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Autonomic influence on pharmacologically-induced cardiac arrhythmogenesis

Edward L. Chan
The University of Montana

AUTONOMIC INFLUENCE ON PHARMACOLOGICALLY-INDUCED CARDIAC ARRHYTHMOGENESIS

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Edward Chan

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Autonomic Influence on Pharmacologically-induced Cardiac Arrhythmogenesis.

Director: Charles L. Eyer, Ph.D.

Intravenous infusion of arrhythmogenic drug solutions was used as a pharmacological tool to measure the ventricular fibrillation threshold. Reserpinized animals increased the fibrillation threshold of McN2165. Animals reserpinized two weeks prior to the experiment still demonstrated an increased fibrillation threshold. Pronethalol, norepinephine, phenoxybenzamine, and neostigmine all increased the fibrillation threshold of McN2165, while atropine produced no change. Isoproterenol was the only drug that lowered the threshold. Reserpinized animals pretreated with norepinephrine still demonstrated an increased fibrillation threshold. Isoproterenol decreased the threshold even when the animals were reserpinized. Pronethaloel was able to suspend the fibrillation induced by McN2165. *Beta* receptors appear to be involved in the induction ventricular fibrillation by McN2165.

Reserpine and pronethalol both increased the fibrillation threshold of strophanthin K, while isoproterenol lowered the threshold. Pronethalol was not able to suspend the fibrillation induced by strophanthin K.

Reserpine, pronethalol, and isoproterenol all increased the fibrillation threshold of barium chloride and pronethalol could not suspend the fibrillation induced by it.

Normal administration of reserpine and isoproterenol did not change the threshold of aconitine. Pronethalol and intraperitoneal injection of reserpine increased the fibrillation threshold. Pronethalol was also unable to stop the fibrillation induced by aconitine. The arrhythmogenic action of McN2165 is different from any other arrhythmogenic agent used in this study.

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CHAPTER 1

INTRODUCTION

The heart contains four chambers--two thin walled atria and two thicker-walled ventricles. The atria lie above the ventricles and are responsible for forcing the blood they receive into the ventricles. The ventricles do most of the work of the heart, expelling the blood into the circulation. In the mammalian heart the S.A. (sino-atrial) node located in the right atrium is the pacemaker (Keith and Flack, 1970). This region initiates a depolarizing current which spreads over the atria. The atrioventricular or A.V. bundle, begins with the A.V. (atrioventricular) node at the base of the atrial septum and passes along the upper edge of the ventricular septum. The A.V. bundle then divides into two branches which run down each side of the ventricular septum to the right and the left ventricles. Each branch forms a reticulated layer under the endocardium of the ventricles, called Purkinje tissue. The excitatory process is transmitted from the S.A. node to the A.V. node and then through the A.V. bundle to the Purkinje tissue. The wave of excitation allows calcium to reach the myofibrils and initiate contraction (Brody, 1964). In this way, the two atria can contract

almost simultaneously and empty the blood into the two ventricles. The ventricles then contract and expel the blood into the circulation.

Cardiac output, the amount of the blood expelled per heart beat, depends on the heart rate and stroke volume. Cardiac output is regulated by the autonomic nervous system and by venous return. The sympathetic branch of the autonomic nervous system contains the accelerator fibers that go to the heart. Those in the right outflow are distributed mainly to the S.A. node, and those from the left outflow supply the A.V. node. Stimulation of these accelerator nerves causes the release of catecholamines, which in turn cause an increase in rate (chronotropism) and force of contraction (inotropism). This applies both to the atria and to the ventricles. Randall (1956) showed that the right and left cardiac sympathetic innervation are different in many respests. The right and left stellate ganglia have predominantly chronotropic and inotropic effects respectively and the right and left stellate ganglia have opposite effects on the T wave of the electrocardiogram (Yanowitz, 1966).

The other part of the autonomic nervous system is the parasympathetic nervous system. The vagus nerves are cardioinhibitory, conveying impulses from the cardioinhibitory center in the medulla to the S.A. node and A.V. node of the heart. Both S.A. node and A.V. node are influenced by the autonomic nerves (Urthaler, *et al*, 1973). Stimulation of

vagus nerve slows the heart rate (negative chronotropism) and the conductivity of the atria to the ventricles (negative dromotropism). Strong stimulation of the vagus can completely stop the rhythmic contraction of the S.A. node or completely block the transmission of the cardiac impulse through the A.V. junction. The parasympathetic nervous system dominates the S.A. node while the sympathetic dominates the A.V. node (Bell, *et al*, 1970).

Cardiac output is a function of Starling's Law which states that the force of contraction (systole) of the heart is related to the degree of stretching of the muscle prior to contraction. The heart also responds to the blood concentration of catecholamines. These hormones are released by the adrenal medulla upon stimulation by the autonomic nervous system. The central nervous system also plays an important role in controlling blood pressure and heart rate (Laubie, 1976).

The S.A. node controls the rate of the heart. The rate of depolarization of the S.A. node is considerably greater than that of any other region of cardiac tissue. Each time the S.A. node discharges, its impulse is conducted both into the A.V. node and into the Purkinje fibers, discharging their excitable membranes. Then all the tissues begin their cycle of recovery. But the S.A. node recovers much more rapidly than does any other part of the heart, and emits still another impulse. Under pathological conditions, other areas of

the heart develop a rhythmic discharge rate which is more rapid than that of the S.A. node. These parts can be the A.V. node, Purkinje fibers, or any points in the atrial or ventricular muscle. These additional pacemakers are called ectopic foci. An ectopic pacemaker causes an abnormal sequence of contractions.

