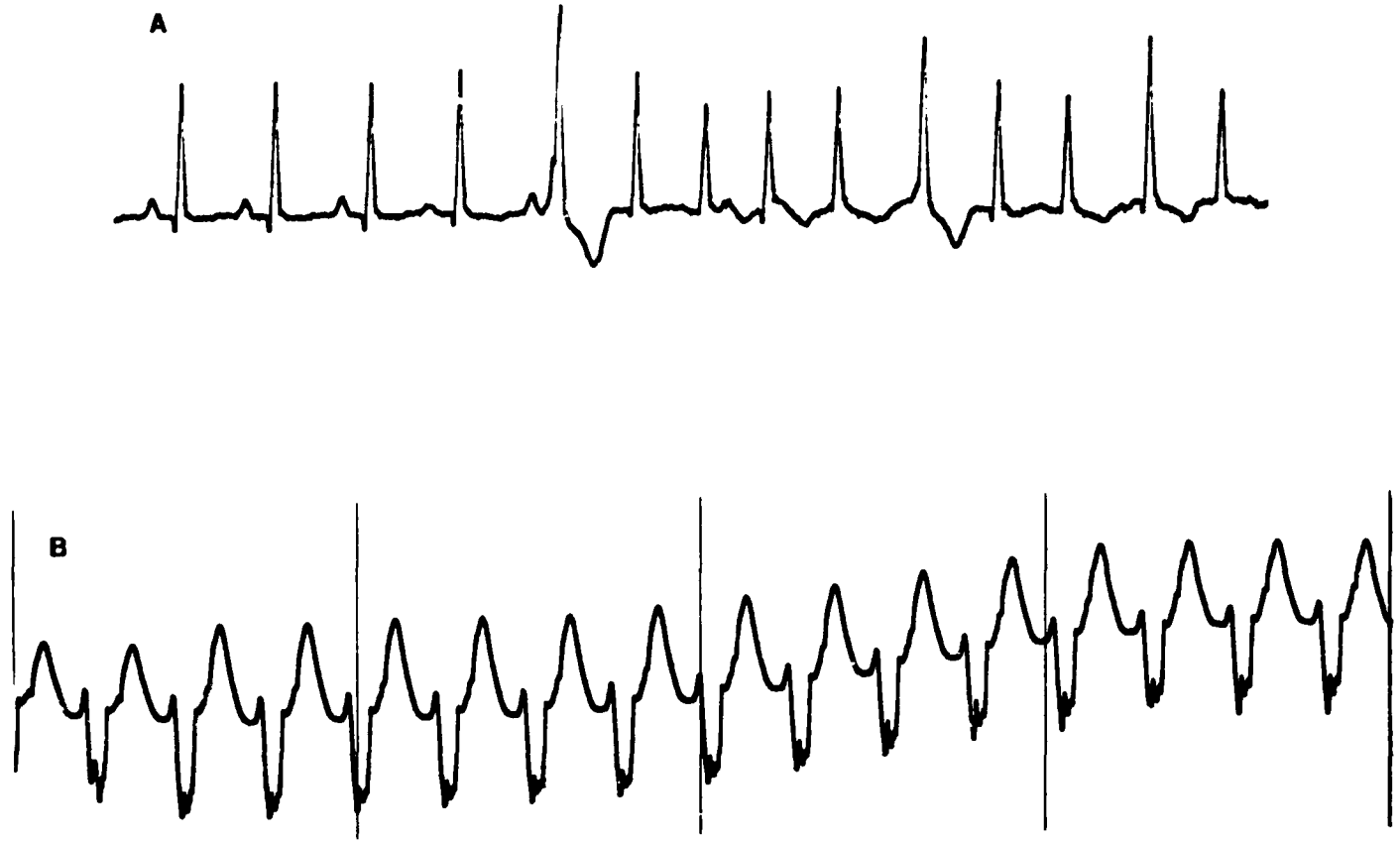


Figure 32

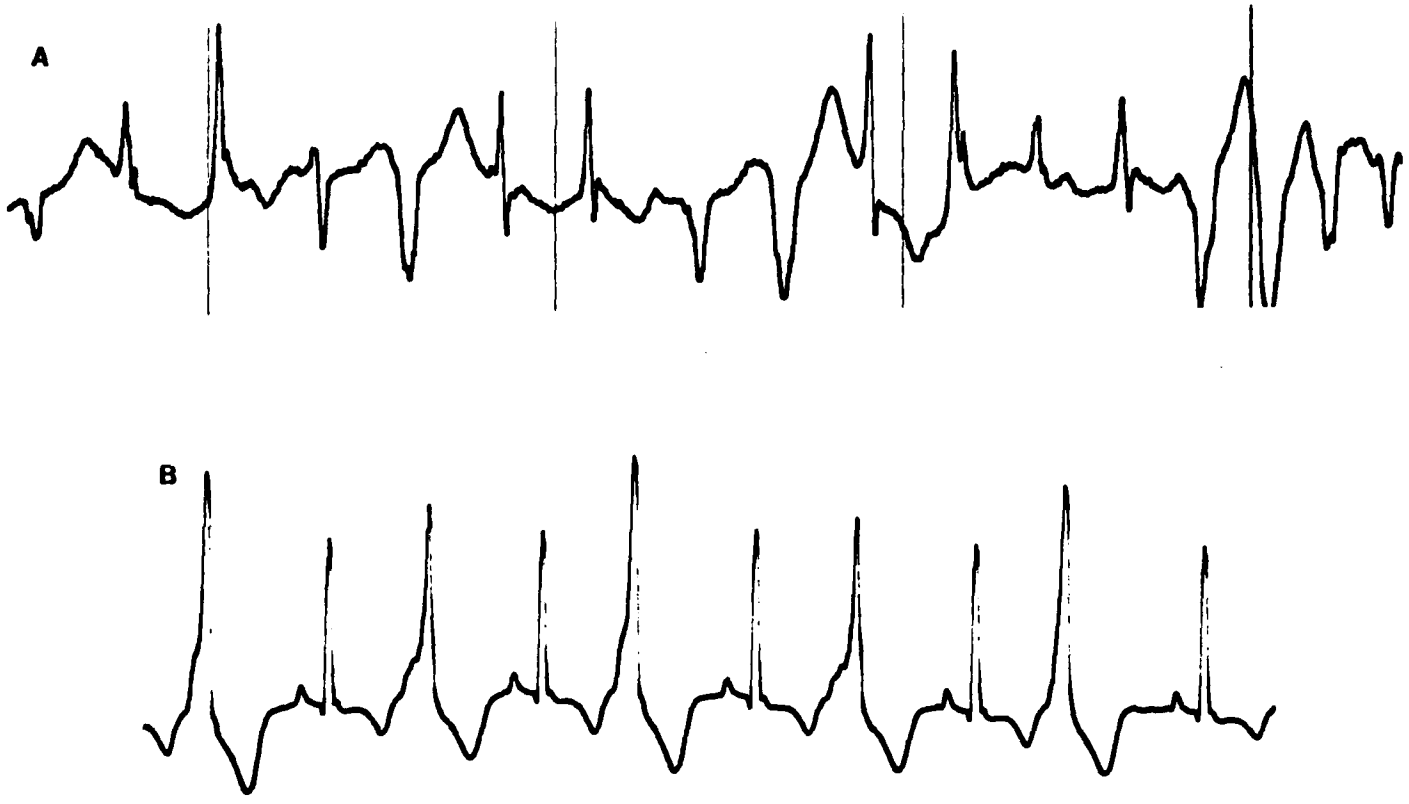


Figure 33

Table 1--CANINE PURKINJE AP - 1211 (bromochlorodifluoromethane) Dose Response
(mean ± S.E.)

Concentration	N	RP (mVolts)	AP (mVolts)	ADP_{50} (msec)	ADP_{90} (msec)	V_{max} (V/sec)	ERP (msec)
Control	6	96.3 ± 1.1	137.9 ± 5.0	284.6 ± 51.9	380.1 ± 51.6	480.2 ± 47.3	339.2 ± 63.9
50 µg/ml	6	94.9 ± 0.9	136.2 ± 3.3	252.9 ± 57.6[b]	353.5 ± 51.9[b]	477.7 ± 47.4	321.6 ± 52.1
100 µg/ml	6	92.8 ± 3.6	133.3 ± 2.8[b]	205.4 ± 52.0[b]	332.7 ± 47.1[b]	456.2 ± 34.7[b]	305.4 ± 44.1
150 µg/ml	6	91.3 ± 4.7[b]	130.2 ± 2.2[b]	175.7 ± 57.8[b]	325.0 ± 48.2[b]	440.5 ± 35.4[a]	296.6 ± 41.8[b]
200 µg/ml	6	90.5 ± 5.5[b]	128.4 ± 2.3[b]	147.2 ± 55.7[b]	317.5 ± 49.3[b]	424.0 ± 47.0[b]	302.3 ± 51.4[b]

a $p<0.05$

b $p<0.01$

Table 2--CANINE PURKINJE AP - Isoproterenol/Isoproterenol - 1211 (bromochlorodifluoromethane)
(mean ± S.E.)

	N	RP (mVolts)	AP (mVolts)	ADP_{50} (msec)	ADP_{90} (msec)	V_{max} (V/sec)	ERP (msec)
Control	6	93.7 ± 3.7	130.0 ± 2.1	231.8 ± 37.7	312.1 ± 29.8	424.7 ± 70.9	279.5 ± 31.1
ISO 10^{-9} M	6	93.7 ± 3.7	130.0 ± 2.4	230.3 ± 38.4	309.8 ± 29.5	424.7 ± 70.9	278.0 ± 30.2
ISO 10^{-8} M	6	94.0 ± 3.8	131.0 ± 2.9	210.6 ± 34.3[a]	285.6 ± 24.1[a]	436.0 ± 68.6	258.3 ± 25.0
ISO 10^{-7} M	6	94.5 ± 3.4	131.3 ± 2.8	194.8 ± 25.6[a]	259.1 ± 21.7[a]	439.7 ± 66.3	235.6 ± 19.4[a]
Washout	6	94.3 ± 4.4	129.9 ± 2.5	236.2 ± 41.5	319.6 ± 32.7	414.8 ± 64.5	285.6 ± 29.4
1211 11.0 ml/min.	6	92.9 ± 3.9	126.7 ± 2.2[b]	228.0 ± 37.8	322.0 ± 31.7	399.5 ± 68.9	286.4 ± 28.4
ISO 10^{-9} M + 1211	6	93.1 ± 3.1	126.3 ± 4.0[a,c]	217.0 ± 42.1	318.1 ± 29.8	416.3 ± 68.9	283.2 ± 22.6
ISO 10^{-8} M + 1211	6	93.5 ± 5.3	128.3 ± 3.0	198.5 ± 31.9[a,d,f]	287.1 ± 17.8[a,c]	423.8 ± 45.9	255.3 ± 17.6[a,c]
ISO 10^{-7} M + 1211	6	93.4 ± 5.0	128.1 ± 3.1[d]	174.2 ± 23.1[a,c,e]	248.5 ± 15.4[a,e]	429.3 ± 48.6	222.0 ± 12.5[a,d,e]

a P<0.01 when compared to control
b P<0.05 when compared to control
c P<0.01 when compared to same ISO concentration
d P<0.05 when compared to same ISO concentration
e P<0.01 when compared to 1211 alone
f P<0.05 when compared to 1211 alone

Table 3--CANINE PURKINJE AP - Freon 12 Dose Response
(Mean ± S.E.)

Concentration	N	RP (mVolts)	AP (mVolts)	ADP_{50} (msec)	ADP_{90} (msec)	V_{max} (V/sec)	ERP (msec)
Control	6	89.3 ± 7.3	117.8 ± 8.6	282.0 ± 55.9	367.3 ± 62.0	436.7 ± 119.1	330.8 ± 45.0
20 µg/ml	6	97.3 ± 6.6	117.5 ± 8.9	253.5 ± 52.0	341.3 ± 40.9	403.8 ± 109.8	306.4 ± 37.4
40 µg/ml	6	90.2 + 7.6	117.4 ± 5.0	242.2 ± 37.0[a]	343.0 ± 44.8	414.0 ± 110.1	304.3 ± 38.9
80 µg/ml	6	87.9 ± 5.5	119.8 ± 15.2	222.3 ± 23.5[b]	323.0 ± 42.2[b]	375.9 ± 96.0	290.7 ± 22.5[b]
Washout	6	83.4 ± 4.4	118.1 ± 6.7	264.0 ± 50.2	376.9 ± 77.5	428.1 ± 90.8	326.8 ± 59.2

a $p<0.05$

b $p<0.01$

Table 4--CANINE PURKINJE AP - Isoproterenol/Isoproterenol - Freon 12
(mean ± S.E.)

	N	RP (mVolts)	AP (mVolts)	ADP_{50} (msec)	ADP_{90} (msec)	V_{max} (V/sec)	ERP (msec)
Control	6	83.3 ± 4.6	122.7 ± 7.9	262.5 ± 27.5	360.5 ± 22.3	511.7 ± 83.3	313.5 ± 49.7
ISO 10^{-8} M	6	86.7 ± 6.4	123.3 ± 6.4	236.8 ± 16.1[b]	307.2 ± 12.9[a]	523.3 ± 92.7	275.2 ± 51.6[b]
ISO 10^{-7} M	6	87.8 ± 6.9	124.5 ± 8.5	227.8 ± 24.3[b]	301.7 ± 24.8[a]	553.3 ±101.1	265.8 ± 55.3[b]
ISO 10^{-6} M	6	85.8 ± 6.2	124.3 ± 7.9	228.2 ± 18.3[b]	309.0 ± 17.8[a]	548.3 ±121.7	286.5 ± 51.9
Washout	6	84.2 ± 5.1	125.0 ± 7.8	264.8 ± 30.9	364.2 ± 28.3	512.5 ±150.7	302.7 ± 34.2
F12	6	79.2 ± 7.1	119.5 ± 8.0	217.2 ± 25.8[a]	337.3 ± 17.2[b]	491.7 ±122.7	287.8 ± 24.3
F12 + ISO 10^{-8} M	6	78.2 ± 13.4	117.7 ± 19.1	219.7 ± 36.9[a]	316.3 ± 29.5[a]	53[illegible].3 ±175.5	270.0 ± 32.7[b]
F12 + ISO 10^{-7} M	6	85.7 ± 2.8	123.8 ± 7.0	194.3 ± 24.2[a,d]	276.3 ± 16.5[a,e]	533.3 ±160.9	244.7 ± 38.5[a,f]
F12 + ISO 10^{-6} M	6	85.7 ± 2.2	123.0 ± 8.8	202.7 ± 21.6[a]	288.2 ± 10.0[a,e]	520.0 ± 160.9	247.2 ± 40.1[a,d,f]

a P<0.01 when compared to control
b P<0.05 when compared to control
c P<0.01 when compared to same ISO concentration
d P<0.05 when compared to same ISO concentration
e P<0.01 when compared to F 12 alone
f P<0.05 when compared to F 12 alone

Table 5--Means, Standard Errors of the Mean (S.E.M.), and Ranges of Canine Cardiac Purkinje Fiber[a] Electrophysiologic and Cyclic Nucleotide Parameters for Control Periods

Parameter	Mean ± S.E.M.	Range
Threshold voltage (V)	9.6 0.6	2.0 - 42.0
Escape time (sec)	36.4 5.0	1.1 - 194.5
Spontaneous rate (beats/min)	16.04 1.67	3.09 - 63.16
Effective refractory period (msec)*	315.0 3.0	203.0 - 491.0
Overshoot (-mV)*	34.2 0.4	16.0 - 52.3
Resting membrane potential (-mV)*	86.7 0.3	72.2 - 102.5
Action potential amplitude (mV)*	120.8 0.5	100.0 - 142.4
Action potential duration @ 50% (msec)*	240.9 3.1	124.4 - 394.1
Action potential duration @ 75% (msec)*	309.5 3.6	179.8 - 482.7
Action potential duration @ 90% (msec)*	342.7 3.6	219.2 - 517.2
dV/dt (V/sec)*	469.0 9.0	150.0 - 900.0
Maximum diastolic potential (-mV)*	84.9 0.4	68.2 - 96.0
Activation voltage (-mV)*	70.8 0.8	42.9 - 95.4
Slope (mV/sec)*	8.51 0.50	0.67 - 40.16
cAMP conc. pmol/mg protein	2.49 0.11	1.23 - 4.48
cGMP conc. pmol/mg protein	0.21 0.04	0.03 - 0.98
cAMP:cGMP ratio	22.5 3.9	2.0 - 90.0

[a]Purkinje fibers dissected from either ventricle were equilibrated for 45 minutes prior to each 20 minute treatment period. Standard electrode technique was used to measure electrophysiologic parameters. Cyclic nucleotide levels were determined by radioimmunoassay.
*Electrode location (proximal, middle, or distal) is not taken into consideration.

Table 6--Means and Standard Errors of the Mean of Threshold Voltage, Escape Time, and Spontaneous Rate for Each Treatment Administered to Canine Cardiac Purkinje Fibers[a]

Treatment	Threshold (V)	Escape time (sec)	Spontaneous rate (beats/min)
1. Control	9.6 ± 0.6*	38.4 ± 4.9	16.04 ± 1.67
2. FC 1211[b]	9.4 ± 0.9	123.6 ± 15.1**	5.05 ± 2.51**
3. Isoproterenol 10^{-7} M	7.3 ± 1.0	10.3 ± 4.3	22.56 ± 7.72
4. Isoproterenol 10^{-6} M	13.9 ± 5.2	8.7 ± 7.3	38.46 ± 5.92
5. Atenolol	5.4 ± 0.8	38.2 ± 9.1	12.85 ± 2.78
6. FC 1211[c] + Iso 10^{-7} M	10.4 ± 1.7	31.3 ± 29.7	58.82 ± 6.92
7. FC 1211[d] + Iso 10^{-6} M	9.6 ± 2.8	41.3 ± 29.5	48.76 ± 4.98
8. Atenolol + Iso 10^{-6} M	7.3 ± 1.5	12.8 ± 11.0	41.10 ± 1.97
9. Atenolol + FC 1211[e]	5.4 ± 1.5	103.3 ± 27.3**	16.57 ± 4.53
10. Atenolol + FC 1211[f] + Iso 10^{-6} M	3.4 ± 0.5	81.4 ± 13.9	16.46 ± 4.70

[a]For detail, see Table 5.

FC 1211 concentration means:
[b]165.6 ± 12.5 µg/ml
[c]165.4 ± 9.1 µg/ml
[d]150.8 ± 6.3 µg/ml
[e]151.6 ± 4.6 µg/ml
[f]151.9 ± 6.5 µg/ml

*No threshold value for treatments 2-10 is significantly different ($p<.05$) from the control value.
**Values are significantly different ($p<.05$) from control values.

Table 7--Means* and Standard Errors of the Mean of Action Potential Overshoot, Resting Membrane Potential (RMP), and Amplitude for each Treatment Administered to Canine Cardiac Purkinje Fibers[a]

Treatment	Overshoot (+mV)	RMP (-mV)	Amplitude (mV)
1. Control	34.2 ± 0.4*	86.7 ± 0.3	120.8 ± 0.5
2. FC 1211[b]	26.5 ± 1.1**	81.4 ± 0.7**	107.1 ± 1.6**
3. Isoproterenol 10^{-7} M	36.0 ± 2.4	83.1 ± 2.2	119.1 ± 3.5
4. Isoproterenol 10^{-6} M	29.2 ± 1.4	80.9 ± 1.6	111.8 ± 2.4**
5. Atenolol	34.8 ± 0.7	86.7 ± 0.6	121.4 ± 0.9
6. FC 1211[c] + Iso 10^{-7} M	22.8 ± 1.9**	79.7 ± 2.0**	102.4 ± 3.1**
7. FC 1211[d] + Iso 10^{-6} M	25.4 ± 2.1**	76.3 ± 1.9**	100.9 ± 3.3**
8. Atenolol + Iso 10^{-6} M	30.7 ± 1.0**	85.8 ± 1.8	116.4 ± 1.7
9. Atenolol + FC 1211[e]	31.3 ± 1.2	82.0 ± 0.6**	113.1 ± 1.3**
10. Atenolol + FC 1211[f] + Iso 10^{-6} M	33.1 ± 1.4	82.8 ± 1.2**	115.8 ± 1.5

[a] For detail, see Table 5.
[b-f] Concentration of FC 1211 are as listed in Table 6.
* Electrode locations (proximal, middle, or distal) is not taken into consideration.
** Values are significantly different ($p < .05$) from control values.

Table 8--Means* and Standard Errors of the Mean of Action Potential Duration (APD) at 50%, 75%, and 90% Repolarization for each Treatment Administered to Canine Cardiac Purkinje Fibers[a]

Treatment	APD_{50} (msec)	APD_{75} (msec)	APD_{90} (msec)
1. Control	240.9 ± 3.1	309.5 ± 3.6	342.7 ± 3.6
2. FC 1211[b]	144.3 ± 3.8**	209.7 ± 3.4**	248.4 ± 3.8**
3. Isoproterenol 10^{-7} M	215.4 ± 17.0	285.3 ± 11.1	326.6 ± 9.3
4. Isoproterenol 10^{-6} M	189.4 ± 9.5**	238.8 ± 12.5**	263.0 ± 12.1**
5. Atenolol	247.7 ± 3.9	309.1 ± 3.8	341.0 ± 3.9
6. FC 1211[c] + Iso 10^{-7} M	143.7 ± 11.8**	186.7 ± 15.1**	211.6 ± 16.0**
7. FC 1211[d] + Iso 10^{-6} M	117.8 ± 4.9**	157.0 ± 4.1**	180.0 ± 4.2**
8. Atenolol + Iso 10^{-6} M	244.8 ± 9.7	288.3 ± 9.7	316.5 ± 9.0
9. Atenolol + FC 1211[e]	152.9 ± 4.2**	227.1 ± 3.5**	271.6 ± 5.0**
10. Atenolol + FC 1211[f] + Iso 10^{-6} M	155.4 ± 6.1**	219.3 ± 5.2**	255.6 ± 6.8**

[a] For detail, see Table 5.
[b-f] Concentration of FC 1211 are as listed in Table 6.
* Electrode locations (proximal, middle, or distal) is not taken into consideration.
** Values are significantly different ($p<.05$) from control values.