Abnormal cardiac rhythms can be caused by:

1. Abnormal rhythmicity of the pacemaker itself.
2. Shift of the pacemaker from the S.A. node to other parts of the heart.
3. Blocks at different points in the transmission of the impulse through the heart, usually from a myocardial infarction.
4. Abnormal pathways of impulse transmission through the heart, and
5. Spontaneous generation of abnormal impulses in almost any part of the heart.

In any general discussion of the initiation of cardiac arrhythmias, the relative merits of the ectopic focus and reentrant theories, is bound to arise.

Segers (Dawes, 1952) first described the possibility of an ectopic focus as the origin of arrhythmias. The ectopic focus is not limited to one single cell. It can be a small group of cells sending rapid impulses in all directions. This results in rapid rates of contraction, leading to tachycardia, flutter and even fibrillation.

The reentrant activity theory consists of two elements; the circus movement and the focal reexcitation. McWilliam (1908) first described the possibility of circus movement as the underlying mechanism of cardiac arrhythmias. It is due to propagation of the impulse in a circular path. A fundamental requirement for such a circus movement is an area of refractory tissue which initially fails to respond to an impulse propagating in one direction. This is due to the presence of a unidirectional block. Because this area then recovers its excitability, it accepts reentry of the same impulse when it returns in the opposite direction. Such a continuous circus movement will be possible only if an excitable area of tissue always exists in front of the excitation process. Mauritis and co-workers (1973) demonstrated the circus movement as a mechanism of tachycardia in rabbit atrial muscle preparation. The other type of reentrant activity is the focal reexcitation. It is not associated with a circus movement loop. It may occur if there is a disparate rate of repolarization in neighboring myocardial fibers, so that fibers already repolarized are reexcited by fibers still depolarized. The potential difference of the depolarized fibers exceeds the threshold potential of the repolarized fibers. This would result in closely coupled ventricular ectopic beats. Reexcitation of this type may generate rapid, repetitive responses in the ventricle. Han and co-workers (1964) demonstrated that focal reexcitation may occur following coronary occlusion.

Ventricular fibrillation is a major cause of sudden deaths from heart diseases. Evidence indicates that catecholamines may be involved in the genesis of the early ventricular arrhythmias that accompany an acute myocardial infarction. In 1911 Levy showed that epinephrine could induce ventricular fibrillation in cats under chloroform anaesthesia. Raines and co-workers (1970) showed that diphenylhydantoin increased the fibrillation threshold of ouabain-induced ventricular arrhythmias by suppression of the sympathetic influence. The use of *beta* receptor blocker seemed to both prevent and cure the fibrillation precipitated by cardiac glycosides (Vaughn Williams, *et al*, 1963). It was found that catecholamines secreted by the sympathetic ganglia have several biochemical effects on cardiac muscle. These hormones stimulate the formation of cyclic 3'5' adenosine monophosphate by the enzyme adenyl cyclase (E. W. Sutherland, *et al*, 1961). The cyclic AMP serves as a "second messenger" to initiate lipolysis, glycogenolysis and inhibition of glycogen synthesis. High cyclic AMP levels in myocardial muscle have positive inotropic effects on the heart (Burthon, 1973). It has been suggested that catecholamines increase the concentration of cyclic AMP which in turn acts at the sarcoplasmic reticulum to accelerate the inflow of calcium ions and cause the positive inotropic effect. Further studies showed that cyclic AMP concentration was increased during ventricular fibrillation caused by ischaemic heart disease or a myocardial infarction (Wollenberger *et al*, 1969).

Tsien and co-worker (1972) found that cyclic AMP increased the pacemaker activity in normal Purkinje fibers in the heart. This indicated that increases in cyclic AMP level might lead to repetitive excitation in the partially depolarized, ischaemic heart muscle. Further evidence showed that the higher cyclic AMP level produced by catecholamines decreased the ventricular fibrillation threshold in isolated rat hearts (Lubbe, *et al*, 1976). It is possible that the ventricular fibrillation produced by catecholamines is due to elevated cyclic AMP levels in the myocardial tissue (Murad, *et al*, 1961).

There are several techniques employed to study the mechanism by which an arrhythmogenic drug induces cardiac arrhythmias. The isolated heart preparation has been widely used (Stafford, 1963). Another preparation is the heart-lung preparation. This technique was first used by Krayer in 1931 (Burn, *et al*, 1958). The use of the whole animal to measure the fibrillation dose or fibrillation threshold of barium chloride was employed by Smith *et al*, (1940). Since then, intravenous infusion of arrhythmogenic substances to measure the fibrillation threshold has become one of the classic experiments to measure the efficacy of antiarrhythmic agents. Also, other drugs were employed in the whole animal preparation to pharmacologically modify the fibrillation threshold. A disadvantage of this preparation is that a given drug may have multiple effects on the organism. The advantage of

using this preparation in this research is that the nervous system, hormonal secretion, and the physiological reflexes are all present.