Table 9--Means* and Standard Errors of the Mean of Action Potential Effective Refractory Periods (ERP) and phase 0 dV/dt for each Treatment Administered to Canine Cardiac Purkinje Fibers[a]

Treatment	ERP (msec)		dV/dt (V/sec)	
1. Control	315 ± 3		469 ± 9	
2. FC 1211[b]	257 ± 5	**	328 ± 21	**
3. Isoproterenol 10^{-7} M	265 ± 14	**	466 ± 63	
4. Isoproterenol 10^{-6} M	250 ± 9	**	371 ± 26	
5. Atenolol	308 ± 4		493 ± 18	
6. FC 1211[c] + Iso 10^{-7} M	215 ± 14	**	297 ± 40	**
7. FC 1211[d] + Iso 10^{-6} M	221 ± 12	**	256 ± 42	**
8. Atenolol + Iso 10^{-6} M	297 ± 10		476 ± 42	
9. Atenolol + FC 1211[e]	258 ± 7	**	410 ± 21	
10. Atenolol + FC 1211[f] + Iso 10^{-6} M	235 ± 5	**	363 ± 21	

[a] For detail, see Table 5.
[b-f] Concentrations of FC 1211 are as listed in Table 6.
* Electrode locations (proximal, middle, or distal) is not taken into consideration.
** Values are significantly different ($p<.05$) from control values.

Table 10--Means* and Standard Errors of the Mean of Spontaneous Action Potential Maximum Diastolic Potential (MDP), Activation Potential (AV), and Slope of Phase 4 for each Treatment Administered to Canine Cardiac Purkinje Fibers[a]

Treatment	MDP (-mV)	AV (-mV)	Slope (mV/sec)
1. Control	84.9 ± 0.4***	70.8 ± 0.8	8.5 ± 0.5
2. FC 1211[b]	81.8 ± 1.0	58.7 ± 2.9**	5.5 ± 2.0
3. Isoproterenol 10^{-7} M	85.1 ± 0.9	70.0 ± 2.6	16.0 ± 3.6**
4. Isoproterenol 10^{-6} M	84.8 ± 1.6	72.6 ± 3.3	12.6 ± 1.9**
5. Atenolol	85.9 ± 0.6	74.1 ± 1.2**	5.0 ± 0.6**
6. FC 1211[c] + Iso 10^{-7} M	87.3 ± 1.4	71.8 ± 3.7	20.1 ± 4.0**
7. FC 1211[d] + Iso 10^{-6} M	85.0 ± 1.4	68.6 ± 2.9	19.9 ± 2.5**
8. Atenolol + Iso 10^{-6} M	88.0 ± 1.8	79.6 ± 2.7**	7.4 ± 1.7
9. Atenolol + FC 1211[e]	83.6 ± 0.8	67.6 ± 1.5	6.7 ± 2.6
10. Atenolol + FC 1211[f] + Iso 10^{-6} M	87.7 ± 1.0	70.3 ± 3.6	5.5 ± 0.3

[a] For detail, see Table 5.
[b-f] Concentrations of FC 1211 are as listed in Table 6.
* Electrode locations (proximal, middle, or distal) is not taken into consideration.
** Values are significantly different ($p<.05$) from control values.
*** No MDP value for treatments 2-10 is significantly different ($p<.05$) from the control value

Table 11--Means* and Standard Errors of the Mean of Purkinje Fiber Cyclic Nucleotide Values for each Treatment Administered to Canine Cardiac Purkinje Fibers[a]

Treatment	cAMP (pmol/mg protein)	cGMP (pmol/mg protein)	cAMP:cGMP ratio
1. Control	2.49 ± 0.11	0.207 ± 0.045**	22.5 ± 3.9**
2. FC 1211[b]	3.35 ± 0.32*	0.242 ± 0.050	20.1 ± 5.1
3. Isoproterenol 10^{-7} M	[illegible]24 ± 0.57	0.220 ± 0.064	11.0 ± 2.9
4. Isoproterenol 10^{-6} M	[illegible]44 ± 0.45*	0.376 ± 0.047	13.6 ± 3.0
5. Atenolol	2.62 ± 0.23	---	---
6. FC 1211[c] + Iso 10^{-7} M	5.94 ± 0.59*	0.335 ± 0.155	27.0 ± 10.0
7. FC 1211[d] + Iso 10^{-6} M	5.35 ± 0.68*	0.135 ± 0.005	29.0 ± 2.0
8. Atenolol + Iso 10^{-6} M	3.19 ± 0.69	---	---
9. Atenolol + FC 1211[e]	2.26 ± 0.12	---	---
10. Atenolol + FC 1211[f] + Iso 10^{-6} M	3.04 ± 0.33	---	---

[a] For detail, see Table 5.
[b-f] Concentrations of FC 1211 are as listed in Table 6.
* Values are significantly different ($p<.05$) from control values.
** No cGMP or ratio value for treatments 1, 2, 3, 4, 6, and 7 is significantly different ($p<.05$) from the control value.

Table 12--Control Means and Standard Errors of the Mean of Right Ventricular Purkinje Fiber[a] Electrophysiologic and Cyclic Nucleotide Parameters

Parameter			
Thres (V)	9.7 ± 1.0		
E.T. (sec)	51.0 ± 9.4*		
S.R. (beats/min)	11.45 ± 1.88*		
cAMP (pmol/mg protein)	2.70 ± 0.16		
cGMP (pmol/mg protein)	0.19 ± 0.08		
Ratio of cAMP:cGMP	29.6 ± 6.7		
Electrode location:	**Proximal**	**Middle**	**Distal**
ERP (msec)	298.3 ± 7.6*	305.0 ± 7.7*	309.3 ± 8.3*
o.s. (+mV)**	33.3 ± 1.1*	31.9 ± 1.0*	33.1 ± 1.2*
RMP (-mV)**	87.0 ± 0.9	86.7 ± 0.8	88.8 ± 0.9
AP AMP (mV)	120.1 ± 1.2	118.6 ± 1.2	121.5 ± 1.3
APD_{50} (msec)**	224.3 ± 6.7*	232.3 ± 7.2*	241.6 ± 9.6*
APD_{75} (msec)**	292.7 ± 8.3*	295.4 ± 8.4*	309.6 ± 10.7*
APD_{90} (msec)	323.2 ± 8.5*	325.7 ± 8.6*	339.7 ± 10.6*
dV/dt (V/sec)**	436.1 ± 20.9	426.1 ± 22.8	499.4 ± 32.7
MDP (-mV)	84.9 ± 1.1	83.5 ± 1.0	85.8 ± 1.1
AV (-mV)	70.0 ± 1.6	67.9 ± 1.9	69.2 ± 2.4
Slope (mV/sec)	7.1 ± 1.1	7.5 ± 1.2	7.6 ± 1.6

[a] For detail, see Table 5.
* Right ventricular Purkinje fiber value which is significantly different ($p<.05$) when compared with the corresponding left ventricular Purkinje fiber value.
** Significant differences ($p<.05$) exist among proximal, middle, and distal values.

AD-A128 424 THE ELECTROPHYSIOLOGIC MECHANISMS OF HALOGENATED ALKANE ARRHYTHMOGENESIS(U) OHIO STATE UNIV RESEARCH FOUNDATION COLUMBUS S M STRAUCH 01 MAR 83 AFOSR-TR-83-0436 2/2

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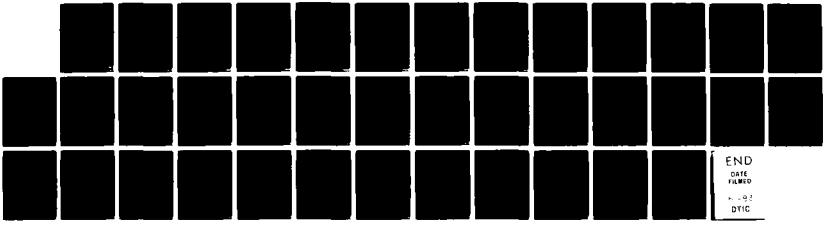

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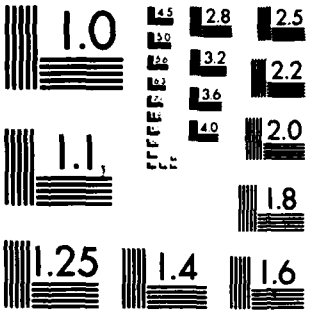

Table 13--Control Means and Standard Errors of the Mean of Left Ventricular Purkinje Fiber[a] Electrophysiologic and Cyclic Nucleotide Parameters

Parameter	
Thres (V)	9.5 ± 0.7
E.T. (sec)	29.6 ± 5.1*
S.R. (beats/min)	21.35 ± 2.13*
cAMP (pmol/mg protein)	2.30 ± 0.14
cGMP (pmol/mg protein)	0.23 ± 0.05
Ratio of cAMP:cGMP	15.4 ± 3.4

Electrode location:	Proximal	Middle	Distal
ERP (msec)	320.6 ± 7.0*	322.5 ± 7.0*	328.1 ± 7.6*
o.s. (+mV)**	37.7 ± 1.0*	34.4 ± 0.9*	33.7 ± 1.0*
RMP (-mV)**	85.4 ± 0.8	86.2 ± 0.7	87.4 ± 0.7
AP AMP (mV)	122.8 ± 1.1	120.3 ± 1.3	121.1 ± 1.1
APD_{50} (msec)**	239.7 ± 6.6*	244.8 ± 6.5*	264.4 ± 9.5*
APD_{75} (msec)**	312.5 ± 7.4*	313.8 ± 7.9*	333.7 ± 9.9*
APD_{90} (msec)	347.3 ± 7.7*	350.3 ± 7.9*	368.8 ± 9.8*
dV/dt (V/sec)**	497.5 ± 17.7	458.4 ± 19.8	504.5 ± 20.5
MDP (-mV)	85.0 ± 0.9	85.0 ± 0.7	85.6 ± 1.1
AV (-mV)	71.9 ± 1.8	71.4 ± 1.6	73.4 ± 1.9
Slope (mV/sec)	8.8 ± 0.9	10.0 ± 1.3	8.7 ± 1.5

[a] For detail, see Table 5.
* Left ventricular Purkinje fiber value which is significantly different ($p<.05$) when compared with the corresponding right ventricular Purkinje fiber value.
** Significant differences ($p<.05$) exist among proximal, middle, and distal values.

Table 14--Summary of the Effects of FC 1211, Isoprotrerenol (Iso), and Atenolol (Aten) on Electrophysiologic Parameters and cAMP Concentration in Canine Cardiac Purkinje Fibers

Parameter[a]	FC 1211	Iso 10^{-6}	Aten	1211 + Iso 10^{-6}	Aten + Iso 10^{-6}	Aten + 1211	Aten + 1211 + Iso
cAMP conc.	↑	↑↑	0	↑↑↑	↑	sl↓	sl↑
Thres	0	0	0	0	0	0	0
ET	↑↑↑	↓	0	0	↓	↑↑	↑
SR	↓↓↓	↑	sl↓	↑	↑	0	0
OS	↓	↓	0	↓	sl↓	sl↓	0
RMP	↓	↓	0	↓↓	0		↓
AP AMP	↓↓	↓↓	0	↓↓↓	↓		↓
APD_{50}	↓↓	↓	0	↓↓↓	0		↓↓
APD_{75}	↓↓	↓	0	↓↓↓	sl↓		↓↓
APD_{90}	↓↓	↓	0	↓↓↓	sl↓		↓↓
ERP	↓↓	↓↓	sl↓	↓↓↓	sl↓	↓↓	↓↓↓
dV/dt	↓↓	↓	↑	↓↓↓	sl↑	↓	↓↓
MDP	sl↓	0	0	0	sl↑	0	sl
AV	↓↓↓	0	↑	0	↑	0	0
Slope	↓	↑	↓	↑↑	0	sl↓	sl

[a] Abbreviations are defined in Materials and Methods Section. Amount of change from control is graded ↑ to ↑↑↑ to indicate increase, ↓ to ↓↓↓ to indicate decrease, and 0 to indicate no change; sl = slight.

Table 15--Blood FC 1211 Concentrations of One Dog Who Inhaled 3 Concentrations of FC 1211 in Oxygen (2%, 5% and 10%) for 60 Minutes.

Time (min.)	10% Blood conc. (µg/ml)	5% Blood conc. (µg/ml)	2% Blood conc. (µg/ml)
1	12.90	43.20	4.62
3	98.54	56.88	19.28
5	111.57	77.38	46.62
10	173.01	108.14	46.62
20	223.27	104.72	50.02
30	240.03	121.80	56.88
40	240.03	139.14	53.46
50	269.82	135.46	50.04
60	303.32	145.72	46.62
Off	--	--	--
10	50.13	39.78	19.28
20	27.79	29.54	5.62
30	22.21	19.28	0
40	9.17	12.44	0
50	5.45	5.62	0
60	1.73	0	0

Table 16--Ten Conscious Dogs Pretreated with Phenobarbital Inhaled One of Four Concentrations of FC 1211 (2%, 5%, 10% or 20%).

Dog no.	Sinus rate (beats/min.)	FC 1211 blood conc. (20 min) (μg/ml)	EKG
Control			
1	74	-	Normal
2	61	-	Normal
3	90	-	Normal
Mean ± S.D.	75±15		
4	73	-	Normal
5	73	-	Normal
6	90	-	Normal
Mean ± S.D.	79±10		
7	130	-	Normal
8	80	-	Normal
Mean ±S.D.	105±35		
9	150	-	Normal
10	160	-	Normal
Mean ± S.D.	155±7		
2% FC 1211			
1	115	46.48	Inc. T wave amp.
2	148	51.15	Inc. T wave amp.
3	103	48.38	Normal
Mean ± S.D.	122±23[a]	48.67±2.35[c]	
5% FC 1211			
4	170	(1 min.)43.23	Sinus tach with RBBB*
5	172	76.81	Inc. T wave amp.
6	210	74.07	Dec. T wave amp.
Mean ± S.D.	184±23[b]	64.70±18.65[d]	
10% FC 1211			
7	280	125.97	Dec. T wave amp.
8	230	140.95	Dec. T, Inc. P wave amp.
Mean ± S.D.	255±35[a]	133.46±10.59[e]	
20% FC 1211			
9	290	320.40	Dec. T and P wave amp.
10	240	293.12	Dec. T wave amp.
Mean ± S.D.	265±35[a]	306.76±19.29[f]	

Table 16--Continued

* Sinus tachycardia with right bundle branch block.
[a] The value is larger than corresponding control mean ± S.D. (P less than 0.05).
[b] The value is larger than corresponding control mean ± S.D. (P less than 0.01).
[d] The value is larger than c (P less than 0.01).
[e] The value is larger than c or d (P less than 0.01).
[f] The value is larger than c, d or e (P less than 0.01).

Table 17--Representation of the Threshold Dose of Isoproterenol Needed to Induce Ventricular Arrhythmias During the Inhalation of 100% Oxygen or FC 1211 (2%, 5% or 10%).

No. reaching end-point	Sinus rate prior to iso. chall. (beats/min.)	FC 1211 blood conc. (µg/ml)	Threshold dose of iso. (µg/kg)	EKG arrhy.
100% oxygen (control)	150	-	1.0	IP
	127	-	1.0	IP
	180	-	3.0	VT
	160	-	3.0	IP
6/9	110	-	3.0	IP
	134	-	5.0	IP
	180	-	10.0	-
	80	-	10.0	-
	114	-	10.0	-
Mean ± S.D.	137±34[a]		2.7±1[f]	
2% FC 1211	145	36.96	.020	B
	190	38.64	.040	B
6/6	170	46.47	.040	B
	125	36.69	.080	IP
	160	42.55	.080	B
	250	38.71	.160	B
Mean ± S.D.	173±44[b]	40.0±3.80[c]	.070±.050[g]	
5% FC 1211	138	73.09	.005	IP
	190	84.55	.010	IP
	210	96.19	.010	IP
8/8	120	83.17	.010	IP
	156	96.69	.080	VT
	144	82.21	.080	B
	220	85.74	.080	VT
	140	94.36	.080	MVT
Mean ± S.D.	165±37[b]	87.0±8.21[d]	.044±.038[g]	
10% FC 1211	200	139.89	.020	IP
	180	84.11	.040	IP
4/4	170	108.40	.080	IP
	190	128.00	.160	IP
Mean ± S.D.	185±13[b]	115.1±24.4[e]	.08±.069	

Table 17--continued

B -Bigeminy
IP -Isolated premature ventricular contractions
VT -Ventricular tachycardia
MVT-Multiple form ventricular tachycardia

a Mean sinus rate of 9 control animals.
b The value is larger than a (P less than 0.01).
d The value is larger than c (P less than 0.02).
e The value is larger than c (P less than 0.01), and larger than d (P less than 0.05).
f Mean threshold dose of isoproterenol in six animals developing arrhythmias.
g The value is smaller than f (P less than 0.05).

Table 18--Determination of an Atenolol Dose to Create Sufficient B_1 Adrenergic Blockade to Resist Changes in Sinus Rate Upon a Challenge Injection of 3 µg/kg Isoproterenol.

Table A

Atenolol followed by isoproterenol challenge (3 µg/kg)	Sinus rate (beats/min.)	Systolic Pressure (mm Hg)	Diastolic pressure (mm Hg)
.25 mg/kg	160	181	146
Iso. challenge	222	203	75
.5 mg/kg	170	194	153
Iso. challenge	216	200	76
1 mg/kg	170	194	153
Iso. challenge	200	200	100
2 mg/kg	170	200	153
Iso. challenge	170	200	97
4 mg/kg	170	200	156
Iso. challenge	174	208	110
6 mg/kg	120	228	156
Iso. challenge	120	212	153

Table B

Atenolol followed by isoproterenol challenge (3 µg/kg)	Sinus rate (beats/min.)	Systolic Pressure (mm Hg)	Diastolic pressure (mm Hg)
6 mg/kg	114	181	126
Iso. challenge	115	178	75

Table 19--Dogs Pretreated with Atenolol (Cardiospecific Antagonist) Inhaled Four Consecutive FC 1211 Concentrations (2%, 5% or 10% and 20%), each for 10 Minutes. At the End of the 10 Minute Inhalation Period of Each FC 1211 Concentration, the Animal was Challenged with 1 µg/kg Isoproterenol.

Dog no.	BEFORE ISO. CHALLENGE				AFTER ISO. CHALLENGE (1 µg/kg)			
	Sinus rate (beats/ minute)	BCF blood conc. (µg/ml)	Sys. press. (mmHg)	Dia. press. (mmHg)	Sinus rate (beats/ minute)	Sys. press. (mmHg)	Dia. press. (mmHg)	Arrhy.
Control (Atenolol)								
1	120	-	159	118				
2	130	-	165	133				
3	120	-	172	141				
4	110	-	165	130				
5	120	-	175	128				
6	135	-	184	135				
Mean	123		170	131				
± S.D.	± 9		± 9[e]	± 8[h]				
2% FC 1211								
1	120	46.29	159	117	120	135		-
2	130	46.00	170	137	130	153	115	-
3	125	46.47	162	134	125	140	110	-
4	110	42.55	159	125	120	109	66	-
5	120	44.23	156	112	140	123	65	-
6	130	46.33	166	122	130	147	94	-
Mean	123	45.65	162	125	127	135	90	
± S.D.	± 8	± 2.00[a]	± 5[f]	± 10	± 8	± 16*	± 23*	
5% FC 1211								
1	120	106.30	155	118	120	137	87	IP
2	130	95.75	153	115	135	137	83	-
3	140	96.51	144	122	150	122	92	-
4	110	87.35	156	115	125	128	65	-
5	130	87.26	181	128	150	138	59	-
6	130	86.16	128	78	140	128	78	-
Mean	127	93.20	153	113	136	132	77	
± S.D.	± 10	± 7.85[b]	± 17[f]	± 18[i]	± 13*	± 7*	± 13*	

Table 19--(Continued)

Dog no.	BEFORE ISO. CHALLENGE: Sinus rate (beats/minute)	BCF blood conc. (μg/ml)	Sys. press. (mmHg)	Dia. press. (mmHg)	AFTER ISO. CHALLENGE (1 μg/kg): Sinus rate (beats/minute)	Sys. press. (mmHg)	Dia. press. (mmHg)	Arrhy.
10% FC 1211								
1	115	187.92	159	119	190	141	79	B,MF,VT
2	130	159.18	150	114	210	120	84	B
3	140	173.84	144	115	170	129	78	B
4	110	142.82	153	112	150	125	55	-
5	140	122.09	153	119	210	121	61	MF
6	130	144.60	150	112	160	125	66	MF
Mean	128	155.08	152	115	182	127	71	
± S.D.	± 13	± 24[c]	± 5[g]	± 3[j]	± 26*	± 8*	± 12*	
20% FC 1211								
1	115	368.17	125	97	180	81	62	IP
2	150	273.36	112	90	210	103	78	IP
3	145	314.86	112	85	150	115	82	-
4	115	258.02	125	97	215	97	75	MF
5	140	204.05	122	87	210	106	81	-
6	130	214.30	106	86	145	103	75	-
Mean	133	272.13	117	90	185	101	76	
± S.D.	± 15	± 62.02[d]	± 8[g]	± 5[j]	± 32*	± 11*	± 7*	

IP = isolated premature contraction, B = bigemeny, MF = multifocal ventricular ectopic beats, VT = ventricular tachycardia.

b The value is larger than a (P less than 0.01).
c The value is larger than either a or b (P less than 0.01).
d The value is larger than a, b or c (P less than 0.01).
f The value is smaller than e (P less than 0.05).
g The value is smaller than e (P less than 0.01).
i The value is smaller than h (P less than 0.05).
j The value is smaller than h (P less than 0.01).
* The value is significantly different (P less than 0.01) than the corresponding parametrer measured before the isoproterenol challenge.