Aconitine is a classic arrhythmogenic drug. Local application of aconitine to the atrial appendage, in the "intact" anesthetized dog produces atrial flutter and fibrillation (Sharma, 1963). Intravenous injection of aconitine nitrate solution can produce ventricular tachycardia and fibrillation. It is believed that these arrhythmias are due to the rapid discharge of an ectopic focus (Scherf, *et al*, 1949).

Barium chloride is an inorganic compound that has the effect of stimulation of all smooth muscles. Intravenous injection causes a sharp rise of blood pressure at the same time as extrasystoles occur. Barium causes depolarization and a considerable increase in the after potential. Sufficient dose can cause ventricular fibrillation (Smith, *et al*, (1940).

Storphanthin K, one of the cardiac glycosides, has the ability to increase the force of myocardial contraction. Cardiac glycosides have been shown to inhibit a sodium and potassium-stimulated adenosine triphosphatase (ATPase). In sufficiently high dose, cardiac glycosides may precipitate arrhythmias, including ventricular fibrillation (Sekiya, *et al*, 1963).

McN2165 is an experimental compound that can produce ventricular arrhythmias. It has the effect of increasing

the QRS interval and cyclic AMP level (Pryss, 1969; Eyer and Johnson, 1977). The terminal effects of the compound in anesthetized rat included ventricular tachycardia, ventricular flutter, ventricular fibrillation, and death. The mechanism of induction of ventricular fibrillation by this agent has not been determined. In order to determine the role of the autonomic nervous system in the cardiac arrhythmias caused by McN2162, the following autonomic agents were used:

Reserpine is an alkaloid found in extracts of *Rauwolfia serpentina*. In the central nervous system reserpine depletes serotonin and norepinephrine from neurons. Other peripheral neurons as well as the adrenal medulla are also depleted. The catecholamine uptake mechanism is also antagonized (Matti, *et al*, 1958). Initially, the responses to indirect acting sympathomimetic amines are potentiated, but later are absent. The effects of reserpine remain for several weeks.

Neostigmine is an anticholinesterase. This drug binds to cholinesterase and is hydrolyzed extremely slowly. Neostigmine has a direct depolarizing action on the neuromuscular junction. The predominant effects on the heart will be bradycardia due to enhanced vagus nerve influence on the S.A. node.

The antagonist to neostigmine is atropine. Atropine competitively antagonizes the action of cholinergic drugs at the muscarinic receptors. It increases the heart rate by blocking the effects of vagus nerve on the S.A. node.

Phenoxybenzamine is a selective *alpha* adrenergic blocker, totally without effects on the *beta* responses. It is a very potent irreversible blocker of norepinephrine. Therefore, this drug potentiates the *beta* action of norepinephrine. Phenoxybenzamine reflexively increases the heart rate because of its vasodilating action (Moran, *et al*, 1962).

Pronethalol is a *beta* adrenergic blocker. This drug is a potent blocker of catecholamine action on the heart, and also has a depressant action on adrenergic nerves, (Standaert, *et al*, 1967).

Norepinephrine is the postganglionic neurotransmitter of the sympathetic nervous system. It is more active on *alpha* receptors than on *beta* receptors, but is by no means devoid of *beta* activity. Norepinephrine increases heart rate and blood pressure. A sufficiently high dose can cause cardiac arrhythmias.

The actions of isoproterenol are exerted almost entirely on the *beta* receptors. This drug produces strong positive intropic and chronotropic actions on the heart. Isoproterenol stimulates adenyl cyclase and is about ten times as potent as either epinephrine or l-norepinephrine (Sutherland, *et al*, 1960).

The effects of these autonomic agents on the arrhythmogenic action of McN2165 was also compared with their action on the other arrhythmogenic agents.

CHAPTER 2

MATERIALS AND METHODS

Methods

Male Sprague-Dawley rats (350-400 grams) were used for all the experiments. Animals were anesthetized with sodim pentobarbital (30-35 mg/kg i.p.), supplemented by additional doses as needed. The rats were connected to a physiograph from Narco Bio Systems, Inc. The lead II electrocardiograms (EKG) were recorded continuously with an ink-writing Oscillograph (paper speed 5 cm/sec) and monitored on a type 502 Dual-Beam Oscilloscope. McN2165, aconitine, barium chloride, and strophanthin K were administered through the femoral vein by means of an infusion syringe pump (Model 255-2 Sage Instruments) until ventricular fibrillation occurred. The rate on constant infusion of the arrhythmogenic agents was 1.80 mg/kg/min for McN2165, 4.32 mg/kg/min for barium chloride, 0.014 mg/kg/min for aconitine nitrate, and 2.7 mg/kg/min for strophanthin K. The infusion was discontinued as soon as the ventricular fibrillation started. A timer was used to time the onset of ventricular fibrillation.

A separate series of experiments, the animals were infused to the point of ventricular fibrillation by the

arrhythmogenic agents. As soon as the heart started to fibrillate, pronethalol 5 mg/kg was given intravenously to see if the fibrillation could be suspended.

Preparation and Administration of Drugs

These arrhythmogenic agents are prepared as follows:

Because of its low water solubility, McN2165 (McNeil Laboratories, Inc.) was dissolved in 75% alcohol and 25% normal saline. This drug was agitated approximately 15 minutes until it all dissolved, yielding a solution of 10 mg/ml. Aconitine nitrate (S. B. Penick and Company), barium chloride (J. T. Baker Chemical Co.), and strophanthin K (Nutritional Biochemical Corporation) were dissolved in distilled water to give a solution of 0.5 mg/ml, 40 mg/ml, and 25 mg/ml respectively.