Table 20--Frequency Comparison of the Arrhythmogenic Effect of FC 1211 and Isoproterenol in the Presence and Absence of B_1 Adrenergic Blockade

FC 1211 inhalation concentration	No. of arrhythmogenic epidodes upon iso. challenge without B_1 blockade	No. of arrhythmogenic episodes upon iso. challenge with B_1 blockade
2%	6/6	0/6*
5%	8/8	1/6**
10%	4/4	5/6
20%	-	3/6

*Arrhythmogenic frequency is significantly decreased (P less than 0.01
**Arrhythmogenic frequency is significantly decreased (P less than 0.05).

DISCUSSION

Action potential threshold occurs when enough sodium channels open to allow an inward sodium current which is large enough to cause further depolarization and the rapid phase 0 upstroke (Mandel, 1980). Since none of the treatments changed threshold voltage significantly ($p<0.05$), it would appear that FC 1211, isoproterenol, atenolol, and combinations of these compounds do not affect the ability of sodium channel to react to external stimuli.

The escape time and spontaneous rate determinations simulate an interruption in normal impulses reaching the Purkinje fibers from the sinoatrial node (SA node). Causes of such an interruption in an intact heart include SA node dysfunction or a conduction block due to injury, ischemia, necrosis, or pharmacologic agents. When the SA node is the dominant pacemaker, it exerts an overdrive suppression of subsidiary pacemakers (cells, including Purkinje fibers, which have the property of automaticity, but normally fire spontaneously at a rate slower than SA node cells). Overdrive suppression is caused by driving a pacemaker cell at a greater frequency than its intrinsic spontaneous rate (Mandel, 1980). Sodium ions enter the cell with each action potential and this causes increased activity of the sodium-potassium exchange pump. Because this pump is electrogenic, that is, more sodium is pumped out than potassium pumped in, a hyperpolarization of the cell membrane occurs which suppresses spontaneous activity of the Purkinje fiber. During the escape time determination, when the external stimulus is absent, spontaneous action potentials do not occur until intracellular sodium concentration has become small enough to allow the cell to depolarize to threshold (Mandel, 1980). In Purkinje fibers exposed to FC 1211, the

mean escape time was much longer than control (Table 2). In many cases, individual fibers exposed to FC 1211 did not fire spontaneously for more than 3 minutes after cessation of the stimulus.

The firing rate of an automatic cell is related to the maximum diastolic potential (MDP), the activation voltage (AV), and phase 4 slope (Katz and Epstein, 1968). Decreased automaticity may be a consequence of hyperpolarization of the membrane: This was not the case with FC 1211 in this study. RMP and MDP were less negative than the control (Tables 3 and 6). Decreased automaticity in FC 1211-exposed fibers may emanate from depressed activation voltage (Table 6). Since the potential at which the cell will fire spontaneously is decreased, a greater depolarization of the membrane is necessary to bring the membrane potential to threshold. In a normal cell, as partial depolarization of the membrane occurs, sodium channels partially open in a voltage-dependent manner. Sodium influx due to the opening of some channels results in further depolarization which causes additional opening of channels. The exponential rise in sodium conductance generates phase 0 of the action potential (Katz, 1977). FC 1211 may cause sodium channels to open more slowly than normal, thus producing a more positive AV. When the Purkinje fiber is pretreated with the beta blocker, atenolol, then exposed to FC 1211, escape time is increased, but AV is not significantly different from control. Decreased phase 4 slope is another cause of depressed automaticity in Purkinje fibers. Diastolic depolarization in normal fibers is caused by a gradual decline of the outward potassium current (i_{K_2}) which allows the background inward sodium current to decrease membrane potential (Mandel, 1980). There is evidence that the potassium

pacemaker current ($^{i}K_2$) may be regulated by the beta receptor (Hashimoto, et al., 1979). If phase 4 slope is decreased, the membrane will not reach threshold potential as fast, escape time will be prolonged, and spontaneous rate will decrease.

Isoproterenol alone shortened escape time and increased spontaneous rate, although these changes were not significant at $p<0.05$. Because isoproterenol is believed to stimulate beta receptors and therefore increase $^{i}K_2$ (Reuter, 1974), this result was expected. When Purkinje fibers are treated with isoproterenol following FC 1211 exposure, escape time shortened toward control and spontaneous rate quickened (Fig. 6). Atenolol pretreatment partially abolished these effects. Atenolol at the 1.0×10^{-5} M concentration did not completely block the effects of isoproterenol on escape time and spontaneous rate. However, higher concentrations of atenolol prolonged escape time; 1.0×10^{-5} M atenolol had no significant effect on any of the parameters except AV and slope.

FC 1211-treated fibers exhibited depressed overshoot, resting membrane potential, amplitude, and dV/dt (Tables 3 and 5). RMP is dependent primarily upon the potassium ion gradient and the diffusion of these ions out of the cell. Outward movement of positive charges causes a net negative charge buildup within the cell. Perhaps FC 1211 affected the cell membrane so that it was less permeable to potassium. In this case, more positive charges would remain within the cell and RMP would be lowered. Depolarization could also be caused by enhancement of the background inward sodium current.

Purkinje fiber dV/dt is determined by the intensity of the sodium inward current which is dependent upon the size of the sodium

electrochemical potential gradient and the fraction of available sodium channels (Mandel, 1980). Depression of dV/dt by FC 1211 may have been caused by a partial inhibition of the rapid opening of fast sodium channels. The rapid sodium current that is responsible for the phase 0 was not affected greatly by catecholamines (Mandel, 1980). From that finding, one may infer that beta receptors do not control the rapid sodium current or dV/dt. A disparity exists in reports on the effects of catecholamines on dV/dt. Davis et al. (1969) showed a slight fall in dV/dt in Purkinje fibers superfused with epinephrine. However, Rosen et al., 1977 reported that epinephrine and isoproterenol each cause increases in dV/dt. The results presented in Table 5 concur with the Davis et al. study. Further decreases in dV/dt were caused by administration of FC 1211 + isoproterenol. If dV/dt is governed by the beta receptor, these findings indicate that beta receptor stimulation leads to a decrease in dV/dt. Another possibility is that atenolol caused a dV/dt increase and FC 1211 and isoproterenol cause a dV/dt decrease by a mechanism independent of the beta receptor. This mechanism could involve direct interaction with fast sodium channels.

Experiments using voltage-clamp,[a] tetrodotoxin (a fast channel blocker), verapamil (a slow channel blocker), and superfusing solutions in which a specific ion (potassium, sodium, or calcium, for example) is set at very high or very low concentrations may allow determination of

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[a] Voltage-clamp is a technique that permits control of the membrane potential by holding ("clamping") it at chosen values. During voltage-clamp experiments ionic currents can be determined (Noble, 1979).

the effects of FC 1211 on membrane ion currents. Although conduction velocity was not measured, FC 1211 may cause it to decrease. Conduction velocity is determined by several factors including dV/dt and AP AMP, both of which decreased with FC 1211. Disturbances in conduction are an important determinant in arrhythmogenesis.

The mean values of OS, RMP, and AP AMP in fibers superfused with isoproterenol 1.0 X 10^{-6} M are significantly lower than control. Reports of previous studies show conflicting results: in some experiments, beta agonists were shown to increase RMP and amplitude (Gilman, et al., 1980; Tsien, 1973; Noble, 1979) in other experiments, a decrease or no change from the control occurred (Katz and Epstein, 1968; Davis, 1969). OS, RMP, and AP AMP after exposure of the fiber to FC 1211 and isoproterenol were lower than FC 1211 alone.

Action potential duration (APD) and effective refractory period (ERP) were reduced by FC 1211 and further decreased by isoproterenol 1.0 X 10^{-6} M in the presence of FC 1211 (Table 4 and 5). An increase in flow of the repolarizing potassium current (i_x) may explain decreased APD due to FC 1211. This is believed to be the cause of the isoproterenol-induced APD abbreviation (Noble, 1979). Within the normal ventricular conduction system, APD progressively lengthens from the proximal His bundle to peripheral Purkinje fibers (Reiser and Anderson, 1980; Myerburg, et al., 1970; Gautier and Coraboeuf, 1980). The long refractory period of the gate cells serves a protective function by increasing the probability that a supraventricular premature impulse will encounter fibers which have not repolarized completely and are still refractory. In this study, ERP, APD_{50}, APD_{75}, and APD_{90} increased from

proximal to distal locations on the Purkinje fiber as measured during the control period (Tables 8 and 9). By decreasing APD and ERP, aberrant impulses may more easily traverse the conduction system and stimulate ventricular myocardium.

The action potential phases 2 and 3 shortened, gained a steeper negative slope, and therefore lost the plateau feature after FC 1211 treatment (Fig. 7). Phase 2 in a normal Purkinje fiber action potential is the result of inward currents nearly balancing outward currents (Tsien, et al., 1972, Mandel, 1980). The major inward current is conducted through slow calcium-sodium channels which are not yet completely inactivated. Outward currents include a potassium current, an inward flow of chloride (inward movement of negative charges produces a net outward current of positive charges), and an outward sodium current due to the activity of the sodium-potassium exchange pump (Mandel, 1980). The outward potassium current terminates the plateau and influences APD (Tsien et al., 1972). The absence of a distinct plateau in an action potential produced by an FC 1211-exposed fiber would indicate a lack of inward currents or an excess of outward currents. Perhaps the fast sodium channels or the slow calcium-sodium channels were more rapidly inactivated by FC 1211. More rapid inactivation of the slow calcium-sodium channels would also explain the negative inotropic effect of FC 1211. Beta receptor blockade by atenolol did not prevent extensive shortening of APD and ERP and steepening of phase 2 due to FC 1211. Atenolol completely blocked the shortening of APD_{50} due to isoproterenol. Although APD_{75}, APD_{90}, and ERP measured during control and atenolol + isoproterenol periods were not significantly different, it appears that

atenolol had not blocked the effects of isoproterenol completely because these 3 measurements were 6-8% shorter than control. Because beta blockade inhibits shortening of APD and ERP by isoproterenol (Tables 4 and 5), the beta receptor is at least partly responsible for control of repolarization current flows and fast channel reactivation.

Refractory periods are not necessarily dependent upon APD. Functional recovery of excitability may lag behind repolarization when reactivation of fast sodium channels is impaired. During exposure to FC 1211 and FC 1211 + isoproterenol, ERP had occurred after APD_{90}. During the other treatments and control, ERP had occurred before APD_{90}. Although beta blockade by atenolol did not prevent shortening of APD and ERP due to FC 1211, it did prevent lag of ERP beyond APD_{90}.

The electrogenic sodium-potassium exchange pump is responsible for production of an outward current. Stimulation of the sodium-potassium exchange pump by epinephrine is one explanation for a more negative maximum diastolic potential (Noble, 1979; Vassalle and Barnabei, 1971). Hyperpolarization of MPD by epinephrine may also be a result of an increase in the repolarizing potassium current, i_x (Katz, 1977). Isoproterenol did not cause an increased MDP in this study (Table 6).

Increased cAMP concentration after FC 1211 treatment was an unexpected result in light of the negative inotropic effects and decreased automaticity caused by the fluorocarbon. Cyclic AMP has been associated with beta receptor stimulation and the effects of positive inotropy, increased automaticity, and abbreviation of APD and ERP (Tsien, et al., 1972; Tsien, 1973). Using theophylline, a substance which raises cAMP levels by inhibiting the cAMP-breakdown enzyme, phosphodiesterase,

Tsien et al, (1972) showed that two effects of cAMP elevation, APD decrease and a shift toward more positive potential of the initial plateau level, could be dissociated. The mechanism which controlled rate of repolarization was more sensitive to cAMP level than the current component that determined the initial level of the plateau (Tsien, et al., 1972). A possible explanation for the effects seen with FC 1211 is that cAMP levels were elevated by the fluorcarbon just enough to induce shortening of APD. However, APD shortening was not blocked by atenolol, therefore, it would seem that FC 1211-induced APD abbreviation was not mediated through the beta receptor. The other cardiac effects caused by FC 1211 could have been a result of direct effects upon membrane currents.

Cyclic AMP concentration in tissues exposed to isoproterenol in the presence of FC 1211 was increased to a level above that produced by FC 1211 alone (Table 7 and Fig. 11). This increased cAMP level indicates that combined effects of FC 1211 and isoproterenol are probably additive and not synergistic. Synergism between FC 1211 and isoproterenol would suggest that the two compounds act at separate sites within the Purkinje fiber to bring about a rise in cAMP level. Since isoproterenol is known to produce its action through the beta receptor, FC 1211 would not act there, but at some point "downstream" if synergism had actually occurred. Possibilities for site of action of FC 1211 include the enzymes adenylate cyclase and phosphodiesterase. Several substances act directly on adenylate cyclase without affecting the beta receptor: fluoride ion (Constantopoulos and Najjar, 1973; deHaen, 1974), prostaglandin E1 (Constantopoulos and Najjar, 1973; deHaen, 1974), glucagon (Fricke, et al., 1980), guanosine triphosphate (Fricke, et al.,

1980), adenosine (Baumann, et al., 1981), and forskolin (Seamon et al.,1981). Methylxanthines and papaverine raise cAMP levels by inhibiting phosphodiesterase, the enzyme which breaks down cAMP (Argel et al., 1980; Tuganowski, 1977; Stirt et al., 1981).

Evidence in favor of FC 1211 acting on the beta receptor is the outcome of experiments with atenolol. Atenolol completely prevented the rise in cAMP due to FC 1211 alone and blocked about 80% of the rise in cAMP due toFC 1211 + isoproterenol 1.0×10^{-6} M (Table 7). Because atenolol is relatively specific for cardiac beta receptors (Hashimoto, et al., 1979; Singh et al., 1975; Barrett, 1977; Heel et al., 1979, Harry et al., 1974, Robinson et al., 1978), FC 1211 probably did act through the beta receptor to increase cAMP and that this increase was additive with the increase due to isoproterenol.

The poor correlation between cAMP concentration and electrophysiologic parameters was unexpected. One explanation for the lack of good correlation is that cAMP may not have changed to the same extent that electrophysiologic parameters changed after exposure to one of the pharmacologic agents. Perhaps the change in cAMP was not linear with compound concentration. Although the inotropic action of cAMP has been well documented (Azuma et al., 1981; Drummond and Severson, 1979; Pastan, 1972), its function of cAMP as a mediator of adrenergic chronotropic effects is still subject to dispute (Nawrath et al., 1980; Borasio and Vassalle, 1974). A single FC 1211 flow level sufficient to produce a mean bath concentration of 158.65 $\pm$ 5.09 µg/ml was used in this study. Previous work has shown that dogs which inspire a 5% mixture of FC 1211 in air develop a concentration of 93 µg/ml in the blood. This level was

sufficient to sensitize the heart to the arrhythmogenic actions of isoproterenol. A dose response study of FC 1211, using several different flows of the fluorocarbon could clarify the relation of FC 1211-induced cAMP increase to change in electrophysiologic parameters.

The yin yang hypothesis outlined by Goldberg et al. (Goldberg et al., 1975) does not appear to apply to the data collected in this study. This hypothesis would require that cGMP levels decline as cAMP level rises. None of the cGMP concentrations listed in Table 7 are significantly different from control, therefore, the data do not support the yin yang hypothesis.

Values of certain right ventricular Purkinje fiber parameters were found to differ from values of the corresponding left ventricular Purkinje fiber parameters (Tables 8 and 9). The right side showed a longer excape time and slower spontaneous rate than the left. This finding is in agreement with results from another set of experiments performed on canine Purkinje fibers (Reiser and Anderson, 1980). Significant differences between right and left were not observed during treatment periods. Lack of observed significant differences may have been caused by 1) actions of FC 1211 and isoproterenol which lessened disparity between left and right fibers; or more probably, 2) the sample size was too small and variation too large to allow detection of differences. OS was slightly lower and RMP slightly higher in right ventricular fibers than in left ventricular fibers. AP AMP was not observed to differ. This could be an indication that the whole right ventricular Purkinje fiber action potential was shifted slightly in the negative potential direction compared with the left. APD_{50}, APD_{75},

APD_{90}, and ERP were found to have a shorter duration on the right side than the left, although the differences for APD_{90} and ERP were not statistically significant. A study by Myerburg et al. (1970) revealed results which were inconsistant with these observations. They noted that Purkinje fibers from the right ventricle exhibited longer APD's and ERP's than those from the left ventricle. The interpretation of their finding was that a longer functional refractory period on the right side of the ventricular conduction system was an explanation for the common occurrence of right bundle branch block in aberrant ventricular ventricular conduction of premature supraventricular impulses. Several factors could account for the discrepancy in results between this study and the one conducted by Myerburg et al., (1970). The Tyrode's solution used in the experiments of the above mentioned group contained 3.0 mM KCl, while 4.0 mM KCl was used in the experiments described here. The higher KCl concentration may have caused more shortening and possibly changed the relation of right to left Purkinje fiber APD and ERP. No attempt was made in this study to identify the physiologic gate. Relative locations of cell impalements from one fiber to another were not necessarily the same. Myerburg et al. (1970) did identify the physiologic gate and did make comparisons between analogous locations of cell impalements.

A possible mechanism for the FC 1211-induced sensitization of the heart to catecholamines may lie with the ability of both catecholamines and FC 1211 to shorten APD and ERP. With shorter refractory periods, cells would tend to be reactivated and would be vulnerable to premature impulses over a greater period of the cardiac cycle. Premature supraven-ptricular impulses could more easily traverse the intraventricular

conduction system and stiumulate the myocardium. Impulses emanating from ventricular ectopic sites could more easily set up re-entrant loops because distal Purkinje fibers would be receptive to re-entrant stimuli.

Because dV/dt and AP AMP were lowered by FC 1211, conduction velocity may be decreased (Mandel, 1980). Addition of isoproterenol to FC 1211-exposed fibers further decreased dV/dt and AP AMP and, therefore, probably also decreased conduction velocity. A decrease in conduction velocity can produce decremental conduction (Mandel, 1980). This could cause a unidirectional block in conduction and favorable conditions for re-entrant loops.

Figure 7, A and B, shows baseline oscillations in a FC 1211-treated fiber which occurred during the escape time determination. This pattern may have been caused by fluctuations in the background inward sodium current, calcium-sodium slow inward current, or the pacemaker potassium current, $^{i}K_{2}$. In an intact heart, oscillations such as these could initiate a tachyarrhythmia, and combined with the other actions of FC 1211, perhaps lead to ventricular fibrillation.

FC 1211 and halothane may act through similar mechanism to sensitize the heart to catecholamines. Halothane has been found to decrease conduction velocity, shorten ERP and APD, decrease dV/dt, and decrease automaticity (Price and Ohnishi, 1980; Hauswirth, 1969; Zink et al., 1975; Turner et al., 1980). Halothane was discovered to effect a decline in slow channel conductance (Lynch et al., 1980). This is believed to be a cause of the negative inotropic effect of halothane, and may also help explain the negative inotropic effect of FC 1211 and the shortening of APD by both compounds (Kapur and Flacke, 1961).

Variability in Purkinje fibers played an important role in this study. Control Purkinje fibers had a wide range of values in many of the parameters measured (Table 1). This range may have been caused by many diverse factors, including size of the heart, health history of each dog, blood concentrations of catecholamines, glucagon, thyroid hormone, and other substances just before excision of Purkinje fibers from the heart, Purkinje fiber morphology (length, width, amount of connective tissue), and inherent biological variability.

Cyclic nucleotide values determined by radioimmunoassay are subject to a small degree of error. Purkinje fibers were removed from the tissue bath twenty minutes after the last pharmacologic agent had been added to the superfusing solution. A time course study was not performed to determine when cAMP reached its maximum level after exposure to a compound. It is possible that 1) cAMP concentrations peak before 20 minutes, then remain at that level or decrease; or 2) at 20 minutes cAMP has not yet reached its maximum concentration. A time course study would be important if further research is to be done on FC 1211 and cyclic nucleotides. Also, to transfer the tissue from the tissue bath to the homogenizer required some manipulation of the fiber and about 10 seconds. It is possible that tissue manipulation may have affected cyclic nucleotide content by causing stimulation or otherwise affecting the membrane. Great care was taken to treat the tissue in a uniform manner and to minimize tissue handling and the time from tissue bath to homogenization. Some Purkinje fibers contained more connective tissue than others. If cAMP content of connective tissue is low, then the cAMP content of a Purkinje fiber with much connective tissue will appear

low. This may be deceptive if, in fact, cAMP exists in high concentrations in discrete locations rather than a uniform concentration throughout the fiber. Cyclic nucleotides are present in Purkinje fibers in very low concentrations (pmol/mg protein range). Purkinje fibers contain high concentrations of substances which may potentially interfere with the assay including noncyclic nucleotides, of which ATP is an example (Steiner, 1979). However, antibodies used in the assay were screened to minimize possible cross-reactivity. Sensitivity of the assay was greatly increased by acetylating the cyclic nucleotides, a process which improved the affinity between antibody and cyclic nucleotide (Steiner, 1979; Harper and Brooder, 1975). Cyclic GMP levels for atenolol experiments were not reported because of possible cGMP contamination within the tissue bath and reservoir system. Use of dibutyryl-cGMP in the system for a separate study is believed to be the cause of contamination.

Variation in sample sizes among treatment groups was a problem with statistical analysis of the data. This resulted in a very conservative treatment of the data: several comparisons appear to be significantly different, but have not been so labeled because of small sample size, large variation, or both.

The toxicological effects of FC 1211 inhalation has been shown in this study to be two-fold. In agreement with findings related to other halogenated alkanes (Van Stee, 1974), the effect of FC 1211 is observed primarily on the central nervous and cardiovascular systems. The compound causes depression of the central nervous system creating effects ranging from tremors to convulsions, and sensitization of the heart to the arrhythmogenic effect of adrenergic amines.

The affects on the central nervous system appeared to be dose dependent. The time of FC 1211 inhalation prior to the onset of the initial convulsive seizure decreased with increasing concentration. Likewise, the severity or time duration between seizures also decreased with the increase in inhaled FC 1211 concentration. While EEG activity indicated central nervous system depression, it retained its functional ability to be stimulated by outside auditory and visual effects.