Proenthalol (Ayerst Laboratories) neostigmine bromide (Hoffmann-LaRoche, Inc.) and atropine sulfate (Mallinckrodt Chemical Works) were dissolved in distilled water and administered rapidly via the femoral vein ten minutes prior to infusion of the arrhythmogenic agent.

L-Isoproterenol-D-Bitartrate (Sigma Chemical Co.) and norepinephrine (Levophed^{R} Bitartrate, Winthrop Laboratories, Inc.) were dissolved in distilled water and were given intravenously twenty seconds prior to the arrhythmogenic agents.

Phenoxybenzamine hydrochloride (Smith Kline and French Labs.) was dissolved in distilled water and given intraperitoneally one hour before infusion.

Reserpine (Serpasi^{R}, CIBA Pharmaceutical Co.) was dissolved in distilled water at pH 2.2 by addition of acetic acid. This drug was given to three groups of animals intramuscularly at 1.25 mg/kg 72 hours prior, 2.5 mg/kg 24 hours prior and 2.5 mg/kg 2 weeks prior to infusion in the McN2165 experiment. In the barium chloride, strophanthin K and aconitine experiments, the rats were injected with 2.5 mg/kg of reserpine intramuscularly 24 hours prior to infusion. In the aconitine experiment, one group of rats was given 2.5 mg/kg of reserpine intraperitoneally 24 hours prior to infusion.

Calculations

The heart rates of the animals were recorded before pretreatment to see if the pretreatment had any effect on cardiac rhythm. The time required for the arrhythmogenic agents to induce ventricular fibrillation was converted to dose. The following formula was used to convert the time (sec) to dose (mg/kg):

$$\frac{\text{time (sec)} \times \text{Concentration of drug (mg/ml)} \times \text{rate of infusion (ml/sec)}}{\text{weight (kg)}}$$

The total doses were averaged to give a mean dose plus or minus the standard deviation.

The doses required to produce these changes were compared for the control and drug-treated groups. Student's t-test was used for statistical analysis of the result. The 5% level of significance was arbitrarily chosen to define

whether values were or were not significantly different. The fraction of fibrillating animals was also calculated and recorded.

CHAPTER 3

RESULTS AND DISCUSSION

Preliminary Findings

Anesthetized rats were hooked up to the Lead II electrocardiograph for observation. It was found that the dose of sodium pentobarbital that was just sufficient to anesthetize the animal had little or no suppressant effect on the heart. The average heart rate was 420 beats per minute. There was uniform heart rhythm and no arrhythmias or abnormal beats were found in any anesthetized rats.

Kostis and co-worker (1973) showed that alcohol demonstrated antiarrhythmic properties at high doses and also suppressed ventricular tachycardia. The ventricular fibrillation threshold was increased as the blood alcohol concentration increased and the threshold value decreased as the alcohol level decreased with time. In the present study, control solution of 75% alcohol and 25% saline was infused into ten of the anesthetized animals. The heart rate did not change after infusion of 0.5 ml and a slight decrease in heart rate to about 380 beats per minute occurred after 1.5 ml of infusion. These volumes are above the average volume

(0.3 - 0.4 ml) of infusion of McN2165 used to induce ventricular fibrillation.

The effects of norepinephrine and isoproterenol on reserpinized animals was investigated. A dose of 4 µg/kg of norepinephrine was the highest dose that could be given without causing any cardiac arrhythmias. The maximum dose of isoproterenol was also in the same dosage range.

A dose of 2.5 mg/kg (IM) reserpine was given to the animals for the reserpinized experiment. The animals were sedated and exhibited some diuretic effects after 24 hours. The animals given intraperitoneal reserpine also appeared to have a lower body temperature.

Animals pretreated with atropine showed a mild increase in heart rate which meant the vagal influence was blocked. Likewise, animals pretreated with neostigmine showed decreased heart rate due to an increased vagal influence.

Animals pretreated with phenoxybenzamine had a higher heart rate than the control. This is a common effect of this agent. The animals pretreated with pronethalol had a lower heart rate due to *beta* adrenergic blockade.

Results

Effects of reserpine pretreatment on ventricular fibrillation induced by McN2165 (Table 1). The reserpinized animals were sedated and their heart rate was depressed from 420/min to 360/min and 240/min in a dose-related fashion. Pretreat-

TABLE 1

Effect of reserpine pretreatment on ventricular fibrillation induced by McN2165

Treatment	Fibrillation[a] threshold (mg/kg)	Fraction[b] fibrillating
Control	6.6 ± 1.0	20/30
Reserpine 1.25 mg/kg 72 hrs	7.2 ± 1.9	7/9
Reserpine 2.5 mg/kg 24 hrs	10.4 ± 3.5*	8/10
Reserpine 2.5 mg/kg 2 wks	8.1 ± 1.8*	9/9

[a]Fibrillation threshold is defined as the total dose (mg/kg) of the arrhythmogenic agent that was infused into the animal to cause ventricular fibrillation.

[b]Fraction fibrillating is defined as the ratio of the animals that demonstrated ventricular fibrillation to the total animals used in the experiment.