The ability of FC 1211 to directly induce spontaneous cardiac arrhythmias seems to be slight, if at all. Only one animal breathing a 5% FC 1211 concentration displayed any type of aberrant cardiac rhythm. The blood concentration of FC 1211 in that animal reached only 14% of the level attained in other animals inhaling higher concentrations, who displayed no signs of spontaneous cardiac arrhythmias. This isolated epidosde of sinus tachycardia with right bundle branch block may have been induced by any of a number of factors. These included, an exaggerate release of endogenous catecholamines, a sudden burst of cardiac nerve activity triggered by a direct effect of FC 1211 on the central nervous system or a direct effect of the compound on automaticity or

conduction of cardiac cells. An increase in peripheral plasma catecholamine levels induced by the inhalation of several concentrations of FC 1211 (2%, 5%, 10%) was not demonstrated (Unpublished data). Therefore, one or both of the two latter proposed occurrences may have been the underlying cause of the arrhythmogenic episode.

The sensitization by FC 1211 to isoproterenol induced cardiac arrhythmias was clearly shown. At all inhalation levels, the amount of exogenous adrenergic amine necessary to elicit minor ventricular arrhythmias (bigeminy, ventricular ectopic beats, ventricular tachycardia) was greatly reduced. Investigation has shown that the arrhythmogenic sensitization property of FC 1211 requires a venous blood concentration of 21-23 µg/ml (Hine et al., 1968), and is lost within 10 minutes after cessation of FC 1211 inhalation (Clark, 1970).

The use of atenolol, a cardiospecific adrenergic receptor antagonist devoid of local anesthetic properties (Barrett et al., 1973), completely blocked the sensitization effect of FC 1211 at low inhalation levels (2% and 5%). At higher levels (10% and 20%) the blockade was competitively overcome, and the arrhythmogenic sensitization properties of FC 1211 were again apparent. Comparison of the arrhythmogenic frequency data between FC 1211 inhalation levels of 10% and 20% shows a decrease in the number of arrhythmias displayed at the higher level. At the 20% inhalation level arterial pressure was greatly reduced and the number of aberrant rhythms induced was decreased. This effect is in agreement with studies by Katz (1965) which demonstrated the effects of blood pressure on hydrocarbon-epinephrine arrhythmias. It seems apparent

that a critical arterial pressure is necessary for the induction of hydrocarbon-adrenergic amine induced arrhythmias.

The mechanism underlying this FC 1211 sensitization property to the arrhythmogenic effect of adrenergic amines is indeed complex. Further elaboration of the function of the sympathetic nervous system of the heart, and the effects of B_1 receptor stimulation and the genesis of cardiac arrhythmias is therefore necessary.

The sympathetic nervous system richly innervates the heart with branches arising from the cardiac plexes (Mizeres, 1963). Postganglionic sympathetic fibers from these nerves innervate all types of cardiac fibers with their greatest density in the sinus and atrioventricular nodes. Upon activation of these nerves the catecholamine, norepinephrine, is released from the postganglionic sympathetic nerve terminal. Ahliquist (1948) demonstrated that the released catecholamine reacted with a receptor (B_1) in the heart and created the excitatory response in that effector organ.

Binding of the catecholamine to the beta receptor triggers a series of reactions. Studies have shown that beta receptor stimulation increases the activity of the membrane bound enzyme, adenylate cyclase. This cyclase then catalyzes the conversion of adenosine triphosphate to cyclic adenosine monophosphate (Robison et al., 1971). This compound elicits a majority of the effects of excitation on cardiac cells causing a positive inotropic and chronotropic effect.

A review by Katz and Epstein (1968) briefly describes the electrophysiologic effects of catecholamines on the heart. Catecholamines cause major effects on automaticity of specialized cardiac

conducting cells. They do not greatly alter threshold potential or maximum diastolic potential, however, they sharply increase the slope of spontaneous diastolic depolarization (phase 4) of cardiac pacemaker cells. Toda and Shimamoto (1968) conclude that since innervation density by the sympathetic nervous system as well as sensitivity to that innervation differs among cardiac cells, and since automaticity may be greatly enhanced by large doses of catecholamines, cardiac arrhythmias resulting from multiple ectopic pacemakers may arise. Additional electrophysiological changes include, increase in rate of rise of phase 0 depolarization and a markedly enhanced conduction velocity in the atrioventricular node (Wit et al., 1975). Giotti et al. (1973) described the effect of isoproterenol to cause accelerated repolarization of the Purkinje fiber action potential, and to shorten action potential duration.

The ventricular muscle itself is also affected by catecholamines released by sympathetic stimulation. Adrenergic amines cause a shortened action potential and a decrease in refractory period. According to Han and Moe (1964) the refractory period may become shortened in some ventricular muscle fibers and remain unaltered in other adjacent fibers. The resulting uneven decrease in refractoriness may predispose the heart to re-entry type arrhytymias.

The electrophysiological parameters of isolated cardiac tissue altered by sympathetic stimulation or the presence of adrenergic amines as described above are numerous. Experiments conducted on superfused canine Purjinke fibers display many similar electrophysiological alterations in the presence of FC 1211. We illustrated a dose-response effect of FC 1211 to decrease action potential duration (ADP_{50} and ADP_{90}) and

and to shorten the effective refractory period of these tissues. Isoproterenol also showed a dose-response effect and created decreases in the same parameters. He showed that fibers superfused simultaneously with FC 1211 and isoproterenol demonstrated decreases in these parameters greater than with either agent alone. The halogenated alkane appeared to be affecting the same membrane system or to be potentiating the effect of isoproterenol. The arrhythmogenic action suggested by the decrease in refractoriness and action potential duration either by FC 1211 or isoproterenol appear to be induced via similar mechanisms.

Many investigators have shown beta adrenergic antagonists to consistently and specifically abolish ventricular arrhythmias induced by a large array of halogenated hydrocarbons (Dresel et al., 1960; Schull et al., 1961 and Taylor et al., 1971). From these experiments it is difficult to discern if their conclusions are correct because some beta adrenergic blockers are also local anesthetics (Morales-Aguilera and Vaughan-Williams, 1965; Somani and Lum, 1966) and have direct effects on cardiac membranes (Sekiya and Vaughan-Williams, 1963) which may, in part, account for their antiarrhythmic action.

More recently with the development of new beta adrenergic antagonists devoid of local anesthetic activity this issue was again challenged. Both Sharma (1967) and Price et al. (1970) using beta antagonists with little or no local anesthetic effects were able to demonstrate blockade of catecholamine sensitization arrhythmias induced by the inhalation anesthetic halothane. Doherty and Aviado (1975) compared the beta blocking activity of propranolol (known to display

local anesthetic effects) and sotalol (known to be almost completely devoid of local anesthetic effects) with respect to their protection against sensitization arrhythmias. Both compounds were equally effective in preventing these arrhythmias.

In this study the employment of atenolol was crucial. This beta adrenergic antagonist is not only cardiospecific (B_1), but is also devoid of local anesthetic or intrinsic sympathomimetic actions. Atenolol showed a FC 1211 dose-dependent protection against isoproterenol sensitization arrhythmias. As the inhaled concentration of FC 1211 was increased the B_1 receptor blockade was overcome and the arrhythmogenic sensitization properties of isoproterenol were again apparent. This evidence appears to indicate that the sensitization property of FC 1211 is due to beta adrenergic stimulation and is primarily responsible for the combined FC 1211-isoproterenol arrhythmias seen in this study.

REFERENCES

Ahlquist, R.P.: A study of the adrenotropic receptors. Am. J. Physiol, 153:586, 1948.

Argel, M.I., Vittone, L., Grossi, A.O., Chiappe, L.E., and Cingolani, H.E.: Effect of phosphodiesterase inhibitors on heart contractile behavior, protein kinase activity, and cyclic nucleotide levels. J. Mol. Cell. Cardiol., 12:939-954, 1980.

Azuma, J., Sawamura, A., Harada, H., Tanimoto, T., Ishiyama, T., Morita, Y., Yamamura, Y., and Sperelakis, N.: Cyclic adenosine monophosphate modulation of contractility via slow Ca++ channels in chick heart. J. Mol. Cell. Cardiol., 13:577-587, 1981.

Barrett, A.M.: The pharmacology of atenolol. Postgrad. Med., J., 53 (Suppl. 3):58-64, 1977.

Barrett, A.M., Carter, J., Fitzgerald, J.D., Hull, R, LeCount, D: A new type of cardioselective adrenoceptive blocking drug. Brit. J. Pharmacol, 48:340, 1973.

Baumann, G., Schrader, J., and Gerlach, E.: Inhibitory action of adenosine on histamine- and dopamine-stimulated cardiac contractility and adenylate cyclase in guinea pigs. Circ. Res., 48:259-266, 1981.

Borasio, P.G. and Vassalle, M.: Dibutyryl cyclic AMP and potassium transport in cardiac Purkinje fibers. Am. J. Physiol., 226:1232-1237, 1974.

Clark, D.G.: The toxicity of bromochlorodifluoromethane (BCF) to animals and man. Tech. Report Imp. Chem. Inds., Ltd., Chesire, England, 1970.

Constantopoulos, A. and Najjar, V.A.: The activation of adenylate cyclase: II. The postulated presence of (A) adenylate cyclase in a phospho (inhibited) form (B) a dephospho (activated) form with a cyclic adenylate stimulated membrane protein kinase. Biochem. Biophys. Res. Commun., 53:794-799, 1973.

Davis, L.D., Tempte, J.V., and Murphy, Q.R.: Epinephrine-cyclopropane effects on Purkinje fibers Anesthesiology, 30:369-377, 1969.

Doherty, R.E., Aviado, D.M.: Toxicity of aerosol propellants in the respiratory and circulatory systems. Toxicology, 3:213, 1975.

Dresel, P.E., MacCannell, K.L., Nickerson, M.: Cardiac arrhythmias induced by minimal doses of epinephrine in cyclopropane anesthetized dogs. Circ. Res., 8:948, 1960.

Drummond, G.I. and Severson, D.L.: Cyclic nucleotides and cardiac function. Circ. Res., 44:145-153, 1979.

Fricke, R.F., Queener, S.F., and Clark, C.M.: Cardiac adenylate cyclase: kinetics of synergistic activation by guanosine-5'-triphosphate (GTP) and glucagon. J. Mol. Cell. Cardiol., 12:595-608, 1980.

Gautier, P. and Coraboeuf, E.: The site of gating in the ventricular conducting system of rabbit, dog, and monkey hearts. Experientia, 36:431-433, 1980.

Gilman, A.G., Goodman, L.S., and Gilman, A.: The Pharmacological Basis of Therapeutics, ed 6. New York, MacMillian Publishing Company, 1980, pp 1843.

Giotti, A., Ledda, F. and Mannaioni, P.F., 1973. Effects of noradrenaline and isoprenaline in combination with alpha and beta receptor blocking substances on the action potential of cardiac Purkinje fibers. J. Physiol., 229:99, 1973.

Goldberg, N.D., Haddox, M.K., Nicol, S.E., Glass, D.B., Sanford, C.H., Kuehl, F.A., Estensen, R.: Biologic regulation through opposing influences of cyclic GMP and cyclic AMP: the Yin Yang hypothesis. Adv. Cyclic Nucleotide Res., 5:307-330, 1975.

de Haen, C.: Adenylate cyclase., J. Biol. Chem. 249:2756-2762, 1974.