*significantly different from control ($p < 0.01$)

ment with reserpine (1.25 mg/kg IM, 72 hrs.) showed an insignificant increase in the fibrillation threshold to 7.2 ± 1.9 mg/kg. An intramuscular dose of 2.5 mg/kg (24 hrs. prior) of reserpine increased the threshold significnatly to 10.4 ± 3.5 mg/kg ($p < 0.01$). The same dose given two weeks before infusion raised the threshold to 8.1 ± 1.8 mg/kg ($p < 0.01$) which was still significantly different from the control.

McN2165 was administered by intravenous infusion to 23 rats. Twenty out of 23 animals produced ventricular fibrillation after a period of time. The average threshold dose to induce ventricular fibrillation was 6.6 ± 1.0 mg/kg. Three of the rats never exhibited ventricular fibrillation. Their hearts went into tachycardia then slowed to a stop as the infusion was continued. The McN2165 induced cardiac arrhythmia went gradually from tachycardia to ventricular flutter and terminated with ventricular fibrillation. The fibrillation lasted about 45 seconds to one minute. After the fibrillation the heart exhibited cardiac standstill with occasional beats.

Effects of autonomic drugs on ventricular fibrillation induced by McN2165 (Table 2). Immediately after injection of 0.05 mg/kg isoproterenol, the heart rate went up from 420/min to 540/min. Isoproterenol decreased the threshold dose from 6.6 ± 1.0 mg/kg to 4.4 ± 0.4 mg/kg ($p < 0.01$).

After injection of pronethalol, the heart rate decreased to about 300/min and then came back to about 380/min after

TABLE 2

Effect of autonomic drugs on ventricular fibrillation induced by McN2165

Treatment	Fibrillation[a] threshold (mg/kg)	Fraction[b] fibrillation
Control	6.6 ± 1.0	20/23
Isoproterenol 0.05 mg/kg	4.4 ± 0.4*	10/10
Pronethalol 2 mg/kg	7.3 ± 1.6	9/12
Pronethalol 5 mg/kg	7.5 ± 1.0#	9/15
Norepinephrine 4 µg/kg	7.5 ± 1.2#	9/9
Phenoxybenzamine 1 mg/kg	8.1 ± 1.7*	11/11
Neostigmine 0.1 mg/kg	8.3 ± 2.7#	6/9
Atropine 1 mg/kg	6.4 ± 0.8	6/6

[a]Fibrillation threshold is defined as the total dose (mg/kg) of the arrhythmogenic agent that was infused into the animal to cause ventricular fibrillation.

[b]Fraction filbrillating is defined as the ratio of the animals that demonstrated ventricular fibrillation to the total animals used in the experiment.

*significantly different from control ($p < 0.01$)

#significantly different from control ($0.02 < p < 0.05$)

10 minutes. Pronethalol at a dose of 2 mg/kg increased the threshold slightly but not significantly. Five mg/kg of pronethalol increased the fibrillation threshold to 7.5 ± 1.0 mg/kg ($0.02 < p < 0.05$).

Phenoxybenzamine 1 mg/kg increased the heart rate to 500/min. The fibrillation threshold was also increased to 8.1 ± 1.7 mg/kg ($p < 0.01$). The increased heart rate may be due to reflex vasodilation or potentiation of the effect of catecholamines on heart rate. This is a common effect with phenoxybenzamine (Stafford, 1963).

A dose of 4 µg/kg of norepinephrine and 0.1 mg/kg of neostigmine increased the fibrillation threshold to 7.5 ± 1.2 mg/kg ($0.02 < p < 0.05$) and 8.3 ± 2.7 mg/kg ($0.02 < p < 0.05$) respectively.

There was a slight increase in heart rate after 1 mg/kg of atropine but there was no change in the fibrillation threshold. The increased heart rate may be due to vagal blockage (Goodman and Gilman, 1970).

Effects of reserpine pretreatment and autonomic drugs on ventricular fibrillation induced by McN2165 (Table 3). Pretreatment with reserpine (2.5 mg/kg IM, 24 hrs.) and norepinephrine (4 µg/kg IV, 20 sec.) before McN2165 infusion raised the threshold to 10.5 ± 2.1 mg/kg ($p < 0.01$). While pretreatment with 2.5 mg/kg of reserpine (IM 24 hrs.) plus 0.05 mg/kg of isoproteronol (IV, 20 sec.) decreased the threshold to 4.5 ± 0.7 mg/kg ($p < 0.01$). These data imply

TABLE 3

Effect of reserpine pretreatment and autonomic drugs on ventricular fibillation induced by McN2165

Treatment	Fibrillation[a] threshold (mg/kg)	Fraction[b] fibrillating
Control	6.6 ± 1.0	20/23
Reserpine 2.5 mg/kg 24 hrs	10.4 ± 3.5*	8/10
Reserpine 2.5 mg/kg 24 hrs + norepinephrine 4 µg/kg	10.5 ± 2.1*	8/11
Reserpine 2.5 mg/kg 24 hrs + isoproterenol 0.05 mg/kg	4.5 ± 0.7*	10/10

[a]Fibrillation threshold is defined as the total dose (mg/kg) of the arrhythmogenic agent that was infused into the animal to cause ventricular fibrillation.

[b]Fraction fibrillating is defined as the ratio of the animals that demonstrated ventricular fibrillation to the total animals used in the experiment.

*significantly different from control ($p < 0.01$)

the involvement of the autonomic nervous system in the McN2165-induced arrhythmias.