Han, J., Moe, G.K.: Nonuniform recovery of excitability in ventricular muscle. Circ. Res., 14:44, 1964.

Harper, J.F. and Brooder, G.: Femtomole sensitive radioimmunoassay for cyclic AMP and cyclic GMP after 2'0-acetylation by acetic anhydride in aqueous solution. J. Cyclic Nuc. Res., 1:207-218, 1975.

Harry, J.D., Knapp, M.F., and Linden, R.J.: The actions of a new -adrenoceptor blocking drug, I.C.I. 66082, on the rabbit papillary muscle and on the dog heart. Br. J. Pharmacol., 51:169-177, 1974.

Hashimoto, K., Hauswirth, O., Wehner, H.D., and Ziskoven, R.: The relation between the current underlying pacemaker activity and beta-adrenoceptors in cardiac Purkinje fibers: A study using adrenaline, procaine, atenolol, and penbutolol. Naunyn-Schmiedeberg's Arch. Pharmacol., 307:9-19, 1979.

Hauswirth, O.: Effects of halothane on single atrial, ventricular, and Purkinje fibers. Circ. Res., 24:745-750, 1969.

Heel, R.C., Brogden, R.N., Speight, T.M., and Avery, G.S.: Atenolol: A review of its pharmacological properties and therapeutic efficacy in angina pectoris and hypertension. Drugs. 17:425-460, 1979.

Hine, C.H., Elliott, H.W., Haufman, J.W., Leung, S., Harrah, M.D.: Clinical toxicological studies on freon, FE 1301. AMRL-TR-68-175, Aero. Med. Res. Lab, Wright-Patterson Air Force Base, Ohio, 1968.

Kapur, P.A. and Flacke, W.E.: Epinephrine-induced arrhythmias and cardiovascular function after verapamil during halothane anesthesia in the dog. Anesthesia, 55:218-225, 1981.

Katz, A.M.: Physiology of the Heart. New York, Raven Press, 1977, pp 450.

Katz, R.L.: The effects of alpha and beta adrenergic blocking agents on cyclopropane-catecholamine cardiac arrhythmias. Anesthesiology, 26:289, 1965.

Katz, R.L. and Epstein, R.A.: The interaction of anesthetic agents and adrenergic drugs to produce cardiac arrhythmias. Anaesthesia, 29:763-783, 1968.

Lynch, C., Vogel, S., and Sperelakis, N.: Halothane depresses cardiac slow action potentials. Anesthesiology, 53:S420, 1980.

Mandel, W.J.: Cardiac Arrhythmias, Their Mechanisms, Diagnosis, and Management. Philadelphia, J. P. Lippencott Co., 1980, pp 681.

Mizeres, N.J.: The cardiac plexes in man. Am. J. Anat., 112:141, 1963.

Morales-Aguilera, A., Vaughan-Williams, E.M.: The effects on cardiac muscle of beta-receptor asntagonists in relation to their activity as local anesthetics. Brit. J. Pharmacol., 24:332, 1965.

Myerburg, R.J., Stewart, J.W., and Hoffman, B.F.: Electrophysiological properties of the canine perpheral A-V conducting system. Circ. Res., 26:361-378, 1970.

Nawrath, H., Blei, I., and Gegner, R.: Opposite effects of -adrenoceptor stimulation and 8-bromo-cyclic AMP on potassium efflux in mammalian heart muscle. Experientia, 36:72-74, 1980.

Noble, D.: The Initiation of the Heart Beat. 2nd ed. Oxford, Clarendon Press, 1979, pp 186.

Pastan, I.: Cyclic AMP. Sci. Am., 227:97-105, 1972.

Price, H.L. and Ohnishi, S.T.: Effects of anesthetics on the heart. Fed. Proc., 39:1575-1579, 1980.

Price, H.L., Skovsted, P., Pauca, A.L., Cooperman, L.H.: Evidence for -receptor activation produced by halothane in normal man. Anesthesiology, 32:389, 1970.

Reiser, J. and Anderson, G.J.: Differences in automaticity between Purkinje strands from right and left dog ventricle. Am. J. Physiol. 239 (Heart Circ. Physiol. 8): H247-H251, 1980.

Reuter, H.: Localization of beta adrenergic receptors, and effects of noradrenaline and cyclic nucleotides on action potentials, ionic currents, and tension in mammalian cardiac muscle. J. Physiol. (Lond), 242:429-451, 1974.

Robinson, C., Birkhead, J., Crook, B., Jennings, K., and Jewitt, D.: Clinical electrophysiological effects of atenolol--a new cardioselective beta-blocking agent. Br. Heart J., 40:14-21, 1978.

Robison, G.A., Butcher, R.W., Sutherland, E.W.: Cyclic AMP, Academic Press, Inc., New York, 1971.

Rosen, M.R., Hordof, A.J., Ilvento, J.P., and Danillo, P.: Effects of adrenergic amines on electrophysiological properties and automaticity of neonatal and adult canine Purkinje fibers. Circ. Res., 40:390-433, 1977.

Schull, L.G., Berry, G., Villarreal, P.: Preventin and correction of ventricular arrhythmias by dichloroisoproterenol in dogs anesthetized with cyclopropane. Anesthesiology, 22:444, 1961.

Seamon, K.B., Padgett, W. and Daly, J.W.: Forskolin: unique diterpene activator of adenylate cyclase in membranes and in intact cells. Proc. Natl. Acad. Sci. USA, 78:3363-3367, 1981.

Sekiya, A., Vaughan-Williams, E.M.: A comparison of anticirculatory actions and effects on intracellular cardiac potentials of pronethalol, disopyramide, and quinidine. Brit. J. Pharmacol., 21:473, 1963.

Sharma, P.L.: Specific adrenergic beta-receptor blockade in the prevention of adrenaline-induced ventricular arrhythmias in dogs anesthetized with halothane in nitrous-oxide-oxygen. Brit. J. Anaesth., 39:766, 1967.

Singh, B.N., Nisbet, H.D., Harris, E.A., and Whitlock, R.M.L.: A comparison of the action of I.C.I. 66082 and propranolol on cardiac and peripheral -adrenoceptors. Eur. J. Pharmacol., 34:75-86, 1975.

Somani, P., Lum, B.K.B.: Blockade of epinephrine and ouabain induced cardiac arrhythmias in the dog heart-lung preparation. J. Pharmacol. Exptl. Ther. 152:235, 1966.

Steiner, A.L.: Cyclic AMP and cyclic GMP. In Jaffe, B.M. and Behrman, H.R. (ed): Methods of Hormone Radioimmunoassay, ed 2. New York, H.R. Academic Press, 1979, pp 3-17.

Stirt, J.A., Berger, J.M., Roe, S.D., Ricker, S.M., Sullivan, S.F.: Halothane-induced cardiac arrhythmias following administration of aminophylline in experimental animals. Anesth. Analg., 60:517-520, 1981.

Stirt, J.A. and Sullivan, S.F.: Aminophylline. (Review Article). Anesth. Analg., 60:587-602, 1981.

Taylor, G.J., Harris, W.S., Bogdonoff, M.D.: Ventricular arrhythmias induced in monkeys by the inhalation of aerosol propellants. J. Clin. Inv., 50:1546, 1971.

Toda, N., Shimamoto, K.: The influence of sympathetic stimulation on transmembrane potentials in the SA node. J. Pharmacol. Exptl. Ther., 159:298, 1968.

Tsien, R.W.: Adrenaline-like effects of intracellular iontrophoresis of cyclic AMP in cardiac Purkinje fibers. Nature (New Biol.), 245:120-122, 1973.

Tsien, R.W., Giles, W., and Greengard, P.: Cyclic AMP mediates the effects of adrenaline on cardiac Purkinje fibers. Nature (New Biol.), 240:181-183, 1972.

Tuganowski, W.: The influence of adenylate cyclase inhibitors on the spontaneous activity of the cardiac pacemaker. Arch. Int. Pharmacodyn., 225:275-286, 1977.

Turner, L.A., Zuperku, E.J., Purtock, R.V., and Kampine, J.P.: In vivo changes in canine ventricular cardiac conduction during halothane anesthesia. Anesth. Analg., 59:327-334, 1980.

Van Stee, E.W.: A review of the toxicology of halogenated fire extinguishing agents. AMRL-TR-74-143, Aerosp. Med. Res. Lab, Wright-Patterson Air Force Base, Ohio, 1974.

Vassalle, M. and Barnabei, O.: Norepinephrine and potassium fluxes in cardiac Purkinje fibers. Pflugers Arch. ges. Physiol. 322:287-303, 1971.

Wit, A.L., Hoffman, B.F., Rosen, M.R.: Electrophysiology and pharmacology of cardiac arrhythmias. IX. Cardiac electrophysiologic effects of beta adrenergic receptor stimulation and blockade. Part A. Am. Heart J. 90:521, 1975.

Zink, J., Sasyniuk, B.I., and Dresel, P.E. Halothane-epinephrine-induced cardiac arrhythmias and the role of heart rate. Anesthesiology, 43:548-555, 1975.

PUBLICATIONS

The Electrophysiologic Effects of Bromochlorodifluormethane on Canine Purkinje Fibers. S.M. Strauch in preparation for submission to Toxicol. Appl. Pharmacol.

Cardiac Arrhythmogenesis of Bromochlorodifluoromethane in Conscious Dogs. Peterson, G.H. and Strauch, S.M. in preparation for submission to J. Cardiovas. Pharmacol.

The Role of Cyclic Nucleotides in Bromochlorodifluoromethane Sensitization to Adrenergic Amines. Ernest, H.B. and Strauch, S.M. in preparation for submission to J. Pharmacol. Expt. Ther.

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DEGREES

G. H. Peterson, MSc received Sept. 2, 1982. Thesis entitled: Cardiac Effects of Bromochlorodifluoromethane in Conscious Dogs.

H. B. Ernest, MSc received Sept 2, 1982. Thesis entitled: Canine Cardiac Purkinje Fiber Electrophysiology and Cyclic Nucleotide Content During Fluorocarbon Exposure.

INTERACTIONS

(A) Spoken: Preliminary findings were presented at: Review of Air Force Sponsored Basic Research in Environmental Protection, Toxicology and Electromagnetic Radiation Bioeffects, San Antonio, Texas, 15-17 January 1980.

Arrhythmogenic Properties of Halogenated Alkanes: Review of Air Force Sponsored Basic Research in Enviromental Toxicology, Columbus, Ohio, 2-3 June 1981.

Exchange of ideas and discussion of research with colleagues at The Ohio State University Veterinary College who participate in Cardiovascular and Electrophysiology Discussion Groups. Seminars presented to The Department of Veterinary Physiology and Pharmacology by Peterson and Ernest in conjunction with Thesis requirements.

(B) Consultations: Richard A. Davis, Toxicologist, Celanese Corporation (formerly AMRL/THT) regarding GC quantitation of halogenated alkanes.

Col. Roger Inman and Mr. Jeff Fisher, AMRL/THE regarding electrophysiology instrumentation for studying the effects of toxicants. Met with the above on 2/3/82 in Cols., Ohio and on 5/3/82 at WPAFB, Ohio.