Effects of reserpine pretreatment on ventricular fibrillation induced by various arrhythmogenic agents (Table 4). Strophanthin K induced ventricular fibrillation at 25.4 ± 2.4 mg/kg. Initially, the heart rate was decreased. This was followed by some disturbances of the QRS complex. Further infusion caused ventricular fibrillation and cardiac arrest. Pretreatment with 2.5 mg/kg reserpine (IM, 24 hrs.) increased the threshold to 33.5 ± 3.5 mg/kg ($p < 0.01$).

Barium chloride induced ventricular fibrillation at 21.7 ± 4.0 mg/kg. The heart went through tachycardia for a brief period followed by a sudden onset of ventricular fibrillation. Cardiac standstill occurred in about 30 seconds. Reserpinization raised the threshold dose approximatly 50%.

Aconitine induced ventricular fibrillation at 0.043 ± 0.01 mg/kg. This agent caused ventricular tachycardia and ventricular fibrillation within a short period of time. Pretreatment with reserpine at a dose of 2.5 mg/kg (IM, 24 hrs.) did not change the threshold. The same pretreatment (2.5 mg/kg 24 hrs.) given intraperitoneally raised the threshold to 0.093 ± 0.03 mg/kg ($p < 0.01$), which is a 110% increase.

Effect of pronethalol pretreatment (5 mg/kg IV, 10 min.) on ventricular fibrillation induced by various arrhythmogenic agents (Table 5). A dose of 5 mg/kg of pronethalol increased

TABLE 4

Effect of reserpine on ventricular fibrillation induced by various arrhythmogenic agents

Treatment	Fibrillation[a] threshold (mg/kg)		Fraction[b] fibrillating
Strophanthin K	25.4	2.4	7/7
Strophanthin K + Reserpine 25 mg/kg 24 hrs	33.5	3.5*	7/7
Barium chloride	21.7	4.0	9/9
Barium chloride + Reserpine 2.5 mg/kg 24 hrs	33.7	4.8*	11/11
Aconitine	0.043	0.01	10/10
Aconitine + Reserpine 2.5 mg/kg 24 hrs	0.053	0.02	6/6
Aconitine + Reserpine 2.5 mg/kg IP 24 hrs	0.093	0.03*	9/9

[a]Fibrillation threshold is defined as the total dose (mg/kg) of the arrhythmogenic agent that was infused into the animal to cause ventricular fibrillation.

[b]Fraction fibrillating is defined as the ratio of the animals that demonstrated ventricular fibrillation to the total animals used in the experiment.

*significantly different from control ($p < 0.01$)

TABLE 5

Effect of pronethalol on ventricular fibrillation induced by various arrhythmogenic agents

Treatment	Fibrillation[a] threshold (mg/kg)	Fraction[b] fibrillating
Strophanthin K	25.4 ± 2.5	7/7
Strophanthin K + Proethalol 5 mg/kg	36.4 ± 6.4*	4/10
Barium chloride	21.7 ± 4.0	9/9
Barium chloride + Pronethalol 5 mg/kg	38.5 ± 8.0*	8/8
Aconitine	0.042 ± 0.01	10/10
Aconitine + Pronethalol 5 mg/kg	0.055 ± 0.01#	7/7

[a]Fibrillation threshold is defined as the total dose (mg/kg) of the arrhythmogenic agent that was infused into the animal to casue ventricular fibrillation.

[b]Fraction fibrillating is defined as the ratio of the animals that demonstrated ventricular fibrillation to the total animals used in the experiment.

*significantly different from control ($p < 0.01$)

#significantly different from control ($0.02 < p < 0.05$)

the fibrillation threshold significantly for all three arrhythmogenic agents. The threshold dose for strophanthin K, barium chloride and aconitine after pretreatment with 5 mg/kg of pronethalol (IV, 10 min.) were 36.4 ± 6.4 mg/kg ($p < 0.01$), 35.5 ± 8.0 mg/kg ($p < 0.01$) and 0.055 ± 0.01 mg/kg ($0.02 < p < 0.05$) respectively.

Effect of isoproterenol pretreatment (0.05 mg/kg IV, 20 sec.) on ventricular fibrillation induced by various arrhythmogenic agents (Table 6). Isoproterenol decreased the fibrillation threshold of strophanthin K to 15.7 ± 4.2 mg/kg ($p < 0.01$) and increased the fibrillation threshold of barium chloride to 31.9 ± 4.3 mg/kg ($p < 0.01$). The fibrillation threshold of aconitine was not significantly altered by isoproterenol pretreatment.

Effect of pronethalol 5 mg/kg on cessation of ventricular fibrillation (Table 7). A intravenous dose of 5 mg/kg of pronethalol, given at the onset of ventricular fibrillation induced by McN2165, restored the normal rhythm in 11 out of 11 animals. None of the eight animals stopped ventricular fibrillation after induction by barium chloride. Also, no aconitine-induced fibrillation could be halted with 5 mg/kg of pronethalol.

Discussion

The production of ventricular arrhythmias by McN2165 in anesthetized dogs was first published by Pruss (1969).

TABLE 6

Effect of isoproterenol on ventricular fibrillation induced by various arrhythmogenic agents

Treatment	Fibrillation[a] threshold (mg/kg)		Fraction[b] fibrillating
Strophanthin K	25.4	2.5	7/7
Strophanthin K + Isoproterenol 0.05 mg/kg	15.7	4.2*	8/8
Barium chloride	21.7	4.0	9/9
Barium chloride + Isoproterenol 0.05 mg/kg	31.9	4.3*	8/8
Aconitine	0.042	0.01	10/10
Aconitine + Isoproterenol 0.05 mg/kg	0.051	0.01	8/8

[a]Fibrillation threshold is defined as the total dose (mg/kg) of the arrhythmogenic agent that was infused into the animal to cause ventricular fibrillation.

[b]Fraction fibrillating is defined as the ratio of the animals that demonstrated ventricular fibrillation to the total animals used in the experiment.

*significantly different from control ($p < 0.01$)

TABLE 7

Effect of 5 mg/kg of pronethalol on recovery from ventricular fibrillation

Treatment	Fraction Recovery[a]
McN2165	0/20
McN2165 + Pronethalol	11/11
Barium chloride	0/9
Barium chloride + Pronethalol	0/8
Aconitine	0/10
Aconitine + Pronethalol	0/8

[a]Animals were given sufficient arrhythmogenic agent to induce fibrillation. At this point, 5 mg/kg of pronethalol was given. Fraction recovery is the ratio of those who returned to normal rhythm to the total number that fibrillated

Further studies by Eyer and Johnson (1977) showed that McN2165 slowed the heart rate and increased the cyclic AMP level in isolated fetal rat heart preparations. The same study also found that McN2165 could not induce tachycardia or ventricular fibrillation in such a preparation. One possible explanation could be that the fetal rat did not have a well developed sympathetic nervous system. Since sympathetic activity might be involved, reserpine was used to determine the relationship between catecholamines and fibrillation. This drug has a biphasic effect on the heart rate (Krayer, *et al*, 1958). After reserpine administration, the heart rate increased for the first few hours due to the release of catecholamines, then decreased for about two to three weeks due to the depletion of these amines. About four weeks was required for the animals to return to normal levels of catecholamines. Direct cardiac effects of reserpine include depression of excitable membranes (Innes, *et al*, 1958). The ability of reserpine to increase the fibrillation threshold was most likely due to the depletion of catecholamines. In this study the antifibrillatory action persisted for more than four weeks. The possibility of a direct membrane effect of reserpine can be ruled out because the antifibrillatory action persisted long after the drug had been excreted. A 1.25 mg/kg dose of reserpine caused less sedation than a dose of 2.5 mg/kg and did not significantly raise the fibrillation threshold (Table 1). This was probably due to less depletion of the catecholamines.

The 2.5 mg/kg dose (24 hrs prior) was a sufficient dose to deplete all the catecholamines without much membrane effect (Boyajy, *et al*, 1965). Therefore, the increase in ventricular fibrillation threshold by reserpine implied that catecholamines might play a role in the fibrillatory action of McN2165.

A dose of 0.1 mg/kg of neostigmine increased the fibrillation threshold significantly while atropine did not change the threshold. Large doses of acetylcholine are known to convert the atrial flutter induced by aconitine into fibrillation through the development of multiple re-entries in the atria (Sharma, 1963; Burn, *et al*, 1956). It was also found that acetylcholine or carbachol was able to inhibit the synthesis of cyclic AMP by 20 to 30% in dog cardiac muscle preparation (Murad, *et al*, 1961). This increase in threshold by neostigmine can be explained if McN2165-induced fibrillation requires increased cyclic AMP levels. McN2165 has been shown to elevate cyclic AMP levels (Eyer and Johnson, 1977). The lack of effect of atropine on McN2165 was in agreement with Pruss' (1969) finding.

Blockade of the *alpha* and *beta* receptors by 1 mg/kg of phenoxybenzamine and 2 mg/kg or 5 mg/kg of pronethalol increased the fibrillation threshold. This implied that both *alpha* and *beta* receptors were involved in the induction of fibrillation. Only 9 out of 15 rats pretreated with pronethalol fibrillated. The other six animals demonstrated no

arrhythmias whatsoever. All the animals pretreated with pnehoxybenzamine underwent ventricular fibrillation. This indicated the involvement of *beta* receptors in the arrhythmias induced by McN2165, although the agent might have acted on both *alpha* and *beta* receptors. Pronethalol itself had some transient cardiac depressant actions similar to quinidine (Sekiya, *et al*, 1963). It is also known that *beta* receptor blockers are effective in preventing the *beta*-stimulated elevation of cyclic AMP levels in the cardiac tissue (Dobson, *et al*, 1973).

The norepinephrine data was difficult to explain because it increased the fibrillation threshold (Table 2). This could be due to the biphasic effects catecholamines have on the fibrillation threshold. In low doses, catecholamines increased the ventricular fibrillation threshold and in high doses it lowered the threshold (MacConaill, *et al*, 1967, Hoffman, *et al*, 1955). Evidently the high dose that lowered the threshold was not attained. Another explanation could be that norepinephrine acted more on the *alpha* receptors than the *beta* receptors. *Alpha* receptor activation may be related to decrease in the intracellular level of cyclic AMP (Turtle, *et al*, 1967). Isoproterenol, a potent *beta* agonist, decreased the fibrillation threshold (Table 2). *Beta* agonists also effectively increase cyclic AMP levels in the heart (Sutherland, *et al*, 1960). In reserpinized animals, isoproterenol was capable of lowering the fibrillation threshold.

This was not possible with norepinephrine. This was further evidence that *beta* receptors are more involved than *alpha* receptors in the arrhythmogenesis produced by McN2165.

McN2165 has been shown to slow conduction of the action potential in the heart, block the A.V. node (Pruss, 1969), and increase the cyclic AMP level (Eyer and Johnson, 1977). High cyclic AMP levels have been implicated in ventricular fibrillation (Opie, *et al*, 1976). Neostigmine and pronethalol, agents that lower the cyclic AMP levels in the heart, increased the McN2165 fibrillation threshold. Isoproterenol increased the level of cyclic AMP and decreased this threshold. Only isoroterenol could decrease the fibrillation threshold of reserpinized animals to the control value. Thus, elevation of cyclic AMP levels in the heart are implicated in the fibrillatory response to McN2165.

Of the three arrhythmogenic agents compared with McN2165, only strophanthin K and barium chloride elevated the fibrillation threshold in reserpinized animals (2.5 mg/kg 24 hrs prior to treatment). Tatsuno (1976) also found that sympathetic activities were involved in the production of arrhythmias by strophanthin K. Blockage of strophanthin K-induced arrhythmias by a *beta*-adrenergic antagonist is well documented (Somani and Lum, 1965; Sekiya and Vaughan Williams, 1963). Lowered fibrillation threshold following isoproterenol pretreatment further demonstrated that *beta* sympathetic activities are involved in the induction of ventricular fibrillation.

Pretreatment with reserpine, pronethalol and isoproterenol all increased the fibrillation threshold of barium chloride. Barium chloride causes arrhythmias by direct myocardial irritation. It resembles calcium in its ability to produce extrasystoles, increase cardiac automaticity and induce ventricular fibrillation (McMillan, *et al*, 1928). It is possible that the slight membrane effect of reserpine or pronethalol was able to suppress the irritation of barium chloride, so that it took more barium chloride to irritate the myocardial muscle, while isoproterenol increased the heart rate and force which made it more difficult for barium chloride to disturb the increased rhythm.

A dose of 2.5 mg/kg of reserpine (IM 24 hrs. prior) did not alter the fibrillation threshold of aconitine. The same dose given intraperitoneally increased the threshold significantly. This showed that either catecholamines may not be involved in the arrhythmia or only a low level of catecholamines is necessary for aconitine-induced fibrillation. The increase in threshold might be due to the enhanced membrane activity of reserpine because intraperitoneal injection produced higher blood levels of the drug (Goldstein, 1974). Another explanation could be that a minute amount of catecholamines is required for aconitine to induce fibrillation. With a higher blood concentration of reserpine, there will be a greater depletion of catecholamines, which could increase the amount of aconitine required to induce fibrillation.

Isoproterenol pretreatment did not change that threshold. This is further evidence that catecholamines were not involved in the arrhythmias or that only small amounts are needed. Pronethalol increased the aconitine fibrillation threshold, contrary to the data of Tatsuno, *et al*, (1976). The quinidine-like or local anesthetic effect is unlikely because administration of pronethalol failed to suspend the fibrillation induced by aconitine. Pronethalol could have some other unknown effects on suppressions of ectopic activities of aconitine.

A dose of 5 mg/kg of pronethalol was injected intravenously during the ventricular fibrillation induced by McN2165, barium chloride, and aconitine (Table 7). Only McN2165-induced fibrillation was suspended. These hearts returned to normal rhythm. Barium chloride-and aconitine-induced fibrillation were not suspended by pronethalol. The fibrillation continued until cardiac arrest occurred.

CHAPTER 4

SUMMARY

McN2165 is an arrhythmogenic agent that produces ventricular fibrillation in rats. In order to investigate the mechanism of action of this agent, autonomic agonists and antagonists were employed to modify the physiological states of the rat heart. The influence of these autonomic drugs on McN2165-induced ventricular fibrillation was compared with other arrhythmogenic agents. Pretreatment with either reserpine, pronethalol, norepinephrine, phenoxybenzamine or neostigmine increased the fibrillation threshold of McN2165. Atropine pretreatment did not change the fibrillation threshold, while isoproterenol pretreatment decreased the fibrillation threshold. Norepinephrine failed to decrease the fibrillation threshold of the reserpinized animals. Isoproterenol was the only autonomic drug that could decrease the McN2165 fibrillation threshold in the reserpinized animals. When fibrillation was induced by McN2165, administration of pronethalol restored normal cardiac rhythm.

The fibrillation threshold of strophenthin K was increased by either reserpine or pronethalol pretreatment. Isoproterenol was able to lower the fibrillation threshold

but pronethalol was not able to stop the fibrillation induced by the agent.

Pretreatment with either reserpine, pronethalol or isoproterenol raised the fibrillation threshold of barium chloride. Pronethalol was not able to stop the fibrillation induced by barium chloride.

Intraperitoneal injection of reserpine and pretreatment with pronethalol increased the fibrillation threshold of aconitine. Isoproterenol did not change the fibrillation threshold of aconitine. Aconitine-induced ventricular fibrillation could not be halted by pronethalol.

These data demonstrate a stronger involvement of the *beta* receptor in the action of McN2165 than for the other arrhythmogenic agents studied. Although these agents resembled McN2165 in some respects, certain differences in their mechanism of action have been demonstrated.

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